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MITES AND HOUSE DUST ALLERGY

By

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INTRODUCTION

Despite many publications in favour of an identity of the *Dermatophagoïdes pteronyssinus* mite and the house dust allergen or at least a very close association of this mite with the allergenicity of house dust (*Voorhorst et al.* (15, 16, 17), *Spieksma* (13), *Bachofen et al.* (1), *Maunsell et al.* (10) and *Pepys et al.* (12)), the final proof of this is still lacking.

This proof would be the demonstration of a chemical and immunological identity between the two allergens after isolation of the specific mite allergen. This condition has not been satisfied so far. The idea of identity or close relationship between the two allergens has only come from the results of skin tests. These are however far from ideal for this purpose. This may be illustrated by the example of the allergens from old kapok and old feathers. Whereas *Voorhorst* (18), working with skin tests, came to the conclusion that old feathers and kapok contain the same allergenic factor as house dust, *Berrens et al.* (3, 4) concluded after the chemical isolation of the specific allergens, that the allergens in house dust, old kapok and old feathers are distinctly different from each other, though frequently giving rise to cross-reactions in the skin test, as do many inhalant allergens.

We too started our work with mites and house dust using skin tests, when the „Haarlems Allergenen Laboratorium“

("H.A.L.") kindly supplied us with extracts of *D. pteronyssinus*, which had been prepared as described by *Spieksma* (13).

Our next step was the performance of inhalation provocation tests with extracts of *D. pteronyssinus* in patients who showed positive skin reactions to this extract. Little has been published so far about the results of such inhalation provocation tests.

Bachofen et al. (1) performed these tests in 20 asthmatic subjects, eight of whom gave asthmatic reactions to inhalation tests with *D. pteronyssinus* and two only to the house dust extract.

Pepys et al. (12) found in 5 asthmatic subjects an immediate asthmatic reaction to an inhalation test with *D. culinae*. Inhalation tests with house dust extract in 4 of these asthmatic reactors gave only an asthmatic reaction in 1 patient.

Miyamoto et al. (11), working with *D. farinae*, carried out inhalation provocation tests in 6 patients who had positive reactions to inhalation of house dust extract. All exhibited positive reactions with inhalation of the mite extract.

So the inhalation provocation test with extracts of mites and house dust seemed to give a less close correlation between the results with both extracts as do the skin tests, the number of these inhalation tests however is too small to draw definite conclusions. Because we believe that the inhalation provocation test is more specific than the skin test and so will be more useful in the differentiation between two allergens (*Bangma* (2), *Young* (20)), we performed inhalation provocation tests with *D. pteronyssinus* extract in 56 patients.

Finally we made a control experiment by comparing the mite culture extract with an extract made from the culture medium alone without mites.

These experiments had been done before by *Spieksma* (14), but he did skin tests only with an extract from the medium made before and not after incubation in a climate cabinet.

In our experiment however we did skin tests with an ex-

tract made after incubation of the medium in a climate cabinet, just as if one would have cultured mites on it.

MATERIAL AND METHODS

Skin tests were made by the intradermal method in a series of dilutions of each test extract. Extracts of *D. pteronyssinus* had been prepared as described by *Spieksma* (13). It were extracts of fullgrown cultures of these mites, derived therefore not only from the mites but also from the nutrient medium. With such extracts also the original work of *Voorhorst* (15, 16, 17), and *Spieksma* (13) had been done.

House dust and human dandruff extract for skin tests were commercial extracts obtained from the "Haarlems Allergenen Laboratorium" ("H.A.L."). The strength of all extracts is given on a weight/volume basis. Inhalation provocation tests were performed with nebulized extracts of allergens (10 minutes by spray with an airflow of 6 L./min.) and vital capacity (V.C.) and forced expiratory volume in one second (FEV₁) were measured before and after spraying (resp. 4, 8, 12, 16 and 20 minutes after). The reaction was called strongly positive (++), if the values of V.C. and/or FEV₁ diminished gradually 10 per cent or more within 20 minutes after spraying (*ten Cate* (7)). The reaction was called positive (+), if after getting no reaction immediately after the test, the patient had asthma during the following night and more than 10 per cent reduced V.C. and FEV₁ in the morning of the next day, a so-called late positive reaction.

