

# Hypersensitivity to Inhaled Flour Allergens

## Comparison between Cereals

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Radioallergosorbent testing (RAST) of sera from subjects sensitized to wheat and rye flour indicated that there is significant reaction with seed extracts of 12 cereals (wheat, durum wheat, triticale, cereal rye, barley, rye grass, oats, canary grass, rice, maize, sorghum and Johnson grass). Results were evaluated in terms of taxonomic relationships and of the electrophoretically determined protein composition of the cereal extracts. RAST uptakes were uniformly low in sera from four rhinitic bakers, yet were significantly above the levels for non-allergic and cord sera. Much higher RAST uptakes were obtained with sera from four asthmatic bakers when tested with wheat and its close relatives, but there was still reasonably high reactivity with more distantly related cereals. RAST inhibition experiments indicated in a more direct way the extent of cross-reactivity between grain extracts of wheat, rye, barley and oats. One baker had a history of more severe attacks of breathlessness following inhalation of rye flour compared with wheat flour. This was confirmed by bronchial challenge testing, but the comparison was not obviously consistent with the results of prick testing or estimation of histamine released from his leucocytes. The results as a whole suggested that the bran layers of cereal grains are at least as allergenic as flour.

*Key words:* allergic rhinitis; bakers' asthma; cereal allergens; cross-reactivity; histamine release assay; taxonomic relationships.

*Accepted for publication 2 July 1979*

Allergic reactions following inhalation of cereal flours are well recognized (7, 17, 20, 30, 32, 36). As Ordman (25) has pointed out, references to cereal allergy go back over 60 years. However, until recently, there has been little progress in identifying the specific allergens responsible. Of 40 different antigens detected in wheat flour, Blands et al. (8) identified three major allergens, but their isolation and characterization were not attempted. Using purified and partially purified fractions isolated from wheat flour together with sera from subjects with bakers' asthma, Baldo & Wrigley (5) showed that the water-

soluble proteins and, in particular, the albumin components of flour are important allergens. These studies have now been extended by comparing allergenic reactivity of water soluble extracts from different cereal grains and by initiating clinical studies in subjects allergic to inhaled flour.

## MATERIAL AND METHODS

*Cereals.* Table 1 lists the plants from which grains were examined, giving the cultivar (variety) used for cultivated crops, and the

Table 1  
Relationships between the common cereals and preparations used

| Family                 | Sub-family   | Tribe         | Genus and species                   | Common name   | Genome | Cultivar used | Preparations ground to give "Wholemeal" | Preparations ground to give "Flour" |
|------------------------|--------------|---------------|-------------------------------------|---------------|--------|---------------|-----------------------------------------|-------------------------------------|
| Poaceae<br>(Gramineae) | Festucoideae | TRITICEAE     | <i>Triticum aestivum</i> L.         | bread wheat   | (ABD)  | Tingalen      | whole grain                             | endosperm                           |
|                        |              |               | <i>Triticum durum</i> Desf.         | durum wheat   | (AB)   | Duramba       | whole grain                             | endosperm                           |
|                        |              |               | <i>Triticosecale</i> Wittmack       | triticale     | (ABR)  | Fast grow     | whole grain                             | endosperm                           |
|                        |              |               | <i>Secale cereale</i> L.            | cereal rye    | (R)    | Black winter  | whole grain                             | endosperm                           |
|                        |              |               | <i>Hordeum distichon</i> L.         | barley        | (H)    | Clipper       | grain + lemma + palea                   | endosperm                           |
|                        |              | FESTUCEAE     | <i>Lolium</i> spp. *                | rye grass     |        | —             | grain + husk                            | —                                   |
|                        |              |               | <i>Avena sativa</i> L.              | oats          |        | Irwin         | groat + lemma + palea                   | groat                               |
|                        |              | Aveneae       | <i>Phleum pratense</i>              | Timothy grass |        | —             | —                                       | —                                   |
|                        |              |               | <i>Phalaris canariensis</i> L.      | canary grass  |        | —             | grain + husk                            | —                                   |
|                        |              | ORYZAE        | <i>Oryza sativa</i> L.              | rice          |        | Calrose       | brown rice                              | polished rice                       |
|                        | Panicoideae  | TRIPSACEAE    | <i>Zea mays</i> L.                  | maize (corn)  |        | mainly XL81   | whole grain                             | endosperm                           |
|                        |              |               | <i>Sorghum bicolor</i> (L.) Moench  | sorghum       |        | Goldrush      | whole grain                             | pearled grain                       |
|                        |              | ANDROPOGONEAE | <i>Sorghum halepense</i> (L.) Pers. | Johnson grass |        | —             | grain + husk                            | —                                   |
|                        |              |               |                                     |               |        |               |                                         |                                     |

\*Mainly *L. rigidum*, but also *L. perenne* and *L. multiflorum*

common and scientific names. The taxonomic interrelationships shown are based on classifications by Watson & Clifford (38), Baum (6), and Stebbins & Crampton (35). To distinguish between effects caused by the endosperm and those from the outer bran layers, two preparations were made for each grain. "Wholemeal" (Table 1) indicates that the whole grain (together with any naturally adhering husk) was finely ground, whereas the term "flour" is used for a finely ground preparation of the white endosperm from which the outer husk layers had been removed as far as possible, for example by using a laboratory flour mill (Quadrumat) for wheat, triticale, barley and rye. The flour prepared from maize (corn) was ground after removing both the germ and the bran layer from the grains.

*Cereal extracts.* Wholemeal and flour were extracted by rotating 5–10% w/v suspensions of the preparations in water, first at room temperature for 1 h and then overnight at 4°C. After centrifugation (15 min at 10,000 g), the supernatant was freeze-dried and used in the *in vitro* and *in vivo* experiments described. Wheat water-soluble proteins, albumins and globulins were isolated as previously described (5).

