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Radioimmunoassay of Serum Pancreatic Amylase in Normal and Pancreatectomized Pigs[1] (38504)

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Serum amylase levels are influenced by the pancreas, salivary glands and the liver (1). Their individual contributions, however, have been determined only qualitatively. To date no physiological or biochemical technique can provide a quantitative analysis of the serum amylase isoenzymes.

In this investigation we sought to develop a radioimmunoassay specific for porcine pancreatic amylase in order to determine the concentration of the enzyme in normal pig serum. Pancreatectomies were performed to evaluate the specificity of the assay and to further define the contribution of the pancreas to the normally circulating amylase levels.

Methods. Antibody production. Four random-bred white, New Zealand rabbits were immunized with porcine pancreatic amylase (Calbiochem) containing 37 mg of protein (25,900 amylase units) per ml. Each rabbit received an initial subcutaneous injection of 10 mg of amylase mixed with Freund's complete adjuvant. After 2 mo each rabbit was reimmunized with a 3 mg preparation. Twelve weeks from the time of the initial injection, the rabbits were exsanquinated by drawing blood from the ear vein under negative pressure. The serum was collected, divided into 1 ml aliquots and frozen at $-10°$. The concentration of antibody in the rabbit serum was determined according to the method of Campbell (2).

Antigen labeling and purification. Commercial porcine pancreatic amylase also served as the source of the standard and the tracer antigen. Radioiodination was achieved by labeling the enzyme with ^{125}I (New England Nuclear) as developed by Hunter and Greenwood (3). Purification of the labeled enzyme was accomplished by immediately transferring the mixture to a Sephadex G-50 (Pharmacia Fine Chemicals) column (1 cm × 35 cm).

Conditions of the assay. The radioimmunoassay developed was a modification of Morgan and Lazarow's double antibody technique (4). All dilutions of amylase antiserum, normal rabbit serum and enzyme were made in 0.05 *M* sodium borate at pH 7.4, containing 5% bovine serum albumin with merthiolate (1/1000) as a preservative. All incubations were performed at 4°. Figure 1 outlines the conditions which permitted maximal precipitation of the antigen–antibody complex. In the first reaction, unlabeled antigen (1.0 ml of standard or unknown), ^{125}I-labeled antigen (0.1 ml containing 0.5 μg) and diluted antiserum (0.1 ml, 1/100 dilution) were mixed and incubated for 24 hr. In the second reaction, goat anti-rabbit globulin (Miles Laboratories, 0.1 ml) and normal rabbit serum (0.1 ml, 1/100 dilution) were added to the reaction mixture and the incubation continued an additional 24 hr. Upon completion of the incubation, the free and the bound antigen were separated by centrifugation at 1000 *g* for 20 min. Each tube was then counted for 1 min in a Packard Auto Gamma Counter. The percent binding of the tracer antigen was expressed as the number of counts in the precipitate divided by the combined counts in the supernatant and precipitate multiplied by 100.

Sensitivity and specificity of the assay. The sensitivity of the assay was determined by varying the concentration of the standard amylase and measuring the amount of tracer antigen precipitated. The concentration of amylase in the standard solution ranged from 0.1 to 24.0 μg/ml.

The ability of the antibody to combine with porcine elastase, lipase, trypsin, carboxypeptidase B and canine pancreatic amylase was also examined. The porcine

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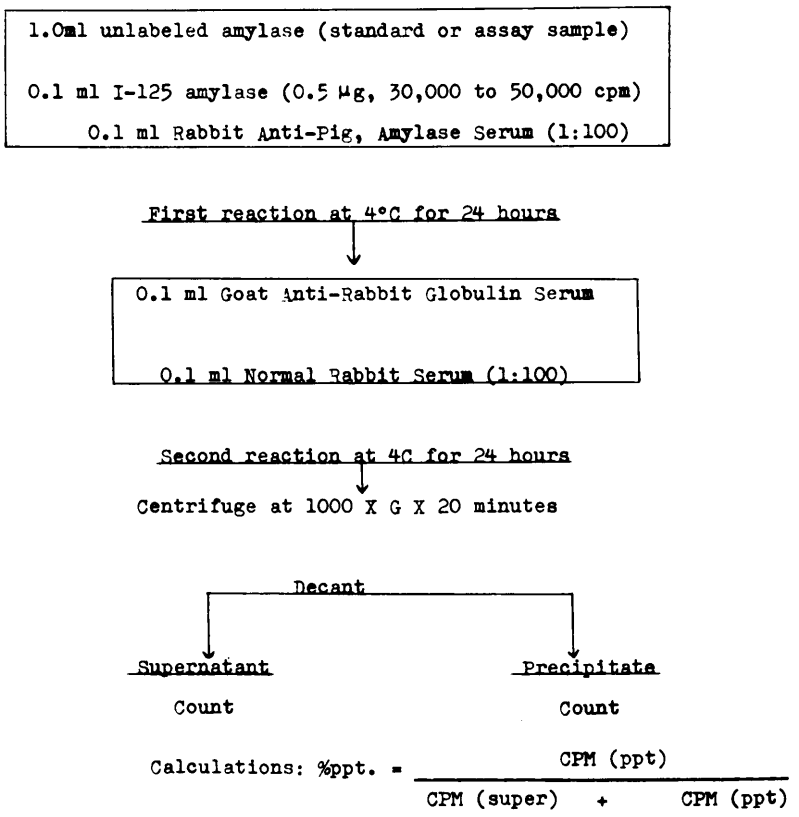

