

Quantitative Immuno-electrophoretic Analysis of Extract from Cow Hair and Dander

Characterization of the Antigens and Identification of the Allergens

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Quantitative immuno-electrophoresis used for the analysis of a dialysed, centrifuged and freeze-dried extract from cow hair and dander revealed 17 antigens. Five of these were identified as serum proteins. Partial identity to antigens of serum and extract from hair and dander of goat, sheep, swine, horse, dog, cat and guinea pig, and to antigens of house dust was demonstrated. Sera from 36 patients with manifest allergy to cow hair and dander selected on the basis of case history, RAST, skin and provocation test, were examined in crossed radioimmuno-electrophoresis (CRIE); sera from five persons with high serum IgE, but without allergy to cow hair and dander, and sera from five normal individuals were controls. 31/36 of the sera contained IgE with specific affinity for two of the antigens of the extract. Further, two major and six minor allergens were identified. The control sera showed no specific IgE binding. A significant positive correlation was found between RAST and CRIE for the first group of patients. The approximate molecular weights of the four major allergens obtained by means of gel chromatography were: 2.4×10^4 , 2×10^4 , 2×10^5 dalton, respectively. Using Con-A and Con-A Sepharose in crossed immunoaffino-electrophoresis, eight of the antigens were revealed to contain groups with affinity for Con-A.

Key words: allergens; characterization; identification; cow dander.

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Mammalian hair and dander are frequently the provoking agents of allergic reactions in man. Extracts of horse (21, 19 and 20) and dog (8) hair and dander have recently been characterized by means of various methods based on RAST and quantitative immuno-electrophoresis. In contrast, only little is known about the composition and potency of extract of cow hair and dander, one of the most frequent offenders seen, not only in the country but also in urban areas.

In a study on milk antigens from bovine whey, 10 of the antigens were found to be in

common with extract of cow hair and dander (18). The purpose of this study has been:

1. To carry out a physical and immunochemical investigation of the extract.
2. To identify the major and minor allergens in order to evaluate future work concerning the isolation of the most important allergens.
3. To compare the allergens of the extract with allergens from related sources.

MATERIAL AND METHODS

The antigens

Extract of cow hair and dander. The extraction was essentially performed as described in (21). Hair and dander were obtained from black and white cattle by simple cutting and were stored at -20°C .

The extraction was performed by gently shaking a 10% (w/v) solution of hair and dander in 0.1 M sodium bicarbonate and 0.015 M sodium azide for 24 h at 5°C . After filtration at 5°C and centrifugation at 0°C the supernatant was dialysed once against a 50-fold volume of 0.025 M ammonium bicarbonate for 24 h and once against a 75-fold volume of 0.005 M ammonium bicarbonate for 48 h. The dialysed extract was freeze-dried and stored dry at 5°C . A 1% (w/v) solution of this extract in 0.1 M sodium bicarbonate and 0.015 M sodium azide, denoted St. C-75, was used for the analysis.

Extract from cow hair and dander obtained from an equal mixture of four different cow races (black and white cattle, red Danish milk breed, Danish Jersey breed and Charolais), was produced in essentially the same way as the freeze-dried product mentioned above. Extract from cow hair and dander for skin and provocation tests and for immunotherapy was obtained from Allergologisk Laboratorium A/S, Copenhagen, Denmark. The protein content of the dissolved extract was determined by the method of Biuret (6).

Other extracts. A number of animal pelts (1:10 w/v), were kindly supplied by Allergologisk Laboratorium A/S, Copenhagen, Denmark. Animal sera were obtained from The Royal Veterinary and Agricultural College, Copenhagen. Bovine whey was isolated from fresh skim milk (18). Human serum was a 1.000 donor serum pool (28).

The antibodies

Antibodies against St. C-75 were raised in three rabbits and purified according to

Harboe & Ingild (16). Antibodies against human serum proteins and bovine whey were obtained from Dakopatts A/S, Copenhagen, Denmark. ^{125}I labelled anti-IgE was obtained from Pharmacia Phadebas®, Uppsala, Sweden.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed essentially as described in (27) using 1% w/v agarose in barbital buffer, batch No. ADS 102 DY (pH 8.6 at 25° , ionic strength 0.02) at 10 V cm^{-1} for 50 min, fixed in picric acid and stained with Coomassie Brilliant Blue®.

