

# Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes, 1961) and house dust as a causative antigen in bronchial asthma

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*Since various kinds of mites are contained in house dust in significant numbers, the possibility of antigenic similarity between house dust and the mites in it was investigated. *D. farinae*, purely cultivated in our laboratory, was used in this study. Although it makes up only 4 per cent of the total mite population in house dust, very close correlation between house dust extract and mite extract was found in skin tests. Most of the antigenic substance stays inside of the Visking tube after dialysis, but a certain amount was dialyzed out. All cases with positive inhalation provocation tests with house dust extract reacted positively to the mite extract. Skin-sensitizing antibody was detected against the mite in sera from the patients allergic to house dust. Skin-sensitizing antibody was almost completely neutralized with house dust extract against the mite extract and vice versa, in vitro as well as in vivo. From these results, it was concluded that the mite is one of the major antigenic substances in house dust, and mites most likely have cross-antigenicity with each other.*

House dust is one of the most important causative antigens in bronchial asthma and allergic rhinitis in Japan. The incidence of positive skin reactions with house dust extract (1:1,000 by weight) is 50 to 70 per cent in asthmatic patients. About half of the patients show a reduction of one-second forced expiratory volume (FEV<sub>1</sub>) after inhalation of house dust extract.<sup>1-3</sup> The incidence of positive skin reaction to house dust is less than 20 per cent in normal subjects. Since house dust is a mixture of various materials, such as detritus of foods, molds, human and animal danders, bacilli, various kinds of fibers, etc., it is a natural corollary to wonder which material is the major

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allergen in house dust. Investigations have been carried out to clarify this point, but the results were not satisfactory until recently. In 1964, one of our coauthors (S. O.<sup>4</sup>) found independently of R. Voorhorst and his co-workers<sup>5</sup> that house dust contained a significant number of mites regardless of the source of the dust. Consequently, it was suspected that mites might be the major allergen in house dust, and allergic investigation was started to clarify the allergenic similarity between house dust and mites living in house dust.

## MATERIALS

### House dust extract

Commercially available house dust extract from Torii Pharmaceutical Company was employed as house dust antigen in this study. In intracutaneous skin testing, 1:1,000 dilution of house dust extract has been used as a standard solution in Japan. Commercial house dust extract was prepared from the mixed house dusts collected by electric sweeper from various parts of Japan. Total nitrogen and protein nitrogen of the 1:10 dilution of house dust extract were 36.49 and 17.63  $\mu\text{g}$  per milliliter, respectively, as determined by a reported method.<sup>6</sup>

### Mite extract

As shown in Table I, various kinds of mites are contained in house dust, but mites of the genus *Dermatophagoides* are the major mite in house dust. R. Voorhorst and his co-workers<sup>5, 7</sup> employed *D. pteronyssinus* in their studies, but *D. farinae*<sup>8</sup> was employed by us since *D. farinae* could be obtained in pure culture in our laboratory, while *D. pteronyssinus* could not at that time. Cultivated material was shaken with ether for one minute and sieved through several meshes (Nos. 60, 100, 150, and 200) and filter paper. Most of the mites were retained on mesh Nos. 100 and 150. Each *D. farinae* was separated from the crude materials obtained by means of sieving, one by one, under the

**Table I.** Average numbers of mites in 1 Gm. of mixed house dust collected for house dust extract in Japan

| Species                                      | No.   | %    |
|----------------------------------------------|-------|------|
| <i>Dermatophagoides</i> Bogdanoff            | 457   | 43.3 |
| <i>Dermatophagoides pteronyssinus</i> (Trt.) | 287   | 27.2 |
| <i>Dermatophagoides passericola</i> Fain     | 92    | 8.7  |
| <i>Dermatophagoides farinae</i> Hughes       | 45    | 4.3  |
| Indeterminable <i>Dermatophagoides</i>       | 33    | 3.1  |
| <i>Euroglyphus maynei</i> (Cooreman)         | 2     | 0.2  |
| Glycyphaginae                                | 335   | 31.8 |
| Tyroglyphinae                                | 38    | 3.6  |
| Trombidiformes                               | 146   | 13.9 |
| Oribatei                                     | 29    | 2.8  |
| Mesostigmata                                 | 17    | 1.6  |
| Indeterminable Acari                         | 30    | 2.8  |
| Total Acari                                  | 1,054 | 100  |

Note: Numbers of mites were counted from 9 Gm. of house dust and averaged per 1 Gm. It was found that the number of mites exceeded 2,000 per gram in some house dust.

microscope,<sup>9</sup> and the extract was made in the same way as house dust antigen. Nondialyzed, dialyzed, and dialysate of dialyzed extract of *D. farinae* were used in skin testing. Protein nitrogen of 1:100 dilution of dialyzed mite extract was 20.60  $\mu$ g per milliliter.

#### **Extract of excreta of mite**

The material sieved through mesh No. 200 in the treatment just mentioned was found mostly to be the excreta. This was used for extraction. Extract was made in the identical way as house dust and dialyzed. Protein nitrogen of 1:100 dilution of dialyzed excreta extract was 31.83  $\mu$ g per milliliter.

