Studies of intestinal lymphoid tissue. IV—The predictive value of raised mitotic indices among jejunal epithelial lymphocytes in the diagnosis of gluten-sensitive enteropathy

MICHAEL N MARSH

From the University Department of Medicine, Hope Hospital (University of Manchester School of Medicine), Salford, Manchester

SUMMARY It has been established that considerable blast-transformation and mitotic activity occurs among epithelial lymphocytes of untreated coeliac mucosa. This paper is concerned solely with the proliferative activity of epithelial lymphocytes (expressed as percentage "mitotic index") in the prospective diagnosis of coeliac disease, in comparison with other conditions such as lymphoma, Crohn's disease and immunodeficiency which are often associated with malabsorption and flattening of jejunal mucosa. The results demonstrate that a high mitotic index ($>0.2\%$) clearly distinguishes, and hence predicts, gluten-associated enteropathies (including dermatitis herpetiformis and malignant histiocytosis) from others in which gluten plays no aetiological role and where the mitotic index differs insignificantly from normal control mucosae ($\leq 0.2\%$). Furthermore, it has been demonstrated that the mitotic index is raised in so-called "non-responsive coeliacs," thus suggesting that such patients may also be gluten-sensitive despite their subsequent failure to respond morphologically to dietary gluten restriction.

Despite considerable investigation over many years, the pathogenesis of gluten-sensitive enteropathy (GSE) still remains unsolved.^{1 2 3} For clinical purposes, diagnosis requires demonstration of a severe lesion of the upper jejunum that responds to a gluten-free diet (GFD) with restoration of villus architecture;⁴ such criteria permit recognition of most symptomatic individuals.

However, in recent years there has been increasing use of gluten challenge as a further means of confirming the diagnosis⁵ once an initial response to dietary treatment has occurred. This additional procedure demands at least three biopsies from the jejunum and the question arises as to whether such practice is reasonable or justifiable for that purpose alone.

Other difficulties arise in practice regarding confirmation of diagnosis of gluten-sensitive enteropathy. Firstly, it is apparent that "flattening" of the upper intestinal villi cannot be regarded as a specific feature of GSE^{6 7} since similar mucosal changes are now known to occur in approximately

30 other clinical and experimental circumstances.⁸ Secondly, there are certain patients who despite reasonably strict adherence to a gluten-free diet fail to regenerate small intestinal villi.⁹⁻¹¹ To label such patients as having "unresponsive coeliac disease" contradicts accepted diagnostic criteria, while if they are indeed gluten-sensitive, the definition should be appropriately modified and other reasons for lack of mucosal regeneration sought.

Thirdly, the difficulty in distinguishing between "lymphomas" complicating pre-existing coeliac disease^{12 13} and the coeliac-like syndrome resulting from primary lymphomas of intestine^{14 15} highlights another controversial aspect of diagnosis to which there is currently no rational solution, or hence certainty in determining whether GFD should be given, or not. Similar problems are encountered in patients with malabsorption and a "flat" mucosa due to immunodeficiency. Thus, if after gluten restriction further biopsy of the intestine fails to show evidence of villus regeneration, it is then difficult to be sure whether the diet should be continued or withdrawn, especially in the face of continuing ill-health, or diarrhoea.

In these circumstances, it is surprising that no further refinements in diagnostic accuracy, or more discriminatory criteria have been proposed since the earliest definitive descriptions of the "coeliac lesion" were given.^{6,7} It therefore seemed timely to re-evaluate critically the cytological features of the small intestine in GSE¹⁶ and in view of its likely immunological basis¹ to monitor changes in the behaviour of lymphoid cells within the epithelium and lamina propria.

In such an evaluation¹⁷ considerable blast-transformation (8%) and mitotic activity of inter-epithelial space lymphocytes was documented: after gluten withdrawal, the proportion of epithelial immunoblasts decreased to control levels (2%), although their mitotic activity sometimes tended to persist above control levels. These observations suggested that certain epithelial lymphocytes might be sensitised to gluten, their blastogenesis and mitotic activity representing a specific, and likely immunological, marker of gluten-sensitivity in the mucosa of affected individuals¹⁷ and those alone.¹⁸

The purpose of the present study was to widen the scope of previous investigations and to test such conclusions more rigorously by determining mitotic indices of interepithelial space (IEL) lymphocytes in a large and unselected series of disease

controls, in addition to patients with coeliac disease and dermatitis herpetiformis. The results presented here confirm that a high mitotic index among IEL is characteristic of coeliac biopsies alone thereby distinguishing them prospectively from all other "flat" lesions of upper jejunum including primary lymphomas, Crohn's disease and certain forms of immunodeficiency. Furthermore, it was found that the proliferative activity of IEL was high in all patients with coeliac-like syndrome but who failed to respond morphologically after gluten withdrawal. These results suggest that so-called "non-responsive" patients also may be sensitised to gluten, at least in terms of this particular histological marker of mucosal lymphocyte activation.

