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A pilot study of fluticasone propionate in untreated coeliac disease

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Abstract

Although gluten withdrawal is likely to remain the mainstay of treatment for adult coeliac disease, many patients find the diet inconvenient and unpalatable and compliance among asymptomatic patients is often poor. Oral corticosteroids have been used for patients who seem to be resistant to gluten withdrawal but preparations with low systemic bioavailability might be preferable. We have given a new glucocorticoid (fluticasone propionate) to 12 adults with untreated coeliac disease for six weeks while they were on a normal diet. One patient defaulted and one suffered a relapse in a pre-existing neoplasm. Excluding these, there was an improvement of symptoms, a mean weight gain of 2 kg, and a rise in albumin of 5·4 g/l. There was a significant improvement in the lactulose/mannitol excretion ratio ($p<0·05$) and in all histological variables examined in paired biopsy specimens (surface and crypt intraepithelial lymphocyte/enterocyte and goblet cell/enterocyte ratios and enterocyte height, $p<0·01$ or better). In six paired specimens sucrase and alkaline phosphatase activity increased in all ($p<0·05$) and lactase in five of six. No appreciable side effects were observed, but two patients had suppressed cortisol values and synacthen responses at six weeks. A further three, with normal pretrial results, had a blunted tetracosactrin response at six weeks. Fluticasone propionate seems worthy of further assessment in the treatment of coeliac disease as an adjunct to gluten withdrawal.

Coeliac disease is a condition where exposure to gluten produces abnormalities in the upper small bowel that lead to malabsorption. Removal of gluten from the diet leads to improvement in most cases but necessitates life long compliance with a socially inconvenient and unpalatable diet. Many patients are non-compliant and thus show poor histological healing though symptoms may be tolerably well controlled. In a proportion of patients the removal of gluten is not sufficient to produce clinical resolution and in these, in particular, corticosteroids have been used with improvements both in clinical state[1-3] and in jejunal morphology,[4] though the benefits do not outlive the period of treatment. Adrenal suppression has been a major worry and might be avoidable if a topically active but non-absorbed corticosteroid could be shown to be effective. Bramble *et al* used clobetasone butyrate or betamethasone valerate over four months and showed biochemical and histological improvements in 10 untreated coeliac patients on a normal diet but eight of 10 developed suppression of the pituitary-adrenal axis and the authors concluded that these agents offered no benefit over prednisolone.[5]

Fluticasone propionate is a new corticosteroid with poor systemic absorption after an oral dose and a high first pass metabolism leading to low bioavailability. Repeated oral doses of up to 20 mg/day in volunteers have not been associated with low cortisol values, neither have these been a problem in clinical trials in patients with ulcerative colitis. We have therefore undertaken an open trial of fluticasone propionate in untreated coeliac disease, looking for objective functional and histological improvement in the small bowel and for evidence of adrenal suppression.

Methods

Patients with untreated coeliac disease, of either sex and over 18 years of age, were considered. A firm diagnosis of coeliac disease including a duodenal or jejunal biopsy was required. Patients with contraindications to corticosteroids, lactating mothers, or those with evidence of other small bowel disease associated with coeliac disease (such as lymphoma) were excluded and women of childbearing age were only included if using adequate contraception.

PRELIMINARY INVESTIGATIONS

Baseline symptoms were assessed, particularly gastrointestinal symptoms of weight loss, anorexia, nausea, bowel frequency and consistency, and abdominal discomfort. Physical examination and routine urine analysis were performed. Full blood count, red cell and serum folate and serum iron (except in those patients on haematinic drugs), urea and electrolytes. glucose, liver function tests (bilirubin, alanine transaminase and alkaline phosphatase), albumin, protein, immunoglobulin, and gliadin antibodies were measured. A short tetracosactrin test was performed.

