

Cat allergen content of commercial house dust extracts: Comparison with dust extracts from cat-containing environment

John L. Ohman, Jr., M.D., Janet R. Lorusso, B.A., and Susan Lewis, B.A.
Boston, Mass.

Eighteen lots of house dust extract from nine commercial sources (obtained as weight per volume or protein nitrogen unit per cubic centimeter) were analyzed for cat allergen content by direct quantitative immunoelectrophoresis after concentration. Cat allergen 1 was measurable (>0.3 units) in 11 extracts with a mean (range) of 5.8 (1.3 to 31.0) U/gm of source material. Cat albumin was measurable (>2.4 units) in 12 extracts with a mean (range) of 53.4 (11.5 to 319.7) U/gm. In order to evaluate whether the cat allergen 1 content is a significant contribution to the allergenic activity of the extract, 17 cat-allergic subjects were tested by prick test with a purified preparation of cat allergen 1. The mean (range) concentration that produced a 3 mm wheal was 0.01 (0.0013 to 1.33) U/ml. Therefore, the commercial house dust extracts studied, when these extracts were diluted to a concentration commonly used for prick testing, would frequently contain enough cat allergen 1 to produce strong prick test reactions in cat-allergic subjects. It is difficult to justify the use of such commercial dust extracts as diagnostic reagents. For comparison purposes, nine dust samples from an apartment housing two cats were similarly analyzed. Cat allergen 1 was measurable in seven samples with a mean (range) of 23.8 (1.8 to 64.3) U/gm. Cat albumin could be measured in all nine samples with a mean (range) of 32.3 (0.16 to 70.8) U/gm. The average amount of cat allergen 1 that could be washed off the surface of the cats was 270 units. Large reservoirs of cat allergen 1 were present. The spread on which the cats commonly slept contained 20,000 units of cat allergen 1. Contamination of a pool of house dust samples with dust from a cat-containing environment would likely be significant. J ALLERGY CLIN IMMUNOL 1987;79:955-9.)

House dust extract is unique among the extracts commercially available for diagnosis and treatment of inhalant allergy in that it is impossible to define the starting material with any precision. Variable amounts of soluble components of mold, bacteria, pollen, animal danders, mites, and even foods and household chemicals might be found in the extracts. When house dust extracts have been studied, evidence has been found of bacterial endotoxin,¹ animal danders,^{2,5} mites,⁶ mold,^{4,7} and green algae.⁸ When different preparations of commercial house dust have been compared, wide variations in allergenic and nonallergenic activity are found.^{1,4,9} Therefore, it is diffi-

cult to evaluate the significance and relevance of positive skin test reactivity to commercial house dust extracts.

We were prompted by several developments to do this study. First, the ability to measure cat allergen 1 with a simple radial immunodiffusion assay is widely available with the use of reagents provided by the Allergenic Products Division of the U.S. National Center for Drugs and Biologics. Second, the clinical significance of specific levels of cat allergen 1 is known (i.e., the concentration of cat allergen 1 that produces a positive prick test reaction in cat-sensitive individuals). Therefore, manufacturers could monitor the levels of cat allergen in their dust extracts before distribution. Our clinical experience has suggested that patients with a strongly positive skin test reaction to cat allergen are almost always also reactive to house dust.

METHODS AND MATERIAL

References to commercial house dust extracts in this article are indicated by number and letter. The number refers to the manufacturer and the letter refers to the particular batch. The following house dust extracts were studied (the

From the Allergy Research Laboratory, Veterans Administration Outpatient Clinic, and Tufts University School of Medicine, Boston, Mass.

Supported in part by the Veterans Administration Medical Research Service, U.S. Public Health Service Grant P50 AI-18023-02, and the Foundation for the Study of Asthma and Related Diseases.

Received for publication Aug. 5, 1986.

Accepted for publication Dec. 21, 1986.

Reprint requests: John L. Ohman, Jr., M.D., 5 Longfellow Pl., Boston, MA 02114.

