

Clinical significance of specific IgE to common allergens

I. Relationship of specific IgE against *Dermatophagoides* spp. and grass pollen to skin and nasal tests and history

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Summary

Serological measurements of specific IgE to *Dermatophagoides farinae*, *D. pteronyssinus* and grass pollen showed statistically highly significant correlations with prick test reactions, the sizes of weals elicited, nasal tests and the clinical history. In subjects with negative prick tests, intracutaneous tests gave reactions which were not associated with specific IgE or reactions to nasal tests. Highly significant correlations were also found between total IgE values and prick test reactions to the allergens tested and to 48/80 but not to histamine; there was a negative correlation with reactions to anti-IgE serum. The reactions to prick tests identified almost all subjects with specific IgE.

Introduction

The discovery of IgE as reaginic antibody by Ishizaka & Ishizaka (1966) and Ishizaka, Ishizaka & Hornbrook (1966) and the development of methods for measuring total IgE in the serum by Johansson, Bennich & Wide (1968) and allergen specific IgE in the serum by Wide, Bennich & Johansson (1967), have made it possible to examine the relationship between amounts of specific IgE in the serum measured directly, and some important problems of Type 1 allergy. Two main sources of allergen were used for examining the correlation between specific IgE and the reactions to prick and intracutaneous tests, nasal tests and the clinical history. One source of allergens consisted of species of the house dust allergy mite genus *Dermatophagoides*, *D. farinae* and *D. pteronyssinus*, shown by Voorhorst *et al.* (1964) to be a major source of the allergens of house dust, since confirmed by many workers (Miyamoto *et al.*, 1968; Maunsell, Wraith & Cunningham, 1968; Pepys, Chan & Hargreave, 1968; Smith *et al.*, 1969; Stenius, 1970; McAllen, Assem & Maunsell, 1970; and others).

Specific IgE to *D. farinae* was found in the serum of house dust allergic patients by Stenius & Wide (1969) and confirmed by Holford-Stevens *et al.* (1970). A close correlation has been reported by McAllen *et al.* (1970) between the history, skin and provocation tests with *D. pteronyssinus* and IgE estimated indirectly by passive sensitization of human lung and by the allergen-induced release of histamine from the patients' leucocytes. Specific IgE to grass pollen, the other source of allergen used here, has been found by Wide *et al.* (1967).

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The possible contribution to the sizes of the weals, elicited with these allergens by prick tests, of individual differences in physiological reactivity of the skin was examined by tests with histamine and a histamine liberator 48/80. Tests with an antiserum to IgE were also made for correlation with the amounts of total IgE, to see whether these were reflected in the amounts of IgE in the skin.

Materials and methods

Subjects tested

A group of ninety-five subjects were tested. They consisted of fifty-four males and forty-one females, of whom sixty-six had asthma, eighty-one rhinitis and seventeen eczema in varying combinations. Two patients had received house dust hyposensitization, none had had grass pollen treatment and none of the patients had received antihistamine for at least 24 hr prior to testing. For the present purposes, and acknowledging a probable degree of error, a history of house dust allergy was recorded if the patient stated that exposure to the dust of bedding or dust in the house caused itching of nose and eyes, sneezing, rhinorrhoea, with or without asthma. A history of grass pollen allergy was recorded if the patient stated that contact with grass provoked rhinitis or asthma, supported by a seasonal history of symptoms in June and July. In some cases the seasonal history alone was taken as suggestive of grass pollen allergy.

Allergens tested

(1) *D. farinae*. A culture of *D. farinae*, on Gaine's Dogmeal plus added yeast, was provided by Mr A. M. Cunningham. The whole culture and a control specimen of dogmeal were extracted for 7 days in Coca's fluid after defatting in ether. The extracts were Seitz-filtered, dialysed and freeze-dried. Low molecular weight material was removed by passage through Sephadex G-25.

(2) *D. pteronyssinus*. A culture of *D. pteronyssinus* on human skin scales was provided by Dr R. Voorhorst. This was extracted as for (1) but without Sephadex filtration.

(3) *Mixed grass pollen*. A freeze-dried extract was provided by Beecham Research Laboratories.

(4) Histamine dihydrochloride (puriss. Koch-Light) and 48/80 (Wellcome Research Laboratory) were dissolved in Coca's fluid for testing.

(5) *Anti-IgE serum*. A guinea-pig antiserum to human IgE was provided by Dr K. Ishizaka, together with a control guinea-pig serum which was used to exclude reactors to guinea-pig serum.

Preparation of test solutions of extracts

The freeze dried extracts were dissolved in Coca's fluid to which 50% glycerol was added for prick testing. The solutions were filtered through a Millipore membrane (0.45 μ) and sterile solutions were made for the intracutaneous tests. The same extracts were used for the serological measurement of IgE.

Methods of testing

Skin tests

Prick tests were made on the volar surface of the forearm using separate No. 20 disposable needles for each test. Intracutaneous injections of 0.01–0.02 ml were made

with the highest concentration of the test extract in a number of subjects who gave negative reactions to prick tests with this concentration.

The prick tests were randomly distributed for the total battery of tests. The diameter of the weals was measured 15 min after the test, using concentric rings on a perspex plate. Weals of 1 mm or more in diameter were regarded as positive for the prick test.

Nasal tests

A control test was made by introducing 0.05 ml of Coca's fluid as drops into one nostril. Where the control was negative, tests were made starting with the lowest concentration of extract in Coca's fluid and allowing a period of 20 min for a reaction. If negative, the next concentration was tested in the same way, and finally the top concentration was used. Positive reactions consisted of unequivocal sneezing, itching of the nose, nasal obstruction and rhinorrhoea occurring within 5–15 min of the test. Extracts of *D. farinae* and grass pollen were used for these tests, not enough *D. pteronyssinus* material being available.

Measurement of total and specific IgE in the serum

Blood was taken from all patients at the time of investigation and the serum stored in the deep-freeze until tested.

The total IgE level of the sera was determined with a solid phase sandwich technique (Wide, 1970). Twenty μ l of the serum was mixed with 0.5 ml of a suspension of cellulose coupled anti-IgE-antibodies. IgE in the serum was bound to the solid phase antibodies during incubation for 24 hr. The solid phase was then washed and, in the second step of the reaction, ^{125} I-labelled anti-IgE-antibodies were added. These labelled antibodies reacted with the IgE bound to the antibodies on the solid matrix. The uptake of labelled antibody on the solid phase after incubation for about 24 hr was directly correlated to the amount of IgE in the test solution. The methods used for coupling antibodies to cellulose particles (Wide, 1969) and for the preparation of ^{125} I-labelled immunosorbent purified anti-IgE-antibodies have been described (Wide *et al.*, 1967). A serum sample with a high IgE level was diluted in cord serum and used as a reference standard. This serum had an activity of 40,000 ng of IgE per ml when tested in a competitive inhibition radioimmunoassay using purified myeloma IgE as a reference (Johansson *et al.*, 1968). The IgE level of this standard serum was set to 40,000 arbitrary units (arb.U) per ml. The IgE level of the cord serum was less than 0.5 arb.U/ml.

