

Mites and house dust allergy

II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) allergens by fractionation methods

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Summary

House dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) dialysed extracts were fractionated by gel filtration and ion-exchange chromatography. The protein and sugar content of each fraction was determined, respectively, by absorbance at 280 m μ and by the orcinol method. Pooled fractions were injected into house dust and mite sensitive patients and their capacity to induce positive skin reactions determined. A certain amount of allergenically active material was dialysable but the greater part remained inside the dialysis bag. The most active non-dialysable house dust and mite extract material was eluted in the excluded Sephadex G-50 fractions and appeared to be located immediately after the Sephadex G-75 excluded fractions. On the basis of calibration curves, its molecular weight was estimated between 10,000 and 50,000.

By further chromatography of active Sephadex fractions on DEAE-Sephadex, the most potent antigen-containing material of house dust and mite extracts was recovered using 0.3 and 0.2 M NaCl as elution media. These data offer further evidence of the existence of a close relationship between the major antigenic substances of house dust and mite extracts.

Introduction

The role of mites as possible house dust allergens has been extensively investigated by comparing the skin test activity of house dust and mite extracts (Voorhorst, Spiekma-Boezeman & Spiekma, 1964; Voorhorst *et al.*, 1967; Maunsell, Wraith & Cunningham, 1968; Pepys, Chan & Hargreave, 1968; Frankland, McEwen & Feinberg, 1970; Frankland & El-Hefny, 1971), by challenge and *in vitro* tests (McAllen, Assem & Maunsell, 1970; Stenius *et al.*, 1971) and by a fractionation method (Miyamoto, Oshima & Ishizaki, 1969). Nevertheless, a chemical similarity between mite and house dust allergens has not yet been definitely proved. This paper reports the results of a study on the allergenic relationship between house dust and mite extracts, carried out by fractionation methods.

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Materials and methods

House dust extract

Commercially available house dust extract (1:100 w/v) was obtained from Diephuis Laboratory (Groningen).

Mite and culture medium extracts

Extracts of *Dermatophagoides pteronyssinus* and its culture medium (1:100 w/v) were obtained from Diephuis Laboratory (Groningen). Extracts of *Dermatophagoides farinae* and its culture medium (1:100 w/v) were provided by Bencard (Brentford, Middlesex).

Chemical determinations

Extract protein was determined by the method of Lowry *et al.* (1951). Total sugar was determined as galactose equivalent according to the orcinol method.

Dialysis

Both *D. pteronyssinus* and *D. farinae* extracts were dialysed in Visking tubes against phosphate buffered saline (PBS) in the cold. The dialysed and non-dialysed extracts, as well as dialysates, were filtered through a millipore filter, diluted to the same concentrations with sterile saline under sterile conditions and injected intradermally in a group of asthmatic patients who showed positive skin reactions to the non-dialysed extracts.

Gel filtration

One per cent house dust, mite and mite culture medium extracts were dialysed against PBS in the cold, freeze-dried and dissolved in PBS to yield a 1/20 dilution. One ml of each extract was applied in 100 × 1.5 cm columns of Sephadex G-200, G-100, G-75 and G-50 (Pharmacia, Uppsala), pre-swollen in PBS. All the columns were eluted with the same buffer at a flow rate of 12 ml/hr and 2.5 ml fractions were collected. Optical densities were determined at three wavelengths: 280, 260 and 215 m μ . The sugar content of each tube eluate was determined as previously mentioned. Before elution the columns were calibrated with blue dextran and bovine serum albumin (BSA) (G-200 and G-100), BSA, cytochrome C and phenol (G-75 and G-50). Ten tube fractions were pooled and tested for skin activity.

