Inhibition of Serum and Urine Amylase Activity in Pancreatitis with Hyperlipemia

ANDREW L. WARSHAW, M.D., CYNTHIA A. BELLINI, B.S.,
PAUL B. LESSER, M.D.

In 6 of 7 patients with acute pancreatitis and hyperlipemia, inhibition of serum amylase activity was detected by dilution of the serum before assaying for amylase and by correcting for the dilution factor. In 4 patients the inhibition phenomenon disappeared within the first few days of hospitalization as the elevated serum triglycerides fell. However, in 2 others there was no relation between triglyceride level and amylase inhibition. Removal of the excess serum lipids by ultracentrifugation did not eliminate the inhibition of amylase activity. Inhibition of amylase activity also occurred in the urine of these patients. No amylase inhibition was demonstrable in lipemic serum from patients without pancreatitis or in pancreatitis serum to which excess lipids were added. The data suggest the presence of a circulating inhibitor of amylase, distinct from the elevated serum lipids, in the serum and urine of patients with acute pancreatitis associated with hyperlipemia. The diagnosis of acute pancreatitis in the patient with abdominal pain and lactescent serum can be facilitated by correcting the serum amylase activity by dilution.

From the Departments of Surgery and Medicine, Harvard Medical School, The Shriners Burns Institute, and the General Surgical Services and Gastrointestinal Unit, Massachusetts General Hospital, Boston, Massachusetts

LIPIDS, especially triglycerides, are abnormally elevated in the sera of 12-38% of patients presenting with acute pancreatitis,[1,3,5,6] gross lactescence being visible in about half of these.[1] In such patients serum amylase activity, the most widely-used laboratory index of acute pancreatitis, may fail to rise.[1,3,4,6,7] It has been postulated that the excessive lipid either interferes with the chemical measurement of amylase activity[4,6] or inhibits the enzymatic action of amylase.[1] Recently Fallat, Vester, and Glueck showed that serial dilution of hyperlipemic pancreatitis serum allows a relative increase in amylase activity and suggested that the excessive serum triglycerides were responsible for the lowered amylase activity.[4] The findings of the present study suggest instead the existence of a circulating inhibitor of amylase in some patients with acute pancreatitis, usually coinciding temporally with hypertriglyceridemia but not being the triglycerides per se.

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Reprint requests: Andrew L. Warshaw, M.D., Department of Surgery, Massachusetts General Hospital, Boston, Mass. 02114.

Materials and Methods

Six patients with lactescent serum and clinically unequivocal severe acute pancreatitis were encountered during a prospective study of 60 patients with acute pancreatitis at the Massachusetts General Hospital. Serum and urine were collected at the time of admission, refrigerated immediately, and assayed within 24 hours. Control specimens were obtained from normal healthy volunteers and from hyperlipemic patients without clinical pancreatitis.

Amylase activity in serum and urine was determined by a direct saccharogenic method in which the liberation of reducing groups (maltose) is detected with 3,5-dinitrosalicylic acid.[11] The period of incubation used in this assay was generally 30 min at room temperature, but was varied in some experiments so that all results would fall within the range of linearity for the assay. Normal serum amylase activity in this laboratory ranges from 20-90 mμmoles of maltose/min.

To detect inhibition of "true" amylase activity, specimens were serially diluted up to 16 times. The value for amylase activity in a diluted sample was multiplied by the dilution factor to give a "corrected amylase activity." The phosphate buffer used in the amylase assay (0.02

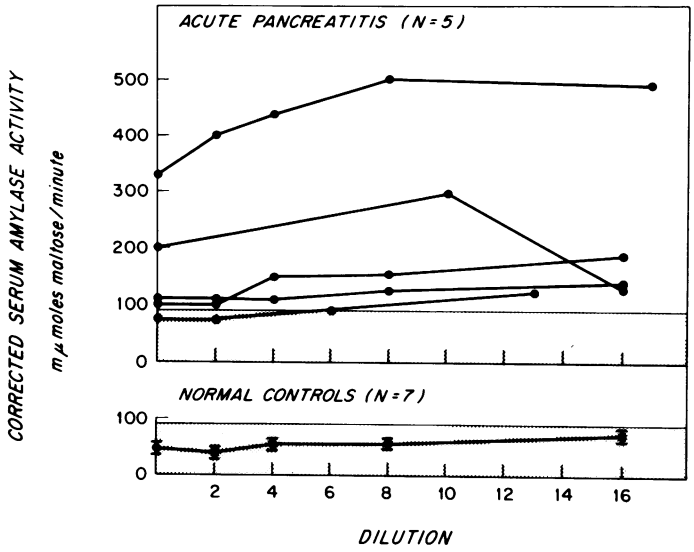

FIG. 1. Unchanged amylase activity after serial dilution of non-lipemic serum. In the upper section each curve is a different patient and each point is the mean of duplicate samples. In the lower section the curve shows the mean for 7 patients and the standard error of the mean at each dilution.

