Gut, 1975, **16**, 89-92

Influence of exocrine and endocrine pancreatic function on intestinal brush border enzymatic activities[1]

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SUMMARY Digestive enzymatic activities (disaccharidases, alkaline phosphatase, peptide hydrolases) have been determined in the mucosa of 14 patients with chronic pancreatitis. All had an abnormal secretin-pancreozymin test. Four patients had insulin-dependent diabetes mellitus, four a pathological glucose tolerance test. Nine patients had steatorrhoea. Maltase, sucrase, and alkaline phosphatase activity was significantly elevated in patients with exocrine pancreatic insufficiency, whereas those of lactase, trehalase, and peptide hydrolase were normal. Patients with steatorrhoea had higher maltase and sucrase activity than those without steatorrhoea, whereas decreased glucose tolerance had no effect on brush border enzymatic activity. It is suggested that decreased exocrine rather than decreased endocrine pancreatic function is responsible for the increase in intestinal disaccharidase and alkaline phosphatase activity, possibly by the influence of pancreatic enzymes on the turnover of brush border enzymes from the luminal side of the mucosal membrane or by direct hormonal stimulation through cholecystokinin.

An increased digestive and absorptive function has been found in the small intestine of rats with experimentally induced alloxan (Crane, 1961; Hossain, Levinson, and Englert, 1970; Olson and Rogers, 1971; Younoszai and Schedl, 1971) or streptozotocin diabetes (Caspary, Rhein, and Creutzfeldt, 1972; Caspary, 1973). Recently, Cerda, Preiser, and Crane (1972) reported increased disaccharidase activity in small intestinal mucosal biopsies from patients with diabetes mellitus and chronic pancreatic disease. In view of the increased mucosal digestive activity in experimental diabetes the raised disaccharidase activity was attributed to the underlying endocrine pancreatic insufficiency rather than to the decreased exocrine pancreatic function.

Since disaccharidase activity in small bowel biopsies from patients with maturity and juvenile onset diabetes (Chaudhary and Olsen, 1973; Ruppin, Domschke, Domschke, and Classen, 1974; Caspary, Winckler, and Creutzfeldt, 1974) was found to be normal the activity of digestive enzymes was estimated in small bowel biopsies from patients with chronic pancreatitis and an abnormal secretin-pancreozymin test with and without diabetes mellitus.

[1]Some of the results were presented at the 28th meeting of the Deutsche Gesellschaft für Verdauungs- und Stoffwechselkrankheiten, Erlangen, September, 1973.

Received for publication 28 November 1974.

Methods

Multiple small bowel biopsies were obtained after the patients' consent with a hydraulic biopsy tube (Quinton Instruments, Seattle, Washington, USA) under fluoroscopic control beyond the ligament of Treitz after an overnight fast. Biopsies for light microscopy and enzymatic analysis were taken from 10 healthy controls without gastrointestinal disease and normal glucose tolerance, and from 14 patients with chronic pancreatitis. All patients in the latter group had exocrine pancreatic insufficiency proven by an abnormal secretin-pancreozymin test. Detailed data concerning age, glucose tolerance, steatorrhoea, and duration of pancreatic disease are given in table I. Patients requiring insulin treatment had the last insulin injection 24-28 hours before the biopsy specimen was obtained.

Mucosal small bowel biopsies were immediately screened under the stereomicroscope. Part of the

Number of patients	14
Mean age (yr)	39·6 ± 12
Duration of chronic pancreatitis (yr)	2·9 ± 2·49
Diabetes mellitus (insulin treatment)	4/14
Pathological oral glucose tolerance[1]	4/14
Steatorrhoea (> 7 g/24 h)[2]	9/14
Pancreatic calcification	4/14
Abnormal secretin-pancreozymin test[3]	14/14

Mean age of control group 44 ± 14 (n = 0), normal glucose tolerance 10/10.

Table I *Clinical data for patients with chronic pancreatitis and exocrine pancreatic insufficiency*

[1]75 g glucose orally, sum of blood glucose levels at 60 and 120 minutes >300 mg/100 ml (Köbberling and Creutzfeldt, 1970)
[2]Treatment with substitutional pancreatic enzymes performed in 10 of the 14 patients was discontinued two days before intestinal biopsy.
[3]Decreased bicarbonate and enzyme output after stimulation with secretin (1 IU/kg) and pancreozymin (1 Ivy dog units/kg).

tissue was fixed in 4% formol. Paraffin sections were stained with haematoxylin-eosin and PAS. Disaccharidase activity (maltase, sucrase, lactase, trehalase) was measured according to the method of Dahlqvist (1964); alkaline phosphatase was determined with p-phenylphosphate as the substrate at pH 10·5. Peptide hydrolase activity was measured by the method of Fujita, Parsons, and Wojnarowska (1972) adapted for human small bowel biopsy specimens (Caspary, 1974). Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall, (1951) using bovine serum albumin as standard. All enzymatic activities were measured at 37°C in triplicate. Results are expressed as specific activity (μ moles of substrate hydrolyzed/min/mg or g of protein).