FIG. 1. Outline of the double antibody radioimmunoassay procedure for porcine pancreatic amylase.

enzymes were obtained commercially (Calbiochem) while the canine amylase was purified using the method of Berndt (5). Solutions of the enzyme were made up in serial dilutions ranging from 0.1 to 24.0 µg/ml.

Serum analysis. Serum from 10 healthy adult pigs was diluted with sodium borate (0.5 and 0.2) and assayed for the presence of pancreatic amylase.

Five pigs weighing 25–40 kg were pancreatectomized under aseptic conditions and blood samples taken at daily intervals for 7 days. A control blood sample was drawn prior to the surgery. All blood samples were analysed for amylase enzyme activity (6) and pancreatic amylase. Blood glucose levels were controlled by daily injections of insulin.

Results. The antibody concentration in the four rabbits injected ranged from 0.5 to 3.0 mg/ml of serum. The rabbit serum having the highest antibody concentration was used for the development of the assay.

The standard curve obtained using unlabeled amylase concentrations ranging from 0.1 to 24.0 µg/ml demonstrated an assay sensitivity of approximately 0.5 µg/ml (Fig. 2). When assaying unknown samples, only those values which fell between 0.5 and 5.0 µg/ml were recorded. Concentrations not within this range resulted in the plateauing of the curve with a decrease in the reliability of the measurement. All such samples were corrected for their proper dilution and reanalysed by radioimmunoassay.

The assay gave a suitable working range compatible with the concentration of pancreatic amylase found in the serum. The mean normal serum concentration of pancreatic amylase in the 10 pigs sampled was 2.4 µg/ml (S.E. ± 0.8).

The cross reactivity of the antibody to the remaining porcine pancreatic enzymes and to canine pancreatic amylase is presented in Table I. With the exception of the porcine pancreatic amylase the amount of tracer antigen bound to antibody was independent

of the concentration of the unlabeled antigen.

Table II shows the effect of pancreatectomy on the serum amylase enzyme activity. Within the first 24 hr, the enzyme activity decreased 46% from a mean of 3100 units/100 ml to a mean of 1680 units/100 ml. After 48 hr, a significant decrease ($P < 0.05$) of almost 60% was recorded. For the duration of the study the serum enzyme activity remained relatively constant.

The decline in serum amylase enzyme activity was reflected by a simultaneous decrease in the amount of radioimmunoassayable amylase in the circulation (Table II). Serum pancreatic amylase declined 38% in the first 24 hr from a mean of 2.4 μg/ml to a mean of 1.5 μg/ml. After 48 hr, the pancreatic amylase in the circulation had been completely removed.

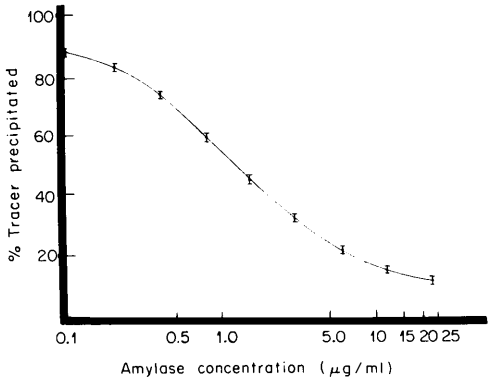

FIG. 2. Standard curve for porcine pancreatic amylase radioimmunoassay. All parameters were those previously determined to give maximal precipitation of the antigen–antibody complex. Each point represents the mean and standard error of 12 determinations.

Two of the pigs were maintained for 3 weeks and the serum analysed daily for changes in amylase enzyme activity and pancreatic amylase concentration. Essentially the same results were observed on day 21 as were recorded 48 hr following pancreatectomy. The concentration of pancreatic amylase remained at zero levels and the serum enzyme activity remained 60% below control values.

