BILE, PANCREATIC CANCER, AND THE ACTIVATION OF PANCREATIC JUICE

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Summary: Bile and pancreatic juice vary in their trypsin inhibitor content. Activation of bile-pancreatic juice mixtures (1:1) occurred when the combined trypsin inhibitory capacity was low. No activation was seen with bile having a high trypsin inhibitor content. Pancreatic juice activated faster where more trypsin inhibitor complex was present. With calcium (25 mM), pancreatic secretions activated in a similar order as with the trypsin-inhibitor-free bile. We propose that activation occurs more readily where large amounts of the trypsin-inhibitor complex are present in pancreatic juice as a result of carcinoma or other etiologies.

It was thought for many years that bile when mixed with pancreatic secretions converted the pancreatic zymogens to their active forms (1) and that these active enzymes mixed with digested bile caused the massive destruction seen in pancreatitis (2). Subsequently, it was shown that bile did not activate pancreatic juice (3,4,5) and that pancreatic juice is normally activated in the duodenum by enterokinase (6). Recently, we found that pancreatic juice from a patient with carcinoma of the pancreas activated when mixed with bile (7). Our investigation of the mechanisms involved is described below.

METHODS

Protein concentrations, trypsin, trypsinogen and pancreatic secretory trypsin inhibitor (PSTI)[1] were determined as previously described (8). The trypsin inhibitor capacity (TIC) of bile was measured as follows: to 200 µl of hepatic bile, at 0°, 50 µl of Tris-calcium buffer, pH 8, was added followed by 25 µl of bovine trypsin (Worthington TRL 3, 1.10 mg/ml in 1 mM HCl) to start the reaction. Final concentrations were 0.023 M Ca++, 0.046 M Tris, 0.04 M NaCl and 0.092 mg/ml of trypsin. 15 µl of specimen B was added to the substrate blank to compensate for the increased absorbancy of this sample. No measurable digestion of the substrate was found with this bile. After 5 min incubation at 0°, 25 µl was removed and assayed for trypsin. The trypsin stock was assayed in duplicate the same day. T.I.C. was expressed as µM TAME inhibited/min/ml of bile. Care was taken to make the same dilutions for each specimen. Pancreatic juice was collected postoperatively (9) in 5 patients (Table I-A) and hepatic bile was obtained from T-tubes placed in the common bile duct (3) in 2 patients. Calcium was determined by atomic absorption spectrophotometry in the Dept. of Laboratory Medicine, Univ. of Washington.

[1] Abbreviations used are: pancreatic secretory trypsin inhibitor, PSTI; trypsin-pancreatic secretory trypsin inhibitor complex, T-PSTI; trypsin inhibitory capacity, TIC; N-tosyl-L-arginine methyl ester, TAME.

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TABLE I. CONTENT OF TRYPSINOGEN AND PSTI IN PURE DUCTAL PANCREATIC JUICE

I-A. Specimens Used For the Bile Activation Experiments

Patient No.	Diagnosis	Protein (mg/ml)	Trypsinogen (μM/min/mg)	PSTI (μM/min/mg)
1	Carcinoma of the head of the pancreas	6.61	39.7	5.0
2	Chronic pancreatitis	9.09	44.7	6.5
3	Gallstone obstruction of the common bile duct	10.9	74.7	7.5
4	Recurrent acute pancreatitis-"normal" pancreatic remnant following Whipple operation.[a]	10.1	31.1	10.0
5	Normal pancreas. Gallstones	7.63	12.3	10.1

I-B. Comparison of Specimens From Patients With and Without Pancreatic Disease

Diagnosis	No. of Patients	No. of Specs.	Trypsinogen (μM/min/mg)	PSTI (μM/min/mg)
Pancreas normal	6	9	47.1 ± 6.3	12.5 ± 3.5
Carcinoma of the head of the pancreas	2	12	24.2 ± 9.4	5.1 ± 1.3

a. All other specimens of pancreatic juice were from patients where the pancreas was unresected.

