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Variability of cat-allergen shedding

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To study the differences in total allergen shed, nine cats were placed individually in a lucite chamber with an air sampler attached for 1 hour. With a RAST-inhibition assay that used specific cat IgE antibodies from cat-sensitive subjects, we ranked the allergen production of each cat. From these nine cats, three high producers and one low producer were studied further. Each cat's allergens were collected for two to four separate 1-hour intervals on 6 different days. The high producers' samples remained high in allergen content, and the low producer's sample remained low. An additional eight cats were selected for similar longitudinal measurements, and allergens from each of these 12 cats were collected during four 1-hour intervals on 2 different days and assayed for total allergy units (AUs) by RAST inhibition and for the major cat allergen, Fel d I, by a two-site assay with a monoclonal antibody. Shedding, particularly by high producers, varied considerably from hour to hour. We found a hundredfold difference in AUs between the mean rate of shedding of the highest and lowest producers and a sixfold difference in Fel d I units. Variation in rate of shedding of Fel d I accounted for about half the variation of shedding of AUs. Allergen shedding varies not only between cats but also in the same cat during the course of a day and between days. Male cats shed more than female cats. (J ALLERGY CLIN IMMUNOL 1990;85:94-8.)

Airborne allergens from cat dander commonly cause allergic symptoms. *Fel d I* is a specific allergen that is believed to be the major cat protein causing these reactions.^{1,2} Immunohistochemical stains reveal that *Fel d I* is produced mainly in hair roots and saliva.³⁻⁶ Many patients report more severe allergic symptoms on exposure to some cats than to other cats. One possible explanation for this experience is that different cats produce different amounts of allergens. To measure rates of allergen shedding, we placed cats individually in a chamber that had an attached air pump that drew air over the cats and caught the airborne particles on a filter. Filter eluates were assayed immunochemically, and variations in rates of total allergen (AU) and *Fel d I* shedding were determined

Abbreviations used

AU: Allergy units, U.S. Office of Biologics
Fel d I: *Felis domesticus* allergen 1 (cat 1)

among 12 cats at different times. We found considerable variation between cats and from time to time in the same cat.

METHODS

Sampling

A lucite chamber measuring 15 by 17 by 66 cm was used to collect air samples from the cats. The air sampler was attached at one end of the chamber and drew air over the animal placed in the chamber and through a polytetrafluoroethylene filter membrane (99% efficiency for particles 0.3 μ m or larger) at a rate of 1 L/sec. The membrane trapped allergenic particles on the filter membrane as they were shed by the cat. Each sampling period was 1 hour in duration. The chamber was cleaned after each sampling.

After completion of sampling, the polytetrafluoroethy-

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lene filters were delaminated from the support backing and placed in 250 μ l of 50% glycerinated RAST-diluent buffer solution (0.1 mol/L of phosphate buffer containing 0.2% bovine serum albumin). The tubes were well mixed to loosen particles from the filter membranes and kept overnight at 4° C and then stored at -20° C until they were ready for assay. Before assay, samples were centrifuged at 10,000 rpm for 3 minutes.

Selection of cats and schedule of sampling

Nine mongrel cats were selected from the animal-care facility of the Mayo Foundation. A single 1-hour sample was obtained from each cat. These samples were assayed for AUs by RAST inhibition. With these results, three high producers and one low producer were selected for further study.

Samples were obtained from each of the four cats for 6 days. The sampler was placed in a hood in the laboratory. The first 3 days and the last 3 days were consecutive. A break of 2 weeks existed between 3-day consecutive samples. Each day, two to four 1-hour air samples were collected. Sampling was done in a laboratory that had no background cat allergens in the air.

Three months later, an additional eight cats were selected for study. Repeated air samples were collected from these eight new cats, along with the four cats studied previously. The same protocol was used. These samples were collected on a different floor of the building in which the cats were housed but in a separate clean room. Because of the possibility of allergens already being present in the air of that room, a background sample without a cat in the chamber was taken immediately before and after sampling each day. When these two values were significantly different, the chamber sample values for that day were not used in calculations, since the variation in the background could elicit inaccurate sample values. In most instances, the prebackground and postbackground values were quite similarly low. In these cases, the two background values were averaged, and the average was subtracted from each sample for that day, 70 AU/hr or 0.01 *Fel d I* units per hour. The remaining five cats were returned to the animal-care facility.

