

# Responses of the Exocrine Pancreatic Secretion to Spontaneous Feeding in Rats with Bile-Pancreatic Juice Diversion (43311)

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**Abstract.** The regulatory response of the exocrine pancreas was examined in rats under unanesthetized and unrestrained conditions. The previous study demonstrated that the pancreatic protease secretion increased 2-fold after spontaneous feeding of a low protein diet in chronically bile-pancreatic cannulated rats (normal rats) whose bile-pancreatic juice (BPJ) was returned to the duodenum. In the present study, we observed the response of the exocrine pancreatic secretion to spontaneous feeding of a low protein diet in rats with chronic diversion of BPJ from the proximal small intestine for 6 days (bypass rat) whose diverted BPJ was returned to the upper ileum. During BPJ diversion, the dry weight and the protein content of the pancreas were increased 2-fold, compared with normal rats. Also, the levels of trypsinogen and chymotrypsinogen in the pancreas were increased several times, but amylase was decreased. The basal secretion of enzymes after a 24-hr fast was enhanced in bypass rats in proportion to the pancreatic enzyme contents. After spontaneous feeding of 8% casein fat-free diet, the increases in the pancreatic secretion of bypass rats were much smaller than those of normal rats. In contrast, the increase of BPJ flow of bypass rats after feeding was greater than that of normal rats. These findings represent that the chronic diversion of BPJ exerts hypergrowth of pancreas and hypersecretion of proteases in the fasting state, and less sensitivity of pancreatic enzyme secretion to dietary feeding.

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The negative feedback regulation by pancreatic endopeptidases in the small intestinal lumen is known as a regulatory mechanism of the exocrine pancreatic secretion in rats (1, 2), pigs (3), and humans (4, 5). In this mechanism, the pancreatic protease in the proximal small intestine is essential to suppress exocrine pancreatic secretion (6), and the mechanism is mediated by cholecystikinin and cholinergic nerve (7–11).

We observed previously that pancreatic protease secretion was increased 2-fold above basal secretion after spontaneous feeding of low protein diets in rats with normal bile-pancreatic juice (BPJ) flow under

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unanesthetized and unrestrained conditions (H. Hara, A. Fujibayashi, S. Kiriyaama. Pancreatic protease secretion profiles after spontaneous feeding of casein or soybean protein diet in unrestrained conscious rats. Submitted for publication.). Recently, Guan *et al.* (12) suggested that the noninvasive state is important for investigation of the pancreatic secretion.

In the present study, the diverted BPJ was continuously returned to the upper ileum through a catheter (bypass rats). Under this condition, the pancreatic proteases did not exist in the proximal small intestine. We investigated the effects of chronic diversion of BPJ from the jejunum on the response of the exocrine pancreatic secretion to spontaneous feeding of a low protein diet, and the changes in the content of pancreatic enzymes. The purpose of the present study was to examine the role of the negative feedback regulation under more physiological conditions.

## Materials and Methods

**Diets and Animal Preparation.** Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan), weighing 220–250 g, were operated on after a 24-hr fast

for implantation of cannula into the common bile-pancreatic duct and duodenum or upper ileum under anesthesia by intraperitoneal injection of Nembutal (sodium pentobarbital: 40 mg/kg body wt; Abbott Co., North Chicago, IL). A tip of polyethylene catheter (SP 28; i.d. 0.4 mm, o.d. 0.8 mm; Natsume Seisakusyo, Tokyo, Japan) was inserted into the common bile-pancreatic duct, connected to silicone tubing (i.d. 0.5 mm, o.d. 1.0 mm; Dow Corning Co., Kanagawa, Japan), and tunneled subcutaneously to the back of the neck region. Another silicone catheter (i.d. 0.5 mm, o.d. 1.0 mm; Dow Corning) for returning BPJ to the lumen was placed through the fistula at 1-cm proximal to the ampulla of Vater (normal rat) or at 45-cm distal from Trize ligament (bypass rat). In bypass rats, BPJ flow bypassed the proximal small intestine through the catheter. The catheter was connected to the bile-pancreatic duct catheter behind the neck to maintain the BPJ flow during a recovery period for 5 days on the 25% casein-sucrose diet (Table I [13–16]). The period is enough to recover from the operation, as reported previously (H. Hara, A. Fujibayashi, S. Kiriya. Pancreatic protease secretion profiles after spontaneous feeding of casein or soybean protein diet in unrestrained conscious rats. Submitted for publication.).

