

Airborne allergens associated with asthma: Particle sizes carrying dust mite and rat allergens measured with a cascade impactor

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Patients with asthma may develop acute symptoms after exposure to domestic or laboratory animal allergens; however, they are usually not aware of a direct relationship between their acute attacks and exposure to pollen or dust mite allergens. The present experiments were designed to study whether the differences in symptoms could be explained by differences in the number or size of particles carrying airborne allergens. Airborne particles were collected with a filter or on the stages of a cascade impactor, and allergens were measured by use of inhibition radioimmunoassays. In rat rooms and during disturbance of rat litter, a large proportion of rat urinary allergen (45.9%) was collected on the second stage of the impactor (mean size $\sim 7 \mu\text{m}$ diameter). When sampled 15 to 35 minutes after disturbance, 16% of these medium-sized particles were still airborne. By contrast, during disturbance of house dust, a significantly larger proportion of dust mite, antigen P₁ ($80.6 \pm 11.8\%$; $p < 0.001$) was collected on the first stage of the impactor, and in keeping with the apparent size of these particles (diameter $> 10 \mu\text{m}$), very little of this allergen ($< 4\%$) was still airborne when sampled 15 to 35 minutes after disturbance. With nebulized diluted rat urine, $\sim 75\%$ of the allergen was collected on the fourth and final stages of the cascade impactor in keeping with the expected size, 0.5 to 3 μm in diameter. These results demonstrate that natural exposure to both allergens is strikingly different from the conditions used for bronchial provocation. The results suggest that the different clinical responses may be explained by differences in the size of the particles on which these two allergens become airborne. (J ALLERGY CLIN IMMUNOL 77:850-7, 1986.)

Many patients with asthma have skin sensitivity and serum IgE antibodies against common airborne allergens. In addition, most of these patients will develop bronchospasm within 20 minutes after bronchial provocation with nebulized extract of the same allergens.¹⁻⁴ With some allergens, particularly those derived from domestic or laboratory animals, the relationship between natural exposure and acute exacerbations of asthma may also be obvious to the patients. Laboratory workers who become allergic to rat urine often develop acute attacks of asthma within minutes of entering an animal facility.⁵⁻⁸ However, with other

Abbreviations used

RIA: Inhibition radioimmunoassay
BBS: Borate-buffered saline, pH 8.0
Antigen P₁: Antigen P₁ of *Dermatophagoides pteronyssinus*

sources of allergen, including pollens and dust mites, a direct association between natural exposure to the allergen and attacks of asthma is usually not clear.⁹⁻¹¹ In order to understand the role that allergens play in both the acute and chronic symptoms of asthma, it is important to know both the quantities that are inhaled during natural exposure and also the size of particles carrying these allergens.

The classic technique for collecting airborne allergens is a pollen count, but this is only applicable to pollens and fungal spores and may fail to recognize allergens associated with smaller particles.¹²⁻¹⁶ When allergen has been collected on a filter, the quantities of allergen recovered have always been small, generally less than $1 \mu\text{g}/\text{m}^3$.¹⁶⁻¹⁸ To measure these airborne

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allergens, either RAST inhibition or RIA have been used.¹⁶⁻¹⁸ Using RIA, it is easier to standardize assays, and because the quantity of allergen can be expressed in absolute units, it is also easier to compare results for different allergens. Sizing of particles carrying allergen can be achieved by use of a series of filters,¹⁵ an impaction device,^{8, 16-18} or by measuring the rate of fall.¹³ We have previously reported that antigen P₁ only became airborne during domestic activity and was predominantly associated with particles >10 µm in diameter.^{17, 19}

In the present studies we have used RIA to measure both antigen P₁ and rat urinary allergens. Airborne allergen was collected with a cascade impactor and on filters both in animal houses and during artificial disturbance of house dust or rat litter. The results confirm that airborne dust mite allergen is predominantly associated with large particles and that these particles fall rapidly after disturbance. By contrast, airborne rat urinary allergen is predominantly carried on particles which are ~7 µm in diameter and that remain airborne for significantly longer periods.

