

Allergens of mammalian origin

II. Characterization of allergens extracted from rat, mouse, guinea pig, and rabbit pelts

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Aqueous extracts prepared from lyophilized, defatted rat, mouse, guinea pig, and rabbit pelts elicited intense wheal-and-flare responses in the skin of a high proportion of patients who were clinically sensitive to these animals. The major allergens in each extract were nondialyzable. Skin test reactions to rat, mouse, and guinea pig serum were common in patients allergic to these animals. The fractions of rat, mouse, and rabbit pelt extract showing maximum allergenic activity contained proteins with the electrophoretic mobility of serum albumin. Fractions of guinea pig pelt extract with maximum allergenic activity were of prealbumin mobility and contained little stainable protein. On Sephadex G-100 gel filtration, most allergen from rat, mouse, and guinea pig pelt extracts was recovered in fractions containing proteins with a molecular weight range of 10,000 to 25,000 daltons. Allergen in rabbit pelt extract had a slightly higher molecular weight range of 18,000 to 38,000 daltons.

Until recently, limited information was available on the properties of mammalian allergens.¹ 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.² A similar estimate of the molecular weight of major cat allergens has recently been reported by other investigators.^{3, 4}

Allergic reactions to rats, mice, guinea pigs, and rabbits are common among people exposed to these animals. These reactions constitute an occupational hazard among laboratory workers and veterinarians. The following report describes some physicochemical and biologic properties of allergen extracted from rat, mouse, guinea pig, and rabbit pelts.

METHODS

Preparation of pelt extracts

Four or more pelts of each species were extracted as previously described.² Fresh, unshaved 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,

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TABLE I. Characteristics of crude pelt extracts

	Rat	Mouse	Guinea pig	Rabbit	Cat*
Prick test reactions to a 1:10 (w/v) extract of pelt	11/11†	9/11	9/9	7/8	19/23
Nondialyzable solid in a 1:10 (w/v) extract of pelt (mg./ml.)	19 mm.‡	21 mm.	12 mm.	18 mm.	22 mm.
	1.3 mg.	2.3 mg.	0.56 mg.	0.98 mg.	0.90 mg.
Optical density at 280 m μ of a solution of pelt extract containing 0.5 mg./ml. (nondialyzable solid) in 0.1 M NaOH	0.57	0.50	0.54	0.57	0.37
Prick test reactions to dialyzable solid in the crude pelt extract§	2/11†	1/9	1/9	0/7	2/8
	12 mm.‡	15 mm.	5 mm.	—	7 mm.
Prick test reactions to a 1:2 dilution of serum	7/11†	8/9	5/9	0/7	7/19
	18 mm.‡	20 mm.	16 mm.	—	28 mm.

*The data pertaining to cat pelt extract are included here for comparison; some of these data have been previously published.²

†Number of subjects with positive responses/total number tested. All subjects gave a history of asthma or rhinitis following specific exposure.

‡Average diameter of the flare among subjects with positive responses.

§Ten milliliters of a 1:10 (w/v) extract of pelt were lyophilized, suspended in 1 ml. 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 50 per cent glycerol for prick testing. Only those subjects who reacted to the crude pelt extract were tested with the dialyzable solid.

thoroughly dried, and extracted with water (1:10 w/v) at room temperature for 6 hours and overnight at 5° C. After the pH had been adjusted to 7.5 with 0.1 N NaOH, the water-soluble extract was separated from pieces of pelt by pressing through Dacron 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.

Serum

Whole blood was obtained by cardiac puncture; rabbits and guinea pigs were exsanguinated under pentobarbital anesthesia and rats and mice were exsanguinated under ether anesthesia. After clotting of the blood, serum was separated and stored at -20° C.

Electrophoresis in agarose

Electrophoresis of pelt extracts in agarose (barbital buffer, pH 8.2) was performed as described previously.⁵ A 50 ml. quantity of a 1/10 (w/v) extract was lyophilized and resuspended in 2 c.c. of water. A 10 μ l quantity of sample was applied to each of nine wells on the gel plate. Following electrophoretic separation, the gels were cut into 5 mm. strips. The strips were separately homogenized and extracted with 1.5 ml. of 0.15 M NaCl.⁶ 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.

Gel filtration

Sephadex G-100 was equilibrated in 0.001 M Tris-buffered saline at pH 8.0 as recommended by the manufacturer. Then 10 ml. of a 1/10 (w/v) extract were lyophilized, resuspended in 0.5 c.c. of water, and dialyzed against this buffer. The sample was applied to 1 by 60 cm. columns at 5° C. and 2 ml. fractions were collected. Bovine serum albumin (BSA), ovalbumin (EA), chymotrypsinogen A, and cytochrome C (obtained from Schwarz/Mann, Orangeburg, New York) were used as markers of known molecular size in calibrating these columns.

