

Specific IgE estimations by RAST in Japanese asthmatics compared with skin, passive transfer and bronchial provocation tests

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Summary

198 asthmatics and twenty healthy persons were studied by RAST and *in vivo* tests with four common inhalant allergens. (a) Higher RAST classes were elicited with mite (*Dermatophagoides farinae*) extract and lower classes with other allergens. The agreement between positive RAST and skin or P-K tests was highest with the mite extract. RAST sensitivity was dependent on the kinds of allergens and was most sensitive to mite extract. Positive RAST was most closely related to the prick test reactions. (b) RASTs to house dust and mite extract were examined in terms of the threshold dosage of house dust and also the types of bronchial response (early, dual and late) induced by a certain amount of house dust; higher RAST classes were found in subjects with bronchial response of the dual or early type, elicited by a threshold dosage of diluted extract, while lower RAST classes were found in cases of the late-type response elicited by the threshold dosage of concentrated extract. (c) Changes in IgE antibodies to house dust and mite extract were estimated in patients with positive house dust provocation. A greater increase occurred in cases of dual or early response, a smaller increase in those with a late response. Despite discordances in skin tests and RAST between house dust and mite extracts, the increases in IgE antibodies to mite extracts as well as house dust were observed in all cases, presumably caused by an allergenic identity between the allergens.

Introduction

The recent development of the radioallergosorbent test (RAST) (Wide, Bennich & Johansson, 1967) has made it feasible to apply the *in vitro* assay of specific IgE antibodies to clinical allergy investigations. The present study, examining asthmatic patients in the Kobe area, Japan, was designed to elucidate the following points, using four common inhalant allergens, i.e. house dust, mite (*Dermatophagoides farinae*), ragweed pollen and *Aspergillus fumigatus*: (a) the relationship between allergen-specific IgE antibodies and reactivities estimated by skin or P-K tests; (b) changes in circulating specific IgE antibodies in comparison with the degree of bronchial allergic response; and (c) the cross-antigenicity between house dust and mite antigens in Japan, with reference to skin reactions and specific IgE antibodies.

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Materials and methods

Subjects

198 asthmatic out-patients, aged 4–70 years and having had no immunological treatment, were investigated. Intradermal and skin prick tests, and passive transfer tests, were made; blood samples were taken for RAST analysis. Sixty out of 198 patients were further examined for suspected house dust allergy by a house dust provocation test (PT). A RAST was also made on sera from twenty healthy individuals, aged 22–58 years.

Skin tests

All cases were examined by the prick test and the intradermal test (IDT) with four common allergens: house dust, mite extract, ragweed pollen and *A. fumigatus* (Torii Co., Japan). Skin tests were performed on the forearm using allergen solutions of appropriate concentrations: for the prick test, 1:10 of house dust, 1:100 of mite extract (*Dermatophagoides farinae*), ragweed pollen and *A. fumigatus*; and for the intradermal test, 1:1000 of house dust and ragweed pollen, 1:10000 of *A. fumigatus* and 1:20000 of mite extract were employed. Skin responses were regarded as positive where the weal was twice the control size by prick testing and the erythema was 20 mm or more in size, or where the weal was 9 mm or more in diameter by the IDT.

Passive transfer test (P-K test)

The test was performed by injecting 0.1 ml of the patient's undiluted serum into a recipient's forearm and 48 hr later 0.02 ml of allergen extract was injected, and assessed by the same score as the IDT.

Bronchial provocation test (PT)

The PT was usually carried out as described in detail in the author's previous paper (Kurimoto, 1975). The threshold dosage of house dust extract to produce the positive bronchial response was determined in terms of the highest dilution of house dust allergen. Reactions provoked by inhalation of 0.5 ml of 1:10 house dust were assessed as early when they developed within 30 min, as late when they developed more than 3 hr later and as dual when the response was biphasic.

Table 1. Frequency of RAST classes to house dust, mite, ragweed pollen and *A. fumigatus* in 198 asthmatic subjects

RAST Class	House dust	Mite (<i>Dermatophagoides farinae</i>)	Ragweed pollen	<i>A. fumigatus</i>
4	0	21	0	0
3	2	41	0	0
2	24	17	7	4
1	21	2	4	0
0	68	34	35	33
Total	115	115	46	37

Total number of subjects = 198; total of tests = 313.