METHODS

Radioimmunoassays

The rat allergen used was the α2 euglobulin fraction molecular weight 16 to 18,000 daltons, purified from male rat urine as described previously.²⁰ Twenty micrograms were radiolabeled with 1 mCi of ¹²⁵I by use of the chloramine-T technique. The labeled protein that was separated on a Sephadex G-25 column had a specific activity of 70,000 cpm/ng. The RIA for rat allergen used IgG antibody in the serum of a patient (J.W.) who was highly allergic to rat urine. This serum bound 225 ng of rat allergen per milliliter in a radioimmune precipitation assay; under comparable conditions nonallergic sera bound <0.5 ng of rat allergen per milliliter. Samples to be assayed were incubated with 0.1 ml of a 1:25 dilution of serum from patient J.W. After 2 hours, 2 ng of radiolabeled rat allergen was added and incubated for a further 2 hours at room temperature before adding goat antihuman IgG. The precipitate was allowed to form overnight, washed three times in BBS, and counted in a four-channel automatic gamma counter (Micromedic Systems Inc., Horsham, Pa.; 4/200). A control curve of serial twofold dilutions of purified rat allergen from 100 to 0.4 ng/ml was carried out in parallel with each assay. Antigen P₁ was measured by RIA as described previously.¹⁹ Briefly, samples were incubated with 0.1 ml of a 1:20,000 dilution of rabbit anti-*D. pteronyssinus* antiserum in 1:50 normal rabbit serum. After 2 hours, 3 ng of ¹²⁵I-labeled purified antigen P₁ was added, and after a further 2 hours, sufficient goat antirabbit gamma globulin was added to precipitate all the rabbit IgG. After 14 hours, the precipitates were washed three times with BBS and counted. A control curve was carried out in parallel with each assay by use of serial dilutions of a *D. pteronyssinus* extract over the range 250 to 1 ng of antigen P₁ per milliliter. This standard extract

TABLE I. Rat and dust mite allergen in house dust and rat litter*

	Rat allergen (ng/gm)	Antigen P ₁ (ng/gm)
Selected house		
dust samples		
I	<8	233,000
II	<8	25,000
III	<8	1,218
IV	<8	340
Samples from		
rat room		
Dirty litter	855,000	<60
Clean sawdust	38	112
Shelf dust	340,000	294
	ng/ml	ng/ml
Skin test reagents		
Rat hair	842	<1.0
<i>D. pteronyssinus</i>	<0.4	61,000
House dust	<1.0	820
Male rat urine	(a) 2,166,000	<2
	(b) 4,660,000	<2
Female rat urine	(a) 30,000	<2
	(b) 540,000	<2

*Allergens measured by double antibody RIA. Values for dust or litter obtained from 100 mg of fine dust extracted with 2 ml of BBS values calculated as nanogram per gram.

had been substandardized from the World Health Organization International *D. pteronyssinus* standard 82/518, available from National Institute of Biological Standards and Control, London, England.²¹

Airborne sampling

Air samples were collected on 3 cm discs of Millipore glass fiber filter (type AP20; Millipore, Bedford, Mass.) held in a filter holder attached to a vacuum pump (Gast Manufacturing Co., Benton Harbor, Mich.; model No. 0322-V4B-G18DX) and run at 17 L/min.¹⁷ Allergen was eluted from filters with 300 µl of 0.1% Tween 20 in 0.3% bovine serum albumin in BBS in glass tubes 30 by 6 mm. For particle sizing, the discs of a cascade impactor (Cassella, London, England) were coated with 2% agarose in BBS with 15% glycerol and run for 5, 10, or 20 minutes at 17 L/min. After sampling, the agarose was transferred to a microtiter plate and extracted with 250 µl of 0.1% Tween 20 in BBS with 0.3% bovine serum albumin. Extracts were stored at -20°C before assay for rat allergen or antigen P₁.¹⁷

