

Allergens of mammalian origin: Characterization of allergen extracted from cat pelts

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An aqueous extract prepared from lyophilized, defatted cat pelts elicited intense wheal-and-flare responses in the skin of patients exhibiting asthma or rhinitis on exposure to cats. On electrophoresis of the extract in agarose, protein bands with the mobility of albumin, α_2 , β -, and γ_1 -protein were observed. Allergenic activity was localized in two distinct regions with albumin and α_2 -mobility, respectively. Allergenic activity remained bound to DE52-cellulose columns equilibrated with phosphate-buffered (0.01M, pH 7.4) saline (0.05M), but could be eluted by increasing the salt concentration to 0.25M. Following gel filtration on Sephadex G-100, allergen was recovered in fractions containing proteins with a molecular weight range of 30,000 to 60,000 daltons. A purified fraction of cat pelt extract obtained by sequential fractionation on DE52-cellulose and Sephadex G-100 columns was subjected to polyacrylamide disc gel electrophoresis; 5 protein bands were observed. Allergenic activity was recovered in fractions containing two of these bands. Five of twelve subjects with strong skin reactivity to cat pelt extract also reacted strongly to cat serum; the major allergen in cat serum was found to have the electrophoretic mobility of α_2 -protein.

Mammalian danders are frequent elicitors of asthma and rhinitis. Of the various animal danders, sensitivity to cat and dog dander is most frequently encountered in urban areas. The incidence of positive skin test reactions to cat and dog dander in an allergic population has been reported to be approximately 25 per cent.¹ The capacity of cat dander to induce asthma was recognized by Salter² in 1864. A recent textbook of allergy emphasizes the peculiar allergenicity of cat dander. "Cats are by far the most highly allergenic animals encountered by most people. Cat dander, like ragweed pollen, has a clinical importance that sets it apart from other allergens in its class."³

Limited information is available on the properties of allergen in mammalian danders.⁴⁻¹¹ The following report describes some physicochemical and biologic properties of allergen extracted from cat pelts.

METHODS

Preparation of cat pelt extract

Initial studies indicated that rat pelts were a good source of allergen capable of eliciting strong skin reactions in patients clinically sensitive to laboratory rats. A comparison of the

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Supported by Grants Nos. RR-05486-10, AI-10129, AM-3564, and AM-5067 from the National Institutes of Health and by grants from the Massachusetts Chapter, Arthritis Foundation and the L. H. Bendit Foundation.

Received for publication March 19, 1973.

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amount of allergen that could be extracted from equal amounts of rat pelts and rat hair indicated that more allergen could be extracted from the former.*

Fresh, unshaved, cat pelts were washed with water and lyophilized (Model USM-15, The Virtis Company, Inc., Gardiner, New York) for 72 hours. The lyophilized pelts were then defatted in ether, thoroughly dried, and extracted with water (1:10 w/v) at room temperature for 6 hours and overnight at 5° C. After adjusting the pH to 7.5 with 0.1N NaOH, the water-soluble extract was separated from pieces of pelt by pressing through cotton gauze. Particulate matter and bacteria were removed by passing the extract through Millipore filters (Millipore Corp., Bedford, Massachusetts) of successively smaller pore size (final pore size, 0.22 μ). Aliquots of crude extract were then lyophilized and stored under sterile conditions at 5° C.

Cat serum

Whole blood was obtained by cardiac puncture from cats anesthetized with amobarbital. After clotting of the blood, serum was separated and stored at -20° C.

Electrophoresis in agarose

Electrophoresis of cat pelt extract and cat serum in agarose (barbital buffer, pH 8.6) was performed as described previously¹²; 10 μ l of sample was applied to each of 9 wells on the plate. Following electrophoretic separation, the gels were cut into 5 mm. strips. The strips were separately homogenized and extracted with 1.5 ml. of 0.15M NaCl.¹³ The two outer wells were left on the plate; the separated protein was fixed and stained with Buffalo black stain in order to localize protein corresponding to the eluted fractions.