After a 24-hr fast, normal and bypass rats were given 2 g of 8% casein fat-free diet (test diet; Table I) for 30 min. Almost all of the given test diet was consumed within 30 min in both the rats. In order to collect BPJ outside of the cage, a polyethylene tube (SP 28; Natsume Seisakusyo) was connected to the pancreatic duct catheter. Bile-pancreatic juice was collected for 3 min at 30 and 60 min before feeding and at 30, 60, 90, and 120 min after feeding of the test diet. In period except 3 min for sampling, BPJ was returned continuously to the intestine during the experiment.

The pancreas was removed 150 min after feeding of the test diet under anesthesia with sodium pentobarbital.

**Analyses of Protein and Enzyme Activities.** The pancreas was freeze-dried and used for the following analyses. The volume of BPJ was measured gravimetrically: 1 mg of BPJ was considered to be 1  $\mu$ l, which is the basis of the measurement of pooled 100  $\mu$ l BPJ weight (100  $\mu$ l = 100 mg). The activity of trypsin, chymotrypsin, and amylase was measured in BPJ diluted adequately with saline containing 0.1% Triton X-100. Zymogens of trypsin and chymotrypsin were activated by purified enterokinase (Sigma Chemical Co., St. Louis, MO). Trypsin (17), chymotrypsin (18), and amylase (19) activities were estimated photometrically using synthetic substrates, *N*- $\alpha$ -*p*-toluenesulfonyl-L-arginine methyl ester, *N*-benzoyl-L-tyrosine ethyl ester, and procion yellow starch, respectively. Protein content was quantified by modified Lowry's method (20, 21).

**Calculation and Statistical Analyses.** One unit of

**Table I.** Composition of Diets

	25% Casein-sucrose diet (%)	25% Casein-nonfat diet <sup>a</sup> (%)
Casein <sup>b</sup>	25.0	9.4
Sucrose	63.1	83.5
Corn oil <sup>c</sup>	5.0	—
Mineral mixture <sup>d</sup>	4.0	4.0
Vitamin mixture <sup>e</sup>	1.0	1.0
Vitamin E <sup>f</sup>	0.1	0.1
Choline chloride	2.0	2.0

<sup>a</sup> Casein fat-free diets were made to contain 8% protein (protein content = N content  $\times$  6.25). That is, 9.4% of casein material was added to the diet.

<sup>b</sup> Casein (ALACID; New Zealand Daily Food, Wellington, New Zealand) contained 13.7% nitrogen, as evaluated by the Kjeldahl method.

<sup>c</sup> Retinyl palmitate (7.66  $\mu$ mol/kg diet) and ergocalciferol (0.0504  $\mu$ mol/kg diet) were added to the corn oil.

<sup>d</sup> The mineral mixture is identical to the mineral mixture (MM2) described by Ebihara *et al.* (13).

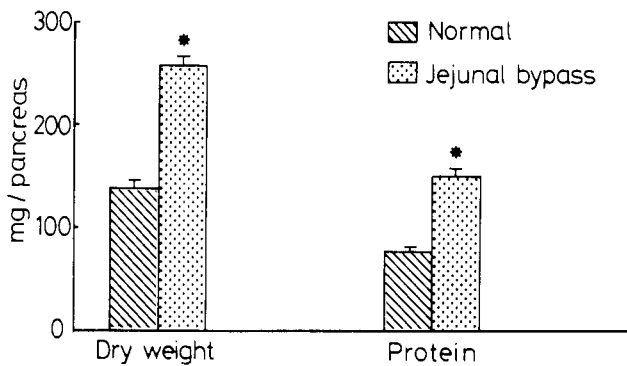
<sup>e</sup> The vitamin mixture was prepared in accordance with the AIN-76 mixture (14), except vitamin K as menadione and L-ascorbic acid were added to give 5.81  $\mu$ mol/kg (15) and 284  $\mu$ mol/kg (16) of diet, respectively.

<sup>f</sup> Vitamin E (Granulated, Yuvela, Eisai Co., Tokyo, Japan) supplied 423  $\mu$ mol all-rac- $\alpha$ -tocopheryl acetate in kg diet.