*Gel electrophoresis of cereal extracts.* Fig. 1 shows the electrophoretic patterns of proteins extracted from flour and wholemeal samples of a range of cereals. Proteins of the albumin, globulin and prolamin classes were extracted from flour or wholemeal (100 mg) with 1 M urea solution (0.6 ml). After 1–2 h contact, the extract was clarified by centrifugation (15 min at 10,000 g), and 10 µl of each extract was applied to a 3–27% polyacrylamide gradient gel (23) (Gradient Pty Ltd, Lane Cove, N.S.W., Australia), equilibrated with sodium lactate buffer (0.005 M NaOH adjusted to pH 3.1 with lactic acid). Cathodic electrophoresis (39) of the samples was performed for 2 h at 200 V. The gel slab (70 × 70 × 3 mm) was stained overnight in 100 ml of Coomassie Blue G250 (0.01%) in 12% trichloroacetic acid.

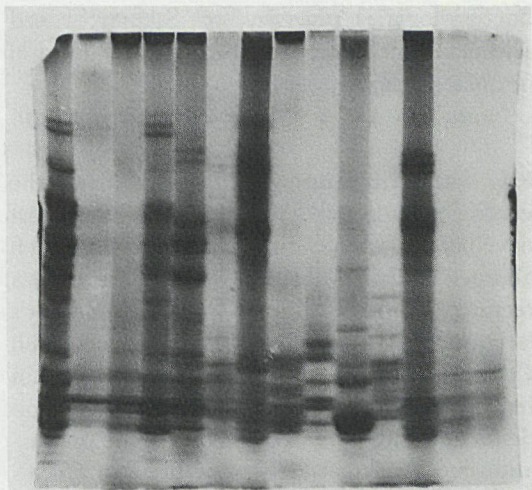


Fig. 1. Gradient gel electrophoresis of extracts of (left to right) wheat flour, wheat bran, wheat pollard, wheat wholemeal, durum wholemeal, triticale wholemeal, rye wholemeal, barley wholemeal, oat wholemeal, rice flour, maize wholemeal, rye flour, sorghum flour, and sorghum wholemeal. Proteins, applied at the top of the gel, migrated downwards towards the cathode.

*Patients and serum samples.* Serum samples were obtained from four bakers, Mas (5), Mos, Nil and Nor, all of whom had a history of episodes of breathlessness after exposure to wheat flour. Patient Nor was a retired baker who had not worked in the trade for 15 years but who continued to suffer symptoms of asthma after inhalation of flour. This patient had shown bronchial hyper-reactivity after inhalation of flour when first diagnosed as having bakers' asthma (A. J. Woolcock, personal communication). Patient Mos, now a bakery instructor with the Bread Research Institute of Australia, had a long history of breathlessness on exposure to flour from wheat or rye. He had not had significant exposure to flour of other cereal grains. This patient reported more severe attacks of breathlessness following inhalation of rye flour compared with wheat flour. Serum samples were also obtained from four bakers But, Dun, Hen and Wal, who experienced rhinitis while handling wheat flour. Sera from non-allergic, healthy adults were provided by Prof. A. Basten, Immunology Unit, Department of

Bacteriology, University of Sydney. Cord serum samples were obtained from St John of God Hospital, Perth.

*Skin tests.* Prick tests were performed on the ventral aspects of both forearms using a short-bevelled 24-gauge disposable needle. Each allergen extract tested was placed in a rectangular area drawn on the patient's forearm, with a distance of 2 cm between each allergen test site. Care was taken to obtain reproducible results with the same person performing all skin tests. The cereal preparations to be skin tested were used in Coca's solution. Prick test responses to Coca's solution alone were also examined. Allergen extracts of house dust 10% w/v, *Dermatophagoides farinae* 2% w/v, cat hair and dander 10% w/v, dog hair and dander 10% w/v, Timothy (*Phleum pratense*) 5% w/v, false (*Franseria acanthicarpa*), giant (*Ambrosia trifida*), short (*A. elatior*), Western (*A. psilostachya*) ragweed pollen mix 5% w/v, perennial ryegrass (*Lolium perenne*) pollen 5% w/v, *Aspergillus* mix 10% w/v and *Alternaria alternata* 10% w/v were obtained from Hollister-Stier laboratories, Spokane, Washington. *D. pteronyssinus* 2% w/v, feather mix 500 PNU/ml and plantain (*Plantago lanceolata*) pollen 5000 PNU/ml allergen extracts were purchased from Dome Laboratories, Mulgrave, Australia. *Tyrophagus putrescentiae* water-soluble extracts were supplied by Mr W. F. Green, Dept. of Medicine, University of Sydney, and used at a concentration of 10 mg/ml in Coca's solution.

Histamine acid phosphate (David Bull Laboratories) (1 mg/ml in Coca's solution) was used as a positive reference (2). Weal sizes were recorded 15 min after testing as the average diameter ( $\frac{D+d}{2}$  where D is the largest diameter of the weal and d the diameter perpendicular to this). Relative potencies of cereal and other allergen extracts to the histamine standard were assessed by comparing the average diameters of weals and by allotting the histamine solution a potency of 1 (3).

