Gut, 1981, **22**, 939–947

In vitro diagnosis of coeliac disease: an assessment

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SUMMARY Jejunal biopsies from controls and coeliac patients were maintained in organ culture for up to 48 hours. The *in vitro* effect of gluten fraction III during the period of culture was assessed by measurement of the activity of the brush border enzymes alkaline phosphatase and α-glucosidase. Mucosa from controls and treated and untreated coeliacs behaved similarly and no reproducible *in vitro* effect of gluten was demonstrated. These results cast doubt on the *in vitro* diagnosis of coeliac disease by monitoring brush border enzyme activity.

During the past 10 years there has been considerable interest in *in vitro* organ culture of human small intestinal mucosa, especially in relation to coeliac disease.[1-5] In 1978 Katz and Falchuk[6] found the technique so reliable that they postulated its use as a predictive test for the definitive diagnosis of gluten sensitivity. Their basis for discrimination between normal and coeliac mucosa in the organ culture system was the degree of change found in mucosal alkaline phosphatase activity during culture in the presence or absence of a peptic-tryptic digest of gluten.

However, there is disagreement in the literature as to the reliability of these changes during organ culture[5] and, consequently, doubt about the *in vitro* diagnosis of gluten sensitivity.

We therefore set out to assess the value of changes in mucosal enzyme activity in coeliac disease during organ culture.

Methods

PATIENTS

Controls

These were 22 patients with normal jejunal histology who had undergone a biopsy to exclude the diagnosis of coeliac disease. It was subsequently shown that none had gastrointestinal disease.

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Received for publication 7 May 1981

Coeliacs

There were 13 patients with a flat jejunal biopsy: eight have subsequently shown a histological response to a gluten-free diet, five have shown a clinical response but have not yet been re-biopsied. These were regarded as untreated coeliac patients.

There were also 14 coeliac patients who had all shown histological improvement after gluten withdrawal which had been continued for at least six months before this study.

BIOPSIES

Multiple biopsies were taken, with informed consent, as part of the routine diagnostic procedure, from the region of the ligament of Treitz using the Quinton hydraulic capsule.[7]

CULTURE MEDIUM

The culture medium consisted of Trowell's T8 medium (6 ml), NCTC 135 medium (2 ml), Glutamine 200 mM (0·2 ml), 1M Hepes buffer (0·1 ml) (all obtained from Flow Laboratories Ltd., Ayrshire, Scotland), 1000 U penicillin (0·1 ml), 1000 U streptomycin (0·1 ml), and fetal calf serum (1·5 ml) (Wellcome Research Labs., Beckenham).[8] This medium (the 'control medium') was mixed one to two hours before use in the organ culture system, and kept at 37°C until the biopsies were obtained.

Gluten-containing medium was similarly constituted with the inclusion of 1 mg/ml of gluten fraction III (GFIII),[9] prepared from gluten (BDH Chemical Co., Poole, Dorset).

CULTURE PROCEDURE

The biopsies, delivered by the Quinton biopsy

apparatus into Ringer-Tyrode solution, were immediately placed in ice-cold control medium and cut into two or three pieces. The pieces were mounted villous surface uppermost on the wire grid of a sterile organ culture dish (Falcon Plastics Ltd., Los Angeles, California), and the central well was filled with approximately 1 ml of culture medium so as to touch the under-surface of the biopsy. The time from excision of biopsy to its being in position in the dish was approximately five minutes. The outer ring of the culture dish contained absorbent paper which was saturated in 0·9% NaCl solution.

The dishes were placed in a sterile anaerobic jar, which was gassed with 95% O_2:5% CO_2 for 30 then sealed and kept in an incubator at 37°C.

Pieces of biopsies from all patients and controls were cultured in control medium and gluten-containing medium. In order to minimise discrepancy due to the patchiness[10] of any lesion only pieces from the same biopsy were compared after culture.

After 24 or 48 hours the biopsies were removed from the jar, washed gently in 1 ml ice-cold 0·9% NaCl and weighed. The biopsies were then homogenised in 2 ml ice-cold sucrose medium (0·3 mol/l) containing disodium EDTA (1 mmol/l, pH 7·4), and ethanol (22 mmol/l) using 10 strokes of a Teflon pestle in a glass homogeniser (Thomas Co., USA). A piece of each biopsy was homogenised when fresh for comparison with cultured pieces.