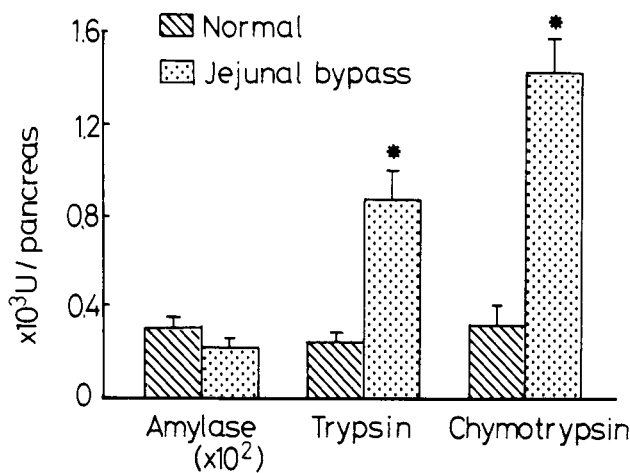
trypsin and 1 unit of chymotrypsin are the activity that hydrolyzes 1  $\mu$ mol of substrate/min at 30°C. The activity of amylase is standardized by purified  $\alpha$ -amylase from porcine pancreas (Type 1A; Sigma) at 37°C. In Figure 3, the values after feeding represent the relative rates of secretion in the fasting state. The statistical analyses were performed by one-way and two-way analysis of variance ("time" and "treatment"). The significant differences among means were determined by Student's *t* test and least significant difference. Values given are mean  $\pm$  SE.

## Results

Body weight gains during the recovery period of rats maintained on 25% casein-sucrose diet were 8.3 g/day and 6.3 g/day in normal and bypass rats, respectively. The dry weight and the total protein content of the pancreas were increased about 2-fold by chronic diversion of BPJ from the proximal small intestine for 6 days in bypass rats, as shown in Figure 1. In Figure 2, the trypsin and chymotrypsin activities of the pancreas of bypass rats were, significantly, 3.7- and 4.6-fold higher, respectively, than those of normal rats. The amylase activity of the pancreas was not changed in bypass rats, compared with normal rats. The specific activity of the trypsin and chymotrypsin of bypass rats was also markedly increased. The specific activity of amylase of the normal rat estimated from the protein content of the pancreas was 394  $\pm$  40 units/mg protein, which is significantly higher than the specific activity of the bypass rat (142  $\pm$  27 units/mg protein, *P* < 0.01).



**Figure 1.** The changes in the pancreatic dry weight and protein content by chronic diversion of bile-pancreatic juice from the proximal small intestine for 6 days. In normal rats, diverted BPJ was returned to the duodenum. In jejunal bypass rats, BPJ bypassed the jejunum by returning the BPJ to the ileum. The values are mean  $\pm$  SE ( $n = 8$ ). Asterisks represent the significant differences between the two groups ( $P < 0.01$ ).



**Figure 2.** The changes in the content of the pancreatic enzymes by chronic diversion of bile-pancreatic juice from the proximal small intestine. The details are described in the legend to Figure 1. Asterisks represent the significant differences between the two groups ( $P < 0.01$ ).

As shown in Table II, the secretions of protein, trypsin, and chymotrypsin in the fasting state were increased 2.2-, 4.2-, and 6.1-fold, respectively, by chronic diversion of BPJ from the proximal small intestine. The amylase secretion and the fluid volume of the bypass rats in the fasting state (0 hr) were similar to those of the normal rats.

After the feeding of the test diet, the volume of BPJ was increased in bypass rats, but not in normal rats, as shown in the top panel of Figure 3. Figure 3 also presents the relative changes in the protein, amylase, trypsin, and chymotrypsin secretions of both the rat groups after the feeding of the test diet. The profiles of the protein and three enzyme secretions after feeding were similar in normal and bypass rats. After feeding of the test diet, the secretions of protein and enzymes were increased about 2-fold at 30 min and peaked at

**Table II.** Secretions of Protein and Enzymes into Bile-Pancreatic Juice after a 24-hr Fast in Normal and Bypass Rats<sup>a</sup>