Ion-exchange chromatography

The active fractions obtained by gel filtration on Sephadex G-75 were pooled, freeze-dried and dissolved in 1 ml of distilled water. 45 × 0.9 cm columns of DEAE-Sephadex A 50 (Pharmacia, Uppsala) were equilibrated with phosphate 0.05 M, pH 7.6, or with Tris-HCl 0.05 M, pH 8.0, buffers. The 1 ml sample was dialysed against the starting buffer in the cold and applied to the column. Elution was performed stepwise by incorporating increasing concentrations of NaCl (0.1–3.0 M) in the buffer. 2.5 ml fractions were collected and the optical density determined at two wavelengths: 280 and 215 m μ . The fractions were pooled, dialysed against PBS and tested for skin activity.

Skin testing

The pooled fractions were filtered through a millipore filter and ten-fold serial dilutions

Table 1. Protein and sugar content of 1% mite and mite culture medium extracts. Protein content was determined by the method of Lowry. Sugar content was determined as galactose equivalent, according to the orcinol method.

Extract (1%)	Protein ($\mu\text{g/ml}$)	Sugar ($\mu\text{g/ml}$)
<i>D. pteronyssinus</i>	130	190
<i>D. pteronyssinus</i> culture medium	100	110
<i>D. farinae</i>	30	140
<i>D. farinae</i> culture medium	30	100

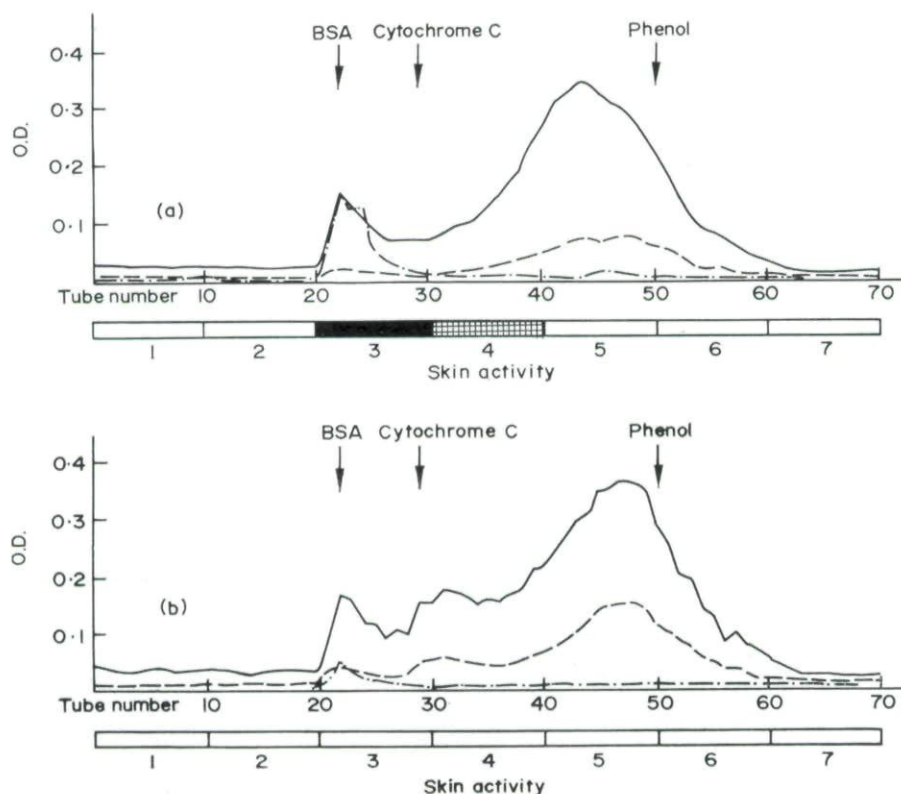


Fig. 1. (a) Gel filtration of 5% *D. pteronyssinus* extract on Sephadex G-50. Column equilibrated with PBS and eluted at a flow rate of 12 ml/hr by the same buffer. Calibration was performed with BSA (mol wt 67,000), cytochrome C (mol wt 13,000) and phenol (mol wt 94). Optical density was determined at 280 (-----), 215 (—) and at 540 (---) m μ , the latter according to the orcinol method, indicating sugar content. Ten tube fractions were pooled and 0.02 ml of each pool were injected intradermally in mite-sensitive patients. Skin activity of the pooled fractions—maximal in pool 3, moderate in pool 4 and absent in the other pools—is indicated by the shaded rectangles. (b) Gel filtration of 5% *D. pteronyssinus* culture medium extract on Sephadex G-50. All column pooled fractions were tested for skin activity in mite-sensitive patients. The skin tests were constantly negative.

