

Dose of cat (*Felis domesticus*) allergen 1 (*Fel d 1*) that induces asthma

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In 10 patients with cat asthma and hay fever, we quantified the doses of cat allergen (expressed as cat allergen 1 [Cat-1] in log Food and Drug Administration [FDA] units) inspired from the ambient air of a room containing living cats required to induce a 20% drop in FEV₁. These doses were compared with the doses required to cause the same change in FEV₁ when the patients were subjected to bronchial challenge with aerosols of cat hair and dander extract in the conventional manner. Patients highly sensitive to cat extract by skin, leukocyte histamine release, and bronchoprovocation tests and with measurable levels of anticat IgE by RAST were exposed for up to 2 hours in a room occupied full-time by two cats. At the same time, room air was sampled by a Durham gravity sampler, by an Andersen sampler, and by lapel fiberglass filters. When hair and dander particles trapped on greased slides by gravity and Andersen samplers were stained, sized, and counted, squamous cell fragments in the <10 mm range were abundant. Assays of extracts from the lapel filters for Cat-1 content by radioimmunoassay and for allergenic activity by leukocyte histamine release demonstrated a significant correlation ($p < 0.05$) between the results of the two assays. All patients developed rhinitis and asthma during exposure. At the time of a 20% drop in FEV₁, when air sampling was stopped, the lapel filters had trapped a total of -1.86 to -3.04 (median -2.74) log FDA units Cat-1. These measurements of Cat-1 exposure in the room correlated ($p < 0.01$) with the doses of cat extract (-1.18 to -3.15 [median -2.26] log FDA units Cat-1 equivalents) required to induce a 20% fall in FEV₁ in the conventional bronchoprovocation tests. (J ALLERGY CLIN IMMUNOL 78:62-75, 1986.)

Cat asthma in humans appears to be an excellent model for studying the effects of allergenic extract immunotherapy on allergic asthma.¹⁻⁶ Patients with cat asthma are numerous and universally distributed, and the cause and effect relationship between cat exposure and asthma is readily demonstrable.³ Highly sensitive patients with cat asthma may require immunotherapy because avoidance of cats is not practical.¹ Cat allergenic extracts have been produced that are stable under recommended conditions, potent, and defined with

reference to their content of cat allergens, especially Cat-1.¹⁻⁷ In several studies¹⁻⁶ immunotherapy with cat extract has been demonstrated to be as safe as immunotherapy with ragweed-pollen extract administered in comparable doses.^{8,9} In randomized, placebo-controlled, double-blind studies, immunotherapy with cat extract has been demonstrated to produce multiple, statistically significant, potentially favorable effects in comparison to placebo. These include a decrease in bronchial reaction to inhalation challenge with cat extract,^{1,3,4,6} a decrease in skin reaction to prick test with cat extract,^{1,3,4} and an increase in IgG antibody to cat antigens including Cat-1.^{2,5,6} These studies have approached the difficult problem of comparing the effects of immunotherapy with cat extract and placebo on severity of symptoms after natural exposure to cat in several ways. The Scandinavian group noted that "as judged by the patients, there was raised clinical tolerance for cat . . . at the end of the study."¹⁴ As a method of evaluating changes in patient sensitivity to cat allergens, Ohman et al.³ introduced deliberate exposure of the patients in a room containing living cats

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Abbreviations used

Cat-1:	Cat (<i>Felis domesticus</i>) allergen 1 (<i>Fel d 1</i> is the name proposed by International Union of Immunological Societies for Cat-1)
Cat-room exposure-PD ₂₀ FEV ₁ :	Dose of cat allergen inspired from cat-room air required for a 20% fall in FEV ₁
FDA unit:	Arbitrary unit for measuring Cat-1 introduced by Anderson and Baer ⁷ of the U.S.A. Food and Drug Administration
HSA saline:	Normal saline with 0.03% human serum albumin preserved with 0.4% phenol
LHR:	In vitro leukocyte histamine release
PIPES-HSA:	Physiologic piperazine- <i>N-N'</i> -bis[2-ethanesulfonic acid]; 1,4-piperazinediethanesulfonic acid buffer containing 0.03% human serum albumin
RIA:	Radioimmunoassay
r _s :	Spearman rank correlation coefficient
Cat-extract challenge-PD ₂₀ FEV ₁ :	Provocative dose of cat extract measured by a standard inhalation challenge that produces a 20% fall in FEV ₁

before and after immunotherapy. They found that, in comparison to placebo-treated patients, extract-treated patients had statistically significant delay in onset of ocular and asthmatic symptoms in the cat-containing room.³

The results of these studies indicate the need for a method to evaluate quantitatively the dose of cat allergen required to produce a specified decrease in pulmonary function (such as a 20% drop in FEV₁) during natural exposure. The inhaled dose of Cat-1 must be measured at each exposure because it is likely to be variable. Indeed, Ohman et al.³ have reported that the quantity of Cat-1 in the ambient air of their cat-exposure room, under apparently constant condition in regard to cats and furnishings, varied from 0.0001 to 0.00046 FDA units (0.25 to 1.14 "ng") per liter of air.¹⁰ Measured natural exposure would result in more discriminating evidence on the effects that immunotherapy with cat extract might produce on the severity of cat-induced asthma. These results might correlate with those of the more convenient and accurate inhalation-challenge test and thus demonstrate that cat extract inhalation-challenge tests are sufficiently com-

TABLE 1. Patient characteristics

	Patients	
	Exposed in cat room	Both Cat-room exposure- and Cat-extract challenge-PD ₂₀ FEV ₁
No.	20	10
M	10	5
F	10	5
Year of birth*		
1931 to 1940	6	3
1941 to 1950	7	4
1951 to 1960	6	3
1961	1	0

*Patients exposed to cat room in 1984.

parable to direct cat-challenge tests to permit regular use of the former as an index of sensitivity to cat allergen.

We report a method for measuring, in FDA units of Cat-1, the provocative dose that produces Cat-room exposure-PD₂₀FEV₁ during natural exposure in a room containing living cats. More importantly, this Cat-room exposure-PD₂₀FEV₁ and the Cat-extract challenge-PD₂₀FEV₁ are significantly correlated.

MATERIAL AND METHODS

Patients

Twenty patients with a history of asthma after exposure to cats and a 2+ end point skin reaction (wheal $\geq$ 1.0 cm in diameter; erythema $\geq$ 2 to 3 cm in diameter) by intradermal skin test end point titration (see skin tests below) to a dilution of cat extract equal to or more dilute than that containing 4.56×10^{-4} FDA units of Cat-1 per milliliter have been exposed to the cat-exposure room one or more times. Demographic data on the patients are presented in Table 1.

Reference cat allergenic extract

Cat-allergen extract (ALK 1209/22952) from Allergologisk Laboratories, Copenhagen, Denmark, studied under FDA BB-IND 1770, was used as the reference preparation throughout. It was prepared by extracting water-soluble material on the hair and dander obtained from overtly healthy cats and was distributed as a stable, freeze-dried extract in vials. When the contents of one vial were reconstituted to 100,000 SQ units per milliliter with 20.0 ml of diluent, the solution contained $4.56 \pm 3.4^*$ FDA units of Cat-1 per milliliter by radial immunodiffusion analysis with the use of Cat-1 reference EI-Ct (35 FDA units per milliliter) and anti-Cat-1 supplied by the FDA. Rocket immunoelectrophoresis performed by the manufacturers demonstrated a cat albumin content of 9 μ g/ml. Further analysis by crossed

*Mean and SD of 29 readings in three separate experiments.

