Blood, Urine, and Tissue Amylase in Depancreatized Rats. (21291)

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Amylase has been reported to occur in all tissues(1) and fluids(2) of animals which have been examined. The widespread occurrence of amylase in the body led to many early studies (3,4) on the site of production of the enzyme. Most of these studies concerned the pancreas, parotid glands, or the liver as the source of blood and urinary amylase. While it has been demonstrated that ligation of the ducts or experimental injury to the pancreas can produce an increase in the level of amylase in the blood and in its excretion in the urine, there is considerable disagreement on the effect of removal of the gland. Some workers, (5,6) have reported an increase in blood amylase, others(4,7) a decrease, and still others(2,8,9) no change following removal of the pancreas. The picture is further complicated by reports(10,11) that there is an initial decrease with a later irregular, but definite, return of blood amylase to control levels. However, the majority of investigators agree that amylase does not disappear from the serum as a result of complete pancreatectomy. Somogyi(12) reported that humans with various impairments in liver function exhibited subnormal amylase levels. This has been confirmed by others(13). In partially depancreatized dogs treated with chloroform (14) there is a distinct decrease in the level of blood amylase. Also low amylase values have been reported in acute alcoholism(15), a condition usually accompanied by liver damage. While the studies mentioned above suggest that the liver may be an important source of blood amylase, Somogyi(16) has reported that all the amylase in the liver is in the extracellular fluid and that the hepatic cells contain no amylase. However, unpublished studies (17) in this laboratory have shown that under certain conditions the liver may contain from 5 to 10 times as much amylase activity as can be accounted for by the blood in the organ.

A technic for complete pancreatectomy in the rat has been developed in this laboratory (18). In view of the lack of agreement among the studies on the origin and significance of blood and urinary amylase it seemed desirable to determine what changes occurred, if any, following pancreatectomy in the rat. The normal rat has a relatively high blood level and 24-hour urinary excretion of amylase, and has not been previously studied in this regard.

Methods. Adult rats of both sexes were housed in individual metabolism cages arranged for the quantitative collection of urine. All animals received the low residue diet, previously described(18), throughout the period of observation. The care of the animals and the operative technic have been described(18). The urine and blood samples were collected successively as follows: urine collected from 4 P.M. to 3 P.M. of succeeding day; food then removed and blood samples (0.1 ml of tail blood) collected 9-11 A.M. following morning. Food was then returned to the cage. The animals were sacrificed and tissue samples taken on the 30th day immediately after a fasting blood sample had been ob-

TABLE I. Amylase Levels in 8 Depancreatized Rats.

	Urine, unfasted		Blood, fasted	
	Mean	± S. D.	Mean	± S. D.
	Preoperative			
Controls*				
A	943†	379		
B	830	428	1386	249
C	716	334	1536	498
	Postoperative			
Days‡				
2, 3	764	364		
4, 5	827	279	1294	227
9, 10	891	355	1418	390
14, 15	1097	328	1379	425
19, 20	883	328	1493	364
24, 25	1118	357	1449	395
29, 30	1113	403	1765	623

* See text.

† Amylase units (19)/24 hr for urine; per 100 ml for blood.

‡ 1st number indicates day of urine collection; 2nd, day of blood collection.

TABLE II. Tissue Amylase Levels in Fasted Rats.

Tissue	Unoperated Mean*	± S. D.	Depancreatized Mean*	± S.D.
Liver	30.1†	4.7	20.6	5.1
Kidney	7.3	1.3	6.0	1.2
Heart	6.8	2.8	4.3	1.0
Muscle	1.0	2.2	.9	.5
Duodenum	26.7	6.9	5.0	1.8
Jejunum	9.5	7.3	4.2	2.3
Spleen	4.5	5.2	7.4	11.0
Pancreas	25799.0	6848.0	53.7	35.8

* 8 animals.
† Amylase units (19)/g wet tissue.

tained. Unoperated control animals were run in parallel and tissue samples taken for comparison with the operated animals. Prior to removal of the pancreas three 24-hour urine samples and 2 blood samples were taken at 5-day intervals as described above and were used as control values for the operated animals. The levels of amylase activity in the blood, urine, and tissue samples were determined by a micromodification of the method of Smith and Roe(19). The data in the tables are given in terms of the "amylase unit" which is defined(19) as the amount of enzyme which will hydrolyze 10 mg of starch in 30 minutes to a stage at which no color is given with iodine at 620 mμ, under the conditions of the procedure.