A differential sugar absorption study was undertaken using lactulose and mannitol as probes: after an overnight fast the subjects drank a test solution of 5 g lactulose and 2 g mannitol in 100 ml of tapwater containing 10·3 g glycerol to achieve a hypertonic solution, osmolarity 1500 mol/l. They continued to fast until the end of the first urine collection. Urine was collected in two consecutive five hour periods and stored in containers holding 0·2 ml sodium methiolate. The total volume was recorded and 20 ml aliquots stored at −20°C. Analysis was by gas-liquid chromatography as previously described[6,7] except that for mannitol methoxime derivatives were formed before trimethylsilylation.

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Endoscopic duodenal biopsy specimens were obtained using a large channel endoscope (3·7 mm channel). Some of the tissue was fixed in formalin for histological assessment, processed in paraffin wax, sectioned at 5 μm intervals, and stained with haematoxylin and eosin and with alcian blue/periodic acid Schiff. Intraepithelial lymphocytes were counted in the villous epithelium or superficial flat epithelium and also in the crypt epithelium. At least 500 enterocytes were counted in each case in sections performed at multiple levels to provide intraepithelial lymphocyte/enterocyte ratios for surface and crypt epithelium. Enterocyte height was measured according to Howdle *et al*[8] and de Ritis *et al*[9]. At least 50 epithelial cells with basal centrally located nuclei were measured using multiple sections at different levels. Cells were chosen at random throughout the epithelium of the villi or superficial flat epithelium. A crossed micrometer eye piece graticule was used for this purpose. Goblet cells in the crypt epithelium were counted and the ratio of goblet cells to enterocytes obtained by counting a minimum of 500 enterocytes. The biopsy specimens were assessed blind.

Intestinal enzyme activities were measured by taking a fresh duodenal specimen and homogenising this in 1 ml of normal saline in a loose fitting Potter-Elvehjem glass homogeniser. The specimen was then frozen until used. Before analysis of protein content,[10] lactase,[11] sucrase,[11] and alkaline phosphatase[12] activities, specimens were defrosted and sonicated three times at 0°C for 10 seconds. The results are expressed as IU/g protein. Biopsy specimens were also obtained from 12 patients having endoscopies for other reasons and in whom routine duodenal histology was normal.

The patients were issued with a diary card to record compliance and stool frequency and consistency. They received fluticasone propionate 5 mg four times a day for six weeks (probably equivalent to 40 mg prednisolone a day). After three weeks they were reviewed for adverse events and the following checked for safety: urine analysis and 9 am cortisol, urea, electrolyte, and glucose concentrations.

POSTTREATMENT INVESTIGATIONS

At six weeks all the preliminary assessments were repeated, full dietary advice on a gluten free diet given, and continuing follow up arranged.

STATISTICAL METHODS

All paired results have been compared using the Wilcoxon signed rank test except for the differential sugar absorption data where, after $\log_{10}$ transformation, Student's t test was used. All p values are two tailed.

This study was performed in accordance with the Declaration of Helsinki and had ethical committee approval. We had thought that it would be desirable to perform insulin stress tests to assess adrenal reserve but permission was not given for this.

Results

Twelve patients were entered into the study, and 10 completed it with good compliance. One patient had a relapse in a pre-existing Hodgkin's lymphoma, necessitating withdrawal, and remained severely symptomatic throughout. Another, with longstanding, mildly symptomatic coeliac disease and known to be entirely non-compliant with dietary measures, defaulted. Except where stated, these patients have not been included in paired assessments of initial and end of treatment data. The Table gives details of the patients.

