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Research Article

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The findings in this study provide insight into several of the following clinically observed phenomena: (*a*) the short serum half-time of amylase accounts for the transient nature of serum amylase elevations in pancreatitis; (*b*) the extra-urinary removal of amylase accounts for the maintenance of relatively normal amylase levels in uremia; and (*c*) the more rapid renal clearance of pancreatic amylase compared to salivary amylase […]

Distribution, Turnover, and Mechanism of Renal Excretion of Amylase in the Baboon

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ABSTRACT Pure amylase was isolated from pancreata and parotid glands of the baboon, an animal which has a serum amylase level and renal clearance of amylase (C_{Am}) similar to man. After bolus injection, both pancreatic and salivary amylase rapidly disappeared from the serum in a monoexponential fashion with a mean serum half-time of approximately 83 min. Only about 24% of the amylase cleared from the serum appeared in the urine indicating that the majority of amylase was removed from the serum by an extra-urinary mechanism. The C_{Am} by the kidney was constant over a wide range of serum amylase levels and the ratio of C_{Am}/C_{In}, which averaged 3.0%, was not influenced by mannitol diuresis. This suggests that the renal excretion of amylase results from glomerular filtration without appreciable tubular reabsorption. Pancreatic amylase was consistently cleared more rapidly by the kidney than was the baboon's endogenous amylase while salivary amylase was consistently cleared less rapidly than endogenous amylase.

The findings in this study provide insight into several of the following clinically observed phenomena: (*a*) the short serum half-time of amylase accounts for the transient nature of serum amylase elevations in pancreatitis; (*b*) the extra-urinary removal of amylase accounts for the maintenance of relatively normal amylase levels in uremia; and (*c*) the more rapid renal clearance of pancreatic amylase compared to salivary amylase may explain the disproportionate elevation of the urinary amylase excretion rate relative to the serum amylase level in acute pancreatitis.

INTRODUCTION

Although measurements of serum and urine amylase activity are important diagnostic tests, little is known about the factors that regulate the levels of amylase in the serum and urine. The little available data concerning the removal of amylase[1] from the serum has been primarily derived from studies in dogs (1, 2). This information is of questionable relevance to the situation in humans since dogs excrete very little amylase in their urine and maintain a serum amylase level 5–10 times higher than that of man (2).

Our preliminary studies indicated that the baboon, unlike most common laboratory animals, has a serum amylase level and a renal clearance of amylase similar to man.

In the present study, pure amylase was isolated from pancreatic and parotid glands of baboons. Observations after bolus injections and during constant infusion of this amylase provided insights into the disappearance kinetics and the mechanism of renal excretion of amylase.

METHODS

Experimental animals. Six Kenya baboons, 3 males and 3 females, ranging in weight from 19 to 50 lb. were studied. 30 min before study, the animals were anesthetized with an intramuscular injection of Sernylan.[2] Subsequently, pentabarbital was intravenously administered to maintain light anesthesia. Urine was collected via catheter, and blood samples were obtained via femoral puncture.

Isolation of amylase. Salivary glands and pancreata were removed from baboons within 1 hr of death and stored until usage at minus 20°C. The glands were homogenized in iced, distilled water and the homogenate was centrifuged at 10,000 *g* for 20 min at 2°C. Amylase was then isolated from the supernate by glycogen precipitation as described by Loyter and Schramm (3).

The purity of the amylase preparation was assessed by (*a*) measurement of specific activity of the enzyme; (*b*) gel filtration on columns packed with polyacrylamide (Bio-Gel P-100)[3] and dextran (Sephadex G-100)[4] and equilibrated

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[1] Amylase and amylase activity will be used interchangeably in this paper.

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

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

[4] Pharmacia Fine Chemicals, Piscataway, N. J.

with and eluted with 0.05 M phosphate-buffered saline (pH 7.0); and (*c*) zone electrophoresis on Pevikon.[5]

Assay methods. All serum and urine samples were refrigerated at 4°C, and amylase and inulin assays were carried out within 48 hr of sample collection.

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 (4). 1 U of amylase activity will liberate reducing groups equivalent to 1 mg of maltose per hr at 37°C.

Preliminary experiments were performed to establish the reliability of the amylase assay technique under the conditions of this study. Recovery studies were carried out by adding known quantities of purified salivary amylase to three serum and three urine specimens. The recovery of amylase activity averaged 98.4% and ranged from 95 to 103%. Amylase recovery averaged 98.8% (range 96–103) from two serum and urine samples to which mannitol and inulin had been added in concentrations greater than that observed in the present studies.

A previous study (5) suggested that dilution of serum resulted in an increase in the observed amylase activity when assayed by a starch-iodine method. We observed no appreciable change in amylase activity over a 30-fold dilution using the 3,5-dinitrosalicylic assay technique. Because measurement of reducing groups is only accurate over an 8-fold dilution, the more concentrated specimens were incubated for a proportionally shorter period of time.

The Folin-Ciocalteau method (6) was used for the determination of protein concentration for enzyme specific activity measurements and for the Pevikon electrophoresis.

