PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 921–925 (1975)

Circulatory Turnover of Pancreatic Amylase[1] (38927)

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(Introduced by R. H. Davis)

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Serum amylase enzyme activities remain remarkably constant under normal conditions, suggesting an efficient balance between liberation of the enzyme from its tissue sources and its elimination from blood. Several investigators have attempted to define the disappearance kinetics of the pancreatic amylase present in serum (1–5). Their studies produced variable findings and have led to considerable confusion concerning the circulatory fate of the enzyme.

In this investigation, we examined the plasma disappearance of iv infused radioiodinated amylase and unlabeled porcine pancreatic amylase into pigs. In the latter studies, we followed the decline in plasma enzyme activity and enzyme concentration. Enzyme concentrations were determined using a radioimmunoassay developed by our laboratory specifically for that purpose (6). Such a technique also allowed quantitation of the pancreatic amylase normally present in the circulation. This information, plus a knowledge of the plasma half-life of amylase enables determination of the circulatory turnover of the enzyme.

Methods. Animal preparation. Eighteen adult pigs of both sexes were anesthetized with sodium pentobarbital (Nembutal, 25 mg/kg) and a catheter placed in the left jugular vein to facilitate the infusion of saline or amylase and the withdrawal of blood samples. Plasma volume was determined following the dye dilution method described by Chinard (7).

Labeled amylase studies. Eight pigs received a 30-sec iv infusion of radioiodinated amylase of known activity (CPM). The enzyme was labeled with ^{125}I (New England Nuclear) using the chloramine T method (8).

Following infusion of the radioiodinated enzyme 5 ml blood samples were drawn at 5, 30, and 60 min postinfusion and then once every hour over a 4-hr period. Immediately upon collection, the blood samples were centrifuged and the plasma separated from the red blood cells. The cells were washed two times with normal saline and resuspended in saline to a final volume of 2 ml.

The radioactivity in a 2-ml sample of plasma and red blood cell suspension was counted for 2 min in a Baird Atomic Well Type Scintillation Counter. All values were corrected for background and converted into cpm/ml of sample. The percentage of initial radioactivity remaining in the plasma was calculated using the formula:

$$\% \text{ Recovery} = \text{cpm/ml} \times \frac{\text{Plasma Volume} \times 100.}{\text{Total cpm Injected}}$$

Unlabeled amylase studies. Ten pigs received a 30-sec iv infusion of unlabeled porcine pancreatic amylase (Calbiochem) of known enzyme activity and concentration. A 5-ml blood sample was drawn at 5, 30, and 60 min postinfusion and then once an hour for 4 hr. The red blood cells were separated from the plasma and resuspended in saline.

Plasma and red blood cell amylase enzyme activities were measured using the Caraway method (9). All samples were corrected for "baseline" enzyme activities determined prior to infusion.

The amount of pancreatic amylase present in the plasma was determined using a radioimmunoassay previously shown to be adequately sensitive and quite specific for porcine pancreatic amylase. All samples were

[1] Supported by Grant AM02723 from The National Institute of Arthritis and Metabolic Diseases.

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corrected for "baseline" concentrations of amylase.

Data analysis. The percentage of amylase activity (concentration) remaining in the plasma was plotted against time upon semilogarithmic graph paper. Following the graphic method of Robertson (10), a straight line was drawn through the linear portion of the curve and extrapolated to time zero. The extrapolated values were subtracted graphically from the remaining points on the nonlinear portion of the curve. This process was repeated until a single monoexponential curve was obtained. In all instances, the amylase removal curves could be reduced to three exponential components.

Results. Labeled amylase studies. The plasma disappearance of radioiodinated amylase is presented in Fig. 1. There was essentially complete recovery of radioactivity when measured 5 min after infusion. Following an initial rapid decline in radioactivity, the remaining portion of the plasma curve could be described by a single monoexponential line. Thus, indicating the radioactivity was being lost at a rate proportional to its activity in the circulation. No radioactivity was found associated with the red blood cells.

Analysis of the original disappearance curves indicated they were actually composed of three individual components each with its own characteristic half-life (Table I).

