ARYLAMIDASES ACTIVITIES IN NORMAL MENSTRUAL CYCLE

THESIS

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By

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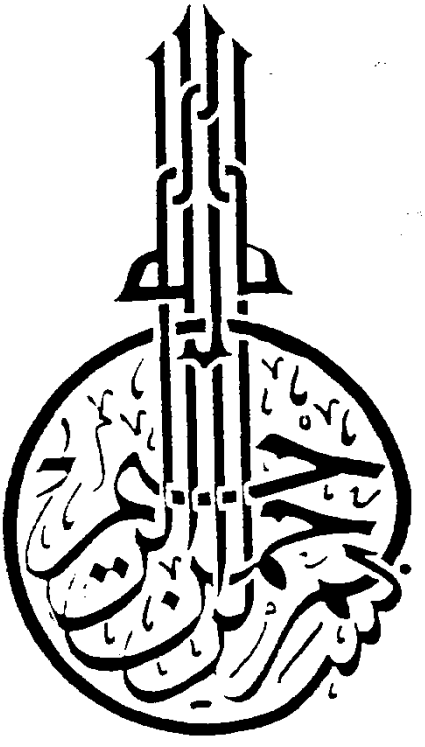

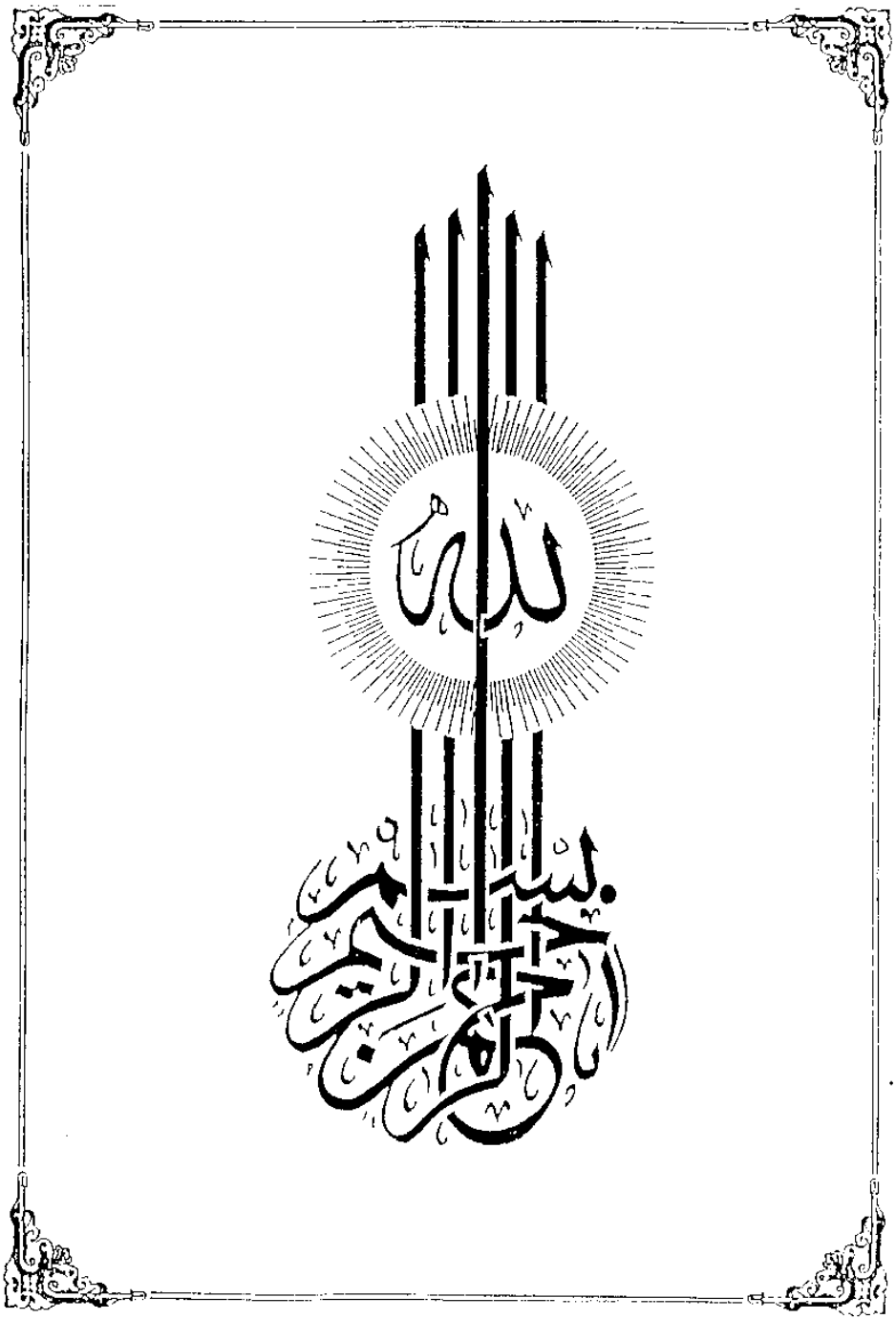

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List of Abbreviations

ANOVA : The analysis of variance.

DEAE : Diethylaminoethyl

FSH : Follicle - stimulating hormone.

GnRH : Gonadotropin releasing hormone.

iso. : Isoenzyme.

Km : Michael's constant.

LH : Luteinizing hormone.

mIU : Milli-international units.

min. : Minute.

rpm : Revolutions per minute.

Prolif. : Proliferative.

Secret. : Secretory.

[S] : Substrate concentration.

T : Absolute temperature

$\mathbf{T_{0.5}}$: Calculated half life time.

Temp. : Temperature (°C).

V : Velocity.

$\mathbf{V_{max}}$: Maximum velocity.

$\mathbf{V_{o\ max}}$: Specific maximum velocity.

vs : Versus.

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INTRODUCTION AND AIM OF THE WORK

I. INTRODUCTION AND AIM OF THE WORK

Arylamidases are a group of enzymes that hydrolyse α-aminoacyl derivatives of β-naphthylamide ***(Patterson et al., 1963)***, 4-nitroaniline ***(Tuppy et al., 1962; Ellis & Perry 1966)***, aniline ***(Kleine et al., 1973)*** or aminonitriles ***(Szewezuk et al., 1965)***. Arylamidases, hydrolyzing amino acid substituted β-naphthylamides and p-nitroanilides, are usually referred to as N, B or A according to whether the substrate possesses a neutral amino acid (L-leucine β-naphathylamide or L-leucine p-nitroanilide), basic amino acid (L-arginine β-naphthylamide or L-lysine β-naphthylamide) or acidic amino acid (L-aspartate or L-glutamate β-naphthylamide) respectively ***(Kar & Pearson, 1976)***. Arylamidases also include L-cystine arylamidase ***(Titus et al., 1960)***, pyroglutamic arylamidase ***(Facklam, 1987)***, L-tyrosine arylamidase ***(De Gandarias et al., 1989 a)*** and L-proline arylamidase ***(Takahashi et al., 1991)***.

The arylamidases possess a capacity to act on certain length polypeptide chains that may result from the action of intracellular proteases ***(Suszkiw & Brecher, 1970)***. It has been found that a free amino group attached to the α-carbon in the substrate is essential for arylamidase activity. Cysteine and histidine appear to participate in the catalytic action of the brain

arylamidase ***(Suszkiw & Brecher, 1970).*** In this respect the brain arylamidase seems to correspond to the rat liver arylamidase for which cysteine and histidine have also been implicated in activity ***(Makinen & Hopsu-Havu, 1967).***

The influence of substrate of different N-terminal amino acid residues on susceptibility to arylamidase catalyzed hydrolysis was studied by ***Little & Behal (1971).*** The highest rates of hydrolysis were observed with substrates having a nonpolar or basic amino acid as the N-terminal residue. Furthermore, substrates with straight side chains on the N-terminal residue were hydrolyzed more rapidly than substrates with branched side chains and substrates with γ branched side chains were hydrolyzed more rapidly than those with β branched side chains.

