

The diversity of allergens involved in bakers' asthma

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Summary

Sera from 35 individuals with suspected allergies to inhaled flour were screened for the presence of immunoglobulin E (IgE) specific for wheat-flour proteins. Sera from nine asthmatic bakers with high wheat RAST scores were selected for further study with the aim of purifying the allergen(s) involved in bakers' asthma and related conditions. However, each of the different techniques applied—ion exchange chromatography, preparative isoelectric focusing and the electrophoretic transfer, or 'Western blotting' technique, showed that serum IgE from different individuals have markedly different specificities and bind to numerous wheat proteins. When three purified wheat proteins were tested—wheat germ agglutinin; a fraction purified using a concanavalin-A affinity column and a putative trypsin inhibitor—all were identified as allergens for some but not all of the allergic bakers.

Introduction

Over many years there have been numerous clinical studies of individuals with bakers' asthma and other immediate hypersensitivity reactions to inhaled flour and bran (see review: Baldo, Sutton & Wrigley, 1982). It has been well recognized that hypersensitivity to flour represents a significant proportion of occupational allergies, at least in Finland (Linko, 1947; Keskinen, Allanko & Saarinen, 1978; Jarvinen *et al.*, 1979), Sweden (Kallos & Kallos-Deffner, 1971), Germany (Herxheimer, 1973) and Romania (Popa, George & Gavanescu, 1970), although the frequency of sensitization is clearly decreasing with the use of modern flour handling processes (Kallos & Kallos-Deffner, 1971; Popa *et al.*, 1970).

A number of detailed case studies have lead to the conclusion that bakers' asthma is the result of an IgE-mediated type-I reaction to wheat- or rye-flour proteins, and that

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the symptoms can be minimized by the use of disodium cromoglycate (Wilbur & Ward, 1976; Hendrick, Davies & Pepys, 1976; Napolitano & Weiss 1978). In many instances a late asthmatic reaction is also observed although the mechanism of this is poorly understood (Nakazawa *et al.*, 1976).

Several studies have assessed the usefulness of skin-prick testing in the diagnosis of flour allergy. Kallos & Kallos-Deffner (1971) found, in a study of 583 bakers, that in all cases a positive skin or provocation test with flour extract was associated with typical clinical signs and symptoms. However other studies have suggested that skin testing is less reliable. Linko (1947) had previously shown in a study of 328 bakery workers that sixty-six had clinical symptoms of whom forty gave positive skin tests to flour. In addition, eighteen people without a clinical history of flour allergy gave 'false' positive skin tests and were described as having a 'latent' allergy. Popa *et al.* (1970) went further and showed that in a group of forty-three bakers, all with rhinitis or asthma and positive skin tests to flour, the flour allergy could only be confirmed in 73% by nasal or bronchial challenge. Of the remaining 12 individuals, four had a positive provocation reaction to insect extracts, five to bacterial extracts and three to none of the challenge tests. Thus, not all individuals with positive skin tests to flour would be clinically diagnosed as having bakers' asthma or rhinitis.

The *in-vitro* radioallergosorbent test (RAST) for flour allergy has not yet been evaluated as extensively as skin testing, but the studies which have been carried out suggest that it is more reliable than skin testing. Bjorksten *et al.* (1977) compared RAST results of twenty-one asthmatic bakers with an appropriate atopic control group; Baur, Dorsch and Liebe (1979) carried out RAST, skin and provocation tests for wheat and rye in thirty-six bakers, and Schwarting (1979) compared case histories, RAST and skin testing, and bronchial, nasal and conjunctival challenges in twenty-five bakers.

All three groups concluded that the RAST assay was useful in the diagnosis of allergy to inhaled flour dust among bakers, and that while borderline positive RASTs are sometimes found in individuals without symptoms, all high RAST results could be confirmed by history and/or challenge. Baur *et al.* (1979) reported that RAST values gave a correlation of 93% with the combined results of provocation tests and history.

Since few, if any, false RAST positive results are observed it can be assumed that wheat-flour allergens may be tentatively identified by testing fractions *in vitro* for IgE binding using sera from allergic bakers. However, despite the apparent clinical interest in bakers' asthma, only a few studies have used this approach to identify the specific allergens involved.

Blands *et al.* (1976) demonstrated the presence of forty different antigens in a wheat-flour extract by crossed immunoelectrophoresis and using sera from twenty-five bakers, they identified eighteen allergens including three major allergens.

Baldo & Wrigley (1978) tested a range of wheat-flour fractions using RAST and RAST-inhibition studies and sera from two bakers with flour asthma. The serum IgE in both cases was bound to a much greater extent by the albumin and globulin fractions compared to the less soluble gluten (gliadin and glutenin) components. This current study commenced with further preparative fractionation of the albumins or water-soluble proteins. In addition the new technique of electrophoretic transfer ('Western blotting') was used to assess the diversity of allergens present in flour extracts fractionated on polyacrylamide gels.

