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CHARACTERIZATION OF EXTRACT OF
DOG HAIR AND DANDRUFF FROM
SIX DIFFERENT DOG BREEDS BY
QUANTITATIVE IMMUNOELECTROPHORESIS.
IDENTIFICATION OF ALLERGENS BY CROSSED
RADIOIMMUNOELECTROPHORESIS (CRIE)

By

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Allergen activity seems to be associated primarily with proteins composed of polypeptides with a molecular weight > 5000 dalton (17), although low molecular weight substances acting as haptens, as well as carbohydrate molecules derived from structural carbohydrates of bacterial spores, cannot be excluded. Rabbit antibodies against proteins with mol. wt. > 5000 dalton and rigid carbohydrate molecules can normally be produced. Thus it seems reasonable to characterize allergen extracts by the use of rabbit antibodies in immunochemical, *in vitro* methods.

The quantitative immunoelectrophoretic methods developed in Scandinavia during the last 10 years (2, 11, 23) provide allergologists with the possibility of identifying and quantifying firstly the antigens, and secondly the allergens of allergen extracts.

These methods have previously been used in the analysis of birch pollen and timothy pollen (4, 19, 24, 15) and recently in the characterization of extracts of horse hair and dandruff

(18, 13, 14). Furthermore, they provide us with the possibility of preparing better extracts for use in specific immunotherapy of the many allergic patients who at present are treated with extracts of great heterogeneity and without biochemical standardization (except for standardization of the PNU content, which gives no measure of the allergenic activity).

In the course of our work we have chosen to analyse extracts of mixed dog hair and dandruff from six different dog breeds. This should be seen in the light of the increasing interest in dogs as pets and the attendant greater risk for sensitisation of persons with a disposition towards allergy.

Earlier studies of extracts of dog hair and dandruff (7, 22) indicated possible qualitative differences between extracts from different dog breeds which might be ascribed to differences in the concentration of the extracts. A later work by Yman, Brandt & Ponterius (26), using a combination of polyacrylamide gel electrophoresis, immunodiffusion and radioimmunoassay, indicated one dog-serum protein in the albumin fraction and one in the γ -globulin region to be the main allergens in dog dandruff. The finding of the albumin-fraction protein is in agreement with the work of Varga & Ceska (22) who found the main allergenic component of dog epithelia to have a mol. wt. of around 65,000 dalton.

The aim of the present study was to use crossed immunoelectrophoresis (CIE) to:

- (1) Characterize the antigens of extracts of mixed dog hair and dandruff from six different dog breeds comprising three long- and three short-haired races, and
- (2) examine possible partial identity between the allergens of different allergen extracts, including extracts from individual dog breeds, as well as of animal and human sera, and furthermore
- (3) to use crossed radioimmuno-electrophoresis (CRIE) to identify the allergens in the mixed dog hair and dandruff extract.

MATERIAL AND METHODS

Allergen extract preparation. Non-deffatted, freeze-dried hair and dandruff from six different dog breeds: alsatian, boxer, collie, poodle, and long- and short-haired dachshund was obtained from Allergon AB, Sweden. The material was prepared by scraping the upperside of the skin, and stored in a closed metal box at -20°C . For the material from each breed the following procedure was employed: A 10 per cent (w/v) suspension of the material (buffer: 0.1 M sodium hydrogencarbonate and 0.015 M sodium azide) was gently agitated for 24 h at 5°C and filtered (to remove the hairs) before being centrifuged at 0°C for 1 h at 50,000 g. Small amounts of the extracts from the individual breeds were stored at -20°C for subsequent analyses. The six supernatants were mixed together and dialysed, once against a 10-fold volume of 0.025 M ammonium bicarbonate and then twice against 0.005 M ammonium bicarbonate, for 24 h at 5°C . (Limiting pore size corresponding to mol. wt. approximately $> 10^4$ dalton.) The product was finally freeze-dried and stored dry at 5°C . The standard extract (denoted St Hu 75) was freshly prepared from the freeze-dried material as 1 per cent (w/v) solution in 0.015 M sodium azide, which was ultracentrifuged at 0°C for 1 h at 50,000 g before each analysis.

The protein content of St Hu 75 was 3.95 mg ml^{-1} . The determination was performed by the method of Lawry et al. (11) using bovine albumin as standard.

Antigens. Freeze-dried extracts of hair and dandruff from cow, cat, dog, guinea pig, and sheep, and freeze-dried house dust extract were obtained from Allergologisk Laboratorium A/S, Copenhagen, Denmark. The extracts were dissolved in a medium of 0.1 M sodium hydrogencarbonate and 0.015 M sodium azide to give solutions of concentrations ranging from 5–20 per cent (w/v). Extract of horse hair and dandruff (St H 75) (18) was also used. Sera from cat, dog, cow, and horse were obtained from the Royal Veterinary and Agricultural College, Copenhagen, Denmark.

Human serum was a 1000 donor pool.

Purified human albumin (Behringwerke, Marburg/Lahn, BRD) and partially purified dog albumin (kindly supplied by Thing Mortensen, Finsensinstituttet, Copenhagen, Denmark) were used.

Antibodies against the extract of mixed dog hair and dandruff were obtained by immunisation of three rabbits with St Hu 75 and were purified according to Harboe & Ingild (2a).

Rabbit antibodies against human albumin, IgA, IgG, ceruloplasmin, transferrin, α_1 -antitrypsin, orosomucoid and haptoglobin were supplied by DAKO-Immunoglobulins, Copenhagen, Denmark.

^{125}I -immunoabsorbed rabbit immunoglobulins against human IgE were obtained from Phadebas RAST Pharmacia, Sweden, or were prepared as described in (16).

