The Distribution of Dust Mite Allergen in the Houses of Patients with Asthma[1-3]

EUAN R. TOVEY, MARTIN D. CHAPMAN, CLIVE W. WELLS, and THOMAS A. E. PLATTS-MILLS

SUMMARY Using an inhibition radioimmunoassay for the major allergen from *Dermatophagoides pteronyssinus* (antigen P_1), we studied the distribution of this dust allergen in the houses of patients with asthma. Both bed and floor dust samples contained a wide range of antigen P_1, 100 to 100,000 ng/g of fine dust, and this concentration correlated well with the number of mite bodies ($r = 0.81$, $p < 0.001$). We were unable to detect antigen P_1 in the air of undisturbed rooms. However, during domestic activity, between 1 and 30 ng were collected on a filter that sampled air for 45 min at 17 L/min. Using a cascade impactor it was shown that > 80% of the airborne antigen P_1 was associated with particles > 10μ in diameter. Some of the particles containing allergen could be identified because they formed precipitin rings when impacted onto agarose containing rabbit antimite antiserum. These particles had the physical appearance of mite feces, which are the major source of antigen P_1 in mite cultures. The results suggested that natural exposure to this dust allergen allows occasional fecal particles to enter the lungs and that these particles contain very concentrated allergen.

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Introduction

Many young patients with asthma are allergic to inhalant allergens, e.g., house dust, pollen, or domestic animal dander. In many studies, the incidence of positive results of skin tests to dust allergens has been so high that it is difficult to avoid the conclusion that there is a causal relationship between asthma and dust allergy (1). When these patients were challenged with aqueous allergen extracts in the skin, nasal passages, or bronchi they generally reacted within 15 min. Some allergic patients with asthma also have described a rapid onset of bronchospasm after natural exposure to domestic or laboratory animals. On the other hand, it is unusual for patients to be aware of a direct relationship between bronchospasm and exposure to either pollen grains or house dust (2-4). Indeed, many patients with asthma are not aware that they are allergic to dust. This absence of a direct relationship between exposure and asthma has led to considerable doubts about the role of dust or pollen allergens in asthma.

In 1873, Blackley studied the airborne distribution of pollen grains and he was struck by how small the quantities were that caused hay fever (5). More recent studies have estimated that the daily exposure to major pollen allergens is in the range of 0.1 to 10 ng/day and that annual exposure may be less than 1 μg (6, 7). In addition, studies using pollen grains have suggested that very few particles of this size enter the lungs (8, 9). Indeed, it has proved very difficult to induce bronchospasm by inhaling even large numbers of whole pollen grains (9, 10). On the other hand, two studies (11, 12) have reported that patients with asthma occurring during the pollen season develop nonspecific bronchial hyperreactivity, which returns to normal after the season. Those results suggested the possibility that repeated exposure to allergens might have a cumulative effect on the lungs without causing acute bronchospasm at the time of exposure. However, other investigators have had difficulty confirming seasonal variations in bronchial hyperreactivity (13).

In Europe and many other parts of the world, house dust mites are a prime source of dust allergens (14). Purification of a major allergen from cultures of *Dermatophagoides pteronyssinus* (antigen P_1) has made it possible to develop a radioimmunoassay for a specific dust allergen (15). Subsequent studies on *D. pteronyssinus* have shown that > 95% of the antigen P_1 accumulating in cultures is in the form of fecal particles that are 10 to 40 μ in diameter and contain very high concentrations of allergen (16). The present studies were designed to assess the quantities of antigen P_1 in the houses of patients with asthma and to determine the size of particles with which the airborne allergen was associated. As with pollen allergens most of the airborne allergen is associated with particles > 10 μ in diameter. The results suggested that during natural exposure to house dust only small quantities of allergen are likely to enter the lungs but that this allergen will be in a highly concentrated form.

