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Wheat gliadin fractions and other cereal antigens reactive with antibodies in the sera of coeliac patients

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SUMMARY

The mixed reverse solid phase passive antiglobulin haemadsorption test (MRSPAH) and the enzyme linked immunosorbant assay (ELISA) were found equally sensitive and fitted for the measurement of serum IgG antibodies against alcohol soluble gliadins. Using the ELISA method, three coeliac sera with elevated antibody titres against gliadins and two control sera with low titres were tested for IgG antibodies against the main groups of wheat proteins (acetic acid soluble glutenins, salt soluble albumins and globulins and alcohol soluble gliadins), eight fractions of gliadin and the alcohol soluble proteins of barley, rye, oat, maize and rice. As rice contained little alcohol soluble protein, a test against acid soluble rice proteins was included. In all three patient sera, titres higher than or equal to that for crude gliadin were found for wheat glutenin and for gliadin fractions seven and eight, both containing alpha gliadins. Similar high titres were found when these coeliac sera were tested against rye, barley and oat prolamines. Maize prolamines gave only low titres and no antibodies could be detected against rice proteins, in line with the tolerance of these latter two cereals by patients with coeliac disease. It would appear that sera from coeliac patients react with more than one antigenic fraction of protein in wheat and other cereals. Also sera from two normal persons appeared to have the same spectrum of reactivity against these cereal proteins as did the three sera from coeliac patients. The titres in normal sera were however much lower.

INTRODUCTION

Shortly after Dicke's original observation on the deleterious effect of wheat and rye in patients with coeliac disease (Dicke, 1950), it was established that the toxic factor was to be found in the protein fraction of grain (Van de Kamer, Weijers & Dicke, 1953). Since then, numerous but so far unsuccessful attempts have been made to separate the coeliac toxic factor.

Wheat, rye and barley proteins are known to be toxic in coeliac patients, while those of maize and rice are harmless (Anand, Piris & Truelove, 1978). There is some disagreement as to the toxicity of oats. Hansted (1955) and Baker & Read (1976) have concluded oats to be harmful, whereas several other workers have found no harmful effect of oats (Sheldon, 1955; Moulton, 1959; Rubin *et al.*, 1962; Dissanayake, Truelove & Whitehead, 1974). Serological studies of cereal proteins by double diffusion agar gel tests (Ewart, 1966) have shown common antigens in wheat, rye and barley, but no significant antigenic similarities between the proteins of wheat, oats or maize.

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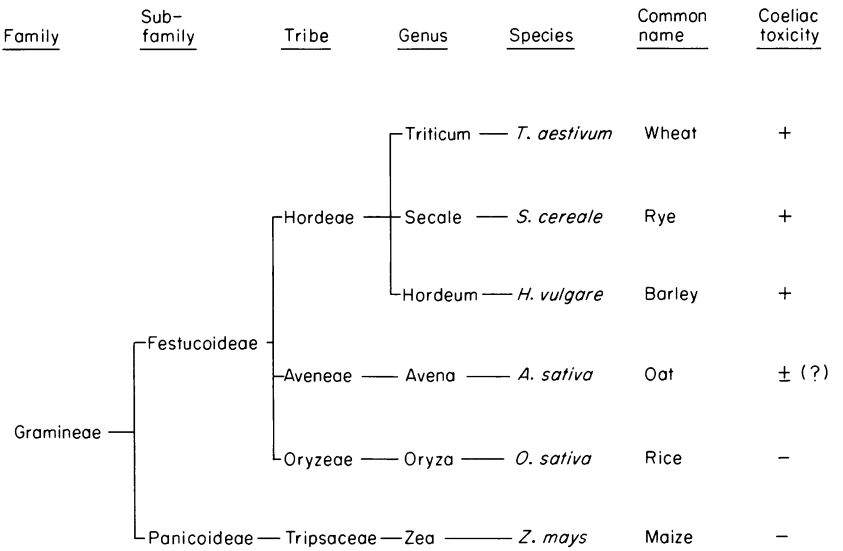

Fig. 1. Taxonomic relationships of important food cereals (Hitchcock, 1950).

