BIOCHEMICAL STUDIES OF STEROIDS ON PROTEIN METABOLISM IN RATS

THESIS

Submitted By

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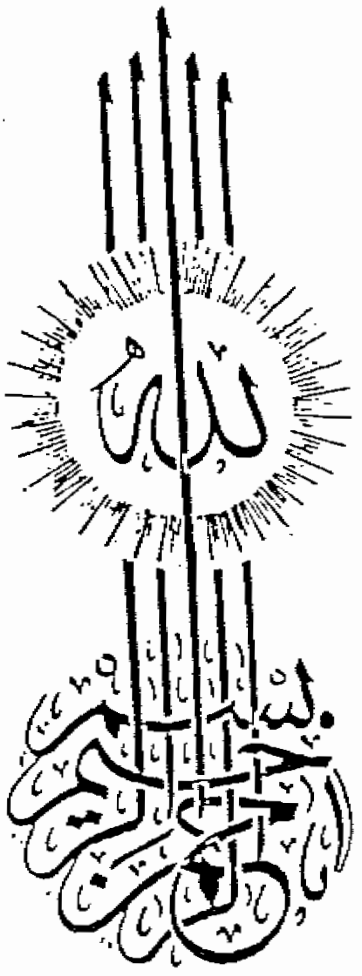

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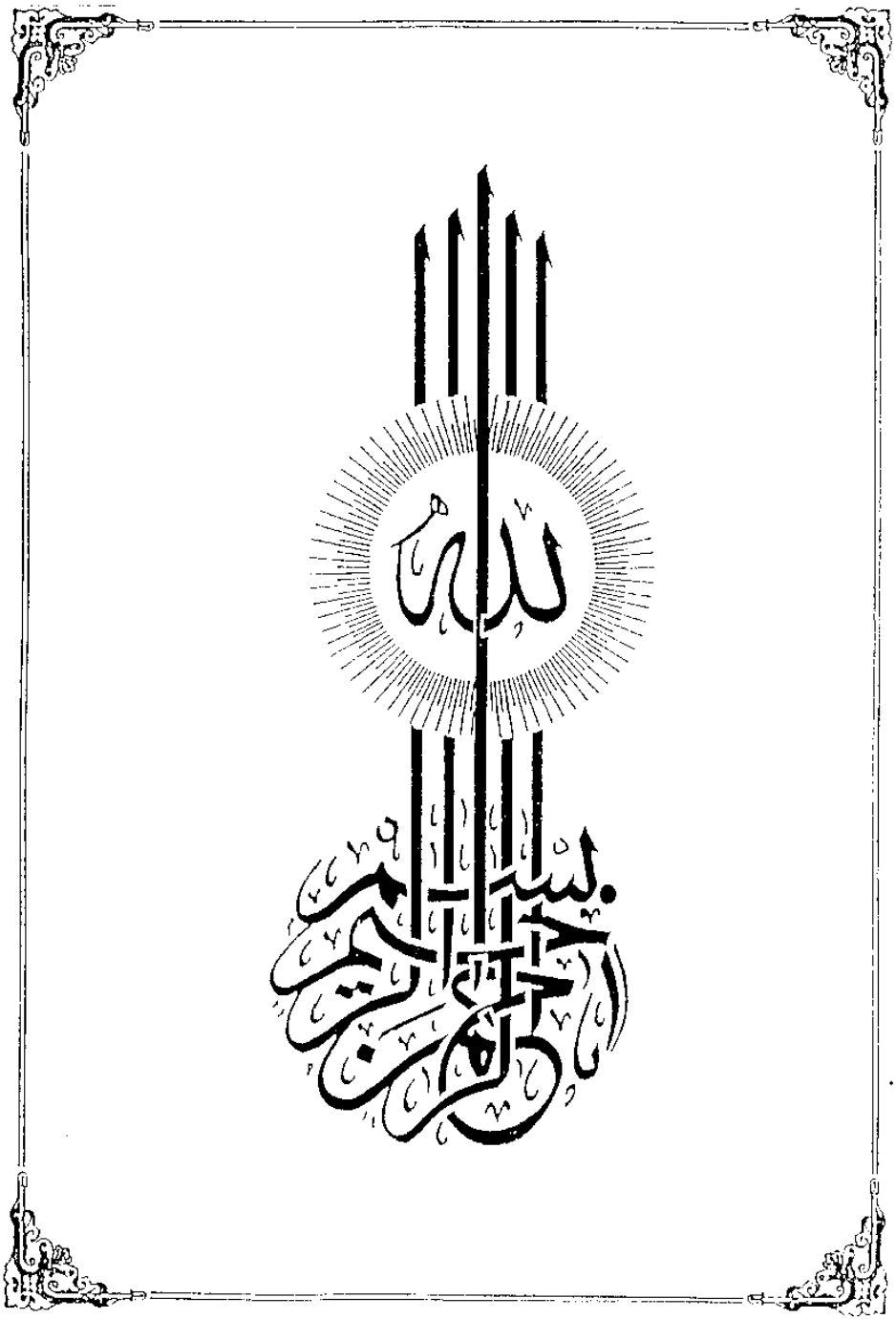

