

ALLERGENS OF MAMMALIAN ORIGIN

III. Properties of a Major Feline Allergen¹

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A purified fraction of cat pelt extract was prepared and subjected to electrophoresis in 7% acrylamide gel. The bulk of the allergenic activity was recovered from the prealbumin portion of the gel that contained a single band of protein (band B). Antiserum to band B reacted with both band B and albumin; after absorption with albumin, only antibody against band B could be detected. Electrophoresis of band B protein in 10% acrylamide gel resolved four protein bands; one of these bands was associated with the bulk of allergenic activity and reacted with absorbed antiserum to band B. The absorbed antiserum (anti-allergen antiserum) reacted in gel diffusion with a single component of crude cat pelt extract; this component had the molecular size characteristics of allergen. Anti-allergen antiserum removed significant amounts of allergenic activity from both crude cat pelt extract and purified fractions of cat pelt extract. It is proposed that the allergen defined by this antiserum be designated cat allergen 1.

The radioallergosorbent test was modified so as to provide an assay for allergen. The modified test provided a useful supplement to the skin test as an assay for allergen and showed that, of various cat tissue extracts, pelt extract was the most abundant source of allergenic activity. Extract of cat brain also contained significant amounts of allergen.

A previous study has shown that allergen extracted from cat pelts has the mobility of albumin and α -2 protein on electrophoresis in agarose and has a molecular weight between 30,000 and 60,000 daltons (1). Polyacrylamide (7%) disc gel electrophoresis of a purified fraction of cat pelt extract, obtained by sequential fractionation on DE52-cellulose and Sephadex G-100 columns, resulted in five protein bands. Allergenic activity was recovered in fractions of prealbumin mobility containing two of these bands (band A and B). Of these, band B has been most consistently associated with allergenic activity.

The present report describes the immunochemical and biologic properties of antiserum to band B. Studies performed with this antiserum

further support the association between a component of band B and allergenic activity. In addition, the radioallergosorbent test was modified for the detection of allergen; application of this test to extracts of various cat tissues revealed the pelt to be the richest source of allergen.

METHODS

Cat pelt extract and purified fractions of cat pelt extract. Cat pelt extract was prepared as previously described from lyophilized, defatted, cat pelts (1). Purified fractions of crude cat pelt extract were prepared by sequential fractionation on DE52-cellulose and Sephadex G-100 columns (1). In brief, portions of cat pelt extract were dialyzed against 0.05 M NaCl in phosphate buffer (0.01 M, pH 7.5) and applied to DE52-cellulose columns that had been equilibrated with the same solution. After elution of the first peak of protein, a second fraction containing allergen was eluted by increasing the

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salt concentration of the buffer to 0.25 M. The material in this second fraction was dialyzed, concentrated by lyophilization, and applied to Sephadex G-100 columns. Two protein peaks were obtained; the portion of the eluate constituting the descending limb of the second peak was pooled and concentrated. Allergenic activity resided chiefly in this fraction.

Cat albumin. Purified cat albumin was obtained from cat serum by sequential fractionation on Sephadex G-100 and DE52-cellulose columns. Albumin was detected in column eluates by gel diffusion with a guinea pig antiserum to bovine serum albumin (BSA)² which was shown to cross-react with cat albumin (1). Cat serum was applied to Sephadex G-100 columns. Two protein peaks were obtained. Eluate from the mid-portion of the second peak which contained albumin was concentrated, dialyzed against phosphate buffer (0.01 M, pH 7.5) and applied to DE52-cellulose columns that had been equilibrated with the same buffer. After elution of the first peak of protein, a continuous ionic gradient was established in the eluting solution by adding 0.3 M NaCl in phosphate buffer (0.01 M, pH 7.5) to a reservoir containing the starting buffer. Two main protein peaks were obtained; albumin was detected in the second peak. The eluate constituting this second peak was pooled and concentrated. The protein in this peak migrated as a single band on electrophoresis in agarose gel (2).

Gel filtration. Sephadex G-100 was equilibrated in 0.001 M Tris-buffered saline (0.15 M) at pH 8.0 as recommended by the manufacturer. Seventy-five milligrams of dialyzed cat pelt extract was suspended in the same solution and applied to 2- x 100-cm columns at 4°C; 4-ml fractions were collected. The optical density of various fractions was measured at 280 m μ . BSA, ovalbumin (EA), chymotrypsinogen A, and cytochrome C (obtained from Schwarz-Mann, Orangeburg, N.Y.) were used as markers of known molecular size in calibrating these columns.