Wheat, rye, barley, oats, maize and rice are all members of the Gramineae family and their taxonomic relationships are shown in Fig. 1. The proteins of the Gramineae are classified by solubility into four groups (Osborne, 1907, 1924; Kasarda, Bernardin & Nimmo, 1976): albumins, soluble in water; globulins, soluble in dilute salt solutions; prolamines, soluble in aqueous ethanol and glutelins, soluble in dilute acids (Fig. 2). The albumins and globulins are usually classified together as a 'salt soluble fraction'. The wheat prolamines are called gliadins and this fraction has been considered of greatest importance in the pathogenesis of coeliac disease. Gliadins can be separated further by gel electrophoresis into 30–50 bands, groups of which have been named alpha-, beta-, gamma- and omega-gliadins in order of decreasing electrophoretic mobility (Woychik, Boundy & Dimler, 1961; Kasarda *et al.*, 1976).

Clinically, alpha-gliadins have been shown to be toxic in patients with coeliac disease (Hekkens, Haex & Willighagen, 1970) and some evidence has been provided that alpha-gliadins may be the only toxic fraction (Kendall *et al.*, 1972; Kasada *et al.*, 1978). This is not supported by the recent work of Ciclitira, Hunter & Lennox (1980), who found that wheat lacking the chromosome coding for some of the alpha-gliadins and containing very few alpha-gliadin components, would still exacerbate coeliac disease.

It has long been established that coeliac patients have circulating antibodies against different fractions of wheat proteins and there is mounting evidence that immune mechanisms are of major importance in the pathogenesis of the disease. The literature concerning this subject was recently reviewed by Stern *et al.*, (1979a, b). Very little has been published about circulating antibodies against other cereal proteins in coeliac disease.

Recently we reported that the mixed reverse (solid phase) passive anti-globulin haemadsorption reaction (MRSPAH) was a suitable test for measurement of class specific antibodies against alcohol soluble gliadins (Kieffer *et al.*, 1981). Moreover we showed that most of the circulating antibodies in coeliac patients were of the IgG class. In the present investigation we first compared the MRSPAH test with another solid phase assay, namely the enzyme linked immunosorbent assay (ELISA). Then using the ELISA method we proceeded to measure IgG antibodies in sera from patients with coeliac disease and in healthy control persons against:

(i) eight fractions of gliadin separated by ion exchange chromatography;
(ii) the main groups of wheat proteins: gliadins, glutenins and the albumins and globulins;
(iii) the alcohol soluble proteins (prolamines) of rye, barley, oat, maize and rice.