The following concentrations were used for the inhalation tests: purified house dust extract, according to the principles of *Berrens & Young* (6), commercially obtained from "Nourypharma" in a concentration of 50.000 Units/ml (for definition of these Units see *Berrens & Young* (6)); *D. pteronyssinus* culture extract 0.01 per cent, commercially obtained from the "H.A.L."; human dandruff extract 5 per cent from the "H.A.L.". Histamine provocation tests were performed by inhalation of increasing concentrations of histamine phosphate by spray for 30 seconds with an airflow of 6 L./min. Reduction of V.C. and/or FEV₁ of 10 per cent or more, immediately after inhalation, was called a positive reaction (*de Vries et al.* (19)).

In our control experiment with culture medium extract the technical part was performed at the "Haarlems Allergenen Laboratorium". Twelve cups containing nutrient medium (*Spieksma* (13)) had been sterilized for one hour at 100 °C. After one week three cups appeared to be contaminated with moulds; these were discarded.

Four of the other cups were then inoculated with *D. pteronyssinus*, the other five remaining closed. All cups were kept in the climate cabinet (*Spieksma* (13)) under weekly control. After 4 months extracts were made from both samples, as described by *Spieksma* (13). These extracts were tested by means of intracutaneous injections in 17 patients.

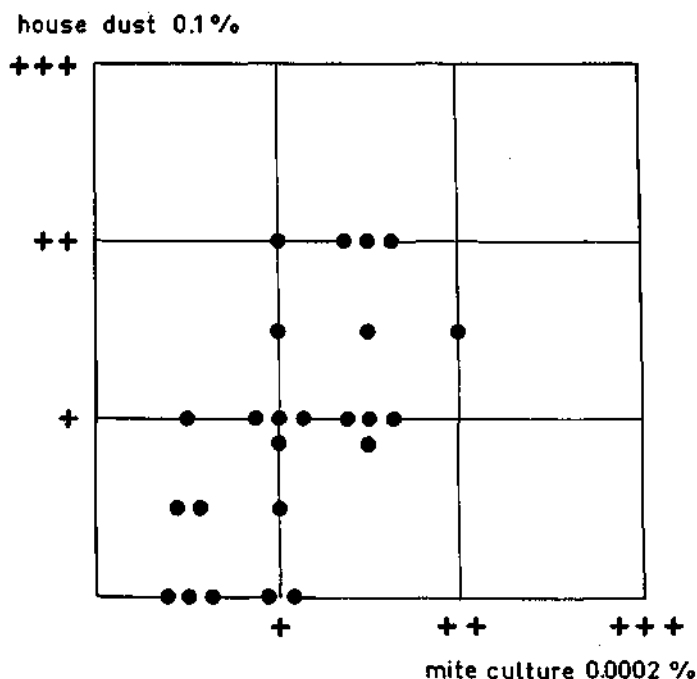


Fig. 1.

Comparison of skin reactions to extracts of house dust and *D. pteronyssinus* culture.

RESULTS

Intracutaneous tests were done in a concentration of 0.002 per cent, 0.0002 per cent and 0.00002 per cent of mite cultures (see Voorhorst *et al.* (17)) in 126 atopic patients.*

These patients were also tested with house dust and human dandruff extracts from the "H.A.L." in a concentration of 1 per cent, 0.9 per cent and 0.01 per cent. Non-atopic individuals generally showed negative reactions to all extracts (including those of the mites).

All of the 126 atopic patients showed positive reactions to one or more of these extracts. From a group of 50 patients with a positive skin reaction to 0.1 per cent house dust and/or

* As defined by Coca (8).