Artificial disturbance for rat studies were carried out in a room, volume ~75 m³, with air ducts and windows closed and the vacuum pump outside the room. Six rat cages with a total of 2585 gm of dirty sawdust litter containing 1.32 mg of rat allergen per gram, total ~3410 mg of rat allergen, were tipped from one cage to another (one cage every 10 seconds) for 20 minutes of disturbance. The room was then left undisturbed for 15 minutes, and a second 20-minute sample was obtained either with a filter or the cascade im-

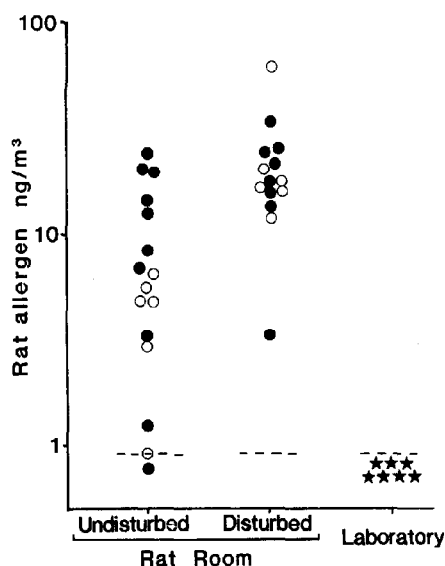


FIG. 1. Airborne rat allergen collected on a filter sampling air at 17 L/min for 20 minutes. Filters extracted overnight in BBS and assayed by RIA; values in nanograms were calculated per cubic meter. Rat rooms were rooms containing rats in cages sampled either when nobody was in the room (undisturbed) or when activity, changing cages, feeding, etc., was going on (disturbed). Rat housing was either cages containing litter (●) or wire cages suspended above the litter (○). Samples were also obtained in a laboratory where no animals were present (★).

pactor. In a separate experiment, six cages containing a total of 1305 gm of woodchips containing 2.1 mg of rat allergen per gram (total 2770 mg of rat allergen) were tipped in exactly the same way. Each cage had contained six rats for 6 days. Air samples were taken 5 feet above the ground and at least 6 feet from the site of disturbance.

For house dust experiments, a pool of samples from vacuum cleaners was used in two separate rooms of 128 and 35 cubic meters. The dust used came from houses both in London, England, and Charlottesville, Va., and the antigen P_1 content of the dust ranged from 13,000 to 200,000 ng/gm. Disturbance of house dust samples was carried out either manually or with a vacuum cleaner without a filter, held so that the exhausted air was not aimed toward the sampling device. Samples were taken 5 feet above the ground and 6 feet from the site of disturbance.

RESULTS

Rat-allergen measurements in rat litter and in the air of rat rooms

The radioimmunoassay for rat allergen has a sensitivity of 0.2 ng and an interassay variation of $\pm 8\%$. With this assay, very high levels of rat urinary protein were demonstrated in rat litter and dust on the floor

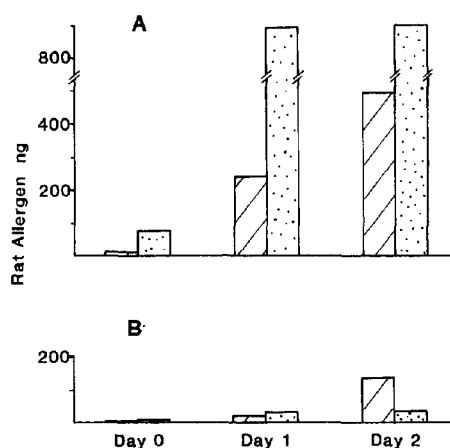


FIG. 2. Airborne rat allergen. A, Total during disturbance and B, after disturbance of rat cages containing wood chips (diagonal lines) or sawdust (dotted bars). The experiment was carried out on the day rats were removed from the cages (day 0) and on the 2 following days. Values are the mean results for two filters run in parallel each at 17 L/min for 20 minutes during disturbance A, and B, from 15 to 35 minutes after disturbance. Filters were eluted overnight with saline, and rat allergen was measured by RIA.

of rat rooms (Table I). By contrast, no rat allergen was detected in house dust samples containing up to 100,000 ng of antigen P_1 /gm (Table I). Similarly, no rat urinary allergen was detected in a range of skin test extracts, including grass pollen, ragweed pollen, cat dander, *Alternaria*, and *Cladosporium* (data not presented). With a filter to sample air at 17 L/min, rat allergen was found in small quantities in the air of rat rooms that were not disturbed except by the rats (Fig. 1). During disturbance, in cleaning or changing cages, slightly higher levels were found airborne. As expected, no airborne allergen was found in offices or in laboratories without animals (Fig. 1). When the air in rat rooms was sampled with a cascade impactor, allergen was detected on all four discs with the largest proportion (43%) on the second disc (Table II).

