

Pure pancreatic juice studies in normal subjects and patients with chronic pancreatitis

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SUMMARY Pure pancreatic juice was obtained from within the pancreatic duct in 54 patients after endoscopic cannulation of the papilla of Vater. In all 20 normal subjects there was a brisk response to intravenous injections of GIH secretin in small dosage (1 and 4 CU). Peak bicarbonate concentrations occurred after a 4 CU stimulus, whereas volumes, and bicarbonate and protein outputs were greatest after 70 CU. Total protein and amylase concentrations were highest in the first specimens collected from each patient, and fell rapidly after stimulation. Plateau levels for all indices were achieved 10-20 minutes after starting infusions of secretin and pancreozymin. When normal patients and those with chronic pancreatitis were compared, there was considerable overlap in all indices (volume, bicarbonate and total protein concentrations) after bolus injections of secretin. Most patients with chronic pancreatitis achieved a peak bicarbonate concentration in excess of 100 mmol/l. The median concentrations were not significantly different from normal after any dose of secretin when pooled 10 minute samples were analysed. However there were significant differences in peak bicarbonate concentrations (after 1 and 4 CU, but not after 70 CU), when one minute samples were compared. There were also statistically significant differences in the median 10 minute responses for volume after 1 and 70 CU, for bicarbonate output after 1, 4, and 70 CU, and for protein output after 70 CU. The results of juice studies in patients believed to have early chronic pancreatitis did not differ significantly from those in normal subjects or those with chronic pancreatitis. Endoscopic duct cannulation cannot guarantee complete recovery of pancreatic secretions, and measurements of volume and output may be inaccurate. When standard biochemical indices are used, the diagnostic role of pure juice studies is limited; further research may reveal more specific disease markers.

Much of our knowledge of human pancreatic exocrine function has been derived from standard pancreatic function tests, which involve the collection of duodenal contents after hormone stimulation. However, recovery in such studies is unpredictable, and pancreatic juice is necessarily contaminated by bile and other secretions. The fiberoptic duodenoscope now allows deep cannulation of the papilla of Vater in conscious subjects. The technique has mainly been used for diagnostic retrograde cholangiography and pancreatography (Cotton, 1977) but also permits the collection of uncontaminated pancreatic juice (and bile) (Cotton *et al.*, 1974). We have explored the pure pancreatic juice response to graded doses of secretin and pancreozymin in normal subjects and patients with pancreatitis.

Methods

PATIENTS

Pure pancreatic juice (PPJ) collections were attempted in 68 patients who had been referred for investigation of known or suspected pancreatic disease. All patients had already undergone diagnostic endoscopic retrograde cholangiopancreatography (ERCP). Fifty-four collections were successful in 52 patients, but five patients were excluded from analysis because the final diagnosis was not clear at the end of the study. The remaining 47 patients have been divided into three groups: normal, chronic pancreatitis, and probable early chronic pancreatitis. The criteria used in classification are shown in Table 1. Among the 19 patients finally judged to have no pancreatic disease, four had peptic ulcers and two were shown to have gallstones. No

Table 1 Diagnostic criteria used in classifying patients

Group	Number of patients	Investigations performed					
		ERCP			Standard PFT	Ultrasound	Laparotomy
		N	Equiv	Abn			
Normal pancreas	6 5 2 2 3 1	6 5 2 2 3 1		1	N N N N	N N N	N N
Total	19	18	1		9	6	3
?Chronic pancreatitis	8 5 1	1 3 1	7 2		Equiv Equiv	N	
Total	14	5	9		6	1	
Chronic pancreatitis	2 3 1 4 2 1 1		1	1 2 1 2 2 1	Abn Abn Abn Abn	Abn Abn Abn	CP CP CP
Total	14	4	1	9	7	4	6
Grand totals	47	27	11	9	22	11	9

N: normal, Equiv: equivalent, abn: abnormal, CP: chronic pancreatitis; PFT: secretin-pancreozymin function test.

