Immunoreactive Forms of Cationic Trypsin in Plasma and Ascitic Fluid of Dogs in Experimental Pancreatitis

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A canine model of bile-induced pancreatitis has been employed to investigate time-dependent changes in the molecular forms of trypsin in blood and ascitic fluid in this disease. The distribution of immunoreactive trypsin as trypsinogen and trypsin bound to plasma inhibitors in ascitic fluid and plasma during the course of the disease has been investigated by means of a radioimmunoassay for canine pancreatic cationic trypsin. In addition, trypsinlike amidase activity was determined in plasma and ascitic fluid using Z-Gly-Gly-Arg-β-Nap as substrate. Early plasma and ascitic fluid samples in four dogs that died contained primarily trypsinogen, while extensive activation of trypsinogen to α_2-macroglobulin and α_1-protease inhibitor-bound trypsin occurred in the course of the disease. A fifth dog survived and showed little activation of trypsinogen. In the four dogs that died, the levels of trypsinlike amidase activity in the ascitic fluid were substantial throughout the course of the disease. The plasma levels of trypsinlike activity in these animals were much lower, but increased during the disease process. The dog that survived had lower concentrations of trypsinlike activity in ascitic fluid and plasma. These results suggest that activation of trypsinogen resulting in inhibitor-bound forms of trypsin in ascitic fluid and plasma is important in the pathogenesis of acute pancreatitis. (Am J Pathol 1981, 105:31–39)

IT HAS LONG BEEN postulated that inappropriate activation of zymogens in the pancreatic parenchyma plays an important role in the pathogenesis of acute pancreatitis. Furthermore, it has been proposed that many of the complications[1-7] of this disease result from release of active enzymes into the surrounding tissues, the abdominal cavity, and the circulatory system.[8-12] However, despite more than 30 years of vigorous investigation, the molecular events concerning the role of proteolytic enzymes in the pathogenetic mechanisms in acute pancreatitis remain largely unknown. Previous investigators have employed enzymatic methods to study the involvement of pancreatic proteases, particularly trypsin, in acute pancreatitis. These attempts have been unsuccessful due to the lack of a specific substrate for pancreatic trypsin, poor sensitivity, and, more importantly, the inability of the enzymatic methods to detect either the zymogen form of the enzyme or trypsin bound to α_1-protease inhibitor (α_1-PI).

In this laboratory, we have employed a highly sensitive and specific radioimmunoassay technique to detect and characterize the molecular forms of immunoreactive pancreatic cationic trypsin in biologic fluids in man. We have demonstrated that the zymogen is the only detectable form of this enzyme in the circulation of normal individuals.[13] Furthermore, the concentration of this immunoreactive material is greatly elevated in random blood samples from patients with acute pancreatitis.[14] In many of these patients, the major circulating form is also trypsinogen.[14] In the more severe cases of acute pancreatitis, however, significant amounts of trypsin are present bound to the protease inhibitors α_2-macroglobulin (α_2-M) and α_1-PI.[14] Since proteases bound to α_2-M remain active toward polypeptides of low to moderate molecular weight,[15-17] it appears possible that the se-

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verity of acute pancreatitis may be correlated with the degree of activation of pancreatic zymogens and the appearance of α_2-M-bound proteases in blood and ascitic fluid.

In the present study, we have employed a radioimmunoassay technique for canine pancreatic cationic trypsin to characterize the molecular forms of this enzyme in the circulation and ascitic fluid of dogs during experimentally induced pancreatitis. The time course for the appearance of trypsinlike amidase activity in plasma and ascitic fluid has also been investigated.

Materials and Methods

Induction of Pancreatitis

Five young female dogs (1-4 years old), each weighing 23-31 kg were deprived of food but not water for 18 hours. Gentamycin (1 mg/kg body weight) and clindamycin (5 mg/kg body weight) were administered intramuscularly at 12 and 2 hours before surgery. The dogs were sedated with 2-2.5 ml of Innovar-Vet (Pitman-Moore, Inc.) and anesthetized with an intravenous dose (15-30 mg/kg body weight) of pentobarbital sodium. Pancreatitis was induced by injection of autologous bile into the main pancreatic duct following the procedure of Geokas et al.[18] After induction of pancreatitis, the pancreas was observed for 5-10 minutes before closure of the abdominal wall. Immediately following injection of bile, the tail and portions of the head and body of the pancreas became edematous with a glassy yellow-brown appearance. With the exception of Dog 5, focal areas of hemorrhage and blue-black discoloration became evident.