Material and methods

PATIENTS

Sixty control patients came to intestinal biopsy (Table 1) for various complaints related to the gastrointestinal tract, but gluten-sensitivity was neither suspected, nor ultimately considered to account for their illness. In this group there were three people with "flat" lesions, two being due to jejunal Crohn's inflammation and one possibly to eosinophilic infiltration of the small intestine. No

Table 1 Details of 60 control patients with miscellaneous gastrointestinal disorders

Males (age range 12-78 yr)		Females (age range 16-76 yr)	
Age (yr)	Diagnosis	Age (yr)	Diagnosis
12	Giardiasis	16	Irritable bowel syndrome
17	*Jejunal Crohn's disease	18	Irritable bowel
21	Intestinal pseudo-obstruction	19	Diabetic steatorrhoea
22	Aphthous ulceration	19	Dietary fat intolerance
23	Aphthous ulceration	20	*Crohn's jejunitis
29	Lactose intolerance	24	Aphthous ulceration
35	Alcoholism	23	Irritable bowel
36	Pancreatic steatorrhoea	29	Irritable bowel
36	Peutz-Jehger syndrome	29	Psychological vomiting
40	Lactose intolerance	32	Irritable bowel
40	Lactose intolerance	38	Nodular prurigo
43	Traveller's diarrhoea	38	Irritable bowel syndrome
46	Irritable bowel	40	Folate anaemia
46	Zollinger-Ellison syndrome	40	Laxative abuse
48	Giardiasis	41	Diarrhoea ?drug
48	Post-gastrectomy anaemia	41	Jejunal Crohn's
50	Asucrasia	48	Abdominal pain
50	Post-gastrectomy malnutrition	52	Dietary folate deficiency
53	Diabetes mellitus	53	Post-gastrectomy symptoms
54	Iron deficiency (gastrectomy)	53	Malabsorption ?cause
58	Polya gastrectomy	54	? polyarteritis nodosa
62	Pancreatic neoplasm	57	Post-gastrectomy B ₁₂ deficiency
62	Post-gastrectomy diarrhoea	58	Diarrhoea ?cause
66	Erythrodermia	60	Diarrhoea ?cause
67	Post-gastrectomy malabsorption	61	Crohn's disease
68	Pancreatic neoplasm	62	Alcoholism
72	Whipple's disease	62	Irritable bowel syndrome
73	Hypocalcaemia	62	Whipple's disease
73	Malabsorption	70	Nodular prurigo
78	Dietary folate deficiency	76	* ?Eosinophilic gastroenteritis

*Denotes associated "flat" jejunal mucosa.

additional jejunal biopsies were performed in any of these individuals.

Tables 2 and 3 contain details of further groups of "proximate" disease controls who were shown to have either (a) malignant disease (lymphoma, carcinoma, or "malignant histiocytosis") or (b) various immunological conditions, especially immunodeficiency, affecting intestinal structure or function. Several of these cases have been reported elsewhere.¹⁹⁻²⁴

There were 21 coeliac patients (age range 18-75 yr). Repeat biopsies were not available in seven of these: the remainder had responded morphologically to GFD. Of 13 patients with dermatitis herpetiformis (DH), four had severe enteropathy: they were studied before and after GFD, to which all responded promptly.

All DH patients were referred for gastrointestinal

investigation by physicians from the Hospital for Diseases of the Skin, Manchester. Diagnosis was based on clinical criteria, immunohistological demonstration of IgA deposits in skin, and response to dapsone. Gluten restriction was not undertaken until biopsy of the small intestine had been carried out.

Table 4 documents nine cases of presumed coeliac disease, but in whom GFD was not associated with a significant degree of mucosal regeneration. A total of 144 intestinal specimens, obtained from 131 patients, was reviewed. The majority of these patients were seen in the University Department of Medicine at Hope Hospital between 1975 and 1981.