To My Parents

THIS THESIS HAS NOT BEEN SUBMITTED FOR A DEGREE

AT THIS OR ANY OTHER UNIVERSITY

Hussam A. A. Mustapha

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INTRODUCTION

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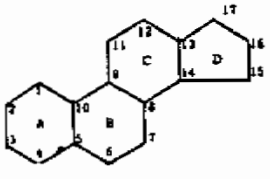
Communication is a most important problem in a multiorgan animal. Thus in the human it is essential that various tissues interrelate so that each can play its particular role in the function of the whole body. Communication involves two types of mechanisms: one is neurological and requires the nervous system, the other is chemical and requires the production of regulatory substances called hormones (Orten & Neuhaus, 1982). The word hormone is derived from a Greek verb meaning "*to stir up or excite*". Hormones; trace substances produced by various endocrine glands (ductless glands) such as the adrenals, ovaries, parathyroids, pituitary, testes, and thyroid serve as chemical messengers carried by the blood to various target organs, where they regulate a variety of physiological and metabolic activities in vertebrates (Lehninger, 1978, 1982). Structurally they are not always proteins, the known hormones include also small polypeptides and steroids (Grodsky, 1979).

The adrenal cortices and the gonads have a common origin in the embryonic coelomic epithelium and, in the adult, they secret hormones all of which are steroids and have very similar structures and many common functions. The steroids belong to a group of compounds found widely in nature and having the characteristic cyclopentanoperhydrophenanthrene nucleus as the chemical basic ring (Montgomery & Welbourn, 1975).



Cyclopentanoperhydrophenanthrene nucleus

The steroid hormones may be divided into 4 main groups: Corticosteroids *(glucocorticoids and mineralocorticoids)*, androgens, estrogens and progesterone. The chemical characteristics of these steroids are indicated in Fig. (1). The shape of the steroid molecule is critical in determining its physiological activity. Thus, the minimal requirement for activity of a *corticosteroid* is the presence of hydroxyl groups at the 11β and 21 positions, ketones at carbon 3 and 20, and a double bond between carbon 4 and 5. *Androgens* have a 17 β-hydroxyl at the 17th carbon and a 3-ketone. In *estrogens* the A ring must be a phenol. **Glucocorticoids** are the steroid hormones of the adrenal cortex, which primarily affect nitrogen metabolism (Bondy, 1985).

The adrenal glands consist of 2 parts; an outer cortex (firm and golden yellow) and an inner medulla (soft and reddish). The former tissue which is derived from the embryonic coelomic epithelium is controlled by adrenocorticotropic hormone; **ACTH** (a polypeptide hormone of 39 amino acids secreted from the anterior pituitary gland), and is essential to life. The latter tissue develops from the neural ectoderm and is a specialized part of the sympathetic nervous system (Montgomery & Welbourn, 1975).

