

IgE antibodies to wheat flour components

Studies with sera from subjects with bakers' asthma or coeliac condition

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Summary

Sera from two subjects with bakers' asthma and six patients with coeliac condition were examined for the presence of IgE antibodies with specificities for wheat flour components. Sera were studied using the radioallergosorbent test (RAST) together with whole flour and thirteen purified and partially purified flour fractions.

IgE antibodies to a number of flour components were demonstrated in the allergic bakers' sera, but the strongest reactivities were observed with wheat albumins and globulins. A more detailed examination of the flour water-soluble proteins using RAST inhibition methods demonstrated that albumins were more reactive with the allergic sera than the globulins. Apart from the results with the water-soluble proteins, the two sera showed a different pattern of reactivity with the other flour preparations.

No IgE antibodies to whole flour or any of the flour components, including A gliadin, were found in the coeliac sera. Failure to detect wheat gluten- or gliadin-specific IgE antibodies indicates that IgE-mediated reactions are not important in the pathogenesis of coeliac condition. Levels of total IgE in the sera from the coeliac subjects were elevated and, with two of the sera, some success was achieved in identifying the allergens responsible for the elevation. We propose that elevation of serum IgE may frequently occur in coeliac condition and may arise due to an increased uptake of antigens via the damaged intestinal mucosa.

Introduction

Hypersensitivity to certain foodstuffs is well recognized (Bleumink, 1970; Goldstein & Heiner, 1970; Freier, 1973) and it is clear that many reactions to a number of foods are true type I IgE-mediated allergic responses (Aas & Lundkvist, 1973; Hoffman & Haddad, 1974; Chua *et al.*, 1976). Amongst a wide range of foodstuffs implicated in food allergy, cereal grains, including wheat, have been shown to evoke IgE-mediated allergic responses in human subjects (Heiner, Goldstein & Rose, 1970; Hoffman & Haddad, 1974; Hoffman, 1975; Caldwell, Tennenbaum & Bronstein, 1975). Not all such responses are initiated by the ingestion of foods. So-called bakers' asthma, for

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example, is a form of allergic asthma induced by the inhalation of cereal flour (Schwartz 1947). Cereal flours, like many natural extracts which provoke allergic reactions, are complex mixtures comprising a wide range of compounds known to have potential immunological reactivity. Although hypersensitivity to flour has been recognized for over 40 years (de Besche, 1929; Stresemann, 1967; Popa, George & Găvănescu, 1970; Blands *et al.*, 1976; Hendrick, Davies & Pepys, 1976; Wilbur & Ward, 1976), little work has been done on identifying the allergens involved in provoking bakers' asthma.

Coeliac condition, or gluten-sensitive enteropathy, is a disorder characterized by the malabsorption of dietary components and by pathological changes in the jejunal mucosa. Reversal of the clinical features and structural abnormalities in the small intestine can be achieved by withdrawal of gluten from the diet (Gryboski, 1975). The toxicity of wheat gluten is thought to be particularly due to the A-gliadin fraction of the α -gliadin proteins (Kendall *et al.*, 1972; Hekkens, Haex & Willighagen, 1970; Falchuk *et al.*, 1974). Although the aetiology of coeliac condition is uncertain, it has been suggested that it has an immunological basis. Evidence given in support of this is the development of 'gliadin shock' in coeliac children after reintroduction of gluten (Krainick *et al.*, 1958). This response suggests anaphylaxis and hence the involvement of gluten-specific IgE antibodies.

In an attempt to obtain information on the possible role of IgE and wheat flour components in bakers' asthma and coeliac condition, whole wheat flour and flour components were used in radioallergosorbent test (RAST) studies with sera from coeliac patients and from two individuals allergic to wheat flour. In RAST studies with coeliac sera, particular attention was paid to the gliadin components of wheat flour.

Materials and methods

Sera

Sera were obtained from two bakers (Mas. and Zir.) who reported asthma-like symptoms after exposure to flour. Clinical diagnosis, including prick-testing with an extract of whole wheat flour, confirmed that the subjects were allergic to wheat flour. Sera from children with coeliac condition were provided by Dr G. L. Barnes, Department of Gastroenterology, Royal Children's Hospital, Melbourne. Three of the sera were from children with acute coeliac disease while three other serum samples were from coeliac patients on a gluten-free diet. Sera from non-allergic, healthy adults and children were provided by the Red Cross Blood Transfusion Service, Perth, West Australia. Sera from cord blood were obtained from the St John of God Hospital, Perth.

Wheat flour and flour components

Flour for fractionation and immunological testing was milled on a Buhler laboratory mill from bread wheat of the variety Timgalen. The flour protein content was 13%.

Gluten was prepared by hand-washing, in water at room temperature, dough made from flour which had been defatted by four successive extractions with chloroform. The gluten was freeze-dried. After removing the starch from the combined washings by centrifugation (10,000 g), a sample of the supernatant was taken ('water-soluble fraction undialysed') and the rest of the supernatant was dialysed against water before freeze-drying.

