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Immunocytochemical studies on the pituitary pars distalis of the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*

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Summary. Immunocytochemical studies were performed to describe the characteristics of cell types and their distribution in the pars distalis of Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*, collected at various stages of the reproductive cycle. Six distinct cell types have been identified in the pars distalis by the unlabeled immunoperoxidase technique and by the ABC method. Growth hormone (GH) and prolactin (PRL) cells were immunostained with antisera against chicken GH and ovine PRL. The GH-immunoreactive cells were round or oval orangeophilic cells distributed throughout the pars distalis with prominent aggregation in the posterolateral region. The PRL cells were pleomorphic carminophilic cells that occurred in small groups within the central and dorsocaudal regions of the pars distalis. They were sparsely distributed in the central region of the pars distalis in the hibernating bats, but increased significantly in the pregnant and lactating bats. The adrenocorticotropic (ACTH) cells were large round or polygonal amphophilic cells in the rostroventral and ventrolateral regions of the pars distalis. The thyrotropic (TSH) cells were small rounded or polygonal and distributed mainly in the ventrolateral region of the pars distalis. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) cells were identified immunocytochemically with antisera against the specific beta subunits of ovine LH and rat FSH. There were two populations of LH and FSH cells, one aggregated in the zona tuberalis and the other scattered singly throughout the rest of the pars distalis. The aggregated cells were immunoreactive with both antisera directed to LH and FSH, while scattered cells were reactive solely with antiserum to either LHβ or FSH and exhibited seasonal variations. In females, the proportional volume of the pars distalis occupied by LH cells was significantly reduced during pregnancy and lactation. No evidence of involution was observed in pars distalis cells except for PRL cells in males or females during hibernation.

Key words: Immunocytochemistry – Hibernation – Pars distalis – *Miniopterus schreibersii fuliginosus* (Chiroptera)

The unusual features of reproduction in hibernating bats, such as delayed implantation, are of special interest due to the various adaptive specializations of the reproductive organs and their functions. Although the hypothalamo-hypophysial system plays a major role in the regulation of the reproductive cycle, there is little information about its structural and functional correlations with reproductive function. The chiropteran pituitary gland has been examined previously by tinctorial and histochemical techniques (Herlant 1964; Purves 1966; Baker 1974). In the pars distalis, five chromophilic cell types, three types of basophils and two types of acidophils, have been distinguished; their secretory functions were deduced on the basis of cytological alterations as correlated with the reproductive cycles. Recently, through the utilization of immunocytochemical techniques, Richardson (1979, 1981a, b) identified cells that secrete prolactin, growth hormone and gonadotropic hormones in the pars distalis of the California leaf-nosed bat, *Macrotus californicus*, and Anthony and Gustafson (1984a) demonstrated seasonal variations in pituitary LH-gonadotropes of the hibernating bat, *Myotis lucifugus lucifugus*. However, very little information is available with respect to the role of the pituitary gland in regulation of specific reproductive events.

The purpose of this study was to explore the role of the pars distalis in the regulation of seasonal reproductive phenomena in *Miniopterus schreibersii fuliginosus*. In this species, copulation and fertilization occur in autumn but implantation is delayed until midwinter. Females give birth in July. Six types of pituitary cells were identified in male and female bats by the light-microscopic immunoperoxidase method. Seasonal variations in cell population and morphology were documented in both sexes in relation to annual reproductive events.

Materials and methods

Six adult male and 12 adult female Japanese long-fingered bats were collected from the Oga peninsula, Akita prefecture, Japan between November 1985 and July 1986. The animals were obtained at their natural hibernacula in November, and at maternity colonies in July. Bats obtained in November (beginning of hibernation) consisted of 3 males and 3 females and among those obtained in July were 3 males and one pregnant, 3 lactating females. The other 5 females collected in July were mostly young non-reproductive individuals. After capture, bats were placed in special cages and transported to the laboratory under conditions designed to minimize stress (cf. King et al. 1984).

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Table 1. Antisera primarily used for demonstration of pituitary cell types

Cell type	Antiserum	Working dilution	Source
GH cell	Anti-chicken GH	1/1000	Dr. C.G. Scanes
PRL cell	Anti-ovine PRL	1/1000	Miles Lab. Inc
ACTH cell	Anti-porcine $ACTH^{1-39}$24H2T	1/3000	Immuno Nuclear Co. Ltd.
FSH cell	Anti-rat FSH S-9	1/200	NIAMDD
LH cell	Anti-rat LH S-4	1/1000–2000	NIAMDD
	Anti-ovine LHβ	1/5000–10000	Dr. T. Yashiro
TSH cell	Anti-rat TSH S-4	1/500–1000	NIAMDD

Pituitary glands were removed from all bats within 24 h of capture; gonads were removed at the same time. The bats were killed by a cardiac puncture to take blood and perfused with Bouin's solution without acetic acid. The pituitary gland was removed together with the brain, and fixed in the same fixative for an additional 4 to 12 h. The tissues were dehydrated in an ascending series of ethanol, cleared in xylene, embedded in paraffin and serially sectioned in sagittal, frontal and horizontal planes at 3 to 4 μm. Serial sections were placed on separate slides to make a series consisting of 6 slides to be stained by different antisera. Serial sections were also stained tinctorially by trichrome (Goldberg and Chaikoff 1952) and tetrachrome (Herlant 1964) methods.

