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Enteral Feedback Control of Pancreatic Hypertrophy: The Role of Pancreatic Biliary Secretions[1] (42245)

B. M. NEWMAN,[2] P. C. LEE, S. BROOKS, D. R. COONEY, AND E. LEBENTHAL

International Institute for Infant Nutrition and Gastrointestinal Disease, Department of Pediatric Surgery, Children's Hospital of Buffalo, and Department of Pediatrics, State University of New York, Buffalo, New York 14222

Abstract. Chronic diversion of pancreatic and biliary secretions away from the proximal small intestine resulted in rapid increases in pancreatic size and protein content in adult rats. The effect was detectable as early as 10 days postsurgery. Differential changes in pancreatic enzymes were evident after bypass. The concentration of trypsinogen remained stable while amylase concentrations showed a marked decrease. Total pancreatic trypsinogen content, however, was increased, while amylase content remained similar to controls. Feeding bypassed rats with chows containing various pancreatic and biliary supplements had no effect on the hyperplastic response of their pancreata. Trypsinogen and amylase levels in supplemented groups remained similar to the bypassed group fed nonsupplemented chow, with the exception of increases in trypsinogen concentration and content in the groups supplemented with bile and cotazyme plus bile acids. The adequacy of oral refeeding of pancreatic biliary components was supported by its effectiveness in restoring mucosal enterokinase activity and trypsin levels in segment 1. However, there was no correlation between tryptic activity in the contents of the bypassed segment and the eventual pancreatic weight. These results indicate that factors other than those supplemented in this study are required in maintaining the steady state of pancreatic growth in normal rats.

Exclusion of pancreatico–biliary secretions from the proximal small intestine has been shown to result in an increase in pancreatic enzyme secretion (1–3) and in pancreatic hypertrophy (4). This has been related to a loss of feedback inhibition due to the absence of luminal pancreatic–biliary components in the duodenal segment of the small intestine. The exact components responsible for this control are not known. Studies with protease inhibitors, particularly trypsin inhibitors, revealed that pancreatic hyperplasia and hypersecretion could also be obtained by chronic feeding of these inhibitors to rats and chicks (5–10). Since purified inhibitors were used in many cases, it was surmised that active pancreatic proteases in the small intestinal lumen might be the controlling factors. The role of biliary factors in the feedback regulation is still controversial. If pancreatic and/or biliary factors are true modulators of pancreatic growth, the reintroduction of these components after their removal should abolish the hypertrophic response of the pancreas. This aspect still has not been examined at length. The present report employs the diversion of pancreatico–biliary secretions in rats to study the hypertrophic effect on the pancreas. Further, by selective oral supplementation of various pancreatic and biliary factors, the relative importance of each of these factors was evaluated.

Materials and Methods. Adult Sprague–Dawley rats weighing between 200 and 275 g were used throughout these experiments. With the exception of dietary supplements following surgery in special groups of rats, environmental conditions were identical with a 12-h light/dark cycle and at an ambient temperature of 25°C. Animals in the experimental groups underwent surgical transposition of a 4-cm segment of duodenum containing the ampulla of Vater to the jejunum, placing the ampulla 30 cm distal to its original site. This was accomplished by dividing the duodenum above and below the ampulla, oversewing the proximal end and anastomosing the distal end to the antimesenteric border of jejunum thus creating a short pouch. The proximal and dis-

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[2] Present address: Department of Surgery, Box 181, University of Virginia Medical Center, Charlottesville, Va. 22908.

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tal ends of the duodenum were reanastomosed, restoring intestinal continuity. Details of the surgical procedure will appear in a separate publication (11). A group of six sham-operated rats underwent transection of the duodenum with reanastomosis. Twenty-seven rats were used as controls. These animals were matched for weight and length of intestinal segments with the experimental rats at the time of sacrifice.

Experimental Groups. *Bypass with standard chow (bypass).* Rats were started on a preoperative elemental diet (Vivonex, Eaton-Norwich) for 2 days. Postoperatively, they were left without oral feeding for Days 1 and 2, and were advanced to the elemental diet on Days 3 and 4. The diet was returned to standard chow from Day 5 until sacrifice. Rats were sacrificed between 10 AM to 12 noon to minimize diurnal enzyme variations.