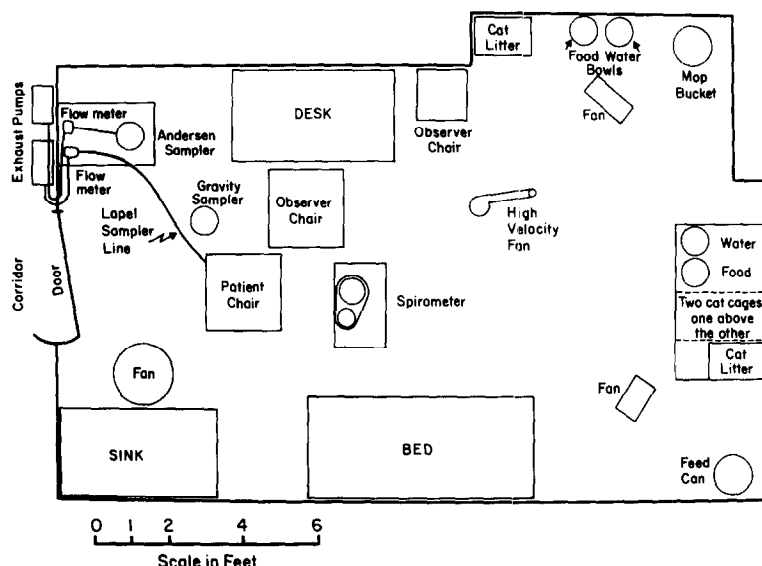


FIG. 1. Diagram of the room used for exposing patients to a natural cat environment.

radioimmuno-electrophoresis indicated that, the extract also contained the cat epithelial allergens designated Ag2, Ag11, Ag12, and Ag13 according to Danish Allergen Standardization 76 for cat hair and dander.¹¹ The freeze-dried extract was stored at -9 to -20°C . For skin and bronchoprovocation tests, the extract was reconstituted with HSA-saline supplied by Hollister-Stier Laboratories, Spokane, Wash., and appropriate dilutions in this buffer were prepared for diagnosis. Diluted extracts were stored at about 4°C and placed in a special shelf ice box¹² during use. They were replaced every 30 days.

Skin tests

Skin test end point titrations, done according to the method of Norman et al.,¹³ used serial tenfold dilutions of reconstituted cat extract and the skin of the patient's back. Testing was carried through to dilutions weak enough to elicit a negative or trace reaction. Disposable 1.0 ml tuberculin syringes with 26-gauge needles were used to inject 0.05 ml of the extract dilutions intradermally. The most dilute dilution required to produce a 2+ skin reaction (wheal ≥ 1.0 cm in diameter; erythema, ≥ 2.0 to 3.0 cm in diameter) was taken as the end point. All skin test end points were expressed in log FDA units of Cat-1 equivalents per milliliter.

LHR

Cat extract-induced LHR was measured by the micro-modification of the release phase of May et al.¹⁴ and the automated measurement of histamine by the technique of Siraganian.¹⁵ PIPES-HSA buffer containing 1 mmol/L CaCl_2 and 1 mmol/L MgCl_2 was used throughout. All sensitivity end points were expressed as log FDA units of Cat-1 equivalents per milliliter required for 50% LHR.

Anticat IgE

Serum levels of anticat IgE were measured by an agarose-based RAST²⁷ with the study cat extract as the source of cat allergen.

Inhalation bronchial challenge with cat extract and methacholine

The study cat extract was administered in increasing concentrations by inhalation until a 20% or greater fall in FEV_1 was observed.¹⁶ Aerosols were delivered to the lung through a DeVilbiss (DeVilbiss Co., Somerset, Pa.) No. 646 nebulizer attached to a dose-metering device* consisting of a breath-activated solenoid valve, timing circuit, and compressed air source.^{17,18} Aerosols are delivered for a duration of 0.6 seconds during submaximal inspirations starting from functional residual capacity. With these techniques we have determined that 0.02 ± 0.002 ml (SD) of solution is aerosolized with each breath and that the mass median aerodynamic diameter of the aerosol particle is 1.5 ± 3.0 μm (geometric mean $\pm$ SD) at a flow rate of 0.5 L/sec. For each subject the initial cat-extract concentration administered by inhalation was at least tenfold higher than that required to produce a 2+ intradermal skin reaction (1.0 cm wheal; 2.0 to 3.0 cm erythema). Ultimately, it was found that a 1:2048 dilution (0.0023 FDA units per milliliter) was a satisfactory starting dilution and saved testing many dilutions that produced no reaction. Subsequent concentrations were administered in twofold increments. A control aerosol of HSA-saline was administered before challenge. Pulmonary function tests (FVC and FEV_1) were performed before and 10 minutes after five breaths of the control aerosol and 10 minutes after five breaths of each of the cat-

*Nebulization dosimeter, model 2A (Laboratory for Applied Immunotherapy Inc., Baltimore, Md.).

extract dilutions. Spirometry was accomplished by standard techniques with a Stead Wells spirometer (Warren Collins, Inc., Braintree, Mass.). The better of two determinations of the FVC and FEV₁ was retained for analysis.

Methacholine was administered as previously described¹⁹ in increasing concentrations by the inhalation of aerosol until a 20% fall in FEV₁ was obtained. Aerosols were delivered by the methods described above that were used for delivery of cat allergenic extract.

Procedure for exposing patients to a natural cat environment

The general plan of the cat-exposure room (room A) is illustrated in Fig. 1. Two cats lived in the room. They roamed freely and had access to all furnishings except for brief periods when the room was cleaned or when a patient was exposed, during which they were confined to individual stainless steel cages illustrated in the drawing.

The room contained a wooden desk, two chairs, and a metal frame bed. To minimize production of allergens other than cat allergens, the bed mattress and the chair pillows were encased in plastic covers that were further covered either by a cotton pad and a sheet or cotton pillow cases or paper sheets. Containers of water, food, and cat litter were located as illustrated. The cinder block walls and concrete floors were painted with glossy enamel paint; the sheet rock ceiling was painted with flat paint. Filtered air was pumped through the room by the ventilating system; the inlets and outlets were adjusted so that the air in the room was at a slight positive pressure with respect to the corridor outside the room, and the room air was changed on an average of 5.1 times per hour.

The room was thoroughly cleaned on Mondays and Fridays after patient exposure. During the periods between these major cleanings, presumed sources of cat allergen such as cat hair, dander, and saliva particles were allowed to accumulate; light brushing was used to clean up scattered cat litter and undesirable dirt. On Mondays and Fridays, 1 hour before patient exposure, the ventilating system was turned off to reduce air exchange. The door was weather stripped so that it provided an effective seal when it was closed. The cats were put into the cages at this time. Three low-velocity air-mixing fans were turned on to provide an even mix of airborne particles in the ambient air. A hand-held high-velocity "leaf-blower" fan (Paramount Rota-Blo Electric Blower, Alleghetti Co., Chatsworth, Calif.) was turned on for 5 minutes to blow into the ambient air large and small particles on wall, floor, bed, and other furniture. The air stream was directed away from the patient and thus did not blow on the patient but did launch particles into the slowly circulating air supplied to the patient by the mixing fans. We expected that the mixing fans would keep small particles suspended and that large particles, such as hairs, would settle to the floor and other surfaces out of breathing range.