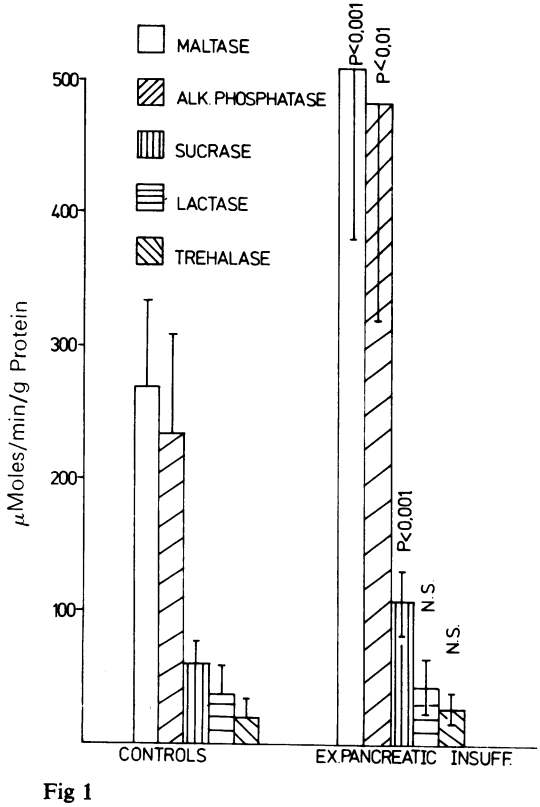

Fig 1

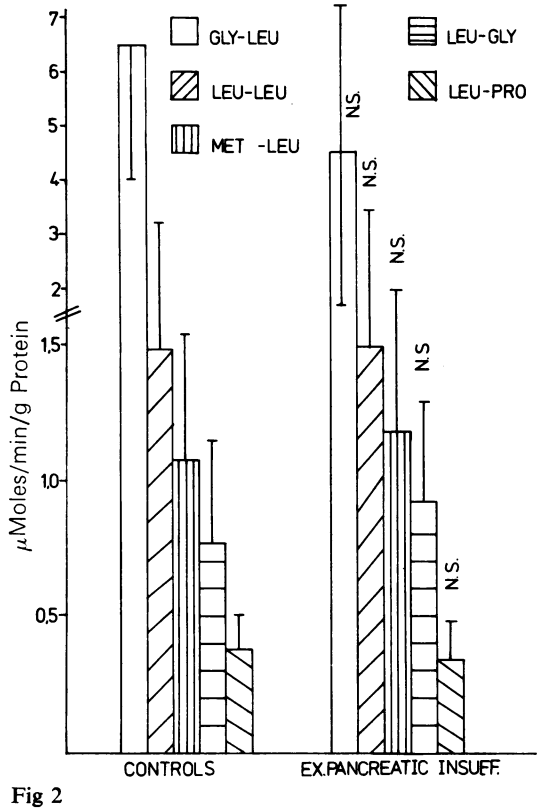

Fig 2

Fig 1 *Brush border enzymatic activities of disaccharidases and alkaline phosphatase in small bowel biopsies of patients with exocrine pancreatic insufficiency and healthy controls.*
Results are means ± STD. Biopsies were taken from 18 healthy controls and 14 patients with exocrine pancreatic insufficiency.

Fig 2 *Peptide hydrolase activity in small bowel biopsies from patients with exocrine pancreatic insufficiency and healthy controls.*
The following substrates were used to measure peptide hydrolase activity: glycyl-leucine (GLY-LEU), leucyl-leucine (LEU-LEU), methionyl-leucine (MET-LEU), leucyl-glycine (LEU-GLY) and leucyl-proline (LEU-PRO). Results are means ± STD.

Enzyme	*Controls* (*n* = 10)	*Exocrine Pancreatic Insufficiency*				
		Total (*n* = 14)	*Diabetic* (*n* = 4)	*Non-diabetic* (*n* = 10)	*Steatorrhoea* (*n* = 9)	*No Steatorrhoea* (*n* = 5)
Maltase	269 ± 65	509 ± 167 (P<0·001)	511 ± 72 (P<0·001)	509 ± 197 (P<0·005)	557 ± 123[2] (P<0·001)	424 ± 56 (P<0·005)
Sucrase	60 ± 18	107 ± 25 (P<0·001)	121 ± 39 (P<0·001)	98 ± 22 (P<0·001)	120 ± 22[2] (P<0·001)	88 ± 16 (P<00·02)
Alkaline phosphatase	235 ± 76	483 ± 243 (P<0·01)	335 ± 63 (P<0·02)	530 ± 301 (P<0·01)	542 ± 223 n.s. (P<0·001)	377 ± 57 (P<0·005)

Table II *Brush border enzyme activity in chronic pancreatic insufficiency*[1]

[1]Results are means ± STD. Specific enzymatic activities are expressed as μmole of substrate hydrolized/min/g of protein.
Significant increases in enzymatic activity in the group with steatorrhoea compared with that without steatorrhoea are indicated as: [2]= P<0·05, [3]=P<0·02, ns = P>0·05.

