Simultaneous Study of the Metabolic Turnover and Renal Excretion of Salivary Amylase-^{125}I and Pancreatic Amylase-^{131}I in the Baboon

WILLIAM C. DUANE, ROGER FRERICHS, and MICHAEL D. LEVITT

From the University of Minnesota Hospital, Medicine Department, Minneapolis, Minnesota 55455, and Hennepin County General Hospital, Minneapolis, Minnesota 55415

ABSTRACT The metabolic turnover of salivary and pancreatic amylase was studied in the baboon, an animal with a serum amylase level and renal clearance of amylase similar to man. Purified amylase was electrolytically iodinated. Although iodinated and uniodinated amylase had similar gel filtration, electrophoretic, enzymatic, glycogen precipitation characteristics, the labeled enzyme was cleared less rapidly by the kidney than was the unlabeled material. However, urinary iodinated amylase which had been biologically screened by the kidney had a renal clearance and serum disappearance rate indistinguishable from unlabeled amylase and thus can serve as a tracer in metabolic turnover studies. Administration of a mixture of salivary amylase-^{125}I and pancreatic amylase-^{131}I made it possible to simultaneously measure the serum disappearance and renal clearance of these two isoenzymes. The metabolic clearance of both isoenzymes was extremely rapid with half-times of about 130 min. This rapid turnover of serum amylase probably accounts for the transient nature of serum amylase elevation which frequently occurs in pancreatitis. Pancreatic amylase-^{131}I was consistently cleared more rapidly (mean clearance ratio: 1.8) by the kidney than was salivary amylase-^{125}I. This more rapid renal clearance of pancreatic amylase may help to explain the disproportionate elevation of urinary amylase relative to serum amylase observed in pancreatitis.

INTRODUCTION

Because measurements of serum and urine amylase levels are frequently used diagnostic tests, clear understanding of serum amylase removal mechanisms is of considerable importance.

Received for publication 1 November 1971 and in revised form 24 January 1972.

Interpretation of previous studies of the turnover of serum amylase are limited by the use of laboratory animals not comparable to man in regard to serum amylase levels and renal amylase clearance (1, 2), by the use of amylase heterologous to the animals studied (2, 3), and lastly, by the need to markedly elevate serum amylase levels in order to measure the removal rate of the unlabeled enzyme from the serum (1–4).

Study of amylase turnover at normal serum levels requires a labeled amylase that accurately traces the unlabeled enzyme. Previous tracer studies (3, 5) using amylase iodinated by the iodine-monochloride technique are of doubtful reliability since this iodination procedure alters the structure of the amylase molecule as evidenced by a marked loss of enzyme activity after iodination (6).

Recently, Katz and Bonorris (6) have shown that a minimal loss of enzyme activity occurred when hog pancreatic amylase was electrolytically iodinated by a technique developed by Rosa, Pennisi, Bianchi, Federighi, and Donato (7). In the present studies electrolytically iodinated baboon salivary and pancreatic amylase were used to study the turnover of amylase in the baboon, an animal which has a serum amylase level and a renal clearance of amylase similar to man (4). Preliminary studies were directed towards the development of an iodinated amylase that could accurately trace unlabeled amylase. This iodinated preparation was then used to elucidate serum amylase disappearance kinetics.

METHODS

Experimental animals. Six Kenya baboons were studied. Just before the study, the baboon was anesthetized with an i.m. injection of Sernylan[1] (1 mg/kg). Subsequently, light anesthesia was maintained with i.v. pentabarbitol.

[1] Parke, Davis & Co., Detroit, Mich.

Urine samples were obtained by urethral catheter and blood samples were obtained by femoral venous puncture.

Isolation of amylase. The glycogen precipitation technique of Loyter and Schramm (8) was used to isolate amylase from baboon parotid gland and pancreas as previously described (4). The purity of amylase was assessed by gel filtration and electrophoresis as described in a previous paper (4).

Iodination of amylase. Amylase was electrolytically iodinated using a technique and apparatus similar to that described by Rosa et al. (7). The basic iodination solution contained 0.05 M phosphate buffer (pH 7.4) and 0.10 M sodium chloride. Amylase to be iodinated was dialyzed against this buffer and then adjusted to a protein concentration between 0.60 and 1.20 mg/ml.

Preliminary studies were carried out to determine the most suitable iodination conditions (see Results). On the basis of these studies the following technique was employed. The molar iodide: protein ratio in the crucible solution was adjusted to 1:1 by the addition of potassium iodide. Carrier-free ^{125}I or ^{131}I was added in a concentration of 5 mCi/mg of protein. Electrolysis was carried out at 3°C for 40 min at a current density of 40–50 $\mu a/cm^2$. Before, during, and after electrolysis, 0.1 ml samples were removed from the crucible and analyzed for amylase activity, protein concentration, and protein-bound radioactivity.

The bulk of the free iodide was removed by dialysis. After dialysis, any residual free iodide was removed by gel filtration. The fractions containing amylase were pooled and mixed with 2 ml of baboon serum to minimize radiation damage.

Assay and chromatography techniques. Amylase was assayed by a saccharogenic technique in which the rate of formation of reducing groups from a 1.5% starch solution was measured using 3,5-dinitrosalicylic acid (9).

The Folin-Ciocalteau method (10) was used for determination of protein concentration.

Protein-bound radioactivity was determined by trichloroacetic acid (10%) precipitation carried out at 3°C. A minimal amount of carrier albumin (about 20 mg/ml of urine) was added to each urine sample. Each precipitate was washed twice with 10% trichloroacetic acid.

Gel filtration was carried out on 2.2 × 50 cm columns packed with polyacrylamide (Bio-Gel P 100).[2] The gels were equilibrated with and eluted with 0.02 M phosphate buffered saline (pH 7.4). Zone electrophoresis was carried out on Pevikon.[3] The block was sectioned in ½ inch segments which were eluted in 5 ml of phosphate buffered saline. After centrifugation, the supernatant was analyzed for amylase and radioactivity.