CLINICAL EVENTS

Initial clinical assessments are shown in the Table. At six weeks seven patients were assessed

Patient details

		Before treatment			*After treatment*			
							Cortisol+tetracosactrin response at 6 weeks (nmol/l)	
Case No	*Age (years)*	*Duration of disease**	*Reason for diagnosis*	*Clinical rating of severity*	*Weight change (kg)*	*Albumin change (g/l)*	*Baseline*	*30 min after tetracosactrin*
1	24	2·5 yr	Diarrhoea, anaemia weight loss	Moderate	+1	+5	<30	99
2	54	4 mth	Diarrhoea, anaemia weight loss	Severe	–	–	‡	–
3	42	2·5 yr	Diarrhoea weight loss	Mild	+4·4	+9	275	733
4	44	33 yr	Steatorrhoea	Moderate	+2	+2	396	519
5	42	6 mth	Anaemia	Moderate	0	+1	138	365
6	51	3 yr	Dermatitis herpetiformis	Mild	+0·5	+3	261	699
7	22	2 mth	Diarrhoea weight loss	Mild	+15	+14	<30‡	<30
8	56	55 yr	Failure to thrive†	Mild	−0·3	+3	524	690
9	35	32 yr	Diarrhoea weight loss	Mild	–	–	–	–
10	66	>30 yr	Anaemia	Moderate	−0·2	−1	338‡	466
11	67	20 yr	Anaemia	Moderate	+0·5	+3	289‡	598
12	68	3 mth	Diarrhoea, anaemia weight loss	Moderate	+2	+15	261‡	465

*Estimated from history; †diagnosed in childhood.
Normal tetracosactrin test: baseline (9 am) >140 nmol/l, 30 minute increment >190 nmol/l, 30 minute value >500 nmol/l.
‡Cortisol response before treatment already abnormal.
Case no 2 and 9 did not complete the trial period.

as in remission and three as having mild symptoms with an improvement having been noticed in bowel frequency (3/3 initially abnormal), stool consistency (6/6 initially abnormal) and weight (mean weight gain 2 kg, $p<0.05$).

ADVERSE EVENTS

There were no major adverse events that could be ascribed to the treatment. One person complained of retrosternal discomfort, another of heartburn, and one of an increase in acne: all could have been due to steroid treatment. For other minor events reported any connection with treatment seemed unlikely. No one developed cushingoid features.

HAEMATOLOGY AND BIOCHEMISTRY

There was a mean rise of 6 g/l in the haemoglobin concentration in the nine patients with paired results (not significant) and of 5·4 g/l in the albumin concentration ($p<0.01$). Liver function tests were initially abnormal in six patients: in five the abnormality was a modest rise in alkaline phosphatase activity (liver *v* bone source not determined) and one had an abnormal alanine aminotransferase activity (70 IU/l, normal <46). After six weeks all patients still had raised alkaline phosphatase activity but the transaminase value was normal. One of the patients with a raised initial alkaline phosphatase activity was the patient with Hodgkin's disease: his liver function tests deteriorated until chemotherapy was introduced and the abnormal tests were almost certainly due to the lymphoma rather than the coeliac disease. Urea, electrolyte, and glucose concentrations were normal in all, and urine analysis showed only slight abnormalities in two of the patients on their first visit only.

Morning cortisol concentrations were initially normal in all. The criteria for a normal tetracosactrin test are shown in the Table, and by these strict criteria five patients failed to show an adequate increment in cortisol after tetracosactrin and one of these also failed to reach the 30 minute minimum concentration of 500 nmol/l. At the end of six weeks of treatment this patient and one other had low morning cortisol concentrations and a very poor response to tetracosactrin, and in a further five patients the response was blunted: two of these were patients whose pretreatment tetracosactrin responses had been abnormal.

IMMUNOLOGY

IgG concentrations were normal throughout, IgM was slightly raised in the one patient who later defaulted (2·72 g/l, normal range 0·75–2 g/l) and IgA in one patient was low but became normal (0·22 g/l, normal 0·65–3 g/l). All but one patient had a positive gliadin antibody initially.