Immediately before the period of patient exposure, the spirometer and sampling lines and flowmeters (Flowrate; Fischer & Porter, Hatboro Pa.) were placed in the room and

attached to exhaust pumps (Gast Mfg. Corp., Benton Harbor, Mich., model No. 05220-V3-G18DX) that were out of the room so that oil fumes from the pumps did not enter the room.

Baseline measurements were obtained for at least 15 minutes in a clean, well-ventilated room (room B) that was relatively free of cat allergen and other allergens. FVC and FEV₁ were measured every 5 minutes with a Collins survey spirometer. Minute ventilation and respiratory rate were measured for 1 minute every 5 minutes by means of a Hans Rudolph valve (Hans Rudolph, Inc., Kansas City, Mo.) and a Collins survey spirometer.

The patient then entered room A and sat in the chair. The sampling filter device (filter cassette, Millipore [Millipore Corp., Bedford, Mass.] MOOO-03-40, with a 37 mm diameter 0.8 µm pore size fiberglass filter, Millipore AP 40-037-05, supported by a cellulose support pad, Millipore AP10-037-00) was attached to the patient's lapel. The outlet of the cassette was connected by a 5/16 by 1/16 inch diameter hose to the flowmeter and exhaust pump, and the flow was set at 30 L/min. The amount of Cat-1 inspired by the patient was calculated by the formula:

$$\text{Cat-1 inspired by patient (FDA units)} = \frac{\text{Cat-1 on filter (FDA units)}}{\text{mean of measurements of minute ventilation of pt in L/min measured every 10 min in exposure rm}} \times \frac{30 \text{ L/min}}{30 \text{ L/min}}$$

A Durham gravity sampler²⁰ was placed on the left side of the patient, containing a 3 by 1 inch microscope slide coated with soft petrolatum (75% Vaseline (Chesebrough Pond's Inc., Greenwich, Conn.), 25% mineral oil). An Andersen sampler²¹ was also placed on the left side of the patient. The Petri dishes on each of the six stages contained 27 ml of agar culture medium. On the surface of the agar was a 3 by 1 inch microscope slide coated with soft petrolatum. The Andersen sampler was attached to an exhaust pump and flow meter as demonstrated, and flow was adjusted to 23.3 L/min (1 cu ft). The Andersen sampler was run until the patient had a 20% fall in FEV₁ or until the patient was removed from the room after 2 hours of exposure.

Every minute during the 2 hours in the cat-exposure room, the presence or absence of specific symptoms of hay fever (eye itch, tears and redness, nose itch, sneeze, discharge, obstruction, and postnasal irritation) were recorded. The duration of these eight manifestations was determined, and the sum of these counts was divided by the number of minutes of observation to obtain the symptom score. FVC and FEV₁ were measured every 5 minutes, and respiratory rate and minute ventilation were measured every 10 minutes. Exposure in the cat room was terminated when the patient had a 20% or greater fall in FEV₁ below the baseline preexposure level. At the time exposure was terminated or at the

*Andersen sampler model No. 0101, Medi-Comp Research and Development, Salt Lake City, Utah.

end of the first hour of exposure, the sampling filter was removed and stored at about -15°C until it was analyzed for cat-allergen content, and the sampling slide from the Durham sampler was placed in a sealed slide carrier.

If there was *not* a 20% fall in FEV_1 during the first hour, the patient stayed in the room for a second hour with a new lapel sampling filter and a new slide in the Durham sampler. In addition to the three mixing fans, the high-velocity fan was used for 1 minute every 5 minutes to blow particles into the air. An effort was made with the air stream to sweep floor, wall, and furniture surfaces and to break up clumps of hair so that individual hairs were airborne. Exposure was stopped when the patient had a 20% or greater fall in FEV_1 below the baseline or at the end of the second hour of exposure.

The patient was taken back to room B. Two inhalations from an albuterol metered-dose inhaler were administered. FVC and FEV_1 were measured every minute for at least 5 minutes and until the values had returned to near baseline levels and every 5 minutes thereafter until the patient had been in a satisfactory condition for at least 15 minutes. Thereafter, the patient was walked for several blocks to ensure that recovery had taken place. The patient was administered Rondec (Ross Laboratories, Columbus, Ohio), 1 tablet every 4 hours if it was necessary for hay fever symptoms and Theo-Dur (Key Pharmaceuticals, Inc., Miami, Fla.), 200 to 400 mg every 12 hours if it was necessary for asthma symptoms.

At the conclusion of the exposure procedure, the fans were turned off, and hair and dander were allowed to settle. Floor, chairs, bed, and desk surfaces were swept. The accumulated hair and dander were put in a plastic bag (Dow Ziploc plastic bag, Dow Corning Corp., Midland, Mich.) $6\frac{1}{2}$ by 5 $\frac{1}{2}$ inch by 1.15 ml) and weighed. Each cat was thoroughly brushed for 4 minutes with a Hartz cat brush, and the hair and dander obtained were stored in a plastic bag and weighed. On those Mondays and Fridays when no patients were exposed and, therefore, the fans were not turned on, cat hair sweepings and brushings were also collected and weighed in the manner just described.

Particle counts

Within 65 minutes after collecting slides from the Durham sampler, three drops of Calberla's solution was applied to a portion of the slide containing soft petrolatum and the sample, and a 24 by 40 mm cover slip was applied. The slide was stored horizontally for 24 hours to permit hardening of the gelatin before examination. Cat-dander particles were counted and sized with a standard compound light microscope with use of $45\times$ objective and $10\times$ ocular lenses and a Porton gradicule²² calibrated for the lens system with the grid of a standard hemocytometer. The cat-dander particles could be recognized by their red stain and granular appearance, and they often contained remnants of nuclei. Some single cells were observed and represented the typical appearance of cornified squamous cells. The largest diameters of these particles were measured in two directions at right angles and recorded in size range columns under the largest of these two diameters. Usually, a fair sampling of

the particles could be obtained by making four passages between the top and bottom of the cover slip in such a way as to sample the contents of each of the four quadrants of the area under the cover slip. The particle count for 1 cm^2 could be obtained by applying an appropriate conversion factor after measuring the transverse axis of the microscope field with the grid of a standard hemocytometer. For the microscope used in this study, the transverse axis of the visual field was 0.036 cm in diameter when $45\times$ objective and $10\times$ ocular lenses were used. Since the width of a slide was 2.4 cm, one passage thereof surveyed 0.086 cm^2 or $1\text{ cm}^2/11.6$. Therefore:

$$\text{Cat dander particle count/cm}^2 = \frac{\text{Total cat-dander particle count} \times 11.6}{\text{number of surveys of width of slide}}$$

Slides were removed from each stage of the Andersen sampler and mounted and stained in the manner described for the gravity slides with the exception that two drops of Calberla's solution were adequate because of the absence of hair and caused less dislocation of the particle deposits than did the three drops used on the gravity slides. The slides contained circumscribed elliptical or round deposits of particles that were representative of the 400 deposits on the slide or the agar under each of the 400 holes in the screen above the Petri dish. The cat-dander particles in a representative deposit were sized and counted in the following manner by use of the microscope and a Porton gradicule, which had been calibrated for each lens combination by use of the grid of a standard hemocytometer. The lengths of the transverse and conjugate axes of the deposit were measured in micrometers by use of the $10\times$ ocular and either the $3.5\times$ or the $10\times$ objective lenses, and the area was calculated by the formula below:

$$\text{Area} = \pi \times \frac{\text{length of transverse axis}}{2} \times \frac{\text{length of conjugate axis}}{2}$$

