

Characterization of Allergens in a Crude Extract of *Dermatophagoides farinae* and Their Identification in a New Purified Preparation

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The allergens of a crude *D. farinae* extract were identified by means of crossed radio-immunoelectrophoresis (CIE). A total of 11 allergens were identified. They were characterized as three major, three intermediate and five minor allergens, based on radiostaining in CIE. In addition to the radiostaining due to allergens, some misleading radiostaining was observed associated with some of the other antigens. A purified commercially available preparation, Spectralgen® Mite *D. farinae*, was compared with the crude extract by means of immunoelectrophoretic techniques and radioallergosorbent test (RAST) based techniques. The purified preparation was found to be fully representative of the allergens in the crude extract. All 11 allergens were identified in the purified preparation. The purification ratio of the allergens varied from 2 to 7, while the total specific allergenic activity was approximately 6 times higher in the purified preparation than in the crude extract, as judged from RAST-inhibition studies.

Key words: allergens; crossed radioimmunoelectrophoresis; *Dermatophagoides farinae*, radioallergosorbent test.

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Allergy to house dust mite is an important cause of asthma, and *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are considered to be the most important house dust mites in this respect (13, 15, 21, 24). A high degree of cross reactivity between the two has been reported (11). Significant cross reactivity between *D. farinae* and several other species of mites has also been reported (16).

The efficacy of allergen extracts in diagnosis and immunotherapy depends on the extract being representative of all important allergens. A purified preparation of *D. farinae* is commercially available (Spectralgen® Mite *D. farinae*). There is, however, always a risk that important allergens may be lost during a purification procedure. Therefore an essential part of the documentation of a purified allergen preparation is to show that it is fully representative of all the allergens in the species.

It has been demonstrated that a major allergen, antigen P₁, is excreted into the medium during cultivation of *D. pteronyssinus* (23). However, this major allergen from the excreta is also identified in isolated *D. pteronyssinus* (20). The high degree of correlation in SPT indicates that also *D. farinae* mite bodies and excreta share a common major allergen (15). A crude extract of isolated *D. farinae* mites was therefore presumed to be representative of the *D. farinae* allergens.

The purpose of the present study was to compare Spectralgen Mite *D. farinae* with a crude extract of this mite to investigate if the purified preparation is a representative extract of *D. farinae* allergens.

CIE and CRIE are the most powerful tools available today for detailed characterization of allergen extracts on the molecular level (12). Accordingly, a detailed CIE/CRIE characteri-

zation of the crude *D. farinae* extract was performed to serve as a basis for comparing the purified preparation with the crude extract. RAST methods were also employed, since they give useful information on total allergenic activity.

MATERIAL AND METHODS

D. farinae extracts

A purified freeze-dried preparation, Spectralgen Mite *D. farinae*, was obtained from Nye-gaard & Co. AS (Norway). The preparation was produced from isolated and crushed *D. farinae* mites supplied by Allergon AB (Sweden). After extraction, a purification procedure was performed, removing material of both high (> 70,000 daltons) and low (< 5,000 daltons) molecular weight.

A crude *D. farinae* extract was prepared from mites of the same quality as Spectralgen Mite *D. farinae* thus: the isolated and crushed *D. farinae* mites (Allergon AB, Sweden) were extracted overnight at 6°C as a 2.5% (w/v) solution in a buffer of 0.125 mol/l ammonium bicarbonate with 2% (v/v) n-butanol (pH 8.0, 25°C). The suspension was centrifuged (27,000 *g* for 1 h at 0°C) and filtered (Acropore 0.8 μ). The clarified filtrate was denoted Crude *D. farinae* and stored freeze dried. The extraction buffer constitutes 2‰ of the freeze dried Crude *D. farinae* preparation determined as ammonia using an ammonia electrode, Orion® model 95-10, and the method recommended by the manufacturer (Orion Research Incorporated, Cambridge).

The protein content of Spectralgen Mite and Crude *D. farinae* was 53% and 22% of the dry weight, respectively, as measured according to Bensadoun et al. (5).

Horse epithelium extract

Horse hair and skin scrapings were obtained from Allergon AB (Sweden). A horse epithelium extract was prepared basically as the crude *D. farinae* extract, but was further concentrated 10 times by using Hollow Fiber equipment

(Amicon, H1DP10) and stored frozen (-20°C) in vials.

Rabbit antisera

Rabbit antisera to *D. farinae* were prepared basically as described by Harboe & Ingild (9), using the Crude *D. farinae* extract at a concentration of approx. 50 g/l dry weight for immunization. Three rabbits were immunized and boosted monthly and bled 14 days after each immunization. Antisera used in these experiments were collected in a period between 10 and 14 months after the first immunization. The antisera were pooled and denoted Anti-*D. farinae*.

Human sera

a) *Sera from mite-allergic patients.* The selection of sera from mite-allergic patients was based on two slightly different criteria: Forty-four sera were obtained from patients with suspected mite allergy and positive Phadebas® RAST to d1 or d2 discs, mostly RAST-class 3 or 4. Twenty-five individual sera were selected randomly, numbered from 1 to 25 and used in the CIE/CRIE characterization experiment. Identical volumes from the remaining 19 of these 44 sera constituted a serum pool. Another 40 individual sera were obtained from mite-allergic patients participating in a clinical evaluation of the Spectralgen preparation (14). The latter sera were used in comparative RAST-experiments.

b) *Negative control serum.* In a CRIE control experiment a serum (N.H.) was employed which contained specific IgE to horse epithelium allergens but not to mite allergens: RAST performed with serum N.H. gave Phadebas RAST-class 4 with e3 disc and RAST-class 0 with d2 disc.