Immuno-electrophoretic methods

Unless otherwise indicated, equipment, reagents and general procedures were as described in (26). Agarose batch No. AGS 102 DY (Litex, Glostrup, Denmark) gel 1% w/v in Tris barbital buffer (Tris 0.073 M, barbital: 0.023 M, calcium lactate: 0.006 M, sodium azide 0.0003 M, pH 8.60 at 20° , ionic strength: 0.025) was chosen for the electrophoresis, crossed immuno-electrophoresis, crossed immuno-electrophoresis with intermediate gel, crossed line immuno-electrophoresis, crossed immuno-electrophoresis with addition of antigen and fused-rocket immuno-electrophoresis were performed respectively as described in (26,3,25).

The thickness of the first-dimension gel, the intermediate gel and the second-dimension gel was 0.15, 0.13 and 0.10 cm, respectively. The first-dimension electrophoresis was performed at 15° for 30 min at 10 V cm^{-1} , the second at 15° for 18 h at 2 V cm^{-1} . In order to avoid proteolytic degradation 1% (relative to antibody volume) of Aprotinin (Novo, Mainz, West Germany) was added to the antibody-containing gel (7).

Affinoimmuno-electrophoresis. Free concanavalin A and concanavalin A coupled to Sepharose were obtained from Pharmacia Fine Chemicals®, Uppsala, Sweden. Con-A is a lectin from jack beans with specific binding properties towards glycoproteins and

polysaccharides containing terminal α -D-glucose or α -D-mannose groups. The equipment, reagents and general procedures were as described in (10, 11, 12).

RAST

(Radioallergosorbent test) (32) was performed as described by Cheska et al. (14), using immobilized St. C-75 as allergosorbent. The results were expressed in sorbent units (SU) and in allergy classes (classes 0, 1, 2, 3 and 4 corresponding to <2 SU, <4 SU, <20 SU, <100 SU and ≥ 100 SU, respectively). Serum from a patient with allergy to timothy, diluted 16 times with incubation buffer, was used as 100 SU reference. This grading system corresponds to Pharmacia Phadebas RAST reference (birch allergen and serum from a patient with allergy to birch pollen).

In order to reveal the occurrence of blocking antibodies the RAST was carried out with undiluted sera and sera diluted 1 + 9 (31).

RIST (Radioimmunosorbent test) was used to determine the serum concentration of IgE. Using Phadebas IgE (Pharmacia, Uppsala, Sweden) and a WHO-standard serum, the IgE concentration was expressed in U/ml (1 U corresponds to 2.4 ng) (4, 23). Values of 30–500 U ml⁻¹ and 16–400 U ml⁻¹ were used as normal range in adults and children respectively (5); based upon double determinations the error of the actual analysis was 6% relatively.

CRIE (crossed radioimmuno-electrophoresis) and autoradiography was performed as described in (19, 29, 30). Considering the radioactive decay of ¹²⁵I, the periods of exposure were 2 (100), 5 (40), 10 (20), 15 (13), and 25 (8) days. The normalized reciprocal values of the corrected exposure time are indicated in parentheses. The specific IgE affinity to the different precipitates was given points according to the exposure time necessary to give a visible radio-staining.

Molecular weight determination by means of gel chromatography

Chromatography was performed at 5°C. A constant flow through the column was ensured by a peristaltic pump. The transmittance of the eluate, which was collected by a fraction collector, was monitored continuously at 280 nm. AcA 54 and AcA 34 Ultrogel® (LKB, Sweden) were used for the chromatography. The packed columns (K 16/100, 2 cm² × 100 cm (Pharmacia, Sweden), were pre-equilibrated with the elution buffer (sodium phosphate 0.1 mol/l, sodium chloride 0.1 mol/l and sodium azide 15 mmol/l, pH 7.2). The elution was performed at a flow rate of 4 ml/h. The following mixtures, made up to a total volume of 1 ml in the elution buffer, were used for the chromatography.

- St. C-75 (50 mg), Blue Dextran® (1 mg) and sodium chloride (60 mg)
- Human serum (400 μ l), Blue Dextran (1 mg), sodium chloride (60 mg)
- α -Chymotrypsinogen A (2 mg), myoglobin (2 mg) and cytochrome C (2 mg) (Sigma®, U.S.A.), Blue Dextran (1 mg) and sodium chloride (60 mg). Samples b and c were used as references for determination of the relationship between elution volume and molecular weight. The elution profile of sample c was based on the transmittance diagram. Using appropriate antibodies the elution profiles of samples a and b were obtained by means of fused-rocket immunoelectrophoresis. By means of crossed immunoelectrophoresis with line gels containing selected fractions, the antigens of mixture a were identified. The elution volume of the following serum proteins was used for calibration curves: α_2 -macroglobulin, albumin, prealbumin, Gc-globulin, α , antitrypsin and orosomucoid; void volume and total volume were determined from measurement of u.v.-absorption at 630 nm and conductance, respectively.

