Secretin Inactivating Enzyme in Liver.* (27670)

ANNA B. BRIDGWATER, Y. KUROYANAGI, TH. CHILES, AND H. NECHELES
Department of Gastrointestional Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Using a preparation of secretin considerably purer than that used by early workers, Necheles *et al.*(1) found that, when this hormone was injected into the portal vein of a dog, it elicited less pancreatic secretion than when injected into a systemic vein. On the other hand, Hart and Clarke(2) in a similar study reported no difference, regardless of route of injection. These investigators did not mention ligating the pylorus or accessory pancreatic duct(s); failure to do so may be the reason for this discrepancy. Recently, our results have been confirmed by Skillman *et al.* (3).

Greengard, Stein and Ivy(4,5) observed that secretin was inactivated or destroyed when it was incubated with whole blood, plasma, serum, or urine. They believed the substance responsible for this phenomenon to be an enzyme and named it secretinase. This paper reports the presence of such a substance in dog's liver, its preparation in partially purified form and some of its properties. For brevity, we are calling it SIE (secretin inactivating enzyme).

Methods. Preparation of SIE. All steps were carried out at room temperature except where noted. Dog's liver was perfused with saline to wash out blood and worked up immediately or kept frozen. One liver only (weight 218-546 g) was used for each preparation; it was minced in a Waring Blendor for one-half minute and stirred with saline (2 ml per g of wet liver) for 15 minutes; the mixture was centrifuged for 30 minutes at 2000 r.p.m., supernatant retained and precipitate discarded; the saline extract was brought to pH 4.7-5.2 by dropwise addition of 3N HCl and centrifuged in 40-ml tubes for 25 minutes at 2600 r.p.m. The supernatant was discarded, precipitate in each tube extracted with 25-30 ml cold 80% ethanol and tubes centrifuged for 15 minutes at 2600 r.p.m. The alcohol extracts, cooled to 0-5°C, were added dropwise with stirring to 5 volumes of cold acetone (0-5°C). After standing overnight in the cold room, precipitate was collected on a Buchner funnel, washed with cold acetone 9-10 times, and dried on the filter for 15 minutes. A light yellow powder was obtained which was stored in the refrigerator.

Assay procedure: For assay of SIE, secretin was used as substrate which in turn was assayed by its effect on pancreatic secretion. Male or female mongrel dogs (dewormed) were fasted for 24 hours, with water available. Under nembutal anesthesia (i.v., 25 mg/kg), pylorus and accessory pancreatic duct(s) were ligated through a mid-line incision; the main pancreatic duct was intubated with plastic tubing, which was exteriorized through the abdominal incision and connected to an electronic drop recorder.† Pancreatic secretion and carotid blood pressure were registered on kymograph; the former was recorded also on a counter. An external jugular vein was intubated for injection of liver extract and/or secretin, which was washed down with a few ml of saline. Secretin Lilly‡ (1.67 clinical or Ivy dog units per mg) was used for most experiments. Toward the end of our work secretin Lilly became unavailable and Boots preparation was used. Assay samples (secretin + SIE) were given after a steady secretory response was obtained with one or more standard doses of secretin. Secretory response to *secretin* or *assay* sample was measured by the number of drops of pancreatic juice recorded for the first 15 minutes following injection. Basal secretion represents drops of pancreatic

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† Made by Elmac Engineering Co., Chicago, Ill. Pancreas secretion displaced a weak electrolyte solution, and drops of the latter passed through the counter; 1 ml of fluid corresponded exactly to 25 drops.

‡ We are obliged to Dr. R. M. Rice, Lilly Laboratories for Clinical Research for generous supplies of secretin.

TABLE I. Effect of Heat on Liver SIE.

Preparation No.	SIE, mg	Buffer pH 6, ml	Secretin, mg	Incubation, min.	Pancreatic secretion (drops) Basal	Secretin + SIE	Secretin control
			Boots				
1A	25	5	5	10	7	79	72
B	"	"	"	"	6	28	72
			Lilly				
3A	50	10	2.5	"	4	56	68
B	"	"	"	"	5	8	37
			Boots				
4A	25	5	5	"	3	31	32
B	"	"	"	"	3	3	29
			Lilly				
6A	50	10	2.5	20	8	62	50
B	"	"	"	"	4	1	42
7A	"	"	"	10	6	43	40
B	"	"	"	"	6	4	41

A. SIE solution heated at 60-70°C for 1 hr; after cooling secretin added, followed by incubation. B. (Control). Treated the same as A but not heated. Samples 4A and 4B were adjusted to pH 4 after heating. Preparation numbers in Tables I and II correspond.

TABLE II. Effect of Dialysis on Liver SIE.