Crossed immunoelectrophoresis (CIE)

CIE was performed on glass slides (5 × 7 cm) essentially as described by Weeke (25) using a 1% agarose gel (Litex, Glostrup, Denmark)

containing a buffer of 0.073 mol/l Tris, 0.024 mol/l barbital, 0.006 mol/l calcium lactate and 0.003 mol/l sodium azide (pH 8.6, 22°C). The first and second dimension electrophoreses were performed at 12°C at $10\text{ V} \times \text{cm}^{-1}$ and at $2\text{ V} \times \text{cm}^{-1}$, respectively. Two patterns of gel sections were used.

Pattern A: $5 \times 3.5 \times 0.11$, $5 \times 1 \times 0.13$, $5 \times 1 \times 0.15$ and $5 \times 1.5 \times 0.13$ cm, respectively, denoted from the anode. 400 μl and 100 μl of Anti-*D. farinae* were added to the gel sections 1 and 4 from the anode. The first and second dimension electrophoreses were run for 35 min and 19 h, respectively.

Pattern B: $5 \times 4.5 \times 0.11$, $5 \times 1.5 \times 0.13$ and $5 \times 1 \times 0.15$ cm respectively, denoted from the anode. 500 μl Anti-*D. farinae* was added to the gel section 1 from the anode. The first and second dimension electrophoreses were run for 45 min and for 19 h, respectively.

The slides were pressed, washed and dried. Heating of slides was avoided. The slides were stored dry at 5°C until used and were stained with Coomassie Brilliant Blue® after completion of the autoradiography.

Crossed radioimmuno-electrophoresis (CRIE)

CRIE was performed essentially as described by Weeke & Løwenstein (26). The CIE preparations were incubated for 24 h at 25°C with patient serum diluted in an incubation buffer (0.05 mol/l phosphate buffer pH 7.5, containing 0.3% (w/v) of sodium azide, 2% (v/v) Tween 20 and 0.1% (w/v) of EDTA) to a volume of 7 ml. 0.4 ml patient serum was used in the characterization experiment, while 0.2 ml (patient sera Nos. 4, 6, 14 and 16) and 0.4 ml (patient sera Nos. 9 and 21) were used in the identification experiment. After washing four times for 10 min with 7 ml 0.9% (w/v) sodium chloride, the slides were incubated with ^{125}I -anti-IgE (Phadebas® RAST isotope) solution, approximately 250,000 cpm (0.5 μCi) in 7 ml of incubation buffer for 24 h at 25°C. After washing four times for 10 min with 7 ml of 0.9% (w/v)

sodium chloride and once with distilled water for 10 min, the slides were dried and laid face down on X-Omat (Kodak) X-ray films for autoradiography.

A paper disc reference system in CRIE with a grading system was used as described by Aukrust et al. (2). The radiostaining was measured and characterized in CRIE classes from A (dense radiostaining) to F (weak radiostaining), using different exposure periods. The allergens were characterized as "major", "intermediate" and "minor" allergens (4), as follows: To qualify as a "major" allergen, an allergen fulfilled two independent criteria: at least 25% of the relevant patient sera gave strong IgE binding (CRIE classes A-D), and at least 50% of all the sera showed at least some specific IgE binding (CRIE classes A-F). To qualify as a "minor" allergen, less than 10% of the patient's sera gave rise to IgE binding in CRIE classes A-F. Allergens not belonging to either of these extreme categories were defined as "intermediate" allergens.

RAST allergosorbents

Phadebas RAST discs d1, (*D. pteronyssinus*), d2 (*D. farinae*) and e3 (horse epithelium) were obtained from Pharmacia Diagnostics AB, Sweden.

The Crude *D. farinae* extract (2.5 g/l dry weight) and Spectralgen Mite *D. farinae* (0.2 and 1.0 g/l dry weight) were coupled to cyanogen bromide activated paper discs according to Ceska et al. (6). The Spectralgen discs were denoted disc 1 and disc 2 respectively.

RAST

RAST was performed as recommended by Pharmacia. The results were expressed as uptake of ^{125}I -anti-IgE (Phadebas RAST isotope, Pharmacia Diagnostics AB, Sweden) in per cent of the total applied, or in the RAST classes recommended by the manufacturer. All results were the means of duplicate determinations.

RAST inhibition

Patient sera diluted to give a RAST-class 3, were used in the RAST inhibition experiments. RAST inhibition was performed as described for RAST, except for an overnight pre-incubation of the sera with allergen extract prior to the addition of allergosorbent. 25 μ l serum and 25 μ l allergen extract were incubated in individual test tubes. The results were expressed as per cent inhibition. The RAST results, obtained with 25 μ l serum in 25 μ l buffer and with only 50 μ l buffer, were used in the calculations as the 0% and 100% inhibition value, respectively. In terms of cpm the 100% inhibition value was insignificantly lower than that obtained with several sera inhibited with high concentration of inhibitor (Table 3).

Estimation of purification ratio

The purification ratio of Spectralgen Mite *D. farinae* relative to the Crude *D. farinae* extract was estimated by both RAST inhibition and CIE/CRIE.

RAST inhibition. 25 μ l pooled serum and 25 μ l of 10-fold dilutions of Crude *D. farinae* or Spectralgen Mite *D. farinae* were incubated in individual test tubes and subjected to RAST inhibition. The extract concentration (dry weight) giving 50% RAST inhibition, was used for calculation of the purification ratio (Fig. 6).

