

27. Ruffin RE, Alpers JH, Crockett AJ, Hamilton R: Repeated histamine inhalation tests in asthmatic patients. *J ALLERGY CLIN IMMUNOL* 67:285, 1982
28. Lemire I, Cartier A, Malo JL, Pineau L, Ghezzi H, Martin RR: Effect of sodium cromoglycate on histamine inhalation tests. *J ALLERGY CLIN IMMUNOL* 73:234, 1984
29. Rosenthal RR, Laube B, Jaeger JJ, Phillips YY, Norman PS: Methacholine sensitivity is unchanged during the refractory period following an exercise or isocapnic challenge. *J ALLERGY CLIN IMMUNOL* 73:179, 1984
30. Black JL, Schoeffel RE, Sundrum R, Berend N, Anderson SD: Increased responsiveness to methacholine and histamine after challenge with ultrasonically nebulized water in asthmatic subjects. *Thorax* 40:427, 1985
31. Ryan G, Dolovich MB, Roberts RS, Frith PA, Juniper EF, Hargreave FE, Newhouse MT: Standardization of inhalation provocation tests: two techniques of aerosol generation and inhalation compared. *Am Rev Respir Dis* 123:195, 1981

## Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: Development of a radioimmunoassay for antigen P<sub>1</sub> equivalent in house dust and dust mite extracts

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*Two species of mites of the genus Dermatophagoides are common in house dust and make a major contribution to the allergen content of house dust. As judged by skin tests and the radioallergosorbent test, these mites, D. pteronyssinus and D. farinae, demonstrate extensive cross-reactivity. When the major allergen from D. pteronyssinus, antigen P<sub>1</sub>, was compared with the equivalent allergen from D. farinae, these two proteins were found to have both species-specific and common antigenic determinants. With specifically purified antibodies directed against the common determinants, we developed a radioimmunoassay for these antigen P<sub>1</sub> equivalent proteins in mite extracts as well as house dust extracts. The quantity of allergen in dust from 63 houses (255 samples) was measured, and values ranged from <100 to >100,000 ng/gm of fine dust. The correlation between antigen P<sub>1</sub> equivalent (ng/gm) and the number of mites per gram of dust (identified by microscopy) was very good ( $r = 0.74$ ;  $p < 0.001$ ). Furthermore, this correlation was not affected by the species of mite in the dust sample. Of 9243 mites identified, 95% were of the genus Dermatophagoides, and of these, 16.4% and 18% could be positively identified as D. farinae and D. pteronyssinus, respectively. When the assay for antigen P<sub>1</sub> equivalent was applied to isolated components of mite cultures of both species, significant allergen was found in the mite bodies, cuticles, and their excreta (fecal particles). For the house-dust samples, <10% of the allergen measured could be explained by the allergen content of mite bodies. Our results demonstrate that a radioimmunoassay for antigen P<sub>1</sub> equivalent can provide an accurate and simple means of assessing the quantity of mite-derived allergen in dust-mite extracts, house-dust extracts, or house dust. (J ALLERGY CLIN IMMUNOL 78: 398-407, 1986.)*

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In 1964 Spieksma and Voorhorst first demonstrated that mites of the genus *Dermatophagoides* were an important source of the elusive "house-dust allergen."<sup>1-5</sup> After that discovery, positive immediate hypersensitivity skin tests and serum IgE antibodies to these mites were found to be very common in patients with asthma or perennial rhinitis in many parts of the

#### Abbreviations used

|                          |                                                                                       |
|--------------------------|---------------------------------------------------------------------------------------|
| Antigen P <sub>1</sub> : | Antigen P <sub>1</sub> from <i>D. pteronyssinus</i>                                   |
| Fr I:                    | Fraction I from <i>D. farinae</i> culture                                             |
| Anti-Dpt:                | Rabbit antiserum to <i>D. pteronyssinus</i>                                           |
| Anti-Df:                 | Rabbit antiserum to <i>D. farinae</i>                                                 |
| Anti-P <sub>1</sub> Eq:  | Anti-Df specifically purified by elution from an antigen P <sub>1</sub> immunosorbent |
| IS:                      | International standard                                                                |
| RIA:                     | Radioimmunoassay                                                                      |
| NIBSC:                   | National Institute for Biological Standards and Control                               |

world.<sup>6-10</sup> There were problems with some early mite extracts because they were low in potency, contained allergens derived from the growth medium, or had nonspecific irritant activity.<sup>11</sup> A further problem with dust-mite allergy is that it is difficult to quantitate mite-allergen exposure in houses. In the last few years, there has been considerable progress. First, it has become possible to produce potent Dpt and Df extracts made from mites or mite culture, grown on nonectodermal, heat-denatured media that are non-allergenic. Second, a major allergen, antigen P<sub>1</sub>, has been purified from Dpt.<sup>12</sup> Third, after a collaborative study in 20 laboratories, an IS preparation of Dpt extract has been established (NIBSC 82/518) and approved by the World Health Organization.<sup>13, 14</sup>

Various techniques have been used to standardize Dpt extracts and measure mite allergens in house dust, including RAST inhibition, RIA, and rocket immunoelectrophoresis.<sup>15-18</sup> These assays may be suitable for areas where Dpt is the dominant mite. However, in the United States and elsewhere, houses contain either Dpt or Df or both, and a mite-allergen assay would only be useful if it was able to measure allergen derived from the two species equally. By use of skin tests or direct RAST, there is a strong correlation between sensitivity to different pyroglyphid mites, and cross-reactivity between them has been reported with immunochemical techniques.<sup>19-23</sup> We report here that antigen P<sub>1</sub> has antigenic epitopes in common with a major allergen produced by Df. We have developed an RIA specific for the genus-specific epitopes on these allergens that is suitable for measuring mite-derived allergen in dust mite- or house dust-allergen extracts and also in dust from the houses of allergic patients.