Monitoring

A systemic arterial catheter was inserted via a cutdown site over the left femoral artery. Systemic arterial pressure, pulse, and respiratory rate were monitored with an Electronics for Medicine VR6 instrument and recorded before surgery and at hourly intervals thereafter. A Foley catheter (No. 12) was inserted into the urinary bladder and attached to a urinary collection bag, which facilitated hourly recording of urinary output and permitted testing for the presence of glucose with urinary dipsticks. Rectal temperature was recorded hourly with a thermistor sensor. Blood samples were obtained to determine venous hematocrit at 4-hour intervals. Serum levels of sodium, potassium, chloride, blood urea nitrogen, and glucose were determined at approximately 12-hour intervals. Throughout each experiment, additional pentobarbital sodium was administered intravenously to maintain light anesthesia.

Management

Vigorous life support was provided for each dog. During abdominal surgery, additional fluid losses were compensated by administering intravenous fluids (0.9% saline/5% dextrose in water) at 150% of maintenance requirements (0.75 ml/kg body weight/hr).[19] Fluid therapy was adjusted each hour, according to alterations in systemic pressure, pulse, and urine output. We attempted to maintain urinary output of 0.12-0.25 ml/kg body weight/hr. In the event of oliguria or hypovolemic shock, intravenous fluids were administered at 2.5 ml/kg body weight/hr for 30-60 minutes and then adjusted according to the response. If 2+ or greater glycosuria was detected, 0.9% saline without dextrose was used and serial blood glucose values were determined. Body temperature was maintained at 37-39 C by using electrically heated water blankets. Gentamycin (1 mg/kg body weight) and clindamycin (5 mg/kg body weight) were given at 8- and 6-hour intervals respectively. If complete renal shutdown occurred gentamycin was continued in the same dose but at 24-hour intervals.

Sampling

A No. 19 butterfly needle, with heparin lock attachment, was inserted into a large vein. Blood samples were drawn in syringes containing 52 μl of 0.32 M EDTA and 500 μg of polybrene before surgery, at 1 and 2 hours after surgical induction of pancreatitis, and at 2-4 hour intervals thereafter. Ascites samples were obtained 2 hours after surgery, and at 2-4-hour intervals thereafter. This procedure was performed by inserting a No. 19 needle into alternate flanks of the abdomen and gently aspirating approximately 5 cc of ascitic fluid. Records of the total amount of blood and ascitic fluid removed were maintained.

Postmortem Examination

The abdominal cavity was examined within 30 minutes of death. The surgical incision was reopened, the ascitic fluid was carefully aspirated, and the volume was determined. The abdominal organs were examined for evidence of edema, fat necrosis, inflammation, hemorrhage, and thrombosis. The pancreas was removed and the extent of disease in its anatomic parts was graded according to the degree of edema, hemorrhage, and fat necrosis. Tissue samples from

the head, body, and tail of the pancreas were fixed in 15% buffered formalin. Microscopic sections were stained in hematoxylin and eosin and Miller's elastic or elastachrome and subjected to microscopic interpretation by a pathologist who was unaware of the clinical outcome in each experiment.

Gross specimens and microscopic sections from the head, body, and tail of each pancreas were graded according to the severity of pancreatitis. A scale of 1+ to 4+ was used to define the degree of infiltration by polymorphonuclear leucocytes, the extent of fat necrosis, and the extent of parenchymal necrosis and vascular changes attributable to acute pancreatitis (hemorrhage, thrombosis, and elastolysis). An average of 1+-2+ was defined as mild acute hemorrhagic pancreatitis, whereas 2+-3+ represented moderate acute hemorrhagic pancreatitis; 3+-4+ changes were interpreted as severe acute hemorrhagic pancreatitis.

Radioimmunoassay for Canine Cationic Trypsin

The procedure for radioimmunoassay of total immunoreactive cationic trypsin in plasma and ascitic fluid was performed in a manner similar to that described previously for human cationic trypsin.[13] Canine cationic trypsin was purified to homogeneity for use as standard, preparation of tracer, and as antigen for preparation of specific antiserum. A dilution of 1:400,000 of specific antiserum was employed for the radioimmunoassay. Samples were diluted with standard assay buffer consisting of 50 mM Tris-HCl (pH 7.6) containing 0.14 M NaCl, 0.4% (wt/vol) bovine serum albumin and 0.12% normal rabbit IgG.

Gel Filtration Experiments

Aliquots of plasma (100–200 μl) or ascitic fluid (50–100 μl) were applied to 1.2 × 90-cm columns of Bio-Gel A-0.5m equilibrated with 50 mM Tris-HCl (pH 7.6) containing 0.14 M NaCl and eluted with the same buffer. The molecular size distribution of immunoreactive cationic trypsin in plasma and ascitic fluid was determined by radioimmunoassay of 100 or 200 μl aliquots of each fraction. Samples were diluted with assay buffer consisting of 0.1 M Tris-HCl (pH 8.0) containing 0.14 M NaCl, 0.4% (wt/vol) bovine serum albumin and 0.12% normal rabbit IgG. The conditions for detection of canine immunoreactive cationic trypsin bound to α-M were established by performing *in vitro* experiments as previously described for human cationic trypsin.[14] To detect α-M bound cationic trypsin, 200 μl aliquots from column fractions containing α-M were adjusted to pH 3.0 by addition of 30 μl of 0.32 M formic acid and incubated for 1 hour at 37 C, followed by radioimmunoassay in duplicate.[14]

