

Standardization of allergen extracts by inhibition of RAST, skin test, and chemical composition

K. J. TURNER,* G. A. STEWART,† A. H. SHARP‡
and D. CZARNY‡.

*Department of Microbiology, University of Western Australia, and †Princess Margaret Children's Medical Research Foundation, Clinical Immunology Research Unit, Subiaco, Western Australia, and ‡Allergy and Respiratory Immunology Unit, Alfred Hospital, Prahran, Victoria, Australia

(Received 5 October 1979; accepted for publication 31 January 1980)

Summary

Five allergen extracts of *Dermatophagoides pteronyssinus*, *Lolium perenne*, *Alternaria tenuis*, *Aspergillus fumigatus* and *Cladosporium herbarum*, obtained from four different manufacturers, were examined by inhibition of RAST, content of protein and carbohydrate, contents of phosphorylcholine (Pc) and tridacnin reactive components, and by skin test. Inhibition of RAST was used as a primary method for establishing allergenic potency and demonstrated wide variations for each preparation supplied by the different manufacturers. The extracts also varied widely in protein and carbohydrate content and in the ratio of these parameters, indicating internal heterogeneity. Pc content was significantly related to RAST potency for extracts of *A. fumigatus* and *A. tenuis*, suggesting that Pc content may be used as a primary standardization procedure for these extracts. Skin test reactions undertaken at a single concentration did not show any significant variation in weal size between preparations of a given allergen extract. However, of particular importance to practising clinicians is the finding that varying numbers of patients showed negative skin reactions to one preparation of a particular allergen yet were positive to the corresponding preparations supplied by the other companies.

Introduction

Much attention has been directed over the past decade to the necessity for establishing adequate methods for measuring the potency of allergen extracts. Methods based on protein content are inadequate since they may not correlate with biological activity. End point skin test titrations overcome this objection in that potency units can be directly related to biological activity, particularly if the procedure is standardized against the weal size produced by 1 mg/ml histamine (Aas & Belin, 1972). However,

Correspondence: Associate Professor K. J. Turner, Clinical Immunology Research Unit, Princess Margaret Hospital for Children, Subiaco, Western Australia, 6008.

the quality of extracts is critical for skin test point titrations since the presence of non-specific irritants may lead to falsely high potency values (Aas, 1975). Leucocyte histamine release (Baer *et al.*, 1970), radial immunodiffusion (Baer *et al.*, 1970), isoelectric focusing (Varga & Ceska, 1972), crossed immunoelectrophoresis (Weeke & Lowenstein, 1973), and inhibition of RAST (Gleich *et al.*, 1974) have found varied support as primary methods for allergen standardization. Passive cutaneous anaphylaxis inhibition in a mouse-to-rat system has recently been developed by Akiyama, Miyamoto & Horiuchi (1979).

In addition to the above mentioned methods, it has been suggested that lectins or other reagents with defined specificity may also be helpful in standardizing allergen extracts (Baldo & Uhlenbruck, 1977). Relevant to this, we used a mouse IgA myeloma with a specificity for phosphorylcholine (Pc) to detect phosphorylcholine-containing components in allergen extracts known to contain Pc such as fungi (Longbottom & Pepys, 1964) and house dust mite (Baldo, Fletcher & Uhlenbruck, 1977; Stewart, McWilliam & Turner, 1979).

We report here the quantification of Pc-containing components in comparison with protein and carbohydrate content, RAST inhibition and skin test for the standardization of five allergen extracts prepared by four different manufacturers. Similarly, we also report on the assay of tridacnin-reactive components present in extracts of *Dermatophagoides pteronyssinus*, since these components have been shown to be allergens (Baldo, Turner & Uhlenbruck, 1976).

Materials and methods

Allergen extracts

Extracts of *Lolium perenne* (rye grass pollen), *Alternaria tenuis*, *Aspergillus fumigatus* and *Cladosporium herbarum*, all at skin (prick) test concentration, were obtained from Beecham Research (Aust.) Pty. Ltd (Bencard allergens), Commonwealth Serum Laboratories, Hollister-Stier and Dome. These are identified, not in the above order, as A, B, C and D. Extracts of *D. pteronyssinus* were supplied by only three companies, A, B and C.

The protein and carbohydrate contents of the various extracts were determined by reaction with Coomassie blue G250 (Sedmak & Grossberg, 1977) and phenol-sulphuric acid (Dubois *et al.*, 1956) respectively.