The adrenal cortex is divided into 3 zones: the outer zona glomerulosa, the zona fasciculata and the inner zona reticularis Fig. (2). The zona reticularis and zona fasciculata are functionally a single unit; the inner zone produces all the steroids except aldosterone, while the zona fasciculata acts as a store house for steroid precursor (cholesterol). During active secretion the clear cells at the junction of the zona fasciculata and reticularis (the interface zone) are converted into compact cells under the influence of **ACTH**. This conversion of clear to compact cells gradually extends outward and the zona fasciculata becomes thinner and depleted of lipids and ascorbic acid, while the reticularis increases in thickness and becomes richer in enzymes and ribonucleic acid (**RNA**). Functional activity is assisted by a peculiar arrangement of the longitudinal muscle of the central vein. When it contracts, blood is trapped in the vascular system of the reticularis. This brings **ACTH**

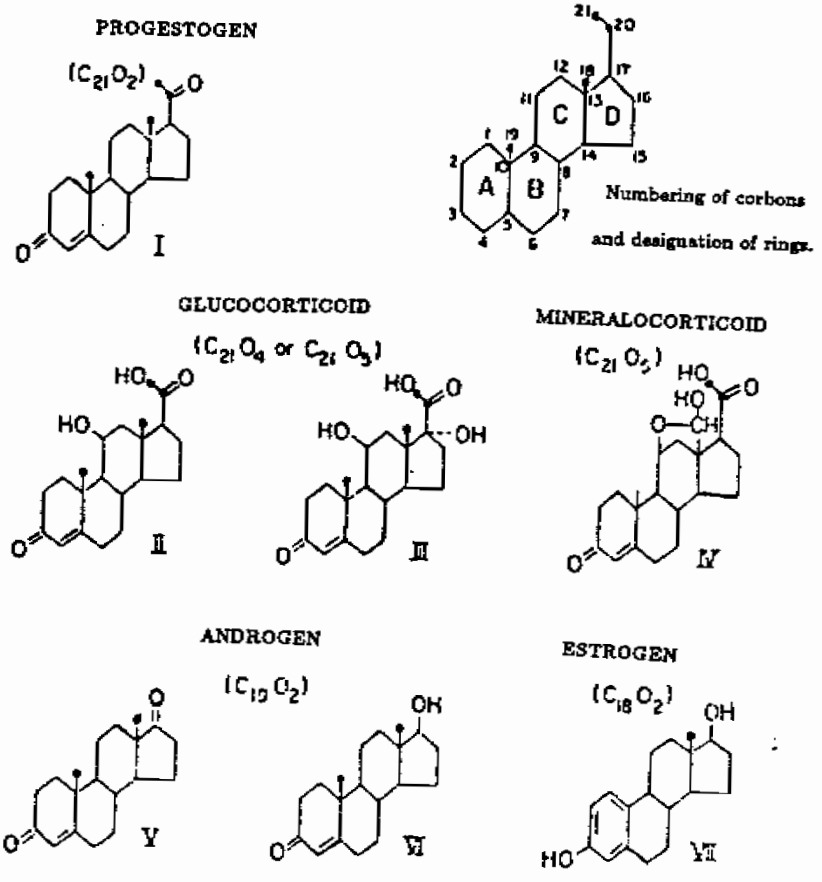


Fig. (1) *Chemical structures of representatives of major classes of biologically active steroids. I = progesterone; II = corticosterone; III = cortisol; IV = aldosterone; V = androstenedione; VI = testosterone; VII = estradiol. A carbon atom is located at each angle of the cyclic structures and at each point on the prosthetic groups where there is a dot. (Adapted from Bondy, 1985).*