DIFFERENTIAL SUGAR ABSORPTION TEST

This test was performed twice in the 10 patients who finished the study, but two patients, on the second occasion, failed to pass any urine in the first five hours. The mean (SE) lactulose excretion on the first test was 66·2 (14·4) mg/vol (n=10) and on the second test 60·9 (18·7) mg/vol (n=8) (not significant, normal range 5–31 mg/vol). Values for mannitol excretion were 128·8 (34·7) mg/vol (n=10) and 259·9 (73·4) mg/vol (n=8) ($p=0.05$, normal range 350–590 mg/vol). Thus, overall, the improvement in the lactulose/mannitol ratio (Fig 1, $p<0.05$) occurred because of increased mannitol absorption. In one case the ratio fell to within the normal range. The first five hour collection of the test is thought to be the most useful part, the second half being more likely to be disturbed by the breaking of the fast and by colonic metabolism of the sugars. In both the cases where the first collection was missing, after pooling the results for urine obtained through the whole 10 hour period and comparing the first and second tests there was a pronounced improvement after treatment: the second lactulose/mannitol ratios being approximately 25% of the first.

HISTOLOGICAL ASSESSMENT

There was a significant reduction in the lymphocytic infiltrate in the 10 paired biopsy specimens (Figs 2, 3): in all cases the intraepithelial lymphocytes, as a ratio of the surface enterocyte count, fell ($p=0.002$), and in comparison with the crypt enterocyte count the intraepithelial lymphocytes fell in all but two cases ($p<0.02$). Enterocyte height improved in nine of 10 ($p<0.01$) and the ratio of goblet cells to enterocytes increased in all cases ($p=0.002$).

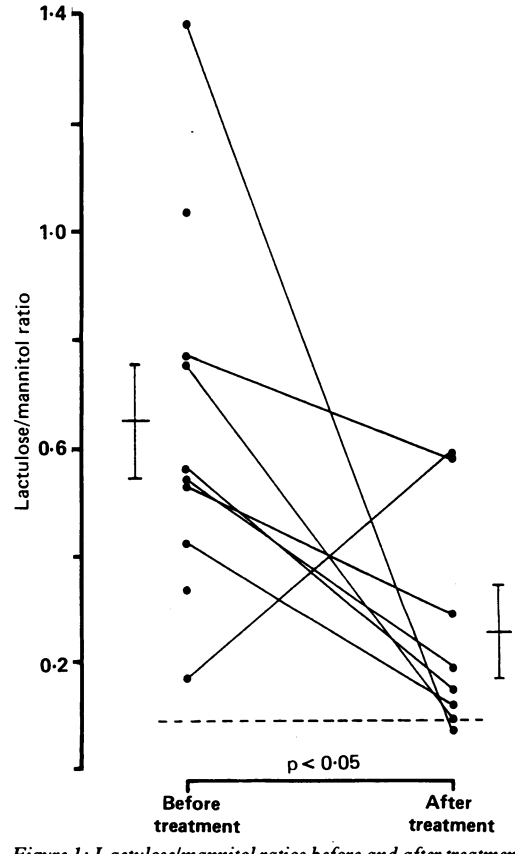

Figure 1: Lactulose/mannitol ratios before and after treatment for the 10 patients who finished the trial. Mean (SEM) and upper limit of normal range shown.