#### **Dried Daphnia extract**

Dried Daphnia (commercially available as a food for tropical fish) was used as nutrition for the mite. Mites were not found in dried Daphnia by microscopic examination. Extracts of Daphnia were prepared and employed in 1:1,000 and 1:10,000 dilutions for skin testing.

### **SUBJECTS**

Forty-two normal and 55 asthmatic subjects (45 males and 52 females) were used in this investigation. The normal subjects were inpatients with diseases other than asthma. Their ages ranged from 18 to 63. None of these asthmatic patients had ever been hyposensitized with house dust previously.

### **METHODS**

#### **Skin test**

A series of twofold dilutions of house dust extract was made from the 1:1,000 concentration, and nondialyzed, dialyzed, and dialysate of mite extract were similarly diluted from 1:10,000. Dialyzed extract of the excreta of the mite was also diluted from 1:10,000. Prior to making the dilutions, the dialysate was concentrated to the original volume of extract. Each of the subjects was intracutaneously injected with 0.02 ml., and the maximum dilution which elicited positive skin reactions was considered to be the end point. Correlation coefficients of end points were calculated among the subjects who showed positive reactions to both house dust and mite extract. Thus correlations of skin reactions between them were compared. In the calculation,  $n$  of  $2^n \times 1,000$  (or  $\times 10,000$ ) of the end point was used.

#### **Inhalation provocation test**

Six asthmatic subjects who showed positive reactions to skin tests and inhalation provocation tests with house dust extract were subjected to the same procedure with mite extract. In addition, two asthmatic patients with strongly positive skin reactions to house dust extract who had not been subjected to inhalation provocation tests with house dust extract were challenged by inhalation with mite extract. The provocation test was carried out in the following way: After determination of the one-second forced expiratory volume ( $FEV_1$ )

as a base line, 0.5 ml. of saline was inhaled by the patients through a nebulizer (Vaponephrine type) with 6 L. per minute of flow, and FEV<sub>1</sub> was measured following this as a control. Then 0.5 ml. of certain concentrations of antigen extract was inhaled by the patients, and FEV<sub>1</sub> was determined 5, 12, and 20 minutes thereafter. Airway obstructive changes were estimated from the following formula:

$$\frac{\text{FEV}_1 \text{ after saline inhalation} - \text{FEV}_1 \text{ after inhalation of antigen}}{\text{FEV}_1 \text{ after saline inhalation}} \times 100$$

#### **Prausnitz-Küstner reaction<sup>10</sup>**

Sera were obtained from the patients with skin-sensitizing antibody against house dust, and two series of P-K sites were made on each recipient with a series of twofold dilutions of the sera. Antigens (1:1,000 dilution of house dust extract and 1:10,000 dilution of mite extract) were injected into each series of P-K sites 48 hours later. The maximum dilution of serum which elicited a positive reaction was considered to be the titer of skin-sensitizing antibody, which was compared with both antigens.

#### **Neutralization of skin-sensitizing antibody**

Two methods were employed.

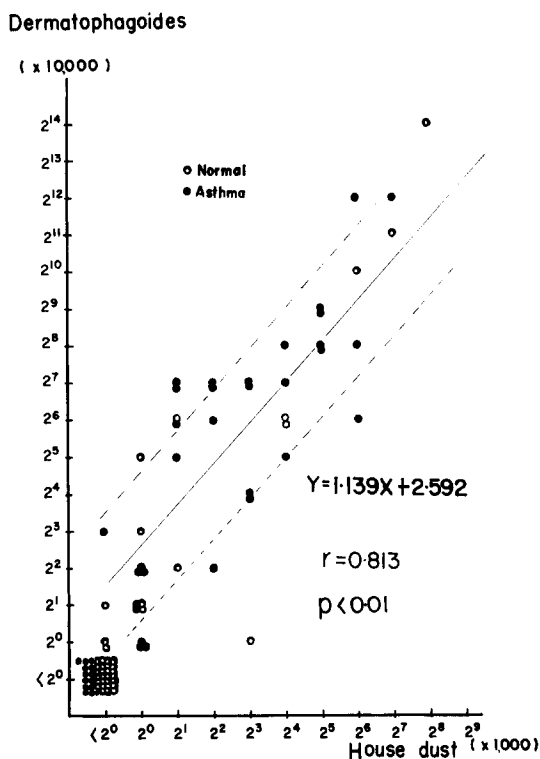
1. *In vitro neutralization.* Three aliquots of allergic serum were mixed with the same amount of house dust extract (1:20 dilution), mite extract (1:200 dilution), and saline, respectively. These were incubated at 37° C. for 1 hour, and a series of P-K sites was made on the normal recipient with twofold dilutions of each sample. Forty-eight hours later house dust extract (1:1,000) was given intracutaneously into the two series of P-K sites made from serum mixed with mite extract or saline. Mite extract (1:10,000) was injected into two series of P-K sites made from serum mixed with house dust extract or saline. This *in vitro* neutralization test of skin-sensitizing antibody was carried out with three different sera. In this neutralization study, a 1:20 dilution of house dust extract and 1:200 mite extract were employed because a preliminary study indicated that these concentrations appeared to provide excess antigen.

