Light and Electron microscope Studies of the Adrenal Glands of the Egyptian Geese (*Alopochen aegyptiacus)*

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ABSTRACT

Twenty mature and immature male and female Egyptian geese, ranged in age from three to eighteen months, were used in this study. The adrenal glands of the Egyptian geese were paired organs weighing approximately 200-250 mg (7.5 mg/100 g body weight) and were situated anterior to the kidneys on each side of the dorsal aorta and inferior vena cava. Each adrenal gland was surrounded from outside by a connective tissue capsule. The interstitial tissue was rich in blood vessels, collagen, and reticular fibers. The parenchyma cells of the adrenal gland were arranged in cords especially at sub-capsular zone (SCZ). Thin layers of connective tissue separated these cords and there were two types of cells: acidophilic and basophilic cells, which intermingle with each other and are separated by sinusoids. The acidophilic cells were large, polyhedral to columnar in shape, with a highly vacuolated and lightly stained acidophilic cytoplasm; while the cells of inner cords were large columnar and are less vacuolated. Ultrastructurally, these cells could be classified into two types, according to the amount of lipid droplets and mitochondria: cells that contained numerous lipid droplets with few somewhat large globular mitochondria, and the other type were cells containing few lipid droplets. Basophilic cells were bluish islets or scattered groups found in-between the acidophilic cells. According to the shape of the secretory granules, these cells could be classified into two types: cells that contained homogenous, polymorphic electron dense secretory granules, and cells that contained secretory granules of electron dense core surrounded by hallow electron lucent coat. With the increasing the age of the geese, the connective tissue capsule became thick and the interstitial tissue was increased. The acidophilic cells of the inner zone were more vacuolated and less acidophilic and slightly numerous in the peripheral, acidophilic cells than in those of the inner zones. The basophilic cells appeared less vacuolated and were smaller.

INTRODUCTION

The role of the adrenal gland is more difficult to be evaluated in geese than in mammals. The difficulties are partly related to the intermingling of the cortical and medullary tissues. It is generally accepted that the cortex of the geese adrenal gland cannot be divided into the three distinct zones (zona glomerulosa, zona fasciculata, and zona reticularis) as in its mammalian counterparts (**Gulmez, Kocamis, Liman and Kukner, 2004)**. The adrenal gland is an indispensable endocrine organ; it is a complex organ concerned with the production of multiple hormones and performs many kinds of physiological functions. The adrenal gland is as important in birds as it is in mammals; and the removal of the adrenal gland in birds eventually leads to death (**Peng, Chen and Liang, 2005)**. Morphological studies of the adrenal gland have been reported in pigeon (**Bhattacharyya, 1975)**, Canadian goose (**Gulmez et al., 2004),** and in Wanxi white geese **(Wang, Zhu and Jin, 1999)**, in fowl (**Siller, Teague and Mackenzie, 1975)**, in quail (**Basha, Vijayaragavan and Ramesh, 2004)**, in duck (**Pearce, Cronshaw and Holmes, 1978)** and in African ostrich chicks (**Li Tang, Peng, Wang, Luo, Cheng, Zhang, Sun, Liu, and Song, 2009)**. However, little attention was paid especially to the morphology of the adrenal glands in Egyptian geese, and the glands ultrastructure remains obscuxre.

The purpose of this study was to provide a concise account of general morphology, the cellular and sub cellular structures of the adrenal glands in Egyptian geese, and to compare them with those observations in other birds. This would hopefully contribute to the understanding of the features of the adrenal gland in birds in general, and of adrenal glands of Egyptian geese morphology, in particular.

MATERIALS AND METHODS

Twenty mature and immature male and female Egyptian geese were collected from EL-Qaliubiya Province in Egypt. Each sex was represented by ten birds that ranged in age from three to eighteen months. The birds were anaesthetized using 10% urethane, (1 g/kg body weight), and were sacrificed. The paired adrenal glands were carefull

dissected and removed from each sample and then cut into 1 mm blocks. Tissues for light microscopy were fixed in 10% neutral buffered formalin solution and Bouin's solution for 72 h, then dehydrated, cleared, and embedded in paraffin. Sections (5 - 6 microns) were cut and stained with haematoxylin and eosin and Crossman's trichrome stain and Gomeri's reticulin and Periodic acid Schiff according to the methods given by (**Crossmon, 1937; Bancroft, Cook, Stirling, and Turner 1994).**