The radioallergosorbent test (RAST) (Wide *et al.*, 1967) was used to assay specific IgE in the serum to *D. farinae*, *D. pteronyssinus* and mixed grass pollen. Antibodies of the IgE type in serum react with the allergen chemically coupled to an insoluble matrix and the allergen bound IgE is then detected by its ability to bind ^{125} I-labelled antibodies directed against IgE. The tests were performed as previously described (Wide *et al.*, 1967) with modifications (Stenius & Wide, 1969) including the use of 'sorbent units'/ml (SU) to express the strength of the reaction. Five mg of the allergen extracts were coupled to 100 mg of CNBr-activated cellulose particles (Wide, 1969) and 0.5 mg of these conjugates were used for the assay of specific IgE.

The values for total IgE and specific IgE for the three allergens are compared in terms of ranking of their values since the results expressed as units do not measure the same amounts of IgE in the different tests.

A test for similarity between allergenic components in the extracts of *D. farinae* and *D. pteronyssinus* was made by inhibition tests. These were made by pre-incubation of 20 μ l of serum, from a patient hypersensitive to these allergens, with 40 μ g of the extracts for 20 hr before assay with the RAST. The results were compared with those obtained when the pre-incubation was made with grass pollen extract and with no extract added.

Results

Specific IgE values (SU, sorbent units) against D. farinae, D. pteronyssinus and grass pollen

The distribution of specific IgE values is shown in Figs. 1(a), 1(b) and 1(c). These show that there were fewer negative values and higher positive values for *D. pteronyssinus* (Fig. 1b) than for *D. farinae* (Fig. 1a). Thus, there were thirteen negatives for *D. pteronyssinus* and thirty-two for *D. farinae*, and the values for *D. pteronyssinus* were above 125 SU in twenty-eight subjects, compared with six for *D. farinae*.

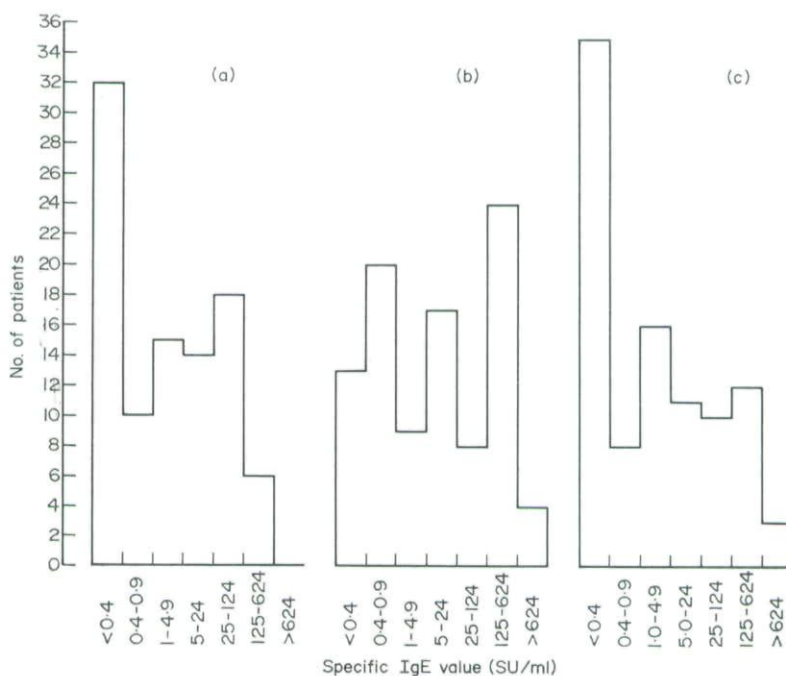


Fig. 1. Distribution of values of (a) *D. farinae* specific IgE; (b) *D. pteronyssinus* specific IgE; (c) grass pollen specific IgE.

The range of values for *D. pteronyssinus* IgE was comparable to that for grass pollen (Fig. 1c) with values over 125 SU in twenty-eight out of eighty-two positives compared with fifteen out of sixty for the grass pollen positives.

Weal sizes to the Dermatophagoides spp. and grass pollen extracts

The distribution of the weal sizes to the three concentrations of each extract is shown in Figs. 2(a), 2(b) and 2(c) and their relative potency in Fig. 3, which shows that the *D. pteronyssinus* at 0.1 mg/ml and grass pollen extract at probably 1.0 mg/ml would be

