

Allergens in Cow Hair and Dander

Origin of Cow Allergens in the Environment

P. PRAHL

Laboratory of Clinical Allergology, Medical Department TA, Rigshospitalet, Copenhagen, Denmark

Quantitative immunoelectrophoretic methods were used for the analysis of the allergens in cow hair and dander and for comparison with related preparations. The immunoelectrophoretic precipitation pattern of an extract from cow hair and dander showed 17 precipitates. Crossed radioimmuno-electrophoresis showed four of these to be major allergens of the extract, and these allergens are common major allergens in four investigated cow breeds. The allergens are associated predominantly with hair and dander, but they were also demonstrated in cow saliva, urine, whey, amniotic fluid and beef, as well as in cow-hide products. Allergens displaying partial immunological identity with the major allergens of cow hair and dander were found in extracts from goat and sheep pelts, in products made of these materials such as carpet and knitting wool, and in carpets made of animal hair.

Key words: allergens; cow dander; environment; immunoelectrophoresis.

Accepted for publication 20 May 1981

CLINICAL ASPECTS

Identification and removal of the offending allergens from the allergic patient's environment is one of the major problems in clinical allergology. In this work the sources of the cow dander allergens are explored.

Many substances are capable of inducing immediate or delayed hypersensitivity reactions in man. Antigens which induce reactions mediated by specific IgE are designated as atopic allergens. Information concerning the nature of the active components of allergenic extracts is still fragmentary, despite the fact that human allergic disease arising from exposure to mammals has been recognized for more than a century. Allergy to epidermal allergens is presumed to be due to contact with skin or hair of various animals.

The allergens of an extract can be divided into major and minor allergens according to the number of patients reacting to them (12). Major allergens are highly active in the majority of allergic individuals, while IgE against the minor allergens is less frequently seen. However, in some patients the minor allergens

can be as active as the major allergens, or even more so (22).

The allergens of mammalian hair and dander can easily be extracted with aqueous solutions (6, 14, 22). They migrate towards the anode on electrophoresis (pH 8.6) and have isoelectric points below 7.0. The molecular weight of the major allergens are generally in the range of 20,000 to 40,000 dalton, while the molecular weights of the minor allergens are highly variable (4, 26).

Only little research has been carried out to determine the sources of mammalian allergens. Allergens of cow hair and dander often cause allergic asthma and rhinitis, and even contact dermatitis in man (23). A pronounced reaction of immunological partial identity has been shown between extract from cow hair and dander and extract from sheep and goat pelts (22).

The work discussed here was undertaken in order to investigate the occurrence of the allergens of cow hair and dander – not only in the cow but also in cow-hide products. Furthermore, allergens showing reactions of immunological identity with those of cow hair and dander have been sought in preparations from sheep and goat pelts. Using present-day immunological and immunochemical techniques, cow allergens and some of the cross-reacting proteins occurring in our environment have been identified and quantified.

MATERIAL AND METHODS

Extracts

The extracts were produced as described by Prahl et al. (22). The extraction was performed by gently shaking a 10% (w/v) solution of the materials to be extracted in 0.1 M sodium bicarbonate and 0.015 M sodium azide for 24 h at 5°C. After filtration, centrifugation and dialysis the extracts were freeze-dried and stored dry at 5°C. The freeze-dried extracts were dissolved in 0.1 M sodium bicarbonate and 0.015 M sodium azide when used for analysis in quantitative immunoelectrophoresis. An extract from hair and dander of black and white cattle, denoted St.C-75, was used as reference extract.

Extracts were prepared from the following material in order to seek allergens with partial immunological identity with those of St.C-75:

Extracts were made from *hair and dander* of red Danish milk breed, Danish Jersey, Charolais and black and white cattle, and further from dander obtained by scraping the skin and hair from the same cow. The hairs were freed from scales by vacuum cleaning the pelt.

Bovine urine, amniotic fluid, beef and saliva. Saliva was obtained from a fasting cow after i.m. injection by placing a receptacle under the mouth. *Bovine whey* was isolated from fresh skim milk (13). These materials were filtered, centrifuged, dialysed and freeze-dried.

Further extracts were made from the commercially available *cow-hide products*: suède, split,

nappe and calf pelt, and from the following *sheep wool products*: Berber, cut pile and Yak yarn for ordinary carpets (Wiltax, Copenhagen, Denmark. Yak yarn contains 20% yak ox wool). Knitting yarn: worsted-, unwashed carded-, ordinary carded-, and superwashed yarn. (The superwashed yarn is rendered shrink-resistant by application of a polymer to form a continuous film over the fibre surfaces). *Mohair* yarn (Turkish goat). An extract was also made from *sheep skin* manufactured for fur coats. Hair from mammals other than sheep is often used in carpets and carpet tiles. We made extracts from two types of carpet tiles containing mammalian hair: Heuga Felt, consisting of 95% hair (donkey, cow, goat and swine) and from Heuga Lux, which contains 40% hair (donkey and goat).

In order to test the amount of allergens given off by carpet tiles made of animal hair during use, the tiles were subjected to mechanical wear simulating ordinary "carpet life". The test was carried out by the Danish Institute for Textile Research (Dansk Textil Institut), Tåstrup. A tetrapod "walked" over the specimen fixed inside a drum, and the specimens were subjected to wear corresponding to 1–2 years of normal use. Worn off hair was picked up on a filter from a vacuum cleaner, weighed, and an extract made for further investigations.

The same vacuum cleaner was used to see if any allergens could be picked up from suède and sheep skin coats.

House dust samples from 26 Danish homes (19 urban and 7 rural) were obtained by vacuum cleaning. An aqueous extract was prepared according to Schwartz et al. (24).

