

Serum Albumin – An Important Allergen in Dog Epithelia Extracts

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Abstract. Allergens from dog serum and extracts of dog hair, dandruff and skin scrapings were studied with respect to some physico-chemical and immunological properties. Serum albumin was found to be an important allergen present in high concentration in epithelia extracts. At least one additional serum protein was found to be a potent allergen.

In spite of the relatively high frequency of animal dandruff allergies comparatively few investigations have been made of the nature and origin of the allergens involved. The presence of serum proteins in extracts of skin scales has previously been reported [SQUIRE, 1950; STANWORTH, 1957] and in some cases certain serum proteins have been suspected of being allergens. However, on this point the results are conflicting due to the lack of adequate methods for identification of the observed antigens as allergens [for a review, see BERRENS 1970, 1971]. In the case of dog epithelia allergens some data have been published, which indicate qualitative differences between allergen extracts obtained from different dog breeds [HOOKER, 1944; FAGERBERG and WIDE, 1970; VARGA and CESKA, 1972]. However, this may as the investigators have pointed out, instead be ascribed to uncontrolled differences in the concentration of the allergen extracts used.

It is obvious that the frequently reported variation in composition and concentration between different batches of allergen extracts is a serious drawback to the diagnosis and treatment of allergy. In our opinion any attempt to standardize such preparations must involve characterization of

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the active constituents with respect to physico-chemical and immunological properties. For this work adequate methods are required for separation, identification and quantitation of the allergenic components of each particular preparation. The present communication shows a combination of polyacrylamide gel electrophoresis, immunodiffusion and radioimmunoassay applied to dog epithelia extracts. The detailed studies are restricted to the apparently dominating allergen, but may easily be extended to the other components as well as other epithelia extracts.

Materials and Methods

Allergen extracts. Four different dog allergen extracts and dog serum were studied. These preparations, subsequently referred to as preparations 1-5, are specified below.

(1) Dog epithelia extract, 10,000 PNU/ml (commercial extract obtained from Dome Laboratories).

(2) Mixed hair and dandruff from a variety of dog breeds extracted with Evans solution after defatting; concentration 1/10, w/v.

(3) Mixed hair from the same batch as No. 2 extracted in the same way after removal of as much dandruff as possible; concentration 1/10, w/v.

(4) Hair and skin scrapings from dachshund extracted with an isotonic phosphate buffer, pH 7.4, containing 0.4% phenol. The material was defatted prior to extraction. The concentration was 1/10, w/v.

(5) Dog serum (beagle).

Estimation of total allergen concentration. The relative concentration of the preparations was calculated from the amount of allergen solution giving half maximal response in a modification of the radioimmunosorbent assay described by CESKA *et al.* [1972]. A serum from a normal non-allergic individual was tested parallel to the patient serum to check that no unspecific reactions occurred.

Polyacrylamide gel electrophoresis was performed essentially according to DAVIS [1964]. Only the small pore separation gel was used and the samples (1-200 μ l) were layered directly on the top of the gel rods. After completion of the electrophoretic run the gel rods were cut longitudinally into two parts. One half was used for detection of allergens as described below. The other half was cut longitudinally once more. One of the parts obtained was stained with amido black while the other part was applied to the surface of a 1.5-percent agar gel (1-mm layer on a microscope slide), permitting the separated components to diffuse into the agar gel. A trough was cut out on each side of the polyacrylamide gel slice. After filling the troughs with the antisera listed below, the gel slides were incubated at +4 °C for 2 days.

Antisera 1: rabbit anti-dog plasma antiserum (Behringwerke AG). Antisera 2: rabbit anti-dog epithelia antiserum obtained by immunization with a commercial extract (preparation No. 1).

These antisera will in the following be referred to as aDP and aDE, respectively.

Detection of allergens after electrophoretic separation. One half of the gel (see above) was cut transversely into thin slices (approx. 1.2 mm). The slices were transferred to separate test tubes and eluted with 500 μ l distilled water at +4 °C for about 20 h. Each eluate was tested for allergen activity using a modification of the radioimmunosorbent allergen assay described by CESKA *et al.* [1972] and VARGA and CESKA [1972]. 200- μ l samples of the eluates were incubated with BrCN-activated paper discs. The discs with their covalently bound proteins were after washing incubated with a patient serum containing a high amount of IgE, specific to dog allergens. The discs were again washed and incubated over night with ^{125} I-labelled anti-IgE. After a final washing the radioactivity of each disc was measured.