In vitro estimation of relative potencies

Inhibition of RAST. Inhibition of RAST (radioallergosorbent test) was done with allergen-conjugated discs and ^{125}I anti-IgE purchased from Pharmacia (South Seas) Pty. Ltd. Pooled human sera, from patients with clinically established hypersensitivity to the respective allergens, were diluted with phosphate buffered saline (PBS) to a concentration which lay on the linear portion of a graph relating concentration to percentage uptake of radioisotope on each of the respective allergen-conjugated discs. Sera at this optimal concentration were then incubated for 180 min at 20°C with various concentrations of the respective allergen preparations. The appropriate allergen-conjugated paper disc was then introduced and RAST performed in the conventional manner.

Potency was then defined as the reciprocal dilution of allergen extract which, on prior incubation with test sera, induced 50% inhibition of the radioisotope uptake

shown by unabsorbed sera. In those extracts failing to give a 50% inhibition value, a lower inhibition value was chosen for comparison.

Phosphorylcholine-containing components. Phosphorylcholine (Pc) content of the various allergen preparation was assayed by inhibition of haemagglutination of sheep red blood cells (SRBC) conjugated using glutaraldehyde with *Ascaris suum* body fluid which contains high concentrations of Pc-bearing macromolecules. A mouse IgA myeloma serum (S-107) with specificity for the phosphorylcholine moiety was diluted in phosphate buffered saline containing 1% bovine serum albumin (PBS-BSA) to give a titre of 1/4 against the conjugated SRBC. The diluted S-107 sera were then incubated for 1 hr at 37°C with doubling dilutions of the various allergen extracts. The sensitized SRBC were then added at a concentration of 1% (v/v) and incubated for a further 1 hr at 37°C. The end point titre was taken as the reciprocal dilution of allergen extract which just failed to inhibit haemagglutination.

Tridacnin reactive components. The content of tridacnin reacting components present in the four extracts of *D. pteronyssinus* was assayed by inhibition of agglutination of human O red blood cells as described by Stewart *et al.* (1979).

In vivo estimation of relative potencies

Consecutive, unscreened patients presenting at (a) a large public hospital allergy clinic, and (b) a private allergy practice, were skin prick-tested over a ten-week period with the five common allergens from the four commercial companies A, B, C and D. Anti-histamine medication was discontinued at least 48 hr prior to testing. One hundred and fourteen subjects were tested with each allergen with the following exceptions: (1) company A, *A. tenuis*, 107 patients tested, and (2) 120 subjects were tested with the *D. pteronyssinus* extract.

The skin test technique used was as described by Aas & Belin (1972), using the volar aspect of the forearm. Both positive (histamine 1 mg/ml solution) and negative (PBS) controls were incubated.

Skin reactions, recorded 20 min after application of allergen, were measured as the mean weal diameter of two readings made at right angles across the lesion. A weal of 3 mm or greater was regarded as positive.

Results

Potencies reported by the four manufacturing companies for the allergen preparations used throughout this study are shown in Table 1.

Table 1. Manufacturers' reported potencies for five allergen extracts.

Extract	Manufacturer			
	A (u/ml)	B (%)	C (PNU/ml)	D (w/v)
<i>D. pteronyssinus</i>	10 000	1.2	1500	1:50
<i>L. perenne</i>	10 000	2.5	5000	1:20
<i>A. tenuis</i>	10 000	10	5000	1:10
<i>C. herbarum</i>	10 000	10	5000	1:10
<i>A. fumigatus</i>	10 000	5	4000	1:10

Dermatophagoides pteronyssinus. Assayed potencies for *D. pteronyssinus* extracts by inhibition of RAST (Table 2) showed close agreement for preparations B and A, both of which were approximately twice as potent as C. Protein content, which was not related to RAST potency showed close agreement between preparations B and A. A larger disparity was found for carbohydrate content, which also was not related to RAST potency. Preparation B, which was the most potent by inhibition of RAST, contained the highest level of phosphoryl choline (Pc) containing components but the Pc levels for the other preparations did not relate to the level or order of RAST potency. The levels of Tridacnin reacting components were very low in all three preparations. Close agreement was found in the mean diameter of skin test reactions between all three preparations, notwithstanding the variation in RAST potency. The percentage of patients who showed positive skin reactions to the various preparations followed the same order as RAST potency but the relationship was not statistically significant.