Airborne rat allergen during and after artificial disturbance of rat cages

In order to study the behavior of airborne rat allergen, we carried out artificial disturbance of dirty rat litter. Preliminary investigations demonstrated large differences in the amount of rat allergen that became airborne, depending on the nature of the litter and how long the cages had been stored. When disturbance was studied on 3 successive days after removal of the rats, the amount of airborne allergen

TABLE II. Airborne rat allergen in rat rooms collected with a cascade impactor*

Impactor stage	Approximate diameter of particles (μm)	Rat rooms					Cage wash†	Laboratory office†	Mean%‡ rat allergen (n = 5)
		Undisturbed	Some disturbance						
1	>20 to 6	0.46	1.7	1.35	0.6	2.2	<0.2	<0.2	26.9
2	15 to 2	3.36	3.0	2.0	1.2	1.5	<0.2	<0.2	45.4
3	5 to 1	0.49	0.5	0.4	0.64	0.5	<0.2	0.2	11.8
4	2.5 to 0.3	0.2	0.36	0.3	0.2	0.3	<0.2	0.2	5.7
Final filter‡	<0.5	1.8	0.26	<0.2	<0.2	0.5	<0.2	0.2	10.3

* Allergen collected for 20 minutes onto agarose, eluted into 0.3 ml of saline, and assayed by RIA; all values are nanograms collected on that stage.

† Samples obtained adjacent to cage washer during washing of rat cages and in an office 100 m from the animal facility.

‡ Mean percent on each stage is for the five cascade studies in rat rooms that elicited positive results.

§ Final filter and parallel filters are 3 cm discs of Millipore glass fiber paper eluted with saline overnight.

TABLE III. Cascade impactor analysis of airborne rat allergen during and after artificial disturbance of partially dried rat litter*

Impactor stage	20 Min disturbance		15 to 35 Min after disturbance		% Remaining‡
	(ng)	(%)†	(ng)	(%)	
Wood chips					
1	108	57.4	0.55	2.4	0.5
2	71	39	19.6	84.1	27
3	4.8	2.6	2.7	11.4	55
4	0.5	0.3	0.26	11.2	51
Final filter	0.9	0.5	0.24	1.0	27
Parallel filter	289		21		
Sawdust					
1	331	56	0.64	2	0.2
2	223	38	26.4	82	12
3	31	5.3	4.2	13	13
4	0.7	0.1	0.6	1.5	89
Final filter	1.2	0.2	0.5	1.5	46
Parallel filter	1201		27		

* Results of cascade impactor studies carried out 1 day after rats were removed from cages.

† Percentage was calculated from the total on all five stages of the impactor.

‡ Percent remaining is the quantity on that stage 15 to 35 minutes after disturbance expressed as a percent of quantity collected on that stage during disturbance.

increased steadily (Fig. 2). It was also clear that on the first 2 days, larger quantities of allergen became airborne from cages containing sawdust than from those with wood chips as litter (Fig. 2). The increased levels of allergen presumably reflect progressive drying of the litter. When the air was sampled for 20 minutes starting 15 minutes after disturbance, significant allergen remained airborne (Fig. 2). Simultaneous cascade impactor analysis demonstrated that during disturbance a large proportion of allergen was present on both first and second stages of the impactor (results on day 1 are illustrated in Table III). By contrast, in sampling 15 to 35 minutes after disturbance,

only a small proportion of the allergen detected was present on the first stage (Table III). Expressing the quantities of allergen on each stage after disturbance as a percentage of the allergen collected on that stage during disturbance confirmed that the percent remaining airborne was inversely related to the apparent particle size (Table III).