In addition, commercial dry gluten, 'devitalized' by heat treatment, was used in studies on the effect of deamidation. The gluten (30 g dry weight) was treated in one experiment with sodium hydroxide (2 g) in 1 litre water at 100°C. After 3 min the mixture was chilled, neutralized and freeze-dried. Estimation of its non-dialysable alkali-labile nitrogen content, by a modification of the procedure of Ronalds (1974), indicated that this preparation retained 80% of its original amide groups. More extensive deamidation was produced by heating 30 g gluten with 4 g sodium hydroxide in 40 ml water at 100°C for 10 min. The preparation contained 40% of its original amide groups.

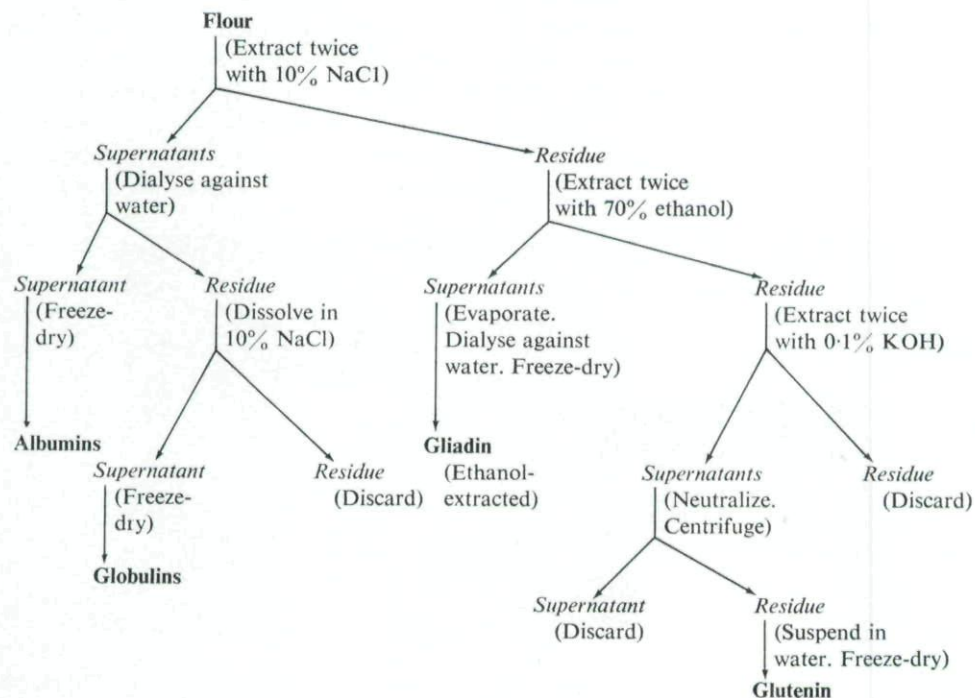


Fig. 1. Fractionation procedure for preparing albumins, globulins, gliadin and glutenin using the solvent systems originally used to define these protein classes by Osborne (1907). For each extraction the residue was dispersed by brief sonication followed by magnetic stirring for 1 hr.

Albumin and globulin proteins were extracted (Fig. 1) from flour (20 g) with 400 ml of 10% sodium chloride solution. This and a second extract were combined and dialysed against water. The dialysate was centrifuged clear (30,000 g) and the supernatant, freeze-dried, was designated 'albumin fraction'. The residue from dialysis was redissolved in 10% sodium chloride, centrifuged (30,000 g) and again dialysed against water before freeze-drying to produce the globulin fraction.

Four gliadin fractions were examined. One was a commercial preparation obtained from the Sigma Chemical Co., St Louis, U.S.A. A second was extracted from hand-washed wet gluten with 2 M urea. The clarified extract was dialysed against water and freeze-dried. A third gliadin sample was prepared by extraction with 70% (v/v) ethanol (400 ml) of the flour residue left after preliminary treatment with 10% NaCl solution (Fig. 1). Two successive ethanol extracts, clarified by centrifugation

(30,000 g.) were vacuum-evaporated to half volume, dialysed against water and freeze-dried. A gliadin (Platt, Kasarda & Qualset, 1974), kindly provided by Dr D. D. Kasarda, was prepared by the method of Bernardin, Kasarda & Mecham (1967).

Glutenin was extracted with 400 ml of 0.1% potassium hydroxide from the residue left after gliadin extraction (Fig. 1). Two combined clarified extracts were neutralized promptly with solid sodium dihydrogen phosphate to pH 7.0. The precipitated protein, separated by centrifugation (30,000 g), was suspended in water and freeze-dried.

An arabinogalactan-peptide was also studied. It was prepared by the method of Fincher, Sawyer & Stone (1974) and was kindly supplied by them.