Statistical analysis was performed by the Student t test. The P values are based on the two-tailed test.

Results

All biopsies showed normal villous structure and light microscopy revealed no signs of mucosal atrophy or inflammation.

Determination of disaccharidase and alkaline phosphatase activity in small intestinal biopsies from patients with exocrine pancreatic insufficiency due to chronic pancreatitis showed that maltase, sucrase, and alkaline phosphatase activity was significantly raised compared with levels in healthy controls (fig 1). Lactase and trehalase were, however, in the normal range. Similarly no significant differences from controls could be observed for peptide hydrolase activity against various substrates (fig 2).

Levels of maltase, sucrase, and alkaline phosphatase were raised independently of the glucose tolerance in patients with exocrine pancreatic insufficiency (table II). Diabetics, as well as patients with normal glucose tolerance, had raised levels of disaccharidase activity within the group of 14 patients with exocrine pancreatic insufficiency. Since all the patients had an abnormal secretin-pancreozymin test, but only nine out of 14 had steatorrhoea, brush border enzymatic activities were compared between patients with and without steatorrhoea. Maltase, sucrase, and alkaline phosphatase activity was significantly elevated in both subgroups compared with controls, but patients with steatorrhoea had significantly higher maltase and sucrase activities than those without steatorrhoea, suggesting that a more severe disturbance of exocrine pancreatic function has a more marked effect on brush border enzymatic activity (table II). However, there was no significant correlation between the reduction of bicarbonate, amylase, and trypsin output during the secretin-pancreozymin test and mucosal enzymatic activity.

Discussion

In agreement with recent observations (Cerda, Preiser, and Crane, 1972; Arvanitakis and Olsen, 1973) an elevation of disaccharidase and alkaline phosphatase activity was observed in the small intestine of patients with chronic pancreatitis. Since only four of 14 patients were diabetic, and diabetics, as well as patients with normal glucose tolerance, showed increased enzymatic activity we assume that exocrine rather than endocrine pancreatic function affects intestinal mucosal enzymatic activity. This corresponds with the findings of normal disaccharidase (Chaudhary and Olsen, 1973; Ruppin *et al*, 1974; Caspary *et al*, 1974) in patients with maturity onset and juvenile diabetes mellitus.

Normal brush border enzyme activity has been observed also in a juvenile diabetic with ketonuria who did not receive any insulin before the small bowel biopsy was obtained (Caspary *et al*, 1974).

The findings of normal disaccharidase activity in human diabetes mellitus are in contrast to the markedly elevated mucosal digestive activity in experimental diabetes (Hossain *et al*, 1970; Olsen and Rogers, 1971; Younoszai and Schedl, 1972; Caspary *et al*, 1972; Caspary, 1973). The reason for this discrepancy is unknown. A possible explanation could be that in experimental diabetes mellitus not only endocrine but also exocrine pancreatic function is impaired. Recent observations from the laboratory of Alpers (1972, 1973) suggest that the turnover of disaccharidases in the brush border membrane is affected by pancreatic enzymes and bile salts from the luminal side of the membrane. Experiments in rats with ligated common bile and pancreatic ducts showed that the absence of bile and pancreatic enzymes leads to an increase in specific enzymatic disaccharidase activity (unpublished observations). The fact that disaccharidases with a high specific activity (maltase, sucrase) were increased in patients

with exocrine pancreatic insufficiency, but brush border enzymes of lower specific activity (lactase, trehalase) were normal might be due to a different turnover rate of these particular enzymes. As in juvenile and maturity onset diabetes mellitus (Caspary *et al*, 1974), peptide hydrolase activity was not increased in exocrine pancreatic insufficiency. This might be due to the predominantly intracellular site of peptide hydrolase activity (Heizer, Kerley, and Isselbacher, 1972) which is not in contact with intraluminal pancreatic enzymes. Also the increased mucosal enzymatic activity may be induced by the intestinal hormone cholecystokinin which has been found to be markedly raised in patients with chronic pancreatitis (Harvey, Hartog, Dowsett, and Read, 1973).

The intravenous administration of cholecystokinin did induce an increase of intraluminal alkaline phosphatase and disaccharidases (Warnes, 1969; Götze, Adelson, Hadorn, Portmann, and Troesch, 1972; Dyck, Bonnett, Lasatter, Stinson, and Hall, 1974). Thus the hormonal effect of cholecystokinin in releasing brush border enzymes does exist, but it is not yet clear whether cholecystokinin exhibits an effect on any specific mucosal disaccharidase activity.

Since hydrolysis and glucose absorption from disaccharides are closely integrated processes at the brush border membrane (Malathi, Ramaswamy, Caspary, and Crane, 1973), it will be of interest to determine whether glucose absorption from disaccharidases is increased in exocrine pancreatic insufficiency.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Ca 71/2). The expert technical assistance of Mrs Balfanz and Mrs Windemuth is much appreciated.

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