Ion exchange chromatography

DE52-cellulose microgranules (Reeve Angel, Clifton, New Jersey) were prepared and equilibrated against phosphate buffer, (0.001M, pH 7.5) as recommended by the manufacturer. One hundred milliliters of cat pelt extract was lyophilized, reconstituted in 3 ml. of water, and dialyzed against phosphate buffer for 48 hours at 5° C. before application to chromatography columns measuring 2 $\times$ 30 cm. Stepwise elution with the first two buffers (0.001M, pH 7.5; 0.01M, pH 7.5) was carried out. Thereafter, the remaining protein was eluted by stepwise addition of NaCl to the 0.01M phosphate buffer as indicated in the section entitled "Results."

The optical density of the various fractions was measured at 280 m μ in 0.1N NaOH. It was found that nondialyzable solid from these fractions in a concentration of 1.4 mg. per milliliter had an optical density of one unit.

Gel filtration

Sephadex G-100 was equilibrated in 0.001M Tris-buffered saline at pH 8.0 as recommended by the manufacturer. Two millimeters of sample, which had been dialyzed against this buffer, were applied to 2 $\times$ 100 cm. columns at 4° C., and 4 ml. fractions were collected. Bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome C (obtained from Schwarz/Mann, Orangeburg, New York) were used as markers of known molecular size in calibrating these columns.

Polyacrylamide disc gel electrophoresis

Polyacrylamide disc gel electrophoresis was performed as previously described¹⁴ using glass tubes measuring 6 $\times$ 100 mm. The separator gel contained 7 per cent acrylamide. A current of 4 mA. per tube was applied until the bromphenol blue indicator had migrated 50 mm. into the small pore gel. The gel columns were then removed from the glass tubes and stained with 1 per cent Amidoblack in 7 per cent acetic acid. Excess stain was removed by

*Ohman, J.: Unpublished observations.

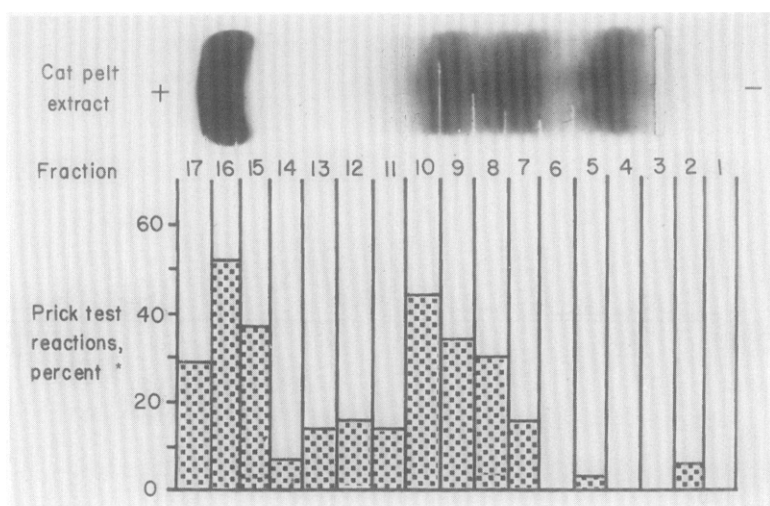


FIG. 1. Electrophoresis of cat pelt extract in agarose (barbital buffer, pH 8.6). Protein bands with the mobility of albumin, α_2 -, β -, and γ_1 -protein were obtained; allergenic activity, as assayed by skin tests in 10 patients clinically sensitive to cats, was largely recovered from two regions with the mobility of albumin (fraction 16) and α_2 -protein (fractions 8 to 10), respectively. *, Each subject's maximum flare diameter was taken as 100 per cent; the other reactions were expressed as a per cent of the maximum reaction. Average values are indicated.

leaching in 7 per cent acetic acid for 48 hours. Fractions of the gel columns were prepared by embedding the columns in 1 per cent agar immediately after electrophoresis and slicing the gel into 5 mm. cylinders beginning at the bromphenol blue indicator. The cylinders were then homogenized and fractions were eluted with one milliliter of 0.15M NaCl at 20° C. for 6 hours and overnight at 5° C. An identical gel column that had been prepared at the same time was marked at the level of the indicator dye and stained. A photograph of the stained gel column was marked so as to permit correlation between eluted fractions and specific protein bands.

