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Grass Allergens, with Particular Reference to Cereals¹

Brian A. Baldo², Rosemary Sutton, Colin W. Wrigley

Roche Research Institute of Marine Pharmacology, Dee Why, N.S.W.
CSIRO Wheat Research Unit, North Ryde, N.S.W., Australia

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Introduction

Many species of the grass family are important sources of allergens. Various anatomical parts of the plant are involved – the pollen, straw, chaff or the pericarp or endosperm of the grain. Contact may be with the skin, through inhalation or ingestion. The aim of this chapter is not only to give a perspective of man's contact with grasses and cereals as allergens, but also to provide the important background information needed to appreciate their role in allergy in contrast to other sources of hypersensitivity.

The chapter sets out to explain taxonomic relationships between cereals, other grasses and other plants, to indicate the occupations and situations where they are most likely to occur as allergens, and to describe the parts of the plant and likely contaminants suspected of producing hypersensitivity. The review is also designed to correct various botanical misconceptions amongst allergists. These include the common division of pollens into those from grass or weed species (when many grasses are themselves weeds), the use of trivial or confusing plant names (e.g. rye, couch) with no clear meaning beyond the locality, and the failure to indicate the anatomical source of plant allergens.

In reviewing cereal-induced hypersensitivities, we have concentrated on IgE-mediated allergic reactions and emphasized, where possible, those studies designed to isolate and characterize important allergens. Although little progress has been made in identifying and isolating allergens from grain contaminants such as fungi, beetles, moths and mites, a discussion of the role of these organisms in initiating allergic disorders is presented in some detail. This has been done since the allergenic properties of organisms that infest grain do not appear to have been reviewed before and because some of the organisms, such as storage mites, are being increasingly recognized as potent allergen sources for man.

*Ubiquitous Grasses**Characteristics of Grasses*

Grass allergens are everywhere. We all breathe their pollens and eat their proteins. The grasses are in fact amongst the most prolific of the plant families, with respect to numbers of species (about 9,000). More significantly the grass family contains the plants of greatest agricultural importance to man, since the various members of this family provide food (from their grains, stems, leaves, roots and shoots), building materials, paper pulp, lawns, pasture, hay and even perfumes. Grasses dominate vast areas of the earth's surface, both cultivated and native areas, extending from the tropics through temperate savannas, prairies and even marshes, to Arctic tundra. Their success is such that there are probably more individual grass plants than there are of all other vascular plants combined. This success also means that many of the most troublesome weeds are grasses.

In size they range from tiny lawn grasses to giant bamboos which may grow to a height of 30 m. However, the grasses share the same general pattern of having ribbon-shaped leaves with parallel veins, and a hollow cylindrical stem which is largely a leaf-sheath with leaves arising alternately from opposite sides.

Cereal Grains

The cereals are amongst the most important of the grass species. The term 'cereal' tends to have a functional connotation (a food grain of the grass family), rather than a strict taxonomic meaning. The common cereals are listed in table I. Other non-cereal grains used for food or feed include peas, beans, soybeans, lupins, rapeseed, cottonseed, linseed, safflower and sunflowerseed. Buckwheat is not a cereal. It is sometimes called a pseudo-cereal, but is quite distant in its relationship to the grasses (table II).

Cereal grains constitute the staple food for most of mankind. They contribute about half the world's supply of human dietary protein and an even higher proportion of the energy needs in man's diet. Annual world production of the three main cereal grains (wheat, rice and maize) averages nearly 300 kg per person. The cultivation of cereals accompanied the rise of civilization in various centres between 5,000 and 8,000 years ago. Cereals are now grown on a large scale in all continents and at virtually all latitudes. The distribution of individual cereals is spread even more widely by the international trade which continues on a massive scale.

Table 1. The cereal grains: world annual production and food uses

Cereal	Megatonnes produced ¹	Major uses in food ²
Wheat	441	bread (leavened and unleavened); pasta (spaghetti, macaroni); noodles; breakfast cereals; cakes; biscuits; cookies; scones; chappatis; porridge; extruded snackfoods; bulgur; beer; flavourings; many canned, packaged, frozen and convenience foods and sweets
Rye	32	bread; crispbread; spirits
Barley	196	porridge; cooked grains; malt; beer; spirits
Oats	50	porridge (rolled oats, oatmeal); muesli; oatcakes
Rice	376	cooked grains; breakfast foods; extruded snack foods; sake
Maize	363	hominy; tortillas; cornbreads; cooked grains; polenta; breakfast foods; popcorn; extruded snack foods; corn syrup; many manufactured foods
Sorghum	69	cooked grains; porridge; unleavened bread; flatcakes; beer
Millet	38	

¹ World production in 1978 according to FAO [77].

² From *Saxelby and Venn-Brown* [210].

Uses of Cereal Products

Table I lists many of the food uses for the individual cereal grains. The range of products extends well beyond the obvious short list of baked foods and breakfast foods. Nor is table I exhaustive since wheat and maize (corn) products in particular are used in an endless variety of processed foods. Cereals are often used in confectionery, spreads and condiments, in processed meat products, as dusting or batter around foods, as thickening agents, fillers and binders, and in many drinks (e.g. malted milk, hot chocolate).

A considerable proportion of the cereal crop is processed in developed countries to separate the starch from the protein. The starch, either partly or completely freed of protein, is widely used in food processing often after chemical modification or degradation [99]. The cereal protein fraction is incorporated in a wide variety of baked and processed foods. Wheat gluten, in particular, is used to manufacture flavourings such as monosodium glutamate, but in addition the elastic properties of gluten suit it to the development of new foods and textured products which mimic animal

protein [96]. The expected expansion in producing liquid fuel (mainly ethanol – 'gasohol') from cereal starch and bran [215] is likely to greatly extend the incorporation of cereal protein in foods where its presence will become impossible to recognize.

Fuel production is only one of many industrial processes reliant on cereal starch and protein for raw materials. The increasing price of petroleum products will lead manufacturers to change to cereal products for many processes [99]. Already they are widely used in the paper and textile industries, to manufacture glues and adhesives, plywood and particle board, in refining metals, and in the polymer industry (where incorporation of starch in plastic products offers the promise of biodegradability).

Add to these uses of cereal products, the feeding of whole grain and mill by-products to animals, and it is obvious that cereals enter our lives at many points. This contact is both general and also specific for certain occupational groups involved in the industries mentioned.

Airborne Products

Inhalation is another way in which cereal and grass allergens are transmitted. Pollens, the most obvious example, are produced by all the grasses – cultivated cereals, lawn and pasture species and weeds – since all are flowering plants. Details of the flowering times have been published for species in many localities [40, 205, 220, 256]. Pollen of one type or another may be present at almost any time of the year, except winter in cold climates. Less obvious inhalation of a cereal product occurs when flour is used in the home. In some cases, such exposure has been sufficient to produce severe allergy to the grain proteins.

However, occupational exposure increases the incidence of inhalant allergy to cereals. Groups at risk are farmers associated with the growing and harvesting of the grain, workmen involved in the handling and transport, and millers and bakers who may inhale bran and flour.

Taxonomy

Table II shows relationships within the grass family, and between the grasses and other plants. Knowledge of these relationships provides a basis for predicting similarities and differences in plant anatomy, chemical composition and immunological cross-reactivity. The major cereals have been

Table II. Relationships between prominent grain- and pollen-bearing plants. A. Monocotyledons – family Poaceae (Gramineae)

Subfamily	Tribe	Genus and species	Common name
Festucoideae	Triticeae	<i>Triticum aestivum</i>	bread wheat
		<i>Triticum durum</i>	durum wheat
		<i>Triticosecale</i>	triticale
		<i>Secale cereale</i>	rye
		<i>Agropyron repens</i>	English couch grass
		<i>Hordeum leporinum</i>	barley grass
		<i>Hordeum vulgare</i>	barley
	Aveneae	<i>Avena sativa</i>	oats
		<i>Avena fatua</i>	wild oats
		<i>Avena barbata</i>	bearded oats
		<i>Anthoxanthum odoratum</i>	sweet vernal grass
		<i>Holcus lanatus</i>	Yorkshire fog (meadow velvet)
	Agrostideae	<i>Holcus mollis</i>	creeping fog
		<i>Phleum pratense</i>	timothy grass
		<i>Agrostis species</i>	bent grass
	Phalarideae	<i>Phalaris canariensis</i>	canary grass
		<i>Phalaris arundinacea</i>	reed canary grass
	Festuceae	<i>Lolium perenne</i>	perennial rye grass
		<i>Poa annua</i>	winter grass
		<i>Poa pratensis</i>	Kentucky blue grass (June grass)
		<i>Dactylis glomerata</i>	cocksfoot (orchard grass)
		<i>Bromus catharticus</i>	prairie grass
		<i>Festuca pratensis</i>	meadow fescue
Eragrostoideae	Chlorideae	<i>Cynodon dactylon</i>	couch grass (Bermuda)
		<i>Chloris gayana</i>	Rhodes grass
Oryzoideae	Oryzeae	<i>Oryza sativa</i>	rice
	Zizanieae	<i>Zizania aquatica</i>	wild rice
	Ehrharteae	<i>Ehrharta erecta</i>	veldt grass, panic
		<i>Ehrharta calycine</i>	veldt grass, perennial
Panicoideae	Tripsaceae	<i>Zea mays</i>	corn or maize
	Andropogoneae	<i>Sorghum sudanense</i>	Sudan grass
		<i>Sorghum halepense</i>	Johnson grass
		<i>Sorghum bicolor</i>	grain sorghum
		<i>Themeda australis</i>	kangaroo grass
	Eu-panicoideae	<i>Echinochloa spp.</i>	Japanese millet
		<i>Panicum miliaceum</i>	common millet
		<i>Pennisetum typhoides</i>	pearl millet
		<i>Paspalum dilatatum</i>	paspalum grass
		<i>Stenotaphrum secundatum</i>	buffalo grass
Arundinoideae	Arundineae	<i>Phragmites species</i>	reed grass

Table II. Relationships between prominent grain- and pollen-bearing plants. B. Dicotyledons

Superorder	Family	Genus and species	Common name
Asteridae	Asteraceae (Compositae)	<i>Taraxacum officinale</i>	dandelion
		<i>Leucanthemum vulgare</i>	ox-eye daisy (marguerite)
		<i>Artemisia vulgaris</i>	mugwort
		<i>Solidago canadensis</i>	Canadian golden rod
		<i>Ambrosia</i> species	ragweeds
		<i>Helianthus annuus</i>	sunflower
	Plantaginaceae	<i>Plantago lanceolata</i>	ribwort (plantain)
	Boraginaceae	<i>Echium plantagineum</i>	Paterson's curse
Rosidae	Umbelliferae	<i>Foeniculum vulgare</i>	fennel
	Linaceae	<i>Linum usitatissimum</i>	linseed
	Leguminosae (Fabaceae)	<i>Trifolium</i> species	clover
		<i>Lupinus</i> species	lupin
		<i>Glycine max</i>	soybean
		<i>Pisum sativum</i>	pea
		<i>Arachis hypogaea</i>	peanut
	Dilleniidae	Brassicaceae (Cruciferae)	<i>Sisymbrium orientale</i>
<i>Brassica napus</i>			rape
Urticaceae		<i>Urtica</i> species	nettles
Malvaceae		<i>Gossypium</i> species	cottonseed
Caryophyllidae	Salsoloideae	<i>Salsola kali</i>	prickly saltwort (Russian thistle)
	Polygonaceae	<i>Fagopyrum esculentum</i>	buckwheat
		<i>Rumex crispus</i>	dock
		<i>Rumex acetosella</i>	sorrel
	Chenopodiaceae	<i>Chenopodium album</i>	fat hen
		<i>Chenopodium cristatum</i>	crested goosefoot

Non-approved names appear in parentheses.

Non-approved names appear in parentheses.

included in table II, together with the grasses commonly associated with pollen allergy. Also included by way of contrast are some of the common pollen- and grain-producing plants that are not grasses.

All the plants listed in table II are flowering plants (Angiosperms) and are thus pollen producers. These in turn are subdivided into monocotyledonous plants and dicotyledonous plants (often abbreviated to monocots and dicots). Use of these names should be encouraged instead of 'weeds' and grasses. Monocots and dicots are distinguished by the number (one or two,

respectively) of seed leaves (cotyledons) in the embryo. The grasses represent only one of many families of monocotyledons. Others include orchids, tulips and lilies. The family name for grasses, approved by the Twelfth International Botanical Congress in 1975 [225], is Poaceae but Gramineae has also been in common usage. The scientific names given in table II have been checked as far as possible to be the approved ones, and may differ from other versions still in use. Common names are even more difficult to standardize. A list of standard plant names [106] was used to compile table II, but different common names for some species are likely to be encountered.

Taxonomic classifications also undergo revision and consequent change. The relationships between the grasses in table II are based on classifications by several authors [27, 229, 244] with personal advice on some aspects from Crampton and from Clifford. Relationships between dicots are taken from Heywood [117] and Stebbins [228]. Table II is set up to primarily illustrate relationships to wheat. It shows the close relationship between the four cereals generally associated with coeliac condition (wheat, rye, barley, oats – in decreasing order of importance). Durum wheat (*Triticum durum*) is a tetraploid species containing two (A and B) of the three genomes of common wheat (*T. aestivum*) (hexaploid, genomes A, B and D). Triticale (genomes A, B and R) is a man-made hybrid of wheat with rye.

Table II shows the close relationship in some cases between species generally associated with pollen allergy, and those grown for grain production. Such instances are English couchgrass with wheat and barley, and ragweed with sunflowers. However, it should not be suggested that there is a clear distinction between pollen- and grain-producing plants; many grain-producing species may be significant sources of pollen allergy because of the large areas given to cultivating them, e.g. rapeseed in Sweden [40].

Inhaled Cereal Allergens

The extent to which we inhale air-borne particles depends on their sizes, in particular, and also on their shape and density. Figure 1 shows the degree of penetration into the respiratory tract of particles of various sizes. Pollens are the most obvious of these inhaled particles of grass origin. In addition, the harvesting, storage, transport, and processing of cereals produce a wide spectrum of inhalable particles which have a potential to provoke hypersensitive reactions in the nasal passages and lungs.

