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# Identification, Localization and Distribution of Different Cell Types in Adenohypophysis of Female Vespertilionid Bat, *Scotophilus heathi*: A Combined Histochemical, Immunocytochemical and Electron Microscopic Study

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Six distinct cell types have been identified in the pars distalis (PD) of *S. heathi* on basis of specific morphological characters, staining reaction and immunocharacteristics. The thyrotropic (TSH) cells were small, round or polygonal and distributed mainly in the antero-ventral or medio-ventral region of the PD. The gonadotrophic (GTH) cells were generally large, round or irregular in shape and distributed throughout the PD. The GTH cells have been distinguished into luteinizing hormone (LH) and follicle stimulating hormone (FSH) cells on the basis of immunoreactivity to anti h LH and anti h FSH sera. Comparison of adjacent sections stained with antisera to βLH or βFSH revealed that some of the cells react both βLH and βFSH antisera. The adrenocorticotrophic (ACTH) cells were rounded, polygonal or irregular in shape and observed mainly in the antero-ventral and ventro-lateral regions of the PD. Prolactin (PRL) cells were rounded or pleomorphic and primarily located in the central and dorso-posterior regions of the PD. Somatotrophic (STH) cells were rounded or ovoid. They were the most abundant types of cells distributed singly or arranged in palisades mainly in the postero-lateral region.

**Key Words: Cell types, Adenohypophysis, Vespertilionid bat, *Scotophilus heathi***

## Introduction

Much of our knowledge about pituitary cytology is based on studies conducted on species belonging to Orders Rodentia, Carnivora, Primates and Artiodactyla, particularly in ruminents. Other groups of mammals, including the Order Chiroptera, have not received adequate attention in the past. Since most of the earlier studies on pituitary cytology of bats were based on conventional histochemical techniques, it would be interesting to compare the results of immunostaining with histochemical staining. The present study was designed to elucidate the cytology of adenohypophysis of female *S. heathi* (Order: Chiroptera; Family: Vespertilionidae), based on immunocytochemistry (ICC), histochemical staining and electron microscopy.

## Materials and Methods

Adult female of *S. heathi* were collected in Varanasi (25°N, 83°E), India. Bats were transported to laboratory and sacrificed under ether anaesthesia within few hours of the capture. The pituitaries along with brain were fixed in various fixatives (table 1) and preserved in 70% alcohol. The tissues were embedded in paraffin wax and serial sections were

**Table 1** *Tinctorial affinity of pituitary cell types in pars distalis of* ***S. heathi***

| Staining technique | Suitable fixative | Pars Distalis (Cell-types) | | | | |
|---|---|---|---|---|---|---|
| | | TSH | GTH | PRL | STH | ACTH |
| PAS-OG | Bouins Sublimate | Negative | Redish pink | Orange | Yellow orange | Negative |
| AB-PAS-OG *p*H (3.0) | Bouins sublimate | Light blue | Deep blue | Orange/ yellow | Yellow | Negative |
| Pb H (Lead hematoxylin) | Sublimate formal | Negative | Negative | Negative | Negative | Dark blue or black |
| Herlants tetrachrome | Bouins Holland Sublimate | Blue (small) | Blue | Vermilion red | Yellow | Negative |
| Modified AB-PAS-OG (*p*H 1.0) | Sublimate formal | Blue | Deep blue | Yellow | Orange | Negative |
| Mallory's Triple stain | Bouins sublimate | Deep blue | Light blue | Negative | Orange | Negative |
| Cleveland & Wolfe trichrome | Sublimate formal | Light green | Dark green | Orange red | Yellow | Negative |
| AF-PAS-OG | Sublimate containing fixatives | Violet or blue | Negative | Negative | Negative | Negative |

(after Patil 1974 and Richardson 1979)

cut at 5 to 7 μm in horizontal and transverse planes.

*Histochemical Study*

For routine histochemical study, the sections were stained with Ehrlich's haematoxylin and eosin. Several histochemical staining procedures were employed (table 1) to identify the different cell types because specificity was not attainable by any one technique.

*Immunostaining*

Pituitary cells were identified by using peroxidase anti-peroxidase (PAP) method of Sternberger et al. (1970). Sections were treated with 3% $H_2O_2$ in 10% methanol for 5 min in order to inactivate endogenous peroxidase activity. Thereafter the slides were treated with 2% normal goat serum to eliminate non-specific binding. The sections were incubates with primary antibody in moist chamber for overnight at 4°C. After washing twice in buffer for 10 min each, the sections were incubated with anti-rabbit gamma globulin (IgG) (1:100 dilution) followed by incubation with PAP complex (1:50 dilution). The immunoreactive cells were visualized by using 3'3' diaminobenzedin tetrahydrochloide (DAB). Control immunocytochemical stainings were performed by replacing primary antiserum with normal rabbit serum.

*Electron-Microscopy*

Bats were sacrificed by perfusion fixation. The fixative consisted of 1% glutaraldehyde

and 1% paraformaldehyde in 0.1 M phosphate buffer (*p*H 7.4). Tissues were post fixed in 2% osmium tetraoxide in 0.1 M phosphate buffer and 3% sucrose. After dehydration in graded series of ethanol, the tissues were embedded in araldite. Ultrathin sections from selected areas were then cut on LKB ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate and examined in Phillips EM 400.