Materials and methods

Sera

Serum samples were kindly provided by a number of general practitioners and by Dr S. Krilis of the Allergy Clinic, Royal Prince Alfred Hospital, Sydney and were stored at -20°C until testing. Different sera are identified in tables and figure legends by a three-letter code.

Radioallergosorbent tests

Allergen discs for RAST assays and ^{125}I -labelled anti-IgE were supplied by Pharmacia (Uppsala, Sweden). Alternatively discs were made by coupling protein extracts to cyanogen-bromide-activated paper discs (Ceska *et al.*, 1972). In Table 1, the data are expressed as RAST scores (0–4) representing undetectable, low, moderate, high and very high levels of uptake. The levels of total circulating IgE were determined by the paper radioimmunosorbent test (PRIST) (Pharmacia, Uppsala, Sweden), and were expressed as units per ml of serum.

Wheat-flour proteins

A grain sample of Australian wheat (*Triticum aestivum*, cultivar Timgalen) was milled to produce flour. For preparative fractionation, extracts were made with 4 volumes of 0.05 M sodium phosphate buffer pH 6.8 and the gliadin was precipitated with 1.0 M or 0.4 M ammonium sulphate. The extract was dialysed against the starting buffer (for ion exchange chromatography) or distilled H_2O (for isoelectric focusing) before application to columns.

A glycoprotein fraction was purified from a saline extract of Timgalen wheatmeal using a concanavalin-A affinity column (Lloyd, 1970). The elution buffer was 0.15 M NaCl with 0.1 M α -methyl-mannoside. A putative trypsin inhibitor was prepared from a water-soluble flour fraction by affinity chromatography on a trypsin-Sepharose 4B column as described by Stewart & Doherty (1970). Purified wheatgerm agglutinin was obtained from Calbiochem (Rice & Etzler, 1975). For the RAST assay, 20 μg of these protein preparations was used per cyanogen-bromide-activated disc.

Ion exchange chromatography

The flour extract was fractionated on a carboxymethyl cellulose column (CM 23, Whatman, England) using an elution gradient of ammonium acetate, starting at 0.05 M, pH 4.5 and rising to a limit of 1.5 M, pH 5.2. Further protein was eluted with washes of 0.5 M NaOH followed by 0.5 M HCl. Fractions were individually dialysed and freeze-dried.

Density gradient isoelectric focusing

Proteins in a flour extract were separated according to their isoelectric point in the range pH 3–10 using an LKB Ampholine column with a continuous sucrose gradient (5–40% w/v) according to the method of Wrigley (1971). Nineteen fractions were collected, dialysed and freeze-dried.

Polyacrylamide gel electrophoresis

Isoelectric focusing of a dialysed water extract of flour was carried out in a 7.5% polyacrylamide slab gel ($90 \times 120 \times 1.5$ mm) with 1 M urea and 2% carrier ampholytes

with a pH range 3.5–10 (Pharmacia, Uppsala, Sweden), as described previously (du Cros & Wrigley, 1979).

For two-dimensional electrophoresis, proteins were separated in the first dimension by isoelectric focusing in 7.5% polyacrylamide tube gels (60 × 3 mm, pH 5–9). The second dimension was run using commercially prepared 3–27% gradient polyacrylamide gels (70 × 70 × 3 mm, Gradient Pty Ltd, Pyrmont, Australia) in a sodium lactate buffer (5 mM Na, pH 3.1).

Western blotting

After fractionation of the proteins on the isoelectric focusing or gradient gel, the protein zones were transferred by transverse electrophoresis onto a nitrocellulose membrane (type GWSP, pore size 0.22 µm, Millipore, Massachusetts) using 0.7% acetic acid as electrode buffer (Towbin, Staehelin & Gordon, 1979; Sutton, Wrigley & Baldo, 1982b). The membrane was then washed in 0.05 M sodium phosphate buffer pH 6.8 with 0.15 M NaCl (PBS), incubated with 1% bovine serum albumin to block