Trypsinlike Amidase Activity

The trypsinlike activity was determined using Z-Gly-Gly-Arg-β-Nap.[20] The Z-Gly-Gly-Arg-β-Nap was dissolved at a concentration of 2.2×10^{-5} M. For each plasma or ascitic fluid sample, incubation times of 15, 30, 45, and 60 minutes were employed in order to demonstrate the linearity of each assay. A 0.5 ml aliquot of substrate in H_2O was mixed with 1 ml of 40 mM Tris-HCl, 10 mM $CaCl_2$ (pH 8.2), and 0.5 ml of H_2O containing 5–15 μl of plasma diluted 1/10 or 1/50 or with ascitic fluid diluted 1/1000. After the appropriate incubation period at 37 C, the reaction was stopped by addition of 0.2 ml of 1 M sodium citrate (pH 4.5), and the fluorescence was read at an emission of 415 nm and excitation of 335 nm. The trypsin equivalents/ml were obtained by comparing the change in fluorescence/60 min/μl of sample with that obtained for a solution of bovine trypsin which had been titrated[21] to establish the concentration of active trypsin. Statistical significance was determined using the Student *t* test.

Amylase Assays

Plasma and ascitic fluid amylase values were determined with the Amylochrome test kits (Roche Diagnostics, Nutley, NJ).

Results

Clinical Course

Four of the animals died at 17.0, 17.5, 27.6, and 29 hours, respectively, but the remaining dog (Dog 5) survived and was sacrificed at 66 hours after surgery. All dogs developed sustained tachycardia within 2 hours of surgery. In the animals that died spontaneously, pulmonary/systemic blood pressures were adequately maintained until 3–6 hours before death, when hypotension unresponsive to fluid therapy occurred. All dogs developed glycosuria, but blood glucose levels were controlled by eliminating dextrose from intravenous fluid therapy until blood glucose returned to normal. Severe oliguria or complete renal shutdown occurred in all dogs that died spontaneously. Dog 5, however, maintained adequate urine output until 54 hours after surgery, at which time oliguria ensued. Ascites fluid accumulated, producing gross distention of the abdominal wall within 10 hours, and at postmortem examination 1200–1800 ml of

ascitic fluid was recovered, except from the dog that survived. Plasma hematocrit increased by up to 60% or greater by 10 hours in all dogs. In the animal that survived (Dog 5), plasma hematocrit fell significantly at 30-40 hours after surgery, at which time the volume of ascitic fluid also decreased noticeably. Within 2 hours of induction of pancreatitis, ascitic fluid was clinically apparent within the peritoneal cavity.

Postmortem Findings

The gross appearance of the abdominal contents in 4 of the 5 animals (except Dog 5) revealed moderate (2 dogs) or severe (2 dogs) changes. Abdominal organs were discolored and edematous with large focal areas of inflammation, hemorrhage, and fat necrosis. The ascitic fluid was sanguineous. In contrast, the appearance of the abdominal organs of Dog 5 showed mild (1 +) edema and minimal inflammation, hemorrhage, and fat necrosis. In all dogs except Dog 5, the pancreas was discolored and had large areas of necrosis and hemorrhage, particularly in the body and the tail. The pancreas of Dog 5 was edematous with minimal hemorrhage and necrosis. Histologic examination of the head, body, and tail of each pancreas revealed that 4 of 5 dogs had developed moderate (2 dogs) or severe (2 dogs) acute hemorrhagic pancreatitis. Microscopic sections from Dog 5, however, showed mild to moderate (1 + -2 +) acute hemorrhagic pancreatitis.