Table 2. Agreements between RAST, intradermal, prick and P-K tests

Test	House dust	Mite (<i>Dermatophagoides farinae</i>)	Ragweed pollen	<i>A. fumigatus</i>
Positive RAST	45	76	11	4
Positive IDT	97	95	36	27
	46%	80%	31%	15%
Negative RAST	52	19	25	23
Positive IDT	97	95	36	27
	54%	20%	69%	85%
Positive RAST	2	6	0	0
Negative IDT	18	20	10	10
	11%	30%	0%	0%
Negative RAST	16	14	10	10
Negative IDT	18	20	10	10
	89%	70%	100%	100%
Agreement	61	87	21	14
	115	115	46	37
	53%	76%	46%	38%
Positive RAST	28	52	4	3
Positive prick test	46	52	7	6
	61%	100%	57%	50%
Negative RAST	18	0	3	3
Positive prick test	46	52	7	6
	39%	0%	43%	50%
Positive RAST	12	17	7	1
Negative prick test	54	43	35	31
	22%	40%	20%	3%
Negative RAST	42	26	28	30
Negative prick test	54	43	35	31
	78%	60%	80%	97%
Agreement	70	78	32	33
	100	95	42	37
	70%	82%	76%	98%
Positive RAST	43	68	10	3
Positive P-K test	77	73	23	14
	56%	93%	43%	21%
Negative RAST	34	5	13	11
Positive P-K test	77	73	23	14
	44%	7%	57%	79%
Positive RAST	4	9	2	0
Negative P-K test	35	37	23	22
	11%	24%	9%	0%
Negative RAST	31	28	21	22
Negative P-K test	35	37	23	22
	89%	76%	91%	100%
Agreement	74	96	31	25
	112	110	46	36
	66%	87%	67%	69%

RAST

Serum samples were analysed by the Phadebas RAST to the following allergens: house dust, mite extract, ragweed pollen and *A. fumigatus*. In Phadebas RAST the allergen, which is covalently coupled to paper discs, reacts with the specific IgE in the test serum. After washing away non-specific IgE three times with saline, 50 μ l of ^{125} I-labelled anti-IgE was added to a disc-allergen-IgE complex. The radioactivity of this complex was measured with a gamma-counter. The results were scored into five classes according to the Phadebas RAST reference system; class 0 was negative and classes 1–4 were positive for circulating IgE antibody.

Results

198 asthmatics and twenty healthy subjects were examined by RAST with four allergens (Table 1). There was a tendency for the RAST to the mite extract to be in the higher classes, while ragweed pollen and *Aspergillus* were in the lower classes. Control RASTs, in twenty persons, to the four allergens were all negative.

The RAST was correlated with skin tests (IDT and prick test) and the P-K test as shown in Table 2: (a) the positive skin or P-K tests showed the highest correlation with the positive RAST to mite extract, and to house dust, ragweed pollen and *Aspergillus* in decreasing order, while a negative skin or P-K test was correlated with a negative RAST in the order of *Aspergillus*, ragweed pollen, house dust and mite extract; (b) a positive RAST was shown to be related most closely to a positive prick test, to a P-K test and an IDT, in that order, while a negative RAST related most closely to a negative IDT or P-K test; (c) with reference to house dust, ragweed pollen and *Aspergillus*, the agreement between negative RAST and negative skin or P-K tests was higher than that between positive RAST and positive skin or P-K tests, being lower for the mite extract; (d) the correlations between negative RAST and positive skin or P-K tests were found to be highest for *Aspergillus* and lowest for the mite extract; and (e) the correlations between positive RAST and negative skin or P-K tests were greatest for the mite extract and lowest for *Aspergillus*, opposite to (d). The sensitivity of the RAST to the mite extract was demonstrated to be the greatest; with the mite extract, positive RAST was found in 30% of cases with a negative IDT, in 40% of cases with a negative prick test and in 24% of cases with a negative P-K test. The sensitivity of RAST to *Aspergillus* was lowest; positive RASTs associated with negative IDT or P-K tests were not elicited (0%), and those associated with negative prick test were observed in only 3% of cases (Table 2).

RAST was studied in combinations of P-K, IDT and prick tests (Figs 1 and 2). For all four allergens, higher RAST classes were mostly associated in cases with positive P-K, IDT and prick tests. For mite allergen, all the cases with positive P-K, IDT and prick tests were also positive by RAST (100%). However, for the three other allergens, only 50–67% of cases with positive P-K, IDT and prick tests were positive by RAST. Except for the mite allergen, the RAST was not sufficiently sensitive.

