

Immunological Cross-reactions between a Cat Hair and Skin Scraping Extract and a Cat Serum and Investigation of Common Allergenic Molecules of Cat 1 with Cat Serum

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ABSTRACT

Investigations of cross reactions between a cat hair and skin scraping extract (CHSS) and a cat serum (CS) were done by several biochemical and immunochemical methods. Furthermore, we looked for common allergenic molecules in a cat 1 reference preparation and in a CS. In crossed (radio) immunoelectrophoresis (CIE/CRIE) of CHSS vs. anti CS-serum one to eight allergen bands could be detected depending on the human serum employed. By constructing allergograms from the CRIE patterns of 17 patients' sera one out of eight allergens could be defined as a major allergen. CRIE of the cat 1 reference vs. anti CS-serum, using the sera of two cat allergic patients, displayed four to nine allergen bands, indicating that the cat 1 reference is not a pure preparation but rather an enriched fraction.

INTRODUCTION

D ebris from cat hair and skin is an important causative agent for inhalant allergies. Murray et al.¹ found that children tend to be more frequently sensitive

to cat allergens than to dog allergens. It was Ohman et al.² who identified cat 1 (Fel d 1) as the major allergen in a cat hair extract. This allergen was later also identified in cat saliva,³ cat washes⁴ and sebaceous glands of cat skin.⁵ Additional allergens were detected in cat urine⁶⁻⁸ and cat serum.^{7,8} However, until now there exists only little information on the importance of cat serum in cat allergy. It is also of interest to know about the cross reactivity of allergens in a cat hair and skin scraping extract with allergens of a cat serum preparation.

We here demonstrate data on the presence of common allergens in a cat hair and skin scraping extract and a cat serum preparation. We examined these two sources of cat allergens for common allergenic molecules by several biochemical and immunochemical methods. Furthermore, we investigated whether a cat 1 reference preparation shares common allergenic molecules with a cat serum.

MATERIALS AND METHODS

Allergen Sources

A s allergen sources a dialysed cat hair and skin scraping extract (CHSS)⁹ (Allergopharma, Reinbek, FRG), a cat serum (CS) which was obtained by separation from whole blood of a healthy cat and a cat

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Table I

RAST Classes of the Sera of 29 Cat Allergic Patients and One Control Person Using Allergen Disks Prepared from CHSS and CS

Allergen Disks	Patients' Sera	RAST-classes	%-Binding
CHSS	25	3-4	15.6-48.0
CHSS	4	0-1	0.77-3.3
CS	12	2-4	4.4-43.7
CS	17	0-1	0.5-2.8
CHSS	cat non allergic serum	0	0.73
CS	cat non allergic serum	0	0.66

allergen 1 (cat 1) reference (Lot E2-Ct; Office of Biologics, NCDB, Bethesda, USA) were used.

Patients' Sera

The sera of 29 patients showing a positive history of cat allergy were examined. As negative control we took the serum of a patient not allergic to cat allergens.

Determination of Specific IgE

Specific IgE was determined by RAST (Pharmacia, Freiburg, FRG) using allergen disks prepared from CHSS and CS.¹⁰

Crossed (Radio) Immunoelectrophoresis (CIE/CRIE)

CIE was performed as described by Løwenstein¹¹ employing 10 µg protein¹² of CHSS and CS. 28 µl of cat 1 (10 units/ml) were employed. We used 60 µl of a rabbit anti cat dander-serum (ALK, Copenhagen, Denmark) and an anti CS-serum (DAKO, Copenhagen, Denmark) per ml separation gel. CIE plates were stained with Coomassie brilliant blue R 250.

CRIE was done as described by Weeke and Løwenstein.¹³ As tracer for specific IgE we used the individual sera and a serum pool (n = 14) (1:24) of cat allergic patients. Bound IgE was detected with 125 I-anti IgE (Pharmacia) (400 nCi for each CIE plate). Autoradiographs were produced by exposing Kodak-X-Omat AR-films at -20°C for three, seven and 14 days to the CRIE-plates.