organic cause for the presenting symptoms was found in the remainder, and no evidence of pancreatic disease has become evident in a follow-up period of one to three years. The 14 patients classified as suffering from chronic pancreatitis all had evidence of irreversible pancreatic damage (Table 1). Only two patients showed calcification on plain radiographs, and none suffered from diabetes mellitus. The third and intermediate group of patients classified as ? early chronic pancreatitis consisted of 14 patients who had suffered recurrent attacks of pancreatic type pain for at least two years, but in whom standard investigations (Table 1) showed no clear-cut evidence of chronic pancreatitic damage. Eight of these patients had suffered well-documented attacks of acute pancreatitis (with serum amylase levels greater than three times the normal) on one or more occasions before the study (but not within two months of investigation).

PPJ collections were performed in a standard manner, with the patient lying in the left lateral semi-prone position. All patients were starved overnight and sedated with intravenous injections of diazepam (Valium) in a dose of 5-20 mg. No anticholinergic agents were used. Cannulation was performed with a standard side-viewing duodenoscope and Teflon catheter (1.6 mm external and 1 mm internal

diameter) with a single end-hole. The catheter was primed with dilute indocyanine green to mark its dead space. Deep cannulation was judged to have been achieved when the catheter tip had passed more than 2 cm through the papilla. PPJ was collected by syphonage at 75 cm below the mouth, in one minute samples for up to one hour.

Purified porcine secretin and cholecystokinin-pancreozymin were obtained from the Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm. Hormones were dissolved in a standard mixture of 16.5 ml 0.9% saline, 1 ml Trasylol for stabilisation and 2.5 ml human serum albumin to prevent aggregation of molecules when using very small doses. Sufficient diluent was added to each ampoule of secretin or cholecystokinin-pancreozymin to provide a concentration of 10 CU/ml. Individual doses were then withdrawn. A 1 ml insulin syringe was used for small doses, which were further diluted to 5 ml with the diluent. All doses were prepared immediately before injection.

Injections were given after a five minute basal collection period. The first 34 patients received bolus intravenous injections of secretin of increasing strength at 10 minute intervals (1, 4 and 70 CU, approximating to 0.014, 0.057 and 1.0 CU/kg). Nine patients also received an initial dose of 0.5

CU (approximately 0.007 CU/kg) and five patients were given an additional injection of the diluent solution alone during the sequence. In the remaining 15 studies, an initial bolus intravenous injection of 4 CU GIH secretin was followed after 10 minutes by an infusion of secretin plus cholecystokinin-pancreozymin (both 1 CU/kg/hour) for up to 40 minutes.

PPJ samples were collected over ice, frozen, and maintained at -20°C before analysis. Volumes were measured by weighing; concentrations of sodium and potassium by flame photometry; chloride with a corning Eel chloride meter; bicarbonate by the method of Van Slyke using a Natelson microgasometer; total protein by a modified Lowry method (Huemer and Lee, 1970) and amylase activity by an iodometric method (Pimstone, 1965). Between batch coefficients of variation ranged from 0.5% for sodium to 6.3% for total protein, and the within batch coefficient of variation for amylase estimations was 11%. In comparisons between groups of patients, the results were expressed for each 10 minute period in terms of volume, mean bicarbonate concentration, bicarbonate output, mean protein concentrations, amylase, and protein output. Since data were not normally distributed, statistical analyses were performed using the Wilcoxon Rank test.

Results

NORMAL SUBJECTS

Bolus injections

A brisk flow of juice followed within minutes of the

intravenous injection of secretin in all patients, even at the lowest doses (Figs 1 and 7). The response increased in size and duration with increasing doses. Within individuals, the sodium and potassium concentrations remained almost constant throughout the studies, whereas the bicarbonate and chloride concentrations closely approximated to the sum of the sodium and potassium concentrations (Fig. 2). Bicarbonate concentration rose less rapidly than the volume after the initial stimulus, but this partly reflects the deadspace of the catheter. In most normal subjects the peak concentration was achieved after 4 CU, and was lower after 70 CU (Figs 3 and 8). The bicarbonate output, however, rose progressively with increasing stimulation (Figs 4 and 10). In normal subjects the highest total protein concentrations were seen in the first specimens collected (Fig. 5), with a rapid fall to a plateau as stimulation continued. Each secretin injection, however, resulted in a transient but marked rise in protein concentration (Fig. 5). Amylase and protein concentrations were significantly correlated ($r_s = 0.88$, $n = 98$, $P < 0.00001$). Ten minute protein outputs rose progressively after each stimulus.