2. *In vivo neutralization.* Six series of P-K sites were made in the same recipient with a series of twofold dilutions of allergic sera. House dust extract (1:20) and mite extract (1:200) each were injected into two series of P-K sites 48 hours later. Seventy-two hours thereafter, when local reactions had completely disappeared, house dust extract (1:1,000) was given at a series of P-K sites where mite extract had been injected previously and at a series of nontreated P-K sites. Mite extract (1:10,000) was administered to a series of house dust-preinjected P-K sites and a series of nontreated P-K sites. Furthermore, house dust extract (1:1,000) and mite extract (1:10,000) were injected at the series of P-K sites which had been preinjected with the same antigen, to ascertain the neutralization of skin-sensitizing antibody by each antigen. Two sera were employed for *in vivo* neutralization tests.

**RESULTS****Skin tests**

1. *Relation between house dust extract and nondialyzed mite extracts.* In 46 asthmatic and 42 normal subjects, 31 (67.4 per cent) and 12 (28.5 per cent) showed positive reactions to a 1:1,000 dilution of house dust extract and 32 (69.6 per cent) and 15 (35.7 per cent) to a 1:10,000 dilution of mite extract, respectively. All subjects who showed a positive reaction to house dust invariably reacted to mite. Only 4 subjects had a positive test to mite but negative to house dust. End points of skin reactions among the subjects with positive skin tests to both house dust and mite showed a linear relation, and the correlation coefficient between them was 0.813, thus showing a good correlation (Fig. 1). Antigenic potency of mite was calculated to be 40 to 130 times stronger than house dust from the skin test results.

2. *Relation between house dust extract and dialyzed mite extract.* The results were very similar to the relation between house dust extract and nondialyzed mite extract. All 27 subjects who showed a positive reaction to 1:1,000 dilution of house dust extract showed positive reactions to 1:10,000 dilution of dialyzed

**Fig. 1**

Results of skin tests with house dust and mite extracts. The  $< 2^0$  indicates negative skin reaction with 1:1,000 dilution of house dust extract or 1:10,000 dilution of mite extract.

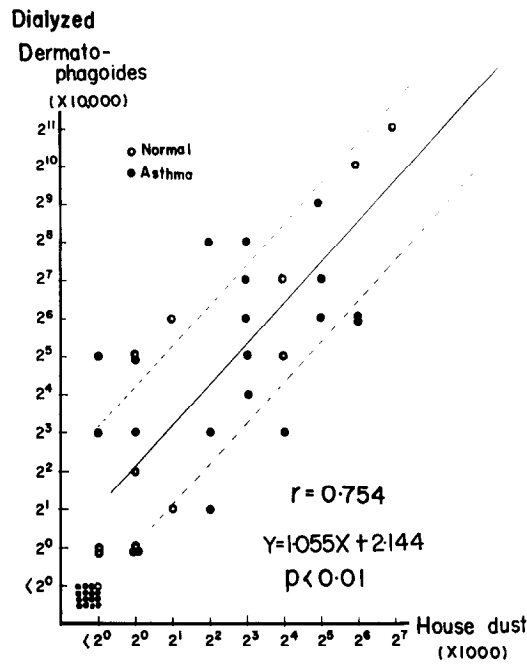


**Fig. 2**  
Results of skin tests with house dust and dialyzed mite extract.

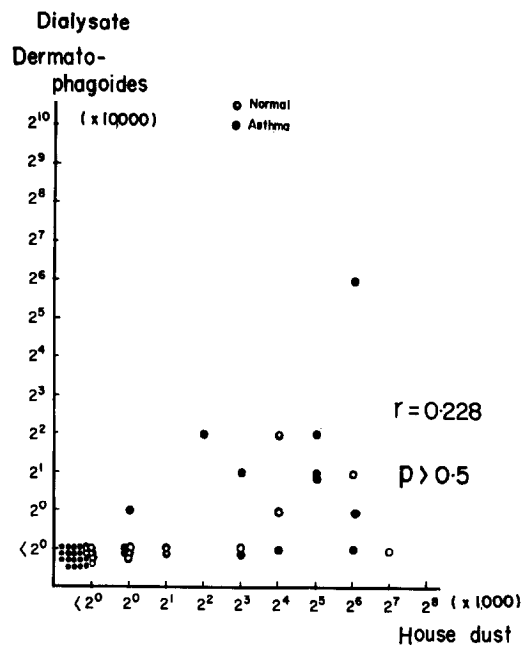


**Fig. 3**  
Results of skin test with house dust extract and dialysate of dialyzed mite extract.

mite extract. Four subjects were positive to dialyzed mite extract but negative to house dust extract. The correlation coefficient of end point among subjects positive to both was 0.754 (Fig. 2).

