PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 897–903 (1975)

Factors Involved in the Intestinal Feedback Regulation of Pancreatic Enzyme Secretion in the Rat[1] (38656)

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Previous studies have shown that pancreatic enzyme secretion in the rat is controlled by a negative feedback mechanism (1, 2). Trypsin, chymotrypsin, or bile-pancreatic juice, infused intestinally, suppressed the secretion of pancreatic enzymes. Diversion of bile-pancreatic juice from the intestine as well as intra-intestinal infusion of soybean trypsin inhibitor (SBTI) or protein stimulated the pancreatic enzyme output several-fold. It was suggested that in the course of digestion, SBTI or protein bound the proteolytic enzymes, which in effect removed them from the intestine, thereby removing the negative feedback on pancreatic enzyme secretion exerted by the enzymes. Hydrolyzed protein did not stimulate secretion presumably because it would not interact with the enzymes. Corring (3) has recently shown that diversion of pancreatic juice from the intestine in pigs caused an increase in pancreatic enzyme secretion. This high enzyme secretion was suppressed by returning the pancreatic juice to the intestine but not by infusion of NaCl plus $NaHCO_3$. Therefore, a pancreatic regulatory mechanism similar to that of the rat appears to exist in the pig.

The purpose of the present study was to investigate further factors involved in the intestinal stimulation of pancreatic secretion in the rat with the aim, ultimately, of elucidating the mechanism(s) involved.

Methods. Animal Preparation. Rats were prepared with bile-pancreatic or pancreatic and intestinal cannulae for use in experiments. Details of the operative procedure have been presented by Green *et al.* (1). Briefly, 300–350 g male Wistar rats (Hilltop Lab Animals, Chatsworth, CA) were anesthetized and the duodenum delivered through an abdominal incision. A cannula was inserted into the common bile-pancreatic duct at the ampulla for the collection of bile-pancreatic juice.

For the collection of pure pancreatic juice, one end of a 5 cm piece of Silastic Medical Grade Tubing (Dow Corning, Midland, Mich., 0.64 mm i.d. × 1.19 mm o.d.) with a 5 mm Teflon tip (Becton-Dickenson, 0.68 mm i.d. × 0.98 mm o.d.) was inserted into the bile duct toward the hilum of the liver. The bile duct was ligated below this point prior to entry into the pancreatic tissue. The other end of the tubing was inserted into the duodenum 1 cm above the sphincter of Oddi, thereby shunting bile directly into the intestine. The common duct was then cannulated as described previously and pure pancreatic juice could now be collected. Additional cannulae were inserted into the duodenum for the return of the juice to the intestine and for infusion of substances to be tested. To infuse substances into various segments of the intestine, additional cannulae were inserted at measured distances from the ileocecal junction (the exact distance was determined after the animal was sacrificed). For intravenous infusions, a cannula (Silastic 0.51 mm i.d. × 0.94 mm o.d., with a bevelled Teflon tip) was inserted into the jugular vein.

Animals were maintained in a modified Bollman restraint cage and allowed 48 hr to recover. They were fed Purina Laboratory Chow and water *ad libitum*. When an animal's juice was diverted for 10 hr or longer, it was given physiological saline and a semi-purified diet containing 18% hydrolyzed casein. Bile-pancreatic juice was analyzed for chymotrypsin according to Hummel (4), using the synthetic substrate *n*-benzoyl-L-tyrosine ethyl ester (BTEE). The zymogen was activated by incubating 25 μl of the juice

[1] Supported in part by U. S. Public Health Service Research Grant No. AM 16259-01.