**Observation**

The pars distalis (PD) consists of cords of parenchymatous cells surrounded by a thin layer of connective tissue. The chromophobes or Follicular cells were spherical or pear-shaped cells with a vesicular nucleus. They were stained deeply with haematoxylin. Apart from the chromophobes,six functionally distinct types of cells (TSH, LH, FSH, ACTH, PRL, STH) were identified in the PD on the basis of specific morphological characters, staining reaction and immunocharacteristics. Except STH cells, the other five cell types viz. LH, FSH, PRL, ACTH and TSH were identified in the PD by immunoreactivity with corresponding antisera. The tinctorial property of various cell types has been studied by employing conventional histochemical techniques (table 1). The EM study also revealed six morphologically distinguishable cells types (figure 5, 6).

*Cell type* 1 (*Thyrotrophs or TSH cells*)

TSH cells were normally small, rounded or polygonal (figure 1). They were usually found in small groups in the antero-ventral or medio-ventral regions of the PD (figure 2). Occasionally, they were seen scattered throughout the PD. The cytoplasm of these cells were finely granular and nuclei eccentrically located. These cells stained deep or light blue with alcian blue (AB) (figure 3) and violet with aldehyde-fuschin (AF). They stained light green with trichrome (figure 4) but remained negative with Lead hematoxylin (PbH) and Periodic acid-Schiff-Orange G (PAS-OG). Immunoreactivity to anti h βTSH was observed in a single cell types which was identified as TSH cells. These cells did not show cross reactivity with any of other antisera used in the present study including anti h βLH or anti h βFSH.

Ultrastructurally, these cells showed the presence of a few small secretory granules which ranged in diameter from 100-160 nm. The cytoplasm contained rounded or elongated mitochondria, free ribosomes and poorly defined Golgi complex (figures 5, 6).

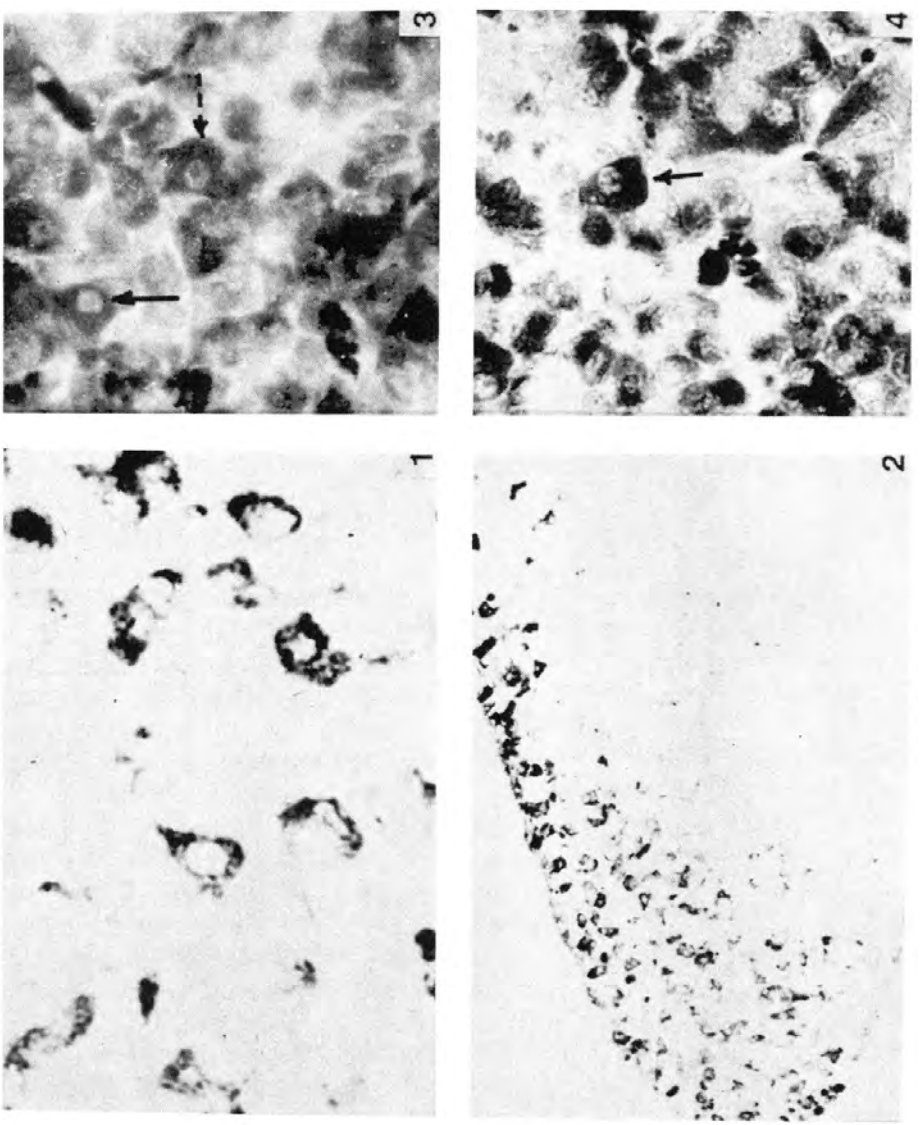
*Cell type II* (*Lactotrophs or PRL cells*)

