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Differentiation between the Anterior Pituitary Cells of the Egyptian Insectivorous Bats *Rhinopoma hardwickei* using Transmission Electron Microscope

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Abstract

In the pituitary gland of some species of the Egyptian bats there were several similarities and differences between the external shape and the distribution pattern of the anterior cells, but these data were insufficient, because we don't known what about with other species, so the present study was carried out new insectivorous species *Rhinopoma hardwickei* in order to elucidate the similarities and the differences. The results indicate that, the gland is irregular in shape and two types of cells appeared by the semi thin sections. The acidophilic and the basophilic cells distributed heterogeneous in the body of the gland. (STH) Somatotropin Hormones are most numerous, the nucleus is irregular and eccentric with endoplasmic reticulum. The secretary granules are large with dense granules. Luteotropic Hormone (LTH): The nucleus is eccentrically near the plasma membrane, the mitochondria are spherical or elongated, the endoplasmic reticulum is rough and the granules are more. (ACTH) Adreno Cortico Tropic Hormone: These cells are found singly, irregular with eccentric nucleus. Secretary granules are small and spherical shaped, while the (TSH) Thyroid Stimulating Hormone: with small secretary granules but the (FSH and LH) Follicle Stimulating Hormone and Luteinizing Hormone: are found singly, angular in shape with eccentric nucleus and the secretary granules are spherical or ovoid in shaped and exhibit variation in electron density than STH cells. The differences in shape and distribution may be related to the phylogeny

Keyword: Pituitary cells; Insectivorous bats; Electron microscope

Introduction

The Bats (order Chiroptera) are the only group of mammals that have the capacity of echolocate and powerful flight.

In bats, such as *Myotis lucifugus lucifugus* [1,2] *Vesperugo savi and Vesperugo picolo* [3] *Cynopterus sphinx* [4], *Rousettus leschenaultia* [5], *Scotophilus heathi* [6,7] and *Hipposideros lankadiva* [8] as in all mammals, the hormones of the anterior pituitary glands regulate the activities of the gonads. By using the light microscope, the authors reveals six cell types of anterior pituitary based on granulation and staining reaction in the cytoplasm.

In littlebrown bat [9] the gonadotrophic cells were aggregated singly in the pars distalis which represent a subpopulation of gonadotropes that receives LHRH.

There were various cell types of the pituitary gland of the plains viscacha (*Lagostomus maximus*) was investigated by Patil [10] which distinguished from each other on the basis of their morphological characteristics. Madkour et al. [11] studied the histological characterizes of the pituitary gland of common bats from Egypt. They observed that there is a wall developed pars nervosa, pars intermedia and infundibular stalk but the residual lumen is poorly developed in some species than others.

In others mammals, El-Desouki and Selim [12] studied the pituitary gland of the carnivore *Vulpes zerda* and the herbivore *Oryctologus cunniculus*, and observed that the gland in *V. zerda* was pyramidal in shape with an apex directed dorsally and its base is cleft and directed ventrally while in *O. cunniculus*, the gland was pyramidal in shape but with a slight long apex directed posteriorly and a smooth base directed anteriorly.

There are randomly distributed extracellular colloidal accumulation which were observed in the pars distalis of Viscacha (*Lagostomus maximus maximus*) located in the peripheral zone of the gland and showed variability in shape and size [13]. In vespertilionid bat, *Scotophilus heathi* the electron microscopic study revealed six distinct cell types in the pars distalis on basis of specific morphological characters, staining reactions and immune characteristics [6,7].

Nerkar and Gadegone made a report on the "(LTH)" cells in some species of bats in which the mitochondira were round with lamellar cristae, rough endoplasmic reticulum was in the form of elongated and short tubular profiles, Golgi complex is inconspicuous, the spherical or ovoid secretary granules 200-250 μm in diameter of variable electron density were present just below the plasma membrane. Nerkar et al. reported that the gonadotrophs exhibit cyclical changes in structure and number during late pregnancy [14,15].

In *R. aegyptiacus*, the pituitary gland is roughly triangular in shape with abroad base directed anteriorly and the pars nervosa found between the two lobes of glandular part (pars distalis), so there was no residual lumen but in Taphozous. *nudiventris* the pituitary gland was dorsoventrally compressed and is semicircular in shape. By using electron microscope, the nucleus of the STH of the two species was circular in shape with a little indentation and centrically placed mostly euchromatic with varying amount of heterochromatin. Mitochondria were spherical and relatively distributed throughout the cytoplasm [16].