Polyacrylamide disc gel electrophoresis. Polyacrylamide disc gel electrophoresis was performed as previously described (3) with glass tubes measuring 6 x 100 mm. The separator gel

contained either 7% or 10% acrylamide (plus 0.18% N, N'-methylenebisacrylamide). A current of 4 mA per tube was applied until the bromphenol blue indicator had migrated 50 mm into the separator gel. The gel columns were then removed from the glass tubes and stained with 1% amidoblack in 7% acetic acid. Excess stain was removed by leaching in 7% acetic acid for 48 hr. Fractions of the gel columns were prepared by embedding the columns in 1% 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 1 ml of phosphate-buffered (0.01 M) saline (0.15 M), pH 7.5; (PBS) for 6 hr at 20°C and overnight at 5°C. A gel column that had been prepared under identical conditions was marked at 5-mm intervals with India ink and stained; this procedure permitted us to correlate the activity of eluates with specific bands of protein.

In order to react antisera with protein separated by disc gel electrophoresis, gel columns were embedded immediately after electrophoresis in a 2-mm layer of 0.9% agarose gel on 8 x 10 cm glass slides. Troughs were cut on both sides of the gel and filled with various antisera, the precipitin lines formed were recorded at 24 and 48 hr.

Immunoabsorbent columns. Cat albumin or the γ -globulin fraction (4) of various antisera were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as previously described (5); the resulting conjugates were poured into columns with a bed volume of 10 ml. Approximately 20 mg of protein was coupled per milliliter of Sepharose 4B. Antisera or allergen containing solutions were applied to the top of the columns, which were then eluted with PBS. One-milliliter fractions were collected. The recovered protein was pooled and concentrated.

Radioallergosorbent test. P.S. myeloma serum (kindly supplied by Dr. K. Ishizaka) was applied to Sephadex G-200 columns and eluate constituting the IgE myeloma protein peak was collected and concentrated. Rabbits were immunized with P.S. myeloma protein emulsified in complete Freund's adjuvant. Antiserum was obtained 6 weeks after the start of immunization. Antisera were absorbed with human IgG coupled to Sepharose 4B, in order to remove

² Abbreviations used in this paper: BSA, bovine serum albumin; EA, ovalbumin; PBS, phosphate-buffered saline; BBS, borate-buffered saline.

anti-light chain activity. The absorbed antiserum developed a single precipitin line on immunoelectrophoresis with P.S. myeloma serum. No precipitin line was observed after gel diffusion against IgG. Purified anti- ϵ antibody was prepared by passing the absorbed antiserum through an immunoabsorbent column consisting of 15 mg of P.S. myeloma protein coupled to 10 ml of Sepharose 4B. After washing the column with PBS, the purified antibody was eluted with glycine-HCl buffer, pH 3.0, and the eluate was immediately neutralized with 0.2 M Tris buffer. One hundred micrograms of anti- ϵ antibody was labeled with approximately 1 mCi of ^{125}I as previously described (6).

The radioallergosorbent test was performed as previously described (7). The protein in purified fractions of cat pelt extract was coupled to cyanogen bromide-activated cellulose particles (8). Fifty microliters of serum were diluted with an equal volume of assay buffer and added to plastic test tubes containing approximately 5 μg of purified allergen coupled to 0.5 mg of cellulose. After rotation of the tubes for 12 hr at room temperature, the cellulose particles were washed three times with borate-buffered (0.005 M) saline (0.15 M), pH 8.0, (BBS) and approximately 10 ng of anti- ϵ antibody with an activity of 50,000 to 100,000 cpm were added to each tube. The tubes were again rotated at room temperature for 12 hr and the cellulose particles were washed three times with BBS. The radioactivity remaining bound to the cellulose was measured in a gamma-counter (Packard Instrument Company, Inc., Downers Grove, Ill., Model 5120). The amount of radioactivity bound to particles exposed to non-allergic serum averaged $500 \pm (\pm 1 \text{ S.D.})$ cpm; the amount of radioactivity bound to particles exposed to serum from cat-sensitive subjects ranged from 1000 to 5000 cpm. Ten consecutive tests with one such serum resulted in an average binding of $3532 \pm 49 (\pm 1 \text{ S.D.})$ cpm. Each test was carried out in duplicate. The amount of anti- ϵ antibody bound was considered significant only if it was 2-fold greater after treatment of allergen-cellulose particles with serum from cat-sensitive subjects compared to serum from controls.