Protein fractions were checked for purity by gel electrophoresis. Fractionation in starch gel at pH 3 (Fig. 2) separates the albumins and globulins from the gliadin proteins since the latter have much lower mobilities. A gliadin corresponds in mobility to the α gliadins, which have the highest mobility for this group of proteins. Gel isoelectric focusing (Wrigley, 1971), either alone or in combination with gel electrophoresis (Wrigley, 1976), showed that even A gliadin still contains a large number of proteins. Much of the glutenin, excluded from the starch gel (Fig. 2) because of its

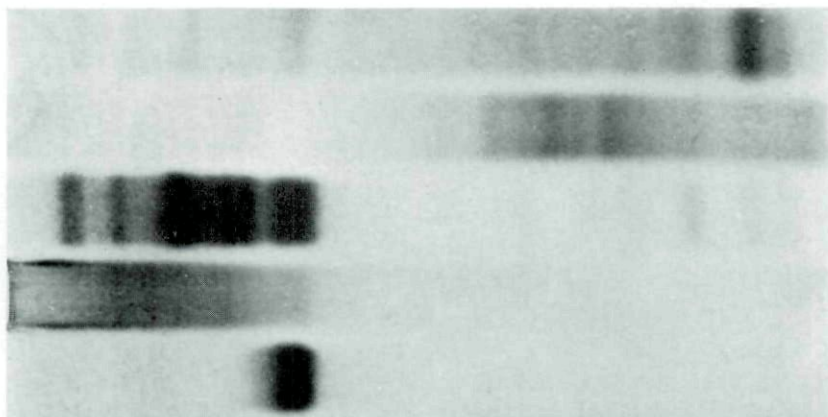


Fig. 2. Gel electrophoresis of flour protein fractions, prepared as shown in Fig. 1, in 12% starch (Connaught Laboratories, Ontario) containing 2 M urea and 0.5% aluminium lactate adjusted to pH 3.1 with lactic acid (Wrigley & Shepherd, 1974). Samples, applied at left, migrated towards the cathode (at right): albumins (top), globulins, gliadin (ethanol-extracted), glutenin, A-gliadin (bottom).

high molecular weight, remains at the point of application. The glutenin protein of intermediate molecular size (Wrigley, 1965) migrates slowly as a diffuse region. The only cross-contamination detectable (at high loadings) in the samples examined by starch gel electrophoresis was between the albumin and gliadin preparations. A more stringent check on cross-contamination between albumins and globulins is provided by polyacrylamide disc electrophoresis according to the method of Davis (1964). Examination by this procedure suggested that the amount of one type of protein in the other was very low.

Allergen discs

Allergen preparations were coupled to Whatman, grade 44, 6 mm paper discs using cyanogen bromide as described by Ceska, Eriksson & Varga (1972). *Aspergillus fumigatus* and *Candida albicans* extracts were coupled to discs as previously described

(Baldo & Pepys, 1976). Rye grass (*Lolium perenne*) pollen and house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) prick/scratch-testing solutions were obtained from Bencard Ltd, Brentford, England. These preparations were dialysed, concentrated and coupled to discs as described by Baldo, Quinn & Turner (1975), using 25 mg rye grass extract or 20 mg mite extract per 100 activated discs. Dandelion (*Taraxacum vulgare*) pollen, house dust, cat epithelium, dog epithelium, *Cladosporium herbarum*, egg white and milk Phadebas RAST allergen discs were obtained from Pharmacia, Uppsala.

Total IgE estimation

The concentrations of IgE in human sera were measured by the radioimmunosorbent test (RIST) using Phadebas IgE test kits. Incorrectly high values were avoided by using the factor 0.96 in the calculation of serum total IgE concentrations (Phadebas IgE test information booklet, Pharmacia Diagnostics AB, Sweden: Nye *et al.*, 1975; Johansson, Berglund & Kjellman, 1976).

RAST and RAST inhibition procedures

Allergen discs, together with either test, reference or cord sera (50 μ l), were incubated at room temperature for 3 hr and washed three times with phosphate-buffered saline (PBS) before the addition of 20,000–40,000 ct/min per tube of immunoabsorbent-purified ^{125} I-labelled anti-IgE (Pharmacia, specific activity 6.2 μ Ci/ μ g). Tubes were rotated for 15 hr at room temperature, washed five times with PBS containing 0.5% bovine serum albumin and 0.5% Tween 20 and the remaining radioactivity was measured by counting each tube in a Packard Auto-Gamma spectrometer. For inhibition studies, sera or serum dilutions (50 μ l) were rotated overnight at 4°C in a total volume of 200 μ l with the preparation being tested for inhibition. Discs coupled to the appropriate flour component(s) were then added to each tube and the RAST procedure continued as above. Percentage inhibition was calculated with reference to the radioactive uptake in tubes where allergic sera were preincubated with saline instead of inhibitor.

Results

Serum IgE levels

Sera from both allergic bakers contained elevated levels of total IgE. Serum from subject Mas. contained 1250 u/ml while the concentration of IgE in serum Zir. was even higher at 9000 u/ml. IgE levels found in the sera of coeliac subjects Las., Nat., Nol., Pet., Sma. and Smi. were, respectively, 250, 20, 550, 1225, 775 and 230 u/ml. In each case these values are significantly higher than the normal levels found in the sera of healthy children (Kjellman, Johansson & Roth, 1976). Total IgE levels for the cord sera and sera from non-allergic individuals were all within the normal range (Nye *et al.*, 1975).

