

Cytological study of the hypophysis pars anterior of the Indian emballonurid bat *Taphozous kachhensis* (Dobson)

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Summary

The anterior pituitary gland (pars anterior) of the bat *Taphozous kachhensis* was examined cytochemically by employing several cytochemical staining techniques. Six cell types could be recognized, on the basis of their morphological characteristics and tinctorial properties, viz., two types of non-mucoid (acidophilic) STH and LTH, and three types of mucoid (basophilic) TSH, FSH and LH cells; the sixth, ACTH cells, were amphophilic. The cells are arranged in follicles or acini, separated from each other by a thin layer of connective tissue. The non-mucoid cells are much more concentrated in the postero-lateral and postero-medial regions of the pars distalis. ACTH and TSH cells are mostly localised in large numbers in the peripheral region of the pars distalis. FSH cells are concentrated towards the antero-lateral, anterior and median regions. LH cells are distributed throughout the pars anterior but are more abundant towards the posterior and lateral regions. The probable role of these cell types in the physiology of reproduction of this bat is discussed.

Key words: Anterior pituitary gland, bat, cytochemistry, reproductive cycle

Introduction

It is assumed that in bats, as in all mammals studied, gametogenic and steroidogenic activities of the gonads are regulated by hormones of the anterior pituitary gland. The light microscopic study of anterior pituitary reveals the presence of six cell types based on granulation and staining reactions in the cytoplasm in the bats such as *Myotis lucifugus lucifugus* (Guthrie and Sawyer, 1935; Seigel, 1955), *Vesperugo savi* and *V. piccolo* (Azzali, 1971), *Cynopterus sphinx* (Badwaik, 1988, 1991), *Rousettus leschenaulti* (Bhiwgade et al., 1989), *Scotophilus heathi* (Singh and Krishna, 1994, 1997) and *Hipposideros lankadiva* (Seraphim, 2009). However, only cell types were identified in *M. lyra lyra* (Bhiwgade et al., 1982) and *Hipposideros* bats (Patil, 1974). Richardson (1979) and O'Brien et al. (2003) reported that pituitary cells have different morphological features at different reproductive phases. Although the anterior pituitary undoubtedly plays a major role in regulating sexual cycle of bats, there is little information about its structural and functional correlates to reproductive functions. The present investigation was, therefore, undertaken to study the cytological features of different cell types in the anterior pituitary of the Indian emballonurid bat, *T. kachhensis* (Dobson), in relation to the reproductive cycle.

Materials and Methods

For the present study the animals of both the sexes of *T. Kachhensis*, a seasonally monoestrous species, were collected from their roosting place in Kampa-Tempa

District, Chandrapur, Maharashtra, India, for two years, representing different phases of the reproductive cycle. The animals were brought alive to the laboratory with minimum stress and constant supply of food and glucose water. The animals were sacrificed under anesthesia and the brain along with the pituitary was immediately dissected out and fixed for 24 hr in various fixatives such as formal sublimate, Rossman's and Bouin's. After fixation the pituitaries were washed in running water for 24 hr, dehydrated in different grades of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections along horizontal and sagittal planes, at 3-5 μm thickness, were obtained to ascertain the distribution of different cell types in the gland. A battery of histochemical techniques was employed to identify the different cell types since specificity was not attainable by any one technique. The staining methods used and the abbreviations adopted are given in Table-1.

Results

The pituitary gland of *T. kachhensis* is flattened dorsoventrally. It lies parallel to the cranio-caudal axis of the brain. It is lodged in a well-defined sella turcica. It is approximately 2.8 mm long, 2 mm wide and 1.6 mm thick. Some notable morphological characteristics of the gland are illustrated in the schematic diagram (Fig. 1). The infundibulum (hypophysial stalk) is first directed downwards and then bends posteriorly so as to lie horizontally to the axis of the brain. The hypophysial stalk is directed posteriorly and its posterior end enlarges to

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Table 1. The staining methods used and the abbreviations adopted

