THE AMYLASE / CREATININE CLEARANCE RATIO AS AN INDEX OF THE EXOCRINE FUNCTION OF THE PANCREAS DURING DIABETIC KETOSIS

THESIS

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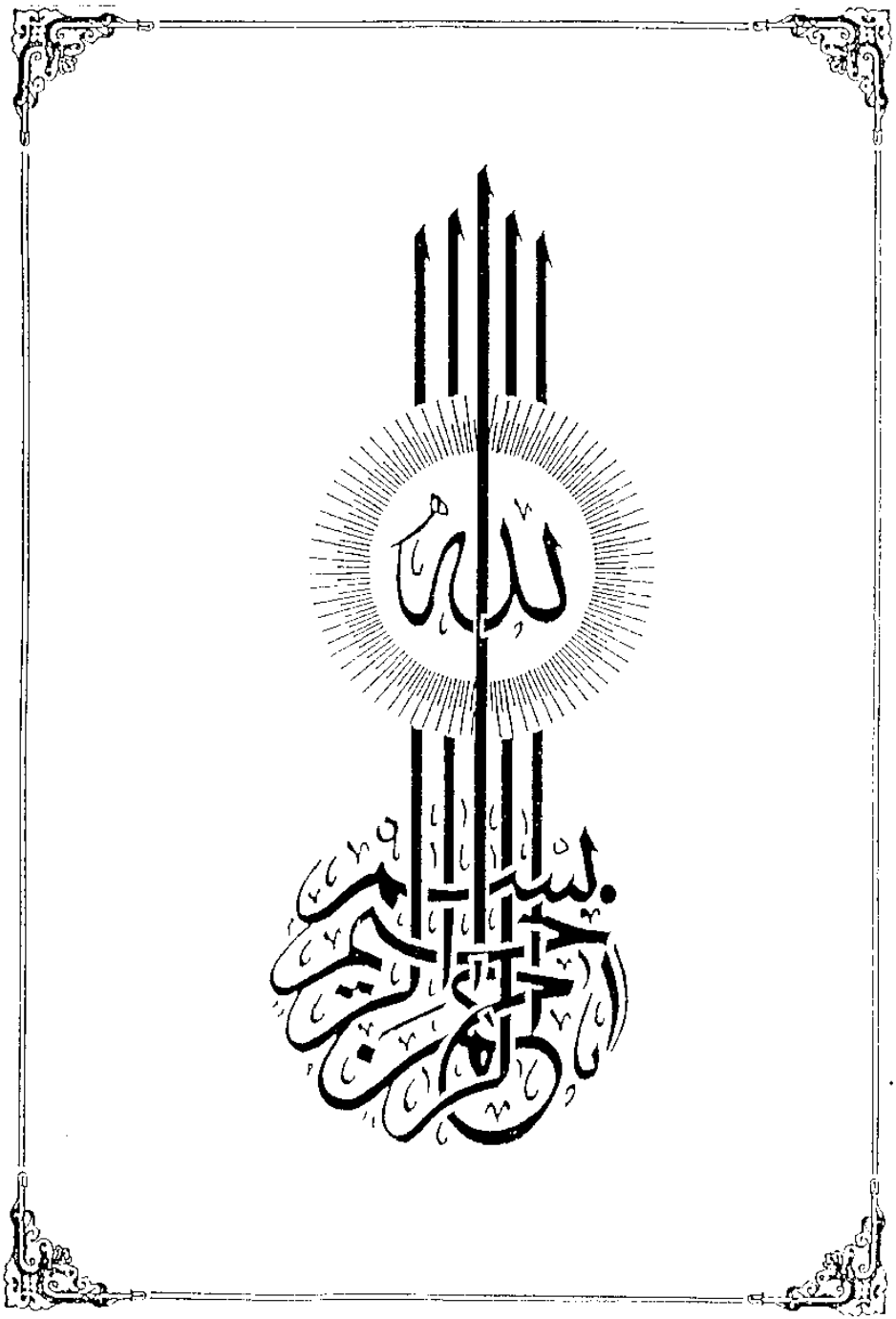
C O N T E N T S



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INTRODUCTION

Many studies have been carried out to explain the abdominal pain that accompanies diabetic ketosis. Some contributed it to stretching of the hepatic capsule (Warren and Le compte,1952), others to gastric distension (Malins, 1968).

Tully and Lowenthal (1958) mentioned that the pain may be due to pancreatitis as evidenced by raised serum amylase. On the other hand, on necropsy for patients who died from diabetic coma, Finn and Cope (1963) found no evidence of acute pancreatitis.