then prepared with sterile saline under sterile conditions. 0.02 ml of each sample were injected intradermally into at least five asthmatic patients who showed positive reactions to the unfractionated extract. Skin reactions were evaluated as previously described (Biliotti *et al.*, 1972). The reactions were read by two workers who had not done the injections, without knowledge of the nature of the sample injected. Non-atopic subjects, tested with various column fractions, always showed negative skin tests.

Results

After dialysis, most of the allergenic material of both *D. pteronyssinus* and *D. farinae* extracts was found inside the Visking tubes but a certain amount was dialysed out.

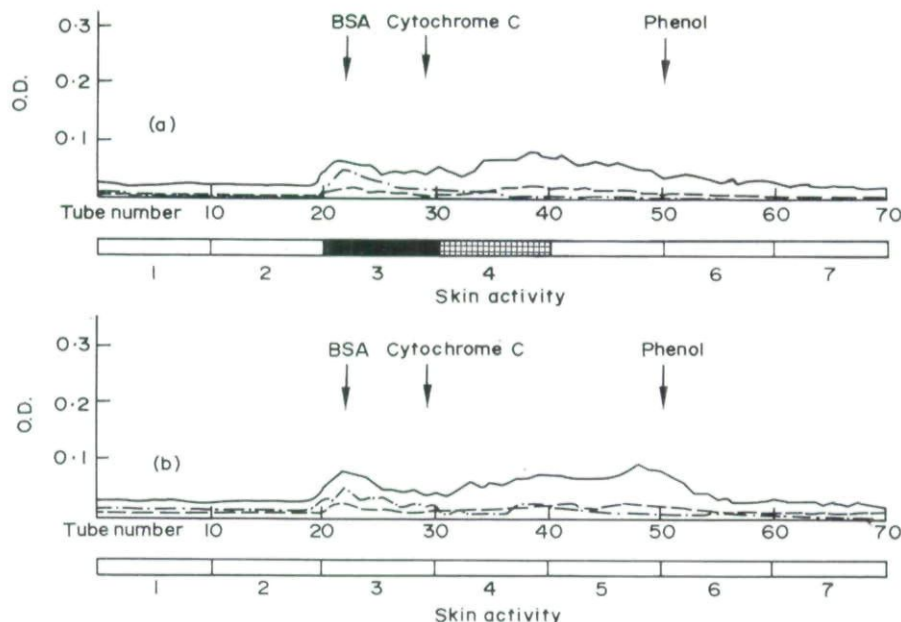


Fig. 2. (a) Gel filtration of 5% *D. farinae* extract on Sephadex G-50. See Fig. 1(a) for explanation. Skin activity was maximal in pool 3, moderate in pool 4 and absent in the other pools. (b) Gel filtration of 5% *D. farinae* culture medium extract on Sephadex G-50. The skin tests were constantly negative.

Miyamoto *et al.* (1968) and Holford-Strevens *et al.* (1970) reported similar results using *D. farinae* extracts. The dialysed extracts of each mite had a protein content similar to that of its respective culture medium but the extracts of *D. pteronyssinus* and its culture medium contained about 3–4 times as much protein found in those of *D. farinae* and its culture medium (Table 1).

Gel filtration of house dust and mite dialysed extracts on Sephadex G-200 and G-100 showed that the allergenic activity was rather widely spread after the excluded peak fractions. For this reason, Sephadex G-50 and G-75 were employed in further experiments.

D. pteronyssinus extract fractionation on Sephadex G-50 resulted in two protein peaks and one sugar peak. The sugar and first protein peak coincided with that of

BSA. The second protein peak was eluted after cytochrome C and before phenol (Fig. 1a). Gel filtration of *D. pteronyssinus* culture medium extract on Sephadex G-50 gave a similar elution pattern (Fig. 1b).