RESULTS

Activation of bile-pancreatic juice mixtures. Five specimens of concentrated pancreatic juice (10) mixed with bile A (1:1) activated at varying rates when incubated at 37° C (Fig. 1). No tryptic activity was found in the corresponding specimens of bile and pancreatic juice incubated alone. The addition of lower amounts (1:9) of the same bile to Specimen #3 slowed the activation rate to 0.06% in 51 hours (data not shown).

In another experiment, an equal volume of a second specimen of bile (B, Table II) was mixed with ductal pancreatic juice (Specimen #1). In this instance no active trypsin was found after 96 hours of incubation at 37° C. Thus the differences in activation rate were related both to the proportion of

TABLE II

Some properties of the hepatic bile used in these experiments.

Specimen No.	A_{280}/ml	A_{405}/ml [a]	Trypsin µM/min/ml	Ca++ mEq/l	TIC [b] µM/min/ml
A	10.0	51.2	0.00	4.6	0.0
B	60.8	337.	0.00	11.2	43.0

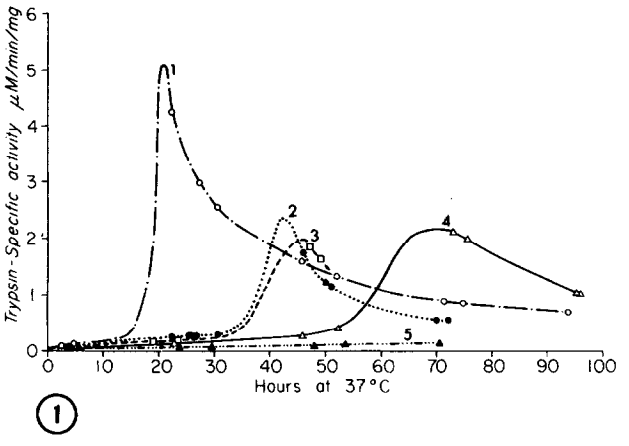
a. Icterus Index
b. Trypsin inhibitory capacity (See Methods)

added bile and to the specimens of bile and pancreatic juice used.

Inhibition of trypsin by pancreatic juice and bile. Lower amounts of PSTI (units/mg) were present in all the samples of pancreatic juice which activated faster with bile A (Table I-A). The activation rates decreased with increasing amounts of PSTI (units/mg). In 2 patients with unresected carcinomas of the pancreas, the amounts of PSTI were significantly lower ($p = < 0.001$) than the values found for the normal pancreas (Table I-B).

Several properties of the biles used for these experiments were also measured (Table II). It was particularly pertinent that bile A, which permitted the activation of pancreatic juice, did not inhibit trypsin. Conversely bile B, which prevented activation, had a high trypsin inhibitory capacity presumably due to the plasma inhibitors, α 1 anti-trypsin and α-2 macroglobulin, found in hepatic bile (11).

Effect of bile on pancreatic juice containing added trypsin. We tested whether bile A reduced the stability of the T-PSTI complex in pancreatic juice causing the release of active trypsin. Bovine trypsin was added to pancreatic juice in amounts to give complete inhibition of the trypsin and thus forming more T-PSTI complex (See Figure 2). The sample containing inhibited trypsin activated the fastest (4 hours) when mixed (1:1) with bile A. Some slight activation also occurred in the trypsin control after 22 hours. No activation was found in bile A or pancreatic juice (5, Table I-A) when incubated alone.



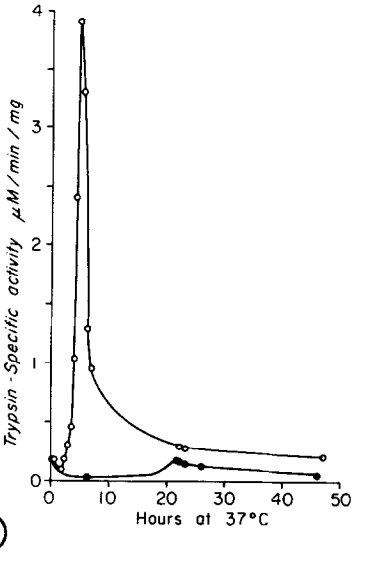


Figure 1. Effect of the incubation of human pancreatic juice with bile on the appearance of tryptic activity. Concentrated specimens of pancreatic juice (Nos. 1-5, Table I) were incubated at 37° C with equal volumes of bile (A, Table II). Aliquots were removed and assayed for trypsin as described in Methods. Corresponding specimens of bile and pancreatic juice incubated alone showed no tryptic activity.

