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**OBSERVATION ON THE HISTOLOGICAL
STRUCTURE OF ADRENAL GLANDS OF QUAIL
WITH REFERENCE TO AGE AND SEX**
(With 14 Figures)

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مشاهدات على التركيب النسيجي للغدة فوق الكليتين في السمان
مع الإشارة إلى تأثير الجنس والعمر على تركيبهما

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أوضحت هذه الدراسات أن نسيج الغدة فوق الكليتين يتكون من خلايا حمضية الصغية وخلايا قاعدية للصيغة مندمجين مع بعضهما تفصل بينهما الأوعية الدموية. الخلايا الحمضية الموجودة تحت غطاء الغدة تكون كبيرة الحجم وتحتوى على فراغات كثيرة فسي حين أن الخلايا الداخلية تكون عمادية وتحتوى على فراغات أقل. يوجد نوعان من الخلايا الحمضية أولهما يحتوى على حبيبات دهنية كبيرة وميتوكوندريا قليلة... أما النوع الثانى فيحتوى على العكس. أيضا يوجد نوعان من الخلايا القاعدية تختلف باختلاف شكل الحبيبات الإفرازية. وأيضاً أوضحت الدراسات أن اختلاف الجنس لا يؤثر على التركيب النسيجي للغدة في حين أن تقدم العمر له تأثير تحلي على الخلايا الحمضية والقاعدية.

SUMMARY

The parenchyma of adrenal glands of quails were formed from acidophilic and basophilic cells intermingled with each other and were separated by blood sinasoids. The subcapsular cells were large with highly vacuolated lightly stained acidophilic cytoplasm, while the cells of inner cords were large columnar with less vacuolated and more acidophilic cytoplasm. The basophilic cells were in form of islets among the acidophilic cords. Two types of cortical cells were demonstrated, some of these cells contained numerous lipid droplets with few large globular mitochondria, and the other type contained few lipid droplets with numerous smaller globular mitochondria. The medullary cells were also of two types of, cells contained homogenous, polymorphic electron dense secretory granules and the other contained secretory granules of

electron dense core surrounded by hallow-electron lucent coat. With the advancement of the age, some degenerative changes were observed in the cortical and medullary cells. No sex differences were demonstrated in the structure of the adrenal gland in the present study.

Key words: *Adrenal Glands, AGE, SEX, Quail*

INTRODUCTION

The adrenal cortex is required for life, its hormones influence numerous essential somatic processes. (Burkitt, 1989; Telford and Bridgmen, 1990 and Ganong, 1993).

In mammals a distinct cortex and medulla are apparent despite the striking interdigitation that may occur. But in birds the cortical and medullary tissue is intermixed. (Hodges, 1974 and Banks, 1993).

The lack of a decisive description of the histological changes in the adrenal gland of birds specially in quail as a result of age and sex in available literature was the principle modulation which evoked our encouragement to carry out the present investigation.

MATERIALS and METHODS

Seventy-five Japanese quail, of both sex, in different ages (from one day to seven months) were obtained from Poultry Breeding Research Farm, Faculty of Agriculture, Kafr El-Sheikh.

The birds were anaesthetized with dimethyle ether. Some of these birds were perfused, through the heart, with 4% glutaraldehyde for electron microscope examination and the others were perfused with 10% neutral buffered formalin or Bouin's fluid for paraffin sections. The adrenal glands were removed and small pieces were taken. After proper fixation, the specimens were dehydrated, cleared and embedded in paraffin wax. Step serial section were obtained at 5 microns thick and stained with haematoxylin and eosin, Crossomom's trichrome and periodic acid Schiff's technique. All stain techniques were adapted to that reported by Bancroft and Stevens (1990).

For semithin sections and electron microscopy, small tissue blocks (1 mm. thick) were cut and immersed in glutaraldehyde (4% in 0.2 M sodium cacodylate buffer, pH 7.2).

The specimens were postfixed in 1% osmium tetroxide. All specimens were dehydrated in graded ethanol solution and embedded in Epon-Araldide. Semithin section, one micron thickness, were cut and stained with toluidine blue. For EM examination, sections were stained

with uracyl acetate and lead citrate and viewed in a JEM-100 X11 electron microscope of up to 15000.

RESULTS

The adrenal gland of quail was surrounded from outside by a thin connective tissue capsule contained collagenous fibres, blood vessels and fibroblasts. Fine septa of collagen fibres were extended from the capsule between the tissue directly underling the capsule.

In H & E stained sections, the parenchyma of adrenal gland was formed from acidophilic and basophilic cells intermingled with each other and were separated by blood sinasoids (Fig. 1).