Direct RAST studies using whole wheat flour and flour components

Estimation of coupling ratios. Sera from wheat flour-allergic subjects Mas. and Zir. were used in RAST experiments with CNBr-activated paper discs which had been reacted with increasing quantities of whole wheat flour. In this way, the optimum quantity of flour which produced maximum uptake of labelled anti-IgE by the coupled

discs was determined. Optimum coupling ratios for each of the flour components investigated were also determined. For example, both A gliadin and the purified ethanol-extracted gliadin fraction were carefully examined over a wide concentration range for binding to the allergic sera. When serum Zir. and serum Mas. were used in RAST experiments with the gliadins (Table 1), maximum uptake of ^{125}I -labelled anti-IgE occurred with 250–400 μg of the ethanol-extracted gliadin preparation per disc. Hence, 250 μg of this preparation was used in all further direct RAST studies with sera from the allergic and coeliac subjects.

Table 1. Estimation of the binding of IgE antibodies from flour-sensitive subject Mas. to paper discs coupled to wheat gliadins

Gliadin preparation	Amount used per disc (μg)	Radioactive uptake (%) with serum:	
		Cord	Mas.*
Gliadins (ethanol-extracted)	5	n.t.	1.0
	10	0.4	1.0
	20	n.t.	1.0
	50	n.t.	1.3
	100	0.5	1.4
	150	n.t.	1.7
	200	n.t.	2.0
	250	n.t.	2.5
	300	n.t.	2.4
	400	0.5	2.5
A gliadin	5	n.t.	0.5
	10	0.5	0.5
	20	n.t.	0.6
	50	n.t.	0.5
	100	0.5	0.5
	150	n.t.	0.5
	200	n.t.	0.6
	250	0.5	0.6

n.t. = Not tested.

* Diluted 1:2.

Approximately the same quantity of flour (450–500 μg) or flour components (150–300 μg) was needed for the reaction with each activated paper disc to produce maximum binding with the IgE antibodies in both of the allergic sera examined. The actual quantities used per disc were 500 μg whole flour, 300 μg water-soluble fraction (undialysed), 150 μg water-soluble proteins, 250 μg gliadins (urea-extracted), gliadins (ethanol-extracted) and gliadins (Sigma), 200 μg A gliadin, gluten (hand-washed), gluten (commercial devitalized), gluten 80% amide and gluten 40% amide, and 250 μg glutenin. Although the wheat arabinogalactan-peptide (5–750 $\mu\text{g}/\text{disc}$) did not react with either allergic serum, this preparation (500 $\mu\text{g}/\text{disc}$) was included in subsequent studies with allergic, non-allergic, cord and coeliac sera. Some other flour preparations reacted with one, but not both, allergic sera. Serum Mas. contained IgE antibodies which reacted with fully amidated gluten, but no significant binding occurred when this serum was examined in RAST experiments with discs coupled to the partially deamidated gluten preparations. By contrast, serum Zir. showed clear reactivity with

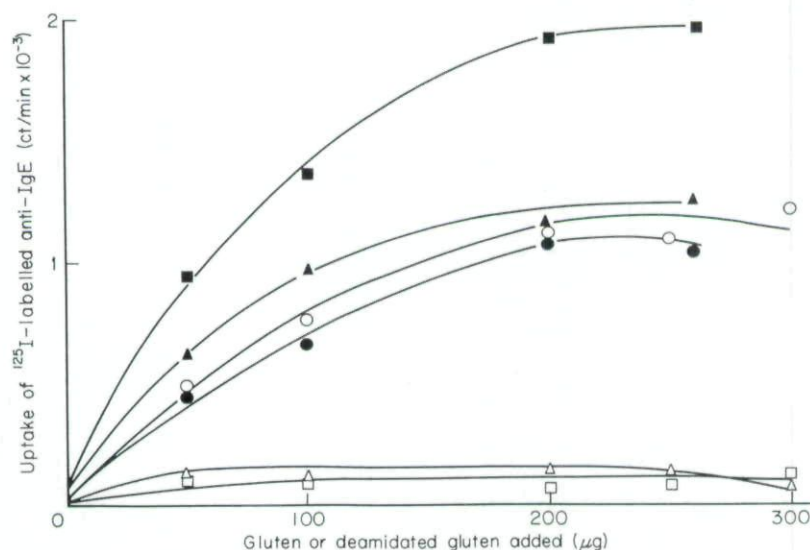


Fig. 3. Curves, obtained from radioallergosorbent test experiments, showing the uptakes of ^{125}I -labelled anti-human IgE after incubating sera from wheat flour-allergic subjects with paper discs which had been reacted with wheat gluten preparations. Serum from subject Mas. used with discs coupled to gluten (○), deamidated gluten containing 80% of the original amide content (□) and deamidated gluten containing 40% of the original amide content (△). Serum Zir. with discs coupled to gluten (●), deamidated gluten containing 80% of the original amide content (■) and deamidated gluten containing 40% of the original amide content (▲). For details of the gluten preparations examined see Materials and Methods section.

unmodified gluten and greater uptake of IgE antibodies occurred with the deamidated gluten discs than with discs coupled to native gluten (Fig. 3).

