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Histopathologic Correlates of Serum Amylase Activity in Acute Experimental Pancreatitis

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The association of serum amylase activity with the extent of pancreatic injury in acute pancreatitis is unclear. To clarify this relationship, we induced acute pancreatitis ranging from mild to lethal in 118 Sprague-Dawley rats (350–450 g). This was achieved by controlled intraductal infusion of low- or high-dose bile salt, with or without enterokinase, followed by intravenous cerulein or saline for 6 hr. Serum amylase was measured at baseline and 6 hr. Pancreatic histopathology was evaluated by two blinded pathologists employing total surface scoring (N = 118) and morphometric 20-field documentation (N = 22). Serum amylase correlated best with edema (r = 0.61) and fat necrosis (r = 0.58), less well with acinar necrosis (r = 0.53) and inflammation (r = 0.50), and poorly with hemorrhage (r = 0.33) and perivascular infiltrate (r = 0.31). Inasmuch as edema and fat necrosis are not important determinants of severity, these observations could explain the poor prognostic value of serum amylase activity in patients with acute pancreatitis.

KEY WORDS: acute pancreatitis; experimental pancreatitis; serum amylase; histopathology; correlation.

Numerous efforts have been undertaken to evaluate the sensitivity, specificity, and predictive value of total serum amylase activity in acute pancreatitis (1–6). The interpretation of these data, however, is hampered by the etiologic heterogeneity of pancreatitis in patient populations, variations of elapsed time between onset of symptoms and blood sampling, and the lack of histopathologic data at a time when the diagnosis of acute pancreatitis has been established. Although frequently elevated, serum amylase activity can be normal or decreased in a substantial number of severely ill patients (7). A potential correlation with pancreatic histopathology and severity of pancreatitis therefore has been challenged repeatedly (3, 8, 9). Despite these drawbacks, many investigators utilize the level of serum amylase activity as laboratory confirmation for severity of pancreatitis in clinical and experimental settings (6, 10, 11). To our knowledge no systematic study has been undertaken to evaluate potential associations of serum amylase with pancreatic histopathology or with specific elements of pancreatic injury.

To study this question, we produced acute pancreatitis in rats with a broad histopathologic spectrum of severity under controlled experimental conditions. Using total surface histopathologic scoring, morphometric analysis, and multiple regression

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TABLE 1. INDUCTION TECHNIQUES

	Group									
Technique	*Control 1*	*GDOC 5 2*	*GDOC 10 3*	*GDOC 34 4*	*CE 5*	*CE/ GDOC 5 6*	*CE/ GDOC 10_1 7*	*CE/ GDOC 10_2 8*	*CE/ GDOC 10_{10} 9*	*EK 10*
Intravenous	Saline	Saline	Saline	Saline	Cerulein	Cerulein	Cerulein	Cerulein	Cerulein	Cerulein
Intraductal	Saline	GDOC 5 mmol/ liter	GDOC 10 mmol/ liter	GDOC 34 mmol/ liter	Saline	GDOC 5 mmol/ liter	GDOC 10 mmol/ liter	GDOC 10 mmol/ liter	GDOC 10 mmol/ liter	GDOC 10 mmol/ liter
Enterokinase (IU/ml)										125
Volume (ml)	0.10–0.15							0.25	0.5	0.5
Duration (min)	1–1.5							2.5	10	10

analysis, we identified and ranked those histopathologic variables that, correlated best with hyperamylasemia.

MATERIALS AND METHODS

Animals. One hundred eighteen male Sprague-Dawley rats (350–450 g) were used and housed individually in hanging wire-bottomed cages in rooms maintained at 21 ± 1° C using a 12-hr dark cycle. Care was provided in accordance with the procedures outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication #85-23, 1985). The animals were given a standard rat chow and fasted overnight prior to the experiment with water allowed *ad libitum*.

Anesthesia and Catheter Placement. Surgical anesthesia was induced with vaporized ether and maintained by an intraperitoneal injection of pentobarbital (20 mg/kg, Anthony Products, Arcadia, California 91006) and ketamine (40 mg/kg, Ketalar, Parke-Davis, Morris Plains, New Jersey 07950). The right internal jugular vein was cannulated using soft polyethylene tubings (Silastic, ID 0.02 in., Dow Corning, Midland, Michigan 48640). Another catheter (Intramedic, ID 0.023 in., Clay Adams, Parsippany, New Jersey 07054) was placed in the left carotid artery for blood sampling. Both catheters were tunneled subcutaneously to the suprascapular area and brought out via a flow-through tether, which permitted free movement.

