

Immunoglobulin E antibodies to ingested cereal flour components: studies with sera from subjects with asthma and eczema

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Summary

The specificity of immunoglobulin E for different cereal grain proteins was investigated using sera from twenty paediatric patients with asthma and/or eczema. Close correlations were observed between radioallergosorbent test values for grain extracts of wheat, rye and barley, and, to a lesser extent, oats. Of the different wheat flour fractions tested, the globulins and glutenins consistently bound higher levels of IgE than the gliadins and albumins. This is in contrast both with bakers' asthma (an allergy to inhaled flour where the albumins are important allergens) and with coeliac disease (in which gliadin is the most toxic fraction). Partial digestion of the flour proteins largely removed their ability to bind IgE. An analytical technique of identifying allergens after gel isoelectric focusing demonstrated that many different flour proteins were involved.

Introduction

Most general surveys of the incidence and causes of food allergy suggest that the ingestion of products made from various cereal grains—wheat, rye, barley, oats, corn and rice—can cause allergic reactions in some individuals (Fontana, Wittig & Holt, 1963; Hoffman & Haddad, 1974; Wraith *et al.*, 1979; Perlman, 1980). However, there have been few attempts to elucidate the precise nature of the particular constituents of cereal grains responsible for allergic reactions because of two main problems common to food allergy research.

Firstly, there is the difficulty of defining what constitutes 'food allergy' (Soothill, 1979; May, 1980; Taylor, 1980). Adverse reactions caused by eating cereals arise through a variety of mechanisms (Soothill, 1979; Bleumink, 1974) and include symptoms ranging from typical atopy, to gastrointestinal reactions and migraine (Perlman, 1980; Gerrard 1979; Anon, 1979; Grant, 1980). Different cereal components

may be involved in each type of adverse reaction or 'allergy', and our study has therefore been restricted to IgE-mediated responses. The second problem is to establish whether there is a cause-effect relationship between the presence of cereal specific IgE and the symptoms observed. In some individuals, positive RAST results to a food allergen are not confirmed by elimination and challenge testing (Wraith *et al.*, 1979).

The spectrum of clinical symptoms which can result from IgE-mediated reactions to food has not been clearly delineated. Hoffman and Haddad (1974) have shown that there are good correlations between high RAST values for a variety of foods, including wheat, and a clinical history of anaphylactoid symptoms, angioedema and asthma following ingestion of these foods. However, the correlation between RAST results and history was not as good in cases of atopic eczema (Hoffman *et al.*, 1975).

The possible role of food allergy in atopic dermatitis (eczema) has been debated for many years (Norins, 1971; Rostenberg & Solomon, 1971; Kahn, 1975; Rajka, 1975). While elevated levels of IgE are usually found in patients with atopic dermatitis (Berg & Johansson, 1969; Juhlin *et al.*, 1969; Clendenning *et al.*, 1973; Gurevitch, Heiner & Reisner, 1973), and correlations have been shown by some workers between the level of IgE and the severity of the symptoms (Ogawa *et al.*, 1971); a causal relationship is yet to be established. However, eczema is frequently associated with other atopic symptoms such as asthma, and the IgE in patients with eczema usually has specificity for a wide range of both inhaled and ingested allergens (Guiang & Speer, 1973; Hoffman *et al.*, 1975).

More recently, Brostoff, Johns and Stanworth (1977) have shown that there are high molecular weight complexes containing IgE present in the sera from patients with atopic dermatitis, and these authors have suggested that eczematous lesions result from the deposition of these complexes in the skin.

The aim of the study reported here was to identify the potentially allergenic proteins in wheat by studying the IgE response to various protein fractions in highly atopic patients, so that comparisons could be made both with coeliac disease and inhaled flour allergies.