Secretion for 3 min	Normal rats	Bypass rats
Volume ( $\mu$ l)	75.4 $\pm$ 7.6	70.4 $\pm$ 7.5
Protein (mg)	0.68 $\pm$ 0.11	1.51 $\pm$ 0.10 <sup>b</sup>
Amylase (units)	1044 $\pm$ 151	966 $\pm$ 115 <sup>b</sup>
Trypsin (units)	8.7 $\pm$ 0.9	36.8 $\pm$ 2.3 <sup>b</sup>
Chymotrypsin (units)	11.4 $\pm$ 1.5	69.5 $\pm$ 4.2 <sup>b</sup>

<sup>a</sup> Values are the amount of protein or enzyme units released from the pancreas for 3 min, and are average of two collections before feeding of a test diet. Values represent mean  $\pm$  SE ( $n = 8$ ).

<sup>b</sup> Significant differences between normal and bypass rats ( $P < 0.05$ ).

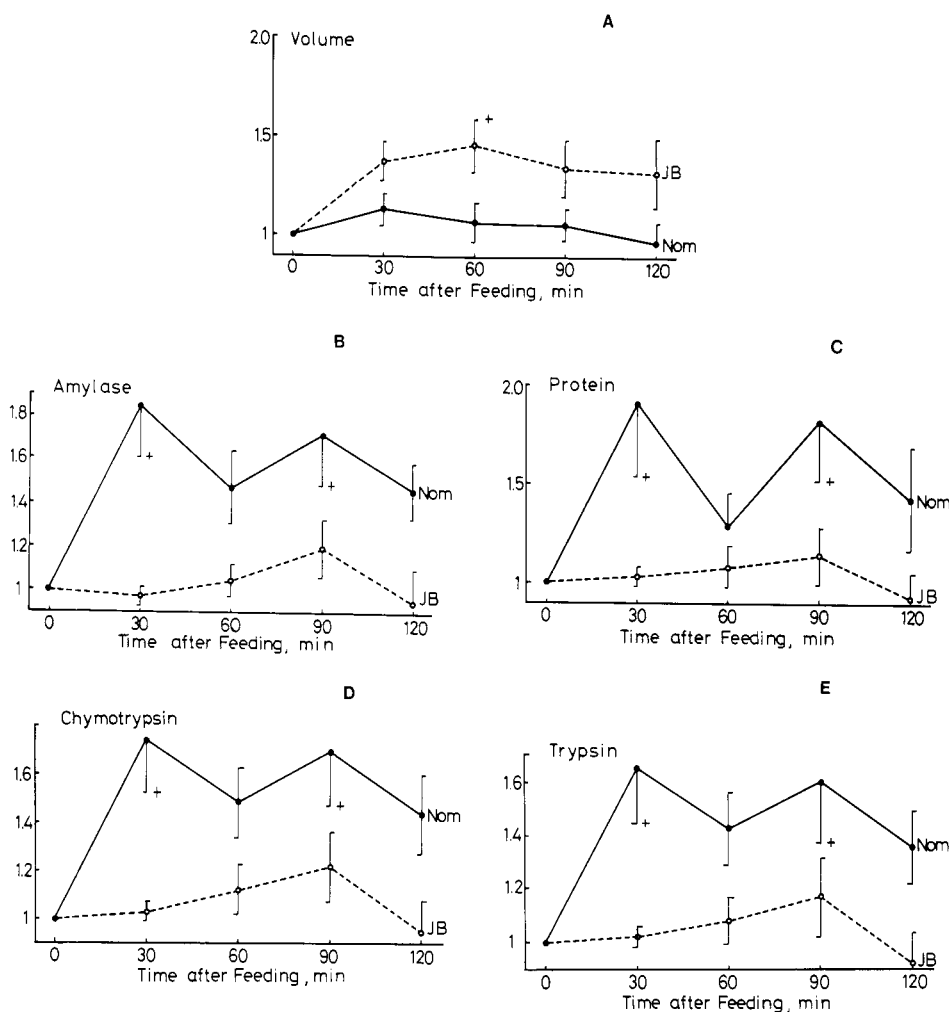
30 and 90 min in normal rats; the increments from the secretions at 0 hr are significant. In contrast, the increases in protein and all enzyme secretions of bypass rats 90 min after feeding were small and were only 1.2-fold the secretions in the fasting state.

