ESTIMATION OF SERUM CHOLINESTERASE ACTIVITY IN LIVER CIRRHOSIS AND NEOPLASM

THESIS

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BY

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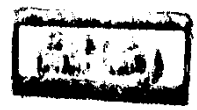

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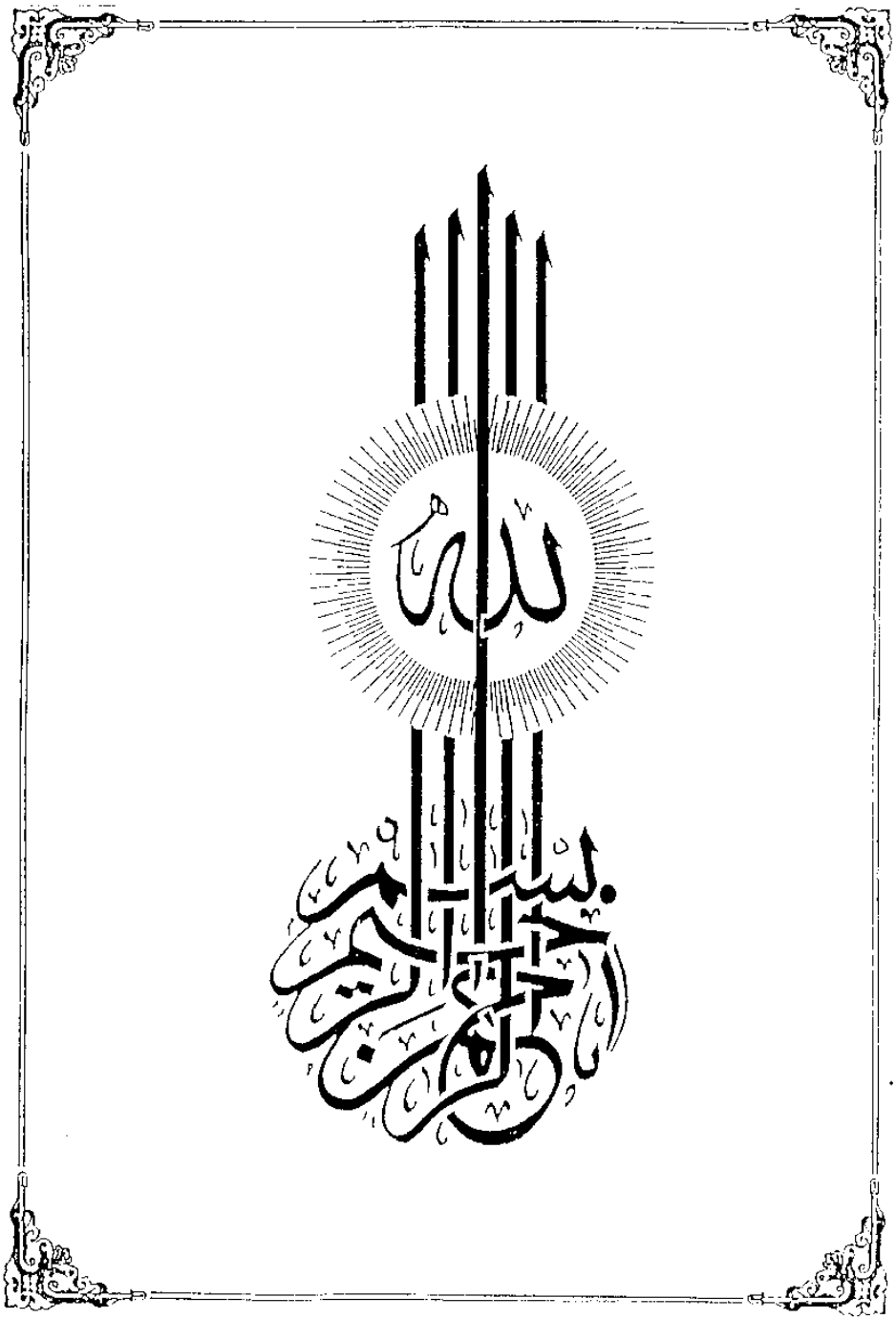

CONTENTS

ABBREVIATIONS

AFP : Alpha fetoprotein .

alb. : albumin.

Alk.Phosph. : Alkaline phosphatase.

CHE : Cholinesterase.

