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FEEDBACK CONTROL OF PANCREATIC SECRETION IN RATS. ROLE OF GASTRIC ACID SECRETION

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Pancreatic secretion in rats is regulated by feedback inhibition of cholecystokinin (CCK) release by proteases in the gut lumen, but little is known about the role of gastric acid in this regulation. This study, carried out on conscious rats with large gastric fistulas (GF) and pancreatic fistulas, shows that diversion of pancreatic juice results in the progressive stimulation of pancreatic secretion only in rats with the GF closed. When the GF was kept open, the diversion resulted in only small increment in pancreatic secretion and this was accompanied by progressive increase in gastric acid outputs. Similar amounts of HCl instilled into the duodenum in rats with the GF open fully reproduced the increase in pancreatic secretion observed after the diversion of pancreatic juice. Pretreatment with omeprazole (15 μmol/kg) to suppress gastric acid secretion or with L-364,718 (5 μmol/kg) to antagonize CCK receptors in the diverted state, resulted in the decline in pancreatic secretion similar to that observed after opening the GF. CCK given s.c. (20—320 pmol/kg) failed to cause any significant rise in the post-diversion pancreatic secretion in rats with the GF closed, but stimulated this secretion dose-dependently when the GF was open. Camostate (6—200 mg/kg) in rats with pancreatic juice returned to the duodenum caused dose-dependent increase in pancreatic secretion, but after opening the GF or after omeprazole this increase was reduced by about 75%. This study provides evidence that gastric acid plays a crucial role in the pancreatic response to diversion of pancreatic juice or inhibition of luminal proteases, and that factors that eliminate gastric acid secretion reduce this response.

Key words: *pancreas, stomach, protease, camostate, cholecystokinin.*

INTRODUCTION

Diversion of pancreatic juice from the duodenum is known to increase pancreatic volume flow and enzyme secretion in rats (1—6) and in other species such as chickens (7), pigs (8) and humans (9). This post-diversion pancreatic hypersecretion was markedly reduced when the pancreatic juice was

returned to the duodenum (1, 6, 10, 11). In addition, trypsin inhibitors, such as camostate, infused into the duodenum produced a rapid augmentation of pancreatic secretion similar to that observed in the post-diversion state while the presence of trypsin or chymotrypsin in the duodenum of rats with diverted pancreatic juice reduced the stimulatory response (1, 4, 10, 12). It has been suggested that the presence of active pancreatic proteases in the duodenum in some way blocks the post-diversion pancreatic hypersecretion, probably by the inactivation of a trypsin-sensitive, putative CCK-releasing peptide present in the pancreatic juice or intestinal content (2, 12—15).

Gastric acid entering the duodenum is known to stimulate pancreatic secretion in vaious species but its role in the regulation of this secretion in rats has been little studied. Noda et al (4), using conscious rats with large GF, found that the drainage of gastric juice to the exterior virtually abolished the post-diversion pancreatic hypersecretion suggesting that this response results from increased acidity in the duodenum (16). Recently, Green and his associates (17, 18) showed that gastric acid has only small augmenting action on the post-diversion pancreatic response and suggested that this acid is not essential for the response because the ligation of pylorus and drainage of the gastric juice to the exterior did not affect the increment in pancreatic secretion or in the plasma CCK levels induced by the diversion of pancreatic juice. Furthermore, duodenal acidification in rats with diverted pancreatic juice or intraduodenal protease inhibition failed to affect the enhanced pancreatic secretion.

The purpose of the present study was to further clarify the mechanisms of the feedback control of pancreatic secretion, especially the role of gastric acid secretion in the regulation of interdigestive pancreatic secretion and that induced by feeding or duodenal administration of protease inhibitor.

MATERIAL AND METHODS

Secretory studies were carried out in conscious male Wistar rats, weighing 250—300 g, and equipped with chronic gastric fistula (GF) and pancreatic fistula (PF). The animals were prepared surgically under ether anesthesia; the abdomen was opened by a midline incision, the stomach was exposed and a large stainless steel gastric cannula (ID 7 mm) was placed in the forestomach to form a gastric fistula (GF). The cannula was secured by a purse-suture and omentum and brought outside through an incision on the left side of the abdominal wall. The bile was redirected by transplanting the common bile duct to the duodenum just above the entrance of the bile-pancreatic duct. For this purpose, bile duct was ligated at the hilus of the liver, cannulated above the ligature with a sialastic tubing and connected to the duodenum. Another

polyethylene cannula (PE 50) was inserted into the distal end of the common bile-pancreatic duct at a point just proximal to its entrance to the duodenum, tied in place and brought out to the exterior through a small incision in the left anterior abdominal wall. A polyethylene cannula was placed in the duodenum in the region of the sphincter of Oddi and also brought outside in the close vicinity of the pancreatic cannula. The cannulas were fixed to the skin with a "snap fastener", connected together to permit an undisturbed circulation of the pancreatic juice into the duodenum and protected by a stainless steel thimble as described previously (19). After surgery, the rats were allowed to move freely in their usual cages. During the experiments, the animals were placed in modified Bollman-type cages to maintain the minimum restraint necessary. Each animal was placed several times in these cages before surgery to allow full adaptation to this restraint. After placing the animals in the individual cages, the metal thimble was removed, the cannulas were disconnected, the pancreatic cannula was used to collect the pancreatic juice and the duodenal cannula for the reinfusion of the juice and test solutions into the duodenum.

The secretory studies usually started after 6—8 days of recovery from surgery, except one series in which the experiments started about 6 h after recovery from ether anesthesia and were repeated on the 2 nd, 4 th, 8 th, 10 th and 12 th day after surgery. Before each experiment, the animals were deprived of food but not water for, at least, 12 h and then placed in individual cages. The pancreatic juice was collected from the PF in small preweighed vials in 30 min aliquots to measure the volume and the protein contents, as described previously (19). The duodenal cannula was used for the constant reinfusion of the previously collected juice (diluted by saline 1 : 2) using a peristaltic pump (Unipan, Poland). The GF was either kept closed or was opened and in the latter instance a small polyethylene tube (ID 2 mm) was inserted through the cannula with the tip in the stomach to facilitate complete drainage of the gastric juice. The drained gastric juice was collected in 30 min aliquots, the volume was measured and the content of HCl was determined by titration of samples with 50 mM NaOH to pH 7.0 using an automatic titrator (Radiometer, Copenhagen, Denmark). In tests with feeding and omeprazole administration, a small polyethylene tube was inserted into the stomach via the cork occluding the gastric fistula and a small sample (0.2 ml) of the gastric content was withdrawn every 5 min period for the measurement of intragastric pH. The sample was then reinstilled into the stomach.

The influence of the presence or absence of pancreatic juice in the duodenum on gastric and/or pancreatic secretion was studied in several series of experiments, each including 8—12 rats. The following tests were performed; 1. Tests with 5 h diversion of the pancreatic juice from the duodenum and the GF closed or open throughout the experiment; 2. Tests with the GF closed or

open throughout the experiment and the pancreatic juice diverted for 3 h, then returned for the next 3 h into the duodenum followed by feeding; 3. Tests performed on day 0, 2 nd, 4 th, 6 th, 8 th, 10 th and 12 th after surgery in rats with the GF closed or open and with pancreatic juice diverted (for 1 h) and then returned (for 1 h) to the duodenum. In this series of experiments performed on the same 10 rats, the pancreatic secretion was also examined for 2 h after feeding. About 6 g of white bread mixed in proportion 1 : 1 (wt/wt) with milk was offered and usually totally consumed during 15—20 min. The GF was kept closed in the postprandial period and the pancreatic juice was returned into the duodenum. Tests with feeding and pancreatic juice either diverted or returned to the duodenum and the GF closed or open throughout the experiment and without or with pretreatment with omeprazole (15 umol/kg) given intraduodenally (i.d.) about 1 h before the experiment. 5. Tests with synthetic protease inhibitor, FOY-305 given i.d. or s.c. after a basal period of 120 min in doses of 6—200 mg/kg with pancreatic juice returned to the duodenum and with the GF closed or open throughout the experiment and without or with pretreatment with L-364,718 (5 μmol/kg i.d.)

Since the diversion of pancreatic juice resulted in the increase in gastric acid secretion, exogenous acid was instilled intraduodenally at 25—100 mM HCl in gradually increasing rates (25—400 μmol/h) but at a constant volume in rats with the pancreatic juice diverted from the duodenum and the GF closed or open and without or with administration of L-364,718 (5 μmol/kg i.d.). For comparison, the tests were performed with s.c. infusion of CCK-8 in graded doses (20—320 pmol/kg-h) without or with administration of L-364,718 (5 μmol/kg i.d.) in rats with the pancreatic juice diverted or returned into the duodenum and the GF closed or open throughout the experiment.

Results are expressed as means ± SEM and were analyzed by paired t-test and by analysis of variance. Differences were considered significant $P < 0.05$.