Immunohistochemistry

All sections of the pituitary series were deparaffinized in xylene, hydrated through a descending ethanol series, and equilibrated in 0.02 M phosphate-buffered saline (PBS; pH 7.4). Immunocytochemical staining was performed by the unlabeled antibody peroxidase antiperoxidase complex (PAP) technique or by avidin biotin peroxidase (ABC) method using antisera to each pituitary hormone. The details of antisera used primarily for demonstration of pituitary cell types are shown in Table 1. In addition to these antisera, many others such as anti-rat PRL S-6, S-8, S-9 (NIAMDD), anti-human PRL (UCB-Bioproduct), anti-mouse PRL (NIAMDD), anti-human TSH β (UCB-Bioproduct) and anti-turkey LHβ (Dr. W.H. Burke) were also used, although they showed only non-specific or weak reactions. The staining procedures by ABC method involved the following steps: (1) incubation in a primary antiserum at 4° C for 12–24 h, (2) washing in PBS for 15 min, (3) incubation in the biotinylated anti-rabbit IgG at 32° C for 30 min, (4) washing in PBS for 15 min, (5) incubation in ABC at 32° C for 30 min, (6) washing in PBS for 15 min, (7) incubation in a medium containing 0.003% 3-3′diaminobenzidine tetrahydrochloride (DAB) and 0.002% hydrogen peroxide (H_2O_2) in 0.05 M Tris-buffer at pH 7.6 for 15–30 min, (8) washing in distilled water for 15 min, (9) treatment with osmium vapor for 15–30 sec.

Control immunocytochemical stainings were performed as follows: (1) use of normal rabbit serum or PBS to replace the primary antiserum, (2) use of specific antisera previously absorbed with each specific antigen, (3) use of PBS to replace the biotinylated IgG or ABC. No specific immunocytochemical reactions were observed in these control stainings.

Morphometric analysis

The percentages of the volume of the pars distalis occupied by each type of secretory cells were calculated using image-processing system (TOSPIX, Toshiba Ltd.) photoanalyzer. Serial tissue sections immunostained by each of the antisera were photographed and enlarged to 12 × 16 cm on high-contrast paper. The photoimages were processed by establishing the threshold to gain two-valued images, where reactive cells were black and non-reactive or non-specifically stained areas were white. Only the black areas were measured mechanically with a TOSPIX image-processing system to gain the percentages of area occupied by reactive cells. Numbers of series used for morphometry were two to five for each animal; at least six sections reacted with different antisera from each series were examined. The cell types were identified by their immunocytochemical reactions using the respective antisera to pituitary hormones. The significance of the difference to value of normal female was determined by Student's *t*-test.

Results

General structure of the pituitary gland

The pituitary gland of *Miniopterus schreibersii fuliginosus* was dorsoventrally compressed and roughly trapezoidal in shape, with the broad wings of pars distalis directed posteroventrally (Figs. 1–4). The gland lay in the sella turcica enclosed in the meninges between the floor of the brain and the basisphenoid bone. The infundibulum was situated on the middorsal surface of the pars distalis and caudally directed to terminate in the pars nervosa. The pars tuberalis formed a thin cell layer covering the ventral surface of the median eminence and surrounding the infundibular stalk. The pars intermedia was well developed between the pars distalis and pars nervosa, practically enclosing the ventral and lateral regions of the pars nervosa. The residual lumen (hypophysial cleft), usually present between the pars distalis and pars intermedia, was surrounded by a single layer of low cuboidal cells (Figs. 1 and 3). Many colloidal cysts were present in the pars distalis, especially in its lateral wings (Fig. 2).

The pars distalis, which consisted of anastomosing cell cords surrounded by a thin layer of connective tissue and interspersed with numerous sinusoids, was roughly divided into rostral and caudal parts, which were distinguishable from each other by difference in distribution of cell types.

