Pancreatic Elastase I. Observations on Cellular Source and Endocrine Influence. (23631)

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Elastase, a pancreatic enzyme characterized by its ability to dissolve elastin, has been isolated and crystallized by Balo and Banga(1). Its physico-chemical and enzymatic properties have been further investigated by Lewis *et al.*(2). Concerning its pancreatic origin, Lansing(3) has claimed that elastase is secreted by the α-cells of the pancreas and this concept has been furthered by Carter(4) who found that cobalt treatment caused a depletion of the elastase content of the dog pancreas. These conclusions are not consistent with the data of Kokas(5) and Lewis, *et al.* (2) who have demonstrated the presence of elastase in the pancreatic juice of the dog. We became interested in elastase as a result of our studies dealing with the possible antiatherogenic effect of the enzyme. During the course of these studies, which included endocrine as well as other forms of experimental manipulation, changes in the elastase content of the pancreas were noted. These studies were then expanded to include parallel measurements of the pancreatic content of one of the known digestive enzymes, *i.e.* lipase, along with elastase.

Material and methods. Male rats of the Sprague-Dawley strain (120-150 g) and male guinea pigs (500 g) were used in our experiments. Upon sacrificing the animals, the pancreas was completely removed, weighed and extracted by homogenizing in a Waring blendor with a measured amount of chilled distilled water so that the final extract contained the equivalent of 5 mg pancreas/ml. Elastase was determined by a modification of the method of Hall(6). Fifty milligrams elastin (prepared from horse ligamentum nuchae) was suspended in 10 ml 0.1 M glycine buffer pH 8.9 and was incubated with the tissue extract for 18 hr at 37°C. At the end of this period, the digest was filtered and the amount of solubilized elastin determined by means of the biuret reaction. A linear response is obtained with both crystalline elastase and pancreatic extracts. The elastase units are expressed in terms of the reading obtained with the Klett colorimeter using a 515 mμ filter. Lipase was measured by a phototurbidometric method, using a commercially available coconut oil emulsion.* The substrate was prepared by addition of 0.2 ml of the oil emulsion to 100 ml of a 0.2 M phosphate buffer, pH 8.1, containing 0.1% sodium desoxycholate (w/v). The substrate was brought to, and maintained at, 37°C in a constant temperature bath. One milliliter of the enzyme solution was added to 4.0 ml of the warmed substrate and the time in seconds for a 50% reduction in optical density was recorded. The enzyme-substrate solution was kept at 37°C between readings. A "Spectronic 20" colorimeter (Bausch and Lomb Co.), at a wavelength of 600 mμ, was used for all determinations. The colorimeter was arbitrarily set at an optical density reading of 0.8 at 0 time by the substitution of 1.0 ml of distilled water for the enzyme solution. The specificity of the substrate for determining pancreatic lipase can further be demonstrated from the data in Table I. Of all the tissues studied, only the pancreatic homogenate caused a rapid clearing of substrate in relatively low tissue concentrations. When the time in seconds for a 50% reduction in optical density is plotted against lipase concentration using logarithmic coordinates, a straight line is obtained.

Results. To determine whether food intake would be a critical factor in evaluating changes in the pancreatic enzymes, a group of animals was starved and the enzyme concentration of the pancreas was compared with that of animals fed *ad lib.* The results (Tables II and III) indicate a marked increase in the enzyme content of the pancreas of both the starved rat and guinea pig. This

* Ediol, Schenley Lab., Inc.

TABLE I. Reduction in Optical Density of a Coconut Oil Substrate for Various Rat Tissue Homogenates.

Tissue	No. of determinations	Homogenate, conc./ml* (wet wt)	Time for 50% reduction in optical density	
			Min.	Sec.
Pancreas	7	5 mg		120 ± 9.9
"	7	2½ mg		211 ±15.4
"	7	1¼ mg		370 ±27.8
Spleen	7	5 mg	95 ± 8.6	
Serum	4	1 ml	150†	
Adrenal	7	5 mg	150†	
Heart	7			
Stomach	3			
Liver	7			
Muscle	3			
Marrow	3			
Lung	3			

* One ml of tissue homogenate added to 4.0 ml of substrate and the time for a 50% reduction in optical density recorded.

† No appreciable substrate clearing observed at this time.

increase in elastase parallels that of lipase both in direction and degree. These data demonstrated the necessity for paired feeding in all subsequent experiments.

Further reference to the data in Tables II and III shows that any experimental procedure which causes an increase or decrease in the elastase concentration of the pancreas results in a similar change in the lipase activity of the organ. This is readily apparent when the ratio exp./control for each enzyme is calculated. Pilocarpine markedly depletes pancreatic elastase and lipase, and causes the appearance of these enzymes in the urine. The duodenal contents were found to contain elastase before and after pilocarpine administration. A decreased concentration of the elastase and lipase content of the pancreas was found following hypophysectomy (Table II).

The guinea pig responds to cobalt administration by depletion of both the elastase and lipase content of the pancreas, whereas the rat—even at near lethal cobalt levels—shows no change. Administration of thiouracil to rats (Table II) caused a marked reduction of the lipase and elastase concentration of the pancreas.