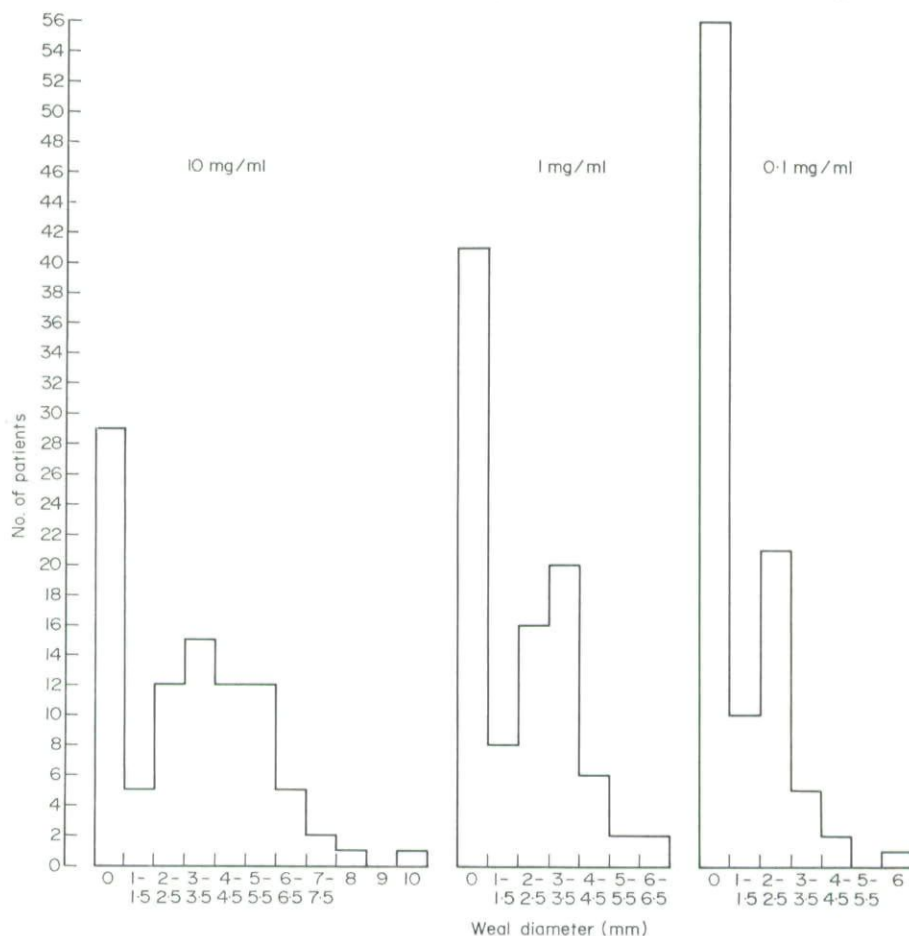


Fig. 2(a). Distribution of weal sizes following prick testing with *D. farinae*: 10 mg/ml, 1 mg/ml, 0.1 mg/ml. One patient not tested at 10 mg/ml.

comparable and that these would be comparable with the 1.0 mg/ml of *D. farinae*. The *D. pteronyssinus* extract is about 10 times more potent than the *D. farinae* extract.

Correlation of specific IgE values and sizes of weals to prick test reactions (ninety-five subjects) (Table 1)

These results show statistically highly significant correlations ($P < 0.001$ to $\ll 0.001$) between the amounts of specific IgE and the sizes of the weals produced by the three concentrations of the corresponding allergens.

Specific IgE, prick test reactions and history

The *D. farinae* results (Table 2) show that in the group with a negative test for specific IgE and in those with < 1 SU the prick test reactions are produced mainly by the highest concentration of 10 mg/ml. In the subjects with more than 1 SU the correlations of the specific IgE and prick test reactions and history is close. This is very marked in those with more than 10 SU, most of whom give prick test reactions to the lowest concentration and a positive history is present in a high proportion.

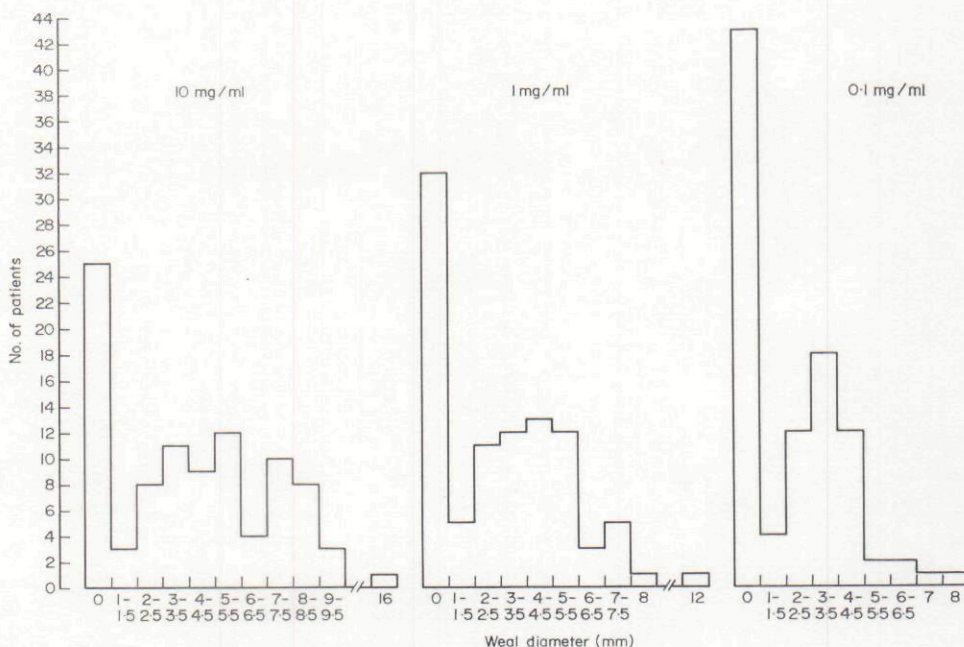


Fig. 2(b). Distribution of weal sizes following prick testing with *D. pteronyssinus*: 10 mg/ml, 1 mg/ml, 0.1 mg/ml. One patient not tested at 10 mg/ml.

The *D. pteronyssinus* results (Table 3) are similar to those for *D. farinae*.

The correlations between specific IgE and prick tests (Table 3) and history becomes more evident in those with up to 10 SU. This is very marked in those above 10 SU, all of whom react to prick tests, and thirty-seven of forty-six have a positive history. Thus very small amounts of specific IgE to *Dermatophagoides* extracts in terms of prick test and history are relevant at values greater than 1 SU and in particular with values of more than 10 SU. In the subjects with negative IgE tests who gave positive prick test reactions, these were elicited mainly by the highest concentration of test extract.

Table 4 shows that grass pollen IgE becomes relevant for prick tests in subjects with 10 SU and that the correlation of prick test and history is very close in those with more than 10 SU. For all of these allergens it is clear that the highest concentration of the test materials used here was necessary for the production of positive prick test reactions in the maximum number of subjects with specific IgE in the serum.

The *D. farinae* extract gave the largest number of subjects (eight) with positive prick test reactions and negative IgE tests, whereas with *D. pteronyssinus* there were three, and with grass pollen five.