The transmission electron microscopy evaluation was conducted at Science collage of Ain Shams University in Egypt. Small pieces of adrenal gland were fixed in 2.5% gluteraldehyde solution with 0.1 M phosphate buffer (pH 7.4) for 24-48 hours, post fixed in 2% osmic acid for 2 hours, dehydrated in ascending grades of alcohols and immersed in propylene oxide. Finally, they were embedded in Epoxy resin. The block was polymerized for 24 hours at 70°C. The ultrathin sections (70 nm) were cut, mounted on copper mesh grids (No. 200) and stained with saturated solution of uranyl acetate dihydrate and lead citrate as described by **Chiu, Schmidt and Prasad (1993)**. Then, the sections were examined with Jeol JEM 100S Transmission electron microscope (70KV).

RESULTS

The adrenal glands of the adult Egyptian geese were paired organs weighing approximately 200-250 mg (7.5 mg/100 g body weight) and were situated anterior to the kidneys on each side of the dorsal aorta and inferior vena cava. The glands measured approximately 7.5 mm in length and 5mm in width, and in transverse section, it appeared either triangular or oval with a thickness ranging from 3.5 to 4.5 mm.

The adrenal gland was surrounded from outside by connective tissue capsule (Fig. 1) that contained mainly collagen fibers (Fig. 2), reticular fibers, with very few elastic elements, blood vessels and fibroblasts. Delicate septa were arising from the capsule and were ramifying between parenchyma tissues form the interstitial tissue. The interstitial tissue was rich in blood vessels, reticular fibers (Fig. 3) which surrounded both types of cells and sinusoids. Groups of ganglionic cells are found both outside (Fig. 4) and inside the gland's parenchyma.

The parenchyma cells of adrenal gland were arranged in cords especially at subcapsular zone, two cells wide (Fig. 5), and the cells were orientated so that their longitudinal axes were transverse to the cord and the nucleus in each cell was situated toward the outer margin. Thin layers of connective tissue separated these cords and there were two types of cells (Fig. 5): acidophilic and basophilic cells. These cells intermingled with each other and were separated by sinusoids (Fig. 6).

The first type of cells, the acidophilic cells, was arranged in two- cells wide cords that rested on PAS positive membrane (Fig. 7&7a). At peripheral (subcapsular) zone, these cells were arranged in clumps forming loops directed opposite to the capsule. These cells were large, polyhedral to columnar with highly vacuolated and lightly stained acidophilic cytoplasm. The cells of inner cords were large columnar cells and are less vacuolated. The nuclei of acidophilic cells were rounded, apically located and contained one or two prominent nucleoli. Ultrastructurally, the acidophilic cells appeared columnar in shape, their cytoplasm contained numerous globular mitochondria, and many ribosomes, lipid droplets (Fig. 8), and smooth and rough endoplasmic reticulum and their nuclei were spherical, large contained prominent nucleoli and coarse chromatin. These cells could be classified into two types, according to the amount of lipid droplets and mitochondria, cells contained numerous lipid droplets (Fig. 8) with few somewhat large globular mitochondria and the other type were cells containing few lipid droplets (Fig. 9).

The second type of cells, the basophilic cells, were found in the form of islets that appeared, with general stain, as bluish islets or scattered groups in between the acidophilic cells (Fig. 10). They were polygonal or rounded in shape with basophilic cytoplasm and large spherical centrally located nuclei that contained two or even three nucleoli. According to the affinity of the cytoplasm to the stain, the basophilic cells could be differentiated into two types: cells with *deeply stained* basophilic cytoplasmic granules, and cells with *lightly stained* basophilic cytoplasmic granules (Fig. 10a). The blood sinusoids were found between the cell cords and islets, and were numerous and wider in the center of the gland than in the peripheral zone of the gland. The peripheral zone were formed mainly from the first type of cells where, the inner zones formed of

large amount of second type of cells and a few of first type of cells.