Examination of allergic sera. Having obtained the optimum quantities of flour and flour components to use with the activated paper discs, whole flour and all of the flour fractions were compared side-by-side in direct RAST experiments with both allergic sera, cord serum and seven different non-allergic (normal) sera. Results with cord and normal sera showed that no significant reaction occurred with flour or any of the flour components examined. The findings with a pooled sample of cord serum and with one serum from a non-allergic adult are shown in Table 2. Sera Mas. and Zir. reacted with whole flour and a number of the flour components, but the patterns of reactivity shown by the two allergic sera were different (Table 2). For example, although both sera showed marked reactivity with the mixed water-soluble proteins, serum Zir. reacted with a wider range of flour components. Major differences between sera Zir. and Mas. were the clear reactivities shown by the former for A gliadin, glutenin and the partially deamidated gluten preparations. Serum Mas. showed little or no reaction with A gliadin, both deamidated gluten preparations, glutenin and the arabinogalactan-peptide. Serum Zir. reacted significantly with all of the preparations tested except the arabinogalactan-peptide (Table 2).

The strongest reactions of both sera were with the water-extractable components of the flour. Removal from this material of small molecules (by dialysis) increased the reactivity, indicating that the water-soluble proteins (albumins and globulins) are the most reactive components. Separate examination of the wheat flour albumins and globulins revealed that each of these preparations reacted strongly with sera from

Table 2. RAST studies on normal adult and cord sera and sera from individuals with bakers' asthma or coeliac condition. Examination for the presence of IgE antibodies with specificity for wheat flour components

Serum	Clinical diagnosis	Age of coeliac subject when serum obtained (years)	Whole wheat flour	Water-soluble fraction (undialysed)	Water-soluble proteins	Gliadins (urea-extracted)	Gliadins (ethanol-extracted)	Gliadins (Sigma)	A gliadin	Gluten (hand-washed)	Gluten (commercial devitalized)	Radioactive uptake (%) with discs coupled to:			
												Gluten (80% amide)	Gluten (40% amide)	Gluten	Arabinogalactan-peptide
Pooled cord	—	—	0.8	0.5	0.7	0.7	0.5	0.6	0.6	0.7	0.7	1.0	0.8	0.6	0.5
Dil.	—	—	0.6	0.7	0.7	0.8	0.5	0.5	0.7	0.8	0.9	0.8	0.8	0.9	0.7
Mas.	Non-allergic adult	—	5.6	8.0	16.9	5.8	3.3	4.4	1.0	7.0	4.2	1.5	0.8	1.1	0.5
Zir.	Allergic to wheat flour	—	9.4	7.0	9.6	7.5	2.0	3.4	2.5	9.1	5.1	8.8	5.3	7.2	0.7
Nat.	Allergic to wheat flour	—	0.6	0.6	0.7	0.7	0.6	0.5	0.5	0.7	0.6	1.1	0.8	0.5	0.8
Nol.	Acute coeliac condition	6.5	0.8	0.8	0.8	1.0	0.7	0.8	0.8	1.0	0.8	1.1	0.9	0.1	n.t.
Sma.	Acute coeliac condition	9	1.2	0.7	0.9	0.7	0.6	0.7	0.7	0.9	0.7	1.1	0.8	0.7	n.t.
Las.	Acute coeliac condition	4	0.8	0.6	0.7	0.7	0.5	0.8	0.7	0.9	0.6	1.1	0.8	0.6	0.6
Smi.	Coeliac condition (on gluten-free diet 2 months)	4	0.7	0.6	0.8	0.6	0.5	0.6	0.8	0.9	0.6	1.1	1.0	0.6	n.t.
Pet.	Coeliac condition (on gluten-free diet 2.5 years)	14.5	0.8	0.7	1.0	0.9	0.7	0.9	0.7	0.9	0.9	1.4	0.9	0.9	n.t.
	Coeliac condition (on gluten-free diet several years)														

n.t. = not tested.