HISTOLOGICAL PREPARATION

All specimens obtained within the Department were fixed in Dalton's chrome-osmium solution,²⁵ or 2.5%

Table 2 *Disease controls: neoplasia and the small intestine*

Lymphoma	
*M 41:	Epigastric pain ?DU. Proximal jejunal mass at operation ? Hodgkin's. Radiotherapy followed by malabsorption. "Flat" mucosa: GFD ineffective. Died from diffuse undifferentiated lymphoma
M 42:	15 yr diarrhoea and malabsorption. Diffuse lesion of jejunum, probably Crohn's. RIF abscess—perforating well-differentiated lymphoma. Postoperative radiotherapy and chemotherapy. Then radiation enteritis. Died from sepsis
M 48:	Abdominal mass? Hodgkin's: radiotherapy. Ten years later, gross malabsorption, "flat" mucosa and diffuse lymphoma. Extensive chemotherapy. Well six years later
M 53:	Massive oedema—protein losing enteropathy and "flat" mucosa. High protein, low fat and GFD + supplements. Developed colic, worse diarrhoea and weight loss. Jejunum now revealed diffuse undifferentiated lymphoma
M 57:	Two year diarrhoea and weight loss. Macroglobulin (19 S) and "flat" mucosa. Marrow: lymphoma. Full recovery after chemotherapy with 6 kg weight gain. Well five years later
F 68:	Chronic lymphatic leukaemia. Developed diarrhoea. Normal villi, treated symptomatically. Alive and well
Carcinoma	
M 65:	Developed tiredness and weight loss. Stricture of upper jejunum—adenocarcinoma. Normal villi adjacent to lesion. Remains well
"Malignant histiocytosis" of intestine	
M 45:	Gross steatorrhoea, weight loss and abdominal pain. Good response to GFD. Died ten years later after progressive deterioration
†M 51:	Six-month watery diarrhoea, pain and weight loss. "Flat" mucosa. Poor response to GFD: lost to follow-up
† F 56:	Diarrhoea, weight loss and pain. Good response to GFD. Died nine years later from perforated jejunum and peritonitis
† F 56:	Steatorrhoea and oedema. Iron deficiency. Good response to GFD. Relapsed—chemotherapy; now fairly well
† F 60:	Malabsorption and weight loss. Excellent response to GFD. Died from peritonitis
† F 60:	Short history of malabsorption with "flat" mucosa. Responded to GFD but died from perforation and peritonitis
F 64:	Weight loss, diarrhoea and abdominal pain. Remains well after chemotherapy, radiation and GFD
F 64:	Weight loss, diarrhoea and abdominal pain, with "flat" mucosa. Died within three months of diagnosis. No further biopsy

Published case reports: *Reference 19, †References 20 and 21.

Table 3 *Disease controls: "immunological" disorders*

M 5:	CVID. "Flat" mucosa. No response to GFD. Globulin helpful
M 16:	CVID. "Flat" mucosa. No response to GFD. Globulin helpful
F 16:	IgA deficiency. Normal villi: no GI symptoms
M 25:	B cell deficiency (Bruton). "Flat" mucosa: GFD no effect
M 33:	CVID. "Flat" mucosa. GFD ineffective
M 33:	CVID. Shortened villi only
F 43:	IgA deficiency. No symptoms. Mother of above (F 16)
F 40:	CVID. No intestinal symptoms
*M 67:	Severe hypogammaglobulinaemia and responsive coeliac disease. "Flat" mucosa responded to GFD
†M 46:	Alpha chain disease. "Flat" mucosa. No effect from GFD. Died despite extensive chemotherapy
‡M 50:	Hypogammaglobulinaemia, nodular lymphoid hyperplasia and severe recurrent giardiasis
M 51:	Pernicious anaemia and mild hypogammaglobulinaemia
M 43:	CVID. No gastrointestinal symptoms

*Reference 22.

†Reference 23.

‡Reference 24.

Table 4 *Coeliac syndrome, with poor morphological response to GFD*

F 28:	Good clinical response to GFD: disaccharides remain low
F 24:	Childhood anaemia and diarrhoea. Fairly strict GFD. Well
M 38:	Iron deficient as child. Good clinical response to GFD. Has coeliac son
F 42:	Diabetes. Megaloblastic anaemia and diarrhoea. GFD religiously strict
F 43:	Glossitis and diarrhoea. 90% strict GFD. Weight gain 14 kg
F 48:	Myxedema and iron deficiency. Poor GFD control, but weight gain 6 kg
M 57:	Glossitis. Diarrhoea and megaloblastic anaemia. Intermittent diarrhoea persists
F 50:	Anaemia and loose bowels. Symptomatically well
F 67:	Ca jejunum resected two years after good response to GFD. Well 15 yr later. Has responsive coeliac daughter

cacodylate-buffered glutaraldehyde and embedded in Epon or Emit resin, sectioned at 1 μ m thickness on Sorvall MT2-B or Reichert OMU-3 ultramicrotomes and stained with toluidine blue.²⁶ Four or five consecutive 1 μ m sections were applied to each slide; a 10 μ m step from the block was discarded between successive slides. Material obtained from other colleagues, or from patients referred direct to the University Department at Hope Hospital, had invariably been processed by local histopathological laboratories. These latter specimens were fixed in "formalin," embedded in wax, sectioned at approximately 4-7 μ m thickness and stained with haematoxylin and eosin.