TEM revealed that the cytoplasm of the basophilic cells contained rod shaped mitochondria with tubular cristea, ribosomes, a few rough endoplasmic reticulum, lipid droplets and secretory granules. According to the shape of the secretory granules, these cells could be further classified into two types: cells that contained homogenous, polymorphic electron dense secretory granules (Fig. 11), and cells that contained secretory granules of electron dense core surrounded by hallow electron lucent coat (Figs. 12 &13).

With the increasing the age of the gees, the connective tissue capsule became thicker, and the amount of the interstitial tissues increased (Fig. 14). The acidophilic cells of the inner zone were more vacuolated and less acidophilic, and the vacuoles were slightly numerous in the peripheral acidophilic cells than in those cells of the inner zones. The basophilic cells appeared less vacuolated and smaller.

FIGURE LEGENDS

Fig. 1. Photomicrograph in geese adrenal gland of three months old female showing the capsule(c), blood vessel (bv), parenchyma of the gland (p), acidophilic cells (a) and basophilic cells (b) H&E. X 100.

Fig. 2. Photomicrograph in geese adrenal gland of seven months old female showing the collagen fibers (cf), blood sinusoids (bs) and blood vessels (bv). Crossman's trichrome stain. X 100.

Fig. 3. Photomicrograph in geese adrenal glands of seven months old female with Gomori's reticulin methods showing the distribution of the reticular fiber (rt) among the of the gland parenchyma X400.

Fig. 4. Photomicrograph in geese adrenal glands of ten months old female showing the capsule (c), parenchyma (p) and ganglionic cells (arrows). H&E. X 200.

Fig. 5. Photomicrograph in geese adrenal glands of ten months old female showing the two cells width forming the cords also, blood sinusoid (bs), acidophilic cells (a) and basophilic cells (b). H&E. X 600

Fig. 6. Transmission electron micrograph from eleven months male of geese adrenal gland showing blood sinusoid (bs) and thin layer of connective tissue between the cells of the glands. X2000.

Fig. 7. Photomicrograph in geese adrenal glands of seven months old male showing the septa between the cell (s) and & **Fig. 7a**.showing the positive membrane (arrows) of the same age and sex. PAS technique X 100 and 1000 respectively.

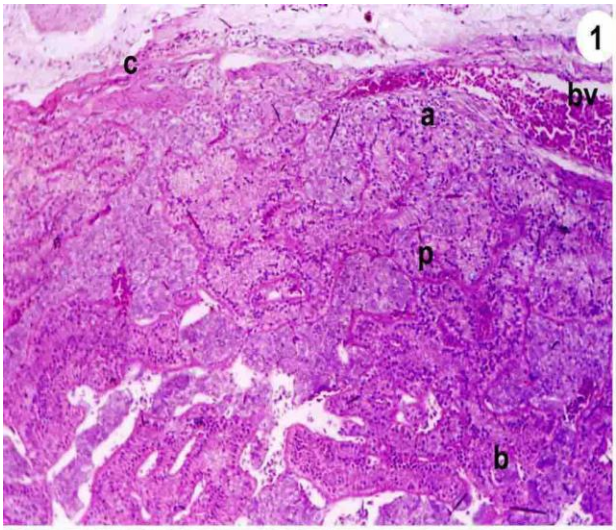
Fig. 8. Transmission electron micrograph from eleven months male of geese adrenal gland showing the subcapsular columnar cells with the nucleus (s), cell membrane (cm), mitochondria (m) and large number of fat droplet (f) in their cytoplasm. X4000.

Fig. 9. Transmission electron micrograph from eleven months male of geese adrenal gland showing the other subcapsular columnar cells with low fat droplet (f) in their cytoplasm and mitochondria (m). X3000.

Fig. 10. Photomicrograph in geese adrenal glands of twelve months old male showing the distribution of basophilic cells (b) in inner zone of the gland between the acidophilic cells (a) also blood sinusoids (bs). **Fig. 10a.** Showing the two types of basophilic, one deeply stain (d) and other lightly stains (l) of the same age and sex. H&E. X100 and 600 respectively.

Fig. 11. Transmission electron micrograph in geese adrenal glands of twelve months old male showing the basophilic cells containing nucleus (n), cell membrane (cm), mitochondria (arrows) and secretory granules (sg). X1000.