Antibodies

Antibodies against a mixture of equal amounts of freeze-dried aqueous extracts from four different cow breeds (black and white cattle, red Danish milk breed, Danish Jersey breed and Charolais) were raised in three rabbits, pooled and purified according to Harboe & Ingild (11). Antibodies against human serum proteins and bovine whey were obtained from Dakopatts A/S, Copenhagen, Denmark.

Blood samples were obtained from patients with allergic asthma to cow. The diagnosis was based upon skin, RAST and provocation tests or on a positive case history. After clotting, serum was withdrawn and kept frozen (-20°C) until analysed in RAST and CRIE.

Quantitative immunoelectrophoretic methods

Unless otherwise indicated the equipment, reagents and general procedures for crossed immunoelectrophoresis (CIE), tandem crossed immunoelectrophoresis, crossed line immunoelectrophoresis, crossed immunoelectrophoresis with intermediate gel, and rocket immunoelectrophoresis were as described by Axelsen et al. (3). Agarose (Type HSA, Litex, Glostrup, Denmark) 1% (w/v) in Tris barbital buffer (Tris 0.073 M, barbital 0.023 M, calcium lactate 0.006 M, sodium azide 0.003 M, pH 8.6 at 20°C , ionic strength 0.025) was used for the electrophoresis plates. The first dimension was performed for 30 min at 10 V cm^{-1} and the second for 18 h at 2 V cm^{-1} , both at 15°C . In order to avoid proteolytic degradation, 1% (relative to antibody volume) of Aprotinin (Novo, Mainz, West Germany) was added to the antibodies (5).

Crossed line immunoelectrophoresis was performed to compare the major allergen content of different extracts with that of St.C-75. To do this CIE was run with St.C-75 against rabbit anti-cow hair and dander with known amounts of St.C-75 poured in line gels (0.001, 0.002, 0.005, 0.01, 0.02 and 0.05 mg/cm^{-2}) and a known amount of an extract to be compared with St.C-75 poured in another line gel. The lines of the major allergens were identified by the fusion occurring between the lines and the crossed immunoelectrophoretic peaks. By comparing the levels of the lines of the major allergens (Ag Nos. 1, 3, 6, 14) the content of the major allergens in the extracts was estimated.

In rocket immunoelectrophoresis the major allergens were identified by performing fused-rocket immunoelectrophoresis using isolated major allergens and the extracts to be examined. The comparison of the content of major allergens in

the extracts was based upon measurement of the length of the individual rockets.

Counter immunoelectrophoresis was performed according to Schwartz (24) with a 0.15-cm thick gel consisting of aliquots of HSA- and LSA agarose (Litex, Denmark) 1% (w/v) in Tris-barbital buffer used for ordinary immunoelectrophoresis. Two rows of wells were punched out opposite each other at a distance of 1 cm. In the anodic row 5 μl of rabbit antibodies against cow hair and dander were applied in each well. In order to avoid unwanted precipitation rabbit antibodies against serum proteins had been eliminated by immunoabsorption. In the cathodic row 5 μl aliquots of two-fold serial dilution of the different extracts were applied. The electrophoresis was run overnight at 2 V cm^{-1} and the gel was then pressed, dried and stained as usual. The titre was given as the reciprocal value of the highest dilution of the extract which still gave rise to a visible precipitate.

The approximate content of "cow major allergens" of the different investigated allergen sources (yarn, leather, etc.) was estimated relative to the amounts of the major allergens in cow hair and dander (raw material). This was done by comparing the amounts of freeze-dried extract made from known amounts of raw materials (i.e. the yield of extraction), combined with the information obtained from crossed line immunoelectrophoresis, rocket immunoelectrophoresis and counter immunoelectrophoresis concerning the amount of cow major allergens in the investigated extracts relative to St.C-75.

Crossed line radioimmunoelectrophoresis (CLRIE)

CLRIE and autoradiography were carried out as for CRIE (15, 29). This was done in order to examine binding of specific IgE in sera from patients with allergic asthma to cow, not only to the allergens of bovine origin, but also to cross-reacting proteins of other extracts. The latter were included in the line gel.

RAST-inhibition experiments (8, 9, 30) were carried out according to Nielsen et al. (17) with St.C-75 coupled to the paper discs. The sera to

be inhibited were the same as those used for CLRIE. Extracts from goat and sheep pelts served as inhibitors.

Skin tests were performed as prick tests with extracts from Allergologisk Lab., Copenhagen, Denmark using cow, goat and sheep extracts (1 HEP* potency) (2). The extracts were dissolved in glycerol/sodium chloride 0.15 M 1:1 (v/v). Histamine dihydrochloride (1 mg/ml⁻¹) served as positive- and glycerol/sodium chloride served as negative controls. The reaction in the prick test was calculated as the sum in mm of two mutually perpendicular diameters of the urticaria element.

Subjects for cutaneous tests were 10 patients with allergy to cow. The diagnosis was based upon a positive cutaneous test with cow (1 HEP), allergen-specific IgE (RAST class ≥ 3) and bronchial provocation test (1). Five persons without any history of allergy, with negative RAST to cow and with normal serum IgE served as controls.

RESULTS

The reference extract

The reference precipitation pattern of an extract of material from black and white cattle (St.C-75) showed 17 precipitates, of which two showed cathodic migration (Fig. 1). It has been shown previously by crossed radioimmuno-electrophoresis that four of the antigens are major allergens (Ag Nos. 1, 3, 6 and 14) (22).

The following experiments show that the quantitative immunoelectrophoretic methods seem suitable for comparing the content of major allergens in different materials from sheep and goat. Crossed line immunoelectrophoresis was performed with St.C-75 against rabbit anti-St.C-75 and with an extract from goat or sheep pelts in the line gel, or reversed using extracts

from goat or sheep pelts against rabbit anti-goat or sheep and with St.C-75 in the line gel. In both experiments, the lines and peaks for the major allergens showed equal staining intensity. Crossed line radioimmuno-electrophoresis was performed with St.C-75 against rabbit anti-cow and with extracts from goat, sheep, suède or sheep skin for fur coats in the line gel. Autoradiography showed equal staining of the peaks and lines of the major allergens. From these results we assume that the number of immunologically active sites in the lines and peaks are the same.