Table 1. Background information on individuals allergic to inhaled flour

	Serum (dilution)	Mas (1/4)	ONE (1/3)	New	Mos	Yin	McM	Spa	Wei	Woo	Mau	Kei	Hen	Gun
Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Occupation*	B	B	B	B	B	P	B	B	B	B	B	B	B	P
Symptom†	A	A	A	A	A	A	A	A	A	A	A	A	R	R
RAST scores														
Grains	Wheat	4	3	3	3	3	3	4	3	4	2	3	1	1
	Rye	4	3	3	3	3	4	4	3	4	3	3	0	2
	Barley	3	3	3	3	2	4	3	2	3	2	3	0	2
	Oats	2	2	2	2	0	1	1	1	2	3	3	0	0
	Rice	3	3	3	2	1	2	3	1	2	4	NT	1	2
	Maize	3	3	3	2	2	2	3	1	1	3	NT	2	2
Pollens	Rye	1	2	0	0	0	0	1	0	0	3	3	0	0
	Ryegrass	2	3	0	0	0	0	2	0	0	3	3	1	1
	Bermuda	2	2	1	0	0	0	1	1	0	3	NT	0	0
	Timothy	1	2	0	0	0	0	0	0	0	2	3	0	0
	Plantain	0	0	0	0	0	0	2	0	0	4	NT	0	2
House-dust mite	3	0	0	0	0	2	0	0	0	0	0	3	4	0
Cat epithelium	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Alternaria	0	0	0	0	0	0	0	0	0	0	4	0	0	0
Aspergillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Egg white	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Milk	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peanut	2	1	2	0	0	0	0	0	0	0	3	3	0	0
Total IgE	880	242	710	224	93		29	347	451	870	376	496	175	121

* Occupation: bakery employee (B), pastrycook (P), flour miller (M), housewife (H), child (C), farmer (F). Superscript r: retired.

† Presenting symptoms: asthma (A), rhinitis (R), eczema (E), conjunctivitis (C), chronic lung condition (L).

additional binding sites and then washed again in PBS and RAST buffer (Pharmacia AB, Uppsala, Sweden), and incubated overnight with diluted serum from allergic bakers. The position of IgE-binding zones on the membranes was detected by incubation of the washed membranes with ^{125}I -labelled anti-IgE (1×10^5 cpm in 1.5 ml PBS per strip in Figure 4; and 7×10^5 cpm in 10 ml for Fig. 5). After extensive washing with RAST buffer, the membranes were air dried and the ^{125}I -labelled zones were detected by indirect autoradiography (Laskey & Mills, 1977).

Results

Examination of allergic sera

Serum samples were collected from a range of individuals (mainly bakers and millers with suspected allergic reactions to inhaled flour) who had been contacted either through the baking industry or through a hospital allergy clinic. For all sera, total IgE level was measured and RAST assays were carried out with commercial discs for wheat flour, house dust mites, and ryegrass pollen. On the basis of the wheat RAST score the sera were divided into high and low groups (Tables 1 and 2).

Sera in the first group, which were used in further experiments reported in this paper, were tested with a wide range of allergens in order to assess the likelihood that these results might be affected by IgE specific for allergens other than wheat (Table 1). Generally these patients showed few sensitivities other than to the cereals. There were significant correlations between the RAST results observed for wheat compared with some other cereals, but not with the other allergens tested (Table 3).

Table 2. Background information on individuals with suspected inhaled flour allergies with low wheat RAST scores

Serum	Ant	Bar	Ben	Bul	But	Cul	Cur	Dea	Dep	Edw	Fry	Gil	Gui
Sex	M	M	M	F	M	M	M	M	F	M	M	M	F
Occupation*	B	B	P ^r	H	B	B ^r	B	B	H	B ^r	P	B	B ^r
Symptom†	A	A	C,E	A	R,E	A	A	A	C,E	L	A	R	
RAST scores													
Wheat	2	2	1	2	0	2	1	2	2	0	0	0	0
Ryegrass pollen	4	0	0	4	0	0	3	0	1	0	0	0	0
House dust mite	1	0	0	4	0	1	4	3	0	0	3	0	0
Total IgE	220	70	20	800	60	30	60	75	180	20	2500	760	40

Serum	Har	Hem	Mak	McE	Mil	Nor	Pep	Pri	Ree	Til	Wal	Whi	Wil
Sex	M	M	M	F	M	M	M	M	M	M	M	M	M
Occupation	M	P	B	H	B	B ^r	B	B	M ^r	B	B	B	C
Symptom	R	C,R	A	R,C	A	A	C	A	L	A	R	A	A
RAST scores													
Wheat	0	0	2	2	0	2	0	0	0	2	0	0	0
Ryegrass pollen	0	0	0	3	0	0	0	0	0	3	0	0	1
House dust mite	0	0	1	0	0	0	0	0	0	3	0	0	3
Total IgE	120	30	260	60	40	82	20	40	20	310	60	40	840

*† see Table 1 for list of abbreviations.

