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Passage of Salivary Amylase Through the Stomach in Humans

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With an inhibitor assay technique rates of passage of salivary and pancreatic isoamylase through the jejunum were measured in six healthy volunteers after different liquid, intragastric meals. In all subjects and in 13/17 experiments, more than 2500 units of salivary amylase were passed over 200 postcibal minutes. Salivary amylase comprised 13.8 ± 3.9% ($\bar{X}$ ± SEM) of the total amylase and appeared predominantly as single, distinct peak. The inhibitor method was validated by isoelectric focusing ($r = 0.988$; $P < 0.001$; $N = 7$). The frequency of detection of salivary amylase in gastric or jejunal samples fell as gastric pH fell below 3.0. In vitro, *amylase was inactivated in gastric juice as pH fell between 3.8 and 3.3. Salivary amylase accounted for 11% of total amylase output in a normal and 27% in an achlorhydric subject after a hamburger meal. We conclude that amylase should not be measured in postprandial studies of pancreatic secretion in humans without correction for salivary amylase.*

KEY WORDS: salivary amylase; stomach; pancreatic secretion; humans.

Salivary amylase is generally thought to be completely destroyed during gastric passage. Conflicting data were reported about the nature of isoamylases in intestinal contents. In one recent study, no salivary isoamylase was found in human intestinal contents (1). Another group reported that more than 75% of duodenal aspirates in healthy human volunteers after a Lundh meal were contaminated by salivary amylase (2). Likewise, in normal infants the majority of amylase in the postcibal duodenal juice was found to be of salivary origin (3, 4).

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Because of its simple assay technique, total amylase activity in duodenal or jejunal aspirates is frequently measured to determine pancreatic function in secretory studies. Passage of high amounts of salivary amylase through the stomach would increase total amylase activity in intestinal contents and render total amylase activity useless as a measure of pancreatic function. In contrast to earlier cumbersome methods, such as electrophoresis (5, 6) and isoelectric focusing (7, 8), a simple technique is available (Kit, Pharmacia Inc.) to distinguish salivary from pancreatic isoamylase using a wheat protein, which is a relatively specific inhibitor of salivary amylase (9). Applying this technique, we determined how much of total jejunal amylase after different intragastric meals was of salivary origin in human volunteers.

MATERIALS AND METHODS

Isoamylase Assay. To distinguish salivary from pancreatic isoamylase, an inhibitor assay (Kit, Pharmacia, Inc.,

TABLE 1. COMPOSITION OF MEALS AND NUMBER OF EXPERIMENTS EVALUATED FOR ISOAMYLASE

Meal (abbrev.)	*Osmolarity (mosm/liter)*	*pH*	*Experiments performed*	*Experiments evaluated*
21% Dextrose (Dex)	1180	7.0	6	2
21% Dex + 7% bovine serum albumin (BSA)	1280	7.0	6	4
21% Dex + amino acids (AmA)	2170	7.0	6	4
4% Dex + BSA (DLo)	240	7.0	5	4
Cholecystokinin octapeptide (CCK-8) (40 ng/kg/hr, IV, with dextrose + BSA (CCK)	1280	7.0	5	3

New Jersey; lots 1553 and 5849) as described by O'Donnell et al (9) was used. This method utilized a wheat protein, which inhibits most salivary (88%) but only a small portion of pancreatic isoamylase (27%). Samples and pure pancreatic and salivary standards were incubated both with and without addition of the inhibitor and amylase activity was then measured by a standard chromogenic method (Phadebas).

As

$$R = aP + bS \quad (1)$$

and

$$T = S + P \quad (2)$$

the actual activity of the isoenzyme in a sample can be calculated by use of formula (3), which is derived from (1) and (2):

$$P = (R - bT)/(a - b) \quad (3)$$

where P (units/liter) is the activity of pancreatic isoamylase; S (units/liter) is the activity of salivary isoamylase; R (units/liter) is the activity remaining after addition of inhibitor; T (units/liter) is the total amylase activity; a is the R/T ratio for pure pancreatic standard; and b is the R/T ratio for pure salivary standard.