Breed specificity?

Extracts of material from four different breeds of cow (black and white cattle, red Danish milk breed, Danish Jersey breed and Charolais) were compared in crossed immunoelectrophoresis. Only minor differences were revealed. By means of tandem immunoelectrophoresis and crossed radioimmuno-electrophoresis the major allergens of the four extracts were shown to be immunologically identical. The commercially available Danish extract from cow hair and dander (Allergologisk Lab., Copenhagen) corresponded to St.C-75 with regard to the major allergens.

Hair or dander?

The precipitation patterns of extracts from dander and from vacuum cleaned hair were identical. The extraction yield was five times greater from dander than from hair. This difference was further confirmed by placing five hairs of equal length from black and white cattle in an agarose gel and performing immunoelectrophoresis at right angles to the hair (Fig. 2). As a result of the greater content of allergens close to the skin the precipitates are higher at this end.

Bovine materials

The major allergens of cow hair and dander were identified in bovine *whey*, *saliva*, *urine*, *amniotic fluid* and, surprisingly enough, in *beef*.

* A 1 HEP potency means that an allergen extract having this biological activity elicits a prick test reaction in most individuals who are allergic to the material, and on average produces a reaction identical to that obtained by a similar prick test using histamine hydrochloride (HCl) 1 mg/ml.

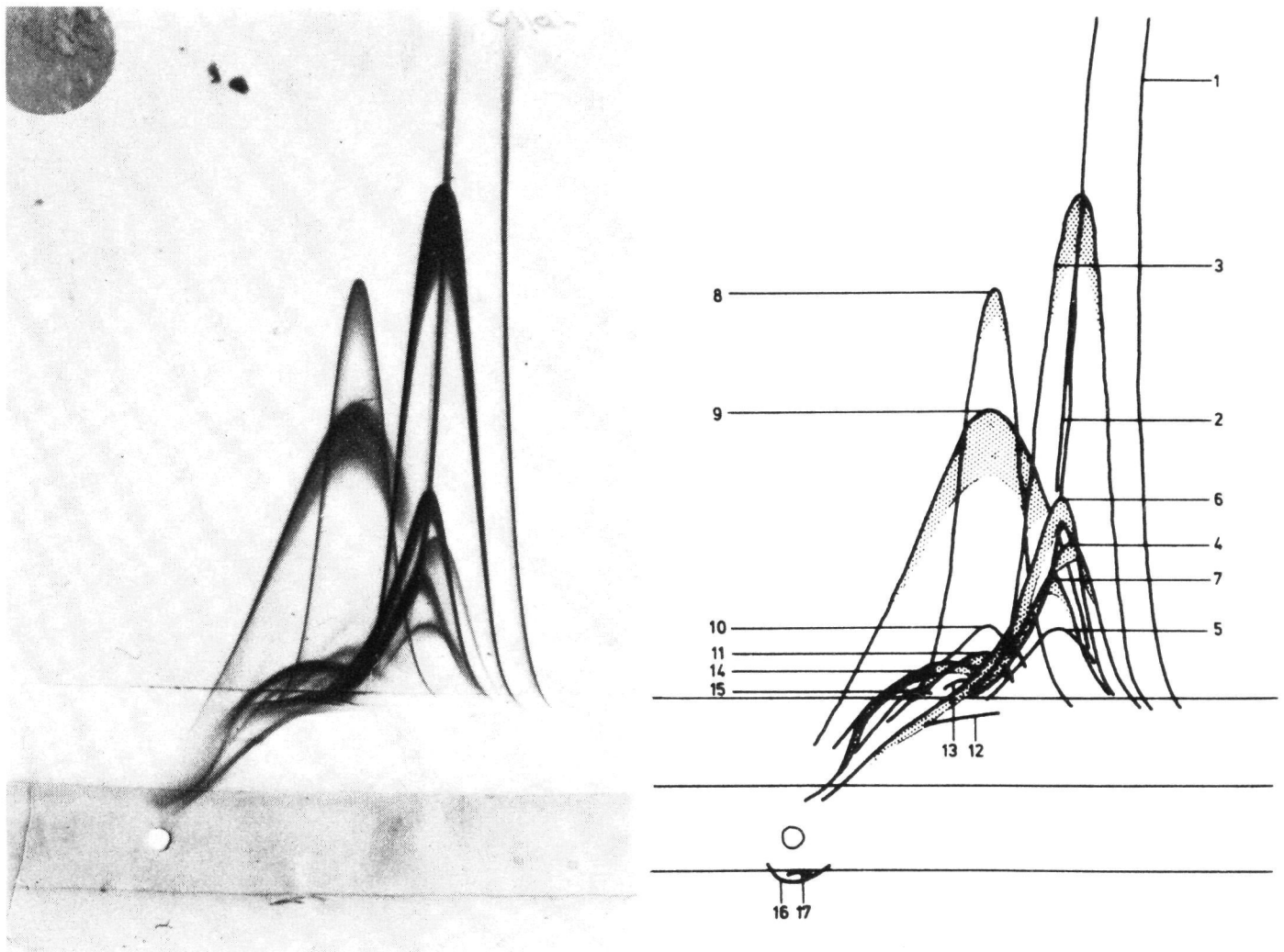


Fig. 1. A: Crossed immunoelectrophoresis of extract from cow hair and dander (St.C-75, 10 mg/ml), 5 μ l St.C-75. Buffer: Tris 0.073 M, barbital 0.024 M, calcium lactate 0.006 M, sodium azide 0.003 M (pH 8.6, 20°C, I 0.025). Gel: 1% agarose (w/v). First dimension: 0.15 cm thick gel for 30 min, 10 V cm^{-1} , 15°C, anode to the right. Second dimension: Below the first dimension and above the intermediate gel a 0.10-cm thick antibody-containing gel was poured in (rabbit antibodies against St.C-75, 8 μ l/cm²). Intermediate gel: 0.13-thick intermediate gel containing no antibodies. Anode uppermost. B: Drawing of the same crossed immunoelectrophoretic precipitating pattern. Classification numbers for the precipitates are indicated.