BIOCHEMICAL ANALYSIS

Alkaline phosphatase and α-glucosidase were measured in the homogenates using the fluorimetric method described by Peters *et al.*[11] Alkaline phosphatase was measured on the day of homogenisation before freezing using 4 methyl umbelliferyl phosphate as substrate at pH 8·7 with 0·05 M borate buffer containing 5 mM $MgCl_2$. α-glucosidase was measured in homogenates which had been stored at −70°C for less than six months using 4 methyl umbelliferyl-α-glucopyranoside as substrate at pH 6·0 with 0·1 M MES buffer. α-glucosidase is known to be stable for at least six months at −70°C (Howdle, unpublished observations). Assays were performed in duplicate at a suitable dilution, and enzyme and substrate blanks were included. Protein was determined on aliquots of homogenate using the method of Lowry *et al.*[12] using bovine serum albumin (Sigma Chemical Co. Poole) as standard.

Enzyme activities were calculated in milliunits, one milliunit corresponding to the hydrolysis of 1 nanomole of substrate per minute at 37°C, and then expressed as specific activity in nanomoles per milligram protein in the same homogenate.

HL-A TYPING

The HL–A status of the coeliac patients was determined by a modification of the microlymphocytotoxic technique of Terasaki and McClelland.[13]

STATISTICAL ANALYSIS

Paired and non-paired *t* tests, correlation coefficient, and test of skewness[14] were used in the statistical analysis.

Results

PRECULTURE ENZYME ACTIVITY

Alkaline phosphatase

There were 34 biopsies from 22 control subjects, 19 biopsies from 14 treated coeliacs, and 15 biopsies from 13 untreated coeliac patients. In those subjects where two preculture values were available, the first preculture value was used in the statistical analysis for comparison between the three groups of patients. The means ±1 SD were 37·4±15·8, 27·9±11·9, and 13·2±7·3 respectively, as shown in Fig. 1. The series of values in each group was not significantly skewed from a normal distribution. There was a significant difference ($P<0·001$) between untreated coeliacs and both controls and treated coeliacs. There was no difference between controls and treated coeliacs.

α-Glucosidase activity

There were 17 biopsies from 13 control subjects, seven biopsies from seven treated coeliacs and eight biopsies from eight untreated coeliac patients. In the four control subjects where two biopsies were available, the first value was used in the statistical analysis. The means ±1 SD were 2·1±0·4, 1·5±0·5, and 0·8±0·2 respectively, as shown in Fig. 2. The series of values in each group was not significantly skewed from a normal distribution. There was a significant difference between each pair of groups; controls versus treated coeliacs $P<0·02$, controls versus untreated coeliacs $P<0·001$, treated versus untreated coeliacs $P<0·01$.

Values for both enzymes were compared in 15 control subjects, seven treated coeliacs, and eight untreated coeliacs and a significant correlation was found, $r=0·65$, $P<0·001$ (Fig. 3).

CHANGES IN ENZYME ACTIVITY DURING CULTURE

Alkaline phosphatase activity

Controls The mean preculture alkaline phosphatase activity for biopsies cultured for 24 hours was 34·8±16·2 and this increased after 24 hours to 65·0±22·8 and 68·2±23·7 without and with GFIII respectively. Both these increases were significant ($P<0·001$) but

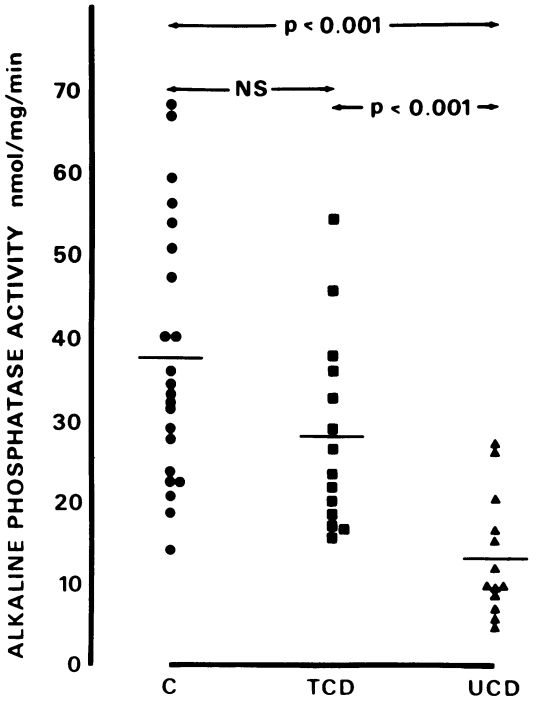

Fig. 1 *Preculture alkaline phosphatase activity in controls (C ●), treated coeliacs (TCD ■), and untreated coeliacs (UCD ▲).*

there was no significant difference between culture with or without GFIII. The mean preculture alkaline phosphatase activity for biopsies which were cultured for 48 hours was 39·7±17·1; this did not differ