PRL immunoreactive cells were visualised by using h PRL antisera. The cells were round or pleomorphic. Their nuclei were large, round to oval and eccentrically located within the cytoplasm (figure 7). PRL cells were distributed throughout the PD. They were primarily located in small groups in central and dorso-posterior regions of the PD (figure 8). PRL cells were generally erythrosinophilic and stained vermilion red with Herlant's tetrachrome (figure 9) and orange red with Cleveland-Wolfe trichrome. These cells stained orange with PAS-OG (figure 10) and yellow/orange with alcian blue-periodic acid-Schiff-orange G (AB-PAS-OG).

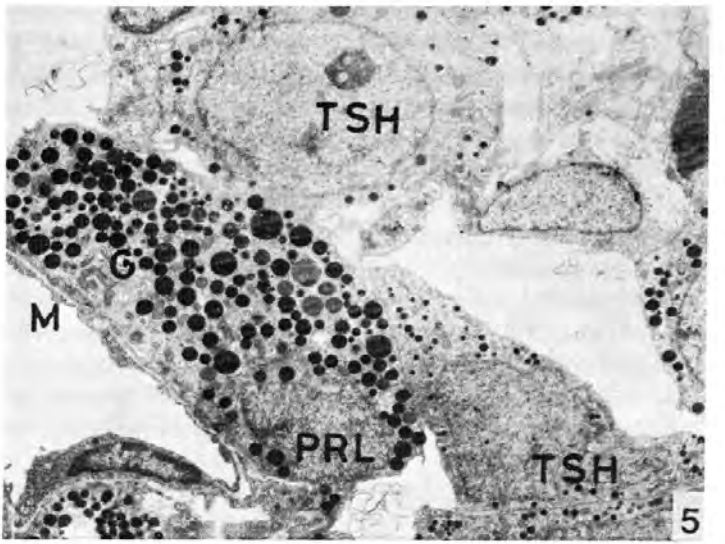
Electron microscopically this cell type is characterised by the presence of abundant larger secretory granules which measured about 300-600 nm in diameter. These cells contained numerous mitochondria, dilated endoplasmic reticulum (ER) and extensive Golgi complex (figure 11).

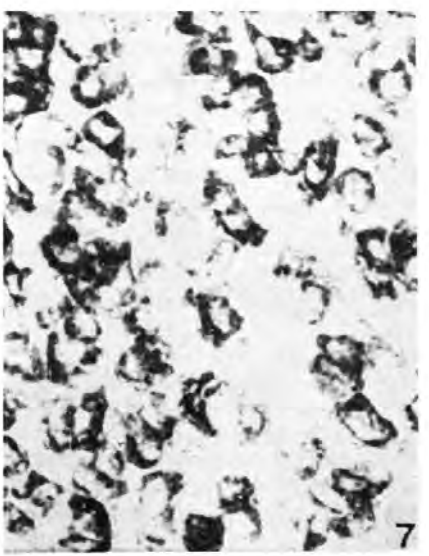
*Cell type III and IV* (*Gonadotrophs or LH and FSH cells*)

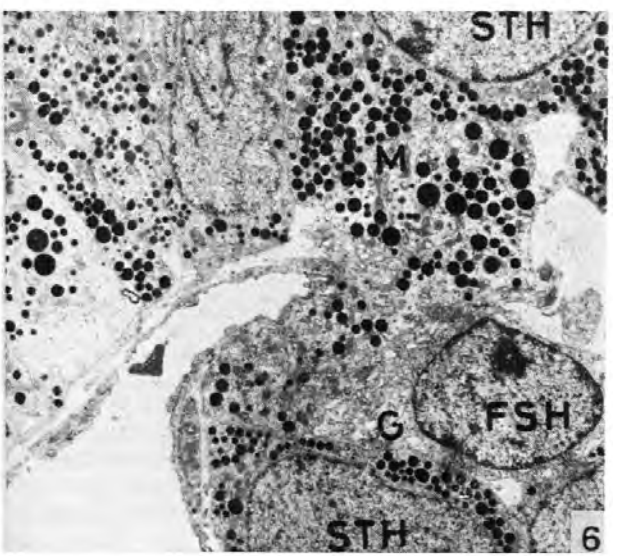
The GTH cells were generally large, oval, rounded or occasionally irregular in shape (figure 12) and are usually seen close to capillaries. The cytoplasm is finely granular with the granules dispersed uniformly throughout the cells. The nucleus is either eccentrically or centrally located. The cells are scatte-