Infusions

Volume response to secretin-cholecystokinin-pancreozymin infusion reached a plateau (of approximately 4 ml/min) after 20-30 minutes, but there was a tendency for bicarbonate concentrations to fall later (Fig. 6). The total protein concentration fell to a constant level over the same period. The plateau value for each index was similar to that

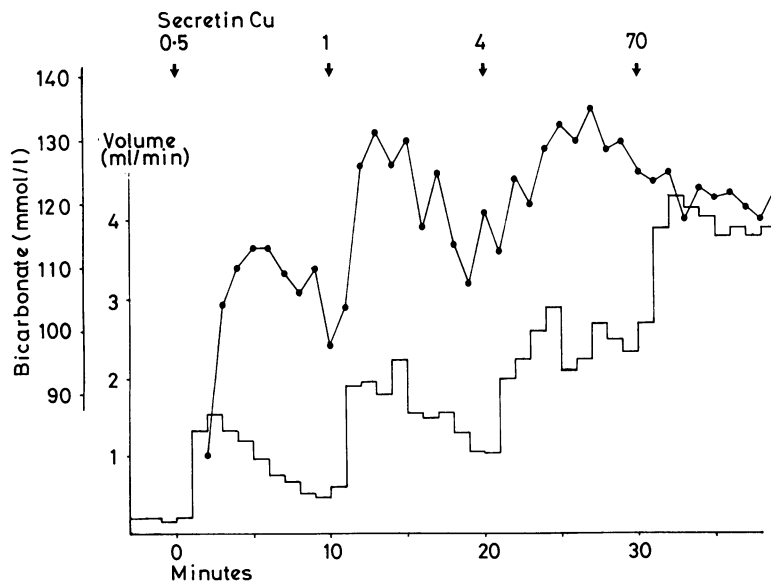


Fig. 1 Volume and bicarbonate concentrations in PPJ after graded bolus intravenous injections of secretin in one normal subject.

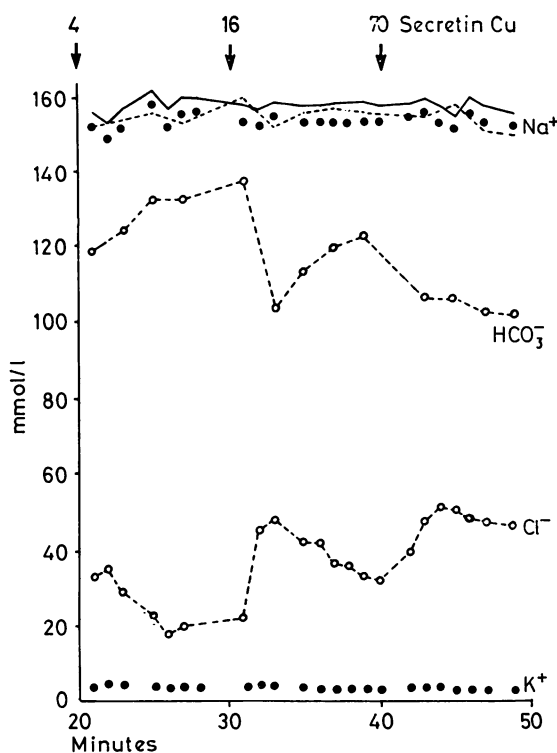


Fig. 2 Electrolyte changes in one normal subject after increasing doses of secretin. The sums of the cations (solid line) and anions (dashed line) are similar, at the top of the graph.

seen after the 70 CU bolus injections in other normal subjects (Fig. 7).