Induction of Pancreatitis. After baseline blood samples had been obtained, the abdomen was opened by a midline incision. The duodenum and the common biliopancreatic duct were identified and the duodenal wall was punctured at its antimesenteric aspect with a 24-gauge Teflon catheter (Critikon, Tampa, Florida 33630). The catheter was carefully advanced into the biliopancreatic duct for 5 mm (there is no main pancreatic duct in the rat) through the papilla of Vater. A fine 6/0 prolene suture was placed around the cannula at the duodenal wall to prevent backflow. Special attention was focused on atraumatic surgical technique. The upper portion of the animal was then elevated 60 degrees for 5 min (with the duct cannula in place) to allow the duct system to empty of bile and pancreatic juice. During the last 2 min of this procedure the main hepatic duct was clamped just below the liver to facilitate complete drainage of pancreatic secretions. This clamp remained in place throughout the intraductal infusion to prevent maldirected flow into the biliary system.

The animals were allocated to 10 different regimens that produce a graded range in severity of acute pancreatitis (Table 1).

Intraductal Infusion. Freshly prepared glycodeoxycholic acid (GDOC, Sigma, St. Louis, Missouri 63178, #G-3258) in glycylglycine–NaOH-buffered solution (pH 8.0, room temperature) at concentrations of 5, 10, or 34 mmol/liter was infused at a pressure of 30 mm Hg controlled via mercury manometer for designated periods with the volume controlled by means of an interposed scaled glass tube. In control group 1 and cerulein (CE) group 5 isotonic saline was infused intraductally instead of GDOC. Animals in group 10 received intraductal porcine enterokinase (EK, 125 IU/ml, Sigma, #E0885) in GDOC (10 mmol/liter). When the infusion volume entered the duct faster than the predetermined time, the infusion was stopped when the required volume was accepted by the duct with the infusion system left in place until the time limit was reached. This technique ensured standardized volume and time exposure to the infusate. The catheter was removed and duodenal wall was closed with a 6/0 prolene suture and the abdomen with 4/0 nylon. The animals were then placed back in cages with minimal environmental stimuli (12).

Intravenous Infusion. Animals in groups 5–10 received a continuous intravenous infusion of cerulein at 5 μg/kg/hr (Farmitalia, Carlo Erba GmbH, 7800 Freiburg, Germany) for 6 hr. Cerulein was reconstituted in normal saline and infused at 1 ml/hr. In control group 1 and groups 2–4 the same volume of normal saline was used.

Serum Amylase Determination. Serum amylase activity was determined at baseline and 6 hr or after death using the colorimetric method initially described by Bernfeld (13) and modified by Warshaw (14), a direct saccharogenic technique using 3,5-dinitrosalicylic acid (DNS) to measure the liberation of reducing groups resulting from

the digestion of starch. The results are expressed in international units (μmol/liter/min).

Histopathologic Analysis. Surviving animals were sacrificed by a lethal dose of pentobarbital (200 mg/kg intravenously) 6 hr after induction of acute pancreatitis and rapidly exsanguinated through the indwelling arterial line. The entire pancreas was removed immediately after death or sacrifice and fixed in 10% buffered formalin in anatomical orientation for histpathologic analysis. After dividing the organ into a duodenal and splenic portion and embedding each in paraffin, one longitudinal section through each of the two parts was stained with hematoxylin–eosin. The total surface of the slides was scored for six different variables determining severity of inflammation (N = 118) by two pathologists with expertise in pancreatic pathology (C.C., K.L.), who were unaware of the induction technique used; in addition careful morphometric documentation for edema, acinar necrosis, inflammation, and hemorrhage was obtained in groups 3 and 7 by mapping the pancreas into 20 fields and evaluating each field independently (N = 22). The following standardized scoring system was applied:

Histopathologic Scoring Criteria

Edema

0 = absent
0.5 = focal expansion of interlobar septae
1 = diffuse expansion of interlobar septae
1.5 = same as 1 + focal expansion of interlobular septae
2 = same as 1 + diffuse expansion of interlobular septae
2.5 = same as 2 + focal expansion of interacinar septae
3 = same as 2 + diffuse expansion of interacinar septae
3.5 = same as 3 + focal expansion of intercellular spaces
4 = same as 3 + diffuse expansion of intercellular spaces

