

From Medical Department TA, Division of Clinical Immunology and Laboratory of Clinical Allergy, Rigshospitalet; and The Protein Laboratory, University of Copenhagen, Denmark.

ALLERGENS IN TIMOTHY POLLEN
IDENTIFIED BY
CROSSED-RADIO-IMMUNO-ELECTROPHORESIS
(CRIE)

By

BENT WEEKE, HENNING LØWENSTEIN & LARS NIELSEN

A timothy pollen (*Phleum pratense*) extract has been shown by examination with crossed immunoelectrophoresis to contain 28 antigens (8). Amongst these antigens are allergens which are capable of stimulating the production of IgE in man, and of reacting specifically with timothy specific IgE in patients with hay fever.

From a clinical point of view, the extracts used in the diagnosis of allergy and immunotherapy should contain the allergens in as pure a form as possible. All other antigens present may, due to antigenic competition, interfere with the precision of the diagnosis and with the immune response during immunotherapy.

By means of crossed-radio-immuno-electrophoresis (CRIE) it is possible to identify those timothy antigens which are able to bind timothy specific IgE from hay fever patients' sera (12). Following identification, the allergens may be characterized and quantified by CIE (10). The CRIE has to be carried out before characterization (8) and purification (6) of the allergens in the extracts can be performed.

In this communication we report an examination by CRIE of a number of timothy allergic patient sera, and compare the

results with those obtained using other procedures for the diagnosis of allergy.

MATERIALS AND METHODS

Timothy allergen extract (St.T 73) diluted 1:10 (w/v) was produced as published elsewhere (8).

Rabbit anti-timothy (KaSt.T 73) was produced by immunizing six rabbits with 0.1 ml of extract together with 0.1 ml of Freund's incomplete adjuvans (5). The gamma globulins from the rabbit sera were isolated by salting out, chromatography and dialysis (5).

Rabbit anti-human IgE (ϵ -chain specific, Behringwerke, West Germany) was used for radiolabelling. After isolation of the rabbit gamma globulins and immunoabsorption of specific anti IgE with polyclonal human IgE, ^{125}I was introduced by electrolytic iodination (kindly performed by Ulf Jacobsen, The Isotope Laboratory, Risø, Roskilde, Denmark). An average of 0.5 atoms of ^{125}I was incorporated per antibody molecule, giving a specific activity of approximately 2.5 mCi mg^{-1} anti IgE.

^{125}I -anti IgE (Phadebas RAST, Uppsala, Sweden), with 1.25 mCi mg^{-1} anti IgE was used alternatively, with comparable results.

Crossed Immunoelectrophoresis (CIE) in microtechnique was carried out on 5 cm $\times$ 5 cm photo glass plates (10). 10 μl of St.T 73 was examined against 300 μl of KaSt.T 73. After electrophoresis the gel was pressed and dried in cold air to a fine film on the glass plate and stored at 4° C in a closed plastic box (5 cm $\times$ 7 cm $\times$ 1 cm) for later use in CRIE.

Crossed-Radio-Immunoelectrophoresis (CRIE) has been described elsewhere. Very briefly the procedure, which was performed at room temperature (18–24° C) was:

1. Incubation with patient serum. 7 ml of 1/15 M phosphate buffer (pH 7.5) and 0.4 ml of Serum added to the plastic box was sufficient to cover the gel film on the glass plate.
2. After being left to react overnight the non-bound serum proteins were removed from the gel by washing it with 10–20 ml of 0.1 M sodium chloride at least 4 times for 10 min periods.
3. ^{125}I -anti IgE in 7 ml of incubation buffer (phosphate buffer 0.05 M pH 7.5 containing 0.3 per cent bovine serum albumin, 0.9 per cent sodium chloride, 0.1 per cent sodium azide and 0.1 per cent EDTA) corresponding to 0.35 μCi and giving rise in our gamma counter to approximately 200,000 cpm was added to the gel and allowed to react for at least 1 d.
4. Non-bound ^{125}I -anti IgE was removed and stored for later experiments.
5. After 4 $\times$ 10 min washings with 10–20 ml of saline and finally one with distilled water, the gel in the glass plate was dried in hot air and placed

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Data on 30 RAST-timothy Positive Sera Arr.