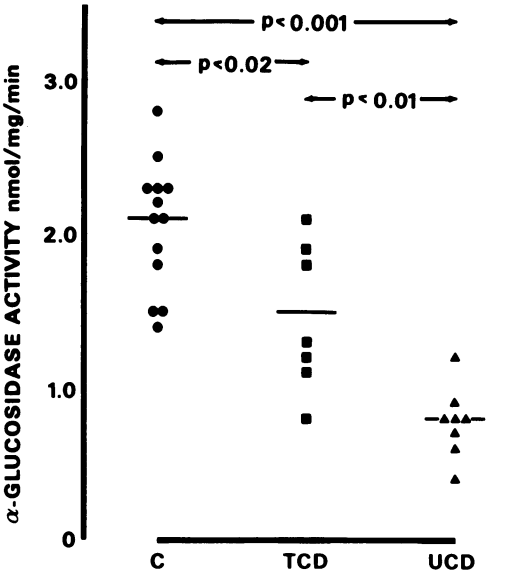

Fig. 2 *Preculture α-glucosidase activity in controls (C ●), treated coeliacs (TCD ■), and untreated coeliacs (UCD ▲).*

significantly from the mean preculture value for biopsies cultured for 24 hours. After 48 hours' culture the mean alkaline phosphatase activity was 80·8± 18·7 without GFIII and 81·0±23·9 with GFIII; there was no difference between these groups but each showed a significant increase from preculture values ($P < 0.001$). The activity after 48 hours was just significantly greater ($P < 0.05$) than that after 24 hours after culture in control medium, but there

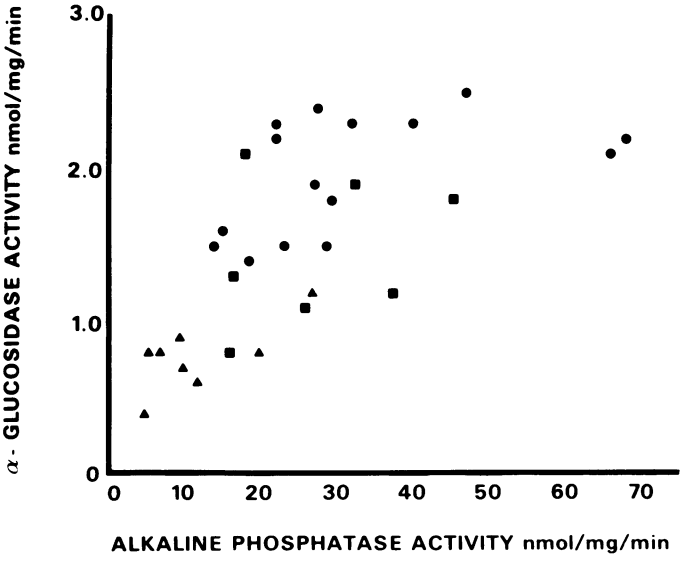

Fig. 3 *Correlation between preculture α-glucosidase and alkaline phosphatase activity in controls (●), treated coeliacs (■), and untreated coeliacs (▲).* $r = 0.65$. $P < 0.001$.

was no difference after culture in gluten-containing medium. Individual values are shown in Table 1, and mean changes in Fig. 4.

Treated coeliacs The mean preculture alkaline phosphatase activity for biopsies which were cultured for 24 hours was 29·2±11·7 and this increased after 24 hours without GFIII to 56·6±22·5 and with GFIII to 56·2±20·0. Both these increases were significant ($P<0.001$), but there was no significant difference between culture with or without GFIII. The mean preculture alkaline phosphatase activity for biopsies which were cultured for 48 hours was 26·3±8·6; this did not differ significantly from the mean preculture value for biopsies cultured for 24 hours. After 48 hours' culture the values were 61·9±9·4 and 67·0±20·4 without and with GFIII respectively. These increases were significant ($P<0.001$), but there was no significant difference between culture with or without GFIII. There was no difference between activity after 24 hours' culture as compared with 48 hours. Individual values are shown in Table 2 and mean changes in Fig. 4.