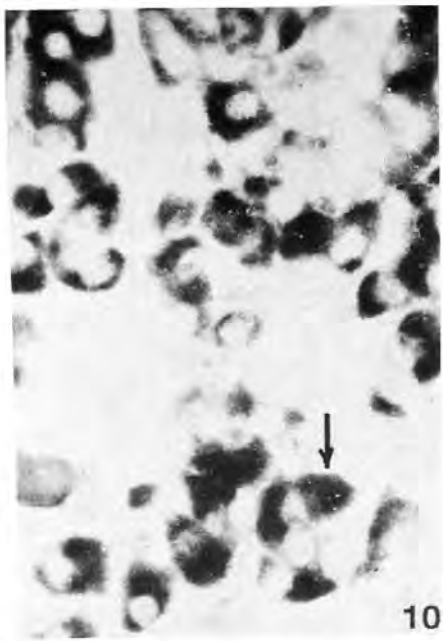
**Figures 1–4 1, TS showing TSH cells stained immunocytochemically with antisera against h βTSH ( × 550); 2. TS showing distribution of** the TSH cell stained immunocytochemically with antisera against h βTSH ( × 60); 3. TS stained with AB-PAS-OG showing two types of AB positive basophils. TSH cells are small and stained light blue (arrow) while GTH are big and stained deep blue (broken arrow) ( × 260); **4**, TS obtained with trichrome showing TSH cells (arrow) ( × 240).

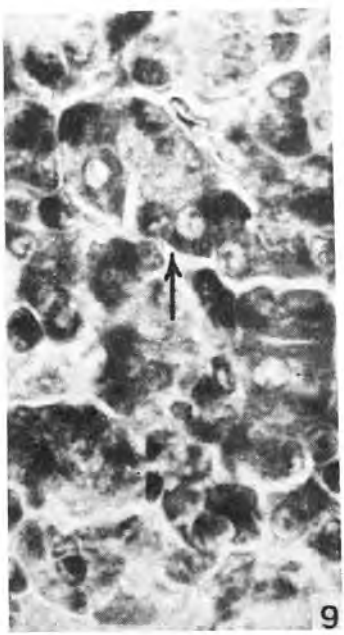


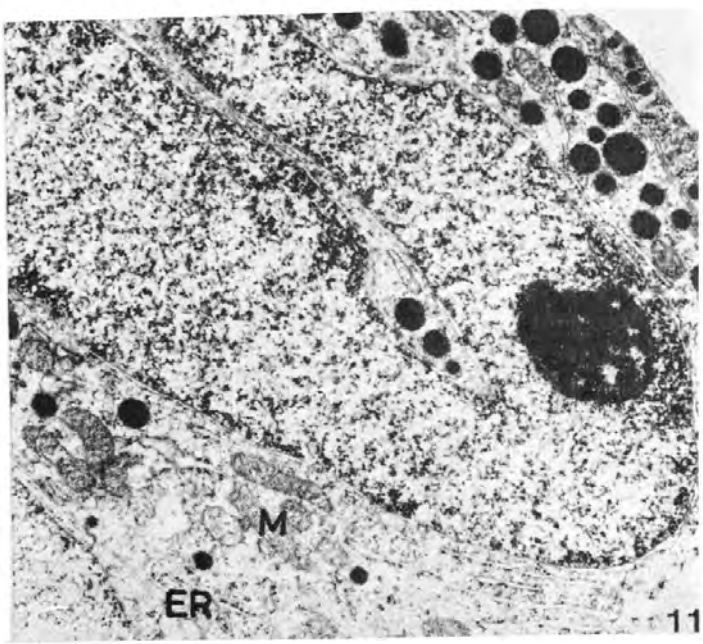




**Figures 5–7** **5**, Low power electron micrograph showing TSH and PRL cells. Note the presence of mitochondria (M) and Golgi bodies (G) ( × 5600); **6**, Low power electron microgaph showing STH and FSH cells. Mitochondria (M), and Golgi bodies (G) are also seen ( × 5600); **7**, PRL cells stained immunocytochemically with antisera against h PRL ( × 250)


**Figures 8-11 8,** Section showing distribution of PRL cells stained immunocytochemically with antisera against h PRL ( × 60); **9,** Section stained with Herlant's tetrachrome showing darkely stained (arrows) PRL cells ( × 240); **10,** Section of pars-distalis stained PAS-OG showing PRL cells (arrows) ( × 275); **11,** PRL cell showing numerous mitochondria (M) and dilated ER (ER) ( × 20000)

red singly or in small groups throughout the PD (figure 13). The FSH immunoreactive cells were numerous in ventral and lateral regions of the PD and the region close to the pars intermedia (PI). The GTH cells were positive to PAS-OG and AB positive in AB PAS-OG(figure 14).They stained light or deep blue with AB-PAS-OG, Mallory's triple and tetrachrome (figure 15), and green with Cleveland-Wolfe trichrome but showed negative reaction with PbH and aldehyde fuschin-Periodic Acid Schiff-Orange G (AF-PAS-OG).

On the basis of immunoreactivity to anti h βLH and anti h βFSH sera, the GTH (LH & FSH) cells of the PD have been identified. Comparison with adjacent sections stained with antisera to βLH or βFSH have revealed that some of the cells react both with βLH and βFSH antisera.