Inulin-^{14}C was measured by adding 0.1 ml of serum or urine to 10 ml of a Biosolv[6]-toluene mixture (1:5). The toluene contained 8.0 g/liter of butyl PBD and 0.5 g/liter of PBBO. Samples were counted in a Beckman LS-250 liquid scintillation counter.

Amylase bolus studies. Before administration of the bolus, the base line serum amylase, the rate of urinary amylase excretion, and the renal clearance of amylase (C_{Am}) of each baboon were determined by measuring the amylase activity of two consecutive 30-min collections of urine and the amylase activity of serum samples obtained at the beginning and end of each urine collection period. A bolus of salivary amylase containing from 10 to 20 times the total serum amylase activity of the baboon was then administered over a 1 min period to each of the six baboons. Blood samples were obtained periodically for 7–8 hr and timed, partitioned urine collections were obtained in four of these baboons. In four of the baboons, this identical procedure was repeated using pancreatic instead of salivary amylase.

The mean base line serum amylase activity was subtracted from the total amylase activity of each serum sample obtained after administration of the bolus. This value, defined as "excess" amylase, was plotted against time on semilogarithmic paper to determine the disappearance curve of amylase.

The use of "excess" amylase rather than total serum amylase activity is based on the assumption that the artificial elevation of the serum amylase level did not influence the endogenously maintained base line level. Control studies carried out in four baboons maintained under anesthesia for 8 hr indicated that base line amylase levels fluctuated by less than ±20% during this period. The possibility remains that the administration of exogenous amylase alters the endogenous level. It seems unlikely that this would elevate endogenous levels and a decrease in endogenous amylase would result in relatively minor errors in view of the high proportion of injected amylase relative to endogenous amylase.

Relation of serum amylase level to C_{Am}. The influence of elevation of the serum amylase level on the renal clearance of amylase was evaluated by two methods. First clearances were calculated by the standard method:

$$C_{Am} = \frac{[\text{amylase}]_{\text{urine}} \times \frac{\text{urine volume}}{\text{time}}}{\text{logarithmic mean } [\text{amylase}]_{\text{serum}}}$$

This C_{Am} for each collection period was then compared with the logarithmic mean serum amylase level for that period. Problems arise with this method because of the rapidly falling serum amylase level. This problem was circumvented by integrating the following differential expression for C_{Am}: $du/dt = C_{Am}(x)$ where u = total amylase excreted in urine from t_0 to t minus the base line amylase excretion between t_0 and t; t = time in minutes; x = serum amylase per ml minus base line amylase per ml ("excess" amylase); and C_{Am} = renal clearance of amylase. If the serum falls off monoexponentially (as will be demonstrated in the results section) then x takes the form of $x = x_0e^{-a_1t}$ (where $x_0 = x$ at $t = 0$ and a_1 = slope of serum semilog disappearance curve), and the integration yields the following expression for u(t): $u(t) = u(\infty) - u(\infty)\cdot e^{-a_1t}$. Rearranging this equation and taking its logarithm yields: $\ln u(\infty) - u(t) = \ln u(\infty) - a_1t$. Accordingly $u(\infty) - u(t)$ was plotted verses time on semilog paper. If C_{Am} is not affected by serum amylase levels, then this plot should yield a straight line with slope equal to the serum amylase fall-off curve.

Studies of the ratio of amylase clearance to inulin clearance (C_{Am}/C_{In}). In an attempt to obtain more precise information concerning the mechanism of renal excretion of amylase, C_{Am} was compared with simultaneous measurements of inulin clearance (C_{In}) at low and high serum amylase levels and also during dehydration and during mannitol diuresis.

Inulin clearance measurements were carried out in the following manner. Inulin-^{14}C was mixed with carrier inulin to give a specific activity of 100 μCi/g. In each of the studies described below, a bolus of inulin-^{14}C calculated to give a serum level of about 0.002 μCi/ml was administered, followed by a constant infusion at a rate that would give an amount equal to the bolus dose of inulin-^{14}C every 90 min. 1 hr was allowed for the serum inulin to stabilize. Serum inulin-^{14}C levels varied by less than ±10% during the course of these studies with the exception that after mannitol infusion, the inulin level fell to approximately 85% of the premannitol level.

In each study described below, three or four base line measurements of C_{Am}/C_{In} were obtained. A 20 min collection of urine and serum samples obtained at the beginning and end of each collection period were used for calculating these base line measurements of C_{Am}/C_{In}. Then either the serum amylase level or the rate of urine flow was altered, 60 min was allowed for stabilization, and the bladder was washed with three 30-ml aliquots of normal saline. Then C_{Am}/C_{In} was once again determined by analysis of three or four 20-min urine collections and serum samples drawn at the beginning and end of each urine collection period.

[5] Mercer Chemical Corp., New York.

[6] Beckman Instruments, Inc., Chicago, Ill.