The radioallergosorbent test was modified for use as an assay for allergen. An inhibition procedure was devised in which 0.1-ml samples of various allergen preparations were added to

the test tube containing the allergen-cellulose particles. Soluble and insoluble allergen were allowed to compete for binding sites on human anti-allergen antibody. After the initial incubation, the cellulose particles were reacted with labeled anti- ϵ antibody; the amount of radioactivity bound to the cellulose particles was compared to a control in which soluble allergen had not been added. The results were expressed as percentage of inhibition of ^{125}I -labeled anti- ϵ antibody uptake.

Skin testing. Prick tests were performed as previously described with allergen fractions prepared in 50% glycerol (1). Informed consent was obtained before testing. The average diameter of the flare in millimeters was noted 15 min after the injection of test substance. Reactions of less than 5 mm in diameter were disregarded. Each subjects' maximum reaction was taken as 100%; the skin test reactions were expressed as a percentage of the maximum reaction.

RESULTS

Immunochemical properties of antiserum to a fraction rich in cat allergen. A purified fraction of cat pelt extract was prepared as indicated in *Methods* by sequential fractionation on DE52-cellulose and Sephadex G-100 chromatography columns. The purified fraction was subjected to electrophoresis in 7% acrylamide gel and the portion of the gel containing band B protein (Fig. 1) was separated and eluted with saline. Two rabbits were immunized with 1 mg of band B protein emulsified in complete Freund's adjuvant; antiserum was collected after 30 days. When these antisera were reacted in Ouchterlony gel diffusion (0.9% agarose) with crude cat pelt extract in various concentrations up to 60 mg/ml, no more than two precipitin lines were observed. Preliminary experiments demonstrated that antiserum obtained from both animals contained antibody to band B and to cat albumin. In order to remove antibody directed against albumin, we passed antiserum through an immunoabsorbent column consisting of cat albumin conjugated to Sepharose 4B.

Unabsorbed and absorbed antiserum from one rabbit was reacted with the purified fraction of cat pelt extract after electrophoresis of the extract in 7% acrylamide gel (Fig. 1). Unabsorbed antiserum to band B protein produced a precipitin line with band B and, in addition, a distinct precipitin line formed with

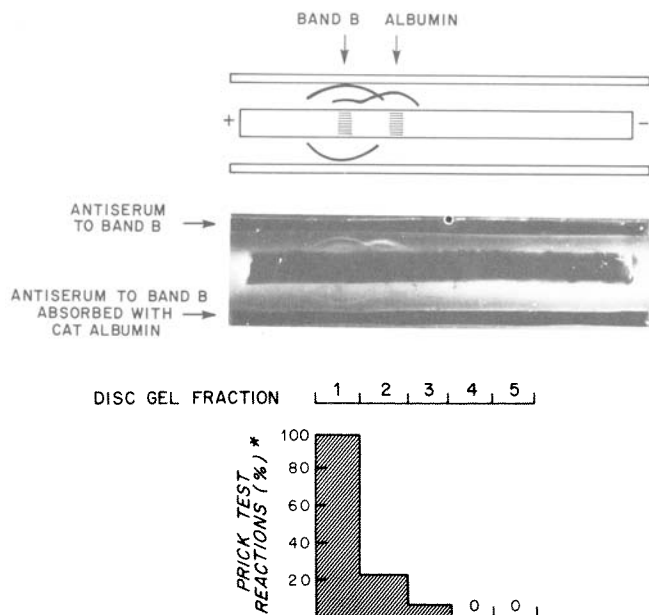


Figure 1. Purified cat pelt extract (150 μ g) was subjected to acrylamide (7%) disc gel electrophoresis. The gel cylinder was embedded in agarose and reacted with antiserum to band B (upper trough) and antiserum to band B absorbed with albumin (lower trough). An identical cylinder was cut into segments, eluates of these segments were tested in the skin of nine cat-sensitive subjects. The unabsorbed antiserum contained antibody which reacted with both band B and albumin. The absorbed antiserum reacted only with band B. A correspondence is seen between band B protein, antibody to band B protein, and the location in the gel of the bulk of allergenic activity. (*, The diameter of the flare of each subjects' maximum reaction was taken as 100%; other skin test reactions were expressed as a percentage of the maximum reaction. Average values are indicated.)

a component present in both band B and albumin. Following absorption with cat albumin, the antiserum produced a single precipitin line against band B.

A 7% acrylamide gel column prepared as above was cut into 5-mm segments and the segments were eluted with PBS. The eluates were tested in the skin of nine cat-sensitive subjects; most of the allergenic activity was located in the portion of the gel column which contained band B protein (Fig. 1, lower portion).

Acrylamide disc gel electrophoresis of other purified fractions of cat pelt extract have in the past yielded an additional faint band of protein with rapid electrophoretic mobility referred to as band A, likewise associated with allergenic activity (1). Band A was not visualized in the present experiments.

