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The Allergens of Cat Epithelia and Cat Serum

Comparative Studies Based on the Radioallergosorbent Technique (RAST)

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Abstract. Cat epithelia extracts were found to contain several electrophoretically separable allergens. Some of these were also detected in cat serum. In addition to serum proteins the epithelia extracts contain allergens with higher electrophoretic mobility than albumin, which was found to be the main allergen of cat serum. The molecular weight of the main cat epithelia allergen was about 32,000. 56 sera from allergic patients were studied by means of the radioallergosorbent test using cat epithelia extracts, a purified epithelia allergen fraction and cat serum as test allergens. About 7% of the patient sera were found to contain IgE specific to cat serum proteins. IgE specific to the epithelia allergen fraction, which was free from serum proteins, was found in 66% of the patient sera, while the rest, about 27%, contained IgE reacting with both kinds of allergens.

Cat epithelia is known to be a frequent cause of atopic allergy [FRANKLAND, 1967]. However, only a few reports have been published on this topic. VOORHORST [1965] studied dander extracts obtained from seven different cat breeds. He found that the extract prepared from Siamese cat contained breed-specific allergens not present in the others. No difference could be demonstrated between the other six extracts (European short-hair, Chestnut brown, Persian, Color point long hair, Manxhair and Abyssinian). SPAIN *et al.* [1942] pointed out that individuals sensitive to animals exhibit local reactions when licked or mouthed by pets. They were able to show the presence of a common allergen in cat saliva and cat epithelia extracts.

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Also cat serum has been found to contain allergens. WALKER [1917] obtained positive skin tests with cat serum in 7 out of 10 cat-sensitive patients. Similar results were obtained by WODEHOUSE [1917]. SIMON [1941, 1942] has reported positive skin reactions in some patients who were sensitive also to sera from other animals. Serum proteins have recently been shown to be important allergens present in high concentration in extracts of dog [YMAN *et al.*, 1973] and horse epithelia [PONTERIUS *et al.*, 1973].

The aim of the present investigation was to study the composition of cat epithelia extracts with respect to allergens originating in serum and epithelia.

Materials and Methods

Allergen preparations. (1) Cat epithelia extract, European shorthair cat (1/10, w/v). A commercial extract obtained from Vitrum AB, Stockholm. (2) Cat epithelia extract (1/10, w/v) prepared from skin scrapings (dry hide) from a European shorthair cat by extraction with Evans solution. (3) Purified cat epithelia allergen. All serum proteins were removed from the cat epithelia extract No. 1 by polyacrylamide gel electrophoresis as described below. The gels were cut into slices. Each slice was eluted and the eluates were pooled as indicated in figure 1. (4) Cat serum.

Estimation of relative allergen concentration. The relative concentration of the allergen preparations and the fractions separated by gel chromatography was estimated by the radioimmunosorbent assay previously described [CESKA *et al.*, 1972; YMAN *et al.*, 1973]. Two patient sera were chosen for these studies. Patient serum A contained IgE mainly specific to cat serum proteins, while patient serum B reacted only with allergens originating in the epithelia.

Gel chromatography. 5 ml of the cat epithelia extract No. 1 was applied to a Sephadex G-75 column (2.5 × 90 cm) equilibrated with 0.05 M phosphate buffer, pH 7.4, containing 0.02% sodium azide. 2.5 ml fractions were collected. The flow rate was about 30 ml/h. The relative allergen concentration of the fractions was estimated as described above and calculated as percent of the concentration of the sample applied to the column. Approximate molecular weights of the allergens were calculated as previously described [PONTERIUS *et al.*, 1973].

Polyacrylamide-gel electrophoresis and immunoelectrophoresis. The procedure was the same as described before [PONTERIUS *et al.*, 1973]. Rabbit anti-cat epithelia antiserum (aCE) was raised by immunization with cat epithelia extract No. 2.