Influence of mannitol diuresis on C_{Am}/C_{In}. The effect of mannitol diuresis on C_{Am}/C_{In} was studied both at normal serum amylase levels and at serum amylase levels artificially elevated by an infusion of salivary amylase. Dehydration was produced in six baboons by complete water restriction for 18 hr before study. After dehydration, urine flow was less than 0.4 ml/min. Base line C_{Am}/C_{In} was determined and then an osmotic diuresis was produced by administering mannitol in a dosage calculated to raise the total body osmolality by 20 mOsm. One-third of this dose was given over 30 min as a 2% solution of mannitol in normal saline. A urine flow rate of 5–10 ml/min was obtained in all baboons.

The influence of mannitol diuresis on C_{Am}/C_{In} at high serum amylase levels was studied in four baboons. Serum amylase was maintained at a level approximately 10 times normal in four dehydrated baboons by infusing a bolus of salivary amylase containing about 10 times the normal total serum amylase activity followed by a constant infusion of amylase at a rate which would replace one-half the bolus dose every 90 min. 1 hr was allowed for stabilization. C_{Am}/C_{In} was then determined before and after the induction of a diuresis with mannitol (administered as described previously). Serum amylase levels varied by less than ±10% before mannitol infusion. After mannitol, the serum amylase usually decreased by about 15%.

Change in C_{Am}/C_{In} induced by elevation of the serum amylase level by salivary of pancreatic amylase. In four baboons, C_{Am}/C_{In} measurements of endogenous amylase (i.e. base line measurements) were compared with C_{Am}/C_{In} measurements obtained after the serum amylase level was elevated 8- to 10-fold by a constant infusion of either salivary or pancreatic amylase. The serum amylase level was maintained at an elevated level as described above.

RESULTS

Isolation of amylase. The average amylase content of a single parotid gland homogenate was about 10 million U while a pancreatic homogenate contained about 1 million U. The yield of purified amylase from these homogenates averaged 15%. The purified amylase from each isolation had a specific activity ranging from 68,000 to 73,000 amylase U/mg of protein which is roughly similar to a previously reported study (3) of human salivary amylase using slightly different methodology.

Fig. 1 shows zone electrophoresis patterns on Pevikon and the gel filtration patterns obtained when purified pancreatic amylase was chromatographed on columns packed with polyacrylamide (Bio-Gel P-100) and/or dextran (Sephadex G-100). Both electrophoresis and

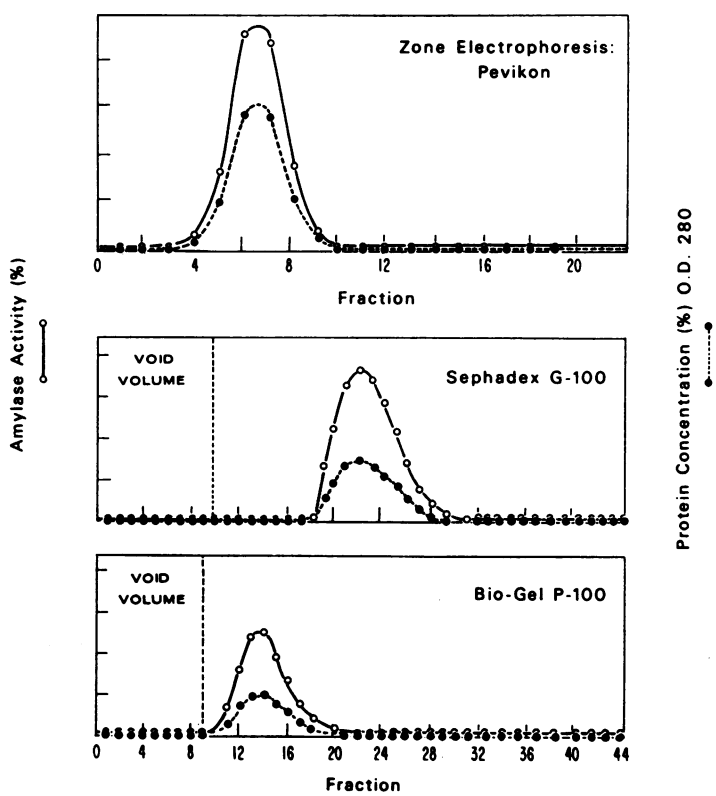

FIGURE 1 Purity of pancreatic amylase preparation demonstrated by electrophoresis and gel filtration. Albumin elutes just after the void volume on each of the gels, i.e., fractions 11, 12, and 13 on Sephadex G-100 and fractions 10, 11, and 12 on Bio-Gel P-100.