Bypass with bile acids (bile). Rats underwent the same feeding regimen as described above, except that the postoperative feedings were supplemented with bile acids, 250 mg taurocholic and taurodeoxycholic acids (Sigma Chemical Co.) in a 2:1 ratio were added to each 100 g of standard rat chow.

Bypass with trypsinogen (Try). These rats had postoperative supplementation with 200 mg trypsinogen (Type I, Sigma) per 100 g of standard chow.

Bypass with trypsinogen and bile acids (T & B). These rats received feeding supplements of trypsinogen and bile acids, each in the same concentration noted above.

Bypass with cotazyme (Cot). These rats had postsurgical supplementation with the contents of one capsule of cotazyme (Organon NS) per 100 g of standard chow.

Bypass with cotazyme and bile (Cot & B). These rats received feeding supplements of cotazyme and bile acids, each in the same concentration noted above.

Preparation of Tissue Samples. Bypassed rats were sacrificed at 10, 23, or 45 days after surgery to follow the sequential changes in their pancreata. Rats on supplemental diets were sacrificed 23 days after surgery. The pancreas was excised, trimmed of fat and mesentery, and frozen immediately. Pancreata were kept at −80°C until ready for analysis. The intestines from these rats were also removed. The proximal bypassed section of duodenum and jejunum (30 cm) was resected. The contents from each segment were collected by flushing with 5 cc of 0.9% NaCl solution. The contents were homogenized with a Potter–Elvehjem homogenizer and homogenates were used for the determination of amylase and trypsin.

Pancreatic tissue was minced with sharp scissors. Minced tissues were then separately homogenized with a Potter–Elvehjem homogenizer using a motor driver Teflon pestle with the vessel immersed in crushed ice. Pancreatic homogenates were sonicated before use for biochemical determinations.

Protein was determined by the technique of Lowry *et al.* (12) using a bovine serum albumin fraction V as the standard. DNA was measured by the colorimetric reaction with diphenylamine reagent according to Burton (13) using highly polymerized calf thymus DNA as the standard. A modified procedure was adapted to minimize the interference by sialic acids according to Croft and Lubran (14).

Trypsinogen was first activated with enterokinase, at a constant ratio of enterokinase: homogenate proteins for 45 min at 25°C; these conditions have yielded optimal and reproducible activation of this zymogen in our laboratory. The enterokinase used was partially purified from the mucosa of the proximal small intestine of rats following the procedure of Baratti *et al.* (15) up to the acidification step. Trypsin activity was then measured from the hydrolysis of *p*-nitroaniline from the substrate benzoyl-DL-arginine *p*-nitroanilide (BAPNA) at pH 8.2 and 25°C (16). Units are expressed as nanomoles of BAPNA hydrolyzed per minute per milligram protein. Lipase activity was determined by potentiometric titration (at a constant pH of 8) of ionized fatty acid liberated from an olive oil emulsion (17). Units are expressed as micromoles of NaOH required to neutralize the free fatty acid liberated per minute per milligram of protein. Amylase was determined by the saccharogenic method (18) using soluble starch as the substrate. Units are expressed as micromoles of maltose liberated per minute per milligram protein.

Statistical analyses were performed using standard analysis of variance for each data set. After determining the error due to treatment,

specific comparisons were made by use of the modified t test (19).

Results. *Development of pancreatic hypertrophy.* Since sham-operated and control rats exhibited no significant differences in the parameters measured, they were combined and used as the control group. Figure 1 indicates the change in the pancreas with time following pancreatic-biliary diversion. The pancreata of bypassed rats showed dramatic increases in wet weight and total protein by 10 days following surgery. Further increases in pancreatic weights and protein contents were seen at 23 days after operation but these were not significantly different when compared to the same parameters in rats at 10 days after surgery. No further increase was seen at 45 days after bypass. Pancreatic DNA content also showed increases but was only significant at 23 days after operation.

