THE EFFECT OF LIVER AND PANCREATIC DAMAGE ON SERUM AMYLASE CONTENT IN THE RAT

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CHAPTER I

INTRODUCTION

Although in recent years a considerable number of investigations have been carried out on the diastatic activity of the blood, the results obtained by various investigators are so conflicting that it is difficult to draw definite conclusions from them. On the one hand there have been reports of experimental evidence which indicate a direct relationship between blood amylase content and blood sugar content, while on the other hand reports of finding no correlation between the blood sugar level and amylase (diastase) content were made.

The site of production of blood amylase has also been the object of considerable investigation from which no definite conclusion has been forthcoming.

It was the purpose of this investigation to determine the relationship between induced diabetes and blood amylase content by causing damage to the beta cells of the pancreas through the use of alloxan.

Another phase of this investigation dealt with the role of the liver, if any, in the production of blood amylase.
CHAPTER II

REVIEW OF LITERATURE

Myers and Killian ('17) found that in several cases of diabetic patients there was increased diastase activity in the blood which was as much as two to 4 times as high as normal. They also found a close relationship between blood sugar and diastase activity.

Reid and Myers ('32) investigated the effect of insulin on the diastatic activity of the blood of diabetic patients. They found that in diabetic patients who received dietetic, but not insulin treatment, the blood diastase was almost invariably high. There also appeared to be some relationship between blood sugar and blood diastase. When insulin treatment was resorted to, however, normal or even sub-normal diastase values were almost always obtained. They concluded that diastase in the blood fulfills no function in carbohydrate metabolism, but when recalled into the liver, it may exert its function with regard to the glycogen-glucose transformation. Insulin appeared to play some part in the transfer of the enzyme from the blood to the liver.

Carlson and Lockhardt ('08), in studies on dogs, cats, pigeons and chickens, made a comparison of the diastases in the blood and body fluids. They reported the following results: (a) pancreatetomized cats had no increase or decrease in serum amylase content; (b) stimulation of the central ends of the transected vagi caused an increase in liver glycogen and blood sugar; (c) slight increase in diastatic activity was observed but not in proportion to the hyperglycemia produced; (d) there was no correlation between the concentration of blood diastase and carbohydrate intake; (e) there was no apparent correlation between blood diastase and oxidation in the body. They concluded
that blood diastase was "discarded" from the body tissues and served no starch-splitting function in the blood.

Hokin ('56) did studies on the formation of amylase by mouse pancreas in vitro. He found that in the unsliced pancreas of control mice, secretion (active extrusion) of amylase occurred during incubation. In the pancreas of normal mice, the rate of spontaneous secretion of amylase was approximately equal to the rate of synthesis. In the pancreas of fasted mice, both the rate of amylase synthesis and the rate of spontaneous secretion were similar to the rates in fed animals, which suggested that in mouse pancreas secretion and syntheses of enzymes were carried on spontaneously in the absence of stimuli (either by feeding or by parasympathetic activity).

Davis and Ross ('21) performed experiments in which they attempted to find the source of blood diastase and got the following results: (a) removal of the pancreas decreased blood diastase markedly; (b) ether anesthesia had no effect on the diastase of normal, partially pancreatectomized, or totally pancreatectomized dogs; (c) chloroform produced a marked fall in the blood diastase of normal dogs, but did not produce any appreciable change in the diastase of animals depancreatized partially or totally.

Otten and Galloway ('09) found that blood diastase fell sharply following pancreatectomy and decreased gradually for several days. It then increased to a sub-normal level and remained constant. It was concluded that the intact pancreas was active in the production of serum amylase, but there must have been another site, e.g., the liver, in order to account for the gradual increase in the diastase content following pancreatectomy.

Reid, Quigley and Myers ('33), in their work on dogs, found that following pancreatectomy blood diastase decreased during the first few days after
the operation, but returned to the preoperational level, or above it, if the diabetic condition was not controlled by insulin. Cessation of insulin caused a decrease in the blood diastase with a subsequent return to normal when insulin injections were resumed. In normal dogs, insulin caused a fall in blood diastase, a rise in liver diastase and no change in muscle diastase.

Wiberg ('55) investigated the contribution by various tissues to serum amylase activity in the rat and obtained the following results: (a) the pancreas appeared to furnish a significant amount as indicated by parallel decreases in pancreatic and serum amylase content associated with fasting and partial pancreatectomy; (b) high dietary concentration of starch and sucrose caused no parallel increase in serum amylase content; and (c) decrease in serum amylase levels and mucosal levels followed fasting and partial pancreatectomy which indicated the mucosa as a possible source of serum amylase.

Morris ('43) performed experiments with human blood samples in which he showed that blood diastase differed from all other diastases in that it hydrolyzed both starch and glycogen at nearly the same rate.

Glock ('38) reported that human liver amylase gave results entirely comparable with other amylases, e.g., salivary, pancreatic etc.

Somogyi ('38) made a statistical study of nearly 6,000 diastase determinations of patients and observed that a general trend toward lowered blood diastase was a sign of disturbances in the liver.