In addition to assaying for protein-bound radioactivity, we attempted to specifically assay for amylase-bound radioactivity in some samples by measuring glycogen-precipitable radioactivity. This technique is similar to that used for purification of amylase (8). 100 U of cold carrier amylase, and 0.50 ml of 0.01 M phosphate buffer (pH 8.0) were added to 0.50 ml of a serum or urine sample containing amylase-^{125}I. A known quantity of amylase-^{131}I was added as an internal standard. By counting the amylase-^{131}I in each precipitate, the percentage yield (about 50%) of amylase could be determined. This correction factor was then applied to the amylase-^{125}I recovered in the precipitate. In some studies the percentage yield of amylase enzyme activity precipitated by this technique was also determined.

Radioactivity was determined on a Picker Autowell II scintillation counter[4] to at least ±2% accuracy.

Comparison of the physiologic behavior of radiolabeled and unlabeled amylase (before biological screening of the labeled amylase). The serum disappearance curves and the renal clearances of salivary amylase-^{125}I and unlabeled salivary amylase were simultaneously determined in two baboons. A bolus containing amylase-^{125}I and sufficient cold amylase to raise the serum amylase level 10 to 15 times normal was injected i.v. 30-min urine collections were obtained over a 2 hr period, and serum samples were obtained at the beginning and end of each urine collection period and in addition at hourly intervals for up to 6 hr.

Each serum and urine sample was analyzed for protein-bound radioactivity and amylase activity. Serum disappearance curves were constructed by plotting amylase activity and protein-bound radioactivity against time on semilogarithm paper. The renal clearance of protein-bound radioactivity and amylase activity were calculated using the logarithmic mean serum concentration of amylase activity and protein-bound radioactivity for each urine collection period.

In order to facilitate renal clearance measurements, two baboons were studied during a constant infusion of a mixture of iodinated amylase and cold amylase. Initially, a bolus containing radiolabeled amylase and about 12 times the total estimated serum amylase activity of the baboon was administered, followed by a constant infusion of the mixture at a rate which would replace the bolus dose every 100 min. 1 hr was allowed for equilibration, and then 20-min urine samples were collected with serum samples obtained at the beginning and end of each urine collection period. It was obvious from the renal clearance studies (see Results) that radiolabeled amylase was cleared more slowly by the kidney than was unlabeled amylase.

Biological screening of labeled amylase. The possibility was then examined that radiolabeled amylase that had been biologically screened via renal excretion might be cleared at the same rate as unlabeled amylase. A bolus of radiolabeled amylase was injected into a baboon and his urine collected on ice for 5–6 hr. This urine was exhaustively dialyzed at 3°C against 0.001 M phosphate buffered saline (pH 7.4), and centrifuged at 10,000 *g* for 30 min. Gel filtration of this supernatant demonstrated that virtually all ^{125}I eluted as amylase-^{125}I. Five bolus studies of the renal clearance and serum disappearance of unlabeled amylase and this biologically screened, radiolabeled amylase were then carried out in the two baboons previously employed for the study of the unscreened amylase-^{125}I. In addition renal clearance studies were carried out on two animals during constant infusion of a mixture of screened amylase-^{125}I and cold amylase as described above. These studies (see Results) indicated that screened, iodinated amylase, and unlabeled amylase had similar renal clearances and disappearance curves. Therefore, the remainder of the experiments described in this paper were carried out using the biologically-screened radioactive amylase.

Simultaneous tracer studies of catabolism and renal excretion of pancreatic and salivary amylase. Serum disappearance curves and renal clearances were studied at normal serum amylase levels in three baboons after bolus injection of tracer quantities of iodinated amylase. In these

[2] Bio-Rad Laboratories, Richmond, Calif.

[3] Mercer Chemical Corporation, New York.

[4] Picker Scientific Apparatus Div., Cleveland, Ohio.

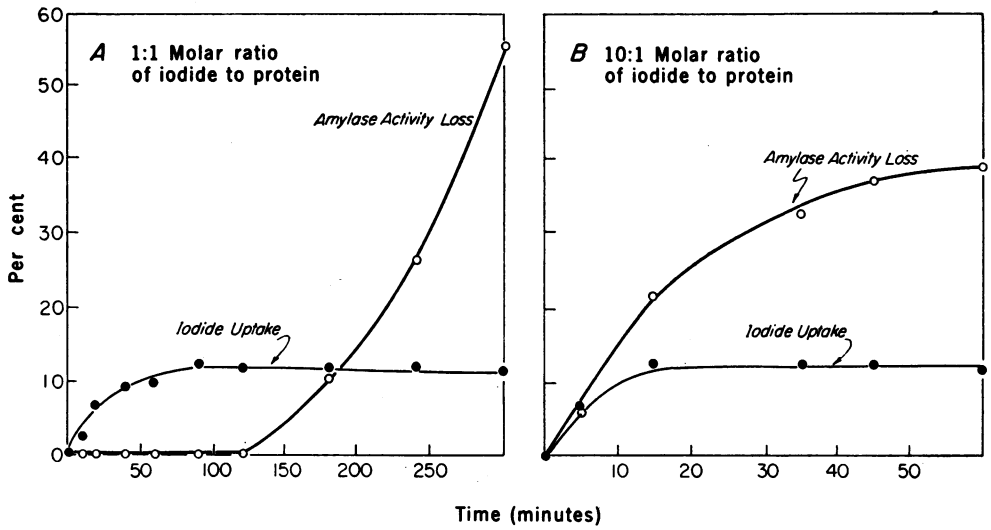


FIGURE 1 Per cent loss of amylase activity and per cent uptake of iodide during electrolytic iodination.

studies salivary amylase-^{125}I and pancreatic amylase-^{131}I were simultaneously administered.

A four-compartment system was postulated as a model for prediction of radioiodinated amylase kinetics (see Appendix).

RESULTS

Iodination of amylase. The effect of electrolytic iodination of salivary amylase enzyme activity at a molar ratio of iodide: protein of 1:1 and 1:10 is shown in Fig. 1A and 1B, respectively. It is apparent that loss of amylase activity continues after iodide uptake has reached a plateau indicating that loss of enzyme activity is not simply related to the attachment of iodine to the protein molecule.