## METHODS

Antigen P<sub>1</sub> was purified from Dpt culture (Bencard, Ltd., Betchworth, Surrey, U. K.) by aqueous extraction for 4 hours and precipitated with 50% saturated ammonium sulphate, and preparative electrophoresis was on Pevikon and Sephacryl (Pharmacia, Piscataway, N. J.) S200 gel filtra-

tion.<sup>12</sup> The antigen P<sub>1</sub> was fully purified (>95% pure) as judged by crossed immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.<sup>12, 24</sup> Df culture (Hollister-Stier, Spokane, Wash.) was extracted with saline and precipitated with 50% saturated ammonium sulphate and S200 gel filtration. The fraction eluting at ~20,000 daltons, Fr I of Df, demonstrated a single line on immunodiffusion with rabbit antisera and demonstrated a single strong line with a weak second line by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Fr I is at least 85% pure by examination of gels. As judged by direct RAST experiments and skin testing with the fully purified material, the dominant protein in Fr I is a major allergen of Df.\* By use of antisera to Df 6 (kindly provided by Dr. Lind in Copenhagen) and to Antigen 11 (kindly provided by Dr. Dandau at Institute Pasteur in Paris), Fr I was demonstrated to have complete identity with these proteins on immunodiffusion.

Proteins (~15 µg) were radiolabeled with 1 mCi <sup>125</sup>I (Amersham Searle Corp., Arlington Heights, Ill.) with the chloramine-T technique, eliciting a specific activity of 17 to 40 µCi/µg.

## Antisera and specifically purified antibodies

New Zealand white rabbits were immunized with crude extract of Df culture (Bencard Ltd.). Four milligrams of protein in complete Freund's adjuvant was injected into footpads and repeated, intramuscularly, four times during 10 weeks. This antiserum (anti-Df) demonstrated six lines on immunodiffusion against Df extract and 12 lines on crossed immunoelectrophoresis. Anti-Dpt was the same as described previously.<sup>12</sup> For specific purification, 12 mg of antigen P<sub>1</sub> was bound to Sepharose activated with CNBr. Ninety milliliters of anti-Df was passed over a column containing 17 ml of antigen P<sub>1</sub> immunosorbent. After extensive washing, the bound antibody was eluted with glycine HCl, pH 2.8. This eluate contained 15 mg of purified antibody (anti-P<sub>1</sub> Eq). Anti-P<sub>1</sub> Eq demonstrated a single line on immunodiffusion against both Dpt and Df crude extracts. Goat antiserum to rabbit gamma globulin was raised as described previously.<sup>12</sup>

## RIAs

The methods used for the RIA for antigen P<sub>1</sub> have been described elsewhere, and the assays described here use a similar procedure<sup>12, 17</sup> (Fig. 1). For each assay a dilution of antiserum or antibody was incubated with mite extract (or house dust) before the radiolabeled mite allergen was added. Finally, the rabbit antibodies were precipitated with anti-RGG, and the precipitate was washed and counted.

The quantity of radiolabeled protein that was added largely determines the sensitivity of the assay. In these experiments we used the same quantity of <sup>125</sup>I-labeled antigen P<sub>1</sub> as in previous assays (i.e., 2 ng), and the quantity of <sup>125</sup>I-Fr I was ~5 ng. The dilution of each antiserum or antibody eliciting 60% of maximum binding was used in the RIAs. In order to create a precipitate large enough to wash, normal

\*Heymann P, Chapman MD, Platts-Mills TAE: In preparation.

0.1 ml, Anti-P<sub>1</sub> Eq. 1/15,000 in Normal Rabbit Serum 1/50

+ 0.1 ml Dilution of Mite or House Dust Extract

2 Hours Room Temperature

+ 0.1 ml <sup>125</sup>I Antigen P<sub>1</sub> ~2 ng

2 Hours Room Temperature

+ 0.1 ml Goat Anti-RGG 1/15

Overnight 4°C

Wash Precipitate 3X with BBS

Count Radioactivity

**FIG. 1.** Radioimmunoassay for antigen P<sub>1</sub> Eq.

rabbit serum was added (Fig. 1), and the quantity of goat antiserum to rabbit gamma globulin necessary to precipitate all the rabbit antibodies present was judged by a qualitative test for the zone of equivalence.<sup>25</sup> In each assay a control curve of serial twofold dilutions of antigen P<sub>1</sub> over the range 300 to 0.5 ng was carried out. Results were extrapolated from this control curve, and where it was necessary, assays were repeated at higher or lower dilutions. With this standard we estimate that the IS (NIBSC 82/518) contains 12.3 µg of antigen P<sub>1</sub>.<sup>14</sup>

### Specificity of the assay

The antisera to mites and the specifically purified antibodies were tested by immunodiffusion against a range of allergen extracts and demonstrated no reactivity. The other allergen extracts were also tested for their ability to inhibit the RIA for antigen P<sub>1</sub> Eq (Table I). Most of the nonmite extracts demonstrated no inhibition of the assay, i.e., <0.01 µg of antigen P<sub>1</sub> Eq/ml. Some of the concentrated extracts, particularly the fungal extracts, demonstrated minor degrees of inhibition, but the maximum apparent antigen P<sub>1</sub> content was 0.05 µg/ml (Table I).

### Collection of dust samples

Houses visited were all in central Virginia and included those of patients with asthma, rhinitis, atopic dermatitis, and also normal individuals. The group included both patients with positive skin prick tests (>5 by 5 mm wheal) to Df (35 µg of antigen P<sub>1</sub> Eq per milliliter [Hollister-Stier]) and also those with negative skin tests. Samples of house dust were collected with a modified hand vacuum cleaner (Douglas hand vac, model L6701, Douglas Products, Walnut Ridge, Ark.) with a filter holder behind the cleaner head (MS filter type 681, Cambridge, U. K.). Samples were collected onto tissues from an area of 1 m<sup>2</sup> for 2 minutes

or by vacuuming bedding for 2 minutes and stored at 4° C. Generally, three samples were obtained from each house, from bedding, bedroom floor, and family room floor.