Results. The data are shown in Tables I and II. Control levels for blood and urine and the tissue levels in unoperated rats agree closely with comparable data on normal rats obtained by Smith(17). Removal of the pancreas did not produce significant increase or decrease in the level of blood amylase or in the 24-hour excretion, either immediately or for 30 days following the operation. The absence of a change in urinary excretion after pancreatectomy is especially significant since it is usually assumed that the level of urinary amylase parallels the amylase activity of the pancreas. Kidney, heart, muscle, and spleen of depancreatized animals had essentially the same levels of amylase activity as normal rats. Sections of the small intestine in the depancreatized rats exhibited a definite decrease in amylase activity as would be expected. The residual amylase in the small intestine may be derived from the salivary glands. The pancreatic tissue indicated in Table II as analyzed for amylase in the depancreatized rats was the fragments of mesentery which normally contain the pancreas. The amylase activity of the liver was lower in the depancreatized rats than in the normal controls. This decrease can not be explained on the basis that liver amylase is in the blood contained in the organ inasmuch as the blood level was the same in the 2 types of animals. It is possible that a part of the liver amylase in the normal rat is derived from the pancreas or, more likely, that the depancreatized animals had the fatty livers, characteristic of such animals; this would lower the amylase level when calculated on a weight basis.

The finding that the amylase activity in the blood, urine, and tissues (except the liver and G.I. tract) of the rat is maintained at the same levels after pancreatectomy as before suggests that in the normal rat the pancreas is not an important source for the enzyme occurring in these fluids and tissues or that in response to removal of the pancreas other factors, *i.e.*, increased extrapancreatic production or diminished rate of destruction, compensate for the loss and thereby maintain the normal levels. The latter possibilities can not be evaluated at the present time but appear unlikely. These suggestions apply only to the role of the pancreas in the *normal* rat for, as reviewed above, the pancreas is an important source of the enzyme in blood and urine in conditions where the levels are elevated above normal. There is no conclusive evidence(16) that other tissues than the pancreas and parotid glands produce amylase. The literature(16) indicates that normally blood amylase does not originate either directly or indirectly in the salivary glands. However, during the 30-day postoperative period of observation each of the depancreatized rats excreted approximately 30,000 units of activity. From this it seems clear that some other organ or organs can produce appreciable quantities of amylase.

Summary. The levels of amylase in the blood, urine, and tissues of the rat were not significantly altered by pancreatectomy. It is suggested that some organ or organs, other than the pancreas, is the principal source of

blood, urinary, and tissue amylase in normal rats.

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Simultaneous Use of I^{131}-Albumin and Cr^{51}-Labelled Red Cells in Blood Volume Studies in the Goat. (21292)

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Several methods have been used to determine red cell and plasma volumes in various animals simultaneously(1-8). T-1824 dye and I^{131}-albumin are generally used for plasma labels. Frank and Gray(9) have recently reported the use of Cr^{51}-labelled plasma. Radioisotopes of P, Cr, and Fe are generally used for red cell labels. In many experiments here it was desirable to obtain plasma and red cell volumes simultaneously using labels which were available for other volume studies and which readily permitted several successive determinations. Small samples of blood for assay were also desired to reduce the blood loss due to frequent sampling. Most procedures require a considerable sampling loss where several volume determinations are made. The method of Gamble *et al.*(1), using I^{131} and P^{32}, although successful in the dog, was not used in the goat since goat red cells did not retain the phosphate.

In the method described below I^{131}-albumin and Cr^{51}-labelled red cells were used simultaneously. Hematocrit tubes were used for counting containers. Plasma was separated by centrifugation of blood and counted separately. The red cells were partially separated and counted. By correcting the cell plus plasma counts for the plasma counts, the red cell counts were obtained. The use of the hematocrit tube facilitates the separation of plasma and cells and requires no more than 1 cc of blood per sample. Volume determinations were made on normal goats using this method. Two *in vitro* recovery experiments were also performed.

Methods. Samples of blood were drawn from goats and the red cells were labelled with $Na_2Cr^{51}O_4$ as described by Gray and Frank (3). I^{131}-human serum albumin (Abbott) was diluted in isotonic saline to provide 0.5-1.0 μc/cc solutions. Wintrobe "EXAX" (Kimble Glass) hematocrit tubes were calibrated and used as containers for samples assayed for ra-