The Aim of the Work

Differentiation the pituitary cells of the insectivorous bats in the world has attracted the attention of many authors, however in

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Egypt may be low, so my article concentrated on pituitary cells of *Rhinopoma hardwickei* as example of insectivorous bats because in the Egyptian bats there were several similarities and differences between the shape and the distribution pattern of the anterior pituitary cells, but these data were insufficient, therefore the present study was carried out new insectivorous species *Rhinopoma hardwickei* in order to elucidate the similarities and the differences.

Material and Methods

Bats

The specimens of *Rhinopoma hardweickei* (Microchiroptera) used in this study were procured alive from Abu-Rawash, Giza Governorate through the years 2010 and 2011.

Measurements

The mean of 20 measured adult male specimens in millimeter were as follows:-

Total length: 57.0(54-61); Tail:14(12-16);Fore arm:61(57-62);Hind foot:12(10-14); Ear:18(16-19) and Tragus: 7.5(5.5-8.5) in Figure 1.

Collected the animals

The adult specimens collected for transmission electron microscope, and small pieces of 1 x 1 x 1 mm of pituitary gland of bats were obtained and were rapidly processed as follow:-

Fixation: Small pieces of fresh specimens were fixed in a mixture of Formaldehyde / Glutraldehyde (4:1) at ph 7.4 at room temperature for 4 hours, and then rinsed twice in 0.1M phosphate buffer (15 minutes for each).

Post-fixation treatment: Specimens were post fixed in 2.0% buffered Osmic acid for half an hour at 4˚C. The tissue specimens were then washed twice in phosphate buffer for 30 min.

Dehydration: The tissues were dehydrated in ascending grades of ethyl alcohol (50, 70, 80, 90 and 100%) for two changes each of 15 minutes (2 x 15 minutes) in each grade. Specimens then cleared in propylene oxide for 2 changes 5 min, each.

Infiltration: Dehydrated tissues were initiated in 1: 1 solution of propylene oxide and epon mixture. Infiltration was continued with 1: 3 (propylene oxide: epon mixture) overnight at room temperature.

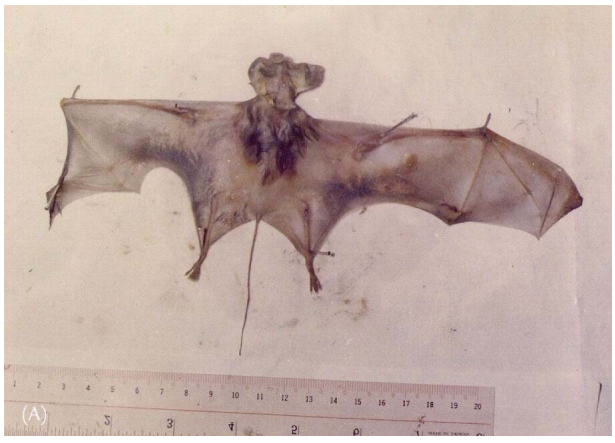

Figure 1: Photographs show external feature of adult insectivorous bat *Rhinopoma hardweickei.*

Embedding: Embedding was carried out using freshly prepared araldite epon mixture in capsules pre-dried for 1-3 hours.

Polymerization: The capsules were polymerized at 60˚C and then the polymerized capsules were cured at room temperature for at least a day before attempting to section.

Sectioning and staining of semithin sections for light microscopy: Blocks were trimmed under the binocular microscope of ultra-cut Reichert jung ultramicrotome. Semithin sections of 1 µm thickness were obtained with the aid of glass knives made on Leica EM KMR (Knife marker). Semi-thin sections were stained by toludine blue and examined for general orientation with the light microscope.

Sectioning and staining of ultrathin sections for electron microscopy: Specimens were then retrimmed to the selected region and ultra-thin section 60 nm thickness was cut and picked up on copper grids. Sections mounted on grids were double stained using uranyl acetate and lead citrate. Seven percent uranyl acetate solution in methyl alcohol was prepared and centrifuged at 5000 rpm for 10 minutes. A drop of uranyl acetate solution was placed on a dental wax sheet placed in a Petri-dish. Grids were then placed on the uranyl acetate drop (the surface grids loaded with section being facing the stain).Staining was done in the dark for about 20 mins. Grids were washed in three successive glass bottles containing distilled water. After the last bath, grids were dried on a filter paper. Sections were then stained with freshly prepared lead citrate that centrifuged at 5000 rpm for 10 minutes and used for staining for 10 minutes as in uranyl acetate, then washed with 0.02 N Noah and finally with freshly distilled water.