### Extraction of dust-mite counts and identification of mites

Dust was sieved through a 0.3 mm mesh screen (to remove large particles and fibers) and weighed, and 100 mg was placed in a 75 by 12 mm test tube.<sup>17</sup> After addition of 2.0 ml of 0.1% Tween 80 in borate-buffered saline, pH 8.0, the dust was rotated end over end for 2 hours on an orbital rotator (Multipurpose Rotator, model 151, Scientific Laboratories, Bohemia, N. Y.). The dust particles were removed by centrifugation at 1200 × g for 20 minutes, and the supernatant was stored at -20° C. The dust pellet was resuspended and transferred to an 18 ml tube with 10 ml of saturated NaCl with 20% sucrose (flotation fluid), taking care to transfer all the particles. After centrifugation at 1200 × g for 7 minutes, the supernatant with floating mite bodies was transferred with 80 ml of H<sub>2</sub>O with two drops of 2% crystal violet into a 70 mm Buchner funnel lined with graduated Whatman No. 1 filter paper. Mite bodies were identified under incident light microscopy and transferred to a microscope slide with C-M mountant.<sup>26</sup> Identification under phase contrast microscopy (200 to 900× [Leitz Ortholux]) was by morphologic criteria.<sup>26</sup> In many samples nymphs and adults were present that could be identified as *Dermatophagoides* but were not assigned to any species. Thus, >95% of the mites were classified to genus and ~40% to species.

### RESULTS

When purified antigen P<sub>1</sub> and the Df allergen preparation (Fr I) were examined by immunodiffusion by use of anti-Dpt and anti-Df, precipitin lines indicating

TABLE I. Specificity of RIA for antigen P<sub>1</sub> Eq

| Extracts                       | Source*            | Antigen P <sub>1</sub> Eq<br>( $\mu$ g/ml) |
|--------------------------------|--------------------|--------------------------------------------|
| Dpt                            | Bencard            | 62; 115                                    |
|                                | U. of Virginia     | 1500                                       |
| Df                             | Hollister-Stier    | 35.4; 54                                   |
|                                | U. of Virginia     | 486                                        |
| House dust†                    | Commercial (n = 4) | 0.04 to 2.3                                |
|                                | From houses        | <0.01 to 20                                |
| Acaridae                       |                    |                                            |
| <i>Tyrophagus putrescentia</i> | Pharmacia          | <0.01                                      |
| <i>Acarus siro</i>             | Pharmacia          | 0.02                                       |
| <i>Dermacentor variabilis</i>  | Old Dominion U.    | <0.01                                      |
| Insects                        |                    |                                            |
| Cricket                        | U. of Virginia     | $\leq 0.05\ddagger$                        |
| Cockroach                      | Hollister-Stier    | $\leq 0.05$                                |
| Fungi and yeast                |                    |                                            |
| <i>Alternaria alternata</i>    | Hollister-Stier    | $\leq 0.05$                                |
| <i>Cladosporium herbarium</i>  | Hollister-Stier    | $\leq 0.05$                                |
| <i>Trichophyton</i>            | Hollister-Stier    | $\leq 0.05$                                |
| <i>Candida</i>                 | Hollister-Stier    | $\leq 0.05$                                |
| Mammalian allergens            |                    |                                            |
| Cat and dog dander             | Greer              | <0.01                                      |
| Rat urinary protein (1 mg/ml)  | U. of Virginia     | <0.01                                      |
| Pollen                         |                    |                                            |
| Ragweed                        | Greer              | <0.01                                      |
| Grasses                        | Greer              | <0.01                                      |
| Others                         |                    |                                            |
| Mite growth media§             | Hollister-Stier    | <0.01                                      |
|                                | Bencard            | <0.01                                      |
| Human IgG (1 mg/ml)            | U. of Virginia     | <0.01                                      |

\*Extracts were obtained commercially from Hollister-Stier, Spokane, Wash; Greer Laboratories, Lenoir, N. C.; Bencard Ltd., London, England; Center Laboratories, Ohio; Miles, Ltd., Stoke Poges, England; or were made locally (U. of Virginia). All extracts were assayed at the maximum strength available, i.e., 20,000 PNU or 1:10 extract. Glycerinated extracts were dialyzed before assay. We are grateful to Dr. Rolfsen, of Pharmacia, for providing extracts of the storage mites, *Tyrophagus putrescentia* and *Acarus siro*, and to Dr. D. Sonenshine, Old Dominion University, Norfolk, Va., for providing ticks, *Dermacentor variabilis*.

†House-dust extracts were obtained from four different companies: Greer, Hollister-Stier, Bencard, and Center Laboratories. At least two lots were assayed from each source, and the range of results observed is listed. Results varied by up to fortyfold from a single laboratory. Dust samples are the results observed extracting 100 mg of dust into 2 ml of saline.

‡Values are indicated as  $\leq$  because dilutions did not inhibit in parallel with the control curve.

§Each mite-culture medium, when tested by intradermal skin testing in 20 allergic individuals, elicited negative responses (wheal <6 by 6 mm) at a protein concentration of 10  $\mu$ g/ml).

partial identity were observed (Fig. 2, *upper* and *middle panel*). The purified antibody made by absorbing anti-Df on an antigen P<sub>1</sub> immunosorbent (anti-P<sub>1</sub> Eq) elicited a single line of identity between the two proteins (Fig. 2, *lower panel*). By use of these same two proteins radiolabeled with <sup>125</sup>I in RIAs, results were obtained that also implied that there are both species-specific and common determinants on the two proteins (Fig. 3). With anti-Dpt and radiolabeled antigen P<sub>1</sub>, the RIA was fully inhibited with Dpt extracts, whereas Df extracts demonstrated a much flatter curve that

never reached >60% inhibition (Fig. 3, *A*). A similar but reversed situation was observed with anti-Df and radiolabeled Fr I (Fig. 3, *B*). Thus, it appeared that an RIA designed for the allergen derived from either species would not be suitable for quantitative assays of the allergen produced by the other species. By contrast, with either anti-Dpt with radiolabeled Fr I or anti-Df with radiolabeled antigen P<sub>1</sub>, the assays were equally inhibited by extracts from either mite. These results establish that there is an allergen produced by Df that has both species-specific and deter-

