

Surface washes of living cats: an improved method of obtaining clinically relevant allergen

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Washing the surface of live anesthetized cats with water results in a solution rich in allergen 1 and low in albumin. CIE and CRIE analysis showed the presence of at least seven antigens. All were shown to be immunologically identical to antigens that occur in pelt extracts and saliva. The only allergens that could be detected in surface washes were allergen 1 and albumin. The animals could be washed at monthly intervals to yield approximately the same quantity of allergen 1. This simple procedure could provide an excellent source of potent cat allergen extracts. (J ALLERGY CLIN IMMUNOL 72:288-293, 1983.)

Allergenic extracts for the diagnosis and the treatment of cat allergy in humans have traditionally been prepared from hair and/or epidermal scrapings or from whole pelts that are dried and ground. These materials contain a variable amount of the major allergenic component, cat allergen 1.^{1, 2} A number of other allergenic proteins such as albumin and nonallergenic proteins are present. These are probably derived from saliva or serum. Only a minority of cat-allergic individuals are sensitive to allergens other than allergen 1. Skin test and RAST inhibition correlate strongly with the allergen 1 content of cat epithelial extracts.^{1, 3, 4}

This report describes the properties of a preparation derived from the surface washes of live cats. At least seven proteins were found in the wash, by CIE, compared to approximately 11 proteins in pelt extract. An apparent difference between washes and pelt was the higher ratio of allergen 1 to albumin in the wash. Washes have a higher ratio of allergen 1 to albumin than whole pelt extracts. The possible advantages of such a preparation for therapy and diagnosis of cat

allergy in man are discussed in the light of our present knowledge of cat allergens.

METHODS

Surface washes of live cats

Mature cats (1.5 to 3 kg), shown to be in good health by a veterinarian, were lightly anesthetized with ketamine HCl (Bristol-Meyers, Syracuse, N.Y.) (10 mg/kg body weight). One liter of water was poured over the animal as the hair was gently massaged. Wash water was pressed out of the hair and collected in a basin; this procedure was repeated twice by using the collected wash. Only the trunk and the extremities were washed. The regions around the mouth and urethral and anal orifices were not washed, to avoid contamination with saliva, urine, or fecal material. The washing procedure was repeated at different time intervals. Cats of both sexes were used. The animals were housed individually in cages with separate ventilating systems.

Each wash was freeze-dried, resuspended in 10 to 20 ml of water, and dialyzed by using membrane tubing with 10,000 MW cutoff (Arthur H. Thomas Co., Philadelphia, Pa.) against 100 vol of water at 5° C for 72 hr with one

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Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
RAST:	Radioallergosorbent test
BGG:	Bovine gamma globulin
BSA:	Bovine serum albumin
RID:	Radial immunodiffusion
RIE:	Rocket immunoelectrophoresis