The HL-A type was determined in 13 of the 14 treated coeliacs. Eleven were B8 positive and two B8 negative. The mean of the values in the B8 positive subjects before culture was 30·4±11·9, increasing to 61·5±22·4 after 24 hours in control medium and to 62·6±18·6 after 24 hours' culture in GFIII. The equivalent values in the B8 negative subjects were 29·1±7·9, 52·2±14·5 and 44·9±13·1. There were insufficient numbers of B8 negative patients for statistical comparison.

Table 1 *Controls: alkaline phosphatase activity (nmol/mg/min)*

Patient no.	*Culture time (hours)*				
	0	*24 Control*	*24 +GFIII*	*48 Control*	*48 +GFIII*
1	53·3			115·3	93·9
	22·7	64·8	62·5		
2	55·9	108·7	102·9		
	58·9			83·3	103·8
3	33·7	76·7	125·4		
	28·0			91·3	87·3
4	40·1	85·2	52·0		
	46·9			105·0	103·2
5	32·7	87·8	86·4		
	66·2			88·0	114·2
6	20·5			60·1	45·9
	17·1	24·1	41·3		
7	31·6	54·4	61·6		
8	35·7	54·2	80·5		
	33·8	74·5	66·9		
9	28·7	50·5	55·9		
	29·3	44·4	46·2		
10	67·8	102·4	86·3		
	65·8	84·5	98·7		
11	27·4	63·9	69·4		
	27·0	70·8	53·9		
12	13·9	28·0	28·8		
	15·0	54·9	63·0		
13	23·2	44·5	48·9	67·2	53·3
14	50·4			81·2	76·4
15	47·1			103·3	120·6
16	66·7			81·3	70·0
17	40·0			55·4	70·9
18	18·5			80·4	71·3
19	22·2			81·8	63·7
20	22·1			48·3	45·3
21	31·8			70·7	95·5
22	59·0	61·2	64·8		
	42·0				
Mean± 1 SD	37·4±15·8	65·0±22·8	68·2±23·7	80·8±18·7	81·0±23·9

Untreated coeliacs The mean preculture alkaline phosphatase activity for biopsies which were cultured for 24 hours was 14·1±7·8, increasing after 24 hours without GFIII to 39·6±14·6 and with

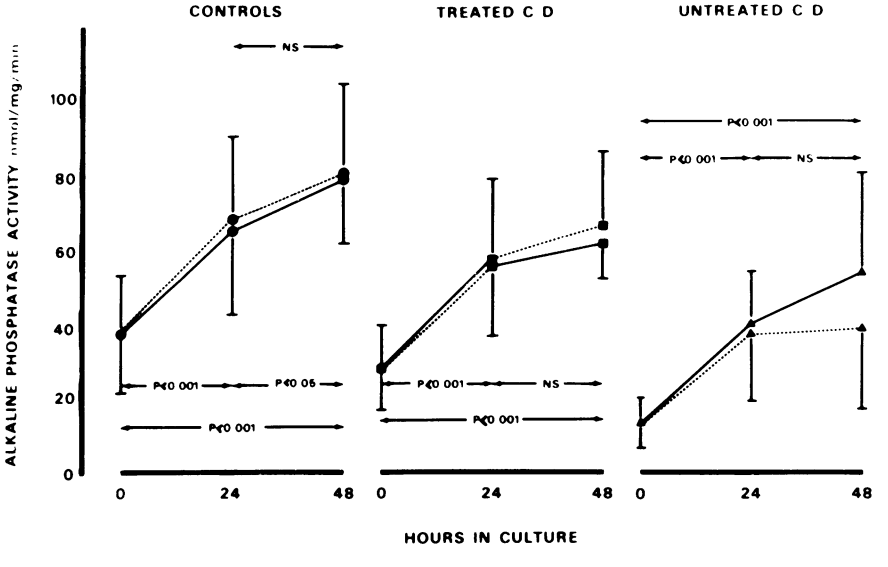

Fig. 4 *Changes in alkaline phosphatase activity during culture in controls (●), treated coeliacs (■), and untreated coeliacs (▲) in the presence and absence of gluten (Means ± ISD, dotted line indicates +GFIII).*

