Gut, 1977, **18**, 373-376

Enterochromaffin cells in the duodenal mucosa of children with coeliac disease

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SUMMARY A cell counting technique was used to count enterochromaffin (EC) cells in the duodenal mucosa of 10 children with coeliac disease and 10 controls, and significantly greater numbers of EC cells were found in children with coeliac disease. In four children with a clinical history suggestive of coeliac disease, but with minor histopathological changes in the duodenum, gluten challenge resulted in increased numbers of EC cells. Abnormalities of 5-hydroxytryptamine metabolism in coeliac disease may result from hyperplasia of EC cells in the small intestine.

Increased urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-hydroxytryptamine (5-HT), has been reported in both adults and children with untreated coeliac disease (Haverback and Davidson, 1958; Kowlessar *et al.*, 1958; Haverback *et al.*, 1960; Pimparker *et al.*, 1961; Scriver, 1961; Sleisenger, 1961; Kowlessar *et al.*, 1964; Benson *et al.*, 1964; Challacombe *et al.*, 1972; Challacombe *et al.*, 1975). Clinical recovery after the introduction of a gluten-free diet is accompanied by a fall in urinary 5-HIAA (Sleisenger, 1961; Benson *et al.*, 1964; Kowlessar *et al.*, 1964; Challacombe *et al.*, 1972). Raised blood levels of 5-HT in coeliac disease also return to normal after gluten is withdrawn from the diet (Pimparker *et al.*, 1961). As 5-HT is synthesised by enterochromaffin (EC) cells in the small intestine, hyperplasia and/or hyperactivity of these cells could explain raised levels of both blood 5-HT and urinary 5-HIAA in patients with untreated coeliac disease. To examine this hypothesis we used a morphometric method (Piris and Whitehead, 1975) to count EC cells in samples of duodenal mucosa obtained by peroral biopsy from children with coeliac disease. The findings have been compared with EC cell counts in a group of children with normal duodenal biopsies on light microscopy.

Methods

PATIENTS

COELIAC DISEASE

Peroral duodenal biopsies were performed on 10 children (six males, four females) aged between 5 months and 7 years. Light microscopic examination of the duodenal mucosa showed flattened villi and increased cellular infiltration of the lamina propria. Introduction of a gluten-free diet in these patients resulted in clinical recovery.

CONTROLS

Ten children (seven males, three females) aged between 3 months and 2½ years underwent peroral duodenal biopsies to investigate suspected malabsorption. Morphology of the duodenal mucosa on light microscopy in all patients was within normal limits.

GLUTEN CHALLENGE

Four children (two males, two females) aged between 5 months and 2 years, with a clinical history suggestive of coeliac disease, were submitted to biopsy and initial examination of the duodenal mucosa by light microscopy showed only minor villous abnormalities. After 10 days on an oral gluten challenge of 10 g of gluten powder (Energen Foods Ltd) three times a day, a second biopsy from the same part of the duodenum showed more severe histopathological changes. These were characterised by increased cellular infiltration in the lamina propria and flattening of the villi. Introduction of a gluten-free diet in these patients resulted in clinical recovery.

TECHNIQUES

Small intestinal biopsy

Tissue from the small intestine was obtained by peroral biopsy from the third or fourth part of the

Received for publication 19 November 1976

duodenum under fluoroscopic control, using a Watson adult intestinal biopsy capsule.

Histology

Duodenal tissue was removed from the biopsy capsule, viewed under a Wild M 7 dissecting stereomicroscope, and orientated, mucosal surface uppermost, onto a small square of black card. The tissue was fixed in 10% formol saline for two hours, processed conventionally, and embedded in paraffin wax, using a magnifying lens to ensure that sections were cut perpendicular to the mucosal surface (Risdon and Keeling, 1974). Sections of duodenal mucosa were cut at a thickness of 5 μ and were stained with alkaline diazonium and Schmorl's ferricyanide reaction to demonstrate EC cells (Pearse, 1960). The alkaline diazonium reaction is specific for 5-HT or its condensation product with formaldehyde. Schmorl's reaction is more sensitive but unspecific, and can be taken to demonstrate only 5-HT in EC cells, in the absence of any strong reducing agent, other than 5-HT.

