

Duodenal endocrine cells in adult coeliac disease

K. SJÖLUND, J. ALUMETS, N.-O. BERG, R. HÅKANSON, AND F. SUNDLER

From the Departments of Internal Medicine, Histology, Pathology, and Pharmacology, University of Lund, Lund, Sweden

SUMMARY Using immunohistochemical techniques we studied duodenal biopsies from 18 patients with coeliac disease and 24 patients with normal duodenal morphology. We had access to antisera against the following gastrointestinal peptides: cholecystinin (CCK), gastric inhibitory peptide (GIP), gastrin-17, glucagon-enteroglucagon, motilin, neurotensin, pancreatic peptide (PP), secretin, somatostatin, substance P, and vasoactive intestinal peptide (VIP). The somatostatin, GIP, CCK, and glucagon cells were increased in number in coeliac disease. The number of motilin cells was slightly increased, while secretin cells were reduced. Cells storing gastrin-17, substance P, or neurotensin were rare in all patients regardless of diagnosis. No PP immunoreactive cells were found and VIP was localised to neurons only. In biopsies from patients having a mucosa with ridging of villi the number of the various endocrine cell types did not differ from that in the control group.

Previous reports have described abnormalities in certain intestinal endocrine cell populations in coeliac disease. Thus Polak and co-workers (1973) found an increased number of secretin immunoreactive cells in children with this disease. Results have also been presented indicating a greater number of enterochromaffin cells (Challacombe and Robertson, 1977) and raised 5-HT concentration (Challacombe *et al.*, 1977) in the duodenal mucosa of such children.

The present report deals with the occurrence and frequency of several peptide hormone-producing cell types in duodenal biopsies taken from adults with coeliac disease. For comparison we examined duodenal biopsies taken from patients with other types of abdominal disturbances but with normal intestinal morphology.

Methods

TISSUE MATERIAL

Biopsies were obtained from the mucosa of the most distal part of the duodenum (at the ligament of Treitz) using a large size Watson capsule. Only those biopsies covering the whole depth of the mucosa were included in the material. Biopsies were taken from 18 patients with coeliac disease (15 females and three males: median age 42.5 years, range 19-60 years) and from 24 patients with other abdominal

disturbances but having a normal mucosal morphology (18 females and six males: median age 33.5 years, range 21-60 years). All patients had been subjected to a thorough gastrointestinal examination including radiography and absorptive function tests. In 21 of the latter patients there were no signs of malabsorption, while three of them had steatorrhoea of unknown aetiology. These patients had a normal exocrine function of the pancreas. Details of the patients with normal intestinal morphology are shown in Table 1. The enteropathy of the patients with coeliac disease was graded histologically and classified according to Alexander (1975) (Table 2). None of the patients received drugs.

IMMUNOHISTOCHEMISTRY

Antisera

Antisera against the following peptides were

Table 1 *Clinical symptoms of patients with normal duodenal morphology*

Symptoms causing examination	Number	Age (yr)	
		Median	Range
Abdominal pain	7	30	27-50
Diarrhoea	7	25	21-60
Steatorrhoea	3	33	33-52
Fatulence	4	35	21-37
Liver dysfunction	3	54	39-56
Total number	24	33	21-60

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Table 2 Classification of enteropathy in patients with coeliac disease according to Alexander (1975)

Enteropathy	Number	Age (yr)		Gluten-free diet (no.)
		Median	Range	
Flat mucosa	10	35	19-60	2
Convuluted mucosa	4	47	41-54	3
Ridging of villi	4	41.5	40-60	4
Total number	18	42.5	19-60	9

available: cholecystokinin (CCK), gastric inhibitory peptide (GIP), gastrin-17, glucagon-enteroglucagon, motilin, neurotensin, pancreatic peptide (PP), secretin, somatostatin, substance P, and vasoactive intestinal peptide (VIP). Details of the antisera are given in Table 3.

Fluorescein isothiocyanate (FITC)-conjugated and unconjugated sheep anti-rabbit-IgG sera were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. Peroxidase-antiperoxidase (PAP) complex was purchased from Cappel Laboratories, Downingtown, Pennsylvania, USA.