Quantification of CRIE

The CRIE patterns of the sera of 17 patients were classified as described by Østerballe et al.¹⁴ These patients took part in a standardization program and complete data on history and skin testing were available. The results of CRIE of the individual sera of 17 patients were summarized in an allergogram classifying major, intermediate and minor allergens.¹⁵

RAST-inhibition (RI)

In order to examine whether common allergenic molecules exist in CHSS and in CS independent RI experiments were performed using allergen disks prepared with CHSS and CS.¹⁶ Furthermore the CS was measured with allergen disks prepared with cat 1. The pooled sera (RAST-class 3, with CHSS-disks) of three cat allergic patients served as specific IgE source. 125 I-anti IgE was purchased from Pharmacia. The 50%-inhibition values and parallel line assay were computed according to a program suggested by the Bureau of Biologics.¹⁷

Isoelectric Focusing (IEF) and Immunoprint (IP)

Agarose IEF and IP were performed with CHSS, CS and cat 1 in principle as described by Peltre et al.¹⁸ Briefly 50 µg protein of CHSS and CS and 5 µl of cat 1 (10 units/ml) were focused at 10% D-Sorbit and 2% Servalyt 3-10 (SERVA, Heidelberg, FRG). The focused proteins were stained with Coomassie brilliant blue R 250. As marker proteins a commercially available kit from Pharmacia was used.

For IP the proteins were transferred to nitrocellulose strips by capillary blotting and the obtained prints were incubated overnight with 5 ml of a serum pool (RAST-class 3, with CHSS-disks) (1:5) of cat allergic patients (n = 14). As tracer for bound IgE 125 I-anti IgE (590 nCi/print) was used. Autoradiographs were produced by exposing Kodak X-Omat AR-films at -20°C for three and 14 days to the print.

RESULTS

Determination of Specific IgE

When using CHSS allergen disks 25 out of 29 patients showed RAST-classes (R-cl.) 3-4 and only four patients showed R-cl. 0-1. When using CS disks in the same patient group 12 patients showed R-cl. 2-4 and 17 patients showed R-cl. 0-1. The serum of the

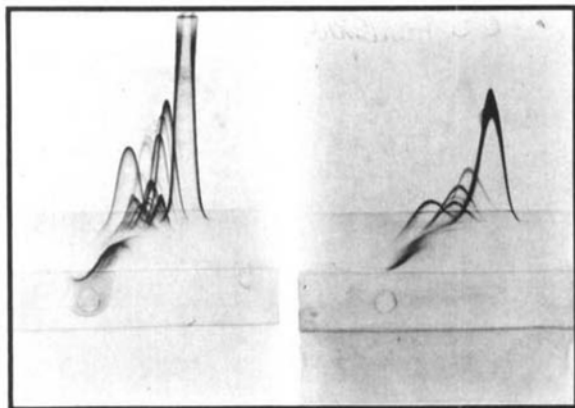


Figure 1. CIE of CS (on the left) and CHSS (on the right) vs. anti CS-serum.

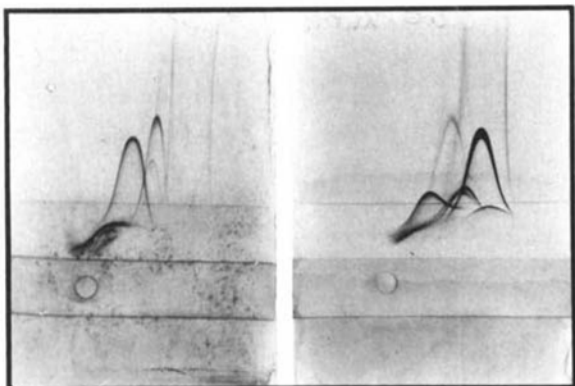


Figure 2. CIE of CS (on the left) and CHSS (on the right) vs. anti cat-dander serum.

control patient showed R-cl. 0 with both allergen disks (Table I).