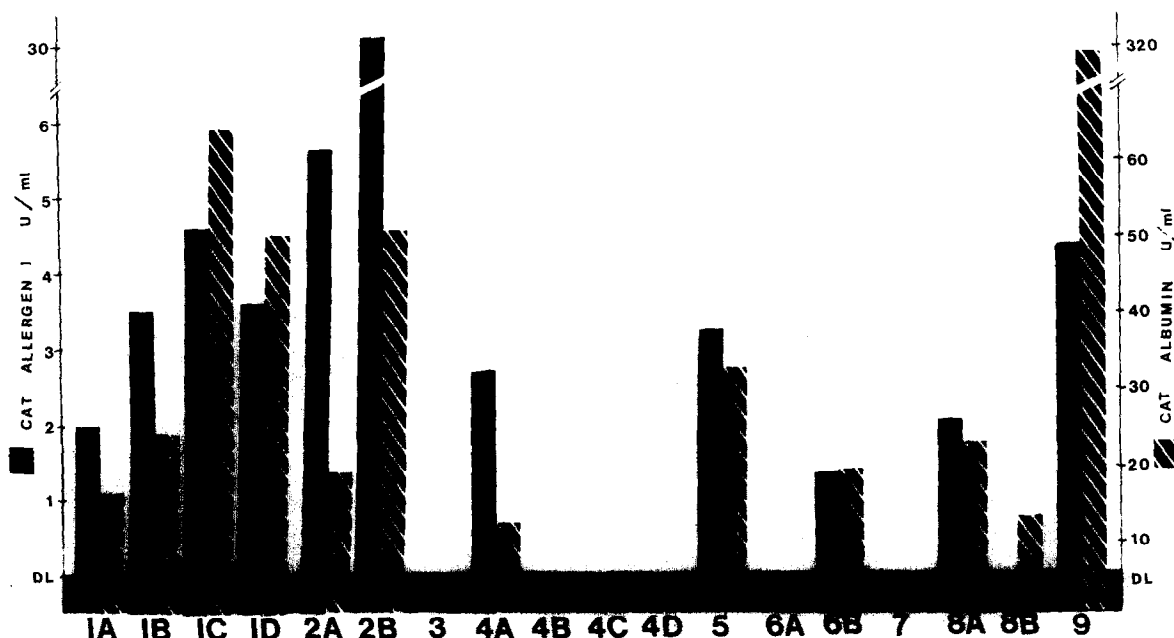


FIG. 1. Cat allergen and cat albumin content of the 18 commercial house dust samples. Numbers and letters below the bars indicate the manufacturer and batch, respectively. Solid bars represent cat allergen 1 content and striped bars represent albumin content. DL indicates the detection limit of the immunoelectrophoresis assay.

number and letter designation are in parentheses): Hollister-Stier Laboratories, Spokane, Wash.: batch I52061M (1A), E599834 (1B), H72735M (1C), and L62472M (1D); Allergy Laboratories, Inc., Oklahoma City, Okla.: batch Y3598 (2A) and 3817 (2B); Allmed, San Diego, Calif.: batch A850422 (3); Allergy Laboratories of Ohio, Columbus, Ohio: batch B404111C (4A), B3080211 (4B), B404111C (4C), and B404111H (4D); Meridian Bio-Medical, Denver, Colo.: batch 4H1B (5); Greer Laboratories, Lenoir, N.C.: batch GD9-311B (6A) and GD9-319B (6B); Center Laboratories, Port Washington, N.Y.: batch 85932 (7); Nelco, Deer Park, N.Y.: batch 850244 (8A) and 856724 (8B); and Veterans Administration Medical Center Allergy Laboratory, Pittsburgh, Pa.: batch D7486 (9). The extracts were each dialyzed in 8000 molecular weight cutoff membrane tubing against 2000 volumes of distilled water for 24 hours at 4° C and were then concentrated by lyophilization. All extracts were concentrated to 1:1 w/v with the exception of three extracts that were supplied as 20,000 PNU (1A, 1B, and 7). These extracts were concentrated tenfold for analysis. All extracts were therefore tenfold more concentrated than what is commonly used for prick testing.

Vacuum cleaner dust was obtained from an apartment in which two cats (a female and a neutered male) had been housed for at least 3 years. Dust was collected by vacuum from nine areas in separate vacuum cleaner bags. The nine apartment dust samples were eluted 1:10 w/v in distilled water for 24 hours at 4° C and then were concentrated tenfold for analysis. The spread on which the cats slept that had remained unwashed for 6 months was extracted in 60 L of water as described above and concentrated to 1 L for analysis with a 10,000 molecular weight cutoff hollow fiber cartridge with a CH2 concentrating apparatus (Amicon Corp., Dan-

vers, Mass.). The dust from a 9 by 12 foot area of rug was vacuumed and contained 20 gm of solid material; this solid material was extracted with water (1:10 w/v) and concentrated tenfold as described above.