Direct two-site assay

Immulon II wells (Dynatech Laboratories, Alexandria, Va.) were coated with 100 μ l (1 μ g per well) of affinity-purified rabbit anti-*Fel d I* (Dr. John Ohman, Boston, Mass.) in a 0.02 mol/L of carbonate buffer at pH 9.2. The wells were incubated overnight at room temperature in a humid chamber. After incubation, the wells were washed three times with RAST wash; 100 μ l of *Fel d I* cat standards (Bureau of Biologics)³ or air samples to be tested were added to the wells. Wells were again incubated overnight and then washed three times with RAST wash. To each well, 100 μ l of radioiodinated monoclonal (6F9A4) anti-*Fel d I* was added (M. Chapman, MD, Charlottesville, Va.).⁷ After another overnight incubation, the wells were washed three times with RAST wash. Bound *Fel d I* allergen was detected by gamma counting and was directly proportional to total counts bound.

RAST-inhibition assay

Immulon II wells were coated with 100 μ l (10 AU per well) of cat hair and dander extract (ALK America, Wellford, Conn.) in 0.2 mol/L of carbonate buffer at pH 9.2. The wells were incubated in a humid chamber at room temperature overnight. After incubation, the wells were washed three times with RAST wash (0.1 mol/L of phosphate buffer, pH 7.4). Wells were filled with 50 μ l of air sample to be tested or the above cat extract at varying concentrations from 0.391 AU per well to 3.13 AU per well, and 50 μ l of serum pool was obtained from four subjects with high titers of IgE antibodies to cat. The serum pool was made up of the four individuals with the highest IgE binding to the ALK solid-phase allergens, selected from 20 subjects allergic to cats. The major allergen detected by this serum pool is *Fel d I*, but it reacts also to other unidentified components of the hair-pelt extract. It reacts poorly, if at all, to cat albumin. However, gel electrophoresis demonstrates the dominant protein in the ALK extract, the Bureau of Biologics standard, and also the air extracts have the molecular weight and charge of albumin (data not presented). After another overnight incubation, wells were again washed; 100 μ l of ¹²⁵I-labeled rabbit antihuman IgE was added to each well (20 ng per well). Wells were incubated overnight and then washed. Bound ¹²⁵I-labeled anti-IgE was detected by gamma counting and was inversely proportional to total AU content.

Assay variability

To determine the variation introduced during the extraction and assay procedures, the cat hair and dander extract was diluted to 250 AU/ml, and 50 μ l was pipetted onto each of 10 filter membranes (12.5 AU per filter). The filters were allowed to dry overnight and then were extracted and assayed in the same manner as the air samples.

Variation from assay to assay was found by comparing the standard curve in each assay to determine the amount of antigen required for 50% inhibition.

RESULTS

Assay variability

Results of the assay with the filters with a known amount of 12.5 AU per filter demonstrated a mean of 13.2 ± 1.2 AU per filter ranging from 10.7 to 14.9 AU. *Fel d I* units for these filters had a mean of $208.6 \times 10^{-5} \pm 24.7 \times 10^{-5}$ units per filter with a range of 167.4×10^{-5} to 242.5×10^{-5} (250×10^{-5} *Fel d I* units added). Coefficient of variation of the assay for AU was 9.1%, and for *Fel d I*, 11.8%.

The coefficient of variation of replicate assays for AU was 12.7%, and for *Fel d I*, 1.08%.

Variability among cats

Variability of allergen production rates among cats was calculated for both *Fel d I* production and total allergen production (AU) (Fig. 1, both graphs). The means ranged from 0.005 *Fel d I* units per hour for