## Discussion

We observed pancreatic hypergrowth, hypersecretion of proteases, and low responsiveness of pancreatic enzyme secretion to the spontaneous feeding of diet after chronic diversion of BPJ from the proximal small intestine in rats. The pancreatic hypergrowth by diversion of BPJ was reported by Levan and Green (22) and Newman *et al.* (23). The latter report and the present study demonstrated that the contents of pancreatic proteases were markedly increased and amylase content was slightly decreased by diversion. In contrast, the results with the rats whose jejunum was isolated from the other parts of intestine (24), or was resected (25) in order to eliminate BPJ from the jejunum, revealed that the pancreatic protein or protease contents were not increased. The difference between these observations and our results is probably due to whether the dietary chyme passed through the jejunum. These findings suggest that the pancreatic hypergrowth by BPJ diversion depends on the dietary chyme in the jejunal lumen.

The pancreatic secretion rate of protein or of each enzyme in the fasting state (0 hr) was paralleled by the pancreatic content of protein or of each enzyme. The finding reveals that the basal secretion rates of the pancreatic enzymes are influenced by the contents of the pancreas, and the pancreatic secretion rates of bypass rats during fasting are controlled in the same manner as those of normal rats.

Also, the present study established that the responsiveness of the pancreatic secretion to diet feeding was markedly decreased by the chronic diversion of BPJ. The increase in the pancreatic secretion of normal rats may depend on casein in the test diet, because the diets does not contain any fat. Petersen and Grossman (26) reported that the pancreatic secretion did not respond to trypsin inhibitor in the rats with BPJ diverted from



**Figure 3.** The secretion profiles of the (A) volume, (B) amylase, (C) total protein, (D) chymotrypsin, and (E) trypsin in normal rats (Nom, ●) and jejunal bypass rats (JB, ○). Each point after feeding represents the relative value for the secretion in the fasting state (0 hr) that is the average of two samplings. Vertical bars show the SE. From the results the analysis of variance, time and treatment were significant influences ( $P < 0.05$ ) in all profiles. Means with a plus sign (+) are significantly different from the secretion of 0 hr in each group ( $P < 0.05$ ,  $n = 8$ ).

the jejunum. These results represent that the feeding of casein stimulates pancreatic secretion in the same manner with trypsin inhibitor. In bypass rats, the proteases are absent from the jejunum. Therefore, the response of pancreatic secretion depending on the negative feedback mechanism cannot occur.

Schneeman and Lyman (16) demonstrated the low response of pancreatic secretion to exogenous cholecystokinin (CCK). Another mechanism of the low responsiveness may be that acinar cells were desensitized to CCK. The cholinergic pathway is also known to be involved in the pancreatic hypersecretion by diversion of BPJ from the jejunum (10, 11). Pretreatment with CCK or carbachol, a muscarinic agonist, increases the desensitization of acinar cells to CCK (27, 28). The pancreas of the bypass rat was probably stimulated continuously by the hormone and nerve. It is also possible that the decrease of digestive products of proteins in the proximal small intestine by deprivation of

proteases affects the lower responsiveness of pancreatic secretion in bypass rats.

We observe an increase in the fluid secretion in the jejunal of bypass rats, which may be due to unneutralized gastric acid in the proximal small intestine. Noda *et al.* (29) and Singer *et al.* (30) reported that gastric acid in the intestine enhances pancreatic fluid secretion. Guan *et al.* (12) reported that the mechanism of protease hypersecretion by diversion also depends on gastric secretion. Under our conditions, the response of pancreatic secretion to feeding was almost abolished in bypass rats. The result suggests that that is not the effect of the gastric acid on the response of pancreatic secretion to spontaneous feeding of diet under our conditions.

Recently, Gomez *et al.* (31), Miyasaka *et al.* (32), and Nakamura *et al.* (33) reported that bile acid is responsible for the regulation of pancreatic secretion and growth, directly and indirectly. In the present study, bile is also diverted from the jejunum; therefore, the

pancreatic hypergrowth may also depend on the bile diversion.

We observed the pancreatic enzyme contents and the pancreatic secretion with the same rats. The secretion rates of pancreatic enzymes for 3 min in the fasting state are about 4% of the total enzymes stored in the pancreas of both the normal and bypass rats, which indicates that pancreatic enzymes are exhausted for 75 min if their syntheses is stopped. This finding reveals that the pancreatic protein turnover is very rapid, even during fasting under the conditions adopted by us.

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