Effects of a protein meal, intraduodenal HC1, and oleic acid on portal and peripheral venous secretin and on pancreatic bicarbonate secretion

G. BODEN, R. M. WILSON, N. ESSA-KOUMAR, AND O. E. OWEN

From the Department of Medicine and the General Clinical Research Center, Temple University Health Sciences Center, Philadelphia, Pa. 19140, and the Department of Surgery, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854, USA

SUMMARY   We have studied the effect of a protein meal on secretin (IRS) concentration in dogs and humans using a radioimmunoassay of improved sensitivity (8 pg/ml). After a meal, pancreatic bicarbonate secretion (PBS) increased markedly and proximal duodenal pH decreased from 6·2 to 4·3. Portal and peripheral IRS concentrations, however, remained unchanged in eight dogs and five patients with cirrhosis of the liver. Similarly, an alkaline solution of sodium oleate (pH 9·2) stimulated PBS but not IRS. Intraduodenal administration of various amounts of HCl in dogs demonstrated that acid-stimulated PBS was invariably accompanied by rises in peripheral venous IRS concentration. We conclude that the postprandial stimulation of PBS involves mechanisms more complex than acid-stimulated secretin release.

Attempts to demonstrate a rise in the concentration of peripheral venous immunoreactive secretin (IRS) after intraduodenal administration of amino acids, fatty acids, various sugars, or following meals have largely been unsuccessful (Kolts and McGuigan, 1977; Boden *et al.*, 1974; Boden *et al.*, 1975a; Lee *et al.*, 1976). This has cast doubt on the validity of the time-honoured concept that secretin is the major stimulant for postprandial pancreatic water and bicarbonate secretion. At present, the physiological role of secretin remains uncertain, primarily because the radioimmunoassays used in the above-mentioned experiments were relatively insensitive and small changes in secretin could have been overlooked which, together with rises in cholecystokinin (CCK), might have been sufficient to stimulate pancreatic bicarbonate secretion (PBS) (Brown *et al.*, 1967; Henriksen and Worning, 1967; Meyer *et al.*, 1971).

In this study, we used a new antiserum and a more sensitive secretin assay to reinvestigate (1) the effect of a protein meal on secretin release, intraduodenal pH, and exocrine pancreatic function in dogs and humans; (2) the effects of intraduodenal administration of amino acids and oleic acid on secretin and bicarbonate release in dogs. Further to facilitate detection of a possible rise in secretin, the hormone

was determined not only in peripheral venous but also in portal venous plasma where secretin changes are more than twice as great as in the peripheral circulation (Boden *et al.*, 1974). Lastly, we examined quantitatively the role of HCl in pancreatic bicarbonate secretion by measuring bicarbonate outputs and peripheral secretin concentration in response to different amounts of intraduodenally infused HCl in dogs.

Methods

OPERATIVE PROCEDURES

Pancreatic and gastric fistulae were prepared by a modification of the Herrera technique (Herrera *et al.*, 1968) in healthy, mongrel dogs, weighing between 15 and 20 kg. The modification consisted of (1) ligation of the accessory pancreatic duct and (2) end-to-end anastomosis of the remaining duodenum after removal of the pouch. This eliminated the long, blind duodenal loop of the original Herrera technique. Postoperatively all dogs remained in good health and gained weight.

INSERTION OF PORTAL VENOUS CATHETERS

Two weeks after preparation of the pancreatic pouches polyvinyl catheters were placed into the portal vein during a second operation. The distal

ends of these catheters were exteriorised through incisions on the backs of the animals and were kept open with heparin locks. A one to two week recovery period was allowed before the dogs were used for experiments. In the morning of the day of an experiment, a polyvinyl catheter was placed in a jugular or femoral vein and kept open with a slow saline drip.