Unlabeled amylase studies. The disappearance of amylase enzyme activity from the plasma is shown in Fig. 2. Approximately 95% of the initial activity could be accounted for 5 min after infusion. The disappearance curve closely paralleled the radiolabeled amylase removal curve. There was an initial rapid loss in enzyme activity followed by a slower monoexponential decline. No amylase enzyme activity could be detected in any of the blood cell samples. Analysis of the plasma curves resulted in the determination of three separate component curves (Table II).

The circulatory removal of the enzyme as determined by radioimmunoassay is presented in Fig. 3. The disappearance curve was almost identical to the plasma radioactivity and enzyme activity removal curves. No radioimmunoassayable amylase was detected in the blood cell samples. Upon examination, a triphasic curve could be extrapolated from the initial plasma activity curves (Table III). The concentration

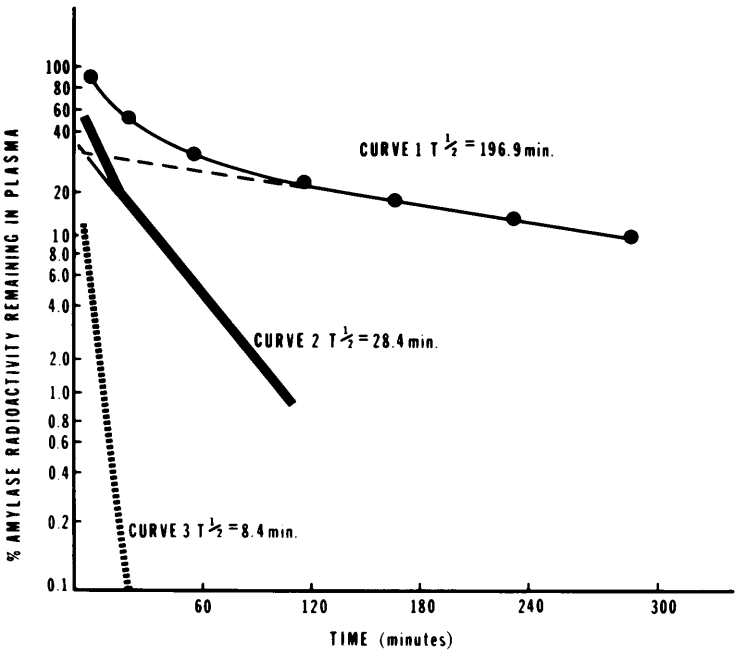

FIG. 1. Analysis of Plasma Radioactivity Curve. Each point represents the mean of eight determinations. A triphasic curve can be constructed from the initial plasma curve, each with a characteristic half-life.

of pancreatic amylase in the plasma prior to infusion was 2.4 μg/ml (SE ± 0.8).

Discussion. The plasma disappearance curves obtained in this investigation can be resolved into three components. Such findings are not unusual and have been reported for a number of enzymes (11–13). The initial rapid fall in amylase activity (concentration) probably represents its distribution between the fast and slow mixing components of the circulatory system. This effect is also seen in studies involving the major protein components of the plasma such as albumin (16). Minutes after labeled albumin is injected into the circulation there is a decline in its activity which can not be accounted for solely on the basis of its circulatory half-life or its loss into extravascular compartments. The second component involved in the removal of an enzyme from the circulation represents its diffusion into and equilibrium with the extravascular fluid spaces (11, 14). Turnover of the enzyme within the circulation, either via tissue catabolism or renal excretion, is represented by the slow-component half-life. The slow component half-lives of this investigation showed no significant differences regardless of the parameter measured. This is important for two considerations. First, it demonstrates that tracer studies involving radioiodinated amylase can give valid results despite the fact iodination renders the enzyme biologically inactive. Secondly, it indicates the decline in plasma enzyme activity is not due to the intravascular inhibition or degradation of the amylase molecule. If such a phenomenon was occurring there would be a noticeable difference in the shapes of the plasma curves representing enzyme ac-