Gel filtration of *D. farinae* and its culture medium extracts on Sephadex G-50 showed a less distinct pattern of optical density (Figs. 2a and 2b). The fractionation of house dust extract on the same column resulted in a large asymmetrical peak when the optical density was read at 280 m μ (Fig. 3).

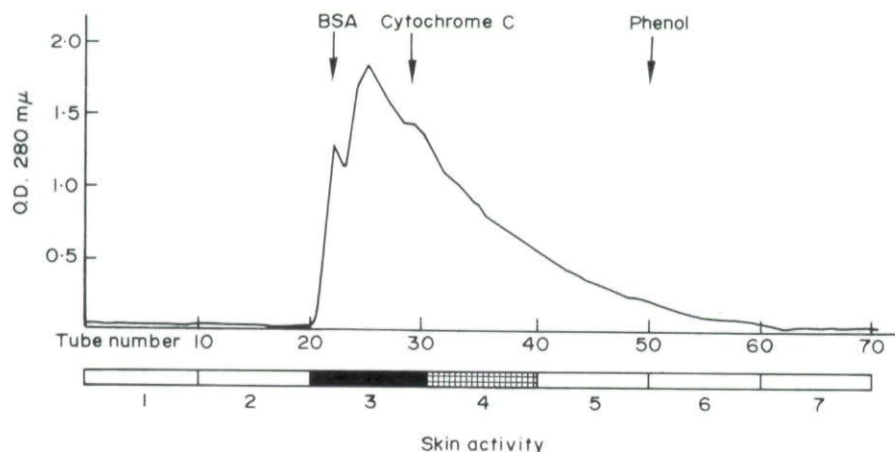


Fig. 3. Gel filtration of 5% house dust extract on Sephadex G-50. Skin activity was maximal in pool 3, moderate in pool 4 and absent in the other pools.

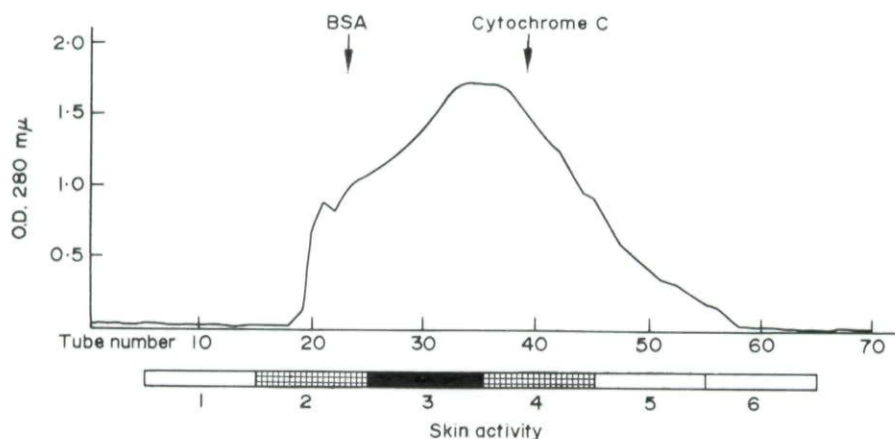


Fig. 4. Gel filtration of 5% house dust extract on Sephadex G-75. Skin activity was maximal in pool 3, moderate in pools 2 and 4, absent in the other pools.

When ten tube fractions were pooled and 0.02 ml of each sample were injected intradermally in those asthmatic patients who showed positive skin reactions to the unfractionated material, the most potent activity of mite and house dust extracts was detected in the excluded fractions (Figs. 1a, 2a and 3). On the contrary, all the corresponding pooled fractions of both mite culture medium extracts never induced significant skin reactions.