FEEDING EXPERIMENTS
All experiments were started in the morning after a 20 hour fast. The dogs were given 600 g of lean, ground hamburger which was eaten within three minutes. In eight experiments, performed on six dogs, pancreatic secretions were collected from the fistulae in 15 minute aliquots for 4¼ hours. In seven of these eight experiments blood was sampled simultaneously from the jugular and portal venous catheters for measurement of IRS. (The portal venous catheter occluded during the eighth experiment.) In all eight experiments 3-5 ml of secretions were aspirated every 15 minutes for 4¼ hours from the proximal one-third of the duodenum via the Herrera cannula (the cannula entered the duodenum about 2-3 cm below the pylorus) for pH measurement.

SODIUM OLEATE AND AMINO ACID INFUSION
Sodium oleate or an amino acid mixture was infused intraduodenally into three dogs (six experiments with Na oleate and six experiments with the amino acid mixture were performed). Sodium oleate was infused as a 50 mM solution (7·8 ml oleic acid was dissolved under vigorous stirring in 500 ml 0·15 M NaOH. Additional HCl was then used to bring the pH to between 9·2-9·4). Amino acids were infused as an 8·5% solution containing eight essential and seven non-essential amino acids (pH 5·5) (Free Amine, McGaw Laboratories, Glendale, CA). Both solutions were infused at a rate of 4 ml/min for 30 minutes using a Harvard pump.

Jugular or femoral venous blood was collected at −10 minutes and then every 15 minutes for one hour for determination of IRS. Pancreatic secretions were collected from the pancreatic fistulas in 15 minute aliquots for 90 minutes, for measurement of pancreatic volume, bicarbonate, and protein output.

INTRADUODENAL HCl INFUSIONS
These experiments consisted of three 30 minute periods; a basal period, an infusion period (HCl or saline), and a postinfusion period. HCl was infused intraduodenally at a constant rate (2 ml/min × 30 min), but in different concentrations into four dogs. Six experiments, one in each of two dogs and

two in each of two other dogs, were performed infusing 0·5, 1·0, 2·5, and 9·6 mmol HCl/30 min, respectively. Six control dogs received saline infusions (2 ml/min). Jugular or femoral venous blood was collected every 15 minutes for measurement of IRS. Pancreatic secretions were collected from the fistulae in 15 minute aliquots for determination of bicarbonate output. Since bicarbonate secretion generally returned to basal levels during the 30 minute postinfusion period the total bicarbonate response to any given HCl dose was obtained by combining bicarbonate outputs during the 30 minute infusion period and the 30 minute postinfusion period.

The gastric fistulae were kept open to prevent the possible entry of gastric acid into the duodenum in all experiments except those with meals.

PORTAL VENOUS SECRETIN AFTER MEALS IN HUMANS
Five patients (three males, two females) who had portal venous catheters were studied. The patients ranged in age from 36 to 58 years. Three had Laennec's cirrhosis proven by biopsy. The remaining two had postnecrotic hepatic cirrhosis. Four patients had oesophageal varices and had previously experienced one or more episodes of gastrointestinal bleeding. One patient had ascites. None of the five patients showed clinical signs of gastritis at the time these studies were performed. The catheters had been inserted previously via the umbilical vein by members of the surgical staff of Temple University Hospital for determination of portal blood flow. This information was used to select the most suitable shunt operation for these patients. After an overnight fast the five patients ate a breakfast consisting of 60 g cooked beef, one can of chocolate Meritene milkshake (Doyle Pharmaceutical Co., Minneapolis, Minnesota), one slice of bread, and one poached egg (total food content: 45 g protein, 42 g carbohydrate, and 20 g fat). Blood was sampled from the portal venous catheter before, and for 60 minutes, during and after intake of the meal for determination of IRS.

