

Cross-reactivity between *Acacia* (wattle) and rye grass pollen allergens

Detection of allergens in *Acacia* (wattle) pollen

B. J. HOWLETT, D. J. HILL* and R. B. KNOX

Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville and *Department of Immunology, Royal Children's Hospital, Parkville, Australia

(Received 12 May 1981; resubmitted 23 September 1981; accepted for publication 7 October 1981)

Summary

Immunoglobulin E (IgE), directed against components of *Acacia* (wattle) pollen, has been detected by radioallergosorbent tests (RAST) in the sera of some children and adults who develop allergic symptoms in the presence of flowering *Acacia* trees in Australia. All these subjects also had high levels of IgE directed against *Lolium perenne* (rye grass) pollen. Inhibition by RAST showed that most of the IgE molecules which bound to *Acacia* pollen components also bound to *L. perenne* pollen extracts, and to Glycoprotein 1, the major allergen of *L. perenne* pollen. In these assays, the allergens have been immobilized on polyvinyl chloride microtitre trays: the sensitivity of this approach is compared to that of commercial RAST kits.

Introduction

Pollen allergens have been isolated and partially characterized from several species of flowering plants including ragweed, rye grass, Timothy grass and birch (King, 1976). In Australia the native tree *Acacia* (wattle) has been implicated in hay fever and seasonal asthma. Its role as an allergen source, however, is controversial since symptoms to *Acacia* have been poorly documented. Its pollen is a minor, seasonal constituent of the atmospheric pollen count in Melbourne (Smart & Knox, 1979) and may be the source of the putative allergens.

In this study *Acacia* pollen has been examined for the presence of allergens. Levels of IgE and IgG, directed against *Acacia*-pollen extracts in the sera of subjects who claim to develop allergic symptoms after approaching flowering *Acacia* trees, have been determined. The IgE directed against *Acacia* allergens has also been examined for its capacity to bind allergens from *Lolium perenne* (rye grass) pollen, a principal seasonal source of airborne allergens in Melbourne (Hill *et al.*, 1979). These

Correspondence: Dr R. B. Knox, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia.

determinations have been performed using a modification of RAST, whereby allergens are insolubilized on polyvinyl chloride microtitre trays (Howlett & Clarke, 1981).

Materials and methods

Pollen was obtained from trees of Australian species of *Acacia* (*A. sophorae* = *A. longifolia* var. *sophorae*, *A. baileyana*, *A. dealbata*, *A. mearnsii* and *A. spectabilis*). Flowering inflorescences of these trees were placed on filter paper over dried silica-gel beads in a closed plastic container for 2 days. This method precludes the possibility of contamination of *Acacia* pollens with other pollens. The shed pollen was sifted several times through fine, metal sieves which separated it from any other debris. It was stored over silica gel at -20°C and was pure as judged by microscopic observation after staining in Calberla's fluid (Ogden *et al.*, 1974). A commercial extract of defatted *A. baileyana* pollen was obtained from Commonwealth Serum Laboratories, Australia. *Lolium perenne* pollen was purchased from Greer Laboratories, U.S.A. *Gladiolus gandavensis* pollen was collected from cut flowers purchased locally.

Pollen extracts were prepared by incubating pollen in 0.1 M sodium phosphate, 0.15 M NaCl pH 7.5 (PBS) (50 mg/ml PBS) for 30 min at 20°C . The suspensions were then centrifuged (10 000 g, *r av.* 4 cm, 2 min) and the protein content of the supernatants determined by the microbiuret technique (Goa, 1953).

Sera were collected from children (10 years of age) and adults, who reported symptoms of wheezing, allergic conjunctivitis or allergic rhinitis on exposure to flowering *Acacia*. These sera were stored in aliquots (200 μl) at -20°C .

The biological activity of the *Acacia* pollen extract (Commonwealth Serum Laboratories, 1.0 mg/ml in 0.15 M NaCl) was assessed by skin prick test in two *Acacia*-allergic and three control subjects. The skin reactivities were read at 15 min and compared to weals elicited by histamine (1.0 mg/ml) (Aas *et al.*, 1978).

