The influence of pancreatic juice on ^{64}Cu absorption in the rat

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1. The absorption of ^{64}Cu from closed duodeno-jejunal loops in anaesthetized rats was significantly less in the presence of pancreatic juice compared with that in the presence of saline (9 g sodium chloride/l). The inhibition of ^{64}Cu absorption caused by pancreatic juice was similar to that achieved with bile.

2. Studies using solutions of electrolytes and of pancreatic extracts showed that the inhibitory effect of pancreatic secretions was due to its protein-enzyme content and not to its bicarbonate content.

3. Pancreatic secretion and bile influence Cu absorption and thereby affect Cu homeostasis.

Little is known of the factors that regulate copper absorption. It is recognized that only a percentage of dietary Cu is absorbed, the percentage decreasing with increasing doses (Owen, 1964) and, furthermore, that the excess Cu which is excreted in bile is poorly reabsorbed (Owen, 1964; Mistilis & Farrar, 1968; Gollan, 1975). The following indirect evidence (Bell *et al.* 1981; Braganza *et al.* 1981*b*) suggests that pancreatic secretions may influence Cu homeostasis by inhibiting Cu absorption: (*a*) the biliary Cu content and serum copper oxidase activity of untreated patients with chronic pancreatitis significantly exceeds the values in controls; (*b*) this increased serum copper oxidase activity correlates inversely with the extent of impairment in pancreatic exocrine function; (*c*) both these indices of Cu metabolism are normal, or reduced, in patients with chronic pancreatitis on long-term treatment with pancreatic extracts.

We undertook the present study to determine whether pancreatic secretions directly influenced the absorption of Cu from closed duodeno-jejunal loops in anaesthetized rats. A preliminary report of part of this work has already been published (Jamison *et al.* 1981).

MATERIALS AND METHODS

The experimental model

Male Sprague-Dawley rats, weighing approximately 200 g, were maintained on a standard diet (containing 14 mg Cu/kg dry weight). Animals were denied food for 12 h before the experiment. They were anaesthetized with a solution of urethane (250 g/l saline (9 g sodium chloride/l)) given as a dose of 2 g/kg body-weight, intraperitoneally. A tracheostomy was performed, followed by laparotomy. The common bile–pancreatic duct was ligated adjacent to the duodenum and a closed duodeno-jejunal loop was constructed by tethering polyethylene tubes (size 10; Portex Ltd, Hythe, Kent) as shown in Fig. 1. In twenty-four experiments, the loop measured 176 (SE 5·4) mm. To remove secreted pancreatic enzymes, the loop was flushed with 20 ml warmed saline, the efficacy of this procedure being confirmed by analyses of duodenal washings. A dose of 0·5 μmol (31·8 μg) ^{64}Cu (100 μg [^{64}Cu]acetate, specific activity 0·5 mCi/mg) in 0·25 ml saline was mixed in a syringe with

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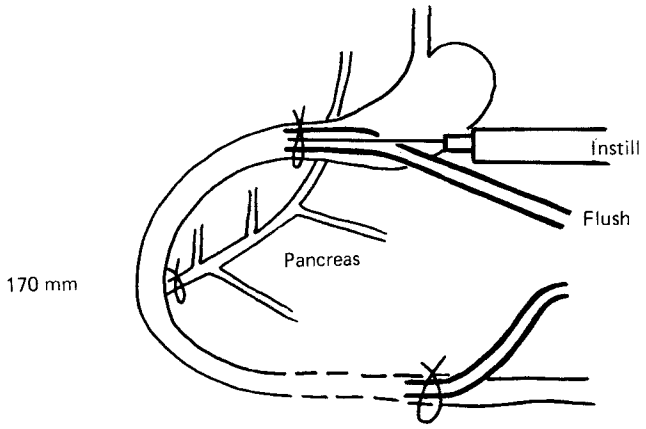

Fig. 1. Rat isolated duodeno-jejunal loop (for details, see p. 113).

0·25 ml saline, or a test solution (Table 1). The mixture was warmed to 38° and instilled into the closed loop. After 2 h the animal was killed; a 2 ml sample of blood was taken by cardiac puncture, the intestinal loop removed and the liver excised, weighed and cut into segments.