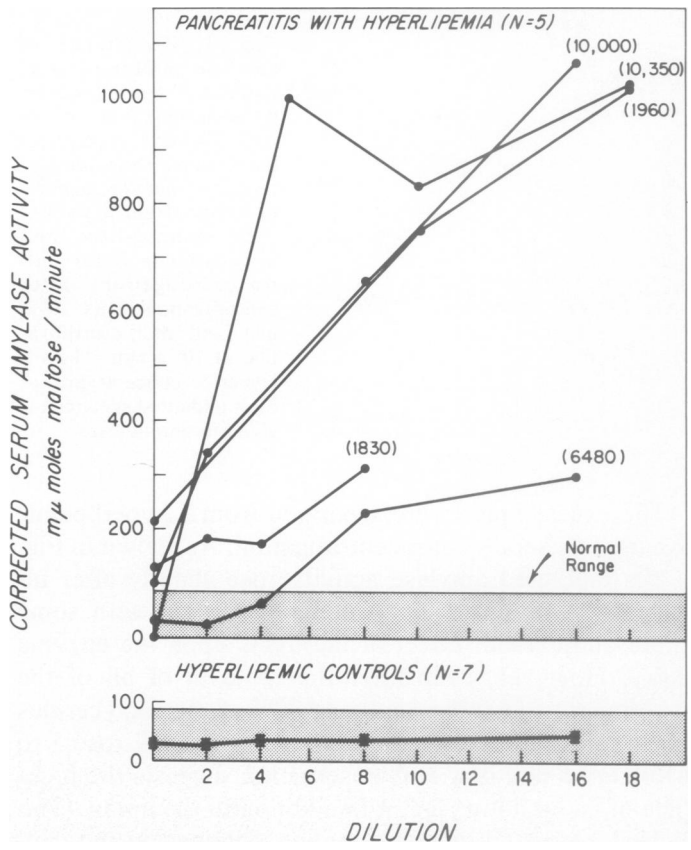

FIG. 2. Increased corrected amylase activity after dilution of hyperlipemic serum from patients with pancreatitis. In the upper section each curve is a different patient and each point is the mean of duplicate samples. The triglyceride concentration (mg/100 ml) of each undiluted specimen is given in parentheses. In the lower section the curve shows the mean for 7 patients and the standard error of the mean at each dilution.

M,pH 6.9, containing 0.006 M NaCl) was used for making serial dilutions of samples.

Triglycerides were measured according to Kessler and Lederer.[8] Ultracentrifugation was performed with a Beckman L-50 refrigerated ultracentrifuge.

Results

The corrected amylase activity in normal serum did not change significantly upon 16-fold serial dilution (Fig. 1). Similarly dilution produced no consistent change in the amylase activity of 5 sera from patients with acute pancreatitis not associated with hyperlipemia (triglycerides < 150 mg/100 ml) (Fig. 1). Dilution of 1 specimen of pure human pancreatic secretions, obtained by cannulation of the pancreatic duct, had no effect upon its corrected amylase activity.

In contrast, the corrected amylase activity in serum from 5 patients with lactescent serum (mean triglycerides = 6100 mg/100 ml) increased strikingly upon serial dilution (Fig. 2). A point of dilution was always reached beyond which no further increase in corrected amylase activity occurred. Control serum (mean triglycerides = 2200 mg/100 ml) from 5 hyperlipemic patients without pancreatitis showed no significant change in amylase activity after dilution (Fig. 2).