[2] Aided by a predoctoral H.E.A. IX-B (formerly NDEA IV) fellowship.

with 475 μl of a solution containing 40 μl/ml trypsin in 0.04 *M* Tris–HCl, 0.01 *M* $CaCl_2$, pH 8.1 for 15 min at 0°. The BTEE units were converted to an amount of bovine chymotrypsin (Worthington Biochem., Freehold, NJ) equivalent in activity to that of the juice. When pure pancreatic juice was collected, the absorbance at 280 nm of the juice was determined and converted to mg protein based on the relationship beween o.d. at 280 nm and mg nitrogen of the juice. Chymotrypsin activity and total protein determinations gave parallel values for the rate of pancreatic secretion.

In some experiments pancreatic juice was returned to the intestine throughout the experiment by collecting the juice for 15 min, measuring the volume, taking 25 μl aliquot for analysis and then infusing the juice into the intestinal cannula during the next 15-min period. Precollected juice was used during the first period. In experiments in which the animal's juice was not returned, it was diverted from the intestine for at least 10 hr prior to the experiment. Therefore an experiment was done after the initial hypersecretion of enzymes and when the output had established a new basal secretion, as described by Green *et al.* (1).

Infusion materials. Lyophilized SBTI was prepared according to Green and Lyman (2) from soybean flour and this powder was dissolved in 2.5 ml of distilled water. It was infused through an intestinal cannula over a 15-min period.

Trypsin, DFP-trypsin, chymotrypsin, or DFP-chymotrypsin (Worthington Biochem., Freehold, NJ) were dissolved in 3 ml of 0.025 *N* $NaHCO_3$, and each infused into the intestine over a 30-min period.

For intravenous infusion, pancreozymin (Sigma Chemicals, St. Louis, MO, containing up to 10% secretin by weight), purified trypsin, and crystalline SBTI (Worthington Biochem.), or chymotrypsinogen (Sigma Chem.) were dissolved in 0.40 ml sterile saline. The solution was infused into the jugular vein over a 30-min period. All enzyme solutions were maintained at 0° during infusion to prevent autodigestion.

Results. Pancreatic enzyme stimulation by SBTI. To test whether the pancreatic stimulatory effect of SBTI was localized or occurred throughout the entire length of the intestine, SBTI was introduced into the intestine at increasing distances from the pylorus in the presence of bile-pancreatic juice. Figure 1 demonstrates that at distances greater than 35 cm from the pylorus, SBTI did not stimulate enzyme secretion, whereas at less than 23 cm below the pylorus it evoked a strong stimulation. Since the average length of the rat's intestine is 100 cm, it is apparent the SBTI stimulated enzyme secretion only in the upper third of the small intestine.

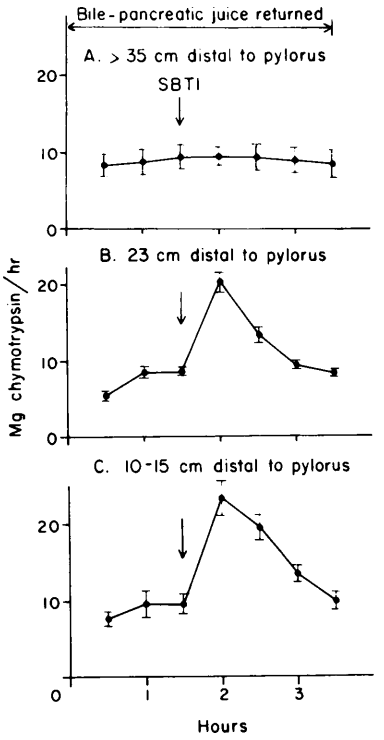

FIG. 1. The effect on pancreatic enzyme secretion of infusing SBTI (soybean trypsin inhibitor) into the small intestine at increasing distances from the pylorus of rats. Fifty mg of SBTI was infused in 2.5 ml water. Enzyme activity is represented as the total amount of equivalent bovine chymotrypsin activity of the secreted juice. Each point is the mean ± SEM, A, $n = 6$; B, $n = 3$; C, $n = 8$. Bile-pancreatic juice was continually returned to the intestine throughout the experiment.