The effects of bypass on pancreatic enzymes are shown in Figs. 2 and 3. No change in the trypsinogen concentrations was seen at any postoperative time. However, amylase concentration showed a rapid decrease by 10 days and remained low throughout the rest of the period studied (Fig. 2). The pancreatic contents of trypsinogen, on the other hand, increased at 10 days after surgery and remained higher than control the rest of the period. Amylase contents showed a decrease at 10 days after bypass but did not reach a significant level. Thereafter, amylase contents remained similar to those found in control rats (Fig. 3).

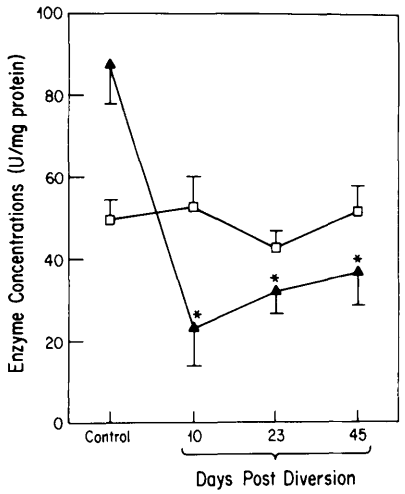

FIG. 2. Effect of chronic pancreatic-biliary diversion on pancreatic concentrations of trypsinogen (□) and amylase (▲). *Significantly different from corresponding control values ($P < 0.05$). All values are means ± SEM. Numbers of rats in each group are as listed in Fig. 1.

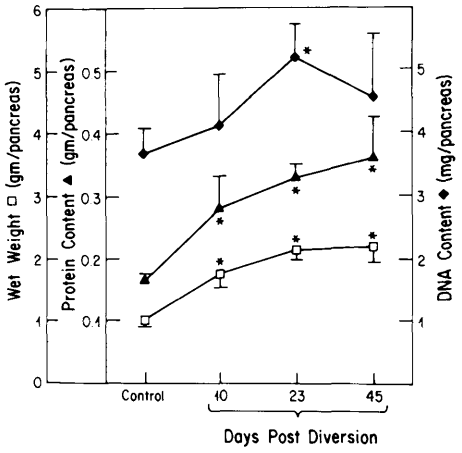

FIG. 1. Effect of chronic pancreatic biliary diversions on pancreatic wet weights, total pancreatic protein, and DNA. *Significantly different from corresponding control values ($P < 0.05$). Control rats ($n = 27$) included both nonoperated and sham-operated rats of similar age. Number of pancreatic-biliary diverted rats at 10 days ($n = 6$) at 23 days ($n = 12$) at 45 days ($n = 6$). All values are means ± SEM.

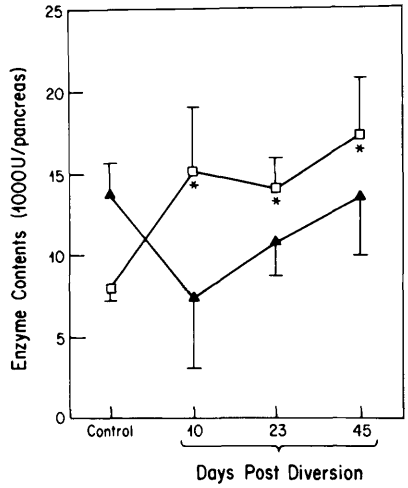

FIG. 3. Effect of chronic pancreatic-biliary diversions on pancreatic contents of trypsinogen (□) and amylase (▲). *Significantly different from corresponding control values ($P < 0.05$). All values are means ± SEM. Numbers of rats in each group are as listed in Fig. 1.