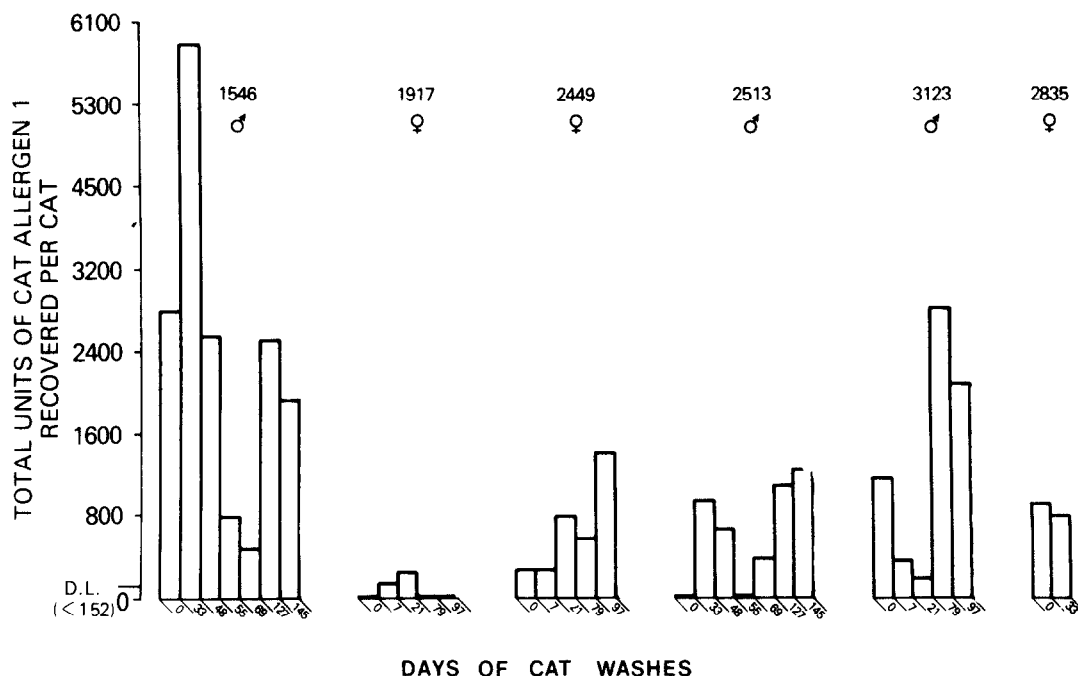


FIG. 1. Quantity of cat allergen 1 in cat washes. Each bar indicates units of cat allergen 1 per wash. Each wash consisted of approximately 300 ml. (D.L. = Lower limit of detection.)

change of water. The dialyzed material was clarified by centrifugation and freeze-dried; the weights of nondialyzable solids were recorded.

Pooled cat wash

Twenty-one separate surface washes from five cats were pooled and freeze-dried for further study; this was designated lot 162

To compare the properties of cat-wash extract with pelt extract, two pelt extracts were employed whose properties were previously described, lots 149¹ and 206.⁴ Protein was determined by the method of Lowry et al.,⁵ the orthophthalaldehyde method,⁶ and by O.D. at 280 nm, with BSA used as the protein standard.

Gel filtration

Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N.J.) was equilibrated in phosphate-buffered (0.01M, pH 7.5) saline (0.15M) and used to prepare a column 60 cm in length with a bed volume of 500 ml. BGG, BSA, chymotrypsinogen, and cytochrome c were used as molecular weight markers for calibration of the column; the column effluent was monitored at 280 nm.

Assay of allergen 1 and albumin

Allergen 1 and albumin were assayed by three procedures: quantitative RID using the same monospecific sheep antiserum and a reference calibrated against pelt 149 as previously described,⁴ quantitative CIE,^{7,8} and quantitative RIE using monospecific antiserum in the upper gel and pelt

149 as the reference. In this assay, the quantity of antigen in the unknowns was determined from the heights of the precipitin arcs.⁷

CPE 149 was prepared in freeze-dried aliquots containing 16 U of cat allergen 1 and 1240 μ g of albumin. It should be noted that a microgram estimate of cat allergen 1 has been used in previous publications.^{2,9} Because of the unavailability of a sufficient quantity of pure allergen 1, the content of this allergen is expressed in units in this publication. The unit is derived from an FDA reference preparation, and 1 U is equal to approximately 25 μ g of the previously published microgram concentration of CPE 149, a value that cannot be verified at this time.

Fused RIE

Seven column fractions resulting from Sephadex fractionation of lot 162 were analyzed by fused RIE⁷ and an adaptation of radioimmuno-electrophoresis. Pelt wash and column fractions of the pelt wash were allowed to diffuse in single wells of a 2 mm agarose gel plate for 1 hr at room temperature. The upper two thirds of the agarose was then removed and replaced with agarose containing antibody to whole pelt extract. The slide was placed on a cooling plate thermostatically controlled at 10° C and electrophoresed at 1.5 volts/cm of gel for 18 hr. The plates were pressed, dried, then overlaid with 500 μ l of a serum pool derived from five untreated allergic individuals in 15 ml of buffer A [0.1M phosphate buffer, pH 7.5, containing 2% BSA, 1% polysorbate 20 (Tween 20; ICI Americas, Inc., Wilmington, Del.), and 0.1% sodium azide]. After overnight