Correlation of prick test, history and specific IgE

Tables 5 and 6 show that the presence of a positive history and prick test is almost always accompanied by the presence of specific IgE above 1 SU for the *Dermatophagoides* extracts, whereas in those with a negative history and prick test, specific IgE above 1 SU was seldom found. Positive skin test reactions in those with a negative history were commonly associated with specific IgE, as shown particularly by *D. pteronyssinus* extract. A positive history and negative prick test to the highest concentration

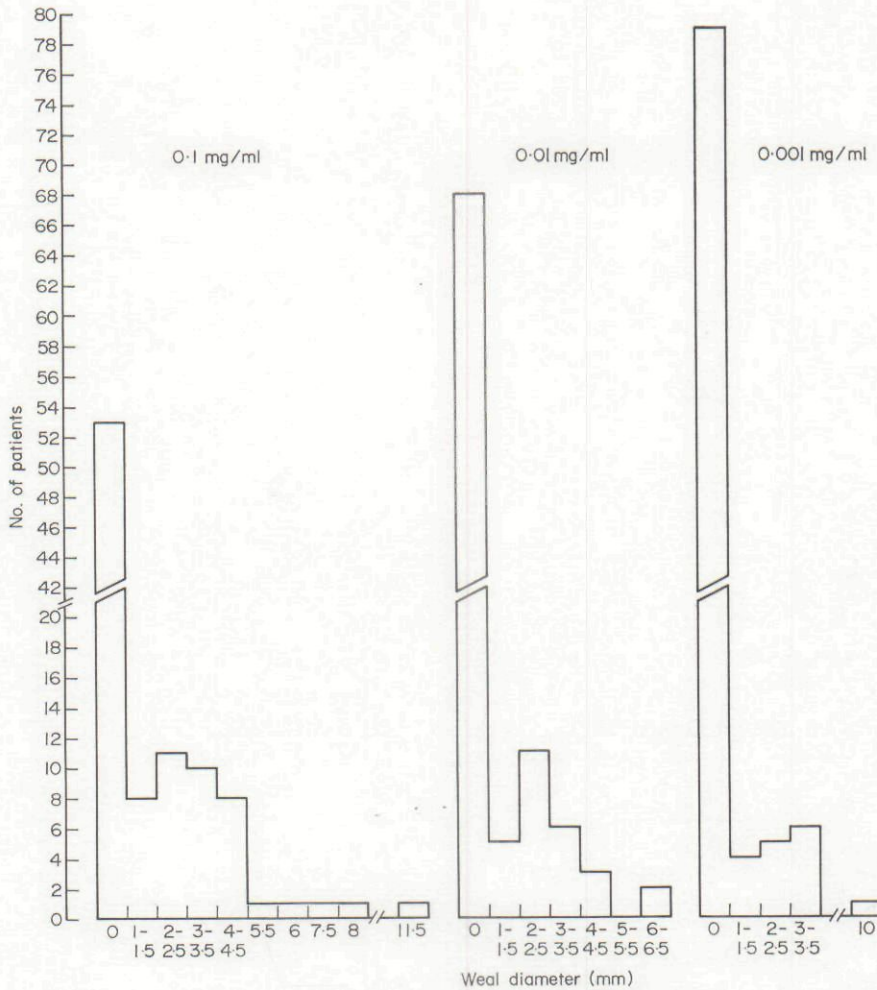


Fig. 2(c). Distribution of weal sizes following prick testing with grass pollen: 0.1 mg/ml, 0.01 mg/ml, 0.001 mg/ml.

was seldom accompanied by specific IgE, though this was not infrequent in relation to prick tests with the lower concentrations. Table 7 shows similar results for the grass pollen extract. There were, however, more subjects with negative skin tests to the top concentration (0.1 mg/ml), a number of whom had IgE levels above 1 SU.

Intracutaneous tests with D. farinae (10 mg/ml) in prick test negative subjects

Table 8 shows that out of fourteen subjects, six gave reactions to intracutaneous tests less than 5 mm in size to *D. farinae* (10 mg/ml) and one of these had specific IgE 0.4 SU to *D. farinae* and one gave a positive reaction to nasal test. Another seven gave reactions of 6-7 mm in size and here too one had 0.5 SU specific IgE and one had a nasal reaction. The intracutaneous test under these circumstances adds little to the prick and nasal test findings, the majority of the subjects with *D. farinae* specific IgE having been identified by the prick tests. The possibility that the positive

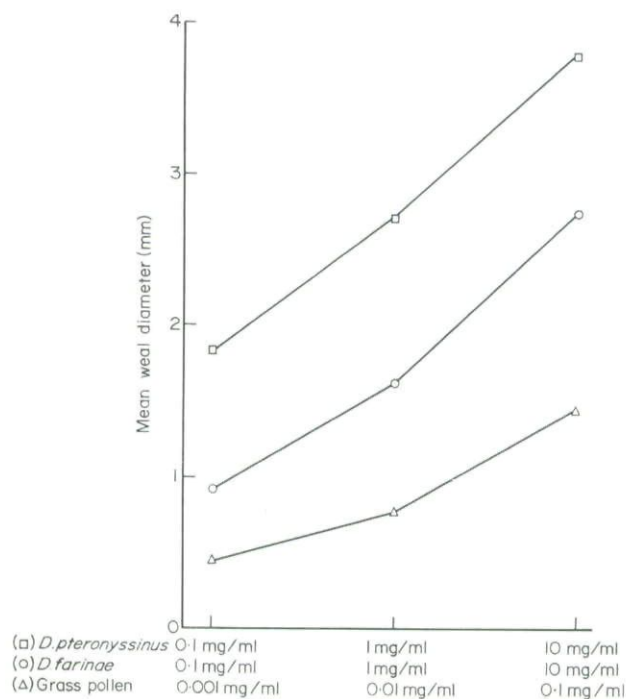


Fig. 3. Comparison of skin test reactions (mean weal diameter) to *D. farinae*, *D. pteronyssinus* and grass pollen extracts.

Table 1. Specific IgE, prick test reactions and weal size in mm (ninety-five subjects)

	<i>D. farinae</i>			<i>D. pteronyssinus</i>			Grass pollen		
	10 mg/ml*	1 mg/ml	0.1 mg/ml	10 mg/ml	1 mg/ml	0.1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
No. positive	69/94	54	39	69/94	64	52	41	27	16
Correlation coefficient of weal size with specific IgE	0.46	0.59	0.62	0.57	0.55	0.65	0.55	0.52	0.58
P value	≤0.001	≤0.001	<0.001	<0.001	≤0.001	<0.001	≤0.001	≤0.001	≤0.001

* Test concentration.