3. *Relation between house dust extract and dialysate of dialyzed extract of mite.* Antigenic material was detected in dialysate of mite extract, but its potency was quite low, and a significant number of subjects were positive to house dust extract but negative to dialysate. Correlation coefficient was 0.228. This lower degree of correlation is probably due to the fact that a part of the antigenic material in mite extract is small in molecular weight and dialyzed out from the Visking tube (Fig. 3).

4. *Relation between inside fluid of dialyzed mite extract and nondialyzed mite extract.* All 16 subjects who were negative to dialyzed extract were negative to nondialyzed extract, and all 26 subjects who were positive to dialyzed extract were positive to nondialyzed extract. Correlation coefficient of the end points of positive skin tests was 0.963 (Fig. 4).

5. *Relation between dialysate of dialyzed mite extract and nondialyzed mite extract.* Antigenic potency of dialysate was quite low in comparison with nondialyzed mite extract. Seventeen subjects were positive to nondialyzed extract but negative to dialysate. Correlation coefficient was 0.582 (Fig. 5).

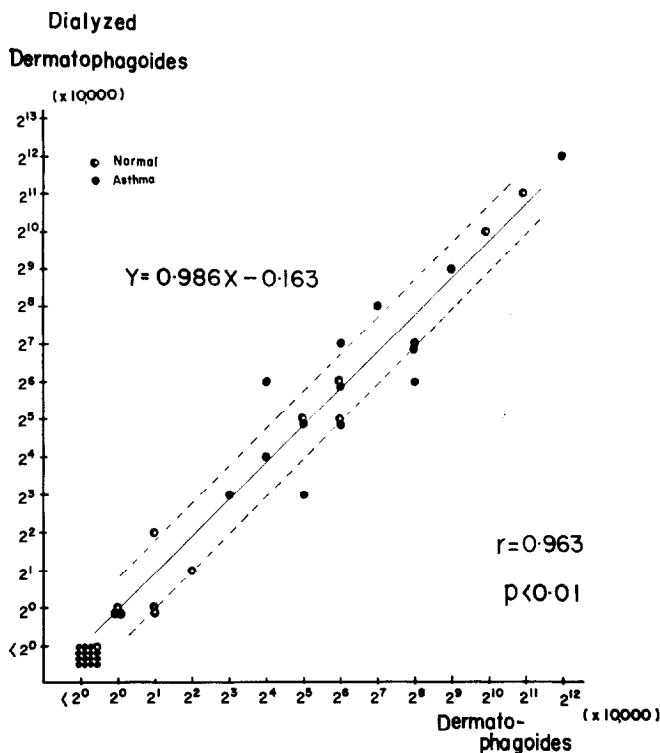


**Fig. 4**

Results of skin test with dialyzed mite extract and nondialyzed mite extract.

6. *Relation between inside fluid and dialysate of dialyzed mite extract.* Obviously, the results were very similar to the relationship between house dust extract or nondialyzed mite extract and dialysate of dialyzed mite extract, as shown in Fig. 6.

7. *Relation between extract of excreta of mite and dialyzed mite extract.* Skin tests were carried out in 10 asthmatic patients with positive skin tests to mite extract. A very close antigenic relationship between them was observed, and the correlation coefficient was 0.957. This is probably due to the fact that the excreta of mite is almost identical with body of mite in antigenicity (Fig. 7).

8. *Dried Daphnia.* Almost all of the subjects showed negative skin reactions to Daphnia extract. Two of the subjects, however, whose skin was highly sensitive to both house dust and mite were also positive to a 1:10,000 dilution of Daphnia extract (Table II).

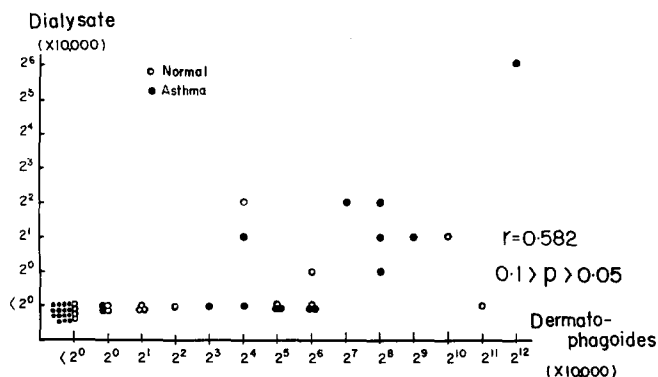


**Fig. 5**

Results of skin test with dialysate of dialyzed mite extract and nondialyzed mite extract.