The grids dried on filter paper and examined with electron microscope (TEM Philips 400 T at 80 Kv). Photos were made on Kodak EM sheet films; developed then enlarged and printed and investigated.

Results

The external morphology

The pituitary gland of *Rhinopoma hardwiekei* is irregular in shape The adenohypophysis occupy large area than the neurohypophysis which it into lobes around the adenohypophysis (AD.H) in Figure 2. By using semi thin sections we can differentiate between two types of cells acidophilic cell and basophilic cell in Figure 3.

Transmission electron observations

Cell identification and differentiation in the mammalian pituitary gland is based on the activity of the Golgi-apparatus, the elaboration of secretary granules, liberation of the granules from the cell membrane and morphology of the mitochondria. Beside this, the shape and the size of the cell, nucleus and the size of the secretary granules are also taken into consideration. All these constitute visible changes which allow us to determine the state of the secretary activity of each cell.

Somatotroph (STH-cells)

These are the most prevalent cell type in the pars distalis of the present bat. These cells are ovoid to polyhedral in shape and are found distributed throughout the pars distalis. The nucleus is different from circular to irregular in shape with varying amount of heterochromatin is seen at the periphery of nucleus. Nucleolus is visible. Mitochondria are poor in some cells. Rough endoplasmic reticulum is seen. Secretary granules are ovoid or irregular in shape with high electron density. They are large and medium mostly seen scattered through one poles of cell. They are seen below the plasma membrane in Figure 4.

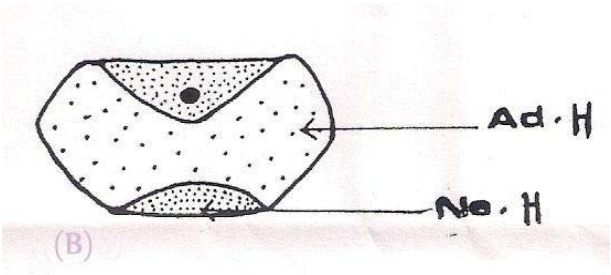

Figure 2: External morphology of the pituitary gland of *Rhinopoma hardweickei* showing the two lobes (Ad. H) adenohypophysis and (Ne.H) neurohypophysis.

Lactotroph (PRL-cells)

LTH cells are easily recognizable with electron microscopy in this bat. Nucleus is eccentrically and a rim of heterochromatin is seen attached to inner surface of nuclear membrane. The rough endoplasmic reticulum is in the form of short and long tubular profiles dotted with ribosomes are seen scattered in the cytoplasm. Large number of electron dense secretary granules is present. They are markedly circular or irregular in shape. These electron dense granules are seen mostly towards the apical cytoplasm, near or attached to the plasma membrane. At some cells there is some collection of mitochondria in Figure 5.

Corticotroph (ACTH-cells)

These cells are found either singly or in groups. These are not very abundant and correspond to a small number of adenohypophysial cell populations. These cells are elongated or angular with long cytoplasmic processes with eccentric nucleus. Nucleus is large, circular or irregular with different shapes. Enormous numbers from mitochondria are present. Golgi apparatus is not well developed. Rough endoplasmic reticulum is present. Granules are spherical or ovoid shaped and exhibit small size than the obvious cells. Large number of small vesicles is present in Figure 6.

Thyrotroph (TSH cells)

These are elongated, polygonal or triangular in shape with large cytoplasmic processes. The nucleus is irregular in outline and shows indentations. It is placed eccentrically; clumps of heterochromatin are seen distributed in nucleoplasm. A rim of heterochromatin is adherent to the inner surface of nuclear membrane. Mitochondria are very few. The secretary granules are very small, somewhat spherical and vary in electron density. In sparsely granulated cells, they show a peripheral distribution in Figure 7.

Gonadotrophs (FSH and LH cells)

In the present study, the identification and characterization of the FSH cell is based on morphological features of the cellular constituents such as secretary granules, ergastoplasm, Golgi apparatus and mitochondria. In the FSH cells the euchromatic nucleus is irregular in outline and shows indentations. Heterochromatin in the form of chromatin granules are seen scattered throughout the nucleoplasm. Mitochondria are very few. Elements of Golgi complex outline a cytoplasmic area nearly as large as the nucleus. Secretary granules are electron dense, spherical, variable size and are distributed in apical cytoplasm. Rough endoplasmic reticulum is present. LH gonadotrophic cells are large circular nucleus occupying maximum part of the cytoplasm. Heterochromatin flakes are distributed throughout the nucleoplasm. A thick rim of heterochromatin is seen below the nuclear membrane. Mitochondria are few. Some mitochondria are hypertrophied with loss of cristae. The cisternae of rough endoplasmic reticulum are dilated and are distributed throughout the cytoplasm, but more towards the nucleus in Figure 8.

