

Inhibition Experiments with Solid Phase Mite or Epithelia in House Dust Hypersensitivity

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The present study was undertaken to verify that mites are not the only allergens in house dust extracts and that other allergens such as cat epithelia can also be responsible for house dust hypersensitivities detected both by house dust skin tests and house dust RAST studies. In order to determine whether mite or epithelia fixed on a solid phase could remove not only the IgE antibodies reactive with the homologous allergens, but also the IgE antibodies reactive with house dust allergens, the authors have absorbed 10 sera of house dust allergic patients with solid phase mite or epithelia. The absorption procedure removed a large part of the IgE antibodies reactive with specific immunosorbent (*Dermatophagoides pteronyssinus* or cat epithelia) and in the same way the IgE antibodies reactive with house dust immunosorbent.

The percentage of RAST inhibition varied from 65% to 92% for *Dermatophagoides pteronyssinus* and from 65% to 94% for house dust in patients allergic to house dust and mite; the percentage of RAST inhibition varied from 67% to 92% for cat epithelia and from 73% to 90% for house dust in patients allergic to house dust and cat epithelia. This is in accordance with the hypothesis that house dust is not an allergen *per se*, but rather a complex mosaic of several allergens including mite, animal epithelia, etc.

Key words: cat epithelia; house dust allergy; inhibition experiments; mite.

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The use of purified allergens in both clinical and experimental allergology is being increasingly recognized and advocated. However, the question of house dust allergens identification is not yet resolved. The present study was undertaken to verify that the mite is not the only allergen in commercial house dust extracts and that, for example, animal epithelia can be responsible for house dust hypersensitivity detected both by house dust skin tests or house dust RAST studies. Amongst a wide range of components implicated in house dust allergy, mites, especially *Dermatophagoides pteronyssinus*, have been shown to evoke IgE-mediated allergic responses in house dust allergic patients (1, 2, 3, 4).

However, it seems clear that mite allergens

are not the only allergenic substances in house dust as has been shown both by clinical and *in vitro* studies (5, 6, 7, 8, 9, 10). House dust hypersensitive patients can in fact be sensitized by other allergens. In a previous study (8), we pointed out that among 186 patients with positive reactions to house dust and negative reactions to *D. pt.* 96% reacted to other known allergens, especially animal epithelia. Similar results were found by direct RAST assays: out of 240 house dust sensitive patients with specific IgE to house dust, 204 also had positive RAST results for *D. pt.* The remaining 36 patients had negative RAST for *D. pt.*, but positive RAST for other allergens, with a high incidence for cat dander (29/36).

Our findings supported the hypothesis

that house dust allergic patients could be classified into several groups, two of which we found important: house dust allergic patients with specific IgE to house dust and mite, and house dust allergic patients with specific IgE to house dust and cat dander. In order to determine whether mite or epithelia fixed on a solid phase could remove not only the IgE antibodies reactive with the homologous allergens, but also the IgE antibodies reactive with house dust allergens, we attempted to absorb samples of serum from house dust allergic patients with solid phase mite or epithelia. The absorption procedure was carried out as described previously by Leiferman & Gleich (11) in a study about the cross reactivity of IgE antibodies with various species of grass pollen allergens.

MATERIAL AND METHODS

Sera

Sera were obtained from 10 patients with positive house dust skin tests and RAST results. In each patient the skin reaction was

positive to house dust for the significant threshold dilution (1/50,000 house dust, Institut Pasteur). The RAST results expressed in RAST scores from 0 to 4 were class 2 in five patients, and class 3 in five patients. Among the 10 patients, five had positive skin test and RAST results for *D. pt.* (RAST class 4 in all cases, skin threshold dilution 1/100,000 *D. pt.* extract, Institut Pasteur), five had positive skin test and RAST results for cat epithelia (RAST class 4 for two patients, RAST class 3 for three patients, skin threshold dilution 1/10,000 cat extract, Institut Pasteur).

Allergen discs

RAST allergen discs for house dust, cat epithelium and *Dermatophagoides pteronyssinus* were obtained from Pharmacia Uppsala.