Sera obtained from 115 subjects were examined by the RAST to house dust and mite extract (Fig. 3); one or two classes higher were generally obtained with the mite extracts than with the house dust extract. A positive RAST to house dust was found in forty-seven (58%) out of eighty-one cases with a positive RAST to the mite extract, while a positive RAST to mite extract was found not only in all forty-seven cases with a positive RAST to house dust (100%), but also in thirty-four out of sixty-eight cases

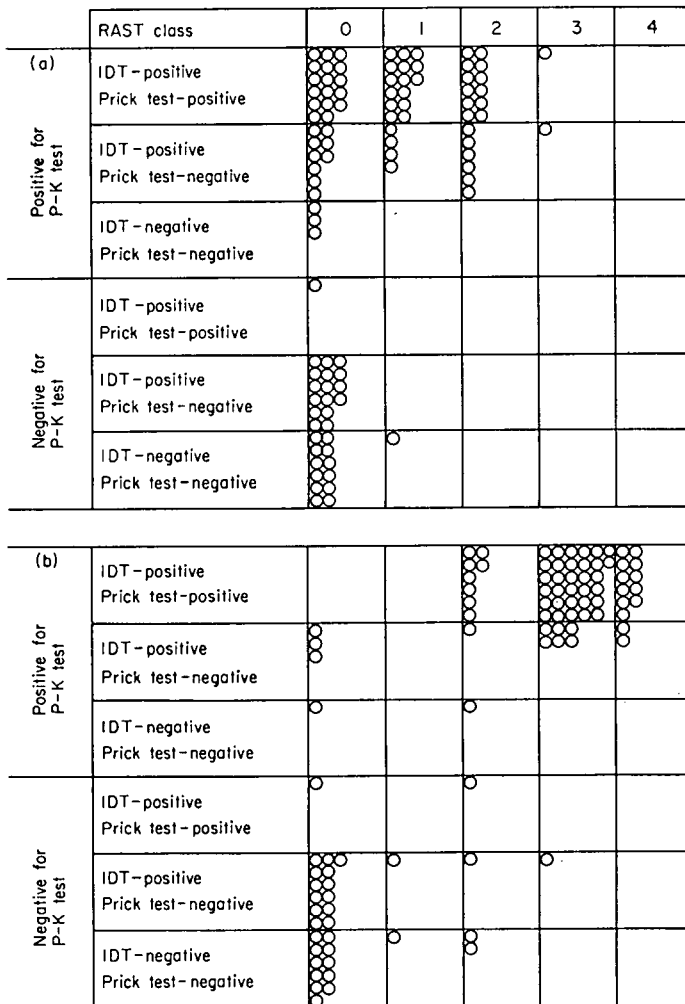


Fig. 1. RAST classes compared with combinations of P-K, intradermal and prick tests; (a) RAST to house dust ($n = 98$); (b) RAST to mite ($n = 93$). See text for details.

with a negative RAST to house dust (50%). There were a number of sera which were RAST-positive to mite extract and negative to house dust, and not vice versa (Fig. 3).

A comparative study between the RAST and IDT was made, in combination, with house dust and mite allergens in 115 patients, who could be classified into four groups (Table 3); group I: 41% were positive by RAST, compared with 72% by IDT, to both house dust and mite allergen; group II: 0% of cases were positive to house dust but negative to mite allergen by RAST, compared with 12% by IDT; group III: 30% of subjects were positive to mite allergen but negative to house dust by RAST, compared with 10% by IDT; group IV: 30% were negative to both house dust and mite by RAST, compared with 5% by IDT. By IDT, the occurrence of positive responses only to house dust (12%) was similar to that only to mite extract (10%), in contrast to the RAST, where the former was 0% and the latter was 30%. Discord-

RAST class		0	1	2	3	4
(a)	Positive for P-K test	IDT-positive	○	○		
		Prick test-positive				
	Positive for P-K test	IDT-positive	○	○		
		Prick test-negative	○	○		
	Negative for P-K test	IDT-negative				
		Prick test-negative				
(b)	Positive for P-K test	IDT-positive	○	○		
		Prick test-positive				
	Positive for P-K test	IDT-positive	○	○		
		Prick test-negative	○	○		
	Negative for P-K test	IDT-negative				
		Prick test-negative				
(c)	Positive for P-K test	IDT-positive	○	○		
		Prick test-positive				
	Positive for P-K test	IDT-positive	○	○		
		Prick test-negative	○	○		
	Negative for P-K test	IDT-negative				
		Prick test-negative				

Fig. 2. The same as Fig. 1 except allergens; (a) RAST to ragweed ($n = 41$); (b) RAST to *A. fumigatus* ($n = 36$).

ances between RAST and IDT were revealed by the combined use of house dust and mite allergens.