Band B protein was isolated by electrophoresis on several 7% acrylamide gels and the eluates containing band B protein were combined. Samples of the pooled eluate were tested to verify their electrophoretic properties and

allergen content (Fig. 2). On electrophoresis in 7% gels, only a single protein band, band B, was visualized. Two identical gel columns were cut into 5-mm segments and the segments were eluted. Eluate from one gel was used for skin testing. Eluted fractions from the second gel column were concentrated 5-fold by lyophilization and applied to each of 12 wells on a glass plate coated with 0.9% agarose gel. Absorbed antiserum to band B was applied to a parallel series of wells. The portion of the gel containing band B protein reacted with absorbed antiserum to band B in gel diffusion, and also contained the bulk of the allergenic activity as determined by prick testing (Fig. 2).

Electrophoresis of samples of pooled band B protein in 10% acrylamide gel resolved four protein bands (Fig. 3). Two identical gel columns were cut into 5-mm segments and the segments were eluted for use in gel diffusion studies with absorbed antiserum to band B and for skin testing. Absorbed anti-band B antiserum precipitated with eluate from fractions 3 and 4; no visible reaction occurred with frac-

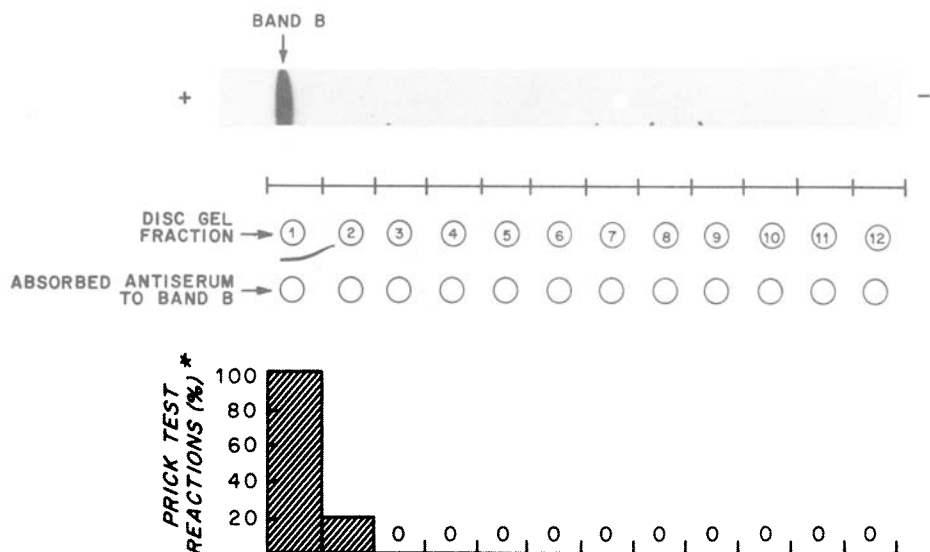


Figure 2. Pooled band B protein (150 μ g) was subjected to acrylamide (7%) disc gel electrophoresis. Only band B protein was visible in the stained gel. An identical gel cylinder was cut into segments; each segment was eluted with water and concentrated 5-fold by lyophilization. Twelve concentrated fractions were then tested by gel diffusion against absorbed antiserum to band B in parallel wells. A line diagram of the resulting precipitin line is indicated. Another cylinder was cut into segments, eluates of these segments were tested in the skin of four cat-sensitive subjects. As in Figure 1, there is a correspondence between band B protein, antibody to band B protein, and the location in the gel of allergenic activity. (*, Prick test reactions were graded as in Figure 1.)

tions 1 and 2. Fraction 3 contained the bulk of allergenic activity as determined by prick testing; appreciable activity was also found in fractions 1, 2, and 4. Since only fractions 3 and 4 formed precipitin reactions with the absorbed antiserum, it is possible that fractions 1 and 2 contained a different allergen or that the concentration of the same allergen was too low to permit detection by the precipitin test.

