

Analysis of Antigens in a Commercial House-Dust Extract by Means of Quantitative Immunelectrophoresis

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Crossed immunelectrophoresis (CIE) of a commercial house-dust extract revealed 43 immunoprecipitates.

Rocket immunelectrophoresis with 106 different allergen extracts from animal hair and dander, feathers, fungi, pollen, foodstuffs, textiles and house-dust mites showed the presence of precipitating antibodies in the rabbit anti-house-dust serum against antigens from 34 of the extracts.

Crossed immunelectrophoresis with the addition of antigen, and crossed line immunelectrophoresis (CLIE) with these 34 allergen extracts, as well as crossed immunelectrophoresis with intermediate gel containing rabbit antibodies against nine human serum proteins, allowed the identification of 14 antigens in the house-dust extract. These antigens were shown to originate from house-dust mites, hair and dander from various animals, fungi, feathers, human hair and dander, and human serum proteins.

Key words: allergens; house-dust allergy; quantitative immunelectrophoresis.

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Extract of house-dust is used extensively in the diagnosis and treatment of allergic diseases. Many studies have been made concerning its allergenic components. By use of skin testing (3, 4, 5, 6, 7, 13, 19, 20, 21, 25, 26, 27, 28), bronchial provocation (20, 21, 25, 26), radioallergosorbent test (RAST) (1, 25, 27), immunodiffusion analysis (6, 8, 13) and histamine release from human leucocytes (13) several allergen sources have been shown to contribute allergenic components to house-dust. Among these, mites (1, 3, 4, 7, 13, 19, 20, 21, 25, 26, 27, 28), feathers (6), moulds (11, 23, 24), animal hair and dander (1, 9), human hair and dander (5, 6, 13) and different textiles (6) are the most common. Results recently obtained by means of quantitative immunelectrophoresis (QIE) and crossed radioimmuno-

electrophoresis (CRIE) on related topics (9, 14, 16, 17, 18, 22, 29) have strongly indicated that these methods may be even more powerful in the analysis of the antigens and allergens in house-dust, because of their high specificity, sensitivity and resolution power.

The aim of this work was to analyse the antigenic composition of a commercial house-dust extract by means of quantitative immunelectrophoresis.

MATERIAL AND METHODS

Antigens

Freeze-dried house-dust extract was obtained from Allergologisk Laboratorium A/S, Copenhagen, Denmark. The extract was

dissolved in 0.1 M sodium chloride to give a 10% (w/v) solution. The solution was then sterile-filtered by means of Millex (TM), pore size 0.22 μm , and denoted St HD 76 before storage at -18°C .

Extraction of human hair and dander (obtained by emptying electric shavers and storing until extraction at -18°C) was performed by agitating a 10% (w/v) solution at 5°C for 20 h; solvent 0.125 M ammonium bicarbonate, 0.015 M sodium azide (pH 7.6). After sterile filtration at 5°C , the solution was centrifuged at 0°C for 60 min at 27,000 g. The supernatant was dialysed twice against a 50-fold volume of 0.005 M ammonium bicarbonate, 0.015 M sodium azide for 20 and 60 h, and then once against water for 20 h, at 5°C . The product was freeze-dried and stored dry at 5°C . A solution (10 g l^{-1}) of the freeze-dried product was then made up in 50% glycerol and 50% 0.9% saline, and stored at 5°C until use.

All extracts listed in Table 1A, except that of human hair and dander, were obtained from Allergologisk Laboratorium A/S, Copenhagen, Denmark, as 5% (w/v) solutions in either a medium of 50% isotonic saline plus 50% glycerol, or in pure isotonic saline.

Antibodies

Antibodies against house-dust were obtained by immunization of rabbits with commercial house-dust extracts during 2–4 years. Four different batches of house-dust were successively used. The immunization and purification were performed according to Harboe & Ingild (2).