Fig. 12. Transmission electron micrograph in geese adrenal glands of twelve months old male showing the distribution of the two types cells of basophilic cells. Cells contained homogenous, polymorphic electron dense secretory granules (1) and cells contained secretory granules of electron dense core surrounded by hallow electron lucent coat (2). X3000.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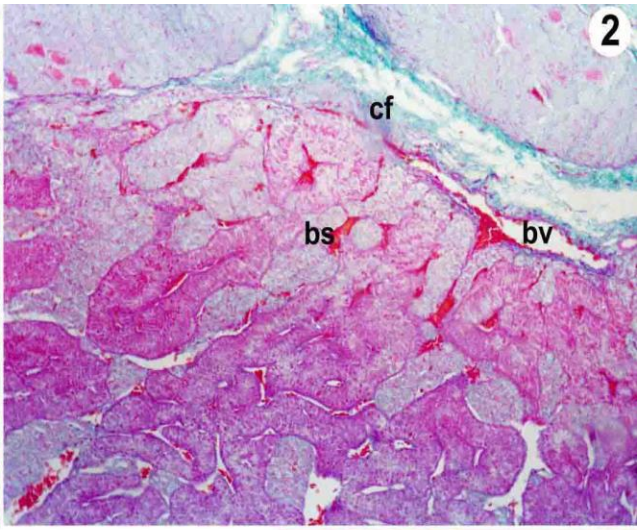
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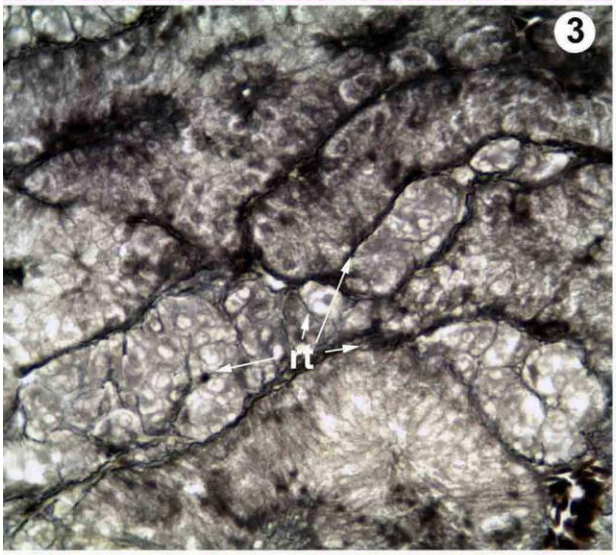
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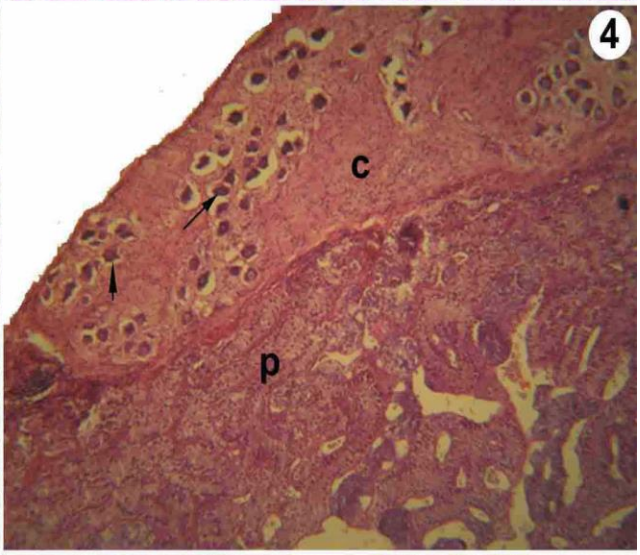