Knight & co-workers in 1973 stated that the hyperamylasemia detected in the serum of such patients may be a mere release of the enzyme without actual cell necrosis as evidenced by normal serum lipase measured simultaneously with serum amylase.

Belfiore and Napoli in 1973 contributed hyperamylasemia to activation of the enzyme in liver cells - together with many lysosomal enzymes present in latency - during the metabolic decompensation of ketosis, a suggestion that has been aborted after that on the basis of the low content of amylase in human liver cells.

In the last few years, amylase could be seperated into two isoenzymes namely salivary and pancreatic types, and so the source of increased amylase enzyme could be more specifically identified.

Warshaw et al in 1977, studying the hyperamylasemia in ketosis using new techniques of seperation of isoamylases, found that the rise in serum amylase is mainly due to salivary type isoamylase, and contributed it to deranged carbohydrate metabolism in many tissues during ketosis.

On the other hand, Dreiling et al in 1974 proposed that measurement of the amylase / creatinine clearance ratio is of diagnostic value in patients suffering from acute pancreatitis, a hypothesis which was supported by Warshaw and Fuller (1975), Marten et al (1977), Johnson et al (1976) and many others.

Using this test in trying to explain the hyperamylasemia of ketosis may be helpful by calculating the ratio of amylase clearance to that of creatinine for both pancreatic and salivary isoenzymes. It may be possible to decide whether it is pancreatitis or not that causes hyperamylasemia and abdominal pain during ketosis.

REVIEW OF LITERATURE

AMYLASE ENZYME

α - Amylase (α - 1,4 - glucan - 4 - glucanohydrolase) is the enzyme which catalyzes the hydrolysis of starch, glycogen and related poly - and oligosaccharides. The end products formed are maltose, small chain dextrins and some glucose. The enzyme hydrolyzes internal α - 1,4-glucosidic bonds in the previously mentioned compunds. β - amylases are of plant origin (α - 1,4-glucan - maltohydrolase) (Salt and Schenker, 1976).

SOURCES

Salivary glands and the pancreas are the two main sources. Human salivary and pancreatic isoamylases differ from each other in electrophoretic migration, the salivary isoamylase migrates faster towards the anode than does the pancreatic isoamylase (Berk and Fridhandler,1977).

Human pancreatic amylase consists of multiple fractions. In saliva, one main fraction as well as a varying number of additional weak zones of amylase are seen. There is no difference in the isoamylase pattern of saliva secreted from the different salivary glands (Merritt and Karn, 1977).

Minute amounts of amylase may be produced by duodenal and bronchial glands, the isoamylase from these two sources

belong to the pancreatic and salivary group respectively. The contribution to the serum isoamylase pattern from these sources is negligible under normal conditions (Skude, 1975 and Otsuki et al 1977).

Isoamylase belonging to the salivary group has been demonstrated in human milk (Berk and Fridhandler, 1974). Also tear fluid contains amylase belonging to the same group (Vanllaeringen et al 1975).

As for the existence of human liver amylases, contradicting reports have been published. None of the reports regarding human liver amylase presents convincing proof (Berk and Fridhandler, 1977 & Rutter et al 1961).

High amylase activity has been recorded in human fallopian tubes and ovarian cysts (Green, 1957). The isoamylase from the fallopian tubes consists of one main fraction which migrates faster than does salivary amylase. In homogenates of cervical mucosa the isoamylase pattern is characterized by one prominent main fraction which can not be differentiated from the salivary main isomylase fraction (Skude et al 1976). The specific group of isoamylases produced in the female genital tract can not be normally demonstrated in serum and urine, although it is clearly detectable in fluid from the cul-de-sac (Skude et al 1976)

METABOLISM AND RENAL EXCRETION

Metabolism

Duane et al (1972) carried out studies on P - and S - type isoamylase in the baboon (an animal with serum level and renal clearance of amylase similar to those of man). They found that :

1. The majority of the serum amylase is cleared via an extrarenal mechanism (Postulated to be the reticuloendothelial system), with urinary excretion accounting for only about 24 % of amylase removed.

2. The metabolic clearance of both isoenzymes is extremely rapid with half-lives of approximately 130 minutes.

3. P -type isoamylase is cleared by the kidneys 80 % more rapidly than salivary isoamylase.

4. Renal excretion of amylase is accomplished via glomerular filtration, without good evidence for tubular secretion or reabsorption.