SIE preparation No.	H of dialysis	Aliquot of dialysate, ml	Pancreatic secretion (drops) Basal	Secretin + SIE	Secretin control
1	67	5	4	4	56
2	68	4	3	4	40
3	22	whole sample	4	5	97
4	72	*idem*	1	1	36
7	144	4	4	3	36
8	168	"	11	10	90

Fifty mg SIE in 10 ml distilled water was dialyzed in cellulose tubing against 1 liter of distilled water at 8°C; an aliquot of the dialysate was incubated with secretin.

juice recorded for the 15-minute period immediately prior to injection of assay mixture.

For the experiments represented in Tables I and II, and Figs. 1-3, a solution of SIE was incubated for 10 minutes at 37°C with 2.5 mg secretin Lilly in 0.5 ml of saline, except where otherwise noted; buffers were prepared according to Clark and Lubs(6).

The per cent of secretin destroyed (Figs. 1-3) was calculated by the formula:

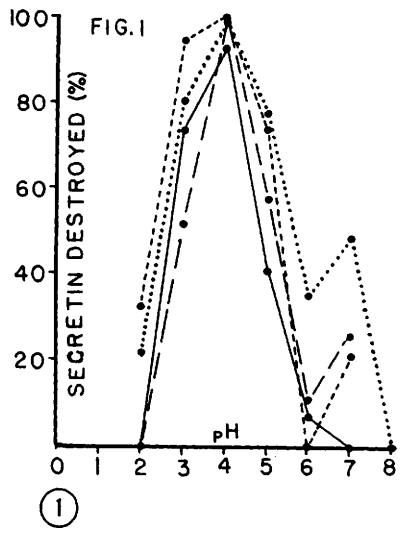
$$\%\ \text{Secretin Destroyed} = 100 - \frac{(\text{secretin+SIE})^* - \text{basal}}{\text{secretin control}^* - \text{basal}} \times 100$$

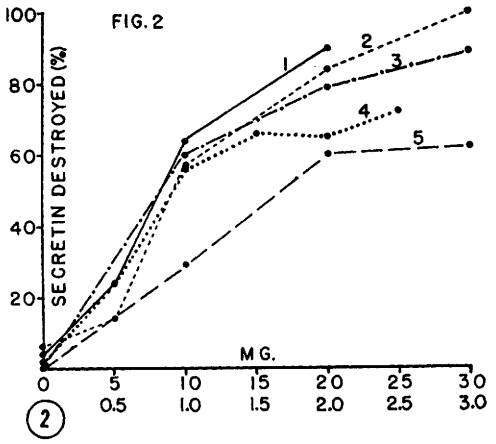
* Drops of secretion.

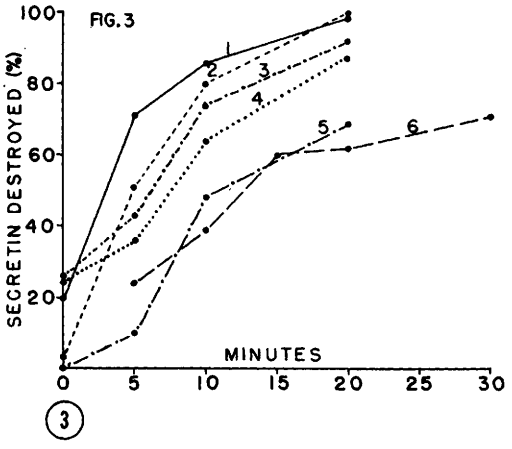
Proteolytic activity of SIE was determined on 2 different protein substrates at pH 3.6; 1) Hemoglobin: Anson's method(7) for determination of cathepsin was used; 2) 1% Casein: 2% Hammarsten casein solution in 0.2 N sodium acetate was added dropwise to an equal amount of 2 N acetic acid. The determination and color development followed the casein method of Kunitz(8) for determination of trypsin. SIE: 2.5 mg in 1 ml of 0.2 N acetate buffer pH 3.6 was used for assay.

Results. Preparation of SIE was based on conventional procedures: isoelectric precipitation of the active substance and extraction with a suitable solvent, followed by reprecipitation with acetone. Fine adjustment of pH of saline liver extract did not seem to be crucial; a range of 4.7-5.2 gave comparable yields (3.9-4.9 mg SIE per g wet liver). Secretin is destroyed or inactivated by this preparation (Table I (Series B)). Employing the same procedure as described for liver, a preparation was obtained from dog's kidney which also inactivated secretin.

Proteolytic activity of SIE was determined With a rare exception where a slight blood pressure drop occurred for 1-3 minutes, no effect was observed with amount of 50-100 mg.







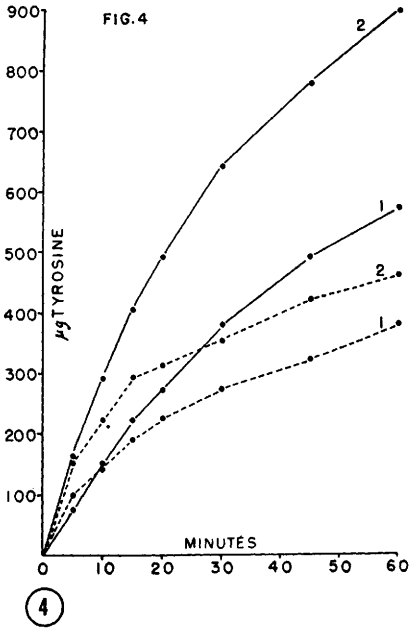


FIG. 1. Effect of pH on SIE. 2.5 mg SIE in 5 ml of appropriate buffer. Each curve in Fig. 1-3 represents a different preparation.