Acinar necrosis

0 = absent
0.5 = focal occurrence of 1–4 necrotic cells/HPF
1 = diffuse occurrence of 1–4 necrotic cells/HPF
1.5 = same as 1 + focal occurrence of 5–10 necrotic cells/HPF
2 = diffuse occurrence of 5–10 necrotic cells/HPF
2.5 = same as 2 + focal occurrence of 11–16 necrotic cells/HPF
3 = diffuse occurrence of 11–16 necrotic cells/HPF (foci of confluent necrosis)
3.5 = same as 3 + focal occurrence of >16 necrotic cells/HPF
4 = >16 necrotic cells/HPF (extensive confluent necrosis)

Hemorrhage and fat necrosis

0 = absent
0.5 = 1 focus
1 = 2 foci
1.5 = 3 foci
2 = 4 foci
2.5 = 5 foci
3 = 6 foci
3.5 = 7 foci
4 = 8 or more foci

Inflammation and perivascular infiltrate

0 = 0–1 intralobular or perivascular leukocytes/HPF
0.5 = 2–5 intralobular or perivascular leukocytes/HPF
1 = 6–10 intralobular or perivascular leukocytes/HPF
1.5 = 11–15 intralobular or perivascular leukocytes/HPF
2 = 16–20 intralobular or perivascular leukocytes/HPF
2.5 = 21–25 intralobular or perivascular leukocytes/HPF
3 = 26–30 intralobular or perivascular leukocytes/HPF
3.5 = more than 30 leukocytes/HPF or focal microabscesses
4 = more than 35 leukocytes/HPF or confluent microabscesses

Statistical Analysis. All data are presented as mean ± SEM. The continuous variable serum amylase activity was analyzed using the Student's t test between groups and the paired Student's t test within groups. Since dynamic changes are of interest the analysis was performed by calculating the difference between the 6-hr and the baseline measurement (increase in amylase activity).

Categorical variables (histopathologic scores) were analyzed with the modified analysis of variance for discrete data (Proc CATMOD, SAS Software Inc., Cary, North Carolina).

For multiple regression analysis, the data were pooled and correlation coefficients calculated using the Spearman rank correlation method (Proc CORR, SAS Software). Data were then stratified according to animals receiving intraductal bile salt at various concentrations alone (groups 2–4), intravenous cerulein alone (group 5), or intravenous cerulein in combination with intraductal bile salt (groups 6–9). The regression analysis was then repeated for the subgroups.

Histopathologic scores of 20 analyzed fields in the morphometric documentation were added within each individual animal to form a sum score for each variable. The data were then entered in multiple regressions with the corresponding Δ-amylase value as for total surface scores.

RESULTS

Total Amylase Activity. Serum amylase levels remained constant in control animals as well as in both low-dose GDOC groups (5–10 mmol/liter) (groups 2 and 3), but increased significantly in animals receiving intraductal GDOC at 34 mmol/liter (group 4). All animals that received intravenous cerulein demonstrated a dramatic increase in serum amylase activity that was most pronounced when intraductal exposure to GDOC was superim-

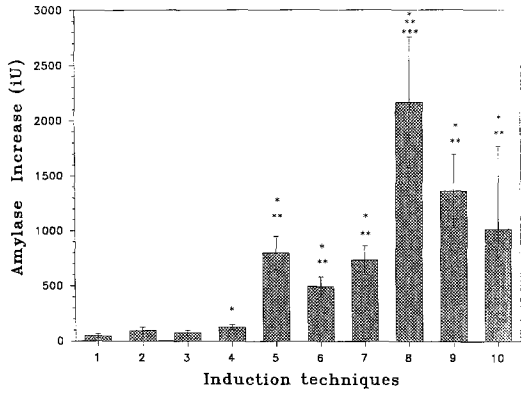

Fig 1. Increase in serum amylase activity over baseline 6 hr after induction of acute pancreatitis with different techniques producing increasing histopathologic severity. The asterisks above the groups indicate significant differences compared to controls (*), compared to GDOC alone (groups 2–4) (**) or compared to groups 6 and 7 (***).