Immunoelectrophoretic methods. Equipment and reagents were essentially as

described in (2 b). One per cent (w/v) agarose gel (batch AGS 102 Dx, Litex, Glostrup, Denmark) containing a buffer of 0.073 M Tris, 0.024 M barbital, 0.006 M calciumlactate and 0.0003 H sodium azide (pH 8.6, 25° C) was used. Crossed immunoelectrophoresis (CIE), crossed-line immunoelectrophoresis (CLIE) and immunoelectrophoresis by the intermediate gel technique were carried out essentially as described in (2 c, d and f). First and second dimension electrophoreses were performed on a 0.15-cm-thick gel at 10 V/cm for 40 min, and on a 0.10-cm-thick gel at 2 V/cm for 15 h, respectively. The thickness of the intermediate gel was 0.12 cm. One per cent (v/v, relative to the antibody volume) of Aprotinin (Novo, Mainz, BRD) was added to the antibody-containing gel (5). The temperature of the cooling water was 15° C. The plates were pressed, washed and dried and finally stained with Coomassie Brilliant Blue® as described in (2 b). Agarose gel electrophoresis was performed according to Johansson (10).

Molecular weight determinations. Gel filtrations were performed on mixtures consisting of a) 50 mg of St Hu 75 + 100 µl of 0.5 per cent (w/v) Blue dextran® + 100 mg of sodium chloride and b) 400 µl of human serum + 2 mg of α -chymotrypsinogen A + 2 mg of myoglobin + 2 mg of cytochrome C (Sigma®, U.C.A.) + 100 µl of 0.5 per cent (w/v) Blue dextran® + 100 mg of sodium chloride, both made up to a total volume of 1 ml in the elution buffer which consisted of 0.5 M sodium chloride, 0.015 M sodium azide, and sodium phosphate (ionic strength 0.05, pH 7.5 at 20° C). The packed column (90 cm $\times$ 3.2 cm²) was pre-equilibrated with the elution buffer and elution was performed at a flow-rate of 10 ml h⁻¹. The immunochemical elution profiles of the dog hair and dandruff antigens were obtained by means of fused-rocket immunoelectrophoresis using rabbit antibodies against St Hu 75. The antigens of the individual fractions were then identified by means of crossed-line immunoelectrophoresis relative to the crossed immunoelectrophoretic reference pattern of St Hu 75.

The immunochemical elution profiles of the individual antigens of serum were obtained using specific antibodies against the following human proteins: α_2 -macroglobulin (mol. wt. 820,000), IgG (mol. wt. 160,000), haptoglobin (mol. wt. 100,000), albumin (mol. wt. 69,000) and α_1 -antitrypsin (mol. wt. 45,000). The profiles of α -chymotrypsinogen A (mol. wt. 12,400), myoglobin (mol. wt. 17,500) and cytochrome C (mol. wt. 12,400) were determined by means of u.v. monitoring. Void volume and total volume were determined from measurement of u.v.-absorption at 630 nm and the conductance, respectively, as a function of volume. From this the relationship between elution volume (fraction No.) and mol. wt. was obtained, and the approximate molecular weights for some distinct antigens of St Hu 75 were determined.

RAST (Radioallergosorbent test) (25) was performed as described by Ceska et al. (7) with St Hu 75 coupled to CNBr-activated filter paper discs. The results were expressed in sorbent units (SU) and in allergy classes (class 0, 1, 2, 3 and 4 corresponding to < 2 SU, < 4 SU, < 20 SU, < 100 SU, and > 100 SU,

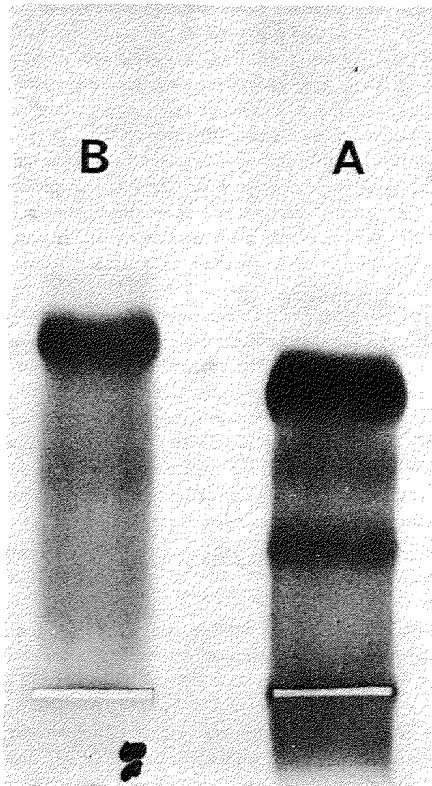


Fig. 1.

Agarose gel electrophoresis of St Hu 75. One per cent (w/v) agarose containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate and 0.0003 M sodium azide (pH 8.6, 25° C) at 15° C, 10 V cm⁻¹, 40 min. Samples: A) 10 µl of human serum deluted with buffer 1 + 4, B) 10 µl of St Hu 75. Fixation with picric acid, staining with Coomassie Brilliant Blue.

respectively), using serum from a timothy-allergic patient (H.D.), diluted 1 + 15 (v/v) with incubation buffer as a 100 SU reference.

Crossed-radioimmuno-electrophoresis (CRIE) was performed essentially as described in (2 d) and (13) respectively except that ¹²⁵I anti-IgE was employed at a concentration corresponding to half the amount used in the former study.

Autoradiography. The exposure times were from day 0 to day 8, from day 8 to day 29 and from day 29 to day 58. After correcting for radioactive decay of ^{125}I , the effective exposure times were 7.6, 16.8 and 17.6 days, respectively. The activity bound to the various precipitates was graded 10, 5 and 4, corresponding to visible radio staining after 8, 29 and 58 days, respectively.