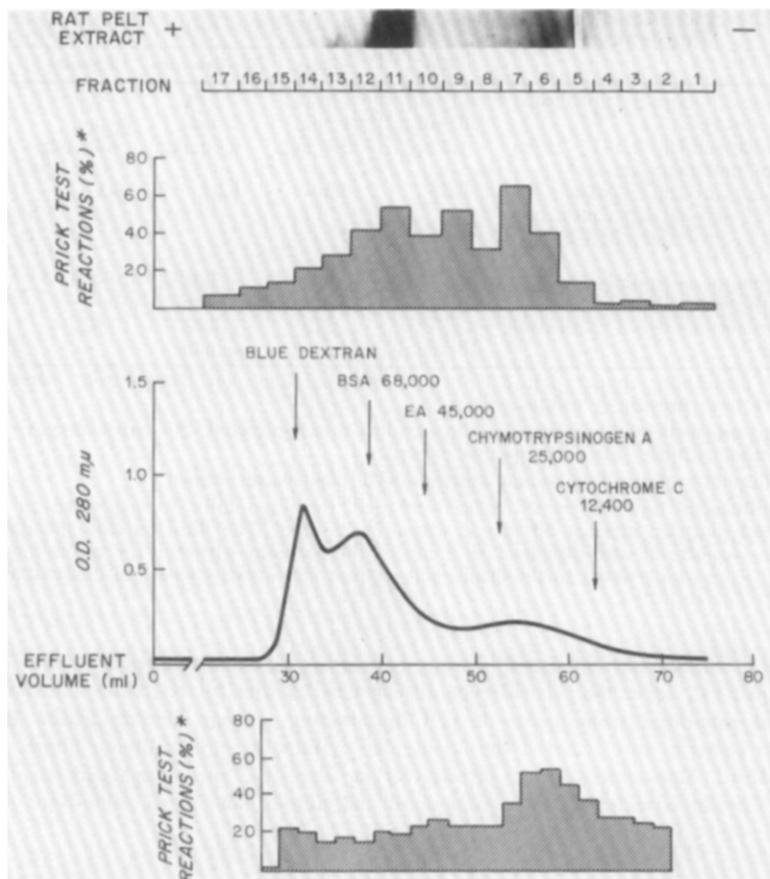


FIG. 1. Fractionation of rat pelt extract. Electrophoresis of rat pelt extract in agarose gel (barbital buffer, pH 8.2) shows protein bands with the mobility of albumin and γ_1 -protein (upper portion of figure). Allergic activity, as assayed by prick tests in 12 subjects clinically sensitive to rats, was recovered from a broad region with mobilities ranging from albumin (fractions 11 and 12) to γ_1 -protein (fractions 6 and 7). The elution profile obtained by gel filtration of rat pelt extract on a Sephadex G-100 column (1 by 60 cm.) is indicated in the lower portion of the figure. Three protein peaks were obtained; maximum allergic activity, as assayed by prick tests in 11 subjects clinically sensitive to rats, was recovered in effluent that contained protein with an approximate molecular weight of 20,000 daltons.

*The diameter of the flare of 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. Average values are indicated.

Skin testing

Prick tests were performed as previously described with fractions prepared in 50 per cent glycerol.² 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; the skin test reactions were expressed as a per cent of the maximum reaction.