Renal Excretion

The normal individual does not exhibit wide variation in the rate of urinary amylase excretion during a given 24-hour period. In acute pancreatitis, however, wide fluctuations in urinary amylase output can occur during

a relatively short period of time (Waller and Ralston, 1971). Fluctuations can be seen within a 2-hour period and usually bears no relation to the clinical condition of patient (Waller and Ralston, 1971).

In pancreatitis the extent to which the urinary excretion of amylase lags behind the serum concentration depends upon the severity of the episode (Saxon et al,1957). Typically, the lag period lasts less than 7 to 10 days from the time the serum amylase concentration has returned to normal.

STABILITY OF AMYLASE

Amylase in normal serum has been found to be stable on storage but there was a loss of enzymatic activity in serum from patients with pancreatitis stored under identical conditions (Howe and Elmslie, 1971). The exact reason for this has not been found, nor is it clear if this happens in stored sera from patients with other diseases.

Prolonged storage at elevated temperature up to 37°C may result in deamidation (Merritt and Karn, 1977).

Urine presents different stability problems. It has long been known that amylase is inactivated in urine at low pH (Berk and Fridhandler, 1975). Below pH 5.0 amylase is rapidly inactivated (Stiefel and Keller, 1973).

It must be considered a possibility that if urine is allowed to rest in the bladder even for a short time, then an acid urine could inactivate amylase.

Amylase activity in serum samples and urine is stable for one week in controlled room temperature, but good practice dictates that samples be stored at 2 - 8°C . For longer periods the samples must be stored at - 20°C (Hegarty et al , 1978). Morton et al in 1976 had stored their samples at - 70°C .

METHODS OF DETERMINATION

Determination of amylase has for a long time been a tool in practical medicine. Some of the determination methods used are amyloclastic in which substrate utilization is demonstrated as changes in the colour reaction between starch and iodine or a reduction of the viscosity of the residual substrate. Also turbidimetric and nephelometric techniques have been used to demonstrate starch digestion. Other methods are saccharogenic i.e the amount of liberated glucoss or maltose is determined.

Among the commonly used methods for isoenzyme seperation and subsequent activity determination can be mentioned electrophoresis, chromatography, isoelectric focusing, temperature inhibition, or activity determination using isoenzyme specific substrates at various pH levels

after immunologic activity inhibition or in the presence of inhibitor.

Early investigations into amylase isoenzymes were confusing, but further works showed that discrepancies in findings were dependent upon the method used (McGeachim and Lewis, 1959 , Ujihara et al 1965 , Wilding, 1963 & Berk and Fridhandler, 1975).

Electrophoresis has been performed in various stabilizing media (Berk and Fridhandler, 1977 , Meites and Rogols, 1971 , Merritt and Karn, 1979 and Skude, 1977). After seperation the amylase activity has been measured in eluates of strips cut out of the electropherogram or with the aid of zymogram techniques using either the electrogram itself or a replica transfer of it. The methods for detecting amylase have been either amyloclastic or saccharogenic. These methods are apt to give rise to false positive reactions, especially in the diabetics due to high serum level of glucose.

Chromatography and Isoelectric Focusing have predominantly been used in the isolation and physicochemical characterization of individual isoamylase fractions (Fridhandler and Berk, 1980 & Meada et al 1978).

Temperature Inhibition in amylase assay is of no value since the salivary and pancreatic isoamylases behave in the same way (Warshaw et al 1974).

Immunologic activity inhibition have not been used due to the close antigenic relationship between salivary and pancreatic isoamylase (Berk and Fridhandler, 1977 & Merritt and Karn, 1977). In the last few years, the purification of an inhibitor of amylase from wheat was described (O'Donnell and McGeeney, 1976). The purified protein inhibitor was found to be 100 times more specific for human salivary amylase in relation to human pancreatic amylase.

REFERENCE VALUES

Normal values for serum and urinary amylase in the adult vary according to the laboratory and method used (Salt and Schenker, 1976).

The most familiar unit is the Somogyi unit (SU.) with normal values in serum of 60-160 SU. / 100 ml and urine of 35-260 SU. / hr. (Somogyi, 1938). The Caraway unit (CU.) is approximately equal to the Somogyi unit, with normal serum values of 95 ± 32 CU. / 100 ml (Salt and Schenker, 1976). One SU. / 100 ml is equivalent to 1.85 International units (IU.) / L (Waller and Ralston, 1971).

NORMAL VARIATIONS

The contribution of the isoamylase from the salivary glands and the pancreatic gland to the total serum amylase