DETERMINATION OF MITOTIC INDICES

To determine mitotic indices (MI), sections were viewed under $\times 100$ oil-immersion objective and the number of mitotic figures within a sample of 3000 IEL per specimen was counted and expressed as percentage of total. If there was doubt about a mitotic figure it was ignored; so also were mitotic figures around the mouths and sides of crypt orifices, particularly in "flat" specimens where the possibility of confusion with epithelial cell mitoses in this region is high.²⁷ In practice, and as discussed elsewhere¹⁷ the majority of positive scores was derived from metaphase-anaphase figures; little attempt was made to recognise and include all prophase. Underestimation was preferred, and thus the final data given are minimal values for each specimen studied. That significant results were thereby obtained justifies the cautious approach adopted.

Although many specimens were reviewed retrospectively, the diagnosis, which was not immediately known to the author, was obtained from the clinical file once the MI was determined. Thereafter, no further modification to the result by revision or recounting the specimen in the light of diagnostic information, was ever carried out. Other specimens, especially those obtained from outside the Department, were assessed "blindly"; in this way, some degree of control over the observations, and hence their validity, was achieved.

Results

MORPHOLOGICAL ASPECTS

Selected mitotic figures taken from specimens observed during this study are illustrated in Figs 1 and 2. Mitotic figures were sometimes less easy to identify in the thicker sections of paraffin-embedded material compared with 1 μ m plastic sections. This difficulty was frequently encountered in routine histological preparations of untreated coeliac mucosae where there was considerable crowding, superimposition and overlapping of epithelial cell and lymphocyte nuclear profiles within the depths of each section. Furthermore, it was not always possible to check doubtful mitotic figures in these preparations, whereas with 1 μ m sections, reference to adjacent sections on the same slide (see Methods) permitted "cross-checking" and hence often a more confident decision. Wherever doubt persisted about a particular configuration, it was ignored.

QUANTITATIVE DATA—MITOTIC INDICES

Controls

No mitotic activity was observed in samples of 3000 IEL from each of 45 control specimens (75%) that is, within a total sample of 135 000 epithelial lymphocytes. In 14 of the remaining specimens, less than three mitotic figures per 3000 IEL were observed. The upper limit for the entire control range was 0.15% (Fig. 3).

From Fig. 3, it can also be seen that the range of mitotic activity of IEL in the other "proximate" disease controls, but excluding malignant histiocytosis of the intestine, was similar to the control group described above. It is important to note that within the second control group were patients with malabsorption syndromes associated with "flat" mucosae—for example, proliferative lymphocytic lymphomas, carcinoma, immunodeficiency and jejunal Crohn's disease, whose mitotic indices did not exceed 0.17%.

These observations contrast sharply with the malignant histiocytosis group, where mitotic indices

Fig. 1 (A) Epithelium from "flat" mucosa in Crohn's disease: there are no mitotic figures $\times 640$. (B) Immunodeficiency and gluten-sensitive enteropathy. Mitotic figure (M) in epithelial lymphocyte. $\times 650$. (C, D) Paraffin sections of jejunal mucosa from untreated coeliac patients showing mitotic lymphocytes (M). C $\times 650$, D $\times 700$.

Fig. 2 Mitotic figures (M) are more easily seen in $1\ \mu\text{m}$ plastic sections of untreated coeliac mucosa: (A) depicts prophase ($\times 1200$) and (B) metaphase plate ($\times 1175$). G = goblet cell; isolated arrowheads locate basement membrane.

Fig. 3 The mitotic index (vertical axis) of mucosal specimens in each group of controls and patients is shown in this diagram. Closed symbols for each group represent "flat" mucosae. In "Neoplasia" column, $\triangle$ refers to lymphomas; $\times$ to carcinoma, and $\blacksquare$ to "malignant histiocytosis." Horizontal dotted line marks arbitrary cut-off between all controls (<0.2) and gluten-sensitive patients. The boxed value in the immunodeficiency column represents a case of combined immunodeficiency and gluten-sensitive enteropathy.

were high, except for one patient whose specimen demonstrated marked lymphocyte depletion both within epithelium and lamina propria; it is perhaps highly significant in these circumstances, therefore, that his mitotic index was 0.17%. None of these patients was believed to have taken a GFD before these particular biopsies were obtained.