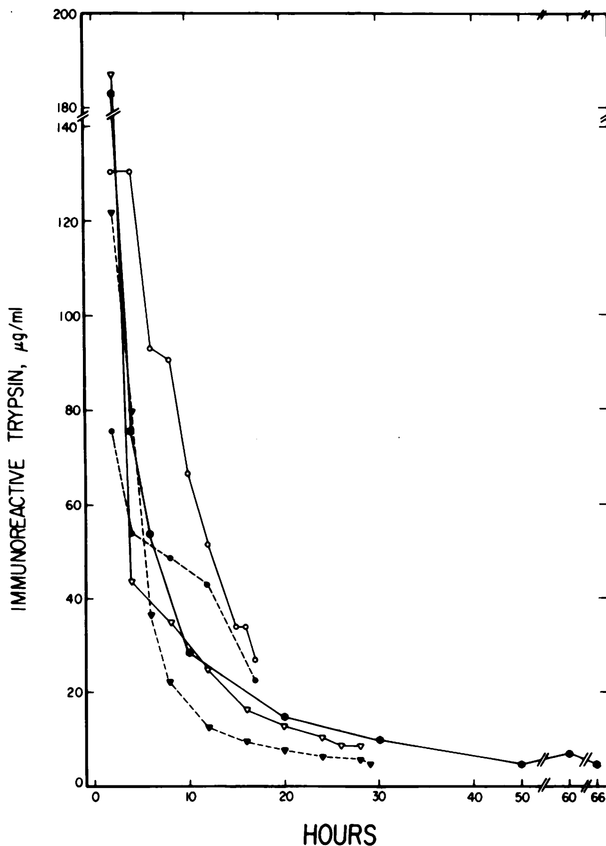

Figure 1—Total immunoreactive cationic trypsin in ascitic fluid. Total immunoreactive trypsin includes trypsinogen and approximately 75% of the trypsin bound to α_1-PI, as described in the text. Trypsin bound to α-M is not detected under the assay conditions employed for these samples. ●——●, Dog 1; O——O, Dog 2; ▽——▽, Dog 3; ▼——▼, Dog 4; ●——●, Dog 5.

Immunoreactive Cationic Trypsin

Total Immunoreactive Cationic Trypsin

As shown in Figure 1, at 2 hours after induction of pancreatitis, total immunoreactive trypsin concentrations in the ascitic fluid were extremely high, with a range of 75-190 μg/ml. There was then a dramatic fall in the concentration of immunoreactive trypsin to approximately 1/10 of the peak concentration. This fall was probably due in large part to the dilution effect of progressively accumulating ascitic fluid.

One hour after induction of pancreatitis, total immunoreactive trypsin concentrations in the peripheral circulation were also greatly elevated (Figure 2). In 4 animals (Dogs 1-4), plasma immunoreactive trypsin levels declined over 4-20 hours and then gradually rose until death. Dog 5, which survived until sacrificed at 66 hours, showed an early decline from the

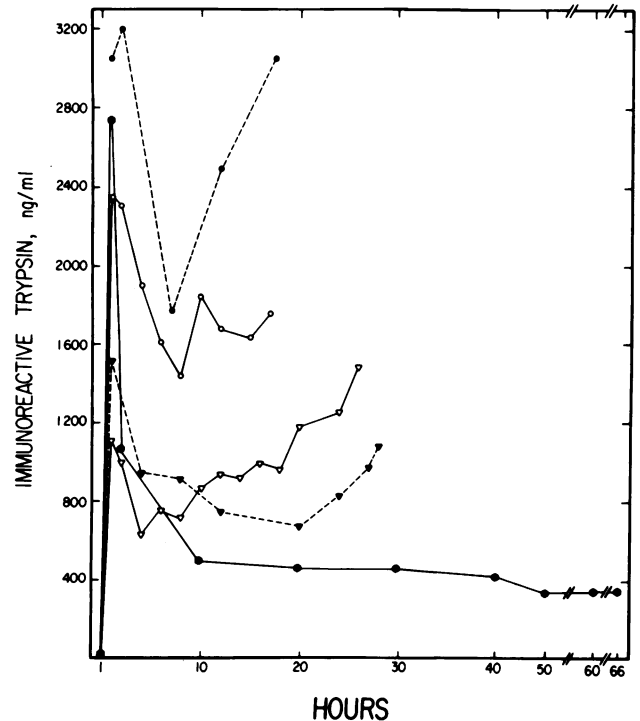

Figure 2—Total immunoreactive cationic trypsin in plasma. See legend for Figure 1 for explanation of total immunoreactive trypsin and for explanation of symbols.

peak concentration of circulating immunoreactive trypsin, but failed to exhibit a secondary rise.

Amylase values were obtained for both ascitic fluid and plasma in 3 animals and these values closely paralleled changes in the concentration of total immunoreactive trypsin.

