Plasma food antibodies during withdrawal and reintroduction of dietary gluten in coeliac disease

F. CARSWELL and A. FERGUSON

From the University Department of Child Health, Royal Hospital for Sick Children, Yorkhill, and the University Department of Bacteriology and Immunology, Western Infirmary, Glasgow

Carswell, F., and Ferguson, A. (1973). *Archives of Disease in Childhood*, 48, 583. **Plasma food antibodies during withdrawal and reintroduction of dietary gluten in coeliac disease.** Out of 55 patients with coeliac disease, 40 had food antibodies in their plasma at diagnosis. The antibodies disappeared in all but 6 of 21 patients after 2 months on a gluten-free diet. 5 out of these 6 patients had high plasma IgA levels at diagnosis. Reintroduction of wheat and rye gluten was accompanied by the intermittent presence of plasma food antibodies in 7 out of 8 patients subsequently shown to have the small bowel biopsy appearances of coeliac disease. Plasma food antibodies did not appear in 4 patients whose biopsies were normal after reintroduction of gluten. A single test for plasma food antibodies did not reliably indicate minor deviations from the gluten-free diet in established coeliac patients.

Circulating antibodies to foodstuffs are present in the majority of patients with untreated coeliac disease (Taylor *et al.*, 1961; Heiner *et al.*, 1962; Kenrick and Walker-Smith, 1970; Visakorpi, Kuitunen, and Pelkonen, 1970; Ferguson and Carswell, 1972) and tend to disappear after a gluten-free diet is started. It seems likely that they result from increased permeability of the small bowel mucosa. Estimation of these antibodies might be useful in assessing the state of the small bowel mucosa. Accordingly, we have studied a large group of coeliac children to investigate the value of food antibody tests in three situations commonly arising in coeliac patients.

Firstly, we studied the time courses of disappearance of food antibodies in the hope that this might prove a criterion of response to a gluten-free diet. Next, we examined the rate of appearance of food antibodies in patients with coeliac disease on readministration of gluten.

As plasma food antibodies might be expected to be present in coeliac patients when they are not adhering to their diet, we have also examined the relation between these tests and the dietary history in the hope that they might provide objective indices of the strictness of the gluten-free diet.

Materials and methods

The patients were admitted to the Royal Hospital for Sick Children for intensive investigation because of clinical suspicion of coeliac disease. The diagnosis of coeliac disease was based on the finding of a flattened upper intestinal mucosa on biopsy when the patient was on a gluten-containing diet and a clinical response to the withdrawal of gluten from the diet. The patients were usually kept in the wards until the clinical response to gluten withdrawal occurred. The clinical response consisted of improvement in temperament and return of stool frequency and character to normal, together with an increase in weight gain (usually >0.4 kg) in the first 4 weeks on the diet. The patients were usually seen in outpatients within 1 month of discharge from hospital and at approximately 3-monthly, 6-monthly, and yearly intervals.

The gluten-free diet contained no wheat or rye products. The gluten-containing diet was a normal diet containing at least one slice of bread per day. All patients and parents were questioned on each visit about the child's gluten intake, and were instructed on the gluten-free diet where appropriate by a dietician.

Fifty-five patients with coeliac disease had food antibody studies performed on their plasma at diagnosis and 28 of these had further antibody tests performed at subsequent outpatient visits. These patients who were followed up were not selected on clinical grounds, but were merely patients whom the consultant allowed to attend the coeliac clinic. 41 other patients, not studied before gluten-free diet was started, were studied later.

12 patients previously on a gluten-free diet had gluten reintroduced to their diet. Precipitating antibodies were detected in capillary blood samples by Crowle's (1958) micro-gel-diffusion technique as modified by Heiner *et al.* (1962). Crude antigens were prepared from gluten (British Drug Houses), wheat flour (white), oatmeal, and rice flour (cereal antigens); cow's milk, bovine calf serum, and sheep serum (ruminant antigens); and hen's egg white and yolk (fowl antigens) as previously described (Ferguson and Carswell, 1972). Plasma antibodies to these antigens are uncommon in normal children but common in children with coeliac disease (Ferguson and Carswell, 1972).

Results

Table I gives the number of coeliac patients who had positive antibody tests at diagnosis. The separation of the antibody results into those which were positive for ruminant antigens (cow's milk, bovine and sheep serum), cereal antigens (gluten, wheat flour, oatmeal, and rice flour), and fowl antigens (hen's egg white and yolk) is appropriate as patients commonly have antibodies to multiple antigens in the one group. In addition, this order (ruminant, cereal, and fowl) reflects the order of decreasing possibility of finding antibody (Table IB). Only 5 out of 27 patients with cereal antibodies did not also have ruminant antibodies. All 9 patients who had fowl antibodies had either cereal (8/9) or ruminant (9/9) antibodies.