Two *in vitro* techniques were used to identify the cereal proteins which bind IgE—the radioallergosorbent test and a new analytical technique for allergen detection following gel isoelectric focusing (Sutton, Wrigley & Baldo, 1982). In addition, the allergenic properties of other cereal grains and bran were compared to wheat flour, to provide a basis for clinicians in recommending suitable diets for allergic patients.

Materials and Methods

Sera

Table 1 gives background information on the twenty children whose sera were used in this study. These children are highly atopic with asthma and/or eczema. They were selected for the study on the basis of their high level of wheat-specific IgE. For preliminary studies, a pool was made of the twenty sera with equal amounts of each included. In addition, sera from two adults (Laos and Tur) were kindly provided by Dr E. Tai of the Prince Henry Hospital, Melbourne, and Dr S. Krilis of the Allergy Clinic, Royal Prince Alfred Hospital, Sydney, respectively.

Radioallergosorbent tests (RAST)

Allergen discs for RAST assays were supplied by Pharmacia (Uppsala, Sweden) for Table 1 and Fig. 1, or made up by coupling protein extracts to cyanogen-bromide-activated paper discs (Ceska *et al.*, 1972). In Table 1, the data is expressed in RAST scores (0–4) representing undetectable, low, moderate, high and very high levels of uptake respectively. The level of total circulating IgE was determined by the Phadebas radioimmunosorbent test (PRIST) (Pharmacia, Uppsala, Sweden), and was expressed as units/ml of serum.

Wheat-grain allergen extracts

Wheat grain (*Triticum aestivum*, cv. Timgalen) was either ground as whole grain to produce wheatmeal or milled to separate the flour from the bran on a Buhler laboratory mill. The bran fraction was thoroughly cleaned to remove endosperm (flour), as far as possible, using a Buhler impact bran finisher. The classical set of flour protein fractions (Osborne, 1907) was prepared by sequential extraction (Baldo & Wrigley, 1978) to produce albumins, globulins, gliadins and glutenins, respectively soluble in water, 10% sodium chloride, 70% (v/v) ethanol, and 0.1% potassium hydroxide.

A dilute acid extract of wheat wholemeal was made by combining the dissolved, dialysed material from two sequential extractions with water to which sufficient hydrochloric acid was added to reduce the pH of the suspension to 3.

Vital gluten (containing the gliadin and glutenin) was prepared by extensive washing and kneading of a water-and-flour dough under running water to remove the starch, albumins and globulins. Commercial devitalized (heat-treated) gluten was also used. This was deamidated by treatment with alkali, as described by Baldo and Wrigley (1978). The extent of amidation of both this and the digested glutens was determined by a modification of the method of Ronalds (1974).

Digestion of wheat extracts with pepsin and trypsin

The enzymic digestion was based on the procedures of Frazer *et al.* (1959), Dissanayake *et al.* (1974) and Anand, Truelove and Offord (1977). Vital gluten (10 g) was dispersed in 100 ml of 0.1 M HCl and digested for 3.5 hr at 40°C with 50 mg pepsin. The pH was raised to 7.8 by adding 1 M NaOH, and 50 mg trypsin was added prior to a further 4 hr digestion at 40°C. The digest was acidified with 1 M HCl to pH 4.5, held for 16 hr at 4°C and centrifuged at 12 000 *g* for 30 min to remove insoluble material. Part of the digest was freeze-dried. The remainder was dialysed (exhaustively) against distilled water before drying. The yields of the undialysed and dialysed digests were 80%, and 55% of the original gluten on a dry weight basis. A sample of the dilute-acid-extracted protein was digested in a similar manner.

Gel filtration

The molecular weight ranges of the protein digests were determined by gel filtration on a column of Sephadex G150 by the procedure of Bushuk and Wrigley (1971).

