Some Properties of Trypsin Inhibitor of Pancreatic Juice. (24331)

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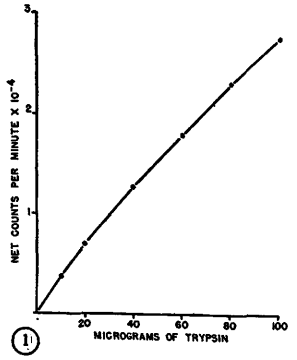
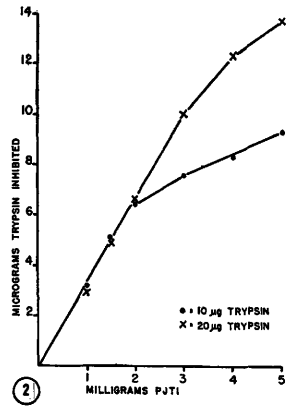
The presence of a trypsin inhibitor in pancreatic juice of dogs was reported by Kalser and Grossman(1). The present study is concerned with further characterization of this inhibitor.

Materials and methods. Materials. Trypsin and chymotrypsin were crystalline products purchased from Worthington Biochemical Lab. Stock solutions of these enzymes were prepared by dissolving in 0.01N HCl and then adding an equal volume of glycerol to give a final concentration of 1 mg/ml. These stock solutions were stored at −20°C and showed no deterioration of activity for 6 months. The trypsin inhibitor prepared from pancreatic tissue of cattle by the method of Kunitz and Northrop (hereafter designated KNTI) was purchased from Pentex Corp. Radio-iodinated (I^{131}) human serum albumin (hereafter designated RISA) was purchased from Abbott Labs. *Measurement of proteolytic activity* was made by a method devised in this laboratory which is based upon release from a RISA substrate of radioactivity soluble in trichloracetic acid. The substrate was prepared as follows. To 80 ml of 0.1N NaOH was added 0.5 g bovine serum albumin (Armour), 10 μcuries of RISA, 36 g of urea and 10 ml of a 0.02% solution of thimerosal. This mixture stood in water bath at 37°C for 1 hour and then 10 ml of 1 M KH_2PO_4 and 4 g of urea were added. To 2 ml of substrate in test tube in water bath at 37°C, 1 ml of enzyme solution was added and mixed by shaking. After exactly 10 minutes 13 ml of 0.185N trichloracetic acid was added, mixed by inversion, allowed to stand 30 minutes and then filtered through Whatman No. 3 paper into a test tube which could be accommodated in the well-type scintillation counter. Blanks were prepared by substituting water for enzyme solution. Standards, prepared by appropriately diluting stock solution of trypsin, were performed with each set of determinations. Radioactivity of samples, blanks and standards was determined by noting time required to make 12,800 counts in a well-type scintillation detector and was expressed as counts/minute. The value for the blank was subtracted from that of the samples and of standards. A standard curve is depicted in Fig. 1. Activity of samples was read from standard curve and expressed as μgrams of trypsin. A similar procedure was followed for chymotrypsin, using chymotrypsin standards. *Preparation of trypsin inhibitor from pancreatic juice.* Pancreatic juice was collected from rats(2) eating rat chow *ad lib.* The juice was collected in tubes placed in ice bath. An equal volume of 0.3N trichloracetic acid was added to the juice and the mixture heated in water bath at 80°C for 5 minutes. After cooling the mixture was filtered through Whatman No. 3 paper. The filtrate was extracted 3 times with an equal volume of diethyl ether to remove trichloracetic acid. The residual ether was removed by exposing the solution to low pressure in vacuum desiccator for 20 minutes. The pH was then adjusted to 7.5 with 0.5N sodium hydroxide. The solution was then lyophilized and yielded approximately 20 mg/ml of pancreatic juice. This pancreatic juice trypsin inhibitor is hereafter designated PJTI. Reagent blanks produced no inhibition of trypsin. In experiments in which the trypsin inhibitor was to be recovered from reaction mixtures the procedure just outlined was used except that lyophilization was not done. *Measurement of trypsin inhibitor activity.* To a solution containing inhibitor was added an equal volume of trypsin solution containing 40 μg/ml. The mixture was kept in ice bath 30 minutes, then proteolytic activity was measured. In another tube water was substituted for trypsin inhibitor solution. The difference in proteolytic activity of the 2 tubes, expressed as μg of trypsin inhibited, was taken as a measure of trypsin inhibitor activity. To take account of dilution by trypsin solution, this value was





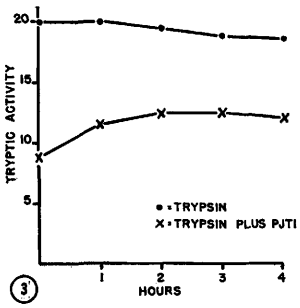


FIG. 1. Standard curve for tryptic activity using RISA substrate.

FIG. 2. Effect of varying concentrations of PJTI and of trypsin on degree of inhibition. Each tube contained either 10 or 20 μg of trypsin/ml plus the indicated amount of PJTI/ml. All mixtures were made up in 0.2M pH 7.5 tris (hydroxymethyl) aminomethane buffer containing 0.05M $CaCl_2$.