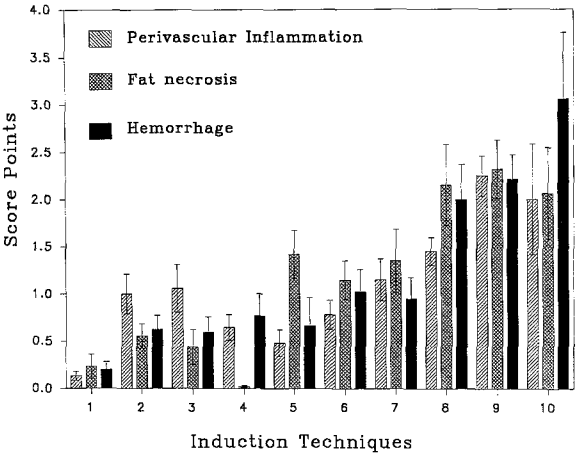

Fig 3. Mean histopathologic scores for perivascular infiltrate, fat necrosis, and hemorrhage in the different induction groups. All pancreatitis groups had significantly higher values than controls.

posed for more than 1.5 min at a volume greater 0.1–0.15 ml (groups 8 and 9) (Figure 1).

Mean Histopathologic Scores. All pancreatitis animals had significantly higher mean scores for all histopathologic variables than controls ($P < 0.05$). Total surface scoring in control animals demonstrated only mild edema, which was predominantly located in the duodenal portion (pancreatic head). With increasing severity of the pancreatitis-inducing stimulus, we observed a gradual increase in the degree of acinar necrosis, inflammation, and edema (Figure 2) with a similar trend for perivascular infiltrate, fat necrosis, and hemorrhage (Figure 3).

Multiple Regression Analysis. The increase of amylase activity (baseline vs 6 hr) correlated best with edema and fat necrosis across the entire histopathologic range of severity. Lower correlation coefficients were noted for acinar necrosis, inflammation, hemorrhage, and perivascular infiltrate (Figure 4). The only subgroup of animals in which there was a moderate correlation with acinar necrosis was that receiving intravenous cerulein alone, and that correlation disappeared when intraductal GDOC (5, 10, or 34 mmol/liter) was superimposed (Table 2). The morphometric analysis demonstrated that the increase of amylase activity correlated best with edema ($r = 0.75$, $P = 0.005$) and moderately well with inflammation ($r = 0.62$, $P = 0.03$) if retrograde infusion of intraductal bile salt (10 mmol/liter) was the sole pancreatitis-inducing agent. The complete results of the morphometric analysis are depicted in Table 3.

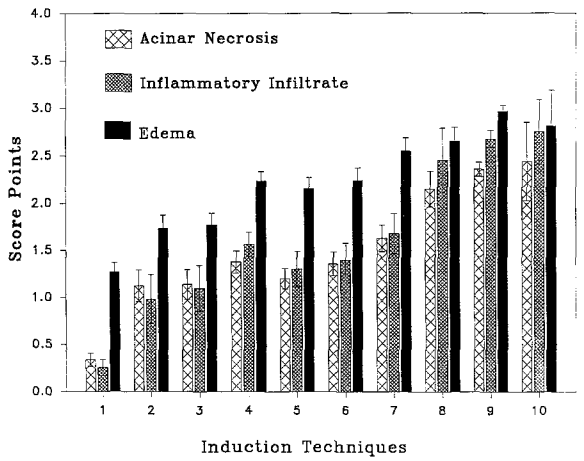

Fig 2. Mean histopathologic scores for edema, acinar necrosis, and inflammatory infiltrate in the different induction groups. All pancreatitis groups had significantly higher values than controls.

DISCUSSION

The mainstay of diagnosis in acute pancreatitis remains the clinical evaluation, supplemented by determination of serum pancreatic enzyme activities. Although other laboratory tests are more specific (4), total serum amylase is still the most important single screening parameter within the first 24 hr after the onset of suspected acute pancreatitis (2, 3, 6, 10, 15, 16).

Numerous attempts have been made in the past to assess sensitivity, specificity, and predictive value of serum amylase (3, 4, 6, 9, 15, 17). While some controversy exists (10), most authors agree that both the specificity and predictive value of the degree of serum amylase elevation are poor (5, 9).