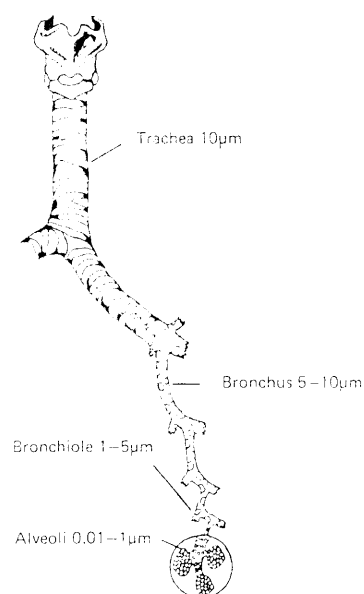


Fig. 1. Extent of penetration of cereal dust particles into the respiratory tract according to particle size.

Pollens

Grass Flower

As illustrated in figure 2, the grasses have flowers although they are not the decorative type borne by other plants. The flowering organs of the grass floret are wholly enclosed by green scale-like structures (glumes, lemma and palea) which look like petals in figures 2 and 3. At the time of flowering these glumes are pushed apart, the stalks (filaments) that support the anthers lengthen rapidly, and each anther begins to split open on both sides, releasing a shower of pollen granules [107, 256]. Some of the pollen (male gamete) falls directly on the feathery stigma where it germinates, leading to fertilization of the ovary (female gamete) at the base of the floret. The ovary develops into the new seed (caryopsis, grain), which remains between the floral glumes (fig. 4) until harvest. In wheat, but not barley, the grain is threshed free from the lemma and palea.

Much of the pollen also falls outside the floret and becomes airborne. Plants that are naturally cross-pollinating depend on the wind (anemophilous



Fig. 2. The flower of beard grass (*Lolium temulentum*) [from ref. 26]. The lemma has been drawn down to the lower right to disclose the ovary with its two feathery stigmas. These will receive the pollen after it is released from the anthers above them at the time of fertilization. (Photo by courtesy of Colin Totterdell, CSIRO, Division of Plant Industry, Canberra.)

Fig. 3. Release of pollen from the anthers of a wheat flower.

plants) or on insects (entomophilous) to carry the pollen from the anther of one flower to the stigma of another. Insect-borne pollen tends to be sticky and thus may not be so readily wind-borne or so likely a source of allergy. In general, the cereals are anemophilous. In some grasses, such as maize, pollen is produced in a completely separate structure (tassel) from that containing the ovary (the ear).

Pollen Structure

The number of pollen grains produced is enormous – over 20 million from one corn tassel or from one panicle of sorghum [107]. In a high wind, some of these may be carried for hundreds of metres. Figure 5a and b illustrates the surface structure of a grass pollen grain. The range of diameters normally encountered is 20–50 μm . Grass pollens tend to be smooth and

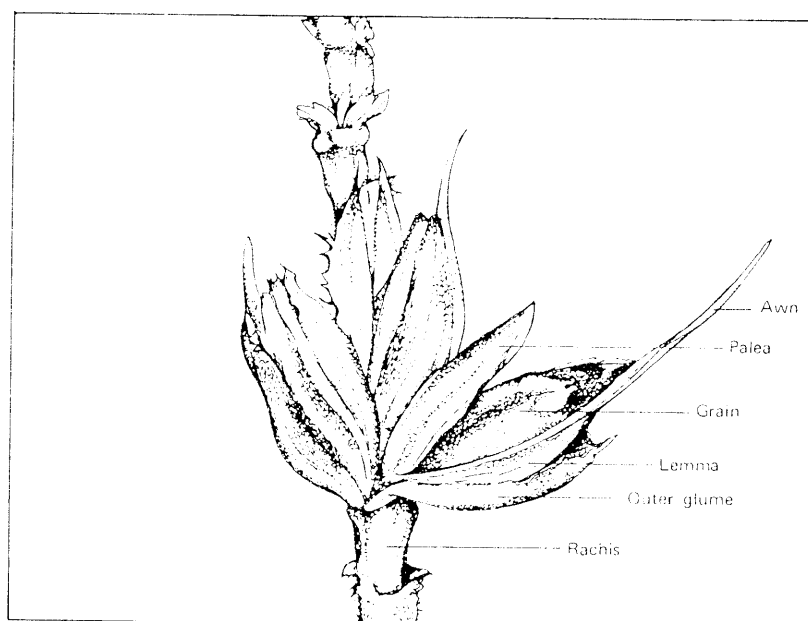


Fig. 4. A single spikelet on a ripe wheat head with other spikelets removed from the rachis. One of the three florets has been drawn open to show how each wheat grain is held between the lemma and palea (husks) [from ref. 79].

round when compared to those of other plants which are pollinated by insects and birds. Careful study of surface structure may permit identification of the plant from which pollen is derived but this is a specialized task [75, 243, 256]. The grass pollen grain is distinctive in having only a single pore, compared to three, in general, for dicots [228]. Viability of grass pollen is short (less than a day) compared to other angiosperm pollen, though its allergenicity is relatively unaffected by storage and is retained until it has been moistened.

When a pollen grain comes in contact with the moist surface of the flower's stigma (or of the bronchial mucosa), the germination process starts (fig. 5c). The grain hydrates and swells, and the allergens (proteins and glycoproteins) which are stored in the walls of the surface of the grain are released [146]. These macromolecules then bind specifically to the surface of the stigma to initiate pollen-stigma communication. They may also bind specifically to IgE molecules on the surface of mast cells of the bronchial mucosa and initiate the allergic response in susceptible individuals [129, 183, 227].

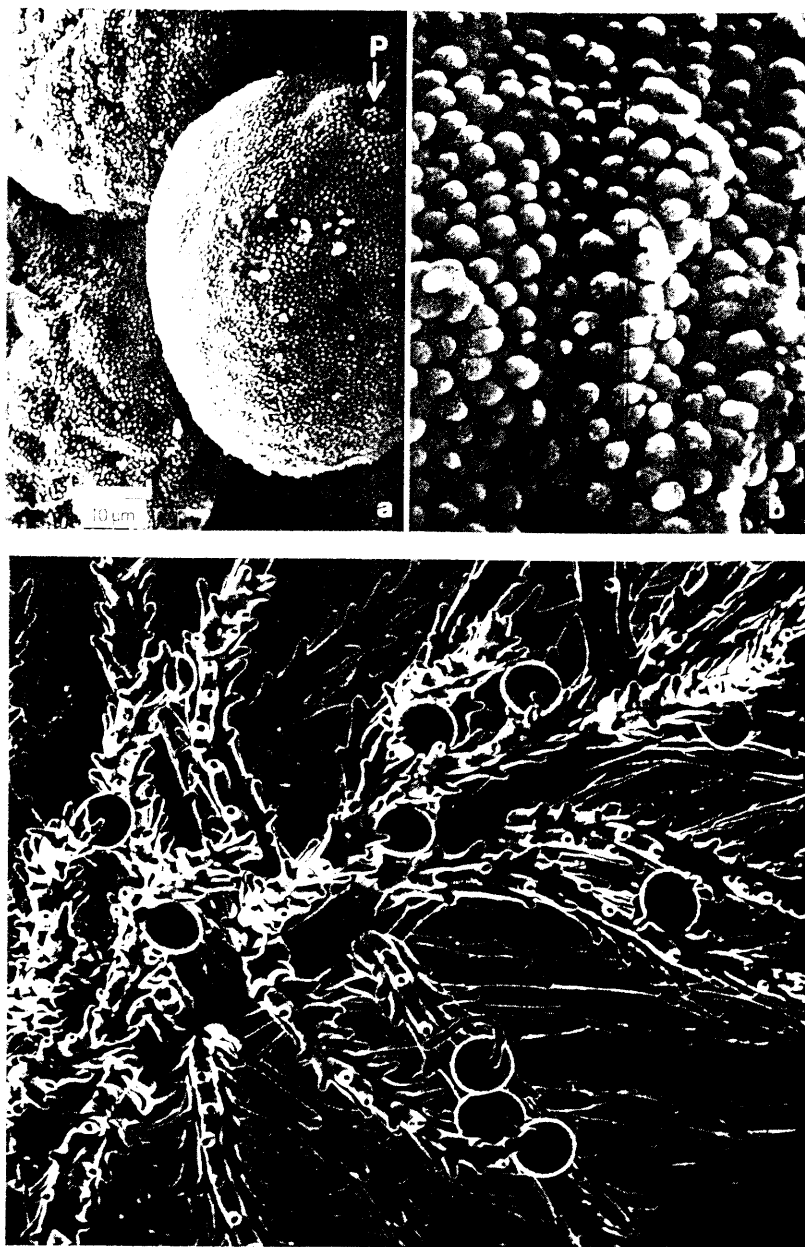


Fig. 5. Scanning electron micrographs of pollen grains from ryegrass (*Lolium perenne*). *a* Whole pollen grains showing pore (p). *b* Detail of surface patterning. *c* Ryegrass pollen germinating on the stigma. Each pollen grain is about 50 μm . In some cases, the pollen tubes can be seen penetrating the feathery stigma to take the male gametes to the ovary of the flower [from ref. 125].

Purified Grass Pollen Allergens

The almost ubiquitous occurrence of pollens, the early realization of their widespread occurrence in 'hayfever' [194, 197, 256], and their accessibility and accumulation in quantities sufficient for study are probably the main reasons why pollens are the most widely studied of all the allergen sources. Despite this, satisfactory progress in allergen identification, isolation and characterization has been made with pollens from only two species of grasses, *Lolium perenne* (perennial ryegrass) and *Phleum pratense* (timothy grass) and one dicot *Ambrosia elatior* (short ragweed). Although many different species of pollen may occur in any one locality and hence be available for sensitization, the position is somewhat simplified by the predominance, pollen-shedding characteristics and allergenic potency of a few species and by the fact that a number of pollens exhibit allergenic cross-reactivity. An indication of likely cross-reactivity may be provided by consideration of taxonomic relationships (table II). This survey covers important allergenic pollens from the few grasses that have been studied so far and reviews results obtained on the antigenic and allergenic cross-reactivity that occurs between the pollens.

A number of grass pollens have been fractionated with a view to purification of the important allergens [11, 13, 32, 49, 184, 191, 194, 226, 237] but, with only perennial ryegrass and timothy grass has the work led to a fairly comprehensive physicochemical characterization of the important allergens.

Ryegrass Pollen. Perennial ryegrass (*L. perenne*) is widely distributed throughout the temperate zones of the earth and sensitivity to the pollen is likewise widespread. Four different allergens, designated groups I, II, III and IV have been isolated from ryegrass pollen by *Johnson and Marsh* [133-135], *Marsh* [177] and *Marsh et al.* [178]. Allergen groups I, II and III each exist in more than one isoallergenic form, group I having four such forms I-A, I-B, I-C and I-D and groups II and III at least two each. The major components of group I, I-B and I-C have isoelectric points of 5.25 and 5.15, respectively, and are *D*-mannose- and *D*-galactose-containing glycoproteins with molecular weights (MW) of 27,000. Studies with enzymes have demonstrated that the carbohydrate moiety (5% of the macromolecule) does not contribute to the allergenic determinants, but allergenic activity is lost following proteolytic digestion. The allergenic properties of the group I molecules are resistant to boiling at neutral pH for 30 min [179]. Group II and III allergen molecules have molecular weights of 11,000 but the former is an acidic and the latter a basic protein. Like group I, group II allergenic

activity is destroyed following proteolytic digestion. Group IV allergen, a basic protein of 50,000, does not give rise to multiple bands following electrophoresis and, as yet, little is known of its stability and biological potency.

In reviewing studies of the biological activities of the different ryegrass pollen allergens, Marsh [177] reported that over 95% of 250 grass pollen-allergic individuals were sensitive to ryegrass group I allergen. 60% of 75 grass pollen-allergic subjects were sensitive to group II allergens and each of these individuals was either more sensitive, or equally sensitive, to group I allergen. Use of allergen groups I and III in 61 subjects revealed that 70% of the subjects were equally or more sensitive to group III than group I allergens. Only 20% of 50 subjects allergic to group I allergens were also sensitive to group IV allergens.

Timothy Grass Pollen. Detailed investigations of the allergens of pollen from timothy grass (*P. pratense*) commenced with the work of Augustin and Hayward [13]. Allergens A and B and antigen D were isolated by Malley et al. [175] and Malley and Dobson [173] who showed that A and B, and B and D were antigenically related. In addition, allergen A was shown to have at least one allergenic determinant not shared by allergen B [174]. Determinations of molecular weights gave sedimentation coefficients of 2.7 S and 1.4 S, respectively, for allergens A and B, although a subsequent examination of allergen B in the analytical ultracentrifuge gave a MW of 10,500. The average MW of antigen D, prepared from the dialysate of timothy pollen, was estimated to be approximately 5,000. Gel filtration of antigen D revealed 2 smaller fragments, antigen D₂ (MW approximately 2,500) and antigen D₃ (MW approximately 1,000). Prior intravenous challenge with antigen D₃ up to 3 h before challenge with antigen B inhibited passive cutaneous anaphylaxis responses to antigen B in guinea-pigs [171, 174]. Antigen D₃ also inhibited allergen-induced histamine release from passively sensitized monkey lung by up to 78%. Attempts to chemically characterize antigen D₃ revealed the presence of the flavanoid quercetin and a disaccharide, probably cellobiose, apparently linked to threonine or serine by an O-glycosidic linkage [171, 172]. The authors believe their studies suggest that quercetin represents the major portion of the antigenic determinant of antigen B.

By combining the radioallergosorbent test (RAST) with isoelectric focusing in polyacrylamide gel, Varga and Ceska [238] studied the distribution of allergens in four commercial timothy pollen extracts and in an extract prepared by the method of Malley et al. [175]. The latter extract

Table III. Allergens of timothy pollen [from ref. 161]

Antigen number ¹	Protein content ²	Extractable material ²	Approx. MW	Isoelectric point	Total allergenic activity, % ³
Ag 3	96	39	10,000	3.9	20
Ag 19	100	4	15,000	4.9	80
Ag 25	100	13	15,000	4.5	
Ag 30	50	4	34,000	9.4	30

¹ Numbers from crossed immunoelectrophoresis patterns.

² Percent (w/w) of dry weight.

