

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Anti-inflammatory Steroid (Prednisolone) and its Effect on the Adrenal Gland

A.E. Essawy¹, S.A. Sakr², A.T. Saleh², F. El-Salmy³

¹Zoology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

²Biology Department, Faculty of Applied Sciences Umm Al-Qura University, Makkah, Saudi Arabia

³Biology Department, Faculty of Education, Umm Al-Qura University, El-Taif, Saudi Arabia

Abstract: Administration of an anti-inflammatory steroidal drug-Prednisolone- (0.85 mg/kg body weight, daily for 10 days) to adult toads *Bufo tibamicus* resulted in suppression of the function of the adrenal gland, as shown by a significant increase in the lipid droplets, autolysis of mitochondria and disorganization and degranulation of rER in the steroidogenic cells. Furthermore, chromaffin cells (catecholamines secreting cells), showed a marked decrease in the number of chromaffin granules and degenerative changes in the nuclei. These serious side effects should be taken into consideration when using this drug.

Key words: Steroid, adrenal gland, anti-inflammatory

Introduction

Prednisolone, the anti-inflammatory steroid, is one of the four Potent synthetic corticoids produced by altering the molecular structure of cortisol, which is one of the naturally occurring glucocorticoids (Haynes and Larner, 1980; Heiman *et al.*, 1994). Although the corticosteroids have gained an important and reasonable well defined place in therapy, the successful use of these chemicals has been hampered by a variety of side effects. They influence protein, carbohydrate and fat metabolism; electrolyte and water balance and the functions of many organs including the adrenal gland.

Administration of prednisolone was found to suppress the adrenal function and cause the adrenal cortex of mammals to regress (Lange and Doorenbos, 1975; Olejniczak and Lee, 1984). Suppression of adrenal function in prednisolone treated animals was attributed to the dimension of the output of adrenocorticotrophic hormone (ACTH) from the pituitary gland (Nicholson *et al.*, 1987; Brockus *et al.*, 1999).

In Amphibia, the presence of a functional pituitary-adrenocortical axis and a negative feed back influence of corticosterone on pituitary ACTH secretion were documented (Piper and deRoos, 1967; Sandor, 1972; Verma, 1977).

The adrenal gland of amphibia is composed of two types of tissues, of separate origins, the interrenal and chromaffin tissues. The former type secretes steroid hormones which affect the regulation of water and saline balance and glucidic and lipidic metabolism (Hanke, 1978). The chromaffin tissue produces catecholamines (adrenaline and noradrenaline) which enable the organism, by various mechanisms, to overcome difficult environmental conditions (Coupland, 1971). In contrast to mammals, the anuran amphibian adrenal gland is without morphological zonation and the adrenocortical tissue is intermingled with chromaffin tissue (Accordi, 1981; Accordi and Grassi-Milano, 1990).

In view of these findings, the present study was constructed to find out, if the adrenal hypofunctional activity, induced by prednisolone, is accompanied by any cytological changes in the adrenal tissue of the toad *Bufo tibamicus*.

Materials and Methods

Sexually mature male and female toads, *Bufo tibamicus*, weighing approximately 25 gm each were used. The experimental animals were collected from El-Taif, kingdom of Saudi Arabia. The toads were maintained in glass tanks at a temperature of 20-22°C. The experimental animals were

divided into 2 groups of 25 toads each. Animals of first group were injected subcutaneously in the dorsal lymph sac with prednisolone at a dose level of 0.85 mg/kg body weight, dissolved in 1 ml distilled water, daily for 10 days. This dose represents what is equal to the human therapeutic dose. Each animal of the second group was administered with 1 ml distilled water and used as control. After 10 days, all the animals were pithed, and biopsies 1mm thick were taken from the mid-portion of each kidney with the attached adrenal tissue. For electron-microscopical observation, the specimens were fixed in 2.5% glutaraldehyde for 1 hr, then rinsed in 0.1 M phosphate buffer. This was followed by post fixation using 1% O₃O₄ for 2 hr at 4°C, then the specimens were dehydrated through graded ethanol, and treated with propylene oxide and embedded in Araldite-Epon mixture. Semithin (1 µm) and ultrathin (50 nm) sections were cut with a glass knife on LKB ultramicrotome. After being double stained with uranyl acetate and lead citrate, the sections were examined by Jeol 100 CX electron microscope.