TISSUE PROCESSING

The biopsies were frozen at the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then exposed to gaseous diethylpyrocarbonate (DEPC) (Pearse and Polak,

1975) or formaldehyde (Björklund *et al.*, 1972) and embedded in paraffin *in vacuo*. Sections were cut at 5 μ m, deparaffinised in xylene, and carried down to water through graded ethanol solutions. They were then exposed to each of the antisera in the appropriate dilution for three hours at room temperature (immunofluorescence) or for 24 hours at 4°C (PAP staining). The site of antigen-antibody reaction was revealed by fluorescein-labelled anti-rabbit-IgG (diluted 1:20, 30 minutes' incubation) or by unlabelled anti-rabbit-IgG (diluted 1:100, 30 minutes' incubation) followed by incubation for one hour with PAP (diluted 1:320) and five minutes' exposure to 3,3-diaminobenzidine (50 mg/100 ml) (Sternberger, 1974). All solutions used contained 0.25% human serum albumin and 0.25% Triton-X-100. The sections were rinsed and mounted in phosphate buffered glycerine (immunofluorescence) or dehydrated and mounted in Permount (immuno peroxidase) and examined with a fluorescence or light microscope. Controls included sections exposed to antigen-inactivated antiserum (10-100 μ g of the respective peptide/ml diluted antiserum).

CELL AND CRYPT QUANTIFICATION

The sections were cut perpendicular to the mucosal surface. The number of immunoreactive cells was counted in a light microscope or in a fluorescence microscope using $\times 10$ objective and $\times 12.5$ eyepiece

Table 3 Source, dilution and characteristics of the different antisera

Antiserum against	Code	Antigen	Dilution of antisera		Source of antiserum	References
			FITC	PAP		
Cholecystokinin (CCK)		Pure porcine CCK-33	1:10	1:320	W. Schlegel, Dept. of Gynecology Univ. of Münster, Germany	Schlegel and Raptis (1977)
Gastrin-17	L 6	Synthetic human gastrin 1-17	—	1:600	G. Dockray, Dept. of Physiology Univ. of Liverpool, England	Dockray and Taylor (1976)
Gastric inhibitory peptide (GIP)	R-D	Pure porcine GIP	1:80	—	T. O'Dorisio, Div. of Endocrin. and Metabolism, Ohio State Univ., Columbus	O'Dorisio <i>et al.</i> (1976)
Glucagon-enteroglucagon	4304	Porcine pancreatic glucagon	1:20	1:2560	J. Holst, Dept. of Clin. Chem. Bispebjerg Hospital, Copenhagen, Denmark	Holst and Aasted (1974)
Motilin	GP 1103	Synthetic motilin	1:80	1:5120	N. Yanaihara, Dept. of Bioorganic Chemistry, College of Pharmacy, Shizuoka, Japan	Yanaihara <i>et al.</i> (1978)
Neurotensin	HC-8	Synthetic neurotensin	1:40	1:640	R. Carraway, Dept. of Phys. and Lab. of Human Reproduction, Harvard Med. School, Boston, Mass., USA	Carraway and Leeman (1976)
Pancreatic polypeptide (PP)		Pure human PP	1:20	1:80	R. E. Chance Lilly Res. Lab. Eli Lilly, Indianapolis, USA	Larsson <i>et al.</i> (1976)
Secretin	5585	Pure porcine secretin	1:80	1:2560	J. Fahrenkrug and O. B. Schaffalitzky de Muckadell, Dept. Clin. Chem. Bispebjerg Hospital, Copenhagen, Denmark	Fahrenkrug <i>et al.</i> (1976)
Somatostatin	19576	Synthetic ovine somatostatin	—	1:320	M. P. Dubois, Station de Phys. de la Reprod. Inst. Nat. de la Rech. Agronomique, Nouzilly, France	Dubois (1975)
Substance P	K 16	Synthetic substance P	1:20	1:160	G. Nilsson, Dept. of Pharm., Karolinska Inst., Stockholm, Sweden	Nilsson <i>et al.</i> (1975)
Vasoactive intestinal peptide (VIP)	5603	Pure porcine VIP	1:80	1:5000	Same source as secretin antiserum	Fahrenkrug and Schaffalitzky de Muckadell (1977)