³ In most cases the sum of the activities of the individual allergens was > 100%.

retained the low MW fragments. Isoelectric points of the major allergens in all of the extracts were in the range 4.5–5.6 although some of the commercial preparations contained allergens with isoelectric points up to 8.

Recently *Aas*, in collaboration with workers at the Nyegaard Company, Norway [90, 101, 102], has described a 'purified allergen preparation', Timothy N, which 'contains a limited number of proteins' and which consists of two allergens of MW 15,000 and 30,000. No details of the purification of the allergens are given, but the authors state that 94% of the proteins and 99% of the carbohydrate were removed during the purification.

Using crossed immunoelectrophoresis, *Nielsen et al.* [196] found at least 28 antigens in timothy pollen extract, eleven of which were shown by crossed radioimmunoelectrophoresis to be allergenic in humans [246]. Four of these were considered to be major and seven were judged minor allergens. The use of gel filtration, ion exchange and affinity chromatography together with crossed immunoelectrophoresis [164] led to the isolation of two antigens, 19 and 25, which together constituted more than 90% of the detectable antigens but only 15% of the total allergenic activity in timothy pollen extract. In a subsequent study, *Lowenstein* [161] isolated two more pure allergens, numbers 3 and 30, and presented data on the characterization and allergenicity of these two pure pollen components and of antigens 19 and 25. These results are summarized in table III.

Cross-Reactivity of Grass Pollen Allergens

Although there are many studies in the literature that demonstrate antigenic cross-reactivity between various pollens [12, 78, 254, 255], clear-cut experimental evidence to back up the clinical observations that allergenic

cross-reactivity exists [56, 89, 206] has only recently been forthcoming. As with purification of important allergens, a knowledge of the patterns of cross-reactivities of allergens from different sources is important for diagnosis and immunotherapy of allergic subjects and for standardization of extracts.

The belief persists amongst many clinicians that most grass pollens have closely related allergens but there is a paucity of data from experiments designed to investigate this question. In a clinical study notable for the simplicity of its approach and the perceptive interpretation of the results, *Wright and Clifford* [259] examined skin test responses to 12 different grass pollens. Although similar patient responses were generally found between the pollens from grasses within the same taxonomic group, the authors concluded that the skin test response to one species of pollen does not necessarily mean that a patient will respond to another closely related pollen.

Using fractions isolated by electrophoresis in starch gels, *Marsh et al.* [178] investigated the distribution of fractions in extracts of seven different grass pollens. Antigenic components from gel fractions were examined by immunodiffusion against rabbit antisera to crude perennial ryegrass pollen and purified group I, II and III ryegrass allergens. Allergenicity of the fractions was investigated by skin testing grass pollen-allergic individuals. Results showed that antigenically cross-reacting pollens generally exhibited similar allergenic properties when tested in the same allergic subject and the authors concluded that pollen antigens which cross-react in rabbits generally show allergenic cross-reactivity in man. Skin reactions to groups I and III pollen antigens were observed with ryegrass, orchard, meadow fescue, meadow velvet, sweet vernal and timothy grass pollen fractions, and it was concluded that the group I antigens of orchard, meadow fescue and meadow velvet pollens are probably the major allergens in these pollens. Positive skin reactions to group II pollen antigens occurred with ryegrass, orchard grass, meadow fescue and meadow velvet pollen fractions. Although sweet vernal and timothy pollen extracts cross-reacted both antigenically and allergenically with ryegrass group I, II and III antigens, the reactions with these two pollens were less pronounced than was observed with the corresponding antigens from orchard grass, meadow fescue and meadow velvet pollens. Failure to find antigenic and allergenic cross-reactivity between Bermuda (couch) grass pollen extract and other pollen extracts studied agrees with previous reports [12, 87, 254] and subsequent studies (see below), and is reasonable in view of taxonomic relationships (table II).

The RAST [249], particularly when used as an inhibition assay [86], is proving of great value in studies designed to investigate allergen potency and

Table IV. Antigenic and allergenic cross-reactivities between perennial ryegrass (*Lolium perenne*) pollen allergens [from ref. 127]

		Allergens ¹
Antigenic cross-reactivity ²	not detected between	I and II
		I and III
		II and I
		II and III
Allergenic cross-reactivity ³	detected between	II and I
		II and III
		III and I
	not detected or slight reaction between	I and II
		I and III
		III and II

¹ Second written allergen used to inhibit.

² Detected by radioimmunoassay with specific rabbit antibody.

³ Detected by RAST inhibition.

cross-reactivity. This experimental approach has recently been used by a few groups to investigate cross-reactivity of IgE antibodies with a number of grass pollen allergens.

Bernstein et al. [30] observed significant cross-reaction between perennial ryegrass, orchard grass, meadow fescue, June grass and sweet vernal, but not with Bermuda grass and common reed pollen extracts. The thorough study of *Leiferman* and *Gleich* [153], in which both RAST inhibition and repeated absorption experiments with seven different allergenically important grass pollens were undertaken, revealed clear differences between some of the species. Perennial ryegrass, orchard grass, meadow fescue and June grass pollens were allergenically very similar, but sweet vernal, timothy and Bermuda grass pollens appeared antigenically deficient. In addition, timothy pollen appeared to possess some unique determinants not displayed by the other pollen examined.

Ryegrass group I, II and III allergens were examined for antigenic and allergenic cross-reactivities by radioimmunoassay using specific rabbit antibodies and RAST inhibition methods [127]. The most significant finding was the clear demonstration of allergenic cross-reactivity between group II and I, II and III, and III and I allergens (table IV).

Using the techniques of crossed-line immunoelectrophoresis and crossed immunoelectrophoresis [151, 245], *Lowenstein* [162] demonstrated cross-

reactivity between two major allergens of timothy pollen and pollen components from three other grasses, false oat (*Avena elatior*), orchard grass and perennial ryegrass, commonly found in Scandinavia. A clear relationship between the grass antigens and timothy antigen 25 was demonstrated but the relationship was less pronounced with timothy antigen 19. When used together in RAST inhibition studies and compared to the four pollen whole extracts, antigens 19 and 25 showed 97, 91, 92 and 73% inhibition, respectively, relative to the timothy, false oat, orchard grass and perennial ryegrass extracts. The study showed, therefore, that for the serum pools studied, timothy antigens 19 and 25 and their cross-reacting allergens in the pollens studied, account for the greater part of the allergenic activity of the four different pollen species. Two further points arising from this study should be emphasized. The method used for comparing RAST inhibition values is a departure from the normally used procedure of determining the concentrations of inhibitors that produce 50% inhibition of the reaction under study. As the author points out, the latter procedure is only applicable when the extracts being studied have an almost identical qualitative composition. Considerations such as this, however, do not justify introduction into the allergen literature of the term 'partial identity'. Use of this term instead of 'cross-reactivity' is an unnecessary departure from long accepted and still appropriate immunological terminology. 'Partial identity' is itself a contradiction in terms, while at the same time suggesting structural homologies that may not be justified from purely serological evidence.

In a study of antigenic and allergenic cross-reactivity between cereal, grass and dicot pollens currently under way in our laboratory, results support the conclusion that perennial ryegrass and timothy pollen extracts show allergenic cross-reactivity but that this cross-reactivity is less pronounced than one sees between a number of other grass species. Table V shows results obtained in a typical experiment using seven different grass pollen and two different dicot pollen extracts in RAST inhibition studies with serum from a typical ryegrass pollen-allergic subject. Clear-cut cross-reactivity was observed between ryegrass, orchard grass and canary grass pollen extracts, weaker cross-reactivity occurred between ryegrass and timothy and wild oats (*Avena fatua*) extracts, but Bermuda (couch) and the dicot pollens from plantain (*Plantago lanceolata*) and Paterson's curse (*Echium plantagineum*) did not cross-react. Results also show that cereal pollens, for example those from wheat (*T. aestivum*), durum wheat (*T. durum*), triticale (*Triticosecale*), rye (*S. cereale*) and barley (*H. vulgare*), strongly cross-react with the grass pollens mentioned above, except for

Table 1. Allergenic cross-reactivity between some common grass and dicot pollens. RAST inhibition studies with serum from a ryegrass pollen-allergic subject¹

Water-soluble allergen extract ² from pollen from common name	genus and species	Amount (μ g) needed for 50% inhibition
Grasses		
Ryegrass	<i>Lolium perenne</i>	2.0
Canary grass	<i>Phalaris canariensis</i>	2.9
Cocksfoot	<i>Dactylis glomerata</i>	4.4
Reed canary grass	<i>Phalaris arundinaceae</i>	8.0
Timothy grass	<i>Phleum pratense</i>	18.5
Wild oats	<i>Avena fatua</i>	25.0
Couch grass	<i>Cynodon dactylon</i>	∞ ³
Dicotyledons		
Plantain	<i>Plantago lanceolata</i>	∞ ³
Paterson's curse	<i>Echium plantagineum</i>	∞ ³

¹ Discs coupled to ryegrass pollen allergen extract.

² Prepared as described by Katelaris et al. [139].

³ 50% inhibition not attained up to 100 μ g/tube.

Bermuda grass. Cross-reaction between cereal and grass pollens has recently been reported by Kalveram and Forek [136], although the grass pollens used in their study were not identified. These results generally follow the pattern that might be predicted from their taxonomic relationships in table II.

Grain

Grain Structure

When wheat, rye and maize are harvested, the grain separates completely from the husks (lemma and palea in fig. 4). These layers remain attached to grains of barley, oats and rice, and further processing is needed following threshing to uncover the grain itself (the kernel).

The wheat grain is about 6 mm long (fig. 6) with the embryo (germ) near the pointed end of attachment to the plant and there is a growth of hairs (the brush) at the other end. The aim of milling is to crush and separate as much as possible of the endosperm from the bran and germ tissues (fig. 7, 8). The aleurone layer, although strictly part of the endosperm, is removed with

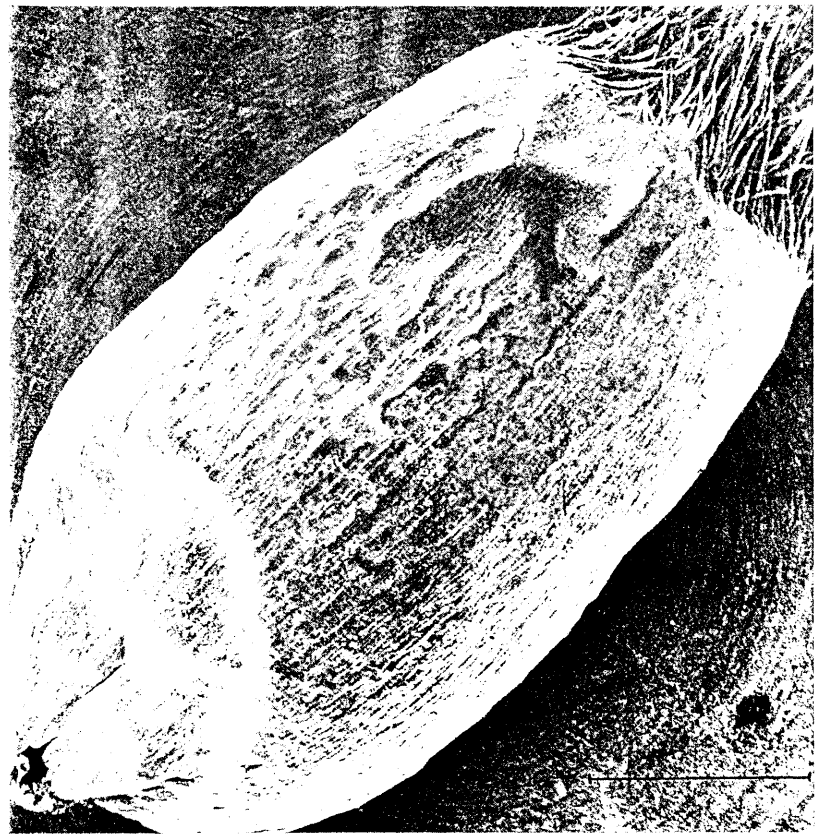


Fig. 6. Scanning electron micrograph of a wheat grain, showing the point of attachment (lower left), the wrinkled area covering the germ above it, and the brush hairs at the opposite end of the grain. The finely textured surface of the bran layer has been damaged at one or two points. The scale bar is 1 mm long.

the bran. Generally about 75% of the grain weight is obtained as white flour. As this extraction rate rises, so does the degree of incorporation of bran, aleurone layer and germ into the flour. It is thus quite possible that white flour contains significant contributions from the bran and the germ (e.g. including wheat germ agglutinin).

Figure 8 shows the internal structure of a wheat grain that has been broken in half to reveal the outer bran layer surrounding a single row of aleurone cells. The main volume of the grain is occupied by the starchy endosperm. About 70% of endosperm is starch in the form of granules, from

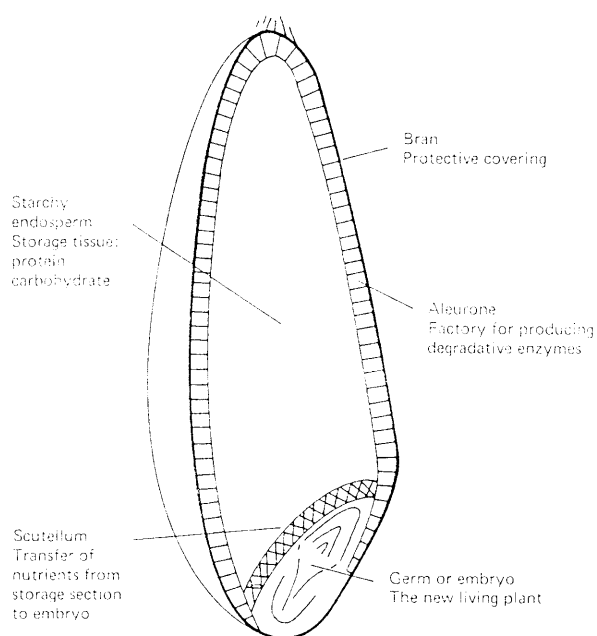


Fig. 7. The anatomical features of a wheat grain.

1 to 30 μ m in diameter, embedded in a matrix of protein. The larger starch granules can be seen in figure 8. Also visible are cell walls separating the endosperm cells.

