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Comparative Fine Structural Studies on the Adrenal Glands of the Toad, (*Bufo tibamicus*) and the Desert Reptile, *(Uromastyx philbyi)*

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Abstract: Fine structure of adrenal gland were studied in the toad, *(Bufo tibamicus)* and the desert reptile, *(Uromastyx philbyi)*. The adrenal glands of both animals are composed of adrenocortical and chromaffin tissues. In *B. tibamicus*, the adrenocortical tissue is comprised of two main types of cells, the steroid cells with abundance of lipid inclusions and eosinophilic cells with eosinophilic granular cytoplasm. The chromaffin tissue is intermingled with adrenocortical tissue. They are formed of adrenaline and nor-adrenaline secreting cells with granules of different electron density and variable sizes. The adrenocortical cells of *U. philbyi* constituent the main bulk of the inner gland and they are typical steroid-secreting cells. They are also characterized by their rich amount of lipid droplets. Cords or islets of inner chromaffin cells are scattered throughout the adrenocortical cells. The outer portion of the gland is composed of chromaffin cells surrounded by the capsule. Adrenaline and nor-adrenaline secreting cells were distinguished by means of different electron density of their chromaffin granules either in the outer or inner portion of the gland.

Key words: Adrenal gland, amphibia, reptiles, TEM

Introduction

Adrenal gland is one of the most important organ because it plays a principle role in the body activities and are essential for the maintenance of the whole life. This gland has undergone conspicuous morphological changes throughout the evolutionary history of vertebrates. Histological examination of the adrenal gland of vertebrates revealed that it is composed of two chief tissues, the adrenocortical and chromaffin tissues. Concerning the amphibian animals, the adrenal gland was found to be composed of two types of tissues, of separate origins, the interrenal and chromaffin tissues (El-Banhawy *et al.*, 1993). In contrast to mammals, the anuran amphibian adrenal gland has no morphological zonation, and the interrenal (adrenocortical) tissue is intermingled with chromaffin tissue (Accordi, 1981; Accordi and Grass-Milano, 1990). The adrenal gland of different amphibian was studied by many investigators (Volk, 1972 a, Verma, 1977, Al-Mouslemani, 1987) and their results revealed that the adrenocortical tissue is comprised of two main types of cells. The first type markedly rich in lipid inclusions and is called steroid cells. The second type is smaller in size than the steroid with a marked affinity to eosin dye and are called eosinophilic cells. The chromaffin tissue of amphibian adrenal gland is comprised of relatively large-sized cells containing a considerable amount of fine granulated cytoplasm (Volk, 1972 b).

As regards, the reptilian adrenal gland was described as a compound organ consisting of an outer distinct layer of chromaffin cells surrounding an inner group of interrenal cells with slight intermingling of two cell types (Wright *et al.*, 1957). This pattern of arrangement of inner adrenocortical cells and an outer chromaffin cells of reptilian adrenal gland is in an opposite to that of the mammalian adrenal that contains an outer cortex (adrenocortical cells) and an inner medulla (chromaffin cells), (El-Desouki,1996).

Varano *et al.* (1978) demonstrated the lizard adrenal gland, *Lacerta siculae* emphasizing on the morphological distinction of the adrenocortical tissue that occupied the largest part of the gland, while the peripheral areas contained namely chromophilic cells. Moreover, Hammouda *et al.* (1983) investigated the adrenal gland of two reptiles living in the Egyptian desert namely *Varanus griseus* and *Agama stellio*. They found that the histological structures of the gland in both animals resembled those of other lacertilians. Madkour *et al.* (1997) demonstrated many differences in the histological structure and histochemical contents of the adrenal glands of some reptiles including: *Chalcides ocellatus, Mabuia quinquetaeniata, Chamaeleo chamaeleon* and *Varanus griseus*. The experiment was conducted to compare the fine structure of adrenal glands of the amphibian and reptilian represented by *Bufo tibamicus* and *Uromastyx philbyi*, respectively.