Staining method	Abbreviation used in text	Reference
1. Martius-Scarlet-blue	M.S.B	Lendrum et al. (1962)
2. Modification of Mallory's trichrome stain	—	Crossomon (1937)
3. Carmoisin-L-orange-G-aniline blue-acid alizarin blue	—	EI Etreby and Tushaus (1973)
4. Periodic acid Schiff-orange G	PAS-OG	Pearse (1967)
5. Luxol fast blue-periodic acid	LB-PAS-OG	Shanklin, et al. (1959) modified by Kerr (1965)
6. Alcian blue (pH 0.2)-Periodic pH 3.0 acid schiff-orange G	AB-PAS-OG	Herlant (1960)
7. Alcian blue (pH 0.2)-Periodic pH 3.0 acid Schiff-orange G	AB-PAS-OG	Herlant (1960)
8. Periodic acid Schiff-methylene blue-orange G	PAS-MB-OG	Wilson and Ezrin (1954) Rennels (1957)
a) 1% aqueous methylene blue		
b) 0.25% aqueous methylene blue		
c) 0.1% aqueous methylene blue		
9. Paraldehyde Fuchsin-light green-orange-G	AF-LG-OG	Halmi (1952)
10. Lead haematoxylin	—	MacConaill (1947)

form the pars nervosa. The recessus hypophyseus extends as a distinct canal throughout the hypophysial stalk and ends at the junction between the hypophyseal stalk and pars nervosa. The pars intermedia is well developed and forms a compact layer abutting the pars nervosa on its anterior and lateral sides.

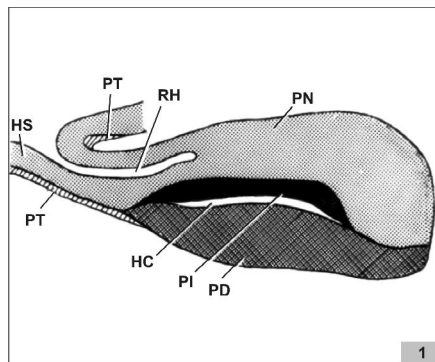


Fig. 1. Schematic diagram of the sagittal section of the pituitary gland of *Taphozous kachhensis*. HC, hypophysial cleft; HS, hypophysial stalk; PD, pars distalis; PT, pars tuberalis; PI, pars intermedia; PN, pars nervosa.

The pars anterior (distalis) lies below the level of the hypophysial stalk. In transverse section the pars distalis has a lateral wing-like expansion on either side of the pars nervosa. The pars distalis is separated from the pars intermedia by a hypophysial cleft in the lateral regions (Fig. 2). The cells are arranged in follicles or acini,

separated from each other by a thin layer of connective tissue. Two types of acidophilic cells, three types of basophilic cells and one type of amphophilic cells could be identified. The zonation of the pars distalis into acidophilic and basophilic is based on the histochemical reactions of the cell types that are predominant the respective areas. Classification of the cell types is based on the staining properties of the cytoplasmic granules, their shape, size and distribution, and functional designations, as proposed by the International Nomenclature Committee of Van Oordt (1965).

Type-I (Probably STH cells)

The secretory granules of these non-mucoid or acidophilic cells are stained with acidic dyes only. Large numbers of these cells are seen distributed throughout the pars distalis but are more abundant towards the posterior, lateral and median regions. These cells are usually present in the form of clusters of 2 to 4 cells or sometimes they occur singly. Most of these cells are oval or round, measuring 9.2-11.0 μ m diameter. In all the cells, the plasma membrane is clearly defined. Nuclei are round and prominent, and measure 3.8-6.5 μ diameter. Cytoplasmic granules are fine, uniformly distributed and stained orange with orange-G, yellow with Martius searlet blue and blue green with Luxol fast blue. These are AB-negative, AF-negative and PAS-negative (Fig. 3).