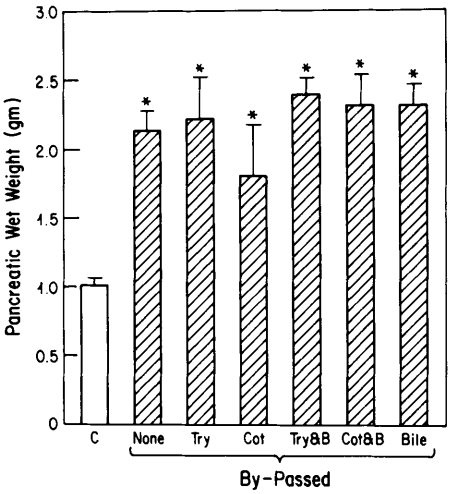

FIG. 4. Effect of chronic pancreatic-biliary diversions and dietary supplementation on pancreatic wet weights of pancreata. C = control rats. Bypassed rats were fed either one of the following six diets: None—regular chow; Try—regular chow supplemented with trypsinogen (200 mg/100 g); Cot—regular chow supplemented with cotazyme (content of 1 capsule/100 g); Try and bile—regular chow supplemented with trypsinogen (100 mg) and bile (250 mg taurocholic and taurodeoxycholic acids 2:1) in 100 g chow; Cot and B—regular chow supplemented with cotazyme (1 capsule) and bile (250 mg) in 100 g chow; bile—regular chow supplemented with bile (250 mg taurocholic and taurodeoxycholic acid/100 g). Numbers of rats for each group equal 6. Bar heights represent means with SEM represented by height of lines on top of bars. *Significantly different from control values ($P < 0.05$).

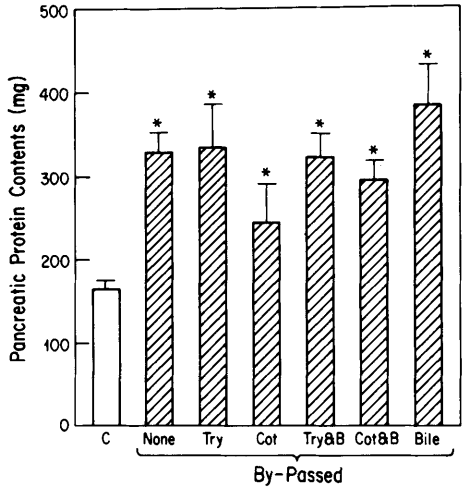

FIG. 5. Effect of chronic pancreatic-biliary diversions and dietary supplementation on pancreatic protein contents. All symbols are similar to Fig. 4.

Effect of supplementation. The response to bypass of the pancreas was stabilized at 23 days postoperatively, and thus this time period was chosen to investigate the effect of oral supplementation of pancreatico-biliary components on the development of pancreatic hypertrophy following pancreatico-biliary diversion from the proximal small intestine. Figs. 4–8 summarize the results.

Pancreatic size (Fig. 4) and total protein content (Fig. 5) were higher in bypassed rats compared to those in control rats whether they were fed a standard diet or diets supplemented with various pancreatic-biliary components. The pancreatic DNA content (Fig. 6) in the bypassed rats remained higher than those of control rats fed standard chow or chows supplemented with trypsinogen and trypsinogen plus bile acids. Bypassed rats were fed on chow with cotazyme, bile, or a combination of cotazyme and bile showed no difference in pancreatic DNA content when compared to control rats. Pancreatic trypsinogen concentrations were the same in control and bypassed rats fed regular diets and diets supplemented with trypsinogen, cotazyme, or trypsinogen plus bile. In bypassed rats fed cotazyme plus bile or bile alone, the pancreatic trypsinogen concentrations were, however, significantly

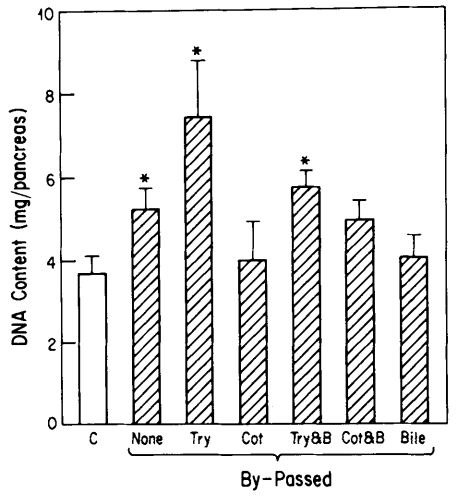

FIG. 6. Effect of chronic pancreatic-biliary diversions and dietary supplementation on pancreatic DNA contents. All symbols are similar to Fig. 4.

