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Effect of Diversion of Bile–Pancreatic Juice to the Ileum on Pancreatic Secretion and Adaptation in the Rat (42235)

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Abstract. Cannulas were implanted to collect bile and pancreatic juice, and the collected secretions were pumped back into the intestine at the level of the duodenum or the proximal ileum. The effect of 6 days of such treatment on pancreatic secretion and on pancreatic growth was determined. The effect on pancreatic secretion was studied by measuring the pancreatic secretory response to a stimulus, provided by acute diversion of bile–pancreatic juice from the proximal intestine. Trophic effects were studied in a separate group of rats by measuring pancreatic weight, protein content, and chymotrypsin activity after an overnight fast. Stimulated pancreatic secretion was 2.1 times greater for protein output and 3.4 times greater for fluid output in rats with chronic diversion of bile–pancreatic juice to the ileum. Pancreatic weight, protein content, and chymotrypsin activity were increased 2.6, 2.9, and 4.8 times, respectively, by chronic diversion of bile–pancreatic juice to the ileum. These results indicate that pancreatic hypertrophy and hyperplasia reported in rats with bile–pancreatic duct transposition to the ileum are the result of loss of feed-back inhibition from bile–pancreatic juice in the proximal intestine.

Transposition of the duodenum to lie between the jejunum and ileum in the rat provokes pancreatic hypertrophy and hyperplasia (1). The cause for this may be bypass of bile–pancreatic juice (BPJ) from the jejunum, since it has been shown that diversion of BPJ to the exterior stimulates pancreatic enzyme secretion (2) and trypsin in the proximal intestine inhibits pancreatic enzyme secretion (3). To explore this connection, we determined the effect of diversion of BPJ to the ileum on pancreatic secretion and on pancreatic growth and adaptation.

Materials and Methods. Rats were prepared with Silastic (Dow-Corning) cannulas draining bile and pancreatic juice separately (Experiment 1, pancreatic secretion studies) or combined (Experiment 2, pancreatic growth studies) and with two intestinal cannulas, one in the duodenum just proximal to the ampulla of Vater and one in the ileum at the midpoint of the intestine in each rat. Details of surgical procedures are described elsewhere (4, 5). Following surgery, rats were placed in Bollman-type restraint cages and bile and pancreatic juice were collected externally and continuously returned by a servo-system (5) into either the duodenum (chronic BPJ to duodenum) or ileum (chronic BPJ to ileum). They were fed by continuous intraduodenal infusion of a liquid, partially hydrolyzed, defined formula diet (Vital, Ross Laboratories, Columbus, Ohio) at a rate of 4 ml/hr, ~0.8 calorie/ml, and fasted 5 hr before experiments, which began at ~0800 hr. Proximate analysis of Vital is 15.8% protein (partially hydrolyzed whey, meat, and soy protein), 4.11% fat (Medium-chain triglycerides), 70% carbohydrate (hydrolyzed corn starch), the remainder vitamins, minerals, and moisture. Measurement of secretory response and pancreatic growth was conducted on the sixth day postoperatively. To measure stimulated pancreatic secretion (Experiment 1), bile and pancreatic juice were diverted back to the duodenum for 3 hr in the group with chronic ileal return in order to place the pancreas under the same resting conditions as the control (chronic BPJ to duodenum) group. Secretory response was then studied in both groups by acute diversion of BPJ to the ileum. The rationale for measuring pancreatic secretory response by acute diversion of BPJ to the ileum is as follows: (a) acute diversion of BPJ to the ileum has approximately the same stimulatory effect as acute

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diversion to the exterior, observed in earlier studies (2), and (b) the maximal pancreatic secretory response to acute diversion of BPJ to the exterior is approximately equivalent to the response to maximally effective doses of CCK, based on results of Petersen and Grossman (6). In pancreatic growth studies (Experiment 2), the same rationale was applied, BPJ was returned to the duodenum in rats with chronic ileal return for 3 hr before removal of the pancreas.

In an additional experiment, the rate of pancreatic secretion after 3 and 4 days of chronic diversion of BPJ to the ileum was determined. As in the protocol described above, rats were fasted 5 hr on the morning of the third day postoperative before monitoring of secretion began. Bile and pancreatic secretion, which had been collected and returned to the ileum automatically by servo-system since surgery, were monitored for 1.5 hr by collecting it at 30-min intervals and returning it to the ileum, followed by infusion of the secretions into the duodenum for 3 hr. Subsequently, the rats were placed back on the servo-system returning BPJ to the ileum and the same procedure was repeated on the following day on the same rats.