Milling finely pulverizes the endosperm, releasing many free starch granules and small protein fragments, making both readily inhalable. The miller aims to remove the bran in large pieces, but fine particles are also produced and become air-borne. The bran and germ fractions generally contain some endosperm material also.

Compared to wheat, only small quantities of barley are milled for human food. The process involves removing the lemma, palea and bran (fig. 15a) by abrasion (pearling). Some of the pearled barley is further milled into flour, but most is sold as such or reduced to flakes after steam cooking. Rice processing first involves removal of the lemma and palea in a huller, followed by pearling to remove the bran layer. Bran and endosperm particles produced in these operations are also likely to be sources of occupational allergies. On the other hand, dust production is not so great a problem in the case of maize, since much of it is processed by wet milling.

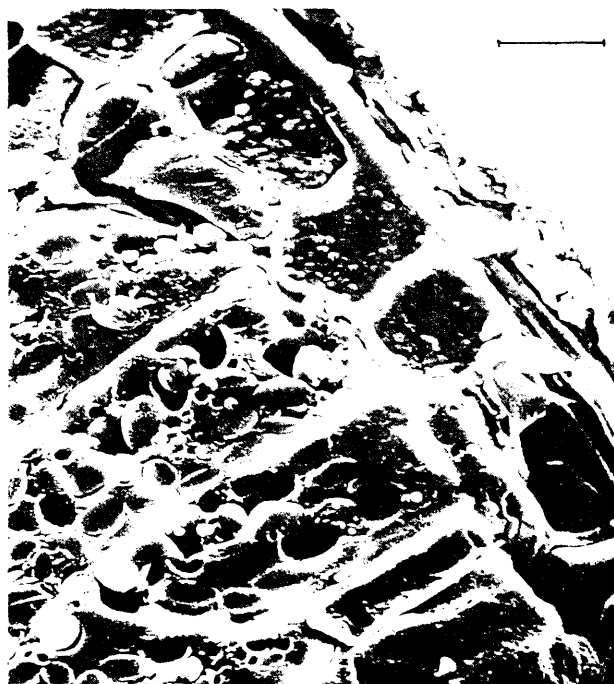


Fig. 8. Scanning electron micrograph showing the internal structure of a wheat grain that has been broken in half. The outer bran layer (top right) lies next to a row of aleurone cells. These in turn adjoin the starchy endosperm cells containing starch granules embedded in a protein matrix. Some endosperm cell walls can be seen. The scale bar is 50 μm .

Grain Composition

As shown in table VI, all the starch, most of the protein and half of the lipid of the grain are located in the starchy endosperm of wheat. The endosperm protein contains all the gluten of the grain, accounting for about 80% of the protein of flour. Its presence can readily be demonstrated by kneading a lump of flour-water dough in a stream of running water. The starch and water-soluble proteins wash out to leave a rubbery ball. This gluten is the protein complex responsible for the unique ability of wheat flour to be made into leavened bread.

Gluten can be further fractionated into the protein groups gliadin and glutenin, each composed of a large number of protein components. Gliadin,

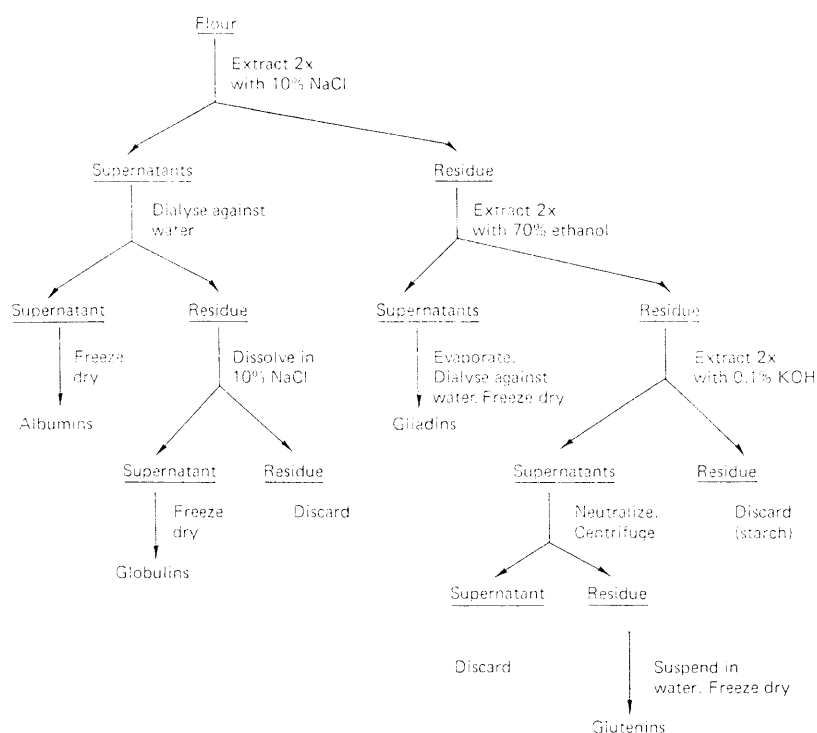


Fig.9. Classical Osborne extraction sequence for preparing albumins, globulins, gliadins and glutenins from wheat flour [25].

sparingly soluble in water, is a member of the prolamin class of proteins characterized by being soluble in 70% aqueous ethanol. Glutenin, a glutelin protein, is insoluble in this solvent, and requires mild acid or alkali for its extraction. Such solvents as these are the basis of the classical procedure (fig.9), devised by *Osborne* early this century, for fractionating cereal grain proteins into four classes – albumins, globulins, prolamins and glutelins [138]. The proportions of these in wheat endosperm protein are approximately 15, 5, 40 and 40%, respectively. The proportions differ considerably from one cereal grain to another [214]. Specific names have been given to the main grain proteins from wheat (gliadin), rye (secalin), barley (hordein), oats (avenin), rice (oryzin), sorghum (kafirin), and maize (zein). These names, derived from the respective generic names (table II), are not in general use, except for gliadin, hordein and zein.

Table VI. Distribution (%) of chemical constituents in the wheat grain [adapted from ref. 143]

	Weight	Starch	Protein	Fibre	Fat	Mineral
Bran, aleurone	15	0	20	93	30	67
Endosperm	82	100	72	5	50	23
Germ, scutellum	3	0	8	3	20	10

The heterogeneity of all these groups of proteins can be demonstrated (fig. 10, 11) by analytical fractionation in starch or polyacrylamide gel electrophoresis, isoelectric focusing or a two-dimensional combination of both [70]. On electrophoresis at pH 3, the gliadin proteins migrate more slowly than the albumins and globulins, so that the gliadins (upper half of fig. 10a and 11) are separated from the water-soluble proteins (lower half of fig. 10a and 11). This procedure can thus indicate the degree of cross-contamination between protein fractions following preparative fractionation. Under these conditions, some of the glutenin does not enter the gel and some migrates as an unresolved streak (fig. 10a). Discrete zones may be resolved for glutenin after breaking disulphide bonds with 2-mercapto-ethanol, particularly if sodium dodecylsulphate gel electrophoresis is used. The experiment in figure 11 was designed to examine contamination of one protein fraction with another. If instead, resolution of individual components of each protein class is desired, a longer time for electrophoresis should be used, preferably in a 3–27% gradient gel (fig. 11) [70]. Gel isoelectric focusing (fig. 10b) further demonstrates the heterogeneity of these protein classes, and indicates that each has components covering a wide range of isoelectric points. Combined gel electrophoresis and isoelectric focusing indicate that there is more than one protein in many of the apparently single zones resolved by either method used above. Analytical procedures such as these provide better resolution than most preparative methods, and are thus well suited to the detection of individual iso-allergen zones by a direct staining procedure referred to on pp. 2–29. Such a method will also prove valuable in studying cross-reactivity between cereal grain proteins, since comparison of the electrophoretic patterns (fig. 11) shows the presence of many components with overlapping electrophoretic mobilities in extracts of bran and flour from a variety of cereals.

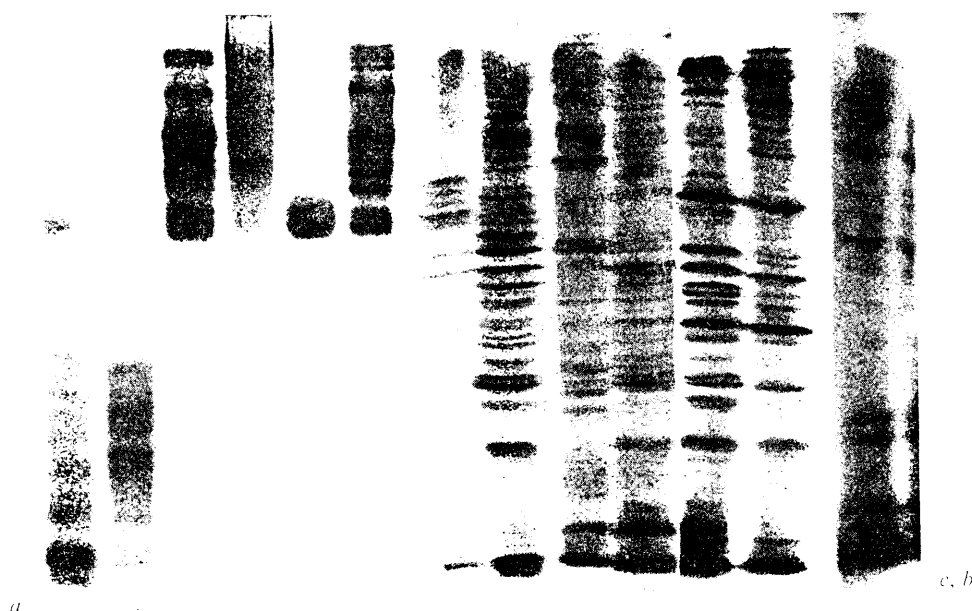


Fig. 10. Analytical fractionation of protein preparations obtained from wheat flour by sequential extraction (fig. 9). *a* Gel electrophoresis at pH 3; origin and anode at top: albumins (left), globulins, gliadins, glutenins, A-gliadin (kindly provided by *D.D. Kasarda*), and whole flour extract. *b* Gel isoelectric focusing in the range pH 3.5 (top) to 10 of wheat-flour globulins (left), wheat-flour gliadins, rye-grain proteins (pH 3 extract), wheat-grain proteins (pH 3 extract), wheat-flour water-soluble proteins and wheat-flour albumins. *c* Autoradiograph showing IgE-reactive regions of the water-soluble protein pattern from *b* after incubation with the serum of a flour-allergic baker.

Flour and Bran as Inhaled Allergens

References to immediate hypersensitivity reactions following inhalation of flour go back over 60 years [22, 33, 37, 93, 116, 159, 199, 204, 233] and so-called bakers' asthma, a form of allergic asthma induced by the inhalation of cereal flour, continues to attract the interest of researchers [25, 115, 130, 192, 193, 250]. Despite the numerous clinical examinations of bakers' asthma, until recently there has been little progress in identifying the specific flour allergens responsible for the condition. Using immunoelectrophoretic methods, *Blands et al.* [35] demonstrated 40 different antigens in wheat flour and showed that 20 of these antigens cross-reacted with rye flour extract. Crossed radioimmuno-electrophoresis examinations with sera from 13 allergic

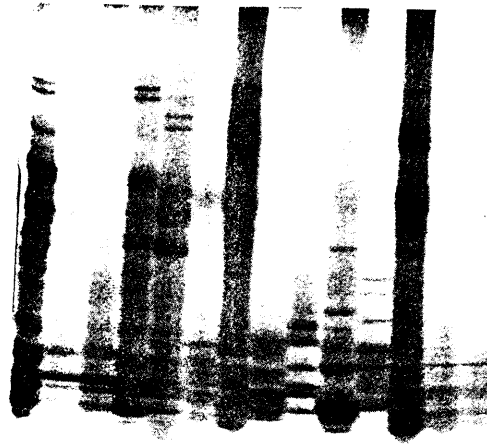


Fig. 11. Gradient gel electrophoresis in sodium lactate, pH 3 [70] of 6% urea extracts (left to right) of wheat flour, wheat bran, wheat pollard (fine bran and some germ), wheat wholemeal (flour and bran), durum wholemeal, triticale wholemeal, rye wholemeal, barley wholemeal, rice flour, maize wholemeal, rye flour, sorghum flour, and sorghum wholemeal. Proteins, applied at the top of the gel, migrated towards the cathode. Gradient range: 2.5% polyacrylamide at top to 27% [from ref. 22].

bakers revealed 18 different allergens although only three of these were classified as major allergens. No attempts were made, however, to isolate and characterize the allergens.

Our attempt [25] to identify major allergens in wheat flour was based on solvent extraction (fig. 9) of purified and partially purified flour fractions which were then used in RAST studies with sera from subjects with bakers' asthma. Examination of whole wheat flour, an undialysed water-soluble fraction, unfractionated water-soluble proteins and gliadins, A-gliadin, albumins, globulins, gluten, deamidated gluteins, glutenin and purified wheat arabinogalactan-peptide [81] showed that the water-soluble proteins and, in particular, the albumin components of flour are important allergens. RAST inhibition procedures revealed that wheat water-soluble proteins and purified albumins were approximately equally active in inhibiting the binding of flour-specific IgE antibodies in sera from patients with bakers' asthma (table VII).

Although wheat albumins, and to a lesser extent globulins, are clearly important allergens in bakers' asthma, all of the reactions studied so far cannot be wholly explained on the basis of albumin and globulin sensitivity.

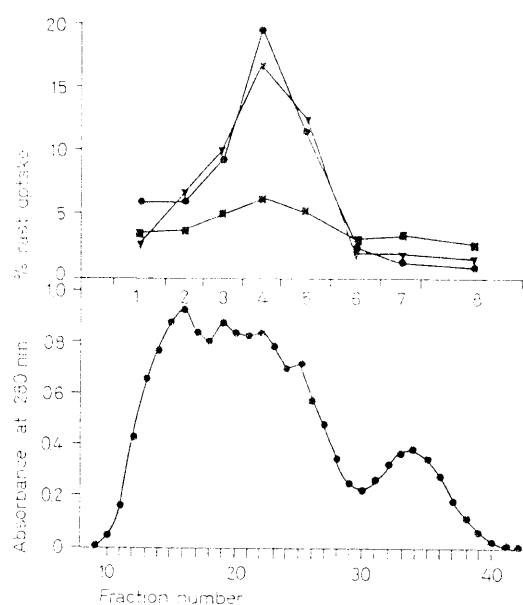


Fig. 12. Gel filtration of flour water-soluble proteins in 0.05 *M* ammonium acetate (pH 4.7) on Sephadex G-100. The lower curve shows the elution of protein as absorbance at 280 nm. The upper graph indicates RAST (as percentage uptake) for sera from three allergic bakers – diluted tenfold (circles), threefold (triangles) and twofold (squares) – for the eight sets of fractions pooled as indicated. The average molecular weights, estimated by gel filtration of ten standard proteins, were 87,000 (pool 2), 43,000 (pool 3), 28,500 (pool 4), 18,500 (pool 5) and 15,500 (pool 6).