*Bronchial provocation tests.* Subject Mos was admitted to hospital for the bronchial provocation testing (1). Baseline values for peak expiratory flow rate (PEFR), vital capacity and forced expiratory volume ( $FEV_{1sec}$ ) were recorded using an Autospirometer (Minato Medical Co. Ltd.) prior to inhalation challenge. Inhalation challenge was performed using a modified version of the "occupational" method as described by Pepys et al. (29). The flour sample (250 g) or lactose, was shaken by the patient from one plastic tray into another. This was performed in a confined space which allowed for saturation of the atmosphere. The patient was closely supervised during the procedure with recordings of his PEFR and  $FEV_1$  every 5 min. The inhalation challenge was terminated when there was a greater than 10% fall in the subject's  $FEV_1$ . Peak flow measurements were then recorded every 10 min for the next 2 h and at frequent intervals over the next 24 h.

*Allergen discs.* Cereal preparations were coupled to Whatman grade 44 6-mm paper discs using cyanogen bromide as described by Ceska et al. (11). Wheat, rye, barley and oat RAST allergen discs were obtained from Pharmacia, Uppsala, Sweden.

*RAST and RAST inhibition procedures.* These were carried out as previously described (4, 5).

*Histamine release assay.* Histamine released from human leucocytes following allergen challenge was determined with the enzymatic-isotopic microassay (34, 37) using whole human blood, S-adenosyl-L-[methyl- $^3H$ ]methionine, 500 mCi/mmol (Radiochemical Centre, Amersham) as the methyl donor and mouse brain as a source of histamine methyltransferase. Assays with heparinized blood were carried out within 4 h of collection, and release of histamine was determined in duplicate on 100  $\mu$ l aliquots in 400  $\mu$ l Beckmann Microfuge tubes. After addition of allergen extracts, tubes were incubated at 37°C for 60 min and centrifuged at 2000 g for

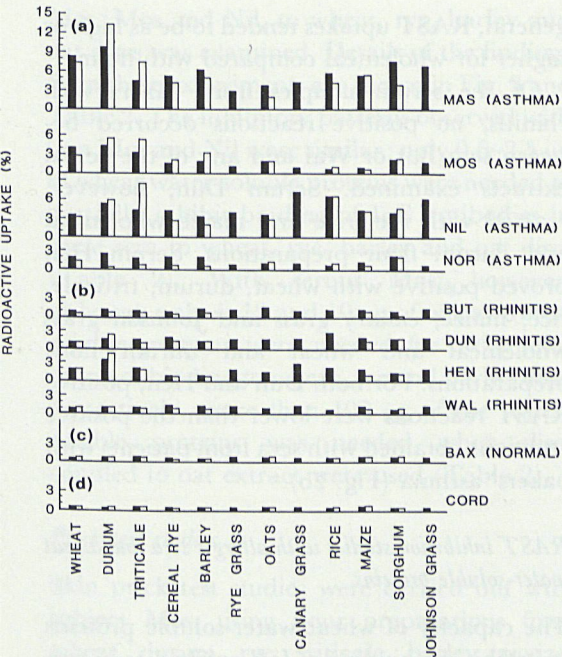


Fig. 2. Estimation by RAST of the presence of IgE antibodies with specificity for extracts of wholemeal (black bars) or flour (white bars) from a range of cereals (arranged from left to right in order of their relatedness to wheat), using serum samples from (a) adults with bakers' asthma, (b) adults with rhinitis due to cereal flour, (c) a normal adult (this set of results is representative of those for six non-allergic individuals) and (d) umbilical cord. Serum samples from Mas, Mos and Nil were diluted 5-, 2- and 2-fold.

10 min before removing 20  $\mu$ l aliquots of plasma for estimation of the liberated histamine. With each assay, blanks and internal and external histamine standards were included (37).

## RESULTS

### Direct RAST studies

Cereal extracts were compared side-by-side in direct RAST experiments with allergic sera, pooled cord serum and six non-allergic (normal) sera (Fig. 2). Results with cord and normal sera showed that no significant reactions occurred with any of the cereal extracts examined. Radioactive uptakes ob-

tained with the normal sera were not significantly different from cord uptakes (Figs. 2c, d). The findings with cord serum and sera from allergic subjects Mas, Mos, Nil and Nor are shown in Fig. 2a.

Undiluted serum from subject Nor, who had not worked as a baker for 15 years, showed

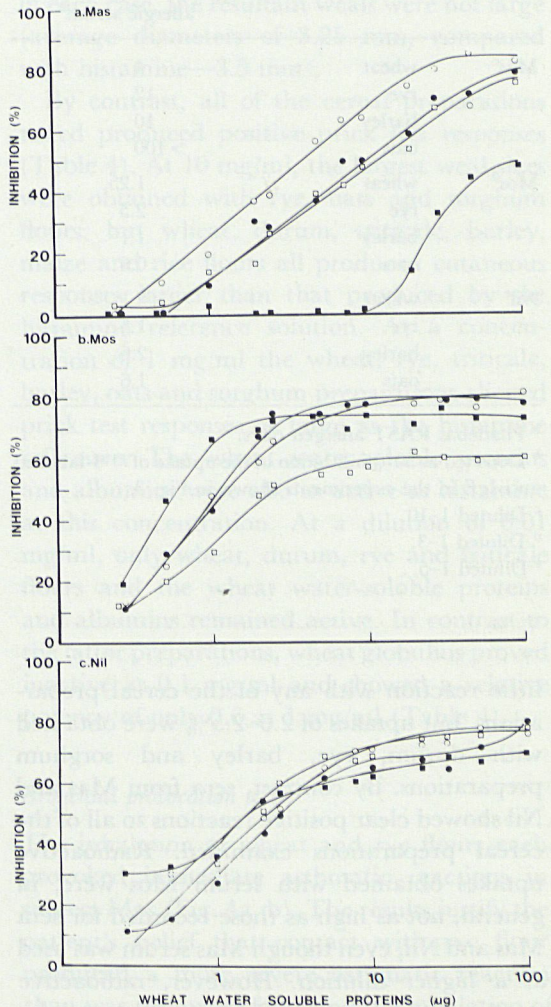


Fig. 3. Radioallergosorbent test inhibition results using Phadebas discs coupled with aqueous extracts from grain of wheat (open circles), barley (closed circles), rye (open squares) and oats (closed squares), together with sera from (a) Mas, (b) Mos, and (c) Nil, diluted 10-, 3- and 5-fold. The graphs show the extent to which IgE binding to the discs was inhibited by pre-incubation of the sera with wheat water-soluble proteins. Table 2 shows the amount of wheat water-soluble protein needed to produce 50% inhibition.