CIE/CRIE. Determination of the purification ratio of individual antigens was based on CIE/CRIE performed at constant antibody concentration. The area enclosed by an immunoprecipitate corresponding to one particular antigen was therefore proportional to the amount of that antigen applied to the plate (12). In more detail, the purification ratio of Ag 19/20 was determined thus:

CIE/CRIE were performed in triplicate with both Crude *D. farinae* and Spectralgen Mite *D. farinae* so that the immunoprecipitate No. 19/20 on the various plates was of appropriate and corresponding size.

The purification ratio of Ag 22 was de-

termined as described above for Ag 19/20, while the purification ratio of Ag 3, Ag 4, Ag 2, Ag 15, Ag 1, Ag 5, Ag 13, Ag 14 and Ag 24 was roughly estimated from the experiment identifying individual allergens in the Spectralgen Mite *D. farinae* (Fig. 4).

EXPERIMENTS AND RESULTS

Characterization of allergens in the Crude D. farinae extract

CRIE was performed with the Crude *D. farinae* extract and 25 mite allergic sera, negative control serum (N.H.) and buffer. The immunoprecipitation pattern is shown as a photograph of the Coomassie Brilliant Blue stained glass-plate and as a drawing with the precipitates arbitrarily numbered (Fig. 1). Some of the precipitates seemed to exist as double precipitates and were therefore given double numbers. 28 precipitates were demonstrated, several only after radiostaining (shown with broken lines in Fig. 1B). The CRIE results are summarized in Table 1, showing that half of the 28 immunoprecipitates gave rise to radiostaining. RAST with the d2 disc demonstrated the high concentration of specific IgE to *D. farinae* in these sera.

A precipitate in the cathodically placed antibody gel formed a confluent line with a precipitate in the anodic gel. The upper part of the anodic precipitate gave rise to a broad and diffuse radiostaining with some sera, and sometimes the radiostaining seemed to indicate a splitting of this part of the precipitate (Fig. 1B). This complex precipitate with its anodic and cathodic part is referred to as precipitate No. 19/20 and the corresponding antigen as Ag 19/20. Radiostaining was frequently obtained with this precipitate.

The intense protein staining precipitate, No. 10/11 radiostained positively with all sera. Even buffer and negative control serum (N.H.) radiostained weakly (CRIE-class F) with precipitate No. 10/11. Two other precipitates, Nos. 3 and 22, also frequently radiostained, while 10 additional precipitates radiostained with only some patient sera (Table 1).

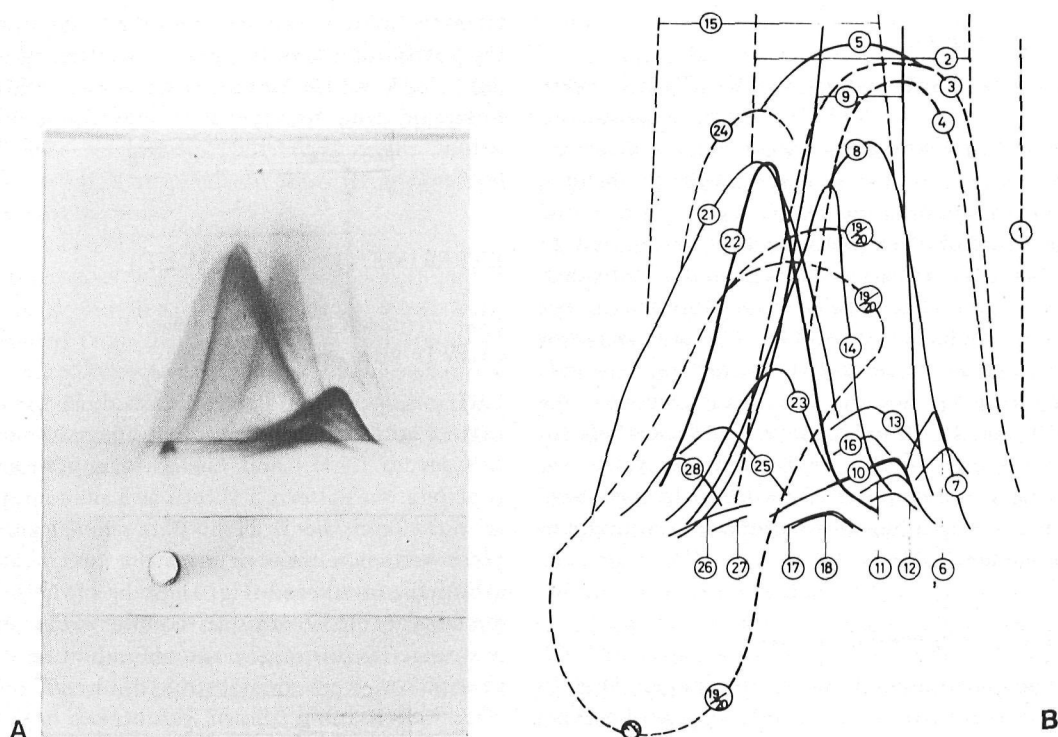


Fig. 1. Reference pattern of the Crude *D. farinae* extract, 90 μ g dry material, in CIE/CRIE. Coomassie Brilliant Blue-stained glass-plate (A) and drawing with the precipitates Nos. 1–28 (B). Some precipitates were demonstrated only in CRIE (shown with dotted lines).