Results

Control animals: The adrenal gland of a control toad *Bufo tibamicus* is composed of yellowish red discontinued islets located on the ventral surface of the kidney. The gland is made up of anastomosed cellular cords among which sinusoidal vessels are found.

The present electron microscopical studies revealed that these cords are of three types of cells.

Steroid cells (Sc): are small, situated in close contact with each other, have polygonal shape and centrally located round nuclei (Fig. 1, 2). The cytoplasm is filled with liposomes of variable sizes. Mitochondria, the most populous cytoplasmic organelle, are large spheroidal or ovoid with tubular cristae (Fig. 3).

Chromaffin cells (Cc): are elongated or elliptical in shape, have oval eccentric nuclei and many granules evenly distributed in the cytoplasm (Fig. 1). According to the shape, electron density and size of chromaffin granules, two different types of chromaffin cells are recognized.

Noradrenaline-cells (N-cells): contain strongly electron dense granules with elongated or roundish shape and diameter with an average of 260 nm (Fig. 4). The cells have elongated shape with more or less round nuclei.

Essawy *et al.*: Anti-inflammatory steroid (Prednisolone) and its effect on the adrenal gland

Adrenaline-cells (A-cells): Rounded or polygonal with a central round nucleus. The chromaffin granules were mostly rounded and their electron density is lower than that of the N-granules. The average diameter of these granules is 280 nm (Fig. 4).

Eosinophilic Stilling's cells (Ec): are oval to round, 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).

Treated animals: Electron microscopy of adrenal gland of prednisolone treated toads reveals interesting cytological changes which can be appreciated only at the ultrastructural level. These alterations involve both the nucleus and cytoplasmic organelles.

In steroid cells, the most marked effect was an increase in the number of liposomes (Fig. 6). The liposomes were not essentially round, as was true of the control animals, but possessed a highly irregular outline and commonly were coalesced into large masses (Fig. 6, 7). On the other hand, prominent degeneration of the nucleus was observed, where dimension of the heterochromatin content and fragmentation of the nucleoli were seen (Fig. 8). These features were also associated with severe disorders of the nuclear envelope.

The mitochondria are small, pleomorphic and exhibit the condensed configuration, however, some mitochondria have excessively dense matrices which obscure the depiction of cristae (Fig. 8).

Close association between mitochondria and lipid droplets was observed (Fig. 9). A single mitochondria appear close to, spread out over, or fused to the surface of small liposomes, and numerous mitochondria were seen surrounding a large lipid droplet. In some cases the lipid droplets lie in deep invagination of the mitochondrial envelope (Fig. 7, 9).

The rough endoplasmic reticulum was deteriorated with degranulation of its cisternae. The detached ribosomes were scattered or aggregated forming clusters of polyribosomes. Degranulation of the rough endoplasmic reticulum is accompanied by its dilatation and vesiculation (Fig. 10).

Chromaffin tissue also appeared degenerative with a significant decrease in chromaffin granules (Fig. 11, 12). Not only there is a highly reduction in the hormones storage granules, but also the remaining granules appeared with large electron lucent halos around the small core material (Fig. 11). In parallel with the loss of the granules, large vacuoles appeared in the cytoplasm (Fig. 11, 12).

Furthermore, the nuclei are pale, predominantly euchromatic with irregular nuclear envelope and diluted nucleoplasm (Fig. 12).

On the other hand, the cells of both cortical and chromaffin tissues show a tendency to lose cohesiveness with neighbouring cells, thus creating conspicuously wide intercellular spaces (Fig. 8, 11). This decreased adhesiveness is incontrovertibly manifested by significant irregularities in the plasma membrane.

Discussion

The present investigation has revealed that, 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 (1972a, 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 large quantities of lipid material 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). In Amphibia, corticosterone and aldosterone are the principal secretory products of the steroidogenic cells (Sandor, 1972).

The function of eosinophilic cells is still under discussion although several speculations have been forwarded to elucidate their functional significance (Chester Jones, 1957; Volk, 1972b).