The other notable exception in this group is the case of immunodeficiency and presumed GSE;²² here the mitotic index among IEL in the initial biopsy on presentation with weight loss and malabsorption was markedly raised (Fig. 3).

Responsive coeliac patients

In this group were 21 untreated patients: the mitotic indices of IEL were markedly raised compared with controls, and ranged from 0.20-0.77%. Similar findings were seen in DH patients with enteropathy and "flat" mucosae. In DH patients without intestinal symptoms and structurally "normal" appearances, mitotic indices lay within the control range.

Non-responsive coeliac syndrome

The remaining data shown in Fig. 3 refer to nine cases of "non-responsive coeliac syndrome." In these patients, in contrast to other disease control

patients with severe malabsorption and "flat" mucosae, mitotic indices among IEL were high (range 0.25-0.47%), and thus identical to those obtained for coeliac patients.

Effect of GFD on mitotic indices

Data are available on four groups of patients treated with GFD: (i) classical coeliac patients, (ii) DH patients with enteropathy, (iii) patients with malignant histiocytosis of intestine, and (iv) the "non-responsive coeliac syndrome" group (Fig. 4). Here the dramatic fall in the mitotic indices of responsive coeliac and DH patients is obvious and striking, despite the preponderance of higher values after treatment compared with the majority of controls. It is also apparent from these data that the value of determining mitotic indices in patients, once gluten has been excluded from their diet, is completely lost.

Fig. 4 This diagram shows the effect of GFD on mitotic index of epithelial lymphocytes (vertical axis) in small intestine. Successive pre- and post-diet values are joined. Significant reductions in mitotic activity occurs in coeliac disease, dermatitis herpetiformis and one case of malignant histiocytosis; the response of the gluten-sensitive immunodeficient patient (stars) is also apparent. The closed circles in the coeliac column are mitotic indices in a group of patients studied after GFD treatment. There is a marked downward trend in indices of "unresponsive" patients, although not all had reached "control" values, when the latest biopsy for this study was obtained.

Of particular interest, however, is the consistent fall in the mitotic indices of IEL in patients with failure of villous regeneration after GFD. It is

clearly of considerable relevance to remember that the only change in these patients was removal of one dietary antigen (gluten) from their intestinal environment.

Discussion

These results confirm previous observations,¹⁷ provide a firm data base relevant to the mitotic activity of IEL in a wide variety of gastrointestinal conditions, and give support to the following conclusions: (i) that IEL in the majority of miscellaneous human control jejunal mucosae show little tendency to divide; (ii) that a similar range of mitotic activity occurs within IEL of disease-control mucosae, including primary intestinal lymphomas, various infections, inflammations, immunodeficiencies and epithelial malignancy; (iii) that in untreated coeliac disease, the mitotic activity of IEL is raised and greatly exceeds that found in various control and disease mucosae; (iv) that after a response to gluten restriction, the mitotic index of IEL falls within the control range, and (v) that a mitotic index of $>0.2\%$ provides a marker which prospectively distinguishes "flat" lesions due to gluten-sensitivity from other disorders, and thus identifies those patients for whom a GFD is rational therapy.

The demonstration in patients with intestinal malabsorption ("idiopathic steatorrhoea") of severe mucosal lesions that recovered after dietary gluten restriction (as invariably occurs in childhood coeliacs^{28 29}) led to the view that they also were suffering from (adult) coeliac disease. In view of the non-specificity of the lesion, however,⁸ the ultimate diagnosis of coeliac disease in adults rests heavily on the demonstration of a mucosal response to gluten restriction, and of mucosal deterioration after a further period of gluten ingestion. Such procedures are necessarily long, drawn-out, retrospective, and require two and often more, jejunal biopsies before the diagnosis can be affirmed. The data provided here in several responsive adult coeliac patients (Fig. 3) clearly indicate that a prospective, and confident, diagnosis can be made when the first biopsy is performed, provided no dietary manipulation takes place beforehand. Furthermore, determination of the mitotic index of epithelial lymphocytes within any mucosal specimen requires no specialised technique or methodology, and can be accomplished within 45-60 min of receipt of prepared sections of mucosa: this is an important practical issue raised by this study.