A representative area of the sample was examined for a width of 22.5 to 90 mm along the entire conjugate axis, and the area of this sample was calculated by the formula: $\text{area} = \text{length} \times \text{width}$. Cat-dander particles within the sample were counted and sized by use of $10\times$ ocular and $45\times$ objective lenses. The number of skin scales in any size range on the slide was calculated by use of the following formula:

$$\text{Cat-dander particle count per stage} = \frac{\text{actual cat-dander particle count in sampled area of deposit}}{\text{total area of deposit}} \times 400 \text{ deposits}$$

After slides from all of the six stages were examined, the numbers of cat-dander particles within a specific size range on each stage were added to determine a total count for each size range trapped by the Andersen sampler in the time

period sampled. The number of cat-dander particles inspired by the patient was calculated by multiplying the Andersen sampler count by the formula presented below:

$$\begin{aligned} \text{Number of cat-dander particles} &= \frac{\text{number of cat-dander particles counted by Andersen sampler}}{\text{mean of measurements of minute ventilation of pt in L/min measured every 10 min in exposure rm}} \\ &\times \frac{\text{air flow through Andersen sampler (28.3 L/min)}}{\text{air flow through Andersen sampler (28.3 L/min)}} \end{aligned}$$

Extracting cat allergens from the cassette-filter assembly

Before commencing the study, we assessed the recovery of Cat-1 from both fiberglass and cellulose acetate (Metricel type AA 0.80 μm pore size, Millipore Corp., Bedford, Mass.) filters in two separate sets of experiments; 4.56 FDA units of the reference cat extract in 3 ml of PIPES-HSA buffer was placed in a cassette that contained either a single fiberglass or cellulose acetate filter. The samples were rocked for 18 hours at 4° according to the same procedure used to extract particulate matter from the air of the cat room (see below). The Cat-1 contents of serial dilutions of "filter extracts" were assayed by comparison against similar dilutions of the untreated reference cat extract. The data from these experiments led us to select the fiberglass filter (see Results).

As already noted, the cassette assembly used in the actual air-sampling experiments contained both a fiberglass and a cellulose support filter. Because of the high absorptive capacity of the cellulose pad, it was first carefully removed from the outlet side of the cassette, and the cassette was reassembled. Three milliliters of extracting fluid (PIPES with 0.03% HSA) was added through the inlet. The inlet was closed by reinserting the blue plug. The cassette was sealed with tape and parafilm and placed on a rocking platform for 18 hours at 4° C. Thereafter, the extracting fluid was aspirated and injected into a storage tube together with residual fluid squeezed out of the filter.

Measurement of Cat-1 by RIA

Purified Cat-1 was isolated from an extract of cat dander kindly supplied by Allergologisk Laboratories. This extract was purified by diethylaminoethyl-Sephadex chromatography followed by repeated chromatography on Sephadex G-50 (fine) and Sephadex G-75 (superfine). The resultant material contained primarily Cat-1 along with three minor impurities. Monospecific rabbit anti-Cat-1 was kindly provided by Dr. John Ohman. This antiserum elicited a single immunoprecipitate when it was tested against the Cat-1 preparation. The reference preparation used as a Cat-1 standard was the same lyophilized extract used for skin testing, bronchial challenge, and other studies.

The double-antibody inhibition RIA used to measure the Cat-1 content of cat extracts obtained from the filters used



FIG. 2. Cat hair and dander. The cat-hair shaft has serrated edges and central, transverse, intermittent deposits of pigment. Five dander particles are observed above the hair.

^{125}I -labeled Cat-1 (about 0.0001 FDA units) together with sufficient rabbit anti-Cat-1 to cause precipitation of about 70% of the maximum precipitable counts per minute after addition of a slight antibody excess of goat antirabbit IgG.

^{125}I -Cat-1 and anti-Cat-1 (in $1/50$ normal rabbit serum) plus either the unknown extract or a solution of the standard were incubated for 4 hours at room temperature. At least two dilutions of the test extract were used, and all dilutions of the test and standard extracts were assayed in triplicate. Goat antirabbit IgG was added, the mixture was allowed to stand for 18 hours at 4° C, and the resultant precipitate was washed and counted. The amounts of Cat-1 in the unknowns were computed from the inhibition curve plotted for eight serial dilutions of the standard. The assay permitted direct measurement of Cat-1 levels down to 4.5×10^{-3} ($\log = -0.235$) FDA units.

Computation of cat-allergen contents of filter extracts by LHR assay

A single, highly cat-sensitive donor was selected. Serial dilutions of the reference extract and appropriate dilutions of each of the filter extracts were assayed in duplicate by LHR assay by methods described in the section on LHR. The filter-extract contents were determined from the standard curve of the reference extract and are expressed as Cat-1 equivalent FDA units per filter.

TABLE II. Diameter and number* of cat-dander particles on gravity sides

	Largest diameter of particle in microns							Nonrespirable	Respirable
	50 +	40 to 49	30 to 39	20 to 29	15 to 19	10 to 14	5 to 9	<5	10 to 50+ 0.1 to 9.0
First hour of exposure									
No. samples	33	33	33	33	33	33	33	33	33
Mean	3.1	1.8	5	10	8.7	13.6	33.8	25.6	59
Median	3	0	4	6	9	12	30	21	49
SD of mean	3.5	2.1	4.1	10	6	12.8	21.2	18.6	36.7
Second hour of exposure									
No. samples	31	31	31	31	31	31	31	31	31
Mean	33.2	21	35.6	62.9	32.8	48.7	96	67.2	236.6
Median	30	15	29	58	31	41	81	51	220
SD of mean	15.3	15.4	22.2	35.6	19.8	27	62.4	58.3	94.7

*Number of cat-dander particles per square centimeter slide per hour.

TABLE III. Number of cat-dander particles inhaled at time of cat-room exposure-PD₂₀FEV₁ and largest diameter thereof in microns*

Diameter (μm)	No. of particles						Total
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	
50 +	860	0	0	0	0	0	860
40 to 49	0	0	0	0	0	0	0
30 to 39	1720	0	0	0	0	0	1720
20 to 29	2007	2296	0	0	0	0	4303
15 to 19	1147	0	0	574	0	0	1721
10 to 14	2580	16070	4592	574	0	0	23816
5 to 9	573	10330	8034	1148	0	0	20085
<5	0	0	2296	19510	0	0	21806

*Patient 8, Andersen sampler results.

Analysis of data

Data were analyzed by the use of nonparametric statistical methods²³ such as the r_s with the use of the CLINFO computer system supplied by the National Institutes of Health Division of Resources to The Johns Hopkins University School of Medicine.

RESULTS

Measurements of cat hair and dander in room sweepings and cat brushings

The cats were a continuing source of hair and dander throughout the year. The weight of hair sweepings on Mondays and Fridays was more on days when no patient was exposed (number of measurements, 15; range, 0.04 to 1.0; median, 0.57 gm per 3- to 4-day period) than when hair sweepings were collected on exposure days at the conclusion of patient exposure (number of measurements, 32; range, 0.02 to 0.46; median, 0.10 gm per 3- to 4-day period). The weight

of cat brushings on Mondays and Fridays was variable but indicated a continuing shedding of hair and dander (Cat A number of measurements, 51; range, 0.08 to 0.35; median, 0.19 gm per 3- to 4-day period; Cat B number of measurements, 49; range, 0.01 to 0.13; median, 0.04 gm per 3- to 4-day period). Analysis of the Cat-1 content of two sets of matched paired aliquots of combined room sweepings and cat brushings kept at room temperature and at -14.5°C for 30 days elicited comparable results (room temperature about 22°C ; 126.9 FDA units per gram; -14.5°C ; 113.8 FDA units per gram) and indicate the stability of Cat-1 in such a room.