(visual field diameter 2.8 mm). Cells were counted in 10 randomly selected visual fields (entire thickness of the mucosa visible) in at least five sections from each biopsy and with each of the antisera. Cell counts are expressed as number of cells per visual field. The number of crypts per visual field was counted and their lengths measured in 12 patients with a normal mucosa and in seven patients with a flat mucosa (at least six sections from each biopsy were examined). Mean values, standard deviations, and statistical significance according to Student's *t* test were calculated.

Results

Cells displaying somatostatin, GIP or CCK immunoreactivity were fairly numerous in all specimens. The number of these cells was almost doubled in the patients having a flat mucosa (Table 4; Figs 1 and 2). In normal mucosa the somatostatin cells and the GIP cells were mainly localised in crypts, while the CCK cells were fairly evenly distributed between villi and crypts (Table 5).

Glucagon-enteroglucagon cells, motilin cells, and secretin cells occurred in moderate number in all

Table 4 Number per visual field of various immunoreactive endocrine cells in coeliacs and in patients with normal duodenal mucosa

Hormone	Mucosa		
	Normal	Flat and convoluted	Flat
Somatostatin	8.1 ± 2.3 n = 24	13.6 ± 6.1* (68%) n = 14	15.3 ± 6.2* (89%) n = 10
GIP	8.2 ± 4.5 n = 22	16.4 ± 9.7† (100%) n = 13	17.5 ± 9.4* (113%) n = 9
CCK	11.1 ± 7.6 n = 24	19.3 ± 11.6† (74%) n = 14	20.1 ± 7.6† (82%) n = 10
Glucagon	4.2 ± 2.6 n = 20	6.2 ± 2.1‡ (48%) n = 10	7.2 ± 1.6† (71%) n = 6
Motilin	3.2 ± 1.8 n = 24	4.7 ± 3.5 NS (47%) n = 14	5.7 ± 3.6‡ (78%) n = 10
Secretin	5.6 ± 2.3 n = 24	3.9 ± 1.4‡ (-30%) n = 14	3.7 ± 1.5‡ (-33%) n = 10

* = P<0.001, † = P<0.02-0.01, ‡ = P<0.05, NS = not significant, Mean ± SD, Percent change in parentheses, n = number of patients.

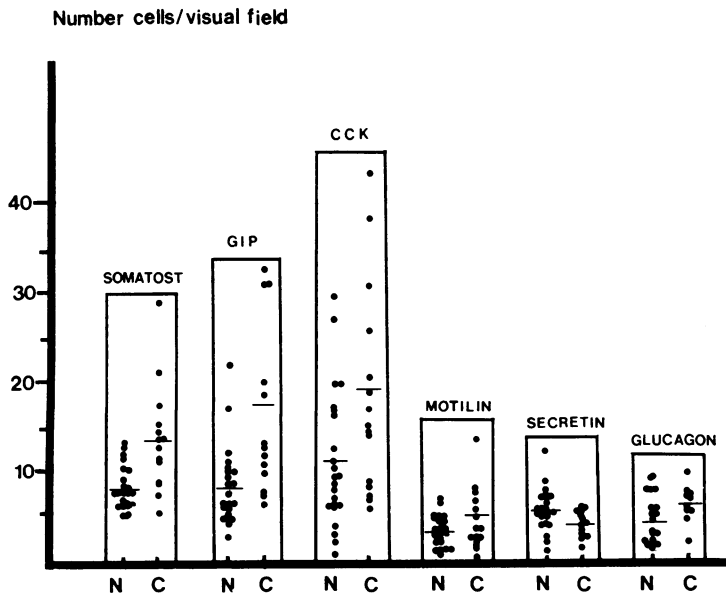


Fig. 1 Number per visual field of various immunoreactive cell types in duodenal mucosa of coeliacs (C) versus patients with normal mucosa (N). Mean values indicated by horizontal lines.