RESULTS

The effects of 5 h diversion of pancreatic juice from the duodenum on pancreatic protein output in rats with the GF closed or open are presented in Fig. 1. Immediately after opening the PF, the rate of protein secretion averaged about 10 mg/30 min. In tests with the GF closed and saline infusion, the protein secretion showed a progressive increase reaching about 40 mg/30 min at the end of an experiment. In tests without saline infusion, pancreatic protein also showed a progressive increase to a peak after 3 h of experiment but then showed a sharp drop back to initial levels. When the GF was kept open and gastric juice was drained to the exterior throughout the experiment, the protein output showed only a small increment reaching at the end of 5 h expeiment about 25% of that attained in tests with the GF closed.

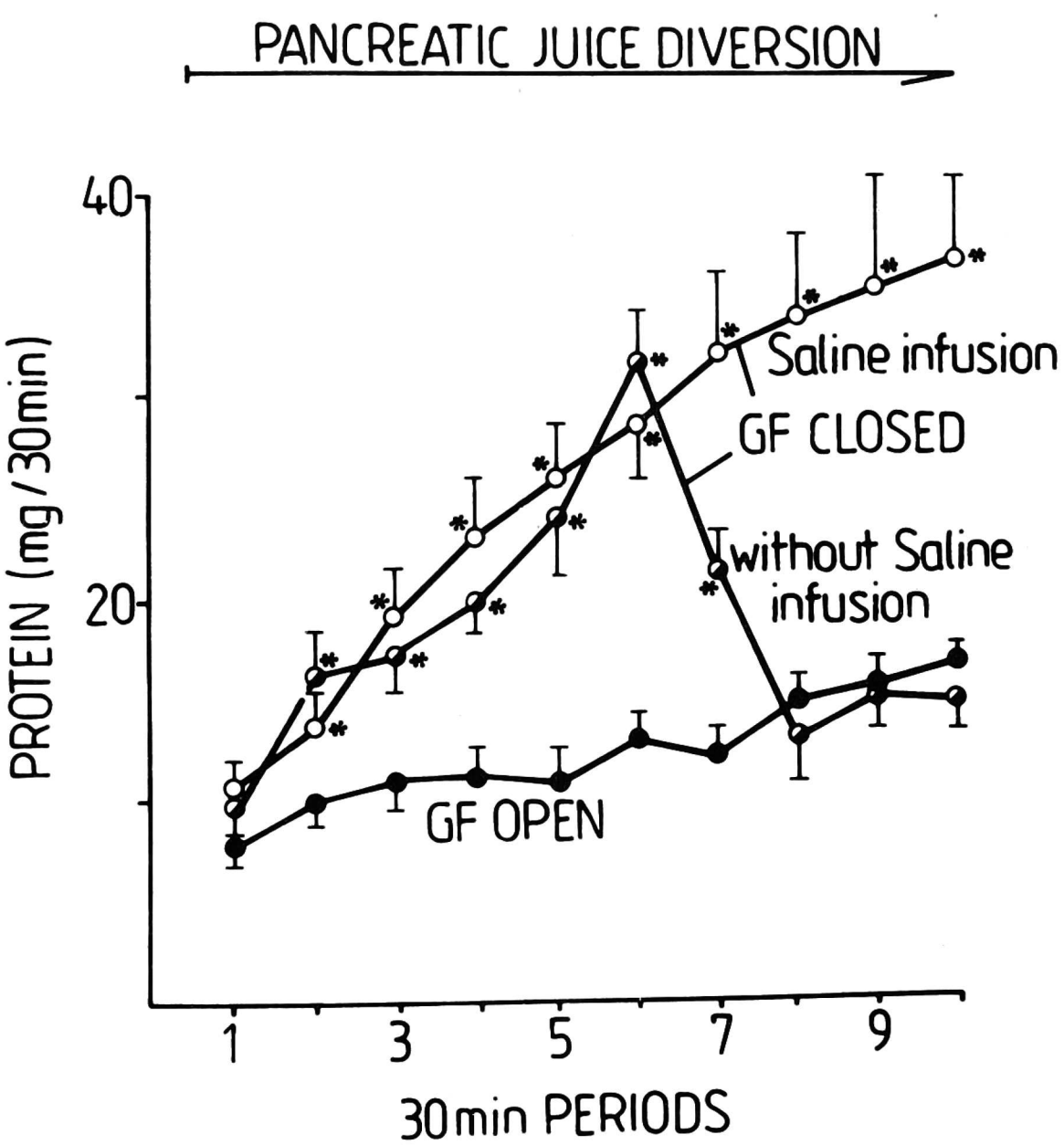

Fig. 1. Pancreatic protein secretion from pancreatic fistula during 5 h diversion of the pancreatic juice from duodenum in rats with the GF closed without or with s.c. infusion of saline and with the GF open throughout the experiment. Studies with diversion or return of pancreatic juice were carried out on separate test days. Mean ±SEM of 8 tests on 8 rats with GF and PF prepared 6 days before the experiments. Asterisk indicates significant increase as compared to those obtained in rats with the GF open.

Gastric acid output collected through the open GF during experiments with the diversion of pancreatic juice showed a progressive increase reaching a peak after 3 h of experimentation both in tests with and without saline reinfusion (Fig. 2). The increment in acid output was about 250 umol/30 min in tests with saline infusion and about 200 umol/30 min in tests without saline infusion. In experiments with the pancreatic juice returned to the duodenum no significant alterations in gastric acid secretion were observed.

The changes in pancreatic protein secretion in tests with diversion followed by return of the pancreatic juice into the duodenum and finally by feeding are shown in Fig. 3. With the pancreatic juice diverted for 3 h and the GF closed, the protein output showed a progressive increase but then immediately after

the return of the juice back into the duodenum the protein output declined towards the basal value. In tests with the GF open, the protein secretion was low and showed only small changes during the diversion or return of the pancreatic juice back into the duodenum. Gastric acid secretion in these tests with the GF open showed a progressive increase from initial value of about 65 ± 12 μmol/30 min to about 205 ± 32 μmol/30 min at the end of 3 h diversion. After the return of pancreatic juice to duodenum, gastric acid secretion decreased towards initial value during 60 min period. Feeding in animals with the pancreatic juice returned to the duodenum caused an immediate increase in protein output reaching a peak of about 40—50 mg/30 min (Fig. 3). In tests with the pancreatic juice diverted for 60 min and the GF open, feeding for 15 min (with continuous drainage of the gastric content to the exterior) caused an immediate increase in pancreatic protein secretion from the initial value of about 12 ± 2 mg/30 min to the peak of about 32 ± 4 μmol/30 min in the first 30 min postprandial period. Protein secretion then slowly declined to about 12 ± 3 mg/30 min at the end of 2nd h of postprandial period. Gastric acid output increased from initial value of about 78 ± 6 μmol/30 min to 125 ± 22 μmol/30 min immediately after feeding and then returned during about 60 min to the basal value. In similar tests with the pancreatic juice returned to duodenum, feeding (with the GF open) resulted in the increase in protein output from initial value of about 7 ± 2 mg/30 min to about 17 ± 3 mg/30 min and acid output rose from initial 24 ± 4 to 51 ± 6 umol/30 min.

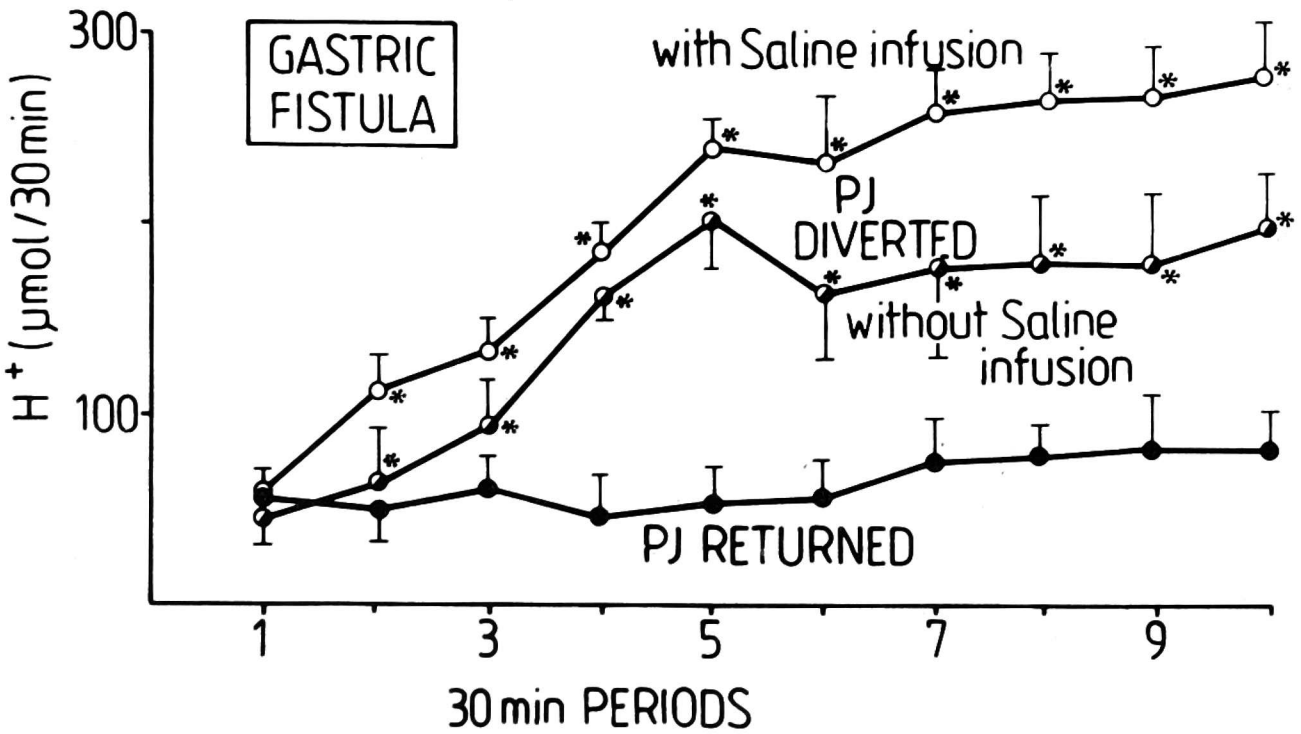

Fig. 2. Gastric acid outputs in rats with chronic GF and PF in experiments with the pancreatic juice diverted without or with s.c. infusion of saline and with the pancreatic juice returned into duodenum. Results were obtained from rats with the GF open throughout the experiments performed on separate test days. Mean ±SEM of 8 experiments from 8 rats. Asterisk indicates significant increase above the values obtained from rats with the pancreatic juice returned to duodenum.