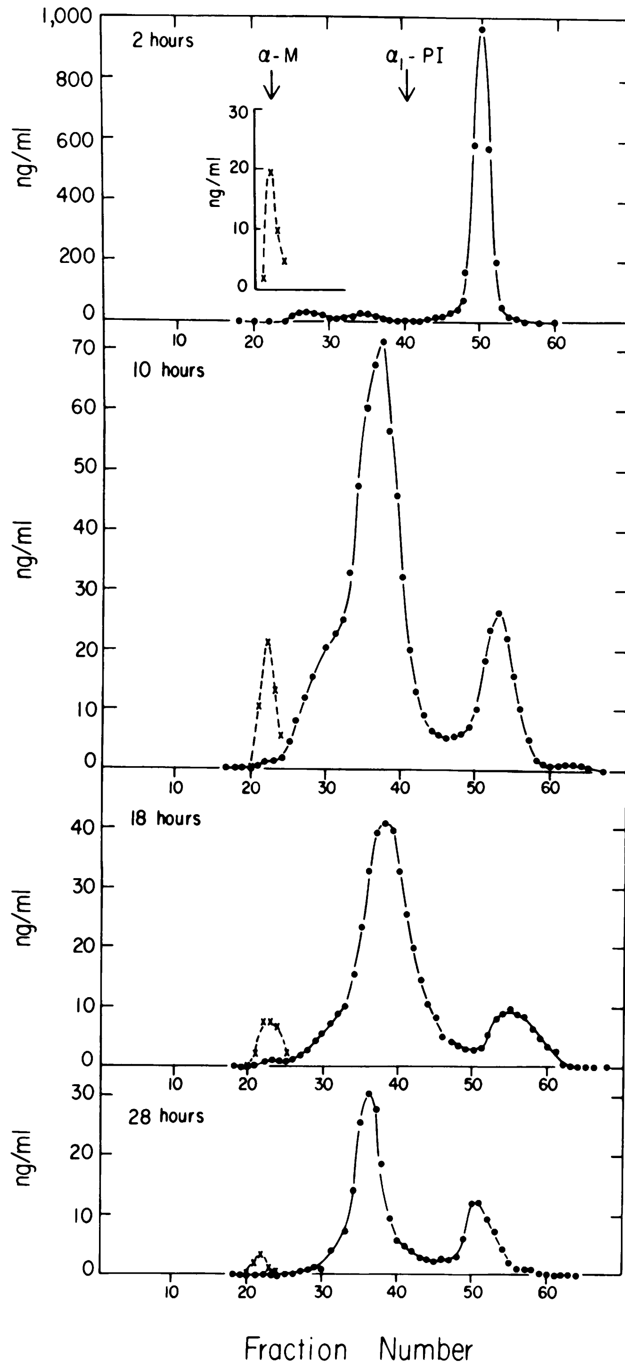

Figure 3—Molecular forms of immunoreactive cationic trypsin in size-fractionated ascitic fluid. Ascitic fluid samples from dog 4 were subjected to gel filtration on Bio-Gel A-0.5 m as described in Materials and Methods. The *arrows* show the elution positions of α-M and α_1-PI. Free trypsinogen elutes between fractions 48-55. Trypsin bound to α-M was detected by adjusting fractions containing this inhibitor with formic acid as described in Materials and Methods.

Gel Filtration Studies

Gel filtration was performed in order to characterize the molecular forms of immunoreactive trypsin in plasma and ascitic fluid. Canine cationic trypsin added to canine plasma *in vitro* is partitioned between α-M and α_1-PI in a ratio of 4:1.[21] Previous studies have demonstrated that a 1:1 complex of trypsin and α_1-PI with a molecular weight of 77,000 daltons is separated from free trypsinogen in Bio-Gel A-0.5 m,[14] while trypsin bound to α-M elutes in the void volume on this column. Canine cationic trypsin bound to canine α_1-PI crossreacts by 75% in this radioimmunoassay system, while trypsin bound to α-M can be detected in approximately 25% yield following acid denaturation of the α-M.

Figure 3 is representative of typical elution patterns for immunoreactive cationic trypsin in ascitic fluid (Dog 4) and shows samples taken 2, 10, 18, and 28 hours after induction of pancreatitis, respectively. At 2 hours, the major portion of the immunoreactive material eluted in a position consistent with trypsinogen. The free trypsinogen peak then declined at successive sampling points, with approximately 30-fold less trypsinogen present at 10 hours. In addition, it is apparent that, with duration of pancreatitis, the ratio of free trypsinogen to immunoreactive trypsin bound to protease inhibitors decreased dramatically, and two major peaks of immunoreactive material in association with α-M and α_1-PI became evident. The ratio of inhibitor-bound immunoreactive trypsin to free trypsinogen appeared to increase in each successive sampling period.

When plasma samples were fractionated under similar conditions, all the immunoreactive material in the 1-hour sample eluted as a single peak of approximately 23,000 molecular weight, in a position consistent with free trypsinogen. Little or no immunoreactive trypsin was detected in association with plasma protease inhibitors. The free trypsinogen peak in plasma declined with the duration of pancreatitis, while there was a corresponding increase of immunoreactive trypsin complexed to α_1-PI. A much smaller peak of immunoreactive trypsin could be detected in association with α-M.

Relative Proportions of the Forms of Immunoreactive Trypsin in Plasma and Ascites fluid

Gel filtration experiments identical to that shown in Figure 3 were performed in 4-8 samples of plasma and ascitic fluid from each animal. Total immunoreactive material in each α-M peak was corrected by a

factor of 4 to account for partial cross-reactivity. The total immunoreactive trypsin in the α_1-PI peak was also corrected by a factor of 1.33, while total trypsinogen was uncorrected, since the zymogen cross-reacts approximately 100% in this assay.