Rabbit antibodies against human albumin, ceruloplasmin, transferrin, α -1-antitrypsin, fibrinogen, prealbumin, orosomucoid, IgG and IgM were supplied by DAKO Immunoglobulins, Copenhagen, Denmark.

Normal rabbit serum (immunoglobulin-fraction) (Code: X 903 batch 097) was also supplied by DAKO Immunoglobulins, Copenhagen, Denmark.

Immunoelectrophoretic methods

Equipment and reagents were essentially as described in (2). The electrophoresis was performed in 1% (w/v) agarose gel (batch 102 Dy, Litex, Glostrup, Denmark) containing a buffer of 0.073 M Tris, 0.024 M barbital, 0.006 M calcium lactate and 0.003 M sodium azide (pH 8.6, 25°C).

Rocket immunoelectrophoresis, crossed immunoelectrophoresis (CIE), crossed line immunoelectrophoresis (CLIE) and crossed immunoelectrophoresis with intermediate gel were performed as described in (2). Rocket immunoelectrophoresis was performed in a 0.15 cm-thick gel at 2 V cm^{-1} for 18 h.

Because of cathodic migration, both an anodic and cathodic second dimension gel were used in CIE. Unless otherwise stated, first and second dimension electrophoreses were performed in a 0.15 cm-thick gel at 10 V cm^{-1} for 30 min and in a 0.10 cm-thick gel at 2 V cm^{-1} for 15 h, respectively. The thickness of the intermediate gels was 0.13 cm; $11 \times 20.5\text{ cm}^2$ or $9 \times 11\text{ cm}^2$ glass plates were used for CIE and $7 \times 5\text{ cm}^2$ glass plates for the CLIE and the intermediate gel immunoelectrophoresis. The plates were pressed, washed and dried, and finally stained with Coomassie Brilliant Blue® as described by Weeke (2).

RESULTS

Reference precipitation pattern for the extract of house-dust

Crossed immunoelectrophoresis of St HD 76 with rabbit antibodies against house-dust revealed 43 precipitates. This precipitation pattern is shown in Fig. 1A.

Two additional precipitates were demonstrated during preliminary crossed radioimmunoelectrophoretic experiments giving a total of 45 precipitates. Fig. 1B is a drawing of the reference precipitation pattern; the precipitates are numbered arbitrarily from anode to cathode. Antigens

