

A study of serum immunoglobulins including IgE and reaginic antibodies in patients with bronchial asthma in relation to the spectrum of the disease

KUNAL SAHA, D. D. KULPATI, R. PADMINI, D. N. SHIVPURI
and P. L. SHALI

Department of Bacteriology and Medicine, Maulana Azad Medical College and associated Irwin and G. B. Pant Hospitals, New Delhi and the Department of Allergy and Applied Immunology, Vallabhbhai Patel Chest Institute, Delhi, India

Summary

Immunoglobulins G, A, M, D, and E were studied in the sera obtained from sixty-nine bronchial asthma patients, who were graded objectively according to atopic score. Among other associated atopic diseases, they often had allergic rhinitis. Ninety-one per cent of these patients had elevated serum IgE levels and the mean level of serum IgE was more than 3.5 times higher than that observed in the normal subjects. Moreover, as their atopic score increased, the serum IgE levels were also elevated, and every patient with high atopic score, had elevated serum IgE levels. This indicates the association of atopic state with serum IgE level.

Furthermore, reaginic antibodies were studied in some of these patients by intradermal tests, Prausnitz-Küstner (PK) reaction, conjunctival and bronchial provocation tests. Intradermal and PK tests were comparable and proved to be the most sensitive method of detecting reagins. The intensity of these reactions correlated significantly with serum IgE level, although this relationship is not invariably present. The reaginic activities in the sera of the patients with atopic bronchial asthma also compared well with the positive bronchial test (induced asthma) by inhalation of specific aerosol although this association is also not always present.

Eleven patients with high atopic score had isolated serum IgA deficiency. There was a significant rise of mean serum IgM in comparison to the controls.

Introduction

The association of high level of serum IgE with extrinsic asthma has drawn the attention of many authors (Loeffler, Cawley & Moeder, 1973). Johansson has advocated use of serum IgE as a diagnostic tool, while Spitz *et al.* (1972) feel that value of IgE estimation is limited. Recently Spitz and his associates (1972) have studied serum IgE levels in asthmatics in relation to the spectrum of the disease, and observed that as the atopic score increases serum IgE levels also rise. The aim of the present communication is to report our observations and also to compare the occurrence of reaginic antibody with (i) serum IgE levels, (ii) incidence of positive bronchial sensitivity tests by

provocation (induced asthma) and (iii) atopic scores of the patients. Immunoglobulins G, A, M, and D have also been estimated in these patients to study their role in extrinsic asthma.⁵

Materials and methods

Selection of atopic patients

Sixty-nine patients with bronchial asthma (forty-one males and twenty-eight females), with ages varying from 12 to 60 years, were selected on the basis of their personal allergic history (asthma, rhinitis and eczema), family history of allergy, skin tests with a battery of about seventy common pollen, fungal, insect and food allergens, degree of eosinophilia, age of onset of illness and absence of parasites in stool. The above findings were assessed objectively and were graded according to the method proposed by Spitz and his associates (1972) (see Table 1).

Table 1.

Clinical examination	Atopic score
1. Age of onset of illness less than 20 years	1
2. Personal history of other allergy (Eczema, rhinitis, urticaria)	3
3. Family history (Atopic diseases in first and second degree of relations)	2
4. Eosiniphilia more than 500/mm ³	1
5. Positive intradermal test (0.01 ml 1:500 antigen)	
2+ by one allergen	1
3+ by one allergen	1
more than 3+ by two or more allergen	3

Maximum score possible in a subject = 10.

The criteria of positivity of skin tests were the same as proposed by Shivpuri & Agarwal (1969). Steroids were not given to any patients before collecting blood. Anti-histaminics were withdrawn 2 days before collecting blood. Sera were separated and were stored at -20°C.

Selection of control subjects for determining the normal levels of immunoglobulins

Thirty-five apparently healthy adults from high socioeconomic strata with no history of family and personal allergy were selected for determining normal serum IgE levels. Their ages varied from 18 to 36 years and their stools had no ova, cysts or parasites. Seventy normal healthy subjects were included for determining normal serum levels of immunoglobulins G, A, M and D.

Estimation of IgE by dual technique

Serum IgE were first estimated in duplicate on direct IgE plates (Meloy Laboratories, U.S.A.). Four dilutions of standard IgE in bovine albumin saline, which had been previously calibrated by the manufacturer against the research standards cited by the

World Health Organization (1970), were used to compare the ring diameters produced by the test samples.