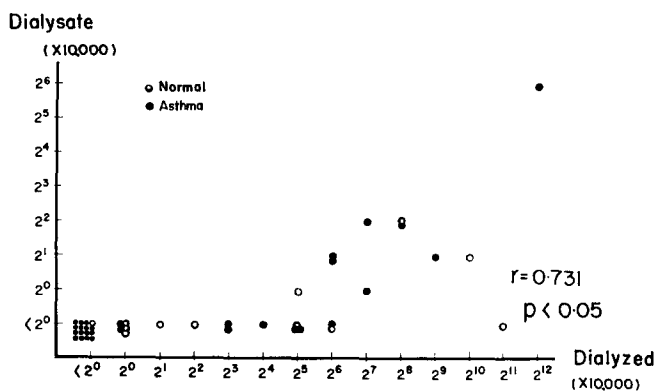
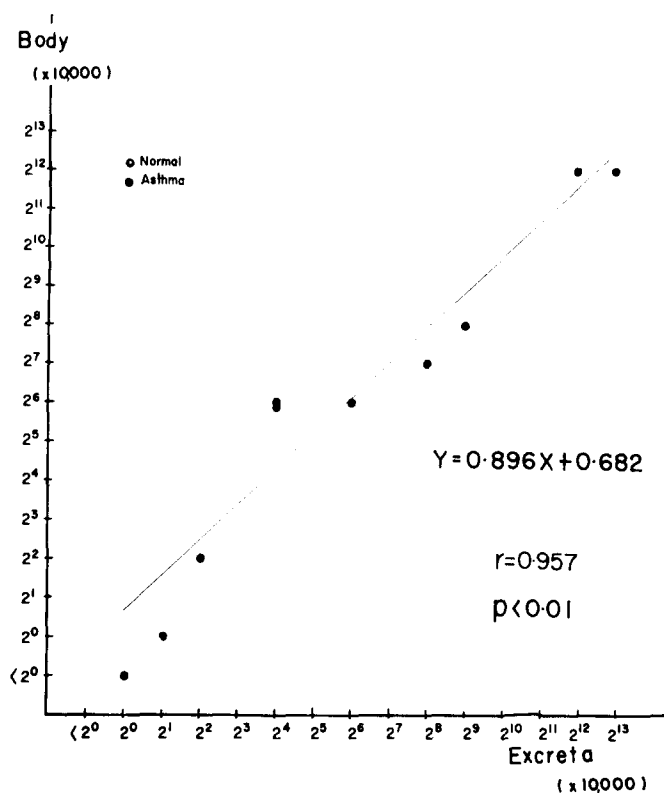


**Fig. 6**

Results of skin test with inside fluid and dialysate of dialyzed mite extract.

**Fig. 7**

Results of skin tests with extract of excreta of mite and dialyzed mite extract.

**Table II.** Relationship of skin test results between house dust, mite, and *Daphnia* extract in asthmatic patients

| Case | Age | Sex | End point of skin reaction |                        | Skin reaction to dried <i>Daphnia</i> |           |
|------|-----|-----|----------------------------|------------------------|---------------------------------------|-----------|
|      |     |     | House dust                 | Mite                   | 1:1,000*                              | 1:10,000* |
| 1    | 24  | M   | $2^1 \times 1,000$         | $2^6 \times 10,000$    | 0                                     | 0         |
| 2    | 34  | M   | $2^2 \times 1,000$         | $2^6 \times 10,000$    | 0                                     | 0         |
| 3    | 29  | M   | $2^3 \times 1,000$         | $2^8 \times 10,000$    | 0                                     | 0         |
| 4    | 32  | M   | $2^2 \times 1,000$         | $2^7 \times 10,000$    | 0                                     | 0         |
| 5    | 36  | M   | $2^5 \times 1,000$         | $2^8 \times 10,000$    | 0                                     | 0         |
| 6    | 39  | M   | $2^7 \times 1,000$         | $2^{12} \times 10,000$ | +++                                   | ++        |
| 7    | 32  | M   | $2^6 \times 1,000$         | $2^{12} \times 10,000$ | +++                                   | ++        |

\*Dilution of extract by weight.

### Inhalation provocation test

All 6 subjects who had positive reactions to inhalation of house dust extract exhibited positive reactions with inhalation of mite extract. In one case (No. 5), the reaction was so severe that bronchodilator therapy was immediately administered after the first measurement of FEV<sub>1</sub>, shortly after the inhalation of the antigen. Mite extract appeared to be 10 to 100 times more potent than house

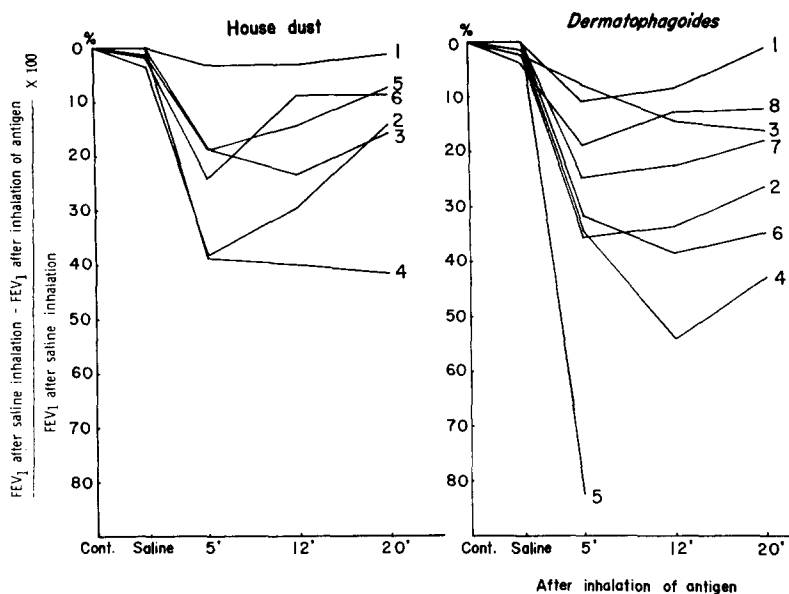


**Fig. 8**

Results of inhalation provocation tests with house dust and mite extracts in asthmatic patients.