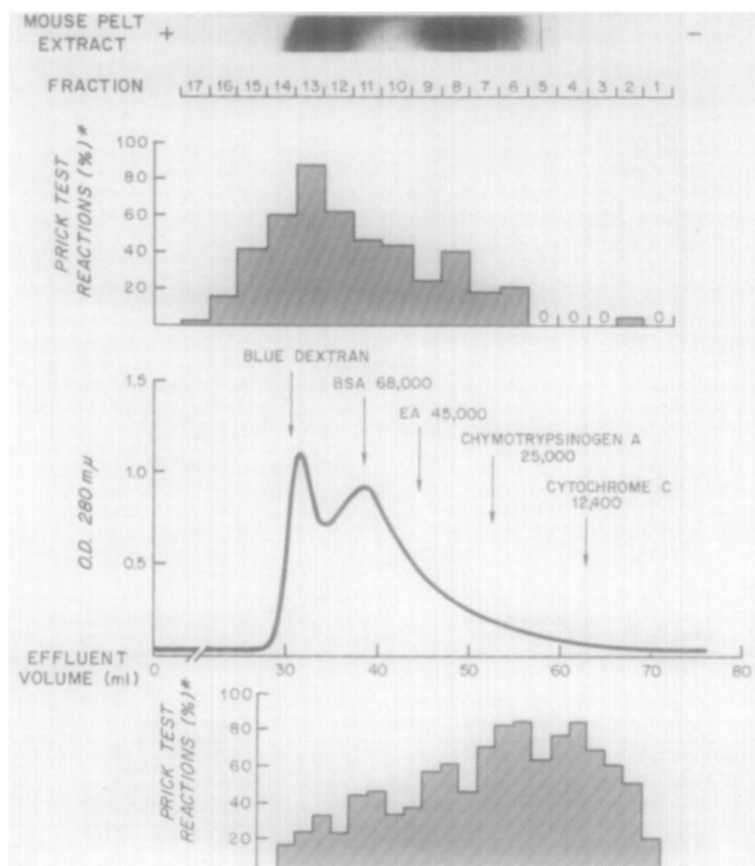


FIG. 2. Fractionation of mouse pelt extract. Electrophoresis of mouse pelt extract in agarose gel shows protein bands with the mobility of albumin and β -protein (upper portion of figure). Maximum allergenic activity, as assayed by prick tests in 8 subjects clinically sensitive to mice was recovered in fraction 13, which had the mobility of albumin. The elution profile obtained by gel filtration of mouse pelt extract on a Sephadex G-100 column is indicated in the lower portion of the figure. Two protein peaks were obtained; maximum allergenic activity, as assayed by prick tests in 8 subjects clinically sensitive to mice, was recovered in effluent that contained protein with a molecular weight ranging from 25,000 to 12,000 daltons.

*Prick tests were graded as in Fig. 1.

RESULTS

Some properties of each pelt extract are summarized in Table I. The data for cat pelt extract are also included for comparison.² There was a high proportion of positive reactions to each crude extract among subjects who were thought to be clinically sensitive to the animal on the basis of a history of symptoms following specific exposure. Some of the individuals with negative prick test responses were probably not allergic to the animal, although others might have exhibited a positive response if intradermal testing had been performed.

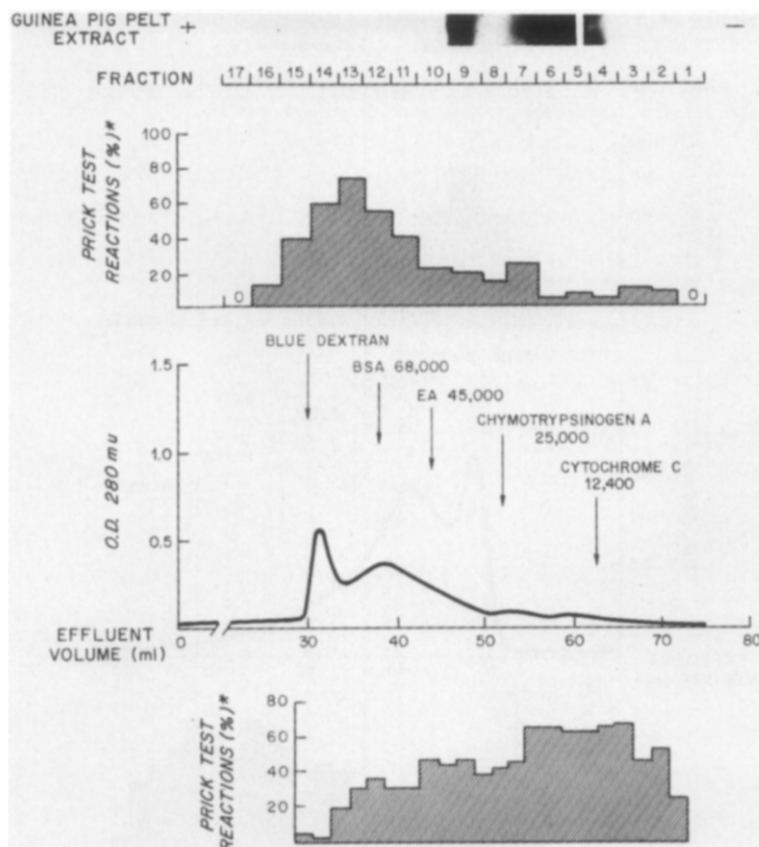


FIG. 3. Fractionation of guinea pig pelt extract. Electrophoresis of guinea pig pelt extract in agarose gel shows a protein band with the mobility of albumin and additional protein with α , β , and γ mobility (upper portion of figure). Maximum allergenic activity, as assayed by prick tests in 9 subjects clinically sensitive to guinea pigs, was recovered from fractions with prealbumin mobility (fractions 12 to 14). The elution profile obtained by gel filtration of guinea pig pelt extract on a Sephadex G-100 column is indicated in the lower portion of the figure. Two main protein peaks were obtained; maximum allergenic activity, as assayed by prick tests in 7 subjects clinically sensitive to guinea pigs, was recovered in effluent that contained protein with a molecular weight ranging from 22,000 to 10,000 daltons.