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, 1965; Accordi and Grassi-Milano, 1977), Two types were recognized: Adrenaline-cells (A-cells) and Noradrenaline cells (N-cells). These are the cells having the responsibility for secreting the hormones of emergency, adrenaline and noradrenaline.

Prednisolone was reported to suppress the adrenal function by inhibiting the Pituitary ACTH production (Nicholson *et al.*, 1987; Brockus *et al.*, 1999).

Herein, it is demonstrated that, prednisolone has induced signs of decreased adrenal function in the toad *Bufo tibamicus*. The increase, enlargement and coalescence of lipid droplets in the steroidogenic cells observed with the drug administration, indicate decreased synthesis and discharge of the adrenal hormones which reflects diminished secretory potency (Penny and Brown, 1971; Nussdorfer *et al.*, 1978). A marked decrease in adrenal lipid following adrenal stimulation in mammals was demonstrated by Armato *et al.* (1974).

Normally, the mitochondria appear to be randomly scattered in the cytoplasm, but in prednisolone treated toads, there is a close association between mitochondria and lipid droplets. These observations are in agreement with those elucidated by Napolitano and Fawcett (1958) and Ghadially (1978) in which they mentioned that, in situation where lipid droplets is accumulated, close association between mitochondria and lipid droplets is seen. The intramitochondrial lipids, observed in this study, was said to lead to the dissolution of the mitochondria (Ghadially, 1978). In addition, mitochondria with dense matrices and irregular, disoriented cristae were demonstrated. Such morphological alterations in mitochondria is considered to reflect impairment in protein synthesis and hence enzyme content and function (Ghadially, 1978). Dilatation, vesiculation and degranulation of rough endoplasmic reticulum were also recorded after prednisolone treatment. Similar result had been described in kidney of mice treated with hydrocortisone (Hamed, 1989). Thus alterations on the rER cisternae indicate an altered protein synthesis and considered to be one of the factors responsible for decreased hormonal synthesis by endocrine glands (Whitehead and Alleyne, 1972). Rubin *et al.* (1973) pointed out that steroidogenesis depends upon new protein synthesis, stimulated by ACTH that mediates the effect of the hormone.

All these histopathological changes reflect suppression of the adrenocortical activity and a probable adrenocortical insufficiency.

In addition to the dramatic alterations in the cortical cells, prednisolone was found to induce degenerative effects in the chromaffin cells. The nuclei showed decreased amount of heterochromatin, dilution and lysis of the nucleoplasm and severe irregularity in the nuclear envelope. Such nuclear changes are associated with necrosis (Ghadially, 1978) and were demonstrated in mice administered with hydrocortisone (Hamed, 1989).

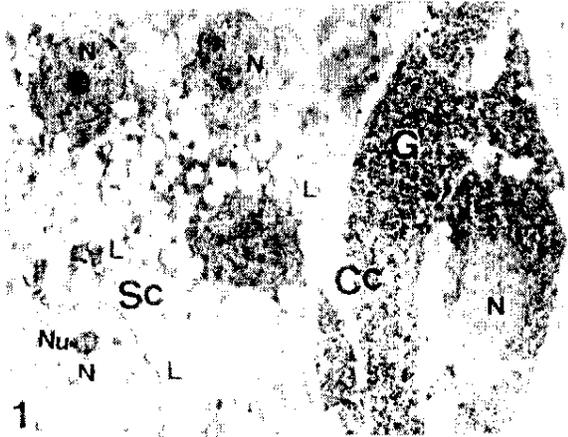


Fig. 1: An electron micrograph of a section of adrenal gland of control toad, showing chromaffin cells (Cc) with chromaffin granules (G) and steroid cells (Sc) with liposomes (L). N: Nucleus, Nu: Nucleolus. (X 2,500)