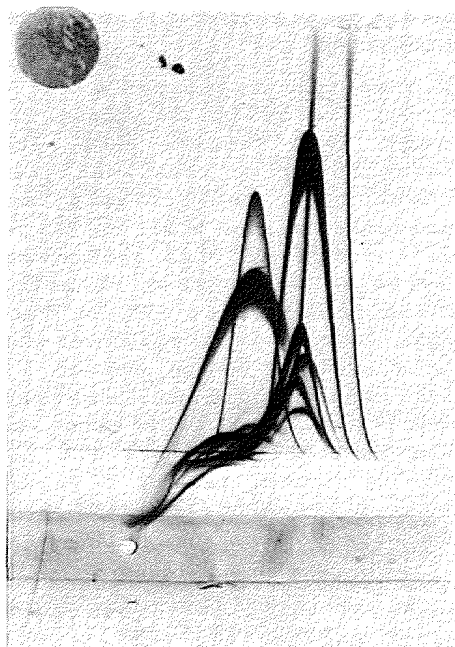
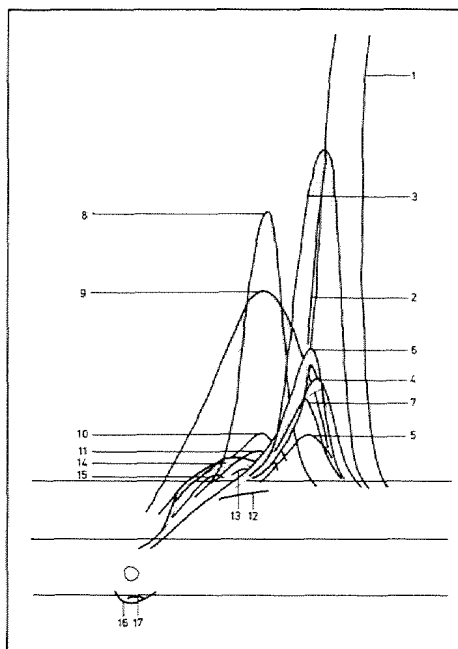


Fig. 1. A. Crossed immunoelectrophoresis of extract from cow hair and dander (St. C-75). Antigen: 5 μ l St. C-75. Buffer: Tris 0.073 M, barbital 0.024 M, calcium lactate 0.006 M, sodium azide 0.0003 M (pH 8.6, 20°C), I: 0.025. Gel: 1% (w/v) agarose gel containing the buffer. First dimension: 0.15 cm thick gel 30 min., 10 V/cm at 15°C, anode to the right. Second dimension: 0.13 cm thick intermediate gel containing no antibodies.



Below the first dimension and above the intermediate gel, a 0.10 cm thick antibody-containing gel was poured in (rabbit antibodies against Sg. C-75, 8 μ l/cm²). Anode uppermost.

B. Drawing of the same crossed immunoelectrophoretic precipitation pattern. Classification numbers for the precipitates are indicated.

Patients

Serum from patients with manifest allergy to cow hair and dander, selected on the basis of case history, skin and provocation test, were obtained from:

1. Allergy Departments TA (11 adults) and TG (12 children) out-patient clinic, Rigshospitalet.
2. Allergy out-patient clinic, Children's Hospital, Fuglebakken (10 children).
3. Allergy out-patient clinic, Copenhagen County Hospital, Lyngby (three adults). For comparison, sera from five persons with high serum IgE, but without allergy to cow hair and dander and sera from five

persons without any history of allergy were included in the study. Sixteen of the patients had been treated with immunotherapy for 1 to 4 years.

Skin test, provocation test and immunotherapy

Using "Soluprik", Allergologisk Laboratorium A/S, Copenhagen, Denmark, the skin tests were performed as prick tests and read according to Aas & Belin (2) on all of the patients except the 12 children from department TG allergy out-patient clinic, who underwent a scratch test. The bronchial and nasal provocation tests were carried out as described in (1). Thirty of the patients had been challenged with extract from cow hair

and dander; 29 were positive, 16 of them by bronchial provocation (10 patients were positive with extract strength 1:100 and 6 with 1:1000 v/w) and 13 by nasal provocation. One had negative bronchial provocation test; the case history indicated allergy to cow hair.

RESULTS

The protein content of St. C-75 was determined as 10.4 g/l.

Electrophoresis and crossed immunoelectrophoresis of St. C-75

In agarose gel electrophoresis all the proteins of St. C-75 migrated against the anode showing two bands with a velocity greater than human albumin.