Table 2 *Coeliacs: alkaline phosphatase activity (nmol/mg/min)*

Patient no.	*Culture time (hours)*					*HLA B8 status*
	0	*24 Control*	*24+GFIII*	*48 Control*	*48+GFIII*	
Treated						
1	16·1	20·1	26·2			
	23·8			62·1	53·5	
2	17·9			46·9	43·1	+
3	16·6	65·2	96·3			+
4	45·6	100·0	70·1			+
5	19·2	30·9	37·5			+
	23·2			69·3	64·8	+
6	28·4	46·9	47·3			—
	37·4	68·6	56·7			—
7	32·5	64·6	73·1	68·2	98·2	+
8	35·8	65·2	67·5			+
	36·3	84·4	67·0			+
9	21·6	41·1	30·8	70·4	73·2	—
10	26·1	53·3	54·9			+
	40·8			59·3	80·4	+
11	22·9	56·4	54·5	67·8	69·3	+
12	37·5			67·1	85·5	+
13	16·2	24·7	32·4	46·0	35·1	+
14	54·3	70·8	72·4			+
Means ± 1 SD	27·9 ± 11·9	56·6 ± 22·5	56·2 ± 20·0	61·9 ± 9·4	67·0 ± 20·4	
Untreated						
1	16·3	60·5	56·9			+
2	8·4	29·3	30·0			+
	16·4			57·1	44·9	+
3	15·1			54·0	49·0	+
4	5·5			22·0	8·4	+
5	9·6			26·7	46·2	
6	26·2	36·2	54·0			
	18·9			119·4	43·1	
7	9·8	14·1	12·3			
8	5·0	27·4	13·6	43·6	40·0	+
9	11·8	42·7	40·0	67·8	32·4	
		27·1	30·7			
10	9·8	55·9	60·9	72·5	89·0	+
11	7·0	41·3	21·2	29·4	35·0	+
12	26·7	43·2	49·0	50·4	27·5	+
13	19·9	58·2	57·2	50·8	14·6	+
			26·1			+
Means ± 1 SD	13·2 ± 7·3	39·6 ± 14·7	37·7 ± 17·6	54·0 ± 27·1	39·1 ± 21·0	

GFIII to 37·7 ± 17·6. Both these increases were significant ($P < 0·001$), but there was no significant difference between culture with or without GFIII. The mean preculture alkaline phosphatase activity for biopsies which were cultured for 48 hours was 13·2 ± 6·8; this did not differ significantly from the mean preculture value for biopsies cultured for 24 hours. After 48 hours' culture increases were to 54·0 ± 27·1 and 39·1 ± 21·0 in the absence and presence of GFIII respectively. These increases were significant ($P < 0·001$ and $P < 0·005$), but there was no significant difference between culture with or without GFIII. There was no difference between activity after 24 hours' culture as compared with 48 hours. Individual values are shown in Table 2 and mean changes in Fig. 4.

From Table 2 it can be seen that, particularly after 48 hours' culture, there is a wide variation in the increase in alkaline phosphatase activity between individual patients. There is also generally less of an increase after culture with GFIII, reflected in the two means of 54·0 and 39·1, even though this trend did not reach statistical significance.

Interestingly, in patient no. 13, two pieces of one biopsy were cultured with and without GFIII for 24 hours and almost identical increases in alkaline phosphatase activity were obtained; however, a piece of another biopsy taken at the same time was cultured with GFIII and showed only a small increase over the preculture value. This stresses the importance of only comparing pieces from the same biopsy after culture with and without GFIII.

HL-A type was determined in the 13 untreated patients. Nine were HL-A B8 positive and four were B8 negative. The mean of the values in the B8 positive subjects before culture was 13·0 ± 7·1, increasing to 45·1 ± 13·6 after 24 hours' culture in control medium and to 39·4 ± 18·7 after 24 hours' culture in GFIII. The mean activities after 48 hours' culture were 47·5 ± 15·9 in control medium and 38·6 ± 24·8 in gluten-containing medium. The equivalent values in the B8 negative subjects were 15·3 ± 7·2; 30·0 ± 12·4 and 34·3 ± 17·5; 71·3 ± 46·5 and 40·5 ± 7·2. There was no significant difference between the two groups before or after culture, with or without GFIII.