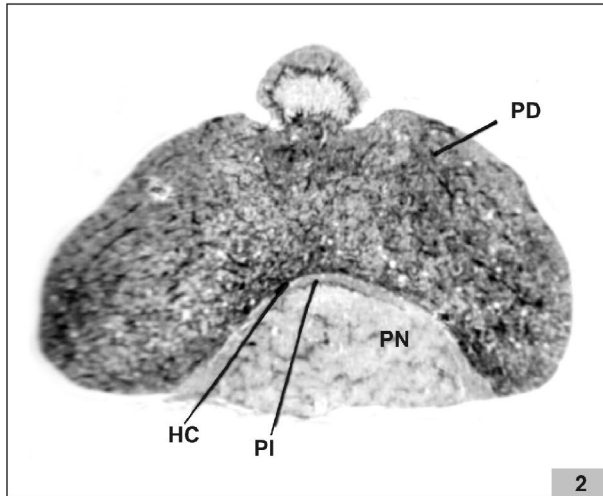


Fig. 2. Horizontal section of the pituitary gland showing various regions.

HC, hypophysial cleft; PD, pars distalis; PI, pars intermedia; PN, pars nervosa.

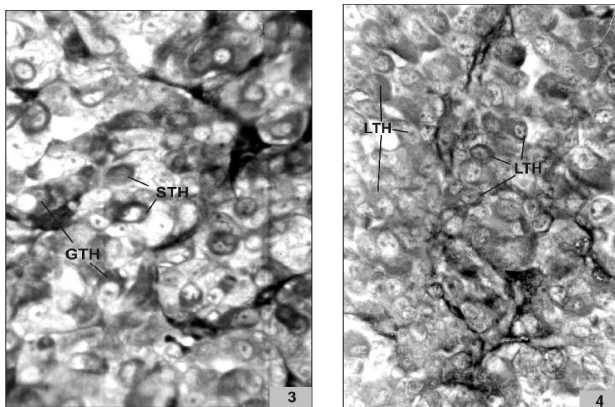


Fig. 3. Pars anterior of oestrus female showing numerous blue green STH cells selectively stained with Luxol fast blue and red coloured GTH cells. x 900. LB/PAS/OG staining.

Fig. 4. Pars anterior of mid-pregnant female showing prominent Carmosin-L-positive lactotrophs (LTH). x 900. CL/OG/aniline blue/acid alizaline blue.

Type-II (Probably LTH cells)

The LTH cells appear singly or in clusters. The cells are distributed throughout the gland but are more abundant towards the postero-lateral and postero-median regions of the pars distalis. They are oval, round or elongated, sometimes appearing irregular in outline. The diameter of the cell in an adult specimen in both the sexes is 9.9 to 18.6 μ m. In most cells, nuclei are round, oval or sometimes eccentrically placed. One or two nucleoli are

observed towards the centre of the cell. The Golgi zone is juxtannuclear. Cytoplasmic granules are numerous and stained orange-red with PAS/OG and red with CL/OG/aniline blue/acid alizarin blue, Crossman's and Martius scarlet blue in the combined histochemical techniques used (Fig. 4, 5). These are AB-negative and AF-negative in *T. kachhensis*. This cell is less common than type I and seems to be associated with pregnancy and lactation.

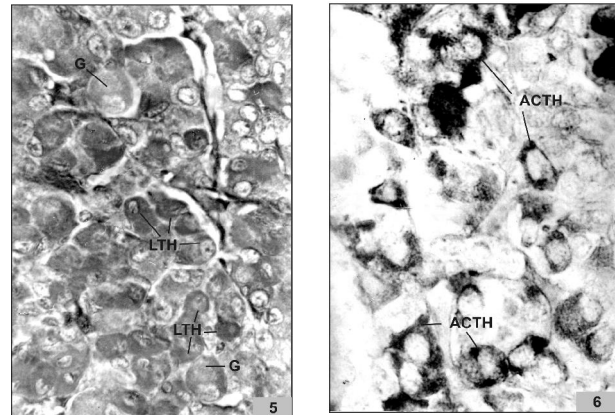


Fig. 5. Pars anterior of female bat during lactation showing the hypertrophy and hyperplasia of LTH cells. Note the Golgi zone in some LTH cells. x 900. CL/OG/aniline blue/acid alizaline blue.