CIE of CHSS and CS vs. Anti CS-serum

Fig. 1 shows the CIE patterns of CS and CHSS vs. anti CS-serum. CHSS produced eight precipitates and CS 20 precipitates with anti CS-serum.

CIE of CHSS and CS vs. Anti Cat-Dander Serum

The CIE patterns of CHSS and CS vs. anti cat-dander serum are shown in Fig. 2. In the heterologous system (CS vs. anti cat-dander serum) eight precipitates were detected, whereas 12 precipitates were found in the homologous system (CHSS vs. anti cat-dander serum).

CRIE of CS vs. Anti CS-Serum and Anti Cat-Dander Serum

Fig. 3 shows the CRIE patterns of CS vs. anti CS-serum and anti cat-dander serum using a serum pool

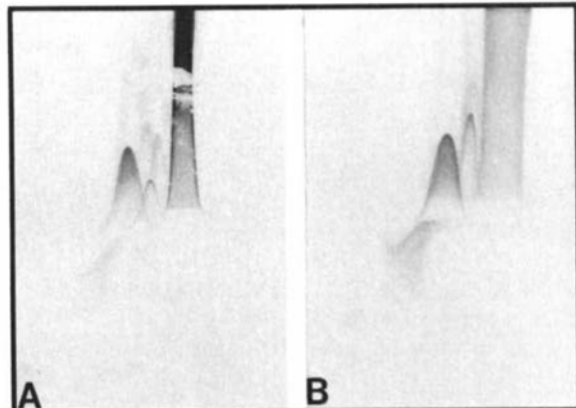


Figure 3. CRIE of CS vs. anti CS-serum (A) and anti cat-dander serum (B) with a pool serum of cat allergic patients ($n = 14$).

of cat allergic patients ($n = 14$) as tracer for the separated allergens. In both cases the patterns show some similarities. CS detects six allergenic molecules in anti cat-dander serum (B) and eight in anti CS-serum (A).

CRIE of Patients A-D Performed with CHSS vs. Anti CS-Serum and Anti Cat-Dander Serum

Using the sera of patients A-D we analysed CHSS vs. anti CS-serum and anti cat-dander serum (Fig. 4). In all four cases allergen bands were detected, however, in different numbers. The allergen patterns of the homologous system were more complex than the ones of the heterologous system. In the sera of patients A-D between one and eight allergen bands were detected in the heterologous system, whereas three to ten allergen bands were found in the homologous system.

Allergogram for CHSS Using Anti CS-Serum and Anti Cat-Dander Serum

The total number of allergens which occurred in the individual sera of the 17 patients was ten in the homologous system and eight in the heterologous system (Fig. 5). For the determination whether some allergens are of major origin we constructed an allergogram (Fig. 6). When using the heterologous system 59% of the patients showed a strong IgE binding to allergen 1* after three days of autoradiography, thus defining it as major allergen.¹⁵

In the homologous system almost all patients' sera recognize allergen 3 (cat 1) (s. Fig. 5). 94% of the patients showed a strong IgE binding to this allergen after three days of autoradiography (s. Fig. 6). Also in CRIE-classification the highest CRIE-class could be detected for allergen 1* ($\Sigma = 51, 6$) and for allergen 3 ($\Sigma = 152, 1$) (s. Fig. 5).