Inhibition studies. Samples of electrophoretically separated allergen fractions were incubated for 3 h with patient serum samples. The mixtures were then incubated for another 3 h with isolated allergens coupled to paper discs. The IgE of the human serum was thus partly bound to free allergens before the incubation with the immobilized allergens. After careful washing the discs were incubated with ¹²⁵I-labelled anti-IgE. Cross-reactions between free and immobilized allergens result in a decrease in the uptake of anti-IgE of the paper discs. The inhibition was expressed

as percent decrease in radioactivity of the disc compared to an identical allergen disc where the serum sample was preincubated with one of the inactive fractions obtained in the same experiment.

Demonstration of specific IgE in patient sera. 56 patient sera were studied by a modification of the radioallergosorbent test (RAST) as previously described [PONTERIUS *et al.*, 1973]. The sera were obtained from several hospitals over a period of several years. All of them were previously found to contain cat allergen-specific IgE by RAST screening employing commercial cat epithelia extracts [LUNDKVIST and BERGLUND, personal commun.]. No information was available concerning the properties of those extracts. The allergens of the cat epithelia extracts No. 1 and 2, cat serum and a purified epithelia allergen preparation (No. 3) were immobilized by coupling to BrCN activated paper discs. The allergen discs were incubated with patient sera and the IgE thus attached to the paper discs was detected by subsequent incubation with ¹²⁵I-labelled anti-IgE. The result of each test was expressed as the anti-IgE uptake of the disc calculated as a percentage of the total amount of immunoreactive anti-IgE added in the last step of the test procedure. A 5-percent uptake was considered as a positive response.

Results and Discussion

Some Properties of the Allergens of Cat Epithelia and Cat Serum

Some of the allergens of dog and horse epithelia extracts have recently been shown to be serum proteins [YMAN *et al.*, 1973; PONTERIUS *et al.*, 1973]. In both cases the extracts contain also other allergens not originating in the serum. Extracts of cat epithelia were found to have a similar composition. Figure 1 and 2 show polyacrylamide-gel electrophoresis of cat epithelia extract No. 1 and cat serum, respectively. The allergen activity, i.e. the ability to bind to specific IgE, was detected by a modification of RAST. Two patient sera containing IgE specific either to cat serum proteins (A) or cat epithelia proteins (B) were chosen for these studies. The main protein zone of the cat epithelia extract, possessing allergen activity detectable by patient serum A, was electrophoretically and immunologically identical to cat serum albumin (fig. 3). The composition of the epithelia extracts No. 1 and 2 was identical with respect to the allergens originating in serum. The pI of the main component, detectable by a patient serum with a specificity similar to serum A, has previously been reported to be about 4.7 [VARGA and CESKA, 1972]. The total activity of cat serum was about 150 times higher than that of the epithelia extract No. 1 as estimated with patient serum A (table 1). Like the extracts of horse epithelia, the cat epithelia extract No. 1 was found to contain allergens apparently originating in the epithelia (fig. 1). No allergens of this kind

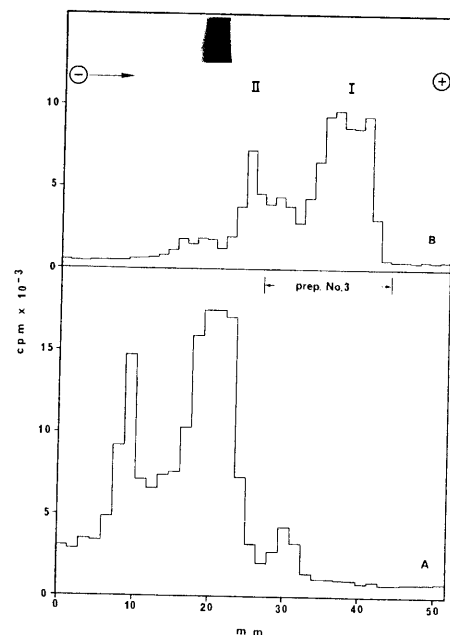


Fig. 1. Polyacrylamide gel (10.5%) electrophoresis of cat epithelia extract No. 1. The upper part of the figure shows the distribution of proteins and the lower part the distribution of allergen activity detectable with the patient sera A and B. For further details, see under Materials and Methods.

could be detected after electrophoresis of epithelia extract No. 2. The concentration of epithelia allergens differed in the two extracts by a factor of about 100. None of these allergens could be detected in cat serum. The epithelia allergens I and II (fig. 1) were immunologically closely related. The binding of IgE to immobilized allergen II could be completely inhibited (95%) by preincubation of the patient serum B with pure allergen I. The epithelia allergens did not show any relationship to the serum allergens. The molecular weight of the main epithelia allergen was calculated to be about 32,000 by gel chromatography on Sephadex G-75 (fig. 4).