Figure 1A shows the standard reference picture of the precipitates generated in crossed immunoelectrophoresis with St. C-75 against rabbit antibodies to this antigen.

Figure 1B is a drawing of the same precipitation pattern. The precipitates were given numbers arbitrarily from 1 to 17.

Antigens 16 and 17 which were moving towards the cathode were found only during the first 18 months after the preparation of the standard extract St. C-75.

Extracts from related sources

St. C-75 was compared with extracts from related sources by means of crossed immunoelectrophoresis with addition of antigens and by means of intermediate gel and crossed line immunoelectrophoresis. The results are given in Table 1. Antigen 7 was common to all except horse and guinea pig hair and dander. Extract from goat and sheep pelts contained antigens which showed partial identity with almost all of the antigens in St. C-75. Antigens 1, 2 and 3 showed partial identity with antigens from extract of cat hair and scale, antigens 1, 3 and 5 were found in extract of house dust as well. Bovine whey showed identity as for antigens 3, 7, 14 and 15. When St. C-75 and extract of guinea pig hair and scale were compared by means of the addition technique in crossed immunoelectrophoresis, a fan-like tail precipitate was seen above the application hole of the first dimension. This

Table 1
Comparison between extracts of cow hair and dander and other allergens of animal origin

Allergen sources	1*	2	3*	4	5	6*	7	8	9	10	11	12	13	14*	15	16	17
1 Cow serum							x			x		x	x	x	x		
2 Bovine whey			x				x								x	x	
3 Goat hair and dander	x	x	x	x	x	x	x	x	x	x	x	x	x				
4 Goat serum						x			x				x	x			
5 Sheep hair and dander	x		x	x	x	x	x	x	x	x	x				x		
6 Sheep serum							x			x					x	x	
7 Swine hair and dander							x								x		
8 Swine serum							x								x		
9 Horse hair and dander																	
10 Horse serum							x			x							
11 Dog hair and dander							x			x							
12 Dog serum							x										
13 Cat hair and dander	x	x	(x)				x										
14 Cat serum							x										
15 Guinea pig hair and dander	(x)		x														
16 Human serum							x			x		x			x	x	
17 House dust	(x)	(x)		x													
18 House dust mites																	

* Major allergens. Parentheses mean low degree of partial identity.

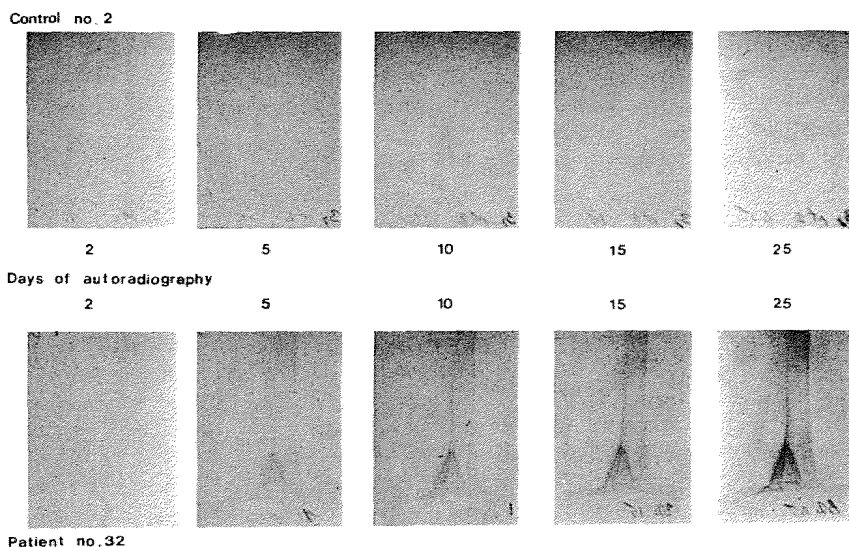


Fig. 2. Autoradiographs of the crossed radioimmuno-electrophoresis (CRIE) from control 2 and patient 32 with allergy to cow hair and dander. The native precipitates of crossed immunoelectrophoretic reference plates were allowed to react with IgE from patients' sera, washed and subsequently reacted with ^{125}I -anti IgE, washed and autoradiography performed for various spaces of time.