On the basis of a series of such studies, all subsequent iodinations of amylase for use in the present studies were carried out at a 1:1 molar ratio for 40 min. The per cent of enzyme activity lost in these iodinations ranged from 1.2 to 4.7%. The per cent uptake of iodide ranged between 4.2 and 12.8%.

Comparison of gel filtration and electrophoretic characteristics of labeled and unlabeled amylase. After dialysis against phosphate buffered saline (pH 7.4), the iodinated amylase was chromatographed on columns packed with polyacrylamide. As shown in Fig. 2, a portion of the amylase apparently aggregated during the iodination procedure and eluted more rapidly as a shoulder on the major peak of amylase radioactivity which eluted in a volume similar to that of unlabeled amylase. The peak tubes containing the normally eluting material were combined and used in the remainder of studies. Repeat chromatography of this fraction yielded single coincident peaks for amylase activity and radioactivity, and the specific enzymatic activity of this material (67,000–72,000 U/mg protein) was indistinguishable from that of unlabeled amylase.

Electrophoresis of the labeled material demonstrated that amylase activity and radioactivity migrated as single peaks (see Fig. 3). It appeared that the radioactivity migrated slightly more anodally than did amylase activity suggesting a minimal difference in the electrophoretic mobility of the labeled and unlabeled molecules.

Comparison of the glycogen precipitability of labeled and unlabeled amylase. Glycogen was added to a mix-

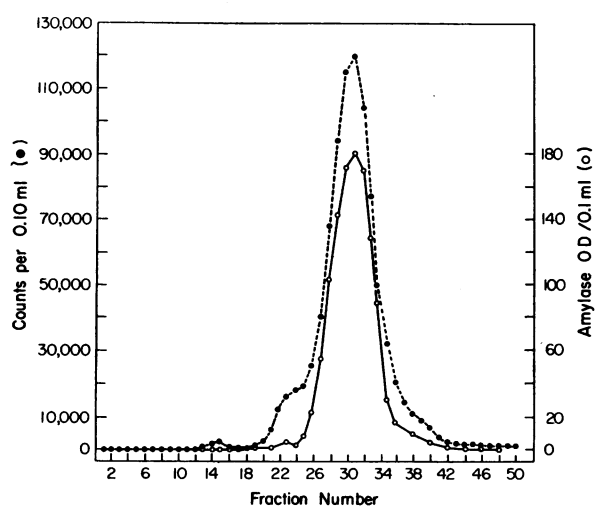


FIGURE 2 Gel filtration elution pattern on polyacrylamide of iodinated amylase. A shoulder of radioactivity precedes the coincident peaks of amylase activity and radioactivity.

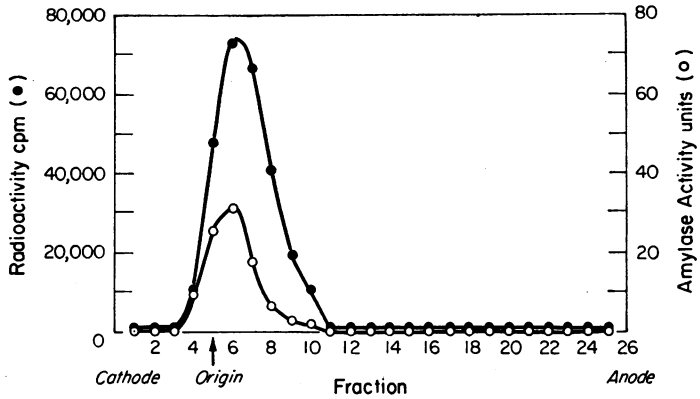


FIGURE 3 Electrophoretic pattern of iodinated amylase.

ture of salivary amylase-^{125}I, pancreatic amylase-^{131}I, and cold salivary amylase as described in the Methods section. Approximately 50% of each form of amylase was precipitated. The percentage precipitation of amylase-^{125}I and amylase-^{131}I (measured as protein-bound radioactivity) averaged 97.8 and 98.3% of the percentage precipitation of unlabeled amylase (measured as amylase activity).

Renal clearance studies. The renal clearance of iodinated amylase was compared with that of the unlabeled enzyme by determining the simultaneous clearance rates of protein-bound radioactivity and amylase activity. Fig. 4 demonstrates that after bolus injection of sufficient amylase to raise the serum level 10- to 15-fold or constant infusion of mixtures of unscreened amylase-^{125}I and unlabeled amylase, amylase-^{125}I (measured as protein-bound radioactivity) was consistently cleared less rapidly than was the unlabeled enzyme (measured as amylase activity). However, as shown in Fig. 5, the amylase-^{125}I which had been screened by renal excretion was cleared at approximately the same rate as was the unlabeled enzyme. The decreased renal clearance of the unscreened amylase-^{125}I did not appear to result from binding of the labeled amylase to serum proteins since gel filtration of serum yielded a single peak for amylase-^{125}I which eluted in its usual position.

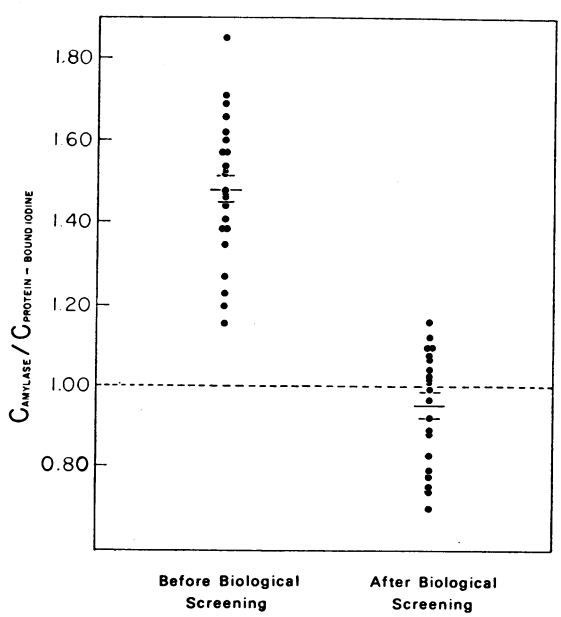


FIGURE 4 Ratio of renal clearance of amylase activity to renal clearance of protein-bound radioactivity before and after biological screening of the iodinated amylase.