Gel filtration of house dust extract on Sephadex G-75 also resulted in an asymmetrical peak starting immediately after the void column (Fig. 4). The fractionation of *D. pteronyssinus* extract on the same column resulted in two protein peaks and one sugar peak. The sugar and first protein peak coincided with the BSA peak (Fig. 5). A very similar elution pattern was found when *D. pteronyssinus* culture medium extract was fractionated on the same column. A less distinct pattern of optical density was obtained by fractionating extracts of *D. farinae* and its culture medium (Fig. 6). In

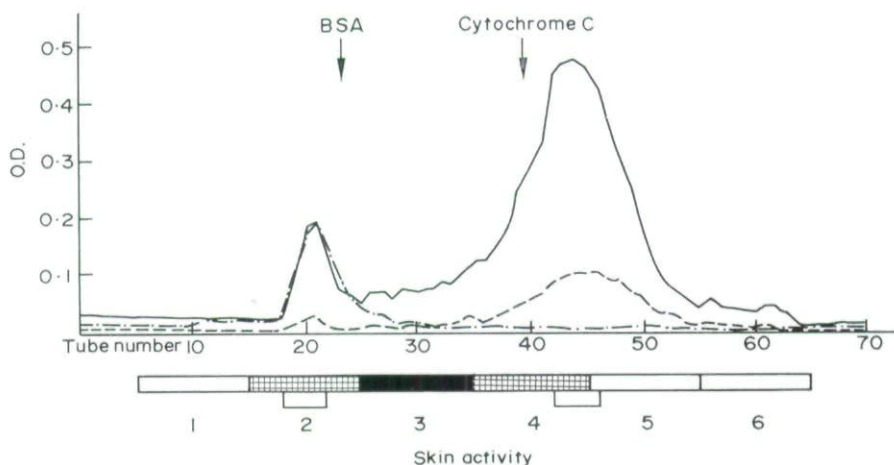


Fig. 5. Gel filtration of 5% *D. pteronyssinus* extract on Sephadex G-75. See Fig. 1(a) for explanation. Skin activity was maximal in pool 3, moderate in pools 2 and 4, absent in the other pools. No activity was found in the fractions corresponding to the mid-portion of both peaks (small white rectangles).

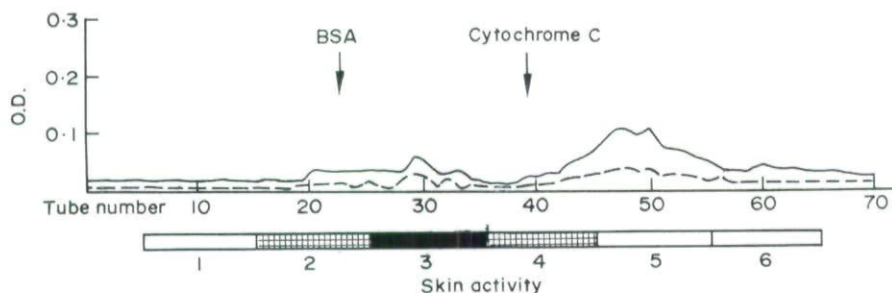


Fig. 6. Gel filtration of 5% *D. farinae* extract on Sephadex G-75. Skin activity was maximal in pool 3, moderate in pools 2 and 4, absent in other pools. Optical density was determined at 215 (—) and 280 (---) m μ .

asthmatic patients injected intradermally with pooled fractions, the most potent activity of all the extracts was seen to be in the material eluted between the BSA and cytochrome C peak (Figs. 4, 5 and 6).

When the most active fractions, obtained by gel filtration on Sephadex G-75, of *D. pteronyssinus* extract were applied on a DEAE-Sephadex A-50 column equilibrated with Tris-HCl 0.05 M, pH 8, and eluted by incorporating increasing concentrations of NaCl in the starting buffer, it was possible to demonstrate that the most potent