In studying pancreatic secretion, the volume of pancreatic juice was determined every 30 min, a 0.01-ml sample was taken for the measurement of protein (by optical density at 280 nm), then the remaining BPJ was pumped into the intestine at a uniform rate. (Results are expressed as ml fluid and mg protein/kg body wt per 30 min.)

For studies on the pancreatic growth response, the pancreas was removed and freed of fat and lymph nodes under a dissecting scope, frozen (−70°C), and lyophilized to constant weight. Samples of lyophilized pancreas (15–20 mg dry wt) were homogenized at 0°C in 2 ml of 0.1 *M* Tris buffer, pH 8.0, containing 0.02 *M* $CaCl_2$ and 1% Triton X-100, using a Duall tissue grinder with Teflon pestle. For activation of chymotrypsinogen, 0.1-ml aliquot of the uncentrifuged homogenate was diluted with 2.4 ml of the above Tris buffer without Triton X-100, but contained purified bovine trypsin at 5 μg/ml. Activation was carried out at 0°C for 90 min, producing maximal activity. Chymotrypsin activity was assayed by spectrophotometric methods (7), using *N*-benzoyl-L-tyrosine ethyl ester as substrate, and purified bovine chymotrypsin as standard. Chymotrypsin activity is expressed as milligram of purified bovine chymotrypsin of equivalent activity. For determination of protein content of pancreatic tissue, the original homogenate was diluted 1 → 100 in Tris buffer without Triton X-100 and analyzed by the method of Lowry *et al.* (8) with bovine serum albumin as standard.

Results were analyzed by Student *t* test for unpaired values and differences were considered significant if $P < 0.05$.

Results. *Experiment 1. Pancreatic secretion studies.* The pancreatic protein and fluid secretory responses to diversion of BPJ to the ileum are shown in Figs. 1 and 2. Pancreatic protein and fluid secretion were increased in both groups following diversion, but output was greater in the chronic diversion group than in controls. Secretion during the period prior to diversion (resting phase secretion) was significantly *lower* in the chronic diversion group compared to controls. Taking into account the lower resting secretion, the increment in secretion (average output following diversion minus average output before diversion) during

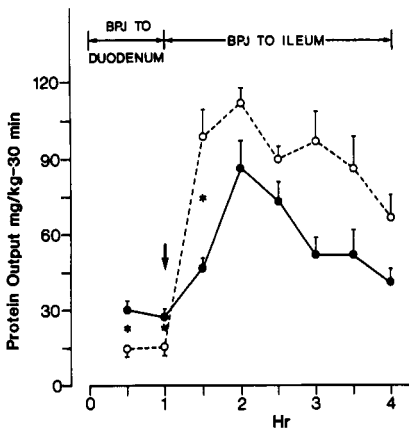

FIG. 1. Effect of chronic return of bile + pancreatic juice to duodenum or ileum on pancreatic protein secretion. Bile and pancreatic secretions were directed by cannulas into the duodenum (●) or ileum (○) for 6 days. Prior to testing, rats were fasted and secretions redirected from the ileum to the duodenum for 3 hr in chronic ileal return rats. Pancreatic secretion was then stimulated by acute diversion of BPJ to the ileum in both groups. Results from six rats per group. Asterisks indicate statistically significant differences between groups ($P < 0.05$).

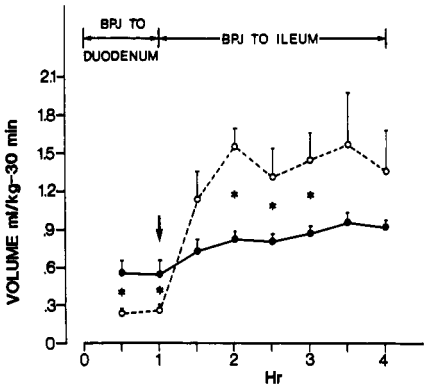

FIG. 2. Effect of chronic return of bile + pancreatic juice to duodenum or ileum on pancreatic fluid secretion. Results from same experiments as Fig. 1. Symbols as in Fig. 1.

the 3 hr following diversion is 79.2 ± 11.3 vs 30.2 ± 7.3 mg protein/kg·30 min and 1.12 ± 0.22 vs 0.31 ± 0.12 ml/kg·30 min for chronic diversion rats compared to controls, respectively (significant for both). Incremental values for fluid and protein output at individual 30-min periods were also significantly different between groups for four of the six periods following diversion (data not shown). Absolute output of fluid and protein during the 3 hr following diversion was also significantly greater in the chronic diversion group (data not shown).

