Quantification of enterochromaffin cells with serotonin immunoreactivity in the duodenal mucosa in coeliac disease

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SUMMARY Enterochromaffin cells in the duodenal mucosa were stained by using a monoclonal antibody against serotonin immunoreactive sites and an indirect immunoperoxidase technique. A semi-automatic image analysing system showed increased numbers of these cells in patients with untreated coeliac disease compared with a control group. The number of serotonin positive granules in individual enterochromaffin cells also seemed to be increased in patients with coeliac disease, a finding which may be related to the pathogenesis of this disorder.

Serotonin (5-hydroxytryptamine) in the gastrointestinal tract is synthesised from dietary tryptophan, mainly by enterochromaffin cells in the upper small intestinal epithelium. Standard histological techniques or fluorescence histochemistry have shown increased numbers of these cells in patients with untreated coeliac disease. Abnormalities of serotonin metabolism have also been described in coeliac disease and have been attributed to increased synthesis and release of this amine by either increased numbers of enterochromaffin cells or by individual enterochromaffin cells. The development of a monoclonal antibody against serotonin immunoreactive sites has now made an assessment of the serotonin content of individual enterochromaffin cells possible and in the present study this antibody was used with the indirect immunoperoxidase reaction to stain enterochromaffin cells in the duodenal mucosa from patients with untreated coeliac disease and a group of controls. Enterochromaffin cells in previous studies have been counted by point counting, using a graticule inserted in the eyepiece of a light microscope, or by counting cells in randomly selected microscopic fields and relating the counts to a unit length of duodenal mucosa. In the present study the accuracy of counting these cells, which lie on the epithelial cell basement membrane, has been improved by relating the counts to a unit length of this membrane using a semi-automatic image analysing system.

Patients and methods

Coeliac disease. Peroral biopsies were performed on 12 patients (7 boys and 5 girls) aged between 7 months and 16 years with untreated coeliac disease. Light microscopy examination of the duodenal mucosa in these patients showed flattened villi and increased cellular infiltration of the lamina propria. The introduction of a gluten free diet led to clinical recovery and the restoration of normal villous architecture in these patients.

Gluten challenge. Five girls with coeliac disease who had been treated successfully with a gluten free diet for four years were challenged with gluten for three months to determine their continuing need for the diet. Biopsy specimens from all these patients were normal on light microscopy before gluten challenge but all biopsy specimens after challenge showed villous flattening and increased cellular infiltration of the lamina propria compatible with a positive challenge. These patients subsequently responded well to a gluten free diet and were advised to remain on this indefinitely.

Controls. Twelve children (6 boys and 6 girls) aged between 3 months and 5 years who had undergone duodenal biopsies to investigate suspected malabsorption were used as controls. The morphology of the duodenal mucosa on light microscopy in all these patients was considered normal by independent observers.

Small intestinal biopsy. Tissue from the third to fourth part of the duodenum was obtained by peroral biopsy under fluoroscopic control using a Watson paediatric biopsy capsule.
Histology. Specimens of the duodenal mucosa were carefully flattened on a piece of card and were fixed in either 10% buffered formalin (pH 7.2) or in acetified formal sublimate. After routine processing and infiltration with paraffin wax, the tissue was embedded in paraffin wax and sections were cut perpendicular to the mucosal surface at a thickness of 5 μ. The sections were then mounted consecutively, two or three at a time, on serially numbered glass slides. After drying thoroughly, slides representing three different depths in the block were selected to count enterochromaffin cells. All sections from material fixed in buffered formalin were predigested in 0.01% pronase in phosphate buffered saline at pH 7.3 for 10 minutes at 37°C to unmask the antigen, but this was unnecessary for material fixed in acetified formal sublimate. All sections were then stained by an indirect immunocytochemical method to show serotonin immunoreactive sites, using a monoclonal antibody. Non-specific, endogenous immunoreactive activity and non-specific binding were reduced by treating the sections with 0.5% hydrogen peroxide in methanol and normal rabbit serum diluted 1/10 with phosphate buffered saline. The primary monoclonal antibody YC 5/45 (Sera Lab, UK) was diluted 1/600 with phosphate buffered saline and was then applied at room temperature for one hour. After washing for 15 minutes in several changes of phosphate buffered saline the second antibody, rabbit anti-rat IgG horseradish peroxidase conjugated antibody (Miles, UK), was diluted 1/50 with phosphate buffered saline and was applied for half an hour at room temperature. The colour at the sites of serotonin immunoreactivity in the enterochromaffin cells was then developed using the diaminobenzidine reaction. Sections were counterstained with Harris’s haematoxylin followed by a dilute, acetified light green to lightly stain other tissue constituents without obscuring the brown colour of the immunoperoxidase reaction. Using this technique the junction between the epithelial cells and the lamina propria was clearly seen as was the basement membrane. Sections of the normal duodenal mucosa were used as positive controls of immunoreactivity and sections processed in the absence of the primary antibody were used as negative controls.

Quantification of enterochromaffin cells. One section from each slide was randomly selected for morphometric analysis and for cell counting, which was performed by using a semi-automatic image analysing system (Kontron MOP/AMO 2). Sections were viewed with a Leitz Dialux 20 EB light microscope fitted with a teaching side arm. This enabled camera lucida drawings of each section and of the epithelial cell basement membrane to be made on a sheet of white paper at low objective magnification (×6-3). The drawing was then placed on the evaluating tablet of the image analysing

Fig. 1  Enterochromaffin cells in a section of duodenal mucosa in coeliac disease. (Magnification ×240).

