Occurrence and Activation of an Elastase Precursor in Pancreas. (22002)

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Lansing and coworkers(1) have found that in teleost fish elastase occurs in pancreatic islet tissue but not acinar tissue. They cited, among reasons for studying this question, the findings that an herbivore (bovine) pancreas produces elastase and that the enzyme has not been detectable in human pancreatic juice. Kokas and coworkers(2) reported the presence of elastase in dog pancreatic juice, but occasionally it could not be found even when proteolytic activity could be measured.

Obviously, the establishment of an islet origin for elastase would indicate an endocrine function. Before an acinar origin and an occurrence in pancreatic juice could be unequivocally excluded, the possibility of an activatable zymogen was explored. The present study indicates that in dog pancreatic juice and in hog pancreas extracts the elastase does exist in the zymogen form, and a duodenal factor or trypsin is capable of converting this precursor to active elastase.

Methods and materials. Activations were carried out at 24°C for 20 minutes with no shaking. Each system contained 0.4 ml of dog pancreatic juice or hog pancreatic extract in a total of 1.4 ml. Other components and their concentrations per tube were: crystalline trypsin and crystalline α-chymotrypsin, 0.2 mg; crystalline soybean trypsin inhibitor, prepared according to Kunitz(3), 2 mg; amorphous pancreatic trypsin inhibitor, prepared according to Kazal, *et al.*(4), 2 mg; 0.4 ml of a 20% aqueous extract of the dried-defatted hog duodenum; and 0.02M Sorensen phosphate buffer, pH 7.4. The juice, which was kindly supplied by Dr. H. Necheles of Michael Reese Hospital, was collected by cannulation after the dog was treated with 100 units secretin, 3 mg mecholyl, and nembutal. The extract was a clarified 2% solution of a lyophilized 25% aqueous extract (pH 7.0) of fresh hog pancreas. The assay system was a modification of that of Balo and Banga(5). Elastin was prepared from beef aorta after removal of collagen with boiling 1 N NaOH. The tubes contained 20 mg elastin, 0.5 ml of 0.5 M Sorensen phosphate buffer pH 7.4, 1.0 ml enzyme (activation mixture), and H_2O to 5.0 ml. Assays were performed for 30 minutes at 37°C with shaking. The tubes were chilled to 1°C. and their contents were filtered (S and S 595 paper). Total protein was determined in the filtrate by a Folin phenol method(6) in which crystalline bovine albumin was used as the standard.

Results. The activation studies are shown in Table I. Elastase is secreted in pancreatic juice in an inactive state capable of being converted to the active enzyme by trypsin or a duodenal factor but not by α-chymotrypsin. The duodenal effect is abolished by soybean and pancreatic trypsin inhibitors if the inhibitors are present from the beginning of the activation period. If present only during the assay they do not completely block the activated elastase. In control experiments, these inhibitors very effectively blocked the hydrolysis of denatured hemoglobin by trypsin; with an inhibitor to enzyme weight ratio of 1, the percent inhibitions by the soybean and pancreatic inhibitors were 87 and 80, respectively.

Discussion. The foregoing data point to an

TABLE I. Activation of Pro-elastase.

Activator	Elastase activity*	
	Dog pancreatic juice	Hog pancreatic extract
None	.7	.9
Trypsin	6.3	4.3
α-Chymotrypsin	.9	.0
Duodenal extract	5.8	3.7
Duodenal extr. + soybean trypsin inhibitor	.8	.4
Idem†	1.9	2.8
Duodenal extr. + pancreatic trypsin inhibitor	.6	.5
Idem†	1.3	2.6

* mg net solubilized protein.

† Inhibitors were added to activation tubes immediately before the aliquot was withdrawn for assay.

indirect role for duodenum—one of activating trypsinogen. Nascent trypsin appears to be the direct activating agent for pro-elastase. The absence of elastase activity in trypsin has been shown by Balo and Banga(5), and we have found it to be absent in both trypsin and α-chymotrypsin.

Several investigators have reported active elastase in extracts of desiccated pancreas without a duodenum component(5,7,8). These observations might be reconciled in the light of varying amounts of active protease in fresh mammalian pancreas tissue(9). Unless the starting tissue has an exceedingly low concentration of active trypsin or the investigator has taken special steps to avoid autoactivation, a given pancreas extract may be expected to contain sufficient trypsin to activate pro-elastase.

The data shown in Table I suggest that the duodenal factor is enterokinase. The activation mechanism strongly resembles that of chymotrypsinogen(10) and pro-carboxypeptidase(11) and may be represented as

(A) Trypsinogen $\xrightarrow{\text{enterokinase}}$ trypsin

(B) Pro-elastase $\xrightarrow{\text{trypsin}}$ elastase

Summary. Dog pancreatic juice and extracts of hog pancreas contain elastase in an inactive form. Trypsin or a duodenal factor is capable of activating pro-elastase. The activation is blocked by pancreatic and soybean trypsin inhibitors, an indication that the duodenal factor is enterokinase.

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Clostridium perfringens Iota Antitoxin Levels In Convalescent Sera from Hemorrhagic Fever Patients. (22003)

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The resemblance of the anatomic-morphologic changes in men who died of hemorrhagic fever (HF) to those in animals which succumbed to enterotoxemias caused by *Clostridium perfringens* led to an inquiry into the possible role of *Cl. perfringens* in the Korean variety of that human disease(1-4). The occurrence of *Cl. perfringens* in the human intestine is well known. Past studies concerned with the distribution of toxigenic types of *Cl. perfringens* have demonstrated that types A and F constitute all of the human intestinal isolates with a few exceptions(5-8). The classification of the strains isolated from the colon of HF patients has not yet been undertaken due to the present limited supply of typing antitoxin on hand. It seemed, however, that the demonstration of serum antitoxins against known species of *Cl. perfringens* in convalescent HF patients would be another approach to this problem.

Materials and methods. Sera. Sera were obtained from 17 patients on the Hemorrhagic Fever Wards of an Army Hospital in Korea, during their convalescence from a disease answering the clinical criteria of HF. These