The precipitation patterns of these extracts are shown in Fig. 3. Saliva appeared to contain a high concentration of proteins showing partial immunological identity with those in St.C-75. The relative amounts of major allergens are given in Table 1. St.C-75 contains five *serum* proteins: No. 7 albumin, No. 10 transferrin, No. 13 α -2-macroglobulin, No. 14 IgG and No. 15 IgA. When *cow-hide products* were examined in quantitative immunoelectrophoresis, suède in particular was found to contain a large quantity of "cow allergens" including all of the major allergens of St.C-75. Split and nappe also contained detectable amounts of allergens, but this was not the case with calf pelt.

Sheep and goat

Extracts from sheep and goat pelts contained allergens showing partial immunological identity with almost all those of St.C-75. Therefore extracts from the products mentioned below were examined for their content of cross-reacting major allergens. When an extract from sheep skin for fur coats was poured in the line gel all the allergens except allergen Nos. 2, 4, 5 and 7 showed partial immunological identity (Table 1).

To see if this finding could be compared with ordinary exposure of allergens *coats made of suède and sheep skin* were vacuum cleaned. No

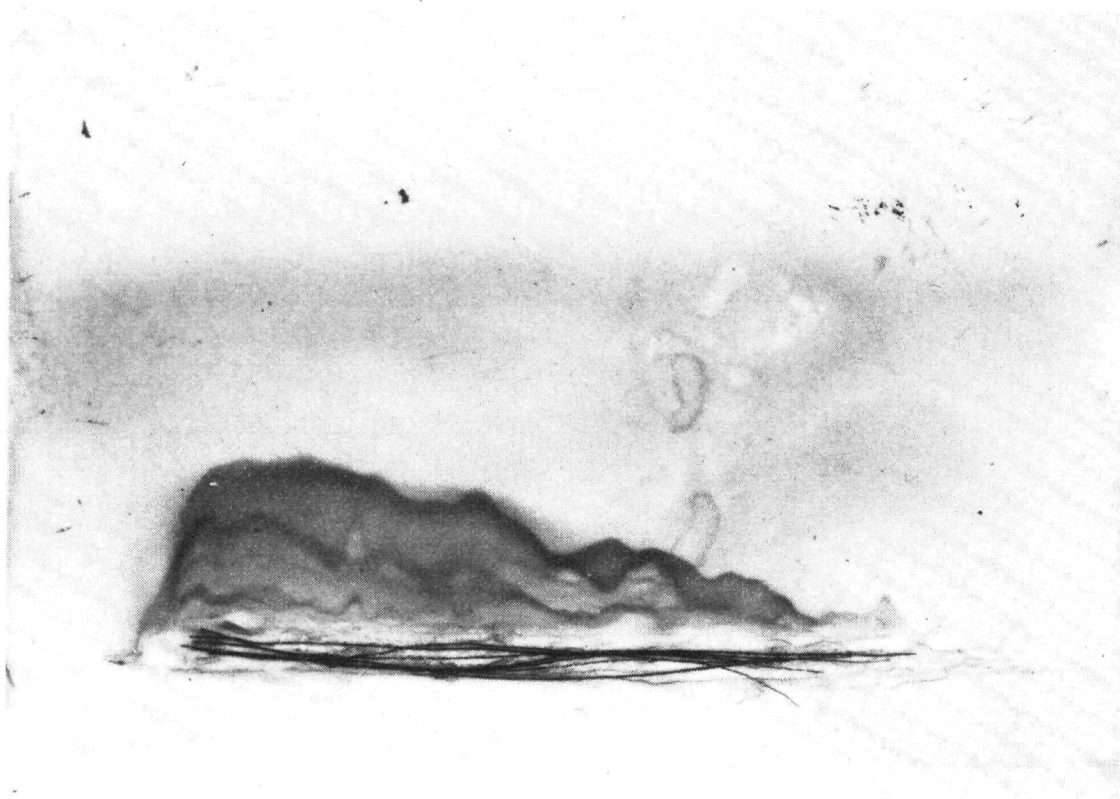


Fig. 2. Immunoelectrophoresis precipitation pattern for five hairs from black and white cattle run against rabbit anti-St.C-75, 5 µl/cm². Dermis to the left. Anode uppermost.