Skin testing

Fractions used for prick testing were generally dialyzed in water, lyophilized, and suspended in 50 per cent glycerol. Prick tests were performed as described previously¹⁵ on subjects exhibiting either rhinitis or asthma on exposure to cats. Informed consent was obtained prior to testing. The average diameter of the flare in millimeters was noted 15 minutes after the injection of test substance. Reactions of less than 5 mm. in diameter were disregarded. Each subject's maximum reaction was taken as 100 per cent; other skin test reactions were expressed as a per cent of the maximum reaction.

Intradermal tests were performed as described previously.¹⁶ Solutions for intradermal testing were passed through a Millipore filter (pore size: 0.22 μ), and 0.03 ml. was injected intradermally at each skin site. Intradermal reactions were graded by adding the average diameter of the wheal to the average diameter of the flare.

Antiserum to albumin

Guinea pig antiserum to bovine serum albumin was found to form a single precipitin line with cat pelt extract on gel diffusion in agar. This antiserum reacted with a protein present in cat pelt extract and cat serum, which had the same electrophoretic mobility and molecular size as bovine albumin (BSA). A reaction of partial identity was observed in precipitin tests with

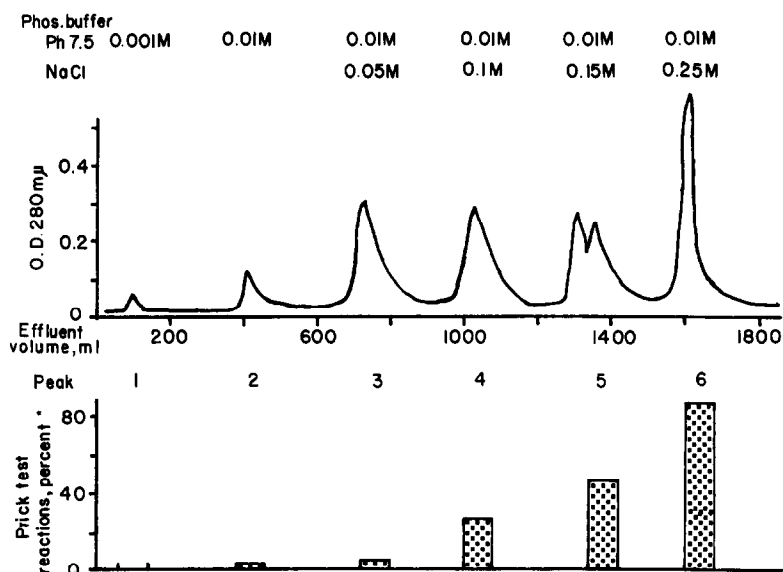


FIG. 2. Elution profile obtained by DE52-cellulose chromatography of 150 mg. of cat pelt extract. Allergic activity, as assayed by skin tests on 13 patients clinically sensitive to cats, was largely recovered in peaks 4, 5, and 6. The most intense activity was recovered in peak 6. *, Prick test reactions were graded as in Fig. 1.

BSA and the constituent of cat extract and serum; hence the antiserum appears to cross-react with cat albumin.

RESULTS

Comparison of allergenic activity in cat pelt extract and commercial extracts of cat hair

Thirteen subjects who were clinically sensitive to cats were skin-tested with a 1:10 extract (w/v) of cat pelt and also with a 1:10 extract of cat hair (Hollister-Stier Laboratories, Yeadon, Pennsylvania). Some of the subjects were tested intradermally with a 1:100 dilution of both extracts. In both procedures, the two extracts produced reactions of equal size and intensity.