Suppression of pancreatic enzyme secretion by intestinal trypsin. After diverting pancreatic juice from the rat's intestine for several hours, the pancreas appears to be unresponsive to intestinal stimulants (1, 5). How-

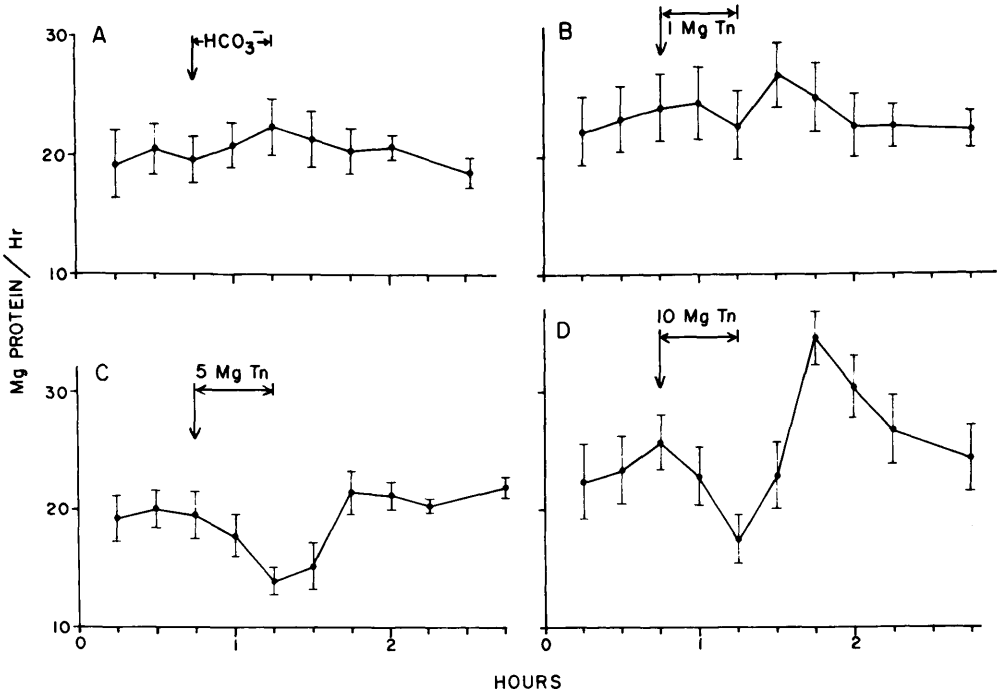

FIG. 2. The effect on pancreatic enzyme secretion of intestinal infusion of 1, 5, or 10 mg trypsin into rats whose pancreatic juice has been diverted for 10 hours. (*Tn* = trypsin.) One, 5, or 10 mg trypsin was infused in 3 ml of 0.025 *N* $NaHCO_3$ for 30 min. Enzyme output is expressed as the total protein of the secreted juice. Each point is the mean ± SEM, $n = 5$. The differences in output at 0.75 hr and 1.25 hr, and at 1.25 hr and 1.75 hr were significantly different in A and B, B and C, and C and D when compared by a paired *t* test ($P < 0.05$), $n = 5$.

ever, the pancreatic output can be suppressed by trypsin or chymotrypsin. These experiments were designed to investigate aspects of this suppression. Figure 2 shows the pancreatic protein output during infusion of three levels of trypsin into the intestine of rats whose juice had been diverted for 10 hr prior to the experiment. A paired *t* test was used to compare two components of each graph, i.e., the difference between the output at 0.75 hr and 1.25 hr, which represents the suppression during the trypsin infusion, and the difference between the output at 1.25 hr and 1.75 hr, the rebound after the infusion was stopped. When the infusion was carried on for a longer time at the same rate of 10 mg trypsin/0.5 hr, there was no further decrease in the pancreatic output, therefore the output at 1.25 hr represents the lowest secretion rate due to a particular trypsin infusion. From Fig. 2, it is apparent that 3 ml of 0.025 *N* $NaHCO_3$ had little effect on the enzyme secretion, whereas 5 or 10 mg of trypsin sharply depressed the basal secretion. The difference between the output at 0.75 hr and 1.25 hr and at 1.25 and 1.75 hr increased significantly with each increase in the amount of enzyme infused ($P < 0.05$) when Figs. 2A and 2B, 2B and 2C, 2C and 2D were compared by means of the paired *t* test. Intestinal trypsin at doses above 10 mg trypsin had no increased effect on the pancreatic response. Chymotrypsin has also been tested in a similar manner and exerts the same effect as trypsin (6).