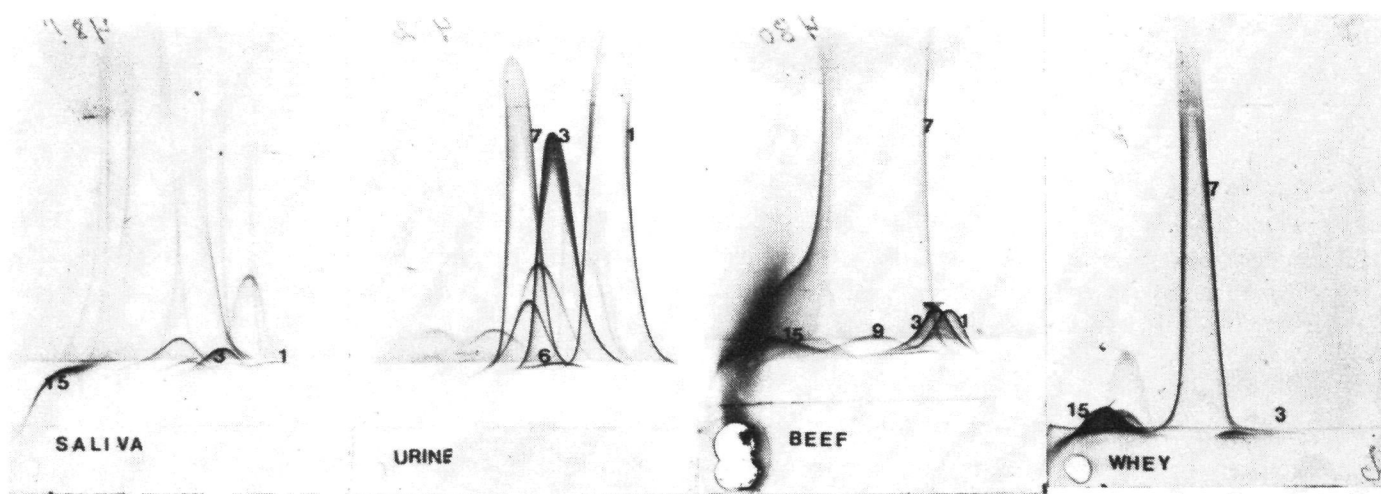


Fig. 3. Crossed immunoelectrophoretic precipitation patterns for extracts from bovine urine, -saliva, -whey and beef run against rabbit anti-St.C-75. Identified allergens showing reaction of immunological identity with those of St.C-75 are indicated. Crossed immunoelectrophoresis has been performed as described in Fig. 1. The following quantities were used: urine, saliva and whey, 5 µl (10 mg/ml) and beef, 100 µl (10 mg/ml), run against anti-St.-75, 3 µl/cm².

detectable allergens were picked up, not even after a very careful vacuum cleaning of the two big coats.

In an extract from woollen knitting yarn a few "cross-reacting" allergens were detected in very small quantities. The only exception to this was unwashed carded yarn, and in order to see if the allergens could be washed out, the same

unwashed carded yarn was extracted repeatedly. After the third wash no detectable allergens were present.

Woollen yarn for carpets (Berber and cut pile) contained a few "cross-reacting" allergens none of which were the major or minor allergens of St.C-75. However, a special quality called Yak (because of its content of 20% yak ox wool)

Table 1

The occurrence of antigens showing partial identity in quantitative immunoelectrophoresis with those of extracts from cow hair and dander

Extract	Allergens in the extract cross-reacting with those of St.C-75	Content of major allergens relative to cow hair and dander judged by yield of extraction			
		Counter immuno- electrophoresis	Rocket immuno- electrophoresis	Crossed-line immuno- electrophoresis	
St.C-75		Cow hair and dander	100 %	100 %	100 %
Bovine saliva	All of St.C-75	14	10	3	
-urine	All except Ags 5 and 9	1	1	0.5	
-serum	7, 10, 12, 14 and 15	—	—	—	
-whey	3, 7, 14 and 15	—	—	—	
-amniotic fluid	1, 3, 4, 5, 6, 7 and 14	8	6	0.8	
Beef	All except 4, 5 and 6	2	2	0.2	
Suède	All of St.C-75	1.5	2	3	
Split	1, 2, 3, 5 and 8	0.2	0.4	0.2	
Nappe	1, 2, 3, 6 and 7	0.7	0.5	0.2	
Calf pelt	None	—	—	—	
Sheep skin for fur-coats	1, 2, 3, 4, 5, 6, 7, 8, 10, 14 and 15	0.2	0.3	0.4	
Berber yarn ¹	7	—	—	—	
Cut pile yarn ¹	6, 7, 8 and 14	—	—	—	
Yak ²	3, 4, 6, 7, 8, 9 and 14	0.02	0.00	trace	
Unwashed carded yarn ³	1, 4, 5 and 7	0.00	0.00	trace	
Carded yarn ³	7	—	—	—	
Worsted yarn ³	3 and 15	0.02	0.00	trace	
Superwashed yarn ³	1 and 3	0.02	0.00	trace	
Mohair yarn ³	7	—	—	—	
Carpet tile Felt ⁴	1, 3, 6, 8, 9 and 15	0.8	1.2	0.1	
Carpet tile Lux ⁴	1, 7, 8, 14 and 15	0.8	0.6	0.1	
Goat 1:10 ⁵	All except 14, 15, 16 and 17	—	—	—	
Sheep 1:10 ⁵	All except 12, 13, 15, 16 and 17	—	—	—	

Counter immunoelectrophoresis with extracts and rabbit antibodies against cow hair and dander. The titre is given as the reciprocal value of the highest dilution of the extracts still giving rise to a visible precipitate. The estimation provides that the dominating antigens are the major allergens.

Rocket immunoelectrophoresis with extracts against rabbit antibodies against cow hair and dander. Major allergens identified by means of purified allergens. Comparison is based upon measurement of the length of the individual precipitates.

Crossed line immunoelectrophoresis with extracts from cow hair and dander or other extracts against antibodies against cow hair and dander. The content of major allergens of the extracts in question was evaluated relative to the effect of cow hair and dander in the line gel by comparing the position of the lines.

¹ Woollen yarn for ordinary carpets.

² Carpet woollen yarn containing 20 % yak ox wool.

³ Knitting yarn.

⁴ Felt: 90 % animal dander (donkey, cow, swine and goat). Lux: 40 % animal hair (donkey and goat).

⁵ Allergologisk Lab. Copenhagen, Denmark.

Trace = change of peaks but without lines in crossed line immunoelectrophoresis.

contained significant amounts of allergens including three of the major allergens (Nos. 3, 6 and 14) (Table 1).