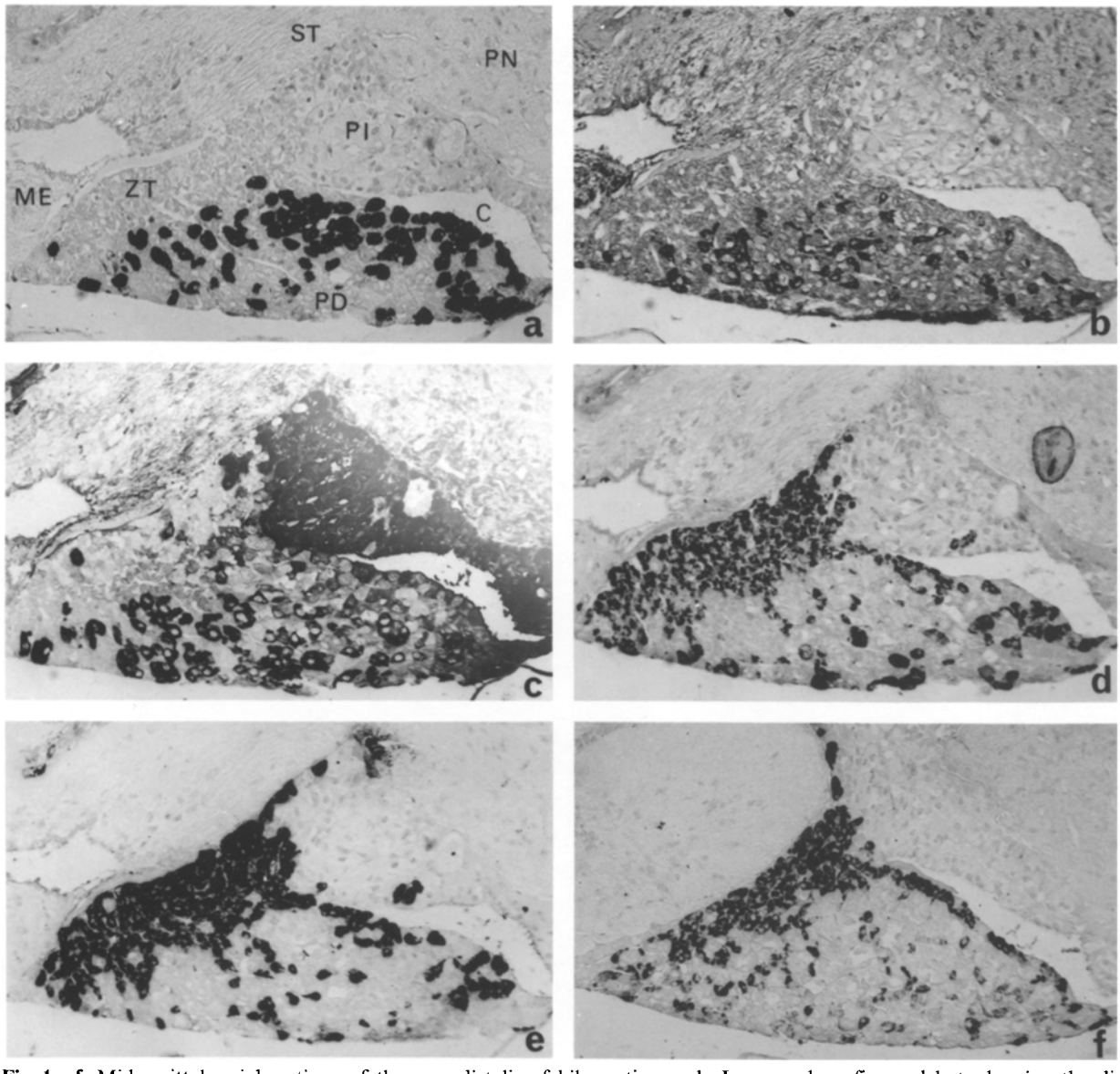

Fig. 1 a–f. Mid-sagittal serial sections of the pars distalis of hibernating male Japanese long-fingered bat, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH (**a**), ovine PRL (**b**), porcine ACTH (**c**), rat TSH (**d**), ovine LHβ (**e**) and rat FSH (**f**), respectively. *c* hypophysial cleft; *ME* median eminence; *ST* infundibular stalk; *PD* pars distalis; *PI* pars intermedia; *PN* pars nervosa; *ZT* zona tuberalis. × 210

The rostral part was characterized by the presence of numerous basophils and a paucity of acidophils, whereas the caudal part had a predominance of acidophils. In addition to these two parts, there was a special region consisting of small basophilic cells closely adjacent to the infundibular stalk (Fig. 1). This area, designated as the zona tuberalis, consisted of LH- and FSH-immunoreactive cells.

Cell types of the pars distalis

Six types of glandular cells, GH, PRL, ACTH, TSH, LH and FSH cells, were readily identified in the pars distalis of the bats by immunocytochemical methods. The percentage of an area occupied by each type of secretory cell in a section of the pars distalis, estimated by the image-processing system, is shown in Table 2.

Growth hormone (GH) cell. Cells immunoreactive to GH were identified and immunocytochemically distinguished from other pituitary cells. They were round or oval in shape and contained a small round nucleus and coarse orangeophilic secretory granules in the cytoplasm. They were the most abundant type of cells, distributed singly or tending to be arranged in palisades along the capillaries. They were distributed throughout the pars distalis, with prominent augmentation in the cell population of the dorsolateral and posterolateral regions. The distribution of GH cells was very similar to that of the PRL cells. The mean percentage of an area occupied by GH cells in the pars distalis of normal female bats was 29.9%, while those of hibernating, pregnant, and lactating bats were 22.2%, 24.3% and 22.3%, respectively (Table 2), showing the slight decrease in the percentage of GH cells in the hibernating and lactating bats.