The acidophilic cells arranged in two cells wide cords rest on PAS positive membrane (Fig. 2). The peripheral (sub capsular) acidophilic cells were arranged in clumps forming loops against the capsule. These cells were large, polyhedral to columnar with highly vacuolated and lightly stained acidophilic cytoplasm. In trichrome stain, they contained few acid fuchsin positive granules, while the cells of inner cords were large columnar with less vacuolated, more acidophilic cytoplasm contained numerous acid fuchsin positive granules (Fig. 3). The nuclei of acidophilic cells were rounded, apically located and contained one or two prominent nucleoli.

The basophilic cells were in the form of islets that found among the acidophilic cords. These cells were polygonal, triangular or rounded in shape with basophilic cytoplasm and large spherical centrally located nuclei contained two or even three nucleoli (Fig. 4). According to the affinity of the cytoplasm to the stain, these cells could be differentiated into two types, cells with deeply stained basophilic cytoplasmic granules and cells with lightly stained basophilic cytoplasmic granules. The blood sinasoids were found between the cell cords and islets, they were numerous and wider in the center of the gland than in the peripheral.

Ultrastructurally, the acidophilic cells appeared columnar in shape, their cytoplasm contained numerous globular mitochondria, many ribosomes, lipid droplets, smooth and rough endoplasmic reticulum and their nuclei were spherical, large contained prominent nucleoli and coarse chromatin. According to the amount of lipid droplets and mitochondria, these cells could be classified into two types, cells contained numerous lipid droplets with few somewhat large globular mitochondria and the other type were cells contained few lipid droplets with numerous somewhat smaller globular mitochondria (Figs. 5, 6).

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 few of first type of cells.

The basophilic cells appeared polyhydal or triangular in shape, their cytoplasm contained rod shaped mitochondria with tubular crista, ribosomes, few rough endoplasmic reticulum, lipid droplets and secretory granules (Fig. 7, 8). According to the shape of the secretory granules, these cells could be classified into two types, cells contained homogenous, polymorphic electron dense secretory granules and the other contained secretory granules of electron dense core surrounded by hallow-electron lucent coat (Figs. 9, 10).

At early ages, in both sex (Premature, one day to one month old), frequent mitotic figures were observed in both acidophilic and basophilic cells (Fig. 11).

With increasing the age in both sex (at maturation, one to four months old), the acidophilic cells of inner zones were more vacuolated and less acidophilic than that in the previous ages, but still lesser vacuolated than that of the peripheral cells. Also, few of acidophilic and basophilic cells showed pyknotic nuclei (Fig. 12, 13).

With the advancement of the age (four to seven months old) in both sex, the acidophilic cells of peripheral and inner zones became more vacuolated and less acidophilic than that in the previous ages and the vacuolation was slightly numerous in the peripheral acidophilic cells than in those of the inner zones. The cells showing pyknotic nuclei were more numerous, specially in the peripheral, than in the previous age and some cells showed cytolysis. The basophilic cells appeared less vacuolated and smaller than that in the previous ages (Fig. 14).

DISCUSSION

The present study revealed that the parenchyma of adrenal gland of quail is formed from acidophilic and basophilic cells intermingled with each other. The acidophilic type is representing the cortical tissue while the basophilic cells represented the medullary tissue (Sinha & Ghosh, 1961 and Ghosh, 1962). The peripheral cortical cells are arranged in clumps forming loops against the capsule, their cells were large, polyhydal to columnar with highly vacuolated lightly acidophilic cytoplasm contained few acid fuchsin positive granules, while the cells of inner cords were large columnar with less vacuolated, more acidophilic cytoplasm contained numerous acid fuchsin positive granules. The peripheral cortical zone were named by Grignon, Haiter,

Guedent, and Dollander, 1972; Kondies & Kjaerheim, 1966; Haak, Abel and Rhees 1972; Unsicker, 1973; Peaurce, Graonshaw and Holmes, 1978 and Shin, Toshike & Donaled, 1980, as subcapsular zone (subcapsular zone), where Homes and Cronshaw, 1984 showed two regions of steroidogenic tissue in the birds adrenal gland: a subcapsular zone 40-60 cells thick and inner zone. Also, Klingbeil, Holmes, Pearce and Cronshaw, 1979 and Holmes & Cronshw, added that in duck the interrenal cells of subcapsular zone produce relatively more aldestrone and relatively less corticosterone than the cells of inner zone. Knouff and Hartman, 1951, and Klingbeil *et al.*, 1979 suggested that the subcapsular zone and inner zone in adrenal gland of birds are homologous of the zona glomerulosa and zona fasciculata, respectively.

In the present study, two types of medullary cells 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 cytoplasmic 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 the cells synthesize epinephrine and the remainder of the cells produce norepinephrine.