Table VII. Inhibition of the binding of sera from wheat flour-allergic subjects to discs coupled with wheat water-soluble proteins [from ref. 25]

Serum	Amount of protein (μ g) needed to inhibit the uptake of IgE antibodies from allergic serum by 50% ¹		
	water-soluble proteins	albumins	globulins
Mas	1.5	1.6	28.0
Zir	3.0	3.2	7.0

¹ Detected as 50% inhibition of the uptake of ¹²⁵I-labelled anti-IgE in the RAST.

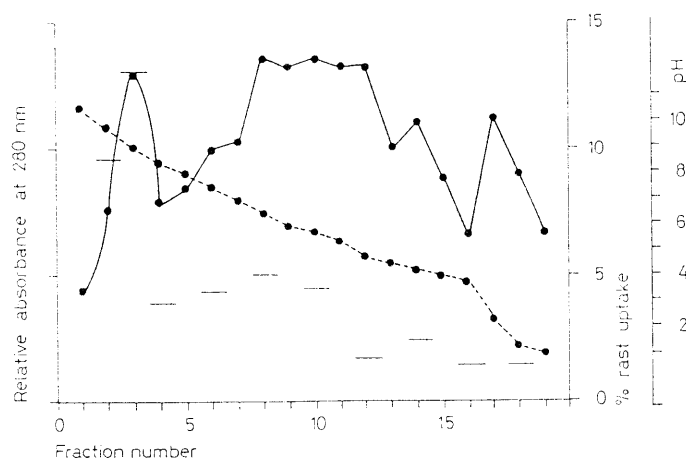


Fig. 13. Density gradient isoelectric focusing of flour water-soluble proteins in the range pH 3.5–10. pH is indicated by a dotted line and protein content of fractions (absorbance at 280 nm) by the solid curve. The horizontal bars show the RAST results (as percentage uptake) for alternate fractions.

Clearly, some reactions occur with other flour components as well and it must be remembered that some of the grain proteins are insoluble under the conditions used for testing. Nevertheless, our attention has been particularly directed towards fractionation of the albumin and globulin proteins. Gel filtration of this fraction (fig. 12) shows that proteins with molecular weights in the range 25,000–35,000 are the most allergenic on the basis of RAST. Figure 13 shows that the most allergenic fraction of those separated on an isoelectric focusing gel for one particular asthmatic baker has an isoelectric point of about 9.1. We have obtained different combinations of isoallergens when the sera of other allergic bakers were used. In the study by Blands et al. [35], three of the 40 antigens (numbers 38, 39 and 40), detected by crossed immunoelectrophoresis, migrated cathodically at pH 8.6 in the first dimension, indicating an isoelectric point higher than 8.6. Both antigens 39 and 40 were identified as allergens in 7 of the 20 bakers for whom specific allergens were identified by crossed radioimmunoelectrophoresis. These allergens may correspond to our basic protein fraction.

The involvement in bakers' asthma of a range of allergens with different isoelectric points can also be demonstrated by their specific reactions with serum IgE following gel isoelectric focusing (fig. 10b, c). The fractionated

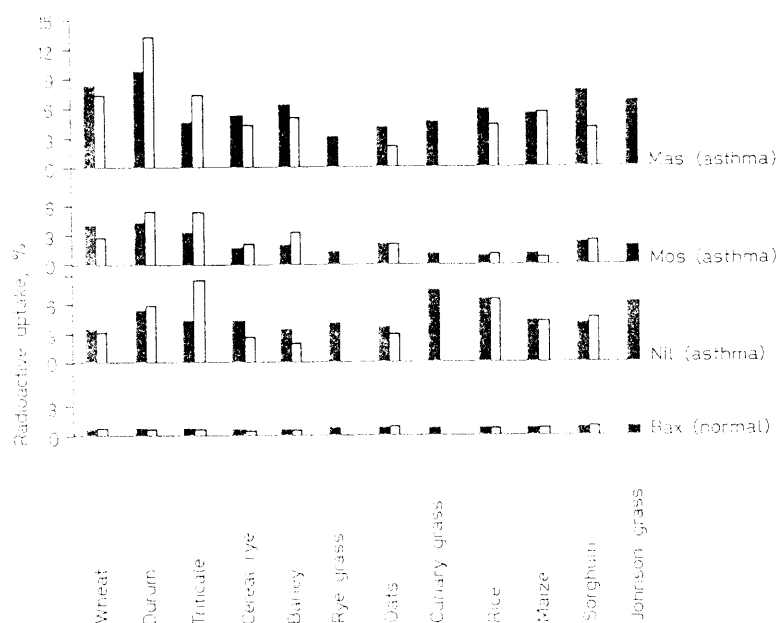


Fig. 14. Estimation by RAST of the presence of IgE antibodies with specificity for extracts of wholemeal (black bars) or flour (white bars) from a range of cereals (arranged from left to right in order of their relatedness to wheat), using serum samples from adults with bakers' asthma, and a normal adult.

proteins, transferred and fixed to a membrane, were incubated with the serum of an allergic baker (the same used for fig. 13) and the position of the IgE was detected with ^{125}I -anti-IgE followed by autoradiography. This method is more sensitive than the analysis of column fractions and shows more allergens than would be predicted from figure 13.

Cross-Reactivity between Grain and Pollen

Intergeneric Comparison of Grain Allergens

These studies of cereal allergens have recently been extended to examine and compare wholemeal (flour, germ and bran) and flour from a wide range of different cereals, and to assess the extent of cross-reactivity within the range of cereals selected (fig. 14) [22]. Until data such as this is accumulated,

one cannot be sure that it is even necessary to isolate pure allergens from cereal grains such as durum, triticale, barley, sorghum and so on, since close examination of one of these cereals may provide results that are typical of the rest. Table II shows the taxonomic relationships and figure 12 shows the electrophoretic similarities and differences between the cereal extracts examined. Even though wheat and rye are classified in two separate genera, intergeneric hybridization between them (to produce triticale) is possible. The most obvious similarities in the electrophoretic patterns of wheat and rye extracts are to be seen in the albumin proteins separated in the lowest third of the gel in figure 11, and this is the class of protein in wheat reported to contain the most potent allergen involved in bakers' asthma. There have been a number of reports of allergenic cross-reactivity between different cereals [34, 35, 120, 159, 211]. We have performed direct RAST (fig. 14), RAST inhibition, prick test and allergic histamine release with a wider range of cereals than has previously been studied. The results strengthen the conclusion that marked cross-reactivity exists between many of these species. In particular, wheat water-soluble proteins showed marked cross-reactivity with the rye, barley and oats cereal extracts tested [22].

No information is available on the incidence of hypersensitivity to bran or on the antigenic and allergenic composition of this constituent of whole grains. Bran, together with the germ, accounts for about 20% of the dry weight of cereal grains [143]. In our study, direct RAST results were often higher for wholemeal preparations (fig. 15) which, unlike flour, contained the outer bran layers of the grain. This would not be expected if the bran layers made no contribution to the allergenicity of the inhaled wholemeal cereal preparation. Figure 11 shows obvious electrophoretic differences between wheat flour and wheat bran and this is, presumably, a reflection of the different protein composition of the two preparations. The conclusion is therefore, that the bran layers of cereal grains are at least as allergenic as flour and efforts should be made to identify and isolate the important allergens in bran as well as in flour.

Cross-Reactivity of Pollen with Grain Antigens and Allergens

Cross-reactions occur between cereal grain and grass pollen antigens and allergens [2], although the nature of the cross-reacting grain and pollen components has not been studied. *Hoffman* [120] found that ryegrass group I pollen allergens removed IgE antibodies from the serum of an allergic patient that reacted with wheat flour, but absorption of the serum with flour did not remove IgE antibodies that reacted with grass pollens. With

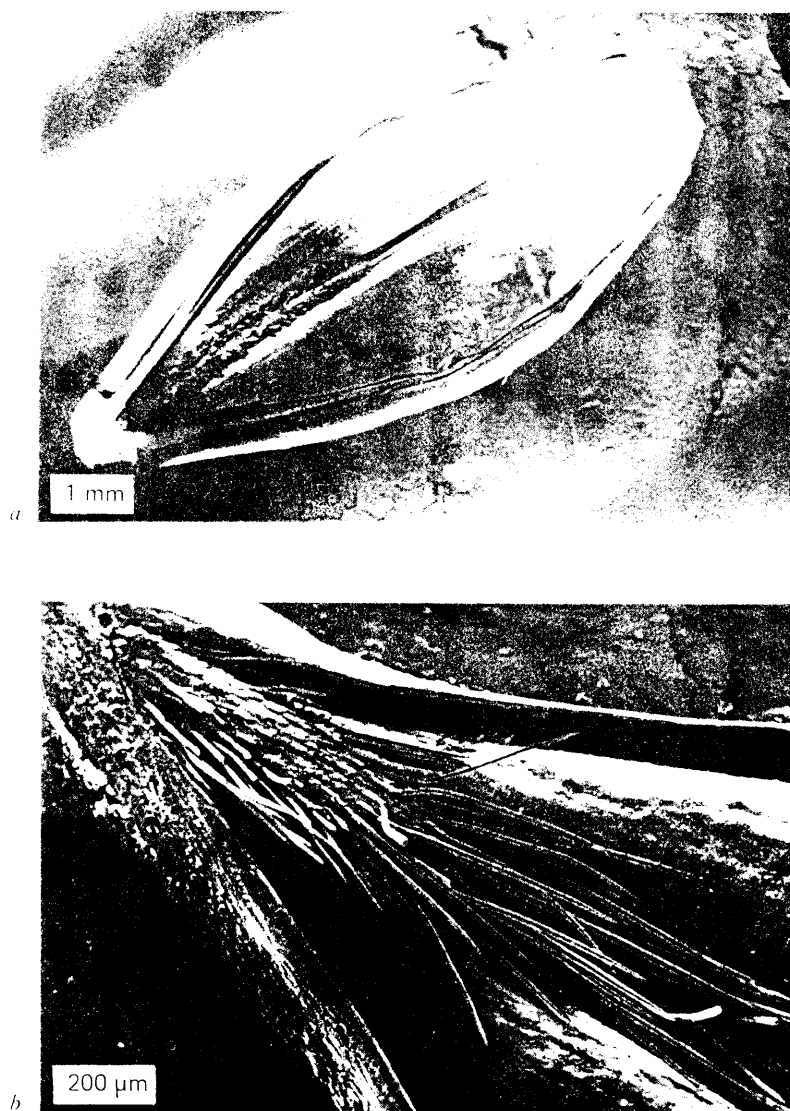
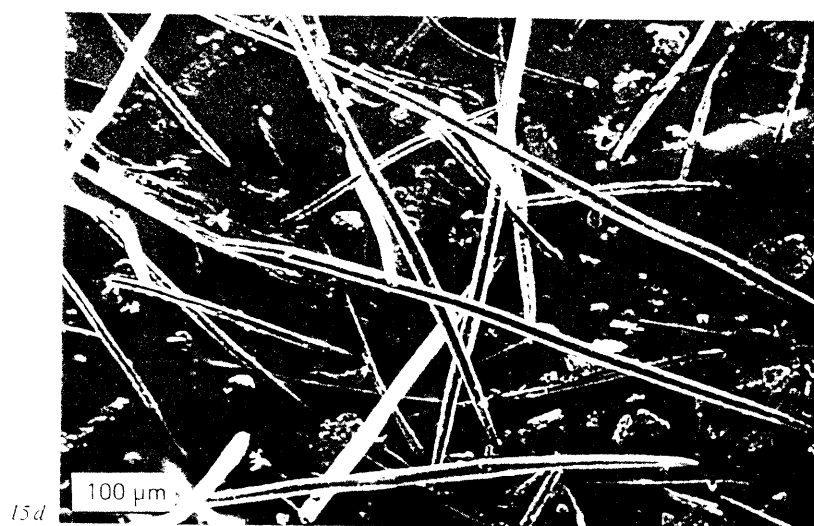


Fig. 15. Scanning electron micrograph of: *a* whole barley grain of a variety (Weeah) with a short-haired rachilla, *b* base of barley grain of a variety (Clipper) with a long-haired rachilla, *c* barley-grain dust, *d* grain dust from Clipper barley, *e* and *f* grain dust from wheat. The black scale bar in *a* is 1 mm, in *b* is 200 μ m, and in *c-f* is 100 μ m [from ref. 260].



sera from some other subjects, absorption with flour did remove the antibodies reacting with pollen but the pollen allergens did not remove the flour-reactive IgE antibodies. No allergenic cross-reactivity between flour and grass pollens was observed with some other sera that reacted with both flour and grass pollens.



In a study of bakers' apprentices, *Herxheimer* [116] found a higher incidence of pollen hypersensitivity in flour-allergic apprentices than in apprentices who showed no hypersensitivity reactions to flour. *Blands et al.* [35] detected 40 different antigens in wheat flour and showed that 7 of these cross-reacted with some pollen antigens. Of the 7 different cross-reacting

antigens, 4 (numbers 1, 6, 8 and 26) were detected in timothy, blue grass, false oat and orchard grass pollens, 1 (number 19) in ryegrass, timothy, blue grass, false oat and orchard grass pollens, 1 (number 21) in ryegrass, timothy and blue grass pollens and 1 (number 2) was common to timothy and false oat pollens. *Wraith et al.* [258] compared RAST scores for grass pollen and cereal flour in food-allergic patients. Some of the patients exhibited symptoms following ingestion of flour while others did not. RAST scores for pollen and flour were similar, or higher for flour, in the group with symptoms. The group without symptoms showed higher pollen than flour scores. The study showed that sera may be positive to both grass pollen and flour or positive to one only. Cross-reactivity between grain and pollen allergens was considered to be a possible explanation of the RAST findings. The authors believe that the presence of IgE antibodies that react with flour does not necessarily indicate allergy to ingested cereal grain. However, they suggest that a flour RAST score of two or more will be significant when the grass pollen score is similar or less.