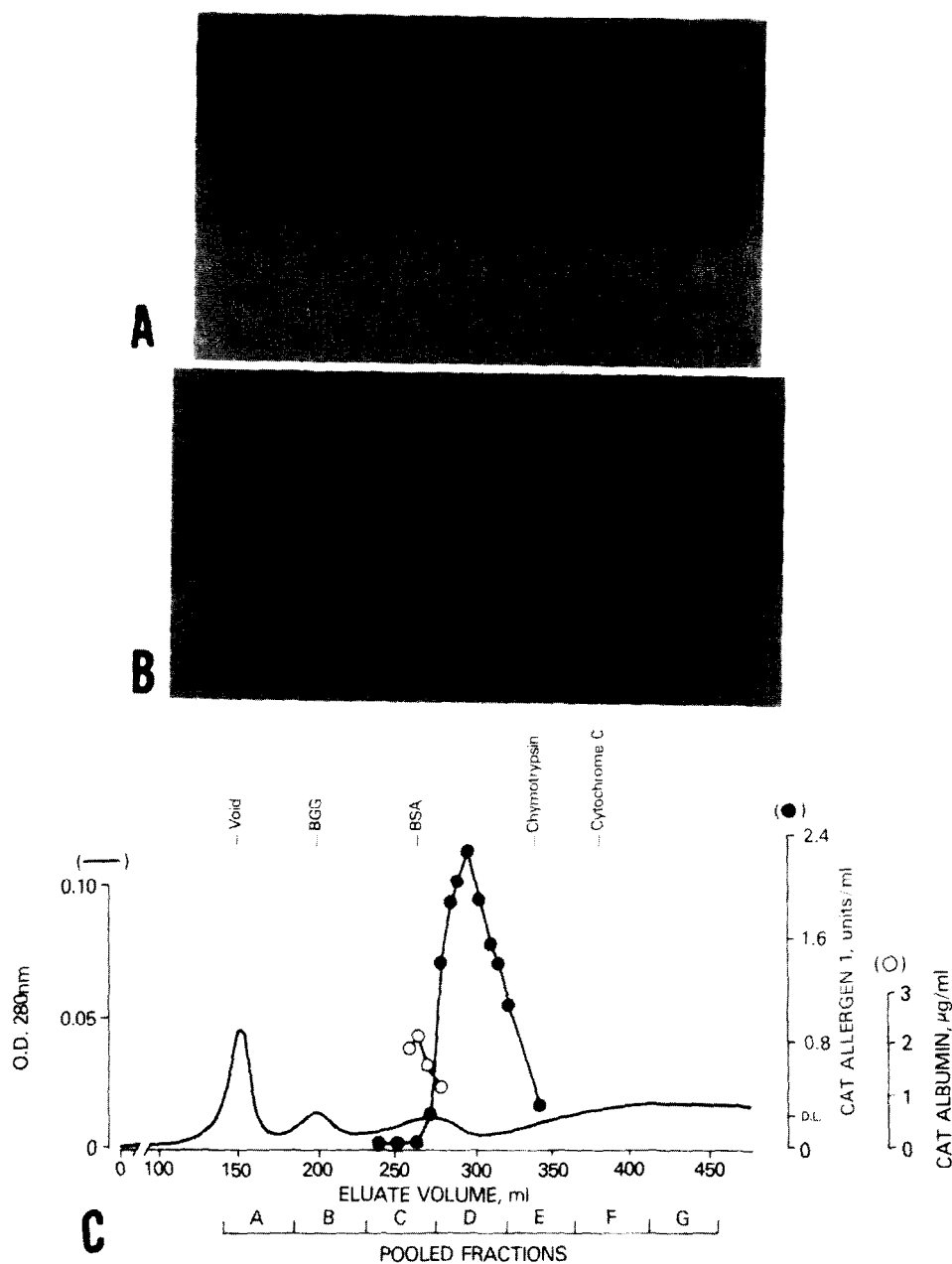


FIG. 2. Analyses of column-fractionated cat wash 162. Immunoelectrophoretic (a and b) and chromatographic (c) analyses of pooled cat wash. Upper gel of a contains rabbit anti-whole pelt. Results of fractionation of pooled cat wash on G-200 Sephadex column is displayed in c, and levels of cat allergen 1 and albumin were determined in the collected fractions. D.L., Lower limit of detection.