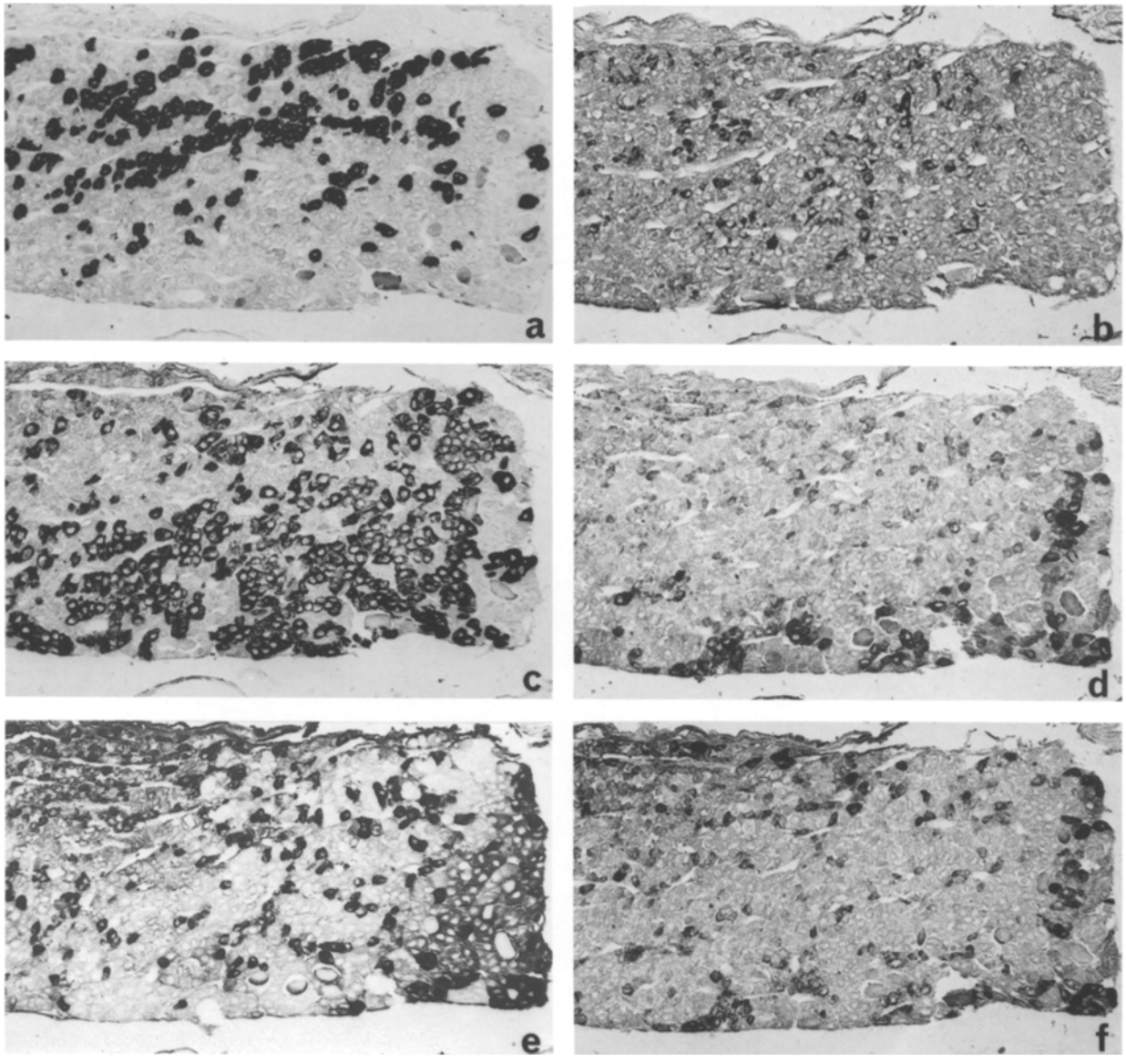

Fig. 2a–f. Serial frontal sections through the anterior portion of the pars distalis of hibernating female Japanese long-fingered bat, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH (**a**), ovine PRL (**b**), porcine ACTH (**c**), rat TSH (**d**), ovine LHβ (**e**) and rat FSH (**f**), respectively. ×210

Table 2. Percentage of an area occupied by each type of secretory cell in the pars distalis of various reproductive phases of female bats

Groups	No of bats	Series of sections measured	GH	PRL	ACTH	TSH	LH	FSH
Young normal	4	7	29.9±8.6	13.7±1.9	19.0±5.6	6.2±4.3	19.9±5.6	9.4±3.8
Hibernating	3	5	22.2±2.1*	8.1±1.7***	26.2±2.1**	11.0±2.1*	21.3±2.5	11.4±1.8
Pregnant	1	5	24.3±3.0	37.2±3.5***	14.9±2.6	5.5±2.9	11.4±1.6***	6.7±1.9
Lactating	3	8	22.3±6.3*	27.7±1.4***	19.4±3.8	5.5±2.4	15.4±3.9*	9.7±2.1

Mean±S.D. * $p<0.05$ vs normal female; ** $p<0.01$ vs normal female; *** $p<0.001$ vs normal female

Prolactin (PRL) cell. In non-lactating (normal) bats collected in July, PRL-immunoreactive cells were usually observed as small groups located primarily in the central and dorsocaudal regions of the pars distalis; they were scarce in the rostral region. These cells, which were generally pleomorphic, contained coarse carminophilic secretory granules. Their nuclei were large, round to oval and eccentrically located within the cytoplasm (Fig. 5a).