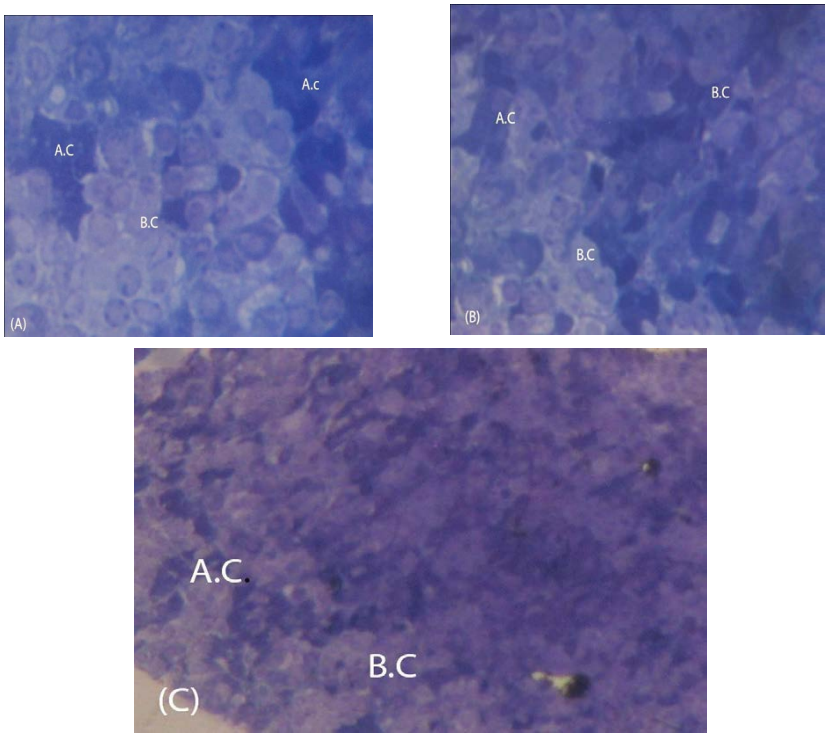

Figure 3 (A-C): ESemi thin sections of the pituitary gland of *Rhinopoma hardweickei* showing the Acidophilic Cells (AC) and the Basophilic Cells (BC) with higher magnifications in (A-B) and the distribution of those cell in (C). Toludiene blue stain.