Methods

Patients. Asthma was diagnosed in patients with a history of intermittent dyspnea and wheezing, and who responded to bronchodilators with or without steroid therapy. Peak expiratory flow rates and FEV_1 measurements were used to document severity and response to treatment. Eight of the 11 patients whose houses were studied in detail (table 1) were first seen after emergency admissions to hospital because of acute attacks of bronchospasm. Patients who were allergic to dust mites were chosen on the basis of a 3 plus (wheal size, > 6mm in diameter) response to prick testing with *D. pteronyssinus* extract (Bencards Ltd, U.K.) and by demonstrating IgE antibodies to antigen P_1 in their serum (15). The prick test reagent contained 10,000 pnu/ml and approximately 60 μg antigen P_1/ml.

Sampling extraction and mite counts on dust. Samples of dust were collected with a handheld vacuum cleaner (Hoover model No. 2614C; Hoover Ltd., U.K.), fitted with

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[1] From the Clinical Research Centre, Division of Immunology, Watford Road, Harrow, England.

[2] Supported by the Asthma Research Council.

[3] Requests for reprints should be addressed to Dr. T.A.E. Platts-Mills, Clinical Research Centre, Division of Immunology, Watford Road, Harrow, HA1 3UJ, England.

filter holder (MS 681 SL; Camlab Ltd., Cambridge U.K.) in front of the motor unit. Dust was collected on a double layer of domestic tissue supported on a wire mesh grid (Bowater-Scott Ltd., U.K.). Tissue and dust samples were stored in a plastic container for as long as 1 wk, at 4° C, prior to extraction and mite counting. Bed dust samples were obtained from mattresses by vacuuming the whole upper surface of the mattress, and from bedclothes by vacuuming each item. Floor dust from the bedroom and a living room (either sitting room or dining room) was collected from an area of approximately one square meter. Usually the collection of bed dust and floor samples required 5 and 1 min, respectively. Dust samples were handled by a modification of previously described techniques (17). Within 1 wk samples were sieved through an 850-mm mesh screen to obtain the fine dust. Mites were recovered from a 100-mg portion of fine dust by flotation in saturated NaCl with 20% sucrose followed by staining with crystal violet and collection on lined paper in a Buchner funnel. A separate 100-mg sample was extracted with 2 ml of phosphate buffered saline (BBS) (pH, 8.0) with 0.1% Tween® for 2 h with constant rotation, centrifuged at 1,500 *g*. The extracts were decoded and stored at −20° C for subsequent assay of antigen P_1 content. In preliminary studies, we found that both antigen assays and mite counts could be carried out on samples that had been stored for as long as 4 months, and that the repeatability of the assays was about 28% for mite counts and about 9.5% for the allergen concentration. Mites were mounted for identification under high power microscopy, as described previously (17).

Assay for antigen P_1 in dust extracts. The quantity of mite antigen P_1 in dust extracts was determined by a double antibody inhibition radioimmunoassay, using radiolabeled antigen P_1 and specifically purified rabbit antimite antibodies. This assay can detect 0.3 ng/ml of antigen P_1 with an intra-assay variation of about 6.4%. A control curve using a standard preparation of purified allergen was carried out in parallel with each assay. Two dilutions of each extract were assayed; where necessary, extracts were further diluted and the assay repeated (15).

Airborne dust samples. Airborne dust was collected on 3-cm discs of millipore glass fiber paper held in an Anderson filter holder connected to a vacuum pump (flow rate 1.17 L/min). For overnight samples a different pump (Dymox 2; Austen Pumps Ltd., Cambridge, U.K.) with a flow rate of 3.4 L/min was used. The filter was supported 15 cm above the surface of the floor. For extraction the filter was folded and placed in a glass durham tube (30 × 6 mm), and 2 ml of elution buffer (BBS, 0.1% Tween) was added slowly. The tube was periodically attached to a vacuum line to facilitate the penetration of the solution into the paper.

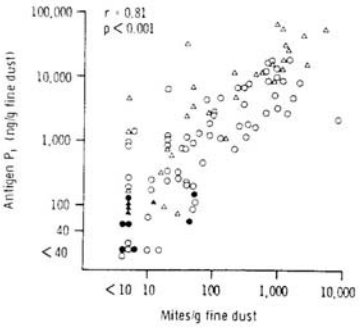

Fig. 1. Dust samples sieved for the fine dust were examined for mites microscopically and also extracted and assayed for antigen P_1 by inhibition radioimmunoassay. Samples were obtained from beds (△) and floors (○) of houses and in addition a few samples were obtained from schools (●) and hospitals (▲). Linear regression analysis on the logs of the results showed a direct correlation between antigen P_1 content and number of mites ($r = 0.81$, $p < 0.001$).