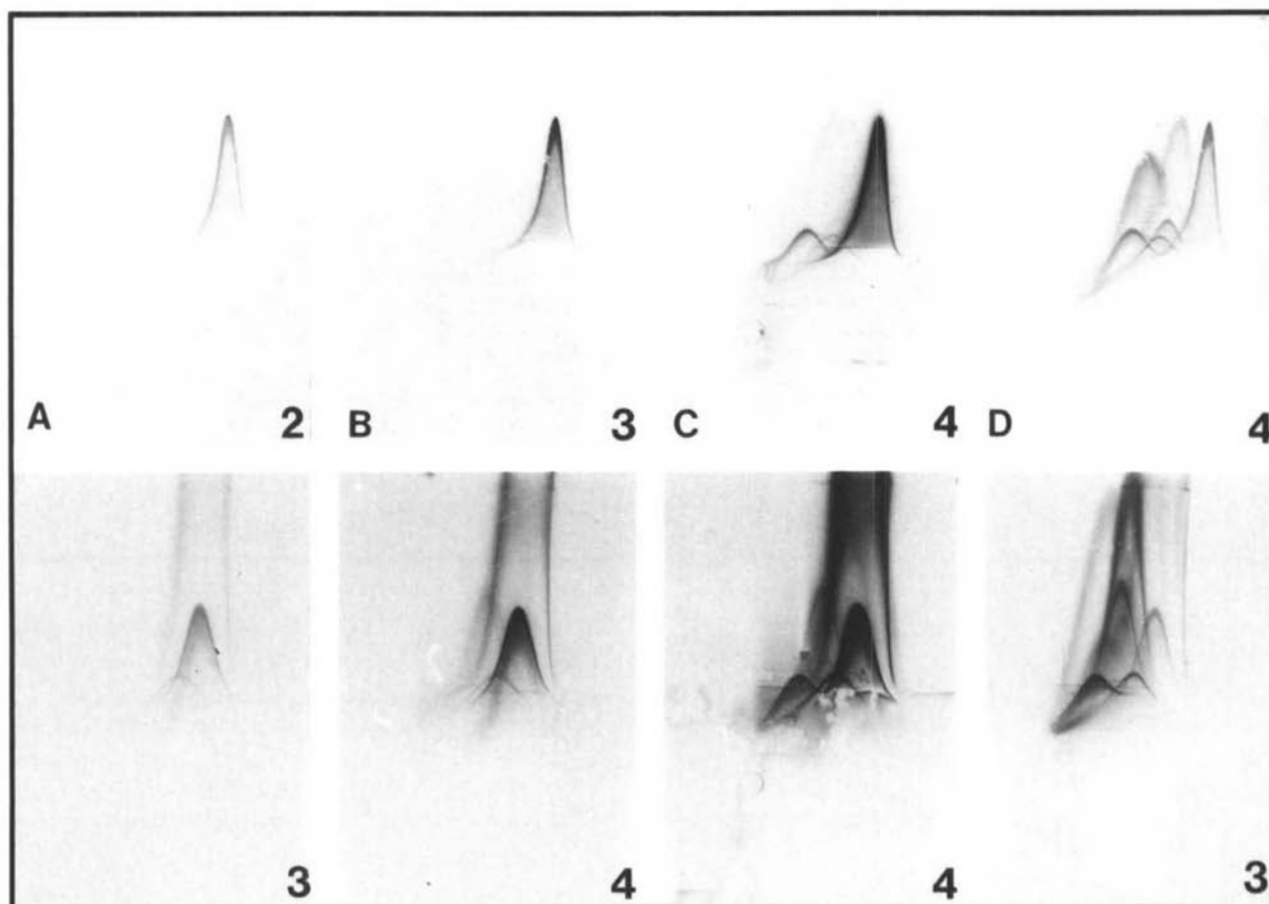
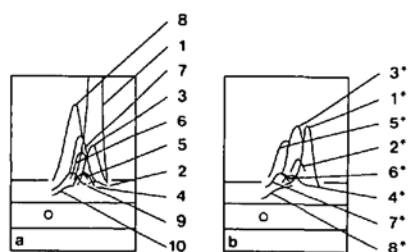


Figure 4. CRIE of CHSS vs. anti CS-serum (upper row) and anti cat-dander serum (lower row) with the serum of cat allergic patients A-D. RAST-classes obtained with CS-disks (upper row) and CHSS-disks (lower row) are indicated on the corresponding CRIE plates.