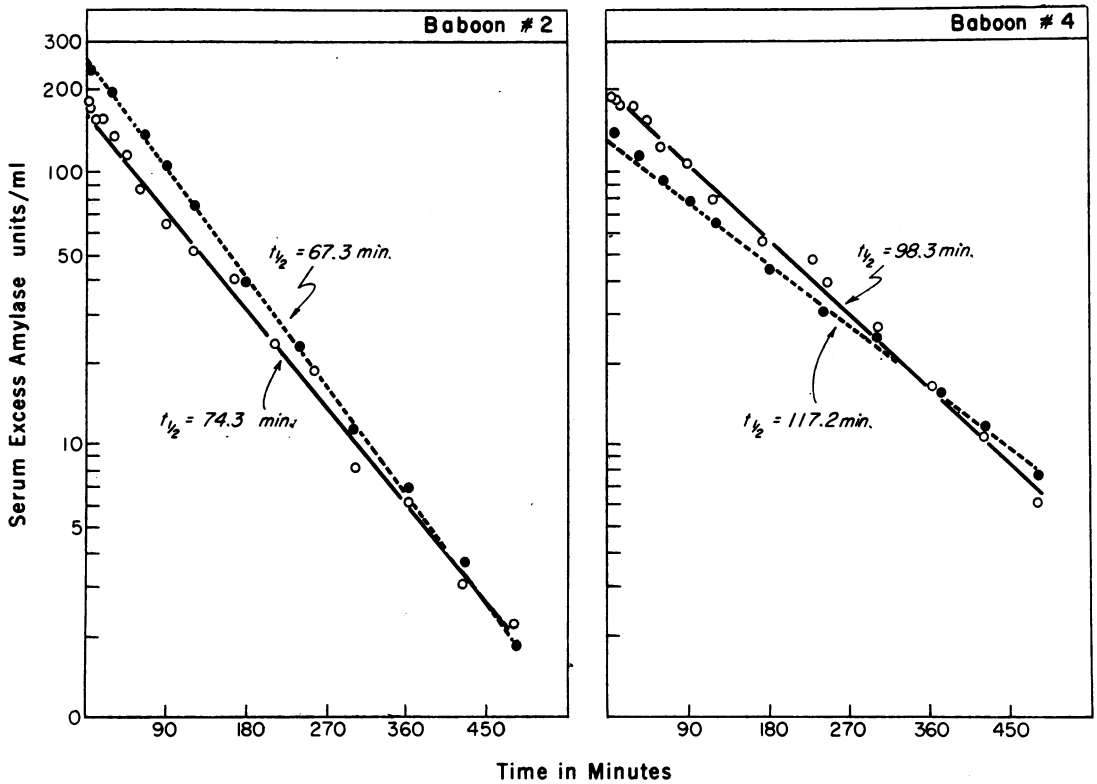

FIGURE 2 Serum disappearance curves for salivary (○) and pancreatic (●) amylase activity after bolus injections into two baboons.

gel filtration yielded single, coincident peaks for protein and amylase activity. Amylase elutes off polyacrylamide in a volume consistent with its molecular weight (7) (approximately 55,000 [8]) while it apparently binds to dextran (9) and elutes with proteins which have a molecular weight of 5000–10,000. The coincident peaks obtained for amylase and protein on each of these two gels suggests that all protein in purified material represents amylase.

Zone electrophoresis on Pevikon also revealed single coincident peaks for protein and amylase activity.

Serum amylase disappearance curve. After a bolus injection of salivary or pancreatic amylase, the fall-off curve for serum amylase activity was always monoexponential. Fig. 2 shows typical fall-off curves obtained after injection of boluses of salivary and pancreatic amylase in each of two baboons. The half-time of salivary amylase activity in six baboons ranged from 39 to 117 min with an average of 83 min. Repeat study of three baboons with salivary amylase yielded half-times within ±22% of the previous measurement. The half-time of pancreatic amylase in a given baboon was within ±20% of the salivary amylase half-time.

Role of renal excretion in serum amylase removal. The rapid clearance of amylase from the serum could not be accounted for solely on the basis of urinary excretion of the enzyme. Urinary amylase amounted to only 24% ±3 (1 SD) of the total quantity of amylase calculated to have disappeared from the serum during the course of the study (see Fig. 3).

Relation of serum amylase level to C_{Am}. The influence of the serum amylase level on the renal clearance of amylase was studied by several different methods. The C_{Am} was relatively constant despite 15-fold changes in serum amylase activity produced by the injections of amylase boluses. A typical example of such a study is shown in Fig. 4 which compares the C_{Am} for each urine collection period with the logarithmic mean serum amylase level for that collection period. The clearance measurement obtained by this technique is somewhat inaccurate because of the short urine collection periods and the rapidly changing serum amylase level. For this reason, a second method of analyzing C_{Am} measurements after the bolus injection was employed in which $u(\infty) - u(t)$ was plotted against time on semilog paper. When graphed in this manner, data from each of four baboons yielded monoexponential curves with slope within ±5% of the corresponding serum amylase fall-off curve (see Fig. 5). This indicates that C_{Am} was relatively constant through an 8- to 15-fold drop in serum amylase concen-