RAST inhibition procedures

Dilution curves To quantitate IgE-type reaginic antibodies in our 10 sera, we determined for each serum the RAST values

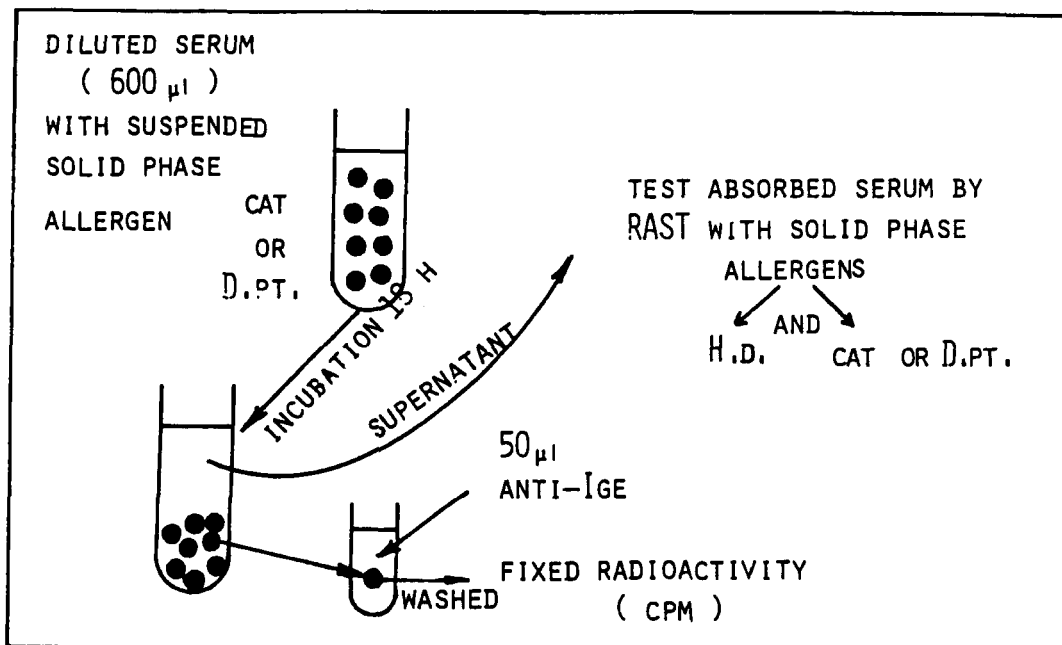


Fig. 1. Procedure of absorption of house dust allergic serum with solid phase cat epithelia or *Dermatophagoides pteronyssinus*

for pure serum and four serial dilutions (1/4, 1/8, 1/16, 1/32). The serum was diluted with RAST diluent PBS (phosphate buffered saline, pH = 7.2). When the suitable dilution was determined, inhibition experiments could be realized.

Protocol employed for the absorption procedure (Fig. 1). Samples of 600 μ l of the suitable dilution of allergic serum were incubated with four or eight solid phase discs coupled to mite or epithelia allergen. The absorption with a greater number of solid phase discs reduced the quantity of supernatant suitable for RAST assays. The tubes were capped and rotated for 18 h. 50 μ l of the supernatant was aspirated and tested by RAST with the homologous allergen *D. pt.* or animal dander, and with house dust extract. In the same experiment, 50 μ l of the diluted native serum was also tested by RAST with the same allergens.

In two cases, repeated absorptions were performed on two sera with specific IgE for house dust and *D. pt.* (Fig. 2): at the first absorption 600 μ l of a 1 to 4 dilution of allergic

serum was added to eight discs and rotated for 18 h. 50 μ l of the supernatant was aspirated and tested by RAST with the homologous allergen *D. pt.* and with house dust extract. If the RAST was still positive, a second absorption with eight discs was made on 300 μ l of the remaining supernatant, and 50 μ l of the second supernatant was tested against by RAST after 18 h incubation. In the same experiment, 50 μ l of the diluted native serum was also tested by RAST with the same allergens.