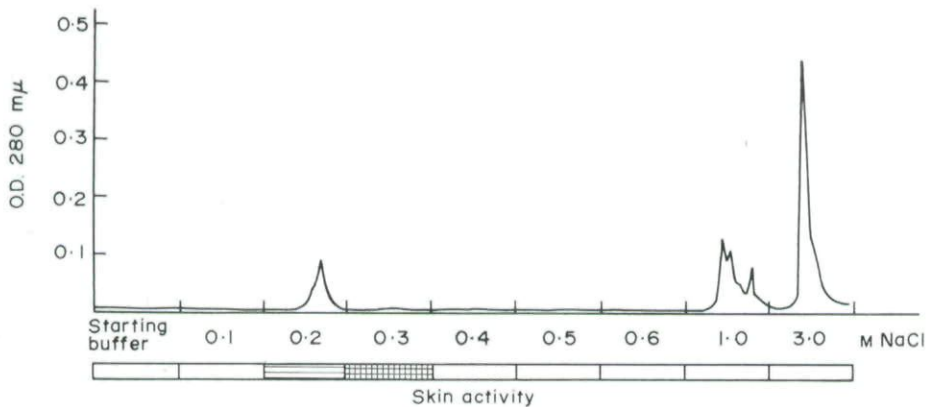


Fig. 7. Chromatography of Sephadex G-75 active fractions of house dust extract on DEAE-Sephadex A-50. Column was equilibrated with phosphate buffer 0.05 M, pH 7.6, and elution performed stepwise by incorporating increasing amounts of NaCl in the starting buffer. The fractions eluted with the same molarity were pooled and 0.02 ml of each pool were injected intradermally in house dust sensitive patients. The skin activity was moderate in the fractions eluted by 0.3 M NaCl, slight in those eluted by 0.2 M NaCl. It should be noted that most of the pigmented material became firmly bound to the gel.

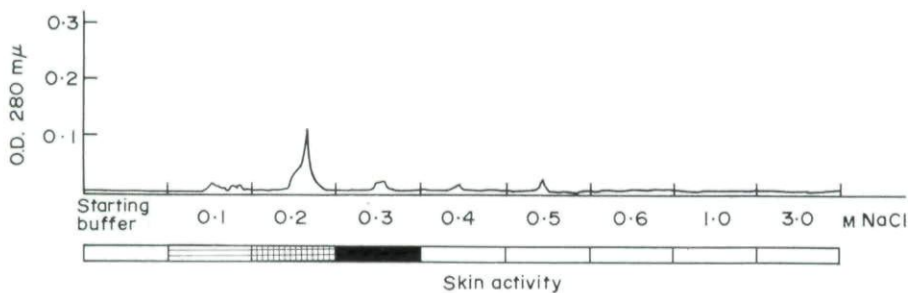


Fig. 8. Chromatography of Sephadex G-75 active fractions of *D. farinae* extract on DEAE Sephadex A-50. See Fig. 7 for explanation. Skin activity was maximal in the fractions eluted by 0.3 M NaCl, moderate in those eluted by 0.2 M NaCl, slight in those eluted by 0.1 M NaCl and absent in all the other fractions.

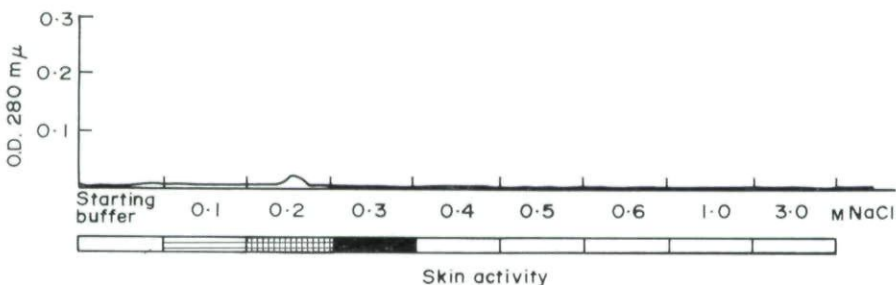


Fig. 9. Chromatography of Sephadex G-75 active fractions of *D. pteronyssinus* extract on DEAE-Sephadex A-50. See Fig. 7 for explanation. Skin activity was maximal in the fractions eluted by 0.3 M NaCl, moderate in those eluted by 0.2 M NaCl, slight in those eluted by 0.1 M NaCl and absent in all the other fractions.

activity was located in the fractions recovered by using 0.3 M NaCl as elution medium. However, a marked brown precipitate occurred in house dust extracts G-75 fractions, freeze-dried, resuspended in 1/5 the original volume and dialysed against Tris-HCl buffer. For this reason, phosphate 0.05 M, pH 7.6, was used as starting buffer in ion-exchange chromatography.