PATIENTS WITH CHRONIC PANCREATITIS AND ? EARLY CHRONIC PANCREATITIS

Bolus injections

There was considerable overlap in the response of these two groups of patients when compared with the normal subjects, in terms of volume (Fig. 7), 10 minute bicarbonate concentration (Fig. 8), and bicarbonate output (Fig. 10). Statistically significant differences were found between normal subjects and those with established chronic pancreatitis for volume after 1 and 70 CU, for 10 minute bicarbonate concentration only after 4 CU, and for bicarbonate output after all stimuli (Table 2). When one minute peaks of bicarbonate concentration were compared, however, significant differences were found between normal subjects and patients with chronic pancreatitis after 1 and 4 CU doses of secretin (Fig. 9). In patients with chronic pancreatitis the protein concentration in the first juice collected (after 1 CU)

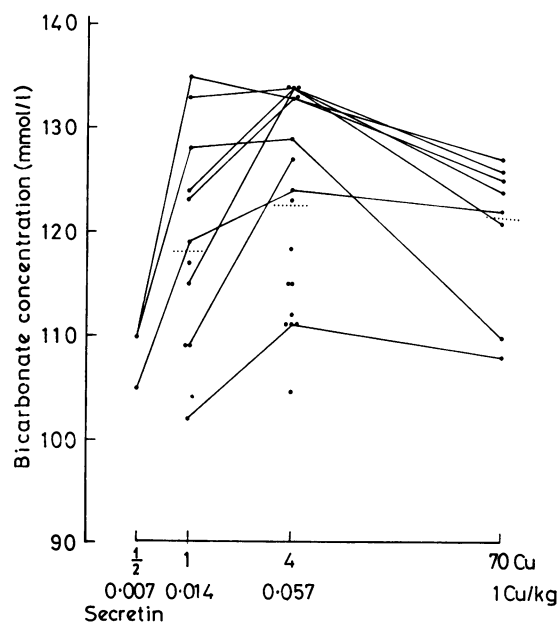


Fig. 3 Bicarbonate concentrations in all normal subjects after graded bolus intravenous injections of secretin. The median values are shown by dotted lines. The median values between 1 and 4 CU, and between 4 and 70 CU are significantly different ($P < 0.05$).

appeared higher than in normal subjects, but the differences did not reach statistical significance (Table 2); the protein output was significantly less after 70 CU among patients with chronic pancreatitis. No significant difference for any index was seen between normal subjects and those with probable early chronic pancreatitis, nor between the latter and those with established chronic pancreatitis.

Infusions

The numbers in the three groups of patients were small, therefore no statistical comparison could be made. There was a tendency for volume, bicarbonate output, and protein output to be higher in normal subjects than in those with chronic pancreatitis (Table 3) but no other obvious difference was observed.

Discussion

Human pure pancreatic juice studies are technically demanding, and few results have been published. Our studies provide some base line information about the response of the normal pancreas to secretin, and the results with very small doses (0.5 and 1 CU) are particularly interesting. When

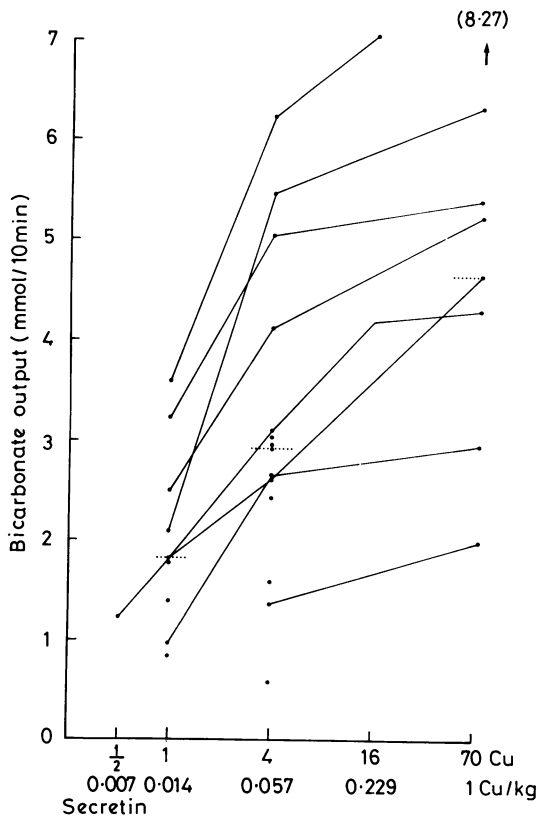


Fig. 4 Bicarbonate outputs in all normal subjects after graded bolus intravenous injections of secretin.