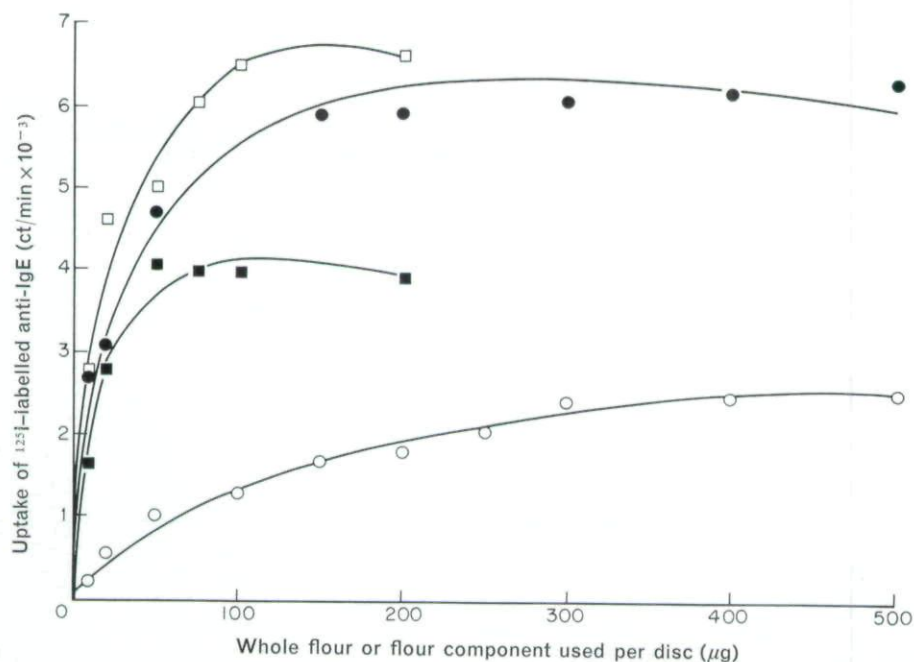


Fig. 4. Curves, obtained from radioallergosorbent test experiments, showing the uptakes of ^{125}I -labelled anti-IgE after incubating serum from wheat flour-allergic subject Mas. with paper discs which had been reacted with whole wheat flour (○), wheat water-soluble proteins (●), wheat albumins (□) and wheat globulins (■). Serum Mas. was used at a dilution of 1:2.

flour-allergic subjects (most strongly with albumin), but not with cord or normal sera. With serum Mas., radioactive uptakes (Fig. 4) in direct RAST experiments with whole flour, the water-soluble protein preparation, albumins and globulins were 12%, 29.4%, 31.1% and 19.1% respectively. Radioactive uptakes with pooled cord sera and with sera from seven non-allergic individuals were all less than 1%.

Examination of coeliac sera. No positive reactions were obtained when the six sera from subjects with coeliac condition were used with the whole flour or any of the flour components. In particular, radioactive uptakes by discs coupled to gliadins were not significantly different from the uptakes observed with normal and cord sera (Table 2).

Inhibition studies with allergic sera and wheat water-soluble proteins

The marked reactivity of the two sera from flour-allergic subjects for the wheat water-soluble proteins was further investigated using RAST inhibition procedures. Activated discs were reacted with the water-soluble protein preparation and the capacities of the mixed soluble proteins and the purified albumins and globulins to inhibit the binding of sera Mas. and Zir. to the discs were determined. The water-soluble protein preparation and the purified albumins were approximately equally active in inhibiting the binding of the IgE antibodies in both allergic sera. Significantly more globulins than albumins or mixed soluble proteins were needed to inhibit the binding of the allergic sera (Fig. 5). For 50% inhibition, approximately eighteen times as much globulins as albumins or mixed soluble proteins were needed to inhibit serum Mas. For serum Zir., however, the ratio globulins:albumins (or mixed soluble

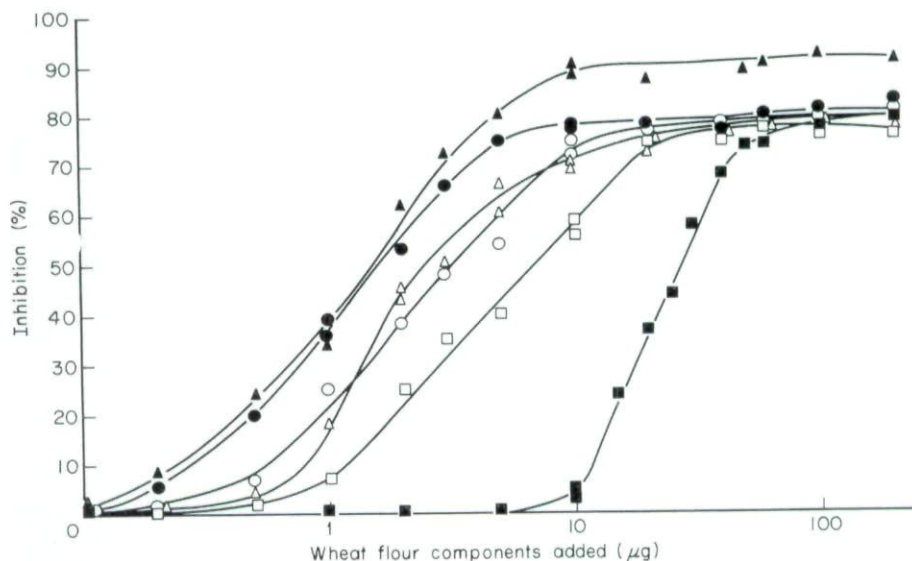


Fig. 5. Radioallergosorbent test inhibition results using paper discs coupled to wheat flour water-soluble proteins together with sera from flour-allergic subjects Mas. and Zir. Each disc was reacted with 150 μ g of the water-soluble protein preparation and incubated with 50 μ l of a 1:10 dilution of serum Mas. or Zir. Uptake of IgE antibodies by the discs was inhibited by preincubation of the sera with the wheat water-soluble proteins, albumins or globulins. IgE antibodies which bound to the discs were estimated by the addition of ^{125} I-labelled anti-IgE. Inhibitors for serum Mas.: (●) albumins, (■) globulins, (▲) water-soluble proteins. Inhibitors for serum Zir.: (○) albumins, (□) globulins, (△) water-soluble proteins.