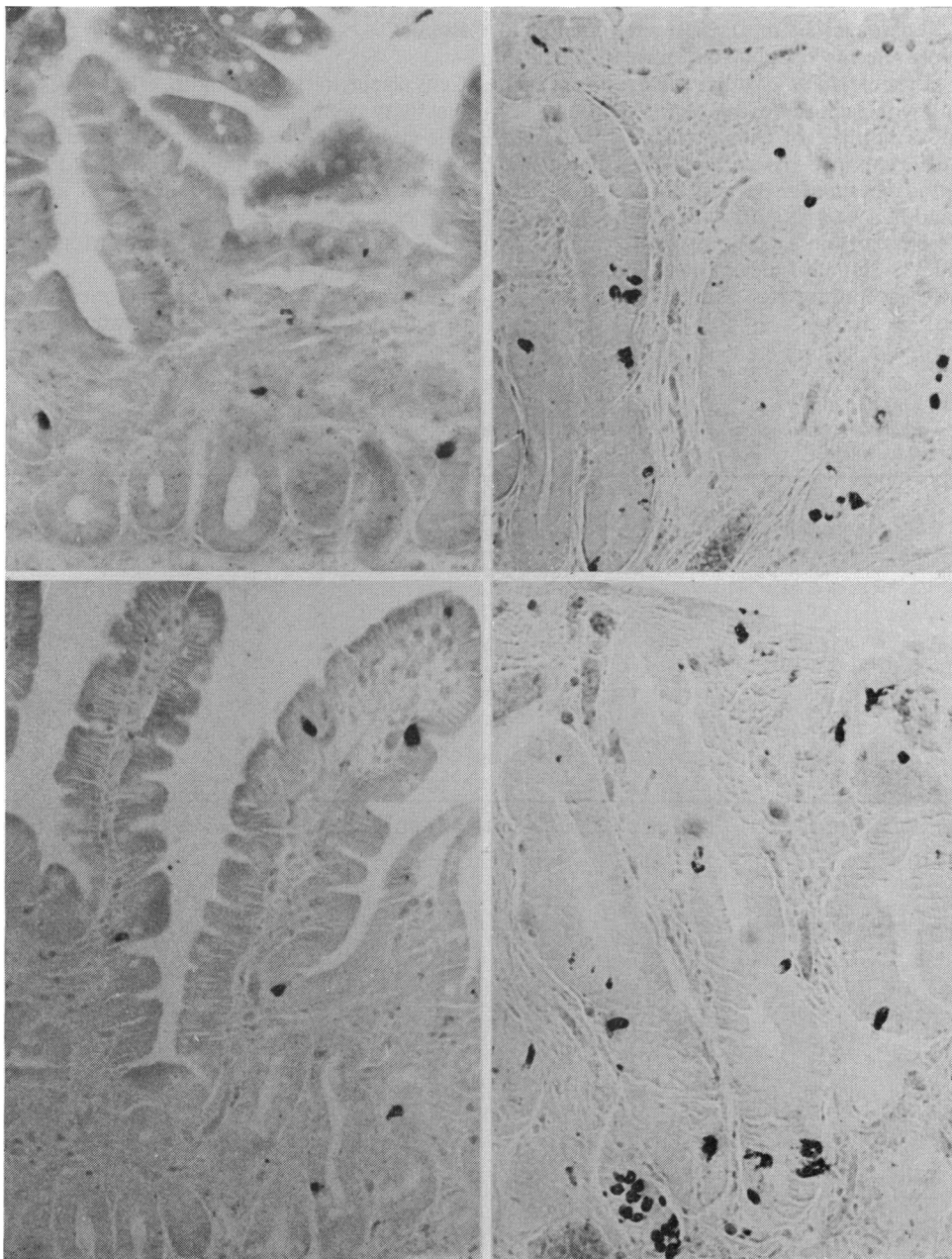


Fig. 2 Sections of duodenal biopsies (DEPC fixation) stained for CCK (above) and somatostatin (below). PAP technique. Left, patient with normal mucosa. Right, coeliac (flat mucosa). $\times 200$.

specimens. The glucagon-enteroglucagon cells characteristically displayed a variable intensity of immunostaining. Their number was slightly increased in coeliac disease (Table 4). In some other mammalian species these cells seem to be identical

with the cells storing GIP immunoreactivity (Alumets *et al.*, 1978). In man, however, only a proportion of the GIP immunoreactive cells displays glucagon-enteroglucagon immunoreactivity.