Fig. 6. A part of the pars anterior of female in estrus. Note the intensely stained lead hematoxylin-positive angular or polygonal ACTH cells. x 960. Lead hematoxylin staining.

Type-III (Probably ACTH cells)

These cells are oval or polygonal in shape, measuring a diameter of 6.5-9.9 μ m, and are localised more in the peripheral region of the pars distalis. These cells are stained orange-red in PAS/OG and brick red in AB/PAS/OG. They are AB-negative, AF-negative and weakly orange-positive in various combined histochemical techniques used. They are selectively stained with lead hematoxylin in blue-black colour (Fig. 6).

Type-IV (Probably TSH cells)

Generally, these cells are angular or polygonal in shape, measuring a diameter of 11.2 to 16.5 μ m. These cells are distributed throughout the pars distalis but more localised towards the periphery. The nuclei are round or oval in shape, centrally or eccentrically placed, and possess a prominent nucleolus. The nuclear diameter is 5.2 to 6.6 μ m. The Golgi zone appears juxta-nuclear in position. The cytoplasmic granules are fine and are uniformly distributed. They are stained red with PAS/OG, dull blue with AB/PAS/OG, and violet with AF/LG/OG. They are, thus, AB-positive, PAS-positive and AF-positive (Fig. 7).

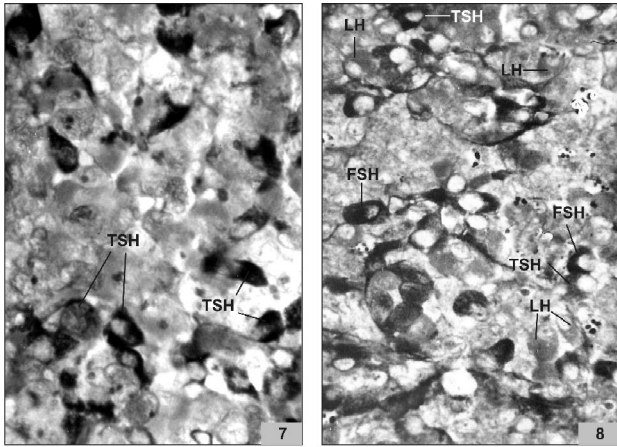


Fig. 7. Pars anterior of male during the preparatory period showing TSH cells stained with aldehyde Fuchsin. Cells are seen distributed throughout the pars anterior. x 900. AF/LG/OG staining.

Fig. 8. Pars anterior of female during early pregnancy showing TSH cells in dull blue colour. Note the distinction between reddish LH cells and bluish purple degranulated FSH cells. x 900. AB/PAS/OG.

Type-V (Probably FSH cells)

These cells are oval, round or elongated in shape and are the largest basophiles measuring 13.2 to 17.5 μ m in diameter. These cells are distributed throughout the pars distalis but are more abundant towards the posterior and lateral regions. The Golgi zone is juxta-nuclear. Cytoplasmic granules are fine and uniformly distributed in the cytoplasm. Some cells show medium coarse granules. The nuclei, possessing 1-3 nucleoli, are large and round, measuring 6.5 to 8.5 μ m in diameter and placed centrally or eccentrically. These cells are stained yellow red with PAS/OG, purple or blue purple in combined histochemical reactions of AB/PAS/OG, MB/PAS/OG, faint violet with AF/LG/OG, and positive to PAS, AB, MB (methyl blue), AF and aniline blue but negative to orange G (Fig. 8).