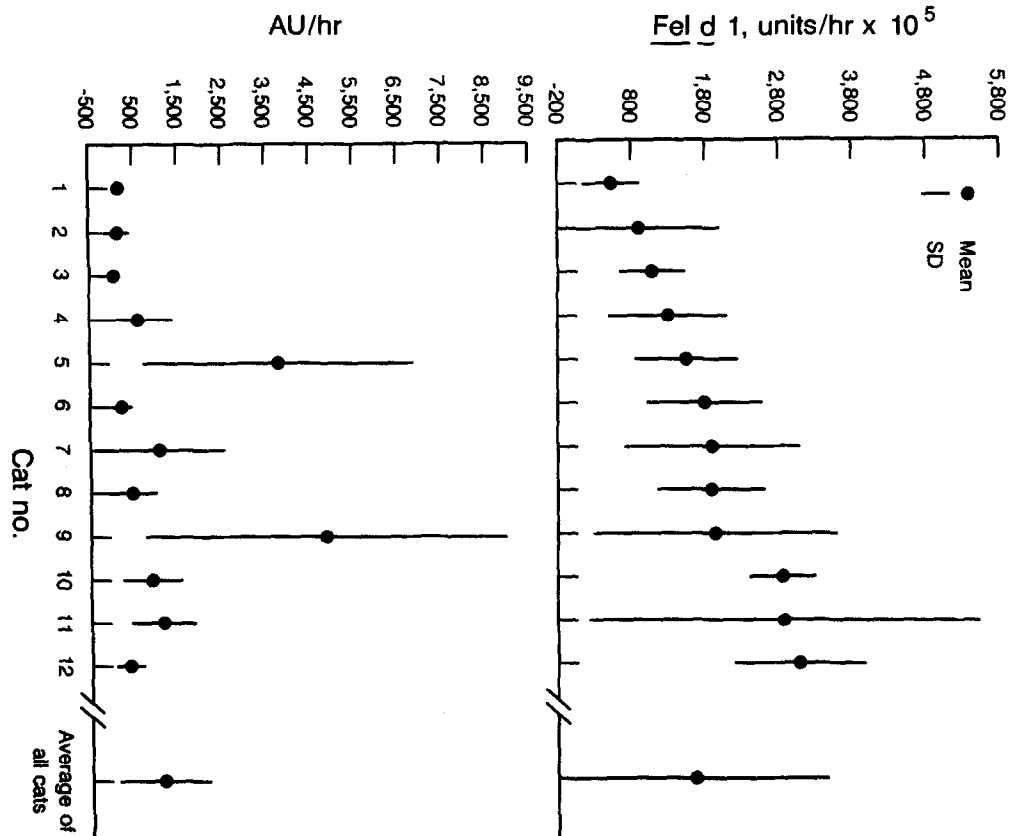


FIG. 1. Means and standard deviations for each cat. Cats are listed consecutively from lowest to highest producers of *Fel d 1* for both graphs. The center point of each line is the mean of all samples for that cat, and the line indicates 1 SD above and below the mean.

TABLE I. Shedding by male and female cats

Cats	AU/hr	<i>Fel d 1</i> × 10 ⁻⁵ /hr
M		
N	4	4
Mean	2600	2400
SD	2100	800
F		
N	8	8
Mean	500	1500
SD	400	700
p	0.009	0.04

the lowest producer to 0.031 *Fel d 1* units per hour for the highest producer. This difference represents a sixfold variation in rates of *Fel d 1* production among the 12 cats we studied. Total allergen production varied from a mean of 49 AU/hr for the lowest producer to 4900 AU/hr for the highest producer. This is a hundredfold difference in AU production rates for these 12 cats. Analysis of variance (StatPac Gold, Walenick Associates, Inc., Minneapolis, Minn.) indicated a *p* value of 0.0003 for variations of *Fel d 1*

production and a *p* value of 0.0001 for total allergen production. The range of *Fel d 1* shed per hour from the lowest of the lowest producer to the highest of the highest producer was 0.003 to 0.043 U. For AU, this value was 10 to 12591 U. Male cats shed more than female cats (Table I).

Variability within individual cats

Three of the cats had been selected as high producers (cats 5, 9, and 11), whereas the fourth (cat No. 1) was selected as a low producer (Fig. 2). In general, the high producers were consistently high and the low producer was consistently low, but the high-producing cats varied considerably from day to day and at different times during the day. At some hours, even the high-shedding cats shed only small amounts of allergen. The hour-to-hour variability of the high shedders was greater than the variability between assays and is unlikely to be due to varying technical factors, such as electrostatic trapping of particles in the chamber.

Correlation of total allergen with *Fel d 1*

To examine the relationship between AU production and *Fel d 1* production, the log₁₀ of these values was