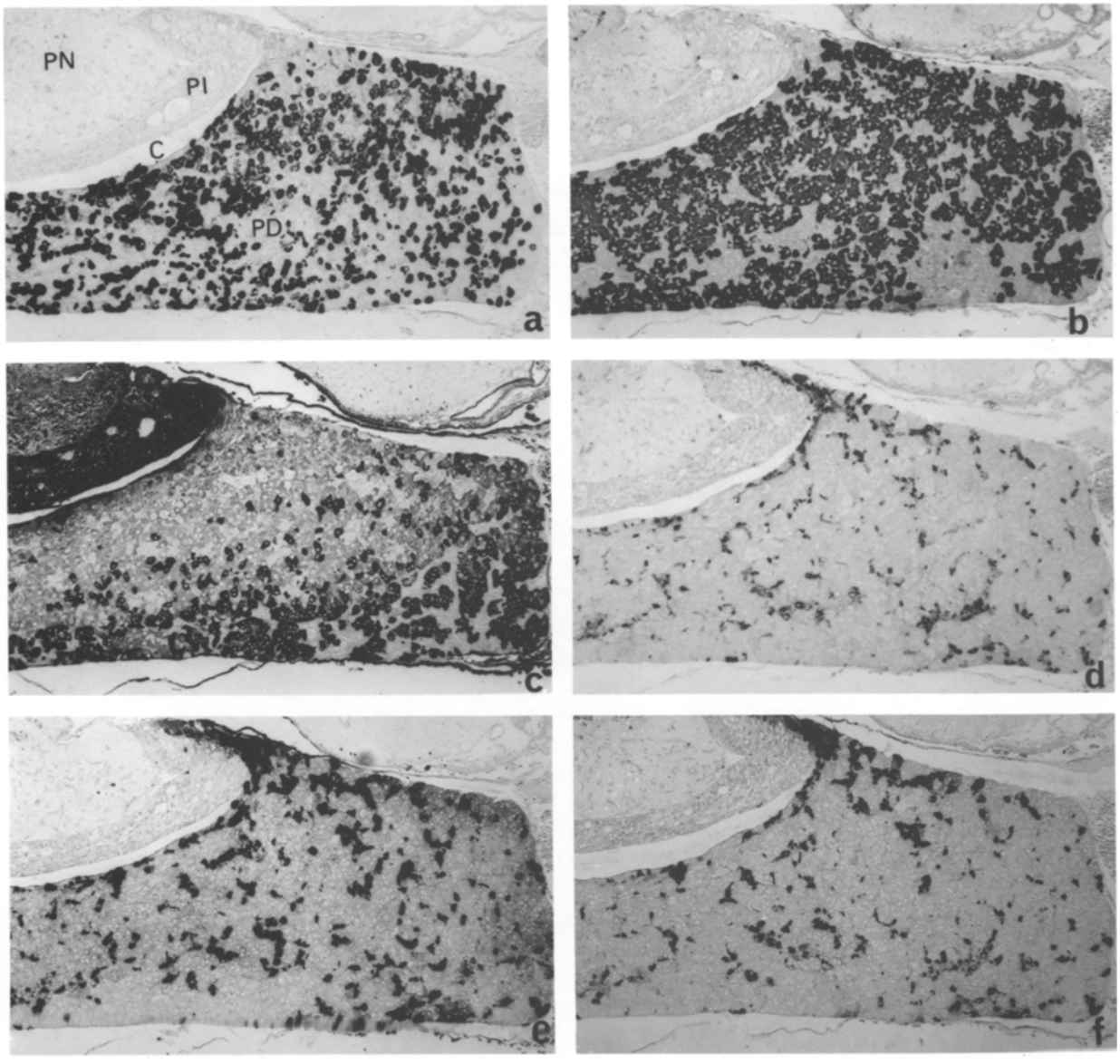

Fig. 3a–f. Serial frontal sections through the anterior portion of the pars distalis of pregnant Japanese long-fingered bat, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH (**a**), ovine PRL (**b**), porcine ACTH (**c**), rat TSH (**d**), ovine LHβ (**e**) and rat FSH (**f**), respectively. *c* Hypophysial cleft; *PD* pars distalis; *PI* pars intermedia; *PN* pars nervosa. ×110

In hibernating bats, the pituitary gland was almost devoid of intensely immunoreactive PRL cells. PRL-immunoreactive cells were very small in size and sparsely distributed in the central area of the pars distalis (Figs. 1b, 2b). In the pregnant bat, the PRL cells, which were enlarged remarkably, increased in number to form large groups of cells located throughout the gland except for its small ventral area (Figs. 3b, 5b). In lactating bats, the PRL cells were large, polygonal in shape, and extremely numerous throughout the pars distalis (Figs. 4b, 5c). The mean percentage of area occupied by PRL cells varied remarkably as a function of phase of reproduction from a minimum of 8.1% in the hibernating bats to a maximum of 37.2% in the pregnant bat. In lactating bats 27.7% of the area was occupied by PRL cells (Table 2). The PRL cells decreased significantly in hibernating bats, but significantly increased in pregnant and lactating bats.

Adrenocorticotropic (ACTH) cell. ACTH-immunoreactive cells were large, rounded or polygonal and usually observed as small groups in the rostroventral and ventrolateral regions of the pars distalis (Figs. 1c, 2c, 3c, 4c). They contained coarse amphophilic secretory granules which were immunopositive to anti-pACTH serum. The mean percentage of the area occupied by the ACTH cells of the pars distalis varied from a minimum of 14.9% in the pregnant bat to a maximum of 26.2% in hibernating bats. The lactating bats showed 19.4% of the area to be occupied by ACTH cells (Table 2).

Thyrotropic (TSH) cell. TSH cells were small rounded or polygonal cells but variable in form and number according season. Though found throughout the pars distalis, they were prominent in the ventrolateral region of the pars distalis (Figs. 1d, 2d, 3d, 4d). In hibernating bats, the TSH