Figure 2. The effect of Bile A on the release of added trypsin from the T-PSTI complex present in pancreatic juice. Bovine trypsin (see Methods) was added to pancreatic juice (5, Table I-A) to give a trypsin concentration of 0.18 mg/ml and a final protein concentration in the pancreatic juice of 6.36 mg/ml. The sample was incubated for 30 min at 0° C and assayed to ensure that the added trypsin was combined with the inhibitor. Two aliquots were removed and incubated at 37° C; one with an equal volume of bile (A, Table II), —o—, and another used as a control, —●—. Aliquots were removed at intervals and assayed for trypsin (see Methods). Additional controls (a 1:1 bile-pancreatic juice mixture and pancreatic juice alone (7.73 mg/ml)) showed no significant tryptic activity in agreement with the data of Figure 1.

Effect of calcium on the rate of activation of pancreatic juice. Calcium causes the release of trypsin from T-PSTI complex. In this process, called temporary inhibition, the inhibitor is destroyed (12,13). Because bile contains calcium, ranging in hepatic bile from 2.5 - 4.8 mEq/1 to 25-30 mEq/1 in gallbladder bile (14), we tested whether calcium caused the same specimens of pancreatic juice (Table I-A) to become active.

With calcium (Figure 3) a rapid activation of pancreatic juice (1, Table I-A)

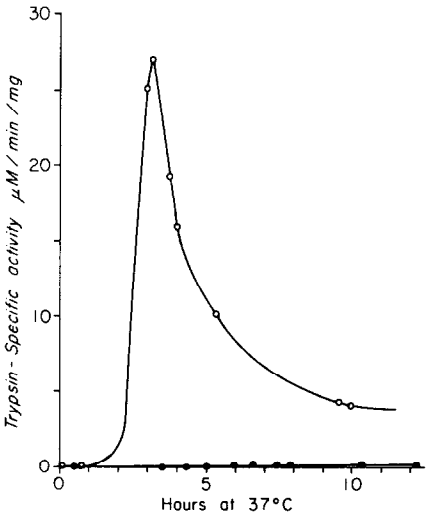


Figure 3. Effect of added calcium on the activation of pancreatic juice. Two aliquots of pancreatic juice from the patient with a carcinoma of the pancreas (1, Table I-A) were incubated with equal volumes of (1) hepatic bile (A, Table II), —o— , and (2) a Tris-calcium buffer, pH 7.4, —●— , which gave concentrations in the bile-pancreatic juice mixture of 0.025 M Ca++, 0.05 M Tris and 0.04 M NaCl. Aliquots were removed at intervals and assayed for trypsin.

occurred after 3 hours at 37° C reaching 68% of the total potential units of trypsin present. In the same experiment, Specimen #4 of pancreatic juice activated after 7 hours and Specimen #5 was not activated after 46 hours. The order of activation was the same as with bile A (Figure 1). Not enough Specimen #3 was available for testing. Specimen #2 from a patient with chronic pancreatitis formed a heavy precipitate with calcium and did not become active. The other specimens did not form these precipitates.

DISCUSSION

Previous investigators have found in humans (3,5) and in animals (4) that bile does not activate pancreatic juice. These findings were attributed to the fact that the enzymes capable of this activation are not present in normal bile. From our studies in humans an equally important factor is apparent, which is that bile can inhibit significant amounts of trypsin, and in this way