TABLE I. ANALYSIS OF PLASMA ACTIVITY CURVES FOLLOWING IV INFUSION OF ^{125}I-AMYLASE

Pig No.	Half-life (minutes) Curve 1	Curve 2	Curve 3
1	190	33	7
2	162	27	8
3	120	36	12
4	210	24	8
5	200	36	9
6	240	24	8
7	200	20	8
8	210	28	8
Mean	196.9	28.4	8.4
SE	±12.5	±1.9	±0.5

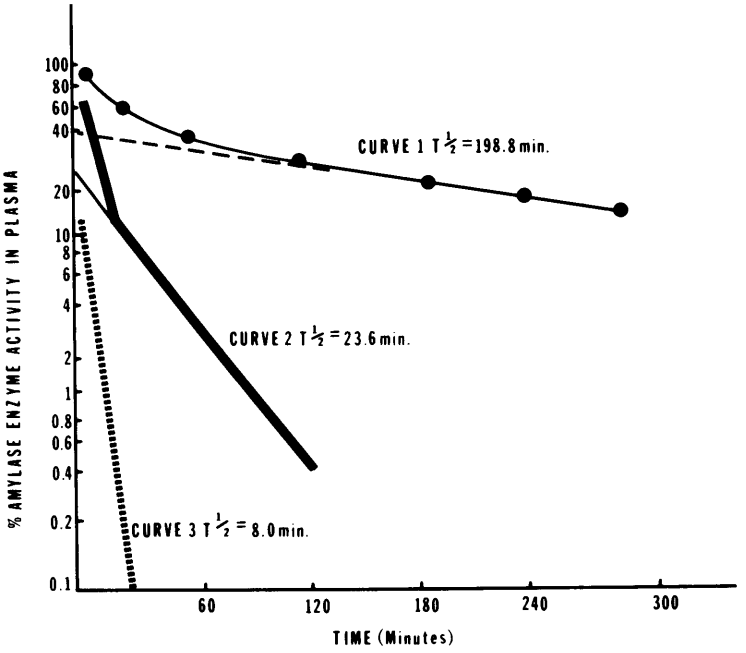

FIG. 2. Analysis of Plasma Enzyme Activity Curve. Each point represents the mean of five determinations. A triphasic curve can be constructed from the initial plasma activity curve.

tivity and enzyme concentration. The similarity of the two curves can be interpreted as indicating the decline in enzyme activity is due to the actual removal of amylase from the circulation. The mechanisms responsible for the plasma removal of amylase are not evident from this study. However, the liver and the reticuloendothelial system are possible sites since each has been implicated in the catabolism of circulating proteins such as albumin (14) and lactic dehydrogenase (15).

The plasma half-life of amylase in these experiments was 3–3.5 hr. This is in good agreement with results reported by Appert and his associates (1, 2) following iv infusion of pancreatic juice into dogs. Unlike Appert's findings, however, almost 100% of the administered enzyme activity could be accounted for in the plasma 5 min after infusion.

TABLE II. ANALYSIS OF PLASMA ENZYME ACTIVITY CURVES FOLLOWING IV INFUSION OF AMYLASE.

	Half-life (minutes)		
Pig No.	Curve 1	Curve 2	Curve 3
1	198	20	9
2	174	30	8
3	192	20	6
4	210	24	8
5	220	24	9
Mean	198.8	23.6	8.0
SE	±7.9	±1.8	±0.5

Duane and his co-workers (3) also reported almost complete recovery of enzyme activity 5 min after the infusion of pancreatic amylase into baboons. They described a monoexponential disappearance curve with a half-life of 83 min for the plasma removal of amylase. This type of curve is unusual in that most plasma removal curves are multiexponential (13). Since amylase has a molecular weight comparable to many of the previously investigated enzymes, it seems reasonable to assume it should be subject to the same distribution processes.

