Exocrine pancreatic secretion and immunoreactive secretin (IRS) release after intraduodenal instillation of bile in man

M. OSNES, L. E. HANSSEN, O. FLATEN, AND J. MYREN

From the Laboratory of Gastroenterology, Ullevål Hospital, Oslo 1, Norway

SUMMARY   Six subjects with a normal endoscopic pancreatogram were investigated after an overnight fast by means of a side-viewing duodenoscope. After cannulation of the main pancreatic duct, juice was collected in five-minute samples for 20 minutes. An iso-osmolar solution of 6 g cattle bile was then infused into the duodenum through a separate catheter attached to the outside of the duodenoscope, and pancreatic juice collected in five-minute samples for another 20 minutes. Blood was frequently drawn from an arm vein through an indwelling catheter for estimation of immunoreactive secretin (IRS) by radioimmunassay. The flow rate of pancreatic juice and outputs of bicarbonate, amylase, and protein increased significantly after intraduodenal infusion of bile. A significant rise in plasma IRS was also found after instillation of bile in the duodenum.

The effect of bile on the exocrine pancreatic secretion is not well understood. Previously, it has been shown that bile in the duodenum augments the stimulating effect of exogenous secretin on pancreatic enzyme secretion (Wormsley, 1970; Forell *et al.*, 1971). The effect of bile on the endogenously stimulated enzyme secretion (Malagelada *et al.*, 1976) and the effect on the exogenously stimulated bicarbonate secretion (Wormsley, 1970; Forell *et al.*, 1971) is uncertain.

The purpose of the present investigation was to study the effect of intraduodenal instillation of bile on the basal pancreatic secretion obtained from the main pancreatic duct after endoscopic cannulation (Osnes *et al.*, 1976), and its effect on immunoreactive secretin (IRS) release in man.

Methods

Six patients, three men and three women, aged 27-73 years (mean 54 years) were selected for the study. After an overnight fast they were examined by means of a side-viewing duodenoscope (Olympus JF B2). After visualisation of the papilla of Vater, 15-20 mm of the main pancreatic duct was cannulated with a Teflon catheter (external diameter 1·9 mm, internal diameter 1·7 mm). Before the examination a
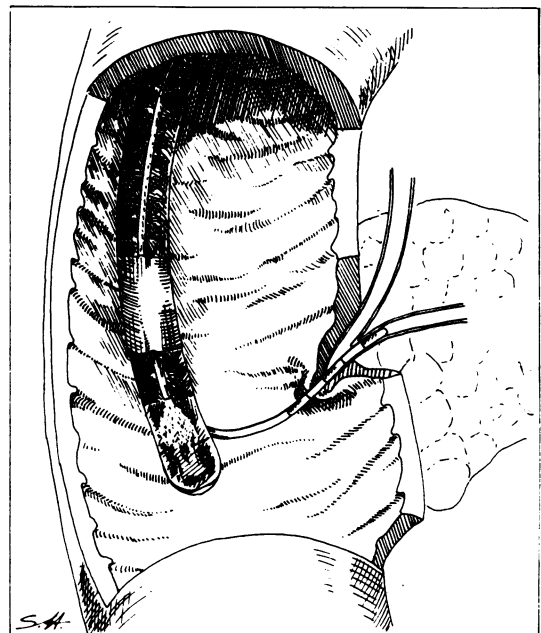
Fig. 1   *The drawing shows one catheter within the main pancreatic duct after endoscopic cannulation. Another similar catheter is attached to the outside of the duodenoscope for infusion of bile into the duodenum.*

similar catheter was attached to the outside of the duodenoscope for instillation of a solution of dried cattle bile (Fig. 1). In all subjects a normal endoscopic retrograde pancreatography had been demonstrated some days before the examination. Pancreatic juice collection was indicated for cyto-diagnosis, and a functional assessment. One patient was refused from the study because of findings indicating malignancy. At operation an adenoma was found in the pancreatic tail. One patient who was disturbed by the endoscopic procedure was given 5 mg diazepam during the examination; other medication was not given.

