HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE ADRENAL GALAND OF THE ALBINO RAT AFTER ADMINISTRATION OF DEXAMETHASONE

Thesis
Presented by

ABDEL HALIM ZEIN SAIED
(M.B., ch.B.)

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Supervised by

Prof. Dr. AHMED SAID EL-MORSY
Professor of histology
Head of Histology Department
Faculty of Medicine
Ain Shams University

Prof. Dr. OLFAT SAIED AHMED
Professor of histology
Faculty of Medicine
Ain Shams University

Prof. Dr. MOHAMMED ESAM ANWAR
Professor of histology
Faculty of Medicine
Ain Shams University

AIN SHAMS UNIVERSITY
FACULTY OF MEDICINE
DEPARTMENT OF HISTOLOGY
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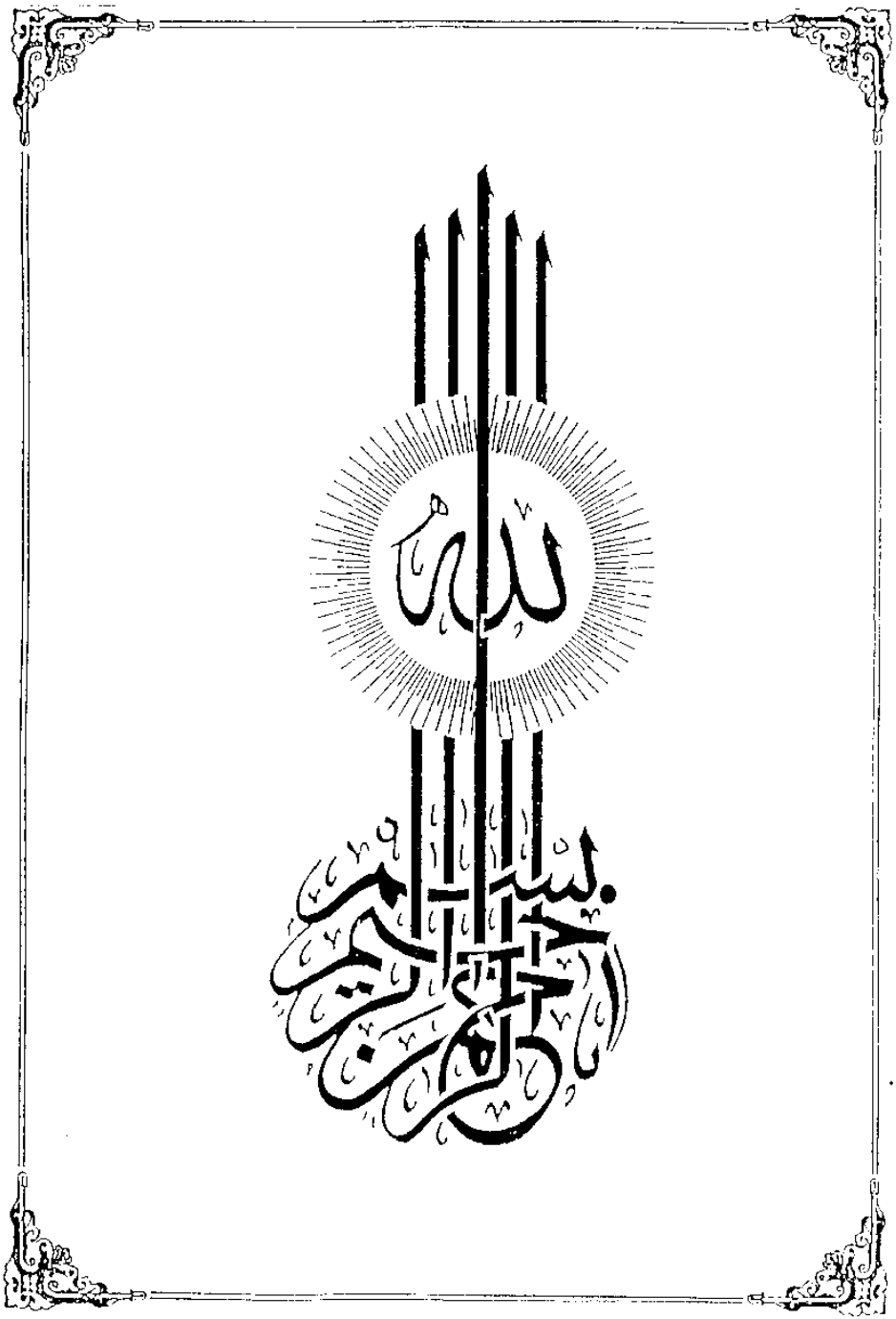

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INTRODUCTION AND AIM OF THE WORK