Pancreatic secretion during chronic diversion. The rate of pancreatic protein and fluid output during diversion in rats with chronic ileal diversion on the third and fourth days following surgery is shown in Fig. 3. The results are from four rats, and the results on the third and fourth days were virtually identical and so were combined (eight experiments on four rats). Pancreatic protein and fluid output during diversion to the ileum averaged 67.7 mg protein/kg·30 min and 1.35 ml/kg·30 min, respectively. Upon return of BPJ to the duodenum, protein and fluid output fell, in a remarkably parallel fashion, to steady-state values that were less than 20% of prior output.

Experiment 2. Growth and adaptation studies. Results presented in Table I show the body weight and pancreatic dry weight, protein content, and chymotrypsin activity per 100 g body wt. Pancreatic weight and protein content were increased 2.6 times and 2.9 times, respectively, in the chronic diversion group. Chymotrypsin activity was increased 4.8 times in the chronic diversion group.

Discussion. The results described here demonstrate that acute diversion of BPJ to the ileum has the same stimulatory effect on pancreatic secretion as we previously demonstrated with acute diversion of BPJ to the exterior (2). This was not surprising inasmuch as Schneeman and Lyman (3) demonstrated that negative feedback regulation of the pancreas by luminal trypsin was limited to the proximal third of the small intestine, and the effect of absence of BPJ from the intestine on pancreatic secretion is attributed to loss of trypsin and other pancreatic proteases (9). Therefore, the ileum appears to have no role in negative feedback regulation of pancreatic secretion by luminal proteases.

Chronic diversion of BPJ to the ileum markedly increased pancreatic growth and protease content and likewise increased pancreatic secretory output as measured by the response to acute diversion of BPJ to the ileum. These results may help to explain the pancreatic changes induced by transposition of the duodenum in the studies of Miazza *et al.* (1), in which BPJ was diverted to the ileum, bypassing the jejunum. Specifically, removal

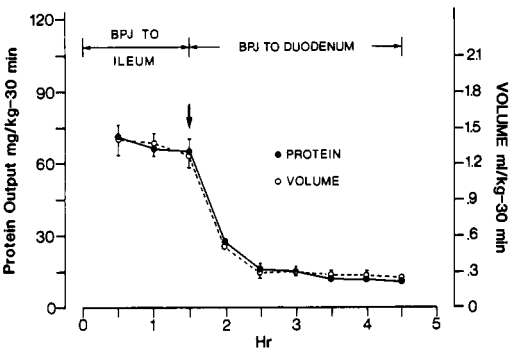

FIG. 3. Pancreatic secretion in rats after 3 and 4 days of continuous diversion of bile + pancreatic juice to the ileum. Bile and pancreatic juice were continuously diverted to the ileum and secretion was monitored for 1.5 hr on the morning of the third day postoperative, followed by return of secretions to the duodenum for 3 hr. This procedure was repeated the following day and the results of the two trials were combined. Results are from four rats. All values during diversion of bile + pancreatic juice to the ileum are significantly different from all values during return of bile + pancreatic juice to the duodenum.

TABLE I. TROPHIC EFFECTS ON THE PANCREAS OF CHRONIC RETURN OF BILE-PANCREATIC JUICE TO DUODENUM OR ILEUM

	Chronic BPJ to duodenum ($N = 5$)	Chronic BPJ to ileum ($N = 6$)
Body weight (BW)	345 ± 8.9	380 ± 2.6
Pancreas dry weight, mg/100 g BW	89.1 ± 7.7	231 ± 53.4*
Chymotrypsin, mg/pancreas/100 g BW	5.04 ± 0.8	24.3 ± 6.2*
Protein, mg/pancreas/100 g BW	58.5 ± 1.7	171 ± 35*

Note. Bile-pancreatic juice (BPJ) was directed either to the duodenum or ileum for 6 days after cannulating the common bile-pancreatic duct and small intestine. Pancreases were removed after fasting and 3 hr after BPJ was redirected from the ileum to the duodenum in chronic ileal return rats.