Negative CRIE control experiment

Preliminary experiments had indicated a discrepancy between the antigenic and allergenic identity associated with precipitates Nos. 9, 10/11, 17/18 and 21 (7). One possible explanation was that allergens were coprecipitated in the precipitates Nos. 9, 10/11, 17/18 and 21. To test this hypothesis a CRIE control experiment was performed:

Horse extract was added to the Crude *D. farinae* extract in equal dry weight proportion, and CIE was performed against Anti-*D. farinae* serum. The mite immunoprecipitates were incubated with the negative control serum (N.H.) which contained specific IgE to horse allergens, but not to mite allergens. The presence of horse allergens in the mite extract led to a marked increase of radiostaining from the mite precipitate No. 10/11, and even precipitates Nos. 9,

17/18 and 21 gave rise to some radiostaining (Table 1). This control experiment demonstrated that the specificity of radiostaining from the mite immunoprecipitates Nos. 9, 10/11, 17/18 and 21 could be doubtful. These precipitates are marked with asterisks in Table 1.

The antigens corresponding to the other radiostaining immunoprecipitates were regarded as allergens.

Identification of individual allergens in the purified D. farinae preparation by means of CRIE using selected sera

Some sera from the CRIE characterization experiment were selected to enable identification of the individual allergens in the purified *D. farinae* preparation.

Eleven allergens were thus identified, and are demonstrated in the following on the photo-

graphs of the CRIE films obtained with patient sera Nos. 4, 6, 9, 14 and 16. Identification of the individual antigens 3 and 4 was based on the results with patient sera Nos. 4 and 16 (Fig. 2A, B, C, D). Antigens 5 and 24 were demonstrated with patient serum No. 14, while antigens 13, 14 and 15 were identified with serum No. 9 (Fig. 3A, B, C, D). The identification of antigens 1 and 2 was based on the results with patient serum No. 6 (Fig. 4C, D). Antigens 19/20 and 22 were demonstrated with all the sera in this experiment. Serum No. 21 confirmed the identification of Ag 2 and Ag 15 (results not shown). A drawing illustrates the results of CIE/CRIE with the six patient sera (Fig. 4E, F). Ag 10/11 and Ag 17/18 are not present in the purified *D. farinae* preparation as

the two dubious, but intense protein staining precipitates, Nos. 10/11 and 17/18, are absent in CIE with this preparation.

RAST-based investigations of allergenic identity between the purified and the Crude D. farinae preparation

Comparative RAST. The RAST results obtained with the purified (disc 1) and the Crude *D. farinae* preparation are compared in Fig. 5 and the correlation coefficient between the results given in Table 2. The RAST results with the two different preparations, Crude and purified *D. farinae*, correlated, as did the results obtained with two slightly different types of discs ($r_1 = 0.97$ and $r_2 =$

Table 1
CRIE radiostaining corresponding to the various precipitates in the *D. farinae* reference pattern

Experiment	Patient serum	RAST class Phadebas, d2	CRIE radiostaining corresponding to precipitates no.:																												
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
CIE/CRIE charac- terization	No. 1	4																													
	2	4																													
	3	4																													
	4	4																													
	5	4																													
	6	4																													
	7	4																													
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	22	4																													
	23	3																													
	24	4																													
	25	3																													
Buffer	-																														
Negative control NH	0																														
CRIE control experiment	Negative control NH	0																													

The hatched squares indicate CRIE classes from A (dense radiostaining) to F (weak radiostaining). *Precipitates giving radiostaining in the CRIE control experiments performed with the addition of horse extract to the Crude *D. farinae* extract.