Gel isoelectric focusing and allergen detection

Proteins extracted, with 4 M urea from the flour sample, were fractionated by isoelectric focusing in a flat polyacrylamide gel containing 2 M urea, according to the procedure of du Cros and Wrigley (1979). Protein zones, transferred and fixed to a nitrocellulose

acetate membrane, were incubated with sera from some of the patients and the positions of IgE-binding proteins were detected using a ^{125}I -anti-IgE (Pharmacia) and indirect autoradiography (Sutton *et al.*, 1982).

Results

Patterns of response to cereal and other allergens

The background clinical information for the children used in this study is summarized in Table 1. All showed IgE antibodies to many common allergens, and most had high levels of total IgE in their sera. The patients are numbered in decreasing order of the RAST score to wheat grain extract in order to facilitate a comparison of their reactions to other allergens (Table 1) and to grain extracts of related cereals (Fig. 1).

Correlations were most evident between the various cereals (Table 2). The sera tested reacted very similarly to wheat (*Triticum aestivum*), rye (*Secale cereale*) and barley (*Hordeum* spp.) while the results with oats (*Avena sativa*) gave lower correlations with the three other cereals. The pattern of reaction to wheat grain showed little relationship to the results for the non-grain allergens tested including rye-grass pollen (Table 2).

IgE binding to wheat-grain fractions

Material from the outer bran-layers of the wheat grain proved to react at least as strongly as flour (the white inner-endosperm), when tested against the pooled children's sera or with the adult serum (Table 3). This conclusion is borne out by the result for wheat wholemeal which contains bran and flour in a ratio of about 1 : 5. However, the study concentrated particularly on flour both because it is quantitatively more important in whole grain, and because it is often eaten without the inclusion of bran.

RAST results obtained with the pooled children's sera showed globulin to be the most reactive fraction (Table 3). This result was verified by testing these sera individually (Fig. 2). In all but three cases, the RAST result for the globulin fraction was higher than that for any of the other flour fractions. The results with the glutenin

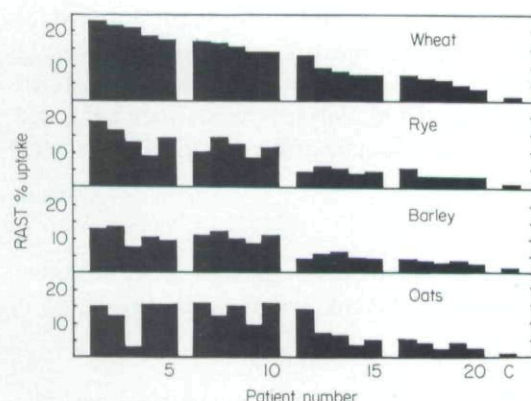


Fig. 1. Comparison of the levels of IgE antibodies, indicated by RAST, to grain proteins of four cereals, using sera (diluted to half concentration) from the twenty patients listed in Table 1.

Table 1. Background information about atopic children and RAST scores to common allergens

Patient	Sex	Age	Atopic symptoms*	Total IgE	Wheat	Egg	Milk	Peanut	Fish	Mites	Pollen†	Other‡	Food allergy diagnosis
1	M	3½	A,E	1077	4	4	0	3	nt	4	4		Egg allergy
2	M	1	A,EE	1208	4	2	2	2	1	1	2		Tolerates all foods
3	F	2	U	142	4	2	0	2	nt	3	4	CO,NO,MO,AO	Previous milk, oatmeal allergy
4	M	4	A,EE	5720	3	2	2	4	1	3	2		Eczema healed on elemental diet
5	M	1½	E	631	3	4	4	4	0	2	1		Milk allergy
6	M	2½	A	1846	3	1	0	3	nt	4	4	C3,DO	Tolerates wheat, peanut
7	M	2½	A,E	841	3	3	4	3	1	0	3		Wheat, milk, egg, peanut allergy
8	M	1½	E	958	3	1	0	4	nt	3	2		Milk, egg, peanut and other food allergies
9	M	1		230	2	2	2	0	0	2	2		Milk, egg, and other food allergies
10	F	5	A,E	852	2	3	2	3	0	4	3	C3,D3,A3,M3	Previous egg allergy, peanut allergy
11	M	1	E,U	363	2	3	0	3	0	0	2		Egg, peanut allergy
12	F	12	A,E	316	2	0	0	3	nt	4	4	CO,D4,MO,AO	Orange juice, tomato eczema
13	F	9	na	1052	2	2	1	3	nt	2	3	A2,M2	
14	M	13	na	959	2	1	0	0	1	3	2		
15	M	4	A,E	1322	2	4	1	2	1	4	2		Egg allergy
16	M	4	A,EE	4170	2	4	2	3	0	4	3		Egg allergy; eczema healed on elemental diet
17	M	14		nt	2	0	0	2	0	0	1		Possible milk allergy or lactose intolerance
18	M	2½	A,E	936	2	2	2	2	nt	4	4	C4,D1,H4,AO,M2	Tolerates all foods
19	F	7	AA,E	589	2	0	1	2	nt	4	4	C4,D1,AO,M2	Tolerates all foods
20	F	2	E,U	36	1	2	0	0	0	2	0	CO,DO	Wheat, egg, orange, lamb & other food allergies