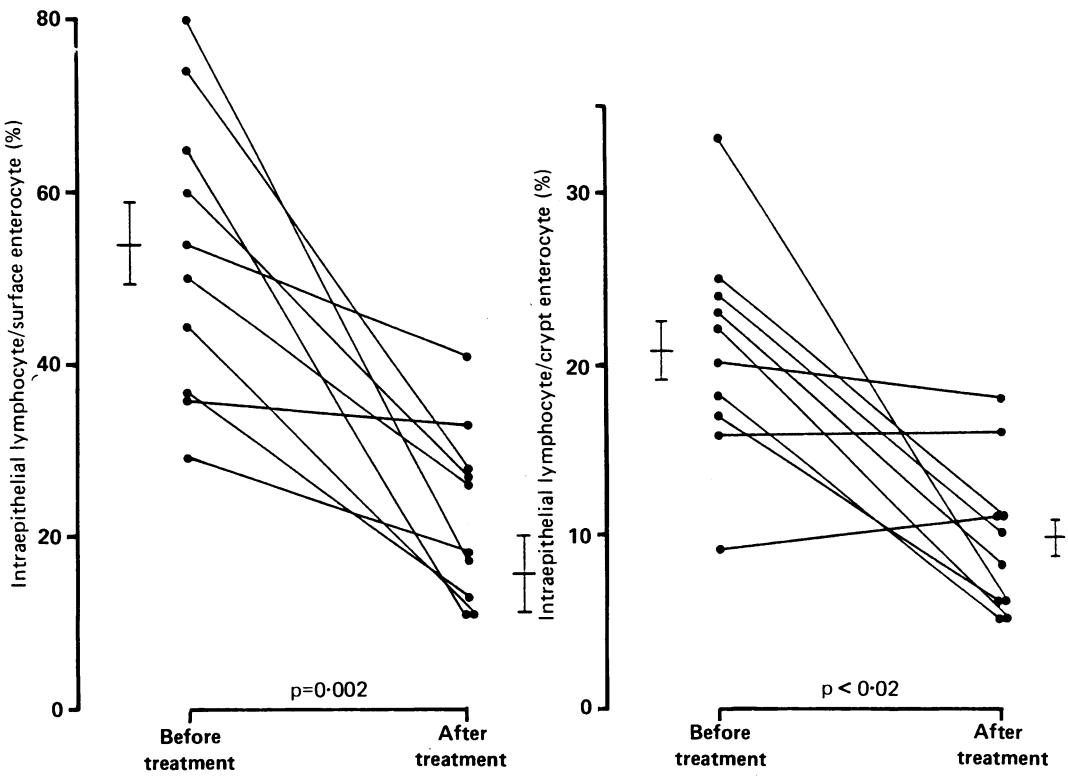

Figure 2: Intraepithelial lymphocyte/enterocyte percentage for surface and crypt epithelium before and after treatment in the 10 patients who finished the trial. Mean (SEM) shown.

INTESTINAL ENZYME ACTIVITIES

Biopsy specimens from 12 normal patients gave the following enzyme activities: alkaline phosphatase: mean (SE) 955 (103) IU/g, range 325–1520; lactase: 52·7 (6·5) IU/g, range 23–105; sucrase: 103·6 (11·8), range 46–179. Six paired samples from trial patients were available for comparison (Fig 4). In no case did pretreatment values fall within the range of the normal biopsy specimens ($p<0·001$). There was a mean improvement in all three enzyme activities measured and with respect to alkaline phosphatase and sucrase the activity increased in every case after treatment ($p<0·05$). The lactase activity rose in five of six cases. Four of the posttreatment results for alkaline phosphatase were within the range of the normal biopsy specimens.

Discussion

Among 10 untreated coeliac patients with villous atrophy confirmed by biopsy who completed six weeks of treatment with fluticasone propionate while continuing with a normal diet, there was an improvement in symptoms, weight, and serum albumin concentration, and in three parameters of gut damage: differential sugar absorption,