To test whether the active site of trypsin is necessary for it to suppress secretion, the effect of 10 mg of trypsin, treated with diisopropylfluorophosphate (DFP), which blocks the active site, was compared with the same level of active trypsin. From Fig. 3, it can be seen that active trypsin suppressed secretion similar to that seen in Fig. 2, however DFP-trypsin had no such effect. DFP-chymotryp-

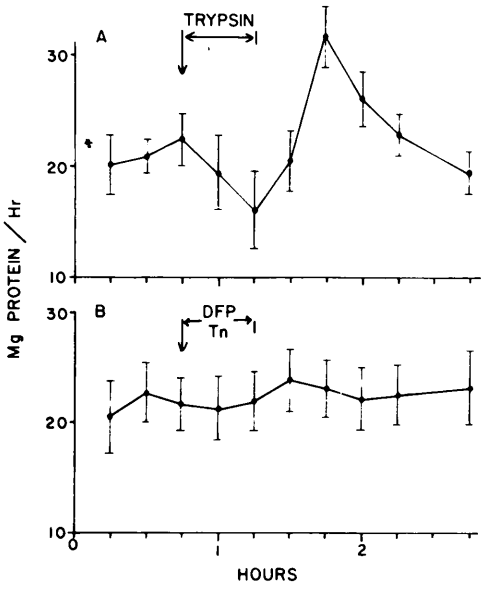

FIG. 3. The effect on pancreatic enzyme secretion of intestinal infusion of trypsin or DFP-trypsin into rats whose pancreatic juice has been diverted for 10 hours. (DFP-tn = diisopropylfluorophosphate-trypsin). Ten mg of either trypsin (A) or DFP-tn (B) was infused. Infusion rate and data presentation are the same as Fig. 2. The variance in Fig. A is significantly greater than that of B ($P < 0.05$), $n = 4$.

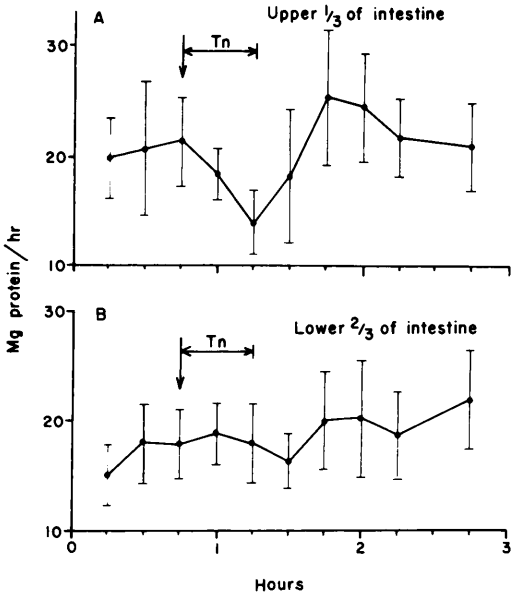

FIG. 4. The effect on pancreatic enzyme secretion of infusing trypsin into the upper one-third or the lower two-thirds of the intestine of rats whose pancreatic juice has been diverted for 10 hr. (Tn = trypsin.) Ten mg of trypsin was infused at either 10 cm below the pylorus (A), or greater than 30 cm below the pylorus (B). Infusion rate and data presentation are the same as Fig. 2. The variance in A is significantly greater than that of B ($P < 0.05$), $n = 3$.

sin did not suppress pancreatic output either (6).