**Table III.** Result of inhalation provocation tests with house dust and mite extracts in asthmatic patients

| Case* | Age | Sex | House dust extract |                                       | Mite extract |                                       |
|-------|-----|-----|--------------------|---------------------------------------|--------------|---------------------------------------|
|       |     |     | Dilution           | Maximum change FEV <sub>1</sub> † (%) | Dilution     | Maximum change FEV <sub>1</sub> † (%) |
| 1     | 48  | M   | 1:10               | 6.0                                   | 1:100        | 11.0                                  |
| 2     | 16  | M   | 1:100              | 38.4                                  | 1:1,000      | 35.3                                  |
| 3     | 27  | F   | 1:10               | 23.8                                  | 1:1,000      | 17.5                                  |
| 4     | 25  | F   | 1:100              | 42.5                                  | 1:500        | 54.5                                  |
| 5     | 33  | F   | 1:100              | 24.0                                  | 1:100        | 82.3                                  |
| 6     | 22  | F   | 1:100              | 43.3                                  | 1:1,000      | 39.0                                  |
| 7     | 25  | M   | ND                 | ND                                    | 1:100        | 32.5                                  |
| 8     | 25  | M   | ND                 | ND                                    | 1:200        | 19.0                                  |

ND—not done.

\*Case number corresponds to case number in Fig. 8.

†Change of FEV<sub>1</sub> was expressed by following formula:

$$\frac{\text{FEV}_1 \text{ after saline inhalation} - \text{FEV}_1 \text{ after inhalation of antigen}}{\text{FEV}_1 \text{ after saline inhalation}} \times 100.$$

dust, to judge from the result of the provocation tests. In two other cases with positive skin reactions to house dust extract, the inhalation provocation test was also positive to mite extract (Fig. 8 and Table III).

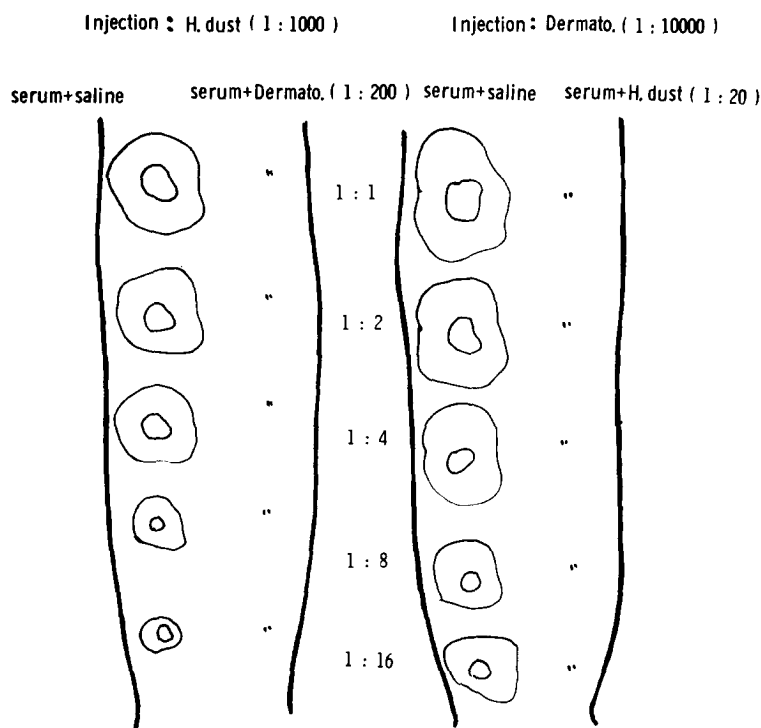
#### Prausnitz-Küstner reaction

In all patients who proved to have skin-sensitizing antibody against house dust, their sera showed positive P-K test to mite, as shown in Table IV. P-K

**Table IV.** Comparison of P-K titer\* against house dust and mite extract

| Serum | House dust (1:1,000) | Mite (1:10,000) |
|-------|----------------------|-----------------|
| 1     | 2                    | 8               |
| 2     | 16                   | 64              |
| 3     | 32                   | 64              |
| 4     | 128                  | 512             |
| 5     | 16                   | 64              |

\*P-K titer was expressed as reciprocal of maximum dilution of serum which elicited the positive reaction.