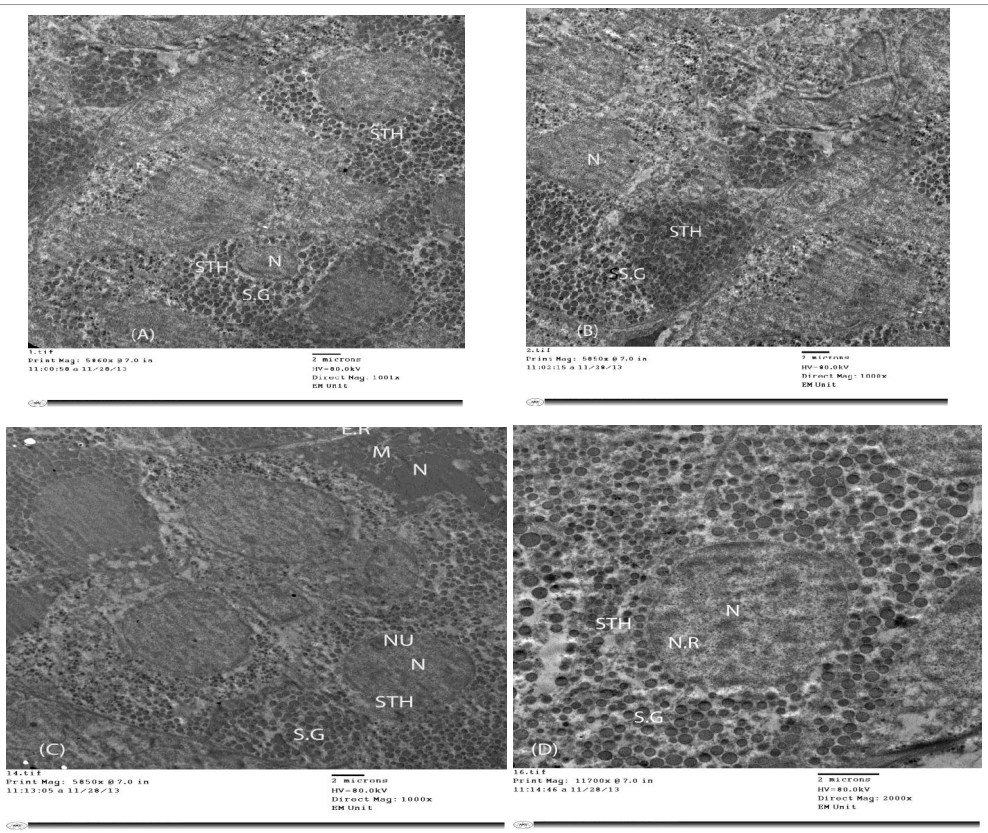

Figure 4 (A-C): Transmission electron microscope of the adult pituitary gland of Rhinopoma hardweickei showing the Somatotrophic Cells (STH) with dense Secretary Granules(SG), spherical Mitochondria(M), different shape of the nucleus, nucluoleus (NU) and Nuclear Rim(N.R).

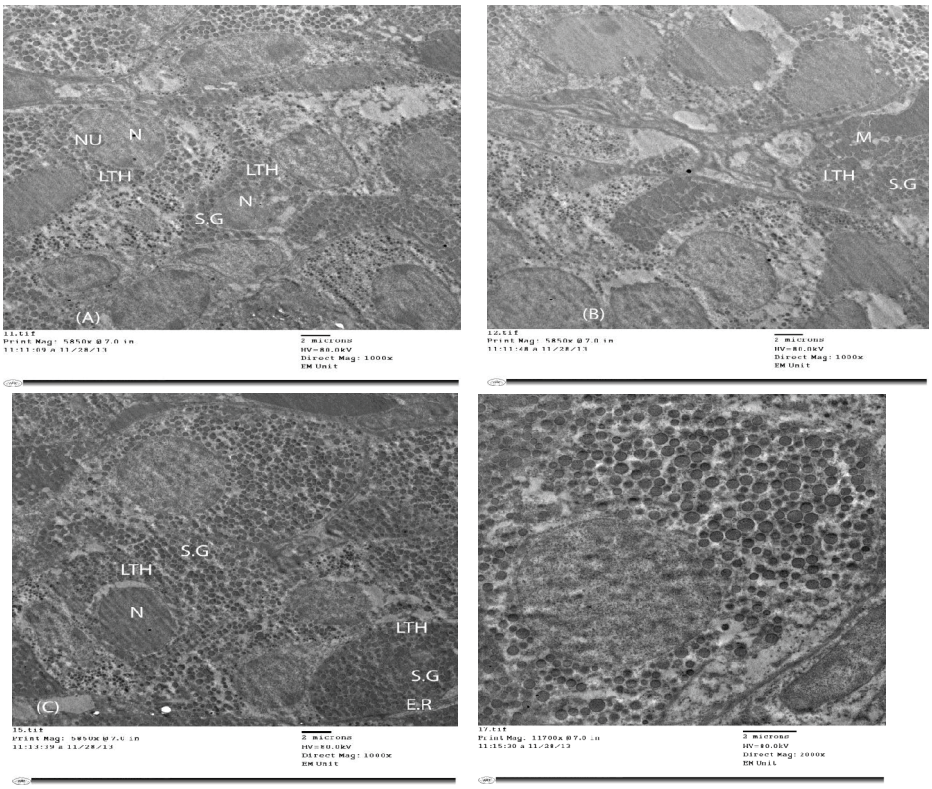

Figure 5 (A-C): Transmission electron microscope of the adult pituitary gland of T Rhinopoma hardweickei showing the Lactotrophic Cells (LTH). With dense Secretary Granules (SG). The nucleus (N), the Mitochondria (M) is spherical, the endoplasmic reticulum (ER) is rough.