Ultrastructurally, the GTH cells contained secretory granules ranging in diameter from 150 to 350 nm. The FSH cells contained spherical secretory granules of approximately 150 to 300 nm in diameter which were distributed regularly throughout the cells. The mitochondria were large with well developed cristae. Rough ER and conspicuous Golgi bodies were usually present (figure 16). In the LH cells, secretory granules measured 175-350 nm in diameter which were irregularly distributed throughout the cytoplsam. The mitochondria were usually elongated or round and Golgi complex was usually conspicuous (figure 17).

*Cell type V (Adrenocorticotrophs or ACTH cells)*

ACTH immunoreactive cells were mainly observed singly or in small clusters in anteroventral region of the PD (figure 18). They were sparsely distributed in other areas too. These cells showed a somewhat irregular shape, being round or oval to polygonal (figure 18). The ACTH cells did not show cross-reactivity with antisera against h βLH, h βFSH, h βTSH or h PRL. These cells can be easily seen by PbH with which they stained dark blue or black (figure 19). Ultrastructural examination of ACTH cells revealed the presence of small secretory granules of about 100-190 nm in diameter (figure 20, 21). The nuclei were ovoid to irregular. The mitochondria with spaced cristae were also seen.

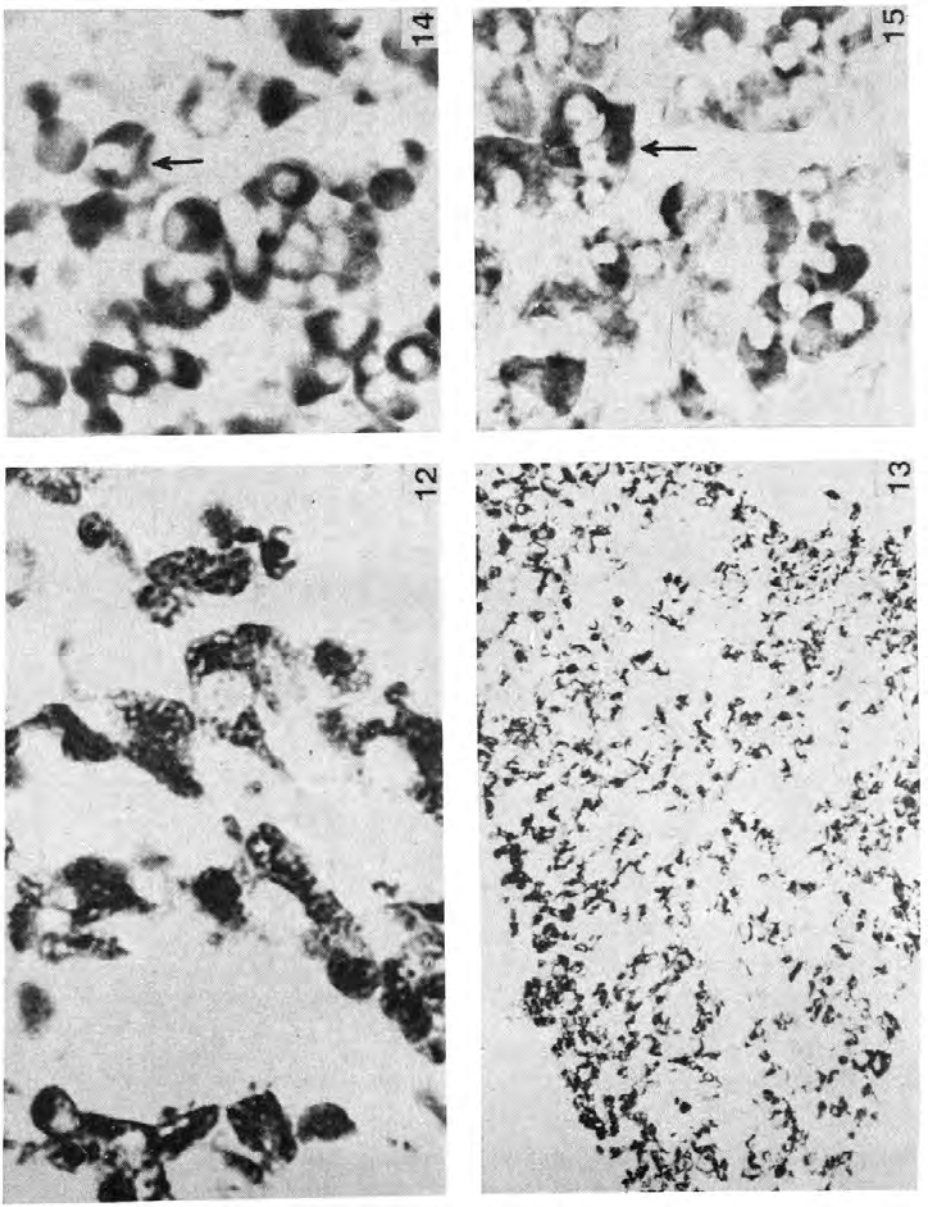
*Type VI (Somatotrophs or STH cells)*

These cells were identified by using histochemical staining techniques (table 1). They were evenly distributed throughout the PD except in the postero-lateral region (figure 22) where they were present in slightly larger number and in clusters. STH cells are rounded or ovoid with finely granular cytoplasm and small rounded nuclei (figure 23). They were the most abudant cells distributed singly or arranged in Palisades along the capillary. These cells stained yellow with tetrachrome (figure 24) and Cleveland-Wolfe trichrome, orange with modified AB-PAS-OG, and Mallory's triple and orange/yellow with PAS-OG. EM study revealed numerous, round to oval dense secretory granules of 240-480 nm in diameter. The mitochondria were usually round and scattered and the Golgi apparatus inconspicuous (figure 20, 21, 25).