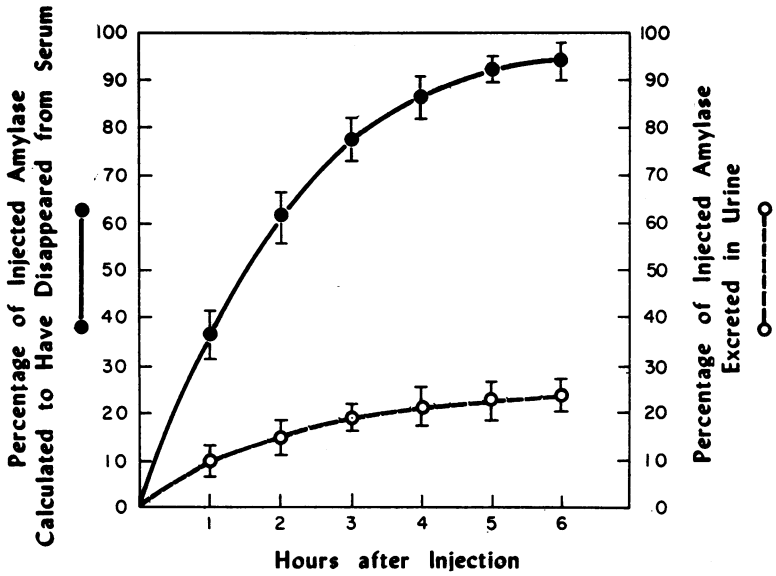

FIGURE 3 Role of urinary excretion in serum amylase removal. Urinary amylase excretion O——O averaged 24% of the total amylase calculated to have disappeared from the serum (●——●). Mean ±1 SE are shown for each time interval.

tration and that there is no tubular reabsorptive (or secretory) mechanism which is saturable at the amylase levels achieved in these studies.

Influence of mannitol diuresis on C_{Am}/C_{In}. The relatively constant C_{Am} observed over a wide range of serum amylase levels suggests that amylase is being filtered at the glomerulus but does not rule out the possibility that the renal tubules reabsorb amylase in proportion to the quantity that is filtered at the glomerulus. In an attempt to rule out this possibility, amylase clearance was measured during hydropenia and then after mannitol diuresis (10, 11). Mannitol diuresis should increase the rate of passage of amylase through the tubule and decrease the concentration of amylase within the tubule, thus decreasing the reabsorption rate. At normal serum amylase levels C_{Am}/C_{In} during mannitol diuresis aver-

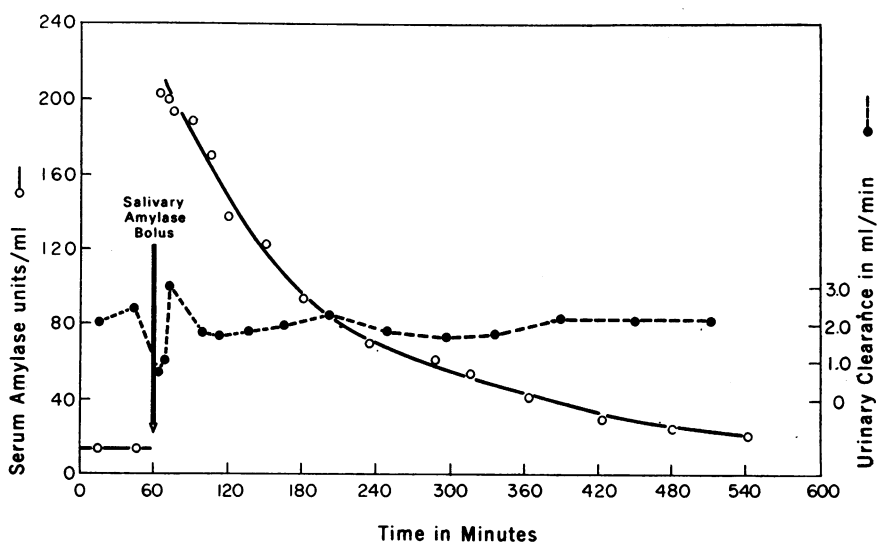

FIGURE 4 Comparison of C_{Am} (●) to serum amylase level (O) before and after bolus injection in a baboon. The sharp fall in C_{Am} seen immediately after bolus injection results from a short lag period between glomerular filtration and appearance of urine in the bladder.