FIG. 3. Tryptic activity of a mixture of PJTI (3 mg/ml) and trypsin (20 μg/ml) at various intervals after incubation at 37°C. Activity of a solution of trypsin without inhibitor is shown for comparison. Both mixtures were made up in 0.2M pH 7.5 tris (hydroxymethyl) aminomethane buffer containing 0.05M $CaCl_2$.

multiplied by 2 to give trypsin-inhibitor activity/ml of inhibitor solution.

Results. Effect of varying concentrations of PJTI and of trypsin on degree of inhibition. Fig. 2 gives the results of an experiment showing that at the lower concentrations of PJTI, the amount of trypsin inhibited is proportional to concentration of PJTI and independent of concentration of trypsin.

Digestion of PJTI by trypsin and chymotrypsin. Pancreatic juice trypsin inhibitor was completely destroyed by incubation with trypsin or chymotrypsin. The Kunitz-Northrop inhibitor was resistant to such treatment. Results of an experiment illustrating this point are given in Table I. Carboxypeptidase did not inactivate either inhibitor. The time course of digestion of PJTI by trypsin is indicated by the experiment depicted in Fig. 3 in which increase in activity of a mixture of PJTI and trypsin with time is taken as a measure of rate of digestion of the inhibitor.

TABLE I. Susceptibility of KNTI and PJTI to Digestion by Trypsin or Chymotrypsin.

Reaction mixture				μg trypsin inhibited/ml reaction mixture
KNTI, μg/ml	PJTI, mg/ml	T, mg/ml	CH, mg/ml	
80				27
80		.5		29
80			.5	30
	10			29
	10	.5		0
	10		.5	0

KNTI = Kunitz-Northrop trypsin inhibitor.
PJTI = Pancreatic juice trypsin inhibitor.
T = Trypsin.
CH = Chymotrypsin.

All reaction mixtures were made up in 0.2M pH 7.5 tris (hydroxymethyl) aminomethane buffer. They were incubated at 37°C for 2 hr and then extracted for trypsin inhibitor.

Inhibition of chymotrypsin by PJTI. PJTI failed to inhibit the activity of chymotrypsin even when an amount twice as great as that needed to inhibit an equivalent amount of trypsin was used. Under the same circumstances KNTI produced marked inhibition of chymotrypsin. Table II gives the results of experiment documenting these statements.

Trypsin inhibitor from pancreatic tissue. An homogenate of dog pancreas in water was treated by the same method as that used for

TABLE II. Inhibition of Trypsin and Chymotrypsin by KNTI and PJTI.

Reaction mixture				
T, mg/ml	CH, mg/ml	KNTI, μg/ml	PJTI, mg/ml	Proteolytic activity
20				20
20		10		0
20			5	6
	20			20
	20	10		9
	20		10	20

T = Trypsin.
CH = Chymotrypsin.
KNTI = Kunitz-Northrop trypsin inhibitor.
PJTI = Pancreatic juice trypsin inhibitor.

preparing PJTI. This material inhibited trypsin (102 μg of trypsin inhibited/g wet weight of pancreas), was destroyed by incubation with trypsin or chymotrypsin, and failed to inhibit chymotrypsin. Thus, in regard to these properties, it was identical to PJTI. Similar extracts prepared from liver, small intestinal mucosa, muscle, and blood serum produced no inhibition of trypsin.

Discussion. In the original report on PJTI (1) the inhibitor could not be found in pancreatic juice which had been activated by enterokinase. This can now be explained by our finding that PJTI is susceptible to digestion by trypsin and chymotrypsin. Laskowski and associates(3,4) have systematically studied a variety of trypsin inhibitors including KNTI, another inhibitor prepared from pancretatic tissue by the method of Kazal *et al.*(5), blood plasma inhibitor, ovomucoid, soy bean inhibitor, and colostrum inhibitor. Only 2 of these inhibitors, Kazal's and ovomucoid, exhibited the properties described here for PJTI, namely inactivation by trypsin and failure to inhibit chymotrypsin. It is noteworthy that the inhibitor obtained from pancreatic tissue by simple extraction with trichloracetic acid has the properties of PJTI and not of KNTI, since both of these inhibitors are soluble in trichloracetic acid. On the basis of the properties studied to date Wazal's inhibitor and PJTI appear to be identical.

Summary. The trichloracetic acid filtrate of rat pancreatic juice contains an inhibitor of trypsin which does not inhibit chymotrypsin. The inhibitor is inactivated by trypsin and chymotrypsin.

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Effect of Time of Injection on Removal of CA-45 and SR-85 by Peritoneal Lavage.* (24332)

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Prior studies of ability of bone to supply calcium continuously to extra-osseous areas using the technic of peritoneal lavage, indicated that following parathyroidectomy in nephrectomized rats, amounts of calcium removed/hour fell to about 60% of that removed in parathyroid - intact controls(1). Additional studies(2) demonstrated that amounts of calcium removed could be increased significantly by addition of citrate to lavage rinse. In contrast, no significant changes in amounts of radiocalcium removed could be detected when radioactivity was injected 24 to 48 hours prior to start of lavage

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