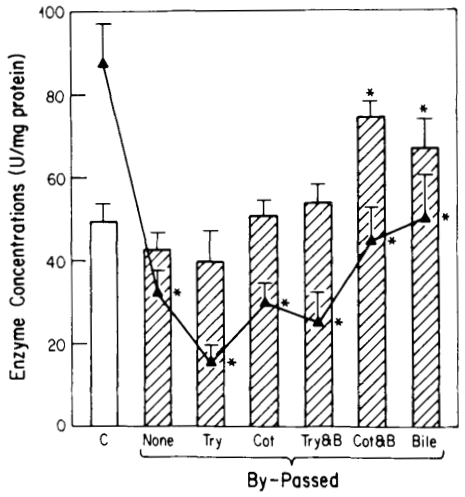

FIG. 7. Effect of chronic pancreatic-biliary diversions and dietary supplementation on pancreatic trypsinogen (□) and amylase (▲) concentrations. All symbols are similar to Fig. 4.

increased over the control and bypassed groups fed the other supplements (Fig. 7). Amylase concentrations showed the typical drop in bypassed rats and remained lower than controls in all the supplemented bypassed rats (Fig. 7). Pancreatic trypsinogen content was increased following bypass and remained so in all the supplemented bypassed rats (Fig. 8). Pancreatic amylase content did not differ from nonoperated rats in bypassed rats fed regular chow, and chows supplemented with trypsinogen plus bile, cotazyme plus bile, and bile alone (Fig. 8). However, in the two bypassed groups fed trypsinogen and cotazyme alone, the pancreatic amylase content was significantly decreased.

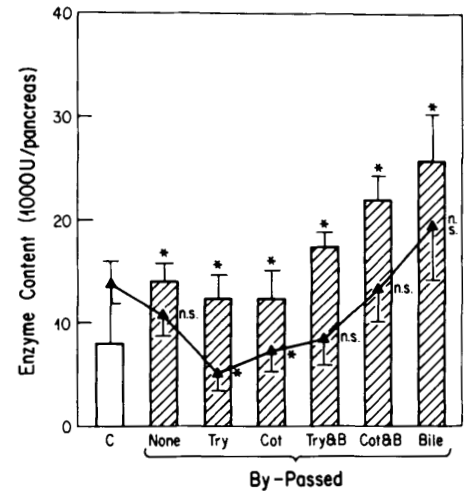

FIG. 8. Effect of chronic pancreatic-biliary diversions and dietary supplementation on pancreatic trypsinogen (□) and amylase (▲) contents. All symbols are similar to Fig. 4.

To evaluate the efficacy of enzyme replacement, particularly that of trypsin and its relationship to pancreatic hypertrophy, the luminal trypsin activity in the entire bypassed segment was plotted against pancreatic weight (Fig. 9). In cotazyme-supplemented groups, the amount of luminal trypsin was evidently less than those found in control groups. In the trypsinogen-supplemented group, the luminal trypsin activity was found to be equivalent or greater than in control rats. Despite the fact that the trypsin activity was similar to that found in normal rats, pancreatic hypertrophy still developed in the trypsinogen-supplemented group. Furthermore, when all the groups are considered, there is no correlation between the trypsin activity in the proximal segment and the pancreatic weights ($r^2 = 0.06$).

Discussion. A direct relationship between pancreatic function and activities of pancreatic enzymes in the small intestinal lumen has long been known to exist in some animals. Both trypsin inhibitor feedings and pancreaticobiliary diversion have been shown to lead to

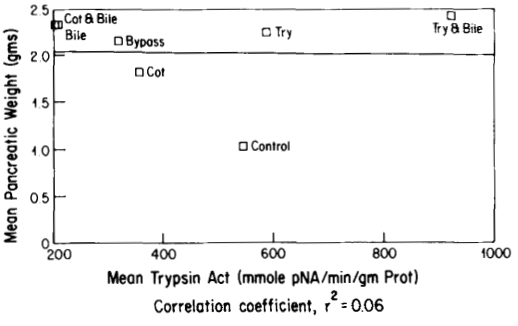

FIG. 9. Correlation between pancreatic weights and trypsin activity in luminal contents in proximal segment of rats. Data represent means of pancreatic weights and means of trypsin contents recovered from proximal segments of small intestines of rats from the same treatment group. The regression line shown was obtained by regression analysis.