Effect of dialysis on allergenic activity of cat pelt extract

There was no loss of skin reactivity on dialysis of cat pelt extract for 72 hours against water. In order to detect low molecular weight allergens, 10 ml. of cat pelt extract was lyophilized, suspended in one milliliter of water, and dialyzed against 50 ml. of water for 3 days. The fluid outside the dialysis bag was then lyophilized and dissolved in 2 ml. of water. Eight subjects clinically sensitive to cats and exhibiting strong reactions to a 1:10 dilution cat pelt extract (prick test reactions greater than 10 mm. in diameter) were prick-tested with the concentrated dialysand; negative results were obtained in 6 subjects and a minimal reaction was obtained in 2 subjects (5 mm. and 9 mm. in diameter, respectively).

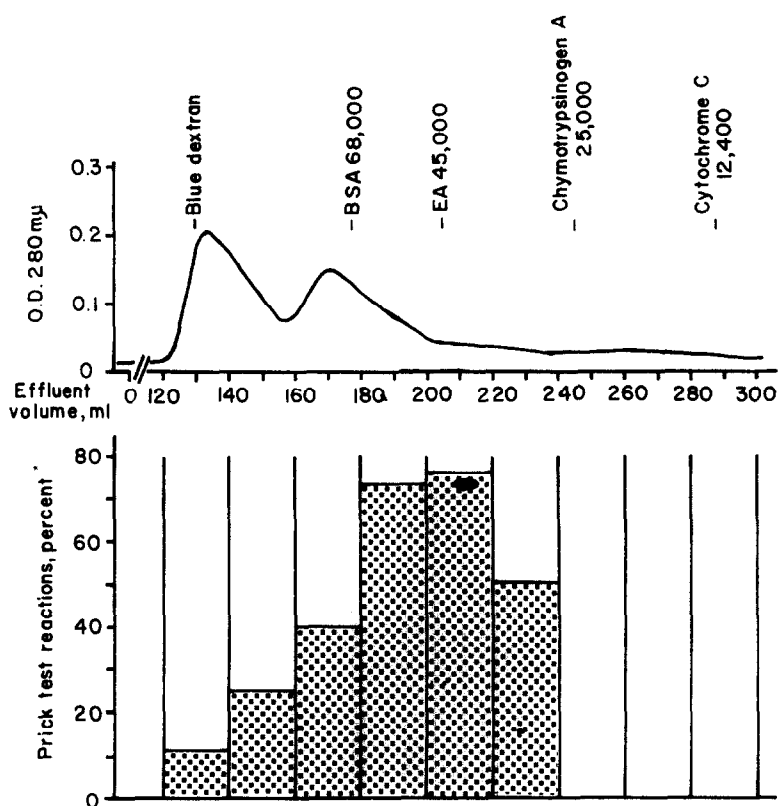


FIG. 3. Elution profile obtained by gel filtration of 2 ml. (75 mg.) of dialyzed cat pelt extract on a Sephadex G-100 (2×100 cm.) column. Two protein peaks resulted; allergenic activity, as assayed by skin tests on 4 patients clinically sensitive to cats, was largely recovered from eluate constituting the descending limb of the second peak. Eluate with maximum allergenic activity contained proteins with a molecular weight range of 30,000 to 60,000 daltons. *, Prick test reactions were graded as in Fig. 1.

Electrophoresis of cat pelt extract in agarose

Electrophoresis of cat pelt extract (30 mg. per milliliter) revealed protein bands with the mobility of albumin, α_2 -, β -, and γ_1 -protein (Fig. 1). Allergenic activity, as determined by prick tests on 10 subjects clinically sensitive to cats, was recovered largely from two regions with the mobility of albumin and α_2 -protein, respectively. Two of the subjects reacted only to fractions with the electrophoretic mobility of albumin.