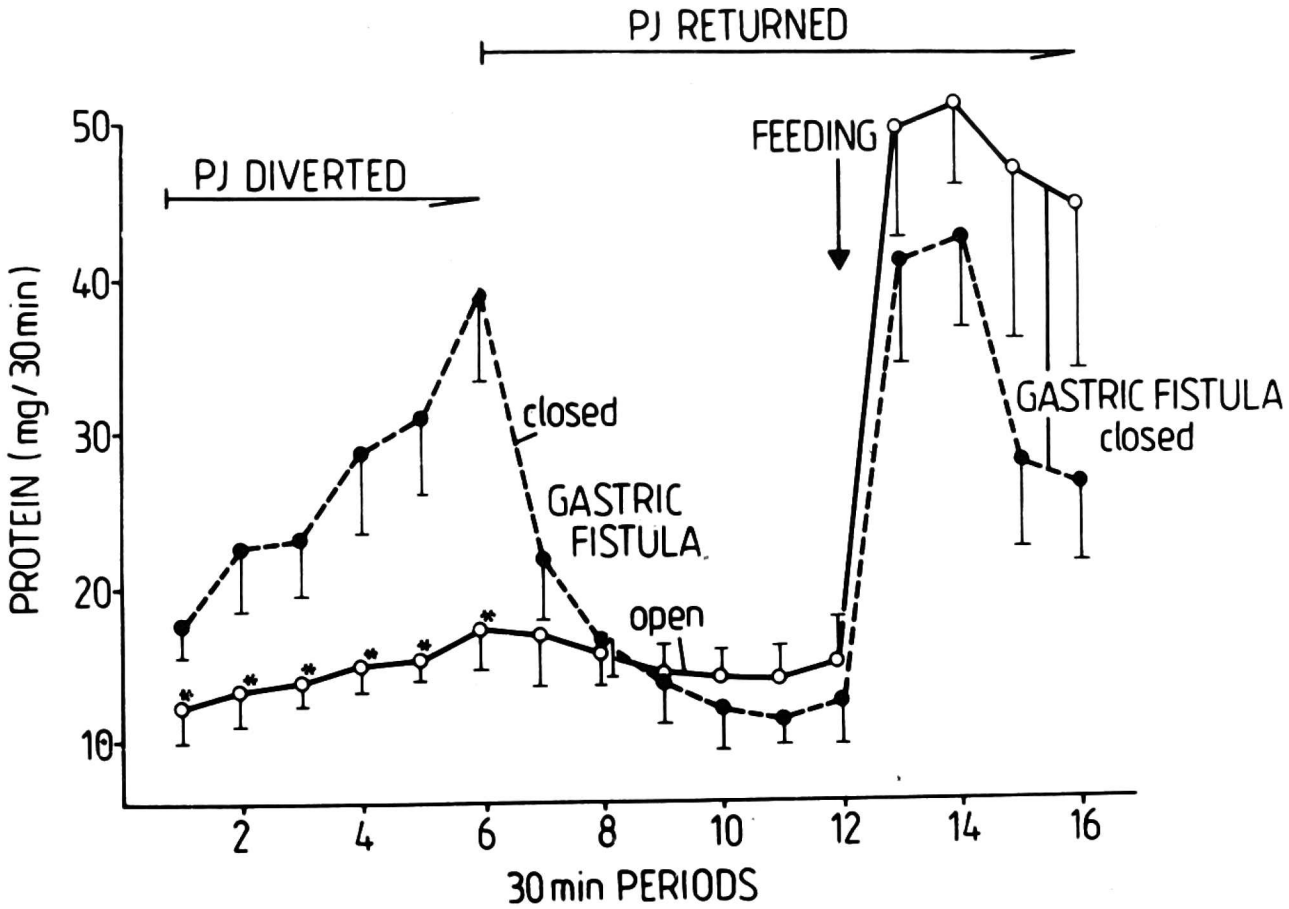

Fig. 3. Pancreatic protein secretion in response to the diversion (for 3 h) then return (for 3 h) of pancreatic juice to duodenum without and with feeding in rats with the GF closed or open. After feeding the GF was closed in tests with pancreatic juice returned to duodenum. Asterisk indicates significant decrease below the value obtained in rats with the diversion of pancreatic juice. Experiments with the GF closed or open were performed on separate test day at least 6 days after the preparation of the GF and PF. Means ±SEM of 10 tests on 10 rats. Asterisk indicates significant decrease below the values obtained in rats with the diversion of pancreatic juice.

In tests with examination of pancreatic protein secretion repeated every second day in rats with the GF closed or open and with the pancreatic juice returned or diverted, a gradual increase in pancreatic protein secretion was observed from day 0 to day 6 th after surgery (Fig. 4). In tests with the GF closed and the diversion of pancreatic juice, the increments in protein outputs were significantly higher after the 4 th day than in tests with the pancreatic juice returned. The peak protein output in response to pancreatic diversion was reached on the 6 th day after surgery and then remained at a well-sustained level in the next test days. In tests with the GF open, the pancreatic protein output also increased from day 0 to day 6 th after surgery and then plateaued but no significant difference in protein outputs was seen between tests with the pancreatic juice diverted and returned. Gastric acid secretion in rats with the diverted pancreatic juice showed similar values from the 2 nd day throughout all consecutive days of examination. On all test days after surgery, except the second, the acid output was about twice as high in experiments with the pancreatic juice diverted than when the juice was returned to the duodenum.