MEASUREMENTS
Blood was collected into iced test tubes, allowed to clot, and then centrifuged at 4°C. Serum was stored at −15°C until assayed. IRS was measured as described previously (Boden et al., 1974) with the following modifications: (1) the sensitivity of the assay was increased to 2 pg/tube or 8 pg/ml serum by use of a new antiserum.[1] This antiserum had been generated in rabbits against pure porcine secretin

[1]The antiserum was kindly donated by Dr Byron Kolts, University of Florida, Gainesville, Fla.

(GIH, Karolinska Institutet, Stockholm, Sweden). It has a high degree of specificity for secretin (Kolts and McGuigan, 1977) and was used in a final dilution of $1:10^6$. (2) Separation of bound from non-bound ^{125}I-secretin was achieved with dextran-coated charcoal. Four-tenths of a millilitre of a charcoal-dextran mixture (468·75 mg charcoal plus 31·25 mg dextran in 100 ml borate buffer, pH 8·0 containing 0·1% BSA) and 0·1 ml 1% gelatin in borate buffer were added to the incubation mixture. The test tubes were then gently mixed and incubated for another 30 minutes at 4°C. After that they were centrifuged at 4°C and decanted. Basal IRS values obtained with this assay are generally higher than those measured in extracted plasma (Schaffalitzky *et al.*, 1977), while IRS changes after HCl are comparable using unextracted or extracted plasma. The reason for this difference may, at least in part, be due to removal of high molecular weight IRS (void volume IRS) (Boden *et al.*, 1975b) by the extraction procedure.

Pancreatic volume was measured in graded conical tubes. Bicarbonate and protein were determined as described previously (Boden *et al.*, 1974). pH was measured immediately after collection in 3-5 ml samples of duodenal aspirate using a Beckman pH meter.

Statistical analysis was performed with Student's *t* test for small paired and unpaired samples (Snedecor and Cochran, 1967). Results are given as means ± SEM.

Results

PANCREATIC SECRETIONS AFTER MEAT MEAL (Fig. 1, upper panel)
Volume and bicarbonate output rose markedly during the first 15 minutes after feeding ($p < 0.01$). Both reached a peak at 75 minutes and then declined but were still above pre-feeding levels after four hours. Protein output also rose immediately after feeding, and peaked at 30 minutes—that is, 45 minutes earlier than the bicarbonate output.
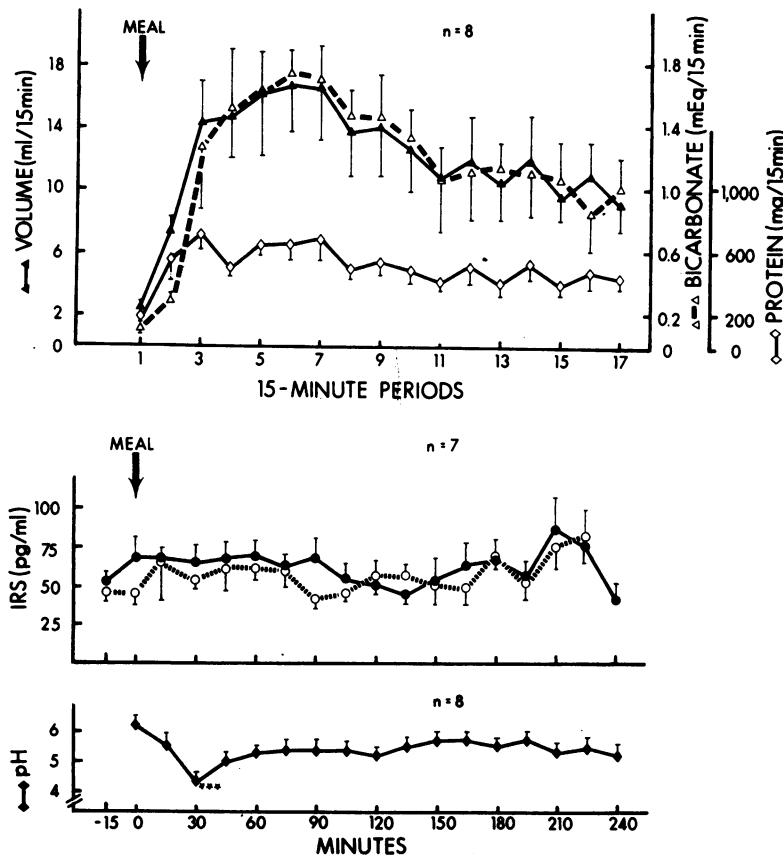