In order to characterize further the absorbed anti-band B antiserum, we tested this antiserum by gel diffusion against fractions obtained by filtration of crude cat pelt extract through a Sephadex G-100 column (Fig. 2). Two protein peaks were obtained by this method; the second peak contained albumin as assayed by gel diffusion against antiserum to albumin. Portions of the eluate were combined for skin testing as indicated in Fig. 4. Allergenic activity, assayed by skin tests in four patients clinically-sensitive to cats, was recovered largely in eluate constituting the descending limb of the albumin peak. It has been shown previously that this portion of the eluate contains proteins with a molecular weight in the range of 30,000 to 60,000 daltons (1). Eluted fractions were concentrated 4-fold by lyophili-

zation and tested by gel diffusion *versus* antiserum to band B absorbed with albumin and unabsorbed antiserum to band B protein. The unabsorbed antiserum reacted with many fractions and the absorbed antiserum produced a precipitin line with those fractions which contained maximum allergenic activity. Thus it appears that anti-band B antiserum absorbed with cat albumin precipitates with a single component of cat pelt extract; this component has both the electrophoretic and molecular size characteristics of allergen. The absorbed antiserum will be referred to as anti-allergen antiserum.

Anti-allergenic activity of anti-allergen antiserum. Ten milligrams of crude cat pelt extract or 500 μ g of a purified fraction of cat pelt extract was applied to immunoabsorbent columns prepared with normal rabbit γ -globulin or the γ -globulin fraction of anti-allergen antiserum. The effluent from these columns was concentrated and used for skin testing or for measurement of allergen by the radioallergosorbent test.

Table I summarizes the skin test results obtained with absorbed crude cat pelt extract and with absorbed purified cat pelt extract.

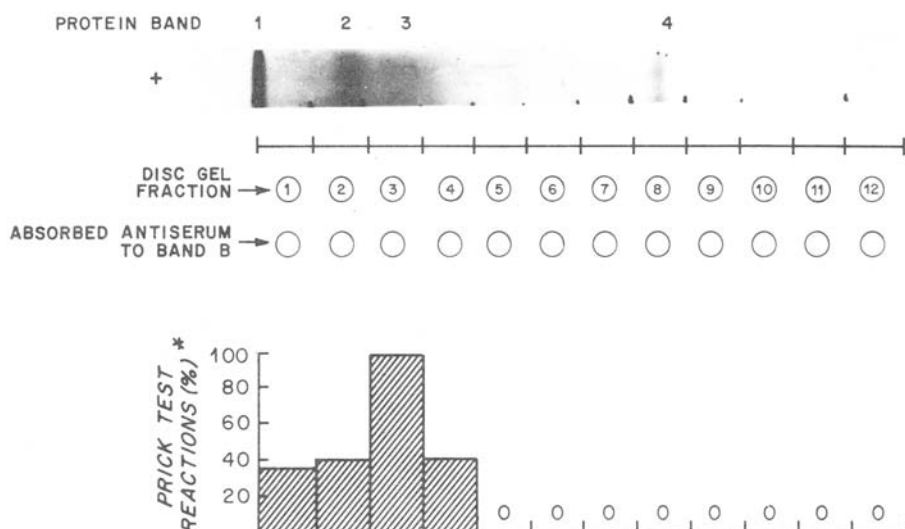


Figure 3. Pooled band B protein (150 μ g) was subjected to acrylamide (10%) disc gel electrophoresis. Four distinct protein bands were visible. Twelve fractions were prepared from two identical gel cylinders and tested as in Figure 2. Antibody to band B protein and the bulk of allergenic activity corresponded to a portion of the gel (fractions 3 and 4) that contained one protein band (band 3). (*, Prick test reactions in six subjects were graded as in Figure 1.)