Validity of microtitre-tray RAST

A microtitre-tray RAST was developed and, in order to test its validity, was performed in parallel with a commercial RAST to determine levels of IgE directed against a reference pollen, *L. perenne*.

Lolium perenne pollen extract (40 μl of 200 μg protein/ml in PBS) was added to individual wells of a microtitre tray. After 4 hr at room temperature, the solutions were removed and the wells washed with 1.0% BSA (bovine serum albumin, Sigma, U.S.A.) in PBS ($3 \times 200 \mu\text{l}$). Sera (50 μl) were added to the coated wells, and to tubes containing discs coated with *L. perenne* pollen extract (Phadebas RAST, Pharmacia Ltd, Sweden). After 5 hr at room temperature (or 16 hr at 4°C), the sera were removed and the wells and discs washed (three times with 1.0% BSA in PBS [200 μl]; eight times with water [200 μl]). [^{125}I]-anti-human IgE (Phadebas RAST-Kit, Pharmacia Ltd) was dissolved in 1.0% BSA in PBS (20 ml) and added to each well and to the tubes containing the discs (50 μl , 15 000 ct/min). After 5 hr incubation at room temperature, the discs and wells were washed (three times with 1.0% BSA in PBS [200 μl]; eight times with water [200 μl]) and drained on tissue paper. The wells were cut from the plates and placed in tubes, as well as the commercial discs, and counted in a gamma counter.

Reference discs were incubated with reference sera (Phadebas RAST, Pharmacia Ltd), and then treated with [^{125}I]-anti-IgE as described above. The results were graded 0–4 as recommended by Pharmacia.

Detection of Acacia- or L. perenne-specific IgE and Acacia-specific IgG in allergic serum

Wells of a microtitre tray were coated with solutions of *Acacia* or *L. perenne* pollen extracts (40 μ l of 200 μ g protein/ml in PBS), then washed with 1.0% BSA as described above. Sera from *Acacia*-allergic and control subjects were diluted (1:18 in 1.0% BSA in PBS) for determination of *Acacia*-specific IgG, and were undiluted for measurement of *Acacia*-specific IgE. These sera (50 μ l) were added to the coated wells and incubated for 5 hr at room temperature (or 16 hr at 4°C). The antisera solutions were then removed and the wells washed three times with 1.0% BSA in PBS and eight times with water. Either [¹²⁵I]-Protein A (50 μ l, 45 ng, 20 000 ct/min) or [¹²⁵I]-anti-IgE (50 μ l, 2 ng, 15 000 ct/min) were added to the wells. After 5 hr at room temperature, the wells were washed as described previously, cut from the plate, placed in individual tubes and counted. Absolute values for both IgG and IgE concentrations could not be determined as reference sera with IgG and IgE specific for *Acacia* are not available.

A preliminary experiment was performed to examine the relationship between specific IgE in the serum and the amount of [¹²⁵I]-anti-IgE bound to the wells. Three-fold serial dilutions of serum from an *Acacia*-allergic subject (D.N., Table 3) were incubated in wells coated with *A. sophorae* pollen extract; the wells were then washed and [¹²⁵I]-anti-IgE applied. The level of counts bound was proportional to the antiserum concentration up to a 1:9 dilution.

The experiments require complete saturation of the IgE-antigen complexes with anti-IgE; and anti-IgE concentration (0.04 μ g/ml) which is one-fifth of that used in other systems (Gleich *et al.*, 1975) was chosen: for each experiment, an assay was set up using this concentration and twice this concentration. In each case there was no significant difference in the levels of anti-IgE bound to the wells.

Several controls in which particular steps were omitted from the protocol, were performed in these experiments. Firstly, wells were coated with BSA, rather than the pollen extracts; sera, then anti-IgE were added. Secondly, pollen-coated wells were reacted with 1.0% BSA in PBS, instead of sera; then anti-IgE was applied. In these cases, a background level of counts was measured (150 ct/min).