L-leucine, L-lysine and L-tyrosine arylamidases activities have recently shown significant changes in rat serum during its estrus cycle. These activities suggest direct participation, probably linked to the hormonal changes that occur during ovarian cycle ***(De Gandarias et al., 1988 and 1989 a).***

Arylamidases were found at the surface of the uterine cells (epithelial and decidual) and in uterine fluid during the peri-

implantation period. They play significant roles in the process of blastocyst-uterine interaction. In the rabbit, one of the earliest indications of embryo recognition by the endometrium is that arylamidase becomes depleted specifically from the luminal epithelium and stroma subjacent to the blastocyst. In the guinea pig, the phenomenon of depletion is also observed, but exclusively in the stroma surrounding the implantation site, while the epithelium remains largely negative throughout, whereas in the rat a cycle of increase and decrease appeared to occur in the stroma. In addition, arylamidases are secreted into the uterine lumen in various species, their concentration in the luminal fluid of the rabbit peaks shortly before implantation is initiated. The secretion of arylamidases into the uterine fluid is modified by ovarian steroid hormones***(Mitchell & Denker, 1991)***

The present study was undertaken in the human to determine if, as estrus cycle in the guinea pig and rat, serum arylamidases activities fluctuate during the different phases of the menstrual cycle and if enzyme levels are susceptible to modification by follicle-stimulating hormone (FSH).

To achieve this purpose the activity of L-leucine and L-lysine arylamidase activities and FSH levels were determined in

Egyptian females before puberty, and during the different phases of the menstrual cycle. According to uterine and hormonal changes, the menstrual cycle is divided to (early & late proliferative or follicular phases) and (early & late secretory or luteal phases). Also, serum L-leucine arylamidase isoenzymes were separated. Further investigations were extended to isolation & partial purification of serum L-leucine arylamidase and some kinetic studies were done during the different phases of the menstrual cycle.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

1. Arylamidases

Bressler et al., (1960) and Sciarra & Burress (1960) found that the serum L-leucine arylamidase level is raised gradually through the second and third trimesters of pregnancy. L-leucine arylamidase is increased during the second month of pregnancy but its level is lower than that in patients with hepatocellular diseases, at the termination of pregnancy the serum L-leucine arylamidase level is significantly higher than that in patients with hepatocellular diseases.

Sciarra & Burress (1960) and Miller et al., (1964) reported that serum L-leucine arylamidase activity was significantly increased during the third trimester in women with twin pregnancy when compared with women with a single fetus of the same gestation age.

Cystine aminopeptidase "L-cystine arylamidase" which is presumptively a measure of oxytocinase was determined according to ***Titus et al. (1960)*** with L-cystine di-β-naphthylamide as substrate ***(Miller et al., 1964).*** Oxytocinase is identical with L-cystine arylamidase. Oxytocinase hydrolyses the peptide bond between N-terminal cystine and adjacent tyrosine, resulting in the loss of the biological effect of oxytocin ***(Melander, 1965 and Lampelo & Vanha-Perttula, 1979).***

Miller et al. (1964) found that serum L-cystine arylamidase attain higher levels in twin pregnancies than in singleton pregnancies and their activities are increased during pregnancy. The mean levels of the L-leucine and L-cystine arylamidases are increased as the length of gestation increases. At each period of gestation, the mean values for two enzymes in twin pregnancies exceed the corresponding value in singleton pregnancies.

Marks et al. (1968) reported that the various cerbral peptidases and arylamidases act sequentially in the degradation of oligopeptides in the brain. In addition to their possible role in pathways of protein degradation, they may involved in the relative or activation of neurosecretory materials.

Hopsu-Havu et al. (1964), Hopsu-Havu et al. (1966), Makinen & Hopsu-Havu (1967) and Makinen & Makinen (1971) demonstrated arylamidase B in various rat tissues. ***Makinen (1969)*** has identified arylamidase B in mammalian tissues and in human liver. It also found in adult human periodontal tissues ***(Makinen & Paunio, 1970),*** in certain inflammatory exudates and in gingivial tissues ***(Makinen & Paunio, 1972).***

Kurtz & Wachsmuth (1969) reported that arylamidase has a role in regulating certain pressor substances such as angiotensin.

Makinen & Paunio (1972) reported that arylamidase B has a possible role in wound healing and inflammatory processes. The enzyme has been considered to be inactive in the intracellular compartment where phosphate concentration is high and the chloride concentration is low. When it liberated into the extracellular compartment. the enzyme becomes activated and carries out its function.

Herrmann & Schneider (1976) identified arylamidase in various tissues of ectodermal, mesodermal and endodermal origin.

Virtamen et al. (1977) reported that denature stomatitis increased the activity of arylamidase B in the connective tissue of the oral mucosa when compared to the control tissue. The increase was considered to be a result of subepithelial inflammation and mechanical trauma.

Hayashi & Oshima (1977) isolated L-leucine arylamidase from monkey brain extract and purified. ***Hayashi (1978)*** reported