Since the direct method was not sensitive enough to detect serum IgE levels below 700 iu/ml, test samples showing no measurable ring diameter were reassessed in duplicate by the indirect radioimmunodiffusion method (Rowe, 1969), which was able to detect serum IgE levels as low as 40 iu/ml. If the results in duplicate tests by both direct as well as indirect methods varied by more than 10%, the tests were repeated.

The standards used in the direct test were also employed in the indirect test, but due to the higher sensitivity of the indirect test as mentioned above, the standard with lowest concentration of IgE (Meloy Laboratories, U.S.A.) was further diluted to two, ten, and twenty times. I^{131} was obtained from Bhaba Atomic Research Centre, Bombay and anti-human IgE antiserum was kindly donated by Dr D. Rowe, Immunoglobulin Reference Centre, Lausanne, Switzerland. Anti-sheep gammaglobulin was raised in rabbits by a technique described by Campbell *et al.* (1964), and then coupled with I^{131} following the method described by Hunter (1967). Kodak fast dental X-ray films Morlite occlusals (2.25 × 3 in) were used to take the pictures. One 10 lambda Hamilton syringe was used to deliver the samples and the standards in the wells of the plates. An optical magnifier (Flubacher & Co., Horgen-Zch, Switzerland) measuring up to one tenth of a millimetre, was used to measure the ring diameters, which were plotted against the concentrations of IgE levels in a semi-log graph paper.

Serum IgE levels of patients were compared with those of normal subjects and also with their atopic status. The coefficient of correlation (r) between IgE levels and atopic scores was determined. In order to compare serum IgE levels with the reaginic antibodies in atopic patients, PK tests were performed with the sera from twenty-one patients according to the method described by Mittal *et al.* (1971). The intensities of positivity of PK reactions and intradermal tests were then compared with their serum IgE levels. These data were statistically evaluated.

Estimation of serum immunoglobulins G, A, M, and D

Serum immunoglobulins G, A, M, and D were estimated in the test samples by single radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965) using monospecific anti-immunoglobulin (heavy chain specific) antisera (Meloy Laboratories, U.S.A.) and immunoglobulin reference standards (Melpar, U.S.A.). The serum immunoglobulin levels of the asthmatic subjects were compared with those of the normal subjects and these data were statistically evaluated.

Comparison of the techniques for the detection of reagins in vivo

In a separate study, nine patients with atopic bronchial asthma, showing various grades of intradermal tests (1+ to 4+) were selected for the study of reaginic antibodies. PK, bronchial sensitivity, and conjunctival tests were performed on them by challenging with allergens to which they showed positive intradermal reactions. The gradings of skin and PK tests were described by Mittal *et al.* (1971).

Bronchial sensitivity tests were performed on symptom-free patients in a closed circuit, containing a nebulizer system connected with a Collin's spirometer of 13.5 l capacity fitted with a kymograph drum having a fast speed of 160 mm/min. Baseline forced expiratory volume (FEV) and first second forced expiratory volume (FEV₁ sec) were recorded. Thereafter similar records were obtained at 2 min and 10 min intervals after the patient breathed spirometer air saturated with an aerosol of buffered saline

for 1 min. After a short rest, similar records were obtained after breathing spirometer air saturated with an aerosol of a 1:500 (w/v) concentration of the antigen for 1 min. The bronchial response was interpreted and graded according to the criteria proposed by Shivpuri & Agarwal (1969). Conjunctival tests were performed and their results were graded according to the criteria proposed by Shivpuri (1962).

The results of the intensities of PK, bronchial sensitivity, and conjunctival tests were correlated for the comparison of their sensitivities for detecting reagins. The severity of asthma in the patients induced by provoking with specific antigens was compared with the reaginic activity in their sera.

Results

Of the sixty-nine cases with bronchial asthma, forty-four patients (64%) had asthma with rhinitis, seventeen (25%) had asthma alone, five (7%) had asthma with rhinitis and eczema, one had asthma with eczema and urticaria, one had asthma, rhinitis and urticaria, and in one asthma was associated with eczema. This therefore indicates that atopic asthma is frequently associated with rhinitis. According to Warwick (1971), 54% extrinsic asthmatics have association of other allergies. In the present study 46%, 36% and 18% of the asthmatics had seasonal, non seasonal and perennial types of asthma respectively.

The results of serum IgE levels in normal Indian subjects and patients with bronchial asthma are given in Table 2. Figure 1 depicts the distribution of serum IgE levels in the patients with bronchial asthma and normal subjects.