A house dust PT was performed in sixty subjects with a suspected allergy for house dust out of the 198 patients, and positive reactions were elicited in forty-nine (Table 4). The positive RAST to house dust and mite correlated with the positive house dust PT in 59% and 92% of cases respectively; a negative RAST to house dust and mite associated with a positive house dust PT occurred in 41% and 8% of cases respectively. With house dust, the agreement (59%) between a positive RAST and a positive PT was similar to that (61%) between a positive RAST and a positive prick test. There was a closer agreement, as shown in Table 4, between the RAST to mite extract and the house dust PT than that between the RAST to house dust and the house dust PT. Positive RAST to house dust and mite extract in cases with a negative house dust PT

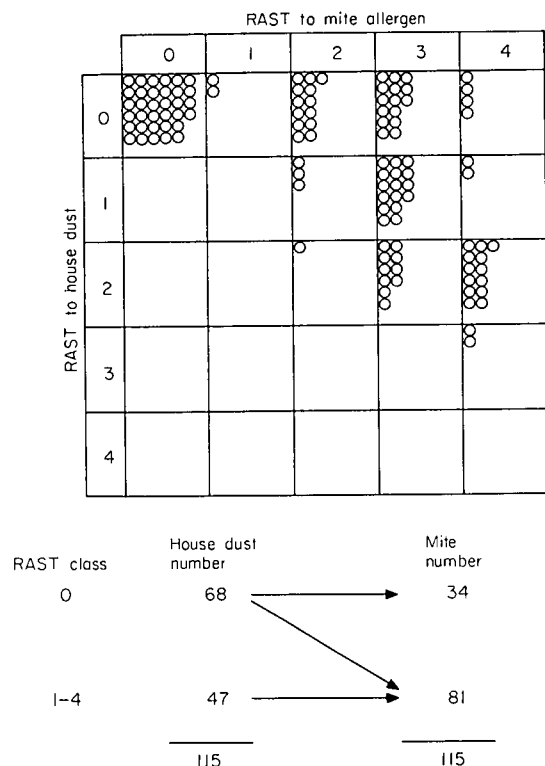


Fig. 3. Comparison of RAST to house dust and mite allergen ($n=115$). Positive RAST to house dust was found in forty-seven (58%) out of eighty-one cases with positive RAST to mite allergen. Positive RAST to mite allergen was found in all cases with positive RAST to house dust and thirty-four (50%) out of sixty-eight cases with negative RAST to house dust.

Table 3. Comparison of RAST and intradermal test, in combination, with house dust and mite allergens

Patients	Group I*		Group II†		Group III‡		Group IV§	
	RAST	IDT	RAST	IDT	RAST	IDT	RAST	IDT
Number	47	83	0	14	34	12	34	6
Total	115	115	115	115	115	115	115	115
Percentage	41	72	0	12	30	10	30	5

* Positive for house dust and mite allergen.

† Positive for house dust, negative for mite allergen.

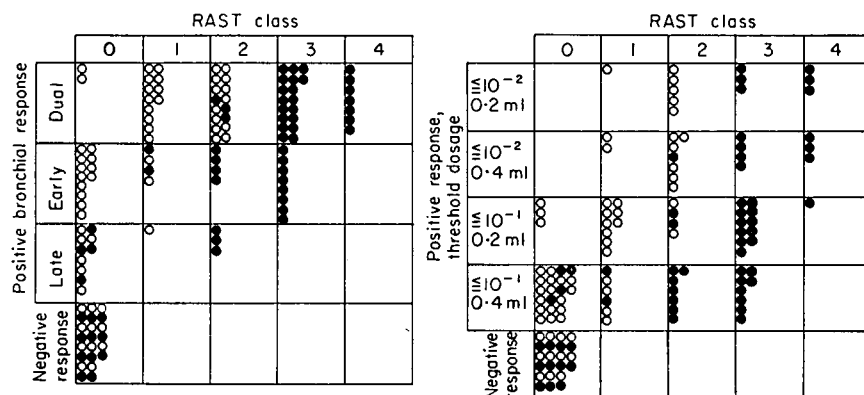
‡ Negative for house dust, positive for mite allergen.