RIST (Radioimmunosorbent test). Phadebas IgE (Pharmacia, Uppsala, Sweden) was used for the determination of total IgE in the analysed sera. The total IgE concentrations were expressed in U/ml by comparison with a WHO standard serum (16). One U corresponds approximately to 2.4 ng of IgE (3). The reproducibility of the determinations was about 10 per cent. Values between 26–630 U/ml were taken as corresponding to a normal 95 per cent range for adults.

Patients followed in the Allergy Department TA for adult out-patients, Rigshospitalet were selected. For comparison purposes serum from a patient without any history of allergy was used. The data for the patients are summarized in Table 3. Intracutaneous tests were performed on the volar side of the antebachium, using a histamine solution (0.1 mg/ml) as reference.

A mixed extract of dog hair and dandruff (Allergologisk Laboratorium A/S, Copenhagen, Denmark) was used for skin testing. The area of the urticaria element was determined by multiplying the mutually perpendicular diameters (in mm) and the result given as a skin index according to

$$\frac{\text{IC dog mm} \times \text{mm}}{\text{IC histamine mm} \times \text{mm}}$$

According to the Scandinavian Allergy Standard (1) a 3+ reaction corresponds to 1 HE (histamine equivalent).

RESULTS

Agarose gel electrophoresis of St Hu 75. Fig. 1 shows the agarose gel electrophoresis pattern of St Hu 75. Human serum was employed for a comparison. All the stained protein components, a distinct band with migration velocity virtually identical to that of human albumin and two bands with migration velocities similar to those of α - and β -globulins, are seen to have migrated towards the anode. No cathodically migrating proteins could be detected with the method used here.

The crossed immunoelectrophoresis reference pattern of extract of mixed dog hair and dandruff (St Hu 75). Fig. 2

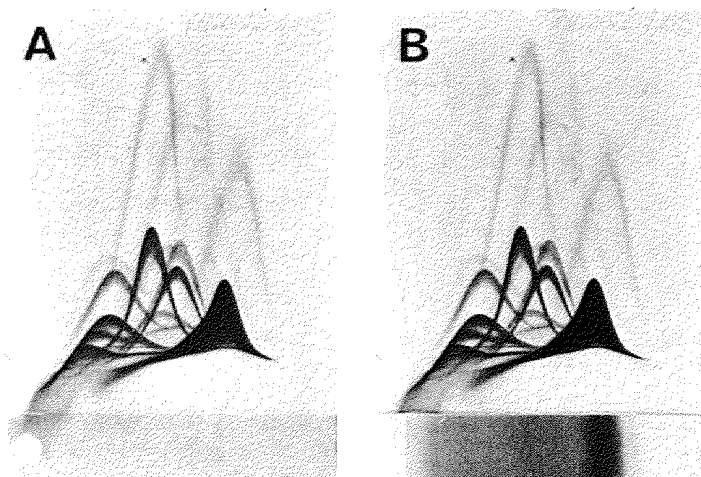


Fig. 2.

Reference pattern for mixed dog hair and dandruff extract, St Hu 75, obtained by crossed immunoelectrophoresis in 1 per cent (w/v) agarose gel containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate and 0.0003 M sodium azide (pH 8.6, 25° C) at 15° C. A.: *Antigens*: 7 μ l of St Hu 75 was applied. *First dimension*: 10 V cm^{-1} in a 0.15-cm-thick gel for 40 min. Anode to the right. *Antibodies*: 6 μ l cm^{-2} of rabbit antibodies against extract of mixed dog hair and dandruff. *Second dimension*: 2 V cm^{-1} in a 0.10-cm-thick gel for 15 h. Anode at the top. *Intermediate gel*: A 0.12-cm-thick gel. B.: Agarose gel electrophoresis superimposed by photomontage on the first dimension gel. 10 μ l of St Hu 75 was applied. Experimental conditions as described above.

shows the crossed immunoelectrophoresis (CIE) pattern for St Hu 75, obtained using rabbit antibodies against St Hu 75.

Fig. 3 is a drawing of the latter precipitation pattern, the precipitates being numbered arbitrarily from anode to cathode. The presence of 25 antigens is indicated, 20 of these being easily seen while the remaining ones (Nos. 3, 4, 5, 15, and 16) could only be detected by varying the concentrations of antigen and antibody. Proteins with mobility between that of human albumin and γ -globulin were seen, the most common ones being antigen No. 2. Antigen No. 3 seemed to be partially identical

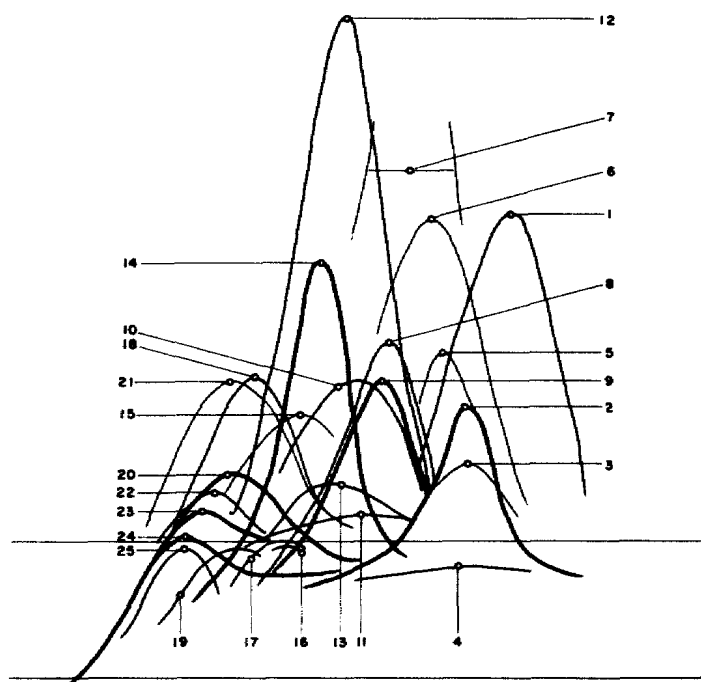


Fig. 3.