* Asthma (A), eczema (E), urticaria (U) (double letter indicates severity); † pollen from rye grass (*Lolium* spp); ‡ results for other allergens tested: cat (C), dog (D), horse (H), *Cladosporium* mould (M), *Alternaria* (A); nt = not tested; na = not available; symptoms also included vomiting and diarrhoea in many cases.

Table 2. Correlations between allergic reactions, based on RAST, to cereal allergens for the patients in Table 1.

	Correlation coefficient		
	Wheat	Barley	Oats
Rye grain	0.92	0.92	0.70
Barley grain	0.89		0.80
Oats grain	0.72		
Egg	0.19		
Milk	0.12		
Peanut	0.41		
Mites	-0.02		
Ryegrass pollen	0.32		

Table 3. Allergenicity of wheat-grain preparations indicated by RAST examination of twenty sera (Table 1) pooled in equal proportions (diluted to half concentration) and of the serum of patient Tur.

Preparation tested	μg coupled/disc	% Radioactive uptake		
		Pooled sera	Tur	Cord
Flour	500	5.0	6.4	1.4
Wheatmeal	500	6.3	8.0	1.4
Bran	200	5.3	nt	0.8
Albumin	150	3.7	4.3	1.1
Globulin	150	6.1	nt	0.8
Gliadin	150	4.1	1.3	0.9
Glutenin	150	2.7	2.2	0.9
Devitalized gluten (100% amide)	250	2.1	1.3	0.6
Deamidated gluten (80% amide)	250	2.8	1.3	0.7
Deamidated gluten (40% amide)	250	3.1	2.9	0.7
Vital gluten	250	4.2	2.2	0.7
Gluten digest, undialysed	250	2.1	1.3	0.7
Gluten digest, dialysed	250	2.0	0.9	0.7
Wheatmeal, HCl extract	250	3.4	5.5	0.7
HCl extract digest (soluble)	250	1.7	0.9	0.8
HCl extract digest (insoluble)	250	1.6	1.0	0.6

nt = not tested.

fraction were almost as high, showing first or second highest RAST figures for sixteen sera. Gliadin, on the other hand, gave particularly low RAST values.