given by slow intravenous infusion, these doses cause rises in serum levels in immunoreactive secretin, comparable with those achieved by endogenous release after intraduodenal infusion of acid (Ward and Bloom, 1974); this suggests that they are within the physiological range. Similar pure juice responses were reported by Domschke *et al.* (1976) using low dose secretin infusion.

The volume response to 70 CU (roughly 1 CU/kg) in our normal subjects was similar to that reported by Gregg and Sharma (1975) using Boots secretin but greater than that recorded by Kawanishi *et al.* (1975a). The finding of a higher bicarbonate concentration after 4 CU than after 70 CU accords with the findings of Domschke *et al.* (1976), and early work on duodenal juice (Wormsley, 1968).

To clinicians, lack of convincing differences between normal subjects and patients with chronic pancreatitis will be surprising and disappointing. There was considerable overlap in all the measured indices.

Only two patients with chronic pancreatitis failed to achieve a bicarbonate concentration higher than 90 mmol/l, the commonly accepted criterion for abnormality in standard duodenal drainage function tests. This suggests that the much lower figures normally reported in duodenal juice result from the dilution of smaller quantities of pancreatic juice within the duodenum by bile and other intestinal contaminants. The differences also reflect the selection of patients. Many studies of pancreatic function in chronic pancreatitis contain a high proportion with calcification of the gland on

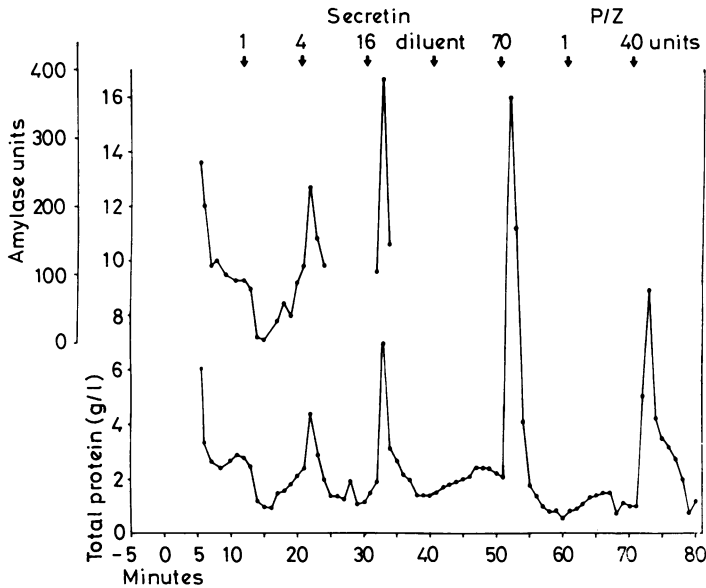


Fig. 5 Total protein and amylase concentrations in one normal subject after graded bolus intravenous injections of secretin, and cholecystokinin-pancreozymin (P/Z).