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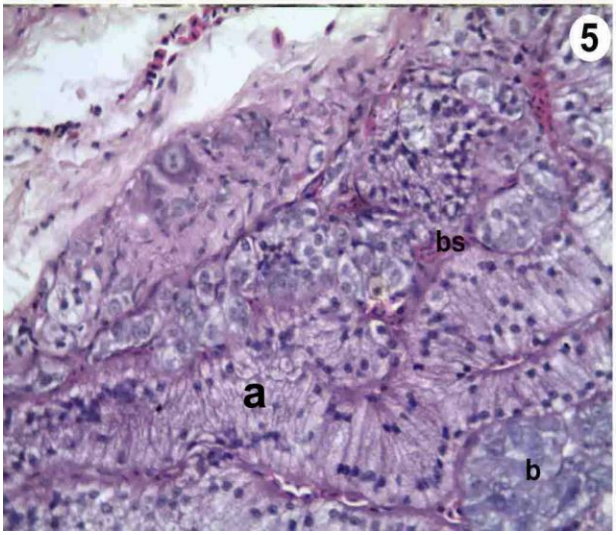
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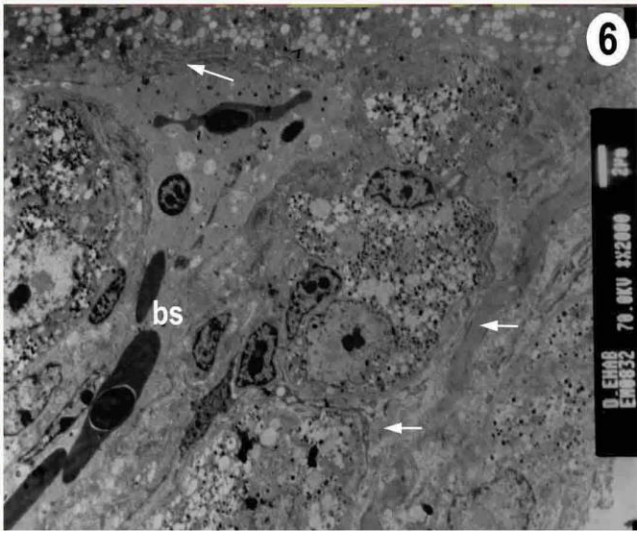
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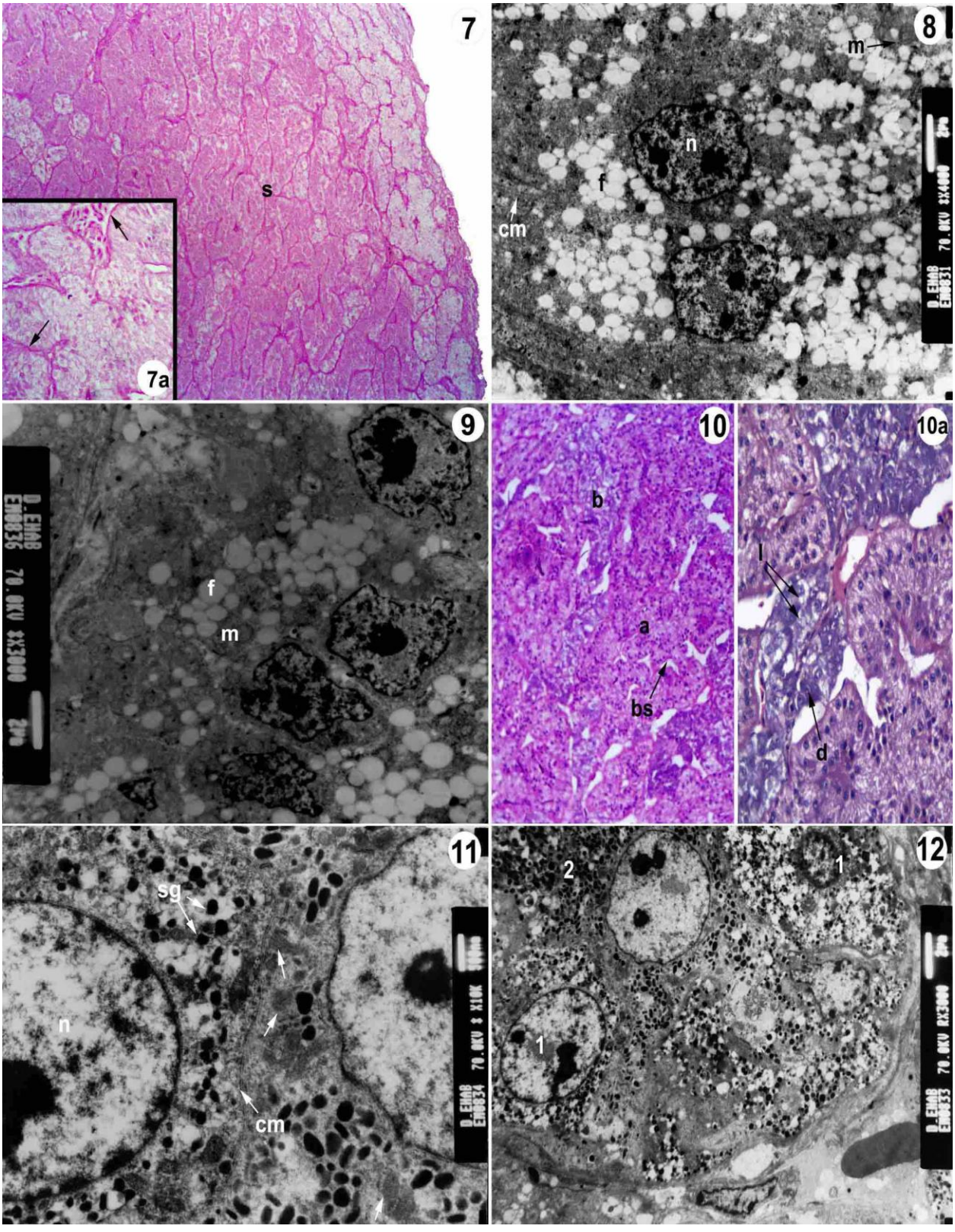
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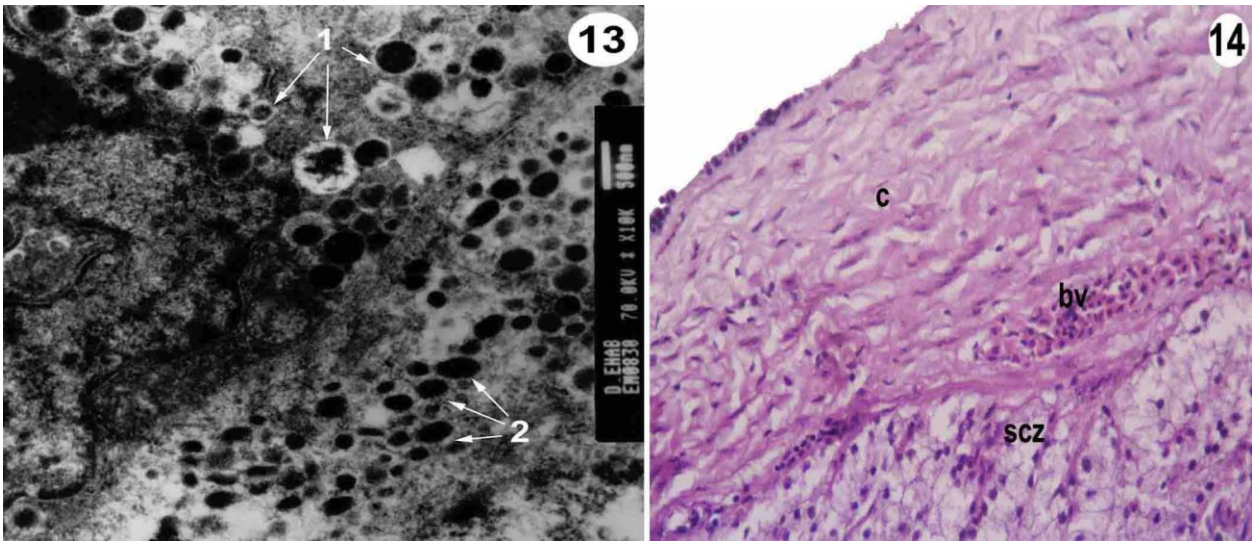