House dust mite allergen airborne during and after artificial disturbance

Our previous results implied that the "large" particles carrying antigen P₁ in house dust would only remain airborne for short periods.¹⁷ In order to study

TABLE IV. Cascade impactor analysis of antigen P₁ airborne during and 15 to 35 minutes after disturbance

Impactor stage	10 Min sample during disturbance		15 to 35 Min after disturbance		% Remaining†
	(ng)	(%)*	(ng)	(%)	
Experiment No. 1					
1	60	85.2	1.2	17.3	1
2	6	8.5	2.1	30.4	17.5
3	2.4	3.4	2.1	30.4	43
4	2	2.8	1.5	21.7	37
Final filter	<1.5	0	<1.5	0	—
Parallel filter	103		2		
Experiment No. 2‡					
1	94.5	77.7	3.6	12.7	0.95
2	14.9	12.2	15.6	55.1	26
3	5.2	4.3	3.7	13.2	17.8
4	4.5	3.7	2.7	9.7	15
Final filter	2.5	2.0	2.6	9.2	26
Parallel filter	270		22		

*Percentage was calculated from the total on all five stages of the impactor.

†Percent remaining airborne was calculated for each stage of the impactor relative to what we calculate would have been collected during 20 minutes of disturbance. Disturbance was continued for 10 minutes, but sampling time was restricted in some experiments to reduce the risk of overloading the impactor with dust particles.

‡Experiment No. 2 airborne allergen during 10-minute disturbance was only collected for 5 minutes.

the rate of falling of dust particles carrying antigen P₁, we carried out artificial disturbance of house dust in a laboratory. On each occasion, when the quantity sampled during disturbance was <18 ng in 10 minutes, none was detected 15 to 35 minutes later. In experiments in which the quantity of mite allergen varied from 31 to 985 ng during disturbance, the proportion remaining airborne 15 to 35 minutes after disturbance was 4% ± 3.8 (n = 12). Analysis of the particle size of the artificially disturbed dust demonstrated that a large percentage of the antigen P₁ was collected on the first stage. In keeping with its apparent size, very little allergen was collected on the first stage 15 to 35 minutes after disturbance (two representative experiments are presented in Table IV). Although the quantities were small, a larger proportion of the allergen collected on the second and third stages was airborne 15 to 35 minutes after disturbance (Table IV).

Particle sizing of nebulized rat urinary allergen

In order to assess the function of the cascade impactor with small particles, we sampled nebulized rat allergens with a disposable nebulizer that is in use both for therapy and challenge procedures at the University of Virginia Hospital. With the use of either

agarose or filter paper on the cascade stages to trap the droplets, we obtained very similar results with a mean of <1% on the first stage and >75% collected on the fourth stage and the final filter (Table V). These results are in keeping with the predicted size of droplets produced by a modified Wrights nebulizer, i.e., ~0.5 to 3.0 μm in diameter and are very similar to those we have previously reported for nebulized mite allergen (Table V).¹⁷

Analysis of results for two allergens

From the results in Tables III, IV, and V, it was clear that the percent of antigen P₁ collected on the first stage of the cascade impactor during dust disturbance (mean 80.6 ± 11.8%) was greater than for rat allergen (mean 40.1 ± 19.4%) (t = 5.98; p < 0.001). Measuring the total quantities of allergen airborne during and after disturbance demonstrated that only 3.9% of antigen P₁ was still airborne 15 to 35 minutes after disturbance, whereas 13.1% of the rat allergen was still airborne at this time (p < 0.01). When the allergen remaining airborne was analyzed for the separate stages of the cascade impactor, there was no apparent difference between the two allergens. For the first stage of the impactor, 3.3 ± 5.1% was still airborne at 15 minutes, and in eight out of 12 experiments, <2% of these large particles

TABLE V. Summary of results on the distribution of rat urinary allergen and mite allergens (antigen P₁) on a cascade impactor