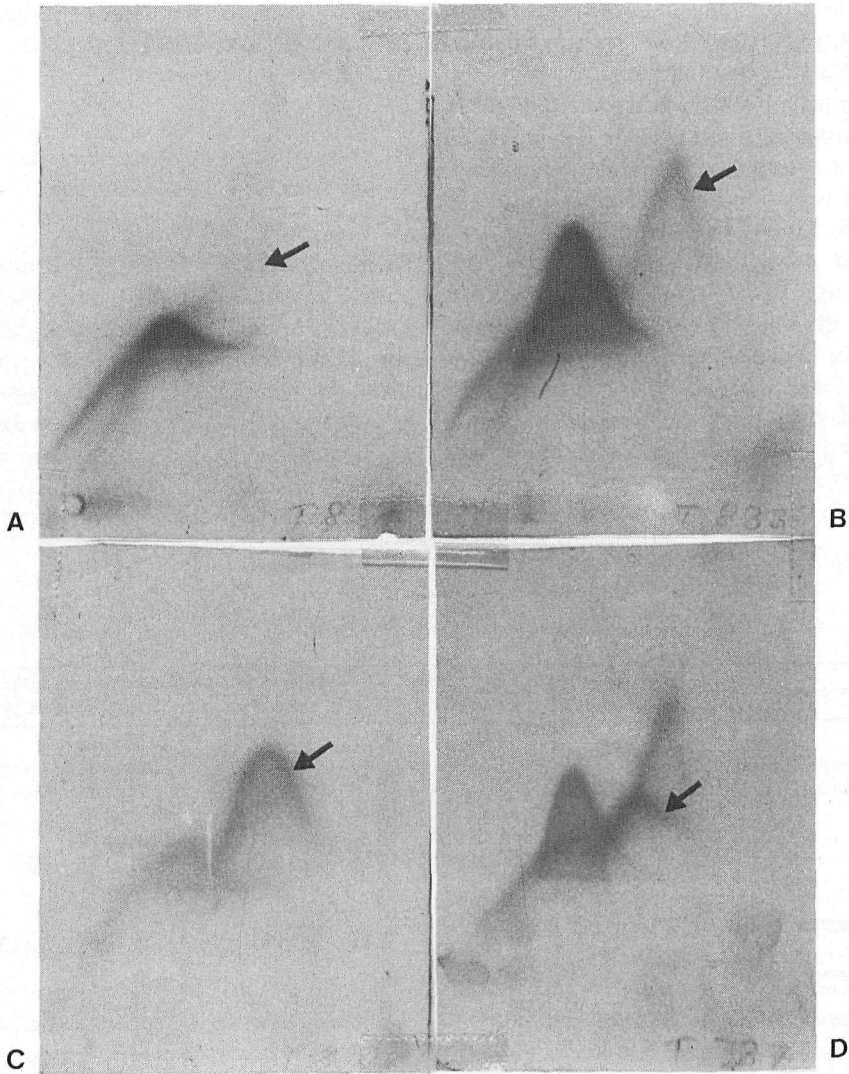


Fig. 2. Autographic films from CIE/CRIE experiments identifying antigens 3 and 4 in the purified preparation. CIE was performed with Crude *D. farinae* extract, 30 µg dry material (A, C) and purified preparation, 10 µg dry material (B, D).

A, B: CRIE films obtained with serum No. 4 giving the identification of antigen 4 (arrows, the radiostaining in A is somewhat indistinct).

C, D: CRIE films obtained with serum No. 16 giving the identification of antigen 3 (arrows).

0.98 respectively). No serum gave an obviously greater reaction to the Crude extract than to the the corresponding purified preparation (Fig. 5).

Preliminary results demonstrated that 2.5 g/l dry weight of Crude extract was optimal for coupling to the cyanogen bromide-activated discs. Comparing the RAST results obtained

with discs 1 and 2 demonstrated that the coupling concentration of disc 1 (0.2 g/l dry weight) was approximately optimal for coupling of the purified preparation to the cyanogen-activated discs (Table 2).

RAST inhibition of individual patient sera. In this experiment the Crude *D. farinae* extract was

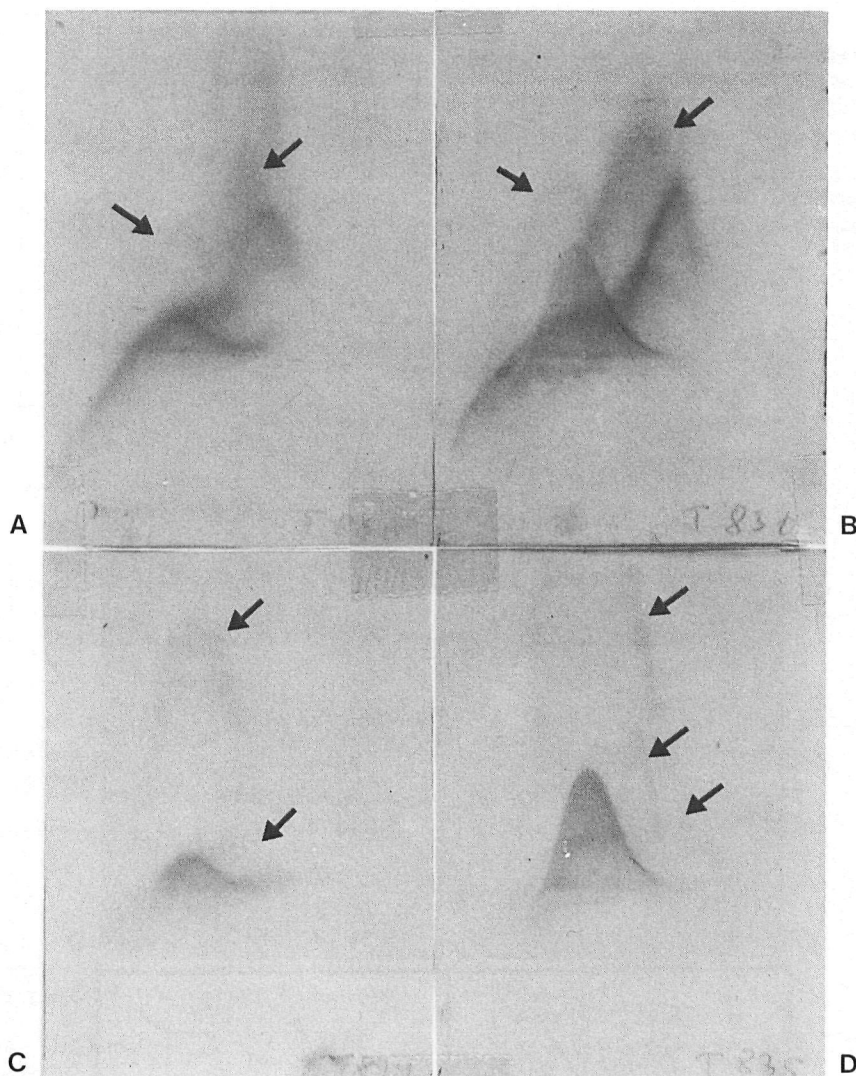


Fig. 3. Autoradiographic films from CIE/CRIE experiments identifying antigens 5, 13, 14, 15 and 24 in the purified preparation. CIE was performed with Crude *D. farinae*, 30 μ g dry material (A, C) and purified preparation, 10 μ g dry material (B, D).