Table 2. Correlation of specific IgE with prick tests and clinical history: *D. farinae*

Specific IgE	No.	Prick test positive			Total		History positive
		10 mg/ml	1 mg/ml	0.1 mg/ml	Positive	Negative	
Negative	32	8	3	1	8	24	10
<1 SU/ml	10	7	2	0	7	3	6
1-10 SU/ml	19	17	15	9	18	1	13
>10 SU/ml	34	33/33	34	29	34	0	27
Total	95	65/94	54	39	67	28	56

Table 3. Correlation of specific IgE with prick tests and clinical history: *D. pteronyssinus*

Specific IgE	No.	Prick test positive			Total		History positive
		10	1	0.1	Positive	Negative	
Negative	13	3	1	0	3	10	3
< 1 SU/ml	20	6	3	1	7	13	10
1-10 SU/ml	16	15	4	6	15	1	7
> 10 SU/ml	46	45/45	45	45	46	0	37
Total	95	67/94	53	52	71	24	57

Table 4. Correlation of specific IgE with prick tests and clinical history: grass pollen

Specific IgE	No.	Prick test positive			Total		History positive
		0.1 mg/ml	0.01 mg/ml	0.001 mg/ml	Positive	Negative	
Negative	35	2	2	2	5	30	9
< 1 SU/ml	8	1	0	0	1	7	2
1-10 SU/ml	22	12	3	0	12	10	8
> 10 SU/ml	30	27	22	16	29	1	24
Total	95	42	27	18	47	48	43

Table 5. *D. farinae*: prick test, specific IgE and history

	No.	IgE -ve	IgE 0.4-0.9 SU/ml	IgE > 1 SU/ml
(A) Prick test with 10 mg/ml				
History + Skin test +	44	2	3	39
- +	20	6	4	10
+ -	12	8	3	1
- -	17	16	-	1*
(B) Prick test with 1 mg/ml				
History + Skin test +	38	-	-	38
- +	14	3	2	9
+ -	18	10	6	2
- -	23	19	2	2
(C) Prick test with 0.1 mg/ml				
History + Skin test +	29	-	-	29
- +	9	1	-	8
+ -	27	10	6	11
- -	28	21	4	3

* Patient reacts at lower concentration.

Table 6. *D. pteronyssinus*: prick test, specific IgE and history

	No.	IgE - ve	IgE 0.4-0.9 SU/ml	IgE > 1 SU/ml
(A) Prick test with 10 mg/ml				
History + Skin test +	47	—	4	43
— +	21	3	2	16
+ —	9	3	6	—
— —	16	7	8	1
(B) Prick test with 1 mg/ml				
History + Skin test +	43	—	2	41
— +	18	1	1	16
+ —	13	3	8	2
— —	19	9	9	1
(C) Prick test with 0.1 mg/ml				
History + Skin test +	38	—	1	37
— +	12	—	—	12
+ —	18	3	9	6
— —	25	10	10	5

intracutaneous tests were related to small amounts of specific IgE not demonstrable by the *D. farinae* extract was examined. It was found that, of the total of eleven skin test reactors without *D. farinae* IgE, eight had low values of specific IgE to *D. pteronyssinus* ranging from 0.4 to 0.9 SU, and it is possible that these account for the positive reactions to the intracutaneous tests with the *D. farinae* extract.

Nasal tests with D. farinae

A total of thirty-eight subjects (Table 9) had nasal tests with the extracts of *D. farinae*, and positive reactions were obtained in nineteen. Of these nineteen reactors, all but

Table 7. Grass pollen: prick test, specific IgE history

	No.	IgE - ve	IgE 0.4-0.9 SU/ml	IgE > 1 SU/ml
(A) Prick test with 0.1 mg/ml				
History + Skin test +	27	—	—	27
— +	14	2	1	11
+ —	16	9	2	5
— —	33	22	4	7
(B) Prick test with 0.01 mg/ml				
History + Skin test +	20	—	—	20
— +	7	2	—	5
+ —	23	9	2	12
— —	40	22	5	13
(C) Prick test with 0.001 mg/ml				
History + Skin test +	11	—	—	11
— +	4	1	—	3
+ —	32	9	2	21
— —	43	23	5	15

Table 8. Intracutaneous tests with *D. farinae* (10 mg/ml): patients with negative prick tests at 0.1, 1.0 and 10 mg/ml

		Size of weals (diameter)			Total
		Negative	3-5 mm	6-7 mm	
No.		1	6	7	14
Specific IgE		0	1	1	2/14
SU/ml			0.4	0.5	
Nasal test	+	0	1	1	2/9
	-	1	3	4	
History	+	1	2	3	6

Table 9. Nasal test with *D. farinae*

	No.	Specific IgE		Prick tests			History of house dust allergy	
		+	-	Positive				
				10 mg/ml	1 mg/ml	0.1 mg/ml		
Total tested	38							
Positive at 0.1 mg/ml, not tested further	4	4	0	4	4	3	0	4
Specific IgE in SU/ml		13.0, 14.0 36.0, 85.0						
Negative at 0.1 mg/ml, positive at 1.0 mg/ml, not tested further	9	8	1	6	6	4	3	8
Specific IgE in SU/ml		0.5, 1.2 3.3, 12.5 12.5, 21.5 27.5, 34.0						
Negative at 0.1 and 1.0 mg/ml, positive at 10 mg/ml	6	5	1	5	5	4	1	5
Specific IgE in SU/ml		0.6, 4.5 13.5, 88.0 160.0						
Negative at all tests	19	5	14	8	4	2	10	2
Specific IgE in SU/ml		0.8, 0.8 1.0, 1.0 1.05						

two had positive specific IgE tests and all but four positive prick tests. Of the nineteen negative reactors, five had very low specific IgE values up to 1.05 SU, and nine gave positive prick tests. There were nasal reactions to the 1.0 mg/ml concentration in three subjects with positive specific IgE who gave negative prick tests, and to the 1 and 10 mg/ml concentration in two subjects with negative IgE tests.

Intracutaneous tests with grass pollen extract

In nine patients who gave negative prick tests to grass pollen extract, intracutaneous tests with the top concentration of 0.1 mg/ml were negative in seven, none of whom had a positive specific IgE test, and positive in two who had specific IgE values of 0.9 and 4.6 SU. It is probable that the latter would have reacted to prick tests with higher concentrations. As with the intracutaneous tests with *D. farinae* in the prick test negative subjects, the reactions to intracutaneous tests with the grass pollen add little to the prick test findings.

Nasal tests with grass pollen

A total of fifteen subjects (Table 10) had nasal tests and seven gave positive reactions. Of these, all but one had positive specific IgE tests and three gave negative prick tests. Of the eight negative reactors to the nasal test, five had specific IgE up to 1.2 SU, and there was 9.5 SU in one.

Comparison of ranking of total IgE (arb.U) values and specific IgE (SU) values

The distribution of total IgE values is shown in Fig. 4. Values of more than 500 arb.