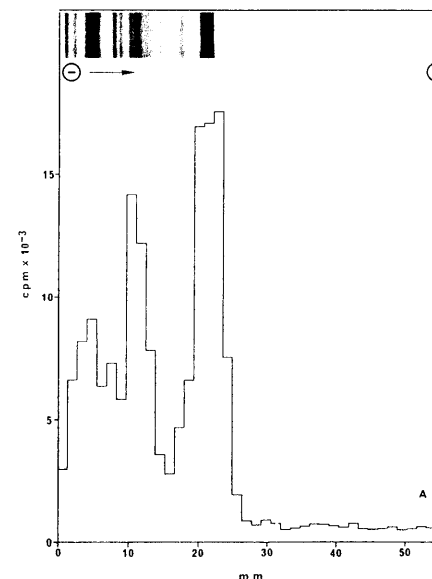


Fig. 2. Polyacrylamide gel (10.5%) electrophoresis of cat serum. The upper part of the figure shows the distribution of proteins and the lower part the distribution of allergen activity detectable with patient serum A. No allergens could be detected using patient serum B.

Comparative Studies on Crude and Purified Cat Allergen Preparations by Means of RAST

The role of animal serum proteins in allergy has been frequently discussed in connection to the possible relations between horse dandruff allergy and serum sickness caused by injection of therapeutic doses of horse serum. However, little attention has been paid to the fact that also serum proteins from other animals may act as potent allergens [WODEHOUSE, 1917; SIMON, 1941]. Allergen extracts for diagnosis of allergy to animals have been and are still prepared from many kinds of raw materials, such as hair, dandruff, fresh epithelia or dry hides. It is obvious that the choice of raw material will have a great influence on the composition of the final

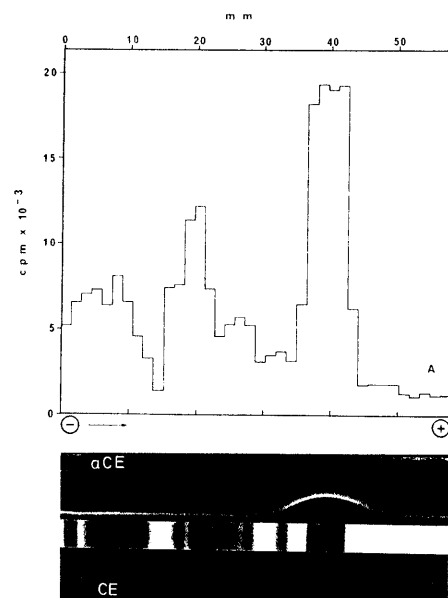


Fig. 3. Polyacrylamide gel (7%) / agar gel immunoelectrophoresis of cat serum. The upper part shows the distribution of allergen activity (patient serum A) and the lower part the antigen detectable by immunodiffusion against rabbit anti-cat epithelia antiserum (aCE). Cat epithelia extract No. 2 (CE) was applied to one of the troughs of the agar gel. A stained slice of the polyacrylamide gel is shown in the position occupied by the polyacrylamide gel rod during the immunodiffusion.