Table 2  
RAST inhibition results using wheat water-soluble protein fraction together with sera from subjects with bakers' asthma and discs coupled to cereal extracts

| Serum            | Discs <sup>a</sup> coupled with extract from | Quantity of wheat water-soluble proteins (µg) needed to produce 50% inhibition of the uptake of IgE antibodies from allergic serum <sup>b</sup> |
|------------------|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Mas <sup>c</sup> | wheat                                        | 4                                                                                                                                               |
|                  | rye                                          | 12                                                                                                                                              |
|                  | barley                                       | 10                                                                                                                                              |
|                  | oats                                         | > 100                                                                                                                                           |
| Mos <sup>d</sup> | wheat                                        | 1.25                                                                                                                                            |
|                  | rye                                          | 2.5                                                                                                                                             |
|                  | barley                                       | 1.1                                                                                                                                             |
|                  | oats                                         | 0.6                                                                                                                                             |
| Nil <sup>e</sup> | wheat                                        | 2.1                                                                                                                                             |
|                  | rye                                          | 1.5                                                                                                                                             |
|                  | barley                                       | 2.6                                                                                                                                             |
|                  | oats                                         | 1.6                                                                                                                                             |

<sup>a</sup> Phadebas RAST allergen discs.  
<sup>b</sup> Detected as 50% inhibition of the uptake of <sup>125</sup>I-labelled anti-IgE in the experiments shown in Fig. 3.  
<sup>c</sup> Diluted 1-10.  
<sup>d</sup> Diluted 1-3.  
<sup>e</sup> Diluted 1-5.

little reaction with any of the cereal preparations, but uptakes of 2.0-2.5% were obtained with durum, rye, barley and sorghum preparations. By contrast, sera from Mas and Nil showed clear positive reactions to all of the cereal preparations examined. Radioactive uptakes obtained with serum Mos were, in general, not as high as those recorded for sera Mas and Nil, even though Mas serum was used at a higher dilution. However, radioactive uptakes with serum Mos indicated the presence of IgE antibodies reactive with wheat, durum and triticale allergenic constituents as well as weaker, but probably significant, reactivity with constituents from rye, barley, oats and sorghum extracts. Only sera Mas and Nil showed clear, positive reactions to ryegrass (*Lolium*), canary grass and Johnson grass wholemeal extracts (Fig. 2a). In

general, RAST uptakes tended to be as high or higher for wholemeal compared with flour.

Of the serum samples from bakers with rhinitis, no positive reactions occurred between sera But or Wal and any of the cereal extracts examined. Serum Dun, however, reacted with rye, rice and maize wholemeal and durum flour preparations. Serum Hen proved positive with wheat, durum, triticale, rice, maize, canary grass and Johnson grass wholemeal and wheat and durum flour preparations. For both Dun and Hen, positive RAST reactions were lower than the positive reactions obtained with sera from patients with bakers' asthma (Fig. 2b).

*RAST inhibition studies with allergic sera and wheat water-soluble proteins*

The capacity of wheat water-soluble proteins to inhibit the binding of IgE antibodies in sera

Table 3  
Prick test responses by subject Mos to some commonly encountered allergens

| Allergen extract <sup>a</sup>   | Cutaneous response <sup>b</sup> and weal size (mm) |
|---------------------------------|----------------------------------------------------|
| <i>Pollens</i>                  |                                                    |
| Ryegrass                        | 2.5 × 2.0                                          |
| Timothy                         | negative                                           |
| Plantain                        | negative                                           |
| Ragweed                         | negative                                           |
| <i>Mites</i>                    |                                                    |
| <i>D. farinae</i>               | negative                                           |
| <i>D. pteronyssinus</i>         | 2.5 × 2.5                                          |
| <i>Tyrophagus putrescentiae</i> | 2.5 × 4.0                                          |
| <i>Dander—fur</i>               |                                                    |
| Cat                             | negative                                           |
| Dog                             | 2.5 × 2.5                                          |
| <i>Fungi</i>                    |                                                    |
| <i>Alternaria alternata</i>     | negative                                           |
| <i>Aspergillus mix</i>          | negative                                           |
| <i>Other</i>                    |                                                    |
| House dust                      | 2.0 × 2.0                                          |
| Feather mix                     | negative                                           |

<sup>a</sup> For details of extracts used, see Material and Methods.  
<sup>b</sup> Prick test response to histamine acid phosphate 1 mg/ml, 3 mm × 4 mm.

Mas, Mos and Nil, to wheat, rye, barley and oat discs was examined. Details of the findings from these experiments are shown in Fig. 3 and Table 2. The inhibitory patterns observed with sera Mos and Nil were similar; only 0.6–2.5  $\mu\text{g}$  of wheat water-soluble proteins were needed to partially inhibit binding of IgE antibodies in these sera to wheat, rye, barley and oat discs (Table 2). With serum Mas, however, although only 4, 12 and 10  $\mu\text{g}$  of wheat water-soluble proteins were needed for 50% inhibition of binding to wheat, rye and barley discs respectively, more than 100  $\mu\text{g}$  of the water-soluble proteins was needed when discs coupled to oat extract were used (Table 2).