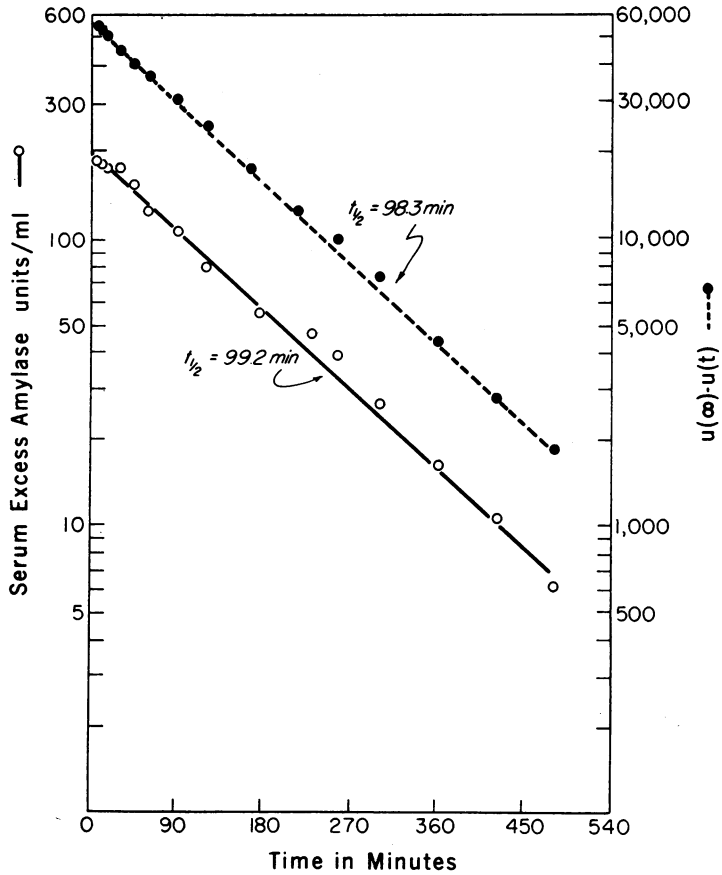

FIGURE 5 Comparison of $u(\infty) - u(t)$ vs. time with excess serum amylase vs. time. Monoexponential form of $u(\infty) - u(t)$ with slope (−0.00307) approximately equal to disappearance curve slope (−0.00304) indicates relatively constant C_{Am} during serum amylase decline.

aged 100.4% of the premannitol ratio suggesting that there is no appreciable reabsorption of endogenous amylase (see Table I A).

In order to exclude the possibility that salivary amylase might be selectively reabsorbed in the tubules, the serum amylase level was elevated by a constant infusion of salivary amylase, and then C_{Am}/C_{In} was determined during hydropenia and then during mannitol diuresis. As shown in Table I B, there was no detectable reabsorption of salivary amylase with the mean post-mannitol C_{Am}/C_{In} averaging 95% of the hydropenic measurement.

Study of C_{Am}/C_{In} of salivary and pancreatic amylase. In a final series of experiments C_{Am}/C_{In} at normal serum amylase levels was compared with C_{Am}/C_{In} after an 8- to 10-fold elevation of the serum amylase level with either pancreatic amylase (Table I C) or salivary amylase (Table I D). In Fig. 6, the mean base line (endogenous) C_{Am}/C_{In} of each baboon has been normalized to 0% change. It is apparent that C_{Am}/C_{In} of each baboon consistently decreased after administration of salivary amylase (mean, −19.8 ±2.2% [1 SE]) and consistently increased after administration of pancreatic amylase (mean, +21.1 ±2.9% [1 SE]). This difference is highly significant ($P < 0.001$).

The baboons tolerated the amylase infusions without any apparent untoward effects. There was no evidence of alterations of blood pressure, pulse, respiration, temperature, coagulation, hemolysis, or renal function.

DISCUSSION

Although many investigators have studied the factors which govern the release of amylase into the blood, there is only limited information concerning the removal of amylase from the serum. In a series of papers, Hiatt and coworkers reported studies dealing with the removal of crystalline hog amylase intravenously administered to dogs and pigs (1, 12–14). Their findings

TABLE I

*Summary of Studies of C_{AM}/C_{In}**

		Serum amylase	Urine amylase	C_{Am}	Serum inulin-^{14}C	Urine inulin-^{14}C	C_{In}	C_{Am}/C_{In}
		u/ml	*u/min*	*ml/min*	*cpm/ml*	*cpm/min*	*ml/min*	
A Influence of mannitol on C_{Am}/C_{In} at normal serum amylase level (6)‡	Premannitol	15.3 ±7.3	26 ±18	1.48 ±0.79	510 ±271	24,500 ±11,200	43.1 ±23.7	3.22 ±0.93
	Postmannitol	13.8 ±6.4	18.4 ±11	1.27 ±0.66	422 ±279	18,200 ±9,300	39.5 ±21.0	3.23 ±0.86
B Influence of mannitol on C_{Am}/C_{In} at high serum levels of salivary amylase (4)‡	Premannitol	244 ±103	280 ±117	1.24 ±0.39	355 ±88	14,000 ±4,100	45.6 ±25.8	3.02 ±0.61
	Postmannitol	186 ±44	238 ±78	1.28 ±0.31	328 ±86	14,800 ±3,040	48.3 ±17.2	2.88 ±1.05
C Change in C_{Am}/C_{In} after elevation of serum amylase level with pancreatic amylase (4)‡	Normal amylase level	18.7 ±5.2	30.0 ±12	1.50 ±0.43	267 ±38	15,200 ±3,500	59.1 ±17.6	2.48 ±0.12
	Elevated amylase level	145 ±13	254 ±81	1.72 ±0.46	257 ±23	14,200 ±2,050	54.0 ±17	3.13 ±0.34
D Change in C_{Am}/C_{In} after elevation of serum amylase level with salivary amylase (4)‡	Normal amylase level	21.8 ±2.7	25.3 ±11	1.32 ±0.30	360 ±84	15,200 ±2,200	41.5 ±14	3.11 ±0.47
	Elevated amylase level	245 ±105	280 ±140	1.25 ±0.39	353 ±117	14,900 ±4,600	47.0 ±26	2.60 ±0.61

* Data expressed as mean ±1SD.