incubation on a rotary shaker, the gels were washed three times with 0.1M phosphate buffer, pH 7.5, containing 1% Tween 20 and then overlaid with 15 ml of buffer C (0.1M phosphate buffer, pH 7.5, 4% calf serum, 1% Tween 20, and 0.1% sodium azide) containing 4 µg of affinity-purified goat anti-human IgE that was radiolabeled by the method of Hunter and Greenwood¹⁰ and contained 300,000 cpm of ¹²⁵I. After overnight incubation, the slides were washed, dried, and placed in a cassette with Kodak Royal Blue x-ray film for 10 days.

CIE and CRIE

These techniques were carried out as previously described.⁴ Antisera to pooled cat wash and cat albumin were raised in rabbits; globulin was prepared from rabbit anti-cat pelt and sheep anti-allergen 1. Cat wash and whole pelt extract were examined by CIE by using anti-whole pelt and anti-pelt wash. Incorporation of anti-albumin or anti-allergen 1 in intermediate gels permitted identification of these antigens in the CIE patterns. Other additions to the intermediate gels included 149, 162, cat saliva, and rag-

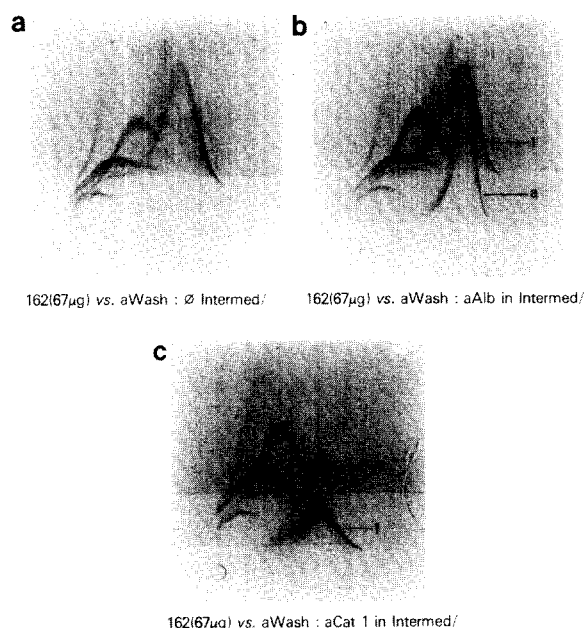


FIG. 3. Lower agarose gel contains first dimension electrophoresis of cat wash 162. **a**, No additive is present in intermediate gel ($\emptyset$). **b**, anti-albumin (aAlb) contained in intermediate. **c** anti-cat allergen 1 (aCat 1) contained in intermediate. All upper gels contain anti-cat pelt. Precipitin arcs of albumin(a) and cat allergen 1(1) are labelled in **b** and **c**.

weed antigen E as a control; all proteins were added in approximately equal concentration. Use of the serum pool from five untreated cat-allergic individuals and a single serum of an untreated patient (B), who was found to have an unusually high titer to many pelt components, permitted identification of those antigens that are allergens in CRIE procedures. Similar procedures were followed for CRIE analyses of the two-dimensional electrophoresis patterns as in the fused RIE.

RESULTS

Analyses of the cat wash preparations

A total of 36 washes from six cats were collected. A summary of the data is found in Fig. 1. It is apparent that there was a wide variation in the amount of allergen 1 recovered. One female cat (No. 1917) consistently produced less than 400 U of allergen 1 per wash whereas quantities above 800 U were recovered in the washes of other cats. Studies with three of the cats (1546, 2513, 3123) showed that repeated washes at intervals of seven days or less showed a tendency toward a progressive decline in the yield of allergen.