#### Prick test studies

Skin prick test studies were carried out with subject Mos, using flour preparations from wheat, durum, rye, triticale, barley, maize, sorghum, oats and rice, together with a battery of allergens known to be important in the Sydney environment, and histamine (1 mg/ml) as reference. The weal size produced by the histamine solution was measured and assigned

a potency of 1. Weal sizes relative to the histamine reference were assessed for all other skin test preparations. Table 3 lists the prick test responses of subject Mos to the pollen, mite, epithelial, fungal and feather preparations tested. Positive reactions were obtained to only five of the 12 extracts; ryegrass, house dust, two mite species and dog dander/fur but, in each case, the resultant weals were not large (average diameters of 3.25 mm, compared with histamine—3.5 mm).

By contrast, all of the cereal preparations tested produced positive prick test responses (Table 4). At 10 mg/ml, the largest weal sizes were obtained with rye, oats and sorghum flours, but wheat, durum, triticale, barley, maize and rice flours all produced cutaneous responses larger than that produced by the histamine reference solution. At a concentration of 1 mg/ml the wheat, rye, triticale, barley, oats and sorghum preparations elicited prick test responses as large as the histamine reference. The wheat water-soluble proteins and albumins were also as active as histamine at this concentration. At a dilution of 0.01 mg/ml, only wheat, durum, rye and triticale flours and the wheat water-soluble proteins and albumins remained active. In contrast to the latter preparations, wheat globulins proved inactive at 0.1 mg/ml and showed a relative potency of only 0.6 at 1 mg/ml (Table 4).

Table 4  
Prick test results using cereal extracts with subject Mos. Relative potency of extracts compared with histamine standard

| Cereal preparation           | Relative potencies of prick test solutions at |         |           |            |
|------------------------------|-----------------------------------------------|---------|-----------|------------|
|                              | 10 mg/ml                                      | 1 mg/ml | 0.1 mg/ml | 0.01 mg/ml |
| Wheat flour                  | 1.1                                           | 1.0     | 0.8       | 0.9        |
| Durum flour                  | 1.3                                           | 0.9     | 0.8       | 0.9        |
| Triticale flour              | 1.4                                           | 1.0     | 0.9       | 0.5        |
| Rye flour                    | 1.5                                           | 1.3     | 0.9       | 0.6        |
| Barley flour                 | 1.4                                           | 1.0     | 0.9       | 0          |
| Oats flour                   | 1.4                                           | 0.7     | 0.5       | 0          |
| Rice flour                   | 1.3                                           | 0.8     | 0.6       | 0          |
| Maize flour                  | 1.1                                           | 0.9     | 1.0       | 0          |
| Sorghum flour                | 1.7                                           | 1.0     | 0         | 0          |
| Wheat water-soluble proteins | —                                             | 1.1     | 0.9       | 0.4        |
| Wheat albumins               | —                                             | 1.1     | 0.8       | 0.5        |
| Wheat globulins              | —                                             | 0.6     | 0         | 0          |

Wheat water-soluble proteins, albumins and globulins were not tested at 10 mg/ml.

#### Bronchial provocation tests

The inhalation of wheat and rye flours each provoked immediate asthmatic reactions in subject Mos (Fig. 4a, b). The results justify the patient's belief that contact with rye flour produced a more severe asthmatic reaction than was produced following the inhalation of wheat flour. Only half the exposure time was required for rye flour to give the same drop in PEFR as was produced by 20 min inhalation of wheat flour and, in addition, the recovery from challenge with rye was slower than in the case of wheat. Both cereal flours also provoked delayed asthmatic reactions about 4 h after the initial exposure. Again this response was also

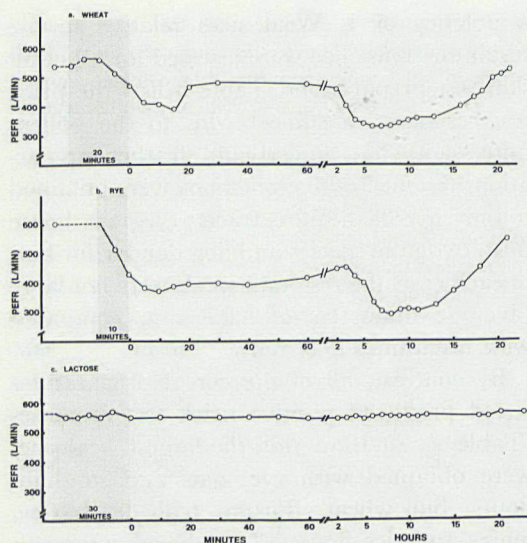


Fig. 4. Bronchial provocation testing of flour-allergic baker Mos, showing PEFr during the 24 h following the inhalation challenge (darkened area), for (a) wheat flour, (b) rye flour and (c) lactose powder.

more severe in the case of rye. No reactions were observed after the inhalation of lactose (Fig. 4c).

### Histamine release studies

Aliquots of whole blood from subject Mos were challenged with different cereal flours. Each of the extracts released histamine although, in the concentration range examined, the oat, rye and triticale extracts provoked the highest percentage release (Fig. 5). There was a contrast between wheat and rye in their relative capacities to release histamine at different concentrations. An increase in the concentration of either antigen from 1 to 10  $\mu\text{g/ml}$  increased histamine release due to rye from 20 to 50%, but had little effect on release due to wheat antigens (33 to 38%). Histamine release due to the wheat-rye hybrid, triticale, was similar to that of rye at the highest concentration tested.