Table 10. Nasal tests with grass pollen

	No.	Specific IgE		Prick tests		
				Positive		
		+	-	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Total tested	15					
Positive at 0.001 mg/ml	0					
Positive at 0.01 mg/ml, not tested further	3	3	0	2	2	1
Specific IgE in SU/ml		0.6*, 46.0 230.0				
Positive at 0.1 mg/ml only	4	3	1†	2	0	0
Specific IgE in SU/ml		0.9, 5.4 48.0				
Negative at all concentrations	8	6	2	1	0	0
Specific IgE in SU/ml		0.5, 0.7 0.9, 1.0 1.2, 9.5				

* Nasal test positive, skin test negative, specific IgE 0.6.

† Nasal test positive, skin test and IgE negative.

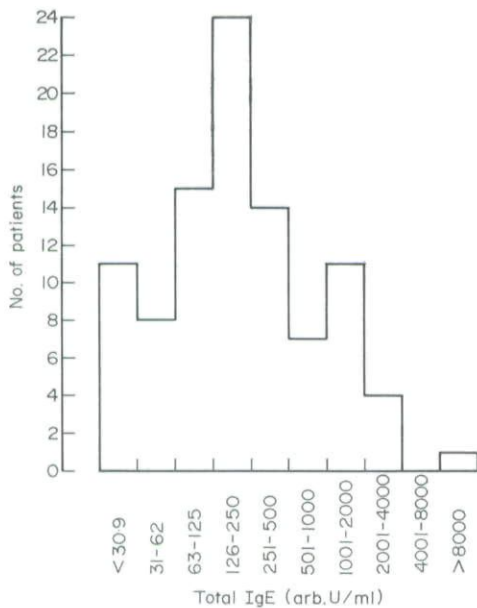


Fig. 4. Distribution of total IgE values.

U/ml were taken to be raised above the level of 'normal' values; many atopic subjects, however, also having values below this.

Total IgE and specific IgE measurements

Correlation of the total IgE values with the specific IgE values (Table 11) shows statistically highly significant correlations with the amounts of specific IgE to *D. farinae*, *D. pteronyssinus* and grass pollen, in terms of ranking of values, though the values themselves are not strictly comparable.

Table 11. Correlation of total IgE with specific IgE to *D. farinae*, *D. pteronyssinus* and grass pollen

Correlation of total IgE with specific IgE to:	Correlation coefficient	P value
<i>D. farinae</i>	0.43	≤ 0.001
<i>D. pteronyssinus</i>	0.42	≤ 0.001
Grass pollen	0.56	≤ 0.001

Total IgE and sizes of weals to specific allergens (Table 12)

A high correlation is shown between total IgE and weal size reactions to all concentrations tested of *D. farinae*, *D. pteronyssinus* and grass pollen.

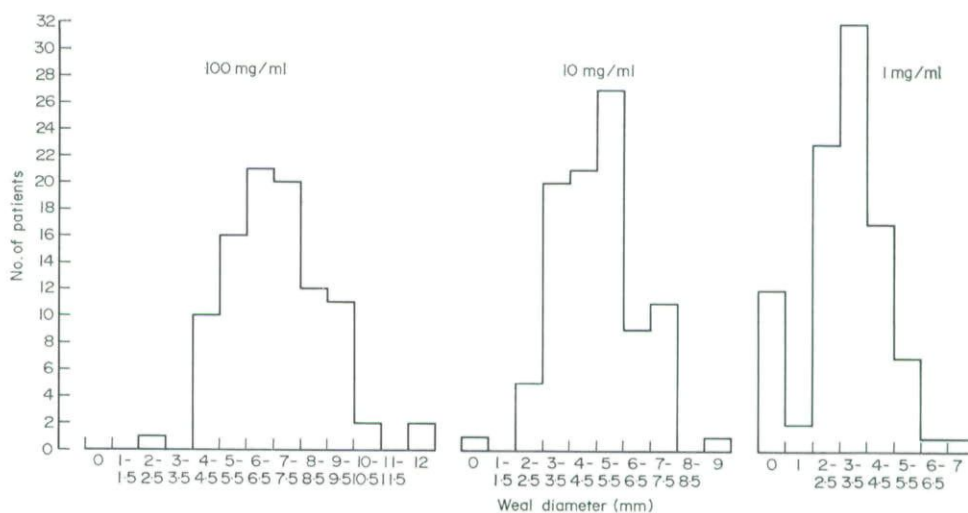
Total IgE and histamine and 48/80 reactions (Table 12)

The distributions of weal sizes to three concentrations of histamine and 48/80 are shown in Figs. 5 and 6. There was no significant correlation of sizes of histamine weals with total IgE.

Table 12. Total IgE and weal size reactions to allergens, histamine, 48/80 and anti-IgE

Total IgE correlated with weal sizes to prick tests with:		Correlation coefficient	P value
<i>D. farinae</i>	10 mg/ml	0.34	<0.001
	1 mg/ml	0.26	0.01
	0.1 mg/ml	0.31	<0.005
<i>D. pteronyssinus</i>	10 mg/ml	0.47	≤0.001
	1 mg/ml	0.46	≤0.001
	0.1 mg/ml	0.36	<0.001
Grass pollen	0.1 mg/ml	0.51	≤0.001
	0.01 mg/ml	0.43	≤0.001
	0.001 mg/ml	0.68	≤0.001
Histamine	100 mg/ml	(0.14)	NS
	10 mg/ml	0.20	0.06
	1 mg/ml	(0.04)	NS
48/80	250 mg/ml	0.40	0.001
	50 mg/ml	(0.10)	NS
	10 mg/ml	(0.18)	NS
Anti-IgE serum diluted	1/2	(0.02)	NS
	1/4	-0.31	<0.005
	1/10	-0.34	<0.001

48/80 is a poor liberator of histamine on prick tests in man, and high concentrations were needed to elicit weals. There was a highly significant correlation between the size of the weals given by prick tests with 48/80 at 250 mg/ml, and the total IgE value.