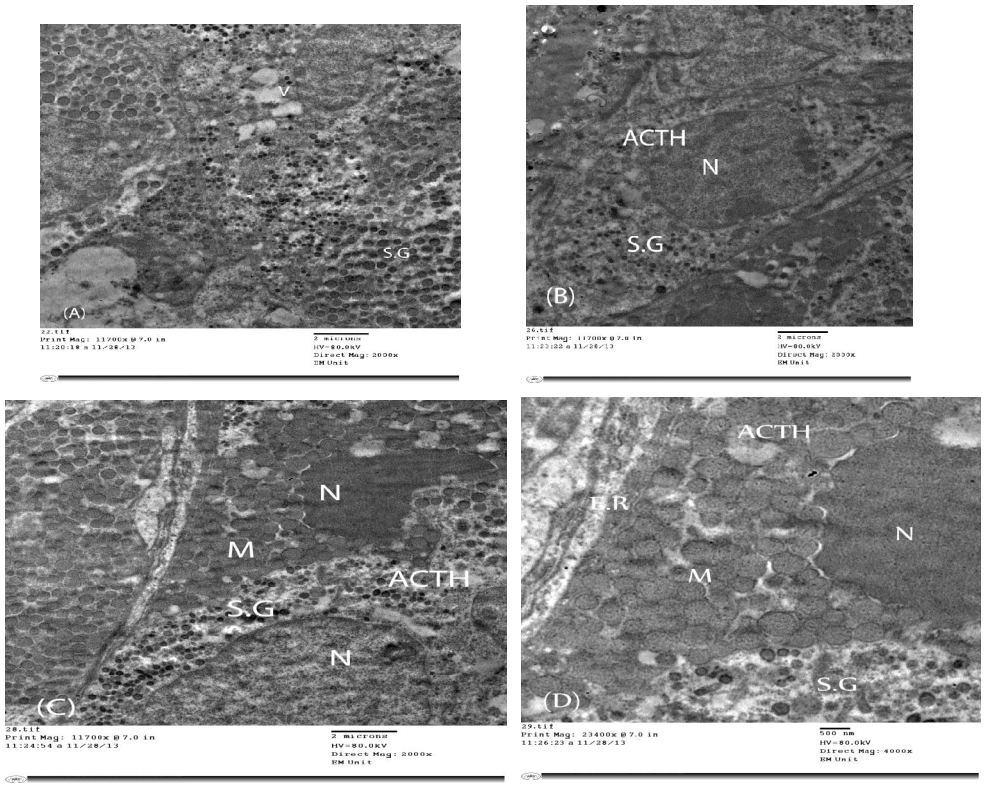

Figure 6 (A-C): Transmission electron microscope of the adult pituitary gland of Rhinopoma hardweickei showing the adrenocortictrophic (ACTH) These cells are found singly, irregular with eccentric nucleus(N). The nucleus is large, Mitochondria (M) round, enormous. vacuoles (v) present . The secretary granules (SG) are small.

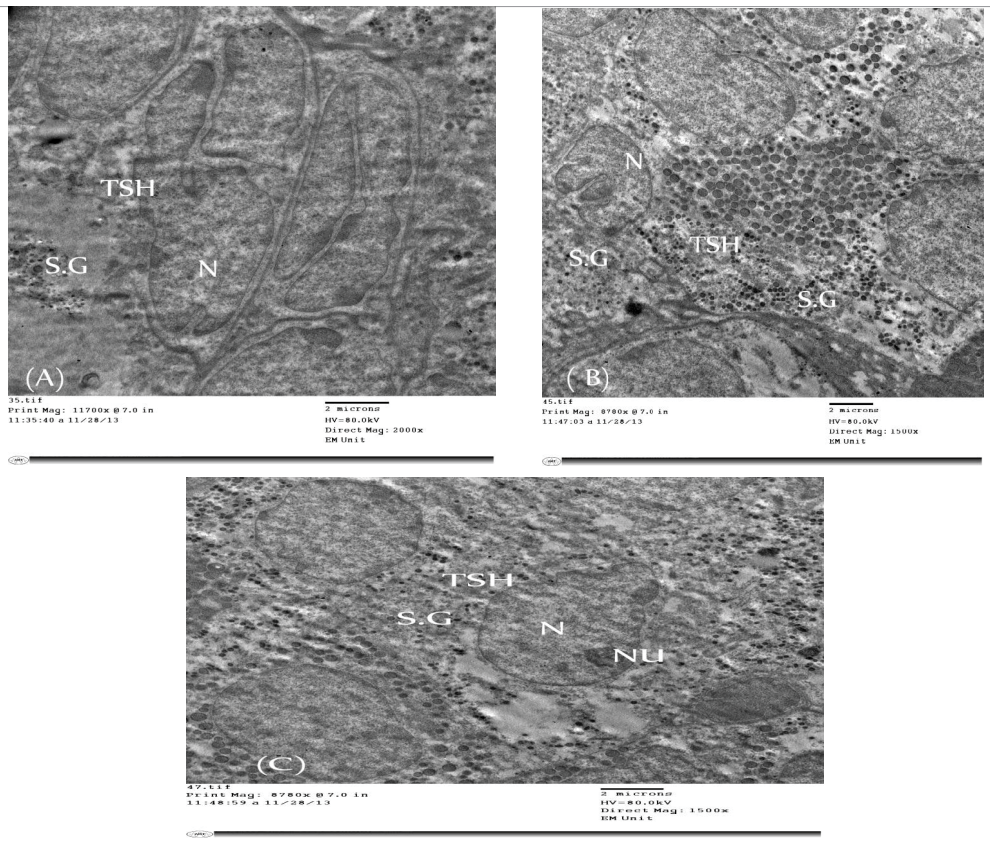

Figure 7 (A-C): Transmission electron microscope of the adult pituitary gland of Rhinopoma hardweickei showing thyrotrophic cells (TSH). These cells are with centric nucleus (N). The Mitochondria (M) are round, Rough endoplasmic reticulum (RER). The Secretary granules (SG) are small spherical.