Figure 4 shows the percentage distribution of the three molecular forms of trypsin in ascitic fluid plotted against duration of pancreatitis. At the initial 2-hour sampling point, at least 80% of the immunoreactive material eluted as free trypsinogen in 4 of 5 animals. In most dogs, there was substantial activation of trypsinogen to inhibitor-bound trypsin at successive sampling points. The percentage activation of trypsinogen to inhibitor-bound trypsin was less striking in Dog 4, but the *total* inhibitor-bound trypsin in this animal at later sampling points exceeded that in all other dogs. The dog that survived (Dog 5) showed less activation of trypsinogen than the dogs that died.

The distribution of the molecular forms of immunoreactive trypsin in plasma closely paralleled that in the ascitic fluid. More than 95% of the immunoreactive material in plasma samples obtained at 1 hour appeared as free trypsinogen. Thereafter, the proportion of free trypsinogen declined to 16–38% of the total trypsin in the plasma, and significant amounts of α_1-PI-bound trypsin became detectable. After 4 hours, low levels of α-M-bound trypsin (2–15%) became apparent in 4 of 5 dogs. Dog 5 had essentially no α-M-bound immunoreactive trypsin.

Trypsinlike Activity in Ascites Fluid and Plasma

Figure 5 shows the mean trypsinlike activity in the ascitic fluid for the 4 dogs that died, as well as the activity in the dog that survived. There are very high levels of equivalent trypsin activity in the samples from the animals that died. The high standard deviation in the samples of these animals at 4 hours reflects the variation in the rate of activation of trypsinogen at the early time points. The dog that survived (Dog 5) had lower levels of trypsinlike activity in the ascitic fluid.

Figure 6 shows the mean levels of plasma trypsinlike activity. The levels of trypsinlike activity in plasma are approximately 100-fold lower than those measured in ascitic fluid. Trypsinlike activity was lower in all the plasma samples and all but the first ascitic fluid sample from the dog that survived.

Discussion

The lack of a clear understanding of the biochemical events which occur during acute pancreatitis has