Table 2. Immunoglobulin E in the sera of normal Indian subjects and patients with bronchial asthma

Groups	IgE iu/ml mean and s.d. (range)	Statistical evaluation	
		<i>t</i>	<i>P</i>
Normal (35)	1025 ± 2103 (below 40–8500)		
Bronchial asthma (69)	3827 ± 2955 (650–12000)	5.5	less than 0.001

Eight normal subjects had serum IgE levels above 700 iu/ml.

In Table 3, patients with bronchial asthma have been classified objectively into three groups according to their atopic score. The mean serum IgE levels have increased with the rise in the atopic score.

Table 4 shows the comparison of mean serum IgE level of the patients with negative skin tests with that of patients with positive skin tests.

Table 5 depicts the comparison of serum IgE levels of asthmatics with the degree of reaginic activity of their sera which have been estimated by PK reaction. Eighty different allergens were tested on twenty-one different recipients at five different sites,

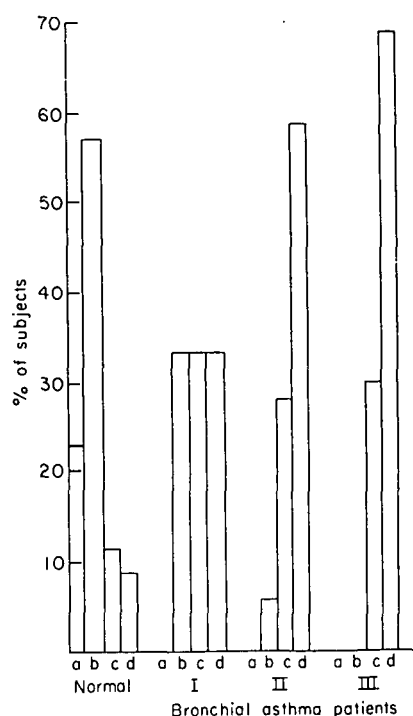


Fig. 1. Histogram showing distribution of serum IgE levels in health and bronchial asthma. Bronchial asthma-patient I, atopic score 0-2; patient II, atopic score 3-5; patient III, atopic score 6-8. a = serum IgE level <40-100 iu/ml; b = serum IgE level 101-1000 iu/ml; c = serum IgE level 1001-2500 iu/ml; d = serum IgE level >2500 iu/ml.

Table 3. Serum immunoglobulin E and atopic status of the patients with bronchial asthma

Atopic status of bronchial asthma patients (69)	Atopic score	Serum IgE iu/ml Mean and s.d. (range)	Statistical evaluation	
			<i>t</i>	<i>P</i>
A. Nonatopic bronchial asthma (15)	0-2	2182 ± 1653 (700-5400)		
B. Questionable bronchial asthma (31)	3-5	3165 ± 2060 (650-8430)	B vs A 1.75	Less than 0.1 more than 0.05
C. Atopic bronchial asthma (23)	5-8	5448 ± 5478 (1200-12000)	C vs A 2.7	Less than 0.01 more than 0.001
			C vs B 1.9	Less than 0.1 more than 0.05

Correlation coefficient (*r*) between serum IgE levels and atopic score = 0.37.

keeping one site as normal control on every occasion. Twenty-one allergens induced no skin or PK test reactions, and were omitted from the table.

Levels of serum immunoglobulins G, A, M, and D in normal subjects have been compared with those in the patients with bronchial asthma (Table 6). Mean level IgA in asthmatics is significantly decreased, while that of IgM is significantly increased in comparison to the control values.

Table 4. Comparison of serum IgE levels and intradermal tests performed on the patients with bronchial asthma

Intradermal tests performed on bronchial asthma patients	No. of cases	Serum IgE iu/ml Mean and s.d. (range)	Statistical evaluation	
			<i>t</i>	<i>P</i>
Negative*	22	2927 ± 2097 (650–8430)		
Positive	47	4212 ± 2097 (1075–12000)	2.37	less than 0.01

* 1+ and 2+ reactions in cases of insect allergens were not considered significant as they are frequently seen in healthy volunteers also.