RAST inhibition studies

RAST inhibition (Howlett & Clarke, 1981) was used to examine inhibition of binding of *A. sophorae* pollen extract to IgE of allergic human serum by pollen extracts of *L. perenne* and several *Acacia* species, and by glycoproteins. Three-fold serial dilutions of the pollen extracts (0.2–1.0 mg/ml protein in PBS) or glycoproteins (rye grass pollen Glycoproteins 1 and 2—Howlett & Clarke, 1981; horseradish peroxidase and fungal glucose oxidase [Calbiochem, U.S.A.]) were prepared in pooled sera (1:3 dilution in 1.0% BSA in PBS) of subjects showing severe clinical symptoms of allergy to flowering *Acacia*. After 2 hr at room temperature the inhibitor-antiserum mixtures (50 μ l) were added to wells of a microtitre tray which had been coated with *A. sophorae* pollen extract (40 μ l of 200 μ g/ml in PBS). The trays were incubated and washed as described above. [¹²⁵I]-anti-IgE (50 μ l) was then added and the wells washed and counted.

Similar experiments were performed where wells were coated with *L. perenne* pollen extract and incubated with allergic sera, in the presence of *A. sophorae* pollen extract as an inhibitor.

Results

Comparison of Phadebas RAST with microtitre tray RAST

The Phadebas RAST and a microtitre tray RAST were used to determine levels of IgE directed against *L. perenne* pollen extract in serum samples from seven individuals. The order of increasing IgE levels of the sera was the same with both assays (Table 1). Using birch pollen as a reference for the Phadebas RAST, four of the sera had significant levels of rye grass pollen-specific IgE. The microtitre tray wells bound up to half as much anti-IgE as the Phadebas discs and the background counts were much lower (150 ct/min compared to 500 ct/min in Phadebas RAST).

Serum antibody levels

IgG antibodies to A. sophorae pollen. The levels of serum IgG directed against *A. sophorae* were measured in *Acacia*-allergic and non-allergic adults and children. Of the *Acacia*-allergic subjects, both adults and half of the children had elevated levels of

Table 1. Determination of serum IgE directed against *L. perenne* pollen extract, by commercial RAST or by microtitre-tray RAST.

Insolubilized extract	Patient	Serum IgE levels [¹²⁵ I]-anti-IgE bound (ct/min)*	
		Phadebas RAST	Microtitre-tray RAST
<i>L. perenne</i> pollen	S.R.	11 114	5902
	G.H.	2402	1170
	P.S.	1896	900
	R.T.	1878	704
	I.S.	774	190†
	B.H.	604	188
	K.J.	600	184
Birch pollen†	A†	5037	ND
	B	2234	ND
	C	857	ND
	D	419	ND

* Serum (50 µl) was added to tubes containing Phadebas RAST discs coated with *L. perenne* pollen extract, or to microtitre tray wells, which had been coated with *L. perenne* pollen extract (40 µl of 200 µg protein/ml). After 5 hr at room temperature, serum was removed and discs and wells washed (three times with 1.0% BSA in PBS (200 µl); eight times with H₂O (200 µl)). [¹²⁵I]-anti-IgE added: 15 000 ct/min.

† Reference discs coated with birch pollen extract were incubated in standard serum samples (A, B, C, D), and treated as described above.

‡ Background: Phadebas RAST, 500 ct/min; microtitre-tray RAST, 150 ct/min.

serum IgG, specific to components of *A. sophorae* (Table 2). Control subjects had only background levels of *A. sophorae*-specific IgG.

IgE antibodies to A. sophorae pollen. Of the *Acacia*-allergic subjects, two of the adults and more than half of the children had elevated levels of IgE directed against *A. sophorae*. These levels were up to twenty times higher than those of control subjects whose IgE levels were not detectable (Table 3).

IgE antibodies to L. perenne pollen. All the *Acacia*-allergic subjects with detectable serum IgE directed against *Acacia* pollen also had elevated *L. perenne* pollen specific-serum IgE: in most cases at least three times higher than IgE levels directed against *Acacia* pollen (Table 3).