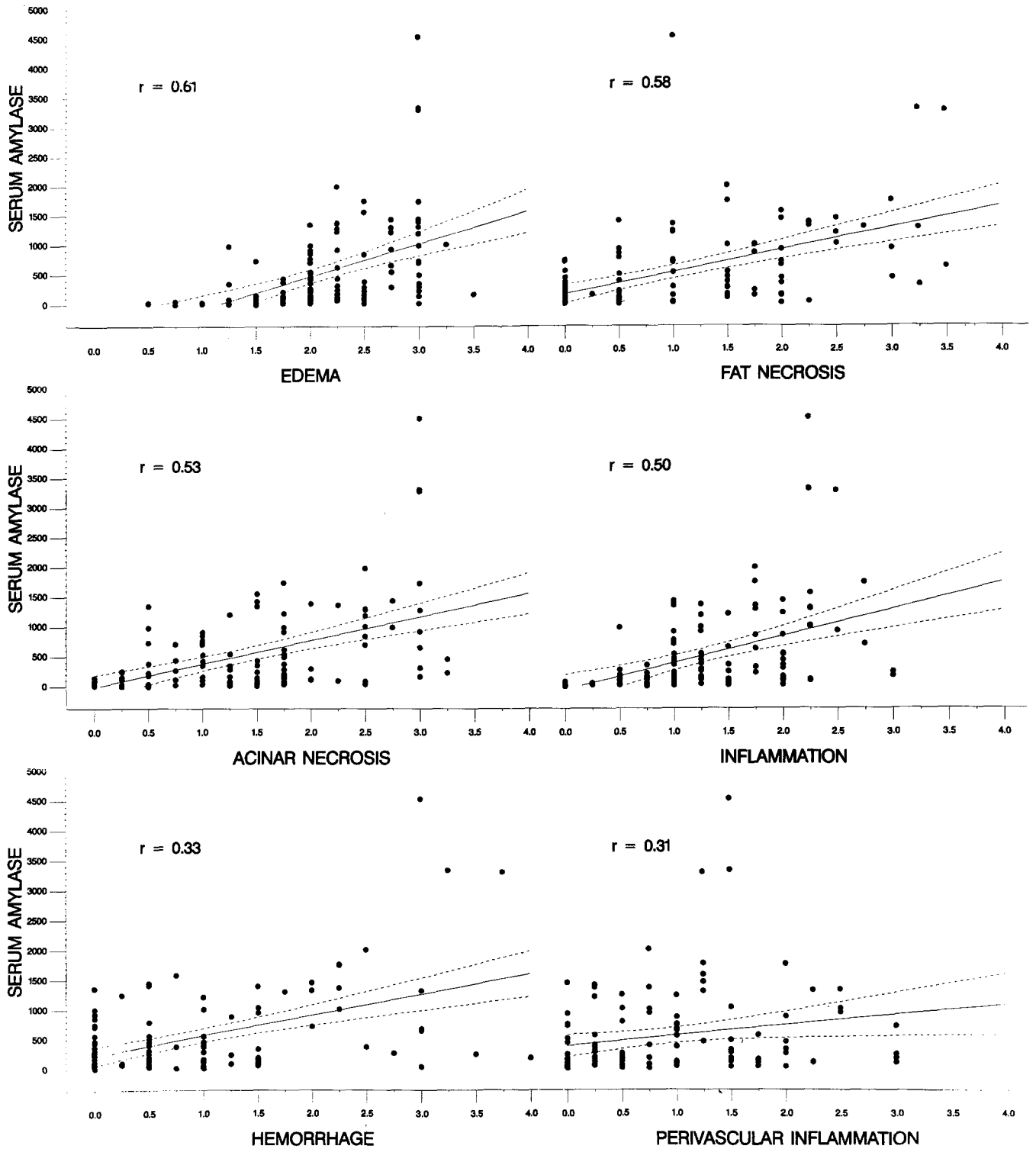

Fig 4. Regression plots of the increment in serum amylase against histopathologic total surface scores for edema, fat necrosis, acinar necrosis, inflammation, hemorrhage, and perivascular infiltrate. Due to the large number of animals studied, all given r values are true with a very low type I error probability (P = 0.0001–0.008). Interrupted lines indicate 95% confidence limits.