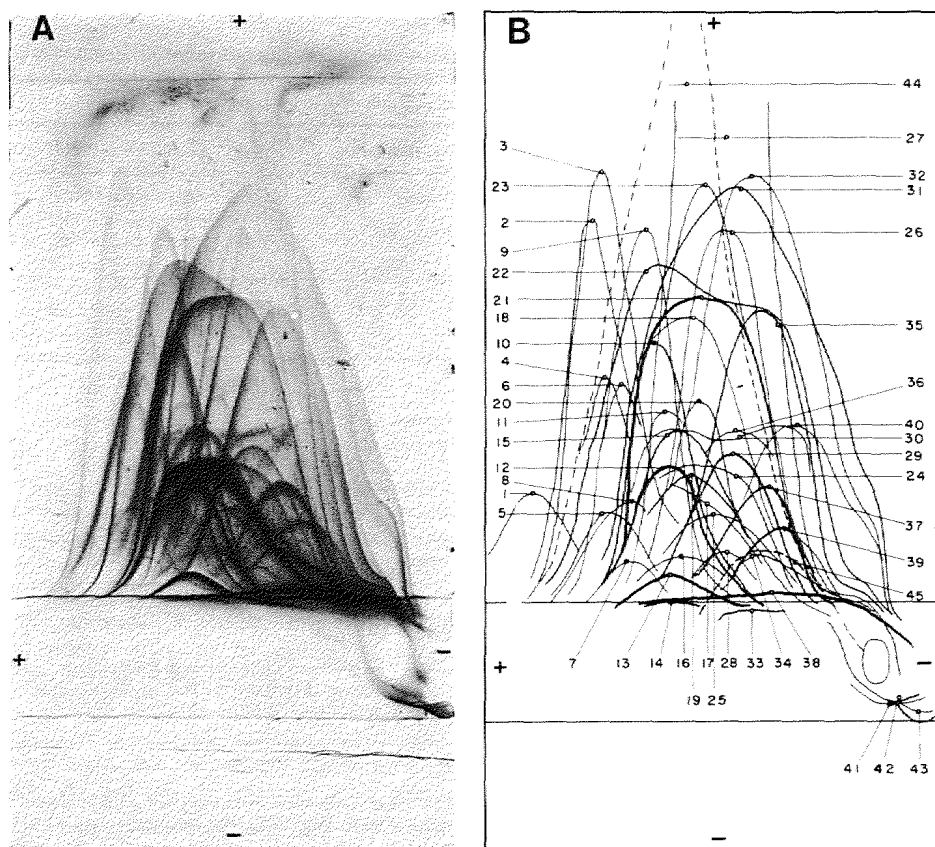


Fig. 1. Reference pattern for house-dust extract, St HD 76, obtained by crossed immunoelectrophoresis in 1% (w/v) agarose gel containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate and 0.003 M sodium azide (pH 8.6, 25°C) at 15°C.

A: Antigens: 80 μ l of St HD 76 was applied. First dimension: 10 V/cm in a 0.15 cm-thick gel for 60 min. Anode to the left. Antibodies: 15 μ l/cm² rabbit antibodies against extracts of house dust. Second dimension: 2 V/cm in a 0.1 cm-thick gel for 18 h. Anode at top. Staining: Coomassie Brilliant Blue[®]. A 11 $\times$ 20.5 cm² glass plate was used.

B: Drawing of the same CIE precipitation pattern of St HD 76. Classification numbers for the precipitates are indicated. Precipitates visualized only in CRIE experiments are drawn with dot and dash lines.

1–40, 44 and 45 precipitated in the anodic antibody-containing gel, antigens 41 and 43 in the cathodic antibody-containing gel, and antigen 42 in both gels.

Both precipitate Nos. 22 and 30 were asymmetrically shaped, possibly due to heterogeneity. No partial identity among individual antigens was seen.

Immunochemical comparison of house-dust antigens with antigens from different sources

By means of rocket immunoelectrophoresis, 34 of the 106 different allergen extracts listed in Table 1A were found to form precipitates with rabbit antiserum against house dust. However, control experiments revealed that

Table 1A

Allergenic extracts used in rocket immunoelectrophoresis. Extracts giving rise to precipitates on the cathodic side of the application well in anti-house-dust gel are marked with *. Precipitation in normal rabbit serum gel is included as †. No extracts precipitated in antibody-free gel