In two experiments standard curves were constructed by mixing known concentrations of pure salivary and pancreatic standards in four different ratios. The fraction of amylase uninhibited in the presence of the inhibitor (R/T) was calculated and plotted against the known P/S ratio. Standard curves plotted by this method and calculated from formula (3) were shown to be superimposable.

In each assay three to five samples of the pure pancreatic and salivary standards were assayed to calculate a and b. In 12 consecutive assays, a and b showed a mean ± SEM of 0.73 ± 0.021 and 0.12 ± 0.006, respectively, with an interassay coefficient of variation of 9.8% and 17.4%, respectively. The intraassay coefficient of variation for the salivary [mean ± SEM amylase activity = 212.9 ± 6.8 (units/liter)] standards was 4.7% and 5.8%, respectively. The interassay coefficient of variation was 12.8% and 11.1% (N = 12), respectively.

***In Vivo* Studies.** After giving informed consent, six normal subjects (age 33–50 years) were intubated with a gastric and a jejunal tube and 500-ml meals of varying composition were given intragastrically on different days. We noted nonparallel jejunal recoveries of more amylase than trypsin. To determine whether this nonparallel recovery of amylase was due to contamination with salivary amylase, we randomly chose for analyses 17 of 23 experiments for evaluation of isoamylase contents in jejunal and gastric juices (Table 1).

Emptying of the meals and total output of trypsin and amylase were determined by a method detailed elsewhere (10, 11). In brief, subjects were intubated after an overnight fast with a triple-lumen tube (OD = 4 mm) of which the distal lumens were attached to a Crosby bubble trap with 1.17-mm holes. The bubble trap was positioned in the jejunal lumen about 30 cm beyond the ligament of Treitz. The third lumen of the tube ended in the intestine 25 cm orad to the bubble trap and was placed about 5 cm beyond the ligament of Treitz. The final position was verified by one to two abdominal radiographs. A second small feeding tube was then placed through the mouth or the nose into the stomach. With the subjects comfortably seated, an infusion of polyethylene glycol 4000 (1.5 g PEG/100 ml 0.15 M saline) was commenced through the proximal port of the jejunal tube at a rate of 1.4 ml/min. After allowing 30–60 min for equilibration, two basal samples were collected from the jejunal site. One of the 500-ml meals was then instilled through the gastric tube (each containing 5–10 μCi ^{99m}technetium-DTPA (^{99m}Tc) as a second nonabsorbable marker). Samples (7–8 ml) of jejunal contents were continuously siphoned on ice from the distal sampling site in 20 consecutive 10-min samples. Samples (5 ml) were taken on ice throughout the experiment each 10 min from the gastric tube beginning 5 min after ingestion of the meals.

Jejunal samples were analyzed for concentration of PEG (12), trypsin (13), and isoamylases. Flow rates passing the jejunal sampling site were calculated based on known infusion rates and PEG concentrations at infusion and sampling ports. Outputs of trypsin and total amount of amylase passing the distal sampling site were calculated from the product of concentrations and flow rates. The rate of ^{99m}Tc passage past the distal jejunal sampling site was accordingly calculated from the product of flow rates and ^{99m}Tc concentrations and was used as a measure of gastric emptying. Gastric samples were analyzed for ^{99m}Tc, PEG, isoamylases (in 12 of 17 experiments), pH, and acid content by titration of 3 ml to pH 7.0 with 0.2 N NaOH, using an automatic titrator.

One of the normal volunteers (male, age 33 years) and a patient with a known idiopathic achlorhydria (male, age 51 years) participated in one additional experiment. The achlorhydria in this subject was demonstrated previously in several gastric secretion studies; he was otherwise healthy. After a 60-min basal period, a hamburger meal consisting of 80 g fried hamburger, 110 g toasted white

bread, and 150 ml water was fed orally. The design of this additional experiment was similar to the above studies, except that no gastric tube was applied and no meal marker used.