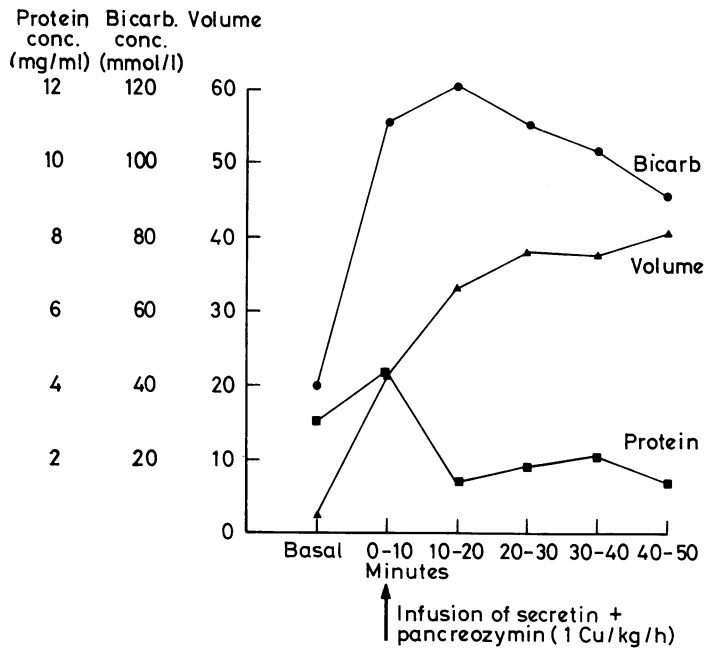


Fig. 6 Median volume, bicarbonate concentration and protein concentration in all normal subjects after the infusion of secretin and cholecystokinin-pancreozymin (each one CU/kg/h).

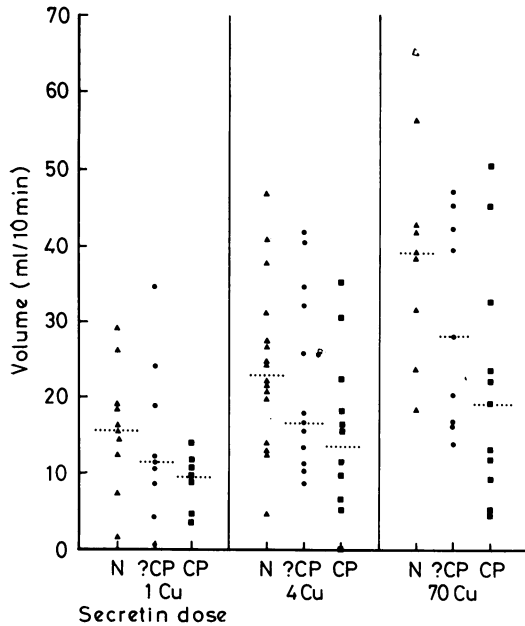


Fig. 7 Median 10 minute volume responses in all patients after bolus injections of secretin (median levels shown by dotted line: N: normal, CP: chronic pancreatitis, ?CP: probable early pancreatitis).

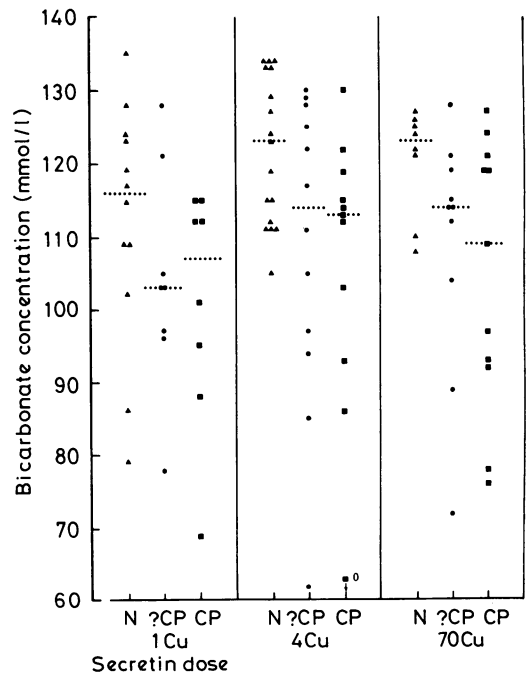


Fig. 8 Median 10 minute bicarbonate concentrations in all patients after graded bolus intravenous injections of secretin.

plain radiographs. This provides a convenient and definite diagnostic end-point, but is applicable only to the most severe cases. Only two of our patients

Table 2 Differences between normal subjects and chronic pancreatitis after bolus injections of secretin