TABLE 2. SUBGROUP ANALYSIS OF TOTAL SURFACE SCORES: SPEARMAN RANK CORRELATION

Pancreatitis groups	N	*Edema*	*Fat necrosis*	*Acinar necrosis*	*Inflammation*	*Hemorrhage*	*Perivascular inflammation*
Bile salt only	39	$r = 0.56$ ($P = 0.0002$)	$r = -0.09$ (P = NS)	$r = 0.19$ (P = NS)	$r = 0.26$ (P = NS)	$r = 0.14$ (P = NS)	$r = 0.33$ ($P = 0.04$)
Cerulein only	21	$r = 0.43$ (P = NS)	$r = 0.32$ (P = NS)	$r = 0.63$ ($P = 0.002$)	$r = 0.55$ ($P = 0.009$)	$r = 0.29$ (P = NS)	$r = 0.28$ (P = NS)
Cerulein + low-dose bile salt	39	$r = 0.28$ (P = NS)	$r = 0.32$ ($P = 0.05$)	$r = 0.44$ ($P = 0.006$)	$r = 0.33$ ($P = 0.04$)	$r = 0.26$ (P = NS)	$r = 0.13$ (P = NS)

Even an inverse correlation between this parameter and severity of acute pancreatitis has been described and was attributed to the decline in secretory capacity when pancreatic cell damage is severe (7, 18). Data confirming this concept were reported by Nordestgaard et al (8), who studied the clinical correlation of serum amylase activities with CT-based morphologic findings, etiology, and outcome of acute pancreatitis in patients and indeed found an inverse relationship.

Perhaps the most comprehensive overview of the dynamics of serum amylase derailment in health and pancreatitis was given by Gullick (19). The author measured serum amylase in 200 patients with either mild, moderate, or severe acute or chronic pancreatic disease and compared the results with those of healthy volunteers. An important finding in that study was that a given amylase level has a bell-shaped clinicomorphologic equivalent in severity of pancreatitis, with low amylase values being indicative of either minimal or extensive injury and highest values being secondary to intermediate acinar damage. This concept, supported by his results, would explain the lack of a correlation between serum amylase activity and severity of inflammation because the interplay of severe and mild injury with similar amylase levels would counteract any positive linear relationship.

Alpha-amylase is produced in all exocrine pancreatic cells with especially high concentrations in periinsular regions (20). Under normal conditions only minor amounts of this enzyme escape the directed apical secretion into the ductal lumen (1). When acute pancreatic inflammation is present, significant elevations of amylase activity in serum and urine can occur via lymphatic, vascular, and peritoneal absorption (21–24). The degree of serum activity increase depends upon both the rate of production and the degree of maldirected release into the interstitial compartment or into the peritoneal cavity, as well as on its circulating half-life, which is mainly determined by renal clearance (1). Due to the enormous secretory capacity of the pancreas [it is the human tissue with the highest protein synthesis rate (25)] there is reason to believe that leaking but still viable acinar cells can cause a greater and longer-lasting serum amylase elevation than necrotic cells, which cease new enzyme production (19). This analysis is compatible with the observation that very high levels of serum amylase are found in cerulein pancreatitis, which is characterized by interstitial edema and minimal cell death (26, 27). Simultaneous leakage of pancreatic lipase could account for the development of fat necrosis, another typical feature of cerulein pancreatitis.

There are very few previous studies of histopathologic correlation with serum amylase in experimental acute pancreatitis. Gibbs and Ivy (28) reported that edema roughly parallels serum amylase elevation in dogs. Sokolowski et al (29) reported a positive correlation with gross morphologic findings after experimental pancreatitis in the rat, but Spormann et al later found no correlation with pancreatic histopathology (30). Schönberg et al. (31) re-

TABLE 3. MORPHOMETRIC ANALYSIS (20 HPF/ANIMAL): SPEARMAN RANK CORRELATION

Pancreatitis groups	N	*Edema*	*Acinar necrosis*	*Inflammation*	*Hemorrhage*
All	22	$r = 0.64$ ($P = 0.001$)	$r = 0.53$ ($P = 0.01$)	$r = 0.59$ ($P = 0.004$)	$r = -0.005$ (P = NS)
Bile salt only	12	$r = 0.75$ ($P = 0.005$)	$r = -0.18$ (P = NS)	$r = 0.62$ ($P = 0.03$)	$r = -0.09$ (P = NS)
Cerulein + low-dose bile salt	10	$r = 0.19$ (P = NS)	$r = 0.18$ (P = NS)	$r = -0.26$ (P = NS)	$r = -0.46$ (P = NS)

cently confirmed that observation. However, none of the cited studies was specifically designed to investigate this relationship, and their findings were merely incidental to other purposes.

