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RAST, skin and nasal tests and the history in grass pollen allergy

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Summary

A comparison of the RAST (Phadebas®), skin prick test, nasal test and a history of hay fever was made in 141 subjects, eighty-seven being prick test positive, with three concentrations of extracts of Sweet vernal (*Anthoxanthum odoratum*), Cocksfoot (*Dactylis glomerata*), Meadow fescue (*Festuca elatior*), Rye (*Lolium perenne*), Timothy (*Phleum pratense*), Meadow (*Poa pratensis*). In adequate concentration any of the species extracts would have sufficed for skin tests, with Cocksfoot pollen the most potent. Correlations found were: positive RAST/prick test, 98%; positive prick test/RAST, 89%; positive RAST/nasal test, 75%, and the higher the RAST the closer the correlation and the higher the nasal sensitivity; nasal test positive/RAST negative, 17%; positive history/RAST, 91%; positive history/prick test, 92%.

Introduction

Since the introduction of the radioallergosorbent test (RAST) for the identification of allergen specific IgE antibody by Johansson (1967) and Wide, Bennich & Johansson (1967), many comparisons have been made of the RAST with skin and provocation tests (Aas & Johansson, 1971; Havnen *et al.*, 1974; Berg & Johansson, 1974; Norman, Lichtenstein & Ishizaka, 1973). The correlations of the RAST with the other tests have been high in most cases, but it is to be expected that there would be differences arising from variability in the test extracts, and differences in the interpretation of the tests and the criteria used for assessing threshold values for positive reactions. In most studies, the skin and provocation tests have been reported in absolute terms, as positive or negative.

In the present investigation of six grass species pollen extracts, a comparison is made between the RAST, skin and nasal tests made with three concentrations of each, and the history.

Materials and methods

Subjects tested

A total of 141 patients suffering from asthma and/or rhinitis attending the Brompton

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Table 1. Number of subjects tested for each grass species

Grass species	Common name	Number of subjects
Anthoxanthum odoratum	Sweet vernal	141
Dactylis glomerata	Cocksfoot	141
Festuca elatior	Meadow fescue	140
Lolium perenne	Rye	141
Phleum pratense	Timothy	141
Poa pratensis	Meadow	140

Hospital were investigated between February and May 1973, that is before the onset of the grass pollen season. Positive skin, prick test reactions to one or more grass pollen extract were elicited in eighty-seven and negative reactions in fifty-four. None of the patients had received injections of grass pollen extracts. A positive history of 'hay fever' was recorded if seasonal rhinitis was present during the recognized grass pollen season, that is from about mid-June, maximal at the end of the month and persisting into July or early August.

Grass pollen extracts

Skin tests. Extracts of the pollen of the six grass species listed were provided for skin, prick tests and for nasal tests by Beechams in three w/v concentrations of 2·5%, 0·5% and 0·1%. With only two exceptions, all the subjects were tested with all the extracts (see Table 1). The 2·5% w/v concentration of these extracts is the one provided in the ordinary way for prick testing.

The weal sizes were taken in mm as the mean of the sum of the largest diameter and that at right angles to it. Weals larger than 1 mm diameter together with erythema and itching were regarded as positive.

Nasal tests. These were made by instilling a volume of 0·05 ml from a graduated syringe into one side of the nose. A control test was made with Coca's fluid. Tests with the grass pollen extracts were made with increasing concentrations starting with the 0·1% w/v and proceeding, if negative, after intervals of 10–15 min to tests with the 0·5 and then 2·5% w/v strengths. A positive reaction was recorded if the test provoked itching of the nose, sneezing, rhinorrhoea and nasal obstruction.

The skin test positive and negative subjects were each divided into 6 groups. A different grass species pollen extracted was used for testing each of six paired groups of skin test positive and negative reactors.

RAST (Phadebas®) tests

Blood was taken at the time of the skin tests and the sera were kept frozen until the RAST determinations were made at the Pharmacia Service Laboratory in Uppsala. In Phadebas RAST the allergens, covalently coupled to paper discs, react with the specific IgE in the serum. After washing away nonspecific IgE, radioactively labelled antibodies against IgE are added, forming a complex. The more bound radioactivity as measured in a gammacounter, the more specific IgE present in the blood sample. The count-rates are compared directly with the count-rates obtained with four references run in parallel with the unknown samples thus enabling a classification of

Table 2. Prick test reactions to three concentrations of six grass pollen extracts in eighty-seven subjects positive to at least one species