After storage overnight at 4° C, the extract was recovered by squeezing the contents of the tube in a 1-ml plastic syringe.

A cascade impactor (Mk 2a; Casella Ltd., London U.K.) operated at 17.5 L/min was used to collect fractions of airborne dust (18). In the impactor, each fraction was collected on a layer of agarose (2% agarose in half strength BBS with 5% glycerol) formed in a trough between a glass disc and a cover slip supported by a double thickness of plastic tape. After collection the agarose was transferred with a fine spatula to the wells of a microtiter plate. Elution buffer (200 μl) was pipetted across the disc into the well and incubated overnight at 4° C. The extract was separated from agarose by drawing it up through the tip of a pasteur pipette stuffed with cotton fiber and transferred to tubes for storage. An aerosol of commercial aqueous mite extract (Bencard, 0.93 μg antigen P_1/ml) and a crude mite extract prepared in the laboratory (14 μg antigen P_1/ml) were nebulized using 8 L of air/min through an Acorn nebulizer (Medic-aid Ltd: Chichester, U.K.). The mist was sampled by placing the inlet orifice of the cascade impactor 1 cm from the outlet of the nebulizer. The collection and handling of the samples was as for house dust.

When particles bearing mite allergen were investigated using the cascade impactor, 1.5% agarose in BBS plus 10% hyperimmune rabbit antiserum against whole mite extract was used. This antiserum gave a strong precipitin line against purified antigen P_1, which showed identity on immunodiffusion with the major line against an extract of mite feces recovered from cultures (15). To minimize the dehydration of the agarose, sampling time did not exceed 10 min at a flow of 17.5 L/min. The precipitin rings around some of the dust particles were visible under a microscope using dark field illumination. In some experiments a peroxidase-linked goat antirabbit IgG reagent was used to enhance the visibility of the precipitin rings (19). For electron microscopy, samples were fixed in 5% glutaraldehyde in 0.1M cacodylate buffer and were processed by a slow procedure and embedded in Spurr resin. Ultrathin sections were cut and were examined with a Philips 300 electron microscope.

Results

Samples of bed and floor dust collected from houses, schools, and hospital wards were found to contain concentrations of mite antigen P_1 ranging

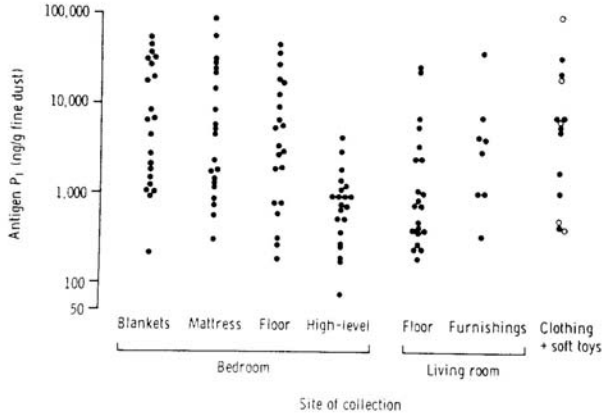

Fig. 2. Antigen P_1 concentrations in samples of dust from various parts of houses. A wide range of concentrations was found at each site. Highly concentrated dust was scraped with a card from the tops of door lintels, pelmets, and wardrobes. Several soft toys, which were kept in bed with patients, were also tested (○).