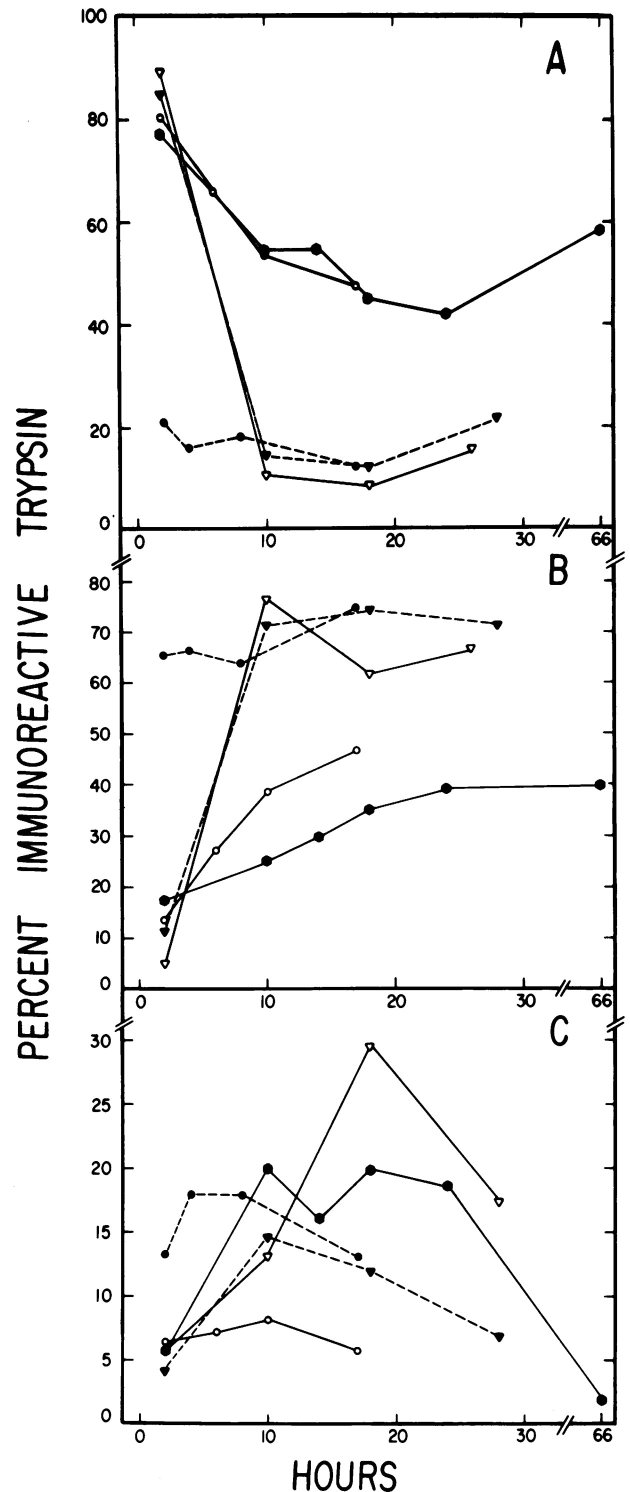

Figure 4—Distribution of molecular forms of immunoreactive trypsin in ascitic fluid. Values for total trypsinogen, α_1-PI-bound trypsin, and α-M-bound trypsin from each column were summed to obtain the total for each from of trypsin. The values for α-M- and α_1-PI-bound trypsin were corrected for partial cross-reactivity as described in Materials and Methods. **A**—Percent trypsinogen. **B**—Percent α_1-PI-bound trypsin. **C**—Percent α-M-bound trypsin. See legend to Figure 1 for explanation of symbols.

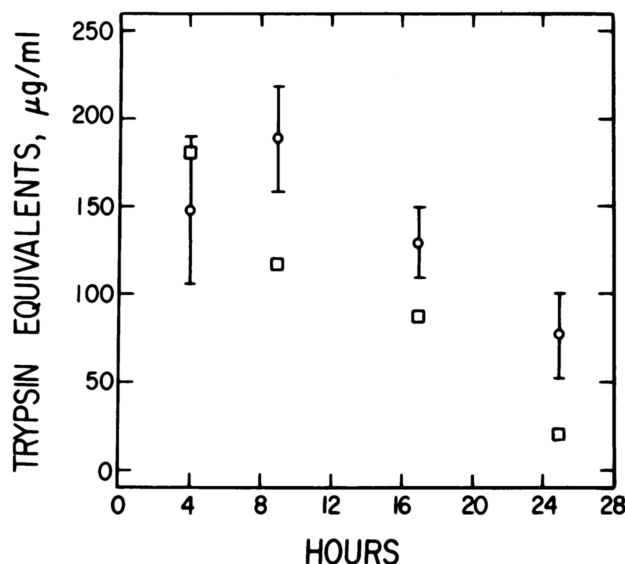

Figure 5—Mean trypsinlike amidase activities in ascitic fluid. Error bars represent one standard deviation from the mean. ○——○, mean values for Dogs 1-4; □——□, values for Dog 5. Time points are 4, 8-10, 16-18, and 24-28 hours.

resulted in the continuing lack of an effective therapeutic modality.[8,23-25] We have approached this problem by attempting to elucidate the biochemical events in canine bile-induced pancreatitis as a prerequisite for developing a rational therapy. The discovery that, at 1-2 hours after induction of pancreatitis, trypsinogen is the predominant form of immunoreactive material in ascitic fluid and plasma is consistent with our previous findings that random plasma samples from most patients with acute pancreatitis contain predominantly trypsinogen.[14]

The time-dependent data from the present study demonstrate that during the course of bile-induced pancreatitis an increasing percentage of the immunoreactive trypsin in plasma and ascitic fluid is present as inhibitor-bound trypsin. This increase may be due to autoactivation of trypsinogen in the pancreatic parenchyma, which occurs during the course of acute pancreatitis,[18,26] with the resultant release of active trypsin into the bloodstream from the gland. Alternatively, inhibitor-bound trypsin might originate from the activation of trypsinogen sequestered in ascitic fluid. Three of the 4 dogs that died had rapid and extensive activation of trypsinogen, while the dog that survived had limited activation, with resultant low levels of inhibitor-bound forms of trypsin. In all of the animals in which there was substantial trypsinogen activation, there were high levels of α-M-bound trypsin in the ascitic fluid, with lower amounts of this material in plasma, probably due to the very rapid clearance of α-M-bound trypsin from the circulation.[27] We have previously reported that random plasma and ascitic fluid samples from some patients with acute pancreatitis contain significant percentages of immunoreactive trypsin as α_2-M- and α_1-PI-bound material.[14] These studies suggested a possible correlation between the degree of activation of trypsinogen and the severity of pancreatitis. The demonstration of a time-dependent activation of trypsinogen during the course of experimental pancreatitis supports this hypothesis. Thus, it is possible that measurement of α_1-PI-bound or α_2-M-bound trypsin in human plasma may be a more useful prognostic tool for assessment of the severity of this disease than the determination of total immunoreactive trypsin.