*Prick tests were graded as in Fig. 1.

The mean diameter of the flare response ranged from 13 mm. (guinea pig pelt extract) to 22 mm. (cat pelt extract). The content of nondialyzable solid in the pelt extracts ranged from 0.56 (guinea pig pelt extract) to 2.3 mg. per milliliter (mouse pelt extract). The optical density at 280 $m\mu$ of a solution of pelt extract containing 0.5 mg. per milliliter (nondialyzable solid) ranged from 0.37 (cat pelt extract) to 0.57 (rat and rabbit pelt extract). The optical density at 280 $m\mu$ provides a crude estimation of protein content. There appeared to be no correlation between solid or protein content of the extracts and the average size of the prick test reactions.

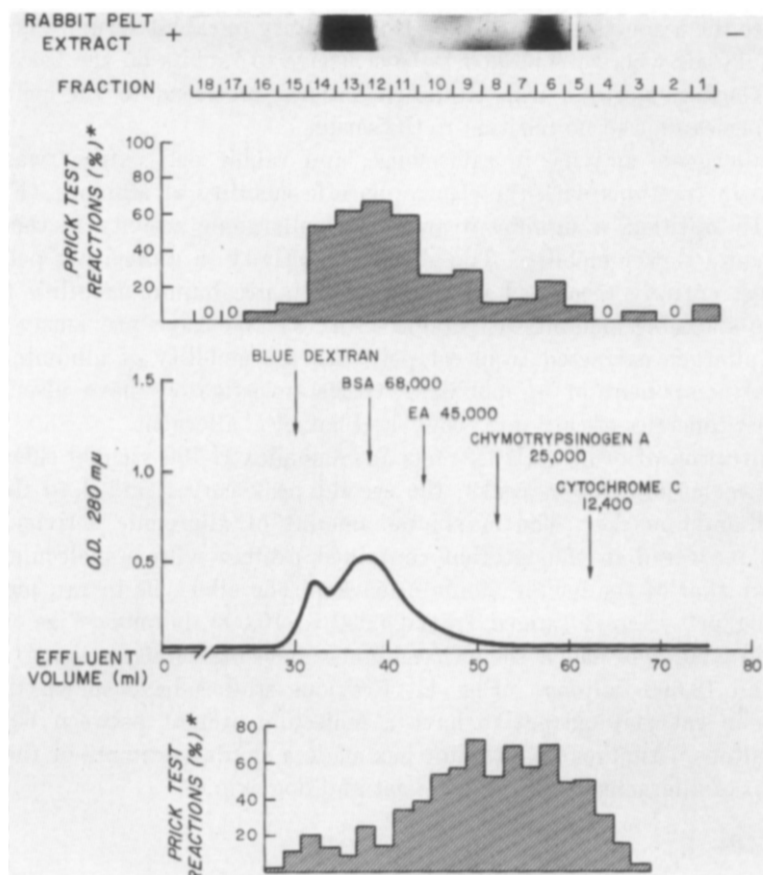


FIG. 4. Fractionation of rabbit pelt extract. Electrophoresis of rabbit pelt extract in agarose gel shows protein bands with the mobility of albumin and γ_1 -protein (upper portion of figure). Maximum allergenic activity, as assayed by prick tests in 5 subjects clinically sensitive to rabbits, was recovered largely from fractions 11 to 14, which had the mobility of albumin. The elution profile obtained by gel filtration of rabbit pelt extract on a Sephadex G-100 column is indicated in the lower portion of the figure. Two protein peaks were obtained; maximum allergenic activity, as assayed by prick tests in 7 subjects clinically sensitive to rabbits, was recovered in effluent that contained protein with a molecular weight ranging from 38,000 to 18,000 daltons.

*Prick tests were graded as in Fig. 1.

There was no loss of skin reactivity on dialysis of each pelt extract. In order to detect low-molecular-weight allergens, the dialyzable solid from each pelt extract was concentrated as indicated in Table I. A minority of subjects reacted to the concentrated dialyzable solid from rat, mouse, and guinea pig pelt extracts. The average diameter of these reactions was smaller than the average diameter of the prick test reactions to the crude pelt extract.