Antisera

Monospecific polyvalent antisera to cat allergen 1 and cat albumin were obtained as previously described.¹⁰

Quantitation of allergen and albumin content

Allergen and albumin content of the 18 commercial dust samples and of the nine apartment dust samples was assayed by Laurel rocket immunoelectrophoresis with the antisera described above. Cat allergen 1 and albumin contents are reported in units per milliliter. Cat allergen 1 and cat albumin units are arbitrary units in our laboratory, developed by comparison with a laboratory reference standard. The cat allergen 1 units are equivalent to the cat allergen 1 units used by the National Center for Drugs and Biologics (Dr. Harold Baer). One cat allergen 1 unit is equivalent to approximately 2 µg of protein. One cat albumin unit corresponds to approximately 0.67 µg of protein. Until an amino acid sequence has been obtained, protein weight cannot be accurately related to units of antigenic activity. Because this information is not yet available, we have chosen to retain the unit designation.

Because of the probability that mammalian albumins cross-react between species, we tested the specificity of our assay. With antisera to cat albumin in the immunoelectrophoresis gel, samples of dog, rabbit, guinea pig, cow, horse, sheep, mouse, and rat serum were tested. No measurable albumin was detected, suggesting that our anticat albumin

TABLE 1. Cat allergen content in dust samples from a cat-containing apartment

Area	Cat contact	Allergen (U/gm)	Albumin (U/gm)
Litter/food	3+	<0.25	0.16
Hall/bath	+	<0.25	0.16
Rug	3+	41.30	57.30
Bed	4+	64.30	70.80
Shelves	2+	13.70	33.40
Heat/air	+	2.50	2.30
Kitchen floor	—	1.80	5.60
Kitchen counter	—	1.80	1.30

3+ Cats often observed in this area.

+ Cats rarely allowed in this area.

4+ Cats observed to spend large amounts of time in this area.

2+ Cats occasionally observed in this area.

— Cats excluded from this area.

antisera did not react significantly to the albumins of these species.

Skin testing of cat-allergic subjects

Seventeen cat-allergic subjects as determined by positive skin test, bronchial challenge, and history were prick tested with serial dilutions of purified cat allergen 1 extract. Wheal response was measured after 15 minutes. Concentration of cat allergen 1 that would produce a 3 mm wheal was determined from the titration curve for each subject.

RESULTS

Cat allergen content of commercial house dust extracts

Fig. 1 summarizes the results of measurements of cat allergen 1 and cat albumin in 18 lots of commercial house dust from nine different manufacturers. Cat allergen 1 was measurable in 11 lots with a mean (range) of 5.8 (1.3 to 31) U/gm. Cat albumin was measurable in 12 lots with a mean (range) of 53.4 (11.5 to 319.7) U/gm. Extracts from manufacturer No. 1 consistently contained large quantities of cat allergen, although different batches exhibited at least twofold differences. Extracts from another manufacturer (No. 4) had measurable amounts of cat allergen 1 and albumin in one batch and not in the other three batches. In the 11 samples in which both albumin and cat allergen 1 could be measured, there was no significant correlation between these measurements.

In order to determine the significance of these measured amounts of cat allergen 1, we prick tested 17 cat-allergic subjects with a purified preparation of cat allergen 1. The mean (range) concentration that elicited a 3 mm wheal was 0.01 (0.0013 to 1.33) U/ml. Fig. 2 illustrates the concentration of cat allergen that produced a positive skin test in cat-allergic individuals

conc. of cat 1 that produces a 3mm prick test wheal in allergic individuals

0.001 .01 .1 1.0 10

conc. in commercial house dust samples *

DL .1 1.0 10

conc. of cat 1, U/ml

* adjusted to concentrations commonly used for prick testing

FIG. 2. Cat allergen 1 concentration of 18 commercial house dust extracts (*lower panel*) compared with the concentrations of cat allergen 1 necessary to elicit a 3 mm wheal by prick test in 17 cat-allergic individuals (*upper panel*). Cat allergen 1 concentration is expressed in a dilution of commercial house dust extract commonly used for prick testing (1:10 w/v or 20,000 PNU/cc). DL indicates the detection limit of the immunoelectrophoresis assay.

and compares that to the amount of cat allergen measured in commercial house dust extracts. Eleven of the 18 dust extracts contained measurable quantities of cat allergen 1 within the limit of detection of this assay. Twelve of the 17 allergic subjects would have reacted to all the commercial dust samples in which cat allergen was measurable. The remaining five sensitive subjects would react to commercial dust samples that contain cat allergen concentrations too low to measure with this assay. Therefore, commercial house dust extracts, when they are diluted to a concentration common for prick testing, will often contain enough cat allergen 1 to produce a positive prick test in cat-allergic individuals on the basis of cat allergen content alone.