Allergen-no.:	1	2	3	4	5	6	7	8	9	10
CRIE-classes (Σ):	22.2	18.5	152.1	3.6	9.5	2.6	141	1.8	29.3	14.8

Allergen-no.:	1*	2*	3*	4*	5*	6*	7*	8*
CRIE-classes (Σ):	51.6	1.3	1.0	74	0.8	0.2	9.2	4.7

Figure 5. Allergen patterns of the CRIE of CHSS vs. anti cat-dander serum (a) and anti CS-serum (b) and the sum of CRIE classes of the corresponding allergens of 17 patients.

RI with CHSS and CS Using Different Allergen Disks

Possible cross reactions between CHSS and CS were investigated by RI. When using CS allergen disks complete RI curves were obtained with CHSS and CS. However, when using CHSS allergen disks only CHSS showed a complete RI-curve whereas CS did not show an inhibition at all (Fig. 7). When using cat I allergen disks in RI CS produced a complete RI-curve (Fig. 8).

CRIE of Cat I Reference vs. Anti CS-Serum

CRIE of cat I vs. anti CS-serum with the sera of two patients (C and E) displayed four to nine allergen bands (Fig. 9). Using the serum of patient C the allergen pattern was more complex and produced stronger radiostainings than the pattern obtained with the serum of patient E.

CRIE of Cat I Reference vs. Anti Cat-Dander Serum

CRIE of cat I vs. anti cat-dander serum with the serum of patient C showed seven strong and two

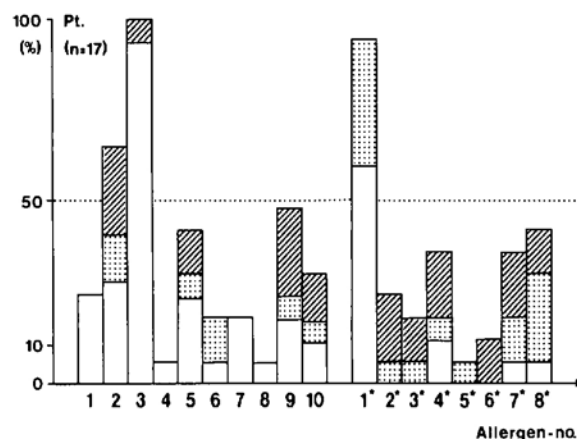


Figure 6. Results of the CRIE of CHSS vs. anti cat-dander serum and anti CS-serum of 17 patients summarized in an allergogram. 1-10: allergen bands which were detected in the homologous CRIE (allergen 3 = cat 1) and 1*-8*: allergen bands which were detected in the heterologous CRIE. Times of autoradiography: □: 3 days, ▨: 7 days, ▩: 14 days. The extent of IgE binding was evaluated visually from autoradiographs of the CRIE plates.

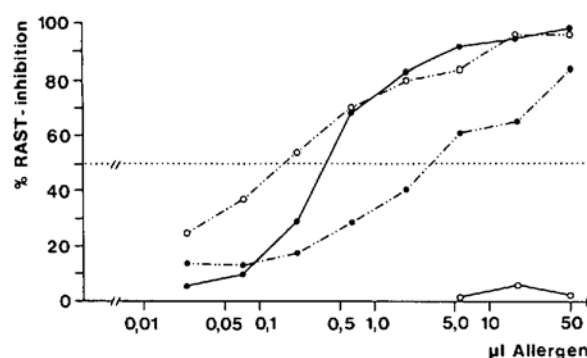


Figure 7. R1 of CHSS (●) and CS (○) with CHSS (—) and CS allergen disks (— · —).

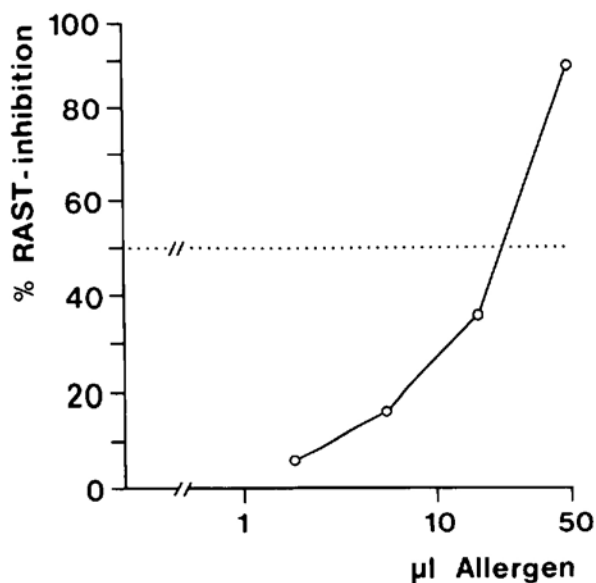


Figure 8. R1 of CS using allergen disks prepared with the cat 1 reference.