Type-VI (Probably LH cells)

These cells are distributed throughout the pars distalis but more localised towards antero-lateral, anterior and medial regions. The cells are round or oval in shape and measure 9.9-15.8 μ in diameter. The cytoplasm contains fine granules, which are uniformly distributed and has a homogenous appearance. The Golgi zone is situated near the nucleus. The nucleus is round and large with a distinct nuclear membrane, either placed centrally or eccentrically. The nuclear diameter is 5.2 to 6.5 μ m. The chromatin matter is clumped and the nucleolus is large and centrally placed. These mucoid or basophilic cells are stained yellow red with PAS/OG, PAS/MB/OG,

red with AB/PAS/OG. They reacted negatively with AB and AF. They could be identified by the combined histochemical reactions of MB/PAS/OG where they are PAS-positive and weakly stained with MB (Fig. 9, 10).

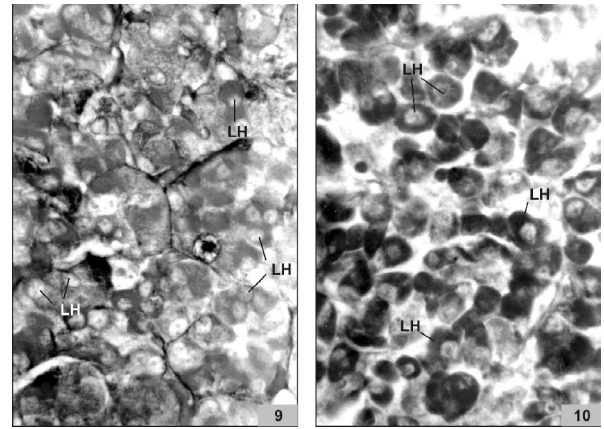


Fig. 9. Pars anterior of female during mid-pregnancy. Note the heavily granulated LH cells (yellow red). X 675. AB/PAS/OG staining.

Fig. 10. The pituitary of female bat during advanced pregnancy. Note the hypertrophy of LH cells. Some cells show signet ring around nucleus. x 675. Carmoisin's technique.

Discussion

The morphology of the pituitary in *T. kachhensis* is similar to that of the emballonurid bat *T. melanopogon* (Badwaik, 1988), and rhinopomatid bats *Rhinopoma hardwicki hardwicki* (Karim and Khan, 1985) and *Megaderma lyra lyra* (Bhiwagade et al., 1982). The tinctorial properties of the different cell types and their interpretation in relation to the endocrine physiology have been discussed in considerable detail by Patil (1974) and Richardson (1979). Whereas Van Oordt's (1965) classification implied that each cell type produces one hormone, the one-cell-one-hormone hypothesis. This classification was contested by some workers (Nakane, 1970; Herbert, 1976; Moriarty, 1976; Richardson, 1979) who, on the basis of immunohistochemical studies, produced evidence to suggest that more than one hormone is produced by the same cell.

The acidophilic cells, due to their solid protein nature of high insolubility, are easily preserved by any method of fixation (Purves, 1961). On the basis of PAS/OG technique, the STH cells are stained yellow or orange with OG in bats (Bhiwagade et al., 1982, 1989; Badwaik, 1988, 1991). Using Crossmon's staining method the two types of acidophils could be distinguished as orange-coloured STH cells and purple or red LTH cells in *Rhinopoma hardwicki hardwicki* (Karim and Khan, 1985). In the present study, orange-coloured STH cells

and red-coloured LTH cells are observed in pars distalis of this bat. This type I STH cells could be specifically stained by Luxol fast blue in the LB/PAS/OG method (Patil, 1974; Badwaik, 1988, 1991; Bhiwagade et al., 1982, 1989; Singh and Krishna, 1994; Seraphim, 2009) and the same differentiation is observed in the present species of bat. This type I cells are observed in the pituitary of mature males and females but are the most predominant cell type in immature animals, thus, proving that they are presumably the STH cells. In the present study, orange-coloured STH and red-coloured LTH cells can be differentiated by CL/LG/OG technique and yellow-coloured STH and red-coloured LTH cells could be differentiated in MSB technique and is similar to that reported in other bats (Patil, 1974; Badwaik, 1988, 1991; Bhiwagade et al., 1982, 1989; Singh and Krishna, 1994; Seraphim, 2009).