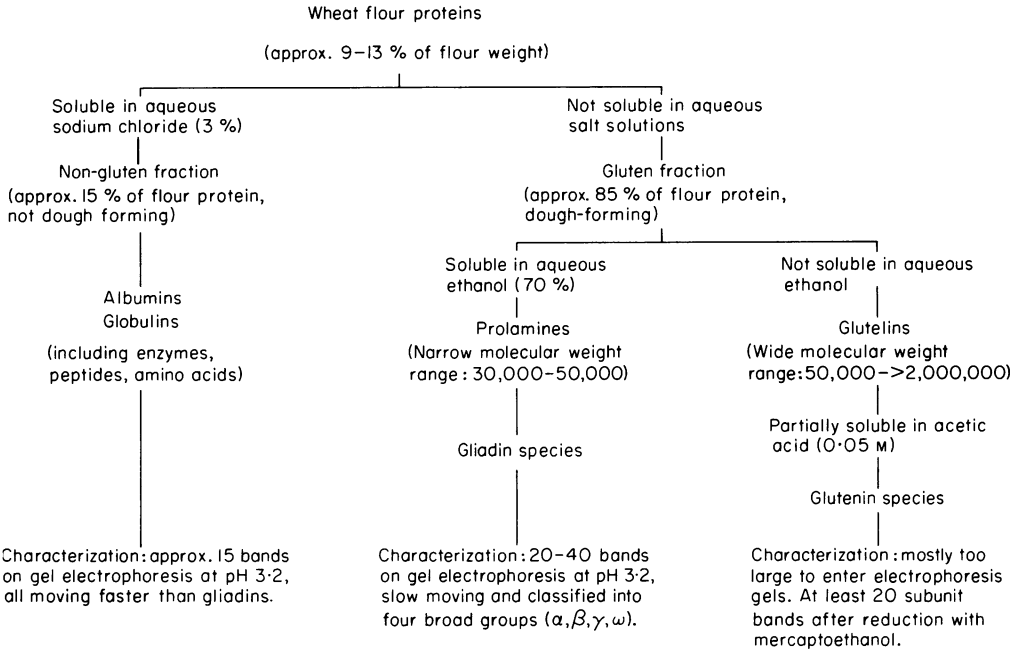

Fig. 2. Classification of wheat flour proteins by solubility.

MATERIALS AND METHODS

Preparation of cereal antigens

Crude total gliadin. This was from the same batch used previously (Kieffer *et al.*, 1981), having been extracted with 70% ethanol directly from wet-butanol-defatted strong breadmaking flour.

Wheat gliadin fractions. Gliadin fractions were prepared from crude total gliadin by ion exchange chromatography following the basic method of Patey & Evans (1973) on 1/10th scale. A column of Whatman CM 52 cellulose was used, eluting with 0·005 M sodium acetate buffer, pH 3·5, containing 1 M dimethylformamide and increasing in sodium chloride concentration from 10 to 500 mM. Eight protein fractions were obtained, designated 1–8 in order of increasing ionic strength of elution, dialysed against 0·1 M acetic acid and freeze dried.

Wheat protein groups. Crude total gliadin contained some albumins and globulins (salt solubles which are also partly soluble in 70% ethanol). In order to separate wheat proteins as rigorously as possible into albumins/globulins, gliadins and glutenins (see Fig. 2) a scheme of sequential exhaustive extraction was used.

Flour (100 g), defatted as before, was stirred for 30 min with 3% sodium chloride solution (400 ml). The supernatant was separated by centrifugation at approximately 1,200 *g* and its absorbance measured at 280 nm. The residue was re-extracted with 3% salt solution, centrifuged and the supernatant absorbance measured again. This procedure was repeated until negligible absorbance was detected in the extract. The salt solubles were then pooled, spun at 10,000 *g* for 15 min to remove any fine particulate material, dialysed against distilled water and freeze dried.

The residue from the salt extraction was stirred in 70% ethanol (400 ml) for 30 min, centrifugally separated and the supernatant absorbance measured as before. Extraction was repeated until negligible protein was found in solution. The pooled ethanol solubles were spun at 10,000 *g* for 15 min, dialysed directly against 0·05 M acetic acid (see below) and freeze dried.

Finally, the residue from ethanol extraction was repeatedly dispersed in 0·05 M acetic acid until no further protein was dissolving. The acetic solubles were spun at 10,000 *g* for 15 min and freeze dried.

Rye, barley and oat prolamines. The ethanol soluble proteins from rye, barley and oat were conveniently prepared from protein concentrates of the cereals. These concentrates were available from earlier work, having been prepared by extraction of the ground whole grains with 2 M citric acid, followed by centrifugation, dialysis and freeze drying. Fat was removed by wet butanol extraction of the concentrates as described for flour. The concentrates were then stirred in 70% ethanol as described before, except that in view of their greater protein content compared with flour, the ratio of solvent to solid was increased from 4 to 10 l/kg, typically 18–20 g concentrate being extracted with 200 ml ethanol. Removal of the ethanol by rotary evaporation was found to cause difficulty in redissolving the extracted prolamines in acetic acid for freeze drying. Instead, direct dialysis of the ethanol solution against 0·05 M acetic acid was found to be satisfactory with the relatively small volumes used.

Rice and maize proteins. Ground rice and ground maize were defatted and ethanol extracted exactly as for wheat flour, the prolamines being recovered by direct dialysis of the ethanolic solutions against 0·05 M acetic acid and freeze drying as above. Rice, as expected, yielded very little ethanol soluble material. The ground residue was therefore redispersed in 0·05 M acetic acid, centrifuged and the extract freeze dried.