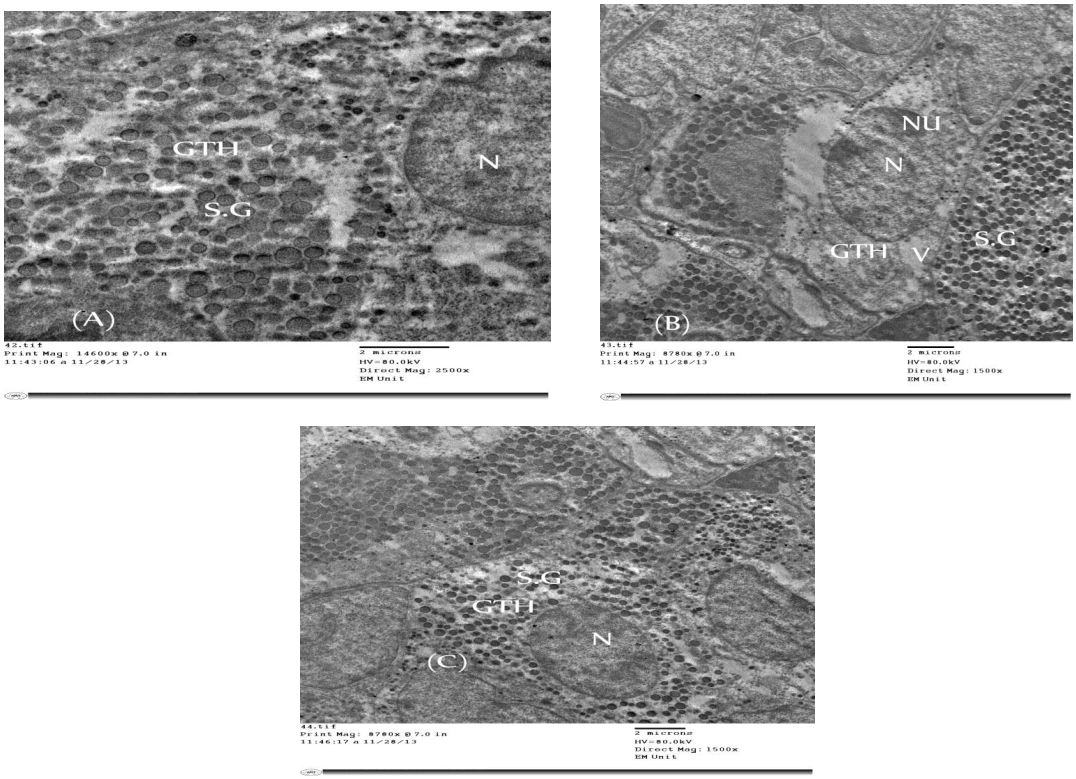

Figure 8 (A-C): Transmission electron microscope of the adult pituitary gland of Rhinopoma hardweickei showing (FSH). These cells are with eccentric nucleus (N). Mitochondria are round and Rough endoplasmic reticulum. Secretary granules (SG) are spherical and larger in FSH than LH.

Discussion

The morphology of the pituitary in *Rinopoma hardwicki* was irregular in shape different from *Taphozous nudiventris* which was semicircular in shape [17] and was that of emballonurid bat, *T. melanopogon* [3]; rhinopomatid bat, *Rinopoma hardwicki hardwicki* [18] and *Megaderma lyra lyra* [5] were semicircular in shape, and also different from *Rousettus aegyptiacus* which was triangular in shape.

The present study demonstrates that the two types of cells (acidophilic and basophilic) in the anterior pituitary undergo changes in number and cytological characters. The acidophilic cells, due to their solid protein nature of high insolubility, were easily preserved by any method of fixation [19]. In the present study, dark blue color of toluden blue stain indicates acidophilic cells while light blue color indicate basophilic cells similar to that reported in *T. melanopogon* and *Cynopterus sphinx* [20].