		0·1%		0·5%		2·5%		
	Total tested	Number positive	Mean weal size (mm)	Number positive	Mean weal size (mm)	Number positive	Mean weal size (mm)	Total positive
Sweet vernal	141	38	2·5	15	3·0	30	4·7	83
Cocksfoot	141	62	5·5	7	3·4	16	5·2	85
Meadow fescue	140	54	5·5	17	2·7	16	4·4	87
Rye	141	56	4·8	15	2·3	15	3·5	86
Timothy	141	53	5·1	9	2·6	22	4·9	84
Meadow	140	45	5·3	19	2·1	20	5·3	84

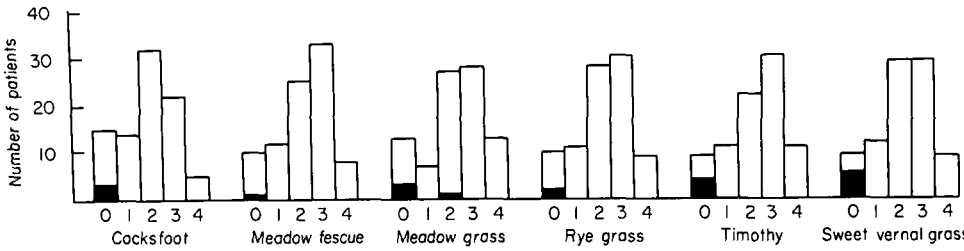

Fig. 1. Frequency of RAST results in the various classes in eighty-seven subjects having positive prick test results to one or more of the six grass species. Shadowed area indicates negative prick test.

the results. Class 0 is considered a negative response and classes 1 to 4 indicate the presence of circulating IgE antibodies in increasing concentrations. In the present study class 1 was regarded a borderline group.

Results

Prick test reactions

Table 2 shows that almost all skin test positive patients gave positive reactions to all 6 grass species pollen extracts. The Cocksfoot pollen extract gave the largest number of positive reactions to the weakest extract concentrations, i.e. was the most potent of those tested, followed by pollen extracts of Meadow fescue, Timothy and Rye. The Sweet Vernal pollen extract was the weakest.

RAST results

For all the six grass species, the majority of the positive RAST responses were found in RAST classes 2 and 3 (Fig. 1). Only cases with positive skin test to one or more of the grass species were considered. Meadow fescue, Meadow, Rye and Timothy showed the highest frequency in class 3, while Sweet Vernal grass had the same number of cases in classes 2 and 3 and Cocksfoot had the greatest number of sera in class 2. The latter grass also had more sera in classes 0 and 1 than any of the other grasses.

Fifty-eight subjects included in the study had a clear cut positive RAST response (classes 2–4) for all the six grasses tested for (Fig. 2), and for eleven others in class 2

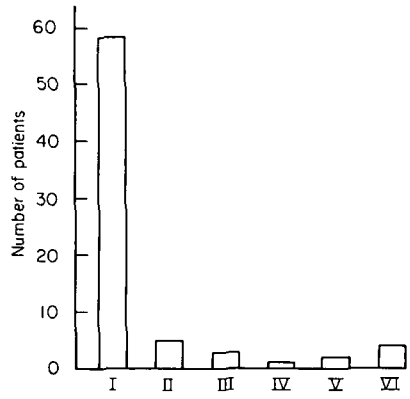

Fig. 2. RAST results from seventy-three patients tested with respect to six grass species.
I: ⩾ class 2 for all species
II: ,, 5 ,, and class 1 for one species
III: ,, 4 ,, ,, 2 ,,
IV: ,, 3 ,, ,, 3 ,,
V: ,, 1 ,, ,, 5 ,,
VI: class 1 for all species.

reaction was obtained for at least one of the grass species. Five subjects were positive to all grasses except Cocksfoot and another two were not positive against Cocksfoot and Timothy. On the other hand, two cases showed a positive response only to Meadow. However, the allergens not giving a clear cut positive response in all cases but one gave a RAST class 1 response, indicating that circulating IgE antibodies were present, but in relatively low concentrations.