Double diffusion was performed according to OUCHTERLONY [1958].

Results and Discussion

Electrophoretic studies on dog epithelia extracts of different origins have shown the presence of a protein with a mobility very similar to that of serum albumin. This protein is often the only one which can be detected after staining with amido black and always constitutes the main part of the protein content of the extracts. Applying a modification of the radioimmunosorbent allergen assay [VARGA and CESKA, 1972; CESKA *et al.*, 1972] this protein can be shown to be responsible for the main part of the IgE-binding capacity of dog epithelia extracts. The similar results obtained using sera from several dog allergic patients indicate that the protein is a common allergen [BRANDT *et al.*, to be published].

A comparative analysis of dog serum and four extracts of hair, dandruff and skin scrapings showed a considerable difference in potency. The relative allergen concentrations of the five preparations are given in table I.

Table I. Relative concentration of five allergen preparations. For details, see materials and methods

Allergen preparation	Relative concentration
1 Dog epithelia extract (10,000 PNU/ml)	0.05
2 Dog hair and dandruff extract (1/10, w/v)	1
3 Dog hair extract (1/10, w/v)	0.003
4 Extract of dog hair and skin scrapings (1/10, w/v)	0.2
5 Dog serum	2,000

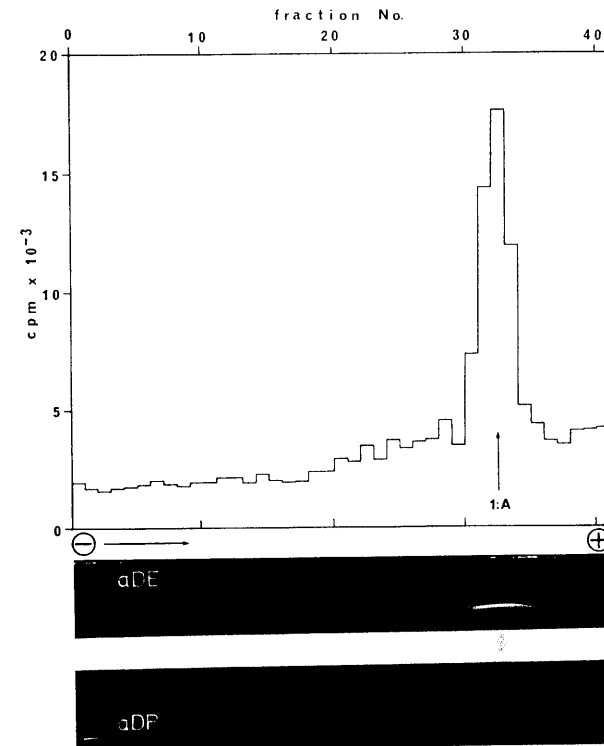


Fig. 1. Polyacrylamide gel electrophoresis of a commercial dog allergen extract (200 μ l of preparation No. 1). The upper part of the figure shows the distribution of allergen activity and the lower part the distribution of antigens detectable by immunodiffusion against rabbit antisera to dog plasma (aDP) and dog epithelia extract (aDE). A stained slice of the same gel is shown in the position occupied by the polyacrylamide gel rod during immunodiffusion. The fraction 1:A was used for inhibition and double diffusion studies.

ble I. Dog serum was found to be about 600,000 times as potent as the extract prepared from purified dog hair.

The four allergen extracts and the dog serum were analyzed by polyacrylamide gel electrophoresis for comparison of the allergenic compo-