C_3 : Complement 3

γ-G T : Gamma-glutamyl transferase.

H.C.C : Hepatocellular carcinoma.

L.C.A.T.: Lecithin-cholesterol acyltransferase.

L.C. : Liver cirrhosis.

L.C.(C) : Liver cirrhosis (Compunsated).

L.C. (D): Liver cirrhosis (Decompunsated).

L.S. : Liver secondaries .

S.G.O.T.: Serum glutamic oxaloacetic transaminase .

S.G.P.T.: Serum glutamic pyruvic transaminase.

Review of Literature

INTRODUCTION AND AIM OF WORK

Serum cholinesterase is an enzyme which is synthetized by the liver and then secreted into the blood, to achieve a constant level in the same indivdual.

It has been observed that the activity of this enzyme is reduced whenever there is impairment of the liver cell function (Vorhaus and Kark, 1953). However, Tajiri et al,(1983) reported that in certain cases of hepatocellular carcinoma the serum cholinesterase activity is increased which constitutes a new paraneoplastic syndrome.

In this work I tried to prove the importance of serum cholinesterase activity estimation in diagnosis of liver cirrhosis and neoplasm. So , the enzyme activity , in this work , was studied in patients with liver cirrhosis; both compunsated and decompunsated, and in patients with liver malignancy; both primary and secondary. In addition ,it was estimated in healthy individuals which were studied as a control group versus patient groups.

Historical Background :

Cholinesterase is an enzyme capable of accelerating hydrolytic cleavage of choline esters ,and then especially of acetyl choline as the only choline ester which occurs physiologically in the human body.

The presence of cholinesterase was first demonstrated in the musculature of the heart by Loewi and Navratil(1926) during their investigations on the vagus action . It has later been found in all tissues of the organism ,though the concentration in nerve tissue is considerably higher than in other places , just as one would expect from what is known regarding the physiology of acetylcholine and its significance for the transmission of nervous effects. It seems, however, that Loewi and Navratil did not differentiate between the two types of cholinesterase which have been described , later on , as erythrocytic or true cholinesterase and serum or pseudo-cholinesterase.

After Galehr and Platner (1928) had shown that normal blood always contains cholinesterase , both in

the plasma and in the red blood cells , interest was aroused in the question of this esterase in connection with pathological conditions. At first, special attention was given to myasthenia gravis since physostigmine , which is a specific cholinesterase inhibitor, was introduced in the treatment of this disease.

More recently , however attention has also been directed towards the connection of cholinesterase with other diseases . It was first documented by Antopol et al , 1938, that serum cholinesterase activity decreases in liver disease., This observation was supported by many investigators later on.

Chemistry , Physiology and Metabolism :

There are two types of cholinesterase enzyme :-

1) The acetyl cholinesterase (Ec. 3.1.1.7) also called specific or true cholinesterase is found in erythrocytes , nervous tissue and brain. It hydrolyses acetyl choline even at low concentrations.

$$CH_3COOH_2N(CH_3)_3 + H_2O \xrightleftharpoons{\text{cholinesterase}} CH_3COOH + HOCH_2 - HC_2N(CH_3)_3$$

acetylcholine water — acetic acid + choline

There, cholinesterase is of high physiological importance because of its possible role in the removal of acetylcholine released at nerve endings during the transfere of nerve impulses to muscle cells in order that further nerve impulses may in turn stimulate muscle contraction. Assay of its concentration is particularly important in industrial medicine for checking workers exposed to insecticides and for checking farmers dealing with such insecticides . The activity of this esterase is measured in the haemolysate.

2) Pseudocholinesterase also called serum cholinesterase (Ec, 3.1, 1.8).

The activity of this esterase can be measured in serum. This enzyme is active only at relatively high concentrations of acetylcholine and is capable of hydrolysing other esters, e.g. simple fats.

In this work I am concerned with serum cholinesterase , whose activity is measured in blood serum.

Enzyme Synthesis and Distribution :

Serum cholinesterase is an alph-globuline formed in the liver , and then released into blood. It is formed of at least eleven isoenzymes whose total activity represents the activity of serum cholinesterase. It is present nearly in all tissues , but mainly in plasma, liver where it is synthetized , pancrease , heart , kidney and skin. It has a short life-span of 28 days.

Serum cholinesterase is destroyed at a rate of about 10 % per day and as the level of the enzyme in any individual is remarkably constant , serial assay rapidly reveals any alteration in enzyme production.