Materials and Methods

Animals used in this investigation were the toad, *Bufo tibamicus* collected from El- Taif and the typical desert reptile, *Uromastyx philbyi* from Riadh, Saudi Arabia. They were killed and quickly dissected. Since the toad's adrenal gland is intermingled with the renal tubules of the kidney, the whole kidney was cut into small pieces and immediately fixed, as the adrenal glands of *U. philbyi* in 2.5% glutaraldehyde for electron microscopical study, then rinsed in 0.1M phosphate buffer. This was followed by post fixation using 1% OsO_4 for 2hr at 4^{0}C, then the specimens were dehydrated through graded ethanol and treated with propylene oxide, and embedded in Araldite-Epon mixture. Semithin (1μm) sections were stained with toluidine blue. The ultrathin (50 nm) sections were cut with a glass knife on LKB ultramicrotome (Gupta, 1983). After being double stained with uranyl acetate and lead citrate, the sections were examined by Jeol 100 CX electron microscope.

Results

Adrenal gland of B. tibamicus: The adrenal gland of toad, *(B. tibamicus)* is spread along the latero-ventral surface of the kidney in the form of small islets scattered among the renal tissues. It is essentially formed of two main types of tissues in semithin sections, the adrenocortical and chromaffin tissues (Fig. 1). The adrenocortical tissue is composed of two main types of cells, the steroid and eosinophilic cells.

TEM examinations revealed that the steroid cells are small, situated in close contact with each other, have polygonal shape and centrally located relatively round nuclei (Fig. 2). The

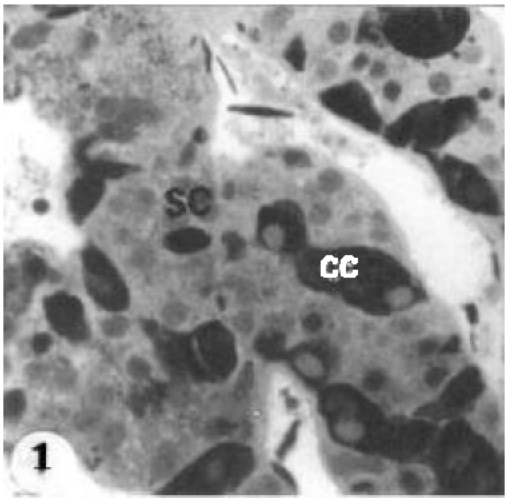

Fig. 1: Semithin section of the adrenal gland of toad showing steroid cells (SC) and chromaffin cells (CC). (Toluidine blue, X 600).

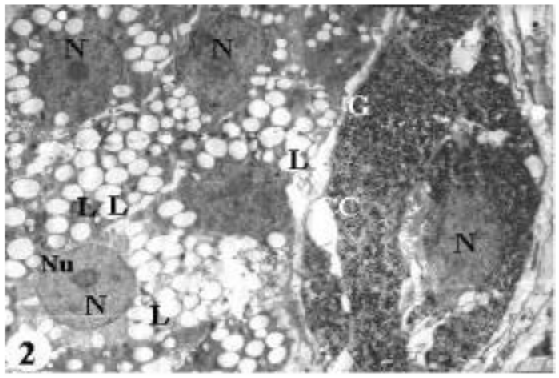

Fig. 2: An electron micrograph of a section of the adrenal gland of toad showing chromaffin cells (CC) with chromaffin granules (G) and steroid cells (Sc) with liposomes (L). N: Nucleus, Nu: Nucleolus. (X 2,5000).