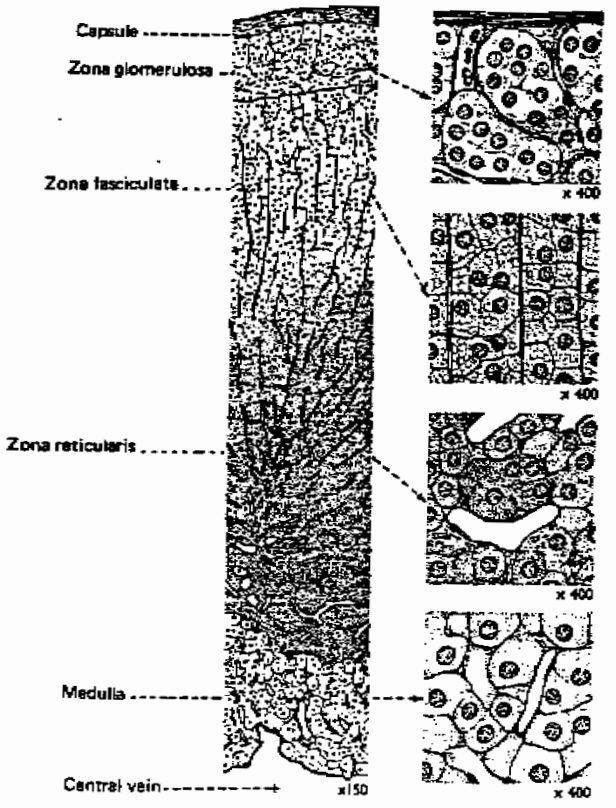


Fig. (2) *Histology of the human adrenal gland (adapted from Briggs & Brotherton, 1970a).*

into contact with the cells of the zona fasciculata closest to the interface zone and the cholesterol in these cells is converted to steroids (Montgomery & Welbourn, 1975).

Liddle (1981) reported that the adrenal cortex was not only able to synthesize cholesterol but also to take it up from the circulation. The cholesterol thus accumulated was then available for conversion into steroid hormones.

Recently, Bondy (1985), stated that the formation of active steroid by the adrenal cortex requires a series of reactions as follows Fig. (3):

a. Provision of the substrate (cholesterol).

b. Cleavage of the side chain from cholesterol to form a C_{21} steroid.

c. Hydroxylation of the resulting steroid in a variety of positions.

d. Oxidation of the 3β hydroxy to a 3-ketone and shift of the double bond from the 5,6 position as in cholesterol to 4,5 double bond found in all secreted hormonal steroids except estrogens.

There are several immediate sources of cholesterol for steroid biosynthesis; the free and the esterified cholesterol associated with the lipoproteins of plasma; the free and the esterified cholesterol stored in adrenal lipid droplets and the cholesterol synthesized by the adrenal cortex. Of these the cholesterol of plasma low density lipoprotein (LDL) appears to be the preferred substrate, at least in humans. Cells of the adrenal cortex have LDL receptors and uptake of LDL is stimulated by ACTH. High density lipoprotein (HDL) can also act as a substrate in some species such as rat, but very low density lipoproteins (VLDL) are not taken up by adrenal cells and thus do not provide precursor cholesterol for steroid synthesis. LDL receptor complexes on the cell surface are internalized by receptor mediated endocytosis, and the protein components as well as cholesterol esters are hydrolyzed in lysosomes releasing free cholesterol some of which are directly used for