At all stages during preparation of rye, barley, oat, rice and maize proteins, great care was taken to prevent any cross-contamination between these species or with wheat flour extracts.

Characterization of cereal antigens. Kjeldahl nitrogen determinations were made on all protein samples to enable MRSPAH and ELISA tests to be carried out at equal protein concentration. Stock antigen solutions were made up to be 1·0 mg dry solid/ml solvent, subsequent dilutions being adjusted according to the actual protein content. Ethanol (70%) was used as solvent for all samples except albumins and globulins (3% salt solution), wheat glutenin and rice acid solubles (0·05 M acetic acid).

Each protein sample was characterized by polyacrylamide gel electrophoresis in sodium lactate buffer, pH 3·2 (Wrigley & McCausland, 1977; Bietz, 1979) using both gradient and single strength gels. The latter (16% total acrylamide, 1·5% bis-acrylamide) were cast with a 4% stacking gel to increase band sharpness. All protein samples were run under the same conditions except that, to ensure complete solubility, the proteins were first dissolved in their respective extracting solvents before being mixed with the sodium lactate running buffer.

For brevity, electrophoretic results are presented diagramatically. A more complete characterization will be reported elsewhere.

Sources of sera. Sera from patients with coeliac disease and healthy control persons were used. All sera had been tested for gliadin antibodies earlier using the MRSPAH technique (Kieffer *et al.*, 1981).

Materials for MRSPAH and performance of tests. These have recently been described in detail (Kieffer *et al.*, 1981).

Materials for ELISA test. Microtitre plates: polystyrene Cook microELISA. Buffers; as for MRSPAH. Antigens: all antigen solutions were diluted to 50 μg protein/ml. Stock solutions were approximately 1 mg/ml. Alcohol soluble antigens were dissolved and diluted in 70% ethanol, acetic acid soluble antigens in 0·05 M acetic acid and salt soluble antigens were dissolved in 3% NaCl and diluted in carbonate buffer 0·05 M, pH 9·6.

Enzyme conjugated antibodies. Peroxidase conjugated rabbit immunoglobulins specific for human γFc were obtained from DAKO Laboratories, Denmark. The recommended working dilution of the manufacturer was 1:200–1:2,000. Checkerboard titrations demonstrated 1:1,000 to be the highest dilution giving optimal results. The enzyme conjugate was diluted in PBS-Tween.

Colour reagent. Eighty milligrams of *o*-phenylenediamine (OPD) were dissolved in 0·15 M, pH 5 citrate buffer (made up of 24·3 ml 0·01 M citric acid plus 25·7 ml 0·2 M Na_2HPO_4). This was diluted 1:1 with water and 40 μl 30% H_2O_2 were added. The reagent was made up just before use and was protected from light.

Performance of ELISA. The wells of the microtitre plates were coated with antigen by adding 75 μl of the antigen solution to each well. The plates were left at room temperature overnight and then washed three times with PBS-Tween 20. Serial doubling dilutions of sera in PBS-Tween 20 were added (75 μl per well) and incubated for 2 hr at 4°C. The plates were again washed three times with

PBS-Tween 20. Peroxidase conjugated anti-human γFc (75 μl) was added to each well and the plates were incubated for 2 hr at 4°C. After another six washes (three times in PBS-Tween 20, three times in PBS), the colour reagent was added, 75 μl per well. After 30 min at room temperature the reaction was stopped by the addition of 30 μl of 3 M H_2SO_4 to each well. The colour in each well was read as absorbance on a 'multiskan through-plate reader' (Flow Lab) at 492 nm. Controls included serum added to antigen free wells and antigen coated wells not treated with serum.

The absorbance readings were plotted versus serum dilution. Using a standard positive serum, a titration curve was constructed and the end point taken as the serum dilution having an absorbance falling in the mid-range of the linear part of the curve. The absorbance given by this 'standard dilution' was then measured in all subsequent tests and used as the 'cut-off' value for end point determinations. The absorbance of the standard dilution was found to vary between 0·70 and 1·20, this being equivalent to ± 1 $\log_2$ dilution, and thus within the limits of experimental error of a doubling dilution procedure.