§ Negative for house dust and mite allergen.

were not found in the present study. RAST to house dust and mite extract were examined with reference to the threshold dosage of house dust extract needed to produce a positive response for the first time (Fig. 4, right), and also the types of bronchial response, early, dual and late, elicited by a certain amount (0.5 ml of 1:10) of house dust inhalation (Fig. 4, left). Patients provoked with the threshold dosage

Table 4. Agreements between RASTs to house dust and mite allergen and bronchial provocation test (PT) with house dust

Test	RAST-positive, PT-positive	RAST-negative, PT-positive	RAST-positive, PT-negative	RAST-negative, PT-negative	Agreement
RAST to house dust	29	20	0	11	40
PT with house dust	49	49	11	11	60
Percentage	59	41	0	100	67
RAST to mite	45	4	0	11	56
PT with house dust	49	49	11	11	60
Percentage	92	8	0	100	93

**Fig. 4.** Comparison between RAST to house dust and mite allergen and bronchial responses induced by house dust inhalation ($n = 60$). Left, RAST to house dust and mite allergen are compared with types of positive response, early, dual and late induced by 0.5 ml of 1:10 house dust inhalation. Right, RAST to house dust and mite allergen are compared with the threshold dosage to produce the positive response for the first time by house dust inhalation. (○) RAST to house dust, (●) RAST to mite.

of the more dilute extract and with a dual type of response were shown to have much higher RAST classes, while patients with an early response were shown to have slightly higher classes and patients reacting to the threshold dosage of concentrated extract with late response were shown to have much lower classes. The more dilute the house dust extract eliciting the bronchial response, the higher the RAST class.

Further investigation of the seventeen out of forty-nine, positive by PT, for changes of specific IgE antibodies to house dust and mite extract were made by RAST before and after PT using 0.5 ml of 1:10 house dust (Table 5). In all the positive PT with house dust allergen, both IgE antibodies to house dust and mite were elevated, even if they differed in response to skin or P-K tests and RAST. There was a tendency for a much greater percentage increase in IgE antibody in cases giving a dual response, with a larger increase in cases of the early and a smaller increase in cases of the late response.

Discussion

The overall agreement (82%) between the RAST and prick test to the mite extract was slightly greater than that (73%) reported by Stenius & Wide (1969). The agree-

Table 5. Percentage changes in IgE antibodies to house dust and mite allergen and changes in total IgE levels, after house dust inhalation (cases 1-17)

Case No.	Sex	Age	House dust			Mite allergen		RAST (% change)		Total IgE (u/ml)	Bronchial response
			IDT	Prick test	P-K test	IDT	Prick test	P-K test	House dust		
1	F	15	+	—	—	+	—	+	+12.8	+28.5	Dual
2	M	22	+	—	—	+	—	—	+10.0	+14.9	Early
3	F	50	+	+	+	+	+	+	+8.1	+9.6	Early
4	F	8	+	+	+	+	+	+	+20.1	+24.6	Dual
5	M	18	+	+	+	+	—	+	+9.3	+8.6	Early
6	M	19	+	—	—	+	+	+	+26.3	+15.4	Dual
7	F	10	+	+	+	+	—	+	+18.9	+13.7	Dual
8	F	36	+	—	—	+	+	—	+22.7	+18.8	Dual
9	F	35	+	+	+	+	+	+	+17.8	+27.3	Dual
10	M	38	+	+	+	+	+	+	—	+12.1	Dual
11	F	17	+	+	+	—	—	—	+15.9	+24.8	Dual
12	F	20	+	+	+	—	—	—	+9.1	+13.9	Early
13	F	7	+	+	+	+	+	+	+5.7	+8.7	Late
14	M	32	+	—	—	+	—	+	+8.0	+8.2	Late
15	M	12	+	+	+	+	+	+	+3.6	+5.2	Late
16	F	25	+	—	—	+	—	—	+9.3	+16.7	Early
17	F	26	+	—	—	+	—	—	+11.1	+8.0	Late
18	F	18	+	+	+	+	+	+	−2.0	−1.3	980–1000
19	M	36	+	—	—	+	+	+	−2.1	+1.8	—
20	F	41	+	—	—	+	—	—	+1.5	−1.2	760–750

Case Nos. 18-20 were asthmatics who did not receive bronchial challenge but whose sera were taken at the two time intervals corresponding to the bronchial challenge. Case Nos. 1-20 were included among the 198 asthmatics shown in Table 1.

ments observed with three other allergens were slightly lower compared with previous reports (Aas & Johansson, 1971; Berg & Johansson, 1974; Pepys, Roth & Carol, 1975). Similar results to this study were reported by Collins-Williams & Bremner (1976).