Comparison of serum disappearance curves of labeled and unlabeled amylase. The disappearance curves of screened radioactive amylase and amylase activity were compared after bolus injection of a mixture of a screened iodinated amylase and unlabeled enzyme. In each of the five studies, protein-bound radioactivity disappeared from the serum at almost exactly the same rate as did amylase activity until normal serum amylase levels were approached (see Fig. 5).

The unscreened amylase-^{125}I disappeared from the serum at a slightly slower rate than did the simultaneously administered bolus of unlabeled enzyme. The mean metabolic clearance rate ($K_{12} + K_{14}$) of unscreened iodinated salivary amylase averaged -0.00668 ± 0.00090 (SE) as compared with -0.00781 ± 0.00035 for the screened iodinated amylase. This difference can be almost completely accounted for on the basis of slower renal excretion (K_{12}) of the unscreened enzyme, and no significant difference was observed in the catabolic rates (K_{14}) for the two amylases which were -0.00532 ± 0.00123 and -0.00587 ± 0.00015 for unscreened and screened amylase, respectively.

Previous studies of iodinated peptide hormones (11) have suggested that a sizeable portion of serum protein-bound radioactivity may actually represent "degradation products" of the peptides rather than the intact

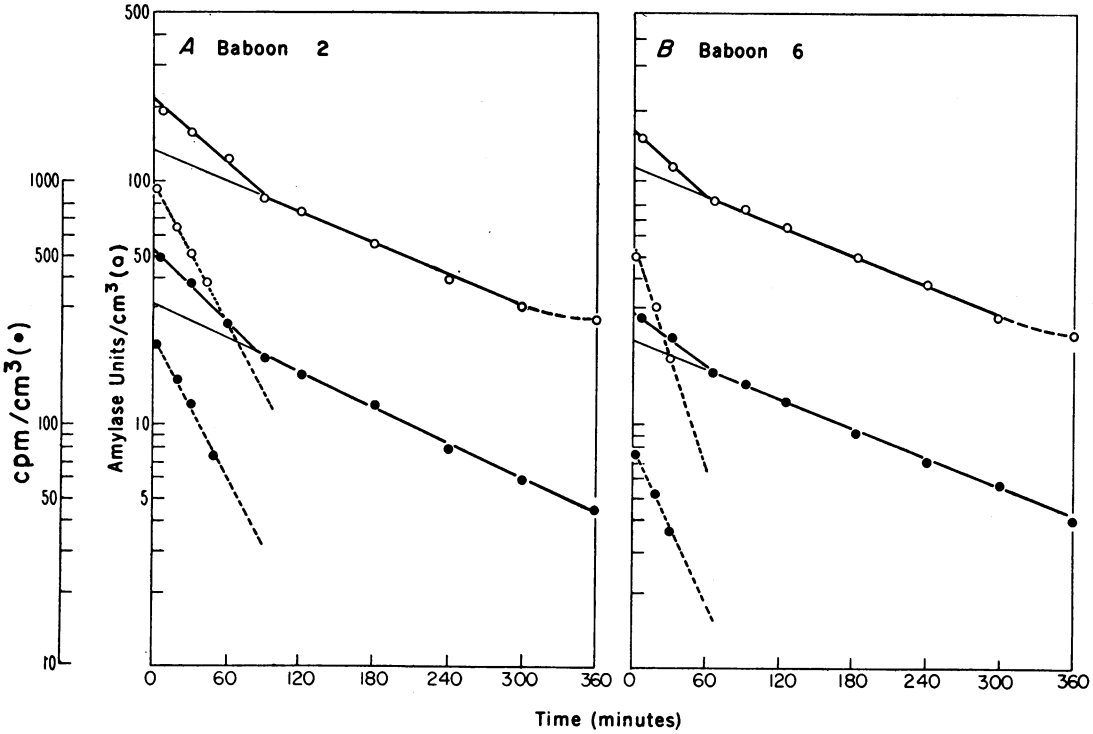


FIGURE 5 Simultaneously measured serum disappearance curves for amylase activity and protein-bound radioactivity after i.v. injection of a bolus of labeled and unlabeled amylase. Salivary amylase was employed in baboon 2 and pancreatic amylase in baboon 6. The solid line represents measured values while the dotted line is computed by extrapolating the steady-state fall-off back to zero time and subtracting the resulting straight line from the observed data.

hormones. To test this possibility with regard to amylase, glycogen-precipitable radioactivity was compared with protein-bound radioactivity in two studies. As shown in Fig. 6, glycogen-precipitable radioactivity was essentially identical to that of protein-bound radioactivity suggesting that no appreciable quantity of "degraded" amylase circulated in the serum. In addition, electrophoresis of serum yielded single coincident peaks of protein-bound radioactivity and amylase activity which migrated similarly to the infused material.

As a final method of assessing the ability of screened, iodinated amylase to trace unlabeled amylase, constant infusion studies were carried out in two baboons. The serum amylase level of the baboons was maintained at about 12 times their normal level for a 2½ hr period. In both studies the serum specific activity (protein-bound radioactivity/amylase activity) remained extremely constant and averaged 98.2±1.1% (1 SE) of the specific activity of the infusate.

Simultaneous tracer studies of serum disappearance kinetics and renal clearance of salivary and pancreatic amylase. Tracer quantities of salivary amylase-^{125}I and pancreatic amylase-^{131}I were simultaneously administered as an i.v. bolus to three baboons. The results of these studies are summarized in Table I. The serum disappearance curves for these two enzymes were quite similar and could be resolved into two exponential components as shown in Fig. 7.

Despite the similar serum disappearance curves, these two isoenzymes were cleared by the kidney at different rates. As shown in Fig. 8, pancreatic amylase-^{131}I was consistently cleared more rapidly than was salivary amylase-^{125}I with the clearance of the pancreatic enzyme averaging 1.8 times that of the salivary enzyme.