Table 1. Relative concentration of the allergen preparations studied

Allergen preparation	Serum allergen (patient serum A)	Dandruff allergen (patient serum B)
Cat epithelia extract (No. 1)	1	1
Cat epithelia extract (No. 2)	1.1	0.01
Purified epithelia allergen (No. 3)	0	not determined
Cat serum (No. 4)	145	0

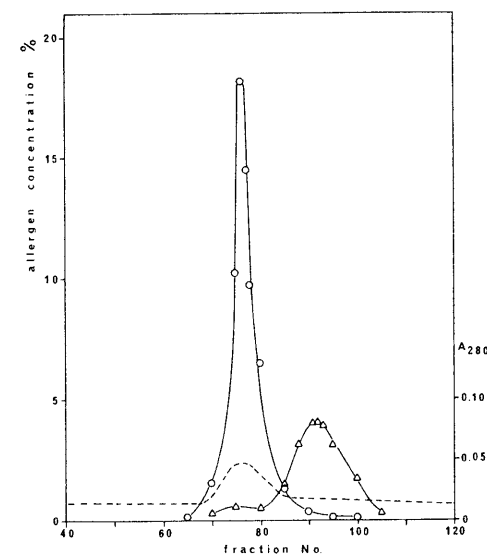


Fig. 4. Gel chromatography on Sephadex G-75 of cat epithelia extract No. 1. The patient sera A (serum allergens) and B (epithelia allergens) were used for the detection of allergen activity. The relative allergen concentration of the fractions is expressed as percent of the concentration of the extract applied to the column. For further details, see Materials and Methods. (-----) = A_{280} ; $\circ$ = allergen concentration (serum A); $\triangle$ = allergen concentration (serum B).

extract. Any attempt to standardize this type of extracts, therefore, has to start with standardization of the raw materials. The influence of the quality of horse allergen extracts on *in vitro* diagnosis (RAST) of horse dandruff allergy has recently been demonstrated [PONTERIUS *et al.*, 1973]. Similar results were obtained when sera from 56 patients, previously classified as cat sensitive, were tested by the same technique with cat serum, two cat epithelia extracts and a purified epithelia allergen preparation free from serum proteins (fig. 5). 37 patient sera were found to contain IgE specific to the epithelia allergens, 4 were sensitive only to cat serum proteins and 15 contained IgE capable of binding both kinds of allergens.

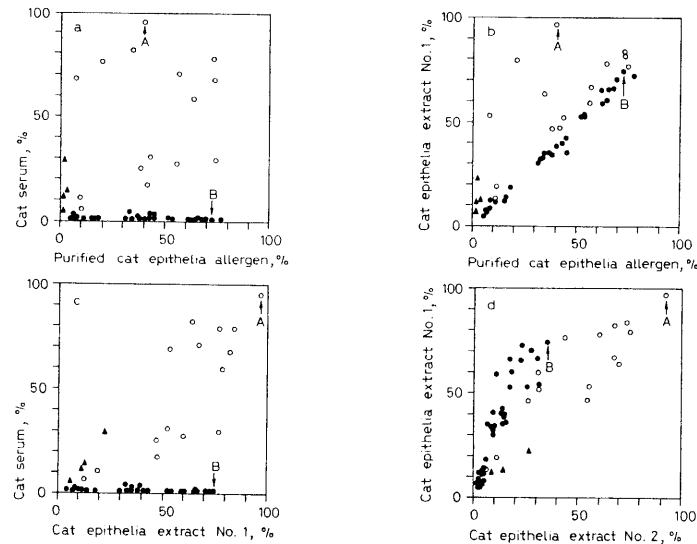


Fig. 5. Correlation between the RAST results obtained with four different cat allergen preparations. 56 patient sera were tested. The patient sera contained IgE specific either to serum proteins (▲) or to epithelia proteins (●) or to proteins of both kinds (○). A and B denotes the sera used for the studies on the properties of the cat allergens. For further details, see Materials and Methods.

As could be expected the test result is highly dependent on the relative concentration ratio of the two types of allergens (fig. 5d). The extract No. 2 which contains about 100 times less epithelia allergen than extract No. 1 and comparable amounts of serum allergens (table I) gives similar results as extract No. 1 for the patients sensitive to cat serum proteins. However, a poor correlation is obtained with the two extracts for patients sensitive only to the epithelia allergens. It is quite clear that differences in composition of the extracts will affect not only the results obtained by *in vitro* diagnostic methods but also the reliability of *in vivo* tests and the therapeutic effect when the extracts are used for desensitization. However, further investigations will be needed to show the clinical importance of the separate allergens of cat epithelia extracts.

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