Impactor stage	Approximate diameter of particles collected (μm)	Rat urinary allergen		Dust mite antigen P ₁				Nebulized aqueous extract	
		Rat rooms* (n = 5) %	Artificial disturbance†		Houses‡ (n = 5) %	Artificial disturbance§		Rat (n = 8) %	Mite (n = 2) %
			During (n = 8) %	After (n = 8) %		During (n = 6) %	After (n = 9) %		
1	<20 to 6	26.9	44.3	8.7	88.0	76.1	26.2	0.6	<2.0
2	15 to 2	45.4	45.9	42.3	6.7	13.2	36.2	9.0	10.4
3	5 to 1	11.8	4.5	17.4	1.8	5.5	15.4	12.3	11.5
4	2.5 to 0.3	5.7	2.6	21.2	1.4	3.9	13.5	55.9	73.5
Final filter	<0.5	10.3	2.7	11.1	1.3	1.2	8.6	22.1	<6.0

The mean percent of nebulized extract collected on the first two stages of the impactor (9.6%) was significantly lower than comparable results for antigen P₁ after disturbance (62.5%, $p < 0.001$) or rat allergen after disturbance (51.2%, $p < 0.005$).

*Values from Table II.

†Values from Table III and six further experiments.

‡Values from Tovey et al.¹⁷ obtained during housecleaning.

§Values from Table IV plus seven further experiments.

remained airborne. For the second stage, significantly more allergen remained airborne, $16.3 \pm 12.1\%$ ($t = 3.43$; $p < 0.005$). For the third and later stages, the percent remaining airborne was generally greater than for the second stage (Tables III and IV), but the quantities after disturbance were generally very low, <2 ng in 11/17 experiments. The results demonstrate that the slower falling rate for rat allergen is explained by the larger percentage of rat allergen associated with smaller particles. The apparent falling rates were in keeping with the size of particles, since particles of 10 to 20 μm with a density of 1.1 would be expected to fall at ~ 5 m/min in still air (by use of Stokes' equation).²²

DISCUSSION

The measurements of rat urinary allergen reported here demonstrate that both in laboratory rat rooms and during artificial disturbance of rat cages, a large proportion of allergen becomes airborne on particles with an apparent size of 5 to 10 μm in diameter. The measurements of mite allergen during artificial disturbance of house dust support our previous finding that a large proportion of this allergen becomes airborne on particles >10 μm in diameter.^{17, 23} For both allergens studied, the falling behavior in still air supported the conclusions reached from the cascade impactor.

In both of the animal facilities where these studies were carried out, patients had reported onset of bron-

chospasm within minutes of entering rat rooms. Furthermore, these patients had positive skin tests to rat urine and had serum IgE antibodies to the urinary allergen used here (data not presented). Our results confirm that very large quantities of allergen are present in rat cages, up to 4 gm per cage. Only a small quantity becomes airborne, and this probably depends on the nature of the litter, the design of the cages, the ventilation of the room, and, particularly, the water content of the litter. It is clear that these factors should be considered in any attempt to control rat allergy. The percent of airborne rat allergen associated with particles <10 μm was unquestionably greater than that observed with dust mite allergen. Comparing absolute quantities suggests that there was more rat allergen airborne in rat rooms than mite allergen in houses. We detected from 0.9 to 24 ng of rat allergen per cubic meter of air in "undisturbed" rat rooms, which compares with <1.0 ng of antigen P₁ per cubic meter of air in undisturbed bedrooms.

Previous results measuring airborne allergens have generally used much larger volume samplers, e.g., an Anderson sampler (Anderson Samplers Inc., Atlanta, Ga.) at 1.1 m³/min (sixtyfold faster than the cascade impactor).^{8, 16, 17, 24} With these high-volume techniques, it has been reported that a significant proportion of both guinea pig and ragweed allergen becomes airborne on small particles. Thus, Swanson et al.⁸ reported 532 ng of guinea pig-derived protein per cubic meter associated with particles <0.8 μm. The

maximum we have found for rat allergen is 21 ng/m^3 associated with particles $<2.5 \mu\text{m}$. The main difference between those experiments and our results is in the sampling device, and we believe that the results obtained 15 minutes after disturbance are strong evidence supporting our measurements. The present results demonstrate that some mite allergen can become airborne on small particles. However, the quantities associated with small particles were very small compared to the quantities on large particles or the quantities necessary for bronchial provocation.