The number of motilin cells was slightly increased

Table 5 Quantitative topographical distribution of various endocrine cell types in normal mucosa together with actual and expected changes in coeliacs if changes were due to crypt hyperplasia only

Cells storing	Normal mucosa		Flat mucosa	
	Crypt	Villi	Expected	Actual
Somatostatin	+++	+	↑	↑
GIP	+++	+	↑	↑
CCK	++	++	↔	↑
Glucagon	+(+)	+	↑	↑
Motilin	+	+	↔	↑
Secretin	(+)	+	↓	↓

(+) rare, + few, +(+) moderate number, ++ many, +++ numerous, ↑ increased, ↔ unchanged, ↓ reduced.

in patients having a flat mucosa, while the secretin cells were slightly reduced in number (Table 4). In the normal mucosa the latter cells were mainly localised on villi, while the glucagon-enteroglucagon and motilin cells were evenly distributed between villi and crypts (Table 5).

Only very few cells storing immunoreactive gastrin-17, substance P or neurotensin were observed in all patients regardless of diagnosis.

No cells displaying PP or VIP immunoreactivity were seen. VIP immunoreactive nerves were numerous in the lamina propria in all specimens.

The crypts in the flat mucosa were 2.5 to three times longer than in the normal mucosa. Thus the length of the crypts in the flat mucosa was almost the same as the distance from the base of the crypt to the tip of the villi in the normal mucosa. The number of crypts per visual field was the same in the normal and the flat mucosa (7.8 ± 0.4 and 7.8 ± 0.4 , respectively).

Four patients on gluten free diet had a mucosa with ridging of villi. Here, the number of the various endocrine cell types did not differ from that of the control group.

Discussion

Available data including those of the present study suggest that certain intestinal endocrine cell populations are affected in coeliac disease. However, the nature of this change is debatable. We found that in the flat mucosa the somatostatin cells, GIP cells, and CCK cells were markedly increased in number, while the number of secretin cells was slightly reduced. The endocrine cell pattern and the intestinal morphology returned to normal upon withdrawal of gluten (four patients). The present results are in agreement with those obtained in earlier investigations as regards the CCK cells (Polak *et al.*, 1978). Where the secretin cells are concerned our findings are at variance with those of Polak *et al.* (1973, 1978). It should be recalled, however, that our material differs from theirs in that we studied

adults only.

Can the observed change in number of the various endocrine cell types be explained by the altered mucosal morphology with loss of villi and elongation of crypts? In fact, two of the cell types which increase in number in coeliac disease, somatostatin cells and GIP cells, normally predominate in the crypts. Secretin cells which are found mainly on the villi tend to be fewer in coeliac disease. However, CCK cells which are evenly distributed between crypts and villi are greatly increased in number. This finding does not support the view that the altered cell pattern simply reflects an altered mucosal morphology. Table 5 shows the topographical distribution of the various endocrine cell types in normal mucosa together with the actual and expected quantitative changes in coeliacs had the above hypothesis been true.

There are reports claiming that the response of the exocrine pancreas to intraduodenal stimulation with amino acids is reduced in coeliac disease (Di Magno *et al.*, 1969). In addition, a decreased emptying of the gallbladder in response to a fatty meal has been observed (Low-Beer *et al.*, 1971). The response to exogenous secretin and CCK was normal (Di Magno *et al.*, 1969; Low-Beer *et al.*, 1971). This may perhaps suggest reduced blood levels of CCK and secretin in coeliac disease. In fact, immunochemical determinations have shown a lowered plasma secretin response to intraduodenal stimulation with citric acid in coeliac disease (Bloom *et al.*, 1976; Besterman *et al.*, 1978). These findings cannot be explained in terms of altered endocrine cell pattern only. Conceivably, impaired release may contribute to the reduced hormone blood levels and to the impaired physiological response to stimuli.

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