Grain Dust and Straw

When cereal grains are harvested, considerable dust is produced, mainly due to the comminution of the dry leaves, stems and husks during threshing. Further dust is produced during the subsequent handling of the grain on delivery to the country silo (elevator), during transfer to rail trucks, into seaboard storage, into ships and at the flour mill. These dusts commonly cause respiratory abnormalities in the occupational groups exposed (farmers and grain handlers). The causal agents may arise from the cereal plant and grain, or from associated organisms such as insects and fungi.

The main accent in this section is on the cereals, but the likely involvement of contaminating organisms is described later. Farmers' lung is an example where the non-cereal factors outweigh the contribution of the straw itself. It is, in fact, the classical example of extrinsic allergic alveolitis, involving the thermophilic actinomycetes *Micropolyspora faeni* and *Thermoactinomyces vulgaris* which accumulate during storage [57, 83, 200, 202, 236]. On the other hand, there is little information available on the effects of straw dust produced during harvest.

Composition of Grain Dust

The dust generated during the handling of cereal grains consists mainly of fragments abraded from the outside of the grain together with particles resulting from the complete disruption of some grains. Figure 15e and f show

the microscopic structure of dust produced from the handling of wheat grain. Particularly prominent are the spear-like fragments that break off from the brush end of the grain (fig. 6). A complete tuft of brush hairs is shown in figure 15f. A second type of fragment consists of flat sections of husk or of bran rubbed off the outside of the grain [216, 260]. In addition, fragments of broken endosperm and individual starch granules are present, as shown in figure 15e and f. The chemical identity of these starch and protein fragments may be confirmed by specific staining methods and light microscopy.

Similar types of fragments can be seen in the illustrations of barley grain dust [260] in figure 15c and d. In this case, the spear-like fragments are mainly rachilla hairs broken from the rachilla which lies, largely protected, in the furrow of the grain (fig. 15a, b). The rachilla hairs of the barley variety Clipper (fig. 15d) are longer than for some other varieties (e.g. Weeah, fig. 15a), and are readily removed from the base of the rachilla where they occur in a tuft (fig. 15b).

The chemical composition of grain dust varies considerably, depending on the relative contributions of husk, hair, bran and endosperm, not to mention non-cereal contaminants. Analyses on a range of dust samples from wheat, oats, corn and sorghum [240] indicate they contain 7–11% protein (presumably based on total nitrogen content), 50–65% starch, 1–2% lipid and 3–10% ash.

Inhalation of Grain Dust

A report on the health of grain workers published by a Canadian Lung Association task force [29], concluded that grain dust may damage the health of those exposed to it. Possible adverse effects identified included cough, wheezing, airflow obstruction, febrile reactions, rhinitis, conjunctivitis and dermatitis.

Although there have been a number of studies, many of them recent, on the effects of grain dust on the health of farmers, dockers and grain handlers [38, 50, 63, 69, 72, 219, 241, 251], there has been no general agreement that allergic reactions are involved. In a study involving nearly 100 grain handlers employed at the major cereal grain terminal in Sydney, Australia [260], grain dust-specific IgE antibodies were not demonstrated by the RAST despite the finding of a higher-than-normal prevalence of respiratory abnormalities among the workers. Positive skin tests were obtained with extracts of grain dust and of flour for a small number of the workers. The positive skin tests did not correlate with respiratory abnormalities. Recent

findings by *Chan-Yeung* et al. [51], however, are relevant to our findings and suggest that careful assessments of grain dust preparations are necessary before concluding that immediate hypersensitivity to dust allergens is absent in the patients or that allergens capable of provoking allergic reactions are not present. Even though positive skin test reactions to grain dust were not observed, *Chan-Yeung* et al. [51] concluded, from results of additional tests such as bronchial reactivity to methacholine and bronchial provocation with crude grain dust, that grain dust asthma probably does have an allergic basis.

More work is clearly needed therefore to establish the presence or absence of allergenic components in different grain dust preparations including water-soluble extracts and dry unextracted dusts. In this regard, patch-testing may also be useful to assess skin reactions. Failure to find positive skin test and RAST reactions to grain dust extracts suggests that extraction of the grain dust preparations may lead to a loss of allergenicity. If this is so, the problem of allergen identification could be approached by injecting rabbits with unextracted grain dust preparations and using hyperimmune antisera in crossed-radioimmuno-electrophoresis studies with sera from suspected grain dust-allergic individuals. Scanning electron microscopy of grain dusts (fig. 15) has revealed that endosperm and bran particles are virtually always present together with structural components of the grain such as brush ends and starch granules [216]. Other possible allergen sources in stored grain may be pollens, fungi, insects, mites and rodents.

The question of the relationship of morphological features of grain to asthma-like symptoms in wheat millers was considered many years ago. *Duke* [71] observed that dust obtained from the first cleaning of wheat contained minute, sharp-pointed wheat hairs and that extracts of the dust were very potent in eliciting both positive skin test reactions and asthma in the affected workers. By comparing the dust extract with extracts of isolated wheat proteins, wheat flour, the germ, bran and wheat fungi, *Duke* [71] concluded that the needle-like hairs initiated the asthmatic responses perhaps by penetrating the mucous membranes and thus allowing access of other grain or dust constituents to the tissues.

Skin Irritation from Barley Dust

The barley variety Clipper has proved very popular with both farmers and maltsters in Australia but it has the disadvantage of causing skin irritation known as 'Clipper itch', during harvesting and handling. As this irritation was more severe in some individuals than in others, we searched for a

possible allergic basis for the itch by examining the sera of severely affected workers for barley dust-specific IgE antibodies using the RAST assay [260]. No positive RAST reactions were obtained indicating that the sera examined did not have IgE antibodies specific for any of the grain dust components we were able to extract. It seemed therefore, that a physical irritant was responsible for Clipper itch and a possible mechanism was suggested by microscopic examination of barley dusts.

An obvious component of barley-grain dust (fig. 15c, d) is the hairs broken from the rachilla which normally lies largely protected in the furrow of the grain (fig. 15a). These hairs are much longer in some varieties, including Clipper (fig. 15b), than in others, e.g. Weeah, (fig. 15a), and this difference in length is reflected in the length of these hairs in dust generated from handling grain of the respective varieties (fig. 15c, d). An additional characteristic of Clipper rachilla hairs is that they form a tuft [82] at the base of the rachilla, where they are unusually susceptible to removal during handling. Figure 15b shows how the hairs in this position have been removed from a Clipper grain that had been through the commercial grain-handling system. There is thus the inference that Clipper itch is associated with the tuft of long hairs at the base of the rachilla; these rub off thereby producing an abnormally large number of long rachilla hairs to cause physical irritation.

Contaminants of Grain and Straw

Fungi, insects and rodents are the major causes of deterioration of stored grain and straw. Many of these contaminants or their products have the potential of provoking allergic responses in man. Hence, in assessing a patient with suspected cereal allergy, it is important to consider likely contaminants as well as flour, bran and grain dust as possible allergen sources.

Fungi

Fungi associated with grain and straw may be present in enormous quantities depending on the cereal species, the kind of fungi, the methods used for harvesting and handling the crop, climatic conditions and storage facilities. In cereal grains, diseases caused by fungi are the most important parasitic diseases. Organisms responsible both for some of the more important diseases of wheat in the field and for some well documented allergic reactions in humans are listed in table VIII.

The bunt fungi *Tilletia foetida* and *Tilletia caries* are seed-borne and, although the plant shows little effect, the seed coverings turn grey-brown and are found to enclose a black foul-smelling powder of fungal spores

Table VIII. Some disease-causing 'field fungi' of cereals implicated in allergic reactions in man

Disease	Fungi responsible
Bunt (ball smut, stinking smut, covered smut)	<i>Tilletia caries</i> and <i>Tilletia foetida</i>
Loose smut	<i>Ustilago nuda</i>
Stem rust	<i>Puccinia graminis</i>
Leaf rust	<i>Puccinia recondita</i> (formerly <i>P. rubigo-vera</i>)
Stripe rust	<i>Puccinia striiformis</i> (formerly <i>P. glumarum</i>)

(fig. 16a). If crushed, the spores are released from the bunt 'balls', spoiling the grain and imparting a smell like that of decaying fish. The smut fungus, *Ustilago nuda*, transforms the grain-containing region of the head into a mass of powdery black spores (fig. 16b) which are partly dispersed before harvest. Rusts, caused by fungi of the *Puccinia* genus, produce conspicuous yellow, red, brown or black pustules on the wheat plants. The spores from these pustules are then carried by the wind to infect other plants. Storage of grain, especially under moist conditions, brings a further opportunity for fungal growth, involving genera such as *Cladosporium* (fig. 16c).

The fungal microflora associated with wheat and other field crops may cause hypersensitivity reactions in man. In 1924, Cadham [45] treated 3 patients suffering from asthma following exposure to rust-infected cereal in Western Canada. Others, including Waldbott and Ascher in 1940 and Jimenez-Diaz et al. in 1941 [quoted in 132], implicated *Puccinia* species as a sensitizing agent in allergic individuals amongst farmers and country-dwelling non-farmers. The authors concluded that the organisms, spread in the atmosphere, sensitized people living in the area of the infected crops.

Hypersensitivity to the fungi that cause bunts and smuts have been observed more frequently than allergic reactions to grass rusts. Early investigations by Wittich and Stakman [253] and Harris [105] implicated smuts in allergy to inhaled grain dusts. Studies carried out in Spain over a number of years by Jimenez-Diaz et al. [132] led this group to conclude that asthma in millworkers and others exposed to grain was almost always due to sensitization by *Tilletia* which was found in great quantities in granary



Fig. 16. Fungal diseases of cereals. *a* Bunt of wheat (*Tilletia caries* and *T. foetida*). The volume of the grain is replaced by fungal spores which are released on harvesting or handling the grain [from ref. 80]. *b* Loose smut (*Ustilago nuda*) on a barley head. The fungus forms a black mass on the rachis of the head in the place normally occupied by the grain. *c* Barley grains infected with black spot (*Cladosporium* species) [from ref. 82].

and mill dusts. Although attention has recently been drawn to the allergenic nature of cereal bunts and smuts [123, 169], reports of hypersensitivity reactions in man to these field fungi are now rare. This has been due to the high standards demanded by grain marketing authorities and the use of effective seed fungicides.

Other common 'field fungi' include *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium* species [41]. All of these fungi have been implicated in immediate hypersensitivity reactions in man [5, 15, 97] and some progress has been made in identifying important allergens from *Alternaria* and *Cladosporium* [14, 16, 54, 163, 262]. Saprophytes such as these readily colonize hay during slow drying [152] but cause less damage in stored grain. A high incidence of allergic reactions caused by 'field fungi' in grain dust was found in a group of English farmers [62]. Nearly 25% of the workers reacted to grain dust with symptoms which suggested a type I immediate hypersensitivity reaction. Symptoms usually developed after several years working on farms. The investigators, concentrating on the airborne fungal flora, found abundant quantities of *Cladosporium*, *Verticillium*, *Aphanocladium album* and *Paecilomyces bacillosporium* spores in the dust around combine harvesters. Extracts of these species produced immediate weal reactions in skin prick tests and rapid decreases in FEV₁ when inhaled by affected workers.

Saprophytic fungi belonging to the genera *Aspergillus* and *Penicillium* are regarded as 'storage fungi' by grain mycologists [41, 55, 84] since species of this group may develop even at low relative humidities and cause the

Table IX. Some important grain-infesting insects

Genus and species	Common name
<i>Sitophilus granarius</i>	grain weevil
<i>Sitophilus zeamais</i>	maize weevil
<i>Sitophilus oryzae</i>	rice weevil
<i>Rhizopertha dominica</i>	lesser grain borer
<i>Oryzaephilus surinamensis</i>	saw-toothed grain beetle
<i>Trogoderma granarium</i>	khapra beetle
<i>Cryptolestes ferrugineus</i>	rust-red grain beetle
<i>Cryptolestes pusillus</i>	flat grain beetle
<i>Tribolium confusum</i>	confused flour beetle
<i>Tribolium castaneum</i>	rust-red flour beetle
<i>Ptinus tectus</i>	Australian spider beetle
<i>Ephestia elutella</i>	cocoa or tobacco moth

'moulding' of grain. Both *Aspergillus* and *Penicillium* species are well known as causative agents in type I allergic reactions in man, while the former organism may also promote type III reactions [64, 200, 208].

Sensitization to *Aspergillus* and *Penicillium* in flour was observed many years ago [131, 231]. The possible involvement of fungi in bakers' asthma has recently been studied by Charpin [52] and Klaustermeyer et al. [145]. The latter group demonstrated pulmonary hypersensitivity to *Alternaria* and *Aspergillus* in 2 cases of bakers' asthma and concluded that the fungi may have contributed to the pathogenesis of the asthma.

Insects and Mites

There are over 150 types of insects known to infest grain and grain products [59, 118, 124]. Insects found in stored grain and grain products are often simply referred to as 'weevils'. In fact, weevils belong to the beetle group of insects. Here we will restrict our discussion to the most important and commonly encountered species of beetles, moths and mites found in stored grain and especially to those species known, or suspected, to have caused allergic reactions in man.

Beetles. These insects belong to the order Coleoptera which includes over 250,000 species, making it the largest order of animals. Species have six legs and a body with three segments. Some of the more important, that

is the most troublesome, species that have exploited the stored grain ecosystem are listed in table IX. Species such as *Sitophilus oryzae*, the 'rice weevil' and *S. granarius* (fig. 17a) are described as primary pests. These pests attack undamaged grain and set the stage for subsequent invasion by other insects, fungi and bacteria. Secondary pests like *Tribolium castaneum* and *T. confusum* (fig. 17b), feed on damaged grain, floury detritus and even faeces of primary pests. A few species such as *Rhizopertha dominica* and *Trogoderma granarium* do not fit into either category since only their late larval instars are able to penetrate undamaged grain.