Drawing of crossed immunoelectrophoresis precipitation pattern of St Hu 75. Classification numbers for the precipitates are indicated.

to antigen No. 2, and there seemed also to be an antigenic relationship between antigens Nos. 11 and 19, and mutually between antigens Nos. 20, 23 and 24.

Crossed-line immunoelectrophoresis (CLIE) with dog serum incorporated in the line gel indicated that all but five antigens (Nos. 1, 10, 11, 13, and 17) in St Hu 75 were serum

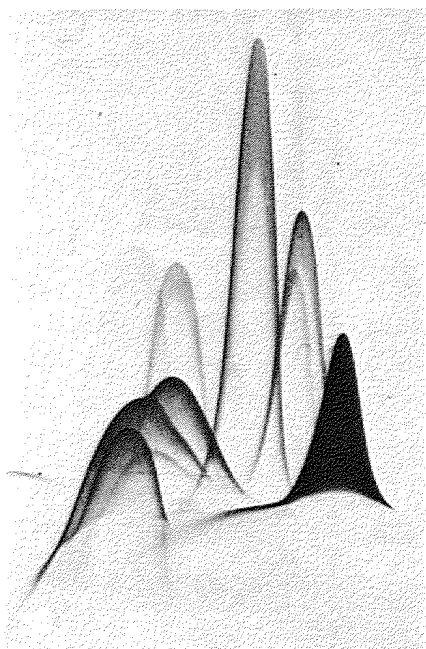


Fig. 4.

Crossed immunoelectrophoresis pattern for dog serum in 1 per cent (w/v) agarose gel. *Antigens:* 5 μ l of dog serum diluted 1 + 9 with Tris-barbital buffer. *Antibodies:* 10 μ l cm^{-2} of rabbit antibodies against extract of mixed dog hair and dandruff St Hu 75. Experimental conditions otherwise as described in Fig. 2.

proteins. Using the same technique with dog albumin incorporated in the line gel, antigen No. 2 was identified as albumin.

Antibodies against human serum proteins, which appear to cross-react with dog serum proteins, were used in the intermediate gel technique and facilitated identification of antigen No. 9 as α_1 -antitrypsin, antigen No. 14 as haptoglobin and antigen No. 20 (23) as IgG.

The serum protein content in St Hu 75 is indicated in Fig. 4, where CIE has been performed using dog serum as

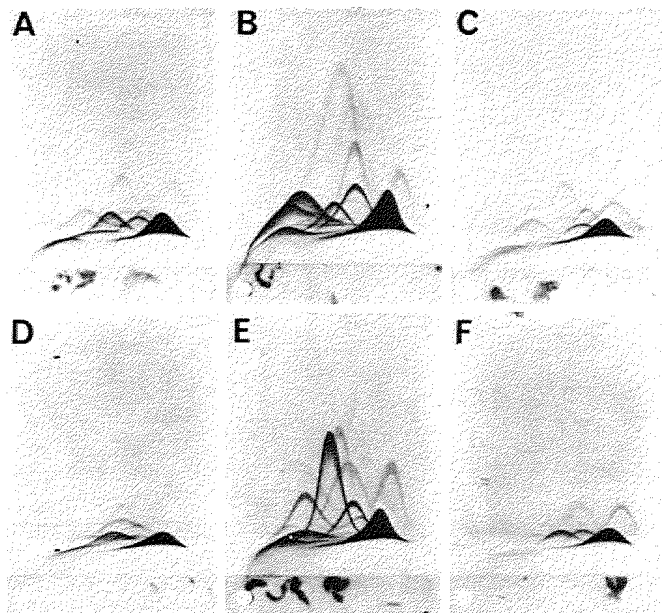


Fig. 5.

Extracts of hair and dandruff from the individual dog breeds examined by crossed immunoelectrophoresis. Antigens applied: 15 μ l aliquots of the individual extracts. A) alsatian. B) boxer. C) collie. D) long-haired dachshund. E) short-haired dachshund. F) poodle. For comparison with St Hu 75 see Fig. 2.

Experimental conditions as described in Fig. 2.

antigen and rabbit antibodies against St Hu 75 as antibody. Antigens Nos. 2, 3, 4, 5, 8, 9, 12, 14, 18, 19, 20, 23 and 24 are easily identified. Three weak, unidentified precipitates were also seen. Incorporation of dog serum in the line gel as explained above seems, however, to indicate an even higher content of serum proteins in St Hu 75.