Secretin dose (CU)	Group	Volume (ml/10 min)	P	Bicarb conc. (mmol/l)	P	Bicarb output (mmol/10 min)	P	Protein conc. (mg/ml)	P	Protein output (mg/10 min)	P
1	N	m = 15.75 n = 12	<0.05	m = 116 n = 12	NS	m = 1.82 n = 10	<0.01	m = 2.8 n = 9	NS	m = 49.5 n = 8	NS
	CP	m = 9.3 n = 8		m = 107 n = 8		m = 1.0 n = 8		m = 9.8 n = 7		m = 44.6 n = 7	
4	N	m = 22.8 n = 18	NS	m = 123 n = 17	<0.05	m = 2.92 n = 13	<0.05	m = 1.8 n = 16	NS	m = 35.8 n = 16	NS
	CP	m = 13.4 n = 12		m = 113 n = 11		m = 1.74 n = 10		m = 2.6 n = 11		m = 26.3 n = 11	
70	N	m = 39.2 n = 9	<0.05	m = 123 n = 8	NS	m = 4.95 n = 8	<0.05	m = 1.8 n = 8	NS	m = 87.4 n = 8	<0.05
	CP	m = 19.2 n = 11		m = 109 n = 11		m = 1.75 n = 11		m = 1.6 n = 11		m = 27.1 n = 11	

N: normal; CP: chronic pancreatitis; m: median value; n: number of patients.

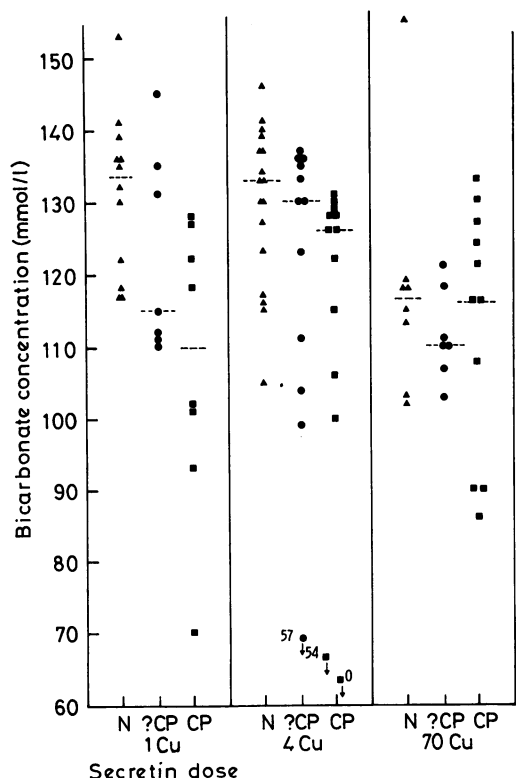


Fig. 9 Peak bicarbonate concentrations in one minute specimens in all patients after graded bolus injections of secretin. There are significant differences between normal subjects and CP after 1 CU ($p < 0.01$) and 4 CU ($p < 0.02$). No differences were seen between normal patients and those with ? early chronic pancreatitis at any dose.

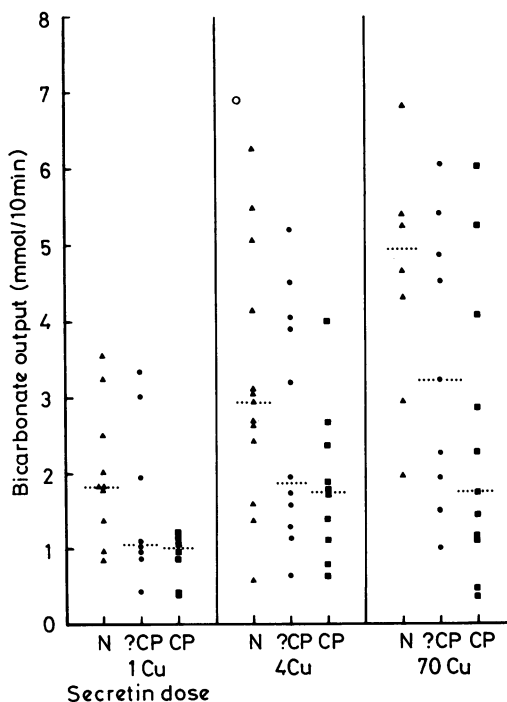


Fig. 10 Ten minute bicarbonate outputs in all patients after graded bolus injections of secretin.