Quantification of EC cells

EC cells were counted using a modification of the method described by Piris and Whitehead (1975). A 1 cm square grid (Graticules, Ltd) was inserted in a $\times 10$ microscope eyepiece and each duodenal section was viewed by light microscopy at a final image magnification of $\times 250$ (Figure). The eyepiece grid contained 81 uniformly distributed points where both horizontal and vertical grid lines crossed. In each field the grid points falling on the epithelium and lamina propria were counted, as were EC cells within the whole 1 cm square grid. By systematically moving the microscope stage and using the muscularis mucosa as a baseline, each section was scanned until EC cells within an area covered by 1000 grid points had been counted. To minimise possible errors due to uneven distribution of EC cells, the whole of any section counted was completed, even if the count of 1000 points was exceeded. Care was taken to avoid recounting the same cells in each section and any two sections on the same slide were taken from levels in the tissue block separated by at least 40 μ. As the number of grid points falling on each section was proportional to the surface area of the tissue (Weibel, 1963), this technique enabled the number of EC cells present to be related to a standard area of mucosa. (Piris and Whitehead, 1975).

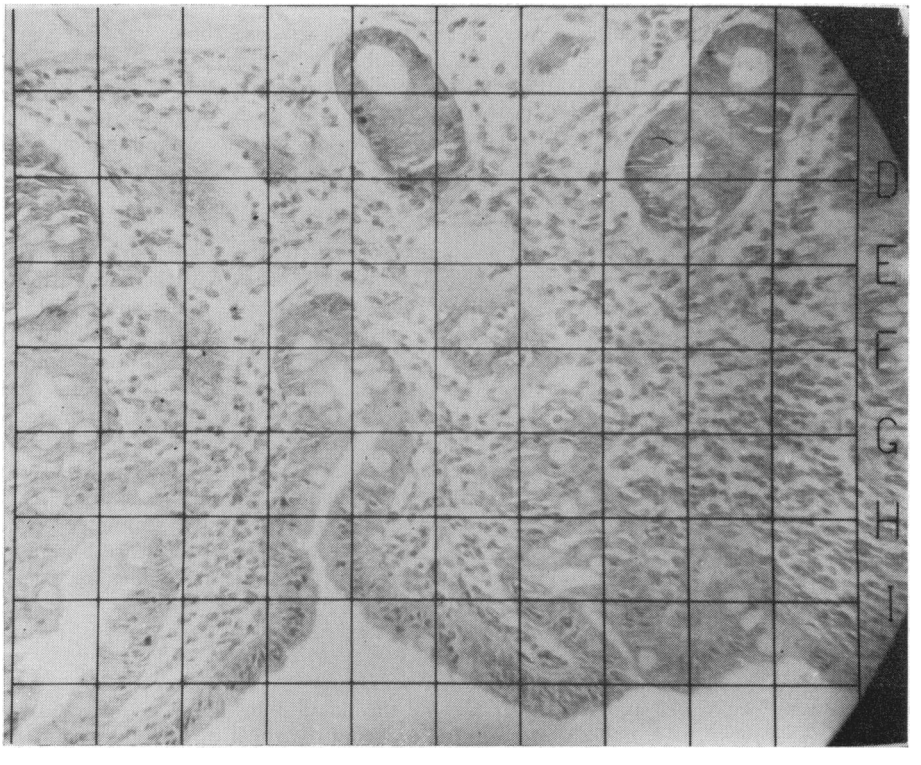

Figure *Enterochromaffin cells in the duodenal mucosa of children with coeliac disease.* $\times$ *250.*