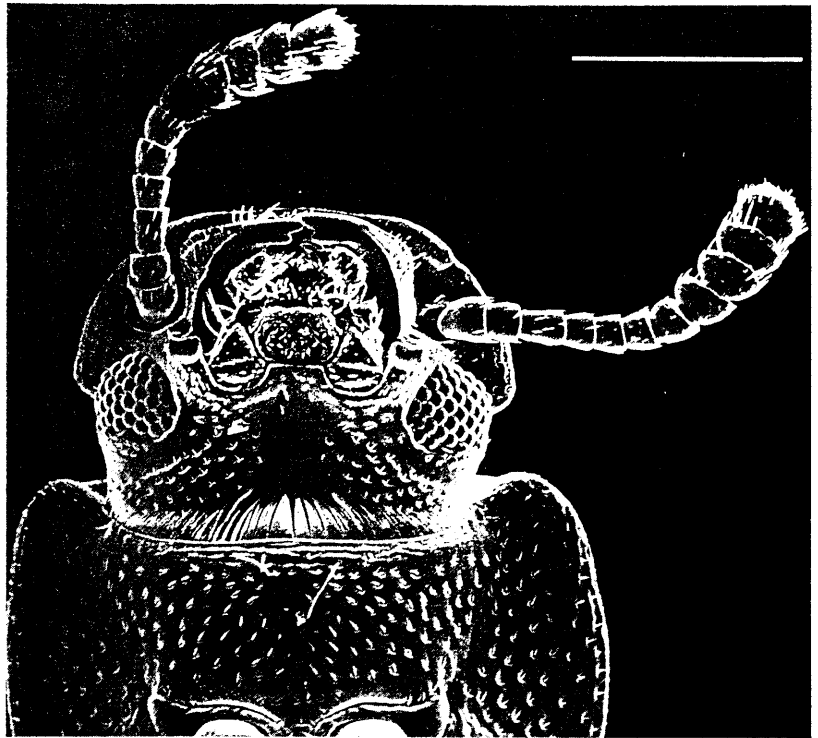
Four distinct stages (egg, larva, pupa and adult) occur in the life cycle of each insect and, unless proved otherwise, each stage must be considered as likely to contribute to the allergenicity of contaminated cereals.

The grain weevil, *S. granarius* (fig. 17a) has been the insect most frequently implicated in allergic respiratory responses [9, 43, 63, 88, 132, 165, 166, 168] and allergic alveolitis [10, 168]. Although numerous publications over the years attest to this, no studies of the origin and nature of *Sitophilus* allergens have yet been reported. Apart from *Sitophilus* species, flour beetles of the genus *Tribolium* appear to be the only grain-infesting beetle to be frequently implicated in the initiation of hypersensitivity responses in man [31, 204]. These findings are consistent with our search so far for insect-specific IgE antibodies in the sera of a small group of laboratory workers who reported respiratory hypersensitivity reactions following exposure to different species of grain-infesting insects. Using the RAST with discs coupled to insect extracts, we have so far detected positive reactions to both *S. oryzae* and *Tribolium* species but not to extracts of *Rhizopertha* and the moths *Ephesia* and *Plodia*.

Moths. Approximately 75 species of moths (order Lepidoptera) are known to infest stored products [58, 118] but species of the family Phycitidae [110] and *Sitotroga cerealella*, from the family Gelechiidae, are the most important members of the Lepidoptera that infest stored products. The high rate of reproduction of these flying insects ensures their efficient and rapid spread through stored cereals and, hence, they can be a considerable problem for millers and others who hold quantities of grain or milled products. The moths do not feed on grain but their larvae eat their way into the endosperm where they pupate. Some larvae attack the germ as well as the endosperm. First instar larvae produce silk and frass and the production of silk by large populations may present problems in mills, warehouses and ships.



a



b

Table X. Some important mites found in stored grain

Suborder Astigmata	
Family Acaridae	<i>Acarus farris</i> <i>Acarus immobilis</i> <i>Acarus siro</i> <i>Tyrophagus longior</i> <i>Tyrophagus putrescentiae</i>
Family Glycyphagidae	<i>Glycyphagus domesticus</i> <i>Lepidoglyphus destructor</i>
Suborder Prostigmata	
Family Cheyletidae	<i>Cheyletus</i> spp.
Family Tarsonemidae	<i>Tarsonemus granarius</i> and other <i>Tarsonemus</i> spp.
Family Tydeidae	<i>Tydeus</i> spp.

The house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* belong to suborder Astigmata, family Pyroglyphidae.

Reports of the involvement of grain-associated moths in respiratory allergic reactions are few. The species involved have usually been from the genera *Ephestia* [28, 44, 53, 116, 185, 186, 188] and *Plodia* both from the family Phycitidae. In one study [187], positive skin test reactions to wing scales of *Ephestia kuehniella* were found in 11% of 70 asthmatics examined.

Mites. Mites have eight legs and a body with two segments. They belong to the subclass Acari, one of several subclasses of the class Arachnida [126]. Other subclasses include spiders and scorpions. The dominant group of mites found in stored products belong to the order Acariformes and the species of most interest for the purposes of this review are in the suborder Astigmata, superfamily Acaroidea (table X). Storage mites are small, translucent, wingless, rapidly breeding species that require high humidities to reproduce.

Fig. 17. Insects of stored cereal grain. *a* Adult granary weevil *Sitophilus granarius*. The hole in the wheat grain (lower left) has been made by the weevil. The larvae, which develop inside the grain, and frass (the insects' excreta) may be more important sources of allergens than the adult insects. The scale bar is 1 mm. *b* Head (underside) and mouth parts of the confused flour beetle (*Tribolium confusum*). The scale bar is 0.5 mm. (Scanning electron micrographs by courtesy of Barry Filshie, CSIRO, Division of Entomology, Canberra.)

Correlations have been found between the percent moisture content of cereals and cereal products and the total numbers of mites present [60]. Although their primitive habitat appears to have been decaying vegetable matter, species have adapted first to damp grain where fungi provided the food source, and then to the grain itself.

In a survey of mites in bulk grain stored on farms in England and Wales, Griffiths et al. [100] found that 90% of the grain examined was infested to some extent with mites. The most common species found was *Lepidoglyphus* (formerly *glycyphagus*) *destructor* [258], a mite implicated in a case of recurrent nocturnal asthma in a farmer after exposure to grain dust [63]. A high mite infestation rate was also found in a wide range of stored products in Ireland [60]. Approximately 75% of the samples examined carried mites and 70 species were identified. Six species – *Acarus siro*, *A. immobilis*, *Cheyletus eruditus*, *L. destructor*, *Tyrophagus longior* and *T. putrescentiae* – were found to be dominant.

Interest in mites has increased enormously over the last 10–20 years and nowhere is this more apparent than in the fields of clinical and experimental allergy. The common pyroglyphid house dust mites of the genus *Dermatophagoides* are now recognized as sources of some of the most widespread and potent allergens which afflict man [19, 39, 248].

Although allergic reactions induced by storage mites are known, or have in some cases been suspected [48, 180, 189, 190, 201, 222, 232, 234, 252, 257], results from some recent studies have suggested that allergy to storage mites may be more important and widespread than previously believed. Green and Woolcock [98] found that positive skin test reactions to *T. putrescentiae* were high in a New Guinea population and occurred as frequently as positive reactions to *D. pteronyssinus* in asthmatics in Sydney. Wraith et al. [257] skin-tested 210 patients with house dust allergy and bronchial asthma or rhinitis, using extracts of *D. pteronyssinus* and four storage mites – *A. siro*, *Glycyphagus domesticus*, *L. destructor* and *T. putrescentiae*. Some patients were included in the study because their occupation or living conditions seemed likely to expose them to some of the mite species. The authors concluded that storage mites may present a considerable occupational hazard to farmers and those who handle infested materials in their work. In addition, the mites may also be an important source of allergens for those living in damp dwellings where the growth of fungi, the storage of food or keeping of pets may favour the growth of *G. domesticus* and other storage mites. Warren et al. [242] have suggested that *L. destructor* may be as important an allergen source in cases of grain dust allergy as *Dermatophagoides*

species are in allergies to house dust. Tests on 28 asthmatics who had worked with grain revealed that 12 of the subjects gave a positive prick test reaction to *L. destructor* extract and 4 responded to *A. siro* extract. Inhalation challenge with *L. destructor* produced asthma in a patient with a positive skin test to the organism confirming its role in the subject's asthma. Allergic differences between *L. destructor* and *D. farinae* were found and the authors concluded that the important *L. destructor* allergens were not shared with *D. farinae*.

Further evidence of immediate hypersensitivity responses to storage mites was recently provided by Cuthbert et al. [61] and Ingram et al. [128] in a study of respiratory disease in an Orkney farming community. Skin test, RAST and serum precipitin examinations revealed that IgE-mediated allergy to storage mites was much more common than farmers' lung in patients whose symptoms were brought on or aggravated by exposure to hay. Nearly 80% of the farming community surveyed gave positive skin test reactions to *A. siro* and *T. putrescentiae* and over 90% reacted to *G. domesticus* and *L. destructor* extracts. The latter mite was also associated with a positive RAST in over half the subjects studied. Cuthbert et al. [61] suggest that the syndrome involving IgE-mediated respiratory symptoms following exposure to non-pyroglyphid grain storage mites should be termed 'barn allergy'.

The apparently increasing importance of mites, and especially storage mites, in human respiratory allergy continues to be emphasized. It has recently been suggested [148] that tarsonimid mites, found in stored grain and cereal products [126], may be responsible for some of the house dust allergies that cannot be ascribed to the *Dermatophagoides* content of house dust.

Rodents

The extent to which rodents may foul stored products is illustrated by the following examples. One rat is said to deposit about 70 droppings and 16 ml of urine a day while a single mouse excretes about 50 droppings a day [104]. Dykstra [quoted in 104] has stated that one rat left free in a warehouse for one year would eat approximately 12 kg of food and deposit about 17,500 droppings (1–2 kg).

Contamination of grain by rodent faeces, urine, hair and carcasses may have health, as well as economic, implications. Hypersensitivity reactions to vertebrate skin, hair, serum, urine and saliva have been reported in a large number of publications. Many different animals have been implicated including the rat and the mouse [32, 198, 209]. Allergies to mice and rats

are not uncommon, especially amongst laboratory workers who handle rodents [158, 167]. Fractionation of rat and mouse pelt extracts indicated that the most allergenic proteins had electrophoretic mobilities of the serum albumins [198]. Mouse albumin was also shown to be a major allergen by *Lery* [154] and *Siraganian and Sandberg* [218]. The latter workers fractionated skin extracts and found allergens of high (67,000) and low (17,000) MW. Both allergens were also found in mouse serum and urine. The high MW allergen appeared to be identical to mouse albumin.

A case of extrinsic allergic alveolitis following exposure to rats was described by *Carroll et al.* [47]. The subject's serum precipitated with rat serum, pelt extract and urine and the authors concluded that rat urine may be an important antigenic and allergenic source in cases of hypersensitivity to rats. Confirmation of this conclusion was obtained in a study of 5 atopic laboratory workers with asthma and other manifestations of allergy to rats and mice [195]. Rodent urine was found to be an important source of allergenic proteins. The major allergens in urine appeared to be low MW urine proteins – a rat α_2 -globulin and mouse prealbumin.

It is likely, therefore, that rodent infestation of grain and grain products could lead to allergic reactions in those who handle the contaminated material. Hence, in cases where allergic responses to grain contaminants are suspected, testing with extracts of mouse and rat pelts, serum, urine and albumins should be considered in addition to the use of extracts of grain dust, grain beetles, moths, mites and fungi.

Ingested Cereal Allergens

General Intolerance to Cereals

Food allergy is both a complex and controversial subject which is probably best viewed with the perspective of a number of recent enlightening reviews [74, 181, 221, 235]. Most general surveys of the incidence and causes of food allergies give evidence that products made from the various cereal grains – wheat, rye, oats, barley, corn and rice – can cause allergic reactions in some individuals [85, 121, 203, 223, 239, 258]. Cereals are so common in man's diet (table I) that there is great potential for developing allergy to cereal foods. However, as with most food allergies, there have been only a few attempts to elucidate the precise nature of the particular constituents of cereal grains responsible for allergic reactions, and in this review we shall concentrate on these.

It is well recognized that the adverse reactions caused by eating cereals and other foods may arise by a number of different mechanisms [36, 221]. Plant seeds often contain a variety of toxic factors – presumably as a survival mechanism aimed at deterring predators including man. Such factors include protease and amylase inhibitors, haemagglutinins and other lectins, goitrogens, cyanogens, phenolic compounds, tannins and substances with unpleasant flavours, all of which have been well reviewed elsewhere [156, 157]. Cereal grains are relatively free of such substances compared with other seeds, particularly those of the legumes, and as a result cereals have become a major food source for man.

Despite the fact that cereals are well tolerated by most people, there are some individuals who react differently and adversely following the ingesting of cereal flours. A well-known example is gluten-sensitive enteropathy or coeliac disease which is discussed below. Another related condition is a severe skin reaction known as dermatitis herpetiformis [207, 212, 247]. This condition can be controlled by drug therapy or sometimes the exclusion of gluten from the diet [103, 176]. It has been suggested that wheat gluten is a pathogenic factor in schizophrenia in certain individuals, probably due to the presence of toxic peptides in digests capable of crossing the gut and brain barriers [65–67]. While there is evidence that some patients do benefit from a gluten-free diet [68, 217], it is too early to assess the extent to which dietary gluten, compared with other factors [114, 122], is important in schizophrenia.

Cereals, especially wheat, are also said to be the most important foods in triggering migraine [94, 95]. However, it should be pointed out that the major causes of migraine are apparently not foods but rather tobacco smoke, tea, coffee, steroids and other drugs [95]. Further, it is not yet known if wheat and other cereals precipitate migraine attacks through an allergic reaction or through changes in amine metabolism.

In addition to these conditions, allergy to ingested cereal products has been described as the underlying cause of a very wide range of symptoms [74, 91, 170, 203]. As with many other foods, there is often a need to establish, by withdrawal from the diet and rechallenge, that cereals are the causative agent of symptoms in a particular patient [2, 46, 182]. As pointed out by *May* [181], the diagnosis of food allergy frequently does not stand up to the rigours of double-blind trials.