Table 3. Patients not giving equivalent RAST results as expressed in RAST classes for the six grass species tested for

Patient	Allergen *Anthoxanthum*	*Dactylis*	*Festuca*	*Lolium*	*Phleum*	*Poa*	Remarks
67	1	0	0	0	0	0	Negative to 5 species
72	0	0	1	0	0	0	
133	1	0	0	1	0	0	Negative to 4 species
51	1	0	1	1	0	0	Negative to 3 species
95	1	0	1	1	0	0	
58	1	0	1	1	0	1	Negative to 2 species
124	1	0	1	1	1	1	Negative to one species
119	2	0	2	2	1	2	
76	1	1	1	1	1	2	Borderline to 5 species
136	1	1	1	1	1	2	
68	1	1	2	2	1	2	Borderline to 3 species
109	2	2	2	2	1	1	Borderline to 2 species
125	2	1	2	2	1	2	
38	2	1	2	2	2	2	Borderline to one species
66	2	1	2	2	2	2	
101	2	1	2	2	2	2	
103	2	1	2	2	2	2	
142	2	1	2	2	2	2	

Comparison of RAST classes for all six grass species. Table 3 shows eighteen of the subjects in whom the RAST results were not definitely positive for all six species.

In ten cases, the RAST result was different for only one of the species. In five of these, it was one class above and in five one below that for the other five grass species. The differences in seven cases were between class 0 and 1 and in the remainder between classes 1 and 2 except for one case in whom four species were class 2, one was class 0 and one class 1.

Comparison between RAST and in vivo *tests*

RAST and skin tests. Skin tests and RAST were performed in a total of 845 instances. Overall there was an agreement of 85% between the two tests (Table 4) with a very high degree of agreement (94%) between negative RAST and negative prick

Table 4. Agreement between skin tests and RAST as expressed in % of number of subjects tested for each allergen. *Figures within parenthesis indicate the number of subjects within each group*

Allergen	Prick test	Agreement with RAST %	*n*	Disagreement with RAST %	*n*	Borderline (class 1 in RAST) %	*n*	Total number of tests	
Anthoxanthum	negative	91·4	(53)	1·7	(1)	6·9	(4)	58	141
	positive	80·7	(67)	4·8	(4)	14·5	(12)	83	
Dactylis	negative	94·6	(53)	0	(0)	5·4	(3)	56	141
	positive	69·4	(59)	14·1	(12)	16·5	(14)	85	
Festuca	negative	94·3	(50)	0	(0)	5·7	(3)	53	140
	positive	75·9	(66)	10·3	(9)	13·8	(12)	87	
Lolium	negative	92·9	(52)	0	(0)	7.1	(4)	56	142
	positive	77·9	(67)	9·3	(8)	12·8	(11)	86	
Phleum	negative	98·2	(56)	0	(0)	1·8	(1)	57	141
	positive	75·0	(63)	11·9	(10)	13·1	(11)	84	
Poa	negative	94·6	(53)	1·8	(1)	3·6	(2)	56	140
	positive	79·8	(67)	11·9	(10)	8·3	(7)	84	
Average all species	negative	94·3	(317)	0·6	(2)	5·1	(17)	336	
	positive	76·5	(389)	10·4	(53)	13·2	(67)	509	
	total	85·4	(706)	5·5	(55)	9·1	(84)	845	

Positive in RAST: classes 2–4.
Negative in RAST: class 0.

tests. In 5·5% there was a disagreement, most of these cases being positive in the prick test but negative in RAST. However, in the majority of these instances, the skin reactions were elicited only by the strongest extract concentration and the skin reaction was weakly or moderately positive.

In only two instances, the RAST was positive but the prick test negative. In both cases RAST was positive for more than one of the grass allergens but in one of the cases the skin test was positive for the other five grass species.

RAST class 1 was obtained in 9%, most in association with positive skin tests. Most of the cases with RAST class 1 and negative skin tests were found in the lower part of class 1 count range (Fig. 3). On the other hand, the cases with positive prick tests were distributed all over the class range. A division of the class would not lead to better discrimination between skin test positive and skin test negative cases.

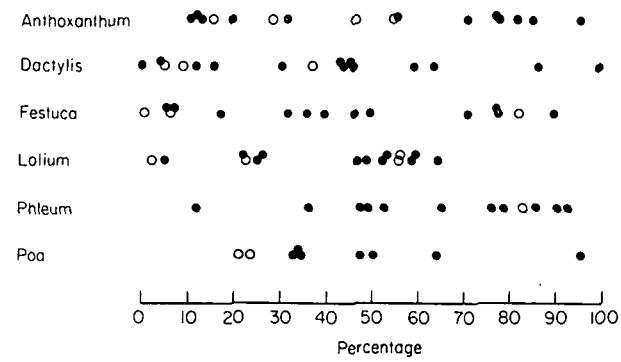

Fig. 3. Localization of cases within RAST class 1 in the RAST test. Counts above the lower class border are expressed as a percentage of the count range for the class. ○, Skin test negative; ●, skin test positive.