Partial identity of antigens from dog hair and dandruff with antigens from related sources. By incorporating extracts of hair and dandruff from cat, cow, horse, guinea pig, and sheep, sera from cat, cow, horse and man and finally extract

TABLE 1

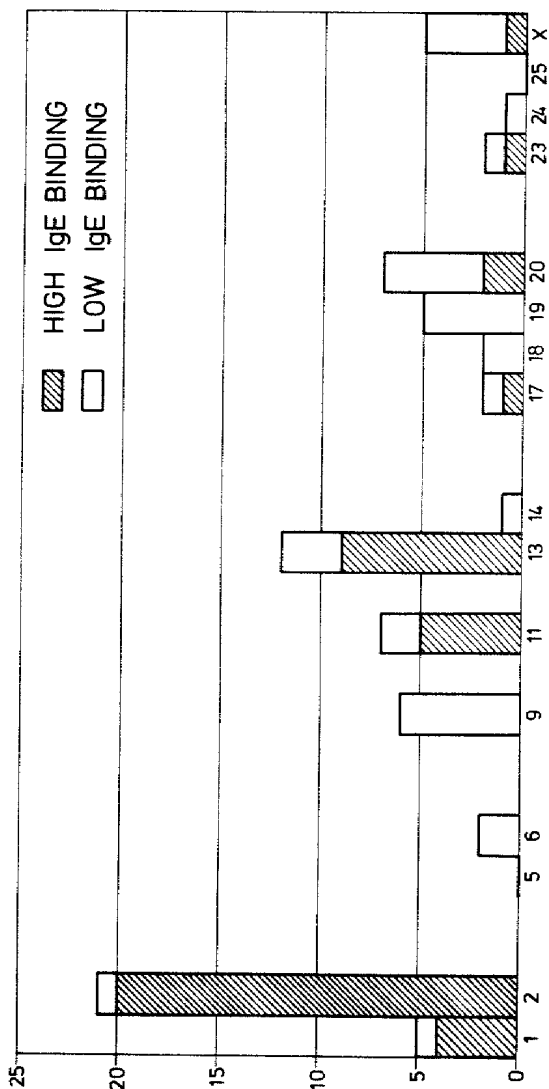
Identity/Partial Identity of Antigens from Mixed Dog Hair and Dandruff with Antigens of Other Animal Origin.

Dog (St Hu 75) Antigen No.	Cat	Cow	Horse	Guinea pig	Sheep	House dander	Dog serum	Cat serum	Cow serum	Horse serum	Human serum
1			(+)			+					(+)
2	+	+	+	+		+	+	+	+	+	+
3							+	+			
4	(+)			(+)			+	+			
5							+	+			
6							+	+			
7							+	+			+
8			+				+	(+)		(+)	+
9			(+)			(+)	+	+		+	+
10		+	+			+			+	+	
11	(+)	+				+		(+)			
12			(+)			+	+	+	(+)		
13						+					
14							+	+			+
15							+	(+)			
16							+	+			
17								(+)			
18							+		+	+	
19						(+)					
20	+	+	+				+	+	+	+	+
21							+	+			+
22							+	+			+
23	+		+				+	+	+		+
24	+		+				+	(+)	+		+
25							+	+	+	+	

of house dust in the line gel for CLIE, antigens partially identical to those of St Hu 75 were identified. The CIE reference pattern of St Hu 75 was used for comparison. The results are summarized in Table 1. All the antigens apart from No. 13 were partially identical to antigens from related sources. The highest degree of partial identity to St Hu 75 antigens was

ALLERGOGRAM DOGHAIR & DANDRUFF

PATIENTS REACTING WITH STHU-75-ANTIGENS



Antigen number

found for serum from dog and cat, whereas no partially identical antigens were found in the extract from sheep. Antigen No. 2 (dog albumin) was present in all the extracts except that from sheep, as well as in all the sera. Antigen No. 20 was present in the extracts from cat, cow, and horse and in all the sera. Six (7) antigens were found in house danders (Nos. 1, 2, 10, 11, 12, and 13 (9)).

Approximate molecular weights. The relationship between molecular weights and fraction No. for some marker proteins applied on the gel filtration column was determined. The corresponding fractions obtained after gel-filtrating St Hu 75 were analysed by crossed-line immunoelectrophoresis, and some distinct antigens identified. Thus the following approximate molecular weights were found. Antigens Nos. 14, 23, and 24 had mol. wt. $> 10^5$ dalton, antigens Nos. 2, 9, and 13 had $3 \times 10^4 < \text{mol. wt.} < 10^5$ dalton and antigens Nos. 3, 10, and 12 had $10^4 < \text{mol. wt.} < 3 \times 10^4$ dalton.

CIE and CLIE of the extracts from individual dog breeds. The individual extracts were compared with the mixed extract St Hu 75 by the use of the CLIE technique. Not all the antigens found in St Hu 75 could be identified in every one of the individual extracts.

Fig. 5 shows a comparison between extracts of hair and dandruff from the individual dog breeds (long- and short-haired dachshund, collie, boxer, alsation and poodle.

Table 2 summarizes the qualitative results. A considerable quantitative and qualitative variation in the antigen content

Fig. 6.

Allergogram for antigens of dog hair and dandruff based upon sera from 21 patients RAST-positive to dog (St Hu 75). Specific IgE-binding to the individual antigens was detected by means of crossed radioimmuno-electrophoresis (CRIE). The extent of IgE-binding was evaluated visually from the autoradiographs of the CRIE plates. The binding was graded as high or low, depending upon whether radio staining was visible after 8 days or more than 8 days, respectively. See text for further details.

TABLE 2
Antigen Composition of the Individual Dog Breed Extracts.