‡ Number in parenthesis indicates number of baboons studied.

were rather bizarre in that within 5 min of injection only about 20% of the administered amylase remained in the serum. Subsequently, the serum amylase level gradually increased, reaching a peak at about 3 hr. Using ^{131}I-labeled amylase they showed that immediately after administration most of the enzyme was bound to the blood cells.

The serum disappearance curves obtained in the present study when pure baboon salivary and pancreatic amylase were administered to baboons stand in sharp contrast to the observations of Hiatt et al. The baboons' serum amylase peaked immediately after injection and thereafter fell off in monoexponential fashion. Approximately 100% of the administered amylase could be accounted for in the serum 5 min after injection. Somewhat similar results have been reported by Appert and coworkers after intravenous injection of fresh canine pancreatic juice to dogs (15, 16), but these studies are difficult to interpret because limited data made clear delineation of disappearance curves impossible; and in addition, physiologic alterations may have arisen as a result of the administered pancreatic juice.

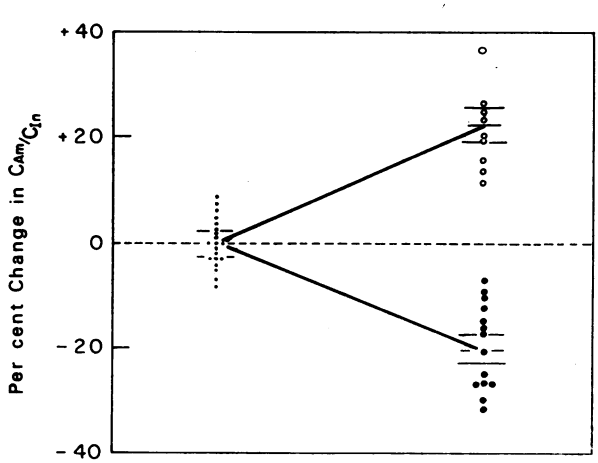

FIGURE 6 Change (mean ±1 SE) in C_{Am}/C_{In} after salivary amylase infusion (●) and pancreatic amylase infusion (○). The mean endogenous (base line) C_{Am}/C_{In} of each baboon (●) has been normalized to zero.

The difference between the results of our study and those reported by Hiatt et al. may simply represent species differences in the handling of amylase. It is also, possible, however, that the injection of heterologous amylase was responsible for the peculiar disappearance curves in his study. It is difficult to imagine a physiologic equilibrium between free and bound amylase such that at first there is only a small amount of the free form, and then later, with less total amylase in the system, an increased amount of free amylase.

The monoexponential nature of our observed amylase disappearance curve is unusual in that most protein fall-off curves are biexponential or even triexponential (17–19). Ostensibly the initial phase of these multiexponential curves represents distribution of the protein into extravascular compartments (20). Since amylase is smaller than many proteins with multiexponential disappearance curves, it should be subject to this same distribution process. However, amylase removal is more rapid than the initial phase of egress for most other proteins (21–23) suggesting that distribution of

amylase is rendered inappreciable by other, more rapid removal mechanisms. For example, the initial disappearance rate of albumin from the serum has a half-time of about 20 hr (18, 23). If a similar rate applied to amylase, a protein of roughly comparable molecular weight, this component would be scarcely visible against the background of our observed mean half-time of 83 min.

Alternatively, it is possible that amylase is rapidly diffusing into a very capacious storage space, one capable of accepting a large quantity of amylase without approaching the saturation point. Since a 15- to 20-fold drop in serum amylase would saturate total body water under isoconcentration conditions, this would have to be a storage space in which amylase existed in a higher concentration than in the serum. Because amylase has a strong affinity for glycogen (24), it is conceivable that amylase-glycogen complexes constitute such a storage mechanism. However, amylase would have to cross cell membranes to combine with glycogen. In addition, this possibility seems unlikely in that the injected bolus of amylase would digest glycogen at a rate of approximately 100 g/hr. After glycogen digestion, amylase should return to the serum, a phenomenon which is not compatible with the observed monoexponential fall-off curve.

The possibility that an amylase inhibitor accounts for the disappearance curve of amylase activity seems unlikely. There is no good evidence that an amylase inhibitor exists in serum or urine (25, 26). Furthermore, preliminary studies in our laboratory using radioiodinated amylase have shown that radiolabeled enzyme and amylase activity are removed from the serum at similar rates.[7]

It is tempting then to assume that all amylase leaving the serum is removed by an irreversible process and that there are no appreciable extravascular storage spaces. If this is the case, some efficient extra-urinary removal mechanism must exist since urinary excretion accounted for less than 25% of the amylase removed from the serum. This observation would explain the finding of normal or only mildly elevated serum amylase levels in patients with severe renal insufficiency (27, 28). In addition, this efficient removal mechanism also explains the transitory nature of serum amylase elevation frequently seen in acute pancreatitis.