## DISCUSSION

Table 1 shows the taxonomic relationships between the range of cereals examined and this

can serve as a basis for discussion. Distance down the Table indicates the degree of relatedness to wheat. In addition, Fig. 1 shows similarities and differences between the cereal extracts examined in terms of protein composition.

Although wheat and rye are classified in two separate genera (Table 1), they are closely related taxonomically. Intergeneric hybridization between them is possible (to produce triticale) and both are presumed to have been derived from the same progenitor. A comparison of the proteins extractable from wheat and rye flour (Fig. 1) shows that differences between the two electrophoretic patterns are most noticeable in the prolamin proteins (22). These are the storage proteins, separated in the upper two thirds of the gradient gel in Fig. 1. Similarity of protein composition between wheat and rye is more noticeable in the albumin proteins (separated in the lowest third of the gel in Fig. 1), and this is the class of

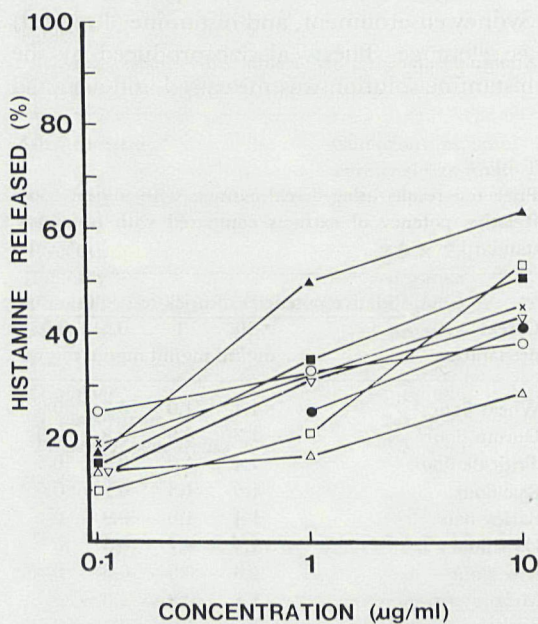


Fig. 5. Release of histamine from leucocytes of subject Mos following challenge at three concentrations with water-soluble material from wheat (open circles), durum (closed circles), triticale (closed squares), rye (open squares), barley (open triangles, pointing up), oats (closed triangles), maize (open triangles, pointing down) and with wheat water-soluble proteins (crosses).

protein reported for wheat at least, to contain the principal allergens involved in bakers' asthma (5). Of course, similarity of electrophoretic mobility does not indicate that proteins are chemically identical, but the possession of some similarities in chemical structure might be expected in view of the close taxonomic relationship between wheat and rye. Clearly, further purification and comparison of the most allergenic fractions from wheat and rye would be valuable.

In immunological studies, mainly involving double diffusion, antigenic similarities between wheat and rye proteins have been demonstrated, both in the albumin (15) and prolamin (13) classes. Minor similarities were evident when wheat was compared with more distantly related cereals. Furthermore, biological similarities between the proteins of wheat, rye and barley, compared with those of other cereals, have been shown in anaphylactic studies in laboratory animals (Wells & Osborne 1911, reported by Ewart (14)).

Our direct RAST studies, in particular with sera from subjects Mas and Nil, showed that cereal-specific IgE antibodies in these sera reacted with water-soluble extracts from each of the species studied. Wheat and its close relatives generally gave higher RAST figures than did other cereals with serum Mos (Fig. 2) but the difference was not great and, in particular, there was no evidence for rye being quantitatively more important. It should be pointed out, however, that some of the grain proteins are insoluble under the conditions used for extraction and would therefore not be expected to bind to the activated paper discs. RAST inhibition findings support the tentative conclusion that allergenic cross-reactivity between cereals exists, although inhibition results with Mas serum indicated the presence of IgE antibodies to oat allergens that cross-reacted only weakly with allergens from wheat, rye and barley. Allergenic cross-reactivity between different cereals has been noticed by Linko (20) and Schwartz (32), and more recently Hoffman (18) concluded that corn (maize) and rice appear to be closely

related antigenically. In a study of cereal protein-specific IgE, Björkstén et al. (7) observed good correlation between RAST results obtained with wheat and rye flour extracts. Using crossed immunoelectrophoresis, Blands et al. (8) demonstrated that 20 different wheat flour proteins cross-reacted with rye flour extract.

In testing a subject sensitized by inhalation of wheat or rye flour, one might expect that reactivity would be lower for distantly related cereals. This contention was borne out in skin testing subject Mos with low antigen concentrations (Table 4). No reactions were observed to the cereals in the lower part of Table 4, that is, to the more distant relatives of wheat such as sorghum, maize, rice, oats and barley. However, skin test responses to the more distantly related cereals were strong at much higher antigen concentrations.

Skin test studies with a panel of important allergen extracts demonstrated that subject Mos reacted weakly to only a few of these (Table 3). Although the patient believed that his most severe symptoms were caused by rye and not other cereal flours, our data, obtained from a number of different tests, at first sight appear to be conflicting on this point. Prick test, histamine release at low allergen concentration and RAST results did not clearly support the patient's belief, although definite reactivity to rye allergens was demonstrated. However, when compared with the response elicited by wheat flour following bronchial challenge, rye flour initiated an earlier and more severe immediate reaction. The subject's PEFR was lower following challenge with rye flour and the delayed reaction was more severe after rye than after wheat flour challenge. In addition, rye extract was more effective at releasing histamine from the patient's leucocytes when used at high (10  $\mu$ g/ml) allergen concentration.