Just as stimulation of pancreatic enzyme secretion occurred only in the upper third of the small gut (Fig. 1), the inhibitory effect of trypsin also occurred only when the enzyme was infused into the upper third of the intestine (Fig. 4).

Intravenous CCK and pancreatic enzyme secretion. Previous studies have shown that following diversion of pancreatic juice from the intestine, pancreatic enzyme output increased sharply over the basal secretion, but after 4–5 hr the output decreased and eventually stabilized at a new but slightly elevated basal secretion rate (1, 2). Experiments were conducted to determine if the pancreas could still respond to exogenous CCK during this period of secretion following diversion. Table I indicates that, although the enzyme output increased from the basal secretion due to CCK infusion, in rats whose juice had been diverted this increase was much less than when CCK was infused into rats whose juice had been continually returned to the intestine. Further analysis of the data indicated that the response to the two levels of hormone (5 and 10 Crick units) was significantly different only when juice was continually returned to the intestine (6).

Intravenous infusion of substances and pancreatic enzyme secretion. Other investigators have proposed that trypsin, SBTI, and chymotrypsinogen may cross the intestinal mucosa and control pancreatic enzyme secretion by their presence in the blood (7–10). Figure 5 demonstrates that trypsin, chymotrypsinogen, or SBTI did not signifidantly alter the pancreatic output when infused directly into the jugular vein.

Discussion. Green and Lyman originally proposed that the presence of trypsin, chymotrypsin, or bile-pancreatic juice in the intestine controls enzyme secretion from the pancreas (2). The results presented here provide further information on the mechanism involved in regulating the secretion.

Cholecystokinin (CCK) is released in cats, dogs, pigs, and humans mainly from the

TABLE I. EFFECT OF CCK[a] ON RATS WITH PANCREATIC JUICE RETURNED OR DIVERTED FROM THE INTESTINE.

	Increase in pancreatic enzyme secretion rate		
	Saline	5 Crick units CCK	10 Crick units CCK
Returned	0.48 ± 3.6	44.4 ± 5.7	99.5 ± 9.0[b]
Diverted	0.43 ± 3.1	31.2 ± 3.5	63.5 ± 7.8[b]

[a] CCK = cholecystokinin, was dissolved in 0.40 ml of 0.9% saline and infused into the jugular vein for 30 min. The response is expressed as the difference between the basal protein secretory rate and the maximal rate which occurred 15 min. after stopping the infusion and is expressed as the mean ± SEM, $n = 4$. A paired t test was used to compare the responses.

[b] The response to 10 units was greater when juice was returned to the intestine than when diverted ($P < 0.05$).

upper part of the small intestine (11–13). In most species the distribution of CCK along the gut parallels that of secretin, and in rats secretin has been shown to be released from the intestine above 10 cm caudal to the ligament of Trietz (14). The results shown in Figs. 1 and 4 indicate that SBTI stimulates secretion only in the upper one-third of the small gut and that trypsin suppresses the secretion only in this area also. Consequently feedback control by trypsin or chymotrypsin operates in the area of the gut from which CCK is most likely released. These results are consistent, therefore, with the concept that trypsin in the upper small intestine somehow prevents the release of CCK into the blood; and SBTI, by binding the trypsin, effectively removes the enzyme from the intestine, thereby releasing CCK and increasing pancreatic enzyme output. It has been proposed for some time (15–17) that CCK may be involved in the SBTI stimulation of pancreatic secretion. The most direct demonstration of the release of a humoral factor by SBTI was reported by Khayambashi and Lyman (15) who showed that plasma from rats fed SBTI stimulated amylase secretion in the isolated perfused rat pancreas. These observations on the involvement of CCK in SBTI stimulation of the pancreas and the results in Figs. 1 and 4 strongly support the proposal that this feedback regulation operates by controlling the release of CCK from the intestinal mucosa.