TABLE 1. *Corrected Amylase Activity After Dilution (16x) of Urine From Normal Volunteers or From Patients with Pancreatitis and Hyperlipemia.*

Normal Volunteers mμmoles maltose/min			Pancreatitis with Hyperlipemia mμmoles maltose/min		
undiluted	diluted	change%	undiluted	diluted	change%
111	110	−1	1176	3880	+230
37	35	−5	670	2150	+221
100	290	+190	61	299	+390
32	39	+22	1600	2240	+40
72	138	+92	365	1388	+280
100	126	+26		Mean change	+232
74	90	+22			
62	64	+3			
113	170	+50			
	Mean change	+44			

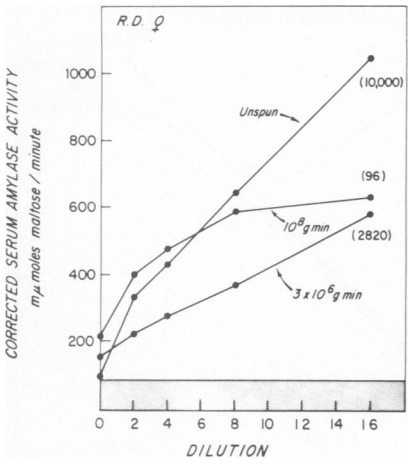

FIG. 3. Persistence of amylase inhibition after removal of serum lipids by ultracentrifugation. In this representative experiment the 3 curves show the correction of amylase activity of serum from a patient with pancreatitis and hyperlipemia before ultracentrifugation, after centrifugation at 3x 10^6 g min, and after centrifugation at 10^6 g min. The triglyceride concentration of each undiluted specimen is given in parentheses.

The excess lipids were separated from 2 hyperlipemic pancreatitis sera by ultracentrifugation. As shown in Fig. 3, the undiluted amylase activity rose slightly after ultracentrifugation alone, a finding consistent with some minor suppressant effect of the lipids upon the enzyme assay. However, even after the removal of all of the chylomicrons (3 x10^6 g min) and 99% of the triglycerides (10^6 g min) the corrected amylase activity still rose 3 to 4-fold upon dilution. This observation suggests the presence of an inhibiting factor in addition to the lipids. (The highest corrected amylase activity obtained in the centrifuged specimens may be a little lower than that in the uncentrifuged specimens because of the interval of two days required for ultracentrifugation and re-assay: after simple storage of unspun "inhibited" serum for several days, the observed rise in corrected serum amylase activity falls off).

Amylase activity was determined in urine obtained from 8 normal individuals and from 5 patients with hyperlipemic pancreatitis and evidence of serum amylase suppression. As shown in Table 1 the mean rise in corrected amylase activity after 16-fold dilution was 44% in normal urine but 232% in urine from patients with serum amylase suppression. This difference is significant ($P < 0.01$ by the Mann-Whitney U Test for non-parametric groups). Since negligible quantities of triglycerides and other

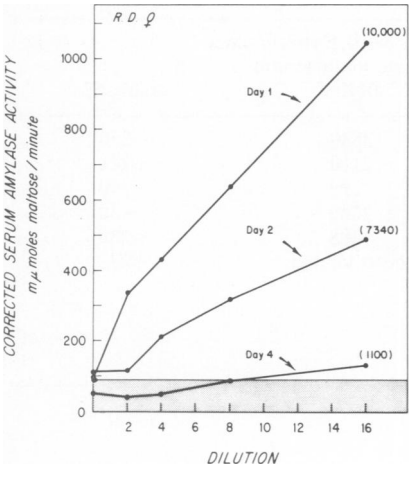

FIG. 4. Loss of amylase inhibition as the pancreatitis wanes. Serial samples taken from this patient, representative of the 4 studied on multiple occasions, showed a progressive decline in the changes of amylase activity detected by dilution. The serum triglycerides for each undiluted sample are given in parentheses.

TABLE 2. *Addition of Triglycerides to Pancreatitis Serum.*

	no incubation	incubation
Pancreatitis serum + buffer	220	240
Pancreatitis serum + normal serum	320	340
Pancreatitis serum + triglycerides	280	340

Pancreatitis serum was mixed in equal parts either with phosphate buffer, normal human serum, or hyperlipemic serum (triglycerides = 2700 mg/100 ml). Amylase activity (mμmoles maltose/minute) was determined before and after incubation at 37° for 1 hr.

serum lipids appear in the urine, the inhibiting factor in urine cannot be the triglycerides.