Measurement of quantity and size of particles of cat hair and dander in the air of the cat room

Light microscopy of the gravity slides revealed that cat hair and dander particles had characteristic mor-

TABLE IV. Number and diameter of particles of cat dander inspired by patient in cat room*

Pt	Exposure time (min)		Largest diameter of particle in microns							
	1st Hr	2nd Hr	50 +	40 to 49	30 to 39	20 to 29	15 to 19	10 to 14	5 to 9	<5
1	59	59	4492	0	17966	34810	11229	83455	35166	43381
2	58	28	0	0	0	19914	8766	12749	66483	220887
3	57	22	6294	0	3148	8498	7558	30237	21630	66997
4	58	60	1120	1287	3529	6104	2408	14842	78912	334077
5	58	33	968	645	3549	4517	1613	11293	17423	35813
6	58	58	4859	1215	4859	13362	2429	9717	40732	36983
7	57	9	0	217	434	1059	96	2188	4672	1345
8	59	41	860	0	1720	4303	1721	23816	20085	21806
9	59	60	1626	904	1626	4625	723	8709	26955	108400
10	42	0	233	0	233	466	466	1440	6117	9949

Pt = patient.

*Andersen sampler results.

phology (Fig. 2) and that they could be identified, sized, and counted. Cat-dander particles ranged widely in size (Table II). Many were in the size range of $<10\text{ }\mu\text{m}$ that would be expected to be respirable and to reach the lower airways in the lung. However, cat-dander particles $10\text{ }\mu\text{m}$ or larger in greatest diameter could be identified more accurately than smaller particles and could be found in the plane of focus more reliably. Therefore, counts of these larger particles were used to obtain the particle-count exposure associated with cat-room exposure- $\text{PD}_{20}\text{FEV}_1$.

Light microscopy of the contents of the stages of the Andersen sampler revealed particles with the morphologic characteristics of cat-dander particles on stages 1 to 4 but not on stages 5 to 6 (Table III). As with the particles on the gravity slides, particles in the $10\text{ }\mu\text{m}$ or larger size range could be identified as cat-dander particles with greater confidence than smaller particles. Most particles of cat dander were in the size range $<10\text{ }\mu\text{m}$ that would be expected to penetrate the lung (Tables III and IV).

Evidence that indicated that levels of cat allergen in room air varied on different exposure days but were uniformly higher in second than in first hour of exposure

Direct measurements were made of the total amounts of cat allergen captured by the lapel filter at air flow 30 L/min . Cat-1 content measured by the RIA (Fig. 3, A) and Cat-1 equivalents by the LHR assay (Fig. 3, B) varied over a wide range on separate exposure days. Where values were large enough to be measured accurately, they were uniformly higher in the second than in the first hour of exposure. Fig. 4 illustrates that when measurements by both methods

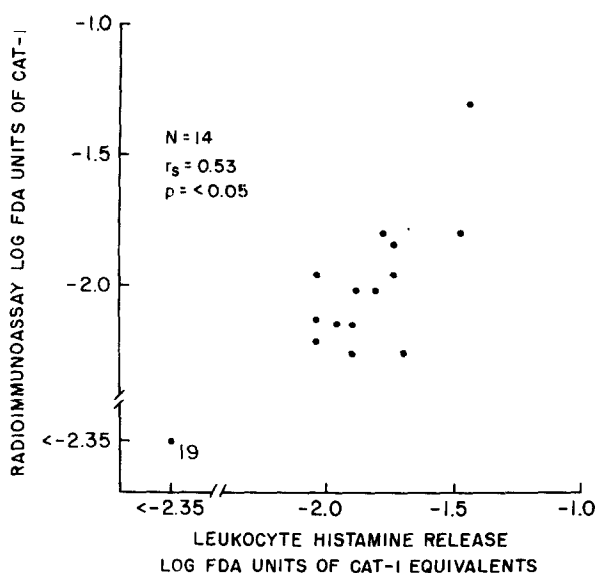
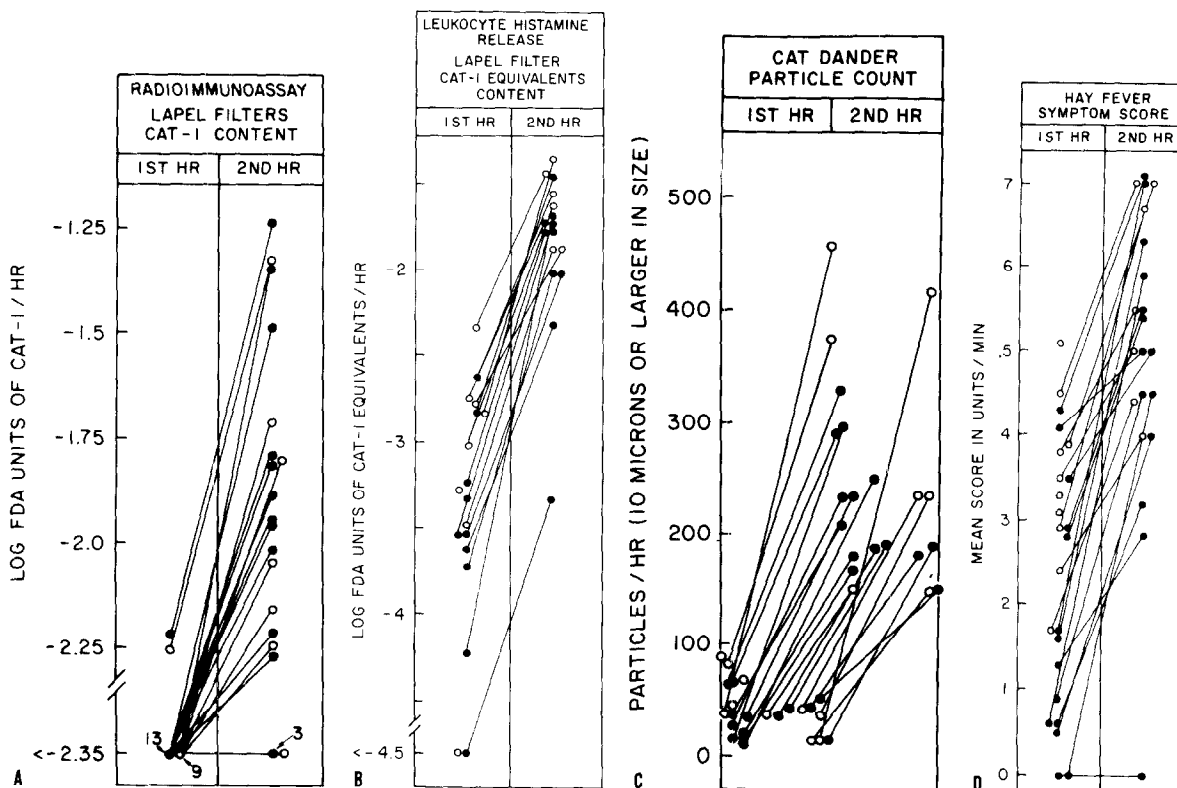
were performed on the same filter samples and were large enough to be measured accurately (14 samples), they correlated significantly ($r_s = 0.53$; $p < 0.05$).