When active Sephadex G-75 house dust extract fractions were eluted on DEAE-Sephadex by incorporating increasing amounts of NaCl in the phosphate buffer three peaks were obtained at 0.2, 1.0 and 3.0 M NaCl. The fractions eluted at 0.2 M NaCl were colourless while those at 1.0 and 3.0 M NaCl were pale brown; however, the greater part of the pigmented material bound tenaciously to the gel as previously reported (Lietze & Reed, 1962).

The intradermal injection of different fractions in asthmatic patients showed that the 0.2 and 0.3 M NaCl fractions possessed skin activity. The elution of corresponding G-75 active fractions of both mite extracts on DEAE-Sephadex resulted in a little peak when 0.2 M NaCl was incorporated in the starting buffer. The fractions of both extracts eluted at 0.3 M NaCl showed the most potent skin activity; a less marked activity was present in fraction eluted with 0.2 and 0.1 M NaCl (Figs. 8 and 9).

Discussion

Our results offer further evidence that a relationship exists between house dust and dust mite (*D. pteronyssinus* and *D. farinae*) allergens. The most potent activity of these extracts is contained in the fractions excluded by Sephadex G-50 or eluted immediately after the excluded peak on Sephadex G-75. Miyamoto *et al.* (1969), after fractionating house dust and *D. farinae* extracts on Sephadex G-200 and G-50, suggested that the most potent antigen was common to both extracts and had a molecular weight between 10,000 and 69,000, probably around 40,000–50,000. From our data it seems possible that the most active substances contained in house dust, *D. pteronyssinus* and *D. farinae* extracts have a molecular weight between 10,000 and 50,000, probably around 30,000.

The optical density elution patterns of both mite extracts seem to be determined by the sugar and protein material of the culture medium as they are similar to those obtained by gel filtration of the media alone. The skin activity of the mite extracts present in the excluded Sephadex G-50 peak fractions does not seem to be related to the sugar-rich material present in the latter, as shown by the results of gel filtration on Sephadex G-75.

Fractionation of house dust extract on different Sephadex did not yield any distinct molecular resolution and resulted in an asymmetrical peak of stained material as previously reported (Lietze & Reed, 1962; Morris, Berrens & Young, 1965). However, most of the allergenic substances seem to be eluted in the fractions corresponding to those in which mite extract activity can be found. Further fractionation of the most active Sephadex G-75 material by ion-exchange chromatography showed that most house dust and mite allergenic activity was located in the fractions eluted with 0.3 and 0.2 M NaCl. As concerns the activity of DEAE-Sephadex house dust fractions, our results seem to agree with those reported by Elias, Voorhorst & Krieken (1964). In fact these authors obtained two colourless and equally active fractions using 0.25 and 0.35 M NaCl as elution media in the fractionation of a cetyl-pyridine-chloride purified house dust extract on DEAE-Sephadex A-25. No information is available on the ion-exchange chromatography of mite extracts. Our data seem to demonstrate

that the most potent allergenic activity is present in the fractions eluted with 0.3 M NaCl where no distinct pattern of optical density was seen and, even if less marked, in those eluted with 0.2 M NaCl where a small peak with an optical density of 280 m μ was observed.

We do not know whether the DEAE-Sephadex fractionation isolated one or more allergens from the mite extracts as the skin reactions do not serve to demonstrate any qualitative difference. Moreover, further investigations are required in order to ascertain the nature of active dialysable substances and their relationship to non-dialysable material. However, the demonstration that the most active material of house dust and mite extracts is represented by molecules with a similar electrical charge and molecular weight seems to be the most significant point emerging from our experiments.

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