One or more precipitates	No precipitates
<i>Animal hair and dander</i>	
†Cat, cow, dog, †goat, †guinea pig, hamster, †horse, *human hair and dander, †pig, †wool	Mouse, rat
<i>Feathers</i>	
†Duck feathers, †goose feathers, *†poultry feathers	
<i>Fungi</i>	
<i>Alternaria iridis</i> , <i>Aspergillus fumigatus</i> , * <i>Cladosporium herbarum</i> , <i>Mucor racemosus</i> , <i>Penicillium glaucum</i> , <i>Trichosporon pullulans</i>	<i>Alternaria tenuis</i> , <i>Aspergillus clavatus</i> , <i>Aspergillus glaucus</i> , <i>Aspergillus ochraceus</i> , <i>Aureobasidium pullulans</i> , <i>Botrytis cinerea</i> , <i>Candida albicans</i> , <i>Mucor mucedo</i> , <i>Neurospora sitophila</i> , <i>Penicillium purpurogenum</i> , <i>Penicillium roqueforti</i> , <i>Rhodotorula rubra</i> , <i>Trichoderma viride</i> , <i>Trichophyton mentagrophytes</i> , <i>Trichophyton rubrum</i> , <i>Ulocladium</i> sp., <i>Verticillium albo-atrum</i>
<i>Pollen</i>	
<i>Trees and shrubs</i>	
	Alder (<i>Alnus glutinosa</i>), birch (<i>Betula pendula</i>), filbert (<i>Corylus avellana</i>), larch (<i>Larix decidua</i>), privet (<i>Ligustrum vulgare</i>), willow (<i>Salix alba</i>)
<i>Grasses, etc.</i>	
Velvet grass (<i>Holcus lanatus</i>)	False oat grass (<i>Avena elatior</i>), meadow foxtail (<i>Alopecurus pratensis</i>), orchard grass (<i>Dactylis glomerata</i>), ryegrass (<i>Lolium perenne</i>), smooth meadow grass (<i>Poa pratensis</i>), timothy (<i>Phleum pratense</i>)
<i>Herbs</i>	
Mugwort (<i>Artemisia vulgaris</i>), nettle (<i>Urtica dioica</i>)	Dahlia (<i>Dahlia variabilis</i>), fuchsia (<i>Fuchsia</i>), oxeye daisy (<i>Chrysanthemum leucanthemum</i>), starwort (<i>Aster chinensis</i>), tulip (<i>Tulipa</i>)
<i>Foodstuffs</i>	
Apple, beef, *cod, †coffee, cow milk (cooked), egg white, plaice, *†potato, *rice, *tomato, †wheat	Almond, barley, carrot, chicken, cocoa, cucumber, egg yolk, flax, grape, green bean, green pea, hazelnut, herring, hops, lamb, lemon, mackerel, maize, mushrooms, oat, onion, parsley, pork, rye, shrimp, soya-bean, strawberry, tea, walnut
<i>Textiles</i>	
	Coir mat, cotton, jute, kapoc, sisal
<i>Miscellaneous</i>	
*House-dust mites (<i>D. pteromyssimus</i> + <i>farinae</i>)	Sawdust from beech, sawdust from pine

Table 1B

Specific rabbit antibodies against human serum proteins used in crossed immunoelectrophoresis with intermediate gel

anti-human albumin
anti-human ceruloplasmin
anti-human transferrin
anti-human α -1-antitrypsin
anti-human fibrinogen
anti-human prealbumin
anti-human orosomucoid
anti-human IgG
anti-human IgM

12 of the 34 allergen sources also were able to form rockets in a gel containing antibodies from normal non-immunized rabbits. No rockets were seen in an antibody-free gel. An example of a rocket immunoelectrophoresis is shown in Fig. 2.

The antigens from these 34 extracts were compared with St HD 76 using either CLIE, or in fewer cases CIE, by adding the allergen extract to be compared.

Allergen extracts giving rise to cathodic precipitates in rocket immunoelectrophoresis were investigated separately; the direction of the current in both first and second dimension in CLIE was reversed. Determination of immunological identity/partial identity between antigens from St HD 76 and antigens from the nine human serum proteins listed in Table 1B was performed by means of CIE with an intermediate gel containing rabbit antibodies against human serum proteins. Fig. 3 shows the reference pattern together with four examples of CLIE and one example of CIE with intermediate gel.

The results are summarized in Table 2. Antigen No. 3 showed partial immunological identity to extracts from cow, horse, goat, wool, duck feathers, *Trichosporon pullulans*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria iridis*, and antigen No. 4 to extracts from goat, *Mucor racemosus*, and probably dog. Antigen No. 5 was possibly immunologically identical or partially

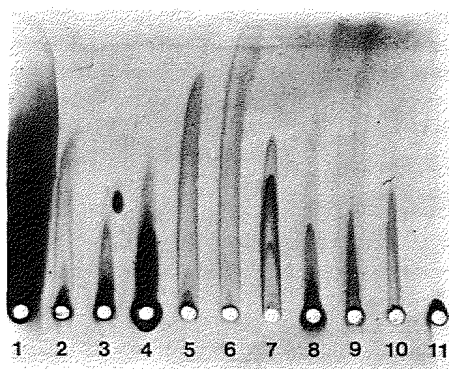


Fig. 2. Demonstration of specific antibodies against 11 different allergen extracts in rabbit anti-house-dust serum, by means of rocket immunoelectrophoresis. Gel and buffer as described in Fig. 1.