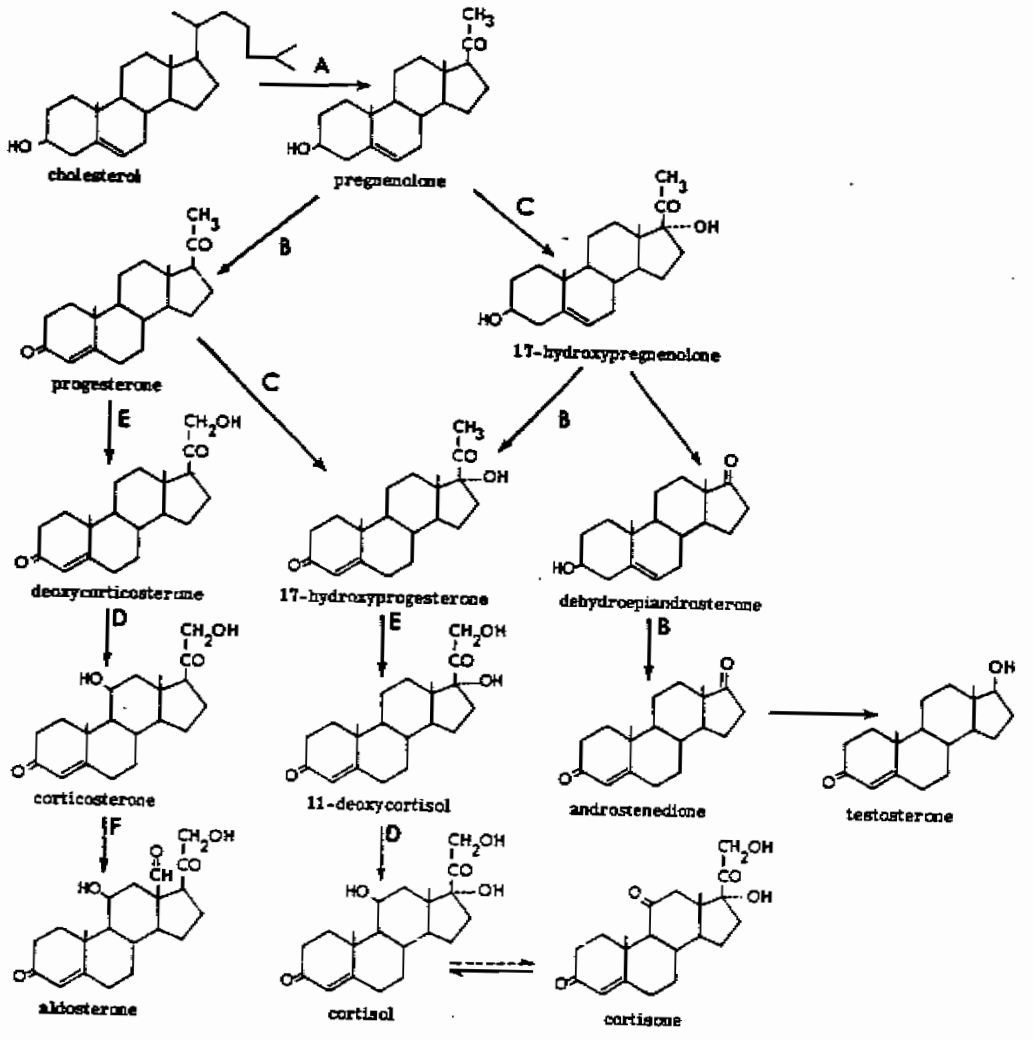


Fig. (3) *Pathways for the synthesis of adrenal steroids. The enzymes involved are as follows: A, desmolase; B, Δ^5-isomerase 3B-hydroxydehydrogenase; C, 17α-hydroxylase; D, 11β-hydroxylase; E, 21-hydroxylase; F, 18-oxidase. (adapted from Bell et al., 1980).*

steroid synthesis. A part is reesterified and stored in lipid droplets as cholesterol ester, from which it can be released later to serve as a substrate (Bondy, 1985). Furthermore, Bondy reported that **ACTH** stimulates steroid secretion in addition to enhancing the uptake of **LDL**-cholesterol, and that the adrenal handles normal steroid production by utilizing free cholesterol from plasma or the labile tissue pool, but drawn on stored cholesterol esters as a reserve when rapid synthesis is required.

The cholesterol is converted to pregnenolone by enzyme systems present in adrenocortical mitochondria, and there is evidence that the 20α-hydroxycholesterol and 20α-22 di-hydroxycholesterol are intermediates. The splitting of the side chain of 20α, 22 dihydroxycholesterol to form pregnenolone and isocaproic aldehyde is mediated by cholesterol desmolase which requires molecular oxygen and nicotinamide adenine dinucleotide phosphate (**NADPH**). The supply of pregnenolone for steroid synthesis appeared to be rate limiting, and it is reasonable to regard the conversion of cholesterol to pregnenolone as a control point for the whole steroid biosynthetic pathway (Briggs & Brotherton, 1970b). The stimulation of corticosteroid biosynthesis by **ACTH** (Stones & Hechter, 1954; Haynes *et al.*, 1959 and Grahame - Smith *et al.*, 1967) takes place at this control point, and more precisely at the first hydroxylation step (Koritz & Kumar, 1970). **ACTH** may stimulate side chain cleavage by inducing the synthesis of a peptide activator of the cleavage reaction (Pederson & Brownie, 1983).

Pregnenolone so formed leaves the mitochondria, where it becoming a substrate for 2 enzymes; NAD^+-requiring 3β-ol dehydrogenase and $\Delta^{4,5}$ isomerase (formed in endoplasmic reticulum, Mustafa & Koritz, 1975; and does not require a cofactor, Neville & Engel, 1968). As a result, progesterone is formed which undergoes a sequential hydroxylation at 17-, 21-carbon in the endoplasmic reticulum and at 11-carbon in the mitochondria to produce the major glucocorticoids; cortisol and corticosterone the relative amounts of which depend in part on the relative rates of 17- and 21-hydroxylation.