Table 5. Comparison of serum IgE levels with the reaginic activities of the sera (from the patients with bronchial asthma), estimated by PK reaction

Degree of PK reactivity	No. of tests (%)	IgE iu/ml Mean and s.d. (range)	Statistical evaluation	
			<i>t</i>	<i>P</i>
A. Negative PK reaction	10(17)	4255 ± 2150 (650–12000)		
B. 1+ and 2+ PK reaction	33(56)	5692 ± 1286 (750–12000)	(vs.A)4.7	less than 0.001
C. 3+ and 4+ PK reaction	16(27)	7025 ± 2825 (1200–12000)	(vs.B)1.7 (vs.A)3.8	less than 0.05 less than 0.001

Grading of PK reaction was same as that of intradermal test (Table 4).

In a comparison of *in vivo* techniques for detecting reaginic antibody (Table 7), PK tests compared well with skin tests, but bronchial sensitivity and conjunctival tests are less sensitive than the PK test in that order.

Table 8 compares the degree of reaginic activity of the sera from asthmatics with the severity of attack of asthma induced by provocative inhalation tests. The data clearly indicate that the degree of reaginic activity in the sera of atopic bronchial asthma patients compares well with the frequency and severity of asthma (bronchospasm), although this association is not always true.

Table 6. Levels of serum immunoglobulins G, A, M, and D in patients with bronchial asthma and normal subjects

Serum immunoglobulin levels mg/100 ml mean and s.d. (range)						
Groups (number of subjects)		G	A	M	D	
Normal adults(70)		1076 ± 262 (250-1828)	122 ± 31 (37-348)	103 ± 22 (42-204)	16.5 ± 6.7 (0.0-33.4)	50%*
Bronchial asthma (45)		1037 ± 238 (280-2080)	83 ± 61 (63-105)	165 ± 69 (21-302)	15.5 ± 7.5 (0.0-30.8)	50%*
Statistical evaluation	<i>t</i>	0.83	4.0	5.9	not done	
	<i>P</i>	less than 0.5	less than 0.001	less than 0.001		

* Percentage of detectable serum IgD. Serum IgD conc. above 1 mg/100 ml have been defined as detectable level.

Table 7. Comparison of the results of intradermal tests with those of PK, bronchial sensitivity and conjunctival tests results in the same patients with the same antigens. (Analysis of data on nine patients)

Various clinical tests performed	Various grades of intradermal reactions, 1+ to 4+ in patients on whom PK, bronchial sensitivity and conjunctival tests were done									
	1+		2+		3+		4+		Total tests done	%+ (+ to 4+)
	TD	%+	TD	%+	TD	%+	TD	%+		
1. PK Test	1	0.0	16	100.0	17	100.0	15	100.0	49	98.0
2. Bronchial test	1	100.0	11	91.0	16	81.2	13	77.8	41	80.5
3. Conjunctival test	1	0.0	11	54.5	16	75.0	14	100.0	42	76.2

TD: total tests done on nine patients who had shown positive intradermal reactions of varying degree.

%+: percentage of positive PK, bronchial and conjunctival reactions in subjects with positive intradermal tests of varying intensity.

However, high reaginic activity is associated with high atopic score and raised serum IgE levels (Table 9), but again these relations do not always occur.

Discussion

Of thirty-five apparently healthy Indians with no family or personal history of allergy, 23% had more than 700 iu IgE/ml serum, in contrast to sixty-two healthy Europeans in whom 7% had more than 700 ng IgE/ml, though amounts stated in units and nanograms are not synonymous. The mean amount of serum IgE (1025 iu/ml) in thirty-

Table 8. Comparison of the intensities of PK reactivity with the bronchial response by provoking tests and severity of attack of asthma in same patients with same antigens. (Analysis of data on nine patients)

Bronchial sensitivity test		Various grades of PK reactivity 2+ to 4+, performed by the sera of nine patients, on whom bronchial sensitivity tests were also done (fifteen PK tests done)		
Reduction in FEV ₁	Bronchial response	2+	3+	4+
No. of patients				
Below 15%	Nil	1	1	0
15–20%	Mild	0	1	0
21–50%	Moderate	0	4	0
Over 50%	Marked	2	2	4

The observed reduction of FEV₁ varied from 0% to over 80%. In one case (PLC), the asthma induced by the provocative tests was so severe, that the reduction of FEV₁ was more than 80%, and his vital capacity became almost equal to his tidal volume. His serum showed 4+ PK reactivity with cockroach antigen.