Gravity slide counts of cat-dander particles (Fig. 3, C) varied over a wide range on separate exposure days but were uniformly higher in the second than in the first hour of exposure.

Hay fever-symptom scores (Fig. 3, D) varied over a wide range on separate exposure days but were almost invariably higher in the second hour than in the first hour of exposure.

Results of patient exposure to cat room

Twenty patients were exposed in the cat room one or more times (Table I). The data on lapel-filter contents of Cat-1, cat-dander particle counts on gravity slides, and hay fever scores presented previously refer to these exposures. Ten of these patients had a 20% drop in FEV_1 achieved during the 2 hours of exposure so that Cat-room exposure- $\text{PD}_{20}\text{FEV}_1$ could be measured and also had measurements of Cat-extract challenge- $\text{PD}_{20}\text{FEV}_1$. The other patients either did not achieve a 20% fall in FEV_1 in the cat room and, therefore, for them Cat-room exposure- $\text{PD}_{20}\text{FEV}_1$ could not be calculated (nine patients) or did not have determination of Cat-extract challenge- $\text{PD}_{20}\text{FEV}_1$ (one patient). The results in these 10 patients are marked with open circles (○) in Fig. 3, A through D. All 10 patients were highly sensitive to the cat extract; the log doses of cat extract for 2+ intradermal skin test end point ranged from -5.64 to -6.64 (median -6.64) FDA units of Cat-1 equivalents and were the end points produced by intradermal injection of 0.05 ml of the 10^{-5} or the 10^{-6} dilutions of the extract. The log doses for 50% LHR ranged from -4.7 to



– 8.0 (median – 6.7) FDA units of Cat-1. All patients had measurable levels of serum anticat extract IgE by RAST ranging from 5.7 to 991 mg/ml (median 111 mg/ml). Nine of the 10 patients were tested by inhalation bronchial challenge with methacholine, and all patients were highly sensitive. The log doses of methacholine required to produce a 20% drop in FEV_1 ranged from – 1.76 to + 1.91 (median + 0.49) units.

Cat-room exposure- $PD_{20}FEV_1$

The results of exposing one patient in the cat room are presented in detail in Fig. 5 to illustrate clearly the methods used for obtaining Cat-room exposure- $PD_{20}FEV_1$. Baseline measurements of minute ventilation, FVC, and FEV_1 , and of hay fever-symptom scores were made in the recovery room. The patient was moved into the cat room and administered a low degree of allergen exposure in the first hour. Throat, nasal, and eye hay fever symptoms developed, but a 20% fall in FEV_1 was not achieved. During the second hour, high exposure was administered by use of the high-velocity fan. A 20% fall in FEV_1 was achieved

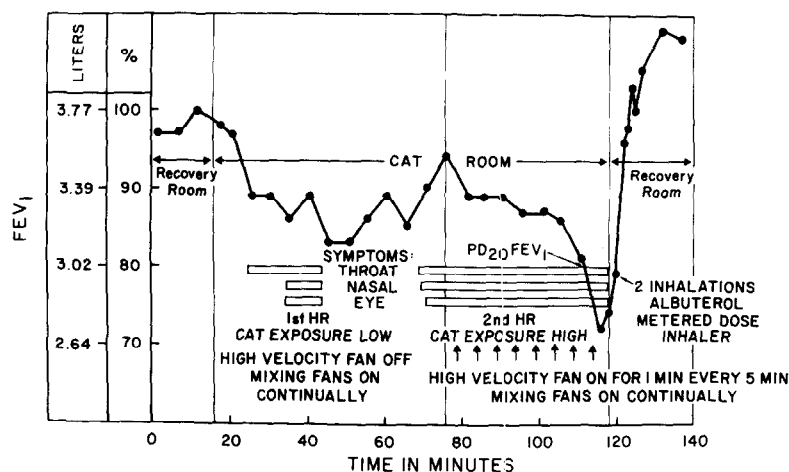


FIG. 5. Exposure of a patient with cat asthma and hay fever to a natural cat environment is presented. The log dose of Cat-1 for $PD_{20}FEV_1$ is -2.42 FDA units.

at a time when the patient had been exposed to particles of cat allergen in the ambient air that permitted the lapel filter to capture -2.42 log FDA units of Cat-1 at filter airflow equal to the patients' mean minute ventilation. The patient was taken to the recovery room and recovered rapidly after two inhalations from an albuterol inhaler.

Fig. 6 illustrates the association between gravity slide counts of cat-dander particles in the morphologically identifiable $>9 \mu m$ size range and lapel-filter contents of Cat-1 (at airflow 30 L/min) at the time FEV_1 fell to 20% of baseline level for each of the 10 patients when they were exposed to the cat room. The results of the morphologic assay and of the chemical assay are significantly correlated ($r_s = 0.77$; $p < 0.01$).

In order to obtain a more nearly accurate index of Cat room exposure- $PD_{20}FEV_1$, the measurement values for lapel-filter contents were adjusted as presented in Table V to account for the fact that the patient sampled room air at a rate equal to the patient's mean minute ventilation during the period of exposure, whereas the filter sampled air at a rate of 30 L/min. The values cited in Table V refer to the total amounts of Cat-1 actually recovered from each filter. These values are probably underestimates of the actual levels. Detailed analysis of Cat-1 recovery from the cat-reference extract exposed to six different fiberglass filters for 18 hours at $4^\circ C$ demonstrate a mean recovery of 71% (range 64% to 80%). This value was calculated from the 50% inhibition points of titration curves for both the reference extract and the extracts exposed to the filters. Six different filters and two standard curves were used in two separate experiments. Similar assessment of the recovery of Cat-1

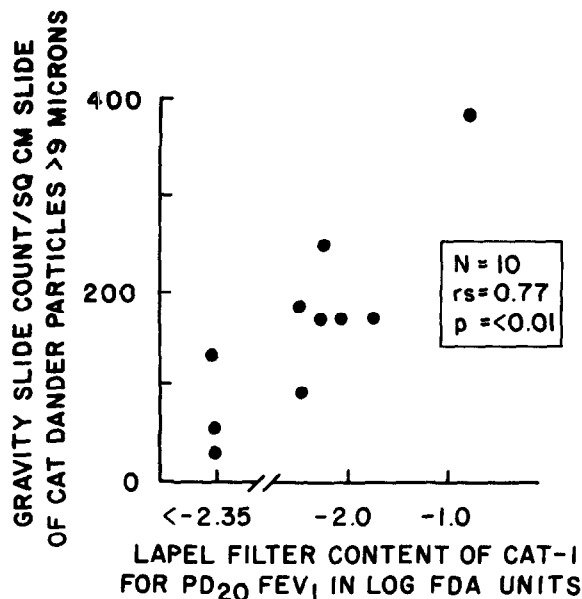


FIG. 6. The association between gravity slide counts of cat-dander particles and lapel-filter contents of Cat-1 at $PD_{20}FEV_1$ is illustrated.

from cellulose acetate filters demonstrated both low and highly variable recovery (range 1.0% to 28%).