While the urinary elimination (K_{13}) of salivary amylase was slower than that of pancreatic amylase, the catabolic rate (K_{14}) of the salivary enzyme was more rapid (see Table I). Thus, there was no significant difference in the over-all metabolic clearance rates ($K_{12} + K_{14}$) of these two isoenzymes.

Comparison of gel filtration and electrophoretic characteristics of pancreatic and salivary amylase. Small, but clear-cut, differences in elution patterns were noted when mixtures of screened salivary amylase-^{125}I and

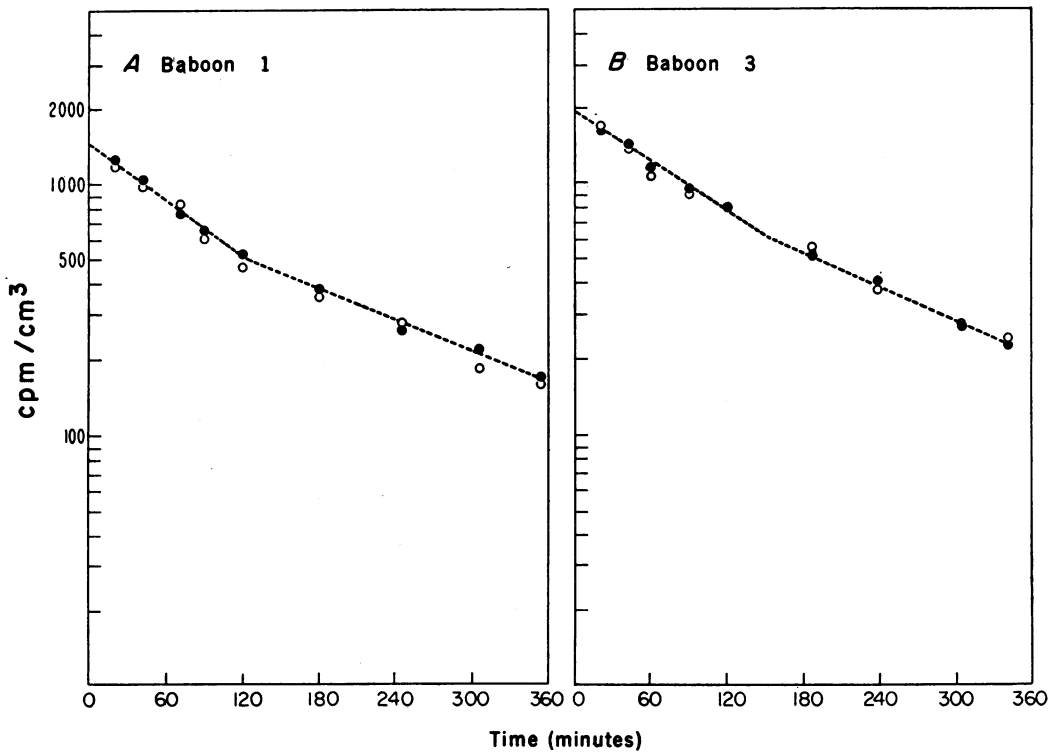


FIGURE 6 Comparison of serum disappearance curves of protein-bound radioactivity (●) and glycogen precipitable radioactivity (○).

screened pancreatic amylase-^{131}I were chromatographed (see Fig. 9). Cold amylase was added to these mixtures such that approximately 95% of the amylase activity in Fig. 9A was salivary amylase and approximately 95% of the amylase activity in Fig. 9B was pancreatic amylase. Pancreatic amylase-^{131}I consistently eluted more rapidly than did salivary amylase-^{125}I.

Electrophoresis of mixtures of pancreatic amylase-^{131}I and salivary amylase-^{125}I showed a slightly more rapid anodal migration of the pancreatic amylase (see Fig. 10).

DISCUSSION

In the present study, the metabolic turnover of amylase in the baboon was investigated using amylase electrolytically labeled with radioactive iodine.

Previous attempts to iodinate amylase using the iodine monochloride (6) technique resulted in a marked reduction in enzyme activity. Tracer studies (5) using such iodine-labeled material suggested that immediately after i.v. injection, most of the radioactivity adhered to blood cells and subsequently was slowly released into the serum. The present study using electrolytically io-

TABLE I

*Simultaneous Measurements of Catabolism and Urinary Excretion of Salivary Amylase-^{125}I and Pancreatic Amylase-^{131}I**

Type of amylase	K_{13}	K_{31}	$K_{12}+K_{14}$	K_{12}	K_{14}	$t_{1/2}$ distribution	$t_{1/2}$ metabolic clearance
	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	*min*	*min*
Pancreatic	−0.00390 ±0.00020	−0.0172 ±0.0031	−0.00682 ±0.00020	−0.00193 ±0.00010	−0.00489 ±0.00030	31±6	137±17
Salivary	−0.00190 ±0.00074	−0.0160 ±0.0040	−0.00730 ±0.00026	−0.00110 ±0.00008	−0.00620 ±0.00020	34±6	130±20

* Mean±1 SE of three studies.