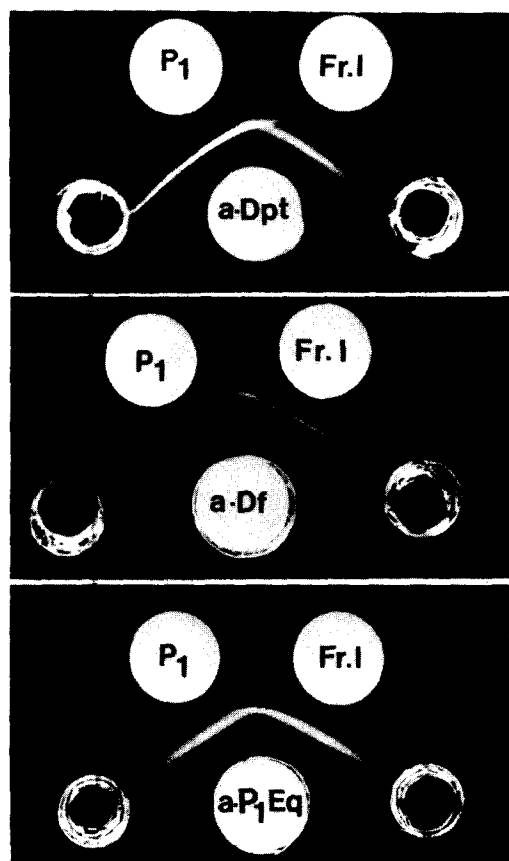


**FIG. 2.** Cross-reactivity between antigen  $P_1$  ( $P_1$ ) and Fr I from Df (Fr I). *Upper panel* illustrates partial identity by use of antiserum to Dpt, *middle panel* illustrates partial identity by use of antiserum to Df, and *lower panel* illustrates almost complete identity by use of antibodies to cross-reacting determinants, anti- $P_1$  Eq. Each of the purified allergens was used at  $\sim 100 \mu\text{g}$  antigen  $P_1$  Eq per milliliter. Immunodiffusion was carried out for 16 hours by use of 2% agarose in borate-buffered saline, pH 8.0, for 16 hours.

minants common with antigen  $P_1$ . We refer to these two proteins as antigen  $P_1$  Eq.

### RIA for antigen $P_1$ Eq

With 2 ng of radiolabeled antigen  $P_1$ , 56% of the radioactivity was precipitable with 1:100 dilution of the specifically purified antibody, anti- $P_1$  Eq, and 60% of this maximum binding was obtained with a 1:15,000 dilution of this antibody (Fig. 1). With 1:15,000 anti- $P_1$  Eq and 2 ng  $^{125}\text{I}$ -labeled antigen  $P_1$ , inhibition curves were very similar with either Dpt extracts, antigen  $P_1$ , or Df extracts (Fig. 4). Because inhibition curves for extracts of Df culture were consistently slightly steeper than those for Dpt, values of antigen  $P_1$  equivalent for a Df extract would be slightly overestimated if values were calculated from

**TABLE II.** Variation in values for antigen  $P_1$  in Df extracts by use of a Dpt control curve

| % Maximum counts bound | Apparent antigen $P_1$ Eq* in Df extract (ng/ml) | Mean % variation† |
|------------------------|--------------------------------------------------|-------------------|
| 90                     | 66                                               | -24.2             |
| 80                     | 75.4                                             | -13.5             |
| 75                     | 80                                               | -9.3              |
| 50                     | 96                                               | 0.0‡              |
| 25                     | 105                                              | 14.7              |
| 20                     | 112                                              | 20                |
| 10                     | 138                                              | 27                |

\*Values obtained from curves on Fig. 4.

†Values are the mean variation observed in four separate experiments in which Df- and Dpt-inhibition curves were run in parallel.

‡Variation at 50% maximum counts bound equals zero by definition. The variations, therefore, indicate the error introduced by use of values obtained at various inhibition levels by comparison with 50% inhibition.

results with  $<30\%$  inhibition and slightly underestimated if values were calculated from results with  $>70\%$  inhibition (Table II). The fact that inhibition curves were consistently slightly steeper with Df extracts may reflect the fact that the antiserum used to purify anti- $P_1$  Eq antibodies was originally raised against Df and may therefore have slightly higher affinity for the cross-reacting determinants on Fr I than on antigen  $P_1$ .

Inhibition curves with serial twofold dilutions of house-dust extracts were parallel to the control curve (Fig. 5). These 15 extracts were chosen because the house dust had been found to contain either one species only or  $\sim 50\%$  of each. In addition, several extracts were available (from other areas) in which the dominant mite was *Euroglyphus maynei*, a closely related pyroglyphid mite, and these extracts also elicited  $>90\%$  inhibition and parallel curves. The intra-assay variability or the interassay variability for the results on a given dust sample were  $\pm 12\%$  and  $\pm 15\%$ , respectively. The numerical values for Df-derived allergen assume that this protein has the same number of common antigenic determinants as antigen  $P_1$  and has the same molecular weight. Preliminary results with fully purified allergen from Df suggest that these assumptions are correct.

### Correlation between the concentration of antigen $P_1$ Eq in dust extracts and numbers of Df and Dpt in house dust

Dust samples from 64 houses in Virginia were examined microscopically for mites and assayed for an-