No.	Sex	Age years	RAST-timothy		Skin test index	IgE U _{1/2} /n
			SU	class		
1	M	29	14	2	0.50	055
2	M	29	19	2	1.17	146
3	M	27	20	3	1.47	081
4	M	30	21	3	1.10	067
5	M	28	21	3	1.78	215
6	M	21	24	3	1.11	070
7	M	22	24	3	0.49	034
8	M	20	32	3	1.18	134
9	F	17	44	3	0.56	113
10	M	29	48	3	0.45	110
11	F	18	54	3	0.60	060
12	M	30	56	3	1.00	105
13	F	28	63	3	0.64	590
14	F	41	75	3	—	114
15	M	32	80	3	1.11	197
16	F	28	86	3	1.10	967
17	M	15	120	4	2.88	444
18	M	32	125	4	1.11	229
19	M	13	142	4	1.00	336
20	F	29	143	4	1.00	200
21	F	21	143	4	1.62	250
22	F	23	167	4	1.30	310
23	M	38	172	4	1.20	210
24	M	36	200	4	1.20	304
25	F	18	205	4	2.05	483
26	M	21	212	4	0.91	681
27	M	15	220	4	2.66	630
28	M	21	230	4	1.67	1094
29	F	19	240	4	1.09	2084
30	M	20	275	4	1.56	3238

Age and sex of the patients are indicated, together with the intracutaneous reactivity to an extract of 3 grasses containing timothy pollen. Total IgE and the specific IgE binding to the individual timothy antigens demonstrated in CRIE are also presented. The IgE-¹²⁵I-anti IgE binding to the timothy antigens in CRIE were semiquantified by means of a point system obtained by multi-

ending Order According to RAST-positivity.

IgE binding to timothy antigen No.									Points	Immuno- therapy (years)
12 (27)	13 (08)	15 (11)	19 (30)	22 (16)	24 (10)	25 (17)	26 (16)	27/28 (5)		
	I				I	I		I	40	3
	I				I	I		I	40	4
	I				I	I		I	40	I
	I					I		I	30	0
	I		I		I	I		I	70	2
	I				I	I		I	40	5
	I				I			I	23	4
	I					I		I	30	I
	I				I			I	23	0
	I				I			I	23	0
	I		I			3	2	I	126	I
	I			I		2		I	63	3
	I		I		I	2		I	87	2
	I					2		I	47	2
	I		I	I	I	2		I	103	4
	I		I			2		I	77	3
	I	I			I			I	34	0
I			I	I		2		I	112	3
	I					4		I	81	0
	I		I			3		I	94	2
	I	I	I	I	I	4		I	148	I
	I			2	I	2		I	89	0
	2	I		I		4		2	121	2
	I		I	I	I	2		I	103	3
	I		I	2	2	3		I	167	2
	2	I		2		4		2	137	I
	I		I		I	4		I	121	0
2	I		I	2	I	2		I	211	I
2	2			3	I	4		2	206	I
2	I	I	3	I	I	4		I	262	I

plication of the grades of radiostaining (grade 4 represented radiostaining of the X-ray film after 1 d of exposure, grade 1 a faint radiostaining after 1 week of exposure) and the length of the stained line (expressed in arbitrary units in parentheses). The number of years the patients had received preseasonal immunotherapy is indicated too.

on an X-ray film in a light-proof box. The exposure time was varied from 1 d to several weeks.

6. Finally the gel on the glass plate was stained for proteins with Coomassie Brilliant Blue.

Reading the autoradiograph. By varying the exposure time of the X-ray film, and including a known serum in all experiments, it was possible to grade the activity bound to a precipitate in grades from 1-4, i.e. grade 4 corresponding to radio-staining after 1 d, and grade 1 corresponding to faint staining after 7 d exposure. This activity grade was multiplied by the length of the precipitation line (the figure in parentheses in Table 1), giving a total number of 'points' for the total bound activity. By comparing the autoradiograph and the protein stained CIE, the activities associated with the individual antigens were estimated.