A, B: CRIE films obtained with serum No. 14 identifying antigens 5 and 24 (arrows).

C, D: CRIE films obtained with serum No. 9 identifying antigens 13, 14 and 15 (arrows, antigen 13 is demonstrated only in D).

used as allergosorbent, and both the Crude and the purified *D. farinae* preparations were used as inhibitors. 13 of the individual sera from the CRIE characterization experiment were inhibited, demonstrating allergenic identity between the two preparations, as both extracts inhibited each of the sera to approximately the same degree (Table 3).

Purification ratio of Spectralgen Mite D. farinae relative to the Crude D. farinae extract

The allergens were quantified in CIE/CRIE experiments. The purification ratio of individual allergens seems to vary from 2 to 7 (Fig. 4, Table 4). The two extracts were further compared in a RAST inhibition experiment using

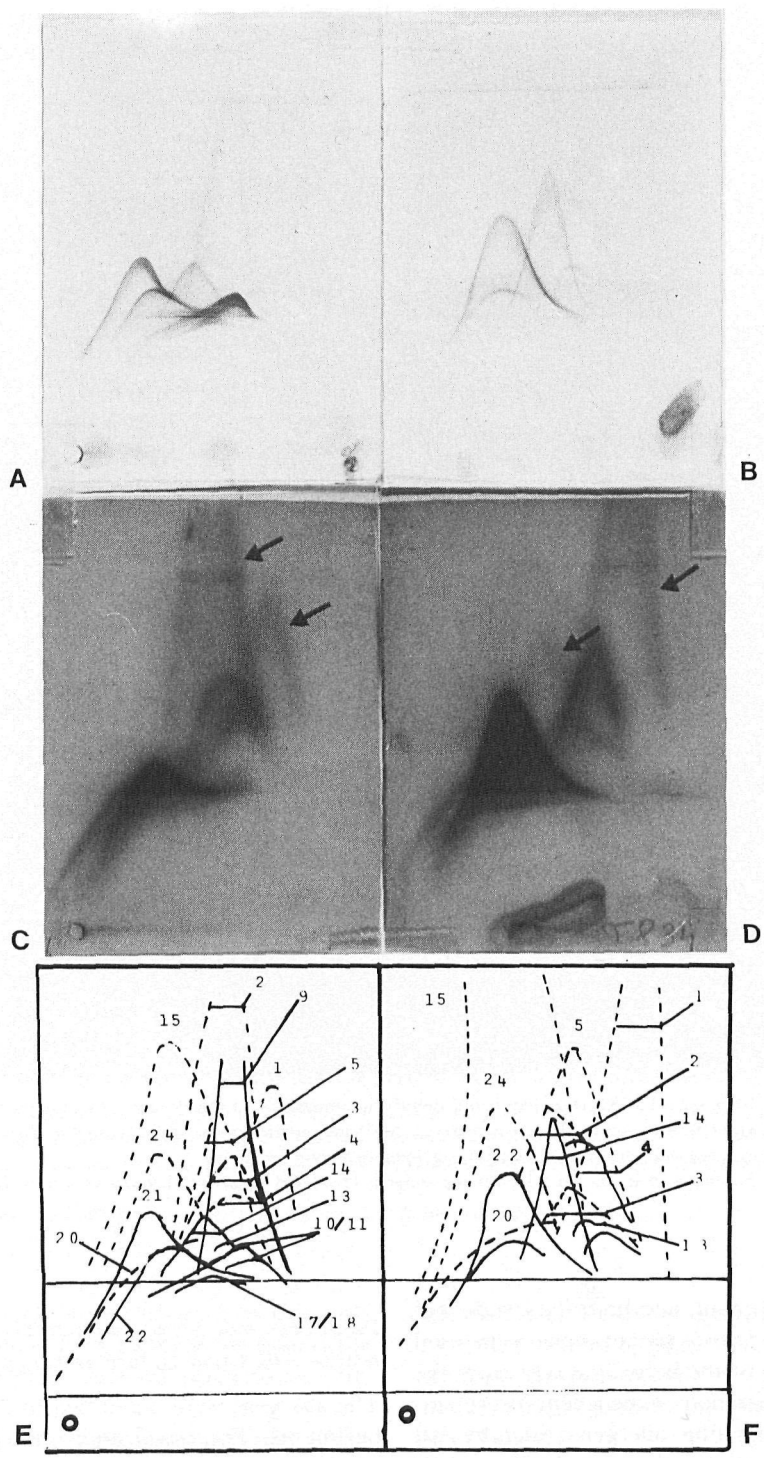


Fig. 4. Comparison of Crude and purified (Spectralgen) *D. farinae* in CIE/CRIE experiments. CIE of A) Crude *D. farinae* 30 µg dry material and B) purified *D. farinae*, 10 µg dry material. C, D). Autoradiographic experiment using CIE plates in A and B, respectively, and patient serum No. 6. Arrows indicate antigens 1 and 2. E, F) drawings summarizing the CIE/CRIE results using corresponding CIE plates as in A and B and six selected patient sera.

