THE EFFECT OF SOME STEROIDS ON CELLULAR ENZYMES

BY

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B.Sc.(Ain Shams University)1968

THESIS

Submitted In Partial Fulfillment Of The Requirements For The Degree Of

MASTER OF SCIENCE

IN

AGRICULTURAL

Agric. Biochemistry

Faculty Of Agriculture

Ain Shams University

Cairo - A.R.E.

1978

APPROVAL SHEET

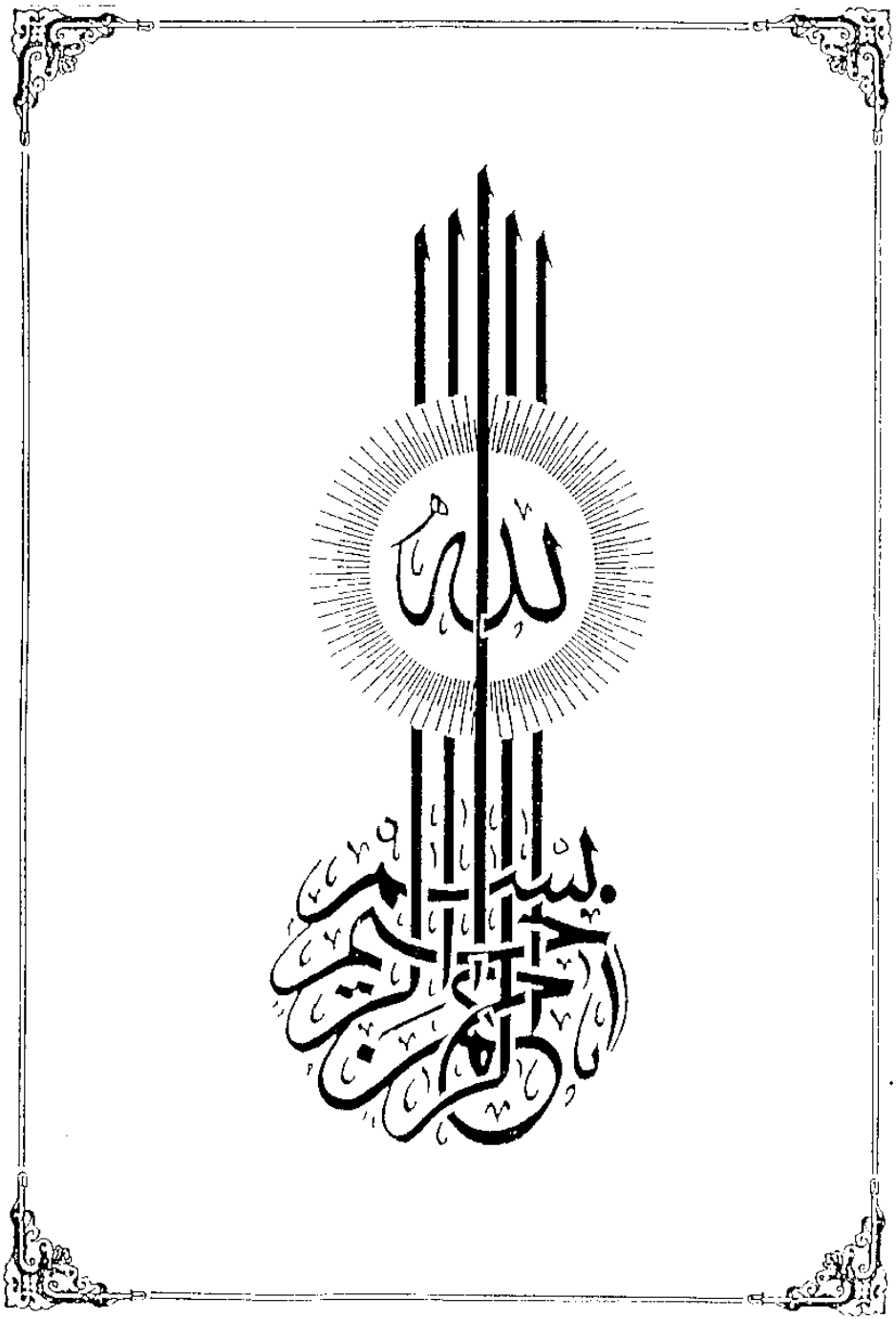
Title Of Thesis : The Effect Of Some Steroids On Cellular enzymes .

Name Of Candidate : Zakaria Ahmed Teleb

Thesis Approved By :

Committee in Charge

Date : / / 1978



To My Wife

ACKNOWLEDGEMENT

The author wishes to express his most sincere gratitude to Dr. Saad El-Hinnawy, Professor of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams University, for his understandable supervision and valuable advice during the course of this investigation.