The results in Fig. 2 indicate that the pancreatic secretion rate responded to a short term infusion of trypsin in proportion to the amount of trypsin infused. At doses higher than 10 mg trypsin/0.5 hr, this relationship

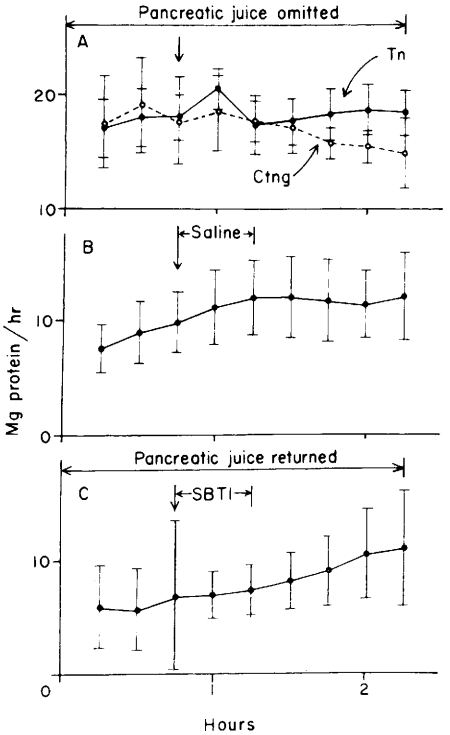

FIG. 5. The effect on pancreatic enzyme secretion of intravenous infusion of trypsin, chymotrypsinogen, saline, or SBTI in 0.40 ml of 0.9% saline for 30 min. A, 2 mg trypsin (tn) ($n = 4$) or 2 mg chymotrypsinogen (ctng) ($n = 4$); B, saline alone ($n = 6$); C, 5 mg SBTI (soybean trypsin inhibitor) ($n = 4$). In A and B the pancreatic juice had been diverted for 10 hr to compare with the effect in Fig. 2, and in C the juice was continually returned to the intestine to compare with previous results (1). Data presentation is the same as Fig. 2.

is lost and the intestine appears to be "saturated." This type of response might be expected if the intestine had a limited number of "receptor sites" that trypsin interacted

with to prevent CCK release. Once the sites were saturated, additional trypsin would have no further effect. Such a receptor site might be CCK-containing cells in the intestine analogous to the secretin containing cells of the intestine or gastrin containing cells in the stomach. The proportionality between pancreatic response (in terms of the suppression and rebound in output) and the dose of infused trypsin when the enzyme was infused through a similar length of segment of the intestine corresponds to the pancreatic volume response noted by Meyer *et al.* (18, 19) when acid was perfused through a given length of bowel in dogs. Their response was a function of the total amount of titratable acid perfused through the intestinal segment. The authors suggested that secretin release from the intestine may be controlled by mucosal receptors sensitive to pH. Perhaps an analogous type of receptor, sensitive to trypsin and chymotrypsin, may control CCK release from intestinal cells containing the hormone.

The need for active trypsin to be present in the digestive tract in order to suppress enzyme secretion is indicated by the fact that DFP-trypsin did not suppress enzyme secretion as did the active enzyme (Fig. 3). Lyman *et al.* (20) have previously reported that the inactive precursor of chymotrypsin, chymotrypsinogen, also was unable to suppress enzyme secretion in the rat as did active chymotrypsin. In both these enzymes the active center of the molecule is blocked, either by DFP, as with trypsin, or by a peptide in the case of chymotrypsinogen. Apparently, trypsin and chymotrypsin require an accessible active site in order to control enzyme secretion. Since the active site is not available in chymotrypsinogen or DFP-trypsin, these molecules also do not have proteolytic activity, which might be necessary to produce a digestion product which suppresses pancreatic output. However, since protein digestion products do not significantly alter the pancreatic output in rats (1, 5), this possibility is unlikely and the suppressive effect on pancreatic output is more likely a direct effect of the active enzyme molecules. Since trypsin inhibitors irreversibly bind trypsin, their effectiveness in stimulating pancreatic secretion in the rat would result from their blocking of trypsin's active center.