Fig. 2  Enterochromaffin cells in a section of duodenal mucosa, from a patient with coeliac disease. (Magnification ×1120).
system and the microprocessor in the system was programmed to measure length. A conversion factor was included in the programme to compensate for the magnification of the drawing. The length of the epithelial cell basement membrane in each drawing, including the basement membrane associated with cross sections of crypts in the lamina propria, was measured by using a cursor connected to the microprocessor. Under higher magnification all positive epithelial enterochromaffin cells in each duodenal section were then counted. The number of enterochromaffin cells in each section from the patients with untreated coeliac disease, from the patients undergoing gluten challenge, and from the controls were counted and expressed per mm length of basement membrane (as enterochromaffin cells lie on this membrane). Morphometric measurements and enterochromaffin cell counts were repeated on three sections from each patient at different depths in the block and means and standard deviations were calculated from these results.

Results

Enterochromaffin cells in the duodenal mucosa from a patient with coeliac disease are shown in Figs. 1 and 2. Enterochromaffin cell counts from patients with coeliac disease and controls are shown in Fig. 3. Counts from patients with coeliac disease undergoing gluten challenge are shown in Fig. 4. Differences between arithmetical means were examined for significance using Student’s t test; mean enterochromaffin cell counts were significantly lower in the patients with untreated coeliac disease than in the controls (P<0.02). Four of five patients who had been treated with a gluten free diet and then challenged with gluten for three months had higher enterochromaffin cell counts before challenge than afterwards, although the differences were not statistically significant (Wilcoxon-White ranking test). It was also observed that the number of serotonin positive granules in individual cells seemed to vary in the controls. In the patients with coeliac disease, however, an even greater degree of variation was present and a larger number of these cells seemed to contain more serotonin positive granules.

Discussion

The highest concentration of enterochromaffin cells in the gastrointestinal tract occurs in the upper small intestine where they are scattered throughout the mucosa, mainly in the lower half of the crypts, and are closely associated with the epithelial cell base-

![Fig. 3 Enterochromaffin cell counts in the duodenal mucosa of patients with coeliac disease and a control group.](image-url)

![Fig. 4 Enterochromaffin cell counts in the duodenal mucosa of patients with coeliac disease undergoing gluten challenge.](image-url)
ment membrane. Serotonin is synthesised by enterochromaffin cells from dietary tryptophan and is stored in the granular cytoplasm of each cell. Increased concentrations of serotonin in blood and duodenal tissue and increased urinary excretion of the major metabolite, 5-hydroxyindoleacetic acid have been reported in patients with untreated coeliac disease and these findings returned to normal after a gluten free diet and with clinical recovery. These metabolic abnormalities may be secondary to the increased number of enterochromaffin cells in the upper small intestinal mucosa that occurs as part of a generalised hyperplasia of crypt cells in untreated coeliac disease. They could also be due, however, to increased synthesis and release of serotonin by individual enterochromaffin cells in a recent cytofluorometric study on the jejunal mucosa of adults with coeliac disease has shown an increase in the median concentration of serotonin per enterochromaffin cell. After the elimination of gluten from the diet in this study1 fluorescence in the enterochromaffin cells returned towards normal, suggesting that gluten was able to cause reversible changes in the ability of these cells to synthesise serotonin. In the present study we have confirmed the finding that enterochromaffin cells are significantly increased in number in the duodenal mucosa of patients with coeliac disease. In addition, a visual assessment of the immunoreactivity of these cells showed that more enterochromaffin cells seemed to contain an increased number of serotonin positive granules in patients with untreated coeliac disease than in the controls. Further studies will be necessary before immunoperoxidase techniques can be used to quantify the presence of antigen in tissue cells, but the results obtained suggest that either an increased synthesis or storage of serotonin, or both may occur in enterochromaffin cells in untreated coeliac disease and may be related to the pathogenesis of this disorder. Small doses of serotonin injected into the rat peritoneum have been shown to accelerate crypt cell production and to shorten cell cycle time in the jejunal mucosa, compared with control animals. Conversely partial serotonin depletion after the injection of 6-fluorotryptophan (a tryptophan hydroxylase inhibitor which depletes serotonin stores in the small intestine) retarded cell cycle time. As a shortened cell cycle time and an expanded proliferative compartment have also been reported in cell kinetic studies on the duodenal mucosa of adults with coeliac disease, the flattened small intestinal mucosa in this disease may be a result of the increased local release of serotonin. An abnormality in either the synthesis or release of serotonin, or both by enterochromaffin cells in coeliac disease may therefore be a factor in the pathogenesis of this disorder. Published family studies suggest that the incidence of coeliac disease is greater among relatives of affected individuals than in the general population; such patients may have an inborn error of serotonin metabolism that may be exacerbated by dietary gluten.

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References

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Editorial committee

J A Davis, G J Ebrahim, J W Scopes, and D G Young have retired from the committee. We are grateful to them for their hard work and loyal help during their term of office.

A G M Campbell, Professor of Child Health, Aberdeen, M L Chiswick, Consultant Neonatologist, Manchester, and L Spitz, Professor of Paediatric Surgery, London, have been elected to the committee.