Table 2
Characterization of 15 allergens in an extract from cow hair and dander

Precipitate no.	Migration velocity relative to human albumin	Approximate mol. wt.	Serum proteins of extract
1*	1.90	2.4×10^4	
2			
3*	1.81	2.0×10^4	
4	1.75	2.3×10^4	
5	1.72	4.0×10^4	
6*	1.72	7.2×10^5	
7	1.64	6.5×10^4	albumin
8	1.39	3.0×10^4	
9	1.39	3.5×10^4	
10	1.39		transferrin
11	1.39		
12	1.39		α_2 -macroglobulin
13	1.39		
14*	0.94	72×10^5	IgG
15	0.94		IgG, IgA
16	0.00		
17	0.00		

precipitate was not seen with guinea pig alone against anti-St. C-75 in crossed immunoelectrophoresis. Antigens 7, 10, 12, 13, 14 and 15 were seen in cow serum. Precipitates 7, 11, 12, 14 and 15 showed partial identity with human serum proteins. These results are given in Table 2. Using crossed immunoelectrophoresis with monospecific antibodies against human serum proteins in the intermediate gel, the following antigens in St. C-75 were identified: no. 7 as albumin, no. 12 as α_2 -macroglobulin, no. 10 as transferrin, nos. 14 and 15 as IgG and no. 15 as IgA.

A mixture of extracts of hair and dander from four different cow races was compared with the St. C-75 by means of crossed immunoelectrophoresis. Apart from two additional high precipitates in the area between precipitates 1 and 3, only a few minor quantitative differences were demonstrated: precipitate 3 was much lower and precipitate 9 a little lower in the mixture than in St. C-75, while 4 and 5 were somewhat higher in St. C-75. The commercially available extract of cow hair and dander that had been used in skin tests and provocation tests was compared with St. C-75 in crossed immunoelectrophoresis. Only quantitative differences were shown. The former showed higher concentration of antigens 6 and 9 relative to the standard.

No contamination from house dust mites was found as determined by means of crossed immunoelectrophoresis. The hair and dander St. C-75 extracted contained a lot of different moulds. The significance of this is being investigated (15).

Identification of the allergens

Crossed radioimmuno-electrophoresis (CRIE). The arbitrarily semiquantitative measure of specific IgE in the sera against the individual antigens of St. C-75 was determined by means of CRIE and is listed in Tables 3 and 4 together with the summed points of CRIE for the different controls and patients. All of the sera from the five normal nonallergic

persons and from the five patients with high serum IgE and with other allergies than allergy to cow hair and dander, showed visible radio-staining after 25 days. As one of the normal control sera also showed staining after 15 days, only autoradiographs after 2, 5 and 10 days of exposure were estimated. Visible staining after 2 and 5 days was defined as high affinity and after 10 days low affinity.

As indicated in Table 4, the sera from 33/36 patients with allergy against cow hair and dander contained specific IgE against the antigens of St. C-75; 31 of the sera contained specific IgE against antigens 1 and 3 (23 showed high affinity and eight low affinity). Nineteen sera contained specific IgE against no. 6 (14 of these showed high affinity and five low affinity), and 19 against antigen 14 (12 of these showed high affinity, seven low affinity). Consequently, these four antigens are considered to represent the major allergens of the extract. In addition, a few sera contained IgE against antigens 4, 7, 8, 9, 11 and 13 which are the minor allergens.

RAST and RIST

The RAST and RIST values for the controls and the individual patients are given in Tables 3 and 4 arranged in descending order of RAST to cow. All of the patients in the control group were in RAST allergy class 0; two of the 36 patients with allergy to cow were in class 3, 17/36 in class 2, 9/36 in class 1 and 8/36 in class 0. 25/36 of the patients showed elevated levels of IgE in serum.

A significant positive correlation was found between RAST expressed in SU, and CRIE expressed in summed points: Spearman coefficient = 0.609 ($P < 0.001$). The correlation between CRIE and total serum IgE expressed in U/ml was less significant: Spearman coefficient = 0.260 ($P < 0.05$). Comparing RIST and RAST the coefficient was 0.474 ($P < 0.001$). We were not able to show any connection between the results obtained in RAST, RIST or CRIE, and previously performed immunotherapy.