Table 3. Correlations between the RAST results for wheat and other allergens in sera from Table 1

Allergen discs		Correlation with wheat score
Grasses		
Grains	Rye (<i>Secale cereale</i>)	0.83*
	Barley (<i>Hordeum vulgare</i>)	0.79*
	Oats (<i>Avena sativa</i>)	0.01
	Rice (<i>Oryza sativa</i>)	0.11
	Maize (<i>Zea mays</i>)	0.06
Pollens	Rye (<i>Secale cereale</i>)	-0.11
	Ryegrass (<i>Lolium perenne</i>)	-0.11
	Bermuda (<i>Cynodon dactylon</i>)	-0.06
	Timothy (<i>Phleum pratense</i>)	-0.07
Other		
Weed pollen	Plantain (<i>Plantago lanceolata</i>)	-0.19
Mites	House dust mite	-0.02
	(<i>Dermatophagoides pteronyssinus</i>)	
Epithelium	Cat	-0.11
Moulds	<i>Alternaria tenuis</i>	-0.21
	<i>Aspergillus fumigatus</i>	0.07
Foods	Egg white	0.36
	Milk	0.28
	Peanut	-0.15
Total serum IgE (units/ml)		0.74*

* Significant at $P < 0.01$; others not significant.

Correlations were calculated from crude RAST % uptake values for the sera shown in Table 1.

In some experiments, sera from four patients were included so that the specificities of their IgE could be compared with those of sera from bakers with high IgE specific for wheat. These individuals were Mau and Kei, who had high RAST scores to wheat as well as other allergens, and Hen and Gun who were bakers with rhinitis and low IgE levels to wheatgrain. Data for these patients are therefore also included in Table 1.

The results for the sera with low or undetectable levels of wheat-specific IgE are shown in Table 2. These individuals tended either to have milder symptoms than those in the first group (e.g. rhinitis instead of asthma) or to have a lower current exposure to flour (e.g. housewives and retired bakers).

Ion-exchange chromatography of flour allergens

A wheat-flour extract, containing predominantly albumin proteins, was fractionated on a carboxymethyl cellulose column using an ammonium acetate gradient and the RAST activity of every second fraction was measured using serum from one asthmatic baker (Mas) (Fig. 1). These fractions were then pooled according to the apparent peaks of RAST activity for Mas, so that these findings could be confirmed using sera from other bakers (Table 4). Relatively low RAST activity was observed for both the 26% of

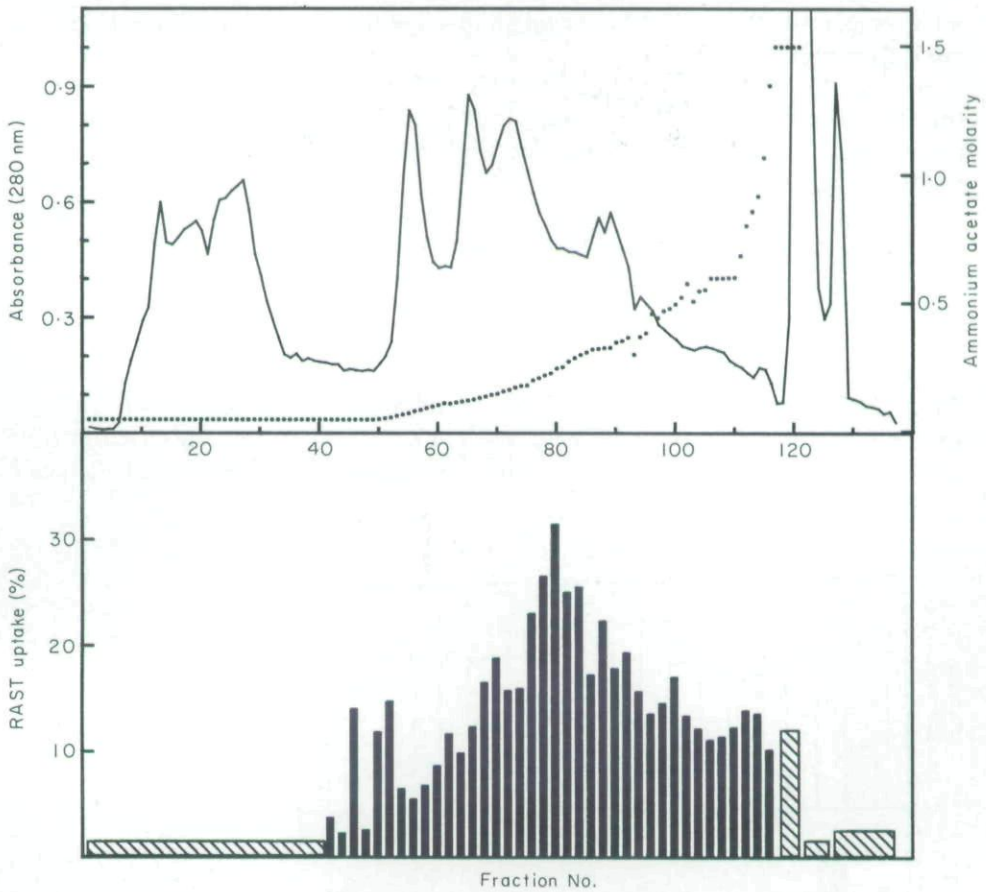


Fig. 1. Ion exchange chromatography of wheat-flour albumins, showing protein elution with respect to eluting salt concentration, and the allergenicity of fractions determined by RAST analysis (100 μ g protein coupled/disc) of Mas serum.