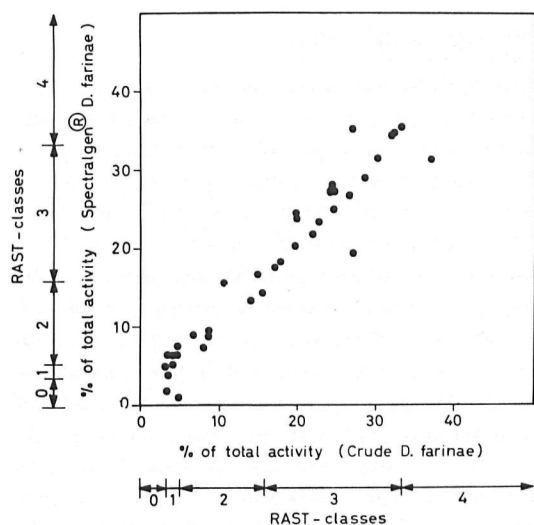


Fig. 5. Comparison of Crude and purified (Spectralgen) *D. farinae* in a RAST experiment using sera from 40 mite-allergic patients. Results are given as a plot of individual RAST results.

Table 2

Comparison of Crude and purified (Spectralgen) *D. farinae* in a RAST experiment using sera from 40 mite-allergic patients

	Mean RAST value	Spectralgen <i>D. farinae</i> (disc 1) 19.7
Crude <i>D. farinae</i>	17.7 %	$r_1 = 0.97$
Control Spectralgen <i>D. farinae</i> (disc 2)	21.1 %	$r_2 = 0.98$

The correlation coefficient (r_1) between the individual RAST results is compared with a control value, which is the correlation coefficient (r_2) between RAST results obtained with two slightly different discs from one preparation (Spectralgen Mite *D. farinae*, disc 1 and disc 2).

the Crude *D. farinae* extract as allergosorbent. The inhibition curves were approximately parallel, showing about 6 times purification of allergens in the purified relative to the Crude *D. farinae* preparation, based on dry material (Fig. 6). This purification ratio corresponded to 2.5 times based on protein content.

Table 3

Comparison of Crude and purified (Spectralgen) *D. farinae* in a RAST inhibition experiment using Crude *D. farinae* as sorbent and individual sera from mite-allergic patients (Table 1)

Patient Serum No.	Inhibitor	
	Crude <i>D. farinae</i> 1 g prot/l (5)	Spectralgen <i>D. farinae</i> 1 g prot/l (5)
1	92.4	95.2
2	84.5	86.4
3	87.6	91.9
4	85.8	83.9
7	86.9	85.2
10	95.9	97.2
11	93.0	92.0
14	90.6	92.6
16	87.2	93.2
17	95.9	97.6
18	96.1	96.2
24	95.6	98.9
25	87.7	83.3

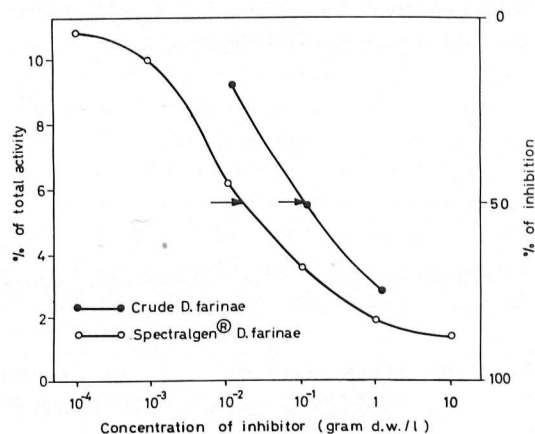


Fig. 6. Comparison of Crude and purified (Spectralgen) *D. farinae* in a RAST inhibition experiment using the Crude *D. farinae* as allergosorbent and a pool of sera from mite-allergic patients.

DISCUSSION

Characterization of the Crude extract

Characterization of the Crude extract of isolated *D. farinae* mite bodies revealed a high number of allergens.

Radiostaining from 11 of the total 14 radiostaining precipitates was regarded as specific, and the corresponding antigens were subjected to further characterization. In accordance with the frequency and density of radiostaining obtained with the 25 patient sera, three of these antigens were characterized as major allergens, three as intermediate and five as minor allergens (Table 4).

The concentration of specific IgE to *D. farinae* allergens was high in the majority of sera used in the present characterization experiment (RAST-class 3 or 4). The demonstration of three major allergens may therefore reflect this selection of sera, as the definition of a major allergen is based on both frequency and strong IgE-binding in CRIE with relevant sera.

Later investigations have revealed that the major allergen Ag 22 in this investigation is excreted into the medium during cultivation of *D. farinae* (10). Therefore, the present Crude extract of isolated *D. farinae* seems to be representative also of excreted allergen(s).

Misleading radiostaining

The addition of horse extract to the Crude *D. farinae* extract in the control experiment demonstrated that especially one antigen, Ag 10/11, and to a lesser extent Ag 9, Ag 17/18 and Ag 21, seemed able to bind other molecules. The binding of allergens may give rise to false-positive radiostaining in the corresponding immunoprecipitate of these antigens. This phenomenon is an extension of the well-known concept of co-precipitation, which gives misleading radiostaining for precipitates within the area of precipitates representing real allergens (3). The dense radiostaining of precipitate No. 10/11 and the weak radiostaining of Nos. 17/18 and 21 in the CRIE characterization experiment were therefore judged as false-positives and not due to specific IgE binding to the antigens in these precipitates.