ALLERGOGRAM COW HAIR AND DANDER

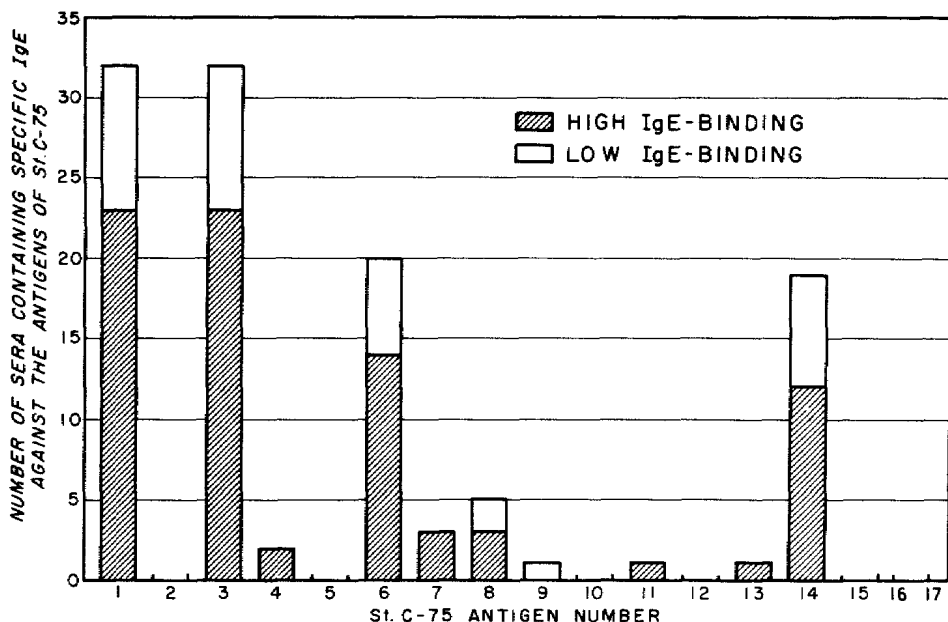


Fig. 3. Allergogram for antigens of cow hair and dander based upon sera from 36 patients allergic to cow hair and dander. Specific for IgE binding to individual antigens was obtained by means of CRIE.

The radioactivity bound to the precipitates was evaluated arbitrarily. Radiostaining visible after 2 or 5 days was taken as high degree of IgE binding and after 10 days as low degree of IgE binding. Visible precipitates after 15 or 25 days only was considered as unspecific and consequently not included. 2 days=100 points, 5 days=40 points, 10 days=20 points.

Approximate molecular weight of the allergens

By means of gel chromatography, the approximate molecular weight of the four major allergens was obtained. No. 1, 2.4×10^4 ; no. 3, 2.0×10^4 ; no. 6, $>2 \times 10^5$; no. 14, $>2 \times 10^5$ D. Besides these, some of the other allergens were also identified by this technique. The individual approximate molecular weights are listed in Table 2.

Concanavalin-A

When Con-A was used in the first-dimension gel, precipitate 1 was shown to consist of three precipitates of which one appeared in a more cathodic position. Precipitate 6 showed a cathodic shift too, while 8 and 13 were completely retarded in the first-dimension gel. Using Con-A and Con-A Sepharose in an intermediate gel, the following antigens

showed affinity for Con-A: 6, 8, 11, 12, 13 and 15.

DISCUSSION

Using Agarose gel electrophoresis, the major part of St. C-75 was shown to have a velocity greater than human albumin. This agrees with the observations made by Osváth et al. (22) using polyacrylamide gel electrophoresis. Using extracts of horse hair and dander, similar results were obtained (21). Using crossed immunoelectrophoresis the extract was shown to consist of 17 precipitates, two of which were moving towards the cathode.

St. C-75 contained seven serum proteins. Five of these were identified as: albumin, α_2 -macroglobulin, transferrin, IgG and IgA. This is very similar to the findings of

Table 3

Data concerning five normal persons without any history of allergy, and five persons with allergy and increased serum IgE without allergy to cow

No.	Sex	Age (years)	Allergy to	Anamnesis indicating allergy to cow	Skin test ^a Cow	Provocation test Bronch.	Nasal	IgE RIST U/ml	RAST		Allergy class SU UD D ^b 1:10	Summed points from CRIE ^c	Points in CRIE of the individual precipitates																
									UD	D			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	F	28	no history of allergy	neg.	not done	not done	not done	126	1	1	0	0	—	13	13	8	8												8
2	M	24	no history of allergy	neg.	not done	not done	not done	105	1	1	0	0	—	—	8	8	8												8
3	M	36	no history of allergy	neg.	not done	not done	not done	32	1	1	0	0	—	—	8	8													
4	F	30	no history of allergy	neg.	not done	not done	not done	49	1	1	0	0	—	—	8	8													
5	M	36	no history of allergy	neg.	not done	not done	not done	90	1	1	0	0	—	—	8	8	8												
6	F	6	rabbit, dog, mould	neg.	neg.	not done	not done	1019	2	1	0	0	—	—	8	8	8												
7	F	9	mites, dog	neg.	neg.	not done	not done	1180	3	1	1	0	—	—	8	8													
8	M	5	rabbit, cat, artemisia	neg.	neg.	not done	not done	1259	1	2	0	0	—	—	8	8	8												
9	F	34	mites	neg.	neg.	not done	not done	955	1	1	0	0	—	—	8	8	8												
10	F	12	housedust, cat, grass	neg.	neg.	not done	not done	1000	2	1	0	0	—	—	8	8	8												

^a Because of differences in the execution of the skin tests the results are given only as pos. or neg.^b D = diluted, UD = undiluted^c Points below 20 are considered as unspecific binding and are not included in the summed points.