In Vitro Studies. Pooled human gastric juice of three healthy subjects was obtained by aspiration through a gastric tube, mixed, and brought to different pHs by adding 1 N HCl or 1 N NaOH. Human saliva was obtained from laboratory personnel by chewing and pooled. Both gastric juice and saliva were centrifuged for 10 min at 2000 rpm and the supernatant filtrated through Whatman No. 3 paper. A constant volume (4.5 ml) of gastric juice of different pH (1.2–5.9) was mixed with 0.5 ml pure saliva at 37°C. Samples (100 μl) were taken out at defined time intervals from 5 to 150 min and immediately mixed with 0.02 M phosphate buffer, pH 6.9 (3.9 ml), and kept on ice. All samples were analyzed for amylase activity using the Phadebas method. The stability of pancreatic and salivary amylases in human jejunal juice was tested by mixing 2 ml jejunal juice with 2 ml saliva at 37°C. (Pooled jejunal juice for this experiment was obtained in one subject after jejunal intubation and intragastric application of an amino acid solution, as described above.) Samples (100 μl) were taken out at time intervals from 10 to 60 min in tubes containing 2.9 ml 0.02 M phosphate buffer, pH 6.9, on ice. All samples were analyzed for amylase activity (Phadebas). Trypsin activity was measured in these samples spectrophotometrically using TAME as substrate (13).

Binding of amylase to starch was tested by mixing 5 ml jejunal juice (obtained in one subject after jejunal intubation and intragastric instillation of a dextrose meal) in three different dilutions (1:1; 1:2, and 1:4, diluted with 0.154 M NaCl) with and without crushed crackers (Sunshine Biscuits, New York, New York). The samples and controls without crackers were incubated for 30 min at room temperature and then centrifuged at 2000 rpm for 10 min. Total amylase and trypsin activity was measured in the supernatants.

Validation. Seven jejunal samples containing different ratios of salivary to pancreatic amylase and five jejunal samples containing only pancreatic amylase (all obtained in the above human experiment) were subjected to isoelectric focusing as described by Renner et al (7). Generally, two major peaks (one between pH 6.18 and 6.43, and other between pH 6.64 and 7.02) were observed. The results were compared with standards containing pure saliva (see above) or pure pancreatic juice. (Pure pancreatic juice was obtained in a patient after surgery of a pancreas divisum by direct cannulation of the pancreatic duct for therapeutic reasons.)

Pure pancreatic juice showed amylase peaks at a pH higher than 6.70; pure saliva was eluted at a pH lower than 6.40. Salivary isoamylase activity was calculated by adding the amylase activity under the peaks eluting at a pH lower than 6.50, and pancreatic activity accordingly at a pH above 6.50. Salivary-to-pancreatic isoamylase ratios were calculated for each sample and compared to ratios derived by the inhibitor method.

RESULTS

In all subjects and in 13/17 experiments more than 2500 units of salivary amylase passed the sampling

TABLE 2. SALIVARY AND PANCREATIC ISOAMYLASE OUTPUTS IN JEJUNAL JUICE AFTER DIFFERENT MEALS IN 6 SUBJECTS*

Subject	*Meal*	*CPA*	*CSA*	*CSA × 100/ CTA*	*PSA*	*PSA × 100/ CSA*
1	BSA	142	176	55	116	66
	Dex	108	101	48	56	5
	AmA	81	21	20	17	57
2	BSA	86	14	14	8	54
	Dex	49	8	14	6	76
	AmA	81	4	4	3	92
	DLo	94	11	11	—	—
	CCK	185	4	2	4	100
3	BSA	84	6	7	3	50
	DLo	121	2	2	—†	—†
	CCK	130	6	4	—	—
4	BSA	123	13	9	8	59
	AmA	72	0	0	—†	—†
5	AmA	75	16	17	8	51
	DLo	89	1	1	—†	—†
6	DLo	48	1	2	—†	—†
	CCK	110	34	24	18	51
	$X \pm$ SEM	98.7 ± 8.4	24.5 ± 11.1	13.8 ± 3.9		

*CSA = cumulative salivary amylase output (KU/200 min); CPA = cumulative pancreatic amylase output (KU/200 min); CTA = cumulative total amylase output (KU/200 min); PSA = salivary amylase peak (KU/30 min). SA peaks are defined as SA outputs in three consecutive periods of >50% of cumulative total salivary amylase output.