showed calcification; in one patient there was no flow of pancreatic juice at all after deep cannulation and secretin stimulation. Patients with calcifying chronic pancreatitis do not require a further diagnostic test—the diagnosis is already established. The diagnostic problem occurs in less severe cases and the main purpose of this study was to see whether

Table 3 Median values (+ ranges) in the groups of patients in 10 minute period starting 20 minutes after start of secretin-cholecystokinin-pancreozymin infusion

	Normal	?CP	CP
Total patients	7	4	3
Volume (ml/10 min)	37.5 (29.3-53.9)	26.5 (21.8-39.8)	20.5 (0.35-9)
Bicarbonate concn (mmol/l)	103 (84-121)	115 (101-116)	105 (0-125)
Output (mmol/10 min)	3.75 (3.1-4.55)	2.85 (2.55-4.6)	2.65 (0.4-35)
Protein concn (mg/ml)	1.85 (0.7-3.2)	1.75 (0.8-5.6)	1.9 (0.3-3)
Output (mg/ 10 min)	70 (46-93)	47 (31-122)	33 (0-78)

pure pancreatic juice function testing provided better discrimination. This was the reason for studying less severe, but definite, chronic pancreatitis, and also a group with probable chronic pancreatitis at an even earlier stage.

In the pure juice studies, the functional defect in patients with chronic pancreatitis was more in volume production rather than bicarbonate concentrating ability. Thus the diagnostic capabilities of PPJ studies depend upon achieving complete recovery of the secreted juice; while this is likely with low flow rates, collections are certainly incomplete after higher doses of stimulants.

Even if it were proved safe to occlude the pancreatic duct orifice with a balloon catheter, some juice could escape in many patients through Santorini's duct and the accessory papilla. Furthermore, the resistance to flow down the long thin catheter might itself exert an influence upon the pancreatic response. It is difficult to measure the recovery fraction during pure juice studies, but this and other technical aspects are being examined. Some idea of recovery can be gained by comparing the results of pure juice studies with those of standard duodenal aspiration studies in the same patients. In our own comparison in 13 patients, the volumes obtained from the duodenum (uncorrected for recovery of markers) exceeded those obtained from the pancreatic duct in a ratio of 1.1 to 1. After correction for duodenal recovery, the ratio was 2.3 to 1. Some of this excess was due to secretions other than pancreatic juice, as evidenced by the fact that the mean bicarbonate concentration in pure juice exceeded that of duodenal juice by a mean of 30 mmol/l (Denyer *et al.*, 1977). Like Escourrou *et al.* (1978), we found that the bicarbonate output as measured in pure juice considerably exceeded that in duodenal aspirates in paired studies.

Total protein concentrations in these studies correlated very closely with amylase levels. The finding of high protein concentrations in the first

juice specimens, and transient increases after each secretin stimulus are open to various explanations. It has been suggested that patients with chronic pancreatitis have high juice protein concentrations, leading to deposition of protein plugs, duct obstruction, and local calcification. A tendency towards higher protein concentrations was seen in our studies after low (physiological) doses of secretin, but did not reach statistical significance (Table 3).

Detailed analysis of individual enzymes and protein fractions in pure juice is technically complex. We were surprised to find active trypsin in the juice of some patients; protein fractions degraded rapidly even on freezing. Statements about protein constituents in pure juice can be accepted only if restricted to studies in which juice was shown not to contain active trypsin immediately on withdrawal. When these problems are overcome, pure juice studies should provide an ideal avenue for further detailed research into constituents which may have diagnostic significance, such as lactoferrin (Colomb *et al.*, 1974; Fedail *et al.*, 1978), carcinoembryonic antigen (Kawanishi *et al.*, 1975b), isoamylases (Skude and Ihre, 1977), various protein fractions (Colomb *et al.*, 1974; Allan and White, 1975), and gamma glutamyl transpeptidase (Renner and Juttner, 1976).

Pure juice studies are technically too difficult for routine clinical practice, but the results obtained can demonstrate the reliance to be placed upon simpler methods, and lead the search for more specific diagnostic markers.

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