Lolium perenne. A wide range of potencies (Table 3) varying one thousand-fold, was found by inhibition of RAST for the various preparations of *L. perenne* (rye grass allergen). RAST potencies were significantly related, by linear regression analysis, to protein ($P < 0.01$) and to carbohydrate content ($P < 0.05$) of the four preparations. The wide range of carbohydrate to protein ratios, however, indicated that this could not be attributed solely to variation in the absolute levels of identical components. None of the preparations of *L. perenne* contained components bearing detectable phosphorylcholine. Close agreement was found in the size of the skin test reactions but the preparations varied in terms of the percentage of patients who demonstrated positive skin reactions. While this trend reflected that found for RAST potencies, the relationship was not statistically significant.

Alternaria tenuis. RAST potency, which varied over a fifty-fold range for the *A. tenuis* preparations, was significantly related to the content of both protein ($P < 0.05$) and phosphorylcholine bearing components ($P < 0.05$) although proteins could not be detected in the preparation of manufacturer A (Table 4). The average magnitude of the skin reactions were again similar for all four preparations. The numbers of patients

Table 2. Potency of *D. pteronyssinus* extracts measured by inhibition of RAST, protein, carbohydrate phosphorylcholine content, content of tridacnin reactive components and skin test reactions

Inhibition of RAST						Skin test		
Preparation in order of potency	Potency*	Protein (µg/ml)	Carbo-hydrate (µg/ml)	Carbo-hydrate/protein	Phos-phoryl-choline† content	Av. diam‡ (mm)	Per-centage positive	Tridacnin content
B	33	68	463	6.8	128	5.0 ± 1.7	78	2
A	29	72	63	0.87	2	5.0 ± 1.6	76	2
C	16	51	250	4.9	4	4.8 ± 1.3	69	2
Significance§		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Reciprocal dilution required for 50% inhibition of RAST.

† Reciprocal dilution of extract which just failed to inhibit haemagglutination of sensitized SRBC.

‡ Positive responders only.

§ By linear regression analysis with respect to RAST potency.

Table 3. Potency of *L. perenne* (rye grass) extracts measured by inhibition of RAST, protein, carbohydrate phosphorylcholine content and skin test reactions

Inhibition of RAST						Skin test	
Preparation in order of potency	Potency*	Protein ($\mu\text{g/ml}$)	Carbo-hydrate ($\mu\text{g/ml}$)	Carbo-hydrate/protein	Phosphoryl-choline† content	Av. diam.‡ (mm)	Per-centage positive
D	950	1350	6960	5.1	—	5.4 ± 2.2	64
B	350	370	4640	12.5	—	5.5 ± 2.6	61
C	120	123	1180	9.6	—	5.3 ± 2.0	61
A	0.95	25	1330	53.2	—	4.8 ± 1.9	52
Significance§		$r=0.99$ $P<0.01$	$r=0.95$ $P<0.05$		—	NS	NS

* Reciprocal dilution required for 50% inhibition of RAST.

† Reciprocal dilution of extract which just failed to inhibit haemagglutination of sensitized SRBC.

‡ Positive responders only.

§ By linear regression analysis with respect to RAST potency.

Table 4. Potency of *A. tenuis* extracts measured by inhibition of RAST, protein, carbohydrate, phosphorylcholine content and skin test reactions

Inhibition of RAST						Skin test	
Preparation in order of potency	Potency*	Protein ($\mu\text{g/ml}$)	Carbo-hydrate ($\mu\text{g/ml}$)	Carbo-hydrate/protein	Phosphoryl-choline† content	Av. diam.‡ (mm)	Per-centage positive
D	500	630	6090	9.7	128	4.0 ± 1.2	35
B	340	275	7420	27.0	64	3.7 ± 0.9	23
A	48	0	485	—	32	3.6 ± 0.6	20
C	11	41	2410	58.9	8	3.7 ± 0.9	16
Significance§		$r=0.96$ $P<0.05$	NS	NS	$r=0.96$ $P<0.05$	NS	NS

* Reciprocal dilution required for 50% inhibition of RAST.

† Reciprocal dilution of extract which just failed to inhibit haemagglutination of sensitized SRBC.

‡ Positive responders only.

§ By linear regression analysis with respect to RAST potency.

exhibiting positive skin reactions were less than those found for *D. pteronyssinus* and *L. perenne* extracts.

A trend, which followed the same order of magnitude as RAST potency but was not significantly related to it, was found for the percentage of patients exhibiting positive skin reactions. Since all of the patients were skin-tested with the same preparations, this showed that 50% of them who were deemed allergic to *A. tenuis* using

the preparation from company D would be classified as unreactive to this allergen if the preparation from company C was used diagnostically.