* Significant differences between groups ($P < 0.05$).

of BPJ or pancreatic protease from the proximal intestine stimulates cholecystokinin (CCK) release (10–12), which in turn stimulates pancreatic secretion and increased growth and protease content (13). If this explanation applies in the interpretation of the results of Miazza *et al.* (1), it suggests that induction of pancreatic hypertrophy by diversion of BPJ does not require removal of BPJ from the duodenum, inasmuch as duodenal transposition in their experiments excluded BPJ only from the jejunum, not the duodenum.

The cause of the decreased resting pancreatic secretion in pancreatic fistula rats with chronic diversion is not known. One possibility is that the proximal intestine chronically deprived of BPJ becomes more sensitive to reinfusion of BPJ, i.e., BPJ inhibits CCK release to a greater extent under these conditions. Another possibility is that the hypertrophic pancreas in chronic diversion rats is less sensitive to, or receives less, basal cholinergic stimuli, as we previously demonstrated that resting pancreatic protein secretion in the rat was predominately controlled by a cholinergic mechanism (14).

Although the experiments represented by Figs. 1 and 2 and Table 1 were carried out 6 days postoperatively, the effects described for chronic ileal return rats may occur earlier than 6 days, based on results illustrated in Fig. 3. In that experiment, steady-state protein and fluid output during chronic diversion on the third and fourth days postoperatively were the same as the output during the final collection period following diversion in 6-day chronic ileal return rats shown in Figs. 1 and 2. If we can assume that outputs after 3 hr diversion in experiments shown in Figs. 1 and 2 represent approximate steady state, then results in Fig. 3 indicate that as few as 3 days diversion of BPJ to the ileum may be sufficient to produce all the changes we have reported here.

Chymotrypsin activity of the pancreas was greatly increased in the chronic diversion studies reported here. This conflicts with the report of Miazza *et al.* (1), in that they reported *decreased* specific activity of trypsin, although they, like we, observed increased pancreas size and protein content. This conflict is not likely due to measuring different proteolytic enzymes, since trypsin and chymotrypsin change in the same direction during pancreatic adaptation (13). The most likely explanation is that the pancreas was removed under resting conditions in our study, but under chronic stimulated conditions in the study of Miazza *et al.* (1), because they did not return BPJ to the proximal intestine before sacrifice. Removal of the pancreas during chronic stimulation could result in lower specific activity of proteases because the newly synthesized enzymes are quickly exported and do not accumulate.

Other factors that may be considered in the interpretation of results reported here are the role of absence of buffering action of BPJ in the proximal intestine of chronic ileal return rats, and the role of the "elemental" diet. Bicarbonate (from bile and pancreatic juice) was not replaced in the chronic ileal diversion rats, but this is unlikely to have influenced the results for the following reasons: (a) the liquid diet infused intraduodenally has considerable buffering capacity due to its high concentration of peptides and amino acids, and (b) acid in the intestine is not a stimulant of pancreatic secretion in the rat based on experiments of

Grossman (15) and our recent study (16). We showed that physiological loads of HCl (60–240 μeq/hr) introduced into the duodenum of pylorus-ligated rats did not affect pancreatic secretion in the presence or absence of bile–pancreatic juice in the intestine (16). HCl augmented the pancreatic response to diversion of bile–pancreatic juice, but only during the initial 2.5 hr of diversion, and HCl did not affect the pancreatic response to trypsin inhibitors (16). Therefore, it is very unlikely that absence of the buffering capacity of bile–pancreatic juice in the proximal intestine had any role in the pancreatic adaptation effects observed. The rationale for the "elemental" diet was to ensure adequate nutrient delivery, in general, and in particular to the area of intestine bypassed by the bile–pancreatic secretion in the ileal-return group of rats. These diets are designed to be absorbed in absence of bile or pancreatic juice. It is well established that intestinal mucosal mass is not maintained in absence of luminal nutrients, and this apparently holds for intestinal endocrine cells as well (17). Therefore, we wanted to ensure that the proximal intestine (the source of CCK) in both groups was well nourished.

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