Table 4. IgE binding to pooled fractions from ion exchange chromatography

Fractions pooled (Fig. 1)	Eluting solution	RAST values (% uptake)				
		Mas (1/15)	Mos	McM	Woo	Cord
1-41	0.05 M AmoAc	2	3	2	3	0.6
42-56	0.05 M AmoAc	17	6	6	16	0.8
58-64	continuous	14	14	5	13	0.9
66-72	gradient	21	16	11	19	1.1
74-82	0.05 M to 1.5 M	24	12	15	18	1.1
84-94	AmoAc	28	7	17	28	1.1
96-116	1.5 M AmoAc	18	6	11	28	1.6
118-121	1.5 M AmoAc	12	5	9	13	1.0
122-126	0.5 M NaOH	2	2	3	4	0.6
127-137	0.5 M HCl	3	3	4	7	0.8

total protein which eluted in the starting buffer and the 17% of the protein which was not eluted by the limit buffer.

For the first baker (Mas), high RAST activity was associated with many of the column fractions, although these peaks did not generally coincide with the major protein peaks in the elution profile. The highest RAST activity was eluted at 0.25 M ammonium acetate pH 5.06.

Using sera from two other bakers (Woo and McM) similar profiles were obtained to that for Mas, but a markedly different pattern was observed for Mos who showed the highest RAST value for a fraction eluting at 0.13–0.17 M ammonium acetate (Table 4).

Density gradient isoelectric focusing of flour allergens

Wheat-flour albumins were fractionated by preparative isoelectric focusing in a sucrose gradient and the fractions were tested by the RAST assay using sera from four different bakers (Mas, ONe, New and Mos). An apparently different set of allergenic

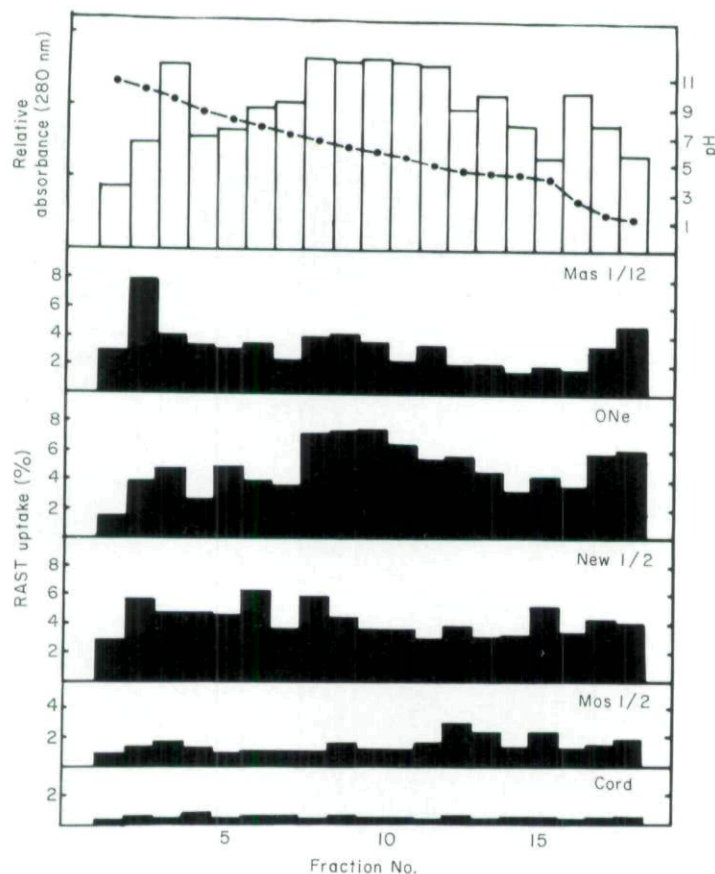


Fig. 2. Density gradient isoelectric focusing of wheat-flour albumins, showing the fractionation of protein with respect to pH (top) and the distribution of allergenicity, determined by RAST, with respect to the sera (dilutions shown) of four allergic bakers and to cord serum.

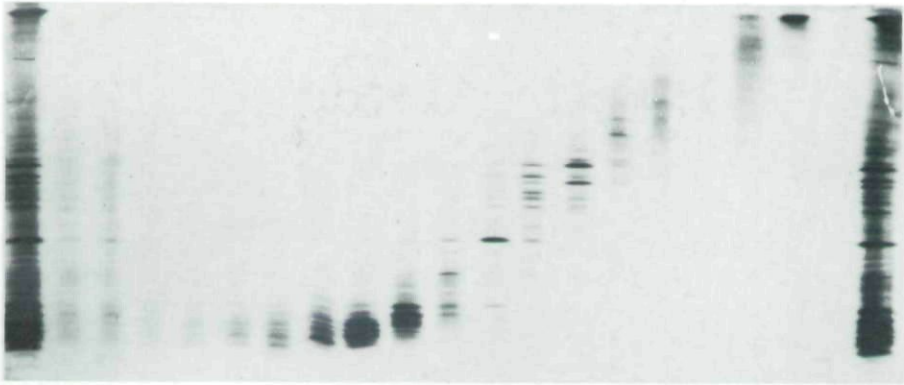


Fig. 3. Gel isoelectric focusing (pH 10, top, to 3; stained for protein) of nineteen fractions (numbered left to right) from density gradient isoelectric focusing of wheat-flour albumins (Fig. 2). The crude extract was applied at both ends.