TABLE 1

DERMATOPHAGOIDES PTERONYSSINUS (ANTIGEN P_1) IN THE AIR OF BEDROOMS AND LIVING ROOMS

Patient No.	Diagnosis	Age/Sex	Airborne Dust* Undisturbed 2 h at 17 L/min (ng)	Undisturbed Overnight at 3.5 L/min† (ng)	Disturbed 45 min at 17 L/min (ng)	Mean Concentration in Fine Dust (μg/g)
Bedroom						Bed and floor dust‡
1	Asthma	38/F	—	< 0.3	7.3	45
2	Asthma	17/M	< 0.3	—	1.24	66
3	Asthma	7/F	< 0.3§	—	4.7	7.5
4	Asthma	56/F	< 0.3	—	1.4	0.67
5	Asthma	32/F	< 0.3	—	30	59
6	Asthma	18/M	—	—	2.2	45
7	Asthma	62/M	< 0.3	—	8.6	7.5
8	Asthma	28/M	< 0.3	< 0.3	7.3	31
9	Asthma	23/F	< 0.3§	—	9.3	41
10	Rhinitis	31/M	< 0.3	< 0.3	2.7	25
11	Rhinitis	22/F	< 0.3	< 0.3	23	19
12	Nonallergic	26/M	< 0.3§	< 0.3	13	34
13	Nonallergic	39/M	< 0.3	< 0.3	< 0.3	0.4
Living room						floor and furniture dust
1	Asthma	38/F	< 0.3	—	2.8	7.2
7	Asthma	62/M	< 0.3	—	1.9	6.8
15	Asthma	33/F	< 0.3	—	< 0.3	1.65
16	Asthma	14/M	< 0.3	—	0.8	21
12	Nonallergic	26/M	< 0.3	—	1.47	12

* Quantities of antigen P, (ng) collected from the air of bedrooms using a single fiber glass filter attached to a vacuum pump.
† Filters run close to the pillow of an occupied bed.
‡ The mean content of antigen P, for bed dust and floor dust determined as in figure 1.
§ In 3 houses, filters were run for 8 h at 17 L/min in the undisturbed room and still contained < 0.3 ng antigen P_1.

from < 100 ng to 100,000 ng/g of fine dust (figure 1). Dust recovered from the floors of schools and hospitals generally contained very little allergen. The number of mites found correlated with the antigen P_1 content of the dust ($r = 0.81$, $p < 0.001$). In houses the most potent source of allergen was usually bed dust although high concentrations were often found in the dust from carpets, furnishings, and clothing in other parts of the house (figure 2). Samples of high level dust concentrations usually contained few or no mites and lower concentrations of allergen than other dust in the same rooms. It was noted that the "cleanliness" of the houses or the presence of central heating was not associated with low concentrations of allergen. Most mites recovered from the dust were dead and not all were identified. However, samples from each dust were mounted and examined by high power microscopy. *Dermatophagoides pteronyssinus* was the most common pyroglyphid species in most houses; in two houses, *Euroglyphus maynei* was dominant; however, *D. farinae* was not observed. Occasional examples of *Cheyletus, Tarsonemus, Tyrophagus*, and *Glycyphagus* species were observed.

Samples of airborne dust were collected in bedrooms using a vacuum pump and a fiber glass filter. The quantities of antigen P_1 recovered after 2 h in an unoccupied room at 17 L/min, or overnight at the head of an occupied bed at 3.5 L/min, or during 45 min of domestic activity, i.e., sweeping, vacuuming, and bedmaking at 17 L/min are shown in table 1. In all cases the color of the filter was darkened by the collected airborne dust, but allergen was detected in the air only when the room

TABLE 2

AIRBORNE ANTIGEN P_1 COLLECTED WITH A CASCADE IMPACTOR*

Impactor Stages	Approximate Diameter of Particles Collected† (μ)	Bedrooms Studied during Domestic Activity‡ 2	7	5	6	14	4, 12, 13	Mean %	Nebulized Allergen Extract§ A	B	Mean %
1	> 20–6	0.78	3.9	28	8.1	17.2	< 0.3	88.8	< 1	< 1	< 2.2
2	15–2	< 0.3	0.48	5.1	< 0.3	1.84	< 0.3	6.7	3.6	19	10.4
3	5–1	< 0.3	< 0.3	1.48	< 0.3	1.12	< 0.3	1.8	3.9	22	11.5
4	2.5–0.3	< 0.3	< 0.3	0.78	< 0.3	1.04	< 0.3	1.4	17	240	73.5
Final filter	< 0.5	< 0.3	< 0.3	< 0.3	< 0.3	1.48	< 0.3	1.3	< 1	28	< 6.6

* Quantities (ng) of mite Antigen P, collected on the 4 stages and the final filter of a cascade impactor.
† The approximate diameters of particles that would be collected on each stage of the impactor.
‡ Dust in the bedrooms of 8 patients with asthma was disturbed by domestic activity, and airborne samples were collected for 40 min. In 3 houses (4, 12, and 13), no allergen was detected on the stages of the impactor.
§ The aerosol mist of 2 aqueous allergen extracts (A and B) generated by a nebulizer and sampled with a cascade impactor.