RIST (Radio-immuno-sorbent-test). Phadebas IgE (Pharmacia, Uppsala, Sweden) was used for determination of total IgE in the analyzed sera. The concentrations are expressed in U/ml by comparison with a WHO standard serum (9). One U corresponds approximately to 2 ng (2). The relative reproducibility of the determinations was about 5 per cent. We used a value of 30-500 U/ml (11) as a normal 95 per cent range in adults.

RAST (Radio-allergo-sorbent-test) was performed with St.T 73 coupled to activated filter paper discs according to Cheska et al. (4). The results were expressed in sorbent units (SU), by using the serum from patient no. 29 in Table 1 (HD), diluted 1 + 15 (v/v) with incubation buffer as a 100 SU reference. After dilution of this reference serum 1:1, 1:5, 1:25 and 1:50 the activity in cpm (y) and concentration of specific IgE in sorbent units (SU) (x) presented a correlation of the first degree in a double log scale. Specific IgE in unknown sera was expressed in SU by interpolation from the cpm, or expressed in allergy grades 1-4 according to the dilution used. Our grading system corresponds with that for Pharmacia Phadebas RAST reference (birch allergen and a birch allergic reference serum).

Patients followed in the "out-patient clinic" for adults, Rigshospitalet, were selected. The sera were taken in the spring before the season in 1971, 1972 and 1973. During the autumn 1973 sera were tested by RAST, and the timothy positive sera were selected. Seven patients were untreated at the time the sera were taken, whereas the remainder had received Allpyral 5 grass mix (Dome Laboratories, England) 10 times as a weekly injection before the pollen season from 1-5 years before (see Table 1). For comparison purposes, the sera of 5 patients with high IgE levels and allergies to allergens other than timothy were included, together with sera from 5 normal persons with no history of allergy. Data are presented in Tables 1 and 2.

Intracutaneous test was carried out on the volar side of the antebrachium, using a histamine solution (0.1 mg/ml) as a reference (Table 1).

An 1:1000 extract (Allergologisk Laboratorium A/S, Copenhagen) containing

TABLE 2
Sera from 5 Normal Persons without Any History of Allergy, and from 5 Patients with Allergy and Increased IgE without Allergy to Timothy Pollen were Included for Comparison.

No.	Sex	Age years	RAST-timothy SU	Skin test index	IgE U/ml	Comments
1	M	37	1	-	166	No history of allergy.
2	F	26	1	-	35	" "
3	F	24	1	-	105	" "
4	M	35	2	-	140	" "
5	M	50	2	-	66	" "
6	F	71	1	<0.25	1602	No allergy demonstrated.
7	F	29	2	<0.25	1260	Allergy to dust and feathers.
8	M	36	2	<0.25	1060	Allergy to dust, artemisia vulg., chrysanthem.
9	F	15	7	<0.25	2136	Allergy to animal dander.
10	M	28	7	<0.25	1300	Allergy to dust and animal dander.

None of these sera demonstrated IgE binding to the timothy antigens in CRIE under the experimental conditions used in Table 1. The intracutaneous test was not carried out in the normals, and was negative in the rest of the patients.

pollens of 3 grasses (*Avena elatior*, *Dactylis glomerata* and *Phleum pratense*) was used for skin testing. The area of the urticaria element was determined by multiplying the mutually perpendicular diameters (in mm) and expressed as

$$\frac{\text{I.C. grass mm} \times \text{mm}}{\text{I.C. histamine mm} \times \text{mm}} \text{ in a skin test index.}$$

According to the Scandinavian Allergy Standard (1), the index 2.00 corresponds to a 4+ reaction, 1.00 to a 3+ reaction, 0.50 to a 2+ reaction, and 0.25 to a 1+ reaction.

RESULTS

RAST-timothy positive sera from 30 patients with grass pollen allergy were examined (Table 1), and the results were compared with those for 10 RAST-timothy negative sera (Table 2).

IgE Binding to Timothy Antigens in RAST and CRIE

Fig. 1 shows the CIE pattern of timothy antigens together with the autoradiograph of the pattern after addition of a serum (patient no. 29, Table 1) containing timothy specific IgE, and labelling of the bound IgE by means of ^{125}I -anti IgE.