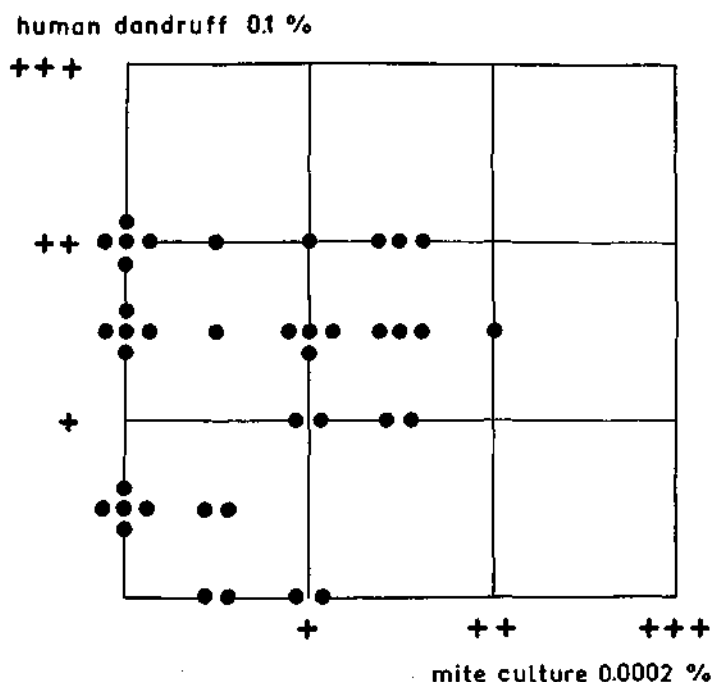


Fig. 2.

Comparison of skin reactions to extracts of human dandruff and *D. pteronyssinus* culture.

0.0002 per cent mite culture, 45 patients showed a correlation between the reactions to these two allergens (on both a one or more + reaction) and 5 others showed no such correlation.

From a group of 80 patients with a positive skin reaction to 0.1 per cent human dandruff and/or 0.0002 per cent mite culture, 49 per cent showed a correlation between the reactions to these allergens (on both a one or more + reaction), and 31 others showed no such correlation. In Fig. 1 and 2 some of these results are shown in a diagram; in Fig. 1 the strength of the skin reactions to house dust extract in a concentration of 0.1 per cent is plotted against the strength of the reaction to the mite culture extract in a concentration of 0.0002 per cent in 24 patients, each dot representing one patient.

In Fig. 2 mite culture extract and human dandruff extract are compared in the same way.

From the above findings a fairly good correlation seems to be present between the skin reactions to the extracts of house dust and mite culture, but not between the reactions to the extracts of human dandruff and mite culture.

The latter is important because *D. pteronyssinus* mites generally are cultured on media containing human dandruff, which allergen could possibly have influenced the results of the skin tests with mite culture extracts.

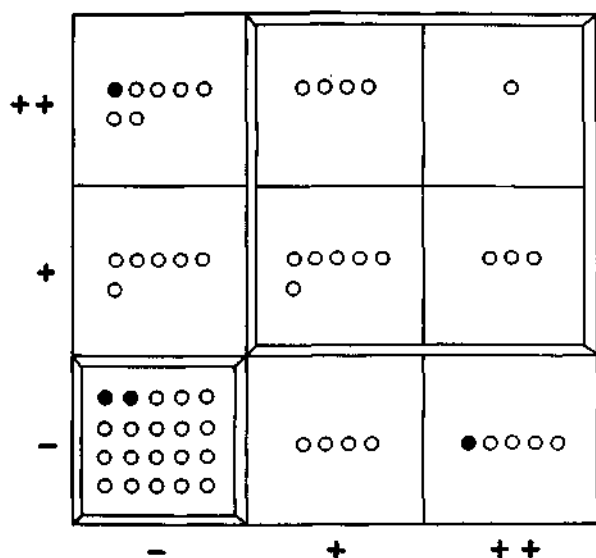
Secondly 56 asthmatic patients with positive skin reactions to *D. pteronyssinus*, house dust and human dandruff extract were tested by means of inhalation provocation tests with these allergens.