†Only experiments with SA outputs of more than 2500 units/200 min are considered.

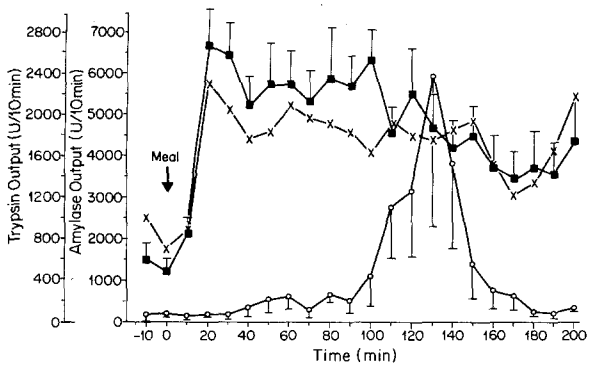

Fig 1. Outputs of trypsin, salivary amylase, and pancreatic amylase after different meals in 17 experiments ($X \pm$ SEM). ■, pancreatic amylase; ○, salivary amylase, ×, trypsin.

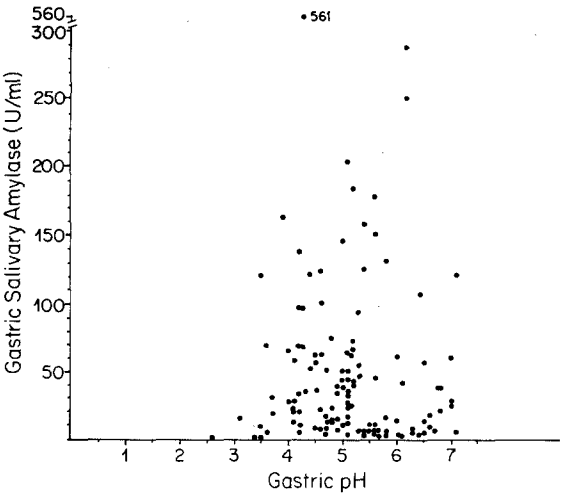

Fig 2. pH and concentration of salivary amylase in gastric aspirates taken every 10 min after different meals.

site in 200 postcibal minutes (Table 2). Salivary amylase comprised 13.8 ± 3.9% (mean ± SEM) of the total amylase output (range 0–55%). In 11 of 13 experiments salivary amylase appeared predominantly as a single, distinct peak (Table 2 and Figure 1). There was no relation between the time course of gastric emptying and the appearance of salivary amylase peaks in jejunal juice (data not shown). When salivary amylase was subtracted from total amylase outputs, parallel secretion of trypsin and pancreatic amylase outputs was recorded as shown in Figure 1 ($r = 0.846$). A significantly lower ($P < 0.05$) correlation was found between total amylase and trypsin outputs ($r = 0.699$). In only 1% of gastric samples (Figure 2) and 11% of jejunal samples (assuming a 10-min passage time from pylorus to jejunum; Figure 3) salivary amylase activity was recorded when gastric pH fell below 3.0 ($P < 0.001$). Likewise, a threshold between pH 3.8 and pH 3.3 was found *in vitro* for rapid destruction of salivary amylase in human gastric juice (Figure 4). Salivary (101 units/ml) and pancreatic (39 units/ml) amylase were stable in jejunal juice (containing 67 units/ml trypsin activity) for at least 60 min.

In the hamburger-meal experiments in both subjects, outputs of pancreatic amylase and trypsin showed an initial peak within the first 40 min and then declined to a plateau. Salivary amylase accounted for 11% of total amylase output in the normal and 27% of the achlorhydric subject. Salivary amylase in the achlorhydric subject appeared continuously during the whole experiment, whereas in the normal subject it was found only between 60 and 110 min after ingestion of the meal. The mean ratios of pancreatic amylase to trypsin for the healthy and the achlorhydric subject were 0.33 and 0.46, respectively. In the liquid meal experiments, a mean ratio of 1.63 was observed. The different ratios of amylase to trypsin may be explained by quantitative binding of amylase but not trypsin to the toasted bread as shown by *in vitro* incubation of jejunal juice with crushed crackers. Compared to the controls without crackers, the ratio of amylase to trypsin in the jejunal juice fell from 7.2 to 0.9 (mean of results with three different dilutions), which was due to a decline of amylase activity.