FIG. 2. Effect of concentration of SIE. Amount and pH of buffer used for each assay: Curves 1 and 2: 3 ml, pH 4. Curves 3 and 5: 10 ml, pH 6. Curve 4: 5 ml, pH 6. On abscissa, concentration in mg: upper line, curves 3-5; lower line, curves 1-2.

FIG. 3. Effect of incubation time on SIE. Curves 1-4: 20 mg SIE in 4 ml of buffer pH 6. Curve 5: 0.2 mg SIE in 4 ml buffer pH 4. Curve 6: 10 mg SIE in 5 ml buffer pH 4.

FIG. 4. Proteolytic activity of SIE. Ordinate: results are expressed as μg of tyrosine released by 2.5 mg SIE at 37°C; 1 and 2 represent 2 different preparations of SIE. —— Hemoglobin, - - - - Casein.

Stability: Preparations stored up to 10 months in the refrigerator (8°C) still had good activity. Activity was destroyed by heating in solution at 60-70°C for 1 hour (Table I). *Effect of dialysis* (Table II): No loss of activity was noted when solutions were dialyzed against distilled water from 22 to 168 hours. *Effect of pH* (Fig. 1): SIE was most active in pH range 3-5, with an optimum at 4. The smaller peak at pH 7 may indicate a second less active substance. *Effect of concentration* (Fig. 2): A linear relationship exists between activity and concentration in the first part of the curve, followed by a relative decrease in

TABLE III. Effect of Plasmin on Secretin.

Procedure	Pancreatic secretion (drops) Basal	Assay	Secretin control
2 ml M/15 phosphate buffer pH 7.0 + 0.1 ml plasmin* + 2.5 mg secretin (Lilly); inc. 50 mins.	4	9	142
Control: 2 ml M/15 phosphate buffer pH 7.0 + 2.5 mg secretin (Lilly); inc. 50 mins.	6	69	94
2 ml M/15 phosphate buffer pH 7.4 + 0.2 ml plasmin + 2.5 mg secretin (Lilly); inc. 30 mins.	6	7	113

* 12 Remmert and Cohen casein units per ml.

activity as concentration increases. No loss of secretin activity was observed after incubation with buffer only. *Effect of time of incubation* (Fig. 3): SIE was most active within the first 10 minutes of incubation; thereafter activity diminished. Inactivation of secretin may begin within the first minute after mixing with liver extract. *Proteolytic activity* (Fig. 4): SIE acted on both, hemoglobin and casein substrates.

Effect of plasmin on secretin: Incubation of secretin with purified plasmin§ showed that this enzyme destroys secretin (Table III).

Discussion. We feel that the preparation herein described has the attributes of an enzyme: heat lability, pH optimum, and non-dialyzable; its activity exhibits an initial linear relationship to increasing concentration and incubation time. Another property is that of proteolytic activity for non-specific protein substrates. Liver tissue, as well as kidney, is a good source of proteinases known as cathepsins which have a pH optimum of 3.5-4.0 and are non-specific. Thus, the enzyme described here may belong to this group, though the possibility of a specific "secretinase" in liver cannot be ruled out here.

"Secretinase" in blood described by Greengard *et al.*(4) with a pH range of 5-8 is probably different from SIE from liver with a pH range of 3-5. Rogers(9) observed that plasma treated with chloroform was more effective in destroying secretin than unactivated plasma, indicating that the active agent might be plasmin, which we found to inactivate secretin at pH 7.0 and 7.4. However, plasmin has a wide spectrum; besides secretin, other hormones of a protein nature are inactivated by it, such as glucagon, ACTH, somatotropin(10) and prolactin(11). Thus "secretinase" in blood may be non-specific also.

As indicated in the introduction, it would seem that secretin is inactivated in the liver in some way. Whether it may be degraded by a tissue proteinase (cathepsin), or bound, inhibited, or inactivated, by other mechanisms, is open to speculation. It is problematic whether SIE with an optimal pH range of 3-5 can act intracellularly.

Summary. The presence and isolation in a partially purified form of a substance from dog's liver which destroys secretin and has the characteristics of an enzyme are described; it is heat labile, does not dialyze, is most active in pH range 3-5 and shows an initial linear relationship of activity to increasing concentration and incubation time. It acts also on non-specific substrates such as hemoglobin and casein. SIE was found also in dog's kidney. The enzyme may be a cathepsin and not a specific "secretinase." Secretin is inactivated by plasmin at neutral pH; it is possible, as indicated by Rogers, that "secretinase" in blood might be identified with this enzyme.

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