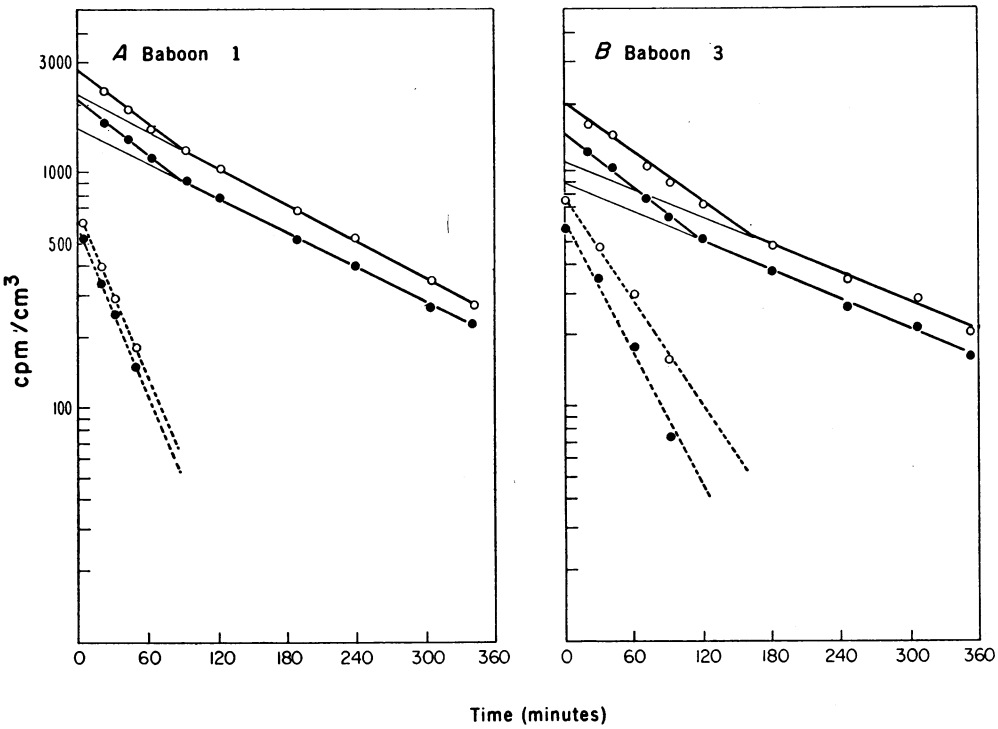


FIGURE 7 Simultaneous determination of serum disappearance curves of salivary amylase-^{125}I (○) and pancreatic amylase-^{131}I (●) studied in two baboons. The solid line represents measured values while the dotted line is computed by extrapolating the steady-state fall-off back to zero time and subtracting the resulting straight line from the observed data.

dinated amylase, as well as previous studies using unlabeled amylase (1, 4), have failed to confirm this adherence to blood cells suggesting that this finding may have been an artifact of iodination.

Use of electrolytic iodination in the present experiments resulted in a 2–4% loss of enzyme activity. Since only about 4–12% of the amylase molecules were iodinated, a sizeable proportion of the iodinated molecules might have lost enzymatic activity without producing readily detectable changes in enzyme specific activity. It is clear from the glycogen precipitation studies, however, that about 98% of the iodinated molecules retained the ability to bind with their substrate, glycogen. It seems likely that these molecules also retained the ability to digest glycogen. In addition, uptake of iodine by an amylase molecule did not seem to be the major factor causing loss of enzyme activity as demonstrated by the iodination studies summarized in Fig. 1.

However, demonstration of antigenic, chromatographic, or functional identity does not necessarily imply that the iodinated material will serve as an accurate in vivo tracer of the native protein (12). Metabolic turnover studies require that the iodinated and noniodinated molecules are catabolized and excreted at the same rate. It would appear that many, if not most, investigations of protein turnover using iodine-labeled protein have failed to conclusively demonstrate this in vivo identity of the labeled and unlabeled material.

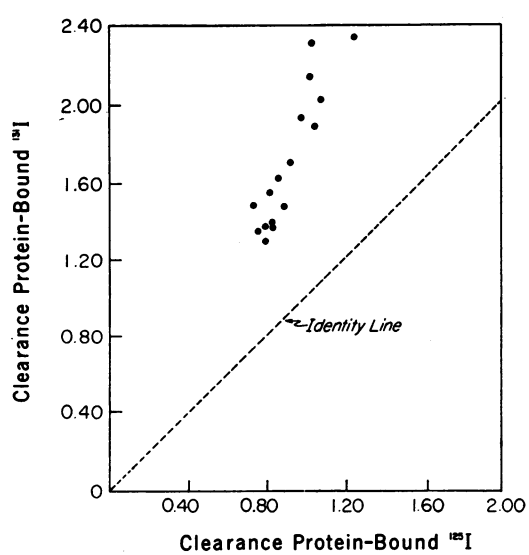


FIGURE 8 Comparison of simultaneous measurements of renal clearance (ml/min) of pancreatic amylase-^{131}I and salivary amylase-^{125}I.

Thus, although the gel filtration, electrophoretic, enzymatic, and glycogen precipitability characteristics of the unscreened amylase-^{125}I and unlabeled amylase were virtually identical, the urinary clearance rate of unscreened amylase-^{125}I was only about 60% of that of unlabeled amylase. In contrast, catabolism of amylase, which accounted for about 85% of the metabolic clearance of salivary amylase was apparently not affected by the iodination process.

The normal rate of renal clearance of amylase-^{125}I that had been screened by the kidneys suggests that iodination resulted in a dual population of labeled molecules: one population that was cleared by the kidney at the same rate as was unlabeled amylase and another that was excreted very slowly, if at all.

In addition to accurately tracing renal clearance, screened, iodinated amylase also appeared to trace the catabolism of amylase. During prolonged constant infusion of mixtures of cold and labeled enzyme the specific activity (cpm/amylase activity) would be expected to rise or fall above that of the infusate if an appreciable difference existed in the rates of catabolism of the labeled and unlabeled enzyme. The specific activity of the serum averaged 98±1.1% of the infusate indicating that the iodinated and uniodinated molecules were removed from the serum at the same rate.

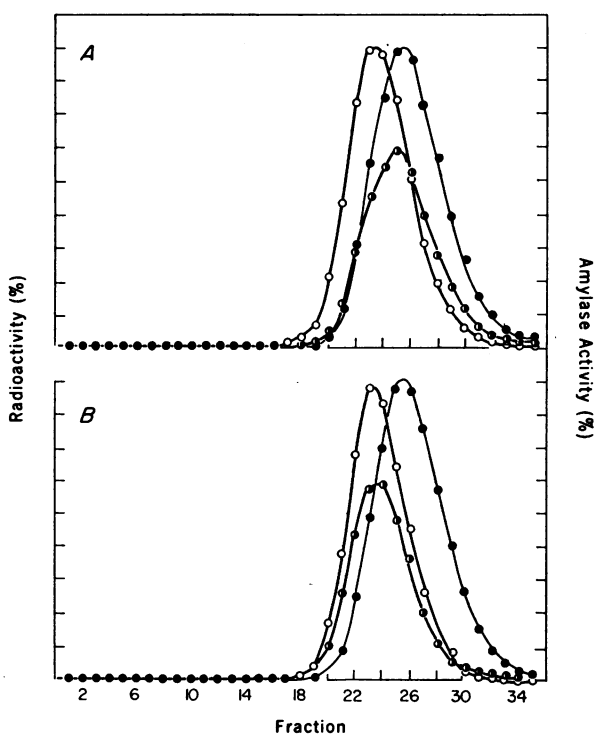


FIGURE 9 Gel filtration elution patterns on polyacrylamide of mixtures of pancreatic amylase-^{131}I (○) and salivary amylase-^{125}I (●). Unlabeled amylase (◑) was added to the mixtures such that about 95% of the amylase activity in *A* was salivary amylase and 95% of the amylase activity in *B* was pancreatic amylase.