Cat-extract challenge- $PD_{20}FEV_1$

Ten patients who had a 20% fall in FEV_1 in the cat room had inhalation challenge with cat extract. Fig. 7 presents in detail the results of inhalation challenge with cat extract of the same patient whose responses to the cat room were demonstrated in a previous slide. Baseline measurements of FEV_1 were obtained after inhalation of five breaths of saline. Sub-

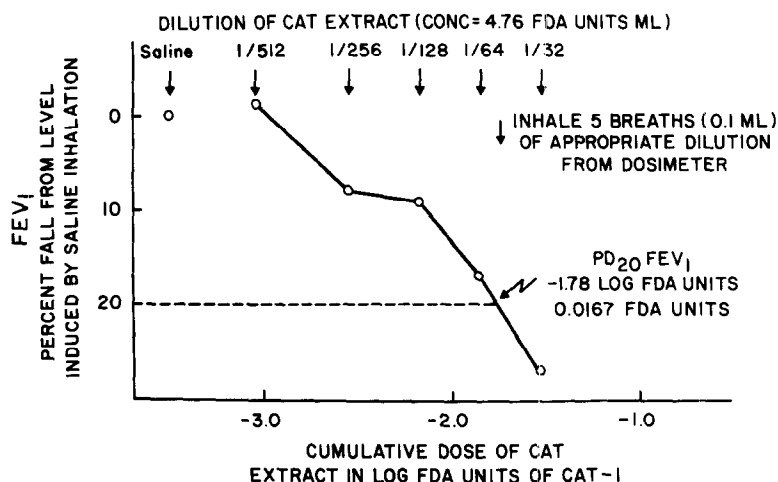


FIG. 7. An example of inhalation bronchial challenge with cat extract is presented.

TABLE V. Cat-extract challenge- $PD_{20} FEV_1$ and Cat-room exposure- $PD_{20} FEV_1$

Pt	Cat-extract challenge— $PD_{20} FEV_1$	Cat-room exposure- $PD_{20} FEV_1$		
		Lapel-filter contents* at airflow 30 L/min	Mean tidal volume during exposure (L/min)	Lapel-filter contents*† at airflow equal to pa- tient's mean minute ventilation
1	-3.15‡	< -2.35‡	6.8	< -3.04‡
2	-3.13	< -2.35	6.18	< -3.04
3	-2.87	-2.26	5.53	-3.03
4	-2.86	-2.26	8.87	-2.79
5	-2.31	< -2.35	7.63	< -3.04
6	-2.21	-2.14	8.55	-2.69
7	-2.13	-2.02	9.14	-2.54
8	-1.78	-1.89	8.95	-2.42
9	-1.73	-2.14	10.2	-2.61
10	-1.18	-1.33	8.96	-1.86

Pt = patient.

*The values for filter contents represent the actual amount of Cat-1 recovered from each filter. It is probable that these values are about 71% of the true values (see text).

†Lapel-filter contents at minute ventilation = lapel-filter contents at 30 L/min $\times$ minute ventilation $\div$ 30 L/min.

‡Cat-1 log FDA units.

sequently, at intervals, five breaths of successively increasing (by twofold) concentrations of the cat extract were inhaled. A 20% drop in FEV_1 occurred when the patient had inhaled a cumulative dose containing -1.78 log FDA units of Cat-1 equivalents.

Association between Cat-room exposure- $PD_{20} FEV_1$ and Cat-extract challenge- $PD_{20} FEV_1$

Fig. 8 illustrates the association between the doses of Cat-1 required for Cat-room exposure- $PD_{20} FEV_1$ (as measured by lapel-filter contents at airflow equal to the patient's mean minute ventilation) and the doses of Cat-1 equivalents required for Cat-extract chal-

lenge- $PD_{20} FEV_1$ in the 10 patients. The results of these two assays of bronchial sensitivity to cat were significantly correlated ($r_s = 0.87$; $p < 0.01$). However, the values of the individual measurements of Cat-room exposure- $PD_{20} FEV_1$ were uniformly lower than those for Cat-extract challenge- $PD_{20} FEV_1$.

DISCUSSION

We have developed a refined model of the cat-exposure room containing living cats developed by Ohman et al.³ that can be readily duplicated. This room is maintained in such a way that it contains allergenic emanations from two cats and probably negligible

amounts of other known allergens such as pollen, mold, and house dust. We have demonstrated that the cats provided continuing, although it was variable, generation of cat hair and dander, that this hair and dander contained Cat-1 allergen, and that the Cat-1 content of the hair and dander as measured by RIA was stable for at least 30 days under the conditions present in this room. The Cat-1 found in hair and dander probably derived from both of the two known sources of Cat-1, cat mucus saliva^{24, 25} and subaceous glands at the base of the hair.²⁵ Many of the particles of cat dander were $<10\text{ }\mu\text{m}$ in size and therefore would be expected to reach small bronchioles and alveoli in the lung. Findlay et al.¹⁰ and Swanson et al.²⁶ have isolated Cat-1 from particles $<10\text{ }\mu\text{m}$ collected by use of an Andersen sampler from a room containing living cats.

We have collected samples of cat hair and dander from the air of the cat room by the use of fiberglass filters and have prepared extracts of this material. By means of a sensitive double-antibody RIA we were able to quantitate the levels of Cat-1 in the extracts. By use of a biologic assay (LHR), we were able to quantitate the allergenic activity (expressed in Cat-1 equivalent per milliliter) of each filter extract relative to a reference cat extract. We provided two different levels of exposure to cat allergen by use of fans to distribute the particulate matter in the air of the room. Low-velocity fans during the first hour of exposure provided a level of Cat-1 about tenfold less than that provided by a high-velocity fan in the second hour of exposure. The lapel-filter extract contents of Cat-1 and Cat-1 equivalents, the cat-dander particle counts, and the hay fever-symptom scores were uniformly lower in the first than in the second hour where the amounts were large enough to be measured accurately. The conditions during the second hour of exposure were designed to simulate in a reproducible manner conditions present at a time of high exposure to particles coming from cat such as provided by brushing the cat, shaking out cat bedding,²⁶ or walking about a room and stirring up particles of cat hair and dander resting on floor and furniture. In our early experiments with cat-room exposure, we provided high exposure by brushing cats and shaking cat sheets. However, we abandoned this technique when we found that the high-velocity fan provided a convenient, reproducible way to launch into the ambient air cat particles at rest on the floor and furnishings. The data in Tables II, III, and IV demonstrate that the use of the high-velocity fan increased exposure to particles in all size ranges.

With the use of LHR, the total allergenic activity collected by each of the filters relative to the allergenic

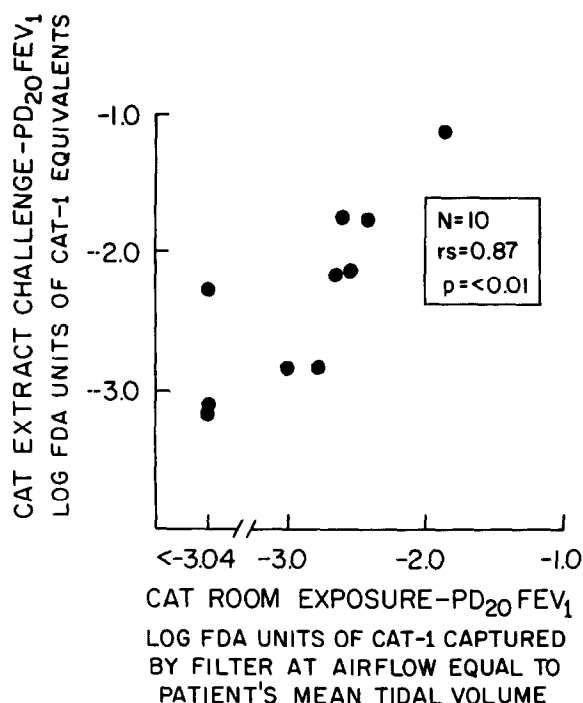


FIG. 8. The association between Cat-room exposure- $\text{PD}_{20}\text{FEV}_1$ and Cat-extract challenge- $\text{PD}_{20}\text{FEV}_1$ is presented.

activity of the reference cat extract is actually being measured. We have made the assumption that, for a given leukocyte donor, Cat-1 is contributing a *constant proportion* of the activity present in each filter extract. Since the Cat-1 equivalent levels by LHR correlated significantly ($p < 0.05$) with the actual levels of Cat-1 (by RIA), this infers that the levels of Cat-1 in each of the filter extracts are, indeed, relatively consistent proportions of the total allergenic activity (at least for the leukocyte donor used).