Table 1 summarises the RAST results for specific IgE binding to the timothy-coupled filter paper, expressed in allergy classes 2-4, or in SU, as a percentage of that for a reference patient (HD), together with the CRIE results for specific IgE binding to the timothy antigens, expressed in a point system based upon the degree of radio-staining of the X-ray film and the length of the staining line. The sex and age of the patients, together with the total IgE in U/ml, and the skin test relative to a histamine reference solution as a skin test index are also indicated.

Most sera contained specific IgE which binds to timothy antigens nos. 13, 25, 27 and 28, while approximately half of the sera showed binding to antigens nos. 19, 22 and 24, and only a few sera contained specific IgE capable of binding to antigens nos. 8, 12, 15 and 26. These results are shown in Fig. 2.

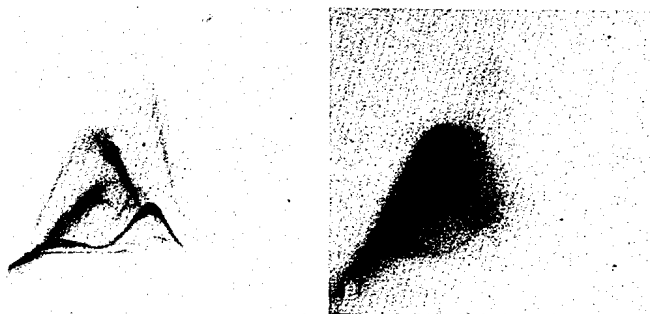


Fig. 1.

The crossed immunoelectrophoresis (left) of timothy pollen extract (St.T 73). The electrophoresis was carried out on 5 cm $\times$ 5 cm glass plates in a 1 per cent agarose (Litex, Copenhagen Ltd.) (w/v) in barbital buffer pH 8.6, ionic strength 0.02 at 15°C. 5 μ l St.T 73 were analyzed in the 1. dimension agarose electrophoresis with a field strength of 10 V cm⁻¹ for 25 min. The electrophoresis in the 2. dimension was performed overnight (18 h) with 1-2 V cm⁻¹ in a gel containing 300 μ l rabbit-anti timothy (15 μ l antiserum per cm² gel). Non-bound proteins were removed from the gel, and the proteins were stained with Coomassie Brilliant Blue. The autoradiograph of the immunoelectrophoresis is shown to the right. Serum from patient no. 29, Table 1, was added to the gel (0.4 ml serum in 7 ml buffer) and allowed to react overnight with the gel. Non-bound serum proteins were removed by repeating washes of the gel. Finally ¹²⁵I-anti IgE was added (0.35 μ Ci in 7 ml buffer), and allowed to react with the gel for 2 d. Non-bound proteins were washed away and the dried gel film was placed on an X-ray film for 7 d. Radioactivity was demonstrated in connection with precipitates of 6 timothy antigens.

In Table 1, the sera examined are arranged according to increasing RAST-timothy positivity.

A significant correlation between the specific IgE binding in RAST, expressed in SU (4) and that in CRIE, estimated in points (x) is demonstrated in Fig. 3. ($y = 1.12x + 6.71$, $r = 0.85$, $P < 0.001$).

On the other hand a significant correlation was also demonstrated between the IgE binding to antigen 25 in CRIE (x) and the total points in CRIE ($r = 0.76$, $P < 0.001$), as well as the SU in RAST (y in Fig. 3, $y = 43.97x + 21.16$,

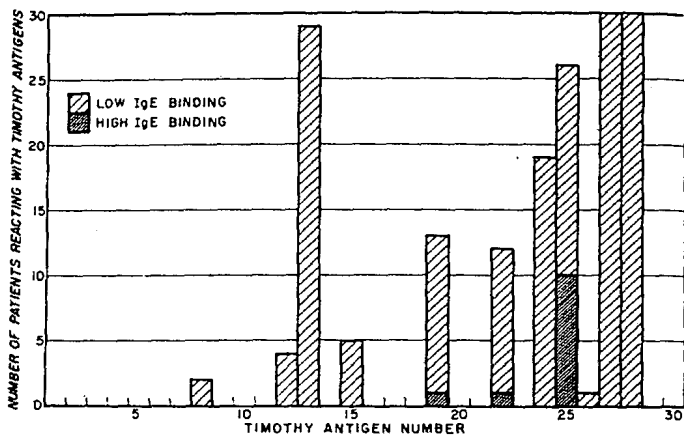


Fig. 2.