RESULTS

Comparison of MRSPAH and ELISA in tests for gliadin antibodies

Seventeen sera were selected so as to represent a wide range of antibody titres against gliadin and were tested in the following assays. The MRSPAH using (1) indicator trypsin treated sheep red cells coupled with sheep anti-human γFc (Z 511G a gift from Dr A. R. Bradwell, Birmingham) and MRSPAH using (2) indicator trypsin treated sheep red cells coupled with rabbit anti-human γFc (DAKO reagent), and the ELISA using peroxidase conjugated rabbit anti-human γFc (ex DAKO). The correlation coefficients were calculated. The results are presented in Table 1. All the comparisons showed correlation coefficients approaching unity and were highly significant ($P < 0{\cdot}001$).

Table 1. Comparison of assays for gliadin antibodies using MRSPAH with two different anti-γFc reagents and ELISA

Assays compared	Correlation coefficient
ELISA–MRSPAH (1)	0·94
ELISA–MRSPAH (2)	0·95
MRSPAH (1)–MRSPAH (2)	0·96

Cereal proteins reacting antigenically in ELISA with the raised antibodies in the sera of coeliac patients

Five sera were tested for IgG antibodies to different cereal protein fractions by the ELISA. One serum had a very high antibody titre to crude gliadin, two sera had intermediate titres and two (from normal healthy persons) had low titres. All the cereal antigens were titrated in checkerboard fashion. The optimal antigen concentrations were found to lie between 10–100 μg/ml and 50 μg/ml gave optimal results for all antigens. The two sera with intermediate gliadin antibody titres turned out to give almost identical results to each other, and so did the two sera with low antibody titres. For simplification the results of only one of each of these two pairs of sera are shown in Figs 3 & 4.

Different fractions of gliadin

The sera were tested for antibody reactivity against eight different fractions of gliadin. Results are presented in Fig. 3 together with a diagram of the electrophoretic characterization of each fraction. Compared with the total gliadin sample (T), the early fractions (1–4) gave relatively low titres.

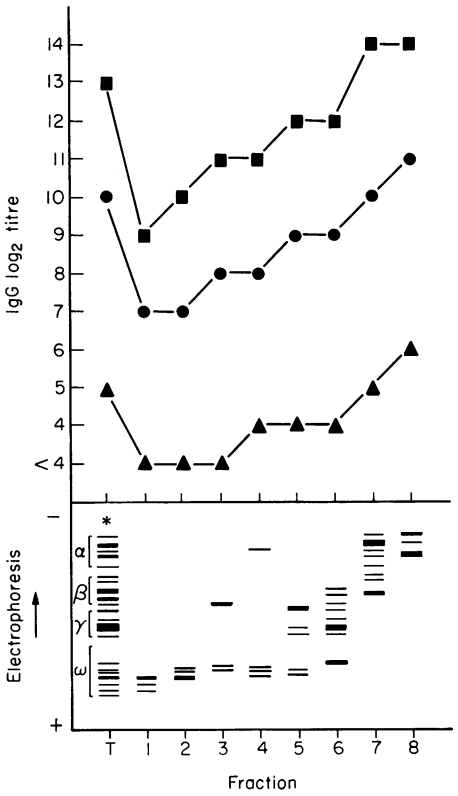

Fig. 3. Serum antibody titres for two coeliac patients (■,●) and a normal healthy person (▲) against crude total wheat gliadin (T) and eight fractions of gliadin (1–8) separated by ion exchange chromatography. A further medium titred coeliac serum and another normal serum gave identical results to those illustrated. Characterization of the protein samples by polyacrylamide gel electrophoresis at pH 3·2 is shown diagrammatically. (*Note crude total gliadin contained further bands, in the albumin/globulin region, not shown in this diagram).

Nevertheless, results from coeliac patients' sera were markedly higher than for normal sera. Gradually the titres increased, following the trend of increasing electrophoretic mobility of the fractions, until the last fraction (8) which revealed slightly higher antibody titres than for the crude (unfractionated) gliadin. It is particularly interesting to note that the normal sera showed a similar pattern of antibody titres, although, of course, at a much lower level.