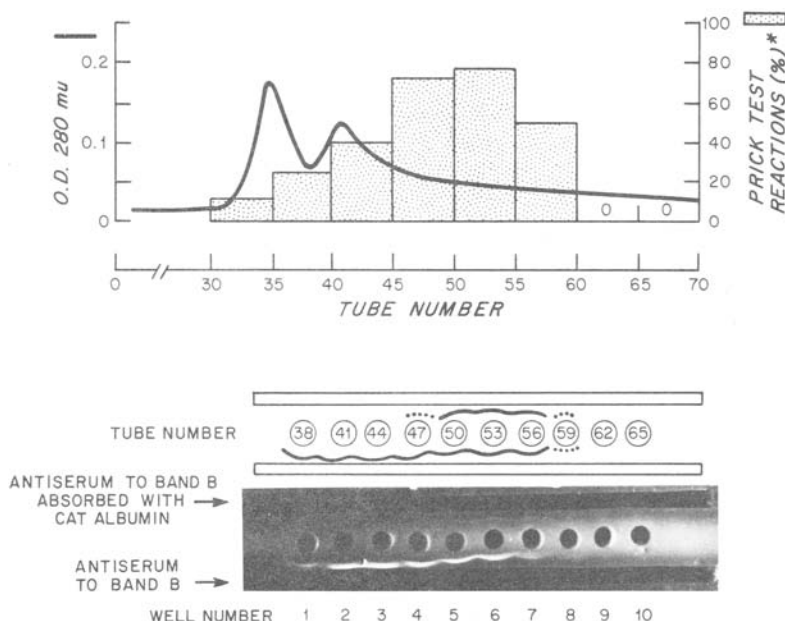


Figure 4. Crude cat pelt extract (75 mg) was subjected to gel filtration on a Sephadex G-100 (2 $\times$ 100 cm) column and 4-ml fractions were collected. Two protein peaks resulted; allergenic activity was largely recovered from eluate constituting the descending limb of the second peak (tubes 45 to 60). Ten of the fractions from this column were concentrated and tested by gel diffusion against antiserum to band B absorbed (upper trough) and unabsorbed with albumin (lower trough). The absorbed antiserum reacted with eluate from tubes 47 to 59 (wells 4 to 8). The unabsorbed antiserum reacted with eluate from tubes 38 to 59 with maximum reactions occurring with eluate from tubes 41 and 44 (wells 2 and 3). Eluate from tubes 41 and 44 also reacted maximally with an antiserum to cat albumin. Thus, absorbed antiserum to band B reacted with a single component of crude cat pelt extract that had the same molecular weight characteristics as the bulk of allergenic activity. (*, Prick tests were graded as in Figure 1.)

TABLE I

Absorption of crude cat pelt extract with normal rabbit γ -globulin or with the γ -globulin fraction of anti-allergen antiserum; residual allergenic activity assayed by prick test

Test Sub- ject No.	Crude Cat Pelt Extract ^a Absorbed with	
	Normal rabbit γ -globulin linked to Sephadex 4B	γ -globulin fraction anti-allergen antiserum linked to Sephadex 4B
1	1/5 ^b	Negative undiluted
2	1/25	Negative undiluted
3	1/5	Negative undiluted
4	Positive undiluted	Negative undiluted
5	1/125	1/125

	Purified Fraction of Cat Pelt Extract ^c Absorbed with	
	Normal rabbit γ -globulin	γ -globulin fraction of anti-allergen antiserum
1	1/25	Negative undiluted
2	1/125	Negative undiluted
3	1/25	Negative undiluted
6	1/625	1/125
7	1/25	Negative undiluted
8	1/125	1/5
9	1/25	1/25

^a Ten milligrams of crude cat pelt extract were applied to each immunoabsorbent column; the effluent was concentrated by lyophilization and reconstituted in 1.5 ml of PBS.

^b Highest dilution of allergen-containing solution eliciting a positive prick test reaction in cat-sensitive subjects.

^c Five hundred micrograms of a purified fraction of cat pelt extract (see *Methods*) were applied to each immunoabsorbent column; the effluent was concentrated by lyophilization and reconstituted in 1.5 ml of PBS.

Crude cat pelt extract absorbed with normal rabbit γ -globulin elicited prick test reactions up to a dilution of 1:125 in five cat-sensitive subjects (Table I, upper portion). Prick test reactions obtained with unabsorbed crude cat pelt extract (not shown) were only slightly more intense than prick test reactions obtained with equivalent dilutions of crude cat pelt extract absorbed with normal rabbit γ -globulin. The diminution in activity observed probably resulted from nonspecific absorption to the columns. Absorption of crude cat pelt extract with the γ -globulin fraction of anti-allergen antiserum resulted in a reduction of allergenic activity as tested in four of five subjects.

Purified cat pelt extract absorbed with normal rabbit γ -globulin elicited prick test reactions up to a dilution of 1:625 in seven cat-sensitive subjects (Table I, lower portion). Absorption of the purified fraction with the γ -globulin fraction of anti-allergen antiserum resulted in a reduction of allergenic activity as tested in six of seven subjects. Three of the subjects (Subjects 1 to 3) were tested with both the absorbed crude cat pelt extract and with the absorbed purified cat pelt fraction. Each of the three subjects demonstrated a decreased prick test response to both the crude pelt extract and the purified pelt fraction absorbed with anti-allergen.