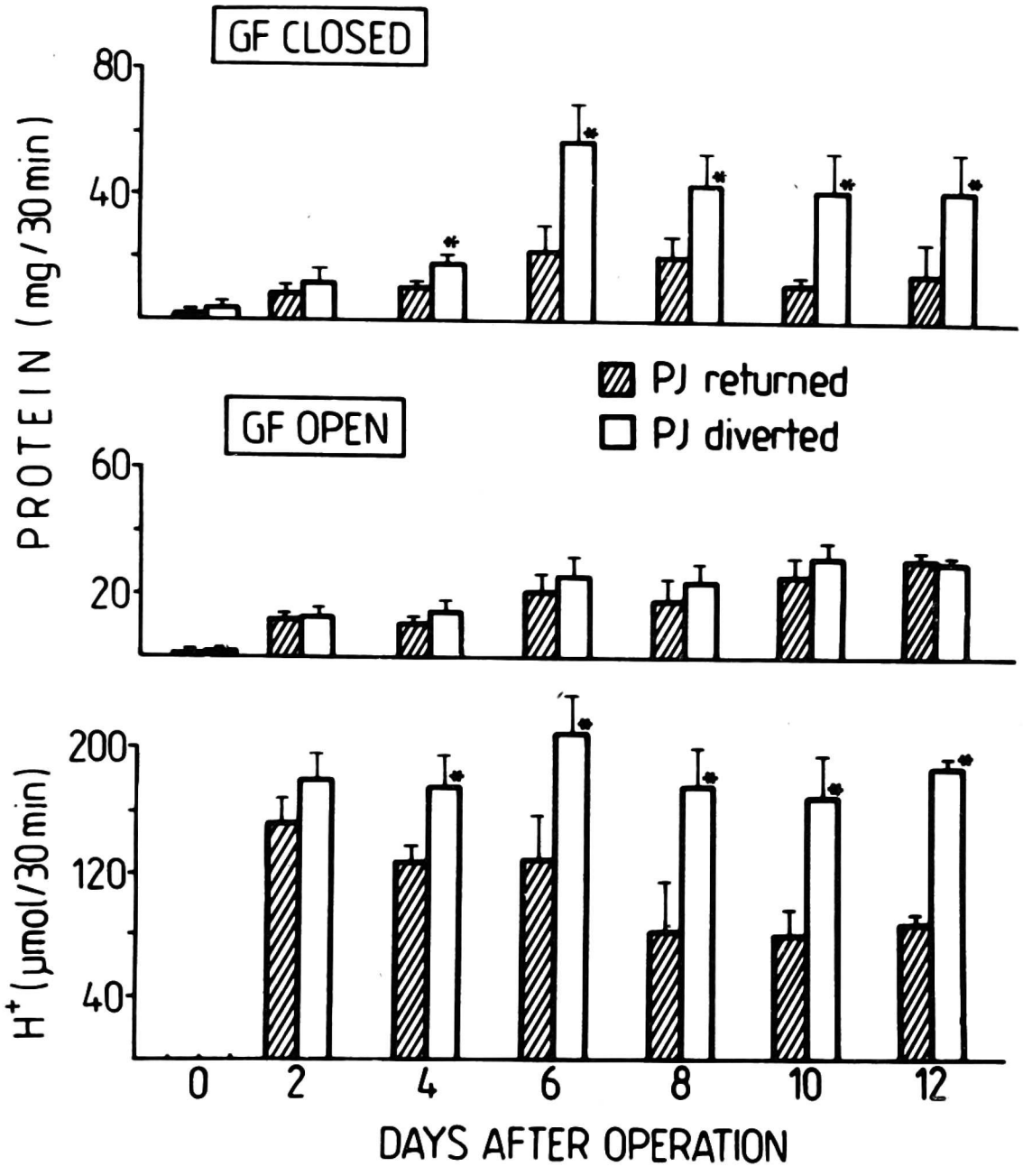

Fig. 4. Effects of alternate closing and opening of GF in rats with the pancreatic juice diverted (for 1 h) or returned to duodenum (for 1 h) on pancreatic protein outputs and gastric acid outputs at day 0, 2 nd, 4 th, 6 th, 8 th, 10 th and 12 th after the preparation of the GF and PF. Means ± SEM of 8 tests on 8 rats. Asterisk indicates significant increase above the value obtained in tests with pancreatic juice returned to duodenum.

The pancreatic protein secretion in response to feeding in rats with the return of pancreatic juice also increased during the first 6 days after surgery and then remained on the same level in the subsequent days of experimentation (Fig. 5). In animals in which feeding was carried out 1—6 h after starting the diversion of pancreatic juice, a significant increment in pancreatic protein secretion was observed only during the first 3 h of the experiment. With the prolongation of pancreatic diversion, the protein response to diversion itself was progressively increased and feeding failed to cause any further increment in pancreatic protein outputs (Fig. 6).

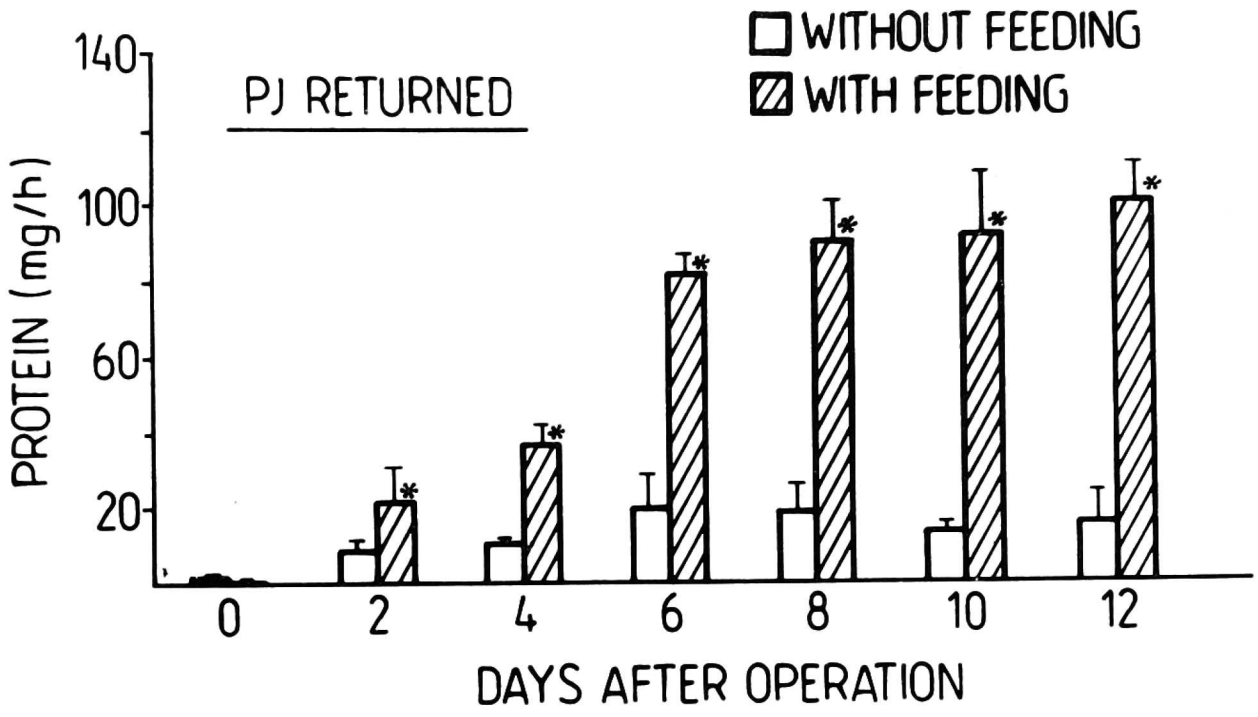

Fig. 5. Pancreatic response to feeding at day 0, 2nd 4th, 6th, 8th, 10th and 12th after surgery in tests as on Fig. 4. The protein output before the start of feeding is also presented. Means ±SEM of 8 tests on 8 rats. Asterisk indicates significant increase above the basal value obtained in these experiments before the start of feeding.

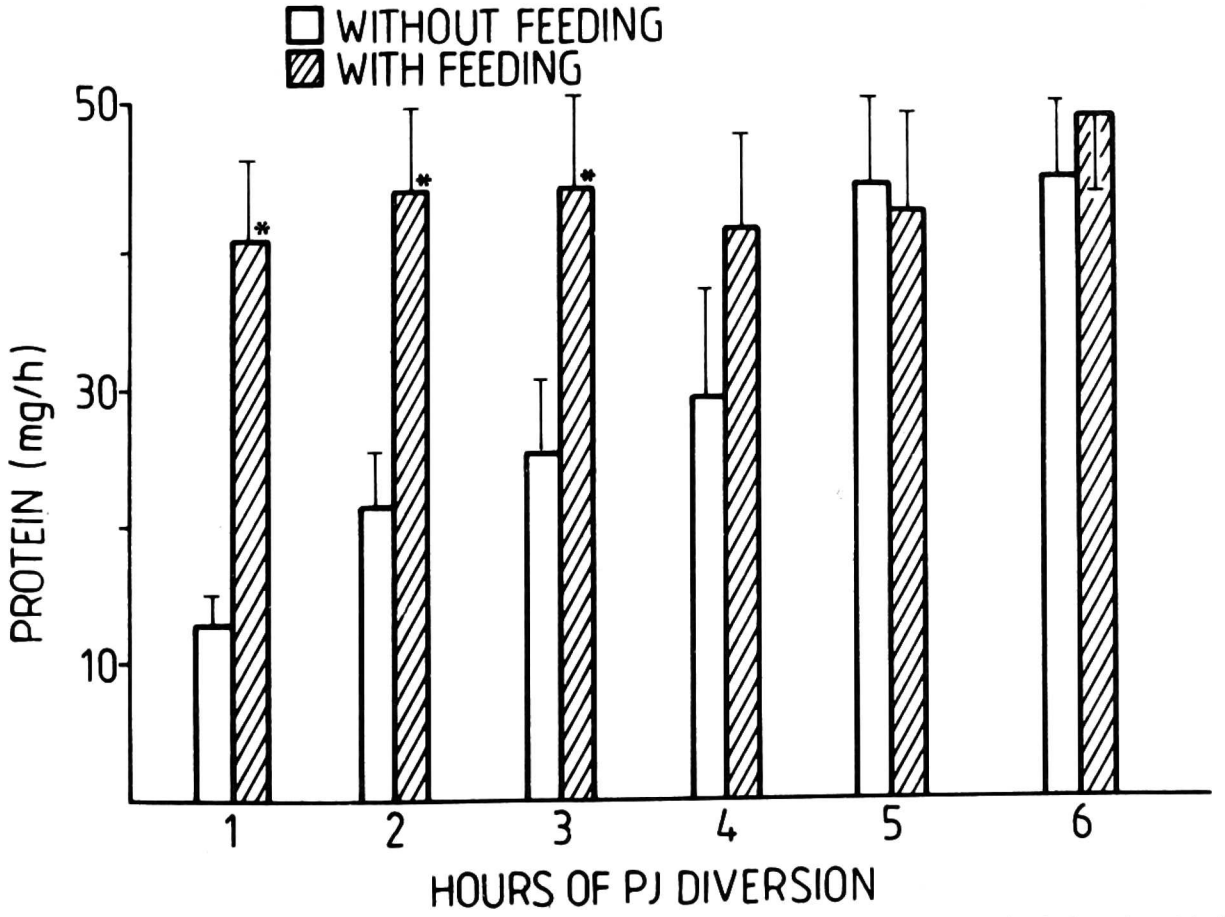

Fig. 6. Pancreatic protein response to feeding in rats with diverted pancreatic juice 1—6 h before the start of feeding. In this series of experiments, rats were used with the GF and PF prepared 6—10 days before the start of experiment. Mean ±SEM of 10 experiments on 10 rats. Asterisk indicates significant increase above the output obtained with diversion alone without feeding.