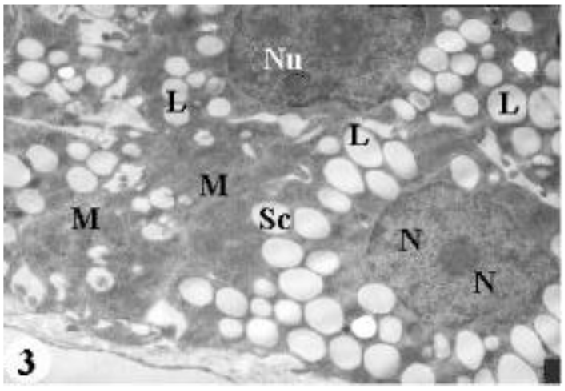

Fig. 3: High magnification of a portion of the previous section showing group of steroid cells (Sc)with liposomes (L), mitochondria (M), nuclei (N) and nucleolus (Nu). (X 5,000)

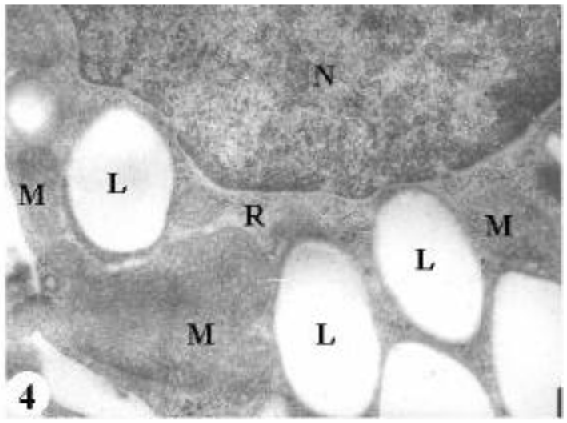

Fig. 4: An electron micrograph of a section of adrenal gland of toad showing steroid cell containing large mitochondria (M) with tubular cristae. L: Liposomes, N: Nucleus, R: Ribosomes, (X 13, 000).

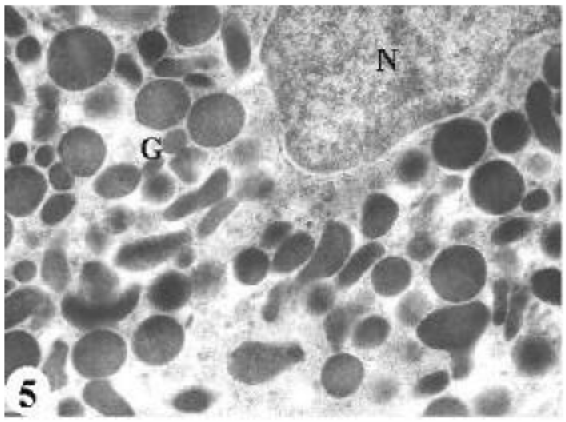

Fig. 5: An electron micrograph of a section of the adrenal gland of toad showing an eosinophilic cell with large pleomorphic eosinophilic granules (G). N: Nucleus. (X 3,000).

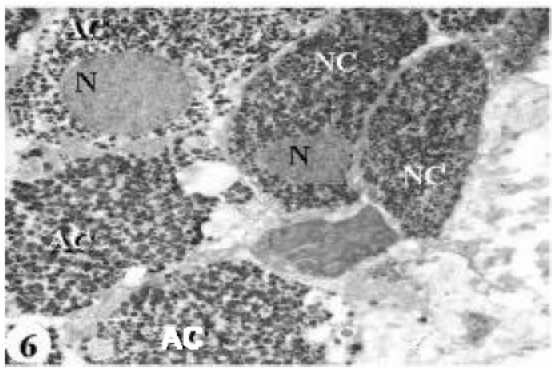

Fig. 6: An electron micrograph of a section of adrenal gland of toad showing noradrenaline cell (NC) with eccentric nucleus (N) with strong electron dense small granules, and adrenaline cells (AC) with rounded nucleus (N) and granules of variable sizes with moderate electron density.(X 5000).