Antigen No.	Alsatian	Boxer	Collie	Long-haired dachshund	Short-haired dachshund	Poodle
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3		+	+		+	
4		+	+		+	
5		+	+		+	
6	+	+	+	+	+	
7	+	+	+		+	
8	+	+	+	+	+	+
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	+	+	+	+	+
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+		+	
16	+	+	+		+	
17	+	+	+		+	
18	+	+	+	+	+	+
19	+	+	+		+	
20	+	+	+		+	+
21		+	+		+	
22		+	+		+	
23	+	+	+		+	+
24	+	+	+		+	
25		+	+		+	

is seen for the six breeds, the relative amount of the various antigens extracted being much higher for the short-haired races (short-haired dachshund, boxer and alsatian) than for the long-haired races (long-haired dachshund, collie and poodle). Antigens Nos. 1, 2, 9, 11, 13, 19, and 20, which appear as allergens by crossed radioimmunoelectrophoresis (see later), are found in the extract from alsatian, boxer, collie and short-haired dachshund, while antigens Nos. 19 and 20 appeared not to be present in the extract from long-haired

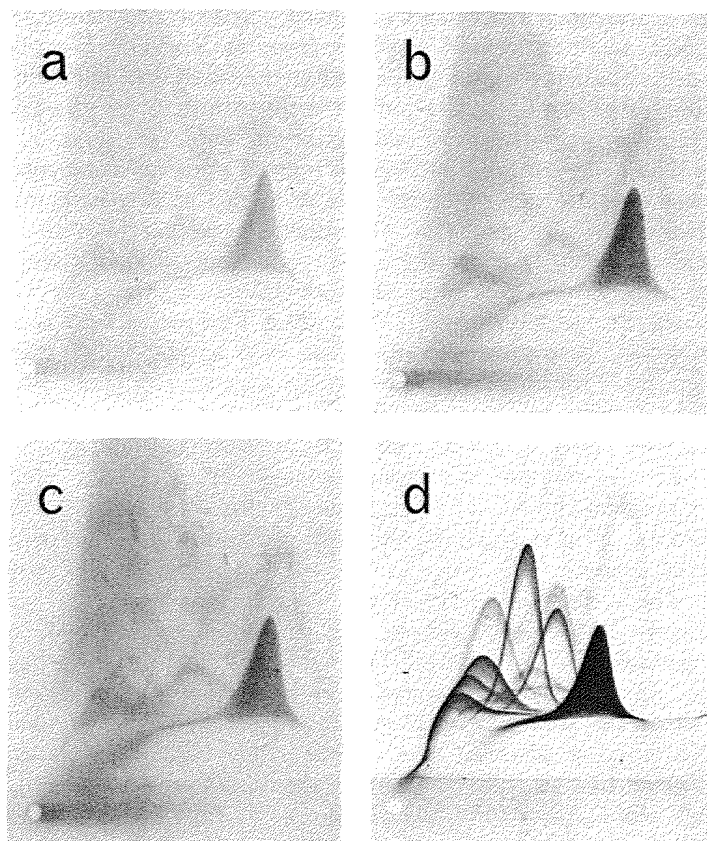


Fig. 7.

Autoradiographs of the crossed radioimmuno-electrophoreses plates for serum from patient No. 1. a) after 8 days exposure, b) after 29 days exposure, c) after 58 days exposure. For comparison d) CIE of St Hu 75.

dachshund, and antigen No. 20 could not be identified in extract from poodle.

Crossed radioimmuno-electrophoresis (CRIE) of extract of mixed dog hair and dandruff. In order to identify the aller-

gens present in the extract of mixed dog hair and dandruff CRIE was performed using sera from 21 RAST-St Hu 75-positive patients with dog allergy together with sera from one RAST-St Hu 75-negative patient with no history of allergy. Data for the patients are summarized in Table 3.

The 21 dog-allergic patients all showed a positive cutaneous reaction (skin index > 1 HE) to an extract of dog hair and dandruff. Furthermore, all the patients were allergic to allergens from other sources (Table 3). Seventeen dog-allergic patients showed allergy to other animal danders, and twelve of these were also allergic to house dust. Two patients (Nos. 4 and 18) showed allergy to house dust as the only other detectable allergy, whilst four patients were allergic to moulds, one of these being in addition allergic to house dust. Two patients (Nos. 15 and 21) showed allergy to grass pollen.

Five of the patients were hyposensitized: three with dog extract plus extract of *Cladosporium herbarum*, *mucor mucedo* and cat, respectively, and one with horse dust and one with grass pollen.

The results of CRIE of the 21 RAST-positive sera are shown in Table 3, where the patients are arranged in descending order of RAST (SU) towards St Hu 75. The sex and age of the patients together with total IgE are indicated. All of the sera contained specific IgE affinity for antigen No. 2 (dog albumin), the affinity being high in 20 cases. Twenty-five to fifty per cent of the sera contained specific IgE with affinity for antigens Nos. 9, 11, 13, 19, and 20, and a few sera contained specific IgE having affinity for antigens Nos. 1, 6, 14, 17, 18, 23 and 25. Finally, five sera possessed specific IgE with affinity for an antigen (X) which we were not able to detect in the reference pattern for St Hu 75.

The allergogram (the summed results of CRIE) for St Hu 75 is shown in Fig. 8. In three cases the control sera showed low affinity for antigen No. 2 (dog albumin), but no cases of affinity for any of the other antigens were observed. With the exception of one serum (No. 19) the CRIE plates of all of the 21 RAST-positive sera showed visible radio

TABLE 3
Data for 21 Sera Arranged in Descending Order of RAST to Extract of Mixed Dog Hair and Dandruff (St Hu 75).