α-*Glucosidase activity*

Controls The mean preculture α-glucosidase activity for biopsies which were cultured for 24 hours was 1·9±0·4; after 24 hours' culture the activity was 1·9±0·4 and 1·9±0·3 in the absence and presence of GFIII respectively. There was no significant difference between these values or from the preculture value. The mean preculture value for biopsies cultured for 48 hours was 2·2±0·5, which did not differ significantly from the preculture value of those cultured for 24 hours. Values at 48 hours were 2·1±0·5 and 2·3±0·4 without and with GFIII respectively; these were not significantly different from each other or from the preculture value. In no patient, control or coeliac, was there a difference between activity after 24 hours' culture as compared with 48 hours. Individual values are shown in Table 3.

Table 3 α-*Glucosidase activity* (*nmol/mg/min*)

Patient no.	*Culture time (hours)*				
	0	*24 Control*	*24 +GFIII*	*48 Control*	*48 +GFIII*
Controls					
9	1·8	1·5	1·8		
	1·5	1·8	1·5		
10	2·1	2·1	2·1		
	2·2	2·3	1·9		
11	1·9	2·5	2·3		
	2·4	2·3	2·1		
12	1·5	1·6	1·6		
	1·6	1·4	1·4		
13	1·5	1·6	1·6	1·5	1·5
15	2·5			2·1	2·5
17	2·3			1·7	2·2
18	1·4			2·3	1·9
19	2·3			2·2	2·5
20	2·2			2·4	2·7
21	2·3			2·6	2·7
23	2·1			1·6	2·6
24	2·8	2·3	2·4	2·9	2·1
Means ±1 SD	2·1±0·4	1·9±0·4	1·9±0·3	2·1±0·5	2·3±0·4
Treated coeliacs					
2	2·1			2·3	1·5
3	1·3	2·2	2·4		
4	1·8	2·8	1·7		
7	1·9	2·2	2·0	1·6	1·8
10	1·1	1·5	1·5	1·2	1·3
12	1·2	0·9	1·4	1·5	0·7
13	0·8	0·6	0·9	1·3	1·1
Means ±1 SD	1·5±0·5	1·7±0·8	1·7±0·5	1·6±0·5	1·3±0·4
Untreated coeliacs					
4	0·8			0·7	0·4
5	0·9			0·8	1·1
8	0·4	0·7	0·5	0·6	0·6
9	0·6	0·8	0·7	0·7	0·4
		0·7	0·5		
10	0·7	1·1	1·0	1·3	1·2
11	0·8	0·7	0·6	0·5	0·6
12	1·2	1·2	0·8	1·1	0·7
13	0·8	1·3	1·4	0·8	0·5
Means ±1 SD	0·8±0·2	0·9±0·3	0·8±0·3	0·8±0·3	0·7±0·3

Treated coeliacs The mean preculture α-glucosidase activity was 1·5±0·5, and after 24 hours the activity was 1·7±0·8 and 1·7±0·5 in the absence and presence of GFIII respectively; there was no significant difference between these values or from the preculture value. After 48 hours activities were 1·6±0·5 and 1·3±0·4 without and with GFIII respectively, and there was no significant difference between these values or from preculture activity.

Untreated coeliacs The mean preculture activity was 0·8±0·2 and after 24 hours' culture was 0·9±0·3 without and 0·8±0·3 with GFIII. Neither of these values was significantly different from the preculture value nor from each other. After 48 hours' culture activities were 0·8±0·3 and 0·7±0·3 without and with GFIII, there being no significant difference between these values or from preculture activity.

Discussion

In 1974 Falchuk *et al.*[2] showed that mucosal alkaline phosphatase activity changed during organ culture of small bowel mucosa. They showed that, in the presence of a peptic-tryptic digest of gluten,[9] coeliac mucosa in exacerbation after a gluten challenge behaved differently from control mucosa and from coeliac mucosa in remission and they suggested that this technique was a useful *in vitro* test of gluten sensitivity. In 1978 Katz and Falchuk[6] used the technique to predict gluten sensitivity in a series of abnormal biopsies from untreated patients with considerable accuracy. In 1980, Falchuk *et al.*[15] again showed gluten sensitivity *in vitro* in patients with 'active' gluten sensitive enteropathy and suggested there are differences according to histocompatibility type. Despite these important results, there is disagreement in the literature about the reliability of such findings[3 5 16] and also about the method of expression of the enzyme activity.[3 17]

We therefore embarked on similar studies, measuring alkaline phosphatase activity, as this had been shown to produce the largest changes of the enzymes measured by Falchuk *et al.*[2] We also measured α-glucosidase, another brush border enzyme, which might be expected to show changes similar to those of alkaline phosphatase. We expressed the results per unit of protein in each biopsy for comparison with the work of previous authors.