The RAST response was obtained with a Phadebas reference serum calibrated against the PRIST system using the international IgE standard as reference. The slope of the curve obtained with the reference serum was determined in each experiment. The RAST values of the supernatant are compared with the RAST values of the native diluted serum. The percentage of inhibition was evaluated as following:

% inhibition:

$$\frac{\text{RAST in PRU native serum} - \text{RAST in PRU after absorption}}{\text{RAST in PRU native serum}}$$

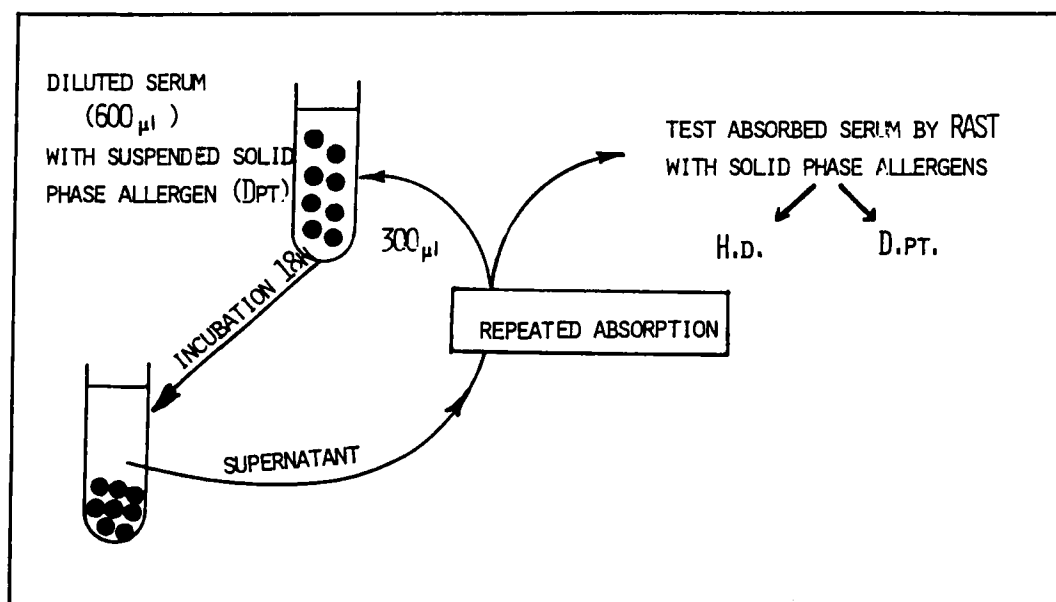


Fig. 2. Procedure for repeated absorption of house dust allergic serum with solid phase *Dermatophagoides pteronyssinus*

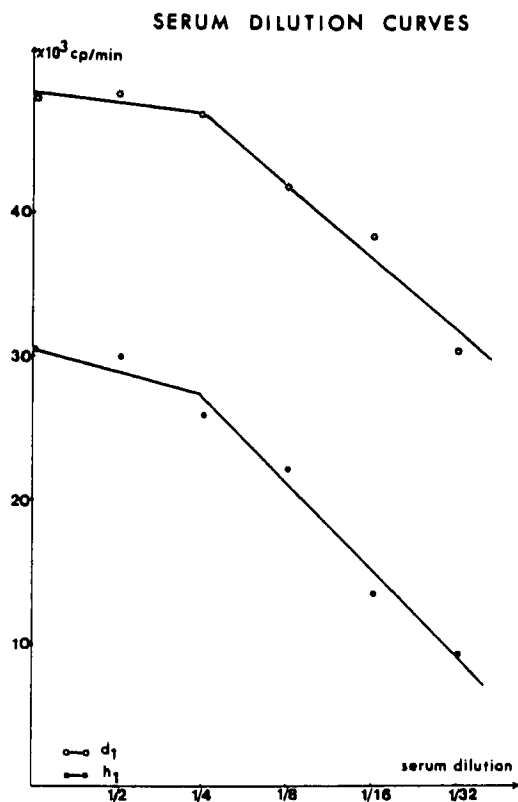


Fig. 3. Serum dilution curves. d1 = *Dermatophagoides pteronyssinus*; h1 = house dust.

0% indicates no inhibition, 100% indicates complete inhibition.