Secretions and solutions

Pancreatic juice or bile was collected from male Sprague-Dawley donor rats, also weighing approximately 200 g, which were anaesthetized with urethane as described previously. In the rat, numerous pancreatic ducts drain into the bile duct. Therefore, in order to collect pancreatic juice free of bile, the bile duct was ligated proximally at the hilum of the liver and cannulated distally through an incision in the duodenal wall with a fine-bore polyethylene tube (0·58 mm bore; Portex). The pancreas was stimulated with carbamylcholine chloride (20 μg/kg body-weight, intraperitoneally; Sigma Chemical Co., Poole, Dorset). Approximately 120 μl of pancreatic juice were collected over 30 min into a polyethylene tube and stored at −30° until required. The electrolyte composition of the juice so collected is given in Table 1. The low bicarbonate concentration (40 mmol/l) is typical of pancreatic juice evoked by cholinergic stimuli in rats and some other species and contrasts with the higher bicarbonate concentration evoked by secretin stimulation, especially in man and carnivores (Case, 1979). Bile, free of pancreatic juice, was obtained by ligating and cannulating the common duct above the level of the pancreas; it was stored at −30° until required.

In order to determine whether the electrolyte or protein content of pancreatic secretions influenced Cu absorption, four additional solutions were prepared (Table 1). Two of these solutions represented protein-free, artificial pancreatic juice and differed only in their content of bicarbonate and chloride: in one, bicarbonate concentration was low (40 mmol/l) and in the other was high (100 mmol/l). The third solution was an electrolyte-free solution of pancreatic extract and was prepared by dissolving 7 g Pancrex V powder (Paines and Byrne Ltd, Pabryn Laboratories, Greenford, Middlesex) in 100 ml deionized water and then dialysing the solution against deionized water at 4° for 3 h. The pH was adjusted to 7·2 with sodium hydroxide and the osmolality to 300 mosmol/kg water with sodium chloride (Table 1). The fourth solution contained albumin in place of Pancrex V powder and was produced by dissolving 1 g bovine serum albumin (Sigma Chemical Co.) in 20 ml deionized water and adding sodium chloride to produce a solution of osmolality 305 mosmol/kg water. Albumin possesses a Cu binding site at its amino terminus (Peters, 1960) and thus retards Cu absorption.

Table 1. *Composition of test solutions*

	Pancreatic juice	Saline (9 g sodium chloride/l)	Bicarbonate buffer		Dialysed pancreatic extract	Albumin electrolyte solution	Bile	Bile–pancreatic juice mixture
			40 mM	100 mM				
Electrolytes (mmol/l)								
Na	156	150*	156*	156*	140	159	146	152
K	7·0		7·0*	7·0*	1	0·2	5·5	6·8
Cl	110	150*	127*	67*	134	154	85	105
HCO_3	40		40*	100*	0	0		
Ca	1·72		1·5*	1·5*	—	—		
Mg	0·78		0·5*	0·5*	—	—		
Zn	0·35		—	—	—	—		
Cu	0·034		—	—	—	—		
Osmolality (mosmol/kg water)	318	281	295	298	300	305		
pH								
Measured	8·1	6·9			7·2	7·2		
At $pCO_2 = 40$	7·61*		7·61*	8·0*				
Trypsin (*EC* 3.4.21.4) (i.u./ml)								
Before activation	0							
After activation	921				98			
Chymotrypsin (*EC* 3.4.21.1) (i.u./ml)								
Before activation	1							
After activation	1098				123			
Lipase (*EC* 3.1.1.3) (i.u./ml)	2795				124			
Amylase (*EC* 3.2.1.1) (Nørby U/ml)	354				48			
Total protein (g/l)	56				25	42	10	40

* Calculated values.

Table 2. *Sample calculation procedure*

	Dose of ^{64}Cu injected (counts/min)	Tissue	Radioactivity: Counts/min	Radioactivity: Counts/min (corrected for background and natural decay)	Corrected radioactivity (% of dose given)
Measurement procedure					
Scintillation probe	9248	Carcass	298·9	222·3	2·40
		Loop	7016	8716	94·2
Automatic well counter		Liver	6538	146·4*	1·58
		Blood (2 ml)	299	6·29*	0·068
Total uptake of isotope					4·048
Radioactivity in loop					94·20
Total recovery of isotope					98·248

* Corrected also for counting geometry.