proteins was identified using each of the sera (Fig. 2), even though analytical isoelectric focusing of these fractions indicated that the initial fractionation was good (Fig. 3).

Investigation of specific flour proteins

Sera from nine of the allergic bakers were tested in the RAST assay against three wheat-flour proteins suspected of being allergenic (Table 5). The proteins tested were wheatgerm agglutinin (a lectin found in wheatgerm which often contaminates wheat flour under commercial milling conditions), a glycoprotein fraction isolated by affinity chromatography on concanavalin-A Sepharose, and a putative trypsin-inhibitor fraction which had been purified on a trypsin-Sepharose column. Whilst non of these preparations is likely to be homogeneous, they are highly purified. The concanavalin-A-reactive protein, for example, showed only one major component on gel electrophoresis and the trypsin inhibitor comprised of a few components (Fig. 4). Each of

Table 5. IgE binding to individual wheat flour proteins

Sera	Wheat-flour extract	RAST values (% uptake)		
		Wheat-germ agglutinin	Con-A-binding protein	Trypsin-binding protein
Mas (1/10)	6	—	3	2
ONe (1/3)	9	3	3	1
New	3	1	3	4
Mos	4	1	2	2
McM	4	6	2	3
Spa	12	—	15	2
Wei	4	—	1	2
Woo	13	—	5	4
Mau	9	2	12	5
Cord	0.7	0.5	0.7	0.7



Fig. 4. Gradient gel electrophoresis of a purified trypsin-inhibitor fraction (left), a concanavalin-A-reactive glycoprotein and a crude wheat grain extract. The glycoprotein was overloaded to accentuate the presence of minor components.

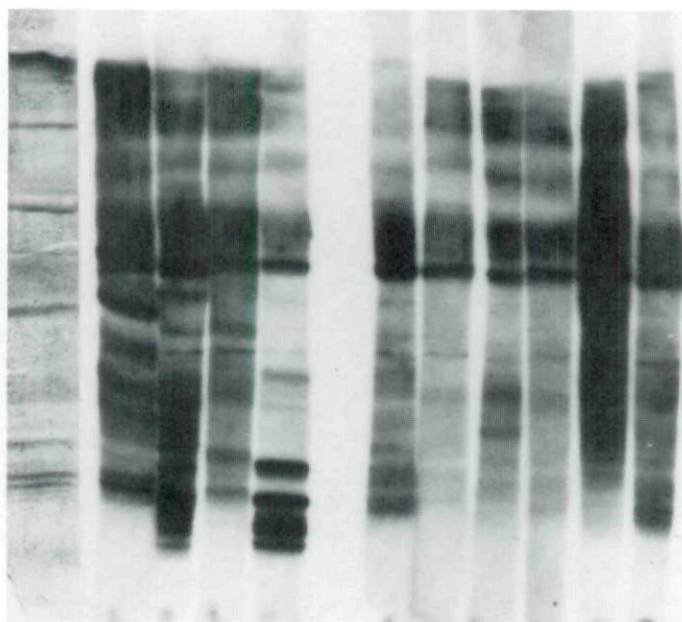


Fig. 5. Gel isoelectric focusing (pH 10, top, to 3) of wheat-flour proteins, showing the protein-stained gel (extreme left) and autoradiographic strips indicating the positions of IgE-reactive zones after incubation with the sera of ten allergic bakers, left to right: Mas (50 μ l), ONe (200 μ l), New (200 μ l), Mos (500 μ l), Mau (200 μ l), Yin (500 μ l), Hen (1 ml), Gun (750 μ l), McM (200 μ l) and Kei (500 μ l), each serum volume being diluted to 1.5 ml with PBS.