The allergen content of absorbed crude cat pelt extract was also measured by the modified radioallergosorbent test as indicated in Table II. The effluent from the immunoabsorbent columns was added to cellulose particles conjugated with cat allergen, followed by the addition of serum from one of five cat-sensitive subjects. In this system, soluble allergen present in the column effluent deviates the subjects' IgE antibody against allergen from combining with insolubilized allergen. Crude cat pelt extract absorbed with normal rabbit γ -globulin inhibited ¹²⁵I-labeled anti- ϵ antibody uptake from 53

TABLE II

Absorption of crude cat pelt extract with normal rabbit γ -globulin or with the γ -globulin fraction of anti-allergen antiserum; residual allergenic activity assayed by a modified radioallergosorbent test

Serum	Crude Cat Pelt Extract ^a Absorbed with	
	Normal rabbit γ -globulin	γ -Globulin fraction of anti-allergen antiserum
	%	%
6 ^b	53	14
8 ^b	53	8
10	80	14
11	54	1
12	92	2

^a Ten milligrams of crude cat pelt extract were applied to immunoabsorbent columns; the effluent was concentrated by lyophilization and reconstituted in 1.5 ml of PBS. A 1:10 dilution of the reconstituted eluate was used for the radioallergosorbent test.

^b Sera 6 and 8 were obtained from subjects 6 and 8 (Table I) who had also been skin tested with purified cat pelt extract before and after absorption with the γ -globulin fraction of anti-allergen antiserum; both subjects had demonstrated a reduction in skin test reactivity following absorption.

to 92%, depending on the serum tested. Absorption of crude cat pelt extract with the γ -globulin fraction of anti-allergen antiserum markedly reduced the ability of the extract to prevent the IgE antibodies in five individual sera from reacting with insolubilized allergen. These experiments confirmed that anti-allergen antiserum contained antibody specifically directed against cat allergen.

Antiserum to cat albumin was also tested for its ability to remove allergen from solutions of crude cat pelt extract and from a purified fraction of cat pelt extract. Ten milligrams of crude pelt extract or 500 μ g of purified fraction were absorbed with the γ -globulin fraction of anti-cat albumin antiserum. The absorbed fractions were assayed for allergenic activity by prick testing in 10 subjects clinically-sensitive to cats; a slight reduction in allergenic activity could be detected in half of the subjects. This reduction was consistently less than the reduction observed after absorption with the γ -globulin fraction of anti-allergen antiserum. The absorbed fractions were also tested in the radioallergosorbent assay employing serum from five subjects. No reduction in allergen content was detected in solutions absorbed with anti-cat albumin antiserum.

Studies of extracts derived from various cat tissues. Cat pelt extract, extracts of 13 other cat tissues, and cat serum were obtained and tested by gel diffusion against anti-allergen antiserum. After exsanguination of a cat anesthetized with pentobarbital, tissues were obtained for the preparation of extracts. Each tissue was lyophilized to dry weight and a 1:10 w/v extract was prepared as previously described (1). For gel diffusion studies against anti-allergen antiserum, the 1:10 w/v extracts were concentrated 5-fold by lyophilization. The extract of parotid gland could not be used in gel diffusion because it produced a precipitin line with normal rabbit serum. Anti-allergen antiserum produced a faint precipitin line against the pelt extract only. No precipitin line could be seen in the reaction between the antiserum and cat serum.

These same cat tissue extracts and cat serum were tested for allergenic activity with the radioallergosorbent test. A 1:10 w/v extract of each tissue and whole cat serum were used to displace the IgE antibody of three cat-sensitive subjects' sera from combining with cat allergen

bound to cellulose particles. The percentage of inhibition of ^{125} I-labeled anti- ϵ antibody uptake onto cellulose particles was greatest with the pelt extract (52 to 81% inhibition). The extract of brain tissue also proved to be a potent inhibitor of anti- ϵ antibody uptake (27 to 67% inhibition). The extracts of other tissues and cat serum inhibited to a variable extent depending on the serum used in the test system; in each case, however, the extract of pelt and brain proved to be the most active inhibitors.

The possibility that cat tissue extracts might nonspecifically inhibit the uptake of anti- ϵ antibody on IgE antibody-allergen-cellulose particles was tested in a second assay system. In this system, serum from a patient with allergy to insulin was reacted with bovine insulin linked to cellulose, followed by the addition of ^{125} I-labeled anti- ϵ antibody, uptake of label was 6 times greater than that obtained with a normal serum control. Addition of cat pelt, brain or parotid gland extract followed by serum of the insulin-allergic patient resulted in a 5, 12, and 0% inhibition of anti- ϵ antibody uptake onto the bovine insulin-cellulose particles. The extent of inhibition observed with pelt and brain extract was considerably less than was observed in the specific test system above.