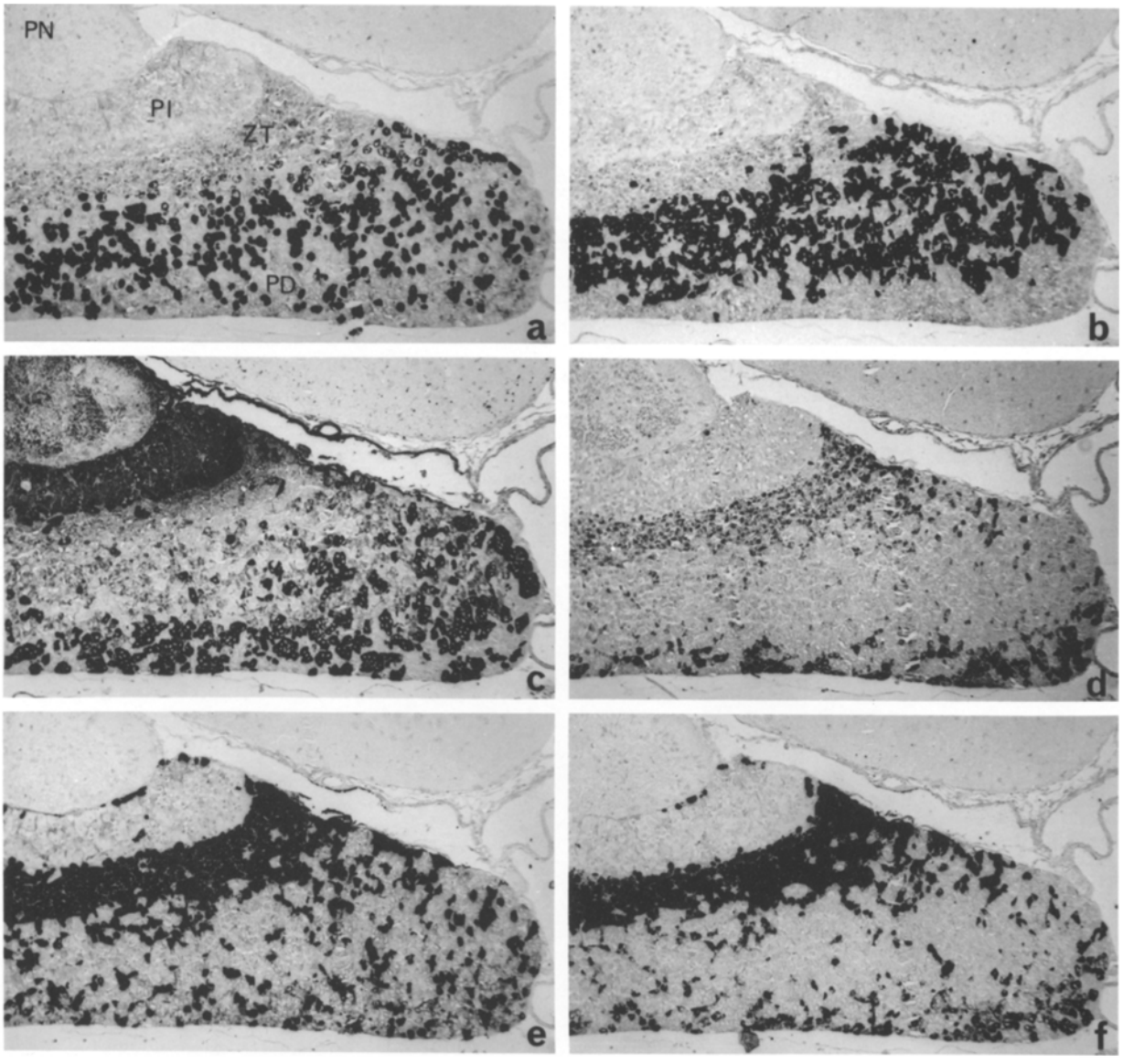

Fig. 4a–f. Serial frontal sections through the anterior portion of the pars distalis of lactating Japanese long-fingered bat, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH (**a**), oving PRL (**b**), porcine ACTH (**c**), rat TSH (**d**), ovine LH (**e**), and rat FSH (**f**), respectively. *PD* pars distalis; *PI* pars intermedia; *PN* pars nervosa; *ZT* zona tuberalis. ×110

cells were relatively numerous (11.0% of area), large and round in shape, while in pregnant and lactating bats, they were small and polygonal, occupying only small percentages (5.5% and 5.5%, respectively) of the area of the pars distalis (Table 2). Some of TSH-immunopositive cells also showed positive reaction to anti-FSH serum, while TSH-immunopositive cells in the zona tuberalis were immunonegative to anti-TSH serum preabsorbed with LH.

Gonadotropic (LH and FSH) cells. Gonadotrophs exhibiting LH-immunoreactivity were irregular in shape and contained a small, round nucleus and fine basophilic secretory granules. Most of these cells also reacted with antiserum to rFSH, as evidenced by comparison of adjacent sections stained with antisera to rLH or rFSH (Figs. 2e, f, 3e, f, 4e, f). However, there were cells that appeared to react solely with anti-rLH serum. The concentration of LH and FSH seemed to vary from cell to cell. They were scattered singly or in small groups throughout the pars distalis in both sexes. An aggregation of LH- and FSH-immunoreactive cells was consistently observed in the zona tuberalis adjacent to the infundibular stalk. These cells were generally smaller and irregular in form than the scattered cells and contained more diffuse reaction product. The FSH-immunoreactive cells were relatively numerous in the ventral and lateral portions of the pars distalis. They were distributed similarly to TSH-immunoreactive cells.