Cat allergen content in the dust of an apartment containing cats

Commercial house dust is likely obtained from a pool of individual house dust samples. We were therefore interested to know how much cat allergen might be found in an indoor location that contained cats. Dust samples were obtained from various locations

and adjusted to a concentration of 1:1 w/v. These results are summarized in Table I. The frequency of direct cat contact in these individual areas was estimated on the basis of observation according to the following scale: -, cats excluded from this area; +, cats rarely allowed in this area; 2+, cats occasionally observed in this area; 3+, cats often observed in this area; and 4+, cats observed to spend large amounts of time in this area.

The highest concentration of cat allergen 1 was observed in the rug and on the bed on which concentrations of >40 U/gm were measured. One commercial house dust extract (2B) contained >40 U/gm, suggesting heavy cat contact with the source material of that extract.

Other areas less frequented by the cats contained less cat allergen 1 and cat albumin. It is of interest that the areas in the kitchen from which the cats were totally excluded contained measurable amounts of cat allergen 1 and cat albumin, suggesting that allergen was carried on cross-ventilated air. In general, areas frequented by the cats contained the most cat protein with the exception of the litter and food area.

In order to appreciate the total quantity of allergen that could be obtained from the two sources with the higher concentration of cat allergen 1, the dust from a 9 by 12 foot area of rug and the spread on which the cats commonly slept was eluted. The dust from the rug yielded a total of 6300 units of cat allergen 1 and 49,000 units of cat albumin. Elution of the spread yielded 20,000 units of cat allergen 1 and <2400 units of albumin. Each of the cats was washed two times as previously described.¹¹ The average yield of cat allergen 1 per individual wash was 270 units. Albumin could not be detected in the washes (<24 units per wash). These results suggest that cat allergen accumulates progressively in rugs and bedding.

DISCUSSION

This study clearly demonstrates that commercial house dust extracts frequently contain quantities of cat allergen 1 that would produce strong prick test reactions in cat-sensitive subjects. Cat albumin, which is a significant allergen in a small percent of cat-sensitive subjects, was also frequently present and would contribute to the allergenic activity of these extracts. The measurement of any allergen does not have practical usefulness if the measurement cannot be performed in a reproducible manner in different laboratories with widely available reagents. The reagents to measure cat allergen 1 with a simple radial immunodiffusion technique are available to manufacturers through Dr. Harold Baer of the National Center for Drugs and Biologics. Therefore, manufacturers are

able to measure levels of cat allergen 1 in batches of house dust during production.

For comparison purposes, the dust from an apartment that contained two cats was analyzed. Dust from areas of the apartment with frequent cat contact contained >40 U/gm of cat allergen 1. Commercial house dust extracts usually contained <6 U/gm with the exception of one extract (2B) that contained 31 U/gm. This particular house dust extract contained more cat allergen 1 than similar weight per volume strengths of some commercial cat epithelial extracts.¹⁰

Some cat-containing environments obviously contain large total quantities of cat allergen. In the apartment studied in this article, 20,000 units of cat allergen 1 could readily be eluted from the spread on which the cats commonly slept. An average of only 270 units of this allergen could be eluted from the surface of the cats. This suggests that a progressive accumulation of allergen occurs in areas that cats frequent. It is likely that beds and stuffed furniture on which cats sleep provide a continuing reservoir for contamination of the environment even after cats are removed. The total duration of time that cat allergen would persist in such areas is not known; however, cat allergen 1 is very stable¹² and would likely persist for long periods. The high quantity of cat albumin in these dusts is somewhat surprising in view of the fact that surface washes of living cats yield very little albumin.¹¹ One possible explanation is that the albumin was derived from cat urine that could have contaminated the dust. Cats are known to deposit urine for territorial marking.

It is obvious that <10% contamination of a house dust pool with a dust sample from a house with cats would likely be significant. The presence of variable amounts of cat allergen 1 in many commercial house dust extracts adds mainly diagnostic confusion because the level of cat allergen in these extracts is not known. It is difficult to justify the continued use of house dust extracts that contain significant and readily measurable amounts of cat allergen 1. More strict control of the source material from which these extracts are made should be exercised.