Table 3. RAST inhibition results using wheat water-soluble protein fraction, albumins and globulins together with sera from wheat flour-allergic subjects and discs coupled to wheat water-soluble proteins*

Serum†	Amount of protein (μ g) needed to produce 50% inhibition of the uptake of IgE antibodies from allergic serum‡		
	Water-soluble proteins	Albumins	Globulins
Mas.	1.5	1.6	28.0
Zir.	3.0	3.2	7.0

* Each disc reacted with 150 μ g wheat water-soluble proteins (see Materials and Methods section).

† Each serum used at a dilution of 1:10.

‡ Detected as 50% inhibition of the uptake of ^{125} I-labelled anti-IgE.

proteins) was only approximately 2:1 (Table 3). Inhibition studies using discs coupled to albumins or globulins further demonstrated specificity differences between the flour-specific IgE antibodies in sera Mas. and Zir. Results clearly showed that serum Zir. contained both albumin- and globulin-specific IgE antibodies while the Mas. IgE antibodies, which reacted with the water-soluble proteins, were largely directed towards the albumins. For example, using discs coupled to albumins, only 1.2 μ g of albumins were needed to produce 50% inhibition of the uptake of Mas. IgE anti-

bodies. When globulin-coupled discs were used with serum Mas., 15 µg of the purified globulins were needed for 50% inhibition.

Examination of coeliac sera for IgE antibodies to some common allergens

The high levels of total IgE found in the sera from children with coeliac condition prompted a search for allergen-specific IgE antibodies in these sera. Allergen extracts from some foods, fungi, pollens, animal epithelia, house dust and mites were examined in RAST experiments with the six coeliac serum samples. Two sera, Nol. and Sma., reacted with one or more of the extracts, but the other four sera did not react with any of the twelve different preparations tested (Table 4). Serum Nol. showed strong reactivity with rye grass pollen and cat epithelial extracts. Serum Sma. reacted with discs coupled to extracts from rye grass pollen, house dust and particularly strongly with allergens from the house dust mites *D. farinae* and *D. pteronyssinus* (Table 4).

Discussion

Although the need for the use of purified allergens in both clinical and experimental allergology is being increasingly recognized and advocated, and the need for allergen standardization has been stressed (Baer *et al.*, 1970; Aas & Belin, 1972; Lichtenstein, 1976; Baldo & Uhlenbruck, 1977), only a few allergens have so far been purified and characterized (Marsh, 1975; King, 1976). The use of highly purified allergens, together with a knowledge of their physicochemical and immunological properties, should greatly assist in the diagnosis and treatment of allergic individuals. The question of allergen identification and purity is as important for allergens from foods as it is for the frequently encountered allergens, such as from the common pollens and mites. Anaphylaxis to foods has been reported (Golbert, Patterson & Pruzansky, 1969). In such cases identification of the food allergens is imperative in order to avoid a subsequent lethal response.

Wheat flour is a complex mixture of peptide- and saccharide-containing substances, many of which must be considered as likely to sensitize man to an immediate allergic reaction. Allergic reactions to wheat flour may occur following inhalation or ingestion of flour (Bleumink, 1970; Caldwell *et al.*, 1975) but, regardless of the route of sensitization, the flour allergens responsible have not been closely studied. Using crossed radioimmuno-electrophoresis, together with sera from thirteen bakers allergic to wheat flour, Blands *et al.* (1976) found that eighteen different wheat flour antigens reacted with IgE antibodies in the allergic sera. Although three of the flour antigens were classified as major allergens, isolation and characterization of these allergens was not carried out. Using sera from two bakers, who in the course of their work became sensitized to flour, we have demonstrated the presence of serum IgE antibodies to a number of purified flour components. For both sera the strongest reactivities were observed with the wheat albumins and globulins. The implication of the albumin fraction as the main allergen was clearest for serum Mas. The moderate reactivity of the hand-washed gluten and possibly also the gliadin preparations might be at least partly explained by the presence in them of some albumin, since they are the ones most likely to contain such contamination. Furthermore, lowest reactions were given by preparations such as A gliadin, arabinogalactan-peptide and glutenin, which are the most likely to be free of albumin. However, all the reactions certainly cannot be wholly explained on the basis of albumin sensitivity, particularly for serum Zir., and there is clearly some reaction with other flour components as well.

Table 4. RAST examination for the presence of allergen-specific IgE antibodies in sera from children with coeliac condition

Serum	Age (years)	Serum total IgE (u/ml)	Rye grass pollen	Dandelion*	House dust*	House dust mites			Cat epithelium	Dog epithelium	Cladosporium herbarum*	Aspergillus fumigatus	Candida albicans	Egg white*	Cow's milk*
						Dermatophagoides farinae	Dermatophagoides pteronyssinus								
Pooled	—	<10	0.5	0.6	0.5	0.5	0.2	0.3	0.8	0.6	0.6	0.8	0.4	1.1	0.7
cord															
Nat.	0.6	20	0.4	0.5	0.4	0.2	0.3	0.2	0.6	0.7	0.6	0.6	0.3	0.9	1.0
Nol.	6.5	550	16.0	0.8	1.2	0.5	0.5	29.3	0.9	0.7	0.7	0.5	0.3	1.3	1.3
Sna.	9	775	2.8	0.7	4.6	23.8	33.1	0.4	0.6	0.7	0.6	0.6	0.4	0.8	1.0
Las.	4	250	1.2	1.0	0.9	0.4	0.6	0.4	0.8	1.0	0.9	0.9	0.5	1.1	0.9
Smi.	4	230	0.6	0.6	0.4	0.3	0.2	0.4	0.8	0.7	0.5	0.7	0.3	1.1	0.8
Pet.	14.5	1225	0.4	0.8	0.4	0.5	0.5	0.4	0.7	0.7	0.7	0.7	0.2	1.3	1.3

* Pharmacia Phadebas RAST allergen discs.