LH-immunoreactive cells in bats collected in July were large, round or oval in shape and distributed throughout the pars distalis. They were conspicuously aggregated in the zona tuberalis. LH-immunoreactive cells in the hibernating bats were also large in size and distributed through-

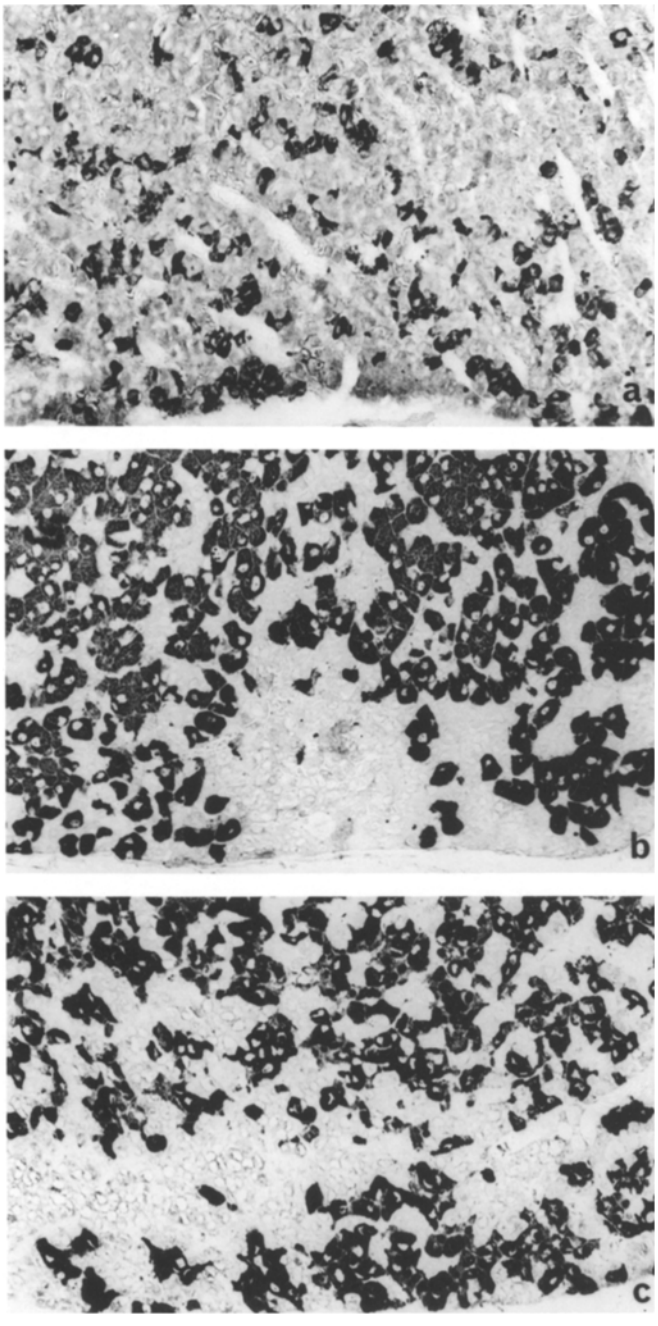

Fig. 5a–c. Frontal sections of the pars distalis of normal female (**a**), pregnant (**b**) and lactating (**c**) Japanese long-fingered bats, showing the distribution of PRL cells stained immunocytochemically with antiserum against ovine PRL. ×240

out the gland (Figs. 1e and 2e). The percentages of area occupied by LH and FSH cells in hibernating bats were 21.3% and 11.4%, respectively (Table 2). In pregnant and lactating bats, LH-immunoreactive cells were very small in size, irregular in form and sparsely distributed throughout the pars distalis (Figs. 3e, 4e). The percentages of area occupied by LH cells in pregnant and lactating bats were 11.4% and 15.4%, respectively, showing significant reduction, but those of FSH cells were 6.7% and 9.7%, respectively.

Discussion

The gross anatomical features of the pituitary of the *Miniopterus schreibersii fuliginosus* were, in general, similar to those described in other bats (*Myotis myotis*, Herlant 1956; *Macrotus californicus*, Richardson 1979, 1981a, b; *Myotis lucifugus*, Anthony and Gustafson 1984a, b). In these species, a well-developed pars distalis and pars intermedia are present, the latter adhering to the pars nervosa. Green (1951) reported the presence of a pars tuberalis, which completely surrounds the median eminence, in *Myotis lucifugus*, whereas Richardson (1981a) was unable to identify any pars tuberalis in the *Macrotus californicus*. In the present study on *M. s. fuliginosus*, the pars tuberalis was observed to surround the median eminence and infundibular stalk completely as a broad collar. The pars tuberalis consisted of one or two layers of the cells, some of which were immunopositive to rLH antiserum. The residual lumen (hypophysial cleft) was always observed to be surrounded by a single layer of cuboidal cells between the pars distalis and pars intermedia, as reported by Patil (1974) in leaf-nosed bats, Hipposideridae.

The histological organization of the pars distalis of *M. s. fuliginosus* resembled that described in other bats, in which the pars distalis is roughly divided into rostral and caudal parts by the difference of regional distribution of cell types. However, there was a special portion enriched in small gonadotropic (LH and FSH) cells in the pars distalis closely adjacent to the infundibular stalk. This portion of the pars distalis may be homologous to the "zona tuberalis" described by Dawson (1937) in the cat and rabbit and by Hanström (1952) in many species of mammals. The zona tuberalis borders the pars tuberalis in most species and continues to a variable extent through the median anteroventral region of the pars distalis. The portal vessels enter the pars distalis in the region of the "zona tuberalis" or "sex zone" in bat (Green 1951; Herlant 1953) as well as in non-chiropteran mammals (Mikami 1980). It is particularly interesting to note that the aggregation of gonadotropic cells occurs in the portions adjacent to the portal vessels and to the infundibular stalk which is rich in immunoreactive LHRH fibers. The proximity of these gonadotrophs to LHRH fibers suggests that these gonadotrophs may receive hypothalamic peptides both via the portal vessels and through simple diffusion (cf. Anthony et al. 1984c).