**Fig. 5.** Distribution of weal sizes following prick testing with histamine: 100 mg/ml, 10 mg/ml, 1 mg/ml.

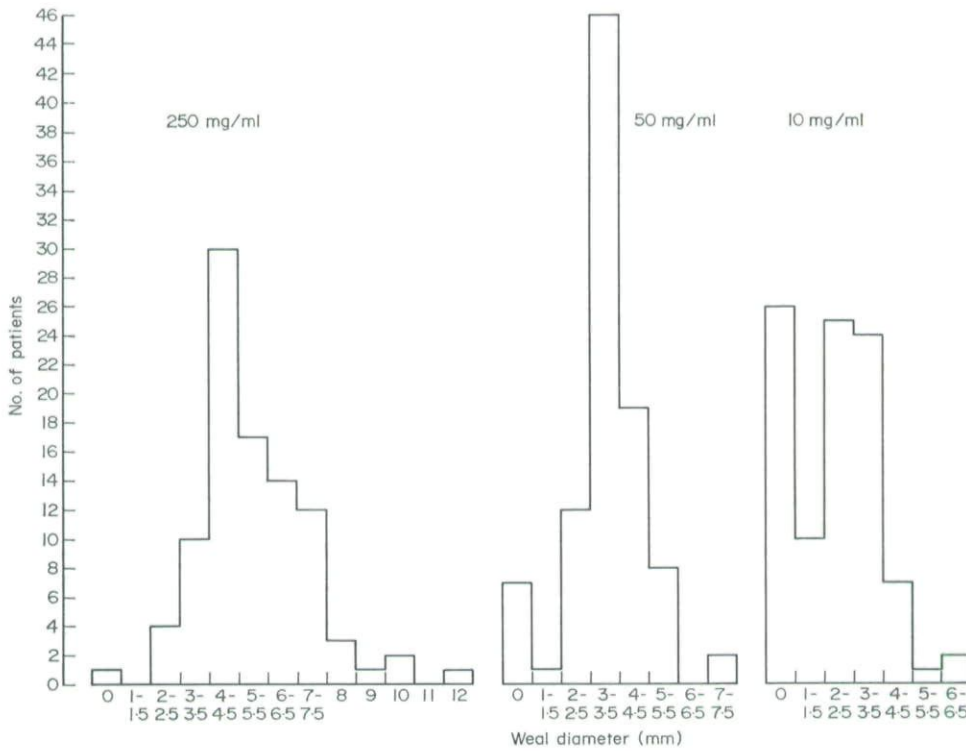


Fig. 6. Distribution of weal sizes following prick testing with 48/80; 250 mg/ml, 50 mg/ml, 10 mg/ml.

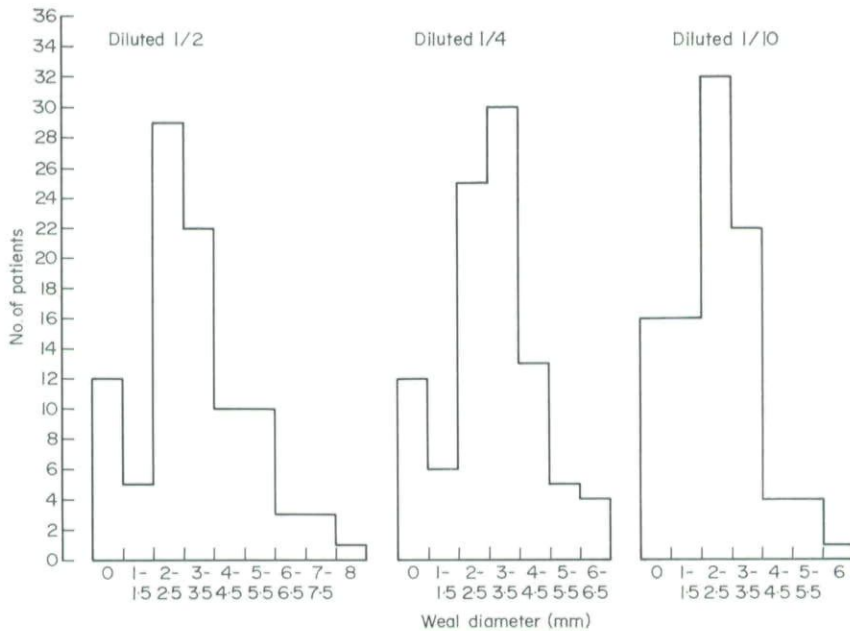


Fig. 7. Distribution of weal sizes following prick testing with anti-IgE diluted 1/2, 1/4, 1/10.

Total IgE and tests with antiserum to IgE (Table 12)

Distribution of weal sizes to three concentrations of anti-IgE serum is shown in Fig. 7.

There was no significant correlation between the total IgE values and the size of the weals produced by the antiserum diluted 1/2, the highest concentration tested. Highly significant negative correlations were, however, obtained with the lower two concentrations, 1/4 and 1/10. Thus, the higher the total IgE, the weaker the reactions to anti-IgE; the opposite of the positive correlation between increasing concentrations of the specific allergens and the size of weals they elicited.

A comparison of weal sizes to histamine, 48/80 and anti-IgE is shown in Fig. 8.

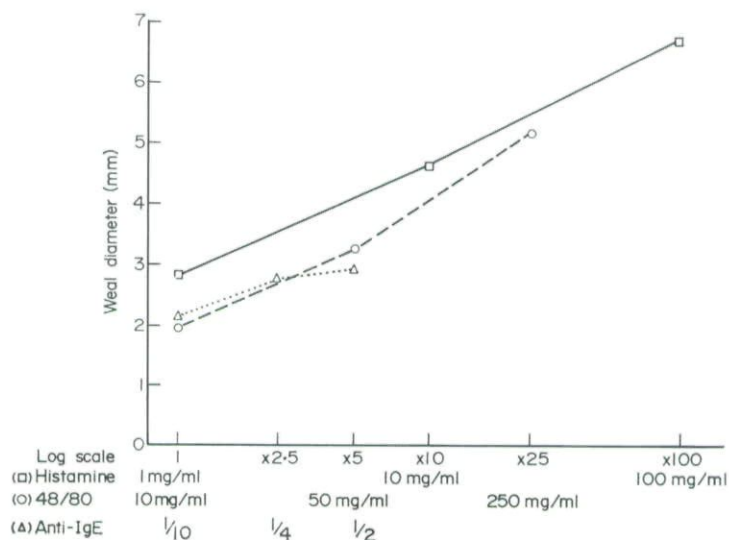


Fig. 8. Mean weal diameters to prick tests with three concentrations of histamine, 48/80 and anti-IgE.

Absorption tests with extracts of D. farinae and D. pteronyssinus

Positive reactions for specific IgE to both types of extract were inhibited by pre-incubation of the serum with either of the two extracts. The capacity to inhibit the reaction was greater for the *D. pteronyssinus* extract than for the *D. farinae* extract. This indicates that the two extracts contain similar allergens which cross-react, and that the *D. pteronyssinus* extract contains at least one cross-reacting component in a higher concentration than in the *D. farinae* extract.