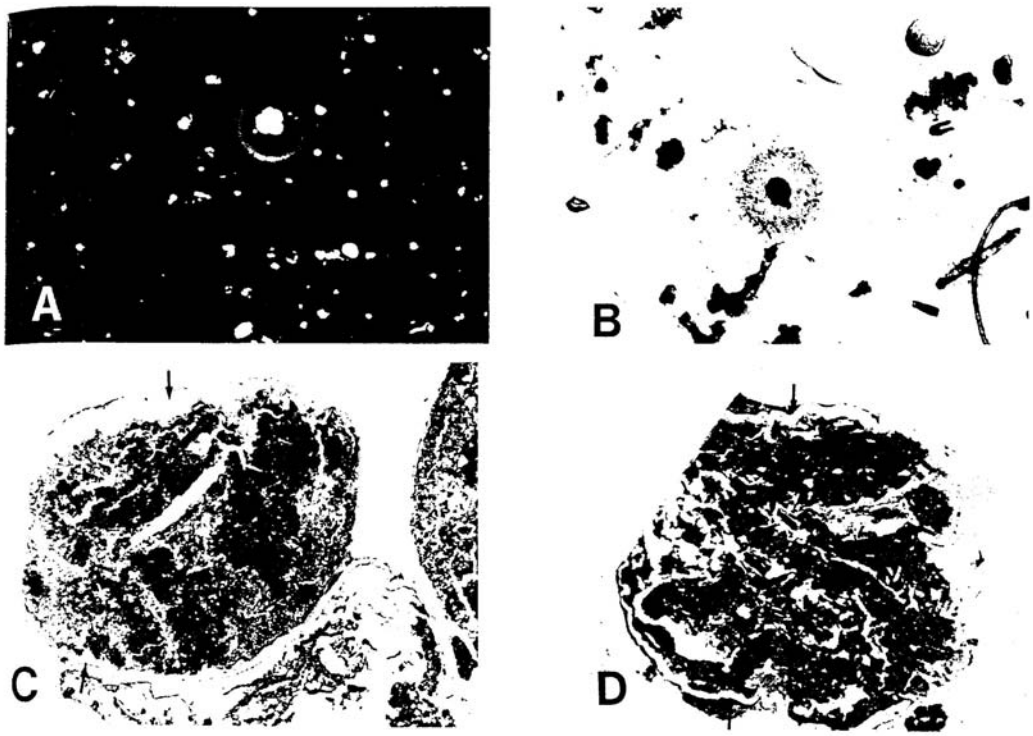

Fig. 3. Particles containing allergen identified on Stage 1 of the cascade impactor. A. A microprecipitin ring produced by an airborne particle impacted onto agarose containing rabbit antimite antiserum. The center of the ring in figure 2A appeared to contain 4 spherical particles stuck together, each approximately 25 μ in diameter (original magnification: × 120). B. A precipitin ring enhanced with peroxidase-linked goat antirabbit IgG (original magnification: × 170). C. Electron micrograph of a fecal particle and part of 2 adjacent particles obtained from *D. pteronyssinus* culture. This particle shows an amorphous center and a distinct peritrophic membrane (*arrowed*) (original magnification: × 7,000). D. Electron micrograph of a particle isolated from the center of a precipitin ring on the first stage of a cascade impactor. This particle also shows an amorphous center and membrane (*arrowed*) typical of mite fecal particles (original magnification: × 7,000).

was actively disturbed. The routine used to disturb bedrooms included making the bed, shaking each piece of bedding, vacuum cleaning the bedding and mattress, followed by vacuum cleaning of the floor. Although a similar routine was used in each house, there was little correlation between the allergen concentration in the air of disturbed rooms and the concentration of allergen in floor or bed dust. Nonetheless, the only house where no antigen P_1 was found in the air had the lowest concentrations of allergen. In 5 houses, similar measurements were made in a living room. In 4 cases, allergen was detectable in the air during vigorous domestic activity but none was detected in undisturbed rooms (table 1).