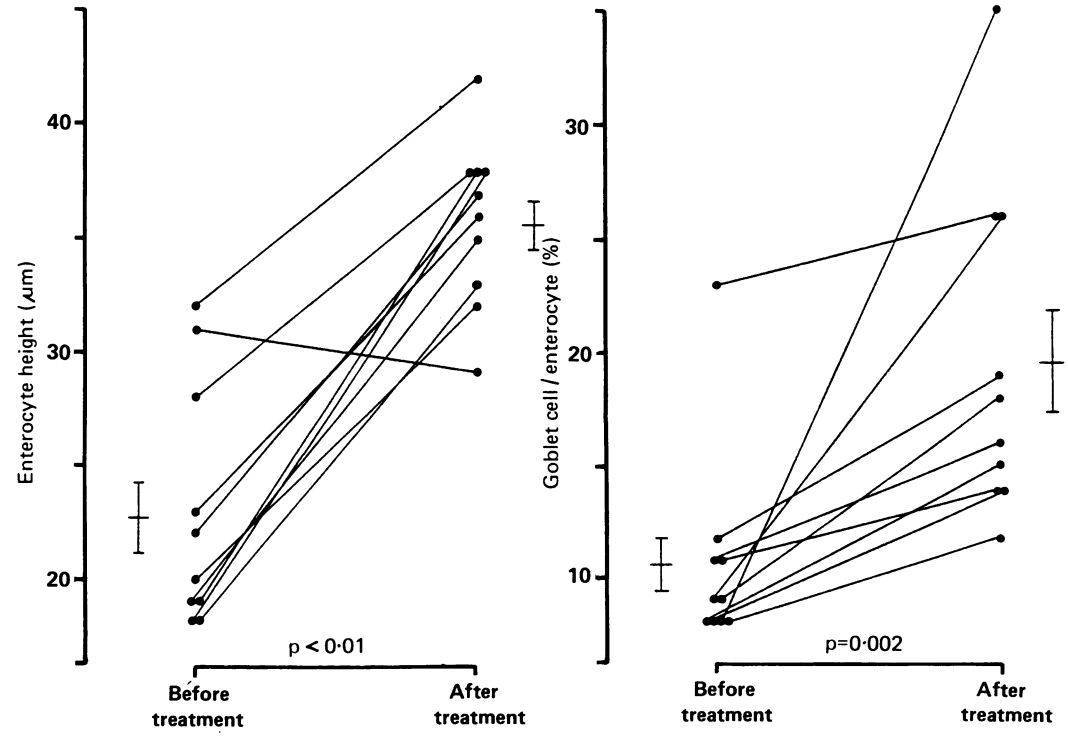

Figure 3: Enterocyte height and goblet cell/enterocyte percentage before and after treatment in the 10 patients who finished the trial. Mean (SEM) shown.