To test for direct inhibition of amylase activity by triglycerides, non-lipemic serum from a patient with pancreatitis was mixed in equal parts with either phosphate buffer, normal serum, or hyperlipemic serum (triglycerides = 2700 mg/100 ml) and assayed for amylase activity. As shown in Table 2, there was no suppression of amylase activity by the lipemic serum. Incubation of the mixture for 1 hour at 37 C before assaying for amylase also did not lower amylase activity (Table 2). These findings are evidence against the inhibiting factor being the serum triglycerides or being the product of interaction between serum lipids and enzymes or other substances in pancreatitis serum.

Serum obtained on successive days after admission from 4 patients with hyperlipemic pancreatitis showed a progressive loss of amylase inhibition as demonstrated by dilution (Fig. 4). This change temporally coincided in 3 of the 4 with falling triglyceride levels.

In the latter days of the study, 2 patients with clear-cut pancreatitis were found who exemplified the dissociation of amylase-inhibition from massive hypertriglyceridemia (Fig. 5). One was a woman with known Type IV hyperlipoproteinemia, mild triglyceride elevation (190 mg/100 ml), and a 4-fold rise in amylase activity. The other was an alcoholic with markedly elevated triglycerides (3970 mg/100 ml) but no amylase inhibition.

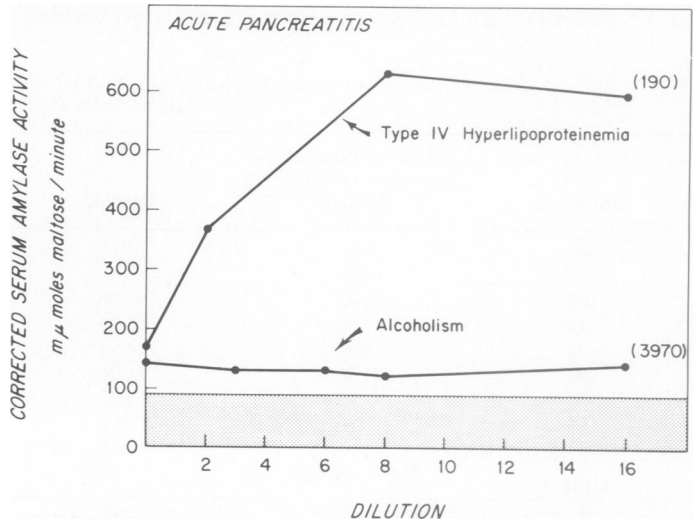

FIG. 5. Dissociation between serum amylase inhibition and triglyceride concentration. Each curve represents 1 patient with acute pancreatitis.

Discussion

An increased incidence of pancreatitis or abdominal pain has been associated with several of the categories of hyperlipoproteinemias, but most reports of bona fide acute pancreatitis have occurred in association with a Type I or Type V hyperlipoproteinemia pattern,[2,5] as was the case for 5 out of 6 of our hyperlipemic patients. Of these 6, 4 were alcoholics, as were a similarly high percentage in Cameron's series.[1,2]

This study confirms that suppression or inhibition of serum amylase activity occurs frequently[1,3,4,6,7] in patients who have acute pancreatitis associated with hyperlipemia. The mechanism of amylase inhibition has been disputed. Fallat, Vester, and Glueck,[4] using a starch-iodine amyloclastic assay for amylase, showed a reduction of apparent amylase activity when an artificial triglyceride suspension was added to the assay medium. They felt that triglycerides directly suppressed the amylase activity, perhaps interfering with light transmission in the colorimetric assay. Indirect support for that view can be drawn from the experience of Farmer and his associates[5] who in their study of 10 hyperlipemic pancreatitis patients noted uniformly elevated amylase activity in centrifuged or ether-washed serum (although they did not measure amylase activity before removing the lipids). Others report that addition of triglycerides does not suppress measured amylase activity.[10] Also, the presence or absence of inhibition has not correlated with the degree of endogenous triglyceride elevation either in our experience or in that of Cameron's group.[3]

Evidence from this study suggests that the serum lipids are not directly responsible for the inhibition of amylase activity. Amylase-inhibition correlated with the period of time, the first few days after admission, during which the triglycerides were elevated, but did not correlate with exogenous manipulation of triglyceride levels. Ultracentrifugation of lipemic inhibited serum allowed only a slight rise in amylase activity, which was further augmented by dilution. Addition of human serum lipids in a concentration theoretically sufficient to suppress amylase activity (1250 mg/100 ml) did not lower the elevated amylase activity of pancreatitis serum. In contrast to the findings of Fallat, Vester, and Glueck,[4] dilution of naturally-occurring lipemic serum from patients without acute pancreatitis did not produce any change in corrected amylase activity. Amylase activity in urine, which has very little lipid content, was also inhibited in patients with serum inhibition. And finally, the dissociation between amylase inhibition and serum triglyceride concentration is graphically illustrated by the 2 patients represented in Figure 5.