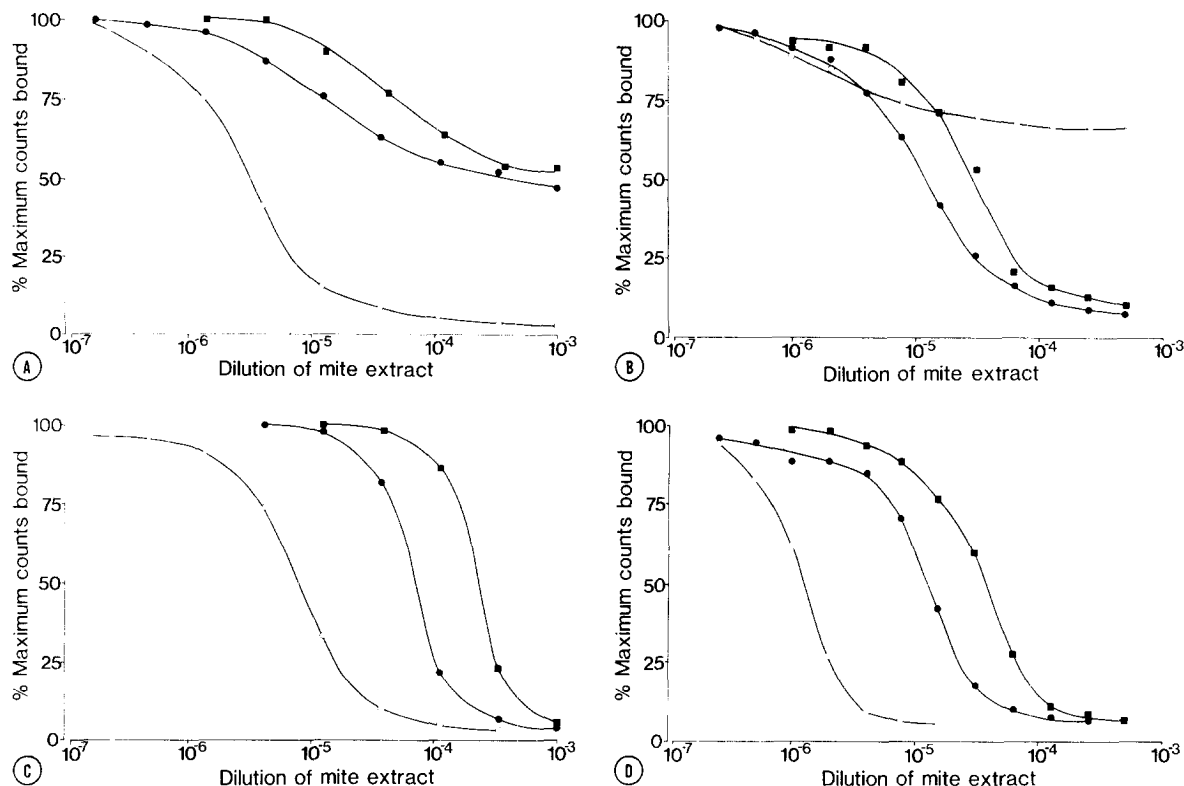


**FIG. 3.** Inhibition of binding of radiolabeled mite allergens by extracts of homologous or heterologous mite extracts. In each panel, **A-D**, the inhibiting extracts are Df (Hollister-Stier) (●—●), Df (Bencard) (■—■), and Dpt extract (Bencard) (○—○). **A**, The radiolabel is on antigen P<sub>1</sub>, and the antiserum is anti-Dpt. **B**, The radiolabel is on Fr I, and the antiserum is anti-Df. **C**, The radiolabel is on antigen P<sub>1</sub>, and the antiserum is anti-Df. **D**, The radiolabel is on Fr I, and the antiserum is anti-Dpt.

tigen P<sub>1</sub> equivalent. The results for the first 40 houses (149 samples) are illustrated in Fig. 6. In Table III, the total results are presented and also separated into samples in which one species was dominant (>66% of identified mites). The calculated slope for the regression line for each species was very similar (Table IIIA). Although there was a good correlation between mite numbers and allergen content, there was considerable scatter. The results demonstrate that on the average, Dpt-dominant samples contained rather more allergen/mite than Df-dominant samples, 147 ng per mite and 86 ng per mite, respectively. Of the mites counted, 95% were *Dermatophagoides*, and a very similar number of each species was identified 16.4% Df and 18% Dpt (Table IIIB). Nine samples with predominantly *E. maynei* (not from Virginia) demonstrated a mean value of 45 ng of antigen P<sub>1</sub> Eq per mite.

In cultures of mites it is possible to identify and separate mite bodies, cuticles, eggs, and fecal pellets. Adult Df contained ~2 ng, eggs contained <0.01 ng,

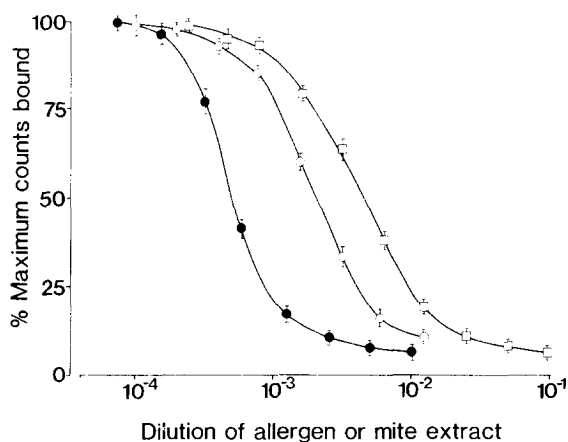


**FIG. 4.** Inhibition curves with use of the RIA for antigen P<sub>1</sub> Eq. Inhibiting extracts were Df (Hollister-Stier) (100 μg/ml) (●—●), Dpt culture extract (NIBSC 82/518) (12.3 μg/ml) (□—□), and partially purified antigen P<sub>1</sub> (24 μg/ml) (○—○). The antigen P<sub>1</sub> curve was used routinely as the control curve. The antibody used for this assay, anti-P<sub>1</sub> Eq, was obtained from antiserum to Df by absorption and elution from an antigen P<sub>1</sub> column.

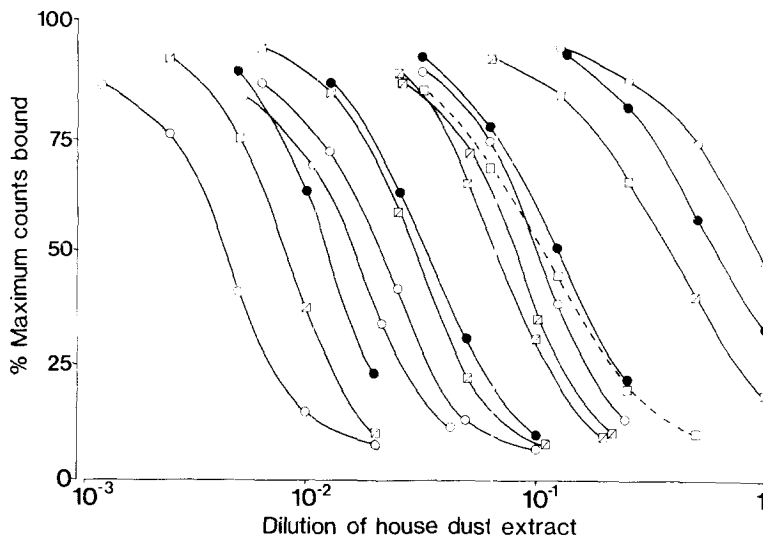


**FIG. 5.** Inhibition curves with 15 extracts of house dust. Extracts were made from house dust that had been demonstrated microscopically to contain either 100% Df (●—●) or 100% Dpt (○—○), or an equal mixture of the two mites (◐—◐). Results for one extract are demonstrated in which the house dust contained 40% *Cheyletidae* and 60% Df (□—□).