When comparing results of the electrophoretic characterization in Fig. 3, it should be noted that the crude total gliadin (T) contained a significant number of albumin/globulin bands of very high mobility which are not shown on the diagram. However, the ion exchange fractionation procedure employed for fractions 1–8 largely eliminated these fast moving proteins from the gliadin fractions. Only fraction 7 appeared to have any trace of albumin/globulin bands and these were very faint.

The two fractions (1 and 2) with the lowest titres both contained three bands exclusively in the omega-gliadin region, but fraction 1 bands were clearly different from fraction 2 bands. Fractions 3 and 4, with the next highest titres, also contained further bands in the omega-gliadin region. However, fraction 3 also showed a major band in the beta-gliadin region while fraction 4 had a major band in the alpha-gliadin region. Titres increased again with fractions 5 and 6 which consisted mainly of bands in the gamma- and the slower part of the beta-gliadin region, although one or two bands were still present in the omega-gliadin region. Fraction 7, with next to the highest titre, on average, consisted mainly of several bands in the beta- and alpha-gliadin region. However, this fraction streaked badly in the gel (not possible to show in the diagram) and, as mentioned above, also contained traces of fast moving albumin/globulin material. The highest titre was shown

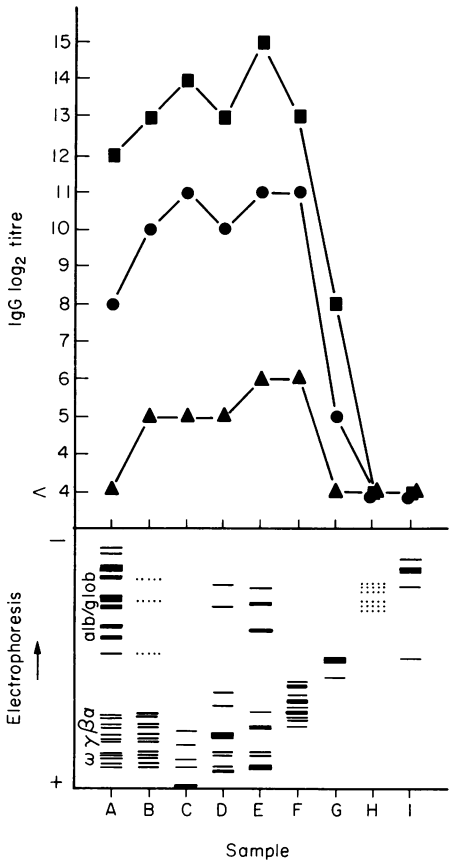

Fig. 4. Serum antibody titres for two coeliac patients (■,●) and a normal, healthy person (▲) against (A) wheat albumins and globulins separated by exhaustive extraction of defatted flour with 3% salt solution; (B) wheat gliadins separated by exhaustive extraction with 70% ethanol after extraction A; (C) wheat glutenins separated by exhaustive extraction with 0·05 M acetic acid after extractions A and B; (D) rye prolamines separated by 70% ethanol extraction of defatted protein concentrate; (E) barley prolamines separated as in D; (F) oat prolamines separated as in D; (G) maize prolamines separated by 70% ethanol extraction of defatted ground maize; (H) rice protein separated by 70% ethanol extraction of defatted ground rice; (I) rice protein separated by extraction with 0·05 M acetic acid after extraction H. A further medium titred coeliac serum and another normal serum gave identical results to those illustrated.

by fraction 8 which was a very clean cut showing one major and two minor bands in the alpha-gliadin region.

These results therefore indicate that the alpha-gliadins are the main antigenic stimulus in coeliac patients, and also, but to a much smaller extent, in normal persons. However, significant antigenic activity is also present in the beta and gamma fractions and it would be difficult to exclude some activity in the omega-gliadin region.