Ion exchange chromatography of cat pelt extract

Two milliliters of cat pelt extract (60 mg. per milliliter), which had been dialyzed against phosphate buffer (0.001M, pH 7.5), was fractionated on a DE52-cellulose column; the elution profile is indicated in Fig. 2. Six protein peaks were obtained by stepwise elution with 0.001M and 0.01M phosphate buffer, followed by the addition of NaCl to the 0.01M buffer in concentrations of

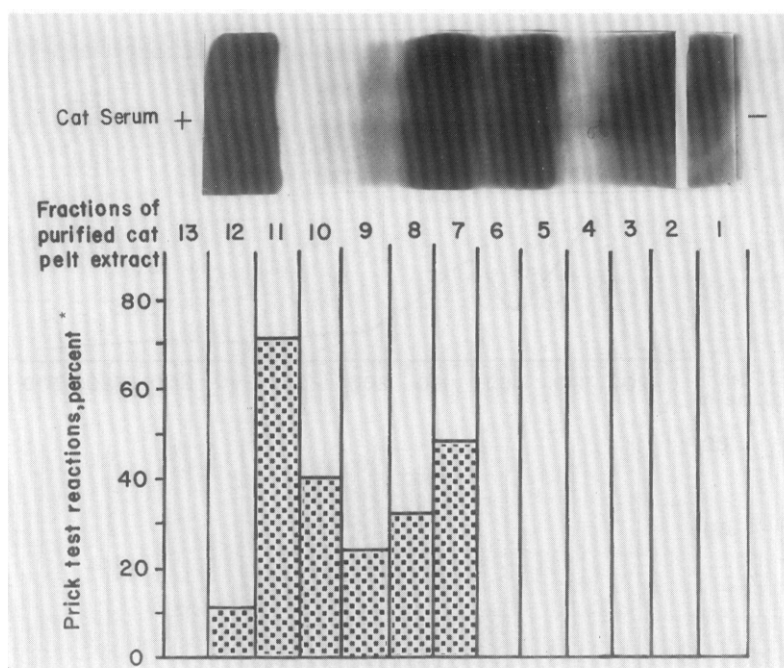


FIG. 4. Electrophoresis in agarose (barbital buffer, pH 8.6) of a purified fraction of cat pelt extract obtained by sequential fractionation on DE52-cellulose and Sephadex G-100 columns. Staining with Buffalo black revealed a faint protein band with albumin mobility. For the purposes of localization, the electrophorogram of cat serum is shown at the top of the illustration. Allergic activity, as determined by skin tests in 5 patients clinically sensitive to cats, was recovered in fractions with both albumin (fraction 11) and α_2 (fraction 7)-mobility. *, Prick test reactions were graded as in Fig. 1.

0.05M, 0.1M, 0.15M, and 0.25M. The effluent from each protein peak was combined, dialyzed against water, and an aliquot of each peak was added to an equal volume of glycerol for skin testing. Allergic activity, as assayed by prick tests on 13 subjects clinically sensitive to cats, was recovered largely in peaks 4, 5, and 6. Ten of the 13 subjects reacted maximally to peak 6. Albumin was detected by gel diffusion in peaks 5 and 6. Elution of the column with 1.0M salt resulted in no further recovery of protein or allergic activity.

Gel filtration of cat pelt extract on Sephadex G-100

Two milliliters of dialyzed cat pelt extract (40 mg. per milliliter) was applied to a Sephadex G-100 column that had previously been calibrated with proteins of known molecular weight. Two protein peaks were obtained; the second peak contained albumin (as determined by gel diffusion against anti-serum to albumin). Allergic activity, as assayed by skin tests on 4 patients clinically sensitive to cats, was recovered largely from eluate constituting the descending limb of the second peak. Eluate with maximum allergic activity contained proteins with a molecular weight range of 30,000 to 60,000 daltons. Although several other gel filtration studies were carried out with Sephadex