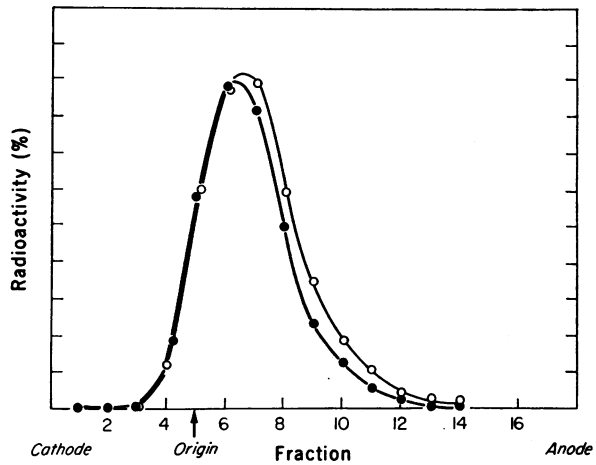


FIGURE 10 Zone electrophoretic pattern of a mixture of salivary amylase-^{125}I (●) and pancreatic amylase-^{131}I (○). Salivary amylase-^{125}I appears to migrate slightly less rapidly towards the anode than does pancreatic amylase-^{131}I.

Also, after bolus injection of labeled and unlabeled amylase, iodinated amylase accurately traced serum amylase activity down its biexponential disappearance curve (see Fig. 5).

The four-compartment model proposed to account for the removal of serum amylase is the simplest model which would predict the observed biexponential serum fall-off curve. The resolution of the disappearance curve into only two exponents suggests that there is single distribution space and that all catabolic and excretory pathways can be summed into a single, exponential removal mechanism. The possibility that amylase also distributes more slowly to a second, more remote, space cannot be entirely ruled out. The irreversible removal of amylase by catabolism and renal excretion is so rapid ($t_{\frac{1}{2}}$ of about 130 min) that the identification of a third exponential component in the disappearance curve would not be possible.

This rapid turnover of serum amylase no doubt accounts for the transient nature of serum amylase elevation often noted in pancreatitis.

The amylase "distribution flux" (K_{13}) indicates relatively rapid movement of amylase from serum to distribution space, averaging 0.00390 min^{-1} for pancreatic amylase and 0.00190 min^{-1} for salivary amylase. By comparison, albumin, which equilibrates with two spaces,

moves at a rate of 0.000841 min^{-1} to the more available space and at 0.0000556 min^{-1} to the more remote space (13). Thus, membranes separating the serum and distribution spaces are a good deal more permeable to amylase than to albumin in much the same way that amylase passes through the glomerular membrane far more rapidly than does albumin.

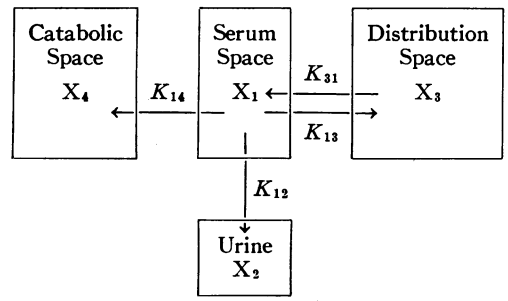
A previous study (4) in which the serum amylase level was elevated by infusion of salivary or pancreatic amylase indicated that the kidney cleared the pancreatic enzyme about 50% more rapidly than the salivary enzyme. The possibility could not be excluded that this finding resulted from trace contaminants in the enzyme preparations which influenced glomerular permeability rather than an inherent different in clearance rates of the isoenzymes. However, the simultaneous measurements of renal clearance of pancreatic amylase-^{131}I and salivary amylase-^{125}I consistently demonstrated that the amylase of pancreatic origin was cleared about 80% faster than was salivary amylase. Thus, the disparity in clearance rates represents a true difference in the renal handling of these two isoenzymes. This difference in renal clearance rates helps to explain why the urinary amylase is elevated out of proportion to the serum amylase level in pancreatitis (14).

Pancreatic amylase also distributed more rapidly than did salivary amylase. The ratio (2:1) of the rate of movement of these isoenzymes into the distribution space was roughly the same as the ratio of their glomerular filtration rates (glomerular filtration rate assumed to equal rate of urinary excretion) (4). This would suggest that the membranes of the glomerulus and the distribution space have comparable sieving characteristics for molecules of the size of amylase.

The use of differentially labeled pancreatic and salivary amylase makes it possible to discern small differences in gel filtration and electrophoretic characteristics. As shown in Fig. 9, simultaneous gel filtration of the two enzymes demonstrated a small but clearly significant difference in elution volumes for pancreatic and salivary amylase. In view of the more rapid renal clearance and distribution of pancreatic amylase it was surprising to observe that pancreatic amylase eluted earlier than salivary amylase suggesting a larger molecular radius for the pancreatic enzyme. Some characteristic such as charge distribution or molecular configuration apparently accounts for the more rapid distribution and renal excretion of the pancreatic enzyme.

APPENDIX

Model of amylase distribution and catabolism. Calculation: the following four-compartment system was postulated as a model for prediction of radioiodinated amylase kinetics:



where X_a = fraction of injected dose in space a, and K_{ab} = fraction at time t of radioiodinated amylase in space a going to space b per minute.