The preculture values of alkaline phosphatase and α-glucosidase showed a significant correlation. The preculture values for the two enzymes in each of the three groups of patients fitted a normal distribution, which is contrary to that found for disaccharidases in the small intestinal mucosa[18] but agrees with that

assumed by other authors for alkaline phosphatase.[19 20] The alkaline phosphatase activity in untreated coeliacs was significantly reduced compared with controls or treated coeliacs. Comparing controls and treated coeliac patients there was no difference in alkaline phosphatase activity between the two groups, suggesting that the coeliacs were well treated. This contrasts with α-glucosidase activity which showed good separations between the three groups of subjects.

The results of the changes in alkaline phosphatase activity during culture of control biopsies agree with those originally described by Falchuk *et al.*[2] There is an increase in activity after culture for 24 or 48 hours. All increases were significant but there was no difference between culture with or without gluten. Similar increases were described by Hauri *et al.*[5] but not by Mitchell *et al.*[3] or Stevens *et al.*[16] The discrepancies may be due to differences in the organ culture technique. The results of the changes in α-glucosidase activity in control tissue do not reflect those of alkaline phosphatase in that there is no significant difference between activity before or after culture at 24 or 48 hours with or without gluten. Perhaps this is not unexpected as Falchuk *et al.*[2] and Hauri *et al.*[5] showed much more change in alkaline phosphatase activity than in other brush border enzymes. Mitchell *et al.*[3] showed no change in α-glucosidase, nor did Stevens *et al.*[16] in any of the enzymes they measured. These different rates of change perhaps reflect varying rates of synthesis or turnover of the different enzymes in the brush border.[21]

In the treated coeliacs the changes in alkaline phosphatase activity were similar to those in controls, there being an increase after 24 and 48 hours' culture, and no difference between culture with or without gluten. These results are in agreement with those reported by Falchuk *et al.*[2] for their coeliac patients in remission.

In untreated coeliac patients there was an increase in alkaline phosphatase activity after culture without gluten for 24 and 48 hours, similar to that reported by Falchuk *et al.*[2] for coeliac patients in exacerbation, by Hauri *et al.*,[5] Stevens *et al.*[16] and Katz and Falchuk[6] for untreated coeliac patients, and by Falchuk *et al.*[15] for challenged and untreated coeliac patients. This increase is numerically similar to that in controls. However, because of the lower initial values, the percentage increase is considerably greater, perhaps reflecting an increased enterocyte maturation in untreated coeliac mucosa when in the gluten-free *in vitro* environment. When tissue was cultured in the presence of gluten the increase in alkaline phosphatase activity was similar to that which occurred when gluten was absent. From Table 2 it can be seen that this increase is generally less after 48 hours' culture in the presence of gluten, reflected in the means of 54·0 and 39·1, but this trend does not reach statistical significance. This finding may support the theory that the mechanism of mucosal damage in coeliac disease is not due to direct toxicity of gluten but that an intermediate mechanism is involved. However, our results are at variance with those of Falchuk's group,[2 6 15] although in agreement with those of Hauri *et al.*[5] This lack of *in vitro* sensitivity to gluten in our untreated coeliac patients might be explained by differences between our study and the studies of Falchuk.[2 6 15] First, there might be subtle differences in the organ culture technique; this, however, is unlikely, as in all other respects we found similar changes in alkaline phosphatase activity to those of Falchuk *et al.*[2 6 15] The difference might be in the preparation of the protein digest of gluten, although we used the same method of Falchuk *et al.*[2 6 15]—that is, the method of Frazer *et al.*[9]—and we have shown other evidence of *in vitro* toxicity of our digest in organ culture.[22] Furthermore, peripheral blood lymphocytes from coeliac patients have been shown to be sensitive to our digest of gluten.[23] Hauri *et al.*[5] used Frazer's preparation[9] together with three other digests of gluten and still failed to show gluten-sensitivity *in vitro*. Perhaps the most likely explanation of the difference in response in alkaline phosphatase activity between ourselves and Falchuk *et al.*[2] resides in the biopsies which were used for culture. Their biopsies came from coeliacs previously in remission who had been challenged for seven to 14 days with a gluten-containing diet before the biopsies were taken. It may be that the *in vitro* gluten-sensitivity of these acutely challenged biopsies is more marked than those from untreated coeliac patients. This accords with the findings of Bullen and Losowsky[23] who detected cell-mediated immunity in the peripheral blood of an acutely challenged patient and suggested that in such circumstances there is a sudden increase in the production of sensitised lymphocytes. This would presumably have an effect on the gluten-sensitivity of the mucosa, whereas in the untreated coeliac the reduction in the number of lymphocytes[24] probably due to chronic enteric loss[25] would be a major factor affecting gluten-sensitivity. However, in their 1978 report, Katz and Falchuk[6] used biopsies from untreated coeliac patients and showed *in vitro* gluten-sensitivity in the majority of cases, whereas we have failed to demonstrate this in our untreated coeliac patients. Katz and Falchuk's[6] patients were all children aged below 20 years, whereas ours were all over 30 years,