Gel filtration of pooled cat wash

The seven fractions (A to F) of lot 162 obtained by gel filtration were analyzed by O.D. at 280, RIE, and fused RIE to detect and quantitate cat allergen 1 and albumin, and the results of these procedures are

shown in Fig. 2. The stained immunoelectrophoretic plate (Fig. 2, a) identified an antigen that was composed of fused arcs from fractions C, D, and E and was shown to be allergen 1. The starting material, in the first and last wells, showed three arcs. Undoubtedly, the increase in volume that occurred during the gel filtration resulted in sufficient dilution to prevent visualization of arcs other than allergen 1. CRIE of the column fractions employing anti-pelt antibody (Fig. 2, b) and subsequent use of anti-allergen 1 identified the allergen 1 peak. The O.D. of the column effluent at 280 nm and the results of the RIE analyses of the fractions are graphically represented in Fig. 2, c.

CIE and CRIE

CIE patterns of wash 162 from rabbit antiwash antibody in the upper gel and the identification of albumin(s) and allergen 1¹ precipitin arcs from intermediate gels containing monospecific antibodies are shown in Fig. 3, a to c; at least seven antigens can be detected in the wash. The apparent doubling of the allergen 1 arc in Fig. 3, c, is an artifact resulting from the large quantity of monospecific antibody required in the intermediate gel to bring the very high allergen 1 peak to the lower part of the gel.

Fig. 4 presents the CIE and CRIE of pelts 206 and 149, and wash 162 with anti-pelt antibody in the

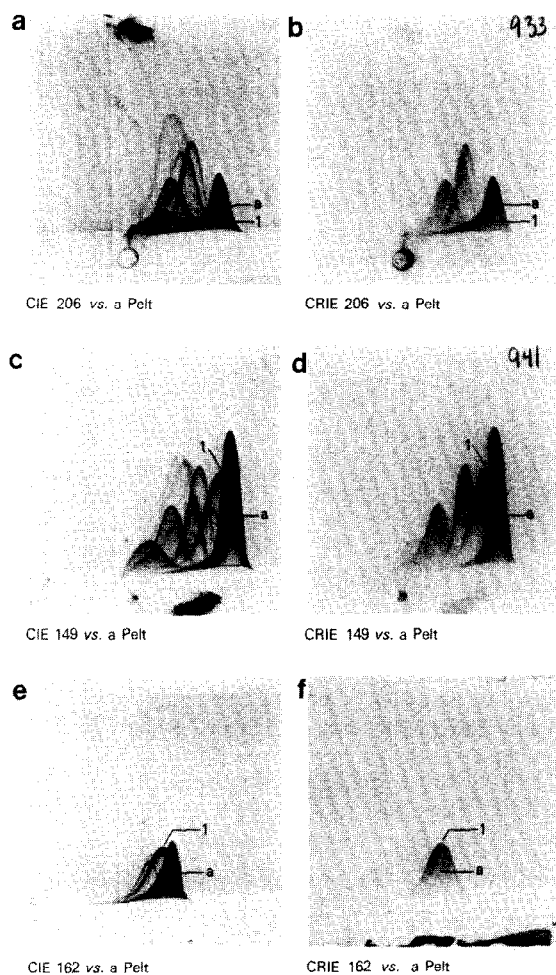


FIG. 4. Extracts 206(a and b) and 149(c and d) are extracts of pelt; extract 162(e and f) is the pooled cat wash. Human serum B was used for the CRIE of all three extracts. Rabbit anti-pelt (*a Pelt*) was used in the upper gel of a, c, and e.

upper gel and with serum B obtained from a cat-allergic individual for the CRIE. Pelt 206 contained at least 11 antigens and five allergens (Fig. 4, a and b); pelt 149 showed at least 12 antigens and six allergens (Fig. 4, c and d); wash 162 had at least seven antigens and two allergens (Fig. 4, e and f). Approximately 12 μg of pelt 206, 20 μg of pelt 149, and 68 μg of wash 162 (total protein) were used in this procedure. The heights of the allergen 1 and albumin peaks shown in Fig. 4 are not proportional to the concentration of these allergens, since the electrophoretic conditions, including the quantity of antibody in the upper gel, were adjusted to yield optimal CIE/CRIE patterns for each preparation.