Table 9. Comparison of reaginic antibody with atopic score. (Analysis of twenty-one patients with bronchial asthma)

Skin and PK tests (no. of cases)	Reaginic anti-body	Serum immunoglobulin IgE level (iu/ml)		
		Below 1000	1001–2500	Above 2500
I. Both PK test and skin test positive (13)	++	No. of cases	1*	2
		IgE mean (range)	750	1150 (1050–1200)
		Mean atopic score (range)	1	5.5 (5–6)
II. Variable PK and skin tests (6)	+	No. of cases	1	1
		Mean IgE (range)	650	1500
		Mean atopic score (range)	4	2
III. Both PK test and skin test negative (2)	–	No. of cases	Nil	1†
		Mean IgE (range)	X	1500
		Atopic score (range)	X	2

Serum IgE above 1000 iu/ml has been defined as elevated levels.

* This patient had high reaginic antibody as evidenced by the positivity of both skin and PK tests, but his IgE level was not elevated; also his atopic status was low.

† Raised serum IgE level with absence of reaginic antibody were observed, which might be due to other causes such as past unnoticed parasitic infections.

five healthy Indian adults is much greater than that observed by Johansson (1968) in normal Europeans (about 160 ng/ml), although in Ethiopian children the mean serum IgE concentration was twenty times greater than that of normal Swedish persons. However, Indians tend to have greater amounts of IgE than Europeans, as Dasgupta (1974) reported a mean concentration of 522 ± 323 iu IgE/ml. These high serum concentrations of IgE possibly reflect past parasitic infections.

On the evidence of these results serum IgE greater than 1000 iu/ml is considered to be abnormally increased, and therefore 91% of the sixty-nine asthmatic persons had increased amounts of IgE. According to Warwick (1971) increased amounts of IgE occur in 60% of cases of bronchial asthma and distinguishes them from intrinsic asthma where no such increase occurs. The mean concentration of serum IgE (3827 iu/ml) in the sixty-nine Indian patients was 3.5 times greater than that of normal persons.

The 'spectral concept' of considering the history, severity and intercurrent signs provided a new approach to differentiate patients with atopic from non-atopic bronchial asthma (Pepys, 1971). McNichol & Williams (1974) arbitrarily graded asthmatic children from mild evanescent to severe persistent asthma into four grades and observed that as the severity of asthmatic attacks increased, the serum IgE concentrations also increased. In the present study, the patients were classified objectively by atopic score in which 22% were at the non-atopic end of the spectrum, 33% were atopic and 55% lay between them.

With the progressive increase in atopic score, the mean serum IgE concentrations increased (Table 3). Furthermore with increasing atopic score the number of sera with IgE concentrations above 2500 iu also increased.

In our series of sixty-nine bronchial asthma patients, the regression coefficient (r) between the atopic scores and serum IgE levels was 0.37 which agreed well with the (r) value of (0.304) obtained by Spitz *et al.* (1972). These results therefore confirm the previous contention that IgE concentrations are related to the atopic status of an individual.

A good, but not exact correlation has been said to occur between serum reagin and skin test reactivity (Flick, 1972). We found (Table 4) that the mean serum IgE concentration (2927 iu/ml) in twenty-two patients who did not respond to intradermal tests with allergens was significantly lower than that (4214 iu/ml) observed in forty-seven such cases who showed positive intradermal tests ($t=2.37$). Similar statistical correlations were reported by Spitz *et al.* (1972) and Wood & Oliver (1972).

Furthermore the intensity of the PK test was also related to the concentration of serum IgE (Table 5) as previously reported by Flick (1972).

Of the other serum globulins, there was a significant decrease ($t=4.0$) in the mean serum concentration of IgA of the sixty-nine asthmatic patients compared to that of normal persons (Table 5). This deficiency of IgA was so profound that in eleven patients with an atopic score of 6 to 8, the concentration was below 20 mg IgA/100 ml serum. This unexpectedly high incidence of IgA deficiency could support the contention of Kaufman & Hobbs (1970) that there is abnormally increased mucosal permeability in highly atopic persons. The mean serum concentration of IgM was significantly increased above normal ($t=5.9$) but there was no significant difference from normal in immunoglobulins G and D in asthmatic persons (Table 6).

Our results show that the PK test is the most sensitive and reliable *in vivo* method of detecting reagins and correlated well with the intradermal test on the serum donors; the bronchial and conjunctival tests are much less sensitive (Table 7). Flick (1972)

stated that the intradermal test was the most sensitive technique though sometimes there are false positive reactions. There is also a good correlation between severity of asthma and intensity of the PK reaction (Table 8), though not in all persons, and indicates a possibility of mediators other than reagents inducing bronchoconstrictions after inhaling allergens to which the persons were sensitized (Warwick, 1971). Similarly there was a good correlation between presence of reaginic antibody and of increased amounts of serum IgE, with high atopic score (Table 9). One patient however was not atopic, his serum IgE concentration was normal, though he apparently had a reaginic antibody in the evidence of a PK and intradermal test. Persons showing immediate reactions on skin tests but without clinical evidence of atopy are often designated as potential or latent atopics, and it is possible that immediate skin reactions in non-atopics may be mediated by short-term sensitizing anaphylactic IgG antibody (Parish, 1970).