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This work aims at studying the alterations induced in the adrenal gland of the rat by the repeated injection of dexamethasone over a period of 30 days. Dexamethasone (9α-fluoro-methyl prednisolone) is a powerful synthetic glucocorticoid. Glucocorticoids are steroid hormones, some of which are produced endogenously by the adrenocortical cells in the fasciculata and reticularis. The major natural glucocorticoid in man is cortisol and in rat is corticosterone. Glucocorticoids are used for a great diversity of conditions. The literature concerned with the effect of these agents on the adrenal gland is voluminous, and this reflects not only the importance taken in it but also the serious disagreement between investigators over this subject. For example, some workers had shown that the administration of glucocorticoids caused cortical atrophy limited to the fascicular and reticular layers (for instance, Winter et al.,1950 and Bransmore,1968), whereas other researchers (Sokoloff et al.,1951 and Wright et al., 1974) demonstrated the morphological signs of inactivity in all the cortical layers. Fraser and Preus (1952) observed that following prolonged administration of cortisone the medulla was likewise atrophic.

The adrenal cortex and the adrenal medulla are two embryologically, histologically and chemically, different glands, but they are anatomically closely associated. The close proximity of these two, seemingly different, glands was the subject of research for many years, and the evidence was provided that the production of adrenaline by

the adrenomedullry cells from its precursor noradrenaline is controlled by the glucocorticoids secreted by the adrenocortical cells and carried to the adrenal medulla via cortico-medullary portal venous system (Wurtman and Axelrod,1966). The study of the adrenal medulla was hampered for long by the lack of a technique which enables the demonstration of both adrenaline and noradrenaline cells in the adrenal medulla, and moreover, the studies concerned with the effect of glucocorticoids on the catecholamine-containing cells in the adrenal medulla were carried out mainly in the hypophysectomized animals and were mostly biochemical (for example, Wurtman,1966; Margolis et al.,1966 and Leach and Lipscomb,1969).

Because of the above mentioned considerations it seemed of interest to study the effect of dexamethasone on both the adrenal cortex and the adrenal medulla.

The adrenal cortex contains the highest concentrations of cholesterol and ascorbic acid in the body (Sayers,1950). Cholesterol is the precursor of cortical hormones (Deane, 1962 and Moses et al.,1969), and although the role of ascorbic acid in the metabolic activity of the adrenal gland is, as yet, uncertain, it was observed that the stimulation of the adrenocortical secretory activity by ACTH results, constantly, in the depletion of ascorbic acid , simultaneously with cholesterol, from the adrenal cortex, and thus it was assumed that the adrenal ascorbic acid also plays, in some way, an important role in the secretion of corticosteroids (Sayers, Sayers, Liang and Long,1947; Meiklejohn,1953; Harding and Nelson,1963). Indeed, the

levels of these two substances in the adrenal were employed as indices for the assessment of the adrenocortical activity (Sayers,1950; O'Donnell et al.,1951 and Passmore and Robson,1976).

Alkaline phosphatase is one of the more important enzymes in the mammalian adrenals (Deane,1962), even though its role in the adrenal physiology is not exactly understood.

Thus, to evaluate better the changes produced by dexamethasone a routine histologic stain (H&E) was supplemented with a battery of histochemical techniques for the demonstration of adrenal lipids, ascorbic acid and alkaline phosphatase, in addition to a method for the identification of adrenaline and noradrenaline cells in the adrenal medulla.

REVIEW OF LITERATURE

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Ingle and Kendall (1937) and Ingle, Higgins and Kendall (1938) observed that the administration of large amounts of cortin produced atrophy in the rat's adrenal cortex and that this atrophy could be prevented by the simultaneous administration of anterior pituitary extract.

Giroud and coworkers (1940) stated that deficiency of ascorbic acid in guinea pigs was accompanied by a definite decrease in cortical hormone production.

Greep and Deane (1947) found that the injection of 2 mg of desoxycorticosterone acetate (DOCA) daily for 3 days completely suppressed the production of desoxycorticosterone in the cells of the zona glomerulosa in both intact and hypophysectomized rats. They concluded that this suppression did not involve the pituitary.

Sayers and Sayers (1947) noted that the drop in adrenal ascorbic acid which followed the application of various stresses could be prevented by pre-treatment of the animal with cortical hormones, however, the administration of ACTH into such pre-treated animals caused rapid depletion of adrenal ascorbic acid. They concluded that cortical hormones prevented the depletion of adrenal ascorbic acid by suppressing the release of pituitary ACTH.

Deane and Morse (1948) reached the conclusion that the presence of ascorbic acid in adrenal cortical cells appeared to be concerned with the capacity of these cells to produce steroid hormones and whenever this capacity was lost, for example after hypophysectomy, ascorbic acid was also lost from these cells.