In the present study we have utilized two factors, an intraductal and an intravenous component, to induce acute pancreatitis with a range of severity resembling the spectrum found in most clinical populations. Previous studies from this laboratory have shown that the combination of low concentrations of intraductal bile salt with intravenous cerulein causes an injury that can be modulated in severity, with 24-hr mortality rates ranging from 8% (group 7) to 20% (group 8) and 42% (group 9) by altering the intraductal infusion time and volume of diluted bile salt solutions (10 mmol/liter) (32). The combination of cerulein and bile salt is synergistic, the product of the combination far exceeding that caused by either component alone. The further addition of enterokinase increases the 24-hr mortality to 100% (group 10). With five fatalities out of 118 animals, the overall mortality was 4.2% at 6 hr. Our prior experience with these techniques has shown the mortality at 24 hr to increase to 9–12%, which is similar to clinical experience in man (33–36).

Multiple regression analysis comparing the increase in amylase activity with various histopathologic variables in these experiments revealed that the amylase increment correlates poorly in general with the extent of pancreatic injury. Among the variables examined, pancreatic edema, not considered to be an index of severity in pancreatitis, had the best correlation with hyperamylasemia, and extrapancreatic fat necrosis was second best. The usual indices of severe pancreatitis—acinar necrosis, hemorrhage, intralobular inflammation, and perivascular infiltrate—correlate poorly with the level of serum amylase. Thus this study provides an experimental basis to support clinical observations that have suggested that the level of serum amylase has no prognostic value in acute pancreatitis.

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REFERENCES

1. Salt WB, Schenker S: Amylase—its clinical significance: A review of the literature. Medicine 55:269–289, 1976
2. Steinberg WM, Goldstein SS, Davis ND, Shamma'a J, Anderson K: Diagnostic assays in acute pancreatitis: A study of sensitivity and specificity. Ann Intern Med 102:576–580, 1985
3. Agarwal N, Pitchumoni CS, Sivaprasad AV: Evaluating tests for acute pancreatitis. Am J Gastroenterol 85:356–366, 1990
4. Tietz NW, Huang WY, Rauh DF, Shuey DF: Laboratory tests in the differential diagnosis of hyperamylasemia. Clin Chem 32:301–307, 1986
5. Puolakkainen PA: Early assessment of acute pancreatitis. A comparative study of computed tomography and laboratory tests. Acta Chir Scand 155:25–30, 1988
6. Robert JH, Meyer P, Rohner A: Can serum and peritoneal amylase and lipase determinations help in the early prognosis of acute pancreatitis? Ann Surg 203:163–168, 1986
7. Clavien PA, Robert J, Meyer P, Borst F, Hauser H, Hermann F, Dunand V, Rohner A: Acute pancreatitis and normoamylasemia. Not an uncommon combination. Ann Surg 210:614–620, 1989
8. Nordestgaard AG, Wilson SE, Williams RA: Correlation of serum amylase levels with pancreatic pathology and pancreatitis etiology. Pancreas 3:159–161, 1988
9. Moossa AR: Diagnostic tests and procedures in acute pancreatitis. N Engl J Med 311:639–643, 1984
10. Dubick MA, Mar G, Mayer AD, Majumdar APN, McMahon MJ, Geokas MC: Digestive enzymes and protease inhibitors in plasma from patients with acute pancreatitis. Pancreas 2:187–194, 1987
11. Evander A, Ihse I, Lundquist I: Influence of hormonal stimulation by caerulein on acute experimental pancreatitis in the rat. Eur Surg Res 13:257–268, 1981
12. Yamaguchi H, Kimura T, Nawata H: Does stress play a role in the development of severe pancreatitis in rats? Gastroenterology 98:1682–1688, 1990
13. Bernfeld P: Enzymes of starch degradation and synthesis. Adv Enzymol 12:379, 1951
14. Warshaw AL: Studies on isoenzymes of Human amylase: Evidence for a circulating amylase not of pancreatic or salivary origin. J Surg Res 16:360–365, 1974
15. Gwozdz GP, Steinberg WM, Werner M, Henry JP, Pauley C: Comparative evaluation of the diagnosis of acute pancreatitis based on serum and urine enzyme assays. Clin Chim Acta 187:243–254, 1990
16. Lin XZ, Wang SS, Tsai YT, Lee SD, Shiesh SC, Pan HB, Su CH, Lin CY: Serum amylase, isoamylase, and lipase in the acute abdomen. J Clin Gastroenterol 11:47–52, 1989
17. Eckfeldt JH, Kolars JC, Elson MK, Shafer RB, Levitt MD: Serum tests for pancreatitis in patients with abdominal pain. Arch Pathol Lab Med 109:316–319, 1985
18. Adams JT, Libertino JA, Schwartz SI: Significance of an elevated serum amylase. Surgery 63:877–884, 1968
19. Gullick HD: Relation of the magnitude of blood enzyme elevation to severity of exocrine pancreatic disease. Am J Dig Dis 18:375–383, 1973
20. Bendayan M, Ito S: Immunohistochemical localization of exocrine enzymes in normal rat pancreas. J Histochem Cytochem 27:1029–1034, 1979