Fig. 1   *Upper panel: pancreatic flow rate, bicarbonate, and protein secretions in dogs before and after a 600 g protein meal. Values represent total output/15 min. Middle panel: portal venous (●) and peripheral venous (○) immunoreactive secretin (IRS) concentration in these dogs. Lower panel: duodenal pH values in these dogs. All values are shown as means ± SEM.* *** p < 0·001 *(Figs. 1-5: mEq = mmol).*

SERUM SECRETIN AND DUODENAL pH
AFTER MEAT MEAL (Fig. 1, middle and lower
panel)
IRS concentration immediately before feeding was
69 ± 13 pg/ml in portal venous serum and 45 ± 8
pg/ml in peripheral venous serum. These values did
not increase significantly during the four hour
post-feeding observation period. The increases in
mean IRS concentration seen between 195 minutes
and 225 minutes were mainly caused by IRS rises
in one dog (data shown in Fig. 2). Mean pH of
duodenal aspirates was 6·17 ± 0·30 before the meal.
Postprandial pH decreased and reached a nadir of
4·27 ± 0·30 (P < 0·001) 30 minutes after feeding.
Thereafter, mean pH rose again and remained
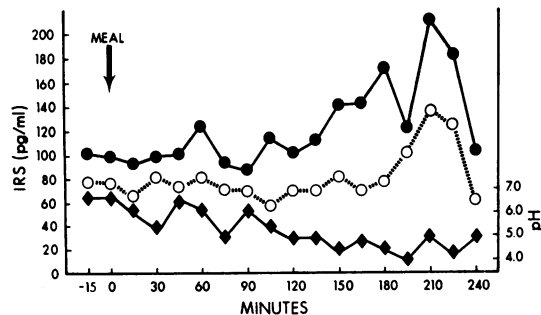between 5·0 and 6·0 for the rest of the observation
period.

Fig. 2 Portal venous (●), peripheral venous (○), immuno-
reactive secretin (IRS) concentrations and pH (◆) in
duodenal aspirates in one dog before and after a 600 g
protein meal.

Whereas mean IRS concentration did not change
in response to a meal we observed a marked IRS
rise in one dog during the fourth hour after feeding.
This is shown in Fig. 2. In this dog, the pH in the
duodenal aspirates started falling continuously 90
minutes after the meal and reached a nadir of 3·90
at 195 minutes postprandially. Concomitant with
this fall in pH there was a steep rise in portal as well
as femoral venous IRS concentration from 108
and 72 pg/ml at 120 minutes to 210 and 135 pg/ml,
respectively, at 210 minutes.

HUMAN PORTAL SERUM SECRETIN AFTER
MIXED MEAL
Figure 3 shows mean portal venous IRS concentra-
tion during and after a meal in five patients with
cirrhosis of the liver. Mean IRS concentration was
58 ± 22 pg/ml at the beginning of the meal. No
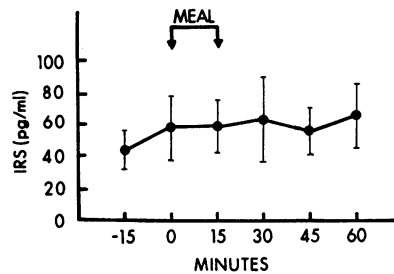significant changes occurred during or after food
ingestion.

Fig. 3 Portal venous immunoreactive secretin (IRS)
concentrations from five patients with hepatic cirrhosis
before, during, and after intake of a meal. Shown are
means ± SEM.