Two kinds of *carpet tiles* made of 95 % (cow, donkey, goat and swine) and 40 % (donkey and goat) *animal hair*, respectively, were investigated

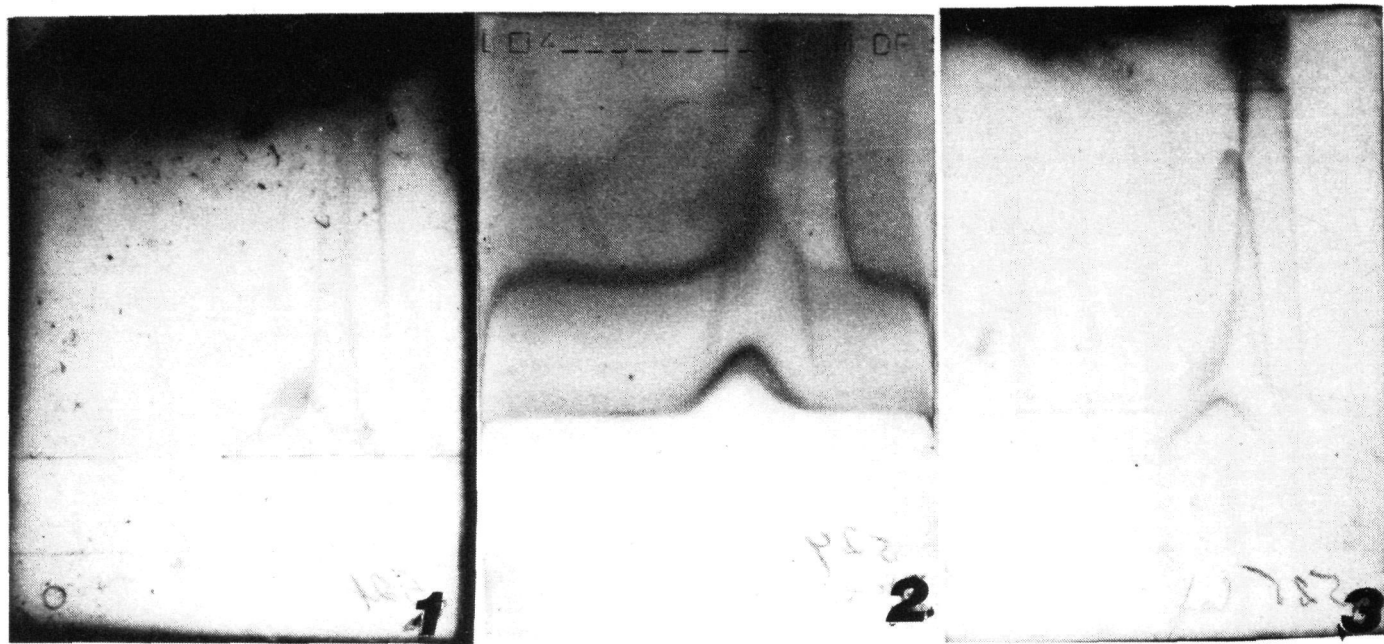


Fig. 4. Crossed line radioimmuno-electrophoresis. Crossed immunoelectrophoresis was performed with St.C-75 against rabbit anti-St.C-75. Autoradiographs of crossed radio-immuno-electrophoresis (1) and crossed line radioimmuno-electrophoresis (2 and 3) with goat and sheep extracts, respectively, in the line gel are shown. The native precipitates of the crossed immunoelectrophoresis were allowed to react with IgE from a serum pool consisting of equal volumes of sera from 10 patients with allergic asthma to cow, washed and subsequently reacted with ¹²⁵I-rabbit anti-human IgE, washing and then autoradiography was performed for 10 days. A normal donor pool served as control (not shown). This pool showed no “staining” in the autoradiography.

Table 2
Results from prick test and RAST-inhibition experiments with cow, goat and sheep allergens

Allergens	Patients	Prick test										RAST-inhibition
		Per cent										Per cent
	Five normal controls	PP	NR	GE	MK	GK	ND	ÅP	BA	KL	GL	
Cow (1 HEP)	0	100	100	100	100	100	100	100	100	100	100	100
Goat (1 HEP)	0	40	40	32	44	32	47	26	36	16	150	50
Sheep (1 HEP)	0	20	26	51	44	8	47	44	16	10	187	15

The extracts were obtained from AL-K, Copenhagen, Denmark.
The cutan reaction of goat and sheep extracts is given as a percentage of that with cow extract:

$$\frac{D \times d \text{ Ag}}{D \times d \text{ cow}} \times 100$$

.D and d are mutually perpendicular diameters of the urticaria element.

The maximum inhibitory capacity of the extracts from goat and sheep pelts (10 mg/ml) is given as a percentage of the maximum inhibitory activity made by an extract from cow hair and dander (St.C-75, 10 mg/ml).
1 HEP: standardized according to biological activity (see text) (2).

for their content of “cross-reacting” allergens. The former contained all the allergens of St.C-75, while the latter contained only major allergen No. 1 (Table 1) (swine and donkey undetectable with this assay). The carpet tiles were exposed to mechanical wear corresponding to 1–2 years of ordinary “carpet life”. After this 0.15% of the mass of pile was picked up by a vacuum cleaner. The presence of allergens “cross-reacting” with the major allergens of

St.C-75 in this material was shown by means of crossed line immunoelectrophoresis.

House dust

House dust collected from the houses of 26 randomly selected patients (Allergy Department (TA) out-patient clinic) were examined by rocket immunoelectrophoresis and counter immunoelectrophoresis. Nineteen of the patients lived in urban and seven in rural areas. Rocket immunoelectrophoresis showed only one sample to contain allergens "cross-reacting" with extracts from cow hair and dander. The patient in question was the only one living on a farm with cows. Crossed line immunoelectrophoresis showed that the two most important major allergens were present in significant amounts. Counter immunoelectrophoresis revealed that 18/26 samples had titre 0, 5/26 had titre 1, 1/26 had titre 4, 1/26 had titre 8 and 1/26 had titre 64. The last mentioned sample was the positive one in rocket immunoelectrophoresis.