It is difficult for the histopathologist to determine clearly whether for a given flat mucosa, lymphoma is secondary to pre-existing gluten sensitive enteropathy, or whether primary lymphoma results in a

secondary intestinal lesion and malabsorption syndrome. The data presented here clearly illustrate that mitotic indices of IEL are only raised in gluten-sensitive patients and not in those with lymphomas, despite the proliferative nature of the tumour itself. Such objective criteria are of considerable value in determining whether a GFD should be instituted, or not. This is of considerable advantage to the patients who, despite repeated courses of radiotherapy or chemotherapy with their attendant side-effects, can be spared the additional problem of adapting to, and maintaining, a major change in dietary habit for no good reason.

The nature of the malignancy arising in coeliac mucosae is still not entirely clear, although may be of the histiocytic cell line.^{20 21 30 31} In this regard, it is of considerable interest that the mitotic index of the cases studied were initially high and fell after gluten restriction, thus indicating their gluten sensitivity (similar to responsive coeliacs). Clearly, much further work is needed to categorise these tumours by appropriate immunological and cytological markers. Nevertheless, the relation of such malignancies to adult coeliac disease and hence to the mitotic activity of IEL, should be of considerable relevance in future explorations, and classifications, in this field.

Although the diagnosis of immunodeficiency can easily be established by immunoglobulin quantification, the association of villous "flattening" with malabsorption and diarrhoea often leads to the use of a GFD, albeit as an additional "non-specific" measure. Experience shows that improvement in symptoms, or regeneration of villi rarely, if ever, occurs in such circumstances.³²⁻³⁶ The finding that no mitotic index in the cases studied exceeded 0.2% firmly excluded the coexistence of gluten-sensitive enteropathy, and thus could have predicted from the outset that GFD would be ineffective.

The recently published case of gluten sensitivity associated with severe hypogammaglobulinaemia²² showed clear evidence of symptomatic, biochemical and histological responsiveness to gluten restriction, with subsequent deterioration within one month of recommencing a normal diet (a conventional 20 g "gluten challenge" having failed to elicit his susceptibility). The mitotic indices in the relevant specimens were high at presentation (0.43%), but low after GFD has been given (0.1%) (Fig. 4). These observations are consistent with a gluten-sensitive enteropathy, and contrast sharply with the low mitotic indices observed in the other immunodeficient patients (Fig. 3).

It likewise follows from this case that the blast-transformation and mitotic activity of IEL are not secondary to activation by antibody, or antigen-

antibody complexes, thus adding further support for the view¹⁸ that a state of T cell hypersensitivity exists within the intestinal epithelium of untreated coeliac patients.

Thus far, it has been shown that high mitotic indices ($>0.2\%$) among jejunal IEL *prospectively* identify gluten-sensitive subjects, and secondly, that absence of a high mitotic index ($<0.2\%$), especially in pseudocoeliac lesions secondary to immunodeficiency, lymphoma, Crohn's disease and various other disorders associated with malabsorption or steatorrhoea, excludes gluten-sensitivity and hence the need for a GFD. Such decisions may be made *prospectively* after analysis of the initial small intestinal biopsy.

Finally, consideration is given to those patients who failed to regrow villi and who, by definition, cannot be regarded as coeliac patients. In these patients (Fig. 4) there was a slower fall in mitotic indices of IEL, compared with the responsive group. If, as has been argued, the blast-transformation³⁷ and raised lymphocyte mitoses³⁸ among epithelial lymphocytes reflects activation by luminal antigen, and since only gluten was withdrawn from the diet, what does the ultimate fall in mitotic indices represent in these patients after treatment with GFD? It is often stated, paradoxically, that failure to regenerate villi necessarily reflects poor dietary control;³⁹ it was difficult to exclude this possibility as many of these subjects were not directly under the care of the author. However, two of these "unresponsive" patients were parents of coeliac children who, by virtue of mucosal regeneration in response to gluten restriction, must be regarded as gluten-sensitive. Should these parents, one of which developed carcinoma of the jejunum, not be regarded as coeliacs in view of the known family prevalence⁴⁰ and if not, to what is their disease due? Despite intensive study of intestinal malabsorption over the last 25 years, few other lifelong dietary hypersensitivities closely mimicking (adult) coeliac disease have been described.^{41 42}

While, in ignorance of cause, current definitions of coeliac disease should be retained⁴⁵ the possibility that such criteria are too restrictive must always be considered.⁸ Such sentiments are embodied in previous reports of persons in whom a mucosal response initially occurred after gluten restriction, but in whom that responsiveness was ultimately lost.^{10 43 44} Thus "unresponsive" coeliac patients (excluding obvious diet defaulters) may continue to have flat mucosae for reasons (and mechanisms) other than ingestion of trace amounts of gluten. High mitotic indices in these patients, although obviously not exclusive to gluten sensitivity, are highly suggestive of the nature of the enteropathy, particularly

when evaluated in parallel with the many other various disease-controls described in this study.