Of the 55 coeliac patients studied at diagnosis, 40 had at least one positive antibody test. 7 of the 15 patients without antibodies who were reviewed after diagnosis had not developed them when tested after 1 to 8 months (mean 3.7 months) on a gluten-free diet. The food antibody results obtained in 21

coeliac patients who had positive antibody tests and were followed up at the outpatient clinic are shown in Fig. 1. 6 of the 21 patients who had antibodies to ruminant protein at diagnosis still had antibodies after 2 months on the gluten-free diet; none of the 13 patients who had antibodies to cereals had antibodies after more than 2 months on the gluten-free diet. 3 of the 4 patients who had fowl antigens still had antibodies after 2 months on the gluten-free diet.

The patients who still had positive food antibody tests after 2 months on the diet (Cases 1, 3, 4, 8, 17, and 18) obviously merit further study. Their ages (mean 49 months) were not significantly different from the rest of the coeliac population (62 months), when compared by Wilcoxon's sum of ranks method. 5 (Cases 1, 3, 4, 8, and 17) of these patients (83%) were female compared with only 5/15 (33%) in the rest of the coeliac population. (Difference not significant by Fisher's test.) When the plasma IgA levels at diagnosis were examined using standard curves for age-related normal values (courtesy of Dr. R. W. Logan), 5 out of these 6 patients had raised values (>95% confidence limit). Only 3 out of 14 other coeliac patients whose antibody tests were positive at diagnosis but did not remain so, had raised levels. This difference is significant ($P < 0.05$ by Fisher's test). The mean IgA concentrations at diagnosis were 158 mg/100 ml (antibody persisted) and 139 mg/100 ml (antibody disappeared). Only 2 of these 6 patients (Cases 4 and 8) were known to fail to keep strictly to the gluten-free diet.

Twelve patients, in whom the diagnosis of coeliac disease had not been firmly established or who had difficulty in keeping to the gluten-free diet, were observed during the reintroduction of gluten and

TABLE I
Number of coeliac patients with food antibodies (55 tested)

(A) Individual antigens								
Cereal				Ruminant			Fowl	
Gluten	Wheat	Oats	Rice	Milk	Calf serum	Sheep serum	Egg white	Egg yolk
20	21	12	4	20	29	24	4	6
(B) Grouped antigens								
Ruminant			Cereal		Fowl			
35			27		9			

χ^2 test shows $P < 0.001$ and thus the order, ruminant, cereal, and fowl, represents the order of antigenicity.

FIG. 1.—Precipitating antibodies to food antigens in coeliac patients with positive tests at week 0, when gluten-free diet was started. + antibodies detected, — antibodies not detected.

food antibody tests were performed (Fig. 2). Small bowel biopsies were taken at a variable time after starting the gluten-containing diet. 8 patients had biopsies indicating active coeliac disease, and

the food antibody tests were positive in 7 of them. Food antibodies were not detected in the 4 patients with normal biopsies.

Food antibodies were estimated in 50 samples from 41 coeliac patients who had been on a gluten-free diet for more than 6 months. A careful dietary history was taken from the patients and their mothers at the time of testing. Diets were classified as lax if the child ate more gluten than one gluten-containing biscuit per week, and as strict if the diet contained not more than one biscuit per week. Table II shows the positive results. Apparently

TABLE II

Established coeliac patients with positive tests (patients allocated to dietary groups on the basis of dietary history)

Food antibodies	Diet	
	Lax	Strict
+	1	9
—	49	41

Yates' χ^2 test shows a significantly ($P < 0.05 > 0.01$) higher proportion of patients on strict diet have positive food antibody tests.

FIG. 2.—Food antibodies in patients undergoing diagnostic reintroduction of gluten from 0 weeks. (Plotted as in Fig. 1.) The time and result of small intestinal biopsy is shown. N, normal; C, coeliac disease.

the presence of food antibodies does not indicate patients who are on lax diets. 2 patients who were thought to be 'oat-sensitive' on clinical grounds did not have food antibodies to oats while on a gluten-free, but oat-containing, diet.

In some cases of children with relapsed coeliac disease, the mother denied that the child was off the gluten-free diet. One child was admitted because she had fallen below the expected centiles in height and weight, and had considerable gaseous abdominal distension. Antibodies to ruminant antigens were present in the plasma. In the ward the child put on 1.3 kg after 3 weeks of strict gluten-free diet and the mother admitted that the child had previously eaten outside her diet.