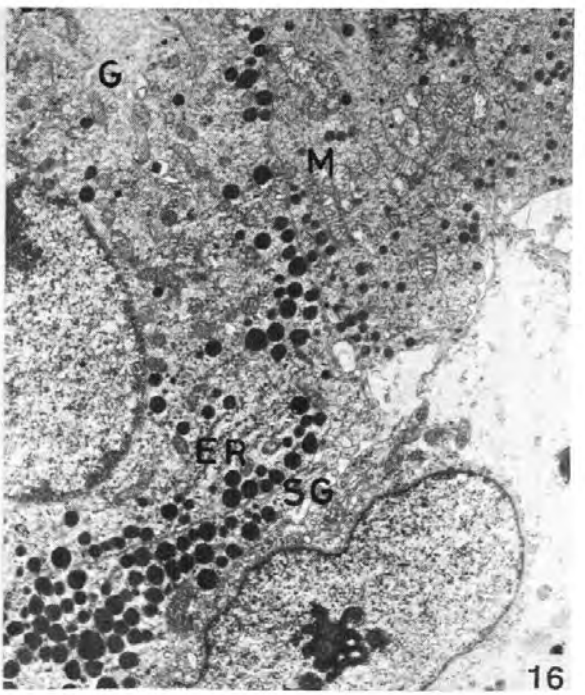
## Discussion

The identification of the TSH, LH, FSH, ACTH, PRL and STH cells in *S. heathi* pituitary by using different histochemical stainings, EM and ICC techniques is in accordance with the earlier findings in other bats (Patil 1974, Richardson 1979, Anthony & Gustafson 1984b, Badwaik 1988, 1989, Mikami et al. 1988, Bhiwgade et al. 1989) and in most other mammals (Girod 1983, Yoshimura & Gorbman 1986, Gopal Dutt 1989).

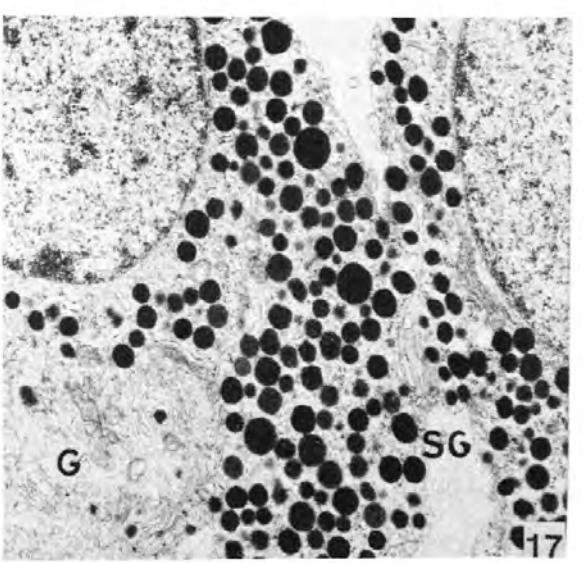
As reported by Herlant (1964) and Patil (1974) in other bat species, the TSH cells of *S. heathi* are AB-, AF- and aniline blue positive. TSH cells with similar staining response

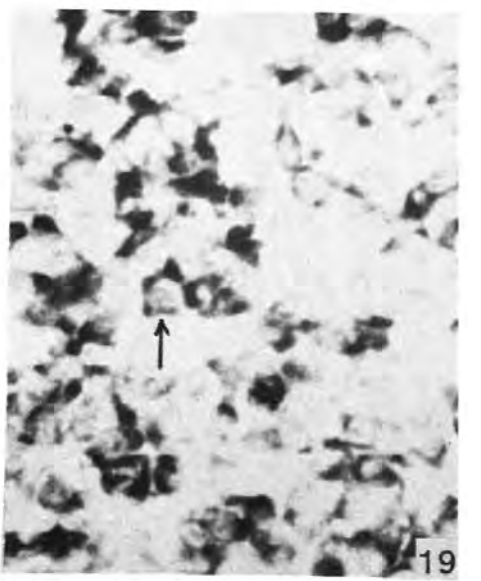



**Figures 12-15 12,** Section showing LH cells stained immunocytochemically with antiserum against h βLH (× 300); **13,** Section showing distribution of the FSH cells stained immunocytochemically with antiserum against h βFSH (× 60); **14,** Section stained with AB-PAS-OG showing two types of AB positive basophil. GTH cells are large and darkely stained (arrow) (× 240); **15,** Section of pars-distalis stained with Mallory showing GTH cells (arrow) (× 275)