Measurement and analysis

Pancreatic juice, bile and the test solutions were analysed for sodium and potassium using a Corning flame photometer; for chloride using a Radiometer chloride titrator; for bicarbonate (as total carbon dioxide) using a Natelson microgasometer; for calcium, magnesium, zinc and Cu by using an Instruments Laboratory 157 atomic absorption spectrometer; and for osmolality by the freezing-point depression method using a Camlab micro-osmometer. Total protein in pancreatic secretion was measured manually by the Biuret method (Varley *et al.* 1980*a*). Lipase (*EC* 3.1.1.3), trypsin (*EC* 3.4.21.4) and chymotrypsin (*EC* 3.4.21.1) activities were measured by pH-stat methods (Varley *et al.* 1980*b*), the two latter enzymes after activation with enterokinase (*EC* 3.4.21.9); amylase (*EC* 3.2.1.1) activity was measured using a modification of Nørby's method (Lagerlof, 1942).

The ^{64}Cu activity in liver segments and blood was counted in a well-shielded automatic gamma counter (1280 Ultrogamma; LKB-Wallac) while that in the isolated duodeno-jejunal loop and in the carcass was counted using a scintillation probe. All counts were corrected for background activity and natural decay by concurrent counting of appropriate standards. The sum of activities in liver, carcass and blood was then compared with the administered dose to provide an index of Cu absorption for each experiment (Table 2).

RESULTS

When [^{64}Cu]acetate, dissolved in saline, was instilled into a closed duodeno-jejunal loop of anaesthetized rats, an average of 4·92% of the dose was absorbed in a 2 h period (Table 3). By comparison, an average of 3·12% of the dose was absorbed when it was administered in pancreatic juice, a reduction of 37%. This inhibitory effect of pancreatic juice is clearly not due to its bicarbonate content since the net uptake of ^{64}Cu from a solution containing 40 mmol bicarbonate was the same as from saline whereas, paradoxically, absorption from a solution containing 100 mmol bicarbonate was increased by 32%. The effect of pancreatic

Table 3. *Percentage uptake of ^{64}Cu in the presence of various secretions and test solutions*†

(Mean values and standard deviations for six determinations except pancreatic juice where values were for ten determinations)

	Saline		Pancreatic juice		Bicarbonate buffer 40 mM		Bicarbonate buffer 100 mM		Dialysed pancreatic extract		Albumin electrolyte solution		Bile		Bile–pancreatic juice mix	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver	1·68	0·28	1·19	0·31	1·65	0·42	3·02	0·84	1·26	0·42	1·22	0·36	1·05	0·20	0·97	0·21
Carcass	3·24	0·95	1·93	0·53	3·42	0·36	3·49	0·59	2·60	0·47	1·95	0·48	2·14	0·32	1·71	0·55
Total	4·92	1·20	3·12	0·77	5·07	0·44	6·51	1·21	3·86	0·84	3·17	0·82	3·19	0·46	2·68	0·63
Statistical significance of difference from saline group: *P*	—		< 0·005		NS		< 0·05		NS		< 0·02		< 0·02		< 0·005	

NS, not significant.

† The results in different groups of experiments were compared using Student's *t* test except in those cases where the variance differs markedly from that for the saline control (i.e. 40 mM bicarbonate and bile groups) where the modification of Cochran & Cox (1957) was employed.

juice was apparently related to its protein content since similar reductions in net ^{64}Cu uptake were observed when the ^{64}Cu was administered in a solution containing dialysed pancreatic extract.

Bile also inhibited net ^{64}Cu uptake and the extent of inhibition was similar to that observed with pancreatic juice (mean 3·19 *v.* 3·12%). Although there was a suggestion that equal portions of bile and pancreatic juice depressed ^{64}Cu absorption still further (mean 2·68%), the difference was not significant. Albumin, as expected, also depressed ^{64}Cu absorption (mean 3·17%). Histological examination of the duodeno-jejunal loop 2 h after instillation of the test substances revealed no significant abnormality, and no obvious differences between the experimental groups.