The inhibition of urinary amylase is of particular interest since it helps to explain the observation by Cameron's group[1] that urinary amylase levels in hyperlipemic patients with pancreatitis, like their serum amylase levels, are often normal and not elevated. In spite of the seemingly low amylase activities in serum and urine, we have found that the amylase/creatinine clearance ratio, which rises in acute pancreatitis,[12] rises also in hyperlipemic patients with pancreatitis and its measurement is an effective aid to diagnosis.[9]

These findings suggest that there is a circulating inhibitor of amylase in the serum of many patients with pancreatitis associated with hyperlipemia. Although the elevated serum lipids may have some minor direct effect, lowering apparent amylase activity by interfering with colorimetric analysis, most of the inhibitory effect appears to be associated with a non-lipid factor which can also be filtered into the urine. The origin and nature of this amylase inhibitor in unknown, but it is reasonable to postulate that it is either a lipid derivative or related to the underlying defect in lipid metabolism which characterizes these patients.[2,6]

The clinical importance of the phenomenon of inhibition of amylase activity lies in its capacity to delude physicians into disregarding the diagnosis of acute pancreatitis. With a normal serum amylase the diagnosis is difficult to make unless one accepts the dictum that abdominal pain in a patient with lactescent serum means pancreatitis.[1,3] As has been found by others also,[4] correction of the amylase activity by dilution (2-16x) and appropriate recalculation unmasks the "true" hyperamylasemia.

Acknowledgments

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References

1. Cameron, J. L., Capuzzi, D. M., Zuidema, G. D. and Margolis, S.: Acute Pancreatitis with Hyperlipemia: The Incidence of Lipid Abnormalities in Acute Pancreatitis. Ann. Surg., 177:483, 1973.
2. Cameron, J. L., Capuzzi, D. M., Zuidema, G. D. and Margolis, S.: Acute Pancreatitis With Hyperlipemia. Am. J. Med., 56:482, 1974.
3. Cameron, J. L., Crisler, C., Margolis, S., et al.: Acute pancreatitis with hyperlipemia. Surgery, 70:53, 1971.
4. Fallat, R. W., Vester, J. W. and Glueck, C. J.: Suppression of Amylase Activity by Hypertriglyceridemia. JAMA, 225:1331, 1973.
5. Farmer, R. G., Winkelman, E. I., Brown, H. B. and Lewis, L. A.: Hyperlipoproteinemia and Pancreatitis. Am. J. Med., 54:161, 1973.
6. Greenberger, N. J., Hatch, F. T., Drummey, G. D. and Isselbacher, K. J.,: Pancreatitis and Hyperlipemia: A Study of Serum Lipid Alterations in 25 Patients With Acute Pancreatitis. Medicine, 45:161, 1966.
7. Havel, R. J.: Pathogenesis, Differentiation and Management of Hypertriglyceridemia. Ad. Intern. Med., 15:117, 1969.
8. Kessler, G. and Lederer, H.: Automation in Analytical Chemistry. New York, Mediad, Inc., 1965; p. 341.
9. Lesser, P. B. and Warshaw, A. L.: Diagnosis of Pancreatitis Masked by Hyperlipemia. Ann. Int. Med., in press.
10. Mishkin, S., O'Hashi, J. and Schneider, P.: Amylase Activity in Hyperlipemic Pancreatitis. JAMA, 228:568, 1974.
11. Warshaw, A. L.: Studies on Isoenzymes of Human Amylase: Evidence for a Circulating Amylase Not of Pancreatic or Salivary Origin. J. Surg. Res., 16:360, 1974.
12. Warshaw, A. L. and Fuller, A. F.: Specificity of Increased Renal Clearance of Amylase in Diagnosis of Acute Pancreatitis. N. Eng. J. Med., 292:325, 1975.