To examine whether the eight samples with measureable titre in counter immunoelectrophoresis contained allergens of importance in connection with cow allergy, the gels with the precipitates were incubated with a pool of sera from patients with allergic asthma to cow parallel with a normal control serum pool. The experiment as a whole was performed as for CRIE (15). Autoradiography showed no staining of the precipitates from those samples with low titre, while the one with titre 64 showed visible staining of two precipitates. Incubation with normal serum caused no staining.

Comparison of allergenic activity

In order to establish the importance of reactions of partial immunological identity between St.C-75 and the extracts mentioned above the following experiments were done.

Crossed line radioimmuno-electrophoresis was performed on St.C-75 against rabbit anti-cow hair and dander with "line gels" containing extracts from goat or sheep pelts or extracts from suède

or sheep skin for fur coats. The sera pool from patients with allergic asthma due to cow showed equal binding of IgE to both the peaks and lines of the precipitates of the major allergens (Fig. 4). The normal control serum pool showed no staining with autoradiography.

In *RAST-inhibition experiments* both goat and sheep extracts were able to inhibit a pool of sera from patients with allergic asthma due to cow by 50 and 15 %, respectively.

Prick test was performed with extracts from goat and sheep pelts. 10/10 patients with reactions $\geq 3+$ to cow hair and dander also showed a positive reaction to both goat and sheep. The sensitivity to goat was the greater (Table 2). This is in accordance with the findings in quantitative immunoelectrophoresis, which has revealed a greater content of "cross-reacting" allergens in extract from goat than in extract from sheep pelts.

DISCUSSION

Crossed radioimmuno-electrophoresis has shown that four of the allergens of an extract from cow hair and dander are major allergens which are common to four different cow breeds. These allergens are also common to both hair and epithelium. Since the content of extractable allergens in dander is greater than in hair it is advisable to cut the hair close to the skin for extraction purposes. As healthy cows desquamate very little it is useless to employ vacuum cleaning instead of cutting.

The major allergens of cow hair and dander were also found in cow saliva, urine, amniotic fluid and in beef. This means that the major allergens are not specific to hair and dander. With the exception of IgG they are not present in serum, implying that they are associated with cells, possibly as membrane proteins. The widespread occurrence in the body of allergens "cross-reacting" with those from hair and dander has been shown with many animals. Allergens have been found in serum (6, 16, 19, 21, 22, 28), saliva (10, 25), urine (7) and brain (20). Recently, the importance of saliva as source of allergens has been stressed (10). Using rabbit antibodies against hair and dander

it was not possible to detect allergens specific to saliva.

The only allergen found in serum was IgG. This is in contrast to findings with cat, dog, horse, mouse and guinea pig (6, 16, 18, 19, 21, 27, 28), which have implicated albumin as an important mammalian allergen. Using the same procedure as employed for extracting hair and dander a 1% (w/v) extract of beef was made. This preparation contained almost all of the allergens of hair and dander, although in very low concentrations. Allergic reactions to beef are rare, whereas reactions to horse meat are seen more often. This difference might be due to the fact that horse albumin is a minor allergen (16), while cow albumin is not (22).

The presence of allergens in amniotic fluid is consistent with the frequent occurrence of eczema among veterinary surgeons who provide obstetric aid. The eczema appears almost immediately, and the presence of IgE against the major allergens of cow hair and dander has been shown by CRIE (23).

Suède, sheep skin for fur coats and unwashed carded yarn were the only products that contained significant amounts of allergens. These products might be allergenic among very sensitive cow allergy sufferers. Carpets made of animal hair except sheep wool contained surprisingly large amounts of allergens and should consequently be avoided by persons with allergic diseases or atopic predisposition.

When house dust samples were tested for cow allergens 8/26 proved positive in counter immunoelectrophoresis. Using the same procedure, Schwartz et al. (24) showed 35/70 dust samples from Danish homes to contain detectable quantities of allergens giving precipitates with rabbit antibodies against cow hair and dander, but only four with a titre greater than 8. The high frequency of faintly positive reactions in counter immunoelectrophoresis might be due to the occurrence of "cross-reacting" proteins in sheep wool and cow-hide products. These proteins are probably without importance as regards allergy, which is supported by the fact that allergy towards cow and sheep is rare.

The use of counter immunoelectrophoresis to quantitate the content of major allergens implies that the antigens which quantitatively dominate the precipitation pattern in crossed immunoelectrophoresis also are the dominating antigens in counter immunoelectrophoresis. This seems to be the fact, as an acceptable accordance was found in the results obtained by the three methods used for quantifying the content of allergens of the extracts. Both rocket- and counter immunoelectrophoresis seem to be suitable for comparison of extracts, but if more detailed information is required it is necessary to perform crossed line immunoelectrophoresis.

In the present study the occurrence of major allergens of cow hair and dander has been shown not only in materials of bovine origin but also in extracts of materials from phylogenetically related animals. The allergenic potency of these "related" allergens was verified in crossed line radioimmunoelectrophoresis, RAST-inhibition experiments and skin test. These findings indicate that goat and sheep products might be able to trigger type I reactions in patients with allergy towards cow hair and dander.

ACKNOWLEDGEMENTS

The work was supported by grants from the Dagmar Marshall Foundation and the Merchant Einer Willumsen Foundation.

Lisbeth Abrahamsen, Lena Baron, Merete Pedersen, Jette Sieben, Inge Hassel and Jonna Theede are thanked for their skilful technical assistance.

Dr. Graven Nielsen of the County Hospital in Ringkøbing and Dr. J. Thygesen of the County Hospital in Nykøbing M. are thanked for their help in skin testing the patients.

The collaboration with the Danish Textile Institute, Copenhagen and Heuga A/S, Denmark, is greatly appreciated.