The disagreements between the RAST and *in vivo* tests are presumably due to the following: (a) the commercial allergens (Berg, Bennich & Johansson, 1971), which are unpurified, heterogeneous and unstandardized; (b) different extracts were used for *in vivo* and *in vitro* tests; (c) the possible involvement of short-term anaphylactic IgG antibody (Parish, 1970) and/or false positive responses in skin tests and PT; (d) the involvement of secreted IgE; and (e) too low a concentration of IgE antibody to be detected by the RAST, in other words, the low sensitivity of the RAST.

RAST classes, and accordingly the correlation between the RAST and skin tests, were demonstrated to be varied, dependent on the kinds of allergens used (Figs 1 and 2, Table 2), as described by previous workers (Aas & Johansson, 1971; Collins-Williams & Bremner, 1976). It was also revealed in this study, in accordance with another report (Miyamoto *et al.*, 1974), that for a sample obtained from the same individual, the RAST classes were higher for mite extract than house dust (Fig. 3). In the present study, the number of cases with a positive RAST to mite associated with a positive RAST to house dust was greater than that reported by Virchow, Roth & Moeller (1976).

Foucard, Aas & Johansson (1973), showing a highly significant correlation between RAST, P-K and CHLT in cod fish allergy, reported that the RAST was about the same as the P-K test in sensitivity. However, a comparison between the RAST and P-K test to house dust, ragweed pollen and *Aspergillus* revealed that the RAST was less sensitive than the P-K test, although the RAST to mite extract was almost the same as the P-K test in sensitivity (Figs 1 and 2). With reference to four allergens, a positive RAST gave the best agreement with a positive prick test; the sensitivities of both tests were assumed to be similar.

In this study, a negative RAST associated with a positive skin or P-K test occurred more frequently than a positive RAST associated with a negative skin or P-K test (Table 2). Such a finding was observed with *Aspergillus*, ragweed pollen and house dust, but not with mite extract. Although in the present study the PT was performed using only house dust since the mite allergen was not readily available, such a tendency of the RAST was also shown in relation to the PT. The agreement between the RAST to mite allergen and the house dust PT with a positive response was greater than that between the RAST to house dust and the house dust PT. A comparative study of RAST and IDT by combined use of house dust and mite allergens revealed some discordances. Those findings might be due to differences in RAST sensitivity to house dust and mite extract rather than short-term anaphylactic IgG antibody or secreted IgE.

There have been controversies about the relationship between RAST and the degree of bronchial allergic response (Berg & Johansson, 1974; Miyamoto *et al.*, 1974; Bryant, Burns & Lazarus, 1975). In the present author's recent work (Kurimoto, 1975) using RIST, the bronchial responses induced by house dust PT were classified into three types, early, dual and late; a rise in IgE level was demonstrated in all three types, but the inter-relationship between the types of bronchial response and the increase in IgE level was not clarified. The present study shows that by analysing RAST to house dust and mite with reference to the threshold dosage of the house dust PT and the

types of bronchial response induced by a certain amount (0.5 ml of 1:10) of house dust, RAST classes to house dust and mite were related to the threshold dosage and types of bronchial response. In addition, some relationship was noted between the increase of IgE antibodies and types of bronchial response. The positive PT with house dust caused not only elevations of IgE antibodies to house dust but also to mite extract, suggesting an allergenic identity common to house dust and mite extract (Mounsell, Wraith & Cunningham, 1968; Pepys, Chan & Hargreave, 1968). PT with mite extract were not made in the present study, and remain to be evaluated.

While regarded as useful and reliable (Wide *et al.*, 1967; Berg & Johansson, 1974), it has been pointed out that as a diagnostic test the RAST is little different from the prick test (Bryant *et al.*, 1975; Pepys *et al.*, 1975). Although it is less sensitive in diagnosing mould allergy, the RAST is invaluable, especially when employing in combination with skin tests, for diagnosing allergy and also predicting the degree of bronchial allergic response.

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