Allergenic components of apparently highly different molecular weight from *D. pteronyssinus* and *D. farinae* have been shown to possess a high degree of identity in RAST inhibition

Table 4

Comparison of Crude and purified (Spectralgen) *D. farinae* with regard to the mite antigens characterized as allergens

Crude <i>D. farinae</i>			Spectralgen <i>D. farinae</i>	
Mite antigens characterized as allergens	No. of sera showing IgE binding (total 25)	Staining in CIE	Identification	Purification ratio
Ag-22 major allergen	24	++	yes	7
Ag-19/20 major allergen	22	(+)	yes	4
Ag-3 major allergen	14	(+)	yes	ca. 2
Ag-4 intermediate allergen	9	(+)	yes	ca. 7
Ag-2 intermediate allergen	7	(+)	yes	ca. 2
Ag-15 intermediate allergen	4		yes	ca. 6
Ag-24 minor allergen	2		yes	ca. 5
Ag-1 minor allergen	1	(+)	yes	ca. 7
Ag-5 minor allergen	1		yes	ca. 6
Ag-13 minor allergen	1	+	yes	ca. 6
Ag-14 minor allergen	1	++	yes	ca. 7

++: Moderate.

+: Weak, but distinct.

(+): Faint.

experiments (7, 19). The ability of Ag 9, Ag 10/11, Ag 17/18 and Ag 21 to bind other molecules might give a reasonable explanation for these observations.

Comparison of the purified with the Crude D. farinae preparation

All 11 allergens demonstrated in the Crude *D. farinae* extract, were also identified in the purified *D. farinae* by means of CRIE experiments with selected sera (Fig. 4, Table 4). The identification of allergens by means of CRIE depends, however, on the quality of the antisera. Comparable RAST and RAST inhibitions were therefore performed to supplement the CIE/CRIE experiments.

It is noticeable that no individual patient serum reacted more obviously in RAST with Crude than with purified *D. farinae* and that the two preparations also correlated in RAST as if the discs were prepared from one extract. The allergenic similarity of the two preparations was further demonstrated in the RAST inhibition experiment, using individual patient sera. Strong IgE binding to precipitate No. 10/11 was obtained with all these sera. (CRIE-class A to D, Table 1). Ag 10/11 is not present in the purified *D. farinae* preparation, and the experiment was performed with the Ag 10/11-containing Crude *D. farinae* as allergosorbent. RAST inhibition results are in accordance with the results obtained by others, showing a high degree of identity between allergenic components of apparently highly different molecular weight (7, 19). The present results demonstrated that there was no specific RAST activity due to Ag 10/11 on the Crude *D. farinae* disc, confirming the conclusion of the CRIE characterization and control experiment, that Ag 10/11 is not an allergen.

The reliability of the RAST experiments depends on the quality of the allergosorbents just as CIE/CRIE depends on the quality of the antiserum. Even though the preparation of RAST discs was performed at optimal conditions, there is always a risk that some allergens were not represented on the disc, and that some allergens were lacking antibodies. The radio-

staining from some immunoprecipitates was judged as false, but the possibility exists that the radiostaining was occasionally partly false and partly specific. In order to compensate for methodological weaknesses, Crude and purified *D. farinae* preparations were compared by means of experiments using both techniques. In total, the experiments gave strong evidence for the conclusion that the purified *D. farinae* preparation is representative for the allergens in the Crude *D. farinae* extract prepared from isolated mite bodies. This conclusion has later been confirmed by others comparing the two extracts in skin prick test (SPT) (14).

Purification ratio of Spectralgen Mite D. farinae

By means of RAST inhibition the purification ratio of Spectralgen Mite *D. farinae* relative to Crude *D. farinae* seemed to be about 6. This is in agreement with the 2–7 degree of purification of individual allergens, demonstrated by means of CIE/CRIE experiments. The present results are also in agreement with the purification ratio of 6 obtained by others using SPT in a clinical evaluation of the purified *D. farinae* preparation (14). As already demonstrated, Ag 10/11 does not contribute to the RAST activity of Crude *D. farinae*. The conformity of RAST and SPT results may indicate that it does not contribute to the SPT results either, supporting further the conclusion that Ag 10/11 is not an allergen.

Why a purified D. farinae preparation?

The purified *D. farinae* preparation was produced for diagnosis and treatment of house dust mite allergy. An allergen extract should be considered as a pharmaceutical speciality (17). Consequently, it should contain as few non-allergic components as possible (1).

The mechanisms responsible for the results of immunotherapy is still obscure, but the treatment seems at least to be specific (18). Some stimulation of the immune response against the allergens is therefore probably necessary to achieve success.

Generally, the efficacy of immunization using

a complex mixture of antigens is limited due to antigenic competition. Antigens in high concentration and strong immunogens will produce antibodies prior to antigens in low concentration and weak immunogens (22). Thalhimer & Freund (22) have recently demonstrated how to awake antibody production against the weak immunogens in a mixture of antigens by using the isolated, weak immunogens for immunization ("cascade immunization"). If the allergens are the weak immunogens in a complex mixture of antigens, it might be of benefit in immunotherapy to use a preparation of the purified allergens in preference to the complex mixture of antigens. It has also been shown that a preparation of purified timothy allergens is more effective than a crude extract in treatment of timothy allergy (8).

It is demonstrated that the Crude *D. farinae* extract is a complex mixture containing at least 11 allergens and 28 antigens. Thus, both for ethical reasons and in order to increase the efficacy in immunotherapy, a purified *D. farinae* was produced removing material of high molecular weight in addition to the more conventional removal of low molecular weight material. In the present investigation the purified preparation was found to contain less antigens and to be fully representative of the allergens in the Crude *D. farinae* extract. The efficacy of this purified preparation in immunotherapy is now under investigation.

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