E.M examination of cortical cells in this study revealed that the cortical cells can be classified into two types according to the amount of lipid droplets and mitochondria, cells contained numerous lipid droplets with few large globular mitochondria, and cells contained few lipid droplets with numerous smaller globular mitochondria. Kalliecharan (1981) revealed that the interrenal cells displayed ultrastructural characteristics common to other steroid-secreting cells. Cronshaw, Holmes and West (1984) said that the stimulated adrenocortical cells has numerous mitochondria and numerous lipid droplets with large amount of smooth endoplasmic reticulum. This may explain the presence of two types of cortical cells in the present study. This suggestion was ensured by Kalliecharan (1981) where, he said that there are a structural functional relationship.

Also, the E.M examination of medullary cells in the present study revealed that the medullary cells could be classified into two types according to the shape of their secretory granules, cells contained homogenous, electron dense secretory granules and the others with secretory granules formed of electron dense core surrounded by hallow-electron lucent coat. Telford and Bridgman (1990) mentioned that the

norepinephrine granules have greater density than the epinephrine granules.

The present study revealed that the difference of sex has no effect on the developmental structure of adrenal gland of quail but with the advancement of the age the cortical cells become more vacuolated and less acidophilic, in addition, some cells showed pyknotic nuclei and cytolysis. The cytoplasmic and nuclear changes (vacuolation & pyknosis) may be attributed to the classical morphological characters of programmed cell death (apoptosis) (Kerr, Wyllie and Curie 1972 and Cohen, 1992). The same result is nearly obtained by (Cronshaw, Collie and Holmes, 1992), where they noted that there is a relationship between the morphological changes in the steroidogenic cells derived from duck embryo and the decline in steroidogenic capacity as the cells aged. Also, they added that the basal unstimulated rates of corticosteroid production also declined as the cells aged.

In conclusion:

From the previous results we can conclude that the organization of the adrenal gland of the Japanese quail is typically avian where its parenchyma is formed from cortical cell cords and medullary cell-islets intermingled with each others, and can be divided to subcapsular zone and inner zones. The sex has no effect on the structure of adrenal gland but the advancement of age has some degenerative effect on the cortical and medullary cells.

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LEGEND OF FIGURES

- Fig. 1:** Paraffine section in adrenal gland of 10 days old female quail showing: Capsul (arrow), septa (1), cortical tissue (2) medullary tissue (3) and blood sinusoids (4). (H & E, X: 100).
- Fig. 2:** Paraffine section in adrenal gland of 2 months old male quail showing: septa (1) and PAS positive membrane (arrows), subcapsular cortical cells (2). (PAS tech. x 100).
- Fig. (3):** Paraffine section in adrenal gland of 50 days old female quail showing: Capsul (1), subcapsular cortical cells (2), inner cortical cells (3), medullary cells (4) acid fuchsin positive granules (arrows) (Crossmen's trichrome, X: 400).
- Fig. (4):** Semi thine section in adrenal gland of 25 days female quail showing: cells of medullary tissue (1), cortical tissue (2), (Toluidine blue, X : 100).
- Figs. 5,6:** Electron micrograph in adrenal gland of one month old female quail showing: cortical cell with numerous lipid droplets (1), cell with few lipid droplets (2), mitochondria (3), lipid droplet (4) ribosomes (5), rough endoplasmic reticulum (6). Notice medullary cell (7). X: 4,000 and 14,000, respectively.

Figs. 7,8: Electron micrograph in adrenal gland of 50 days old female quail showing: two types of medullary cells (1) and (2), notice, mitochondria (3), lipid droplets (4), secretory granules (arrows), endothelial cells of blood sinasoids (5). X: 4,000 and 5,000, respectively.

Figs. 9,10: Higher magnification in the two types of medullary cells showing shape of secretory granules (1) and (2). X: 14,000 and 20,000, respectively.

Fig. 11: Paraffine section in adrenal gland of one weak old male quail showing subcapsular zone (1), inner zone (2), medullary cells (3) and mitotic division in cortical cells (arrow). (H & E x 400).

Fig. 12: Paraffine section in adrenal gland of 50 days old male quail showing the vacuolation in cortical cells of inner zone (1), notice the medullary cells (2) (H & E, X: 400).

Fig. 13: Paraffine section in adrenal gland of two month old female quail showing cells of medullary tissue with pyknotic nuclei (arrow), cortical tissue (1). (Crossman trichrom stain, X: 1000).

Fig. 14: Parafin section in adrenal gland of seven months old male quail showing subcapsular zone (1), inner zone (2) and medullar cells (3) (H & E, X: 400).