Many of the systemic complications of acute pancreatitis, including cardiovascular lesions,[1] hypotension,[2] the acute respiratory distress syndrome,[3] coagulation abnormalities,[4] hypocalcemia,[5] acute renal failure,[6] and shock,[7] have been ascribed to the presence of trypsin and/or other pancreatic enzymes in the circulation. Of the three molecular forms of trypsin that we have detected in plasma or ascitic fluid in experimental as well as in human pancreatitis,[14] trypsinogen is inactive, and trypsin bound to α_1-PI has such a low dissociation constant[28] that there should be no release of active trypsin during the period necessary for clearance of this material.[11] In contrast, trypsin bound to α_2-macroglobulin is enzymatically

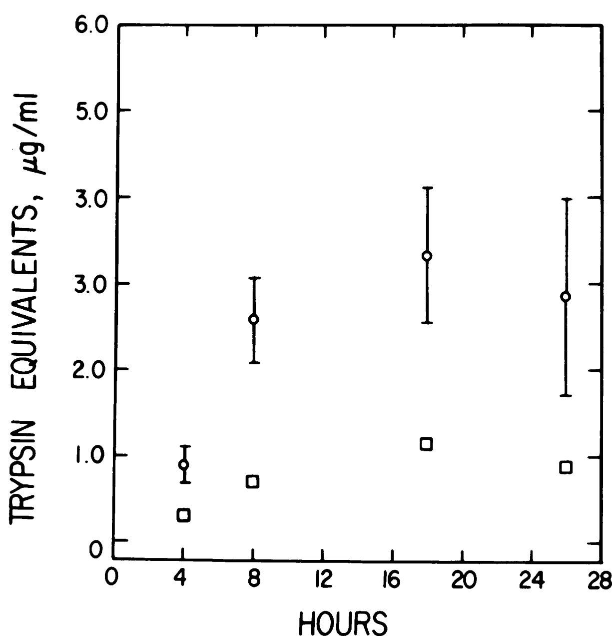

Figure 6—Mean trypsinlike amidase activities in plasma. Error bars represent one standard deviation from the mean. ○——○, values for Dogs 1-4; □——□, values for Dog 5.

active toward low molecular weight ester or amide substrates, as well as low molecular weight hormones, such as angiotensin and vasopressin,[16] and polypeptides, such as proinsulin[15] and parathyroid hormone[17] but has little or no activity toward high molecular weight protein substrates.[29] Thus, α_2-macroglobulin-bound trypsin is the only known source of potential trypsin enzymatic activity in plasma, other than the very small amount of free trypsin present from activation of trypsinogen prior to binding by plasma inhibitors.

Aprotinin (Trasylol, Delbay, Kenilworth, NJ), a trypsin and kallikrein inhibitor from beef lung, has previously been used as specific antiprotease therapy for acute pancreatitis.[8] After much controversy, it has apparently been concluded that Trasylol therapy is ineffective in acute pancreatitis.[23-25] The presence of α-M-bound trypsin in the circulation in the current studies and in patients with severe acute pancreatitis[14] provides a rationale for the ineffectiveness[8,24,26] of Trasylol therapy in this disease. Balldin and Ohlsson[30] have demonstrated that Trasylol does not compete effectively with α_2-macroglobulin for exogenous trypsin. Furthermore, Trasylol, with a molecular weight of about 6000 daltons, inhibits α_2-M-bound trypsin very slowly.*[31] In effect, α_2-M protects trypsin from Trasylol inhibition. In addition, Trasylol does not inhibit human chymotrypsin[32] or elastase 2[33] and does not effectively suppress all of the trypsinlike esterolytic activity in plasma from patients with acute pancreatitis.[34] In this regard, we have recently detected proteolytic activity in plasma and ascitic fluid of patients with acute pancreatitis, using bovine parathyroid hormone as substrate.[17] This activity is only partially blocked by a specific active-site directed trypsin inhibitor, and is more effectively reduced by the addition of an inhibitor against chymotrypsin and elastase 2. Thus, it appears plausible to assume that a more effective therapy of acute pancreatitis might be accomplished by the use of a combination of low-molecular-weight-specific active-site inhibitors for trypsin, chymotrypsin, and elastase that would be capable of inhibiting the α_2-M-bound enzymes, as well as by preventing further activation of trypsinogen. A future communication will describe the use of low-molecular-weight protease inhibitors to treat experimental pancreatitis.

* Free trypsin (10^{-9} M) is inhibited in a pseudo-first-order process by 8×10^{-9} M Trasylol (48 ng/ml) with a half-life of 5 minutes. In contrast, trypsin bound to α_2-M is inactivated very slowly by approximately 100-fold higher concentrations of Trasylol with biphasic kinetics, in which there is a more rapid component followed by slow loss of residual activity. A 2:1 complex of trypsin with α_2-M (fully saturated α_2-M) is half inactivated by 6×10^{-7} M Trasylol (4,000 ng/ml) in approximately 18 minutes, while a 1:1 complex is half inactivated by the same concentration of Trasylol in approximately 24 minutes.

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