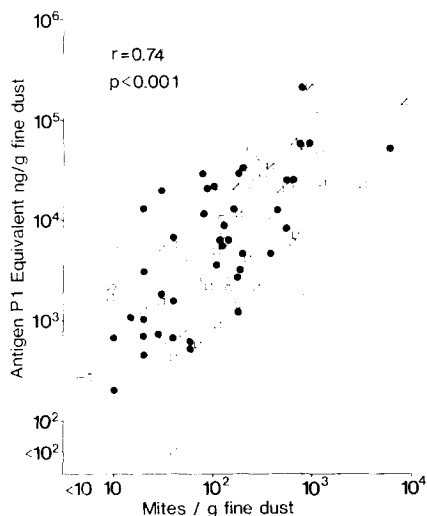


**FIG. 6.** Correlation between the number of mites identified microscopically and the concentration of antigen P<sub>1</sub> Eq in house dust. Results are demonstrated for 144 samples from 40 houses. Samples with >66% Df (●) or >66% Dpt (○) are distinguished from those with a mixture of mite species (◐) or with no mites that were fully identified (□).

and fecal pellets contained ~0.4 ng of antigen P<sub>1</sub> Eq each (Table IV).

## DISCUSSION

Cross-reactivity between closely related allergens is well established for the grasses in which the group I proteins from many different grasses have common antigenic determinants.<sup>27, 28</sup> Our results confirm that a similar situation applies to antigen P<sub>1</sub> and the equiv-

alent allergen from Df and that it is possible to assay specifically for the cross-reacting epitopes.<sup>29</sup> Antigen P<sub>1</sub> has been purified and can be assayed in several different laboratories.<sup>14</sup> Fr I is the same protein that has been identified as Df 6 by Lind et al.<sup>18</sup> and Lind and Løwenstein<sup>29</sup> in Copenhagen, and as Ag 11 by Le Mao et al.<sup>22</sup> in Paris. In each case the allergen was regarded as a major allergen of Df. The assay for antigen P<sub>1</sub> appeared to be highly specific for house-dust mites. Strikingly, we found little or no cross-reactivity with two storage mites, *Tyrophagus* and *Acarus*, nor with the tick, *Dermacentor*. Assays of extracts from house dust containing a high percentage of *E. maynei* suggest that this mite produces an allergen that also cross-reacts with antigen P<sub>1</sub>. Two pieces of evidence suggest that *E. maynei* produces an allergen cross-reactive with antigen P<sub>1</sub> Eq. First, the quantity of allergen found in *E. maynei*-dominant samples was very high if it was only produced by the *Dermatophagoides* species present, i.e., 444 ng of antigen P<sub>1</sub> Eq per mite. Second, with the original assay for antigen P<sub>1</sub>, we obtained much lower results on the *E. maynei*-dominant samples. This latter observation suggests that *E. maynei* produces an allergen that, like antigen F<sub>1</sub>, cross-reacts partially with antigen P<sub>1</sub> and can be measured with the present RIA. There are many advantages to the use of an RIA. The technique is very sensitive and, in our hands, is more rapid and reproducible than either RAST inhibition or mite counts. In addition, it is much easier to standardize RIA. The RIA is also equally applicable to dust-mite or house-dust extracts.

The quantitation of the RIA for antigen P<sub>1</sub> is de-

**TABLE IIIA.** Correlation between numbers of mites in dust and antigen P<sub>1</sub> Eq

|                  | n   | Antigen P <sub>1</sub> Eq<br>ng per mite* | Regression coefficient†     | Slope | Intercept† |
|------------------|-----|-------------------------------------------|-----------------------------|-------|------------|
| All samples      | 255 | 117                                       | 0.74<br>( <i>p</i> < 0.001) | 6.2   | 110        |
| Df‡              | 81  | 86                                        | 0.69<br>( <i>p</i> < 0.001) | 6.0   | 130        |
| Dpt              | 84  | 147                                       | 0.57<br>( <i>p</i> < 0.001) | 5.7   | 200        |
| <i>E. maynei</i> | 9   | 45                                        | —                           | —     | —          |
| Dominant (>66%)  |     |                                           |                             |       |            |

\*Mean of the calculated values for each sample, dividing the allergen content by the total number of mites observed in each sample. The mean value for Df-dominant samples was not significantly different from that for Dpt-dominant samples (*t* = 1.8; *p* > 0.05).

†Regression of log antigen P<sub>1</sub> Eq against log total number of mites in each sample. Intercept indicates the quantity of allergen for one mite per sample.

‡Samples in which >66% of the fully identified mites were Df.

**TABLE IIIB.** Species of mites identified in dust

| Total* | Df              | Dpt           | Dermatophagoide† | Others‡       |
|--------|-----------------|---------------|------------------|---------------|
| 9243   | 1517<br>(16.4%) | 1667<br>(18%) | 5650<br>(61.1%)  | 409<br>(4.4%) |

\*Total number of mites examined microscopically from 255 samples.

†Mites identified by genus but not by species, mainly nymphal forms or partially damaged specimens.

‡Other mites identified included *Cheyletidae*, *Tarsonimidae*, *Acarus*, *Glycyphagus*, and *Gamasina* species. No specimens of *E. maynei* were identified in these samples.

**TABLE IV.** Antigen P<sub>1</sub> Eq (nanograms) in different components of dust mite culture\*

| Component†      | Number<br>assayed | Df         |              | Dpt        |              |
|-----------------|-------------------|------------|--------------|------------|--------------|
|                 |                   | Total (ng) | ng/Specimen‡ | Total (ng) | ng/Specimen‡ |
| Adult mites     | 50                | 91.8       | 1.84         | 230        | 4.6          |
| Cuticle         | 50                | 18.2       | 0.36         | 12.1       | 0.24         |
| Eggs            | 200               | 1.44       | <0.01        | 0.45       | <0.01        |
| Fecal particles | 500               | 190        | 0.38         | 88         | 0.18         |

\*Measured on extracts made from separated culture components (22) by use of RIA for antigen P<sub>1</sub> Eq values in nanograms. Adult mites and eggs were homogenized before extraction.