SERUM SECRETIN AND PANCREATIC HCO3
OUTPUT AFTER HCl
Figure 4 shows the effect of intraduodenal infusion
of increasing loads of HCl on peripheral venous
IRS concentration and on pancreatic bicarbonate
output. Depicted are the mean IRS concentrations
observed during the 30 minute HCl infusion and the
bicarbonate output per one hour (30 minute infusion
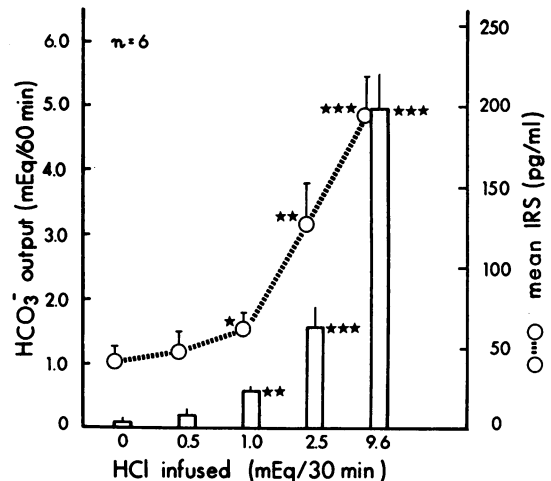period + 30 minute postinfusion period). Control

Fig. 4 Maximal peripheral venous immunoreactive
secretin (IRS) concentrations (○) and total pancreatic
bicarbonate outputs (columns) following intraduodenal
infusion of different quantities of HCl. (Total bicarbonate
output: output during the 30 minute infusion period and the
30 minute post-infusion period.) Physiological saline was
infused as control (0 mmol/30 min HCl). Shown are mean
± SEM. *P < 0·05; **P < 0·01; ***P < 0·001.

IRS concentration was 42 ± 8 pg/ml, while bicar-
bonate output was 0·12 ± 0·04 mmol/h. Infusion of
0·5 mmol HCl over 30 minutes had no significant

effect on IRS concentration or bicarbonate output. When 1·0 mmol HCl was infused IRS concentration rose to 62 ± 10 pg/ml (P < 0·05) and bicarbonate output rose to 0·59 ± 0·06 mmol/h (P < 0·01). The respective values for the infusion of 2·5 mmol HCl were 127 ± 126 pg/ml and 1·53 ± 0·34 mmol/h. Infusion of 9·6 mmol HCl resulted in a peak IRS concentration of 194 ± 25 pg/ml and in a bicarbonate output of 4·97 ± 0·64 mmol/h. This bicarbonate output is comparable to the hourly outputs observed after a meal which were 4·70 mmol, 6·40 mmol, 4·64 mmol, and 4·03 mmol, for the first, second, third, and fourth hour after feeding.

SERUM SECRETIN AND PANCREATIC SEC-RETIONS AFTER Na OLEATE AND AMINO ACIDS

The effect of intraduodenal infusion of sodium oleate and of an amino acid mixture on peripheral venous IRS concentration and on bicarbonate and protein output is shown in Fig. 5. IRS concentration (upper panel) did not change significantly in response to sodium oleate or to amino acids. Bicarbonate output (middle panel) rose from 0·24 ± 0·10 to 1·35 ± 0·34 mmol/15 min during the sodium oleate infusion (P < 0·01) and from 0·10 ± 0·03 to 0·37 ± 0·05 mmol/15 min after amino acid infusion (P > 0·05). Protein output rose from 222 ± 30 to 625 ± 105 mg/15 min (P < 0·01) and from 205 ±

44 to 582 ± 139 mg/15 min (P < 0·01) in response to oleate and amino acid infusions, respectively.