DISCUSSION

This report describes certain properties of an antiserum directed against allergen derived from cat pelt. After absorption with cat albumin, this antiserum reacted in gel diffusion with a single component of cat pelt; this component had a molecular weight between 30,000 and 60,000 daltons and migrated as a single band, band B, in 7% acrylamide gel. Repeat electrophoresis of band B protein in acrylamide gel of reduced pore size (10% acrylamide gel) resulted in a further division of band B protein into four distinct protein bands; one of these bands contained the bulk of allergenic activity and reacted with the absorbed antiserum (Fig. 3, fractions 3 and 4). Two additional fractions (1 and 2) contained some allergenic activity; these fractions may have contained different allergens or they may have contained a low concentration of the same allergen that is predominant in fraction 3. Despite the presence of several protein components in band B (defined by separation in 10% acrylamide gel), two rabbits re-

sponded with the production of antibodies to only two components, albumin and allergen. These results suggest that these two components are immunodominant in the mixture injected.

Antiserum to cat allergen was tested for its capacity to remove allergenic activity from crude extracts of cat pelt and from purified fractions of cat pelt. Both the crude extract and the purified fraction exhibited reduced allergenic activity after absorption as determined by skin testing and the radioallergosorbent test. A few subjects who were skin tested demonstrated no reduction in allergenic activity of crude extract or purified fraction after absorption. These subjects may have been sensitive to allergenic components that were not removed by the antiserum. Alternatively, the prick test may not have been sufficiently sensitive to detect significant reductions in allergenic activity.

A slight reduction in the allergenic activity of crude cat pelt extract and purified fraction of cat pelt extract occurred after absorption with antiserum to cat albumin. Other evidence also suggests that cat albumin may represent a minor allergen. For example, small amounts of allergenic activity were associated with acrylamide gel and Sephadex gel fractions that contained albumin (Figs. 1 and 4). It is possible, however, that allergen is bound to cat albumin. Brandt *et al.* (9) have recently studied cat serum allergen; IgE antibody from one presumably cat-sensitive patient reacted predominantly with a component of cat serum that had the electrophoretic mobility of albumin in acrylamide gel. Our previously published study (1), as well as unpublished observations from our laboratory strongly suggest that albumin is not the major allergen in cat serum. The role of cat albumin as an allergen needs to be further substantiated.

The radioallergosorbent test is a useful procedure for the measurement of specific IgE antibody against allergen (7, 10, 11). The inhibition procedure described in this paper, which has also been used by others (12, 13), adapts the radioallergosorbent test to measure allergen. This test cannot quantitate allergen directly, however, relative amounts of allergenic activity in various fractions can be compared. This *in vitro* assay may be limited by the fact that it can measure only those allergenic components

that are conjugated to cellulose particles. Non-allergenic protein may compete with allergen for binding sites on the activated cellulose. Furthermore antigenic sites could be masked by the coupling process. In spite of these possible limitations, our results to date indicate that the radioallergosorbent assay is in general equivalent to the skin test as an assay for allergen. Skin testing will remain a useful supplement to this *in vitro* test.

The distribution of allergen in various cat tissues and cat serum was studied by two different methods. With the technique of gel diffusion, anti-allergen antiserum reacted only with the concentrated pelt extract. This test is relatively insensitive and may not have detected appreciable amounts of allergen in the other extracts. With the radioallergosorbent test as the assay for allergen (Table III), it was also found that the pelt was the most abundant source of allergen. Extract of cat brain contained significant allergenic activity. The extract of parotid gland and cat serum contained progressively smaller, but probably significant, amounts of allergenic activity.

Preliminary studies from this laboratory have strongly suggested that allergen in cat serum is distinct from the major allergen in pelt with regard to both electrical change and molecular

TABLE III

Allergenic activity in cat serum and various cat tissues estimated by the radioallergosorbent test

Tissue	% Inhibition of ¹²⁵ I-labeled Anti- ϵ Uptake		
	Serum 6	Serum 8	Serum 10
Serum	23 ^a	5	38
Pancreas	8	0	3
Liver	8	0	0
Spleen	11	0	5
Large bowel	11	0	1
Skeletal muscle	11	0	13
Heart	12	0	0
Lung	13	0	7
Kidney	13	0	11
Stomach	15	0	18
Ileum	15	0	10
Parotid gland	27	0	61
Brain	42	27	67
Pelt	65	52	81

^a The allergen content of whole serum and of a 1/10 (w/v) extract of tissues was estimated by radioallergosorbent test. Serum from three cat-sensitive subjects was used in the test system.

size properties. Because it is probable that in the future a number of distinct cat allergens will be isolated, it is suggested that the allergen described in this paper be designated cat allergen 1.

The availability of an antiserum that is directed against a single mammalian allergen will make it possible to develop an assay to measure this allergen quantitatively. Guided by this assay and the separation procedures already developed, it may in the future be possible to purify large amounts of allergen for studies of primary structure and for determination of the sequences that are necessary for allergenicity.

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