‡Very similar results were obtained in a second experiment on isolated components.

†Specimens were isolated from live cultures grown in our laboratory on a mixture of human shavings and heat-denatured, nonectodermal mammalian medium.

pendent on measurements of protein made on the purified protein by use of both the Lowry technique and amino acid analysis.<sup>12</sup> Antigen P<sub>1</sub> was recently measured in the new Dpt IS by six different laboratories eliciting results within a twofold range (12 to 26 µg of antigen P<sub>1</sub>), average value 20 µg per ampule.<sup>14</sup> Our quantitation of the equivalent allergen produced by Df was based on the antigen P<sub>1</sub> control curves. The IS for Dpt has been allotted 100,000 units. By use of our values for antigen P<sub>1</sub>, the IS contains 12.3 µg of antigen P<sub>1</sub> per ampule, and this would suggest that 1

ng of antigen P<sub>1</sub> equals ~7 IU. This unitage could only strictly be applied to Dpt extracts. Clearly, the assay for antigen P<sub>1</sub> Eq does not measure all the mite-derived allergen, since there are other important allergens.<sup>23, 23, 29</sup> However, from all available results, it appears that dust mites always produce this protein. Thus, the concentration of antigen P<sub>1</sub> Eq will accurately reflect the quantity of mite allergens present in a dust or dust-mite extract.

Despite a widespread impression that Df was the North American mite, most published articles have

demonstrated that both mites occur in the United States.<sup>30-36</sup> Our own results in Virginia demonstrate an almost equal number of each. The variation observed in the relationship between mite numbers and the allergen content of house dust is not surprising, since the major source of mite allergen in dust is not mite bodies. Most of the allergen is associated with mite debris, particularly the fecal particles.<sup>17, 37</sup> Live dust mites are strongly photophobic and are only occasionally observed in vacuum cleaner dust; <5% of the mites counted in the present study were alive. Applying our values for the allergen content of mite bodies to the results on house-dust samples suggests that on the average, the mite bodies identified would account for <10% of the allergen observed.

Several published studies from different parts of the world have associated mite counts >300/gr of dust with symptoms of asthma.<sup>17, 38-40</sup> We consider that >10,000 ng of antigen P<sub>1</sub> Eq per gram of dust is a high level of mite allergen.<sup>17, 38</sup> Greater than 2000 ng/gm is regarded as moderate, between 400 and 2000 ng/gm is regarded as low, and <400 ng/gm is regarded as very low. Clearly, these values, like pollen counts, are only a guide to the level of exposure. The houses reported here were those of unselected patients attending an allergy clinic, but they included 37 patients with asthma, of whom 23 demonstrated skin sensitivity and serum IgE antibody to dust mites. In 14 cases (out of 23) house dust from these houses contained >300 mites per gram, and in 16 cases, dust contained >10,000 ng of antigen P<sub>1</sub> Eq per gram. Thus, we consider that at least 16 of the patients with asthma studied were allergic to mites and were exposed to high levels of mite allergen in their houses. We have found similar levels of mites and mite allergen in dust from the houses of mite-allergic children with asthma in Atlanta<sup>41</sup> where the weather is humid for more months than in Charlottesville, Va. It is well established that mite growth is dependent on the relative humidity and that mite numbers rise during the humid summer months in North America.<sup>32, 35, 36</sup> It appears likely that average levels of mite allergen will be lower in houses in which the relative humidity is >50% for less than 4 months of the year.

There is already good evidence that house dust-allergic patients with asthma can improve if they are kept in artificial dust-free conditions.<sup>38, 42, 43</sup> In some studies patients have been improved simply by making their bedrooms dust free,<sup>44, 45</sup> whereas other studies have concluded that standard measures for dust avoidance in houses make little effect on symptoms because it is difficult to reduce the level of mite allergens.<sup>16, 46</sup> The progressive development of standardized diag-

nostic skin test reagents and accurate measurements of allergens in house dust will make it much easier both to decide which patients are being affected by household allergens and to evaluate the measures proposed to modify allergen levels in houses.

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## REFERENCES

1. Voorhorst R, Th F, Spieksma M, Varekamp H, Leupen MJ, Lyklema AW: The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces: identity with the house dust allergen. *J ALLERGY* 39:325, 1967
2. Kern A: Dust sensitization in bronchial asthma. *Med Clin North Am* 5:751, 1921
3. Spivacke CA, Grove EF: Studies in hypersensitiveness XIV: a study of the atopen in house dust. *J Immunol* 10:465, 1925
4. Vannier WE, Campbell DH: A starch block electrophoresis study of aqueous house dust extracts. *J ALLERGY* 32:36, 1961
5. Miyamoto T, Oshima S, Domae A, Takanashi K, Izeki M, Tanaka T, Ishizaki T: Allergenic potency of different house dust extracts in relation to contained mites. *Ann Allergy* 28:405, 1970
6. Maunsell K, Wraith DG, Cunningham AM: Mites and house dust allergy in bronchial asthma. *Lancet* 1:1267, 1968
7. Smith MJ, Disney ME, Williams JD, Goels ZA: Clinical significance of skin reactions to mite extracts in children with asthma. *Br Med J* 2:723, 1969
8. Clarke CW, Aldons PM: The nature of asthma in Brisbane. *Clin Allergy* 9:147, 1979
9. Hendrick DJ, Davies RJ, D'Souza MF, Pepys J: An analysis of skin prick test reactions in 656 asthmatic patients. *Thorax* 30:2, 1975
10. Miyamoto T, Johansson SGO, Ito K, Horivchi Y: Atopic allergy in Japanese subjects: studies primarily with radioallergen sorbent test (RAST). *J ALLERGY CLIN IMMUNOL* 53:9, 1974
11. Kawai T, Marsh DG, Lichtenstein LM, Norman PG: The allergens responsible for house dust allergy. I. Comparison of *Dermatophagoides pteronyssinus* and house dust extracts by assay of histamine release from allergic human leucocytes. *J ALLERGY CLIN IMMUNOL* 50:117, 1972
12. Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P<sub>1</sub>. *J Immunol* 125:587, 1980
13. Ford AW, Rawle FC, Lind P, Spieksma FTM, Løwenstein H, Platts-Mills TAE: Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* 76:58, 1985
14. Ford A, Seagroatt V, Platts-Mills TAE, Løwenstein H: A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J ALLERGY CLIN IMMUNOL* 75:676, 1985
15. Tovey E, Vandenberg R: Mite-allergen content in commercial