Cladosporium herbarum. While all four preparations of *C. herbarum* exhibited high potency by inhibition of RAST, neither the actual potencies nor their order of magnitude were related to any of the other parameters of potency (Table 5).

Aspergillus fumigatus. The *A. fumigatus* extract prepared by company D was, by inhibition of RAST, approximately 35 times more potent than that supplied by any other company (Table 6). This extract also contained the highest content of protein,

Table 5. Potency of *C. herbarum* extracts measured by inhibition of RAST, protein, carbohydrate, phosphorylcholine content and skin test reactions

Inhibition of RAST						Skin test	
Preparation in order of potency	Potency*	Protein ($\mu\text{g/ml}$)	Carbo-hydrate ($\mu\text{g/ml}$)	Carbo-hydrate/protein	Phosphoryl-choline† content	Av. diam.‡ (mm)	Per-centage positive
A	530	0	1290	—	64	3.2±0.4	11
C	160	54	4650	86.1	4100	3.7±1.0	12
B	140	950	5000	5.3	128	3.1±0.5	12
D	110	110	4330	39.3	2050	3.7±1.0	20
Significance§		NS	NS	NS	NS	NS	NS

* Reciprocal dilution required for 50% inhibition of RAST.

† Reciprocal dilution of extract which just failed to inhibit haemagglutination of sensitized SRBC.

‡ Positive responders only.

§ By linear regression analysis with respect to RAST potency.

Table 6. Potency of *A. fumigatus* extracts measured by inhibition of RAST, protein, carbohydrate, phosphorylcholine content and skin test reactions

Inhibition of RAST						Skin test	
Preparation in order of potency	Potency*	Protein (μg/ml)	Carbo-hydrate (μg/ml)	Carbo-hydrate/protein	Phos-phoryl-choline† content	Av. diam.‡ (mm)	Per-centage positive
D	280	450	5260	11.7	32800	3.8 ± 1.2	14
B	8.3	140	2350	16.8	4100	3.6 ± 1.2	10
C	3.3	21	1150	54.7	4100	3.6 ± 0.5	6
A	2.9	0	740	—	16	3.3 ± 0.5	8
Significance		<i>r</i> = 0.96 <i>P</i> < 0.05	NS	NS	<i>r</i> = 0.99 <i>P</i> < 0.01	NS	NS

* Reciprocal dilution required for 50% inhibition of RAST.

† Reciprocal dilution of extract which just failed to inhibit haemagglutination of sensitized SRBC.

‡ Positive responders only.

§ By linear regression analysis with respect to RAST potency.

carbohydrate and phosphorylcholine-containing components. The high RAST potency was apparently not solely attributed to the absolute concentration of soluble material since the ratio of RAST potencies between this preparation and that of company B far exceeded the corresponding ratios of protein, carbohydrate and Pc components. Both protein ($P < 0.05$) and Pc-containing component ($P < 0.01$) concentrations were significantly related to RAST potency but not to carbohydrate content and skin test reactivity, as judged by percentage positive patients, although the order of magnitude for these parameters followed the same general trend as RAST potency.

Skin test reactions

Varying numbers of patients exhibited positive skin reactions to one preparation of a specific allergen but were skin test negative to the corresponding preparations supplied by the remaining companies (Table 7). It was not possible to determine whether this resulted from (1) the presence of non-specific irritants in such extracts (Aas, 1975), (2) the presence of unique skin reactive allergen determinants, or (3) an overall increase in common allergenic components. The extract of *C. herbarum* from company D, for example, was the least potent by RAST, yet yielded the highest number of 'sole' positive skin reactions, suggesting the presence of non-specific skin irritants. On the other hand, the D preparations of *L. perenne*, *A. tenuis* and *A. fumigatus* were both the most potent by RAST and also yielded the highest number of 'sole' positives, suggesting that alternatives (2) and (3) above provide a more plausible explanation than (1).