Antigens: 10 μ l of:

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|--|----------------------------|
| 1: St HD 76 | 7: Horse hair and |
| 2: <i>Dermatophagoides pteronyssinus</i> | dander |
| 3: <i>Dermatophagoides farinae</i> | 8: Sheep wool |
| 4: Human hair and dander | 9: Pig |
| 5: Dog hair and dander | 10: <i>Mucor racemosus</i> |
| 6: Cat hair and dander | 11: Coffee |

Antibodies: 23 μ l cm^{-2} of rabbit antibodies against extracts from house-dust. Electrophoresis: 18 h at 2 V cm^{-1} in a 0.15 cm-thick gel, anode at the top.

identical to extract from *Mucor racemosus*, whereas No. 6 showed only the reaction of partial identity to extract from cow. Antigen No. 9 appeared to be partially identical to extracts from cow, wool, *Trichosporon pullulans*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria iridis*. Antigen No. 10 showed partial identity to extracts from cat, horse, guinea pig, goat, *Mucor racemosus* and probably dog and wool, and antigen No. 11 possibly to extract from cat. Antigen Nos. 12, 13, 31 and 35 were partially identical to extracts from house-dust mites and human hair and dander. In addition, antigen No. 13 showed partial identity to human serum albumin and extracts from dog, cat, guinea pig, goat, *Cladosporium herbarum* and probably poultry feathers. Antigen Nos. 14, 33 and possibly 34 showed partial identity to extract from duck feathers only. Antigen No. 24 was partially identical to extracts from dog, cat,

either to increase the area of any precipitin arch in CIE with the addition of allergen extract, or to elevate any precipitin arch in the reference precipitation pattern in CLIE.

These extracts might nevertheless have antigens in common with St HD 76 as this very extract had not been used to raise the rabbit antibodies against house-dust employed in these experiments.

By means of CLIE and CIE with the addition of allergen extract, only antigens from 19 different sources were shown to be identical or partially identical to antigens from St HD 76. Those extracts which were able to form rockets in anti-house-dust antibodies but were not able to elevate precipitates of St HD 76 were thought to be not present in St HD 76.

Different species of house-dust mites have been shown by many studies to be included in house-dust.

In our experiments *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* turned out to have the same five antigens (Nos. 12, 13, 31, 35, 38) in common with St HD 76. These five antigens, among which antigen No. 13 is seen to be identical or

partially identical to human serum albumin, were also contained in the extract of human hair and dander, which correlates well with previous findings (5, 8, 13, 20, 29).

Surprisingly, human serum orosomucoid was found to be the only substance showing identity/partial identity to antigen No. 26. One might have expected this antigen to be found in extracts from human hair and dander and perhaps in extracts from mites as well.

Allergen extracts from animal hair and dander and extracts from house-dust have been demonstrated by different techniques to share common antigens. Our findings clearly confirmed that animal hair and dander are a significant source of antigenic components in house-dust. The pronounced antigenic similarity among extracts from hair and dander from different animals revealed in other works (9, 18, 22, 29) was verified here as well.