Feeding rats with the GF closed and the pancreatic juice diverted from the duodenum produced the peak protein output similar to that in tests with the pancreatic juice returned, though in the diverted state, the secretion was more

prolonged than in the same animals with the pancreatic juice returned to the duodenum (Fig. 7). Omeprazole caused a significant decrease in basal protein output in tests with pancreatic juice diverted but the increment in protein response to feeding in omeprazole-treated rats was significantly higher than that obtained in rats without omeprazole (Fig. 8). The samples of gastric content withdrawn from the stomach after the administration of omeprazole showed pHs within the range of about 7.0 throughout the postprandial period.

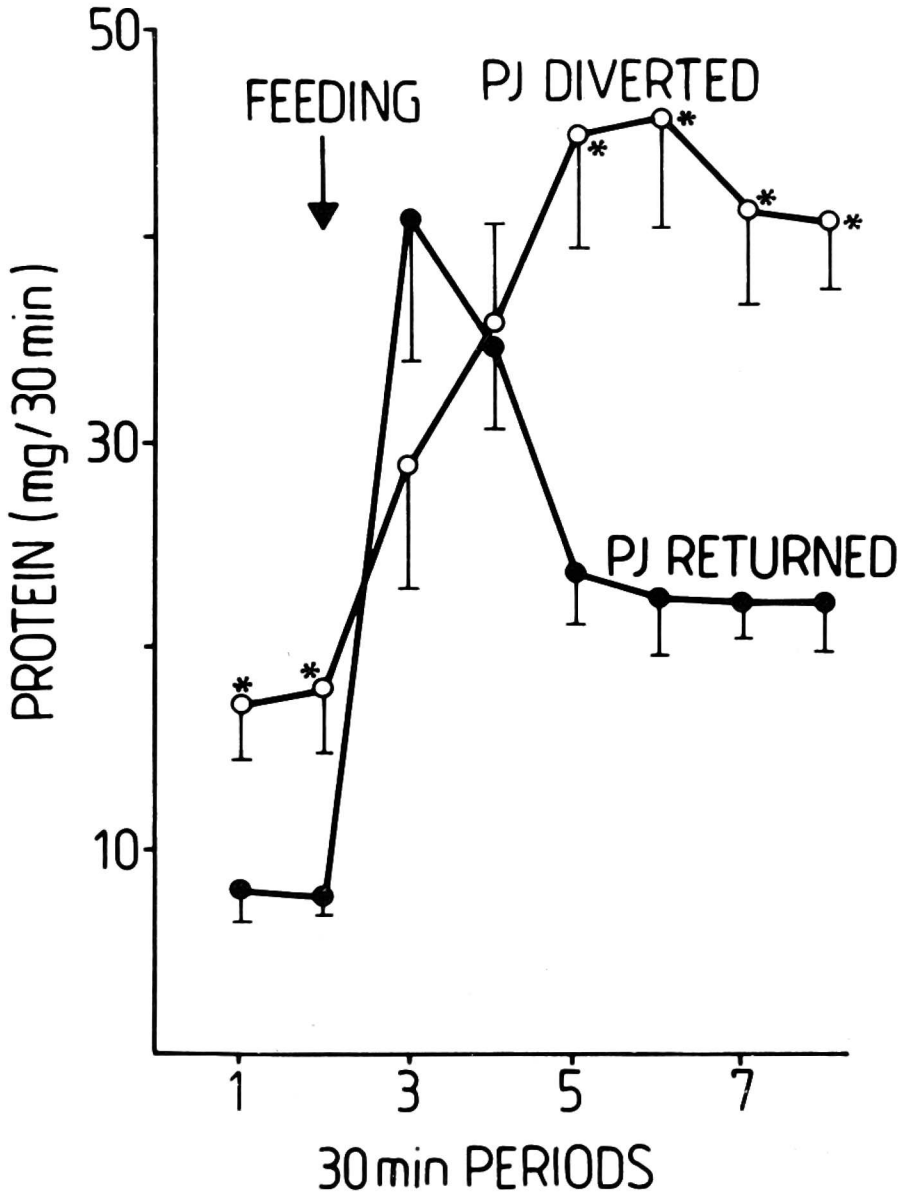

Fig. 7. Pancreatic protein secretion in response to feeding in rats with the GF closed and the pancreatic juice diverted or returned into duodenum. Mean ±SEM of 10 tests on 10 rats in experiments performed 6—8 days after the surgery. Asterisk indicates significant increase above the level obtained in tests with the pancreatic juice returned to duodenum.

Following intraduodenal administration of FOY-305 (camostate) in a dose of 200 mg/kg in rats with the pancreatic juice returned to the duodenum the pancreatic protein output increased 2—3 fold over the basal level in tests with the GF closed (Fig. 9). Opening the GF significantly reduced the protein response to FOY-305 to about 30%of that observed in tests the GF closed and resulted in only transient increase in protein secretion as compared with that

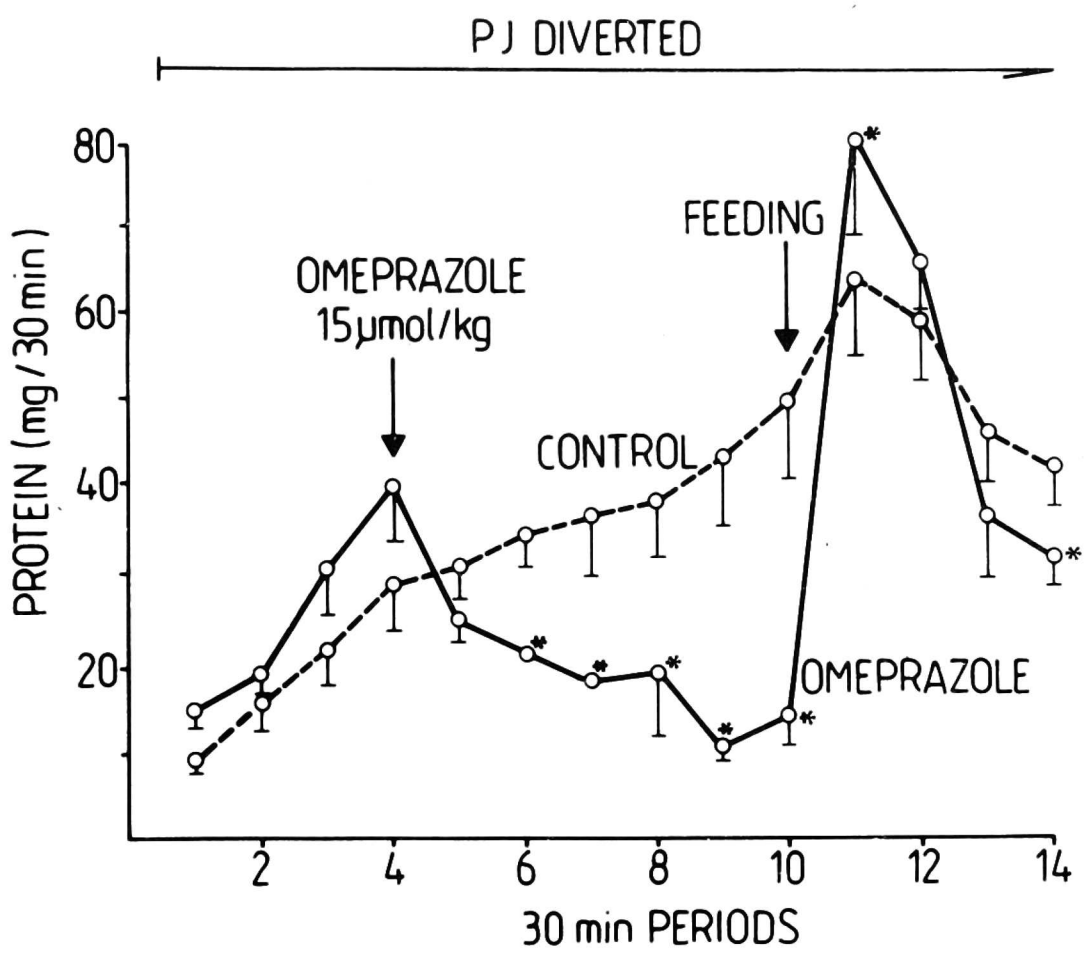

Fig. 8. Pancreatic protein secretion in rats with the GF closed and diverted pancreatic juice from duodenum for 5 h and then with feeding in rats without and with i.d. administration of omeprazole (15 umol/kg). Mean ±SEM of 6 tests on 6 rats. Asterisk indicates significant decrease below the value obtained in tests without omeprazole administration.