The natural function of serum cholinesterase is not definitely known , but since many substances of the enzyme are inhibitors of true cholinesterase, it has been suggested that serum cholinesterase protects true cholinesterase against inhibition (Evans and Lehmann 1971).

Methods of Estimation :

There are three methods of estimation of the serum cholinesterase activity:-

1) Manometric Method :

Also called gasometric (Warbung) technic of Ammon (1934). In this method the reaction is carried out in Warbung apparatus. The enzyme preparation is allowed to react with the substrate in a bicarbonate - carbon dioxide buffer in atmosphere of 95 % nitrogen and 5 % carbon dioxide which gives pH of 7.4 . The acid released during hydrolysis of the substrate reacts with bicarbonate to liberate carbon dioxide , and from the resulting increase in pressure , the enzyme activity is usually calculated in terms of the number of u mol hydrolysed per minute per milliliter.

2) Titrimetric Method :

This rapid and simple procedure is used for emergency diagnosis of suxamethonium sensitivity and of organophosphorus poisoning .

The amount of acetic acid liberated during the incubation of acetyl choline with the enzyme preparation can be determined by continuous titration

with an alkali at a constant pH(Kaufman,1954). In this procedure the pH of the reaction mixture is measured before and after incubation of serum with acetylcholine in the buffer. Results are recorded in terms of the change in pH per hour per 0.02 ml of plasma.

3) Colorimetric Method:

For the definitive investigation of serum cholinesterase , the method of Dietz et al., 1973, is recommended . In this method cholinesterase , hydrolyses the substrate propionyl thiocholine to release thiocholine, which has a reactive sulphhydral SH group . This,in turn, reacts with 5, 5 dithibis - 2-nitrobenzoïc acid to produce a yellow 5-thio -2- nitrobenzoate ion whose absorbance is measured at 410 n m. The determination is standarized by refrence to millimolar absorbtivity of 5- thio-2- benzoate. The reaction is stopped by the addition of quinidine sulphate solution. Ellman et al. colorimetric method (1961); is the method used in this study.

Many substrates are used for measuring serum cholinestrase activity e.g butyrylthiocholine ,

acetyl thiocholine , propionylthiocholine,succinylcholine , etc ... Since butyrylthiocholine is the least labile of thiocholinesters and because it is relatively specific for human serum pseudocholinesterase (Silk et al.,1979) , it has been recommended and used as the substrate of choice for routine purposes (Das and Liddell 1970, Garry et al.,1972 & Silk et al., 1979). Furthermore , O-toluolcholine has been reported to be as stable as butyrylthiocholine and more specific for human serum pseudocholinesterase than butyrylthiocholine. (Okabe et al., 1980). Although propionylthiocholine is recommended as a better substrate than butyrylthiocholine for the differentiation of the phenotypes of the pseudocholinesterase (Dietz et al., 1972 & Evans and Wroe, 1978), succinylcholine may be the most suitable substrate for direct detection of succinylcholine sensitivity (Abernethy et al., 1984)

With respect to specificationof substrates for measuring serum cholinesterase avtivity for the assessment of hepatic function , Prellwitz et al., (1976) have reported that in hepatic cirrhosis and,also in chronic hepatitis the activities of serum cholinesterase

determined at 25 °C with acetyl-,butyryl-, or propionylthiocholine , all showed similar decreases , and revealed no significant differences in the specificationsof substrates. Likewise , Uete et al., 1985 , showed that each of these substrates ,in hepatic cirrhosis , revealed the same decrease of enzyme activity when the assay was conducted at 37 °C , thus confirming the findings of Prellwitz et al , at 25 °C. Furthermore , the serum cholinesterase activity determined with O-toluolcholine and succinylcholine decreases at a similar rate compared with the enzyme activity determined with acetyl-, butyryl-, and propionylthiocholines. As a conclusion,there is no significant difference in specification between various substrates when using cholinesterase activity for the evaluation of hepatic function. Acetyl-thiocholine is the substrate used for assay of enzyme activity in this study.

Merch (Darmstadt, W., Germany) reintroduced a pH based cholinesterase assay in the form of a dipstick. In this assay , filter paper, impregnated with a pH - indicator and acetyl choline , changes colour in relation to the amount of acetic acid released (i.e., the enzymatic activity). The method has been