Fig. 13. High magnification transmission electron micrograph in geese adrenal glands of twelve months old male showing the difference of the secretory granules of basophilic cells, homogenous, polymorphic electron dense secretory granules (1) and secretory granules of electron dense core surrounded by hallow electron lucent coat (2)

Fig. 14. . Photomicrograph in geese adrenal of eighteen months old female showing the thick capsule(c), blood vessel (bv), subcapsular zone (scz) containing acidophilic cells. H&E. X 1000.

DISCUSSION

The birds are quite different from the mammals in that their adrenal glands are distinctly divided into an outer cortex, and a medulla that lies in the center of the gland, because of the scattered chromaffin tissue pervaded with islands between the cortical cells (**Luo, 1983; and Li, Luan, Yue and Zh, 2003).** For geese in this study, the parenchyma tissue was intermingled with each other, which generally agrees with the description of avian adrenal gland provided by **Unsicker (1973), Aire, (1981) and Cronshaw, Holmes, Ely, and Redondo (1989)** for mallard duck**.**

The present study also revealed that the parenchyma of adrenal gland of Egyptian geese consisted of two types of cells intermingled with each other. These cells are

acidophilic and basophilic cells. The former cells are largely found in the outer zone of the gland, while the latter type is concentrated in the center of the gland. These results are in agreement with **Sinha and Ghosh (1961)** in pigeon**, Ghosh (1962)** in avian, and with **Vyas and Jacob (1976)** in Indian avian species.

In ostrich chicks, the interrenal tissues and the tissue of the medulla intermingle with each other, which generally agrees with the description of other avian species. Beesides, the adrenal glands of ostrich chicks appeared to show larger amount of interrenal tissue (**Li et al., 2009)** than other avian species.

The peripheral zone of the adrenal gland is arranged in clumps forming loops reverse to the capsule, which is lined by columnar cells which are highly vacuolated lightly acidophilic while those of the inner cords were large and less vacuolated but more acidophilic. As observed by T.E.M, there are two types of cells according to the amount of lipid droplets and mitochondria, these finding are similar to those described by **Gulmez, Kocamis, Liman and Kukner (2004)** in goose (Anser Anser); while in parakeet, quail, and myna adrenal it was that the subcapsular zone cells in quail continued inside the gland as a double- layered central cords **(Bhattacharyya and Ghosh, 1972)**. The central cords consisted of high columnar cells with nuclei that were localized in adjacent layer of cells. In parakeets, they found that subcapsular zone cells were vacuolated and their nuclei were localized adjacent to the basement membrane. Also, the cytoplasm of internal zone cells consisted of columnar epithelium that was dense and basophilic. In Wanxi white geese, the cell cords in SCZ were arranged tightly, parallel to each other, and were perpendicular to the capsule, which consisted of high columnar cells with a lightly stained cytoplasm and a central nucleus **(Wang et al., 1999).** The arrangement of these cell cords is similar to those of the fascicular zone in mammalian adrenal gland.

The two types of cells, found in the center of the gland, could be differentiated according to the affinity of their cytoplasm to the stain: cells with *deeply stained* basophilic cytoplasmic granules, and cells with *lightly stained* basophilic granules.

Hodges (1974) has related the variation of the basophilia of medullary cells to the physiological activity of the cells. In this respect, **Telford and Bridgman (1990)** showed that there are two cell populations in the medulla of adrenal gland of mammals, about 80% of theswe cells synthesize epinephrine and the remainder of the cells produce norepinephrine. In birds, the interrenal or cortical tissue is of mesodermal origin and secretes the corticosteroid hormones, while the chromaffin or medullary tissue is of ectodermal origin and secretes adrenaline and noradrenaline (**Assenmacher, 1972; and Mori and George,1978).** Medullary cells in duck are characterized by a large population of electron opaque neurosecretory granules. These cells contain fewer mitochondria and cisternae of endoplasmic reticulum than the cortical cells **Cronshaw, Holmes and Loeb (1974)**. In Japanese quails, medullary cells have polyhedral shape and centrally located nucleus. Close to the centrally located nucleus, a moderate number of mitochondria, endoplasmic reticulum and well developed Golgi complex can be found. Catecholamine-containing secretory granules in both Epinephrine and Norepinephrine cells are enveloped by a continuous membrane and granules of Epinephrine are much smaller in size and more in number than that in Norepinephrine, in duck (**Klingbeil, Holmes, Pearce, and Cronshaw, 1979),** in avian (**Manna and Ghosh, 1979)** and in quail **Cigankova, Zibrin, Boda, and Holovska, 2005)**.

The results of the present study for Adrenal glands of Egyptian geese demonstrated that the uses of specific identification techniques (e.g. immunocytochemistry) are required to help identify or verify certain of cell types.

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