Our findings support the conclusion of Hoffman (1975), who examined cereal grain-specific IgE antibodies in the sera of eleven atopic children. Although most of the subjects studied did not have overt symptoms associated with the ingestion of cereals, all the sera showed clear-cut reactions in RAST studies with wheat albumin and globulin fractions. Little or no reactions were observed with the gliadin, gluten and starch fractions, but an informative comparison with our results is difficult since no details of the preparation of the wheat fractions used were given in the Hoffman (1975) study.

Attention should be drawn to the fact that even purified albumin, globulin, gliadin and glutenin preparations are each undoubtedly heterogeneous mixtures of proteins (Wrigley & Shepherd, 1974; Kasarda, Bernardin & Nimmo, 1976). Even purified A gliadin is far from being a homogeneous protein, as reported in the Materials and Methods section. A gliadin is a subfraction of α gliadin, which has been implicated in a case of acute wheat hypersensitivity (Goldstein, Heiner & Rose, 1969). Although that study and the present one identify the protein classes involved, more work is needed to determine whether any single component within a class is more allergenic than others.

IgE antibodies to animal albumins and/or globulins have been demonstrated in the sera of individuals allergic to cats (Brandt, Ponterius & Yman, 1973), dogs (Yman, Brandt & Ponterius, 1973), horses (Chan & Porter, 1967; Ponterius *et al.*, 1973) and birds (Faux *et al.*, 1971). It seems then that albumins from a variety of sources are not infrequently involved in eliciting immediate allergic reactions. Although the ready solubility and molecular size range of this general class of protein fits the requirements which are generally thought to be necessary for allergenicity (King, 1976), comparative amino acid sequence studies are required before any firm conclusions can be drawn on the nature of allergenic groupings on various plant and animal albumins.

Despite widespread support for the theory that coeliac condition has an immunological basis and the existence of some evidence to support this belief (Crabbé, Douglas & Hobbs, 1969; Ezeoke *et al.*, 1974), the underlying mechanisms involved in the pathogenesis of the disorder are not understood. 'Gliadin shock', which is occasionally seen in coeliac children on a gluten-free diet following ingestion of small amounts of gliadin, points to the involvement of a reagin-mediated immediate hypersensitivity reaction. We, and Heiner & Rose (1970), found elevated levels of total IgE in the sera of some children with coeliac condition, but examinations for the presence of gluten-specific IgE antibodies in coeliacs do not appear to have been carried out. No IgE antibodies to any of the wheat flour components examined were detected in the coeliac sera we studied, even though we examined A gliadin, which is reputed to be the main toxic component of gluten. As we examined only six sera, three from subjects with acute coeliac condition and three from subjects on a gluten-free diet, it is possible that examination of a larger number of sera would reveal the presence of flour-specific IgE antibodies. However, our RAST results reported here clearly demonstrate that gluten- and, in particular, gliadin-specific IgE antibodies do not occur in the sera of individuals with coeliac condition as a general rule.

In an attempt to find a simple diagnostic skin test for coeliac condition, Baker & Read (1976) and Anand, Truelove & Offord (1977) recently injected coeliac patients and healthy control subjects intradermally with a range of gluten fractions and subfractions. In the latter study, all of the coeliac patients but none of the controls

gave a positive Arthus-type reaction (type III) to some of the gluten subfractions (Frazer *et al.*, 1959), but no immediate (type I) reactions were observed in any of the injected subjects. The absence of immediate skin test reactions in the coeliac patients (see also Schneider, 1977) is consistent with our failure to detect IgE antibodies to wheat gluten and gliadin components. Taken together, our results and those of Anand *et al.* (1977) indicate that type I IgE-mediated reactions are not important in the pathogenesis of coeliac condition.

Our results and the results of Heiner & Rose (1970) suggest that elevated serum IgE levels may be a consistent finding in coeliac condition. In our attempts to discover the specificities of the IgE antibodies in coeliac sera, some success was achieved with sera Nol. and Sma., but none of the other four coeliac sera reacted with any of the twelve different allergen preparations tested. Radioactive uptakes observed in RAST experiments indicated that subject Nol. was allergic to rye grass pollen and cat epithelia and subject Sma. was allergic to house dust mites. Use of a larger battery of allergen preparations would be necessary to identify the specificities of the relatively high levels of IgE antibodies in the other coeliac sera. Elevation of serum IgE levels may arise in coeliacs following increased uptake of antigens via the damaged intestinal mucosa. Some studies have demonstrated that the small intestine is an important site for IgE synthesis (Tada & Ishizaka, 1970). It would be interesting, therefore, to examine sera from a large number of coeliac patients for both total IgE and allergen-specific IgE antibodies.

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