21. Mayer AD, Airey M, Hodgson J, McMahon MJ: Alimentary tract and pancreas. Enzyme transfer from pancreas to plasma during acute pancreatitis. The contribution of ascitic fluid and lymphatic drainage of the pancreas. Gut 26:876–881, 1985
22. Foulis AK: Acute pancreatitis. Recent Adv Histopathol 12:188–196, 1984
23. Dubick MA, Mayer AD, Majumdar APN, Mar G, McMahon MJ, Geokas MC: Biochemical studies in peritoneal fluid from patients with acute pancreatitis. Relationship to etiology. Dig Dis Sci 32:305–312, 1987
24. Turner MD, Correll WW, Fain WR, Conn JH, Cockrell JV: Enzyme levels in pancreatic tissue and body fluids during experimental pancreatitis. J Surg Res 3:485–490, 1963
25. Case RM: Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol Rev 53:211–354, 1978
26. Adler G, Rohr G, Kern HF: Alteration of membrane fusion as a cause of acute pancreatitis in the rat. Dig Dis Sci 27:993–1002, 1982
27. Klöppel G, Dreyer T, Willemer S, Kern HF, Adler G: Human acute pancreatitis: Its pathogenesis in the light of immunocytochemical and ultrastructural findings in acinar cells. Virchows Arch Pathol Anat Histol 409:791–803, 1986
28. Gibbs GE, Ivy AC: Early histological changes following obstruction of pancreatic ducts in dogs: Correlation with serum amylase. Proc Soc Exp Bio Med 77:251–254, 1951
29. Sokolowski A, Spormann H, Urbahn H, Letko G: Contribution of pancreatic edema and short-term ischemia to experimental acute pancreatitis in the rat. II. Behaviour of serum parameters. Z Exp Chir Transpl Kuenstl Organe 19:331–339, 1986
30. Spormann H, Sokolowski A, Letko G: Experimental acute pancreatitis—a quantification of dynamics at enzymic and histomorphologic levels. Pathol Res Pract 185:358–362, 1989
31. Schönberg MH, Buechler M, Gaspar M, Stinner A, Younes M, Melzner I, Bueltmann B, Beger HG: Oxygen free radicals in acute pancreatitis of the rat. Gut 31:1138–1143, 1990
32. Schmidt J, Rattner DW, Compton CC, Lewandrowski K, Mandavilli U, Warshaw AL: Acute pancreatitis of graded severity. A new experimental tool to study pathogenesis and therapy in pancreatic inflammation. Digestion 46:174–175, 1990
33. Jacobs ML, Daggett WM, Civetta JM, Vasu MA, Lawson DW, Warshaw AL, Nardi GL, Bartlett MK: Acute pancreatitis: Analysis of factors influencing survival. Ann Surg 185:43–51, 1977
34. De Bolla AR, Obeid MI: Mortality in acute pancreatitis. Ann R Coll Surg Engl 66:184–186, 1984
35. Becker V: Pathological anatomy and pathogenesis of acute pancreatitis. World J Surg 5:303–313, 1981
36. Frey CF: Hemorrhagic pancreatitis. Am J Surg 137:616–623, 1979