RESULTS

Determination of suitable dilution

As can be seen in Fig. 3, the serum dilution curves for house dust and *D. pt.* show differing segments:

- in the first segments the immunosorbent is saturated: RAST values do not decrease when the serum is diluted.
- with increasing dilution, the percentage of radioactivity bound on the paper discs is nearly proportional to the dilution. Between these two segments of the curve, the transition zone depends on the degree of avidity of the specific IgE. For each patient, we tried to select the first dilution being beyond the antibody excess zone for

the two allergens tested. However, the suitable dilution for *D. pt.* or cat epithelia did not always correspond to the suitable dilution for house dust. When the dilution was beyond the antibody excess zone for *D. pt.* or cat epithelia, the amount of specific IgE for house dust was not still significant, and house dust antibodies absorption could not be evaluated. For this reason, in all cases but one the 1 to 4 dilution was employed rather than a higher dilution (1/8, 1/16), although in some experiments this selected dilution corresponds to the first segment of the curve for *D. pt.* or cat dander extract.

Results obtained in patients allergic to house dust and D. pt.

The results obtained in patient M.A. expressed in PRU are reported in Table 1. The absorption procedure removed a large part of the IgE antibodies reactive with specific immunosorbent *D. pt.* and in the same way the IgE antibodies reactive with house dust. However, the solid phase discs used for the absorption in the experiments with four discs and eight discs are saturated, which means that the absorption procedure was not complete for *D. pt.* in this case. So it seems more suitable to use eight discs in order to maximize the absorption of the homologous antibodies.

Table 2 shows the results obtained in five patients as in the above example. In patient A.N. two independent experiments show similar results both for *D. pt.* RAST inhibition and house dust RAST inhibition.

Results obtained in patient allergic to house dust and cat epithelia

The results obtained in patient S.O. expressed in PRU are reported in Table 3. Table 4 shows the results obtained in five patients as in the above example.

Results obtained with repeated absorption procedure

These indicate that it is possible to improve the degree of inhibition both for *D. pt.* and house

Table 1
Example of *D. pt.* and house dust RAST inhibition by solid phase *D. pt.* in patient M.A.

	RAST <i>D. pt.</i> (PRU)	RAST H.d. (PRU)
Non-diluted serum	35	8
1/4 Diluted serum	50	6.2
Supernatant after incubation with 4 discs	13	1.5
Supernatant after incubation with 8 discs	6.6	1.4
Radioactivity fixed on discs:		
experiments with 4 discs	saturated	saturated
experiments with 8 discs	saturated	saturated
% RAST inhibition	86	77

Table 2
D. pt. and house dust RAST inhibition by solid phase *D. pt.* discs

Patient		RAST <i>D. pt.</i> (PRU)		RAST H.d. (PRU)	
R.I.	1/4 Diluted serum	32		2.7	
	Supernatant after incubation with 8 discs	5		0.32	
	% RAST inhibition	84		88	
U.T.	1/4 Diluted serum	13.5		2.2	
	Supernatant after incubation with 8 discs	3		0.43	
	% RAST inhibition	77		81	
A.N. ^a	1/8 Diluted serum	18	21	1.55	2.4
	Supernatant after incubation with 8 discs	1.75	1.55	0.58	0.84
	% RAST inhibition	90	92	64	65
S.K.	1/4 Diluted serum	52		18	
	Supernatant after incubation with 8 discs	18		1.1	
	% RAST inhibition	65		94	
M.A.	1/4 Diluted serum	50		6.2	
	Supernatant after incubation with 8 discs	6.6		1.4	
	% RAST inhibition	86		77	

^aDouble control

dust RAST (Table 5). In the first case a complete inhibition of the house dust RAST is obtained, in the second the inhibition is improved (73% instead of 65%).

DISCUSSION

These results indicate that *D. pt.* discs were an efficient immunosorbent for house dust IgE

antibodies in many patients allergic to house dust. It appears that in the five patients studied, the majority of the IgE antibodies reacting with house dust are absorbed by *D. pt.* solid phase discs. The percentage of RAST inhibition varied from 65% to 92% for *D. pt.* and from 65% to 94% for house dust. The procedure used could be improved by repeated absorption as studied in two

Table 3
Example of cat epithelia and house dust RAST inhibition by solid phase cat epithelia in patient S.O.