Pt. No.	Sex	Age	Allergy to ¹	Hyposens. with extracts of	IgE (U ml ⁻¹)	RAST		Summed points of CRIE ²	IgE-binding to dog antigen No. ²																												Target organ
						SU	Class		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	X			
1	F	36	animal dander, house dust	house dust	3300	265	4	88	10	10				4		5			10	4			5		10			10	10		10	lung					
2	F	19	house dust, animal dander		2845	260	4	60	10	10				5		5		5	10					5				5			5	lung, nose					
3	F	20	cow, house dust		251	260	4	49		10							4		10	10				5	5						5	lung					
4	F	23	house dust		804	260	4	42	4	10									10	10				4	4								nose, lung				
5	F	30	cat, cow, horse, house dust		240	195	4	38		10									10	10				4	4								nose, lung				
6	F	24	cat, mould fungus, house dust		570	190	4	44		10									10	10			5		4						5		lung				
7	M	23	cat, <i>Derm. ph.</i> , house dust		1180	170	4	15		10							5															lung, nose, eye					
8	M	34	cat, cow, horse, house dust	dog, <i>Clad. herb.</i>	3200	125	4	35	10	10							5			10												lung, nose, skin					
9	M	41	cat, house dust		190	115	4	28			10									5	5			4	4								lung				
10	F	49	cat, rabbit, goat, house dust, horse		165	112	4	10			10																						lung				
11	F	25	cat, horse, cow, <i>Clad. herb.</i> , <i>Asp. fum.</i>		12612	55	3	69	10	10								4		10	10			10			10				5		lung				
12	F	18	horse		2136	42	3	20			10										5		5										lung, nose, skin				
13	F	19	cow, horse, guinea pig, house dust		1030	29	3	10			10																						eye, nose (lung)				
14	F	24	<i>Mucer mucedo</i> , (cat) (horse) (cow)	dog, <i>Mucer mucedo</i>	434	22	3	10		10																						skin, lung, nose					
15	F	29	guinea pig, pollen	cat, dog	390	18	2	10		10																						nose, eye (lung)					
16	M	18	animal dander, house dust		3115	17	2	20			10									10													lung				
17	F		cat					2	10		10																						lung				
18	M	24	house dust		72	7	2	10			10																						lung				
19	M	33	house dust, alt. 71		240	6	2	5			5																						lung, nose				
20	M	27	cat, cow, horse, <i>Derm. ph.</i> , house dust		172	6	2	10			10																						lung				
21	M	46	cat, house dust, pollen	pollen	309		2	15		10									5													nose					
22	F	27	no history of allergy		72			5		5																						-					
23	F	27	no history of allergy					0																								-					
24	F	27	no history of allergy					5		5																						-					
25	F	27	no history of allergy					5		5																						-					

¹) Allergy to allergens from sources other than mixed dog hair and dandruff.²) Summed specific IgE-binding.

^a) Specific IgE-binding to the individual antigens of mixed dog hair and dandruff.

(cf. Fig. 3) - semiquantified as described in the text.

staining after 8 days. Seven of the sera revealed affinity, for antigen, No. 2 alone.

Fig. 7 shows autoradiography of the CRIE plate for serum from patient No. 1 after 8, 21, and 28 days of film exposure respectively, together with the reference pattern. A significant linear correlation between specific IgE-binding in RAST (SU) and that in CRIE (summed points), was observed. $r=0.7705$, $P < 0.001$.

No linear correlation between specific IgE-binding in RAST and total IgE, or between summed CRIE and total IgE was observed.

DISCUSSION

Characterization of St Hu 75. The CIE reference precipitation pattern for St Hu 75 revealed the presence of 25 antigens. Some of these appeared to be partially identical, reducing the number to 21 distinct antigens. All the antigens except Nos. 1, 10, 11, 13, and 17 were shown to be serum proteins and of these six were identified: No. 2 as dog albumin and Nos. 9, 14 and 20 and 23 as α_1 -antitrypsin, haptoglobin and immunoglobulin, respectively. Antigens Nos. 2 and 13 appeared from CRIE (see above) to be major allergens, while antigens Nos. 1, 9, 11, 19, and X (the latter to be discussed later) are minor allergens. Of these seven, all but antigens Nos. 1, 11, and 13 were found to be serum proteins. The discrepancy between the results obtained by CLIE with dog serum incorporated in the line gel and those obtained by CIE of dog serum, the former analysis, indicating a higher content of serum proteins in St Hu 75 than the latter, could be due to the presence in St Hu 75 of degradation products of serum proteins.

The molecular weights estimated in the present work for antigens Nos. 23, 14, 2, and 9 are close to the molecular weights of the corresponding human serum proteins (immunoglobulin, haptoglobin, albumin and α_1 -antitrypsin, to which these antigens were partially identical. Ceska (7) determined

the molecular weight range of the allergens of dog hair to be 17,000–100,000 dalton. Varga & Ceska (22) found a molecular weight of 65,000 dalton for one dog allergen (albumin) whilst Glovsky et al. (9) found the allergenic activity of dog extract to be associated with allergens in the molecular weight range 20–45,000 dalton. These results are consistent with our findings that both the major allergens (antigens Nos. 2 and 13) have $3 \times 10^4 < \text{mol. wt.} < 10^5$.

Comparison of the extract of mixed dog hair and dandruff with sera, with extracts of hair and dandruff from other animals, and with house dust. Partial identity amongst antigens in various animal dandruff extracts has been demonstrated previously both by means of the Ouchterlony technique and by quantitative immunoelectrophoresis. A recently published study of horse dandruff extract (18), demonstrated the common antigens to include defined horse allergens. The present study demonstrates the partial identity of antigens of St Hu 75 with antigens in hair and dandruff extracts and serum proteins from several animals. This partial identity also extends to include defined allergens as antigens Nos. 2, 9, 11, and 20.

The highest degree of partial identity was found with dog and cat serum. This is in agreement with recently published work by Ohman et al. (20) who used a method involving inhibition of patient-specific IgE antibodies with dog and cat serum bound to cellulose particles. This indicated cross-reaction between the sera. These authors further demonstrated that the inhibition was due to proteins with albumin- and γ -mobility which agrees with our results.