Skin tests. Subjects with elevated levels of IgE directed against *L. perenne* pollen also had positive skin tests to the *L. perenne* pollen extract. Of five subjects tested for skin sensitivity to *A. baileyana* pollen extract, three were positive: of these the patient with the largest skin reaction also had the highest level of *Acacia*-specific IgE (Table 3).

Table 2. Detection of serum IgG directed against *Acacia* pollen.

Patient	Serum IgG levels.* [¹²⁵ I]-Protein A bound (ct/min)	
<i>Acacia</i> -allergic adults	V.V.	1250
	S.R.	1425
<i>Acacia</i> -allergic children	L.P.	1000
	C.M.	600
	T.C.	580
	A.P.	400
	C.B.	200
	J.E.	200
	G.C.	126
	M.P.	<100†
	M.F.	<100
	K.J.	<100
	A.T.	<100
Control adults	B.H.	<100
	G.H.	<100
	I.S.	<100
	S.M.	<100

* Wells of a microtitre tray were coated with *A. sophorae* pollen extract; human sera (diluted 1:18 in 1.0% BSA in PBS) was then added, and bound IgG detected by the addition of [¹²⁵I]-Protein A (20 000 ct/min). Each determination was performed in triplicate. The amount of [¹²⁵I]-Protein A bound is proportional to serum IgG levels.

† Background: 100 ct/min.

Table 3. Skin reactivities and serum IgE levels directed against components of *Acacia* and *L. perenne* pollens in patients who suffer allergic symptoms when exposed to flowering *Acacia*.

Patient	<i>Acacia</i> sensitivity			<i>L. perenne</i> sensitivity	
	Skin tests		Serum IgE* (ct/min)	Skin tests	Serum IgE* (ct/min)
<i>Acacia</i> -allergic adults	V.V.	ND†	3373	+	6900
	S.R.	ND	1157	+	8010
	N.G.	+	230	ND	ND
<i>Acacia</i> -allergic children	L.P.	ND	2757	+	7050
	D.N.	+++	964	++	3940
	T.C.	ND	829	+	ND
	V.F.	ND	821	+++	ND
	A.P.	ND	780	+	3105
	C.B.	ND	731	+	ND
	C.M.	ND	541	+	ND
	A.T.	ND	400	+	ND
	M.F.	ND	180‡	+	ND
	G.C.	ND	125	+++	ND
	K.J.	ND	120	-	ND
	J.E.	ND	100	-	ND
Control adults	B.H.	-	380	ND	180
	R.K.	++	280	++	ND
	G.H.	ND	160	ND	1400
	D.H.	-	154	+++	ND
	I.S.	ND	150	-	180

* Wells of a microtitre tray were coated with either *A. sophorae* or *L. perenne* pollen extract; serum was added and bound IgE detected by the addition of [¹²⁵I]-anti-IgE (15 000 ct/min). Each determination was performed in triplicate. The amount of [¹²⁵I]-anti-IgE bound to the wells is proportional to the serum IgE levels.

† ND: not determined.

‡ Background: 150 ct/min.

Inhibition studies

Inhibition of the binding of A. sophorae pollen extract to IgE. All pollen extracts tested were effective inhibitors of the binding of *A. sophorae* pollen extract to the IgE of allergic sera (Fig. 1).

Pollen extracts of *A. sophorae* and *A. spectabilis* were very effective inhibitors (87% inhibition at 180 µg protein/ml). Extracts of *A. baileyana* pollen and of two unrelated pollens, the monocots, *G. gandavensis* and *L. perenne*, were slightly less effective inhibitors than those described above. Of all the pollen extracts tested, the least effective inhibitors were *A. mearnsii* (69% inhibition at 200 µg protein/ml) and *A. dealbata* (55% inhibition at 400 µg protein/ml).