RAST and provocation tests. The degree of agreement between nasal provocation test results and RAST on the one hand and provocation tests and prick tests on the other was of the same order, 76 and 79% respectively, of a total number of 136 tested subjects (Table 5). Fifteen of the RAST results were found in RAST class 1, but a direct comparison is not possible as no borderline group was recorded for the prick test results. From Fig. 4 however, it is evident that negative nasal provocation test results were, to a fairly large extent, combined with positive skin test and RAST results and also that the provocation was sometimes positive despite negative skin test and RAST. There were two cases where the two *in vivo* tests were positive but RAST was negative. On the other hand, no case appeared where both *in vivo* tests were negative and RAST was positive.

Correlation of RAST, prick and nasal tests and history

In order to correlate the RAST, prick and nasal tests with the history, the findings in the six pairs of groups of skin test positive and negative subjects, each pair of which

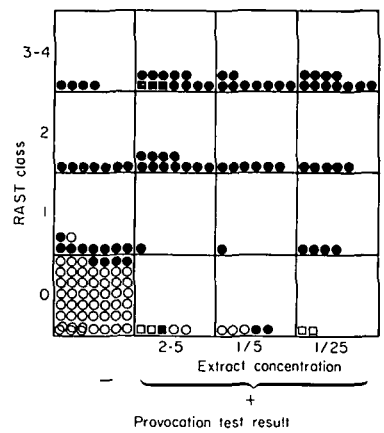

Fig. 4. Comparison between nasal provocation tests, prick tests and RAST. □ and ○, prick test negative; ■ and ●, prick test positive; □ and ■, provocation test result recorded as ±.

Table 5. Agreement between nasal provocation tests and prick tests and RAST respectively as expressed in % of number of subjects in each group. Figures within parenthesis indicate the number of subjects. Provocation test results recorded as ± are excluded from the calculations

Nasal provocation test	Agreement with RAST	Agreement with prick test	Disagreement with RAST	Disagreement with prick test	Borderline (class 1 in RAST)	Total number of tests
Negative	72·2 (52)	68·1 (49)	15·3 (11)	31·9 (23)	12·5 (9)	72
Positive	79·7 (51)	92·2 (59)	10·9 (7)	7·8 (5)	9·4 (6)	64
Average	75·7 (103)	79·4 (108)	13·2 (18)	20·6 (28)	11·0 (15)	136

Positive in RAST; classes 2–4, negative in RAST; class 0.

Table 6. Pollen tests and history

Positive no.		Positive no. (%) Prick test	RAST	Nasal test	Seasonal history
Prick test	87		77 (89%)	61 (70%)	58 (67%)
RAST	79	77 (98%)		59 (75%)	57 (72%)
Nasal test	71	61 (86%)	59 (83%)		49 (69%)
Seasonal history	63	58 (92%)	57 (91%)	49 (78%)	

had nasal tests with a different one of the six grass species pollen extracts, were combined and the totals are given in Tables 6, 7, 8 and 9. These show comparisons of the following: all the tests are positive; all are negative; positive tests with others negative, and negative tests with others positive.

Thus, Table 6 shows close correlations of positive prick and RAST tests with nasal tests and history. Positive nasal tests were elicited in 70–78% of those with positive prick tests and history.

Correlations of RAST and prick tests with the history showed that in those with a positive history there was a close correlation with the prick test (92%) and with the RAST (91%), but less so with the nasal test (78%). The correlations between positive RAST and prick tests with the history were less close, the RAST (72%) showing a better correlation however than the prick test (67%).

Table 7. Pollen tests and history

Negative no.		Negative no. (%) Prick test	RAST	Nasal test	Seasonal history
Prick test	54		52 (96%)	46 (85%)	49 (91%)
RAST	62	52 (84%)		50 (81%)	56 (90%)
Nasal test	70	46 (66%)	50 (71%)		57 (81%)
Seasonal history	78	49 (63%)	51 (65%)	56 (72%)	

Table 7 shows that where all the tests and history were negative, close overall correlations were found between negative prick (91%), RAST (90%) and nasal test (81%) and the history. In those with a negative seasonal history, however, the correlations with negative RAST, prick and nasal tests were lower, ranging from 63 to 72%.

Table 8 shows that positive prick, RAST and nasal tests were associated with a negative history in 28–33%. Positive seasonal histories, however, were associated with lower incidences of negative prick and RAST test, 8 and 10% respectively.