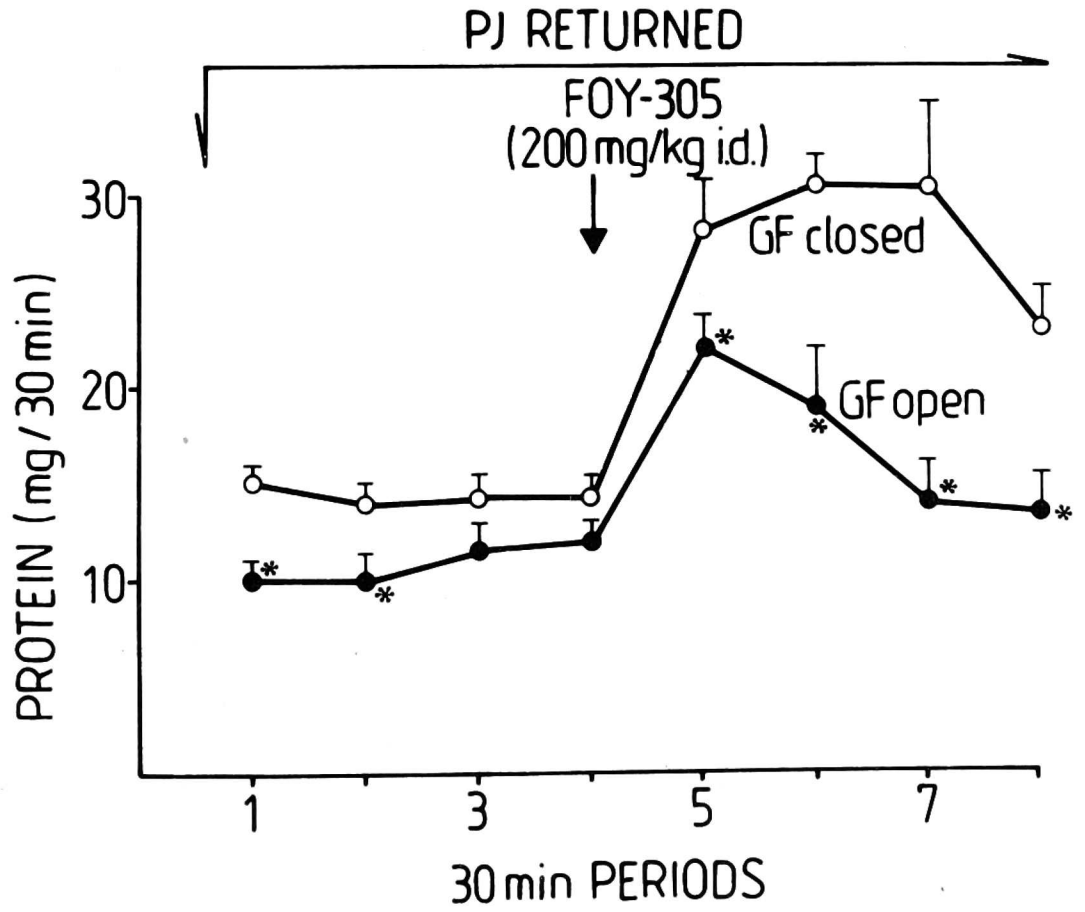

Fig. 9. Pancreatic protein secretion in rats with the pancreatic juice returned to duodenum and the GF closed or open before and after intraduodenal administration of FOY-305 (200 mg/kg). Asterisk indicates significant decrease in protein secretion below the value obtained with the GF open.

obtained in tests with the GF closed. The pancreatic protein responses to FOY-305 administered intraduodenally or subcutaneously in increasing doses in rats with the GF closed or open and after the administration of omeprazole are shown on Table 3. In control tests with the GF closed, FOY-305 given i.d. started to cause significant increase in protein output at a dose of 12 mg/kg to reach the peak of about 30 mg/h at a dose of 200 mg/kg. In tests with omeprazole, the peak protein secretion reached only about 50% of the control value. This increase in protein output in response to FOY-305 was accompanied by a dose-dependent increase in gastric acid secretion. FOY-305 given s.c. also stimulated the protein secretion both in rats with the GF closed and open and this increase reached the value similar to that observed in rats with i.d. FOY-305 and the GF open. Gastric outputs showed similar dose-dependent increase in tests with FOY-305 given s.c. compared to that administered i.d.

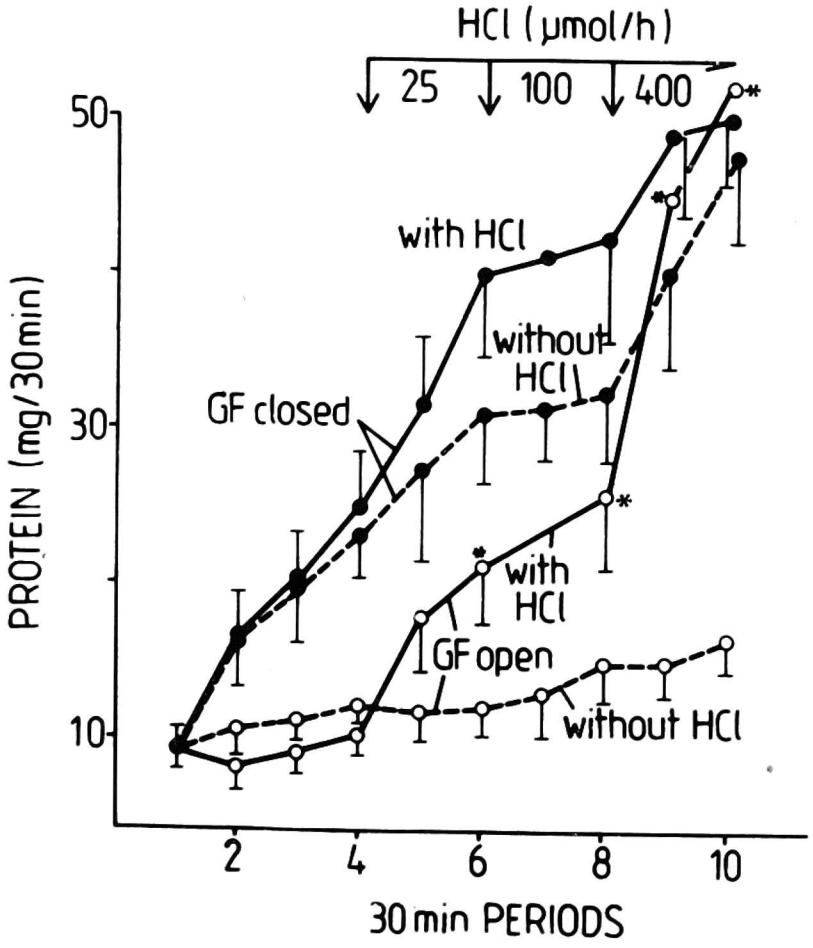

Fig. 10. Pancreatic protein secretion in response to HCl instilled intraduodenally in gradually increasing amounts in rats with the GF closed or open throughout the experiment but with the pancreatic juice diverted from the duodenum. Mean ±SEM of 8 tests on 8 rats. Asterisk indicates significant increase above the level obtained in control tests without HCl instillation.

Intraduodenal instillation of HCl at radually increasing rates ranging from 25 to 400 umol/h in rats with the GF open and pancreatic juice diverted from the duodenum resulted in a gradual decrease in gastric acid secretion and in an increase in protein secretion reaching a peak of about 50 mg/30 min at the highest acid load (Fig. 10, Table 1). Pretreatment with L-364,718 (5 μmol/kg

i.d.) in rats with the GF open and pancreatic juice diverted did not affect gastric acid secretion but almost completely abolished the pancreatic protein response to intraduodenal HCl (Table 1). In control tests on the same animals with the GF open, the i.d. instillation of saline instead of HCl resulted in a small rise in the protein output during the experiment. In tests with the GF closed and pancreatic diversion, the initial value of the protein secretion was significantly higher than those with the GF open and instillation of HCl into the duodenum produced the increments in protein outputs that were not significantly higher than those obtained with the diversion alone without duodenal acidification. Pretreatment with L-364,718 (5 μmol/kg) significantly reduced the pancreatic protein response to diversion of pancreatic juice from the duodenum and abolished the pancreatic responses to intraduodenal HCl.

Table 1. Gastric acid and pancreatic protein secretion in rats with GF open or closed and pancreatic juice (PJ) diverted from duodenum and duodenal instillation of HCl (400 μmol/h) without or with pretreatment with L-364,718 (5 μmol/kg). Means ±SEM of 8 tests on 8 rats.