It is quite likely that there are as many types of immunologically based food allergy as there are varieties of immune response, and that more than one type of food allergy can occur in the same patient [91]. Most authors define at least two categories of food allergy [91, 235, 239]. The first type is

an immediate and obvious reaction, normally initiated by small amounts of antigen, and associated with strongly positive prick tests and RAST values, and elevated total IgE levels. These reactions are clearly type I hypersensitivities with typical symptoms such as asthma, allergic rhinitis, eczema, angio-oedema, anaphylaxis, severe vomiting or diarrhoea, and patients usually are well aware of the causative food.

All other types of food allergy tend to be indiscriminately placed together into a second group, often described as delayed or masked reactions. There is a tendency for these reactions to be caused by the intake of a large amount of antigen and by foods eaten frequently [91]. In some instances these reactions are associated with negative prick tests and RAST values, and low or normal total IgE levels. The reactions are very diverse affecting many parts of the body and may be mediated by immunoglobulins other than IgE, immune complexes, direct activation of complement, or the cellular immune system. Reactions to wheat and other cereals can belong to either of these categories [2, 239, 258].

Little more can be said about the delayed reactions to cereals because there has been no characterization or purification of the particular components responsible. However, these types of food allergies deserve further investigation if only because of the prominence given to them in the popular press [170].

Coeliac Disease

Coeliac condition, or gluten-sensitive enteropathy, is a well known form of wheat-protein intolerance, but it is not a type I allergic reaction. It will be discussed here briefly because it may result from an immunodeficiency or a delayed type III Arthus reaction.

The results of skin testing and RAST indicate that most coeliac patients do not have circulating IgE specific for wheat proteins [25, 34, 108]. We have been unable to find significant levels of IgE in the sera of coeliac children directed against any of 14 different wheat fractions including purified A-gliadin [25]. Elevated serum levels of total IgE are not uncommon in coeliac patients [25, 108, 109], but these may arise as a secondary effect resulting from the increased uptake of antigens via the damaged intestinal mucosa.

Many workers have suggested that an underlying cause of coeliac disease is an immunodeficiency. Coeliac patients have been variously reported to have elevated serum levels of IgA, IgG and IgD and to have lower levels of IgM in comparison with the normal population, though the significance of these results is not clear [17, 18, 119, 140, 142].

Several studies have shown that most, but usually not all, coeliacs show delayed hypersensitivity reactions to dermal testing with gluten fractions [6, 18, 73, 160, 261]. It may be that coeliac disease is dependent on immune complexes of antibody with wheat protein; certainly many coeliac patients do have circulating antibodies particularly of the IgG class, specific for gliadin [42, 76, 230]. However, the results of neither dermal testing nor specific IgG determinations have been sufficiently conclusive for these tests to replace the use of intestinal biopsy in diagnosis.

It is now well accepted that the toxicity of wheat flour to coeliac patients is caused by the gliadin proteins, particularly α -gliadin, and is unaffected by digestion with trypsin and pepsin [111, 112, 137, 141]. It is also of interest that some cross-reactivity does occur with other cereals. As might be predicted from their phylogenetic origins, coeliacs show progressively decreasing reaction to rye, barley and oats [137].

Immediate Hypersensitivity to Ingested Cereals

A small number of studies have attempted to characterize the proteins responsible for immediate hypersensitivity to ingested grains and to assess the cross-reactivity between cereals using both skin testing and RAST. In these studies the patients were generally atopic individuals with asthma, eczema or angio-oedema, who had positive skin tests and/or high IgE specific for wheat or other cereals. One problem faced by research workers in this area is that it is not always possible in these patients to establish a cause-effect relationship between the presence of specific IgE and the atopic symptoms observed [2, 34, 120]. This may be partly because many of these patients are highly atopic with multiple allergies both to inhaled allergens (e.g. house dust mites and pollens) and to foods other than cereals such as milk and egg. In such cases the withdrawal of one allergen may make no difference to the symptoms. Additionally, the development of symptoms after ingesting a food is dependent on the allergen crossing the gut wall. Thus, in some of the patients with high specific IgE in whom oral challenge had no effect, symptoms may be elicited only when some other factor causes a disturbance of the gastro-intestinal barrier. The fact that many antigens do cross the gut in apparently normal animals and people is now established [113, 114]. Clearly a greater understanding of this phenomenon is of key importance to food allergy research.

As in coeliac disease and delayed ingestion reactions to wheat, it is clear that patients who are allergic to one cereal are frequently also sensitive to other closely related cereals [203]. An epidemiological study carried out

in Missouri (USA), involving over 1,000 patients, showed a 50% correlation between positive skin tests to corn and rice, over 45% correlation for the pairs wheat-rye, wheat-corn and rye-corn, while oats tended to show less correlation with other cereals (about 35%) [155]. Recently we have used RAST to look for cross-reactions to other cereals in patients with ingested wheat allergies. The IgE in all sera tested showed specificity for rye, barley and oat proteins as well as wheat, with very close correlations between the wheat, barley and rye RAST scores. *Björkstén* et al. [34] also found a definite relationship between RAST scores for wheat and rye in children suffering from eczema.

It is likely that close correlations between results of testing with different cereal extracts is due to the identity or cross-reactivity of the allergens present, in turn reflecting the close botanic relationships between some cereals (table II). However, at this stage we cannot discount the possibility that these correlations are simply caused by the frequent exposure of patients to a number of different cereal products.

Only a few attempts have been made so far to purify the proteins responsible for allergies to ingested cereals. *Shibasaki* et al. [213] investigated the protein fractions which are important in allergic reactions to rice proteins. They showed that two out of three salt-soluble fractions separated by gel filtration (equivalent to albumins and globulins as defined in this article, fig.9) are probably of greater significance than the salt-insoluble, alkali-soluble protein (prolamin and glutelin).

Hoffman [120] showed that the IgE present in serum from atopic patients with high RAST scores for wheat flour tended to react to a greater extent with albumins and globulins compared with gliadins and glutenins. However, these results were quite variable, probably reflecting the mixed nature of the patients who were with or without demonstrable symptoms to either ingested or inhaled wheat, corn or oats.

Goldstein et al. [92] described experiments on a patient with extreme allergy to wheat, either ingested or inhaled. This patient showed a strong reaction to gliadins, but other fractions such as albumins, globulins and glutenins were not tested.

We have tested the four major fractions of wheat proteins (fig.9), using sera from 22 patients, with asthma or eczema and high levels of wheat-specific IgE. The RAST score was lowest to gliadins and to a tryptic-peptic digest of wheat gluten (Frazer fraction III, a preparation particularly toxic to coeliacs), thus making another clear distinction between cereal food allergy and coeliac disease. The highest RAST scores were consistently

found with the globulin fraction, followed by the glutenins. This contrasts to bakers' asthma and rhinitis where the albumins are most reactive [25].

Research Needed

With a few exceptions, most clinically important allergens have not been identified, isolated and characterized [144, 177]. As a consequence, accurate diagnosis of allergic disorders and the development of effective modes of immunotherapy have been retarded. It has been evident in this review that investigations aimed at purifying allergens are proceeding with relatively few grass pollens, cereal grains and grain contaminants.

Although the number of different allergenic grass pollen species is large [256], identification and isolation of important allergens has been achieved with only two monocots, ryegrass (*L. perenne*) and timothy grass (*P. pratense*). Even though the pollens from these widespread, and allergenically important, species cross-react with many other pollens implicated in allergic reactions, there is still a need to identify and isolate pollen allergens from a number of plants including wild oats (*Avena fatua*), canary grass (*Phalaris canariensis*), cocksfoot (orchard grass) (*Dactylis glomerata*), couch (Bermuda) grass (*Cynodon dactylon*) and so on. Apart from the cross-reacting allergens, there will be major pollen allergens that are species-specific.

Some progress has been made in identifying [25, 35] and isolating [25] the allergens responsible for IgE-mediated rhinitis and asthma following inhalation of cereal flours. Clearly, a number of flour allergens exist and these should be easily identifiable and isolated using available techniques and sera from a large enough panel of flour-allergic subjects.

In general, our studies indicate that there are multiple 'isoallergens' (distinguishable macromolecules with allergenic activity) for each tissue of each plant species. The task of allergen purification and characterization appears quite daunting if the number of plant species requiring investigation is multiplied in turn by the number of different tissues of each (i.e. pollen, endosperm, bran, straw) and by the multiplicity of isoallergens in each tissue. If such a task is to be attempted realistically, preparative fractionation must be combined with analytical screening techniques to provide both depth and breadth of coverage. As in many fields of research, success will depend on suitable techniques becoming available. To facilitate the application of RAST to many different allergen preparations (crude or purified), we are developing a simpler procedure for making allergen discs, based on an

adsorptive membrane. Such a method would also release clinicians from reliance on a limited selection of commercially available discs.

An extension of this method is the membrane-transfer technique, illustrated in figure 10c, which permits allergen identification following analytical fractionation of crude extracts by gel electrophoresis and gel isoelectric focusing, in a manner analogous to the detection of isoenzymes. This method permits comparisons of the specific isoallergens reactive with different sera using a range of allergen preparations, without the prior requirement of raising antibodies to each as is required for crossed radio-immunoelectrophoresis.

Such studies could be expected to provide valuable information about cross-reactivities of allergens from related species, thereby reducing the number of different plant tissues to be considered in diagnostic testing, immunotherapy and preparative investigations. Studies of allergenic cross-reactions between grass pollens and cereal flours should be extended in an effort to understand the clinical implications of the presence of IgE antibodies that react with both pollen and flour in allergic, and apparently non-allergic, subjects.

Progress towards a molecular understanding of allergen action may be sought by searching for and applying specific chemical agents that modify the ability of each particular allergenic determinant to combine with IgE. This might be possible by chemical treatment of the allergens in the electrophoretic gel before incubation with IgE, thereby providing some characterization of the active site of each allergen. Such studies may ultimately alter our classification of atopic individuals from the present diagnosis (as being, say, allergic to ryegrass pollen, wheat flour and cat fur) to a designation of hypersensitivity to allergens in terms of the particular combining sites involved, as defined by the reagents that inhibit IgE-isoallergen combination.

Evidence is emerging that storage mites such as *A. siro*, *I. putrescentiae*, *G. domesticus* and *L. destructor* are potent allergen sources. Of all the possible contaminants of stored grain and grain products, mites may be the most allergenically important. Research is needed to establish the extent of cross-reaction between different storage and house dust mite species and to investigate the distribution of storage mites in houses and their role in promoting allergic responses in humans.

As the reader may have perceived (pp. 2-36), the adverse health effects caused by exposure to grain dust is a complex problem. A variety of disorders may be seen in grain handlers but the components of grain dust that initiate the adverse responses and a number of other questions and clinical obser-

vations related to the adverse effects of grain dust exposure [29] are still little understood. The Canadian Lung Association task force, set up to report on the health of workers who handle grain, identified a number of areas in need of investigation and mentioned the desirability of government support for research in this specialized field of occupational medicine [29]. Clearly, it is important to establish whether or not offending batches of grain dust contain allergens that are involved in provoking allergic reactions in grain handlers. If allergens are detected, interest would immediately focus on their source whether that be from the grain itself or a contaminant.

From a clinical viewpoint, the area of cereal allergy most in need of research is the study of reactions to ingested cereal products. There is clearly a need to establish the reliability or otherwise of cytotoxic tests, skin testing, RAST and other in vitro methods of diagnosing clinically significant food allergy [2, 235].

In purifying the major allergens present in cereals, we should consider not only the native proteins but also denatured products produced by cooking or peptides resulting from incomplete digestion. *Spies* [224] has demonstrated the appearance of 12 new antigens after pepsin hydrolysis of milk proteins. If peptides such as these were important allergens, it could explain why in some cases negative skin tests or RAST results are obtained despite apparent immediate allergic reactions to foods. Similar work is needed on cooked and partially digested cereal products.

The study of the mechanisms involved in the transport of peptides or whole proteins across the intestinal lining is of direct importance to our understanding of food allergy. Certainly the concept that oral immunization reduces later uptake of antigen by raising local IgA levels in the gut [7, 8] deserves further investigation.

There is a need to establish the underlying mechanisms of reactions to ingested cereals occurring in the absence of specific IgE in the serum. Such cases could involve immunological reactions (such as direct activation of complement or type III reactions dependent on immune complexes), or non-immunological reactions (such as changes in amine metabolism or presence of toxic peptides or other substances).

Finally, any review dealing with allergens and allergic disease must confront the vitally important but perplexing problem of allergen standardization. The need for standardized allergen preparations has been convincingly demonstrated [1, 3, 4] but progress in providing such preparations has remained slow. Although a variety of methods has been used or suggested, no convincing, generally applicable procedure has emerged. Few, if any,

qualitative and quantitative controls for allergen extracts therefore exist. This is as true for extracts of grass pollens, grain, grain dust and grain contaminants as it is for other allergen extracts. To overcome the present deplorable situation, selective methods [23] of standardization involving lectins and myeloma proteins have been suggested [19, 20, 24].

Using mouse hybridoma technology [147], there is nothing to prevent the immediate introduction of monoclonal antibody primary standards for purified allergens from ryegrass and timothy pollens and for wheat flour albumins [21]. It should also be pointed out that the production of hybridoma-derived, allergen-specific, monoclonal antibodies does not necessarily rely on the availability of highly purified allergens for immunization of the donor mouse. Following 'immortalization' of spleen cells from the immunized mouse by fusion with a tumour cell, the process of cloning enables one to isolate different homogeneous cell lines secreting monoclonal antibodies with specificities for individual allergens. This approach was used successfully to prepare monoclonal antibody standards for house dust mite allergens [19, 149, 150] and could be applied to prepare allergen-specific antibody primary standards following injection of crude pollen, flour, grain dust or storage mite extracts. It is feasible that hybridoma-derived, allergen-specific monoclonal antibodies could be used as international primary standards for individual allergens. Antibody-secreting hybridomas may be kept viable for long periods under liquid nitrogen and could provide a central panel of reference tumour cell lines. These cells could be exchanged between laboratories throughout the world and used to provide allergen-specific antibodies for qualitative and quantitative standardization of allergen extracts.

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Dr. B.A. Baldo, CSIRO Wheat Research Unit, North Ryde, N.S.W., 2113 (Australia)