DISCUSSION

Several factors may influence the amount of Cu absorbed from a normal diet; the amount and chemical form of Cu (Van Campen, 1971); the presence of other inorganic constituents in food (Underwood, 1977); the efficiency of gastric digestion; the intestinal milieu; interaction with ligands in biliary, intestinal and pancreatic secretions (Gollan, 1975); and competition between Zn and Cu for available binding sites on metalothionines in intestinal mucosa (Van Campen, 1966; Hall *et al.* 1979).

The literature on Cu absorption is small. Since Cu is excreted in bile, the effect of bile on Cu absorption has been more extensively studied than any other influence (Owen, 1964; Mistilis & Farrar, 1968; Gollan, 1975). When Cu is added to bile in vitro, 70% immediately forms a complex from which the Cu is unabsorbable (Owen, 1980). Furthermore, whereas ^{64}Cu can be readily dialysed from saliva, gastric juice and duodenal aspirate, it cannot be so from bile (Gollan, 1975). Hence, it seems clear that biliary Cu is complexed in a form which precludes its reabsorption from the intestine.

The increased biliary Cu content and serum copper oxidase activity in untreated patients with chronic pancreatitis and the reduced or normal biliary Cu content and serum copper oxidase activity in chronic pancreatitis patients on long-term pancreatic supplements suggested to us that pancreatic secretions may normally restrict Cu absorption from the upper intestine (Bell *et al.* 1981; Braganza *et al.* 1981*b*). A study by Abdulla *et al.* (1978) offers some support for this hypothesis: serum Cu concentration increased progressively in the first 10 weeks following pancreatic duct ligation in rats. In unpublished studies we have confirmed the findings of Abdulla *et al.* (1978). We now report that pancreatic secretions do limit Cu absorption from closed duodeno-jejunal loops in anaesthetized rats.

In theory, this effect could have been mediated by any of the constituents of pancreatic juice, including electrolytes (predominately bicarbonate), enzyme proteins, trace elements (notably Zn), or the presence in pancreatic juice of some substance which could reduce Cu to its less soluble cuprous form. Of these the first is clearly inapplicable since net Cu uptake increased with increasing concentration of bicarbonate in the test solution (Table 3). The third also seems unlikely since the small amount of Zn in pancreatic juice (0·35 mmol/l in post-carbachol secretions) is complexed with enzymes (Adler *et al.* 1980). We have no information on the fourth suggestion which, therefore, remains a possibility. The experiments using pancreatic extract suggest that the protein constituents in pancreatic juice can exert an inhibitory effect on Cu absorption with an efficiency equal to bile. In similar experiments in rats, Gollan (1975) found no inhibition of Cu absorption by saliva, gastric juice or an L-histidine solution but significant inhibition by hepatic and gall-bladder bile. Thus, although the magnitude of inhibition of Cu absorption in the experiments with albumin and pancreatic juice was equal, it is unlikely that the inhibition of Cu absorption by pancreatic juice results from non-specific protein binding as one would expect similar inhibition from

the proteins of saliva and gastric juice and a significant negative correlation between the protein concentration of the test solution and Cu uptake and this was not the case ($r - 0{\cdot}3643$, n 5, not significant). Furthermore, the inhibition of Cu absorption by pancreatic juice could not be demonstrated when Cu and pancreatic juice were instilled into the peritoneal cavity, suggesting that a process more specific than simple physical binding was impairing absorption (Braganza *et al.* 1981*a*).

Our studies in closed duodeno-jejunal loops in anaesthetized rats clearly show that pancreatic secretions are capable of inhibiting Cu absorption, as they can inhibit iron absorption (Davies & Biggs, 1965). However, these findings cannot yet be extrapolated to Cu absorption in the intact rat since so many factors interact in normal digestion. We have recently developed a method of studying Cu absorption after an oral meal using ^{64}Cu given orally and intravenously on separate occasions and a computer-derived deconvolution program; using this approach it may be possible to assess the influence of pancreatic secretions on Cu absorption in the intact animal, and in man.

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