Additional case material or histological preparations were kindly provided by Dr Peter Isaacson (Southampton University); Dr Henry Thompson and Dr Gerry Slavin (National Coeliac Lymphoma Panel); Dr E Holmes (Manchester Royal Infirmary); Professor Fred E Pittman (Medical University of South Carolina, Charleston, USA); Dr Roger Gillett (Hope Hospital) and Dr Frank Lee (Royal Victoria Hospital, Blackpool).

This work was supported by grants from the North West Regional Health Authority; the Granada Foundation (Salford); the Smith, Kline & French Foundation; the British Digestive Diseases Foundation, and the Medical Research Council. It was presented in part at the 81st Annual Conference of the American Gastroenterological Association, Salt Lake City, May 1980 and at the Fourth International Congress of Immunology, Paris, July 1980.

References

- Booth CC, Peters TJ, Doe WF. Immunopathology of coeliac disease. In: *Immunology of the gut*. Ciba Foundation Symposium 46. Amsterdam: Elsevier-North Holland, 1977:329-46.
- Strober W. An immunological theory of gluten-sensitive enteropathy. In: McNicholl B, McCarthy CF, Fottrell P, eds. *Perspectives in coeliac disease*. Lancaster: MTP, 1978.
- Marsh MN. The small intestine: mechanisms of local immunity and gluten sensitivity. *Clin Sci Mol Med* 1981; **61**:497-503.
- Booth CC. The enterocyte in coeliac disease. *Br Med J* 1970; **14**:14-7.
- Meuwisse GW. Diagnostic criteria in coeliac disease. *Acta Paediatr Scand* 1970; **59**:461-3.
- Shiner M, Doniach I. Histopathological studies in steatorrhoea. *Gastroenterology* 1960; **38**:419-40.
- Rubin CE, Brandborg L, Phelps P, et al. Studies of coeliac sprue. I—The apparent identical and specific nature of duodenal and proximal jejunal lesion in coeliac disease and idiopathic steatorrhoea. *Gastroenterology* 1960; **38**:28-49.
- Marsh MN. Studies of intestinal lymphoid tissue. V—The cytology and electron microscopy of gluten-sensitive enteropathy, with particular reference to its immunopathology. *Scand J Gastroenterol* 1981; **16**, suppl 70: 87-106.
- Pink I, Creamer B. Response to a gluten-free diet of patients with coeliac syndrome. *Lancet* 1967; **i**:300-4.
- Neale G. A case of adult coeliac disease resistant to treatment. *Br Med J* 1968; **ii**:678-84.
- Barry RE, Morris JS, Read AE. A case of small-intestinal mucosal atrophy. *Gut* 1970; **11**:743-7.
- Gough KR, Read AE, Naish J. Intestinal reticulosis as a complication of steatorrhoea. *Gut* 1962; **3**:232-9.
- Austad W, Cornes JS, Gough KR, et al. Steatorrhoea and malignant lymphoma. The relationship of tumours of lymphoid tissue and coeliac disease. *Am J Dig Dis* 1967; **12**:475-90.