The main groups of wheat proteins

The same sera were next tested (Fig. 4) for antibodies to the salt soluble wheat albumins and globulins (A), the alcohol soluble gliadins (B) separated as completely as possible from the salt solubles, and the acetic acid soluble glutenins (C) separated as completely as possible from both gliadins and salt solubles. In all coeliac sera the highest antibody titres were measured using the acid soluble wheat glutenins. This activity was higher than for the exhaustively extracted gliadins so the glutenin antigenic activity was unlikely to have been due to traces of protein still present in the

gliadin region (compare C and B, Fig. 4). The majority of the glutenin proteins, of very high molecular weight, were too large to enter the electrophoresis gel and remained at the origin.

Antibody titres against the exhaustively extracted gliadins were identical to those obtained earlier for the crude total gliadins (compare Fig. 4B with Fig. 3 T). This activity was therefore almost certainly not due to the presence of contaminating albumin/globulin material since the exhaustively extracted gliadins showed only very faint traces of bands in the fast moving region of the electrophoregram (compare B and A, Fig. 4).

Only slightly lower antibody titres were measured against the salt soluble albumins and globulins. However, interpretation here is more difficult since proteins with the electrophoretic characterization of gliadin were also present in the salt extract (see Fig. 4 A). It appears that exhaustive extraction with 3% salt dissolves significant quantities of material in the gliadin region. An earlier single extraction with salt showed far fewer bands in the gliadin region (not shown in Fig. 4), but this extract also gave very high titres with sera from coeliac patients, thus indicating antigenic activity of the albumin/globulin fraction.

It must be concluded from these results that, although the gliadins have been confirmed as a major antigenic stimulus in coeliac disease, and within this fraction the alpha-gliadins have been shown to have the greatest antigenic activity, nevertheless both the water soluble albumins and globulins and the acid soluble glutenins also have very significant antigenic activity in coeliac patients. This latter result is regarded as particularly important since the titre for high molecular weight glutenins was as high as for alpha-gliadin.

Proteins of other cereals

Finally, the same sera were tested for antibodies against the alcohol soluble proteins (prolamines) of rye, barley, oats, maize and rice, again at their optimal antigen dose. Rice was found to contain considerably less alcohol soluble protein than the other cereals. Consequently the sera were also tested for antibodies against the acetic acid soluble proteins of rice. The results are presented in Fig. 4. Rye, barley and oat (D, E and F respectively) gave antibody titres which were similar to or slightly higher than those measured to wheat gliadin (B). Only low antibody titres were found to maize (G) and no antibodies could be demonstrated to rice proteins (alcohol soluble, H or acid soluble, I).

Electrophoretic characterization of these cereal proteins showed rye prolamines to contain bands in the same region as wheat omega- and gamma-gliadins, but little or nothing in the beta- or alpha-region (compare Fig. 4 D with B). Rye also contained significant bands in the albumin/globulin region. Barley prolamines, with the highest recorded antibody titre, showed strong bands in the omega- and beta-regions (see Fig. 4 E), a faint band in the alpha-gliadin region and quite heavy bands in the albumin/globulin area. Oat prolamines showed many closely spaced bands, but only in, and just ahead of, the wheat alpha-gliadin region (compare Fig. 4 F and B). Maize prolamines with relatively low antigenic activity, showed only one major and one very minor band in the albumin/globulin region (see Fig. 4, G) and no bands in the gliadin region, although some protein material remained in the stacking gel. Only a very small quantity of ethanol soluble protein could be obtained from rice. In had no discernable antigenic activity and gave only very faint bands in the albumin/globulin area (Fig. 4, H). The acid soluble protein from rice also had negligible antigenic activity but produced a broad band in the albumin/globulin region (see Fig. 4, I). Compared with extracts of other cereals, neither the ethanol nor the acid extract of rice had very high protein contents. Dilutions were adjusted to provide antigen solutions at 50 μg protein/ml for the ELISA tests. Since electrophoretic characterization showed bands only in the albumin/globulin region, future work on rice protein antigenic activity would be better carried out on salt or buffer extracted proteins.

The results of this work indicate clearly that the 70% ethanol soluble proteins of rye, barley and oat are similar to those of wheat in antigenic reactivity with coeliac sera. This activity is not apparently due to any single, common protein in the alpha-gliadin band or any other electrophoretic region. The activity of oat prolamines in these tests appeared quite unequivocal despite uncertainties in the literature as to the effect of oats in coeliac disease. Maize prolamines had low but definite activity even though maize is reported to be harmless to coeliac patients. Rice is also

known to be harmless and both ethanol and acetic acid soluble proteins had no discernable activity towards coeliac sera.