Discussion

In this study, ingestion of a protein meal was followed within 15 minutes by a marked increase in pancreatic secretion of water, bicarbonate, and protein. Similar data have been reported previously (Preshaw et al., 1966; Cooke et al., 1967; Henriksen and Worning, 1969). In addition, we observed, as have others (Thomas, 1940; Henriksen and Worning, 1969; Brooks and Grossman, 1970), a decrease in duodenal pH during the initial 30 minutes after feeding, indicating that gastric acid had been emptied into the upper duodenum. However, no rise in mean secretin concentrations in the portal or in the peripheral circulation was detected despite the use of a very sensitive assay. Several questions can be asked about these results. Firstly, the possibility has to be considered that secretin was released after feeding but was destroyed during storage before the serum was assayed. The disappearance of porcine secretin from human serum has been studied in our laboratory. It was found that approximately 20% of added secretin was lost after four hours at 4°C (the maximum time span allowed between collection of blood and separation of the serum in this study), while no loss of secretin immunoreactivity was
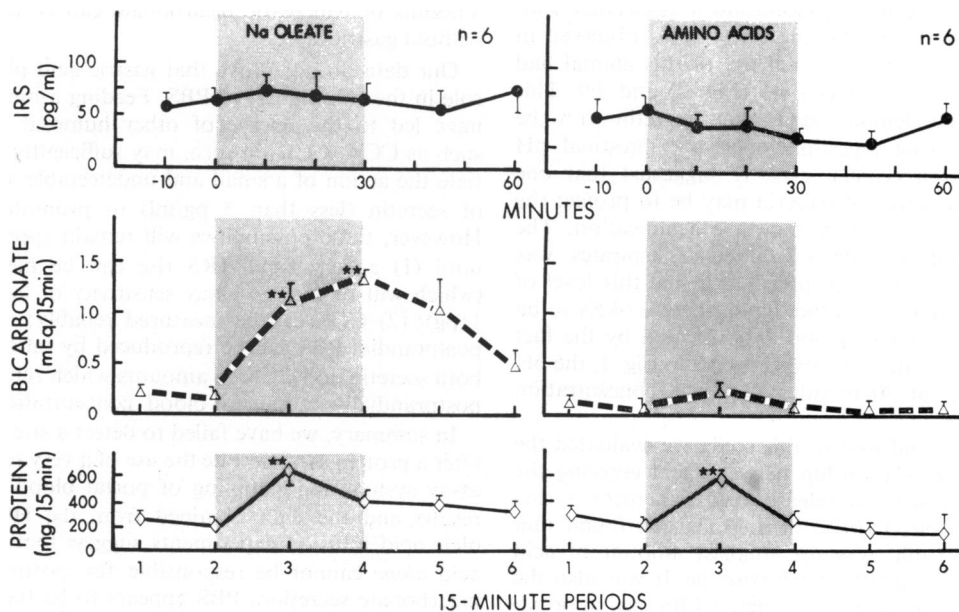

Fig. 5 *Peripheral venous immunoreactive secretin (IRS) concentration (upper panels), pancreatic bicarbonate output (middle panels), and pancreatic protein output (lower panels) in dogs (two experiments per dog) after intraduodenal infusion of Na oleate (6 mmol/30 min) and an amino acid solution (10·2 g/30 min). Shown are means ± SEM.* **P < 0·01.

observed in sera kept frozen for over one year (Boden, 1974). Secondly, could secretin have been released as a molecular species that was bioactive but not immunoreactive? This possibility can be excluded on the following grounds. The antiserum used in these studies, similar to the one characterised previously (Boden and Chey, 1973), recognised the complete secretin molecule and large carboxy terminal secretin fragments but did not react with amino terminal fragments. Thus, if an amino terminal secretin fragment was released after feeding, it would not have been detected; however, all amino terminal secretin fragments studied so far have been practically without bioactivity (Ondetti et al., 1968). A third and very important question concerns the possibility that there may have been secretin rises after meals which were too small to be detected but were sufficient to stimulate the pancreas. The assay used in these studies detected changes in IRS concentration of 8 pg/ml in serum with greater than 95% confidence. Allowing for 20% degradation of secretin in serum (Boden, 1974), our failure to observe a rise in portal venous IRS indicated that a change of up to 10 pg/ml could have occurred undetected. Since IRS changes in the portal circulation are at least twice as great as in the peripheral circulation (Boden et al., 1974), it can be concluded that if there were an increase in peripheral venous secretin concentration it must have been smaller than 5 pg/ml.