The 'sole' negative skin reactions, that is preparations which were skin test negative

Table 7. Numbers of aberrant skin test results

Extract		Manufacturer			
		A	B	C	D
<i>D. pteronyssinus</i>	{ +	4	3	1	4
	{ -	2	2	9	9
<i>L. perenne</i>	{ +	1	2	1	3
	{ -	15	3	4	5
<i>A. tenuis</i>	{ +	4	1	0	10
	{ -	10	3	11	0
<i>C. herbarum</i>	{ +	3	2	3	9
	{ -	6	4	5	1
<i>A. fumigatus</i>	{ +	2	3	1	5
	{ -	5	3	6	1
Total	{ +	14	11	6	31
	{ -	38	15	35	16

+ = 'Sole' positives, i.e. where that manufacturer's extract only is positive.

- = 'Sole' negatives, i.e. where that manufacturer's extract only is negative and two or more other manufacturers' extracts are positive.

in a patient who reacted with positive reactions to two or more corresponding preparations from other manufacturers, are likely to be of more relevance to the consideration of relative potencies. In general, with the exception of *Cladosporium* extracts, the preparations which resulted in the largest number of 'sole' negatives were also least potent by inhibition of RAST (Table 7).

Discussion

Allergen extracts of *D. pteronyssinus*, *L. perenne*, *A. tenuis*, *C. herbarum* and *A. fumigatus* purchased from four different manufacturers showed wide variation within extracts in both protein and carbohydrate content. Moreover, the carbohydrate/protein ratios varied extensively, indicating (by these criteria) heterogeneity of composition and not merely differences in the absolute concentration of identical components in each extract of a given allergen.

Inhibition of RAST was used as the primary method for establishing allergenic potency. This method demonstrated a wide range of potency for the extracts of each allergen prepared for use at allegedly comparable potency by the four different manufacturers. Similar variations between different preparations have been reported elsewhere for ragweed pollen (Gleich *et al.*, 1974). Potencies varied over a six-fold range for *D. pteronyssinus* extracts to a thousand-fold for *L. perenne* (rye grass pollen). RAST potency was significantly related to protein levels for *L. perenne* ($P < 0.01$), *A. tenuis* ($P < 0.05$) and *A. fumigatus* ($P < 0.05$) but not for *D. pteronyssinus* and *C. herbarum*. Potency was also directly related, by linear regression analysis, to carbohydrate content of the *L. perenne* ($P < 0.05$) and *A. fumigatus* preparations.

The CNBr method employed for coupling allergens to paper discs for use in RAST is primarily selective for protein and glycoprotein components since CNBr binds through $-NH_2$ groups. As a consequence, IgE antibodies to carbohydrate allergens lacking such residues may not be detected by conventional RAST (Baldo & Pepys, 1976), hence the potency of such carbohydrate allergens may not have been assayed in the RAST system used in this study. In this context, the trend towards increasing potency by skin testing based on percentage positive for the four *Cladosporium* extracts with increasing carbohydrate, and decreasing RAST potency, is interesting as it suggests that a significant proportion of the allergens in *Cladosporium* extracts are carbohydrate and not glycoprotein in nature.

A correlation was found between RAST potency and the percentage of subjects showing positive (≥ 3 mm weal) skin reactions to a particular extract for all allergens except *C. herbarum* where this association was inversely related. This method of assessing skin test reactions highlights further the inconsistencies existing between different preparations of the same allergen extract, particularly for *A. tenuis* extracts where twice as many patients would be classified as allergic if skin tested with the preparation from manufacturer 'D' compared with that from manufacturer 'C'. Similar considerations apply to *C. herbarum* (D vs A) and *A. fumigatus* (D vs C). We were unable to establish whether any of the preparations gave false positive skin reactions. Of more significance, however, were the large number of patients who were skin test negative to a particular preparation yet positive to preparations of the same allergen from at least two other manufacturers (e.g. *L. perenne* extract from manufacturer A, *A. tenuis* from manufacturers A and C—Table 7). Summation of these 'sole' negatives showed the five allergen extracts prepared by manufacturers B and D to be

more consistent in quality as judged by lower numbers of negative skin reactions than were those from manufacturers C and A.

Previous studies have shown that Pc components are present in the fungi *A. fumigatus*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Fusarium* species (Longbottom & Pepys, 1964; Potter, 1972). Our results show that Pc is also present in extracts of both *Alternaria* and *Cladosporium* species. Phosphorylcholine (Pc) content was significantly related to RAST potency for *A. fumigatus* ($P < 0.01$) and *A. tenuis* ($P < 0.05$) preparations, suggesting that Pc is either an allergenic determinant *per se* or is intimately associated with an allergen. However, other studies in this laboratory on Pc containing allergens from *D. pteronyssinus* and *A. suum* suggest that the latter is the more likely (Stewart *et al.*, 1979; A. McWilliam, G. A. Stewart & K. J. Turner, unpublished observation). Moreover, Pc content was also related to skin test reactivity, assessed by percentage of positive responders, suggesting that Pc-containing components are major allergens in these particular extracts. It follows therefore that Pc content may be used as a primary standardization procedure for these extracts. Phosphorylcholine was not detected in the four extracts of *L. perenne*, notwithstanding their high carbohydrate content, but was present in low concentration in all *D. pteronyssinus* extracts, except that from manufacturer B.