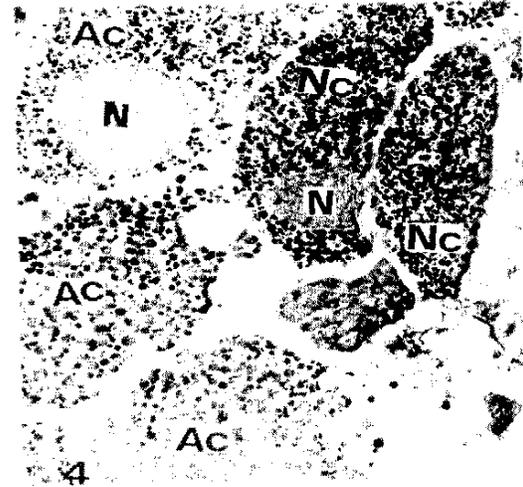


Fig. 4: An electron micrograph of a section of adrenal gland of control toad, showing noradrenaline cells (Nc) with eccentric nucleus (N) and strongly electron dense small granules, and adrenaline cells (Ac) with rounded nucleus (N) and roundish granules of variable sizes and moderate electron density



Fig. 2: Higher magnification of a portion of the previous section, showing showing group of steroid cells with liposomes (L), mitochondria (M) and rounded nuclei (N). Nu: Nucleolus. (X 5,000)

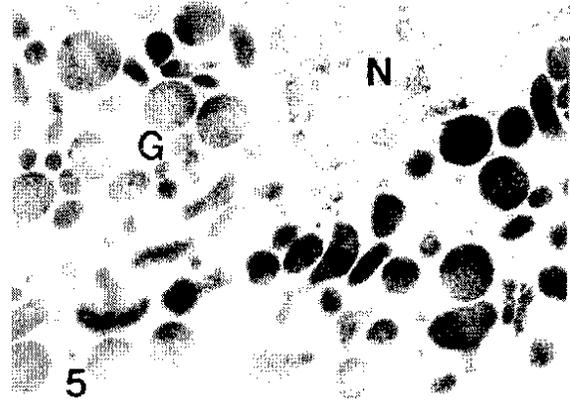


Fig. 5: An electron micrograph of a section of adrenal gland of control toad, showing an Eosinophilic cell with large Pleomorphic eosinophilic granules (G). N: Nucleus. (x 3,000)

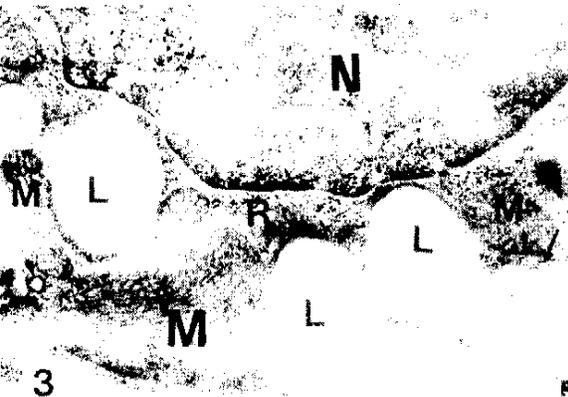


Fig. 3: An electron micrograph of a section of adrenal gland of control toad, showing steroid cell containing large mitochondria (M) with tubular cristae. L: Liposomes, N: Nucleus. Ft: Filibosomes. Arrow points at Golgi body and arrow's head points at coated vesicle (X 13,000)

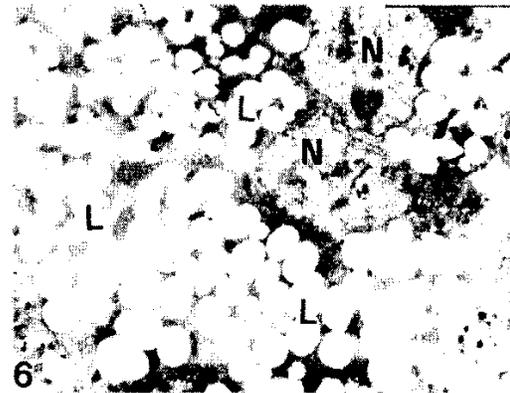


Fig. 6: An electron micrograph of a section of adrenal gland of treated toad, showing steroid cells with cytoplasm, extensively crowded with liposomes (L). Most of liposomes are coalesced. N: Nucleus (X 4,800)