Detection of IgE binding following analytical fractionation

Fig. 3 shows the combinations of proteins reacting to serum IgE for some of the children. The 4 M urea extract of the wheat flour protein used contains the albumin,

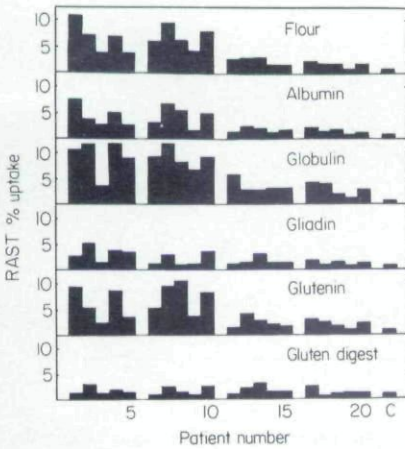


Fig. 2. Comparison of the levels of IgE antibodies, indicated by RAST, to protein fractions from wheat grain. The amounts coupled per RAST disc are shown in Table 3; patient numbering is given in Table 1.

globulin and gliadin, but would exclude the least soluble glutenin. The results clearly show that many flour proteins are bound by the IgE of each serum, and that there are some differences between individuals.

Deamidation and digestion of gluten

Gluten appeared to be rendered less allergenic by heat treatment, since vital gluten gave higher RAST uptakes than devitalized gluten (Table 3). On the other hand, RAST values rose as a result of progressive alkali treatment which removes amide groups and makes the gluten more water soluble.

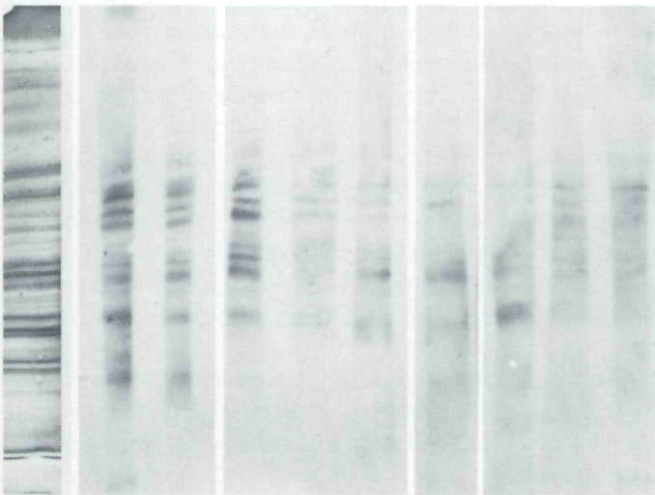


Fig. 3. Gel isoelectric focusing (pH3[top]–pH10) of wheat proteins extracted from wheat flour with 4 M urea: polyacrylamide gel (left) stained with coomassie blue and autoradiographs (right) of the transfer membranes showing zones which bind IgE from 200 μ l serum samples of patients 1, 2, 6, 8, 7, 9, 10, Tur, Laos (left to right).

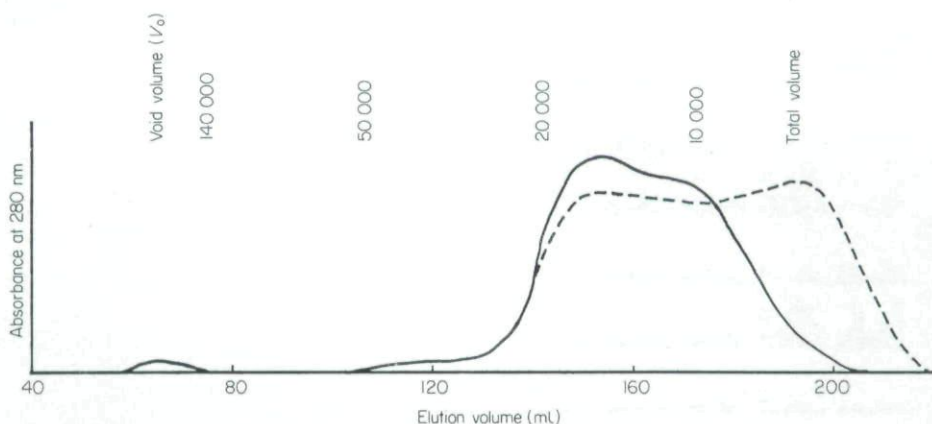


Fig. 4. Gel filtration of gluten digest before (broken line) and after dialysis (solid line). The figures above the elution profiles indicate the approximate molecular weights of proteins eluted at the respective stages.