- extracts and bed dust determined by radioallergosorbent tests. Clin Allergy 9:253, 1979
16. Burr ML, Dean BV, Merrett TG, Neale E, St. Leger AS, Verrier-Jones ER: The effects of antimite measures on children with mite-sensitive asthma: a controlled trial. Thorax 35:506, 1980
17. Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE: The distribution of dust mite allergen in the houses of patients with asthma. Am Rev Respir Dis 124:630, 1981
18. Lind P, Korsgaard J, Løwenstein H: Detection and quantitation of *Dermatophagoides* antigens in house dust by immunochemical techniques. Allergy 34:319, 1979
19. Voorhorst R, Th F, Spijksma M: Comparison of skin reactions to extracts of house dust, mites, and human skin scales. Acta Allergol 24:124, 1969
20. Chapman MD, Platts-Mills TAE, Gabriel M, Ng HK, Allan WGL, Hill LE, Nunn AJ: Antibody response following prolonged hyposensitization with *Dermatophagoides pteronyssinus* extract. Int Arch Allergy Appl Immunol 61:431, 1980
21. Romagnani S, Biliotti G, Passaleva A, Ricci M: Mites and house dust allergy. II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) allergens by fractionation methods. Clin Allergy 2:115, 1972
22. Le Mao J, Dandieu JP, Rabillon J, Lux M, David B: Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. J ALLERGY CLIN IMMUNOL 71:588, 1983
23. Holck AL, Dale S: Allergens of the house dust mite, *D. farinae*. Proceedings of the XI International Congress of Allergology and Clinical Immunology, London, 1982, Macmillan Press, Ltd, p 474
24. Chapman MD: Purification and characterization of the major allergen from the house dust mite (*Dermatophagoides pteronyssinus*)—antigen P<sub>1</sub>. 1981, University of London (Ph.D. thesis)
25. Garvey JS, Cremer NE, Sussdorf DH: Methods in immunology. New York, 1977, WA Benjamin, Inc, p 280
26. Hughes AM: The mites of stored food and houses. London, 1976, Her Majesty's Stationery Office
27. Marsh DG: Allergens and the genetics of allergy. In Sela M, editor: The antigens. New York, 1975, Academic Press Inc, vol III, p 271
28. Kahn CR, Marsh DG: Analysis of grass group I allergens using monoclonal antibodies. J ALLERGY CLIN IMMUNOL 71:95, 1983 (abst)
29. Lind P, Løwenstein H: Species-specific determination of major allergen from *D. pteronyssinus* and *D. farinae*. J ALLERGY CLIN IMMUNOL 69:127, 1982 (abst)
30. Wharton GW: House dust mites (a review). J Med Entomol 12:577, 1976
31. Shamiyeh NB, Hornsby RP, Bennett SE, Woodiel NL: Distribution of house dust mites in the environs of a Tennessee river valley. J Econ Entomol 66:998, 1973
32. Arlian LG, Bernstein IL, Gallagher JS: The prevalence of house dust mites *Dermatophagoides* spp and associated environmental conditions in homes in Ohio. J ALLERGY CLIN IMMUNOL 69:527, 1982
33. Bronswijk JEMH, Sinha RN: Pyroglyphid mites (*Acari*) and house dust allergy: a review. J ALLERGY 47:31, 1971
34. Sharp JL, Haramoto FH: *Dermatophagoides pteronyssinus* (Trouessart) and other acarinae in house dust in Hawaii. Proc Hawaii Entomol Soc 20:583, 1970
35. Lang JD, Mulla MS: Seasonal dynamics of house dust mites, *Dermatophagoides* spp, in homes in southern California. Environ Entomol 7:281, 1978
36. Murray AB, Zuk P: The seasonal variation in a population of house dust mites in a North American city. J ALLERGY CLIN IMMUNOL 64:266, 1979
37. Tovey ER, Chapman MD, Platts-Mills TAE: Mite feces are a major source of house dust allergens. Nature 289:592, 1981
38. Platts-Mills TAE, Tovey ER, Mitchell EB, Mozarro H, Nock P, Wilkins SR: Reversal of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 2:675, 1982
39. Green W, Woolcock AJ, Dowse G: House dust mites in blankets and houses in the highlands of Papua New Guinea. Papua New Guinea Med J 25:219, 1982
40. Korsgaard J: Mite asthma and residency: a case control study on the impact of exposure to house dust mites in dwellings. Am Rev Respir Dis 128:231, 1983
41. Smith TF, Kelly LB, Heymann PW, Wilkins SR, Platts-Mills TAE: Natural exposure and serum antibodies to house dust mite of mite-allergic children with asthma in Atlanta. J ALLERGY CLIN IMMUNOL 76:782, 1985
42. Storm van Leeuwen W, Einthoven W, Kremer W: The allergen-proof chamber in the treatment of bronchial asthma and other respiratory diseases. Lancet 1:1287, 1927
43. Kerrebijn KF: Endogenous factors in childhood CNSLD. Methodological aspects in population studies. In Orie NGM, van der Lende R, editors: Bronchitis III. Royal Vangorcum Assen The Netherlands, 1970, p 38
44. Sarsfield JK, Gowland G, Toy R, Norman AL: Mite-sensitive asthma of childhood: trial of avoidance measures. Arch Dis Child 49:716, 1974
45. Murray AB, Ferguson AC: Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 71:418, 1983
46. Korsgaard J: Preventive measures in house dust allergy. Am Rev Respir Dis 125:80, 1982