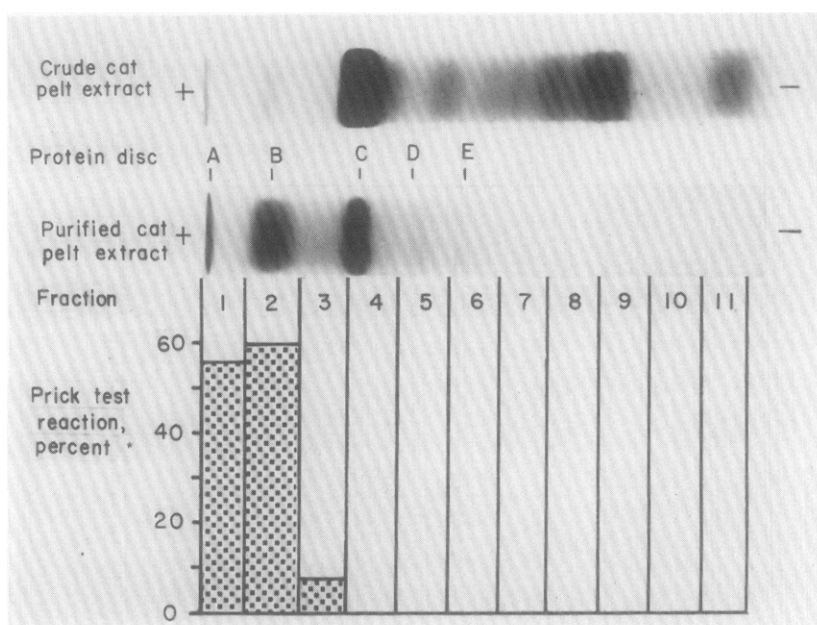


FIG. 5. Polyacrylamide disc gel electrophoresis of 100 μGm of a purified fraction of cat pelt extract obtained by sequential fractionation on DE52-cellulose and Sephadex G-100 columns. Five-millimeter portions of the gel were sliced, homogenized, and eluted with 2 ml. of 0.15M NaCl; a 1:10 dilution of this eluted fraction was used for prick testing. Allergenic activity, as determined by skin tests in 5 patients clinically sensitive to cats, was largely recovered in fractions 1 and 2 corresponding to protein bands A and B. (Disc gel electrophoresis of 300 μGm of crude dialyzed cat pelt extract is included in the upper portion of the figure for comparison.) *, Prick test reactions were graded as in Fig. 1.

G-100 and G-75 columns, a more precise localization of allergenic activity was not attained.

Preliminary studies of a purified fraction of cat pelt extract

Portions of cat pelt extract were dialyzed against 0.05M NaCl in phosphate buffer (0.01M, pH 7.5) and applied to DE52-cellulose columns that had been equilibrated with the same solution. Following elution of the first peak of protein, a second fraction containing allergen was eluted by increasing the salt concentration of the buffer to 0.25M. This second fraction was dialyzed, concentrated by lyophilization, and applied to Sephadex G-100 columns. The elution profile shown in Fig. 3 was obtained; allergenic activity was found to be maximal in fractions constituting the descending limb of the second protein peak. This portion of the effluent was combined and concentrated. Prick testing with this fraction (at a protein concentration of 200 μGm per milliliter) in 3 subjects, clinically sensitive to cats, yielded reactions of average intensity equal to those reactions obtained with crude cat pelt extract (at a protein concentration of 10 mg. per milliliter).