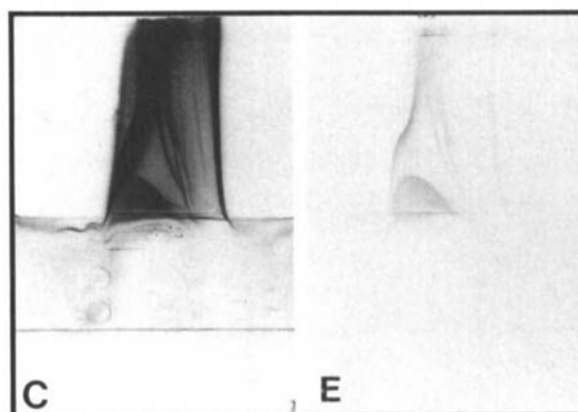


Figure 9. CRIE of cat 1 reference vs. anti CS-serum using the sera of patients C and E.

weak allergen bands. With the serum of patient E only one strong and two weak allergen bands could be found (Fig. 10).

IEF and IP of CHSS, CS and Cat 1 Reference

All three samples showed a complex protein/allergen pattern in IEF/IP (Fig. 11). In all extracts allergen bands could be detected at pI range of 4.4-5.0. Additional strong allergen bands were found in CS at a pI range of 5.15-5.6 and at a pI range of 6.6-7.2, the latter

being hardly visible in the other two extracts. The allergen pattern of cat 1 was comparable to the one of the CHSS. By IEF/IP of cat 1 and CHSS more than ten protein/allergen bands could be detected.

DISCUSSION

Recently Anderson et al.⁸ reported that six out of 43 individuals with a history of cat allergy showed also positive findings in RAST to cat serum. We could

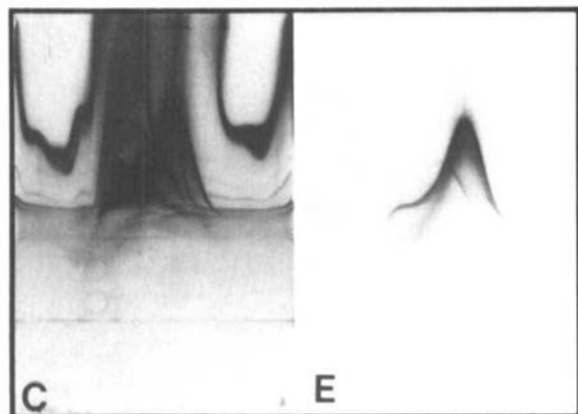


Figure 10. CRIE of cat 1 reference vs. anti cat-dander serum using the sera of patients C and E.

confirm this in our investigations where 12 out of 29 cat allergic patients with a positive history of cat allergy showed R-cl. 2-4 with CS disks. Using CS vs. anti CS-serum eight allergen bands were detected in CRIE. This proves that CS contains allergenic molecules.

Little is known whether CHSS shares allergenic molecules with CS. In CIE/CRIE of CHSS vs. anti CS-serum performed with individual sera of cat allergic patients one to eight allergen bands were detected. By summarizing these results in an allergogram, 59% of the patients showed a strong IgE binding to allergen 1* (Fig. 6). This allergen appeared already after three days of autoradiography, thus, defining it as a major allergen.¹⁵ This allergen showed also the highest CRIE-class in CRIE-classification. By RI we could further prove that CHSS shares common allergens with CS, but apparently in different concentrations. Using CS disks both extracts produced complete RI-curves. When using CHSS disks only CHSS showed a complete RI-curve, whereas CS did not show an inhibition at all. From these results one can speculate that CHSS contains allergens which have a high binding affinity to BrCN activated paper disks. On the other hand, one can assume that these allergens are not present in CS or only in very low concentrations. This could be the reason for the missing inhibitory capacity of CS in the described constellation of RI.