Incorporation of house dust shows in comparison with St Hu 75 content of (partial identity with) antigens Nos. 1, 2, (9), 10, 11, 12, and 13. House dust has previously been shown by means of CRIE (Weeke, Løwenstein & Venov, unpublished results) to contain allergens from both animal danders and mites (*Dermatophagoides pteronyssinus*). The finding of animal danders in house dust makes the validity of hyposensitisation treatment using an extract of house dust

somewhat doubtful. As discussed in the above, St Hu 75 seems to contain a number of serum proteins. This is interesting because it has recently been shown (18) that horse extract does not contain any of these, apart from albumin. This could of course be explained by the use of different methods for obtaining the hair and dandruff material, the horse hair and dandruff being obtained by "vacuum cleaning", and the dog hair and dandruff material by cutting and scraping the upperside of the skin. The amount of sweat and the microflora present also differ from animal to animal. That serum proteins play an important role in allergy to dogs seems, however, to be demonstrated by our CRIE results and by other studies (20, 26) as mentioned earlier.

Comparison of the extract of dog hair and dandruff from the individual breeds with the mixed extract St Hu 75. A quantitative and qualitative variation in the antigen content of the extracts from the different dog breeds was found. However, each of the defined allergens except antigens Nos. 19 and 20 were common to all the breeds investigated.

Judged by the quantitative immunoelectrophoretic results, the antigen content in the extract from the short-haired races was much greater than in that from the long-haired ones. It has recently been shown (15) that solubilized horse hair does not contribute to the allergenic activity of an extract of horse hair and dandruff. The phenomenon demonstrated in our study might then be explained as a concentration phenomenon, there being more scales (dandruff) relative to hair in the short-haired races.

The present study did not reveal with certainty any breed-specific allergens, such as were indicated in earlier studies (8), but has revealed variations in the relative concentrations of antigens and allergens, a phenomenon which perhaps can explain the well-known clinical experience that dog-allergic patients react to only some dogs and not to others.

Identification of the allergens in St Hu 75 by means of CRIE. CRIE demonstrates that particularly antigens Nos. 2 (albumin) and 13 show a great affinity for specific IgE from

the sera of the RAST-positive patients. Twenty-five to fifty per cent of these sera contained specific IgE adsorbed to the immunoprecipitates 1, 9, 11, 19, 20, and X. Antigens Nos. 2 and 13, and antigens Nos. 1, 9, 11, 19, 20, and X are consequently considered as major and minor allergens, respectively.

CRIE also revealed a new immunoprecipitate (X) which was not visible after staining with Coomassie Brilliant Blue. However, use of ^{125}I -labelled antigens may increase the sensitivity of the CIE method by a factor of 10 (H. Løwenstein, unpublished results) an effect which can also be achieved by use of ^{125}I -anti-IgE.

CRIE of the four sera from the control patients showed that some of the IgE uptake of antigen No. 2 was due to non-specific adsorption. However, it is a well-known fact that albumin associates non-specifically with various proteins.

The CRIE method has been discussed thoroughly in two previous publications (12, 23) and has been shown to be a reliable method for identifying allergens. In these studies control experiments involving inhibition of the specific IgE-binding to the allergen extract employed in RAST and CRIE were performed by adding increasing amounts of the allergen extract to the serum before analysis in RAST and CRIE. In both these studies a decreasing RAST positivity was observed.

Four of the allergens (Nos. 2, 9, 19, and 20) were serum proteins: No. 2 (albumin) was a major allergen, and Nos. 9 and 20 (α_1 -antitrypsin and IgG, respectively) were minor allergens. Another minor allergen, No. 19, present in dog serum, was not identified. However, some minor allergens, Nos. 1, 11, 13, and X, appeared not to be serum proteins.

Earlier studies (22, 20) of dog allergen extracts using gel filtration, polyacrylamide gel electrophoresis, immunodiffusion and radioimmunoassay indicated that serum proteins are responsible for a certain amount of the allergenic activity of the extracts. Dog albumin was found to be the most important allergen, but a protein with γ -mobility was also found. Glow-sky et al. (9) have recently obtained similar results in a study

of dog danders by immunoelectrophoresis using whole serum dog antibodies. Thus, the results of our study are in full agreement with earlier reports concerning allergen identification apart from the fact that we have found at least two more serum proteins which act as allergens. One of these, No. 9, has been identified as α_1 -antitrypsin. Furthermore, four non-serum proteins have been shown to be allergens.

Further work concerning the purification and characterization of these allergens is presently in progress, the aim being to prepare a more specific extract of mixed dog hair and dandruff which is free of as many non-allergenic components as possible.

SUMMARY

An extract of mixed dog hair and dandruff from six different dog breeds (alsatian, boxer, collie, poodle, and long-haired and short-haired dachshund) was obtained by mild extraction, centrifugation, dialysis and freeze-drying. Extract of hair and dandruff from the individual dog breeds was obtained in the same way, but the material was not freeze-dried. Examination and characterization of the mixed extract by means of crossed immunoelectrophoresis revealed a precipitation pattern composed of 25 antigens, some of which were mutually partially identical, and a high content of dog serum proteins was found. Quantitative and qualitative differences between the individual dog breeds were demonstrated. Partial identity of the antigens of the mixed extract with antigens of serum, antigens of extracts of hair and dandruff from cat, cow, horse and guinea pig, and antigens from extract of house dust was also observed.

By means of crossed radioimmunoelectrophoresis, using sera from 21 patients who were RAST-positive to dog hair and dandruff extract, the specific IgE-binding to antigens of the mixed extract was examined. On the basis of these results major and minor allergens were identified. Dog albumin was found to be a very important major allergen, but α_1 -antitrypsin

and γ -globulin were also identified. Furthermore, four non-serum proteins were shown to be allergens. No breed-specific allergens could be identified in the extracts from the individual dog breeds.

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