Electrophoresis of 250 μGm of this purified fraction in agarose disclosed a

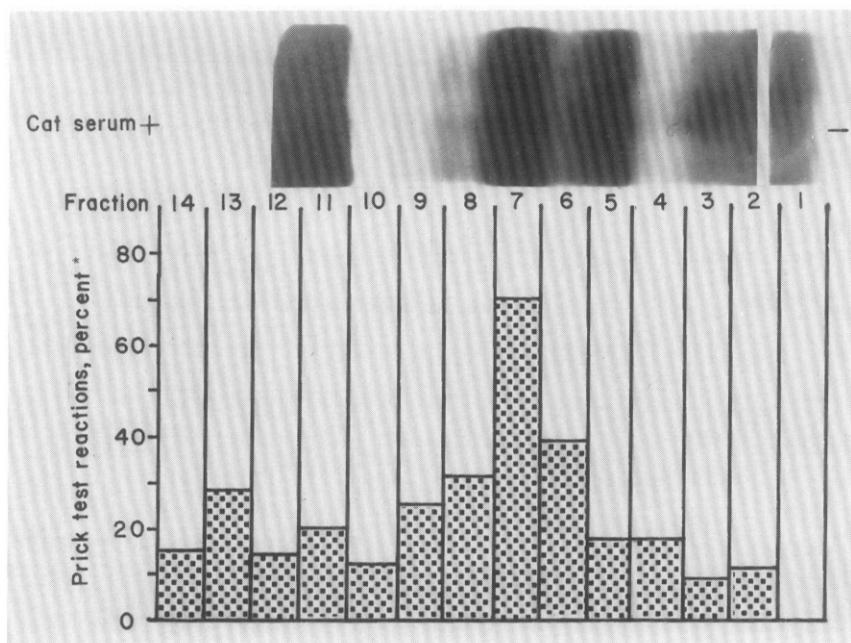


FIG. 6. Electrophoresis of cat serum in agarose (barbital buffer, pH 8.6). Allergic activity was determined by skin tests in 5 patients who were clinically sensitive to cats and who exhibited intense skin reactivity to a 1:10 dilution of cat serum. Fraction 7 (α_2 -mobility) contained most of the allergenic activity; lesser amounts of allergenic activity were obtained from fractions with albumin and prealbumin mobility. *, Prick test reactions were graded as in Fig. 1.

faint protein band with the mobility of albumin. Allergic activity, as determined by skin tests in 5 patients clinically sensitive to cats, was recovered in fractions with both albumin and α_2 -mobility (Fig. 4; note that for purposes of localization, the electrophorogram of cat serum is shown in this figure).

Polyacrylamide disc gel electrophoresis, in 7 per cent acrylamide gels, of 100 μ Gm of purified fraction was carried out (Fig. 5). Five distinct protein bands were seen; bands A and B of rapid mobility, band C, which contained albumin, and several additional faint bands (D and E). Bands A, B, and C were easily visualized after disc gel electrophoresis of 300 μ Gm of crude cat pelt extract (upper portion of Fig. 5). Five-millimeter portions of an unstained gel column containing purified fraction were removed sequentially starting at the bromphenol blue indicator dye. Protein was eluted with one milliliter of 0.15M salt, and a 1:10 dilution of this eluate was used for prick testing. Allergic activity, as determined by skin tests in 5 patients clinically sensitive to cats, was largely recovered in fractions 1 and 2 corresponding to protein bands A and B. Little or no activity was found in fractions 3 to 6, which contained bands C (containing albumin), D, and E.

Numerous attempts were made to localize allergenic activity to either band A or band B. Although such experiments always resulted in recovery of al-

lergenic activity in the prealbumin portion of the gel column, it was not possible consistently to associate allergenic activity with either band A or band B.

Allergenic activity in cat serum

Twelve subjects exhibiting strong prick test reactions to cat pelt extract were tested with cat serum in a 1:2 or 1:10 dilution. Five subjects exhibited strong reactivity to cat serum with flare diameters greater than 10 mm. After electrophoresis of whole cat serum in agarose (Fig. 6), allergenic activity was recovered primarily in fractions with α_2 -mobility. Lesser amounts of allergen were recovered in albumin and prealbumin regions.