Several research works have indicated that allergen sources other than mites, human hair and dander and animal hair and dander might contribute antigenic material to house-dust (6, 11, 23, 24).

dust to antigens from 19 different origins

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
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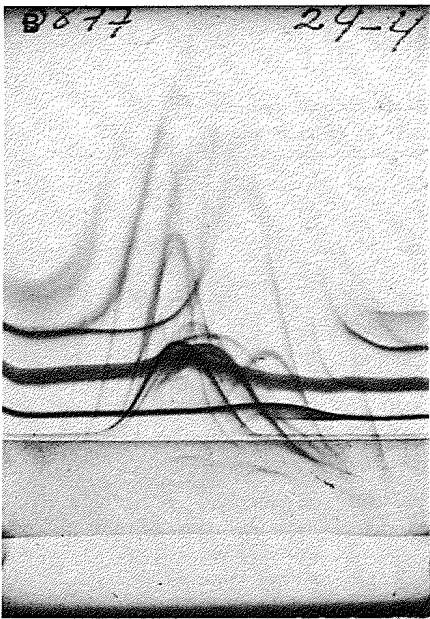
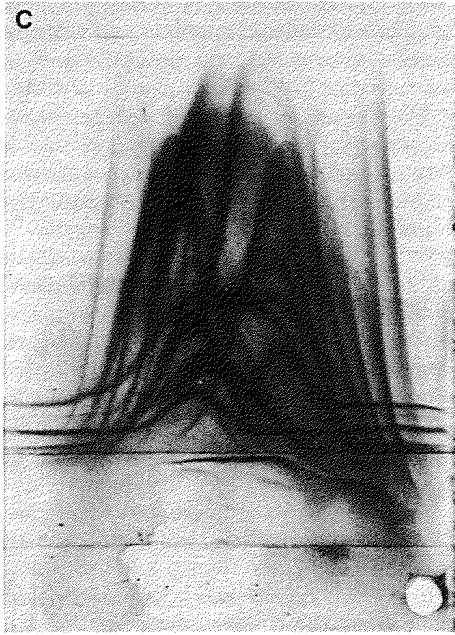
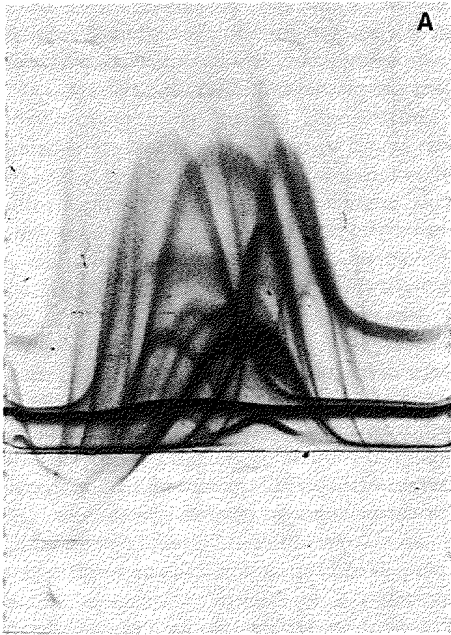
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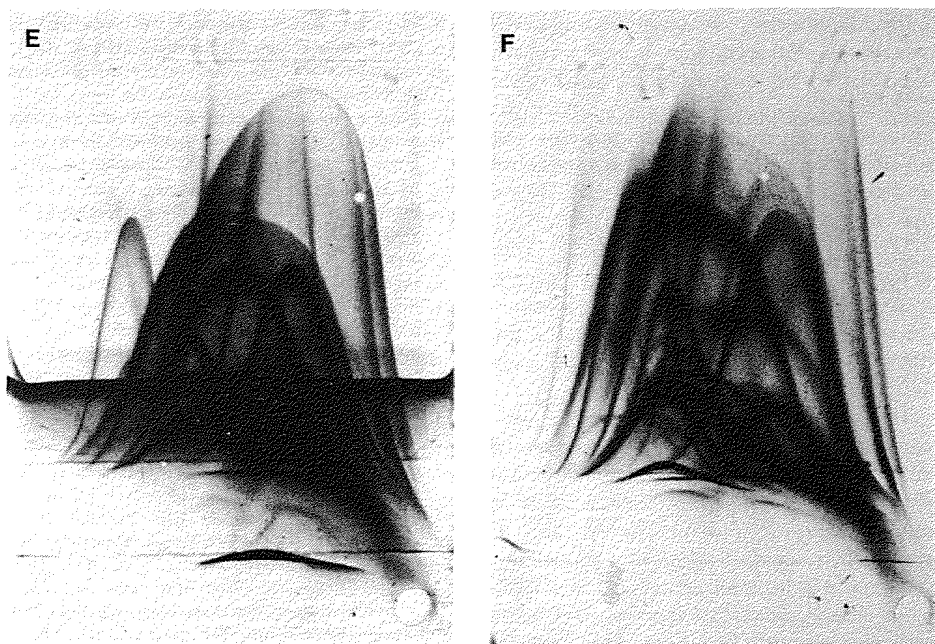