After successful cannulation of the main pancreatic duct, juice was collected in five-minute samples for 20 minutes. During the next five minutes, 6 g cattle bile (Kabi Chemi A/G, Hannover, Germany) (Table 1) in 60 ml distilled water (iso-osmolar, pH 6-7, 37°C) (Table 2) was infused through the external catheter. After the start of the bile infusion pancreatic juice was collected for four five-minute samples (Fig. 2).

The experimental procedure was characterised as successful when clear juice was flowing continuously shortly after cannulation of the main pancreatic

duct, when the catheter remained within the duct during the whole study, and when no colouring indicating contamination with bile was seen. All samples were collected in tubes at 0°C and analysed the same day. When the volume of a sample in the basal period was too small to allow determinations, neighbouring samples were mixed to determine concentrations, but the individual volumes were used for output calculations.

Bicarbonate concentration was determined by the addition of 100 mmol/l HCl, mechanical stirring for 20 minutes, and backtitration to pH 7·0 with 100 mmol/l NaOH (Autoburet, Radiometer, Copenhagen). The output of bicarbonate was expressed as mmol per five-minute sample.

Amylase concentration was estimated by the method of Phadebas (Pharmacia, Uppsala, Sweden), and results given in kilounits per litre (kU/l). The output of amylase was expressed in kU per five-minute sample.

Optical density was read at 280 nm (OD 280), and this was assumed to represent protein, with bovine serum albumen (fraction V, Sigma Chemical Corp, St Louis) as reference. The output was expressed as milligram bovine serum albumen equivalents (mg BSA equiv) per five-minute sample.

Immunoreactive secretin (IRS) was determined by radioimmunoassay employing ^{125}I-labelled synthetic secretin, antibody against synthetic secretin, and standards prepared from pure natural porcine secretin as described previously (Hanssen and Torjesen, 1977). In the present study blood samples were extracted into ethanol instead of methanol, increasing the extraction efficiency to 70%. Blood was drawn from an arm vein through an indwelling catheter at intervals as indicated (Fig. 6) IRS was expressed as pmol/l.

For statistical analysis of differences the Wilcoxon test for paired comparison was used, and stimulated values were compared with the mean of the basal levels. Results were given as mean ± standard error of the mean (SEM), n = 6.

Table 1 *Contents of bile acids, protein, bilirubin, and cholesterol in dried cattle bile (Kabi-Chemi A/G, Hannover)*

	Bile acids	Protein	Bilirubin	Cholesterol
Per cent (W/W)	59	2·8	0·12	0·2

Table 2 *Osmolality, pH, and acid concentration in solutions of 6 g cattle bile in 60 ml distilled water (mean ± SEM n = 5)*

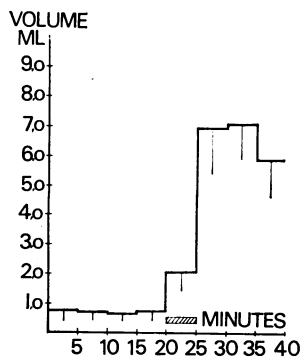| Osmolality (mosmol/kg)	pH	Acid (mmol/l)
308 ± 13·6	6·6 ± 0·1	6·8 ± 1·1

Fig. 2 *Effect of intraduodenal infusion of an iso-osmolar solution of 6 g cattle bile (hatched area) on volume of basal pancreatic juice. Vertical bars indicate SEM, n = 6.*

Results

The mean flow rate of basal pancreatic juice ranged from 0·6 ± 0·2 ml to 0·8 ± 0·3 ml per five-minute sample (mean level 0·7 ± 0·3 ml). After intraduodenal instillation of bile a significant rise in flow rate was found in all samples (P < 0·02). Peak volumes were found in the third sample (7·0 ± 1·2 ml).