Prick test reactions to serum were found in a varying proportion of subjects who reacted to rat, mouse, and guinea pig pelt extracts. The mean prick test reaction to serum, when present, was nearly as great as or greater than the

reaction to the respective pelt extract. No reactivity to rabbit serum was observed in 7 individuals who were judged to be sensitive to rabbits on the basis of their history. Certain subjects who exhibited intense reactions to the pelt extract of each species showed no reaction to the serum.

The allergenic activity in rat, mouse, and rabbit pelt extract was largely recovered in fractions with the electrophoretic mobility of albumin (Figs. 1, 2, and 4). In addition, a significant amount of allergenic activity in the rat pelt extract had a slower mobility. The allergenic activity in guinea pig pelt extract was almost entirely recovered in fractions with prealbumin mobility that contained no stainable amounts of protein (Fig. 3). We have previously reported that the allergen extracted from cat pelts has the mobility of albumin with an additional component of α_2 mobility.² Other investigators have also observed the acidic properties of cat,⁷ dog,⁷ cow,⁸ and horse^{9, 10} allergens.

Gel filtration of crude pelt extracts on Sephadex G-100 yielded effluents containing two major protein peaks; the second peak corresponded to the bovine serum albumin marker. The maximum amount of allergenic activity in each case was recovered in effluent that contained protein with a molecular weight lower than that of the bovine albumin marker. The allergens in rat, mouse, and guinea pig pelt extract ranged from 25,000 to 10,000 daltons (Figs. 1, 2, and 3). Rabbit allergens had a somewhat higher molecular weight range (between 38,000 and 18,000 daltons) (Fig. 4). Previous studies have shown the major allergens in cat pelt extract to have a molecular weight between 60,000 and 30,000 daltons.² Another investigator has made a similar estimate of the molecular weight of allergens extracted from cat and dog skin.³

DISCUSSION

Previous studies of cat pelt extract² were extended to rat, mouse, guinea pig, and rabbit pelt extracts. Strong prick test reactions were observed with the pelt extract in a majority of subjects who were considered to be clinically sensitive to the specific animal. Each extract could be dialyzed without a significant loss of allergenic activity. The dialyzable solid in each extract produced only moderate reactions in a minority of sensitive individuals. These results are similar to the results obtained with cat pelt extract (Table I).

Lyophilized pelts are an abundant source of allergen. It is possible that pelt extracts will also prove to be a more reliable source of allergen than conventional extracts of hair or epidermal scrapings. Animal dander extracts obtained from commercial sources have been shown to exhibit wide variations in allergen content.^{7, 8, 11-13} These variations may be due to differences in extraction procedures or to losses of allergenic potency during storage. Another source of mammalian allergen, full-thickness skin, was first reported by Pepys¹⁴; this source has recently been utilized by a second investigator.³

The major allergens derived from each pelt extract were found to be acidic in nature with a molecular weight lower than that of bovine serum albumin. The separation procedures employed in this study yielded many fractions with allergenic activity, but in each case the bulk of the activity was concentrated in a few fractions. It is not possible to determine as yet whether the extracts contain

one or a few allergens existing in multiple molecular forms or whether many discrete allergens are present.

Some subjects showed strong prick test reactions to rat, mouse, and guinea pig pelt extract, as well as to serum obtained from these species. Other subjects, however, responded to the pelt extract but not to serum protein, suggesting that the pelt extracts contain allergenic components that do not originate in serum. It seems likely that pelt extracts are mixtures of serum- and non-serum-derived allergenic components. Allergenic activity has previously been observed in cat, dog, and horse serum,^{2, 4, 15-22} and serum albumin has been postulated as an allergen in each of these species.^{4, 19, 20}

It has been shown that skin test reactions to different animal dander extracts in allergic patients tend to correlate with one another.²³ This finding suggests that similarities may exist between dander allergens derived from different animal species. The major allergens in rat, mouse, guinea pig, rabbit, and cat pelt extracts share certain physicochemical properties. Future studies may reveal that mammalian allergens also possess common structural properties.

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Select the ONE best answer for the following question from the Allergy Foundation of America Self-Assessment Program:

Question 3. Which of the following is NOT a sign of hypercapnia?

- (A) Disorientation
- (B) Combativeness
- (C) Somnolence
- (D) Fine tremors
- (E) Euphoria

The correct answer and bibliographic reference will be found on page 48 of this Journal.