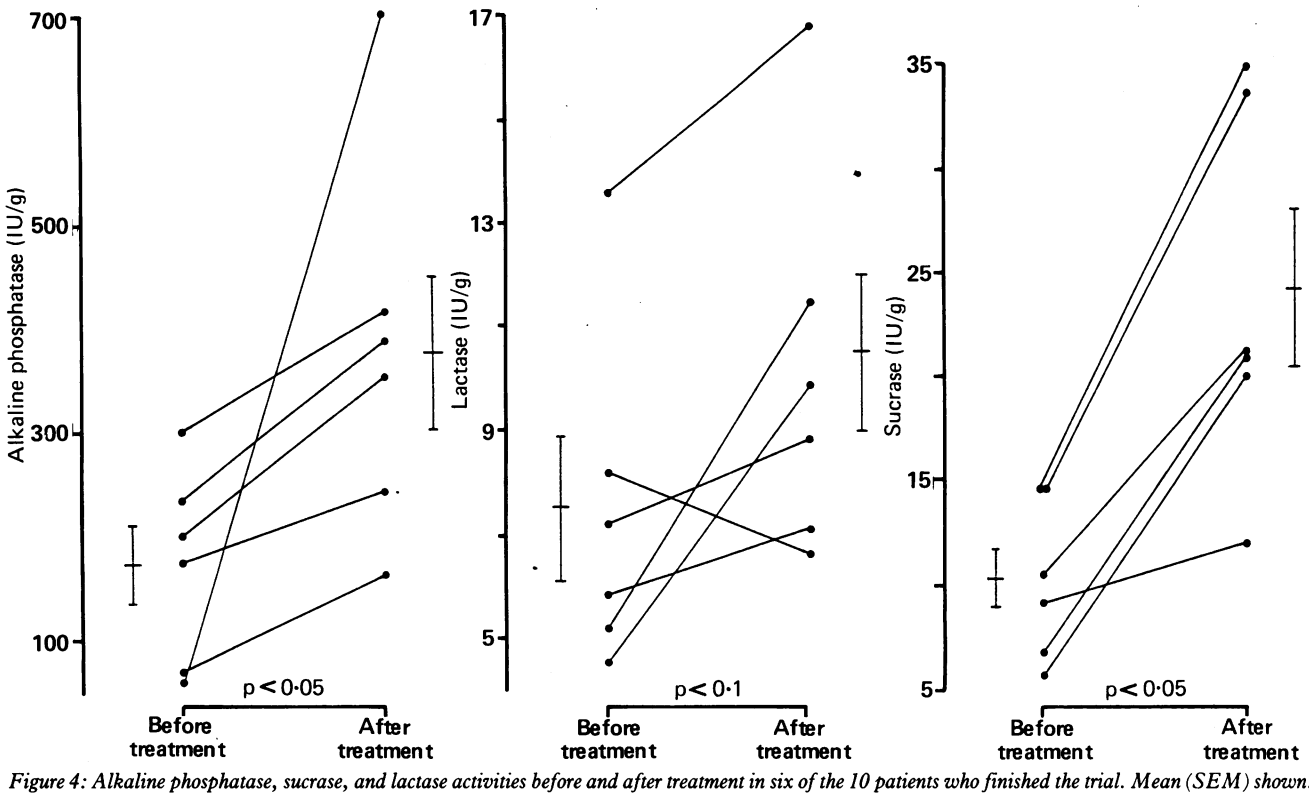

Figure 4: Alkaline phosphatase, sucrase, and lactase activities before and after treatment in six of the 10 patients who finished the trial. Mean (SEM) shown.

small intestinal enzymic activity, and intestinal histology. Even though the number of patients was small many of the changes were consistent enough to be significant.

Longterm follow up of these patients to establish a definitive diagnosis of coeliac disease – that is, clinical and histological response to gluten withdrawal – has been possible in the 10 patients who have been largely compliant with a gluten free diet. All have remained clinically well with no recurrence of the symptoms leading to their original diagnosis. Further duodenal biopsy specimens, taken at intervals of 9–18 months after the study, have shown normal or near normal histology in three, appreciable villous atrophy in one, while in the remaining six, histological appearances were improved compared with the biopsy specimen taken at the end of the study.

The results are in keeping with those of previous studies of steroid use in coeliac disease,[4 5 13] all of which showed an improvement in intestinal enzyme activity and, in two reports,[4 5] clinical and histological improvement. The histological improvement shown here occurred both in the inflammatory infiltrate (intraepithelial lymphocyte count) and in the absorptive surface epithelium (enterocyte height and goblet cell ratio).

Our findings and those of Wall *et al*[4] and Bramble *et al*[5] are in keeping with the rate of recovery seen both clinically and in the mucosa of the gut of coeliac patients after withdrawal from gluten. It is well recognised that full recovery of the villous pattern on gluten withdrawal may take months to occur or, indeed, may never do so. The clinical recovery seen on gluten withdrawal, however, occurs within days to weeks. Yardley *et al*[14] described three patients in whom gluten withdrawal for 10 days or less was associated with an improvement in enterocyte height, and Riecksen *et al*,[15] using serial biopsies in one patient, showed that recovery in enzyme activities was detectable within two weeks and appreciable within four weeks of gluten withdrawal.