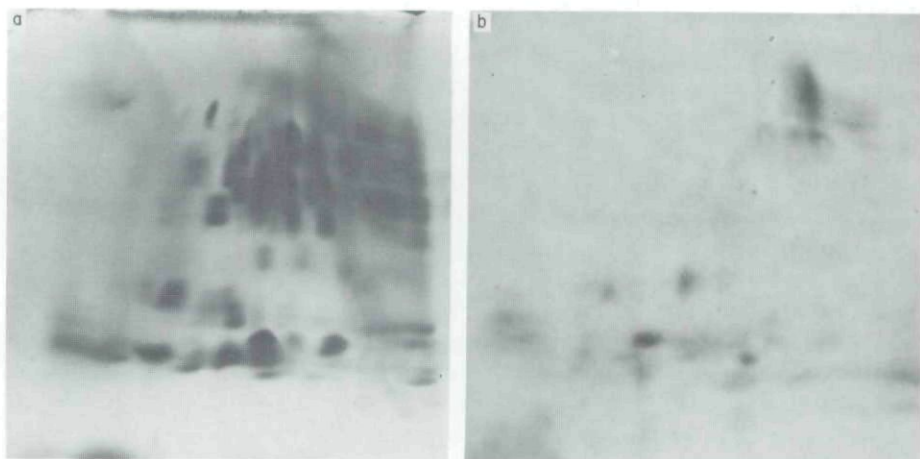


Fig. 6. Two-dimensional fractionation of wheat-flour proteins by gel isoelectric focusing (pH 5, left, to 9) followed by gradient gel electrophoresis (pH 3.1, origin at top). (a) The gel stained for protein; the corresponding autoradiograph (b) shows the positions of proteins which bound IgE from a pool of the sera used in Fig. 5.

these proteins bound IgE from sera of at least three bakers but gave negative RAST scores for at least one other baker and the cord control. These results suggest that the binding of IgE to these wheat proteins is a specific reaction and that there are differences in the specificities of the IgE from different individuals.

The nature of wheat-flour allergens as shown by Western blotting

Western blotting was used to characterize the proteins in wheat flour which bound to the IgE from ten allergic individuals (Fig. 5). Different but to some extent overlapping sets of allergens with a range of isoelectric points can be identified for different bakers. While isoelectric focusing provides good resolution of the wheat proteins, even more components can be identified using a two-dimensional electrophoresis (Fig. 6). In this system, gliadin proteins are found predominantly in the top half of the gel and albumins in the lower half. Glutenins do not enter the gel under these conditions. The corresponding radiograph indicated that the major allergens for the group of bakers tested are albumins and/or globulins with a full range of isoelectric points and slightly basic gliadins.

Discussion

In this study two *in-vitro* methods—the RAST assay and the Western blotting method—were used to assess the potential allergenicity of wheat-flour fractions. Since both of these methods depend on the binding of IgE to wheat proteins and its detection by ^{125}I -labelled anti-IgE, sera with high specific IgE binding to wheat proteins and preferably little binding to other allergens were required.

The data in Table 1, which gives the RAST scores for a variety of allergens for the individuals who were selected for further study, clearly show that these sera reacted to few of the common allergens, except for the cereals. The IgE in all these sera reacted to rye and barley grain as well as to wheat, and a close correlation was found between RAST values for these three allergen sources (Table 3). This apparent cross reactivity

between wheat, rye and barley has been observed previously and can be explained by the fact that these three species are closely related grasses, all being in the tribe *Triticeae* (Baldo *et al.*, 1980; Baldo *et al.*, 1982). The relationship between the wheat allergens and those of oats, rice and maize is less clear, because although there is a high incidence of positive RASTs to grains of these lesser-related grasses, there is no correlation between these results and the ones for wheat (Table 3). Finally it is clear that the RAST values obtained with various grass pollens are unrelated to the wheat results. This reflects the different protein composition of grain (or seed) compared with pollen.

Many patients with wheat-related symptoms had low or undetectable IgE to wheat (Table 2). In these individuals, it is possible that flour is not acting as an allergen but rather as a general irritant to a respiratory tract which has been sensitized by another allergen. The RAST results in Table 2 suggest that the primary antigen for Hen, Cur, Fry and Wil may be house dust mite. Popa *et al.* (1970) observed that nine out of forty-three allergic bakers gave positive challenge results with arthropod or bacterial extracts but not to flour, indicating that contaminants of wheat flour play an important role in some cases of bakers' asthma. The very broad range of mite, insect, rodent and fungal allergens which may contaminate flour or grain has been reviewed by Baldo *et al.* (1982). Thus in clinical cases of suspected bakers' asthma or rhinitis, it would be useful to test not only for allergy to wheat flour but also to potential contaminants of flour and to other common allergens.

Preparative fractionation of wheat allergens

The original aim of this study was to purify the major allergen(s) present in wheat flour which are responsible for bakers' asthma. It was assumed that a small number of proteins would be the important allergens for most individuals allergic to flour, since this appears to be the case for some other inhaled allergenic sources (Marsh, 1975; King, 1976).