cytoplasm is filled with liposomes of variable sizes, the most populous cytoplasmic organelle. Mitochondria, are large spheroidal or ovoid with tubular cristae (Figs. 3 & 4).
Eosinophilic (Stilling's) cells are oval to round in shape, have eccentric nuclei and the cytoplasm is almost completely filled with oval or round large strongly electron dense granules. Individual granules are much larger than the granules of chromaffin cells (600-900 nm in diameter), (Fig. 5)
The chromaffin tissue is formed of elongated cells with oval eccentric nuclei and many granules are distributed in their cytoplasm (Fig. 6). According to the shape, electron density and size of the chromaffin granules, two different types of chromaffin cells are recognized; noradrenaline and adrenaline cells. The noradrenaline-cells (NC) are elongated and contain strongly electron dense granules with an elliptical or rounded shape. The average of their diameters is 260 nm.(Fig. 6). The adrenaline-cells (AC) are rounded or polygonal in shape with central rounded nuclei. The chromaffin granules are mostly rounded and their electron density is lower than that of the NC-granules. The average diameter of these granules is 280 nm bigger than NC- granules(Fig. 6).

Adrenal gland of *U. philbyi*: The adrenal gland of *U. philbyi* appeared as an elongated to elliptic shape in semithin section, and is completely enveloped by a thin connective tissue capsule. Two portions could be easily identified an outer portion composed of chromaffin cells and an inner one formed of adrenocortical cells which represent the main bulk of the gland (Fig. 7). Numerous islets of chromaffin tissue are scattered throughout the adrenocortical tissue. Blood sinuses are numerous and are present between the two types of tissues or occupy the center of the adrenocortical tissue.
The adrenocortical cells are polygonal to oval in shape and are characterized by the presence of numerous lipid droplets of variable sizes. Their eccentric nuclei are spherical or oval in shape and each has one nucleolus. The mitochondria are numerous and have rounded shape with variable sizes (Figs. 8 & 9). The smooth endoplasmic reticulum could be demonstrated.
TEM observations showed that the gland is surrounded by a capsule formed of elongated fibroblasts and scarce mast cells associated with collagenous fibers (Figs. 10 & 11).
The chromaffin tissue is differentiated by TEM into adrenaline-secreting cells (AC) and noradrenaline - secreting ones(NC).
The AC cells are polygonal in shape, exhibit a lighter appearance as they contain lesser pleomorphic electron dense secretory granules than that of the NC ones. The granules of AC are bundled together in groups surrounded by a delicate connective tissue. They have eccentric rounded to oval nuclei with clear nucleoli. The nuclei have homogenous euchromatin materials surrounded by clear nuclear membrane (Figs. 10&11).
The NC cells are oval to elongate in shape, containing dark electron dense granules with different sizes in the cytoplasm. They contained rounded eccentric nuclei with one nucleolus in each. The nuclear membrane consists clearly of double layer surrounds the evenly distributed chromatin materials (Fig. 12).
The mitochondria in both types of cells (AC & NC) are often few in number. They are small, rounded or elongated with relatively slight infolding and more obviously in NC cells than that of AC cells (Figs.11&12). The RER are hardly seen, probably due to the predominance of the surrounding cytoplasmic granules. Moreover, the ribosomes and glycogen are few and demonstrated as minute particles.