Electron microscopic study reveals the state of activity of the cells and their morphological features in the non-pregnant adult females and adult males, which allows formulating the criteria for their functional analysis. In conjunction with the ultra-structure of the mammalian pituitary gland [6,7,21-24] the ultra-structural observations demonstrate the presence of six cell types in the pars distalis of the present bat. The increase in Nuclear size, enlargement of nucleoli and nature of secretary granules of the cells have taken as the indicators of differentiation between the different cells.

Somatotroph (STH- cell)

The Ultra structural characteristics of (STH) cells of Indian fruit bat, *R. leschenaulti* have been studied by Bhiwagade et al. [25] which are round to oval in shape with centrally placed round nucleus. The secretary granules are numerous, uniform, round and very dense about 350-400 nm in diameter and mitochondria are round and scattered in the cytoplasm. The nucleus of the (STH) cells in the present bat is eccentric and irregular in shape different from the case of *R. leschenaulti* and the case of *Taphozous nudiventris* which characterized by different shapes of the nucleus from rounded, irregular to bilobed. But in *Rousettus aegyptiacus* the nucleus rounded or irregular not lobed [17]. The secretary granules are numerous and large, no well-developed Golgi apparatus, but in *S. heathi*, [6] the secretary granules are dense ranging from 240 to 480nm in diameter, round mitochondria and Golgi apparatus present in the (STH) cells.

The present finding study of (STH) cells similar to the case of *Hipposideros lankadiva* [8] which are oval with eccentrically placed nucleus. The secretary granules are numerous, mostly round to oval with uniform electron density.

The lactotrophic cells (LTH cells)

The ultra-structure features of LTH cells in the present animal reveal that mitochondria are numerous, rough endoplasmic reticulum are well developed and the electron dense granules are very large scattered in the cytoplasm similar to the case of Bhiwagade et al. [5]. In *S. heathi,* Singh and Krishna [6] reported numerous mitochondria, dilated endoplasmic reticulum and extensive Golgi complex, large number of secretary granules in the (LTH) cells. Ishibashi and Shiino (1989) identified the (LTH) cells in the pars distalis of *Pipistrellus abramus* conforming to the present study [26].

The proliferation activity of mammotrophs in female bat was observed during pregnancy and lactation [27]. The morph metrical activity and PRL level were mostly low during follicular development

while the PRL level increases after implantation [28].

In the present study, (LTH) cells in par distalis of adult male bat, were. The ultra-structural features of luteal cells in *Taphozous* indicate that these cells are steroidogenically active during pregnancy [15] who suggesting that the (LTH) cells are luteotrophic. Our findings correlate with the findings of other workers on bats.

Corticotroph (ACTH cells)

In the present study the (ACTH) cells are elongated or angular found either singly or in groups in pars distalis. Mitochondria are enormous. Rough endoplasmic reticulum present and the cytoplasm appear vacuolated. The spherical or ovoid secretary granules of variable electron density are present just below the plasma membrane, which similar to the case of the (ACTH) cell of *T. longimanusa* and also in *S. heathi* [6,7] *R. leschenaulti* [5].

Thyrotroph (TSH cells)

The (TSH) cells show ultra-structural features except well-developed rough surfaced endoplasmic reticulum. It is in the form of array of elongated, tubular or lamellar cisternae frequently localized at one pole or periphery of the cell. The profiles of cisternae are parallel to one another or curved. Golgi is inconspicuous. The small secretary granules are very small with electron density scattered throughout the cytoplasm or show peripheral distribution similar to *S. heolhi* [6,7].

Gonadotrophs (FSH and LH cells)

The gonadotrophs in the present bat differentiated into two distinct types the FSH and LH secreting gonadotrophs. According to Bhiwagade et al. [5] the variations observed in the electron density of the secretary granules is sufficient to differentiate two types of gonadotrophs. These cells also differ in their cytoplasmic organelle. The ultra-structure studies on pars distal in pregnant bats of *Taphozous nudiventris* and *Rousettus aegyptiacus* point to the existence of two types of gonadotrophs in bats [17].

The (FSH)-secreting cells described in the present bat correspond to the presumptive rat (FSH) FSH cells of bat [24] and (FSH) cells of mink [29]. In the present study (FSH) cells are large ovoid and the rough surfaced endoplasmic reticulum is well developed. Secretary granules are spherical and show variable electron density

Conclusion

From the previous study we can concluded that there are obvious differences in the cytological structure of the pars distalis cells from the other Egyptian bats and also from the other bats in the world may be related to some extent to the phylogeny, difference of habit and feeding intake.

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