Discussion. Although antibodies have been produced against a number of enzymes (7, 8), relatively little work has been done on the possible use of radioimmunoassay in measuring serum enzyme concentrations. Our study indicates that a radioimmunoassay can be applied to the measurement of pancreatic amylase in the circulation. The assay appears to be quite sensitive and very specific.

No cross reactivity of the antibody could be demonstrated to the remaining porcine pancreatic enzymes or to canine pancreatic amylase. Moreover, following pancreatectomy, there was no evidence of cross reactivity with the remaining serum amylase isoenzymes.

The specificity of the assay is dependent upon the antigenic properties of the enzyme and is different from enzymatic specificity which relies on the affinity of the enzyme for

TABLE I. CROSS-REACTIVITY OF THE ANTIBODY TO THE OTHER PORCINE PANCREATIC ENZYMES AND TO CANINE PANCREATIC AMYLASE.

Concentration μg/ml	% Binding of tracer antigen					
	Amylase (pig)	Elastase	Lipase	Trypsin	Carboxy-peptidase B	Amylase (canine)
24	10.7	83.0	85.4	86.1	85.1	86.2
12	15.4	81.4	85.6	85.2	85.5	85.8
6	18.6	85.5	86.3	86.5	86.5	86.0
3	30.0	85.7	86.5	85.6	88.0	84.0
1.5	49.0	85.0	85.2	86.1	86.2	85.4
0.8	61.3	87.0	86.2	86.2	86.6	86.2
0.4	78.4	86.5	86.4	86.0	86.4	85.0
0.2	85.5	86.7	85.9	86.3	86.2	85.5
0.1	86.3	86.3	85.0	84.5	84.3	84.7
0.0	86.8	86.3	85.7	85.1	85.6	85.3

TABLE II. EFFECT OF PANCREATECTOMY ON SERUM PANCREATIC AMYLASE CONCENTRATION AND SERUM AMYLASE ENZYME ACTIVITY IN PIGS[a].

Days after pancreatectomy	Serum amylase	
	Concentration (μg/ml)	Enzyme activity (units %)
0	2.4 ± 0.4	3100 ± 564
1	1.5 ± 0.2	1680 ± 241
2	0.0 ± 0.0	1253 ± 220
3	0.0 ± 0.0	1204 ± 193
4	0.0 ± 0.0	1210 ± 192
5	0.0 ± 0.0	1214 ± 182
6	0.0 ± 0.0	1222 ± 183
7	0.0 ± 0.0	1212 ± 193

[a] Values represent the means and standard errors of five pigs.

its substrate. This factor, immunological specificity, permits the determination of the enzyme even when it is present in an environment that contains several enzymes exhibiting similar biochemical activity. Such a situation exists for the serum amylase isoenzymes.

Studies on normal pig serum indicate the presence of an amylase capable of reacting with the antibodies specific for porcine pancreatic amylase. This is important because although it has been known for quite some time that the serum contains several amylase isoenzymes, the exact contribution of the pancreas has been a matter of controversy (9).

A pancreatic influence on serum amylase levels was further emphasized by the significant decline in enzyme activity that occurred following pancreatectomy. Within 48 hr, a 60% decrease in the serum amylase enzyme activity could be recorded. This loss in enzyme activity was reflected by a simultaneous decrease in the serum concentration of pancreatic amylase. After 48 hr, pancreatic amylase was no longer measurable in the serum. Thus the pancreatectomy experiments demonstrated two points: (1) they indicated that the pancreas contributes significantly to the serum amylase enzyme activity of pigs under normal conditions, and (2) they demonstrated the antibodies developed were specific for pancreatic amylase and had no apparent reaction with the circulating amylase isoenzymes of extrapancreatic origin. The development of a radioimmunoassay specific for pancreatic amylase offers, for the first time, a quantitative analysis of one of the serum isoamylases.

Summary. A double antibody radioimmunoassay has been developed that is capable of assaying and discriminating small amounts of pancreatic amylase even in the presence of amylase isoenzymes. Assays on adult pigs indicate a mean serum concentration of pancreatic amylase of 2.4 μg/ml. To date, no other technique can offer such a discrimination of the pancreatic amylase in the circulation. Following pancreatectomy, the amount of radioimmunoassayable amylase in the circulation disappeared within 48 hr. This was reflected by a simultaneous decline in the serum amylase enzyme activity. Within 48 hr after pancreatectomy, the serum amylase enzyme activity had declined 60%. Thus, in pigs, the pancreas contributes significantly to the circulating amylase levels.

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