Discussion

The clinical significance of allergen specific IgE, even in small amounts of 1 SU/ml, for *D. pteronyssinus* and *D. farinae*, and of slightly higher amounts of 10 SU/ml for grass pollen, is shown by its statistically, highly significant, correlation with positive reactions to prick tests, nasal tests and a positive clinical history. The strongest of the three test concentrations for each allergen was needed to elicit the maximum number of positive prick test reactions, and it is probable that a higher concentration of grass

pollen extract would have elicited as many positive prick test reactions in subjects with IgE values below 10 SU/ml as was the case with the *Dermatophagoides* extracts. The possibility of excessive numbers of prick test reactions to extracts which are too strong has to be considered in deciding what concentrations should be used. In this investigation, in the patients with negative specific IgE tests, positive prick tests were obtained to the top concentration (10 mg/ml) of *D. farinae* and *D. pteronyssinus*, in eight out of thirty-two and three out of thirteen subjects respectively, and in five out of thirty-five to tests with grass pollen extract. The positive prick test reactions to the *D. farinae* extract in these subjects with negative specific IgE tests may be explained by the fact that far fewer of these subjects gave negative specific IgE tests to *D. pteronyssinus* and, since this has allergens in common with *D. farinae*, the prick test reactions may be attributable to *Dermatophagoides* specific IgE, which the *D. farinae* extract was not sufficiently potent to demonstrate. But, it is also possible that in some cases with negative IgE tests the positive prick test reactions may be mediated by the 'short-term' or 'short-latency' IgG antibody, shown by Parish (1970) to be capable of sensitizing mast cells in man.

Our findings show that high enough concentrations of allergens, which would need to be established for each extract, would give positive prick test reactions in all or almost all subjects with allergen specific IgE in the serum, at least in amounts of 1 SU. An appreciable number of those with less than 1 SU also gave positive prick tests, for example seven out of twenty to *D. pteronyssinus* and seven out of ten to *D. farinae*. Once the basic facts about a particular allergen and its corresponding specific IgE have been established, it seems that prick tests with a range of concentrations could be used to indicate the presence of specific IgE and whether it is present in small, moderate or large amounts. The close relationship between the amount of specific IgE and the prick test reactivity is shown by the statistically very highly significant correlation between the amount of specific IgE and the weal sizes for all three concentrations of all three allergens.

It is common practice for a negative prick test to be followed by an intracutaneous test with the same concentration. In fourteen subjects giving negative prick test reactions to the highest concentration of *D. farinae*, intracutaneous tests with this concentration gave reactions of 6-7 mm diameter in seven of them. Such reactions would be regarded as positive, but only one subject had a low specific IgE to *D. farinae* of 0.5 SU/ml, and one gave a positive nasal test reaction. In six subjects with smaller reactions of 3-5 mm diameter, one had a low specific IgE of 0.4 SU/ml, and one gave a positive nasal test.

The screening off of all the positive prick test reactions before doing intracutaneous tests was very effective in excluding almost all the subjects with specific IgE to *D. farinae*, and under these circumstances the intracutaneous test with *D. farinae* added little to the prick test findings. A possible explanation for these positive intracutaneous tests to *D. farinae* is provided by the presence in all, except three, of low values of specific IgE to *D. pteronyssinus*. For these latter reactions too, it is possible that short-term/latency, IgG antibody may be responsible.

In the subjects giving negative prick test reactions to grass pollen and positive reactions to intracutaneous tests, low values of specific IgE were found, whereas there was none in the subjects giving negative reactions to both tests.

Comparison of the specific IgE values showed that the ranges were comparable for *D. pteronyssinus*, where twenty-eight of eighty-two (34%) of those with specific IgE

had values over 125 SU/ml and for grass pollen, where this was so in fifteen of sixty (25%). There were no *D. farinae* values over this level, showing that *D. pteronyssinus* is the more specific source of allergen in the subjects tested here. The high values for *D. pteronyssinus* emphasize its clinical importance and the importance of house dust allergy.

Although total IgE (arb.U) and specific IgE (SU) values cannot be taken to represent the same amount of IgE, it is possible to compare their ranking. This showed a high correlation of total IgE with the specific IgE values for the different allergens. This may be due to the contribution of the IgE values for the particular allergens described here, or possibly to the presence of multiple sensitivity to some of the large group of common allergens examined in these subjects, in a parallel investigation at present undergoing analysis. The total IgE values also showed a high correlation with the sizes of weals elicited by the allergens tested. This correlation may be evidence only of high specific IgE values which correlate well both with larger reactions and with the total IgE. But an additional possibility is suggested by the finding of a high correlation between the total IgE and sizes of weals to the histamine liberator 48/80. This suggests that in subjects with high total IgE values the mast cells may be more susceptible, as a result of more frequent or severe allergic reactions, to agents capable of causing them to react, whether these be an allergen or a histamine liberator. By contrast with the correlation of total IgE and weal sizes to the allergens and to 48/80, the sizes of weals to prick tests with histamine showed no significant correlation with total IgE. This in turn suggests that individual physiological contributions to the weal size resulting from the allergen/IgE reactions could not have played an important part.

The prick tests with guinea-pig antiserum to IgE, in which the IgE on the cell surface acts as the antigen, and combines with the antibody against it in the anti-IgE serum, showed a highly significant negative correlation, especially with the lower concentrations of the guinea-pig anti-IgE serum. The higher the total IgE values in the serum of the test subjects, and presumably the higher the local IgE values, the weaker the reactions to the anti-IgE. Similar observations were made by Assem & McAllen (1970), who found that human leucocytes, which were highly responsive to the relevant allergen, showed smaller releases of histamine on tests with high concentrations of anti-IgE serum than with low concentrations. It would seem that the ratios of local IgE acting as antigen, to the anti-IgE antibodies used for testing, were important in the liberation of mediators of tissue reactions from the mast cells.

In conclusion, the clinical significance of the *in vitro* assay of specific IgE to *D. pteronyssinus*, *D. farinae* and grass pollen was shown by its statistically highly significant correlation with prick tests, nasal tests and clinical history. It was also found that the prick test is a simple, precise and easily reproducible *in vivo* method for indicating the presence of specific IgE. Preference for the prick test because of its better correlation with the clinical history, rather than the intracutaneous test, has been discussed by Pepys (1968), and this is supported by the present findings. The intracutaneous test properly employed, as by Voorhorst (1970) with serial dilutions of extracts, has its place in investigation, though for most purposes it would seem that the prick test may provide as much information as may be required. Whether the same high correlations will be obtained for other allergens remains to be established, and it is not unlikely that some extracts may be so low in allergen content, or the level of specific IgE may be so low, as to require intracutaneous testing.

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