The mean bicarbonate concentration ranged from 71·5 ± 5·4 mmol/l to 73·3 ± 5·9 mmol/l during the basal period (mean level 72·2 ± 6·2 mmol/l) (Table 3). There was a significant rise in the second (P <

Table 3   *Concentrations (mean ± SEM) of bicarbonate, amylase, and protein during basal period (sample 1-4) and after intraduodenal infusion of 6 g cattle bile in 60 ml distilled water (sample 5-8) (n = 6)*

Sample				Sample			
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Bicarbonate (mmol/l)							
73·0 ± 6·6	72·8 ± 7·0	71·5 ± 5·4	73·3 ± 5·9	80·0 ± 7·9	108·8 ± 9·4	125·1 ± 8·5	124·2 ± 5·2
Amylase kU/l							
464 ± 37	499 ± 39	498 ± 42	508 ± 36	530 ± 25	438 ± 36	299 ± 23	261 ± 26
Protein (mg BSA equiv/ml)							
59·0 ± 12	59·8 ± 12	64·6 ± 13	61·7 ± 11	67·2 ± 7	34·0 ± 5	17·9 ± 5	13·8 ± 5

0·05), third (p < 0·02), and fourth (p < 0·02) sample after the start of intraduodenal infusion of bile. Peak bicarbonate concentration (125·1 ± 8·5 mmol/l) was found in the third five-minute sample (Table 3). There was a significant rise in bicarbonate output in all samples after infusion of bile (p < 0·05 in the first sample, p < 0·02 in the other samples). Peak output of bicarbonate (0·89 ± 0·16 mmol) was found in the third sample (Fig. 3).
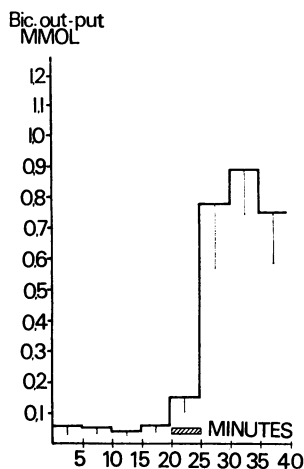

Fig. 3   *Effect of intraduodenal infusion of an iso-osmolar solution of 6 g cattle bile (hatched area) on bicarbonate output of basal pancreatic juice. Vertical bars indicate SEM, n = 6.*
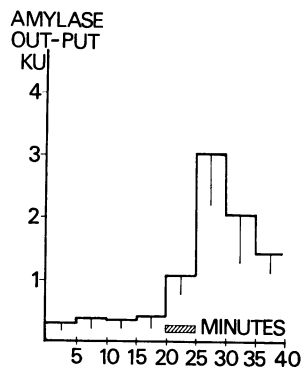

Fig. 4   *Effect of intraduodenal infusion of an iso-osmolar solution of 6 g cattle bile (hatched area) on amylase output of basal pancreatic juice. Vertical bars indicate SEM, n = 6.*

tion was significantly lower in the second (p < 0·05), third (p < 0·02), and fourth (p < 0·02) sample after the start of bile infusion (Table 3). There was a significant rise in protein output in all samples after the start of bile infusion (p < 0·02 for the increase in the first and second sample, p < 0·05 for the third and fourth sample). Peak output was found in the second sample after the start of bile infusion (209·1 ± 38·3 mg BSA equiv) (Fig. 5).

The concentration of amylase in basal pancreatic juice ranged from 464 ± 37 kU/l to 508 ± 36 kU/l, mean level 492 ± 38 kU/l. The amylase concentration was significantly lower in the second (p < 0·05), third (p < 0·02), and fourth (p < 0·02) sample after the start of bile infusion (Table 3), but because of the increased flow there was a significant rise in amylase output in all samples (p < 0·02). The peak output of amylase was found in the second five-minute sample (3·06 ± 0·86 kU) (Fig. 4).