The allergogram of timothy pollen antigens based upon the examination of 30 RAST-timothy positive sera. The IgE-¹²⁵I-anti IgE bindings to one or more antigens were identified by comparing the protein stained CIE and the radio-stained autoradiograph from CRIE. The radioactivity binding was graded as high or low depending upon the appearance of the radiostaining on the X-ray film after 1 d and 1 week, respectively.

$r = 0.74$, $P < 0.001$). The particular significance of the IgE binding to timothy antigen no. 25 becomes more apparent following the visual evaluation of the autoradiograph than it was from the raw semiquantitative point system. As can be seen from Fig. 1, the IgE and radiolabelled anti-IgE binding to antigen no. 25 often give rise to broad and marked radiostaining of the autoradiograph. Inhibition of the specific IgE binding to timothy antigens in RAST and CRIE was carried out in parallel experiments by adding increasing amounts of timothy pollen extract to the serum before analysis by RAST and by CRIE. With decreasing RAST positivity, more and more IgE binding disappears in CRIE. All IgE binding in CRIE disappeared at the same stage as total inhibition occurred in RAST.

RAST-timothy negative sera with high IgE from 5 allergic

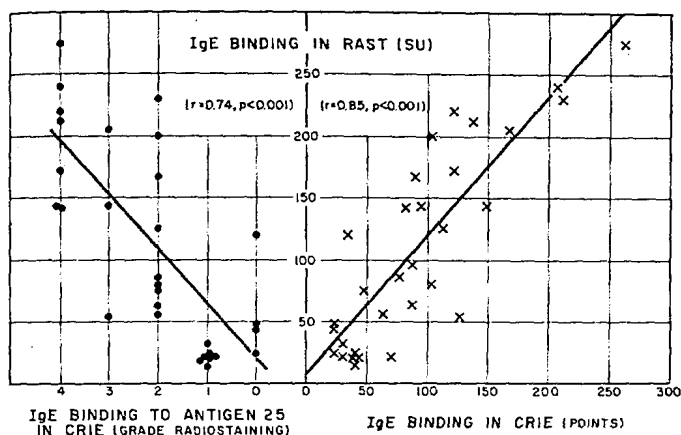


Fig. 3.

The correlation between the IgE-¹²⁵I-anti IgE binding to the timothy coupled filter paper in RAST (y) and the IgE-¹²⁵I-anti IgE bindings to the timothy antigens (x_{right}) and timothy antigen 25 (x_{left}) in CRIE.

patients who were not allergic to grass pollen, and sera from 5 normal persons without any history of allergy (not skin tested) were studied under the same experimental conditions as in Table 1. None of these sera showed IgE binding in CRIE under these conditions (Table 2).

By increasing the exposure time of the X-ray film, it was possible with 5 of these 10 sera to demonstrate a very faint radio-staining associated with timothy antigens nos. 13, 27 and 28. Only in patient no. 9 was a faint staining to timothy antigen no. 25 present after a longer exposure time. The previous immunotherapy on some patients did not influence the RAST or CRIE relative to untreated patients.

Skin tests, carried out as for the intracutaneous test with the extract of grass pollen, were compared with a histamine reference reaction and expressed as a skin test index. As shown in Table 1, an index less than 1.00 was found for 7 out of 29 patients tested; and index 1.00 to 2.00 was found for 19 out of

29; whilst 3 out of 29 tested had an index higher than 2.00. The skin test index did not correlate with 1) the total IgE ($r = 0.21$, $P > 0.05$), and 2) IgE binding in CRIE ($r = 0.28$, $P > 0.05$). A slight correlation between skin test and IgE binding in RAST was found ($r = 0.43$, $0.02 < P < 0.05$).