The apparent inconsistencies of our results with prick test, histamine release and RAST studies on the one hand and bronchial challenge responses on the other, are not necessarily incompatible with the patient's

belief that rye flour provoked more severe asthmatic attacks than other cereal flours. The well-known failure of antihistamines to consistently ameliorate asthma has been attributed to the involvement and bronchoconstricting properties of another mediator, slow reacting substance of anaphylaxis (SRS-A), in human asthma (9). This substance causes a prolonged contraction of circular muscles of human bronchioles and, although the ratio of SRS-A to histamine is high in human lung, the former mediator is difficult to detect in skin (10). Hence, one should not necessarily expect allergic reactions detected by RAST studies on serum, prick test and *in vitro* histamine release investigations to always show close correlation with bronchial provocation tests.

Immediate and non-immediate (dual) asthmatic responses have been reported to a range of allergens including *Bacillus subtilis* enzymes (27), *Aspergillus fumigatus* (21), avian allergens (16), wood dusts (12), ragweed pollen (31) and flour (17, 24). Pepys & Hutchcroft (28) and Pepys (26) claim that there are at least three patterns of non-immediate (late) asthmatic reactions. Our bronchial provocation test results and those of Hendrick et al. (17) and Nakazawa et al. (24), conformed to one of those patterns—a delayed reaction that started at about 2 h, reached maximum intensity at 7 h and lasted for up to about 18 h.

Although the number of individuals we have examined in the present and past (5) studies is still small, our results indicate that flour-specific IgE levels as measured by the RAST are far higher in patients with bakers' asthma than in patients with flour-induced rhinitis. Only two of the four sera taken from bakers with flour-induced rhinitis showed clear positive reactions in the RAST and, even then, radioactive uptakes were significantly lower than the uptakes obtained with sera from the asthmatic subjects Mas, Mos and Nil. These findings demonstrate the need for the use of individually purified cereal allergens rather than whole unfractionated water-soluble ex-

tracts in RAST studies of rhinitis to cereals. As with bakers' asthma, however, identification of the important allergens is the first requirement.

Although hypersensitivity to bran was not specifically examined in this study, it can be inferred from comparison of the results obtained with flour and wholemeal (Fig. 2) that bran proteins, as well as those in flour, may contribute to allergic reactions. Although the germ, bran and husk content of cereal grains accounts for 15 to 25% of the dry weight (19), RAST figures were not, in general, proportionally lower for wholemeal, as might be expected if the bran layers acted merely as an inert diluent. In fact, in many instances RAST uptakes were actually higher for wholemeal samples. Two clear-cut exceptions to this generalization were durum and triticale for which flour extracts were consistently more reactive than the wholemeal preparations. Fig. 1 shows that the protein composition of wheat bran and flour are different in many respects, although differences in electrophoretic behaviour may not necessarily go hand-in-hand with differences in allergenicity. The relative allergenicity of the bran proteins is probably of more significance to grain handlers than to bakers since much of the dust produced when whole grain is moved is due to damage to the outer layers of the grain (33). Further investigation of bran allergens is thus warranted.

One of the possible implications of our results is that an individual who has become sensitized to a cereal allergen by inhalation, for example in a wheat flour bakery, is unlikely to completely overcome the problem posed by his hypersensitivity by transferring to a branch of the trade that handles a different cereal, for example, rice or maize milling. This conclusion presumes, of course, a close relationship between symptoms and immunological testing. On the other hand, if general cross-reactivity between cereals is confirmed in studies using larger numbers of allergic patients, testing with extracts from many different cereal flours would not be necessary. Furthermore, such

information would be useful in designing specific immunotherapy, since it is possible that only one allergen would need to be used, particularly if it is reasonably closely related to the cereal provoking the hypersensitivity.

## ACKNOWLEDGEMENTS

We thank Kevin S. Moses and the Respiratory Laboratory, Royal Prince Alfred Hospital, Sydney, for their co-operation, Dr R. B. Wilson of Cudal, New South Wales, for supplying serum, and Catherine L. Nelson and Paul G. Thomas for technical assistance.