Note that net movement into urine (space 2) and catabolic space (space 4) is irreversible and therefore depends only on X_1 and the appropriate rate constant (K_{12} or K_{14}). Therefore K_{12} and K_{14} can be summed to $K_{12}+K_{14}$ and considered to be one irreversible rate constant. Since K_{12} can be directly measured as the renal clearance of radioiodinated amylase, we can then calculate $K_{14} = (K_{12}+K_{14}) - K_{12}$.

The above model can be described by the following system of differential equations:

$$\frac{dX_1}{dt} = K_{31}X_3 - (K_{13} + K_{12} + K_{14})X_1 \quad (1)$$

$$\frac{dX_2}{dt} = K_{12}X_1 \quad (2)$$

$$\frac{dX_3}{dt} = K_{13}X_1 - K_{31}X_3 \quad (3)$$

$$\frac{dX_4}{dt} = K_{14}X_1. \quad (4)$$

Solving this system of equations by Laplace transformations yields the following general solution where b_1 and b_2 are constants and equal to the slopes of the two exponential components on the graphs of ln X_1 vs. time:

$$X_1 = \left(\frac{K_{31} - b_1}{b_2 - b_1}\right)\exp(-b_1t) + \frac{K_{31} - b_2}{(b_1 - b_2)}\exp(-b_2t) \quad (5)$$

$$X_2 + X_4 = 1 - \left[\frac{(K_{12} + K_{14})(K_{31} - b_1)}{b_1(b_2 - b_1)}\right]\exp(-b_1t) - \left[\frac{(K_{12} + K_{14})(K_{31} - b_2)}{b_1(b_1 - b_2)}\right]\exp(-b_2t) \quad (6)$$

$$X_3 = \left(\frac{K_{13}}{b_2 - b_1}\right)\exp(-b_1t) + \left(\frac{K_{13}}{b_1 - b_2}\right)\exp(-b_2t). \quad (7)$$

Thus, the general solution for X_1 using this theoretical model is biexponential. Since the experimental equation for X_1 is also biexponential, the model proposed would seem to be a reasonable one. From the above equations it follows that:

$$b_1 b_2 = (K_{12} + K_{14})K_{31} \tag{8}$$

$$b_1 + b_2 = K_{12} + K_{14} + K_{13} + K_{31}. \tag{9}$$

If C_1 and C_2 are arbitrarily designated as the coefficients of the exponents in the equation for X_1:

$$C_1 = \frac{K_{31} - b_1}{b_2 - b_1} \tag{10}$$

and

$$C_2 = 1 - C_1 = \frac{K_{31} - b_2}{b_1 - b_2}. \tag{11}$$

Algebraically solving equations 8, 9, 10, and 11 yields:

$$K_{12} + K_{14} = \frac{1}{C_1/b_1 + C_2/b_2}$$

$$K_{31} = C_1 b_2 + C_2 b_1$$

$$K_{13} = \frac{C_1 C_2 (b_2 - b_1)^2}{C_1 b_2 + C_2 b_1}.$$

C_1, C_2, b_1, and b_2 can all be obtained graphically from a semi-log plot of X_1 vs. time.

As stated above K_{12} can be measured as urinary clearance, thus K_{14} can be calculated and represents the rate constant for radioiodinated amylase catabolism. K_{13} represents the capillary permeability into the extravascular distribution space.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Mrs. Terriel Berggren and the help of Miss Nelda Peterson in the preparation of this manuscript.

This work was supported by U. S. Public Health Service Grant AM-13309-02.

REFERENCES

1. Yacoub, R. S., H. E. Appert, and J. M. Howard. 1969. Metabolism of pancreatic amylase and lipase infused intravenously into dogs. *Arch. Surg.* **99**: 54.
2. Hiatt, N., and G. Bonorris. 1966. Removal of serum amylase in dogs and the influence of reticuloendothelial blockade. *Amer. J. Physiol.* **210**: 133.
3. Hiatt, N., G. Bonorris, G. M. Coverdale, and G. F. Lanchantin. 1968. Hepatic deposition of ^{131}I-labelled amylase in dogs; comparison of enzymatic and isotopic measurements. *Proc. Soc. Exp. Biol. Med.* **128**: 125.
4. Duane, W. C., Frerichs, R., and M. D. Levitt. 1971. Distribution, turnover, and mechanism of renal excretion of amylase in the baboon. *J. Clin. Invest.* **50**: 156.
5. Hiatt, N., G. Bonorris, and G. F. Lanchantin. 1967. Influence of reticuloendothelial blockade on the binding of amylase to blood cells. *Amer. J. Physiol.* **213**: 744.
6. Katz, J., and G. Bonorris. 1968. Electrolytic iodination of proteins with I^{125} and I^{131}. *J. Lab. Clin. Med.* **72**: 966.
7. Rosa, U., F. Pennisi, R. Bianchi, G. Federighi, and L. Donato. 1967. Chemical and biological effects of iodination on human albumin. *Biochim. Biophys. Acta.* **133**: 486.
8. Loyter, A., and M. Schramm. 1962. The glycogen-amylase complex as a means of obtaining purified α-amylases. *Biochim. Biophys. Acta.* **65**: 200.
9. Ujihira, I., R. L. Searcy, J. E. Berk, and S. Hayashi. 1965. A saccharogenic method for estimating electrophoretic and chromatographic distribution of human serum amylase. *Clin. Chem.* **11**: 97.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
11. Cameron, D. P., H. G. Burger, K. J. Catt, and A. Doig. 1969. Metabolic clearance rate of radioiodinated human growth hormone in man. *J. Clin. Invest.* **48**: 1600.
12. Yalow, R. S., and S. A. Berson. 1966. Labelling of proteins—problems and practices. *Trans. N. Y. Acad. Sci.* **28**: 1033.
13. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I-labelled plasma proteins. *Phys. Med. Biol.* **2**: 36.
14. Levitt, M. D., M. Rapoport, and S. R. Cooperband. 1969. The renal clearance of amylase in renal insufficiency, acute pancreatitis and macroamylasemia. *Ann. Intern. Med.* **71**: 919.