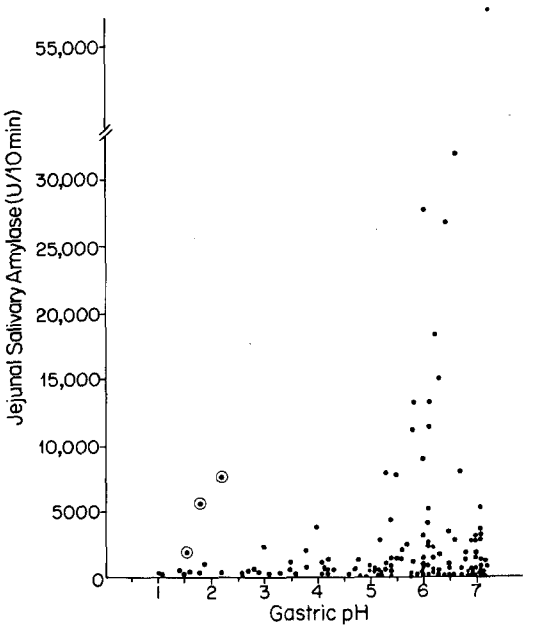

Fig 3. Outputs of salivary amylase in 10-min periods in jejunal juice and gastric pH (with a 10-min compensation for lag time of transit). Circled points are those coming from antecedent peaks.

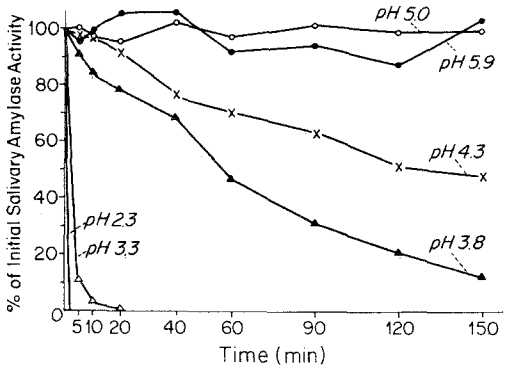

Fig 4. Time course of salivary amylase activity added to human gastric juice (37°C) brought to controlled pH levels.

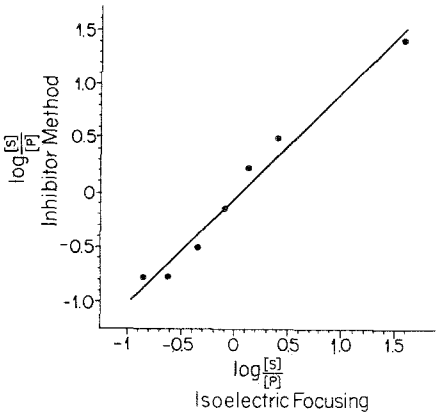

Fig 5. Plot of ratios of salivary to pancreatic isoamylases in seven human jejunal samples obtained by isoelectric focusing and the inhibitor isoamylase assay; $y = -0.041 + 0.975x$; $r = 0.988$; $P < 0.001$.

The results obtained by the inhibitor method were confirmed by isoelectric focusing in jejunal samples obtained in the liquid-meal experiments. Ratios of salivary to pancreatic amylases in seven samples obtained by the two methods were highly correlated (Figure 5; $r = 0.988$; $P < 0.001$). In additional (five) samples both methods correspondingly showed no salivary amylase content.