The protein concentration as determined by OD 280 remained nearly constant during the basal period and ranged from 59·0 ± 12·3 mg BSA equiv/ml to 64·6 ± 13·5 mg BSA equiv/ml. Protein concentra-
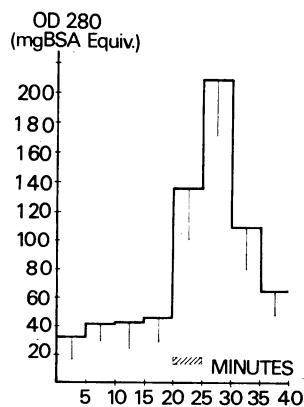

Fig. 5   *Effect of intraduodenal infusion of an iso-osmolar solution of 6 g cattle bile (hatched area) on OD 280. Output given as mg bovine serum albumine equivalent (mg BSA equiv). Vertical bars indicate SEM, n = 6.*
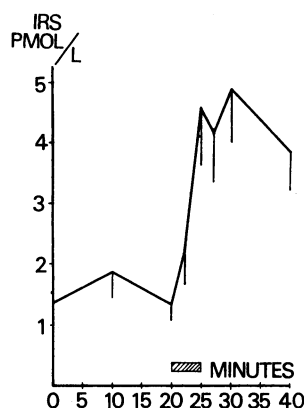
Fig. 6 *Effect of intraduodenal infusion of an iso-osmolar solution of 6 g cattle bile (hatched area) on release of plasma immunoreactive secretin (IRS). Vertical bars indicate SEM, n = 6.*

Immunoreactive secretin concentration in plasma increased significantly ($p < 0.02$) five minutes after intraduodenal infusion of bile from a mean basal level 1.5 ± 0.3 pmol/l to a mean peak level of 4.9 ± 0.9 pmol/l 10 minutes after the start of intraduodenal infusion of bile (Fig. 6).

Discussion

Previous animal experiments have given contradictory results concerning the effect of bile on the exocrine pancreatic secretion. Mellanby (1926) showed that the introduction of bile into the duodenum in cats stimulated the pancreatic secretion at a high degree. Ivy and Lueth (1927) found that bile was only a weak stimulant to the pancreas in dogs. Thomas and Crider (1943) could not find any stimulating effect of bile in dog experiments. It seems that not only species differences account for the contradictory results in these animal experiments. It seems possible that concentrations and amount of active substances in the bile might have played a part in the contradictory results.

Lagerlöf (1942) showed that introduction of bile salts into the duodenum stimulated the basal pancreatic flow rate and secretion of bicarbonate. The secretion of enzymes was, however, not influenced. Wormsley (1970) and Forell *et al.* (1971) have shown that infusion of bile into the duodenum in man augments the stimulating effect of a high dose of exogenous secretin on pancreatic enzyme secretion. In experiments on dogs Konturek and Thor (1973) found that bile was not able to augment the pancreatic secretion of bicarbonate during stimulation with exogenous secretin. However, bile infusion into the duodenum had a stimulating effect on the bicarbonate secretion under basal conditions. These findings are confirmed and complemented by the present study of human pancreatic secretion, which shows

that infusion of bile into the duodenum has a significant stimulating effect on the pancreatic secretion of water, bicarbonate, amylase, and protein in man.

The mechanism by which bile or bile salts stimulate the pancreatic secretion has not yet been defined. Our study confirms the hypothesis of Mellanby (1926) of release of secretin from the upper intestine. Interestingly, the instilled bile was nearly neutral in pH, but the diversion of pancreatic juice from the intestine in our experiments might have made the mucosae more susceptible to the small amount of H^+ present in the bile. The release of secretin documented in the present study is probably not the sole explanation of the observed increase in pancreatic enzyme secretion. It seems likely that other mechanisms, such as release of other gastrointestinal hormones, local nervous reflexes, and circulatory alterations also influence the pancreatic enzyme secretion.

Recent studies in rats (Green and Lyman, 1972) support the view that pancreatic enzyme secretion might be subjected to a negative feedback mechanism of intestinal proteolytic activity. This view was documented by an increase of pancreatic secretion during diversion of bile and pancreatic juice from the intestine. However, such a mechanism cannot alone explain the stimulating effect on pancreatic secretion in our study, as we were not able to document any stimulating effect during the basal period.

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