REFERENCES

1. Siraganian RP, Baer H, Hochstein HD, May JC. Allergic and biologic activity of commercial preparations of house dust extract. *J ALLERGY CLIN IMMUNOL* 1979;64:526-33.
2. Pauli B, Bessot JC, Hirth C, Thierry R. Dissociation of house dust allergies: a comparison between skin tests, specific IgE, and basophil histamine-release measurements. *J ALLERGY CLIN IMMUNOL* 1979;64:245-52.
3. Lorusso JR, Ohman JL, Lewis SJ. Commercial house dust extracts frequently contain significant quantities of cat allergen [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:130.
4. McCullough JA, Ownby DR. Do commercial house dust ex-

- tracts (CHDE) contain unique allergens [Abstract]? *J ALLERGY CLIN IMMUNOL* 1986;77:130.
5. Schwartz B, Lind P, Graff-Lonnevig V, Hedlin G, Heilborn H, Sundin B, Lowenstein H. Sequential quantitative measurements of cat, dog, and mite allergenic molecules in dust from allergic homes [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:203.
 6. Pepys J, Chan M, Hargreave FE. Mites and house dust allergy. *Lancet* 1968;1:1270-2.
 7. Bardana EJ, McClatchy JK, Farr RS, Minden P. The primary interaction of antibody to components of aspergilli. I. Immunologic and chemical characteristics of a nonprecipitating antigen. *J ALLERGY CLIN IMMUNOL* 1972;50:208-21.
 8. Bernstein IL, Safferman R. Clinical sensitivity to green algae demonstrated by nasal challenge and in vitro tests of immediate hypersensitivity. *J ALLERGY CLIN IMMUNOL* 1973;51:22-8.
 9. Kawai T, Marsh DG, Lichtenstein LM, Norman PS. The allergens responsible for house dust allergy. I. Comparison of *Dermatophagoides pteronyssinus* and house dust extracts by assay of histamine release from allergic human leukocytes. *J ALLERGY CLIN IMMUNOL* 1972;50:117-27.
 10. Ohman JL Jr, Lowell FC, Bloch KJ, Kendall S. Allergens of mammalian origin. V. Properties of extracts derived from the domestic cat. *Clin Allergy* 1976;6:419-28.
 11. Ohman JL, Baer H, Anderson MC, Leitermann K, Brown P. Surface washes of living cats: an improved method of obtaining clinically relevant allergen. *J ALLERGY CLIN IMMUNOL* 1983;72:288-93.
 12. Leitermann K, Ohman JL. Cat allergen I: biochemical, antigenic, and allergenic properties. *J ALLERGY CLIN IMMUNOL* 1984;74:147-53.

Nifedipine treatment of patients with bronchial asthma

D. Patakas, M.D., E. Maniki, M.D., V. Tsara, M.D., and
E. Descalópoulou, M.D. Thessaloniki, Greece

Nifedipine, 30 mg/day for 4 weeks, was compared to placebo in a double-blind, randomized, crossover study, as an additional drug added to the usual treatment of 14 patients with bronchial asthma. Nifedipine did not significantly change peak expiratory flow rates or subjective symptoms like cough, sputum, wheezing, shortness of breath, or disturbed sleep. Nifedipine did not decrease the number of salbutamol rotacaps inhaled per day. Arterial blood pressure significantly decreased ($p < 0.01$) after nifedipine treatment, and side effects (headache and flushing) were not uncommon. In this study, long-term treatment with nifedipine had essentially no effect on subjective symptoms at peak expiratory flow rates. (J ALLERGY CLIN IMMUNOL 1987;79:959-63.)

Nifedipine antagonizes calcium ion in smooth muscle cells, thereby inhibiting constriction of certain types of smooth muscles. Nifedipine is used in the treatment of coronary artery spasm,¹ hypertension,² Raynaud's phenomenon,³ esophageal sphincter spasm,⁴ and hypertrophic cardiomyopathy.⁵

Many pathophysiologic events that underlie bronchial asthma and hyperreactive airway disease are calcium-dependent phenomena, and the effects of calcium blocking agents on the prophylaxis and therapy

Abbreviation used

PEFR: Peak expiratory flow rate

of these conditions have been investigated. Most of the published work deals with the effect of calcium blockers on the pharmacologic antigen- and exercise-induced bronchospasm. Nifedipine (20 mg) was demonstrated to protect against histamine-induced bronchoconstriction in subjects with bronchial asthma.⁶ Nifedipine, administered as a single oral dose, blunted grass pollen⁻⁷ as well as ragweed-induced bronchoconstriction in sensitive patients with asthma.⁸ Finally, sublingual nifedipine (20 mg) protected against exercise-induced bronchoconstriction^{6,9} and also blunted the bronchial obstruction induced by isocapnic hyperventilation with dry, subfreezing air.¹⁰

From the Pulmonary Clinic Department of Internal Medicine, University of Thessaloniki, Thessaloniki, Greece.

Received for publication Feb. 14, 1986.

Accepted for publication Dec. 27, 1986.

Reprint requests: D. Patakas, M.D., Pulmonary Department, Internal Medicine, University of Thessaloniki, 1 Patriarchou Ioakim St., Thessaloniki, Greece.