DISCUSSION

This study shows a significant contribution of salivary amylase to the total amylase content in the human small intestine after a variety of meals. Our findings confirm previous reports demonstrating high amounts of salivary amylase in postcibal duodenal juice of healthy infants (3, 4) and adults (2). On average, salivary amylase accounted for 14% of total amylase in our study. This figure corresponds well with a 15% relative contribution after a Lundh meal reported by Skude and Ihse (2), who measured isoamylase activity by gel electrophoresis. In contrast, Banks et al (1), using the same inhibitor assay technique, could not detect salivary amylase in single samples of intestinal juice from 16 patients aspirated from various levels of the intestine. However, the few samples analyzed in this study were obtained from subjects under poorly defined conditions; six patients were fasting.

Gastric pH is an important factor for the intact passage of salivary amylase through the stomach. This is evident in our study, as we found little of salivary amylase in jejunal samples, when gastric pH was lower than 3.0. Also, gastric samples were nearly free of salivary amylase at this low pH. These *in vivo* findings were confirmed by *in vitro* experiments which showed a threshold for inactivation in gastric juice between pH 3.3 and 3.8. Our findings confirm a recent report by Rosenblum et al (14) that salivary amylase is more and more rapidly destroyed in gastric juice as the pH falls progressively below 4.0. Considering the importance of gastric pH, it is possible that the highly buffered protein and amino acids meals in our study facilitated the intact gastric transit of salivary amylase. On the other hand, we found significant amounts of salivary amylase in jejunal samples after two pure dextrose meals. Considerable amounts of salivary amylase in jejunal contents were also found after a hamburger meal in a healthy subject. Malagelada et al (15) demonstrated that gastric pH after a meal consisting of a steak, a sandwich, and ice cream stays above pH 3.0 for 60 min; in this period salivary amylase may pass intact through the stomach. The influence of gastric pH is also demonstrated by the finding of a 2.5-times higher ratio of salivary to total amylase in an achlorhydric compared to a normochlorhydric subject. In our study salivary amylase may be further protected during the hamburger meal by maltriose and other starch digestion products (14).

In most of the subjects salivary amylase in jejunal juice appeared as a single distinct peak. The occurrence of this peak was not predictable, being neither related to changes in gastric pH nor to the time course of gastric emptying (data not shown). The concentration of salivary amylase in saliva floating on top of gastric contents may explain these findings. In analogy to the floating of fat in the stomach (16), it would be preferably emptied at the time of final emptying of gastric contents, but depending on

the configuration of the stomach, an earlier emptying of the floating saliva is conceivable.

Our findings are important for the interpretation of postcibal studies of pancreatic secretion. The contamination of jejunal samples with salivary amylase may be significant after a variety of meals, although the amount of contamination was highly variable within and between the subjects. Furthermore, the time of entry of salivary amylase into the intestine was unpredictable. Amylase should therefore not be used as a measure of human pancreatic secretion postprandially unless specific pancreatic isoamylases are measured. The inhibitor assay technique in jejunal samples was validated by isoelectric focusing and shown to be specific, resulting in an improved correlation between trypsin and pancreatic isoamylase outputs.

Our observation sheds new light on studies of pancreatic secretion after gastric surgery. We found that patients who had undergone truncal vagotomy secreted about half the normal output of trypsin after a meal but maintained normal levels of amylase in the proximal small bowel (17, 18). Others have also noted that the ratio of total amylase to trypsin in the proximal intestinal lumen increases markedly after gastric resection (19). Since these operations reduce the secretion of gastric acid, it is possible that the increased ratio of total amylase to trypsin postoperatively reflected a reduced intragastric destruction of salivary amylase by acid and, thus, a greater contamination of pancreatic amylase in the duodenum with the salivary isoenzyme. This hypothesis is supported by the findings in our achlorhydric patient, but further studies in patients with truncal vagotomy and partial gastrectomy are needed.

In addition, one could argue that, analogous to lingual lipase for lipid digestion (20), salivary amylase may be important as an alternative pathway for the digestion of glucose polymers in situations where pancreatic amylase output is diminished. This concept was already proposed in neonates with a physiologic deficiency of pancreatic amylase and a diminished gastric acidity (4). In adults it is consistent with the observation by Skude and Ihse (2), of a higher relative contribution of salivary amylase (40%) in patients with chronic pancreatic insufficiency.

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