A rise in secretin concentration associated with an increase in bicarbonate output was observed in one dog after the duodenal pH of this animal had fallen to values between 4·5 (Fig. 2) and 3·9. This observation demonstrated that secretin can be released during digestion when the intestinal pH falls below a critical level. It suggested that one physiological role of secretin may be to protect the intestinal mucosa from excessive acidification. The measurement of pH in duodenal aspirates was probably too crude to precisely define this level of acidification. On the other hand, there is likely to be some individual variation as illustrated by the fact that in two of the eight experiments in Fig. 1, the pH fell to 4·0 (at 30 minutes) but IRS concentration did not rise.

In the second part of this study we evaluated the quantitative relationship between acid entering the duodenum, secretin release, and pancreatic bicarbonate secretion (PBS) in dogs. It was found that 1 mmol/30 min was the smallest amount of acid which increased IRS above baseline. It was also the threshold dose which stimulated PBS. An approximately 10 times greater acid load was needed to induce 5 mmol of PBS, a quantity similar to that seen after a protein meal. Such an acid load not only

stimulated IRS maximally but also consistently depressed duodenal pH to levels below 3·0. Neither event occurred after protein meals. Theoretically, intraduodenal acidification could also release an inhibitor of PBS. This could explain why secretin release, for any given amount of PBS, was so much greater after HCl than a possible secretin release after feeding. This argument, however, can be discarded. We have shown that a given increase in IRS concentration results in a PBS response which is the same regardless of whether it is achieved by infusion of exogenous secretin or through release of endogenous secretin by acidification (Boden et al., 1977). These observations provide direct evidence that HCl induced PBS is secretin mediated and exclude the release of an inhibitor for PBS. Infusion of acid into the duodenum cannot be directly compared to the events taking place after a meal. Nevertheless, these data clearly show that postprandial bicarbonate secretion cannot be explained through a simple sequence of events starting with gastric acid entering the duodenum leading to the release of secretin which then promotes PBS. In fact, there is good evidence that PBS can be driven by acid independent mechanisms. Fatty acids and soaps have long been known to be strong stimulants for bicarbonate secretion (Thomas, 1950; Meyer and Jones, 1974). The present study demonstrated a marked stimulatory effect of an alkaline solution of sodium oleate (pH 9·2) on PBS, confirming that considerable amounts of pancreatic bicarbonate can be released without gastric acid.

Our data do not prove that gastric acid plays no role in the stimulation of PBS. Feeding a meal may have led to the release of other humoral factors such as CCK. CCK, in turn, may sufficiently potentiate the action of a small and undetectable amount of secretin (less than 5 pg/ml) to promote PBS. However, these possibilities will remain speculative until (1) a very small IRS rise can be measured (which will require an assay sensitivity of less than 1 pg); (2) CCK can be measured reliably; and (3) postprandial PBS can be reproduced by infusion of both secretin and CCK in amounts which reproduce postprandially measured blood concentrations.

In summary, we have failed to detect a rise in IRS after a protein meal despite the use of a very sensitive assay system and sampling of portal blood. These results and the data obtained from the HCl and oleic acid infusion experiments suggest that gastric acid alone cannot be responsible for postprandial bicarbonate secretion. PBS appears to be the result of a complex interplay between humoral and neural factors, wherein gastric acid, secretin, and CCK may all play modulating roles.

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