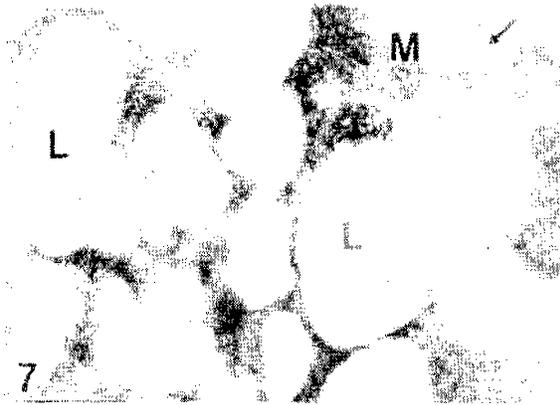


Fig. 7: An electron micrograph of a section of adrenal gland of treated toad, showing large coalesced liposomes (L). Arrow points at mitochondria (M) with intramitochondrial liposome, (X 20,000)

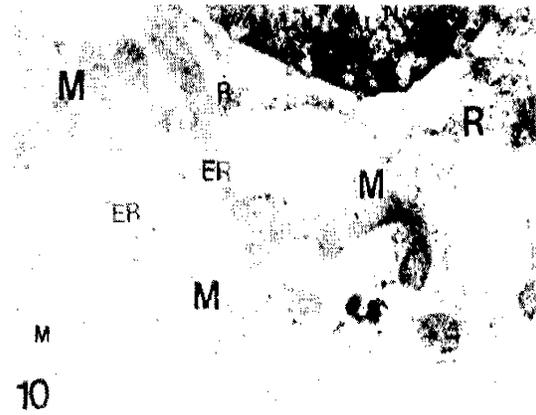


Fig. 10: An electron micrograph of a section of adrenal gland of treated toad, showing steroid cell with highly dilated and vesiculated endoplasmic reticulum (ER) with progressive loss of their adherent ribosomes (R). The mitochondria (M) display varying degrees of pleomorphism and some of them showing autolysis. N: Nucleus (X 13,000)

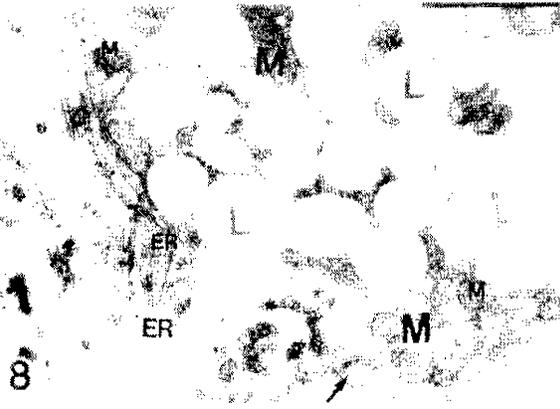


Fig. 8: An electron micrograph of a section of adrenal gland of treated toad, showing steroid cell with unusual number of coalesced liposomes (L). (+) indicates close association between mitochondria and large liposome. Arrow points at intramitochondrial liposome. ER: Endoplasmic reticulum, M: Mitochondria (X 10,000)

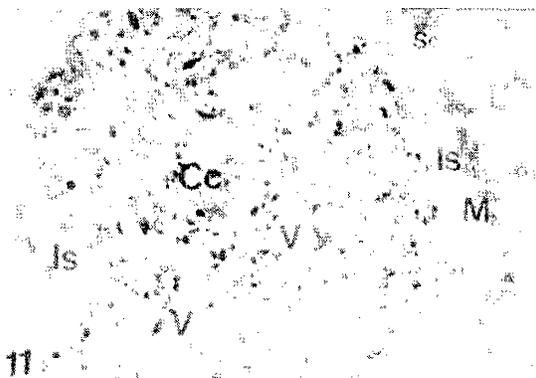


Fig. 11: An electron micrograph of a section of adrenal gland of treated toad, showing chromaffin cell (Cc) and steroid cells (Sc) with wide intercellular spaces (Is) between them. Note the vacuoles (V) in the cytoplasm of chromaffin cells due to regression and disappearance of chromaffin granules. L: Liposomes, M: Mitochondria (X 5,000)



Fig. 9: An electron micrograph of a section of adrenal gland of treated toad, showing steroid cell with euchromatic, degenerative, irregular shaped nuclei (N). Note the wide intercellular spaces (Is) and the small mitochondria (M) with dense matrices. L: Liposome, Nu: Nucleus (X 7,500)