By IP we could show that most of the allergen bands of CHSS and CS were in the range of pI 4.4-5.0. In addition, CS produced strong allergen bands in the pI ranges 5.15-5.6 and 6.6-7.2, which were hardly detectable in CHSS (s. Fig. 11).

Recently it was published that cat 1 is not present in CS.^{7,8} This is in contrast to our results obtained by RI, CRIE and IP. Using cat 1 allergen disks in RI a complete

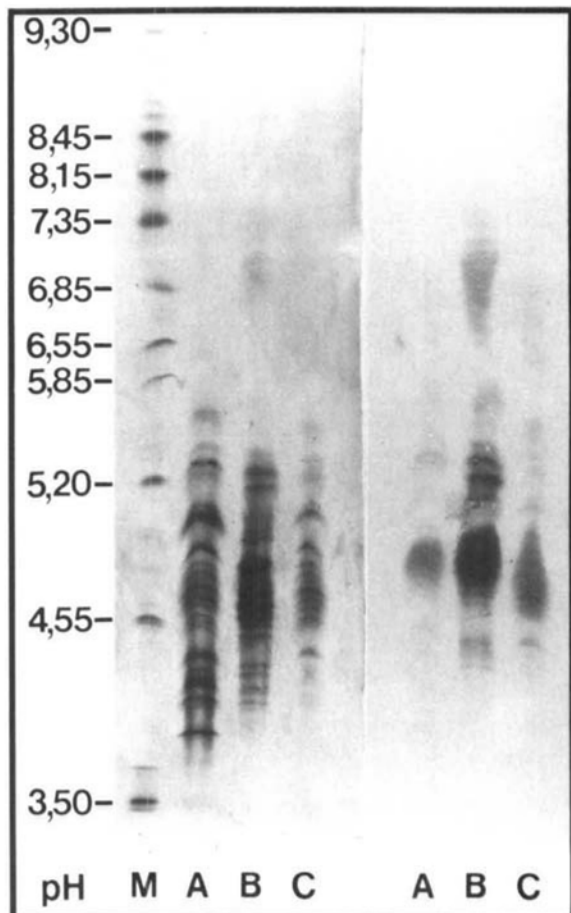


Figure 11. IEF (on the left) and IP (on the right) of CHSS (A), CS (B) and cat 1 reference (C). M = marker protein.

RI-curve was obtained with CS. In CRIE using cat 1 vs. anti CS-serum four to nine allergen bands were detected with the sera of two patients. When investigating cat 1 in IP most of the allergen bands were detected in the same pI range as they were found in the case of CS (pI 4.4-5.0). Furthermore our results showed that the analysis of purity of allergen extracts by CRIE is strongly influenced by the patients' sera employed. With the serum of patient E only one strong and two weak allergen bands were detected, whereas seven strong and two weak allergen bands were found for cat 1 vs. anti cat-dander serum with the serum of patient C.

In conclusion our examinations showed, that CS contains allergenic material and that CHSS shares some allergenic molecules with CS. One of these common allergens is a major allergen. Furthermore, it could be shown that the cat 1 reference shares also common allergenic molecules with CS. These findings suggest that the cat 1 reference is not a pure preparation but rather

a cat 1 enriched fraction. This could be confirmed by IP and CRIE where the cat 1 reference displayed complex patterns. Investigations to isolate the major cross reacting allergens of CHSS with CS are in preparation.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. I. Nohr, Mrs. A. Zieren and Mrs. G. Harders for skillful technical assistance. Furthermore we thank Mrs. L. Bozzardi for preparing the manuscript.

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