Table 8. Pollen tests and history

		Negative no. (%)			
Postive no.		Prick test	RAST	Nasal test	Seasonal history
Prick test	87		10 (11%)	26 (30%)	29 (33%)
RAST	79	2 (3%)		20 (25%)	22 (28%)
Nasal test	71	9 (13%)	12 (17%)		22 (31%)
Seasonal history	63	5 (8%)	6 (10%)	14 (22%)	

Table 9. Pollen tests and history

		Positive no. (%)			
Negative no.		Prick test	RAST	Nasal test	Seasonal history
Prick test	54		2 (4%)	8 (15%)	5 (9%)
RAST	62	10 (16%)		12 (20%)	6 (10%)
Nasal test	70	25 (36%)	20 (29%)		13 (19%)
Seasonal history	78	29 (37%)	27 (35%)	22 (28%)	

Table 9 shows that in those with negative prick test, RAST and nasal tests, 9, 10 and 19% respectively had a positive history. Negative histories were associated with positive prick, RAST and nasal tests in 37, 35 and 28% respectively.

Positive nasal tests and RAST class. Table 10 shows that in those with positive nasal test and negative RAST, the majority had low degrees of nasal sensitivity, requiring higher concentrations to elicit reactions. In those with positive nasal tests and positive RAST, the results show that even in the group with RAST class 1, nasal sensitivity was high and that high degrees of nasal sensitivity were present in the other RAST classes, though with little direct relationship to the RAST classes.

History of hay fever and prick test reactions. A positive history of hay fever was present, in two out of eighteen subjects with low degrees of sensitivity who reacted only to the strongest (2·5% w/v) extract; in three out of five reacting to the 0·5 % w/v and in forty-seven out of sixty-three reacting to the 0·1% extracts. In the latter group a positive history was obtained in three out of eight of those giving the smallest weals

Table 10. Degree of nasal sensitivity inversely proportional to test concentration

RAST	No.	Nasal test positive	Low (2·5% w/v)	Moderate (0·5% w/v)	High (0·1% w/v)
Negative	62	12	5	5	2
Class 1	15	6	1	1	4
Class 2	32	24	13	5	6
Class 3	24	22	10	3	9
Class 4	10	9	2	6	1

1 mm in diameter (approximately); in seven out of ten of those with weals 2 mm in diameter; in seventeen out of nineteen with weals 3–5 mm in diameter and in twenty out of twenty-six cases with weals 6 mm in diameter or more.

History of hay fever and RAST reactions. The subjects were classified into a RAST class where more than one half of the RAST values to the six pollens fell into that class. A positive history of hay fever was given in two out of ten of those giving class 0 reactions; in three out of ten of the class 1 group; in ten out of twenty-seven of the class 2 group; in twenty-five out of thirty-one of the class 3, and in eight out of nine of the class 4 group.

Family history

In Table 11 an analysis is made of the relationship of the different groups of RAST, nasal and prick test reactions to the history of asthma, hay fever and infantile eczema in the total numbers of first degree relatives in each group. This shows that in the subjects giving positive nasal reactions and negative prick and RAST tests, there is a

Table 11. Family history of 'atopic' disease

				First degree relatives		
		No. of patients	Total	Asthma	Hay fever	Infantile eczema
NT PT RAST	+ve −ve −ve	9	52	5	7	5
NT RAST	−ve +ve	20	102	3	6	1
NT PT	−ve +ve	25	135	8	8	2
NT PT RAST	−ve −ve −ve	39	187	15	19	3
NT PT RAST	+ve +ve +ve	55	221	11	27	8

Nasal test, NT; prick test, PT.

significantly higher incidence of infantile eczema ($P<0{\cdot}05$) but not of asthma and hay fever than in the groups giving negative nasal tests, whether or not they were positive or negative to the prick test or the RAST. There was no significant difference between the nasal test positive and prick test and RAST negative group compared with the group who gave positive reactions to nasal, prick and RAST tests. It would seem that nasal test reactivity may have some association with the presence of infantile eczema in the family.

Discussion

The RAST, prick and provocation tests in the present study showed close correlations as have been reported by many authors, and show them, furthermore, for a panel of pollen extracts from six different common grass pollen species, namely, Sweet Vernal, Cocksfoot, Meadow fescue, Rye, Timothy and Meadow. A number of questions in the interpretation of the prick, RAST and nasal tests and of the history have to be considered in assessing their overall relationships and in attempting to explain apparent inconsistencies.