Tables 4

Data concerning 36 persons with manifest allergy to cow

No.	Sex	Age (years)	Allergy to	Anamnesis indicating allergy to cow	Skin test cow ^a	Provocation test		Immu- notherapy IgE	RIS
						Bronch.	Nasal	(years)	U/ml
11	M	10	dog, cat	?	pos.	pos.	not done	—	119
12	F	12	dog, cat, horse, guinea pig, birch	?	pos.	not done	pos.	6	225
13	F	22	horse, dog, cat	pos.	pos.	not done	not done	—	14
14	F	11	horse, cat, guinea pig, house dust	?	pos.	pos.	not done	—	73
15	F	10	dog, cat, guinea pig, house dust	?	pos.	not done	pos.	2	199
16	F	6	horse, cat, dog, guinea pig	pos.	pos.	not done	not done	—	51
17	M	18	cat, guinea pig, goat, timothy	pos.	pos.	not done	not done	—	1
18	F	4	horse, dog, mould, house dust	?	pos.	pos.	not done	—	1
19	M	10	horse, goat	?	pos.	pos.	not done	—	41
20	M	18	horse, cat, dog, guinea pig	?	pos.	pos.	not done	2	46
21	M	5	horse, house dust, mould	?	pos.	pos.	not done	1	83
22	M	8	horse, dog, cat, guinea pig	?	pos.	not done	pos.	4	87
23	M	10	horse, cat, dog, timothy	?	pos.	pos.	not done	—	531
24	F	12	horse, cat, guinea pig	?	pos.	not done	pos.	½	16
25	F	17	dog	pos.	pos.	not done	not done	—	7
26	F	8	horse	pos.	pos.	not done	not done	2	5
27	F	13	horse	?	pos.	not done	pos.	4	48
28	M	12	horse, rabbit	?	pos.	not done	pos.	½	5
29	M	16	horse, cat, dog	?	pos.	pos.	not done	1	169
30	F	35	horse, sheep, mites, house dust, birch	pos.	pos.	not done	not done	½	17
31	F	6	dog, horse, cat, goat, house dust	?	pos.	pos.	not done	2	161
32	M	13	horse, dog, cat, mites, timothy	?	pos.	pos.	not done	—	176
33	F	13	horse, dog, cat, guinea pig	?	pos.	not done	pos.	—	65
34	F	11	horse, dog, cat, guinea pig, art.v.	?	pos.	pos.	not done	—	4
35	M	12	cat, house dust	?	pos.	pos.	not done	—	4
36	M	6	horse, dog, cat, house dust	pos.	pos.	neg.	not done	2	111
17	M	9	horse	?	pos.	not done	pos.	1	63
18	F	7	horse, grass pollen	?	pos.	not done	pos.	2	6
39	M	10	cat, mould	?	pos.	not done	pos.	3	162
40	M	37	0	?	pos.	pos.	not done	1	88
41	M	13	horse, dog, cat, timothy, birch	?	pos.	pos.	not done	3	75
42	M	9	dog, cat, house dust	?	pos.	pos.	not done	—	17
43	M	9	guinea pig, timothy, birch	?	pos.	not done	pos.	—	35
44	F	14	horse, cat, dog, guinea pig	?	pos.	not done	pos.	2z	527
45	M	10	horse	?	pos.	not done	pos.	½	214
46	1	31	horse, cat, goat	?	pos.	pos.	not done	1	64

^aBecause of differences in the execution of the skin tests the results are given only as pos. or ne^bD= diluted 1:10, UD undiluted.