Comparing airborne antigen P_1 with airborne rat allergen and with nebulized extract of either allergen demonstrates some interesting differences. Assuming that mite allergen is airborne as fecal pellets of $20 \mu\text{m}$ in diameter containing 0.2 ng per particle, then 20 ng equals 100 particles. An equivalent quantity of airborne rat urinary allergen on $7 \mu\text{m}$ diameter particles, assuming a concentration of 1 mg/ml , i.e., the concentration in rat urine, represents $\sim 27,000$ particles. With allergen extract nebulized to produce droplets of $2 \mu\text{m}$ in diameter, 20 ng would represent ~ 100 million droplets of extract, assuming $10 \mu\text{g}$ of allergen per milliliter. The predicted deposition of these particles in the lung would be very different. There is disagreement about the fate of particles 10 to $20 \mu\text{m}$ in diameter. Some results suggest that few particles this size enter the lung²⁵; however, from direct examination of human lungs, it is clear that pollen grains enter, and studies in monkeys suggest that up to 12% of particles of $>10 \mu\text{m}$ may enter the lungs.^{26, 27} Almost all of the large particles that entered the lung would be deposited in the bronchi. With particles of $7 \mu\text{m}$, between 20% to 40% would enter the lung.²⁸ For both $7 \mu\text{m}$ and $2 \mu\text{m}$ particles, the number of particles entering the lung would be such that an even distribution in all subsegments should occur. By contrast, during natural exposure of the lung to mite fecal particles or pollen grains (at most 10 particles in 20 minutes), it is possible that events occurring in separate subsegments of the lung would not be recognizable as an overall increase in lung resistance. Nonetheless, the deposition of a particle containing 0.2 ng would produce a very high local concentration of allergen on the bronchial mucosa. We have calculated that the concentration of allergen in fecal particles is $>10 \text{ mg/ml}$, and a single mite fecal particle contains sufficient allergen to elicit a strong positive skin test in many allergic patients.

A variety of different evidence suggests that allergen exposure may contribute to bronchial hyperreactivity, although the mechanism of this contribution is by no means clear. Patients with pollen allergy may demonstrate seasonal increases in hyperreactivity.²⁹

Prolonged allergen avoidance can lead to reductions in bronchial hyperreactivity.^{30, 31} Finally, some patients have demonstrated increased reactivity after bronchial provocation with allergens.³² The provocation experiments suggest that the concentration of allergen necessary to cause increased nonspecific reactivity is higher than the threshold for bronchospasm.³² However, if allergen is inhaled on particles, e.g., mite fecal pellets or pollen grains, the local concentration at the site of impact would be expected to be much higher than any concentration used for bronchial provocation.²³ Thus, inhaling allergen particles during a period of weeks could in theory progressively alter the reactivity of the lung without obvious episodes of bronchospasm triggered by inhaling the particles. Mite-allergic patients with asthma are often unaware of a direct relationship between their attacks and house dust exposure. Nonetheless, these patients usually improve during prolonged allergen avoidance.^{30, 31, 33} By contrast, laboratory workers who report rapid onset of bronchospasm on entering rat rooms do not always have increased nonspecific bronchial reactivity.³⁴ Patients with ragweed hay fever will often develop bronchospasm after bronchial provocation, even though they have never had asthma.³⁵ Thus, a positive bronchial provocation test can only be regarded as evidence that a particular allergen is causing asthma if it is known that the allergen becomes airborne in similar quantities and particle size. In conclusion, provocation with nebulized extract may be a relevant model of the rapid bronchospasm that can occur after exposure to rat urine. However, for dust mite allergens, the clinical response to house dust exposure is not similar to the acute response after experimental provocation.⁹⁻¹¹ Furthermore, the droplets used for bronchial provocation experiments are strikingly different from the size and allergen content of the particles that carry allergen in airborne house dust.

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