## REFERENCES

- Aas, K.: The bronchial provocation test. C. C Thomas, Springfield Ill., 1975.
- Aas, K. & Belin, L.: Standardization of the diagnostic work in allergy. *Acta Allergol.* 27, 439-468, 1972.
- Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radio-allergosorbent tests. *Allergy* 33, 130-137, 1978.
- Baldo, B. A. & Uhlenbruck, G.: Selective isolation of allergens. I. Reaction of house dust mite extracts with tridacnin and concanavalin A and examination of the allergenicity of the isolated components. *Clin. Allergy* 7, 429-443, 1977.
- Baldo, B. A. & Wrigley, C. W.: IgE antibodies to wheat flour components. Studies with sera from subjects with bakers' asthma or coeliac condition. *Clin. Allergy* 8, 109-124, 1978.
- Baum, B. R.: Taxonomy of the tribe Triticeae (Poaceae) using various numerical techniques. II. Classification. *Canad. J. Bot.* 56, 27-56, 1978.
- Björkstén, F., Backman, A., Järvinen, K. A. J., Lehti, H., Savilahti, E., Syvänen, P. & Kärkkäinen, T.: Immunoglobulin E specific to wheat and rye flour proteins. *Clin. Allergy* 7, 473-483, 1977.
- Blands, J., Diamant, B., Kallós, P., Kallós-Defner, L. & Löwenstein, H.: Flour allergy in bakers. I. Identification of allergenic fractions in flour and comparison of diagnostic methods. *Int. Arch. Allergy* 52, 392-406, 1976.
- Brocklehurst, W. E.: Slow reacting substance and related compounds. In Kallos, P. & Waksman, B. H. (eds.): *Progress in allergy*, Vol. 6, pp. 540-558. Karger, Basel, 1962.
- Brocklehurst, W. E.: Pharmacodynamics and mechanisms of asthma. In Weiss, E. B. & Segal, M. S. (eds.): *Bronchial asthma mechanisms and therapeutics*, pp. 117-136. Little, Brown & Co., Boston, 1976.
- Ceska, M., Eriksson, R. & Varga, J. M.: Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunol.* 49, 1-9, 1972.
- Chan-Yeung, M.: Maximal respiratory flow and airway resistance during induced bronchoconstriction in patients with asthma due to Western Red Cedar (*Thuja plicata*). *Am. Rev. Resp. Dis.* 108, 1103-1110, 1973.
- Ewart, J. A. D.: Cereal proteins. Immunological studies. *J. Sci. Food Agric.* 17, 279-284, 1966.
- Ewart, J. A. D.: Immunochemistry of wheat proteins. In Catsimpoolas, N. (ed.): *Immunological aspects of foods*, pp. 87-116. AVI Publ. Co., Westport, Conn., U.S.A., 1977.
- Hall, O.: Immuno-electrophoretic analyses of allopolyploid ryewheat and its parental species. *Hereditas* 45, 495-504, 1959.
- Hargreave, F. E. & Pepys, J.: Allergic respiratory reactions in bird fanciers provoked by allergen inhalation provocation tests. *J. Allergy Clin. Immunol.* 50, 157-173, 1972.
- Hendrick, D. J., Davies, R. J. & Pepys, J.: Bakers' asthma. *Clin. Allergy* 6, 241-250, 1976.
- Hoffman, D. R.: The specificities of human IgE antibodies combining with cereal grains. *Immunochemistry* 12, 535-538, 1975.
- Kent, N. H.: Technology of cereals with special reference to wheat. Pergamon Press, Oxford, 1975.
- Linko, E.: On allergic rhinitis and bronchial asthma in bakers. *Ann. Med. Intern. Fenn.* 36, 98-111, 1947.
- McCarthy, D. S. & Pepys, J.: Allergic bronchopulmonary aspergillosis. Clinical immunology: (2) Skin, nasal and bronchial tests. *Clin. Allergy* 1, 415-432, 1971.
- McCausland, J. & Wrigley, C. W.: Electrophoretic analysis of wheat and rye mixtures in meal, flour and baked goods. *J. Sci. Food Agric.* 27, 1197-1202, 1976.
- Margolis, J. & Wrigley, C. W.: Improvement in pore gradient electrophoresis by increasing the degree of cross-linking at high acrylamide concentrations. *J. Chromatog.* 106, 204-209, 1975.
- Nakazawa, T., Toyoda, T., Furukawa, M., Taya, T. & Kobayashi, S.: Inhibitory effects of various drugs on dual asthmatic responses in wheat flour-sensitive subjects. *J. Allergy Clin. Immunol.* 58, 1-9, 1976.
- Ordman, D.: Cereal grain dusts as a cause of respiratory allergy in South Africa. *S.Afr. Med. J.* 32, 784-788, 1958.
- Pepys, J.: Non immediate asthmatic reactions. In Weiss, E. B. & Segal, M. S. (eds.): *Bronchial asthma mechanisms and therapeutics*, pp. 231-256. Little, Brown & Co., Boston, 1976.
- Pepys, J., Hargreave, F. E., Longbottom, J. L. & Faux, J. A.: Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet* i, 1181-1184, 1969.
- Pepys, J. & Hutchcroft, B. J.: Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am. Rev. Resp. Dis.* 112, 829-859, 1975.

29. Pepys, J., Pickering, C. A. & Loudon, H. W. G.: Asthma due to inhaled chemical agents—piperazine dihydrochloride. *Clin. Allergy* 2, 189–196, 1972.
30. Popa, V., George, S. A. L. & Găvănescu, O.: Occupational and non occupational respiratory allergy in bakers. *Acta Allergol.* 25, 159–177, 1970.
31. Robertson, D. G., Kerigan, A. T., Hargreave, F. E., Chalmers, R. & Dolovich, J.: Late asthmatic responses induced by ragweed pollen allergen. *J. Allergy Clin. Immunol.* 54, 244–254, 1974.
32. Schwartz, M.: Flour allergy. *J. Allergy* 18, 341–350, 1947.
33. Simmonds, D. H., St Clair, M. S. & Collins, J. D.: Surface structure of wheat and its contribution to the dust problem. *Cereal Sci. Today* 15, 230–239, 1970.
34. Snyder, S. H., Baldessarini, R. J. & Axelrod, J.: A sensitive and specific enzymatic isotopic assay for tissue histamine. *J. Pharmacol. Exp. Ther.* 153, 544–549, 1966.
35. Stebbins, G. L. & Crampton, B.: A suggested revision of the grass genera of temperate North America. In *Recent advances in botany*, pp. 133–145. University of Toronto Press, 1961.
36. Stresemann, E.: Results of bronchial testing in bakers. *Acta Allergol.* 22, Suppl. 8, 99–102, 1967.
37. Taylor, K. M., Krilis, S. & Baldo, B. A.: An enzymatic-isotopic microassay for measuring allergic release of histamine from blood and mast cells *in vitro*. *Int. Arch. Allergy* (in press).
38. Watson, L. & Clifford, H. T.: The major groups of Australasian grasses: a guide to sampling. *Aust. J. Bot.* 24, 489–507, 1976.
39. Wrigley, C. W. & McCausland, J.: Variety identification by laboratory methods: Instruction manual for barley, wheat and other cereals. Technical Publication No. 4, CSIRO Wheat Research Unit, North Ryde, 2113, Australia, 1977.

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