	Gastric acid (umol/30 min)	Pancreatic protein (mg/30 min)
PJ diverted + GF open	218±27	10±2
PJ diverted + GF open + duodenal HCl	79±10*	48±7*
L-364,718 + PJ diverted + GFopen + duodenal HCl	96±15*	12±3**
PJ diverted + GF closed	—	47±5
PJ diverted +GF closed + duodenal acidification	—	49±4
L-364,718 + PJ diverted + GF closed + duodenal acidification	—	14±3**

* Significant change as compared to the value obtained in rats with PJ diverted and GF open

** Significant decrease below the values obtained with duodenal acidification.

CCK-8 infused s.c. in gradually increasing doses (20—320 pmol/kg-h) in rats with the GF closed and the pancreatic juice diverted did not affect the rise in pancreatic protein output as compared with that obtained by pancreatic diversion alone (Fig. 11). In rats with the pancreatic juice returned to the duodenum and the GF open, CCK produced a dose-dependent increase in protein output reaching a peak that was significantly lower than that obtained in similar tests with the pancreatic juice diverted and GF closed. Pretreatment with L-364,718 (5 μmol/kg) abolished the increments in pancreatic protein secretion in response to CCK both in tests with the GF closed and open and these results have not been included. Gastric acid output in rats with the GF

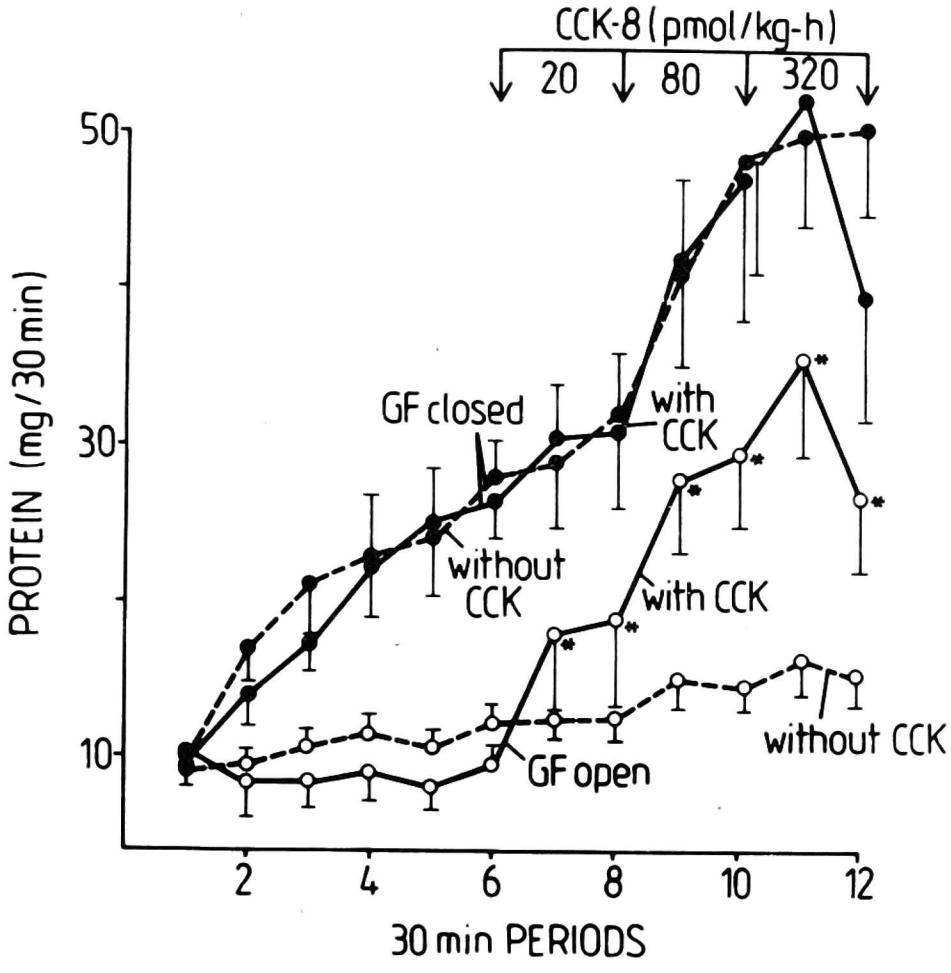

Fig. 11. Pancreatic protein response to CCK infused s.c. in gradually increasing doses in rats with diverted pancreatic juice and the GF closed or with returned pancreatic juice to duodenum and the GF open throughout the experiment. Mean ±SEM of 6 tests on 6 rats. Asterisk indicates significant increase in protein response above the control value obtained with the GF closed or open but without infusion of CCK.

Table 2. Gastric acid and pancreatic protein secretion in response to CCK (320 pmol/kg-h) in rats with the GF open and pancreatic juice (PJ) returned to duodenum or diverted to the exterior without or with pretreatment with L-364-718 given i.d. Means ±SEM of 8—10 tests on 8—10 rats.

	Gastric acid (μmol/30 min)	Pancreatic protein (mg/30 min)
PJ RETURNED		
Before CCK	48±7	8±2
After CCK	142±26*	35±6*
After L-364,718 + CCK	230±42**	12±3**
PJ DIVERTED		
Before CCK	210±32	50±8
After CCK	194±18	54±7
After L-364,718 + CCK	240±28	14±5**

* Significant increase compared to the value obtained before CCK

** Significant change compared to the value obtained in tests with CCK alone

open and pancreatic juice returned to the duodenum was relatively small (48 ± 7 µmol/30 min) throughout the experiment. Addition of CCK resulted in a small but dose-dependent increase in acid output reaching about 142 ± 26 µmol/30 min at the highest dose of CCK. Pretreatment with L-364,718 (5 µmol/kg) resulted in significant enhancement in gastric acid response to CCK reaching higher peak (about 230 ± 42 µmol/30 min) than in response to CCK alone (Table 2).

Table 3. Pancreatic protein outputs and gastric acid outputs in response to intraduodenal (i.d.) or subcutaneous (s.c.) infusion of graded doses of FOY-305 in rats with pancreatic juice returned to duodenum and the GF open or closed during the experiment. Means ±SEM of 6—8 tests on 6—8 rats.

		GASTRIC ACID (µmol/30 min)	PANCREATIC PROTEIN (mg/30 min)
GASTRIC FISTULA OPEN			
FOY-305 i.d.	0 mg/kg	59 ± 7	5.6 ± 0.6
	6 „	68 ± 5	5.6 ± 1.5
	12 „	82 ± 7 *	9.8 ± 1.6 *
	50 „	101 ± 11 *	12.4 ± 2.4 *
	200 „	120 ± 18 *	14.8 ± 2.1 *
FOY-305 s.c.	0 „	65 ± 12	6.5 ± 0.5
	6 „	80 ± 32	7.4 ± 1.0
	12 „	159 ± 24 *	10.2 ± 1.2 *
	50 „	123 ± 28 *	14.2 ± 0.5 *
	200 „	115 ± 30 *	17.5 ± 0.8
GASTRIC FISTULA CLOSED			
FOY-305 i.d.	0 „	NT	9.0 ± 1.3
	6 „	NT	9.5 ± 2.2
	12 „	NT	14.3 ± 3.4 *
	50 „	NT	23.5 ± 5.1 *
	200 „	NT	29.8 ± 5.8 *
FOY-305 s.c.	0 „	NT	5.8 ± 0.5
	6 „	NT	8.0 ± 1.2
	12 „	NT	11.8 ± 1.4 *
	50 „	NT	13.9 ± 1.6 *
	200 „	NT	17.8 ± 1.4 *
GASTRIC FISTULA CLOSED + OMEPRAZOLE			
FOY-305 i.d.	0 mg/kg	pH ± 7.0	7.4 ± 2.2
	6 „	pH ± 7.0	8.0 ± 2.3
	12 „	pH ± 7.0	9.2 ± 2.2
	50 „	pH ± 7.0	11.8 ± 2.4
	200 „	pH ± 7.0	14.5 ± 3.1 *

* Significant ($P < 0.05$) increase above the value obtained during experiment with i.d. or s.c. administration of vehicle saline without FOY-305.

DISCUSSION

The present results confirm that the diversion of pancreatic juice from the duodenum causes progressive increase in pancreatic protein secretion and show that the diversion is accompanied by a rise in gastric acid secretion, whereas the return of pancreatic juice to the duodenum results in the fall in gastric secretion. Since opening of gastric fistula and draining of gastric juice to the exterior or the suppression of acid secretion by omeprazole reduced by over 70% the post-diversion pancreatic hypersecretion, we propose that the gastric acid plays a crucial role in this hypersecretion.

The importance of luminal proteases such as trypsin and chymotrypsin in the feedback control of pancreatic secretion is well known, particularly in rats (1—6, 12). Numerous studies have demonstrated that the removal of these proteases from the intestinal lumen by the diversion of pancreatic juice from the duodenum or by the inhibition of protease activity in the gut lumen results in a potent stimulation of pancreatic secretion, so that neither feeding, exogenous CCK (20) nor intestinal irrigation with amino acids or protein (17) was capable of causing any further increment in this secretion. It has been assumed that the pancreatic secretion was already maximally stimulated under these conditions by the loss of feedback control of CCK release. The hypothesis remains that the absence of trypsin, chymotrypsin and elastase in the intestinal lumen enhances the liberation of CCK possibly via mediation of CCK-releasing peptides produced by the intestinal mucosa or the pancreas itself (2, 12, 14, 15, 21). This hypothesis is supported by numerous studies showing that protease inhibitors can duplicate post diversion pancreatic hypersecretion while the return of proteases or pancreatic juice to the duodenum depresses this hypersecretion. The latter effects are seen only in that part of the intestine from which CCK is released (22, 23). Evidence for a crucial role for CCK is supported by the fact that pancreatic diversion or intraduodenal trypsin inhibitor results in a marked increment in plasma CCK, whereas the reinfusion or pancreatic juice or trypsin in the duodenum lowers plasma CCK levels (9, 22).