The presence of common allergens in the pollens of the six different grass species is suggested by the production, in almost all the sensitive subjects, of positive skin test reactions and, in most of them, positive RAST values of similar class to all six extracts. Milner & Tees (1972) have also reported common reactions to skin tests with twelve different pollen extracts with specific reactions in 19%. Cross reactivity has also been demonstrated between species within classes of aeroallergens other than grasses, such as ragweeds and to some extent trees (Bernstein *et al.*, 1974). The Cocksfoot extract was the most potent for prick test in the present study, though it gave more RAST class 0 and 1 than the others, but it seems as if almost any one of the extracts would suffice, if in adequate concentration, for demonstrating or confirming the presence of grass pollen sensitivity. The present results cannot necessarily be taken as an indication of which grass species extract could be used for testing if only one were desired. A comparison of extracts of Cocksfoot pollen as provided commercially by three different manufacturers, the same extracts being used for skin and RAST tests, showed that the ranking of the potency of the extracts by the skin prick test corresponded with that of RAST based allergen assays. The biological potency of the preparations, the strongest being unity, showed the potency of the other two to be 0·28 and 0·18% respectively (Pepys *et al.*, 1975). In the present study different extracts were used for the skin and nasal tests, prepared by Beechams, and for the RAST, prepared by Pharmacia. It is desirable that in further studies the same extracts should be used for all tests.

Positive RAST correlated well with the prick tests with only few exceptions. Positive prick tests and negative RAST were found however in about 11% of the tests. The decision used here for a definitely positive RAST was based on arbitrary class greater than 1 and derived from values obtained with a birch pollen IgE antibody system. Consideration of the count levels of individual sera and comparison with background activities might have given still better correlation between the two test systems. Among the possible explanations for the disagreement between the prick test and the RAST may be sensitization of mast cells by amounts of IgE too low to be demonstrated in the RAST, differences in the allergen extracts as measured by the two tests, or the participations of other types of antibody, such as the short-term mast cell sensitizing IgG antibody described by Parish (1970).

The use of provocation tests to try and establish the possible clinical relevance of

allergens giving positive skin or RAST tests has shown good correlations (Stenius *et al.*, 1971), but they also have their limitations and apparent inconsistencies. Provocation tests may perhaps provide evidence of local sensitization which does not correspond with evidence of systemic sensitization (McAllen *et al.*, 1970). In the present study, subjects giving class 1 RAST had only limited evidence of nasal sensitivity. Those with RAST class 2 or more showed a high incidence of positive nasal reactions with a tendency to high degrees of nasal sensitivity. One of the limitations of provocation tests is the fact that positive reactions are most likely to be elicited in the more highly sensitivity subjects in response to the concentrations which are acceptable clinically. Thus, some of the 25–30% positive RAST reactors who gave negative nasal tests might have reacted to clinically unacceptable higher test concentrations.

Positive nasal tests in subjects with negative prick and RAST tests were elicited with low as well as high concentrations of extract and would appear to be specific. It is possible here, as with the skin test, that there may be local IgE sensitization in the absence of demonstrable IgE antibody. Donovan *et al.* (1970) have reported higher local concentrations of IgE in nasal polyps than in the serum, indicating local production. Alternatively, the possibility that specific IgE mast cell sensitizing antibody is present has also to be considered.

The use of a clinical history of seasonal symptoms for correlation with the RAST, skin and nasal tests also present problems. A history of symptoms corresponding to the grass pollen season is highly indicative of grass pollen sensitivity, though not absolute proof. A positive seasonal history correlated strongly with the prick test (92%) and RAST (91%), but less so with the nasal test (78%). Possible reasons for negative nasal tests in association with positive RAST and prick tests have been discussed above. Correlations of positive prick test and RAST with a seasonal history were lower, 67 and 72% respectively. Grass pollen specific IgE antibody may, it seems, be present without obvious clinical relevance, though it is probable that, in some cases, patients with perennial symptoms might not have recognized seasonal variations. The clinical significance of skin and RAST tests and, to a lesser extent, of nasal tests is readily evident in more sensitive subjects, and as far as the history is concerned, in those with a definite history, namely in those who do not as a rule pose diagnostic problems at least in relation to grass pollen allergy. The clinical significance of weaker prick test and RAST reactions is however not clear. Prospective studies particularly in children, would be of interest to see whether these values increase with time, and whether clinical manifestations appear 'pari passu'.

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