REFERENCES

1. Aas, K.: The bronchial provocation test. Charles C Thomas, Springfield, USA, 1975.
2. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. *Allergy* 33, 130-137, 1978.
3. Axelsen, N. H., Krøll, J. & Weeke, B.: A manual of quantitative immunoelectrophoresis. Methods and applications. *Scand. J. Immunol.* 2, Suppl. 1, 1973.
4. Berrens, L., Morris, J. H. & Yoing, E.: Studies of the

- human dandruff allergen. *Dermatologica* 132, 432–439, 1966.
5. Bjerrum, O. J., Ramlau, J., Clemmensen, S., Ingild, A. & Bøgg Hansen, T. C.: An artefact in quantitative immunoelectrophoresis of spectrin caused by antibody preparations. *Scand. J. Immunol.* 4, Suppl. 2, 188, 1975.
 6. Blands, J., Løwenstein, H. & Weeke, B.: Characterization of extract of dog hair and dandruff from six different dog breeds. *Acta Allergol.* 32, 147–169, 1977.
 7. Carroll, K. B., Pepys, J. & Longbollone, J. L.: Extrinsic allergic alveolitis due to rat serum proteins. *Clin. Allergy* 5, 443–456, 1975.
 8. Ceska, M., Eriksen, R. & Varga, J. M.: Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunol.* 49, 1–9, 1972.
 9. Foucard, T., Johansson, S. G. O., Bennich, H. & Berg, T.: In vitro estimation of allergens by a radioimmunoantiglobulin technique using human IgE antibodies. *Int. Arch. Allergy Appl. Immunol.* 43, 360–370, 1972.
 10. Guerin, B. & Hewitt, B.: Allergens obtained from cat fur, cat pelt and cat saliva. Annual meeting of the European Academy of Allergology and Clinical Immunology 1979. Helsinki, Finland.
 11. Harboe, N. & Ingild, A.: Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 161–165, 1973.
 12. Løwenstein, H.: Purification and characterization of allergens. *Allergy* 35, 210–211, 1980.
 13. Løwenstein, H., Bjerrum, O. J., Weeke, E. & Weeke, B.: Characterization of bovine whey proteins by crossed immunoelectrophoresis. *Scand. J. Immunol.* 4, Suppl. 2, 155–161, 1975.
 14. Løwenstein, H., Marcussen, B. & Weeke, B.: Isolation and partial characterization of three major allergens of horse hair and dandruff. *Int. Arch. Allergy Appl. Immunol.* 51, 48–67, 1976.
 15. Løwenstein, H., Marcussen, B. & Weeke, B.: Identification of allergens in extract of horse hair and dander by means of crossed radioimmuno-electrophoresis. *Int. Arch. Allergy Appl. Immunol.* 51, 38–47, 1976.
 16. Markussen, B., Løwenstein, H. & Weeke, B.: Allergen extract of horse hair and dandruff. Quantitative immunoelectrophoretic characterization of the antigens. *Int. Arch. Allergy Appl. Immunol.* 51, 25–37, 1976.
 17. Nielsen, L., Løwenstein, H. & Weeke, B.: Quantitative immunoelectrophoresis used in analysis of antigens in timothy pollen extract. *Acta Allergol.* 29, 385–401, 1974.
 18. Ohman, J. L., Block, K. J. & Kendall, S.: Allergens of mammalian origin. IV. Evidence from common allergens in cat and dog serum. *J. Allergy Clin. Immunol.* 57, 560–568, 1976.
 19. Ohman, J. L., Lowell, F. C. & Block, K. J.: Allergens of mammalian origin. II. Characterization of allergens extracted from rat, mouse, guinea pig and rabbit pelts. *J. Allergy Clin. Immunol.* 55, 16–24, 1975.
 20. Ohman, J. L., Lowell, F. C. & Block, K. J.: Allergens of mammalian origin. III. Propertiel as a major feline allergen. *J. Immunol.* 113, 1668–1667, 1974.
 21. Ponterius, G., Brandt, R. & Hedben, E.: Comparative studies on the allergens of horse dandruff and horse serum. *Int. Arch. Allergy* 44, 679–691, 1973.
 22. Prahl, P., Løwenstein, H. & Weeke, B.: Quantitative immunoelectrophoretic analysis of extract from cow hair and dander. *Allergy* 33, 241–253, 1978.
 23. Prahl, P. & Roed-Petersen, J.: Type I allergy from cows in veterinary surgeons. *Contact Derm.* 5, 33–38, 1979.
 24. Schwartz, B., Gravesen, S., Petersen, B. N. & Weeke, E.: Laboratory investigations for allergens in the home. *Ugeskr. Læg.* 141, 882–886, 1979.
 25. Spain, W. C., Gillson, R. E. & Strauss, M. B.: Comparative immunological studies with salivary and epithelial extracts of the dog, cat and rabbit. *J. Allergy* 13, 563–573, 1942.
 26. Stanworth, D. R.: Reagin antibodies. *Adv. Immunol.* 3, 181, 1963.
 27. Tuft, L.: Further studies in serum allergy. VI. Antigenic relationship between horse dander and horse serum sensitivity. *J. Allergy* 6, 25–38, 1934.
 28. Yman, L., Brandt, R. & Ponterius, G.: Serum albumin. An important allergen in dog epithelia extracts. *Int. Arch. Allergy* 44, 679–691, 1973.
 29. Weeke, B. & Løwenstein, H.: Allergens identified in crossed radioimmuno-electrophoresis. *Scand. J. Immunol.* 2, Suppl. 2, 149–154, 1973.
 30. Wide, L., Bennich, H. & Johansson, S. G. O.: Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 458, 1105–1107, 1967.

Address:

P. Prahl

Laboratory of Clinical Allergology

Medical Dept. TA

Rigshospitalet

DK-2100 Copenhagen Ø

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.