In 8 houses, a cascade impactor was used to provide information about the size of airborne particles carrying antigen P_1. The impactor collects particles on 4 discs and a final fiber glass filter in such a way that large particles (> 10 μ) are collected on the first disc and progressively smaller particles on the following discs (table 2). Between 76 to 100% of the total antigen P_1 was found on the first stage, as much as 14% on the second stage, and only a very small proportion was found on the remaining stages and final filter. When two different aqueous extracts of *D. pteronyssinus* culture were nebulized and sampled with the cascade impactor, approximately 75% of the total antigen P_2 was deposited on the fourth disc (Table 2).

When the cascade impactor was modified so that the discs had a coating of agarose containing rabbit antimite antiserum, some of the particles carrying allergen could be identified (figure 3A). Using peroxidase-linked goat antirabbit antiserum as a second antibody, the number of particles forming visible rings was approximately doubled (figure 3B). The particles forming precipitin rings were evident only in disturbed rooms and were most evident on the first stages of the impactor. However, occasional particles with a ring of immune precipitation were seen on the second but rarely on the third and fourth stages of the impactor. Most of the particles giving rise to precipitin rings were similar in size and shape to dust mite feces: mean diameter for 25 of these particles was 25.4 ± 4.0 μ. However, there were many particles present on the first disc of the cascade impactor that were of similar appearance but that did not give rise to precipitin rings. A few of the particles giving rise to precipitin rings were isolated

and studied by electron microscopy. Some of them showed the characteristic appearance of mite feces with an amorphous center and a surrounding peritrophic membrane (figure 3D) (20). A fecal particle from a mite culture is shown for comparison (figure 3C).

Discussion

Our measurements of the quantities of antigen P_1 in dust samples demonstrated that the domestic environment can contain large quantities of accumulated allergen. A house containing a total of 1 kg of dust might well contain several milligrams of antigen P_1, picogram quantities of which are sufficient to induce a skin response. Some houses, schools, and hospital wards were found to be almost free of mite allergen. All the houses studied were within a 3-mile radius; nevertheless, there was considerable variation in the quantity of allergen. Repeated vacuum cleaning, although it affects the total quantity of dust present, probably only has a modest effect on the allergen content (17, 21). Some houses appeared to be much damper than others, but additional factors such as age and type of furnishings and bedding may also account for the variation. We have found that not only bed and flooring but also clothing and soft toys can harbor considerable colonies of mites. In keeping with most previous studies, the quantity of allergen found in dust was proportional to mite numbers (14, 17, 21, 22). Our previous results showed that > 95% of the antigen P_1, which accumulates in mite cultures, is associated with fecal particles, each of which contains approximately 0.1 ng of antigen P_1 (16). The data in figure 1 suggest that dust samples contain ~ 10 ng antigen P_1/mite body. However, live mites only contain 1 to 1.5 ng/antigen P_1, whereas dead mites probably contain much less. The quantities of antigen P_1 found in dust samples probably represent accumulated fecal particles and could be explained if the dust contained ~ 100 fecal particles for each mite body.

A previous study on particles demonstrated that household cleaning was accompanied by a significant rise in the number of small particles in the air (23). The number of large particles in undisturbed air was generally very low but the relative increase after disturbance was greater, i.e., 10- to 15-fold, and these particles settled rapidly. In our studies, we were unable to detect allergen in undisturbed rooms even after prolonged sampling. This suggested that very little allergen was associated with particles that will remain airborne for more than a few minutes. In keeping with this, the allergen found in disturbed rooms was largely associated with particles > 10 μ in diameter. Whole mite bodies and fragments of mites can become airborne during bedmaking (24). We have observed similar fragments of mites on the first two stages of the cascade impactor but very few of these gave rise to visible precipitin rings. The cascade impactor cannot be regarded as a detailed model of the human lung; however, it does allow an assessment of the physical size of allergen particles in the air. The first stage of the impactor collects all particles > 20 μ diameter, whereas smaller particles 6 μ and less are collected with progressively decreasing efficiency. We found most of the allergen in the air on the first stage of the impactor and many studies have shown that particles > 10 μ generally do not enter the lungs (8, 25, 26). In addition, our data showed that nebulized particles designed to enter the lungs are predominantly collected on the fourth disc of the impactor. The lack of allergen associated with small particles in the air suggests that allergen in large particles in floor dust does not transfer to small particles and that the large particles do not generally break up.