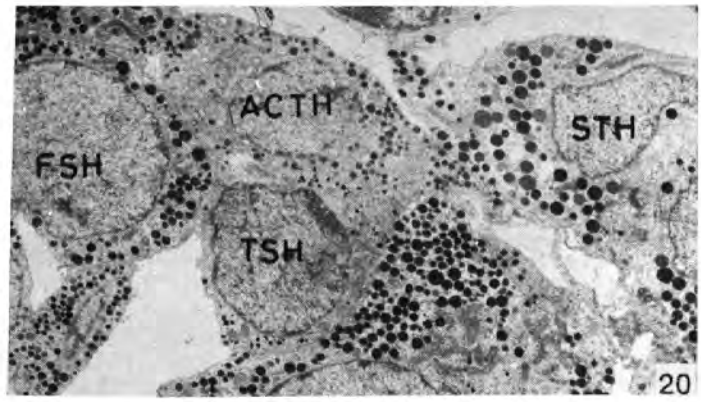


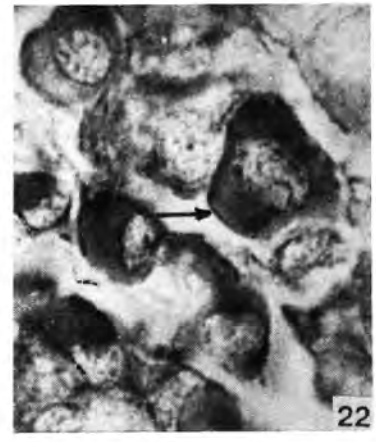


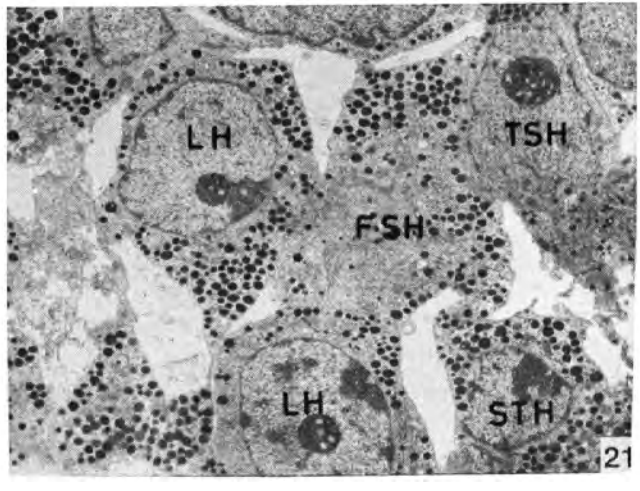


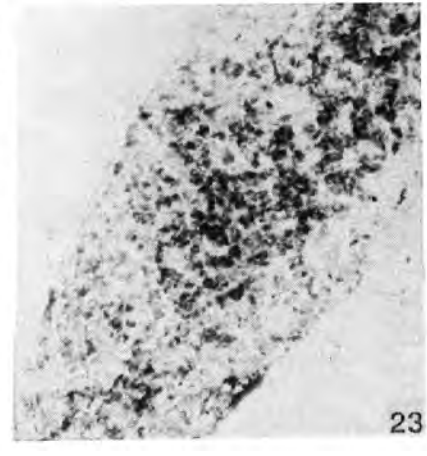




**Figures 16-19** **16,** FSH cells showing mitochondria (M) with well-developed cristae. Rough ER (ER) and Golgi complex (G) are also seen. **16,** (SG) Secretary granules ( × 20000); **17,** LH cells showing mitochondria (M) and conspicuous Golgi complex (G), SG = Secretary granules ( × 20000); **18,** ACTH cells stained immunocytochemically with anitsera against h ACTH ( × 60); **19,** Section of pars-distalis stained with PbH showing ACTH cells (arrows) ( × 240)