In addition to enzyme analyses and detailed histological assessment we used a differential sugar absorption test to assess the efficacy of treatment. This is a more sophisticated version of the simple sugar absorption studies which depended on the passive absorption of water soluble molecules across the gut and their subsequent renal excretion to quantify gut permeability. The absorption of mannitol seems to be transcellular and factors that reduce the enterocyte area, such as coeliac disease, will reduce absorption. Lactulose seems to diffuse via paracellular pathways and diseases that damage the gut and increase its general 'leakiness' lead to increased lactulose absorption. Lactulose behaves in the same way as cellobiose and raffinose, both of which have been used in absorption studies in coeliac disease[16 17] with closely similar results. In one centre the lactulose/mannitol combination has been favourably compared with duodenal biopsy in measuring response to gluten challenge.[18] The lactulose/mannitol ratio clearly improved with treatment, primarily due to an improvement in mannitol absorption rather than to a reduction in lactulose absorption, suggesting that gut healing initially takes the form of increasing enterocyte area rather than of reducing 'leakiness'.

The major problems with the use of oral steroids in coeliac disease have been adrenal suppression[19] and the worry about longterm side effects such as osteoporosis to which the underlying condition increases vulnerability. We found adrenal suppression in two patients after fluti-

casone treatment, although one had been abnormal previously, while there was a blunted cortisol response in a further five, two of whom had been abnormal previously. This suggestion of systemic absorption of fluticasone propionate is not understood as it has not been seen in normal volunteers and only once in patients with colitis: it would be better clarified by more stringent testing with insulin stress tests. The same observations concerning clobetasone butyrate were made by Bramble *et al.*[5] Presumably the greater 'leakiness' of the damaged gut – as shown by the greater than normal absorption of lactulose – encourages absorption of the steroid, but this is not seen with prednisolone where blood concentrations after oral doses were the same in coeliac patients and in normal subjects.[20] If so, as the gut heals a lessening of corticosteroid absorption and adrenal suppression would be expected. This is partially supported by Bramble *et al*[5]: 9 am cortisol concentrations were at their lowest during the four months of treatment, while by the end of the treatment period they had actually risen slightly. Thus their conclusion that the two steroids they were studying had nothing to add to prednisolone treatment may have been unduly severe. The question of longterm side effects would only be answered by longer studies – corticosteroid osteoporosis is not necessarily circumvented by avoiding adrenal suppression.

The use of corticosteroids in coeliac disease seems to relate to three different patient groups. Firstly, there are the patients who are resistant to gluten withdrawal (estimated to be 10% of all coeliac patients), in whom prednisolone has been used with gratifying response and where the benefits clearly outweigh the disadvantages but where substitution with a corticosteroid with lower systemic bioavailability must be theoretically preferable. The second group of patients are those who are clearly non-compliant. It is important to recognise that corticosteroid treatment cannot be justified in these patients as an easy alternative to a gluten free diet: removing the allergen must always be preferable to suppressing the body's response. Among non-compliant and ill patients (a small group) there is an obvious role for steroid use, but there is also an argument for steroid use among non-compliant and well patients now that compliance has been shown to reduce the longterm risks of small bowel neoplasia,[21] presumably by encouraging normalisation of the small bowel mucosa. If a safe corticosteroid could be found then treatment of well but non-compliant patients becomes a valid consideration. Even here corticosteroids are not an easy alternative to a gluten free diet as it cannot be assumed that suppression of the reaction to gluten would have the same beneficial effect on the incidence of neoplasia as gluten removal. The third group of patients are the newly diagnosed in whom the addition of steroids for a short period might hasten healing and well being and improve dietary compliance: an unexpected finding in this study was that at least two patients were sufficiently impressed by the improvement in their health with fluticasone propionate that they were motivated to undertake a gluten free diet conscientiously, something they had been reluctant to consider.

These considerations, as well as the clear benefits associated with fluticasone propionate, make it reasonable to suggest that the drug is worthy of further longterm assessment in coeliac disease, in a controlled trial *v* gluten withdrawal. Such a trial would have to incorporate stringent observations for adrenal suppression.

This study was undertaken with the aid of Glaxo Group Research Ltd, who kindly supplied the fluticasone propionate.

ADDENDUM

The morphological aspects of this study have now been extended using quantitative computerised image analysis. (Zaitoun A, Record CO. *Alimentary Pharmacology and Therapeutics*, in press.)

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