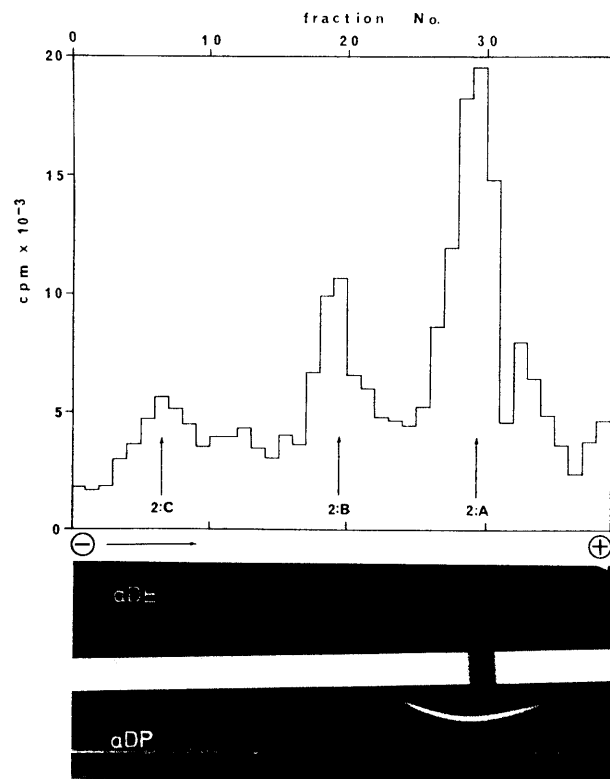


Fig. 2. Polyacrylamide gel electrophoresis of an extract prepared from mixed dog hair and dandruff (50 μ l of preparation No. 2). For details, see the legend to figure 1. The fractions 2:A, 2:B and 2:C were used for inhibition and double diffusion studies.

nents in the five preparations. A main component with a mobility corresponding to serum albumin was observed in all the four extracts (fig. 1-4). The number of additional components varied from extract to extract. However, considering the relative allergen concentration of these extracts (table I), the most reasonable explanation for this variation is

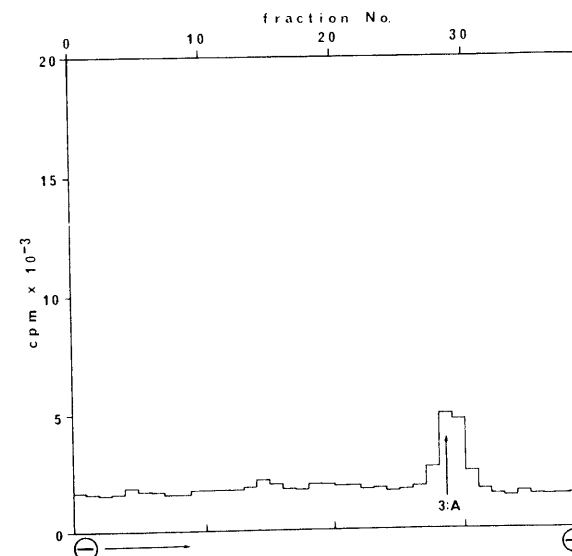


Fig. 3. Polyacrylamide gel electrophoresis of an extract prepared from mixed dog hair (200 μ l of preparation No. 3). Only the distribution of allergen activity is shown. The fraction 3:A was used for inhibition and double diffusion studies.

that some components in the weaker extracts simply are present in too low concentration to be detected. Analysing dog serum in the same way, the albumin fraction was found to be as potent an allergen as the epithelial protein (fig. 5). Additional activity was, as in the case of the most concentrated epithelia extract, found in the γ -globulin region. By immunodiffusion in the polyacrylamide gel – agar gel system heavy precipitation was observed in the position of albumin for both the antisera in all cases but one (preparation No. 3), where the concentration of antigen was too low. The results indicate the presence of the same or a very similar allergen in both dog dandruff and dog serum. This finding is supported by the result of double diffusion experiments, showing at least one component common to all the preparations (fig. 6) and identity between the isolated main components of serum and epithelia (fig. 7).