The identities of the allergen 1 and albumin peaks were established in a manner analogous to that used for wash 162 and confirmed the direct analysis by

TABLE I. Characteristics of cat allergen preparations

Extract	Allergen 1 (units/100 μg of protein)	Albumin (μg /100 μg of protein)
Wash 162	0.18	0.59
Pelt 149	0.68	52.8
Pelt 206	0.10	27.8

showing that pelt 149 had much more allergen 1 than pelt 206. Fig. 4, e is the CIE of wash 162 using anti-pelt in the upper gel that shows at least seven antigens and appears different from Fig. 3, a, which had anti-wash in the upper gel, although the same number of antigens was visible. This is due to the difference of the antibody population of the two antisera. It also shows that wash 162 has approximately as many proteins as pelts 206 and 149 but the relative concentration of the various proteins is different.

To determine the relationship of the proteins of pelt 149, wash 162, and saliva, wash 162 was electrophoresed through intermediate gels containing either pelt 149, saliva, or ragweed antigen E as an unrelated protein control into an upper gel containing anti-pelt antibody. All the bands of wash 162 were shown to be identical to bands in pelt and cat saliva; the control, ragweed antigen E, had no effect on the pattern. These results are analogous to previous findings; therefore the patterns are not shown.⁴

A comparison of the composition of the pooled wash with two pelt extracts is found in Table I. The concentration of allergen 1 per 100 μg of total protein shows that pelt 149 is highest, wash 162 intermediate, and pelt 206 lowest; pelt 149 has approximately six times more allergen 1 per microgram of protein than pelt 206. The concentration of albumin is very low in wash 162 and high in both pelts; pelt 149 has approximately 100 times more albumin than the wash.

DISCUSSION

This paper describes the properties of material obtained from the surface washes of live cats and compares it to extracts of pelts. From Fig. 1, it is seen that allergen 1 can be obtained from all cats by washing, but the quantity is variable. Since allergen 1 can be easily removed by merely pouring water over a cat, it is not tightly bound to the fur or skin but rather appears to be on the surface. Washing at short intervals reduces the yield of allergen 1, but it reappears in its original concentration within a month. The source of such a soluble substance is of special interest. An examination of cat tissues showed that the only sub-

stantial source of allergen 1 was the salivary glands and pelt.¹ It has also been shown that the antigens and allergens of pelt were identical to those in saliva, by the same technique as in an earlier study.⁴ These data together with the well-known propensity of cats to groom themselves seem to point to saliva as the major source of allergen 1.

Table I shows the comparative properties of cat wash pool 162 and two pelt extracts. The pelt extracts are somewhat divergent in their properties but both differ from the wash in having a high concentration of albumin. The pelt and wash extracts have a number of proteins, in addition to allergen 1 and albumin, as demonstrated by CIE. For diagnosis of cat allergy, the allergen 1 content is the determining factor.¹⁻³ This is also probably true for hyposensitization therapy. Whether individuals who have IgE to many components (e.g., the individual from whom serum B was obtained) would benefit from the injection of all allergens is not known, but if such an individual reacted in a manner analogous to those sensitive to timothy grass pollen, he would benefit from the use of multiple components.¹¹

The relative merits of a product of very high or low albumin content cannot be evaluated at this time. The injection of large quantities of albumin will undoubtedly lead to IgG antibody to this protein. It is known that precipitating quantities of antibody to albumin can result from immunotherapy with preparations that contain high concentrations of albumin.⁹ No tests have been carried out to determine whether precipitating antibodies to other proteins are present. This will be examined in future studies. However, since the wash has a large quantity of other proteins, not yet identified or quantitated, it almost certainly will induce other antibodies.

The use of wash as a source material appears to have many advantages. It is obtained from living

animals whose health can be evaluated, and the same animals can be a continuing source of allergens.

The results of the CIE and CRIE studies indicate that the difference between wash and pelt extracts is largely quantitative. Since allergen 1 is the most important component related to allergy to cats, wash and pelt extracts should be interchangeable if adjusted to equal allergen 1 content.

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