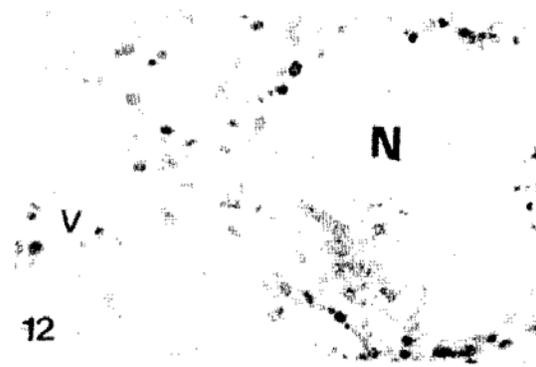


Fig. 12: An electron micrograph of a section of adrenal gland of treated toad, showing a degenerative chromaffin cell. Note the irregularity of the nuclear membrane, lysis of the nucleoplasm, disappearance of the chromaffin granules and vacuolation of the cytoplasm. N: Nucleus, V: Vacuole. (X 10,000)

Essawy et al.: Anti-inflammatory steroid (Prednisolone) and its effect on the adrenal gland

Following treatment with prednisolone, significant decrease in the chromaffin granules and vacuolation of cytoplasm were denoted. Degranulation of chromaffin cells can be taken as an evidence for reduction in the secretory activity of these cells (Turner and Bagnara, 1976).

The degenerative effect of prednisolone on the chromaffin cells appeared to be secondary to the adrenocortical hypofunction. This is supported by the article of Mpoy and Kolanowski (1986) who reported that in adrenocortical insufficiency, the excretion of epinephrine is reduced proportionally to the decrease in adrenocortical activity.

Based on the results drawn of the present investigation, it could be said that treatment with prednisolone resulted in evidence of regression of most of the adrenal cells. These observations are consistent with published physiological data that have established a functional pituitary-adrenocortical axis in anuran amphibians (Sandor, 1972; Verma, 1977).

In conclusion, the present results suggest that prednisolone is injurious to the adrenal gland, both structurally and functionally, and these serious side effects should be taken into consideration when using this drug.

References

Accordi, F. and E. Grassi-Milano, 1977. Catecholamine-secreting cells in the adrenal gland of *Bufo bufo* during metamorphosis and in the adult. *Gen. Comp. Endocrinol.*, 33: 187-195.

Accordi, F. and E. Grassi-Milano, 1990. Evaluation and development of the adrenal gland in amphibians. *Fortschritte Zool.*, 38: 257-266.

Accordi, F., 1981. Histology and ultrastructure of the adrenal gland of *Rhacophorus eucomystax* (Amphibia, Anura). *Bull. Zool.*, 48: 277-284.

Armato, U., G.G. Nussdorfer, P.G. Andreis, G. Mazzochi and E. Draghi, 1974. Primary tissue culture of human adult adrenocortical cells. Methodology and electron microscopic observations on ACTH-deprived and ACTH-treated cortical cells. *Cell Tissue Res.*, 155: 155-180.

Brockus, C.W., A.R. Dillon and R.J. Kemppainen, 1999. Effect of alternate-day prednisolone administration on hypophyseal-adrenocortical activity in dogs. *Anim. J. Vet. Res.*, 60: 698-702.

Chester Jones, I., 1957. *The Adrenal Cortex*. Cambridge University Press, Cambridge, pp: 146-167.

Coupland, R. E., 1971. Observations on the form and size distributions of chromaffin granules and the intensity of adrenaline and noradrenaline-storing chromaffin cells in vertebrates and in man. *Memoirs Soc. Endocrinol.*, 19: 611-635.

Coupland, R.E., 1965. Electron microscopic observations of the structure of the rat adrenal medulla. 1. The ultrastructure and organization of chromaffin cells in the normal rat. *J. Anat.*, 99: 231-254.

El-Banhawy, M.A., M.A. Shahin and W.W. El-Shennawy, 1993. Correlation between tyrosine inclusions and the biological activities of the vertebrate adrenal glands. *J. Egypt. Ger. Soc. Zool.*, 10: 1-16.

Ghadially, F.N., 1978. *Ultrastructure Pathology of the Cell*. Butterworths Inc., London.