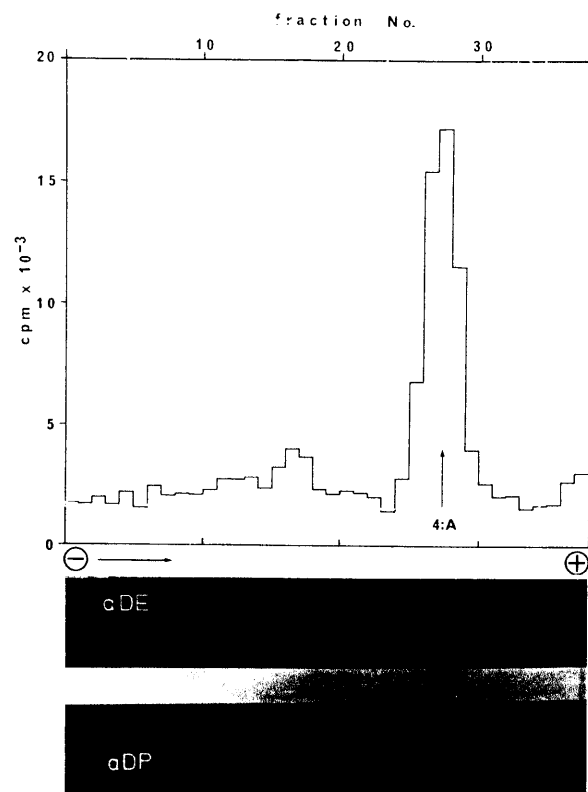


Fig. 4. Polyacrylamide gel electrophoresis of an extract prepared from dachshund hair and skin scrapings (100 μl of preparation No. 4). For details, see the legend to figure 1. The fraction 4:A was used for inhibition and double diffusion studies.

In an inhibition study patient serum was preincubated with certain isolated allergenic fractions (fig. 1–5) before the incubation with allergen fractions coupled to paper discs. The results, expressed as percent decrease in ^{125}I -labelled anti-IgE attached to the discs, are shown in ta-

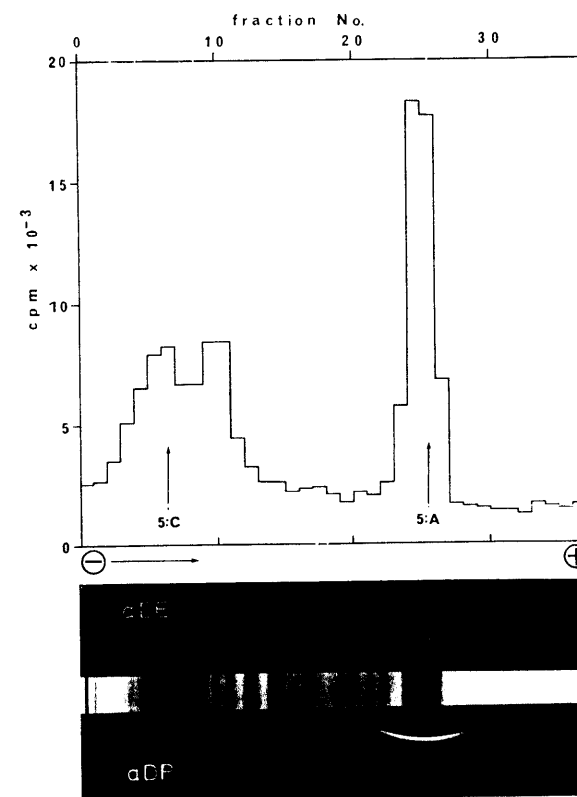


Fig. 5. Polyacrylamide gel electrophoresis of dog serum (1 μl of preparation No. 5). For details, see the legend to figure 1. The fractions 5:A and 5:C were used for inhibition and double diffusion studies.

ble II. The albumin fraction (5:A) obtained from serum was found to inhibit the corresponding fractions from hair and epithelia extracts (1:A, 2:A, 3:A and 4:A) to the same degree as it inhibited itself, i.e. almost complete inhibition. A fraction (5:C) from the γ -globulin region of serum inhibited a corresponding fraction (2:C) from dandruff to about the same

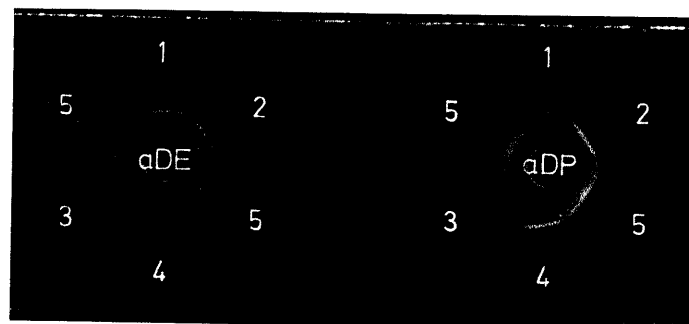


Fig. 6. Double diffusion analysis on five allergen preparations using rabbit antisera to dog plasma (aDP) and dog epithelia extract (aDE). (1) A commercial dog epithelia extract. (2) Extract of mixed hair and dandruff. (3) Extract of mixed hair. (4) Extract of hair and skin scrapings from dachshund. (5) Dog serum.