pancreatic hypertrophy and pancreatic hypersecretion particularly of proteases (1–4). This feedback loop apparently involves the proximal part of the small intestine, as resection of the proximal portion of the small intestine in rats will eliminate the hypertrophic response of the pancreas to trypsin inhibitor feeding (20, 21). Although biliary obstruction can be a stimulus to pancreatic secretion (3, 22), the role of biliary factors in the hypertrophic response of the pancreas is not known.

Our results using the rat as a model confirm that the diversion of pancreatic and biliary secretion from the proximal portion of the small intestine stimulates pancreatic growth as evidenced by the increase in wet tissue weight and protein and DNA content. The growth is a combination of hypertrophy and hyperplasia as there were increases in DNA but not in proportion to the increases in protein. The degree of hypertrophy is progressive with time such that the relative values at 45 days after surgery were all higher than those at 10 days. The pancreas apparently reaches a new steady-state level inasmuch as pancreatic size and protein content seem to stabilize at levels reached by 23 days postsurgery.

We have previously demonstrated that a complete diversion of pancreatico–biliary secretions from the proximal segment of the small intestine was achieved in this operative procedure. The absence of trypsin and amylase from the proximal 15-cm segment of duodenum, and the disappearance of EK were also noted in the bypassed rats in the present study. Thus, the pancreatic hypertrophy observed is most likely due to the absence of some pancreatic–biliary factors in the lumen of the proximal small intestine.

The pancreata in the bypassed rats not only became hypertrophic but also showed changes in enzyme content. Of the two enzymes studied, trypsinogen concentrations were not changed but amylase concentrations were significantly decreased. Similar observations were seen in rats fed trypsin inhibitors (10). This further suggests that the lack of trypsin activity in the proximal small intestinal lumen is the major cause of the pancreatic changes observed.

The results from dietary supplementation of specific components of pancreatic and biliary secretions provide means to look at the varying effects of these factors as modulators for pancreatic growth. Cotazyme supplementation had a partial effect in preventing the hypertrophy, but none of the other components used were effective in abolishing the hypertrophic response of the pancreas. This does not, however, rule out the role of these components as modulators for pancreatic growth. One explanation for the lack of effect could be that oral supplementation is inadequate to duplicate natural secretions. The supplements were given orally, and thus the enzymes in particular might not survive passage through the gastric environment. However, several observations indicate that adequate amounts of the enzymes did get through to the duodenum. The trypsin activities recovered from the lumen of the proximal segment (segment 1) in both trypsinogen and trypsinogen and bile supplemented groups were equivalent if not higher than those found in the control group. Furthermore, there is preservation of enterokinase activity in the mucosa of the bypassed segment of the small intestine in supplemented rats as compared with that of unsupplemented rats (11). The latter evidence is particularly important since enterokinase levels have been shown to be specifically dependent on trypsin activity (10, 1). These results show that following supplementation, the level of trypsin found in the proximal bypassed segment is sufficient to maintain EK levels in the duodenal mucosa. Despite these observations, there is an obvious inadequacy of oral supplementation in duplicating the natural milieu of the duodenum. This is demonstrated by the lack of correlation of trypsin activity in the proximal small intestinal lumen and pancreatic mass. One particular problem with oral delivery is the discontinuity of the supplementation, in that the rats only eat at night for about 6–8 hr and thus the intestine is exposed to the supplement for a maximum of about 10 hr (including 2–4 hr of gastric emptying time). During the balance of the day, the duodenum of these rats was essentially void of pancreatic–biliary secretions. In normal rats, however, even during times of fasting, the duodenum continues to be exposed to a basal level of pancreatic and biliary secretions. Confirmation of these results will require a comparison between intermittent and constant infusion of pancreatic biliary supplements by surgical

means into the proximal duodenum in bypassed rats.

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