Vogt (1948) could show no correlation between the contents of cortical ascorbic acid and of cortical hormone in the plasma obtained from the adrenal blood.

Dempsey et al.(1949) noted that hypophysectomy in the male rat caused disappearance of alkaline phosphatase enzyme from the inner zones of the adrenal cortex but did not alter the distribution in the glomerulosa. Replacement with whole pituitary powder restored normal distribution.

Jones ((1949) revealed that in the mouse the three zones of the adrenal cortex had different integrations with the anterior pituitary. The zona fasciculata was completely dependent on corticotrophin. The X zone was dependent on LH. The zona glomerulosa, though capable of existence independent of the pituitary, the presence of sudanophilic material within this zone depended on corticotrophin.

Yoffey and Baxter (1949) demonstrated that the repeated administration of cortical extract into the adult male rat caused intensified staining by Sudan black in both the glomerulosa and the remainder of the cortex during the first three days, followed by subsequent gradual weakening which by 14 days was very marked.

Sayers (1950) demonstrated that various types of stress caused depletion of ascorbic acid and cholesterol from the adrenal cortex in the intact rats but not in rats subjected to hypophysectomy. However, the administration of ACTH into such hypophysectomized rats caused rapid depletion of the adrenal ascorbic acid and cholesterol.

Sprague et al.(1950) noted, at necropsy, in the adrenal glands of patients treated with cortisone a depletion of lipid material, particularly in the zona fasciculata, which was significantly narrowed. The glomerulosa was well preserved.

Winter and his coworkers (1950) observed in rats treated with cortisone for 6 weeks that the adrenal cortex was markedly decreased in width, due to reduction in size of the cells of the fascicular and reticular zones, with a pronounced fall in concentrations of lipids and ascorbic acid from these cells. Few scattered cells in the reticularis showed hydropic degeneration.

Fortier et al.(1951) studied the effect of cortisone on the release of ACTH, using the depletion of adrenal ascorbic acid as a criterion of ACTH release. Cortisone at dose levels of 0.5, 5, 25 mg/100 gm failed to significantly decrease the depletion of adrenal ascorbic acid resulting from various types of severe stress. They concluded that during severe stresses the release of ACTH did not function as a negative feedback mechanism.

Hyman et al.(1951) observed that the administration of ACTH to scorbutic guinea pigs caused a fall in adrenal cholesterol and lymphopenia (an indication of cortical hormone production), although the ascorbic acid concentration in the adrenal was practically zero.

O'Donnell et al.(1951) showed that prolonged administration of cortisone caused, in the human adrenal cortex, marked atrophy limited to the inner cortical zones (zona fasciculata and zona reticularis) with broadening of the zona glomerulosa. The concentration of the sudanophilic material was reduced and the remaining lipid was localized to the glomerulosa and reticularis.

Proctor and Rawson (1951) described an instance of irreversible cytolytic destruction of the human adrenal cortex resulting from injection of ACTH immediately after discontinuance of cortisone. The zona fasciculata stood out as a narrow pale band. The nuclei in this zone were pyknotic and often absent in the lysed cells. The reticularis was somewhat narrowed and the glomerulosa was somewhat widened and it appeared normal.

Sokoloff et al.(1951) demonstrated that small doses of cortisone did not cause appreciable changes in the human adrenal cortex, and that massive amounts of cortisone over a 4-months period caused definite atrophy in all the zones of the cortex, while comparable quantities of cortisone over a shorter period of time (18 days) caused no atrophy.

Stepto et al.(1951) showed that guinea pigs receiving no ascorbic acid had a marked reduction in adrenal ascorbic acid and cholesterol concentrations, followed by a rapid reduction in liver glycogen (an indication of diminished output of glucocorticoids). There was also a diminution of adrenal sudanophilia and birefringency. The histochemical changes were limited to the zona fasciculata , and were considered an indication of a depletion of cortical steroids.

Fraser and Preus (1952) observed in a patient who died after receiving cortisone for a period of 8 months a marked bilateral adrenal atrophy. The three layers of the adrenal cortex were still identifiable, but each was markedly reduced in width. The cortical cells revealed no appreciable changes. The medulla was, likewise, atrophied.

Salassa et al.(1953) described the changes in the human adrenal cortex characteristic of previous cortisone administration. There was narrowing of the cortex and diminished quantities of lipids throughout all zones. The cortical cells were shrunken, but there was no apparent decrease in the number of cells. The medulla appeared normal.

Spirots (1953) observed in the adrenals of cats given large doses of cortisone a marked atrophy of the zona fasciculata and a slight regression of the zona glomerulosa. There was no demonstrable changes in the zona reticularis.