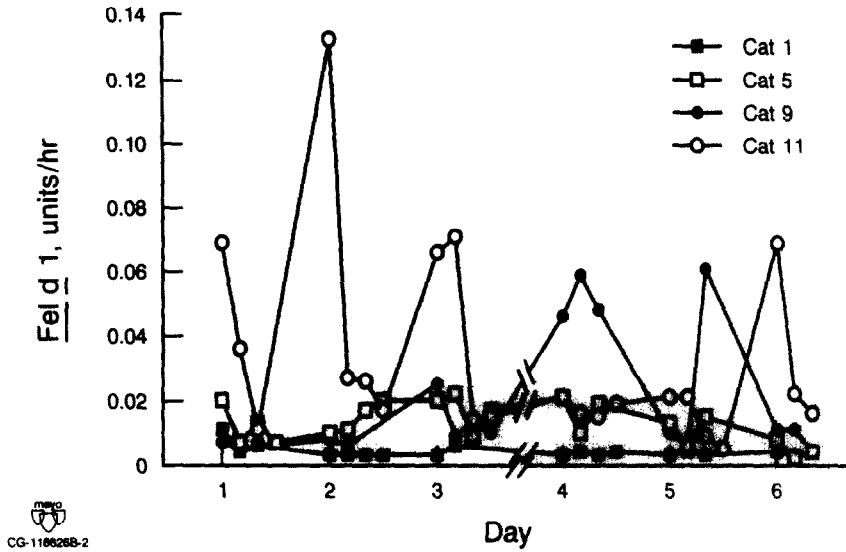


FIG. 2. Hourly samples for three good producers (cats 5, 9, and 11) and one poor producer (cat 1) during 6 days. Days 1 to 3 and 4 to 6 are consecutive, but there is a break in between of about 2 weeks. Each sample is illustrated for each day.

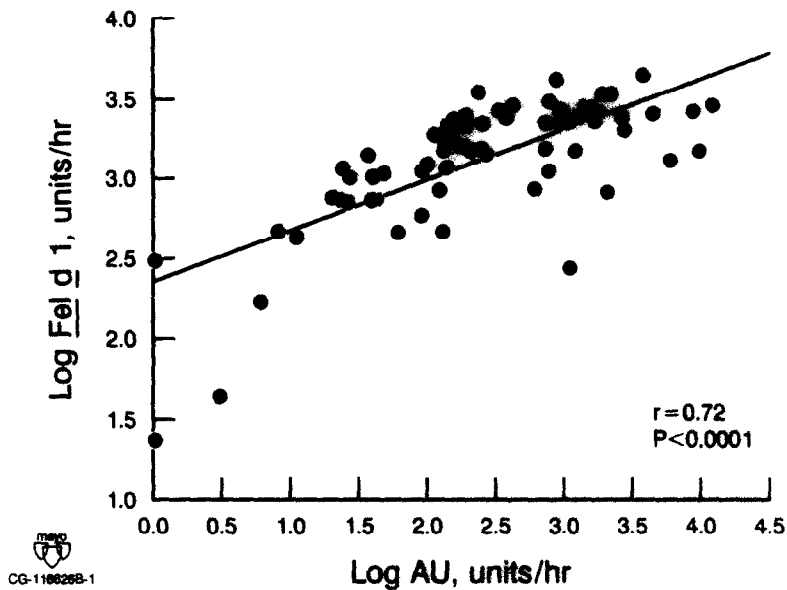


FIG. 3. Correlation between AU and *Fel d 1* units of the individual air samples. The data have been transformed to $\log_{10}$ because the results were not normally distributed.

obtained and plotted for all applicable samples (Fig. 3). Regression analysis produced a Pearson's r of 0.72, an r^2 of 0.52, and a p value of <0.0001 .

DISCUSSION

These results are consistent with cat-allergic patients' observations that some cats provoke more symptoms than other cats. Our data also confirm and extend the observations of Ohman et al.⁸ who found also that the amount of allergen recovered by washing

live cats varied both between cats and in the same cat from time to time. The variation in production rate was greater with total allergen than with *Fel d 1*, which suggests that patients who respond to the minor allergens from cats may be particularly likely to experience differences between cats. These studies do not explain the reason for the variation. It does not appear related to the animals' activity during sampling. Most of the cats slept throughout the procedure, and grooming behavior was quite infrequent. After

the animals had been in the chamber, there was hair on the floor and walls. In addition, it is quite likely there were particles trapped on the walls by electrostatic forces. Inasmuch as temperature and humidity varied only within a narrow range, it is unlikely that small variations in electrostatic forces contributed much to the considerable observed variation of allergen shedding from time to time in the same cat and between cats. To this extent, however, our measurements underestimate the actual amount shed. Male cats did shed more allergen than female cats, particularly when the results were expressed as total AUs. However, the numbers of animals were limited, and the difference is not great enough to support a recommendation that cat-sensitive patients should choose female pets. Further studies might profitably analyze the effect of hair length, breed, age, season, and other variables on the rate of cat-allergen shedding.

It is of interest that the correlation coefficient between shedding of total allergen and *Fel d* I, although it was highly significant, was only 0.72. This indicates that only about half the variation in the rate of total allergen shedding is due to variation in the rate of shedding of *Fel d* I. Again, our sample size is too small to suggest reasons for the variation in this ratio.

Even in individual cats, the ratio varied from time to time.

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