Twenty patients showed a negative reaction to house dust and *D. pteronyssinus* as well. From the remaining thirty-six patients twenty-seven showed a positive reaction ($++$ or $+$) to house dust and twenty-three to *D. pteronyssinus*. Only fourteen patients showed a correlation between the reactions to these allergens (on both a one or more $+$ reaction). The other twenty-two showed no such correlation (Fig. 3). Only in four patients there was a positive reaction by inhalation to human dandruff, but no correlation existed in these cases with the reactions to the two other allergens.

Because it would be possible that part of the positive reactions had not been typical allergic reactions, but had to be considered as aspecific reactions of a bronchial mucosa with a high degree of hyperreactivity, we measured the rate of bronchial hyperreactivity by means of the histamine provocation test (*de Vries* (15)).

As expected all fifty-six asthmatic patients except one, showed a certain rate of bronchial hyperreactivity with a reaction to inhalation of histamine phosphate concentrations < 32 mg/ml (*Kreukniet & Young* (9)).

There is more chance to have an aspecific positive reaction to inhalation of allergens, if there exists a reaction to low histamine phosphate concentrations (0.25–1 mg/ml).

house dust 50.000 μ /ml

D. pteronyssinus culture 0.01%

● = positive reaction to human dandruff 5%

Fig. 3.

Results of inhalation provocation tests in 56 asthmatic patients to house dust, D. pteronyssinus and human dandruff.

The reactions to house dust are plotted against the reactions to D. pteronyssinus, each dot representing one patient.

The points inside the quadrants, circumscribed by double lines, are of patients with either a positive or a negative reaction to both house dust and D. pteronyssinus extract.

In Fig. 4 the histamine phosphate reactions are compared for the group of patients with positive inhalation tests to D. pteronyssinus and negative to house dust (Group I), the group of patients with positive reactions to house dust and negative to D. pteronyssinus (Group II) and the group of patients with positive reactions to house dust and D. pteronyssinus (Group III). The histamine phosphate concentrations at which a positive reaction was obtained were averaged for each group. It was found that a reaction occurred in group I at a lower

concentration (1,7 mg/ml) than in group II (3,1 mg/ml) and in group III (3,2 mg/ml). The difference is not significant. So the chance to a non-specific positive reaction on inhalation of allergen extracts is not essentially different in the three groups.

Histamine concentration	Group I house dust — D. pteron. + or ++ Number of pat.	Group II house dust + or ++ D. pteron. — Number of pat.	Group III house dust + or ++ D. pteron. + or ++ Number of pat.
0,25 mg/ml	1	1	—
0,50	2	—	2
1,0	2	4	—
2,0	2	2	4
4,0	2	4	7
8,0	—	2	1
16,0	—	—	—
32,0	—	—	—
mean histamine concentration	1,7 mg/ml	3,1 mg/ml	3,2 mg/ml

Fig. 4

Results of histamine phosphate provocation tests in 36 asthmatic patients with pos. inhalation tests either to house dust or to *D. pteronyssinus*.

Finally we made the control experiment with incubated culture medium extract.

In this experiment extracts were tested by means of intracutaneous injections in 17 patients in a concentration of 0.002 per cent, 0.0002 per cent and 0.00002 per cent with the mite culture and of 0.2 per cent, 0.02 per cent and 0.002 per cent with the medium without mites.

Moreover these patients were tested with house dust and human dandruff both in a concentration of 1 per cent, 0.1 per cent and 0.01 per cent ("H.A.L.").

The results are given in Fig. 5.