Digestion with pepsin and trypsin produced a dramatic reduction in RAST values for both vital gluten and an acid extract of wheatmeal, when these were assayed with children's sera, either as a pool (Table 3) or when tested individually (Fig. 2) or with adult serum (Table 3). Examination of the molecular-weight distribution (Fig. 4) of the gluten digest (dialysed and undialysed) indicates the extent of proteolysis by comparison with the original gluten protein in which most proteins have molecular weights above 25000 and many are above 100000 (Bushuk & Wrigley, 1971). Enzymic digestion had little effect on the degree of amidation of the protein, as judged by the amide contents of the original gluten, the undialysed and the dialysed digests which were respectively 2.6, 2.2 and 2.8 mmol/g protein.

Discussion

The close correlations between the observed reactions with wheat, rye and barley presumably reflect their close taxonomic relationship to one another. These cereals are grouped together in the tribe, Triticeae, as distinct from oats which belong to a related tribe, Aveneae (Baldo, Krilis & Wrigley, 1980; Baldo, Sutton & Wrigley, 1982). Ryegrass (*Lolium*) is itself a close relative of these four cereals, being a member of another tribe (Festuceae) of the same subfamily (Festucoideae). Yet, there was poor correlation between wheat and ryegrass RAST values (Table 1) for the important reason that different anatomical parts of the plant were involved—grain as distinct from pollen. In fact, correlation was slightly better with peanut (*Arachis hypogaea*), also a seed, even though it is taxonomically quite distant from the cereals (Baldo *et al.*, 1982).

The results suggest that it would be unwise for a clinician to recommend that barley or even oats should replace wheat grain in the diet of a wheat-sensitive patient. Substitution of more distantly related cereals such as rice or corn would be preferable, but these may still cross react to some extent (Lewis & Imber, 1975). Furthermore, the results indicate that wheat-flour sensitive patients should also be warned to avoid cereal bran in their diet. Hypersensitivity to grass pollens, as a result of cross reactivity, seems unlikely, on the other hand.

These recommendations are based on the assumption that a positive RAST result

to cereal grain indicates that the patient would have an allergic reaction to dietary cereals. Final diagnosis of cereal-food allergy should be made on the basis of elimination, and challenge tests (Aas, 1978). Two studies have attempted to confirm that positive wheat RAST are of clinical significance. Wraith *et al.* (1979), in a study which examined 119 patients with a variety of possible food allergies, showed that of the twenty-four patients with positive wheat-RAST values, fourteen showed symptoms after challenges with wheat following a three-week elimination diet. In all fourteen patients, these reactions were delayed, occurring at least 1 hr after feeding.

In an earlier study, Hoffman and Haddad (1974) examined 127 paediatric patients with suspected food allergy and either symptoms of hypersensitivity such as asthma, eczema, urticaria, and angioedema or acute gastrointestinal reactions. Of the patients with a history of food wheat allergy, 61% had positive, 11% borderline, and 28% negative RAST results for wheat. When the eighteen patients in the study with positive wheat-RAST results were examined, thirteen had a clinical history of wheat food allergy. There were no apparent wheat-related symptoms for one patient. The remaining four patients with positive RAST results all lived close to a flour mill and the authors concluded that they had allergic reactions to inhaled flour. Such patients with inhalant allergy usually have no problems eating flour products (Hoffman & Haddad, 1974; Aas, 1978; Baldo & Wrigley, 1978). It is unlikely that any of the children in our present study have been extensively exposed to inhaled flour, so that it has been assumed that the high levels of wheat-specific IgE observed arose because of the ingestion of wheat flour products, and consequent exposure of lymphocytes in the intestinal mucosa or in the blood to wheat allergens. Not all the patients studied (Table 1) have a clear diagnosis of food allergy, though this can be implied in some cases. Despite this variation between individuals, there was a remarkable uniformity in the specificity of their IgE for globulin and glutenin protein. A description of these proteins as allergens thus appeared to be justified at least for some individuals.