Discussion

The adrenal gland of the toad *(Bufo tibamicus)* is distinguished into adrenocortical and chromaffin tissues being rather intermingled together. The adrenocortical tissue is composed of two main types of cells, the steroid cells with abundance of lipid inclusions and eosinophilic cells with eosinophilic granular cytoplasm. These findings reinforce those represented by Volk (1972 b) in the American bullfrog, *(Rana catesbeiana)*. Accordi and Grassi-Milano (1977) in *Bufo bufo* and El-Banhawy *et al.*(1993) in the Egyptian toad, *(Bufo regularis)*.
The eosinophilic cells were identified for the first time by Stilling (1898) in the adrenal gland of *Rana esculenta* and therefore, they are called "Stlling cells". These cells were only apparent during the summer period following the breeding season but completely absent during the rest of the year. These cells were absent in some amphibian species such as *Tritarus torosus*, *Rana temporaria* and *Bufo arenaum* (Burgos, 1959; Piezzi and Burgos, 1968) and appeared in others such as *Bufo viridis* and *Bufo regularis* (Lakshman, 1962 and El-Banhawy *et al.*, 1995).The function of eosinophilic cells is still under discussion although several speculations have been forwarded to elucidate their functional significance (Chester-Jones,1957 and Volk, 1972 b).
The chromaffin cells possess chromaffin granules, which correspond to those isolated from the mammalian adrenal medulla and found to contain adrenaline and noradrenaline hormones (Turner and Bagnara, 1976). In agreement with the major criteria of distinction of chromaffin cells (Coupland, 1971; Accordi and Grassi-Milano, 1977), two types were recognized: adrenaline and noradrenaline cells. These cells are responsible for secreting the hormones of emergency, adrenaline and noradrenaline.
Ultrastructural examination of the adrenal gland of *U. philbyi* showed that the adrenocortical tissue occupied the greatest depth part of the gland and the chromaffin tissue are mainly segregated to the periphery of the gland. Cords or islets of chromaffin cells are scattered throughout the adrenocortical tissue. This is similar to what has been reported on adrenal glands of other reptilian species (Varano *et al.*, 1978; Hammouda *et al.*, 1983 and Madkour *et al.*, 1997).
The adrenocortical cells are typical steroid-secreting cells and are characterized by lipid droplets, mitochondria and SER. These results are in accordance to Dufaure (1969) in four species of reptiles, and Abd-Elsamie (1987) in *Mabouia quinquetaeniata*. They recorded that the SER is known to play a role in steroid and cholesterol synthesis, and in carbohydrate metabolism. The lipids of steroid cells serve as storage deposits for steroid cell products (Kalliecharna, 1981). Orme-Johnson (1990) reported that the mitochondria have enzymes that are important to synthesize and regulating the steroid hormone.
Adrenaline and noradrenaline secreting cells are distinguished in *U. philbyi* in the present study by means of the different electron density of their granules. Such cells were also detected by some investigators in some reptilian species. Unsicker (1976) studied adrenal glands of three species of reptiles (*Testudo graeca, Lacerta dugesi & Natrix natrix*) and reported that the adrenaline (AC) and noradrenaline (NC) storage cells can be distinguished by means of the different electron density of their granules. AC granules are moderately electron-dense, while NC granules showed a core of high electron density. In all species studied, NC granules displayed a remarkable pleomorphism which is most pronounced in the tortoise. In this species, AC-granules are pleomorphic, too.

Both types of granules showed wide variations in size, which are particularly great in the tortoise. Delconte (1977) stated that the adrenaline storing cells were present together with noradrenaline-storing cells in the peripheral chromaffin tissue in the tailed lizard, *(Cnemidophrus lemniscatus)*. The granules of the former type are smaller and regular in shape, surrounded by distinct membranes and showed moderate electron density. The noradrenaline-storing cells are large, irregular in shape, more closely packed & considerably electron-opaque.

Benedeczky *et al.* (1965) reported that the chromaffin tissue of *Natrix natrix* showed a third cell type containing dopamine granules in addition to AC and NC-secreting cells. Such cell types were not observed in the adrenal of *U. philbyi* in the present investigation.

It is clear that the process of steroidogenesis is more or less similar in the adrenocortical cells (steroid cells) of *B. tibamicus* and *U. philbyi* as reflected by their rich amount of lipid droplets. The large amount of lipid materials present in the steroid cells serve not only as a source of substrate but also as a possible storage depots for steroid products(Kalliecharna, 1981). However, the Stilling cells are demonstrated only in the adrenal gland of *B. tibamicus*. The chromaffin tissue of these two types of animals composed of two cell types: AC and NC-secreting cells. These cells produced catecholamines which enable the organism, by various mechanisms, to overcome difficult environmental conditions (Coupland, 1965).

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