Hokin ('51) investigated the synthesis and secretion of amylase by pigeon pancreas in vitro, and reported that amylase content of pancreatic tissue was reduced by feeding the pigeons and injecting them with carbamylcholine prior to pancreatectomy. An increase in the total amylase activity occurred when
pancreas slices were incubated in media which contained glucose. The increase in total amylase activity was greatest in normal saline which contained amino acids, least in normal saline without amino acids, and intermediate in serum.
CHAPTER III

MATERIALS AND METHODS

The animals used in this investigation were adult male albino rats of the Sherman strain obtained from Rockland Farms, New City, New York. The animals were kept in the laboratory for three weeks in order to let them become acclimated to the new environment. They were kept in mesh wire cages and fed on regular laboratory diet. In preparation for experimental procedure, they were arranged into 4 groups, each of which was composed of 6 rats. The first group was treated with alloxan in order to induce damage to the insulin-producing cells (beta cells) of the pancreas, thereby causing diabetes. The second group was treated with carbon tetrachloride in order to induce liver damage. The third group was treated with both alloxan and carbon tetrachloride in order to cause damage to the pancreas and liver. The fourth group was the control group and remained untreated.

The alloxan was dissolved in 0.9% saline solution (50 mg./ml.), and administered in the amount of 170 mg./kg. of body weight. A 40% CCl₄ solution was made by dilution with sesame oil. The animals in group one were injected intraperitoneally with the alloxan solution and kept for 48 hrs. They were then anesthetized with Nembutal, after which, 5 ml. of blood were extracted by cardiac puncture. The animals in group two were injected subcutaneously in the mid-dorsal region between the scapulae and kept for 72 hrs. They were then anesthetized with Nembutal and 5 ml. of blood were extracted by cardiac puncture. The animals in group three were injected intraperitoneally with alloxan and, subcutaneously, with carbon tetrachloride and kept for 72 hrs. The alloxan was administered 24 hrs. after the injection of carbon tetrachloride. These animals were then anesthetized and 5 ml. of blood
collected from each.

The extracted blood was allowed to clot and synerise for 24 hrs. so that maximum serum could be extruded. It was then centrifuged at 5,000 rpm for 10 min. and the serum pipetted off. The serum was analyzed for amylase content by using the method outlined by Andersch ('46) which follows.

1. Five-tenths milliliter of serum was incubated at 37° to 38°C. for 15 min. using β-amylose starch as the substrate in the presence of a phosphate buffer solution (pH 6.8) and 5.7% sodium chloride.

2. Serum proteins were precipitated by the addition of barium hydroxide and zinc sulphate.

3. A second sample, which served as the blank, was prepared in the same manner as the above except that distilled water was substituted for the serum.

4. A third sample which contained unincubated serum was prepared by mixing distilled water, barium hydroxide and serum.

5. The three samples were centrifuged and two milliliters of liquid from each were placed into three correspondingly marked Folin sugar tubes.

6. Two milliliters of alkaline copper tartrate solution were added to each of the tubes and the samples were boiled in a water bath for 10 min.

7. The samples were then cooled under tap water for three minutes.

8. Two milliliters of arsenomolybdic color reagent were added to each of the tubes and the contents diluted to the 25 ml. mark with distilled water.

9. The samples were read on the colorimeter (Bausch and Lomb "Spectronic 20").
CHAPTER IV

EXPERIMENTAL RESULTS

In this investigation it was found that the normal serum amylase level was approximately three milligrams per 100 ml. of blood. In those animals which were injected with alloxan, thereby causing diabetes, the average value of serum amylase was 35.8 mg.% which was considerably higher than that of group 4, as indicated in Table 1. Those animals which had liver damage were invariably found to have a sub-normal serum amylase content (Av. = -14 mg. per 100 ml. of blood). The third group of experimentals, which had both liver and pancreatic damage, was found to have a serum amylase content of -47.3 mg. per 100 ml. of blood.
CHAPTER V

DISCUSSION

The preceding results would appear to agree with those reported by Myers and Killian ('17) and Reid and Myers ('32) in which diabetics always showed high serum amylase content. The hyperglycemia produced by the administration of alloxan was always accompanied by increased serum amylase. This tends to indicate that liver diastase, which during hyperglycemia is not needed, is "discarded" into the bloodstream. The fact that liver damage was invariably accompanied by decreased serum amylase, indicated that the liver was a site of serum amylase production.

It is also thought that glucagon, a hormone believed to be produced by the alpha cells of the pancreas, may have an indirect role in the increased serum amylase content during insulin insufficiency. Since the role of glucagon in the normal intact animal is opposite to that of insulin, it would seem that the two hormones might have an inhibitory or controlling effect upon each other. It is suggested that during insulin insufficiency the transformation of liver glycogen to glucose, due to the action of glucagon, would decrease the need for amylase in the liver. Thus, the excess amylase could be given off into the bloodstream. This theory is substantiated by the fact that insulin insufficiency with simultaneous liver damage produced no increase in serum amylase content.
CHAPTER VI
SUMMARY AND CONCLUSIONS

Colorimetric determinations of serum amylase were made on blood samples obtained from white Sherman rats in which pancreatic and liver damage had been induced. On the bases of these tests, the following conclusions were drawn.

1. Normal serum amylase level in the rat is approximately three milligrams per 100 ml. of blood.

2. There was a simultaneous increase in serum amylase content with induced diabetes.

3. There was a decrease in serum amylase with liver damage.

4. There was also a decrease in serum amylase with simultaneous liver and pancreatic damage.

5. An increase in blood sugar brought about release of amylase from the liver into the blood stream.

6. The pancreatic hormone, glucagon, possibly played an indirect role in the increase of serum amylase content during diabetes.
LITERATURE CITED


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<tr>
<th>Group I</th>
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| Av. 35.8 | Av. -14 | Av. -47.3 | Av. 3.5 |

Adult male Sherman albino rats injected intraperitoneally with alloxan and subcutaneously with \( \text{CCl}_4 \). Amylase content was measured in serum collected from blood obtained by cardiac puncture. Values listed in table above represent milligrams of amylase per 100 ml. of blood.