**Fig. 9**

In vitro neutralization of skin-sensitizing antibody against house dust by mite extract and against mite by house dust extract. The numbers at P-K sites indicate dilution of mixtures of serum.

titer was two to four times higher against mite than house dust with the antigen concentrations employed.

#### Neutralization of skin-sensitizing antibody

1. *In vitro neutralization.* Strong skin reactions were observed when house dust extract (1:1,000) and mite extract (1:10,000) were injected intracutaneously into two series of P-K sites made from serum mixed with saline. Skin reactions, however, were almost completely suppressed when house dust extract (1:1,000) was given intracutaneously into a series of P-K sites made from serum

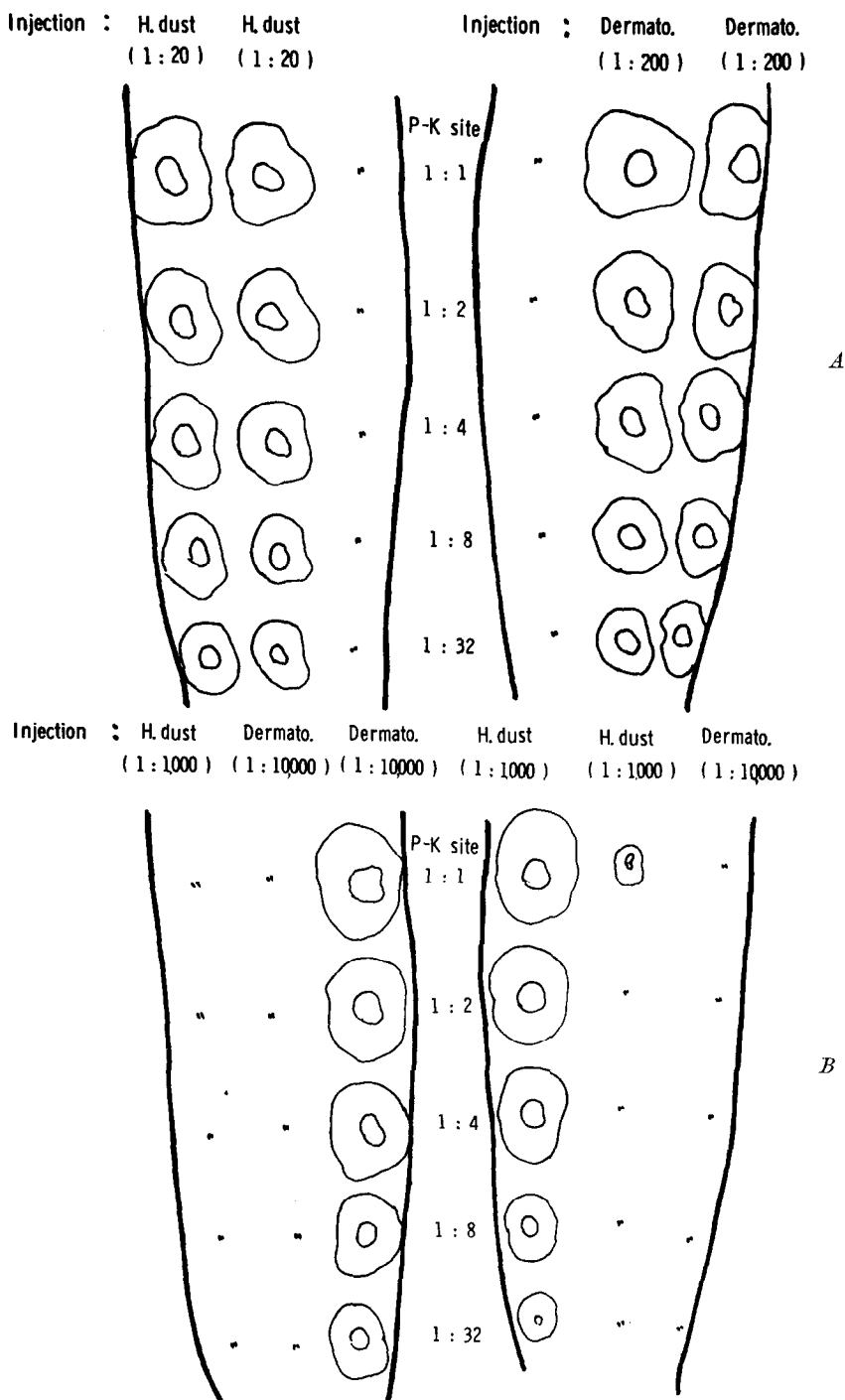


**Fig. 10**

In vivo neutralization of skin-sensitizing antibody against house dust by mite extract and against mite by house dust extract. *A*, P-K sites were injected with 1:20 dilution of house dust extract and 1:200 dilution of mite extract. The numbers at P-K sites indicate dilution of mixtures of serum. *B*, P-K sites preinjected with house dust extract were injected with mite extract (1:10,000) and P-K sites preinjected with mite extract were injected with house dust extract (1:1,000) 72 hours thereafter.

mixed with mite extract (1:200) and mite extract (1:10,000) into a series of P-K sites made from serum mixed with house dust extract (1:20), indicating almost complete in vitro neutralization of each other. Four allergic sera were tested by this method with identical results. A diagram of a typical example is shown in Fig. 9.