The low RAST results obtained with gluten digests suggest another explanation for the occurrence of some false RAST-positive results (i.e. patients with positive RAST but negative oral-challenge results). The digestion of wheat protein with pepsin and trypsin, in a manner designed to mimic *in vivo* conditions, virtually abolished allergenicity. Possibly a positive challenge is achieved only when a disturbance of the gastrointestinal barrier permits reasonably intact allergen to cross the gut wall. Such a disturbance may be important in the initial development of hypersensitivity. These studies should be extended to determine the extent of proteolysis required to eliminate IgE binding, particularly if this could be carried out on individually purified allergens.

The RAST analyses of flour protein fractions (Fig. 2 and Table 3) suggest that the globulin and glutenin groups of proteins would be the most promising fractions for allergen purification. On the other hand, analytical fractionation combined with allergen detection (Fig. 3) indicates that many molecules are bound to IgE and that screening many sera by this method to identify the common, major allergens would be a worthwhile prerequisite to preparative isolation of specific allergens.

It may be significant that the most allergenic fractions appear to be the least soluble flour proteins. This may be because they are present for a longer time in the gut, and thus have greater opportunity to cause hypersensitivity. Increased solubility *per se* did not consistently alter the IgE binding capacity, as shown by contrasting the effects of heat treatment and deamidation on gluten (Table 3). Glutenin may be a more

significant cause of allergy *in vivo* than globulin because it represents a much greater proportion (30–40%) of flour protein than does globulin (5–10%) (Simmonds, 1978).

Relatively low specific IgE binding was found for both the albumin and gliadin fractions. These findings show that even at the level of crude fractions, distinctions can be made between the proteins potentially involved in ingested cereal allergic reactions, compared with coeliac disease and with inhalant allergy such as bakers' asthma. In similar studies using the sera from individuals with bakers' asthma, the highest RAST results were found with the water-soluble proteins (albumins) with lower results for a range of gliadin and glutenin fractions (Baldo & Wrigley, 1978). This difference could be explained by the hypothesis that, in bakers' asthma and related conditions, the proteins most likely to be important allergens are those which are readily soluble in the mucous lining of the bronchial tract.

Several distinctions can be made between the patients in this study and individuals with coeliac condition (gluten-sensitive enteropathy). Firstly, the gliadin portion of gluten is reported to be the most toxic part of flour in coeliacs (Hekkens, Haex & Willighagen, 1970; Kendall *et al.*, 1972; Kasarda, 1978). Secondly, coeliac condition is not marked by the presence of circulating wheat-specific IgE and it is not considered to be a Type I allergic reaction (Heiner, Goldstein & Rose, 1970; Bjorksten *et al.*, 1977; Baldo & Wrigley, 1978). In addition, peptic-tryptic digestion of gluten does not remove its toxicity to coeliacs (Frazer *et al.*, 1959); in fact, such digests have been used with some success in skin testing (Type III reaction) for diagnosis of coeliac condition (Dissanayake *et al.*, 1974; Baker & Read, 1976; Anand *et al.*, 1977; Durve, Kelly & Sen, 1980; Wyeth, Kamath & Wrigley, unpublished).

Conclusion

The results described in this study indicate that the four main groups of flour proteins play different roles in abnormal reactions to flour—albumins for bakers' asthma, gliadins for coeliac condition, and globulins and glutenins, apparently, for IgE-mediated food allergy. However, it needs to be emphasized that in each case, proteins from other fractions may be involved to some extent, and that within each of the main protein groups there are many non-reactive proteins. The individual variation in the sets of protein zones that combine with serum IgE (Fig. 3) indicate the importance of examining IgE-binding for many sera before choosing the proteins that are most worthy of further characterization and isolation.

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