except for two boys of 12 and 18 years (patient nos. 8 and 11 respectively, Table 2) and, interestingly, the alkaline phosphatase values at 24 hours in these two patients might be taken to suggest gluten sensitivity. It may be that the *in vitro* sensitivity of mucosal biopsies from untreated patients differs according to age, perhaps *via* the duration of the enteric loss of lymphocytes.[25] In their latest report, Falchuk *et al.*[15] do report evidence of gluten sensitivity in biopsies from untreated, as well as challenged, coeliac patients up to the age of 52 years, but unfortunately they do not state whether the untreated patients spread throughout the age range of 11 months to 52 years, or were from the younger age groups. A further finding is that Falchuk *et al.*[15] related *in vitro* gluten sensitivity to histocompatibility type, 88% of HLA B8 positive patients demonstrating *in vitro* gluten sensitivity, whereas this was demonstrated in only 25% of HLA B8 negative patients. They do not state whether the HLA B8 positive or negative groups had a preponderance of older or younger patients, or of untreated as opposed to acutely challenged patients. We found no difference between our HLA B8 positive or negative patients in their *in vitro* response to gluten, although the majority were older patients and there was only a small number of B8 negative patients.

We intend to carry out additional studies to establish whether there is a discrepancy in results between ourselves and Falchuk's group by using treated coeliac patients after a gluten-challenge and children with untreated coeliac disease, and relating the findings to HLA status. Nevertheless, for *in vitro* testing of coeliac mucosa to be useful clinically as an aid in the diagnosis of gluten sensitivity in the adult, positive findings would be necessary in the manner in which we have used the test in this study.

It is well documented that the mucosal lesion in coeliac disease is patchy[10] and we compared pieces of tissue from the same biopsy after culture with or without gluten, whereas Falchuk *et al.*[2 6 15] compared pieces from different biopsies. As we showed in patient no. 13 (Table 2), in two pieces from one biopsy alkaline phosphatase activity increased from 19·9 to 58·2 without gluten and to 57·2 with gluten, whereas a piece from another biopsy from the same level in the same patient at the same time increased to only 26·1 in the presence of gluten. This demonstrates the possible variability in gluten sensitivity between different biopsies from the same patient.

The results for α-glucosidase activity in untreated coeliac patients are similar to those for controls and treated coeliacs, with no significant change after culture.

In our hands it was possible to show that alkaline phosphatase activity increases during organ culture of small bowel mucosa. Untreated coeliac mucosa reacts by producing enhanced percentage increases as compared with control or treated coeliac tissue. We have not been able to demonstrate any reproducible *in vitro* effect of gluten on these biopsies by monitoring alkaline phosphatase activity. It may be, however, that a different method of objectively assessing change during culture would reveal *in vitro* sensitivity to gluten—for example, by measuring enterocyte height to assess histological change.[22]

We are grateful to Dr S M Rajah, Regional Blood Transfusion Laboratory, Leeds, for performing the HLA typing in the coeliac patients.

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