This study shows the wide range of potencies to be found in extracts of the same allergen prepared for skin testing by four different manufacturers whose products are widely used for the confirmation of type I hypersensitivity. As such it provides supportive evidence for the suggestion (Aas, 1975) that health authorities should demand effective standardization of allergen extracts according to a restricted range of procedures in order that the physician might know the quality of the extract available to him.

Acknowledgments

The skilled technical assistance of Mrs E. Fisher and Miss A. Butcher is gratefully acknowledged. This work was supported by research grants provided by National Health and Medical Research Council (Australia) and the Princess Margaret Children's Research Foundation (Inc.), Western Australia.

References

- AAS, K. (1975) Standardization of allergen extracts. International WHO-IABS Symposium on standardization and control of allergens administered to man, Geneva, 1974. *Developments in Biological Standardization*, **29**, 341.
- AAS, K. & BELIN, L. (1972) Standardization of diagnostic work in allergy. *Acta Allergologica*, **27**, 439.
- AKIYAMA, K., MIYAMOTO, T. & HORIUCHI, Y. (1979) A new method of allergen standardization. Passive cutaneous anaphylaxis inhibition in a mouse to rat system in comparison with other methods. *Journal of Allergy and Clinical Immunology*, **63**, 354.
- BAER, H., GODFREY, H., MALONEY, C.J., NORMAN, P.S. & LICHTENSTEIN, L.M. (1970) The potency and antigen E content of commercially prepared ragweed extracts. *Journal of Allergy*, **45**, 347.
- BALDO, B.A., FLETCHER, T.C. & UHLENBRUCK, G. (1977) Reaction of house dust mite extracts with mouse anti-phosphorylcholine and anti-galactan myeloma proteins. *Naturwissenschaften*, **64**, 594.
- BALDO, B.A. & UHLENBRUCK, G. (1977) Allergen standardization using lectins: A promising approach. *Lancet*, **i**, 802.
- BALDO, B.A. & PEPYS, J. (1976) Radioallergosorbent test (RAST) studies with insolubilized polysaccharides. *Clinical Allergy*, **6**, 563.
- BALDO, B.A., TURNER, K.J. & UHLENBRUCK, G. (1976) Isolation of a potent allergen from house dust mite by interaction with the lectin tridacnin. *Experientia*, **32**, 641.

- DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. & SMITH, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28**, 350.
- GLEICH, G.J., LARSON, J.B., JONES, R.T. & BAER, H. (1974) Measurement of the potency of allergy extracts by their inhibitory capacities in the radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **53**, 158.
- Longbottom, J.L. & PEPYS, J. (1964) Pulmonary aspergillosis: Diagnostic and immunological significance of antigens and C-substance in *Aspergillus fumigatus*. *Journal of Pathology and Bacteriology*, **88**, 141.
- POTTER, M. (1972) Antigen-binding myeloma proteins in mice. *Annals of the New York Academy of Sciences*, **190**, 306.
- SEDMAN, J.J. & GROSSBERG, S.E. (1977) A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue 9250. *Analytical Biochemistry*, **79**, 544.
- STEWART, G.A., MCWILLIAM, A. & TURNER, K.J. (1979) The isolation of an allergen from extracts of *Dermatophagoides pteronyssinus* using a murine IgA myeloma with a specificity for phosphorylcholine. *Clinical Allergy*, in press.
- VARGA, J.M. & CESKA, M. (1972) Characterization of allergen extracts by gel-isoelectrofocussing and radioimmunosorbent allergen assay. Allergens in Timothy pollen. *Journal of Allergy and Clinical Immunology*, **49**, 274.
- WEEKE, B. & LOWENSTEIN, H. (1973) Allergens identified in crossed radioimmunoelectrophoresis. *Scandinavian Journal of Immunology*, **2**, (Suppl. 1), 149.

Copyright of Clinical Allergy: Journal of the British Allergy Society is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.

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.