The morphological characteristics and the responses of type 1 and type 2 acidophils to the various stains indicate that they are similar to the cells in the *M. myotis* described by Herlant (1956), who suggested that the former secrete somatotrophic hormone and the later lactotrophic (prolactin). In the present study type 1 ovoid or round cells are distributed throughout the pars anterior but more abundant towards the posterior and lateral regions. Round or elongated type 2 red carminophilic, acid fuchsin positive and brilliant crystal scarlet 6B positive cells were observed in the reproductively quiescent males, and increased during the breeding phase. LTH cells are present few in number in non-pregnant females and their number increased during gestation and lactation. These cells indicated hyperplasia and hypertrophy during late pregnancy and lactation.

Amphophilic (ACTH) cells are usually oval to irregular in shape with long cytoplasmic processes and an eccentrically placed nucleus. In many species these corticotrophs are not positively identified owing to their weak reaction with most of the stains used due to the scanty cytoplasm. Hence, they are referred to as intermediate between chromophobes and chromophils. However, with AB/PAS/OG techniques they were observed as red orange or brick red cells as in other bats (Bhiwagade et al., 1982, 1989; Singh and Krishna, 1994, 1997; Seraphim, 2009). In lead haematoxylin method of MacConail (1947) these cells are stained blue black in *T. kachhensis* as in other bats (Bhiwagade et al., 1989; Singh and Krishna, 1994, 1997; Seraphim, 2009). However, these cells stained negative with lead haematoxylin in hipposideros bats (Patil, 1974), *Megaderma lyra lyra* (Bhiwagade et al., 1982), *Cnopterus* and *Taphozous* (Badwaik, 1988, 1999) and *Rhinopoma hardwickei hardwickei* (Karim and Khan, 1985). These cells are recognised by their irregular shape, peripheral distribution and tinctorial affinities to various dyes.

The basophilic (TSH) thyrotrophs are stained blue with AB/PAS/OG in *T. kachhensis*, as is the case in hipposiderid bats (Patil, 1974), *Rousettus leschenaultia* (Bhiwagade et al., 1989), *Megaderma lyra lyra* (Bhiwagade et al., 1982), *T. meganopogon* (Badwaik, 1988), *S. heathi* (Singh and Krishna, 1994) and *H. lankadiva* (Seraphim, 2009). These cells are stained with aldehyde Fuchsin in AF/LG/OG. The AF-positive cells are also reported in other bats (Bhiwagade et al., 1989; Patil, 1974; Azzali, 1971; Badwaik, 1988, 1991; Singh and Krishna, 1994; Seraphim, 2009). The type IV cells, oval, round, polygonal or angular in shape, are positive to PAS, AB and AF in the various techniques used, and thus, corresponded to the TSH cells of mammals including bats.

In *T. kachhensis*, gonadotrophs are round or ovoid, cytoplasm is coarsely and finely granulated and occupies the antero-lateral and the centro-medium regions of the anterior pituitary. FSH cells are stained purple blue in combined histochemical reaction of AB/PAS/OG as in *R. leschenaulti* (Bhiwagade et al., 1989), *M. lyra lyra* (Bhiwagade et al., 1982), *T. melanopogon* and *C. sphinx* (Badwaik, 1988, 1991), *S. heathi* (Singh and Krishna, 1994) and *H. lankadiva* (Seraphim, 2009). LH cells are stained yellow red with PAS/OG, PAS/MB/OG and AB/PAS/OG. FSH cells are stained with methylene blue and, in combination with PAS, they appeared purple blue in PAS/MB/OG. Similar results were obtained in other species of bats (Patil, 1974; Bhiwagade et al., 1982, 1989; Badwaik 1988, 1991; Singh and Krishna, 1996 and Seraphim, 2009). The gonadotrophs are further differentiated into LH cells and FSH cells on the basis of physiological evidences since both the cell types undergo changes in number and size in accordance with the reproductive cycle. LH cells undergo hypertrophy and hyperplasia during ovulation, pregnancy and lactation in this bat.

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