2. *In vivo neutralization.* Injection of house dust extract (1:20) and mite extract (1:200) each into two series of P-K sites resulted in striking wheal-and-erythema reactions. Seventy-two hours thereafter, house dust extract (1:1,000) was given intracutaneously at the P-K sites where mite extract (1:200) had been injected previously. The skin reactions were minimal even at the site of maximum concentration of allergic serum. At the same time, house dust extract (1:1,000) was administered to the P-K sites where house dust extract (1:20) had been preinjected. No reactions were elicited, whereas house dust extract (1:1,000) given into a series of nontreated P-K sites produced strong reactions, demonstrating the persistence of skin-sensitizing antibody at the P-K sites. The identical results were obtained with mite extract.

These results indicated that the employed concentrations of house dust extract (1:20) and mite extract (1:200) were each sufficient for complete neutralization of skin-sensitizing antibody in vivo against themselves, that skin-sensitizing antibody against house dust was almost completely neutralized in vivo by previous injection of mite extract (1:200), and that skin-sensitizing antibody against mite extract was almost completely neutralized by previous injection of house dust extract (1:20). Only two sera were tested by this method since concentrated antigen injection into the P-K sites annoyed the recipients. A diagram of a typical example is shown in Fig. 10, *A* and *B*.

Thus, in the 6 allergic sera employed, skin-sensitizing antibody was almost completely neutralized in vitro as well as in vivo.

## DISCUSSION

House dust is a mixture of various materials. It is quite important to determine which substance is most important as the causative antigen. Recently our studies in this line were focused on the mite in house dust after being stimulated by R. Voorhorst and his co-workers.<sup>5, 7</sup> Skin tests showed good correlation between house dust and mite. So far, no such close correlation has been found between house dust and any other antigenic material such as molds, pollens, animal danders, bacilli, etc.<sup>11</sup>

From the skin testing, it was thought that the mite is probably a very important antigenic material in house dust, although it is obvious that the mite is not the single antigen contained in house dust. To ascertain further whether the mite antigen could be a causative antigen in bronchial asthma, inhalation provocation tests were carried out. The result was quite striking in that all 6 subjects who had positive reactions to inhalation of house dust extract exhibited positive reactions with inhalation of mite extract, as shown in Table III and Fig. 8. Antigenic potency was calculated to be 10 to 100 times stronger by weight in mite than house dust from the results of skin tests and inhalation provocation tests.

Furthermore, to confirm antigenic identity between house dust and the mite, neutralization tests of skin-sensitizing antibody were employed with 6 different allergic sera. Skin-sensitizing antibodies against mite extract were almost completely neutralized by antigen excess of house dust extract and vice versa. This strongly confirmed that the mite is the major antigen in house dust.

At the present time, there is controversy between Berrens and Voorhorst.<sup>12</sup> Berrens and Morris<sup>13-15</sup> strongly believe that human dandruff is the major antigen in house dust, based upon their studies. They reported human dandruff components constitute a considerable portion of the allergenic fraction of house dust. On the other hand, Voorhorst and his co-workers<sup>5, 7, 16</sup> insist that skin reactions to extracts of *Dermatophagoides pteronyssinus* are quantitatively and qualitatively indistinguishable from those obtained with house dust, thus indicating that the mite is an important antigen in house dust. Voorhorst and his co-workers' studies were totally in agreement with our conclusions. Yet their argument is weak since they used human dandruff in cultivation of their mites. In our studies, however, *Daphnia* was used in cultivation instead of human dandruff. Consequently, no argument can be raised with respect to human dandruff as the powerful antigen, although the possibility of antigenic cross-reactivity between human dandruff and mite can not be denied at the present time. From the results of skin tests, provocation tests, P-K tests, and neutralization of skin-sensitizing antibody, we consider the mite to be the most important antigen in house dust.

Excreta of the mite apparently have the same antigenicity as the mite body. This is quite interesting, and the fact that the house dust obtained from old houses is usually more potent in antigenicity is thought to be partly due to this fact, since more excreta might have accumulated in older houses in addition to an accumulation of decayed dead bodies of mites. Since a certain amount of antigenic substance is dialyzable from a Visking tube, the molecular weight of such substance might be quite small, most likely below 5,000, but most antigenic substances must be more than 5,000 in molecular weight as the major portion stays inside the dialysis bag.

*D. farinae* was employed in our current investigation, but it makes up only about 4 per cent of the total number of mites in house dust. However, since such close antigenic identity was found to exist between house dust and *D. farinae*, it is our present feeling that *D. farinae* probably has close cross-antigenicity to other mites, such as *D. pteronyssinus*, *D. passericola*, and indeterminable *Dermatophagoides*, which make up about 33, 10.3, and 8 per cent, respectively, of the mite population of house dust. Further investigation of this aspect is in progress at the present time.

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