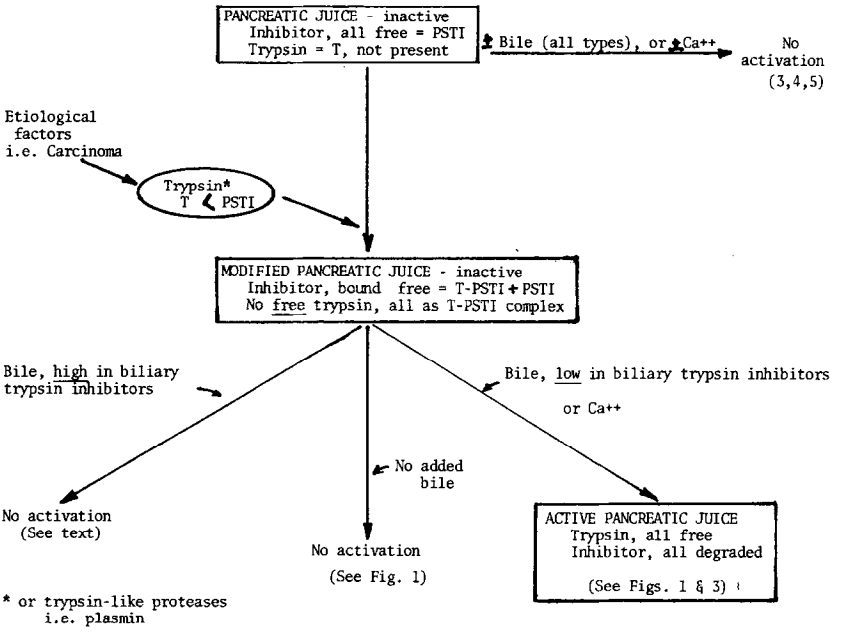


Figure 4. THE ROLE OF BILE AND CALCIUM IN THE ACTIVATION OF PANCREATIC JUICE AT 37° C (in the absence of enterokinase)

can protect human pancreatic secretions from premature activation.

Our data, however, also show that the activation of pancreatic juice can occur in the presence of bile. We have found that this takes place with bile which does not inhibit trypsin. In this regard, the presence of trypsin in some biles in pancreatic disease is an important observation (15). We also found that the rate of activation of pancreatic juice in the presence of bile correlated with the amount of PSTI present (Table I-A); therefore, the rate of activation was faster where the trypsin inhibitory capacity of the bile-pancreatic juice mixtures was low. The data also suggest (See Figure 2 and Figure 3) that increased amounts of the T-PSTI complex were present in the samples of pancreatic juice which activated faster. Thus, activation in the presence of bile depends on both the specimens and proportions of bile and pancreatic juice used.

We were also interested to find that pancreatic secretions from a patient with carcinoma of the pancreas activated much faster than the other specimens (Figure 1). The fact that such an activation may occur in patients with carcinoma of the pancreas (16) may explain the inflammatory hard pancreas often found in areas of the pancreas which are uninvolved, or well away from the tumor process. In carcinoma of the pancreas, it is likely that pancreatic juice contains plasminogen activator, a protease which is secreted by pancreatic tumor cell lines, including one of ductal origin (17,18). This protease may act alone, or through the formation of plasmin, an enzyme similar to trypsin, to modify the pancreatic secretions and to reduce the amount of free trypsin inhibitor. Further studies are being carried out based on these observations to differentiate secretions from patients with carcinoma of the pancreas as opposed to other pancreatic disease.

We have summarized the current status of this problem in Figure 4. The central premise is that pancreatic juice may contain a significant amount of trypsin in combination with the PSTI, and yet remain inactive. Incubated alone this juice will not activate, but should bile low in trypsin inhibitory capacity (or calcium) be mixed with this modified pancreatic juice, then trypsin would be released from the T-PSTI complex and the PSTI digested. Through the continued degradation of the inhibitor, in this manner, sufficient trypsin could become available to activate the trypsinogen present in the pancreatic secretions.

Thus we propose that bile can only activate pancreatic juice following a trigger event of sufficient magnitude where trypsin, or trypsin-like enzymes (i.e. plasmin) have been introduced into pancreatic secretions by pancreatic carcinoma or other etiological factors (16,19) resulting in the formation of large amounts of the T-PSTI complex. The necessary conditions for activations such as this to occur *in vitro* as well as in pancreatitis and carcinoma in man have yet to be defined.

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