The mechanism of extra-urinary removal of amylase is speculative. Both the liver as well as the entire reticuloendothelial system are possible destruction sites since the former has been implicated in the catabolism of serum proteins, such as albumin (29, 30), and the latter seems to play a role in the catabolism of enzymes such as lactic dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) (31, 32). In addition, certain of the smaller plasma proteins such as insulin are filtered by the glomerulus, reabsorbed by the proximal tubule, and catabolized by the proximal tubular cells (33).

Although not conclusive, our data suggest that amylase is not catabolized in the renal tubule. The constant C_{Am} observed over a wide range of serum amylase levels would require that the noncatabolized fraction be a fixed percentage of the amylase filtered. The lack of influence of mannitol diuresis on C_{Am} would further require that this percentage remain constant despite the more rapid passage of fluid through the tubule induced by mannitol. This seems unlikely. Lastly, the relatively normal serum amylase levels observed in anephric patients is not consistent with the kidney being the major site of amylase catabolism.

The results of several previous studies suggest that salivary and pancreatic amylase are isoenzymes separable by electrophoresis (34, 35). In contrast to the isoenzymes of LDH (36) and SGOT (37, 38), which are removed from the serum at markedly different rates, the present study indicates that pancreatic and salivary amylase are cleared from the serum at relatively similar rates.

Despite the frequent clinical use of urinary amylase measurements, little is known about the mechanism of renal excretion of this enzyme. There is evidence to indicate that proteins such as hemoglobin (39) and albumin (40) are filtered at the glomerulus and subsequently reabsorbed in the renal tubules. The extremely constant C_{Am} observed in our studies over a 10- to 15-fold range of serum amylase levels indicates that if an active tubular transport mechanism exists for amylase it cannot be saturated at serum amylase levels observed in clinical situations.

However, C_{Am} would remain constant over a wide range of serum amylase values if the tubules reabsorbed a constant proportion of the filtered load of amylase. Such reabsorption should be appreciably reduced by mannitol diuresis which decreases the contact time between the filtrate and the tubule as well as decreasing the concentration of amylase in the tubular lumen. The failure to detect any significant change in C_{Am} after mannitol diuresis suggests that there is no appreciable tubular reabsorption of amylase. McGeachin and Hargan have previously demonstrated that a water diuresis does not influence C_{Am} in man (41, 42).

Lastly, a tubular secretory mechanism which secreted amylase at a rate proportional to the serum amylase level could also theoretically account for the findings of this study. However, there is no evidence for tubular secretion of proteins. Although not conclusive, our data

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suggest that amylase is excreted via glomerular filtration without appreciable tubular reabsorption. Amylase, which has molecular weight of 55,000 (43), thus appears to be filtered at the glomerulus at mean rate of about 3% of that of inulin, a rate roughly equal to that of hemoglobin which has a molecular weight of 68,000 (44).

Egg albumin which has a molecular weight of 43,000 is filtered at a rate of about 22% of that of inulin (45). The factors which influence the permeability of the glomerulus to proteins are poorly understood, however, the configuration of the protein molecule appears to be extremely important. For any given molecular weight, a cylindrically shaped molecule will be sieved more readily than will a spherical molecule (44, 45). The rapid clearance of egg albumin can be attributed to its cylindrical configuration (44).

While the molecular configuration of amylase has apparently not been determined, the sedimentation coefficient ($s_{20,w} = 4.60S$) of amylase (43) is higher than that of hemoglobin ($s_{20,w} = 4.31S$) (46) which suggests that amylase is a somewhat more spherical molecule than is hemoglobin (46). This difference in molecular configuration may explain why these two proteins are filtered at about the same rate despite the disparity in their molecular weights.

Pancreatic amylase was consistently cleared by the kidney more rapidly than was salivary amylase. The failure of mannitol diuresis to increase C_{Am}/C_{In} when the serum amylase was artificially elevated with salivary amylase suggests that this difference in renal clearance cannot be explained by selective tubular reabsorption of salivary amylase. Therefore, it seems likely that pancreatic amylase is filtered at the glomerulus more rapidly than is salivary amylase. This difference in filtration rate cannot be explained by any known difference in molecular weight of these two enzymes; however, at this molecular weight (55,000), minor changes in molecular radii not detectable by presently available methods could account for the clearance differences observed.

It is known that C_{Am} increases during acute pancreatitis (27, 47), and the rate of urinary amylase excretion is therefore disproportionately elevated relative to the serum level. The present studies rule out the possibility that this increased C_{Am} results from a saturation of a tubular reabsorption mechanism for amylase. The finding that pancreatic amylase is cleared more rapidly by the kidney than is salivary amylase may help to explain elevated C_{Am} observed in patients with pancreatitis.

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