In one patient (no. 14), the histamine reference reaction was not performed. While a low skin reactivity to the grass pollen was demonstrated in a number of patients having sera with a low to moderate IgE binding in RAST and in CRIE, especially to timothy antigen no. 25, patients nos. 26 and 29, with marked seroallergy, demonstrated a low skin sensitivity.

Patient no. 17 demonstrated a marked grass pollen sensitivity in the skin test and in RAST, but only a poor sensitivity in CRIE, mostly due to a lack of IgE binding to timothy antigen No. 25. The patient, who never has been hyposensitized, was allergic to a number of allergens (dust, animal danders, birch), besides being allergic to grass pollen. The timothy specific IgE in this patient, demonstrated by RAST, seems unable to bind to timothy antigen no. 25 in CRIE, and this results in low IgE binding points. The reactivities in the skin test were not influenced by the immunotherapy.

Total IgE was relatively high (over 500 U/ml) in 7 out of 30 sera examined (Table 1). In none of the normal sera was the IgE level elevated (Table 2). Five sera from patients nos. 6-10 were selected as a control of the RAST and CRIE because of the increased IgE. Total IgE correlated significantly with 1) RAST ($r = 0.64$, $P < 0.001$) and 2) the IgE binding in CRIE ($r = 0.75$, $P < 0.001$). A less significant correlation was demonstrated between the total IgE and the IgE binding to timothy antigen no. 25 in CRIE ($r = 0.45$, $P < 0.02$). There was no difference in the total IgE in patients with and without previous immunotherapy.

DISCUSSION

IgE Binding to Timothy Antigens in RAST and CRIE

For the majority of the 30 sera from grass pollen allergic patients reacting positively to timothy in RAST, we have been able to show the presence of specific IgE having affinity for four timothy antigens, nos. 13, 25, 27 and 28, out of the total of 28 demonstrated antigens in timothy pollen extract. Furthermore, half or less of the sera have been shown to contain IgE with ability to bind to antigens nos. 8, 12, 15, 19, 22, 24 and 26 (Fig. 2). We consider these two groups of antigens to represent major and minor allergens, respectively, because of:

1. the significant correlation between the IgE binding to these antigens in CRIE and the IgE binding in RAST,
2. the absence of IgE binding in CRIE for RAST-negative sera from normal persons and allergic patients with high total IgE, and
3. the ability to inhibit completely the IgE binding in both RAST and CRIE by making the serum react with the allergen extract before performing inhibition experiments.

The significant correlation between the specific IgE binding in RAST and CRIE was mostly due to specific IgE binding to timothy antigen no. 25 in CRIE. Although timothy antigens nos. 13, 27 and 28 bound IgE in all sera, the degree of binding was independent of the amount of specific IgE (expressed in SU in RAST and in points in CRIE). The non-specific binding of some radiolabelled anti-IgE was independent of the presence of a normal or increased amount of IgE (Table 2). This can be explained as a non-specific binding of radiolabelled-anti IgE to some of the antigen-antibody complexes. Timothy antigen no. 25 seems to be the most important of the major allergens. This was the only antigen among the 28 demonstrated in this study that correlated positively with the total sero-allergy, expressed in total points, in CRIE and in RAST (Fig. 3). It was not possible to demonstrate anything special about the 4

patients whose sera revealed no binding to antigen no. 25 in CRIE (Table 1). The CIE results did not allow calculation of the absolute amount of antigen no. 25 in the extract. Only a faint protein staining in the vicinity of antigen no. 25 was demonstrated by agarose electrophoresis of the extract (8). Further study and purification work concerning antigen no. 25 is now in progress. Preliminary CIE studies indicate acid phosphatase activity associated with this antigen and its precipitate also resembles the glycoprotein precipitates well known for e.g. orosomucoid in human serum (3).

The skin tests were unfortunately carried out with a mixture of three grass pollens, the timothy extract used in RAST and in CRIE not being included. With reference to a histamine skin reaction, $\frac{3}{4}$ of the patients tested (Table 1) demonstrated a more positive wheal and flare reaction, corresponding to the presence in the skin of cell-bound specific IgE against the grass pollen; whilst in $\frac{1}{4}$ of the patients the reaction was insignificantly less positive than the reference reaction. A slight significant correlation was found between the skin test and the seroallergy as determined by RAST, while no correlation to the CRIE was shown, although the higher cutaneous reactivities were exhibited by the most severely sero-allergic patients.