A number of glycoproteins of known monosaccharide composition were tested for their ability to inhibit the binding of *A. sophorae* pollen extract to IgE of *Acacia*-allergic sera. *L. perenne* pollen Glycoprotein 1 was an effective inhibitor at low concentrations (75% inhibition at 70 µg/ml); Glycoprotein 2 was less potent (25%

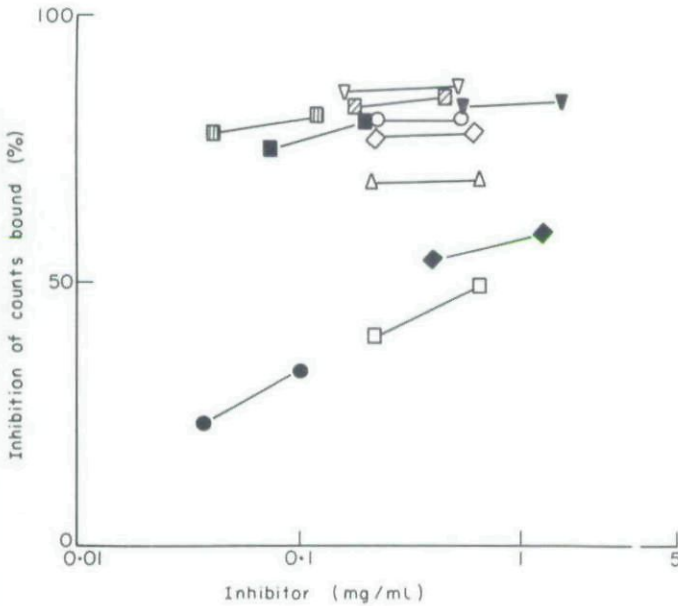


Fig. 1. Inhibition of the binding of *A. sophorae* pollen extract to IgE of allergic human sera by pollen extracts. ▽, *A. sophorae*; ○, *A. baileyana*; ■, *A. spectabilis*; Δ, *A. mearnsii*; ◆, *A. dealbata*; ■, *A. baileyana* (Commercial); ▼, *L. perenne*; ◇, *G. gandavensis*; or by glycoproteins (■, Glycoprotein 1; ●, Glycoprotein 2; □, horseradish peroxidase).

[¹²⁵I]-anti-IgE added: 15 000 ct/min. The allergic sera, in the absence of inhibitors, bound 950 ct/min. Pooled sera from non-allergic subjects, in the absence of inhibitors, bound 130 ct/min. Each point is the mean of three determinations. Glucose oxidase was an ineffective inhibitor at both concentrations tested (150, 450 μg/ml).

inhibition at 35 μg/ml). Horseradish peroxidase also inhibited the reaction (40% inhibition at 200 μg/ml) whilst glucose oxidase was ineffective as an inhibitor at both concentrations tested (Fig. 1).

Inhibition of the binding of *L. perenne* pollen extract to IgE. *L. perenne* pollen extract was an extremely potent inhibitor of the *L. perenne* pollen extract-IgE interaction (95% inhibition at 200 μg protein/ml, Fig. 2). In contrast *A. sophorae* pollen extract was a poorer inhibitor (25% inhibition at 200 μg protein/ml).

Discussion

In this study we have demonstrated the presence of significant levels of both *Acacia*-specific IgG and IgE in sera of patients who developed allergic symptoms when exposed to flowering *Acacia*. Most of the serum IgE molecules which bound *Acacia* pollen components, also bound to components of rye grass pollen. Although the data was obtained with pooled sera from three individuals, it is probably a general phenomenon since all the *Acacia*-allergic subjects in the study, who had elevated levels of serum IgE directed against *Acacia* pollen also had high *L. perenne* pollen-specific IgE.

Although some of these sensitized patients had positive skin tests to *Acacia* pollen, there was not a good correlation with specific serum IgE levels. However, if a larger number of patients were skin-tested, this correlation might be demonstrated.