Both RIA and LHR demonstrated that Cat-1 or Cat-1-equivalent levels in the room air on different patient exposure days varied over a wide range despite apparently constant conditions with reference to the number of cats and the furnishings in the room. Ohman's group has also demonstrated variation in the air content of Cat-1 in their cat room on different days under apparently constant conditions.¹⁰ Therefore, direct measurements of inspired Cat-1 are required to quantitate the amount that produces Cat-exposure room- $\text{PD}_{20}\text{FEV}_1$. One cannot rely on measurements of time spent in the cat room to be a reliable index of this provocative dose.

With the atmosphere of this room as a source of cat allergen, we have produced a 20% drop in FEV_1 in 10 patients with cat asthma. These 10 patients were highly sensitive to cats as measured by skin test end point titration, in vitro LHR, and inhalation bronchial

challenge with a potent cat extract. Furthermore, the nine patients tested had nonspecific bronchial irritability as measured by inhalation challenge with methacholine.

We have attempted to define various indices of Cat-room exposure- $PD_{20}FEV_1$ as accurately as possible and to estimate their limitations (Table V). Lapel-filter contents of Cat-1 captured at an airflow of 30 L/min were suitable for comparison with gravity slide particle counts because both indices were measured under standard sampling conditions. These two different indices of cat exposure, one immunochemical and the other morphological, were significantly correlated ($r_s = 0.77$; $p < 0.01$). Lapel-filter contents of Cat-1 were not a suitable index of Cat-room exposure- $PD_{20}FEV_1$ for comparison with Cat-extract challenge- $PD_{20}FEV_1$ until adjusted to allow for the fact that the patient had sampled room air at a rate equal to his or her mean minute ventilation during the exposure period, whereas the filter sampled room air at 30 L/min. Lapel-filter contents of Cat-1 captured at an airflow equal to the patient's mean minute ventilation were significantly correlated ($r_s = 0.87$; $p < 0.01$) with the values of the measurements of Cat-extract challenge- $PD_{20}FEV_1$. It is likely that corrections also should be made for the amount of inspired Cat-1 on respirable particles $<10 \mu m$ in diameter that would be expected to reach the lower airways. We have not yet measured the amount of Cat-1 on these respirable particles $<10 \mu m$ in diameter but have demonstrated that such particles can be demonstrated by microscopy on both the gravity sampler and the Andersen sampler microscopic slides. Findlay et al.¹⁰ found in their cat-exposure room a variable percentage of total quantity of Cat-1 on particles $<10 \mu m$ in diameter (14%, 30%, and 0% in three experiments) and the remainder (86%, 70%, and 100%, respectively) on particles $>10 \mu m$ in diameter. Swanson et al.²⁶ also found much cat allergen in particles $<10 \mu m$ in diameter. These figures cannot be applied directly to our cat-exposure room, but they suggest, as do our microscopic findings, that the dose of Cat-1 that is in or on $<10 \mu m$ particles and that can be expected to reach the peripheral airways of the lung is considerably less than the total inspired dose of Cat-1. We plan to use our RIA to measure Cat-1 on respirable particles $<10 \mu m$ in diameter and on nonrespirable particles $>10 \mu m$ in diameter obtained from the air of our cat room under patient-exposure conditions by use of the type of Andersen sampler used by Findlay et al.¹⁰ It is important to recognize that Cat-extract challenge- $PD_{20}FEV_1$ was measured by having the patients inhale particles of cat extract that were $1.5 \pm 3.0 \mu m$ (geometric mean $\pm$ SD) in diameter and that would be expected to reach the peripheral airways of the lung.

It is of interest that, although there was a strong, significant rank correlation between the doses of Cat-1 required for Cat-room exposure- $PD_{20}FEV_1$ and those required for Cat-extract challenge- $PD_{20}FEV_1$, the doses of Cat-1 for Cat-room exposure- $PD_{20}FEV_1$ were significantly lower than those for Cat-extract challenge- $PD_{20}FEV_1$ (Table V). As indicated in Table V, the reported values for lapel-filter contents of Cat-1 represented the total amount of Cat-1 recovered from each filter. Since the results of the Cat-1 recovery experiments indicated that we could recover only about 71% of Cat-1 deliberately applied to the filters in the form of the reference cat extract, these reported values are probably only about 70% of the Cat-1 actually on the filter. However, multiplying the values reported by 1.4 to correct for this loss in recovery does not account for the differences observed. These differences may be related in some way to the fact that Cat-room exposure- $PD_{20}FEV_1$ was measured by a continuing exposure to cat antigens, whereas Cat-extract challenge- $PD_{20}FEV_1$ was measured by brief five-breath exposures at about 12-minute intervals. We plan to test this hypothesis in a subsequent study.

The methods used to measure the various indices of Cat-room exposure- $PD_{20}FEV_1$ are cumbersome, expensive, labor-intensive, and demand a series of steps, each of which can introduce error. By contrast, inhalation challenge with cat extract is a much simpler procedure and one that is likely to be more accurate. The patient is put through less discomfort, and there are few distractions to accurate measurements of FVC and FEV_1 . The doses of Cat-1 equivalents administered can be conveniently measured, since the inhaled volume of extract, the dilutions of extract, and the content of Cat-1 in each dilution are known. Therefore, it is of considerable practical importance that the results of this cumbersome yet more natural method of exposing patients to cat demonstrate very significant correlations with the results obtained by inhalation challenge with cat allergenic extract.

The existence of a significant association between measurements of Cat-room exposure- $PD_{20}FEV_1$ and measurements of Cat-extract challenge- $PD_{20}FEV_1$ makes it likely that reports of significant decreases in bronchial responses to inhaled cat extract in immunotherapy-treated patients as compared to placebo-treated patients^{1, 3, 4, 6} can be interpreted with reasonable confidence as indicating that these patients also would have significant decreases in their bronchial response to exposures to living cats. Ohman et al.³ have already presented evidence that indicates that immunotherapy-treated patients in comparison to placebo-treated patients have a statistically significant delay in onset of ocular and pulmonary symptoms in a room containing living cats. A study is yet needed

that demonstrates that both the dose of Cat-1 for Cat-room exposure- $PD_{20}FEV_1$ and that for Cat-extract challenge- $PD_{20}FEV_1$ increase in significant fashion in patients with cat asthma treated with immunotherapy with cat extract but not in matched control subjects treated with placebos. This would provide direct evidence that measurements of Cat-extract challenge- $PD_{20}FEV_1$ can be used in place of measurements of Cat-room exposure- $PD_{20}FEV_1$. Such a study is currently being conducted by our group.

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