I would like to express my sincere thanks to Dr. Ezzat Younan, Head of the Molecular Drug Evaluation Division, The National Organization for Drug Control & Research, for suggesting this research work, sincere guidance and supervision through the course of the present investigation.

The author is deeply grateful to Dr. Salwa Ahmed Hid, Assist. Prof. of Biochemistry, Fac. of Agric., Ain Shams University. For their active helps and kind copperation during the course of this study.

Many thanks to Dr. E.A. El-Haddidy Lecturer of Biochemistry, Fac. of Agric., Ain Shams University, for his kind help and advice.

C O N T E N T S

INTRODUCTION

I N T R O D U C T I O N

Due to the introduction of cell fractionation techniques and the isolation of subcellular organelles in culture media invitro, is an important contributions to the mode of action of some drugs. Thus, studies on cultures in-vitro had led to better understanding of the direct action of certain drugs and of the mechanism underlying resistance of cells to these drugs. Furthermore, detection of the activity of steroidal and non-steriodal anti-inflammatory drug on the stability of the lysosomal membranes and screening of the effects of heavy metals and dyes on the lability and fragility of the membranes had been benifitted to a considerable extent from the application of cell and subcellular particles in culture methods. Further, culture systems apparently represent a useful tool in studies of drugs with the [illegible] of free access of the drug to the cells and cell surface, lack of changes in drug concentration, homogeneity of the media, and strictly controlled conditions.

The aim of the present work was to introduce the lysosomal cultures in-vitro to the field of drug assessment of potentially useful 7 steroids at the molecular level without interference of humeral or nervous factors present in the intact organism.

REVIEW AND LITERATURE

The introduction of most steroidal hormones to the field of biological research had been started at the beginning of this century. These groups of steroidal hormones were used in this investigation namely, estrogens androgens, progesterone and pregnandiol in comparison with cholesterol.

These hormones represent a collection of hormones with different biological functions. Ryan & Smith (1965) reported that estrogens are responsible for the development of the female sex organs and the secondary sex characteristics.

Among these estrogens, estrone, has less activity than estradiol and its activity probably depends upon its metabolic conversion to estradiol. Estriol is very importance during pregnancy. The work carried out by Gower (1972) revealed that androgens produce masculization characteristics, and are capable of stimulating the male secondary sex. They cause increased activity of acid phosphatase but a decrease in alkaline phosphatase in serum.

Bowers (1972) detected that the androgenic acitivity was completely lost if oxidation of the 17-B hydroxyl group to 17-oxo-steroid, as in the case of testosterone. The progestational activity of derivatives of progesterone was usually

associated with the presence of a delta-4 double bond. Varying the position of the double bond changes the progestational activity.

On the other hand, pregnandiol had been detected in urine, blood, faeces by Klopper and Macnaughton (1959). Pregnandiol was one of the main metabolites of progesterone.

Studies with 35 neutral steroids showed that hemolytic action was also associated with 5β-H configuration, although a few delta 4,5 compounds were also active. No 5 alpha- H compounds was hemolytic, as stated by Fateno & Kilbowrne (1954).

Weissmann (1963 a) showed that steroids could affect the cell membrane itself. The studies of De Duve et al. (1961) revealed that both progesterone and testosterone rendered rat liver lysosome more permeable to β-glycerophosphate a substrate for acid phosphatase. Also Scheib (1963) confirmed these findings.

Leonard and Rutstein (1953) had shown that progesterone affected the membranes of marine annelid eggs. Selye (1942) had discovered the anaesthetic effects not only of progesterone and testosterone, but of many of the 5β - H

teroids active on lysosomes and erythrocytes. Another xperiments on the stabilization of the structures by estra- iol, had rather a direct effect of testosterone. In protec- ive action, 17-β estradiol exceeded estriol, and estrone.

Uptake & Distribution and Fate of Steroids in the Body :-

Unhjem and Tveter (1969) reported a selective uptake nd retention of labelled androgens in certain tissues, such s seminal vasicle, hypothalamus, and anterior pitutary gland, ut surprisingly not in the seminal vasicles and prostate pro- eins. Thus androgens were preferentially taken up by their arget tissues but the uptake was comparatively less dramatic than in the case of the estrogens. Early attempts to demons- trate the presence of a high affinity androgen-binding protein in testicular extracts were not particularly successful as stated by Mainwaring and Mangan, (1973).