DISCUSSION

Lyophilized, defatted cat pelts could be extracted with water so as to yield an allergen capable of producing intense skin test reactions in patients clinically sensitive to cats. This extract proved to be approximately equal in potency to a commercial cat dander extract. The yield of allergen from a cat pelt was much greater than that which could be obtained from the hair and dander of a single cat.

After electrophoresis of cat pelt extract in agarose, allergenic activity was obtained in protein fractions with albumin and α_2 -mobility. Ion exchange chromatography resulted in recovery of allergen, at pH 7.5, in fractions containing proteins with relatively high charge properties; several of these fractions also contained albumin. Previous reports have indicated that the allergens in horse dander,^{5, 10, 19} dog dander,⁹ and cow dander¹¹ are also acidic in nature. Similar findings have also been reported for a number of nonmammalian allergens.^{17-21, 31-33} Most of the allergenic activity in cat pelt extract was found to be nondialyzable. On gel filtration, allergen was recovered in fractions containing proteins with a molecular weight range of 30,000 to 60,000 daltons. Nonmammalian allergens, previously studied, were found to have a similar or slightly lower molecular weight.²²⁻²⁶

A purified fraction of cat pelt extract prepared by ion exchange chromatography followed by gel filtration was shown to have high biologic activity; a fiftyfold increase in specific activity was obtained. Allergenic activity in this purified fraction had an electrophoretic mobility in agarose similar to that of allergen in crude cat pelt extract, suggesting that there had been no loss of allergenic components during purification. Polyacrylamide disc gel electrophoresis of this fraction revealed 5 protein bands; 3 of these bands, one of which contained albumin, were associated with minimal allergenic activity. Intense allergenic activity was recovered in fractions of rapid mobility containing 2 protein bands (A and B, Fig. 5). The relation of allergen to these protein bands has not yet been determined. Both protein bands may represent allergen; alternatively, allergen may be present as a trace protein not visualized by this technique. Allergen migrated more rapidly than albumin during electrophoresis in 7 per cent acrylamide gels, while in agarose, allergen had, in part, the same electrophoretic mobility as albumin. It seems unlikely that two different allergens were detected using these two electrophoretic techniques. The smaller molecular

size of the allergen relative to albumin may explain its more rapid mobility in the acrylamide gels, which tend to retard proteins of higher molecular weight.²⁷

The difficulties involved in allergen purification are related to the high biologic potency of allergens, their low concentration in extracts, and the semi-quantitative nature of methods to measure the active components. These problems have been discussed previously.^{15, 17} The prick test provides a useful assay for allergen because large numbers of fractions can be tested for the presence or absence of allergenic activity. The radioallergosorbent assay²⁸ might be an improvement over skin testing, but it has not yet been extensively applied to the problems of allergen purification.

Cat serum was found to be highly allergenic in some, but not all, cat-sensitive subjects who exhibited strong skin test reactions to cat pelt extract. The serum allergen was predominantly of α_2 -mobility with additional components of more rapid mobility. The allergen in cat pelt extract also had a component of α_2 -mobility and in addition had another major component of albumin mobility. The relation of cat serum allergen to the allergen in cat dander has not yet been determined. The finding of an allergen in cat serum is analogous to previous studies of horse serum, which was found to be allergenic in some individuals who were sensitive to horse dander.²⁹ Based on these findings, it was concluded that there were similarities between allergenic components in horse dander and horse serum.^{15, 30-32} Horse albumin was postulated as an allergen¹⁵; however, contamination of albumin preparations with a different serum allergen was not excluded.³²

The present studies have shown that cat pelts are a useful source of an extract that can be used to study allergens of mammalian origin. Further characterization of the allergen or allergens in cat pelt extract may be followed by a better understanding of the origin and distribution of these allergen(s) in cat tissues and may also allow comparison with allergens derived from other mammals. Many previous studies have been concerned with inhalant allergens of plant origin; characterization of a mammalian allergen might help to delineate common physicochemical properties of antigens that predispose to allergenicity.

The authors acknowledge the excellent technical assistance of Mrs. Marsha Williams.

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