Hamed, S.S., 1989. Histological, cytological and cytochemical studies on the effect of Hydrocortisone on kidney of young and adult mice. Ph.D. Thesis, Alexandria University, Alexandria, Egypt.

Hanke, W., 1978. *The Adrenal Cortex of Amphibia*. In: *General, Comparative and Clinical Endocrinology of the Adrenal Cortex*, Chester Jones, I. and I.W. Henderson (Eds.). Vol. II, Academic Press, London, pp: 419-495.

Haynes, R.C. and J. Larner, 1980. *The Pharmacological Basis of Therapeutics*. 6th Edn., The McMillan Publishing Co., New York, pp: 1472-1506.

Heiman, A.S., D. Hong and H.J. Lee, 1994. Receptor binding affinity and antiproliferative activity of new antiinflammatory antedugs: 6-methoxycarbonyl prednisolone and its derivatives. *Steroids*, 59: 324-329.

Kalliecharna, R., 1981. The influence of exogenous ACTH on the levels of corticosterone and cortisol in the plasma of young chick *Callus domesticus*. *Gen. Comp. Endocrinol.*, 44: 249-251.

Lange, W.E. and H. Doorenbos, 1975. *Meyler's Side Effects of Drugs*. 7th Edn., Vol. VIII, American Elsevier Publishing Co. Inc., New York, pp: 812-833.

Mpoy, M. and J. Kolanowski, 1986. Urinary catecholamine excretion in patients with secondary adrenocortical insufficiency. *J. Endocrinol. Invest.*, 9: 253-255.

Napolitano, L. and D. Fawcett, 1958. The fine structure of brown adipose tissue in the newborn mouse and rat. *J. Cell Biol.*, 4: 685-685.

Nicholson, S.A., E.A. Campbell, B. Gillham and M.T. Jones, 1987. Recovery of the components of the hypothalamo-pituitary-adrenocortical axis in the rat after chronic treatment with prednisolone. *J. Endocrinol.*, 113: 239-247.

Nussdorfer, G.G., G. Mazzocchi and V. Meneghelli, 1978. Cytophysiology of the adrenal zona fasciculata. *Int. Rev. Cytol.*, 55: 291-365.

Olejniczak, E. and H.J. Lee, 1984. Systemic effects of chronically administered methyl prednisolone and methyl 17-deoxyprednisolone. *Steroids*, 43: 657-662.

Penny, D.P. and G.M. Brown, 1971. The fine structural morphology of adrenal cortices of normal and stressed squirrel monkeys. *J. Morphol.*, 134: 447-466.

Piper, G.D. and R. deRoos, 1967. Evidence for a corticoid-pituitary negative feedback mechanism in the American bullfrog (*Rana catesbeiana*). *Gen. Comp. Endocrinol.*, 8: 135-142.

Rubin, R.P., S.D. Jaanus, R.A. Carchman and M. Puig, 1973. Reversible inhibition of ACTH-induced corticosteroid release by cycloheximide: Evidence for an unidentified cellular messenger. *Endocrinology*, 93: 575-580.

Sandor, T., 1972. Corticosteroids in Amphibia, Reptilia and Aves. In: *Steroids in Non-Mammalian Vertebrates*, Idler, D.R. (Ed.). Academic Press, New York, pp: 253-327.

Turner, C.D. and J.T. Bagnara, 1976. *General Endocrinology*. 6th Edn., W.B. Saunders Co., Philadelphia, pp: 325-359.

Verma, M.M., 1977. Ultrastructural evidence for aldosterone and corticosterone-secreting cells in the adrenocortical tissue of the American bullfrog (*Rana catesbeiana*). *Gen. Comp. Endocrinol.*, 33: 61-75.

Volk, T.L., 1972a. Ultrastructure of the cortical cell of the interrenal gland of the American bullfrog (*Rana catesbeiana*). *Z. Zellforsch.*, 123: 470-485.

Volk, T.L., 1972b. Morphologic observations on the summer cell of stilling in the interrenal gland, of the American bullfrog (*Rana catesbeiana*). *Z. Zellforsch.*, 130: 1-11.

Whitehead, R.G. and G.A. Alleyne, 1972. Pathophysiological factors of importance in protein-calorie malnutrition. *Br. Med. Bull.*, 28: 72-79.