Acknowledgments

The authors wish to record their sincere gratitude to Dr D. S. Rowe, Immunoglobulin Reference Centre, the World Health Organization, Lausanne, Switzerland, for his kind gift of anti-human IgE antiserum, and to Dr G. Torrigiani, Immunology, the World Health Organization, Geneva, Switzerland for his generous donation of commercially available direct human IgE kits and monospecific anti-human immunoglobulin antisera with reference immunoglobulin standards. Thanks are also due to Dr A. Dasgupta, Microbiology Department, All India Institute of Medical Sciences, New Delhi for his co-operation and guidance.

References

- CAMPBELL, D.H., GARVEY, J.S., CREMER, N.E. & SUSSDORF, D.H. (1964) *Methods in Immunology*, p. 96. W. A. Benjamin Inc., New York.
- DASGUPTA, A. (1974) Immunoglobulins in health and disease. III. Immunoglobulins in the sera of patients with amoebiasis. *Clinical and Experimental Immunology*, **16**, 163.
- FLICK, J.A. (1972) Human reagents: Appraisal of the properties of the antibody of immediate type hypersensitivity. *Bacterial Reviews*, **36**, 311.
- HUNTER, W.H. (1967) The preparation of radioiodinated proteins of high activity. Their reaction with antibody in vitro: The radio immunoassay. In: *Handbook of Experimental Immunology* (Ed. by D. M. Weir), 1st edn, p. 608. Blackwell Scientific Publications.
- JOHANSSON, S.G.O. (1968) Serum IgND levels healthy children and adults. *International Archives of Allergy*, **34**, 1.
- KAUFMAN, H.S. & HOBBS, J.R. (1970) Immunologic defects in an atopic population. *Lancet*, **ii**, 1061.
- LOEFFLER, J.A., CAWLEY, L.P. & MOEDER, M. (1973) Serum IgE levels—Correlation with skin test sensitivity. *Annals of Allergy*, **31**, 331.
- MANCINI, G., CARBONARA, A.C. & HEREMANS, J.P. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- MCNICHOL, K.N. & WILLIAMS, H.E. (1974) Spectrum of asthma in children—II, Allergic Components. *British Medical Journal*, **4**, 12.
- MITTAL, M.M., SAHA, K., SHALI, P.L. & SHIVPURI, D.N. (1971) A study of Pransnitz-Küestner reaction in leprosy. *Clinical and Experimental Immunology*, **8**, 657.
- PARISH, W.E. (1970) Short term anaphylactic IgG antibodies in the human sera. *Lancet*, **ii**, 591.
- PEPYS, J. (1971) Immunological mechanisms in asthma. *CIBA Foundation Study Group*, **38**, 86.
- ROWE, D.S. (1969) Radioactive single radial diffusion: A method for increasing the sensitivity of immunochemical quantification of proteins in agar gel. *Bulletin of the World Health Organization*, **40**, 613.
- SHIVPURI, D.N. (1962) Comparative evaluation of common methods of diagnostic antigen tests in patients of respiratory allergy. *Indian Journal of Chest Diseases*, **4**, 102.

- SHIVPURI, D.N. & AGARWAL, M.K. (1969) Studies on allergenic fungal spores of the Delhi, India Metropolitan area. *Journal of Allergy*, **44**, 204.
- SPITZ, E., GELFAND, E.W., SHEFFER, A.L. & AUSTEN, K.F. (1972) Serum IgE in clinical immunology and allergy. *Journal of Allergy and Clinical Immunology*, **49**, 337.
- WARWICK, M.T. (1971) Provoking factors in asthma. *British Journal of Diseases of the Chest*, **65**, 1.
- WOOD, C.B.S. & OLIVER, J. (1972) Serum IgE in asthmatic children—Relation to ages, sex, eczema and skin sensitivity tests. *Archives of Diseases in Childhood*, **47**, 890.
- World Health Organization (1970) A research standard for human immunoglobulin E. *Bulletin of the World Health Organization*, **43**, 609.

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.