In this paper, we have described an assay for allergen-specific IgE which appears to

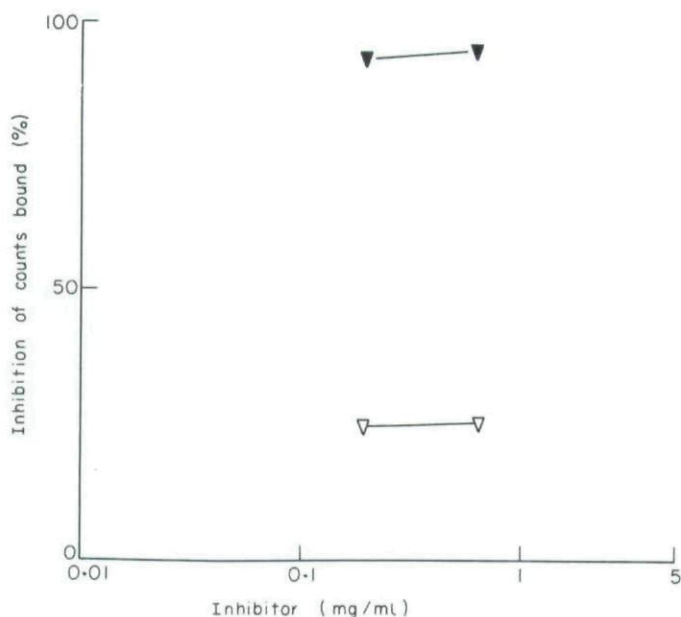


Fig. 2. Inhibition of the binding of *L. perenne* pollen extract to IgE of allergic human sera by pollen extracts of *A. sophorae* (▽) or *L. perenne* (▼). [^{125} I]-anti-IgE added, 15 000 ct/min. The allergic sera in the absence of inhibitors, bound 3650 ct/min. Pooled sera from non-allergic subjects, in the absence of inhibitors, bound 200 ct/min. Each point is the mean of three determinations.

have similar sensitivity to the commercial RAST-kit assays. One advantage of our assay is that solid phase allergen extracts are prepared by adsorption to polyvinyl chloride microtitre-tray wells, obviating the covalent coupling to paper discs or cellulose. The method is inexpensive and rapid (requiring a maximum of 6 hr incubation during each of three steps) and is extremely reproducible (less than 5.0% variation between replicates).

The appreciable cross-reactivities between allergens of quite unrelated pollens (*Acacia*, *L. perenne* and *G. gandavensis*) is unexpected since other laboratories have reported only low levels of cross-reactivity of pollen allergens within species of ragweeds, grasses and trees (Gleich *et al.*, 1975; Bernstein *et al.*, 1976). These authors performed RAST inhibition studies with pollen extracts as inhibitors over a concentration range of 1–2000 μ g protein/ml. The higher concentrations of inhibitors used in the present study (200–1200 μ g protein/ml) may account for the high levels of cross-reactivity between unrelated pollens. Indeed pollens of other families may also be expected to cross-react under these conditions. It is probable that at much lower concentrations, this extensive cross-reactivity would not occur.

The pollen extracts used as sources of allergens and as inhibitors consist of proteins, glycoproteins, polysaccharides and lipids. It is striking that the binding of serum IgE to such a heterogeneous mixture is inhibited, almost completely, by a single glycoprotein (Glycoprotein 1, 95% protein, 5.0% carbohydrate—Howlett & Clarke, 1981; termed Group 1 allergen—Johnson & Marsh, 1965). Either the protein and/or the carbohydrate of this defined glycoprotein has IgE-binding (allergenic) determinants in common with components of the *Acacia* pollen extract. Johnson and Marsh (1966)

have evidence that the protein component of Group 1 allergen contains the allergenic determinants. Furthermore our results suggest that the carbohydrate is not appreciably involved in the cross-reacting allergenic determinants, since three other glycoproteins of higher carbohydrate content than Glycoprotein 1, i.e. Glycoprotein 2, glucose oxidase and horseradish peroxidase, were much less effective inhibitors of the binding of *Acacia* pollen extract to IgE.