	RAST cat epithelia (PRU)	RAST H.d. (PRU)
Non-diluted serum	43	25
1/4 Diluted serum	34	17
Supernatant after incubation with 4 discs	24	9.2
Supernatant after incubation with 8 discs	11	2.3
Radioactivity fixed on discs		
experiments with 4 discs	saturated	saturated
experiments with 8 discs	saturated	saturated
% RAST inhibition	68	86

Table 4
Cat epithelia and house dust RAST inhibition by solid phase cat epithelia

Patient		RAST cat epithelia (PRU)	RAST H.d. (PRU)
S.O.	1/4 Diluted serum	34	17
	Supernatant after incubation with 8 discs	11	2.3
	% RAST inhibition	68	86
S.A.	1/8 Diluted serum	11.5	16.5
	Supernatant after incubation with 8 discs	1.45	4.4
	% RAST inhibition	87	73
H.U.	1/4 Diluted serum	11.5	6
	Supernatant after incubation with 8 discs	1.55	0.6
	% RAST inhibition	87	90
T.R.	Non-diluted serum	7	1.8
	Supernatant after incubation with 8 discs	2.3	0.3
	% RAST inhibition	67	83
L.I.	Non-diluted serum	11	3
	Supernatant after incubation with 8 discs	0.8	0.6
	% RAST inhibition	92	80

patients. In one patient (A.N.), despite an almost complete inhibition for the homologous allergen *D. pt.*, the degree of inhibition for house dust was only 64%. It can then be assumed that this patient's serum contained antibodies directed against other allergenic components of house dust extract.

On the other hand, our findings show also that animal epithelia discs can also be an efficient immunosorbent for house dust IgE antibodies in some patients with house dust hypersensitivity. The percentage of inhibition for house dust is always greater than 70% when absorbed with animal epithelia. Weeke

& Loewenstein (9), using the crossed radio-immunoelectrophoresis, could demonstrate similar findings: specific IgE binding to the animal dander antigens occurred in sera which gave positive RAST and skin tests to both animal dander and house dust. The hypothesis that house dust is always associated with other positive RAST has been suggested by direct RAST studies made by other authors: according to Virchow (6) the offending allergens in patients with "specific" house dust IgE were mite or cat dander. Similarly, Johansson (12) has pointed out that house dust was found in

Table 5

Dermatophagoides pteronyssinus and house dust RAST inhibition by repeated absorption with *D. pt.* solid phase

		<i>D. pt.</i> RAST (PRU)	H.d. RAST (PRU)
Patient			
M.A.	1/4 Diluted serum	50	14.5
	First supernatant after incubation with 8 discs	20	2.2
	% RAST inhibition	60	85
	Second supernatant after second incubation with 8 discs	5	0.5
	% RAST inhibition	90	97
A.N.	1/8 Diluted serum	21	2.4
	First supernatant after incubation with 8 discs	1.55	0.84
	% RAST inhibition	92	65
	Second supernatant after second incubation with 8 discs	0.6	0.64
	% RAST inhibition	97	73

close association with the animal dander cluster.

Preliminary studies using absorption procedures have been reported by Aalbersee et al. (10): these authors have been able to inhibit house dust RAST by absorption with soluble allergen extracts. After preincubation with either mite extract or cat dander extract, they found that inhibition was just as good as preincubation with house dust soluble extract. They could also inhibit cat and mite RAST with house dust soluble allergens. We preferred to use extracts made insoluble by coupling on to paper discs; in contrast to the soluble allergen technique, the absorption of specific IgE is not dependent on the ratio between the antigen and antibody concentrations.

Our previous findings, using skin tests and direct RAST studies, gave no answer to the question whether antigens in house dust have only cross-reactivity with animal dander, mite or other allergens. These absorption experiments showing a complete removal of house dust IgE antibodies, when the responsible allergen is used as immunosorbent, are in accordance with the hypothesis that house dust is not an allergen *per se*, but rather a complex mosaic of several allergens including mites and animal epithelia. Our experiments do not exclude the existence of

allergens other than those mentioned in our study, which can also be found in house dust.

Rather than searching for highly purified house dust allergens, it appears to us that what is necessary is to determine the exact allergens responsible for house dust hypersensitivities. This determination is of importance clinically to avoid wrong diagnosis of the responsible allergen: careful investigations including a panel of allergens varying according to the allergen frequency profile of the local region, both for skin tests and *in vitro* tests, could aid towards a more specific diagnosis. It seems unreasonable to maintain immunotherapy with house dust extract when it would be more suitable to use the allergenic preparation corresponding to the real offending allergens.

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