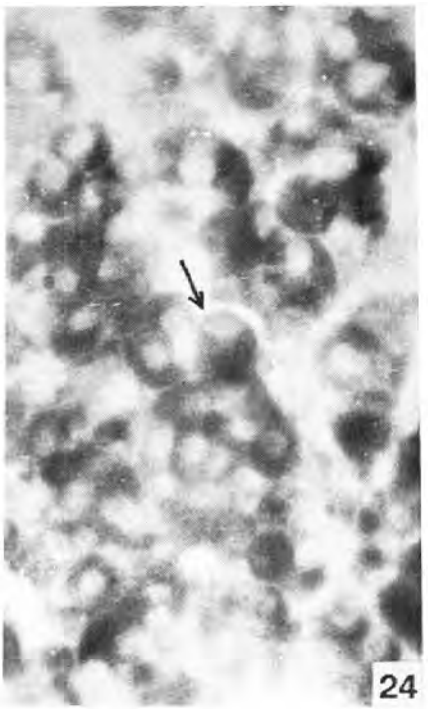


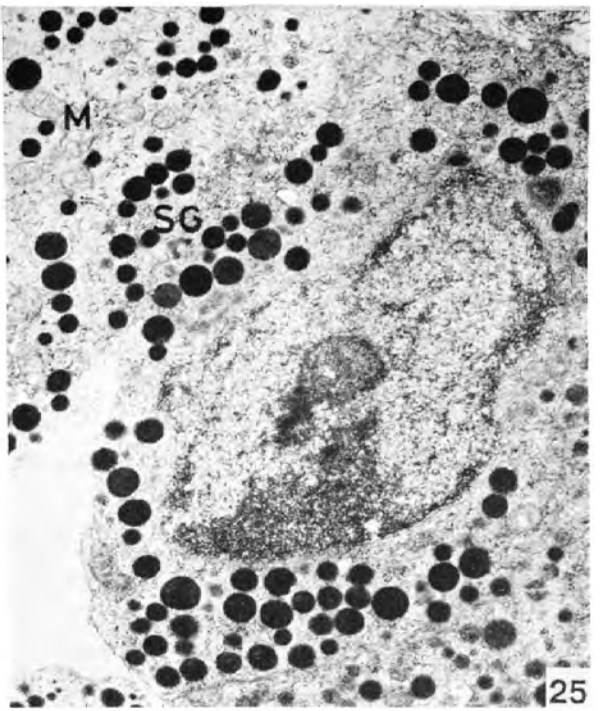




**Figures 20-23 20,** Low power view of electron micrograph showing ACTH, FSH and TSH cells ( × 5600); **21,** Low power view of electron micrograph showing LH, TSH, FSH and STH cells ( × 5600); **22,** Section showing STH cells (arrow) stained histochemically with Mallory ( × 660); **22,** Section **23,** Section showing distribution of STH cells stained histochemically with Mallory ( × 60)




**Figures 24-25** **24,** Section of pars-distalis stained with Herlant's tetrachrome showing STH cells (arrow) ( × 240); **25** STH cells showing round mitochondria (M) and secretary granules (SG) ( × 20000)

have also been identified in several mammalian species including bats (see Herlant 1964, Richardson 1979, Girod 1983). In ICC staining TSH cells appear as a distinct type of cells showing marked differences in immunoreactivity and distribution from other pituitary cell types. Ultrastructural studies have shown that the TSH cells in *S. heathi* contain small secretory granules, which range from 100 to 160 nm in diameter. In *Myotis lucifugus* and *M. velifer* TSH cells range from 100 to 150 nm in diameter (Kobayashi 1966).

As in other bat species (see Patil 1974), GTH cells in *S. heathi* have been found to be large, oval or round and stained positive with PAS, methylene blue, AB and aniline blue. The presence of two distinct types of GTH cells (FSH & LH) in PD of several bats have been reported by earlier workers using cytochemical, ICC and ultrastructural techniques (Herlant 1964, Azzali 1971, Richardson 1979, Anthony & Gustafson 1984a, Mikami et al. 1988, Bhiwgade et al. 1989). In *S. heathi*, by using antisera against h βFSH and βLH on consecutive section of the pituitary, it was found that some of the cells react with both the antisera. At the same time other cells reacted either with FSH or LH antisera. Ultrastructural studies have revealed that as in other bat species (Herlant 1964, Azzali 1971), in *S. heathi* the GTH cells contain dense secretory granules ranging in diameter from 150 to 360 nm. According to Bhiwgade et al. (1989), the variation observed in electron density of the secretary granules may be sufficient to differentiate two types of gonadotrophs. Ultrastructurally, we also noticed two types of GTH cells in the PD of *S. heathi*.

In general, the PRL cells in bats are large (Patil 1974) and show affinity for erythrosine, azocarmine G and carmosine L (Azzali 1971, Richardson 1979) while STH cells are

orangeophilic (Herlant 1964, Richardson 1979). In *S. heathi* also the PRL cells are erythrosinophilic in Herlant's tetrachrome and Cleveland-Wolfe trichrome and STH cells are orangeophilic in the same techniques. Ultrastructurally STH cells in *S. heathi* are characterized by very dense secretory granules ranging from 240 to 480 nm in diameter. In general, STH secretory granules in bat pituitary are dense, membrane enclosed and range from 350 to 400 nm in diameter (Herlant 1964, Azzali 1971). Whereas, the PRL cells in *S. heathi* are characterised by large secretory granules which are about 300-600 nm in diameter. Earlier studies in bat have revealed variations in PRL secretoy granules (Richardson 1979).

Very little information is available on ACTH cells of bats. Herlant (1964) identified these cells in *Myotis myotis* as classical acidophils by the trichrome method. ACTH cells in *Rousettus leschenaulti* were shown to be amphophilic in nature (Bhiwgade et al. 1989). They are weakly PAS-positive and have an affinity for basic dyes such as aniline blue and haematoxylin. PbH positive nature of the ACTH cells in *S. heathi* is consistent with the reports in certain teleost fishes and mammals (Gopal Dutt 1989). Other studies employing PbH technique (Richardson 1979, Gopal Dutt 1989) have also described the presence of PbH positive cells in bats. However, Patil (1974) could not identify this cell type in the three hipposiderid bats. In *S. heathi*, ACTH cells were immunopositive to anti-human ACTH serum. The only other ICC study on the ACTH cells in bats appear to be on *Miniopterus schreibersii fuliginosus* (Mikami et al. 1988). Ultrastructural characteristics of ACTH cells include small size of the granules and mitochondria with spaced cristae. These characteristics correspond to those reported for the ACTH cells in other mammals (Girod 1984).

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