From Fig. 5 it is apparent that also with the culture medium (without mites) positive skin reactions can be obtained, which seem to be generally indistinguishable from those with

Patients	house dust			human dandruff			mite culture			medium		
	1 %	0.1 %	0.01 %	1 %	0.1 %	0.01 %	0.002 %	0.0002 %	0.00002 %	0.2 %	0.02 %	0.002 %
1.	++	+	—	++	±	—	+	±	—	+	—	—
2.	+++	++	+	++	++	+	++	+	±	++	+	—
3.	++	+	±	++	+	+	—	—	—	++	+	—
4.	—	—	—	+	—	—	—	—	—	—	—	—
5.	++	++	+	++	+	±	++	+	±	++	+	—
6.	++	+	±	++	±	±	+	+	—	+	±	—
7.	+++	++	+	++	++	+	++	+	—	++	+	±
8.	—	—	—	—	—	—	—	—	—	—	—	—
9.	—	—	—	—	—	—	—	—	—	—	—	—
10.	++	++	+	++	++	+	++	+	—	++	+	—
11.	++	+	±	++	+	—	+	±	—	+	+	±
12.	+++	++	+	++	++	+	++	++	+	++	+	—
13.	+++	++	+	++	++	+	++	++	+	++	+	±
14.	++	++	+	++	+	+	++	+	±	+	+	±
15.	++	+	+	++	+	—	++	+	+	+	+	—
16.	—	—	—	++	+	±	±	—	—	±	—	—
17.	—	—	—	++	+	—	±	—	—	±	—	—

Fig. 5.

Results of skin tests with extracts from culture of *D. pteronyssinus*, nutrient medium (without mites), house dust and human dandruff in 17 patients.

the mite culture extract provided the former extract is tested in a 100 fold concentration. Non-atopic individuals gave negative reactions to both extracts.

Unfortunately we did not test the culture medium before incubation in the climate cabinet, but afterwards we made this experiment by testing an extract from culture medium which had not been incubated. We then found only a comparable allergenic activity in it, if tested in ten-fold stronger concentrations as mentioned above for the incubated culture medium.

DISCUSSION

The results of our skin tests are in accordance with those of *Maunsell et al.* (10), who found that 94 per cent of the house dust sensitive patients gave skin reactions to *D. pteronyssinus* extracts.

Voorhorst et al. (17) found such a correlation even in 100 per cent of cases, but this seems to be just a too high degree of correlation for a biological experiment.

The results of our inhalation provocation tests which fail to show such a high degree of correlation between tests with mite culture extracts and house dust extracts, in our opinion, strongly suggest that there is a close relationship between these two allergens but not an identity.

How can the positive skin reactions to the incubated culture medium be explained?

Certainly not by the presence of human dandruff allergen in the medium, for according to *Voorhorst* (16) the 0.02 per cent test extract of culture medium, which still gives clearly positive skin reactions in some of our patients, contains only 0.01 per cent human dandruff and this cannot account for the positive reactions, because the human dandruff had been extracted for two days by a salt solution containing 0.5 per cent phenol to remove the allergen in it before addition to the culture medium. In this way one may expect to remove about 90 per cent of the allergen. So the finding of fairly strong positive

reactions to the culture medium after incubation must be considered as a very remarkable one. It seems likely to us that these positive reactions might be explained by assuming that the medium could have been altered chemically during incubation, so as to become more allergenic.

A Maillard reaction in the culture medium might play an important role in this respect, as it is an essential step in the formation of most, if not all, inhalant allergens (*Berrens (5)*).

The presence of mites would possibly enhance this Maillard reaction so as to form more allergen, as can be seen in Fig. 5.

If this is so, the next step would be then to investigate whether this would be a specific function of the *D. pteronyssinus* mite or this could have been accomplished also by other mites or even other (micro)-organisms, such as moulds and bacteria.

Anyhow, in the case of *D. pteronyssinus* culture the result seems to be an allergen closely related to house dust but not identical with it.

SUMMARY

Skin tests show a correlation of the reactions to the allergens of house dust and *D. pteronyssinus* in about 90 per cent of patients.

Inhalation provocation tests with these allergens however fail to show such a high degree of correlation.

Skin tests with the culture medium of the mites were compared to those with the mite culture.

In this latter series of experiments clearly positive reactions were to be found also to the culture medium, provided the latter had been tested in a higher concentration.

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