Results

EC cell counts from the three groups of patients studied are shown in the Table. Differences between arithmetical means were examined for significance using Student's *t* test. Mean EC cell counts (Alkaline diazo and Schmorl's) were significantly higher in patients with untreated coeliac disease than in the controls ($P < 0.001$). Mean counts (Alkaline diazo and Schmorl's) in patients with minor villous changes in the duodenum before gluten challenge were not significantly different from the controls ($P > 0.3$). After gluten challenge, the mean count (Alkaline diazo) became significantly higher than the pre-challenge value ($P < 0.01$) and was also significantly greater than the mean count in the controls ($P < 0.01$). The mean EC cell count (Schmorl's) after gluten challenge was higher than the pre-challenge value but the difference was not statistically significant ($P < 0.3$). The mean post-challenge count (Schmorl's) was, however, significantly greater than the mean count in the controls ($P < 0.02$).

Table *EC and counts from three groups of patients*

Patients	*Age (m)*	*EC cells/1000 grid points*	
		Alkaline diazo	*Schmorl's*
Controls			
1	3	15	46
2	3	63	70
3	4	65	92
4	5	26	54
5	10	60	84
6	1 y, 2 m	52	86
7	1 y, 5 m	19	80
8	1 y, 9 m	29	70
9	1 y, 10 m	44	85
10	2 y, 6 m	41	96
Mean		41	76
SD		18	16
Coeliacs			
1	5	82	126
2	5	67	126
3	6	79	80
4	6	90	122
5	9	100	136
6	9	201	324
7	1 y, 2 m	137	264
8	3 y, 9 m	103	139
9	5 y, 9 m	68	93
10	7 y, 10 m	129	259
Mean		105	167
SD		41	83

		Pre-challenge		*Post-challenge*	
		Alkaline diazo	*Schmorl's*	*Alkaline diazo*	*Schmorl's*
Gluten challenge					
1	5	40	104	82	126
2	9	42	102	100	136
3	15	49	84	70	103
4	20	16	41	61	71
Mean		37	83	78	109
SD		14	29	17	29

Discussion

A cell counting technique has been used to count EC cells in sections of the duodenal mucosa from 10 children with coeliac disease and 10 controls, and increased numbers of EC cells were found in patients with coeliac disease. In four children with a clinical history suggestive of coeliac disease but with minor histological abnormalities on light microscopy, an oral gluten challenge significantly increased the numbers of EC cells present and also produced more severe mucosal changes in the duodenum. EC cells are normally found between epithelial cells in the crypts of the small intestine, and the small intestine of patients with coeliac disease characteristically shows crypt cell hyperplasia. Raised EC cell counts in the duodenum of children with coeliac disease may therefore reflect hyperplasia of all crypt cells. Generalised hyperplasia of endocrine cells and specifically of secretin (S) cells has also been reported in the jejunum of patients with coeliac disease (Polak *et al.*, 1973).

5-HT is synthesised and stored by EC cells and further studies will be necessary to determine whether increased synthesis, storage, or release of this amine occurs in coeliac disease. Increased release of 5-HT into the circulation is suggested by both raised blood levels of 5-HT (Pimparker *et al.*, 1961) and increased urinary excretion of its metabolite, 5-HIAA (Haverback and Davidson, 1958). Small doses of 5-HT (10 μg/kg) injected into the rat peritoneum have been shown to accelerate crypt cell proliferation and to shorten cell-cycle time in the jejunal mucosa when compared with control animals (Tutton, 1974). Conversely, partial 5-HT depletion after injection of 6-fluorotryptophan (a tryptophan hydroxylase inhibitor which depletes 5-HT stores in the small intestine) retards crypt cell-cycle time (Tutton, 1974). As shortened cell-cycle times and an expanded proliferative compartment have also been reported in kinetic studies on the duodenal mucosa of adult patients with coeliac disease (Trier and Browning, 1970; Wright *et al.*, 1973a and b), these findings could result from increased local release of 5-HT from increased numbers of EC cells.

We are grateful to Dr E. J. Harries, Dr J. Harkness, and Mr W. G. Tripp for their help with this project, which was supported by the Research Committee of the South West Regional Hospital Board. The study forms part of a thesis being presented by K.R. to the Institute of Medical Laboratory Sciences.

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