Interactions of pollen allergens of *Acacia* and *L. perenne* were examined by two methods and extensive cross-reactivity was only found using one of these methods: when *L. perenne* pollen was tested as an inhibitor of the *Acacia* pollen extract-IgE interaction. This result is consistent with the finding that serum IgE-levels directed against *L. perenne* pollen were four times higher than those directed against *Acacia* pollen. The most likely explanation is that only a subpopulation of the IgE which bound to the *L. perenne* pollen also bound to *Acacia*, whilst nearly all the IgE which bound to insolubilized *Acacia* also bound to *L. perenne* pollen. An alternative hypothesis is that a lectin is present in *L. perenne* pollen and binds the carbohydrate component of the IgE. This would account for both the high levels of IgE binding by *L. perenne* pollen, and its effective inhibition of the binding of *Acacia* pollen extract to IgE. The binding of lectins to immunoglobulins has been described in several laboratories; for instance, wheat germ agglutinin binds IgG (Bouchard *et al.*, 1976). The presence of lectins in the pollen extracts cannot be ruled out, as the extracts are extremely heterogenous mixtures of uncharacterized macromolecules. Further studies are necessary to establish whether either of these explanations are valid and also to determine the molecular basis for the allergenic cross-reactivity between *Acacia* and rye grass pollen.

Acknowledgments

This project has been supported in part by grants from the Asthma Foundation of Victoria, the Sir A. E. Rowden-White Foundation, the National Health & Medical Research Council, the Australian Research Grants Committee and Pharmacia (South Seas) Ltd. The generous gift of a sample of patient's serum (D.N.) from Dr E. Tai of Prince Henry's Hospital, Melbourne, is gratefully acknowledged. We thank Dr A. Clarke for helpful discussion of techniques, Mrs. S. Harrison, Ms. J. Kenrick and Ms. E. Streat for assistance with *Acacia* pollen collection and preparation of extracts. D. J. Hill is a National Health and Medical Research Council (Aust.) Fellow in Allergy.

References

- AAS, K., BACKMAN, A., BELIN, L. & WEEKE, B. (1978) Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radioallergosorbent tests. *Allergy*, **33**, 103.
- BERNSTEIN, I.L., PERERA, M., GALLAGHER, J., MICHAEL, J.G. & JOHANSSON, S.G.O. (1976) *In vitro* cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *Journal of Allergy and Clinical Immunology*, **57**, 141.
- BOUCHARD, P., MOROUX, Y., TIXIER, R., PRIVET, J. & MONSIGNY, M. (1976) An improved method for purification of wheat germ agglutinin by affinity chromatography. *Biochimie*, **58**, 1247.
- GLEICH, G.J., LARSON, J.B., JONES, R.T. & BAER, H. (1975) Measurement of the potency of allergy extracts by their inhibitory capacities in the radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **55**, 334.
- GOA, J. (1953) A microburet method for protein determination. Determination of total protein in cerebrospinal fluid. *Journal of Clinical Laboratory Investigation*, **5**, 218.

- HILL, D.J., SMART, I.J. & KNOX, R.B. (1979) Childhood asthma and grass pollen aerobiology in Melbourne. *Medical Journal of Australia*, **1**, 426.
- HOWLETT, B.J. & CLARKE, A.E. (1981) Isolation and partial characterization of two antigenic glycoproteins from rye grass pollen. *Biochemical Journal*, **197**, 695.
- JOHNSON, P. & MARSH, D.G. (1965) The isolation and characterization of allergens from the pollen of rye grass (*Lolium perenne*). *European Polymer Journal*, **1**, 63.
- JOHNSON, P. & MARSH, D.G. (1966) Allergens from common rye grass pollen. II. The allergenic determinants and carbohydrate moiety. *Immunochemistry*, **3**, 101.
- KING, T.P. (1976) Chemical and biological properties of some atopic allergens. *Advanced Immunology*, **23**, 77.
- OGDEN, E.C., HAYES, J.V., LEWIS, D.M. & HAINES, J.H. (1974) *Manual for sampling airborne pollen*. Hafner Press, New York.
- SMART, I.J. & KNOX, R.B. (1979). Aerobiology of grass pollen in the city atmosphere of Melbourne: quantitative analysis of seasonal and diurnal changes. *Australian Journal of Botany* **27**, 317.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.