It should be pointed out that little attention has been paid to the possible involvement of gastric acid in the feedback control of pancreatic secretion by luminal proteases. As shown in our study, the diversion of pancreatic juice resulted in an immediate increase in gastric acid secretion whereas the return of pancreatic juice to duodenum was followed by a marked reduction in gastric acid secretion. These results indicate that in rats the presence or absence of pancreatic juice in the duodenum had a potent influence on gastric acid secretion.

Annis and Hallenbeck (24, 25) were the first to show in dogs that the diversion of pancreatic juice enhanced the postprandial pancreatic volume flow and bicarbonate secretion and this has been attributed to the incomplete neutralization of gastric acid in the duodenum. It is of interest that the diversion of pancreatic

juice, pancreatectomy or duct ligation in dogs resulted in a marked increase in gastric acid secretion that could be reversed by feeding pancreatic juice or pancreas (11, 26, 27). Thus, an intricate relationship, similar to that observed in our study, was found to exist between gastric and pancreatic secretory mechanisms in dogs.

In rats, unlike dogs, the diversion of pancreatic secretion caused a marked stimulation of pancreatic enzyme secretion only under basal conditions, whereas postprandial pancreatic secretion reached similar values in tests with the pancreatic juice diverted or returned to the duodenum. As presented in this report, the time course of the post-diversion pancreatic hypersecretion in rats with the GF closed and saline infusion showed the progressive increase to reach the peak about 4 h after opening the pancreatic fistula. This peak attained a level not significantly different from that observed postprandially or following administration of CCK in these animals. When saline was not administered, the post-diversion pancreatic hypersecretion reached the peak somewhat earlier and then declined towards the basal level. This suggests that the compensation of fluid lost during the diversion is an important factor in maintenance of the pancreatic response to diversion. It should be emphasized that this post-diversion pancreatic hypersecretion was observed in our study only in rats with the GF closed. When the gastric acid was drained to the exterior by opening the GF or suppressed by omeprazole, the diversion resulted in only a small increase in pancreatic secretion reaching about 25% of that obtained with the intact gastric secretion.

The question arises as to what is the major factor responsible for the post-diversion pancreatic hypersecretion in rats with the GF closed. Grossman (28) observed that the ligation of pylorus reduced the post-diversion hypersecretion of both volume and protein. Noda et al (4) also found that after opening the fistula and draining the gastric juice to the exterior the post-diversion pancreatic volume flow was abolished and the enzyme output was greatly reduced. These investigators used larger gastric fistulas to secure complete gastric drainage and they found that such a drainage reduced not only the post-diversion pancreatic hypersecretion but also that induced by soybean trypsin inhibitor. Opening the GF combined with atropinization completely eliminated the post-diversion pancreatic hypersecretion. They hypothesized that the presence of trypsin depressed the sensitivity of duodenal receptors to respond to gastric acid and consequently that removal of trypsin increased the responsiveness of these receptors to gastric juice accounting for the pancreatic hypersecretion that occurs following administration of trypsin inhibitor or diversion of pancreatic juice from the gut. More recently, Green (17) clearly demonstrated that acid in the duodenum significantly augmented the pancreatic response to diversion of bile and pancreatic juice, although significant pancreatic stimulation nevertheless occurred in the absence of HCl in the intestine suggesting that gastric acid in the duodenum is not important or necessary for the pancreatic hypersecretion in

response to diversion of the pancreatic juice from the duodenum or to inhibition of luminal protease. The major argument was that intraduodenal instillation of HCl solution at physiological rates failed to affect the pancreatic secretion in pylorus-ligated rats with either diverted or returned pancreatic juice and did not alter the pancreatic response to the inhibition of luminal proteases. In that study, however, no animals without pyloric ligation were used for comparison so it was difficult to assess whether the pancreatic hypersecretion was affected by such ligation. In addition, the animals were held in Bollman cages throughout the study period so the effects of chronic stress on the observed pancreatic secretion could not be eliminated.

Our results obtained from conscious rats, well adapted to the cage restraint and equipped with large gastric fistulas to achieve complete drainage of gastric juice to the exterior, demonstrate that the diversion of pancreatic juice from the duodenum markedly stimulates gastrc acid secretion and that prevention of gastric acid from entering the duodenum actually eliminates the post-diversion pancreatic hypersecretion. Identical effects have been observed following the suppression of gastric acid secretion by omeprazole. These results strongly support the notion that gastric acid is of crucial importance in the mechanism of the post-diversion pancreatic hypersecretion in conscious rats.

Further support for the role of gastric acid in the post-diversion hypersecretion derives from our studies with intraduodenal instillation of HCl solution at the rates similar to those secreted by the stomach in the diverted state. Such duodenal acidification was as effective in pancreatic protein stimulation as the diversion of pancreatic juice from the duodenum but in rats with the juice diverted and the GF closed, duodenal acidification failed to cause any further increase in pancreatic secretion probably owing to the masking effect of endogenous gastric acid entering the duodenum. Endogenous acid entering the duodenum in large amounts in the diverted state seems to be a key factor in the post-diversion pancreatic hypersecretion but whether it is simply due to the release by acid in the duodenum of secretin and CCK and their potentiating interaction on pancreatic secretory cells or to the suppression by acid of residual luminal proteases is an open question (29). Our finding that the antagonism of CCK receptors by L-364,718 completely eliminated the stimulatory effect of the diversion of pancreatic juice and duodenal acidification on pancreatic secretion strongly supports the involvement of CCK. Although duodenal acidification was reported to increase the release of CCK (30) in dogs no evidence was so far obtained that for such acid-induced release of CCK in rats.

As mentioned before, the diversion of pancreatic juice from the duodenum caused a marked stimulation of gastric acid secretion but the mechanism of this gastric hypersecretion is not clear. It is likely that the absence of pancreatic juice in the duodenum triggers the release of gastric secretagogue such as CCK and that this hormone might be responsible for the observed secretion of gastric acid in the

diverted state. However, blocking of CCK receptors with. L-364,718 caused significant increase in the post-diversion gastric acid secretion and augmented gastric acid response to CCK. This could be interpreted that CCK alone is a weak gastric acid secretagogue and that it acts on gastric secretory mechanism through CCK-B rather than CCK-A receptors. The observed increase in gastric acid response to CCK after pretreatment with L-364,718 suggests that CCK may also have an inhibitory component probably mediated by CCK-A receptors on somatostatin-producing cells and involved in local release of gastric inhibitor such as somatostatin (31).

The major argument favoring the implication of luminal proteases in post-diversion pancreatic hypersecretion is the finding that instillation into the duodenum of trypsin or other pancreatic proteases reverses this pancreatic hypersecretion and that the inhibition of luminal proteases by a variety of inhibitors results in the stimulation of pancreatic secretion and CCK release (1—6, 12). The mechanism by which luminal proteases and their inhibitors might affect the release of CCK and pancreatic secretion in rats has not been elucidated. It has been proposed that they may act either directly on the CCK-producing cells or by the CCK-releasing peptides (2, 12, 14, 21). The most direct evidence for implicating luminal proteases in CCK release derives from recent studies using isolated vascularly perfused rat duodeno-jejunum (32). In this preparation trypsin added to the luminal infusion suppressed the release of CCK stimulated by protein digests and this effect was reversed by coinfusion of soybean trypsin inhibitor. However, neither trypsin alone (without protein digests) nor combined with its inhibitor in the intestinal lumen affected CCK release, excluding the direct interaction of proteases and protease inhibitors on the CCK-releasing cells. The finding of this and previous studies (33) that camostate is effective in the release of CCK and in the stimulation of pancreatic growth after parenteral administration also reinforces the notion that CCK release does not require the direct action of this agent on CCK-releasing cells. It is not excluded that soybean trypsin inhibitor stimulates the exocrine pancreas either by direct excitation of the secretory cells or by activating some other as yet undefined stimulatory mechanisms without involvement of luminal proteases.

According to the results presented in this paper and reported by others (4), the pancreatic stimulation induced by the diversion of the juice or by the inhibition of luminal proteases appears to depend upon gastric acid because this stimulation can be greatly attenuated by the drainage of gastric acid to the exterior. Miyasaka et al (34) found in conscious rats with pancreatic fistula that a recovery period of at least 4 days was necessary before the pancreatic response to diversion reached the peak value and no significant stimulation occurred with intraduodenal application of trypsin inhibitor until the third postoperative day. In contrast, the recent study of Li et al (35) performed on anesthetized rats showed that both pancreatic secretion and CCK release can be reduced by intraduodenal adminis-

tration of trypsin or chymotrypsin. It should be noticed that the pancreatic volume flow and protein outputs in the latter experiments were negligible as compared with that obtained in conscious animals several days after surgery (34). According to our experience with pancreatic fistula rats that were completely recovered postoperatively and well-conditioned to the individual cages, the rate of pancreatic volume flow and protein secretion was several times higher than during anesthesia (34, 35). Furthermore, the diversion of pancreatic juice produced a significant elevation of pancreatic secretion about 4 days after surgery. These divergent results obtained from rats immediately after surgical preparation of the pancreatic fistulas and those completely recovered from surgery indicate that in the former instance the results may not be conclusive because of the disturbance of the basic physiological regulatory mechanism in such acute in vivo experiments.

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