Discussion. Lansing(3) has stated that the pancreatic alpha-cell is the source of elastase inasmuch as he could extract it from the islet tissue of the teleost fish, the pancreas of which is divided into distinct islet and acinar zones. Carter(4), using cobalt to destroy the alpha-cells of the dog pancreas, found a marked reduction in the elastase content of this organ, and interpreted this data as confirmation of Lansing's claim(3). Our data indicate that elastase, at least in the rat and guinea pig, is a digestive enzyme secreted by the acinar tissue of the pancreas. This is inferred from the findings that (1) any experimental procedure which causes a change in elastase concentration also elicits a similar

TABLE II. Changes in Pancreatic Elastase and Lipase in the Rat.

Treatment	Pancreatic wt, mg/100 g B.W.		Elastase, units/mg pancreas		Elastase, exp./control	Lipase (time in sec. for 50% lipolysis/2.5 mg pancreas)		Lipase, exp./control¶
Starvation*(5)	250 ± 22	N.S.	105 ± 9.2	P = <.01	2.06	74 ± 31.6	P = <.05	2.34
Control (5)	300 ± 17		51 ± 12.6			166 ± 6.0		
Pilocarpine†(5)	369 ± 62	N.S.	33 ± 3.8	*Idem*	.39	463 ± 62.7	P = <.01	.31
Control (5)	389 ± 24		84 ± 3.9			151 ± 10.3		
Hypox‡ (10)	258 ± 21	P = <.05	44 ± 5.3	"	.59	262 ± 37.4	P = <.05	.54
Control (6)	343 ± 21		75 ± 6.2			138 ± 22.9		
Cobalt§ (8)	348 ± 30	N.S.	98 ± 20	N.S.	—	171 ± 13.7	N.S.	—
Control (8)	322 ± 17		86 ± 4.2			181 ± 8.7		
Thiouracil‖ (10)	284 ± 22	N.S.	17 ± 2.6	P = <.01	.22	616 ± 83.4	P = <.01	.29
Control (10)	316 ± 22		78 ± 6.4			182 ± 19.7		

* 5-day starvation. † 4 mg pilocarpine S.C., pancreas removed 4 hr after inj. ‡ 4-wk hypox. § 7 mg cobalt chloride/7 day S.C. ‖ Thiouracil, 0.2% in diet for 3 wk. ¶ Reciprocal ratio since an inverse relationship exists in the assay between activity and time of lipolysis.

No. in parentheses = No. of animals in group.

TABLE III. Changes in Pancreatic Elastase and Lipase in the Guinea Pig.

Treatment	Pancreatic wt, mg/100 g B.W.	Elastase, units/12 mg pancreas	Elastase, exp./control	Lipase (time in sec. for 50% lipolysis/12 mg pancreas)	Lipase, exp./control
Starvation* (6)	242 ± 25	73 ± 11.3	1.55	228 ± 22	1.54
Cobalt† (8)	213 ± 18	20 ± 1.4	.42	1423 ± 180	.27
Controls (10)	241 ± 19	47 ± 7.4		387 ± 48	

* 5-day starvation. † 25 mg cobalt chloride daily for 7 days.
No. in parentheses = No. of animals in group.

change in lipase concentration both in direction and degree. (2) Elastase is present in duodenal juice and is depleted from the pancreas by pilocarpine. (3) The observation that cobalt causes a depletion of the elastase and lipase content of guinea pig pancreas, whereas the rat pancreas is unaffected, is paralleled by the reports of other investigations (8,9) that the rat is resistant to the hypothyroid effect of cobalt; whereas the guinea pig is markedly sensitive to this activity. Accordingly, we postulated that perhaps the depletion of pancreatic elastase and lipase was the result of hypothyroidism or some mechanism which results in a lowered basal metabolic rate. This postulate was strengthened by the finding that thiouracil administration to the rat results in a depletion of the elastase and lipase content of the pancreas as is also observed after hypophysectomy. Shafer(10) has also implicated the thyroid in the maintenance of the normal appearance and proteolytic activity of the rat submaxillary gland. Perhaps Carter's(4) data may be reinterpreted in this fashion. (4) Our finding that hypophysectomy results in a decreased concentration of elastase and lipase of the rat pancreas, is in accord with the work of Baker (7) who reported a similar depletion of pancreatic proteinase after hypophysectomy. These data are difficult to reconcile with the concept that elastase is secreted by the alpha-cell of the pancreas inasmuch as Volk *et al.* (11) have found that in the rat, hypophysectomized for 4½ months, there is no change in alpha-cell cytology or occurrence.

Summary. 1. Concentration of elastase and lipase in the pancreas of rat and guinea pig responds similarly to various experimental manipulations. The changes are parallel both in direction and degree. Starvation causes an increased concentration, whereas pilocarpine, hypophysectomy, thiouracil treatment result in a marked depletion of these pancreatic enzymes. Cobalt administration to the guinea pig causes a depletion of pancreatic elastase and lipase, whereas in the rat such an effect is not obtained. This is attributed to the relative resistance of the rat to the hypothyroid effect of cobalt. 2. Based on above observations, it is inferred that in the rat and guinea pig, elastase is a digestive enzyme elaborated by the acinar tissue of the pancreas.

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Received September 18, 1957. P.S.E.B.M, 1958, v97.