DISCUSSION AND CONCLUSIONS

Choice of test method for 'coeliac' antibody

In our first report (Kieffer *et al.*, 1981) we used the MRSPAH reaction to measure gliadin antibodies in patients with coeliac disease. The present study has shown that this reaction and the ELISA test gave very comparable results. MRSPAH is very simple to perform and can be read without any elaborate equipment as a microplate haemagglutination test. The ELISA test is probably more economic because the anti-immunoglobulin reagent and the enzyme coupled reagents can be kept for long periods at 4°C while the red cell coupled reagents at present have to be prepared every 2–3 weeks. The two assays are equally sensitive but the ELISA is read on a photometer which makes the results independent of individual judgement. For coating the microtitre plates for these tests the crude gliadin extract in 70% ethanol was a satisfactory antigen for screening sera for 'coeliac' antibody.

Identity of cereal proteins of importance in coeliac disease

The most important question in coeliac disease is, of course, what is the cereal protein component responsible for inducing the intolerance and intestinal lesions? A major problem in attempts to isolate this factor is the complex composition of cereal proteins. As shown in Fig. 2, the main groups of proteins are characterized by their solubility properties. However, it must be emphasized that separation of cereal proteins by solubility does not provide exact or precisely 'pure' fractions. A spectrum of solubility exists and the composition of each fraction can vary appreciably depending on the solubilization procedure employed. In particular, the Osborne method depends on sequential extraction. A considerable proportion of the salt soluble fraction is also soluble, together with the gliadins, in 70% ethanol, and albumins, globulins and gliadins may be extracted by acetic acid along with the glutenins. Unfortunately, as seen in Fig. 4A, exhaustive extraction to remove albumins and globulins completely, tends to extract significant quantities of gliadin. The possibility of carry over of a very active antigen can therefore never be entirely discounted in these fractionation methods.

Sub-fractionation of gliadins by ion exchange chromatography can produce relatively narrow fractions, but only with very lengthy procedures could pure, single proteins be obtained. The alpha-gliadin group of proteins has been postulated to contain the coeliac toxic factor (Kendall *et al.*, 1972), possibly due to the A-gliadin protein within this group (Kasarda *et al.*, 1978). In the present work, alpha-gliadin bands were seen in the crude gliadin and its ion exchange fractions 4, 7 and 8 (Fig. 3), in the salt soluble fraction of wheat proteins and in barley and oat prolamines (Fig. 4). All these fractions gave high antibody titres. However, the titre for barley was disproportionally high for this to have been due to the very faint band in the alpha-region, and wheat glutenins (after prior exhaustive salt and ethanol extraction) and rye prolamines both had high antibody titres, yet showed no bands in the alpha-gliadin region (Fig. 4). Similarly gliadin fractions 3, 5 and 6 (Fig. 3) had moderately high titres but no trace of bands in the alpha-gliadin region.

In conclusion, our findings show that the antibody response in coeliac disease is not restricted to any single fraction of wheat protein. We agree with others that the alpha-gliadins are strongly implicated, but it would take a much more extensive investigation to discount activity *per se* in other gliadin fractions. Likewise activity cannot be excluded from the glutenins or even the albumins and globulins. In addition serum antibodies are found against rye, barley and oat proteins, the first two of which are known to be toxic in coeliac disease whilst the latter is under suspicion. No significant antibodies could be found against maize and rice cereals known to be tolerated by patients with coeliac disease.

Finally, and of considerable interest, is the discovery that sera from two normal persons gave the same pattern of reactivity against the various fractions and samples as did sera from three coeliac patients—the difference being only in the magnitude of the titres. This suggests that the gliadin

antibodies found in coeliacs may not be specially directed against a specific antigen(s) operative in the disease process, but reflect rather the heightened antibody response to raised levels of gut absorbed antigens. If so, the patterns observed are likely to be due to the relative antigenicity of the different cereal proteins.

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