Reports by Hiatt and his associates (4, 5) indicate an initial binding of the enzyme by the red blood cells. This study does not substantiate that claim. Regardless of the parameter measured, no amylase could be detected in the red cell suspensions. It cannot be argued that washing the cells accounted for the differences in results since

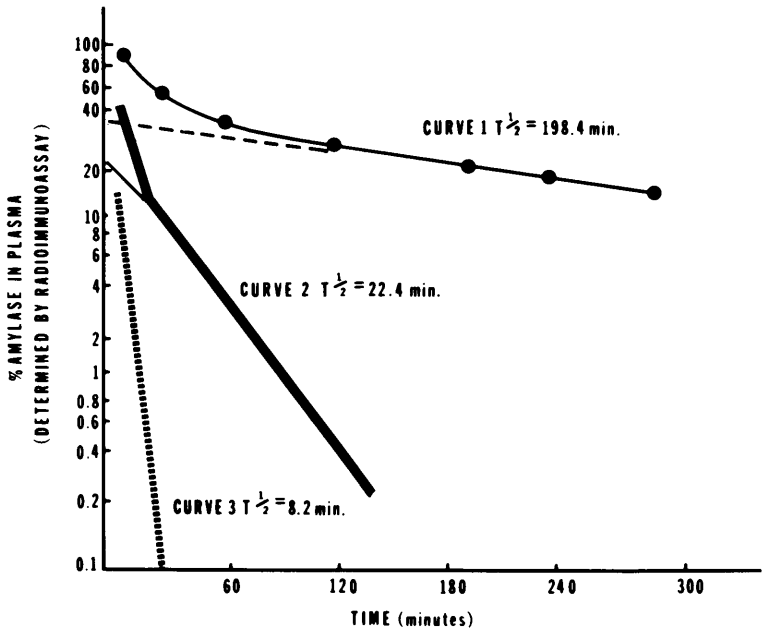

FIG. 3. Analysis of Plasma Enzyme Concentration Curve. Amylase concentrations were determined by radioimmunoassay. Each point represents the mean of five studies. A triphasic curve can be constructed from the initial plasma curve.

TABLE III. ANALYSIS OF PLASMA ENZYME CONCENTRATION CURVES FOLLOWING IV INFUSION OF AMYLASE

Pig No.	Half-life (minutes) Curve 1	Curve 2	Curve 3
1	180	20	9
2	200	30	9
3	200	20	8
4	192	18	6
5	220	24	9
Mean	198.4	22.4	8.2
SE	±6.5	±2.1	±0.6

they also washed the red cells in their experiments.

One possible explanation for Hiatt's findings may involve the enzyme preparation he used. When freshly prepared canine amylase was the infusate it was purified according to the method of Caldwell (16) in which amylase is precipitated several times with an alcohol–ether mixture. Rossing and Jensen (17) have shown that alcohol isolated preparations of albumin are metabolized differently than salt fractionated albumin preparations due to partial denaturation of the protein. Conceivably partial denaturation of the amylase molecule could account for its preliminary attachment to the red cells.

The amount of amylase the pancreas contributes to the circulation can be calculated from a knowledge of the plasma concentration and the circulatory half-life of the enzyme. In this investigation, the plasma concentration of pancreatic amylase was 2.4 μg/ml with a half-life of 3 hr. Since in any 3-hr period 1.2 μg of amylase will be lost per milliliter of plasma, an equal amount must reenter the bloodstream to maintain the enzyme at constant levels. Over a 24-hr period this would require the replacement of 9.6 μg of amylase/ml of plasma.

Summary. Radiolabeled amylase and unlabeled pancreatic amylase were infused into pigs in order to determine the plasma half-life of the enzyme. Regardless of the parameter measured (radioactivity, enzyme activity or concentration), the plasma removal curves could be resolved into three components when subjected to tracer analysis. The plasma half-life was estimated to be approximately 3 hr. Through the use of a recently developed radioimmunoassay specific for porcine pancreatic amylase, the plasma concentration of amylase was calculated at 2.4 μg/ml. Knowing the plasma concentration and half-life of amylase we determined the circulatory turnover of the enzyme. Over a 2.4-hr period, 9.6 μg of pancreatic amylase/ml of plasma must reenter the circulation to maintain the enzyme at constant levels.

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Received January 22, 1975. P.S.E.B.M., 1975, Vol. 149.