Richardson (1981a) has recently demonstrated the presence of GH and PRL cells in the pars distalis of *Macrotus californicus*. He reported that the PRL cells, which were pleomorphic and carminophilic, occurred in small groups within the ventro-, dorsocentral and posterolateral regions of the pars distalis, while the GH cells, which were small and orangeophilic, were randomly scattered throughout the pars distalis. Our immunocytochemical observations confirmed that the GH and PRL cells are two distinct types, by the comparative observations of two adjacent sections, one stained with anti-oPRL serum and the other with anti-GH serum. GH cells were usually ovoid or round cells containing orangeophilic granules and occurred in clusters throughout the pars distalis. They were the most abundant type of cells in both sexes in the various reproductive phases. The pleomorphic PRL cells were located as small groups in the central and dorsocaudal regions of the pars distalis. However, in the pregnant or lactating bats, they were extremely enlarged and located as large groups

throughout the pars distalis. Herlant (1964) indicated that GH cells represent 35–45% of the total number of pituitary cells in mammals. Richardson (1981a), who made a morphometric analysis on the immunoreactive PRL and GH cells in *Macrotus californicus*, revealed a greater volume percentage of GH cells and up to 44% of PRL cells in lactating bats. In the present study, GH cells occupied 22.2% of the area of a section in hibernating bats, and 24.3% and 22.3% of the area in pregnant and lactating bats. The PRL cells were small and occupied only 8.1% of the area in the hibernating bats, while in pregnant and lactating bats, they occupied 37.2% and 27.7% of the area, respectively, showing significant increase.

Very little information is available on ACTH cells of bats. Herlant (1956) identified these cells in *Myotis myotis* as classical acidophils by the trichrome method. However, no immunocytochemical study on the ACTH cells of bats has been performed up to now. In the present study, ACTH cells in *Miniopterus schreibersii fuliginosus* were found to contain coarse amphophilic secretory granules, which were immunopositive to anti-pACTH serum. The volume percentage of area occupied by ACTH cells showed small seasonal variation depending on reproductive phase.

Herlant (1956) and Patil (1974) reported that TSH cells of *Myotis myotis* and hipposiderid bats were small polygonal, PAS- and AF-positive cells and scattered throughout the pars distalis. Siegel (1955), who studied male *Myotis lucifugus* under different physiological states, observed alterations in the TSH cells. Both Herlant and Patil reported little seasonal changes in these cells. No reference is available on the immunocytochemical study on the TSH cells of bats. In the present study on *M. s. fuliginosus* TSH cells were an independent type of cell different in immunoreactivity and distribution from other types of pituitary cells. They were scattered mainly in the ventrolateral region of the pars distalis. They showed little seasonal change in population from a maximum of 11.0% in hibernating bats to a minimum of 5.5% in the pregnant bat and lactating bats.

The gonadotropic cells in bats have been reported to be large, oval or rounded and stained with PAS, methylene blue, alcian blue and aniline blue (Herlant 1956; Patil 1974). They have been divided into two functional groups, FSH cells and LH cells, based on cyclic alterations in number and tinctorial properties as correlated with the reproductive and activity cycles. Immunocytochemical studies on the LH cells and FSH cells performed on *Macrotus californicus* by Richardson (1981b), using antisera against the specific beta subunits of rat LH and rat FSH, revealed that selective immunoreactivity to both antisera was localized primarily in the same cell types, with the exception of some cells that reacted solely with antisera to either LH-β or FSH-β. Recently, Anthony and Gustafson (1984a) demonstrated seasonal variations in pituitary LH-gonadotrophs of the hibernating bat *Myotis lucifugus lucifugus*, but did not mention FSH cells and the relationship between LH and FSH cells.

In the present study, most of small basophilic cells distributed in the zona tuberalis reacted identically with antisera to ovine LHβ and rat FSH, while the LH-immunoreactive cells dispersed throughout the pars distalis did not react with the anti-rFSH serum. Since clustered and dispersed LH cells appear to be different in shape, immunoreactive product and location, it is speculated that they may be two functionally distinct populations. Furthermore, FSH-immunoreactive cells scattered in ventrolateral portion of the pars distalis showed a similar distribution with that of TSH-immunoreactive cells, suggesting the cross-reactivity between anti-rFSH and anti-rTSH serum used in the present study.

Seasonal variations of a population of gonadotropic cells have been reported by Richardson (1981b) and Anthony and Gustafson (1984a). Richardson (1981b) noted that the mean volume fraction of LH-immunoreactive cells varied 8 to 20% in females throughout the year. Anthony and Gustafson (1984a) reported that the mean volume fraction of LH-gonadotrophs in the pars distalis of *Myotis lucifugus* varied seasonally from 2.27% to 8.63% in females, and 6.31% to 10.90% in males. They also mentioned that the volume fraction declined in April, following ovulation, and remained low during pregnancy and lactation. Similar reductions in pituitary LH-immunoreactivity during late pregnancy and lactation have been reported in *Macrotus californicus* by Richardson (1981b) and in rat by Merchant (1974). This investigation also demonstrated a seasonal variation of a population of cells immunoreactive to both anti-rLH and anti-rFSH sera. The most striking seasonal variation in LH- and FSH-immunoreactivity in *M. s. fuliginosus* occurred in females. The mean percentages of the volume of the pars distalis occupied by LH- and FSH-immunoreactive cells were 21.3% and 11.4%, respectively, in the hibernating females, while they were 11.4% (LH) and 6.7% (FSH) in pregnant female, showing significant reduction in the percentage of the volume occupied by LH cells. The presence of the luteal body in the ovary of the November females (unpublished data) is consistent with higher percentage of LH cells.

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