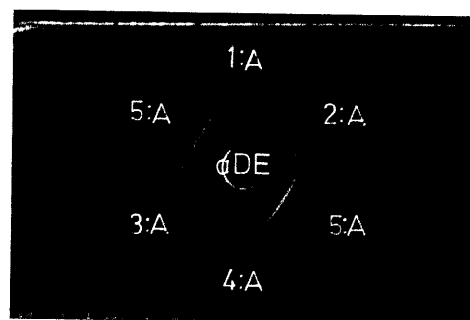


Fig. 7. Double diffusion analysis of the isolated main allergen component from five different allergen preparations. The symbols refer to the fractions indicated in figure 1-5. Rabbit antiserum to dog epithelia (aDE) was used.

extent. The same serum γ -globulin fraction (5:C) was not significantly inhibited by serum albumin (5:A). The second largest peak (2:B) from the dandruff extract was almost completely inhibited by the main peak (2:A) from the same extract while the third fraction (2:C) was not.

Table II. Influence on binding of IgE to isolated and immobilized allergens by preincubation of the patient serum with the same or other allergen fractions. The symbols in brackets refer to fractions obtained in the electrophoretic separations shown in figure 1-5. For further details, see under materials and methods

Allergen fractions coupled to paper discs	Inhibitor	Percent decrease in ^{125}I -labelled anti-IgE uptake
<i>Isolated main (albumin) fraction from</i>		
Extract No. 1 (1:A)	albumin fraction from dog serum (5:A)	88
Extract No. 2 (2:A)		97
Extract No. 3 (3:A)		100
Extract No. 4 (4:A)		97
Dog serum (5:A)		94
<i>Isolated fraction from the γ-globulin region of</i>		
Extract No. 2 (2:C)	' γ -globulin fraction' from dog serum (5:C)	81
Dog serum (5:C)	albumin fraction from dog serum (5:A)	12
<i>Isolated fractions from extract No. 2</i>		
Fraction 2:B	albumin fraction from extract No. 2 (2:A)	85
Fraction 2:C		25

The results clearly indicate the presence of at least two different types of allergens in dog serum, one in the albumin fraction and one in the γ -globulin region. These serum proteins are responsible for the main part, if not all, of the allergen activity of dog dandruff, either in their native state or slightly altered. Peak 2:B from dandruff, which crossreacts with the main peak (2:A) and never has been found in serum, probably represents the dimer of the main component.

On the basis of the data presented in this communication together with preliminary gel filtration, ultracentrifugation [BRANDT and CESKA, personal communications], and gel isoelectrofocusing studies [VARGA and CESKA, 1972], where the main allergenic component of dog epithelia has been shown to have a molecular weight around 65,000 and pI around 4.7, it seems evident that serum albumin is an important allergen in dog dan-

druff. It is also obvious that certain other serum proteins may act as allergens. These are, as could be expected, also found in the epithelia extracts. As pointed out earlier by BERRENS [1970] there is no doubt that the important allergens are found in the skin scales and not in the hair.

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Two Classes of Homocytotropic Antibodies in the Rhesus Monkey

Determinant-Dependent Induction and Contribution to Manifestations of Hypersensitivity¹

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Abstract. Rhesus monkeys injected intravenously with TNP-KLH produced homocytotropic antibody separable into two components on ion exchange chromatography, a heat-labile γ_1 - and a heat-stable γ_2 -immunoglobulin. *Homocytotropic* antibody was directed against the TNP group only, though both groups of determinants gave rise to large amounts of *precipitating* antibody. Arthus reactions induced by TNP on heterologous carrier were more severe than those caused by KLH. Prolonged TNP-induced reactions resembled delayed hypersensitivity reactions grossly and histologically. Anaphylaxis occurred in a few of the animals in response to TNP-KLH, but not to KLH alone, although no relationship to levels of homocytotropic antibody was discernible.

Introduction²

This study was initiated to determine the nature of immediate hypersensitivity reactions of rhesus monkeys. TNP-KLH was selected as the main antigen to obviate the need for adjuvants, and to permit observations of reactions directed against two groups of antigenic determinants.

¹ This work was supported by US Public Health Grant AI-06274 and AI-09519.

² Abbreviations used: TNP = 2,4,6-trinitrophenyl; KLH = Keyhole limpet hemocyanin; BSA = bovine serum albumin; BGG = bovine γ -globulin; Ab = antibody.

Data on the early phase of this experiment were presented at the Midwinter Conference of Immunologists, Palo Alto 1966.

The histological examinations were carried out by Dr. V. MARUFFO, formerly of the ORPRC.

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