This assumption had been strengthened by the demonstration (albeit with a limited number of sera) by both Hoffman (1975) and Baldo & Wrigley (1978) that the albumins and globulins bound more IgE from the sera from allergic individuals than the less-soluble gliadins and glutenins. Further it was shown that when the water-soluble proteins were fractionated by gel filtration, the IgE from three bakers (Mas, Mos, ONe) reacted in a similar way to give a broad peak of high RAST activity for proteins in the mol. wt range 15–35000, suggesting that a single protein or a group of related proteins was responsible for allergic reactions to flour (Baldo *et al.*, 1982).

On the other hand, Blands *et al.* (1976), using the more sensitive technique of crossed radioimmuno-electrophoresis, had demonstrated that eighteen out of forty antigens bound IgE from at least one of twenty-five bakers' sera and that there was little similarity between any of the serum specificities. Therefore, to allow for possible variations between individuals, we used a number of sera to test each set of protein fractions. The application of this approach to both ion-exchange chromatography (Fig. 1, Table 4) and preparative isoelectric focusing (Fig. 2), showed that there are many flour allergens, and that these differ from one individual to another. An alternative interpretation for this data was that the proteins were poorly resolved by these fractionation techniques, but this was not the case at least for the isoelectric focusing (Fig. 3).

Further evidence for the diversity of flour allergens was provided by testing relatively pure samples of suspected allergens. Wheatgerm agglutinin was examined

because it has a similar structure to the ragweed pollen allergen Ra₅ (Drenth *et al.*, 1980). A putative trypsin-inhibitor fraction, isolated by affinity chromatography from wheat flour, was included because wheat trypsin-inhibitor activity elutes from gel filtration columns in a similar position to the highest RAST activity, with a mol. wt of approximately 27000 (Petrucci *et al.*, 1974). Moreover a trypsin inhibitor has been identified as one of the allergens in soybeans (Moroz & Yang, 1980). Thirdly, the glycoprotein fraction purified on a concanavalin-A column was tested because lectins have previously been useful in purifying other allergens (Baldo *et al.*, 1982; Barnett, 1982). The results of RAST assays carried out with a number of sera (Table 5) show that in fact each of these protein preparations is potentially allergenic for some bakers.

An analytical approach to allergen identification

The application of Western blotting to the characterization of allergens separated in polyacrylamide gels provides an easy method for assessing the number and nature of allergens in an extract. Using this technique on gradient gels, it was shown that the IgE from sera from four different bakers bound predominantly to proteins in the albumin region of the gel with both quantitative and qualitative differences in affinities (Sutton *et al.*, 1982b).

Since isoelectric focusing provides better resolution of the albumin proteins than gradient gel electrophoresis, it was used in combination with Western blotting to detect the allergens involved in bakers' asthma (Fig. 5), in suspected ingested allergies to wheat (Sutton *et al.*, 1982) and in house-dust mite allergy (Krilis, Baldo & Sutton, 1983). In the latter two allergic conditions, the patterns of IgE binding established for different individuals were quite similar, suggesting the presence of common or major allergens with occasional rare or minor allergens. In contrast, the bakers' sera gave much more diverse binding patterns. For example Mos and ONe reacted to a set of acidic proteins, which do not bind IgE from the other sera.

One explanation for the diversity of reactions amongst the bakers is that inhaled flour may not be as allergenic as the more common allergen sources, because it lacks proteins present in high levels which evoke a strong IgE response in many people. Individuals who develop allergies to inhaled flour have generally been exposed to a much greater antigen load (than, say, patients with pollen allergy) and for perhaps a greater length of time before the development of symptoms. Popa *et al.* (1970) reported an average of 9 years' exposure before the appearance of allergic rhinitis and 14 years before asthmatic reactions to flour. Hence flour allergy may only become a problem because of the high level of exposures for someone routinely inhaling flour-laden air.

The possible role of 'insoluble' allergens in bakers' asthma

An inherent limitation of the RAST assay associated with the use of cyanogen-bromide-activated discs is that any components in an allergen extract which are insoluble in the sodium bicarbonate coupling solution are unlikely to be coupled to the paper and therefore may not be detected. Blands *et al.* (1976) and Baldo and Wrigley (1978) have recognized that this could present a major difficulty in the assessment of the relative allergenicity of various wheat fractions since 40% of wheat flour protein is the highly insoluble glutenin fraction (Simmonds, 1978). It is unlikely therefore that any allergens which are glutenins would have been detected by the methods used in this paper.

In an effort to circumvent this problem, preliminary experiments have been carried

out using discs of nitrocellulose membrane as the solid phase in the RAST assay. An advantage of the nitrocellulose membrane is that proteins can be solubilized in an appropriate solvent, applied and dried onto the disc and then any remaining solvent components can be washed off with buffer leaving the proteins fixed to the membrane. While this method still requires extensive evaluation, the results using sera of eleven bakers from this study show that the glutenin fraction does bind high levels of IgE. The allergens present in wheat flour which are important in bakers' asthma may therefore be even more numerous and more diverse than the range of water-soluble proteins already identified.

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