Fig. 8. Comparison of St HD 76 with antigen sources by means of CLIE or CIE with intermediate gel. Gel and buffer as described in Fig. 1.

Antigens: 10 μl of St HD 76 was applied. First dimension: 30 min at 10 V cm^{-1} in a 0.15 cm-thick gel. Antibodies: 12 $\mu\text{l cm}^{-2}$ of rabbit anti-house-dust serum. Second dimension: 2 V cm^{-1} in a 0.1 cm-thick gel for 18 h. Line or intermediate gels: In 0.13 cm-thick gels were incorporated:

A) *Dermatophagoides pteronyssinus* (66 $\mu\text{l cm}^{-2}$). B) Human hair and dander (13 $\mu\text{l cm}^{-2}$). C) Cat hair and dander (66 $\mu\text{l cm}^{-2}$). D) *Alternaria iridis* (66 $\mu\text{l cm}^{-2}$). E) Rabbit anti-orosomucoid (5 $\mu\text{l cm}^{-2}$). F) Isotonic saline (66 $\mu\text{l cm}^{-2}$). 5 $\times$ 7 cm^2 glass plates were used.

The possible role of fungi in house-dust allergy has been stressed by others (11, 23, 24). We found reactions of identity/partial identity between St HD 76 and five different moulds which are all shown by other methods (11) to be predominant in house-dust samples from Danish homes. A marked partial identity among the antigens of the individual moulds was revealed. Feathers have also been incriminated as a possible contributor of antigens in house-dust (6). In our study feathers from ducks and possibly poultry turned out to contain antigens which were also present in St HD 76.

Apart from partial identity of antigens from related allergen sources, partial identity of antigens from different groups of sources

was seen.

Mould antigens showed partial identity to antigens from animal hair and dander and feathers.

A possible explanation of this may be mould growth on feathers and animal hair and dander, thus generating degradation products containing both antigenic sites common to mould antigens and antigenic sites common to animal dander antigens. This also explains the partial identity of antigens from animal hair and dander to antigens from feathers. However, a recent study has shown that fungi isolated from horse hair and dander did not contribute significantly to the antigens extracted from horse hair and dandruff (12).

Partial identity of antigens from mite extracts to antigens from other groups of sources may be due to the contents in mite extracts of human hair and dander and human serum proteins which are well known to share antigens with proteins in animal serum and animal hair and dander. Thirty-one antigens of the revealed 45 antigens in St HD 76 remained unidentified. These antigens might originate from allergen sources other than the 106 most common ones incorporated in this research, or they might be degradation products of native antigens from other allergen sources.

House-dust extracts thus seem to be a very complex and heterogeneous mixture of antigens of both known and unknown origin. This makes it less suitable both for exact diagnostic tests in allergy, and for specific hyposensibilization. The documented occurrence in house-dust extracts of antigens from many known allergen sources might very well account for the allergic reactions against this extract. If this is the case it will make no sense to maintain the concept of house-dust allergy. To help clarify this problem, further analysis of house-dust extracts is at the moment being carried out by means of CRIE in order to determine which antigens constitute the major allergens.

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