The production of visible precipitin rings by allergen particles, which was previously reported for birch pollen grains (27), probably requires a very high concentration of allergen, i.e., > 1 mg/ml. We previously estimated that the fecal particles in mite cultures contain concentrations of antigen P_1 of about 10 mg/ml (16). A few particles forming precipitin rings were identified on later discs of the impactor but in each case they were formed round a large particle. Most of these particles have the appearance of mite feces, and some of them were positively identified as mite fecal particles by their appearance on electron microscopy. Clearly, particles containing less concentrated allergen would not be identified. The number of observed precipitin rings did not appear to be sufficient to account for the total quantity of allergen collected. It is possible that mite fecal particles in house dust contain less allergen than particles in mite cultures. However, failure to produce visible rings could occur because of mild desiccation of the agarose and consequent poor elution of the impacted particles.

At present there is no clear answer to some rather fundamental questions. Is allergen required to reach the lungs to produce hyperreactive bronchi? Is allergen necessary to induce asthma in allergic patients? In Europe, many young patients with asthma are allergic to *D. pteronyssinus*. If inhaled mite allergen is contained in large particles most of the allergen will be deposited and eluted in the nose. During natural exposure only picogram quantities would be expected to reach the lungs. Much of our thinking about allergen-induced asthma has been based on experimentally induced bronchospasm using nebulized allergen extracts. However, the quantities of allergen used in bronchial provocation are very different from those occurring naturally. The quantity of allergen entering the lungs during bronchial provocation with pollen allergen has been estimated to be equivalent to the quantity inhaled in 1 month of natural exposure (6, 28). Using commercial dust mite extract it is not unusual to nebulize 1 ml of a solution containing 1 μg/ml of antigen P_1. The maximal quantity of antigen P_1 that we observed on the late stages of the cascade impactor in a patient's house was less than 2 ng. The amounts of antigen P_1 inhaled in an undisturbed room during sleeping appear to be very low; however, the total quantity inhaled during domestic activity in some houses appears to be much higher than the daily exposure that has been calculated for pollen allergens. Thus for both pollen grains and dust particles, natural exposure represents a much slower rate of exposure than bronchial provocation; however, the natural allergen is in a highly concentrated form. Fecal particles contain ~ 0.1 ng of antigen P_1. The droplets produced by a nebulizer, i.e., 3 μ diameter and 1 μg antigen P_1/ml, may contain a million-fold less allergen than a fecal particle. Thus the reason why the quantities of nebulized allergen necessary to cause bronchospasm during bronchial provocation appear to be high relative to natural exposure, may be because the local concentration produced by each droplet is very low.

It seems likely that despite their size occasional pollen grains enter the lungs during the pollen season. Repeated local inflammatory events could well be re-

sponsible for the increase in bronchial hyperreactivity that has been reported to occur during the pollen season (11, 12). Exposure to dust mite antigens in Europe occurs throughout autumn and winter and it may be that prolonged exposure is important in the development of asthma. It is also possible that exposure to mite fecal particles in a dusty house represents a significantly larger quantity of allergen than natural exposure to pollen grains. At present, it is still not possible to predict with any accuracy the quantities of dust mite allergen that enter the lungs of patients with asthma. Our experiments suggested that dust mite fecal particles become airborne during domestic activity but do not remain airborne for long periods. From the size of these particles and their behavior on a cascade impactor we would only expect a small proportion of them to enter the lungs. However, because of their high concentration of allergen it seems likely that a fecal particle entering the lung would give rise to local inflammatory events.

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