Increased pancreatic enzyme secretion in the rat after diversion of the pancreatic juice has been reported by others (5, 21, 22) and more recently by Green *et al.* (1) who followed pancreatic chymotrypsin output in fully recovered, conscious rats for 10 hr after diversion of the juice. A characteristic of the enzyme response after diversion was an immediate increase in enzyme output which persisted for about 3 hr, then declined abruptly to plateau at a new and generally higher basal rate of secretion. Evidence that this cessation of pancreatic enzyme secretion was not due to exhaustion of the pancreatic enzymes is provided by the results in Table I showing that exogenous CCK could still evoke a strong enzyme output. This suggests that the factor limiting secretion after diversion or after feeding SBTI (1) may be the rate at which CCK is synthesized by the intestinal cell. The initial effect of suddenly removing trypsin suppression of pancreatic enzyme secretion by either diversion of the juice from the intestine or by feeding SBTI would be a rapid release of stored CCK from the intestinal cell and induction of a maximal enzyme secretion. Exhaustion of stored CCK would occur upon prolonged removal of trypsin and the new basal secretion would be maximum for the rate at which new CCK could be synthesized and released. This interpretation is consistent with observations that duodenal infusion of SBTI, protein, or protein hydrolysates had no secretory stimulating effect in those animals with juice diverted (1, 5). The rebound effect noted in Figs. 2–4 after stopping the infusion of trypsin into the intestine of these animals probably resulted from CCK that had accumulated during the trypsin infusion which was then released after stopping the enzyme infusion.

All of the evidence we have obtained so far indicates that trypsin or chymotrypsin exert their feedback regulation of pancreatic secretion in the intestine. Laporte (7, 8) have also suggested that a feedback control of pancreatic secretion exists in the rat. However they have proposed that trypsin passes

through the duodenal wall and binds CCK in the blood thus preventing its stimulation of the pancreas, and SBTI would stimulate secretion by removing the trypsin from the blood. Rothman *et al.* (9, 10), using *in vitro* preparations of rabbit pancreas, have reported that chymotrypsinogen crosses the intestinal wall and might also exert some unspecified regulatory effect on the pancreas. The results in Fig. 5 show that intravenous infusion of either trypsin, SBTI, or chymotrypsinogen had no significant effect on pancreatic enzyme secetion. Therefore it seems unlikely that these agents have their effect on the pancreas by acting in the blood rather than in the small intestine.

Summary. Further studies on the feedback regulation of pancreatic enzyme secretion by trypsin were conducted in conscious rats, surgically prepared so that pancreatic juice could be collected or returned. Suppression of enzyme secretion by trypsin as well as its stimulation by SBTI occurred only in the upper part of the small intestine, where the hormone CCK is known to be released. Over a limited range, trypsin suppression of pancreatic secretion was proportional to the dose of trypsin. Higher concentrations had no further effect, suggesting "saturation" of the intestine. Trypsin which had its active center blocked by DFP did not suppress enzyme output.

These results supported the concept that only trypsin (or chymotrypsin) with an exposed active center suppressed pancreatic enzyme secretion in the rat by somehow suppressing the release of CCK from the intestinal cell. Presumably CCK is released from the intestine following "removal" of trypsin from the intestine either by diverting the juice or by feeding SBTI which binds the enzyme. All of the evidence supported the view that the effect of trypsin or SBTI on pancreatic secretion was mediated at the intestinal level and not in the blood as has been suggested.

We are grateful to Dr. Gary Green for many valuable discussions.

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Received October 25, 1974. P.S.E.B.M. 1975, Vol. 148.