- ¹⁴ Eidelman S, Parkins RA, Rubin CE. Abdominal lymphoma presenting as malabsorption. *Medicine* 1966;**45**: 111-37.
- ¹⁵ Novis B, Bank S, Marks I. Abdominal lymphoma presenting as malabsorption. *Q J Med* 1971;**40**:521-40.
- ¹⁶ Marsh MN. The morphology and cellular kinetics of epithelial lymphocytes in coeliac disease—a reappraisal of the jejunal lesion. *Gut* 1979;**20**:A909.
- ¹⁷ Marsh MN. Studies of intestinal lymphoid tissue. III—Quantitative analyses of epithelial lymphocytes in the small intestine of human control subjects and of patients with celiac sprue. *Gastroenterology* 1980a;**79**:481-92.
- ¹⁸ Marsh MN. Abnormal behaviour of epithelial lymphocytes in sprue. *Gastroenterology* 1980b;**78**:1218.
- ¹⁹ Williams EI. Hodgkin's disease arising primarily in the jejunum. *Br Med J* 1962;**i**:158.
- ²⁰ Isaacson P, Wright DH. Malignant histiocytosis of the intestine: its relationship to malabsorption and ulcerative jejunitis. *Hum Pathol* 1978;**9**:661-77.
- ²¹ Hodges J, Isaacson P, Smith CL, *et al.* Histiocytosis of the intestine. *Dig Dis Sci* 1979;**24**:631-8.
- ²² Webster ADB, Slavin G, Shiner M, *et al.* Coeliac disease with severe hypogammaglobulinaemia. *Gut* 1981;**22**: 153-7.
- ²³ Pittman FE, Tripathy K, Isober T, *et al.* IgA heavy chain disease. *Am J Med* 1975;**58**:424-30.
- ²⁴ Williams SE. An unusual inflammatory lesion of the jejunum in a patient with late onset immunoglobulin deficiency. *Postgrad Med J* 1980;**56**:128-32.
- ²⁵ Dalton AJ. A chrome-osmium fixative for electron microscopy. *Anat Rec* 1955;**121**:181.
- ²⁶ Trump BF, Smuckler EA, Benditt EP. A method for staining epoxy sections for light microscopy. *J Ultrastruct Res* 1961;**5**:343-8.
- ²⁷ Wright NA, Watson AJ. Morphology and cell kinetics of the jejunal mucosa in untreated patients. *Clin Gastroenterol* 1974;**3**:11-31.
- ²⁸ Anderson CM. Histological changes in the duodenal mucosa in coeliac disease. Reversibility during treatment with a wheat gluten-free diet. *Arch Dis Child* 1960;**35**:419-27.
- ²⁹ Cameron AH, Astley R, Hallowell M, *et al.* Duodeno-jejunal biopsy in the investigation of children with coeliac disease. *Q J Med* 1962;**31**:125-40.
- ³⁰ Whitehead R. Primary lymphadenopathy complicating idiopathic steatorrhoea. *Gut* 1968;**9**:569-75.
- ³¹ Holmes GKT, Cooper BT, Cooke WT. Malignant lymphoma in coeliac disease. In: McNicholl B, McCarthy CF, Fottrell P, eds. *Perspectives in coeliac disease*. Lancaster: MTP, 1978.
- ³² Johnson RL, van Arsdel P, Tobe A, *et al.* Adult hypogammaglobulinaemia with malabsorption and iron deficiency anaemia. *Am J Med* 1967;**43**:935-43.
- ³³ Hoskins LC, Winawer SJ, Broitman SA, *et al.* Clinical giardiasis and intestinal malabsorption. *Gastroenterology* 1967;**53**:265-79.
- ³⁴ Ament ME, Rubin CE. Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes. *Gastroenterology* 1972;**62**:216-26.
- ³⁵ Ament ME, Oochs HD, Davis SD. Structure and function of the gastrointestinal tract in primary immunodeficiency syndromes. *Medicine* 1973;**52**:227-48.
- ³⁶ Hermans PE, Diaz-Buxo JA, Stobo JD. Idiopathic late-onset immunoglobulin deficiency: clinical observations in fifty patients. *Am J Med* 1976;**61**:221-37.
- ³⁷ Marsh MN. Studies of intestinal lymphoid tissue. I—Electron microscopic evidence of “blast-transformation” in epithelial lymphocytes of mouse small intestinal mucosa. *Gut* 1975;**16**:665-74.
- ³⁸ Marsh MN. Studies of intestinal lymphoid tissue. II—Aspects of proliferation and migration of epithelial lymphocytes in murine small intestine. *Gut* 1975;**16**: 674-82.
- ³⁹ Cooke WT, Asquith P. The diagnosis [in coeliac disease]. *Clin Gastroenterol* 1974;**3**:3-10.
- ⁴⁰ McCrae WM. The inheritance of coeliac disease. In: Booth CC, Dowling RH, eds. *Coeliac disease*. London: Churchill-Livingstone, 1970.
- ⁴¹ Ament ME, Rubin CE. Soy protein—another cause of the flat intestinal lesion. *Gastroenterology* 1972;**62**: 227-34.
- ⁴² Baker AL, Rosenberg IH. Refractory sprue: recovery after removal of non-gluten dietary proteins. *Ann Intern Med* 1978;**89**:505-8.
- ⁴³ Dowling RH, Henry K. Non-responsive coeliac disease. *Br Med J* 1972;**iii**:624-31.
- ⁴⁴ Doe WF, Evans D, Hobbs JR, *et al.* Coeliac disease, vasculitis, and cryoglobulinaemia. *Gut* 1972;**13**:112-23.

Requests for reprints to: Dr Michael N Marsh, University Department of Medicine, Hope Hospital (University of Manchester School of Medicine), Eccles Old Road, Salford, Manchester, England.