Mowen et al. (1970) detected that the elucidation of the process for the binding of androgens in the brain. These studies were initiated to establish whether the binding of androgens control gonadotrophin secretion and male sexual be- haviour. These important functions of androgens must be expressed by their direct effects of androgens on the brain.

Gorski et al. (1968) demonstrated that in most of the major estrogen-responsive tissues, estradiol was located mainly in the nucleus after in-vivo injection, and microsomal fractions from target tissues labelled in-vivo with H^3 estradiol always contained upto 10 % of the total tissues tritium.

Spelsberg et al. (1972) demonstrated that in the progesterone system the specific acceptor was present in one fraction of the non-histone proteins, but similar experiments have not been carried out with the estradiol system. Jensen et al. (1968) advocated the view that the selective, high affinity binding of estradiol-17 β in rat uterine nuclei was proceeded by distinct steps, first the binding of estradiol to cytoplasmic receptor and secondly, the transfer of the entire receptor - estradiol complex into the nucleus.

Brinkmann et al. (1970) demonstrated that, both in-vivo and in-vitro experiments, a small amount of progesterone was relatively weak and its ligand specificity was low. The decreasing order of affinity was progesterone more than either testosterone or estradiol. Furtherrore, the level of the progesterone-binding protein was higher in prostate than in liver or muscle.

The Mode of Action of Some Steroidal Hormones :-

Davis et al. (1949), found that androgens affect the protein metabolism, electrolyte balance, and the effective concentrations of enzymes in sex specific tissues and in other tissues as the liver and kidney. Androgens influence the concentration of β- glucuronidase, and arginase in kidney. The succinic acid dehydrogenase concentration of rat prostate and seminal vesicles were in part controlled by androgens.

Beaulieu et al. (1971) stated that androgens may control the formation of an enzyme in a specific tissues. If the androgen was an effective regulator of enzyme synthesis, by the same process in all tissues in other words the androgens can stimulate the formation of an enzyme in one tissue but not in the other.

[illegible] and other workers found that retention of [illegible] by target organs such as uterus and vagina depend [illegible] presence of [illegible]. However, the steroid [illegible] found by the target organs was mainly progesterone [illegible], Jenson and Jacobson, (1962).

Barnea and Gorski (1970), suggested that the effects of estrogen depended on the changes in the synthesis of one

or few proteins, which were not detectable by measuring the change in the overall rate of the protein synthesis. Baulieu et al. (1971) demonstrated that the estradiol specifically stimulated the accumulation of specific protein.

The Effect of Steroids and Drugs on Lysosomes :-

Fell et al. (1962) examined the effect of a number of compounds such as retinol, other alcohols and terpenes, on the release of cathepsin from both embryonic chick limb cartilage in culture, and from rate liver lysosomes. They found that only retinol and retinoic acid were active. Dingle and Lucy (1962); Glauert et al. (1963) demonstrated that rabbit erythrocytes were hemolysed readily in-vitro by incubation at 37°C in the presence of retinol. The same effect was observed with pig ox, rat , and human erythrocytes.

Weber et al. (1964) showed that vitamin A induced the release of β-glucuronidase. In these experiments, they found that more vit. A was necessary to damage the lysosomes than to rupture mitochondria. Weissmann & Thomas (1963) demonstrated that the excess vit. A, caused an increased release of acid hydrolases from a large granule fraction of homogenates in-vitro. Roels et al. (1964) demonstrated that the

stability of the liver lysosomes of the vit. A-deficient rats were greatly impaired.

Moreover, the specific activity of the lysosomal enzymes increased very greatly in the liver of rats suffering from advanced stages of vit. A-deficiency.

De Duve et al. (1962) demonstrated that the addition of alpha-tocopheryl acetate or alpha-tocopherol, significantly increased the rate of release of acid phosphatase from the mitochondrial lysosome-rich fraction of rate liver.

More [illegible] doses of alpha-tocopherol added in-vitro to rate liver lysosomes fractions stabilized the lysosomal membrane under the same conditions. Wherease vit. A-deficiency in different animals was frequently accompanied by increasing the activity of lysosomal enzymes.

De Duve et al. (1961), studying the effects of several lipid soluble agents upon rat liver lysosomes in-vitro, observed that cortisone, cortisol, and their acetates when admitted at very low doses i.e. 10^{-4} - 10^{-7} M. retarded the access of polyacrophosphate to the granules at acid pH. Since vit-A and cortisone had antagonistic effects, it appeared likely that cortisone might stabilize lysosomes. Dingle (1961) had demonstrated that the vit A released enzymes from lysosomes.