RAST		Allergy class		Summed points		Points in CRIE of the individual precipitates																
0	SU	UD D1:10	UD D1:10	from CRIE	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
65	22	3	3	400	11	100	40	100	40						40		40	40				
34	5	3	2	80	12	40	40				8	8							13			
19	11	2	2	80	13	20	20		40													
16	2	2	1	280	14	100	100	8	40	13	13								40			
15	15	2	2	200	15	100	100		20											40		
12	6	2	2	—	16	8	8													8		
12	1	2	0	100	17	40	40		20	8												
12	4	2	1	140	18	40	100		13	8										8		
11	3	2	1	40	19	20	20		8											13		
9	4	2	1	320	20	100	100	40	40	40	13											
8	6	2	2	120	21	40	40		13	8	10											
8	5	2	2	140	22	40	40	8	8	20	13	8								40		
7	2	2	1	—	23	13	13		13											8		
6	2	2	1	380	24	100	100		40	40	40	20								40		
6	3	2	1	380	25	100	100	40	40	40			8							20		
5	4	2	1	40	26	30	20				8											
5	2	2	1	80	27	40	40		13	8												
4	2	2	1	120	28	40	40		40	8	8											
4	2	2	1	180	29	40	40		40	20										40		
3	2	1	0	120	30	40	40		40	13	8									13		
3	2	1	1	120	31	40	40	13	40	8										8		
3	1	1	0	80	32	20	20		13											8		
3	1	1	0	—	33	13	13		8											13		
3	3	1	1	160	34	40	40		40	13	13									40		
3	1	1	0	40	35	20	20		13											13		
3	3	1	1	140	36	40	40		20											40		
3	3	1	1	160	37	40	40		40	8	8									40		
3	4	1	1	200	38	40	40		40	40										40		
2	3	0	1	60	39	20	20		13											20		
2	2	0	0	120	40	40	40	8	13	20										20		
2	2	0	0	100	41	40	40	8	8	8										20		
1	1	0	0	—	42	8	8		8											8		
1	3	0	1	160	43	40	40	13	40	13	13	13								40		
1	2	0	1	60	44	20	20													20		
1	1	0	0	80	45	20	20		20	8	13	8	8							20		
1	1	0	0	20	46	8	8		8											20		

^c Points below 20 are considered as unspecific binding and are not included in the summed points.

Løwenstein et al. (18) who showed 10 of the antigens of bovine whey, including the above-mentioned serum proteins, to be in common with those of cow hair and dander. In this study, which is based upon antibodies against cow hair and dander, we have shown only four common antigens.

As expected, a marked partial identity was shown between the antigens of St. C-75 and those of extract from goat and sheep hair and dander. Partial identity was found for antigen 7 of St. C-75 with extract from goat, cat, dog, sheep, and swine pelts.

Recently, partial identity among three antigens of an extract from horse hair and dander and extracts from dog, cat, cow and guinea pig pelts have been found by the same technique (21).

Crossed radioimmuno-electrophoresis (CRIE)

The method of CRIE has previously proved to be a valuable tool for identification of allergens in a complex protein mixture. Using this method, the specific serum IgE from 33 of 36 patients suspected to be allergic to cow hair and dander identified antigens 1, 3, 6 and 14 as the major allergens of St. C-75. Four of the patients suspected to be allergic to cow hair and dander showed only unspecific binding in CRIE. None of these had previously had immunotherapy. One had a positive inhalation provocation test with extract of cow hair and dander at 1:1000. One had a 4+ reaction in the scratch test. The third patient showed only a 1+ reaction in the scratch test. Only one showed RAST values indicating allergy to cow.

There are some sources of error: (1) subjectivity in the reading of the autoradiogram, (2) the extract may not contain all the allergens in cow hair and dander, (3) polyspecific antibodies may not be directed against all of the allergens in the extract, (4) unspecific binding of IgE to the precipitates, but we consider the CRIE to be very useful in the research into allergens and allergy.

Like Weeke et al. (30), we were unable to show low RAST-values and slight radio-staining in CRIE caused by possible blocking antibodies. The approximate molecular

weights of two of the most important allergens were between 20,000 and 25,000. Because of the different content of carbohydrate in the proteins, the estimated molecular weights are a little dubious. On the other hand, our results are in agreement with the results obtained by other techniques. Using ultracentrifuge, the average molecular weight of acetone-precipitated antigens of cow dander was 32,000 (24). By means of Sephadex gel thin-layer chromatography the active components of bovine hair extract proved to be substances having a molecular weight of about 20,000 (17).

Concanavalin-A.

The crossed affinoimmuno-electrophoretic analysis revealed antigens 1, 6, 8, 9, 11, 12, 13 and 15 to contain groups with affinity for Con-A. Antigen 1 was able to bind to Con-A and form three different complexes when the lectin was used in the first dimension, but showed no binding to Con-A when the latter was contained in the intermediate gel. The reason for this is now under current investigation. However, the separating power demonstrated for Con-A by means of crossed affinoimmuno-electrophoresis together with the separation obtained by size chromatography will serve as a guideline for future purification studies.

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