Total IgE was increased in 7 out of 30 patients, although the blood was taken before the season. It is well known, that pollens are strong allergens giving rise to a frequent and marked increase in total IgE, especially during the season and in the following months. On the other hand, severe pollen allergy may be present in patients with normal IgE levels. In this study, we demonstrated that the total IgE increased significantly with increasing sero-allergy as determined by RAST and by CRIE.

The objection may be raised that the rabbit antiserum does not contain antibodies against one or more of the antigens, as these are not antigenic to the rabbit. Although the allergens in timothy pollen are believed to be of protein nature (7), and antibodies can be raised in rabbits by the use of suitable im-

nunization techniques (5), the above problem cannot be definitively resolved at the present time. However, the good correlation between the CRIE and RAST results seems to indicate that both methods measure the presence of the same allergens. Furthermore St.T 73 has been shown not to contain any cathodically migrating antigens under the experimental conditions used (8).

Most of the timothy allergens are smaller than the rabbit antibodies (8), and the binding of the allergens with the antibodies may interfere with the subsequent IgE binding due to blocking of the binding site or formation of a spatial conformation which does not leave space for the large IgE molecule. However, a number of unsaturated antigens are present in the environment of an immuno-precipitate leaving place for the IgE-radiolabelled-anti IgE binding. This is demonstrated in Fig. 1.

Furthermore, the presence in patients' sera of blocking antibodies of the IgG class may occupy the IgE binding sites on the allergens in such a way as to inhibit specific IgE binding in CRIE as well as in RAST. In this study we were not able to point out any difference between the IgE binding in RAST and in CRIE due to the previous immunotherapy. Further studies using ^{125}I -anti IgG in timothy-CRIE performed on sera from patients during immunotherapy are taking place. Finally, it should be mentioned that CRIE is here compared to 1) an *in vitro* (RAST) and 2) an *in vivo* (I.C.) test, which themselves may give rise to wrong interpretations: 1) a minor allergen which contains a great number of ϵ -aminogroups gives rise to a disproportionately high RAST value, and 2) an allergen which is not present in *Phleum pratense*, but only in *Avena elation* or *Dactylis glomerata*, could be the major allergen in the skin test performed.

CONCLUSION

The CRIE and RAST techniques seem to be valuable *in vitro* methods, not only for allergen standardization but also for the

specific diagnosis of allergy in individual patients. RAST gives a measure of the total IgE binding to the insolubilized allergen in the test system, whilst CRIE gives more detailed information about the IgE binding to the individual antigens present in the extract. After the initial identification of the allergens in the extract, we may be able to use quantitative immunoelectrophoresis methods in further work on the characterization and purification of the allergens. The demonstration of the presence in the patient's serum of specific IgE having affinity for one or more of the antigens in the extract may result in a more detailed picture of the patient's allergy that can be used to give a more exact diagnosis of the allergy, and thus make it possible to hyposensitize the patient with the purified antigens.

SUMMARY

28 timothy antigens were demonstrated to be present in an extract of timothy pollen (*Phleum pratense*) by CIE. By means of a new CRIE technique the IgE affinity for one or more of these antigens was identified and semiquantified. Most of the 30 RAST-timothy positive sera were found to contain specific IgE which binds to 4 of the timothy antigens (major allergens), while half or less of the sera examined showed affinity for 7 other antigens (minor allergens). No IgE binding was demonstrated for normal sera or IgE-rich sera from RAST-timothy negative samples under the experimental conditions employed. Antigen no. 25 was responsible for most of the IgE binding in CRIE and the latter correlated well with the IgE binding in RAST. This antigen represents one of the major allergens. Total IgE levels correlated well with seroallergy, whereas the cutaneous allergy was unrelated to the total IgE and only slightly to the seroallergy.

CRIE and RAST together are valuable tools in the standardization of allergen extracts and the precise diagnosis of allergy.

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Author's address:

Henning Løwenstein
University of Copenhagen
The Protein Laboratory
34, Sigurdsgade
DK-2200 Copenhagen N.
Denmark

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