Discussion

The series of food antibody results performed at diagnosis confirms our earlier finding (Ferguson and Carswell, 1972) that food antibodies are commonly found in the plasma of patients with undiagnosed and untreated coeliac disease. As 15 of our 55 patients did not have plasma food antibodies at diagnosis, the absence of plasma food antibodies does not exclude the diagnosis of coeliac disease. However, the presence of plasma food antibodies—notably to cereal and fowl antigens, which are not usually found in patients without coeliac disease—makes the diagnosis probable (Ferguson and Carswell, 1972).

Our results show that most coeliac patients responded to the gluten-free diet by losing food antibodies over the next 2 months. The small number of antibodies detected after 2 months were antibodies to ruminant and fowl antigens, presumably reflecting the continuing antigen challenge with milk and egg and the reduction in intake of cereal antigens. Patients who still had positive antibody tests after 2 months may have been more profoundly ill at diagnosis, more intolerant to a normal gluten intake, eating more gluten, or subsequently adhering less closely to the gluten-free diet. The higher plasma IgA concentrations at diagnosis offer some support for reduced tolerance or greater intake of gluten before diagnosis in these patients.

A small proportion of children with coeliac disease may have 'transient gluten intolerance' and do not require the gluten-free diet for life (Walker-Smith, 1970; Meeuwisse, 1970; Walker-Smith, 1972). Very few adequately documented cases of this have been reported. For complete diagnostic clarification, patients in whom the diagnosis of transient gluten intolerance is suspected should have a jejunal biopsy after a period of gluten re-

introduction. It is difficult to decide when to perform biopsy since abnormality may develop at any time from 1 week to 2 years after gluten reintroduction (Meeuwisse, 1970; Hamilton and McNeil, 1971; Walker-Smith, 1972). The longer the child is off the gluten-free diet, the more difficult it is to restart it. Our results suggest that frequent antibody tests may indicate the time for biopsy.

Our results do not suggest that a single testing for plasma food antibodies will reliably indicate the strictness of the gluten-free diet. However, it may be that the dietary histories obtained from the mothers were inaccurate, and the quoted example illustrates that the unexpected presence of plasma food antibodies in a coeliac child should make one suspicious of the strict adherence to the diet. Our finding that a significantly higher proportion of patients on a strict diet have food antibodies is puzzling, but it may be that the patients most sensitive to gluten take the strictest diet.

We thank Professor J. H. Hutchison and Dr. R. A. Shanks for allowing us to study patients under their care. Drs. M. L. N. Willoughby, of Paediatric Haematology, and R. W. Logan, of Paediatric Biochemistry, were most helpful in offering facilities and advice. We acknowledge the technical help of Mrs. S. Campbell of the University Department of Bacteriology and Immunology and Mr. F. Jewell of the Department of Paediatric Haematology.

REFERENCES

- Crowle, A. J. (1958). A simplified micro double-diffusion agar precipitin technique. *Journal of Laboratory and Clinical Medicine*, **52**, 748.
- Ferguson, A., and Carswell, F. (1972). Precipitins to dietary proteins in serum and upper intestinal secretions of coeliac children. *British Medical Journal*, **1**, 75.
- Hamilton, J. R., and McNeil, L. K. (1971). Coeliac disease; response to treatment and to dietary wheat gluten. *Proceedings of the XIII International Congress of Paediatrics*, **2**, 191.
- Heiner, D. C., Lahey, M. E., Wilson, J. F., Gerrard, J. W., Shwachman, H., and Shaw, K. T. (1962). Precipitins to antigens of wheat and cow's milk in celiac disease. *Journal of Pediatrics*, **61**, 813.
- Kenrick, K. G., and Walker-Smith, J. A. (1970). Immunoglobulins and dietary protein antibodies in childhood coeliac disease. *Gut*, **11**, 635.
- Meeuwisse, G. (1970). Diagnostic criteria in coeliac disease. *Acta Paediatrica Scandinavica*, **59**, 461.
- Taylor, K. B., Thomson, D. L., Truelove, S. C., and Wright, R. (1961). An immunological study of coeliac disease and idiopathic steatorrhoea; serological reactions to gluten and milk proteins. *British Medical Journal*, **2**, 1727.
- Visakorpi, J. K., Kuitunen, P., and Pelkonen, P. (1970). Intestinal malabsorption. A clinical study of 22 children over 2 years of age. *Acta Paediatrica Scandinavica*, **59**, 273.
- Walker-Smith, J. A. (1970). Transient gluten intolerance. *Archives of Disease in Childhood*, **45**, 523.
- Walker-Smith, J. A. (1972). Transient gluten intolerance. *Archives of Disease in Childhood*, **47**, 155.

Correspondence to Dr. F. Carswell, Department of Child Health, Royal Hospital for Sick Children, Bristol BS2 8BJ.