

# Antigenic relation between house dust and a dust mite, *Dermatophagoides farinae* Hughes, 1961, by a fractionation method

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*Both house dust and mite (Dermatophagoides farinae Hughes, 1961) extracts were filtrated through Sephadex G-50 and 200 with  $I^{131}$ -labeled human serum albumin and phenol as indicators. Protein and sugar contents of each constituent were determined by absorbance at 280 m $\mu$  and by a slightly modified anthrone method, respectively. The end point of skin test-eliciting capacity with twofold dilutions of each fraction was determined in 6 house dust-sensitive subjects. The tube numbers of the eluent of the house dust or the mite extract containing the most potent antigen eliciting skin reactions in house dust-sensitive subjects agreed with each other, but these fractions did not necessarily match the protein or sugar content. Moreover, these potent fractions did not appear to match the antigen producing precipitating bands against antiserum of the experimental animals. Consequently, it was thought that the most allergenic constituent in house dust and mites is not necessarily the most antigenic in experimental animals. Considering the location of the most potent, active eluate in allergic subjects on Sephadex, the molecular weight of such antigen was thought to be larger than 10,000 but smaller than 69,000, and the composition of such antigen was considered to be probably a protein and polysaccharide conjugate. Further study in this line is in progress.*

It has been concluded that one of the major antigenic substances in house dust is the body and excreta of mites contained in house dust, as previously reported.<sup>1-4</sup> This paper concerns further investigations regarding immunological relationships between these two antigens with the use of fractionation methods.

## MATERIALS

### House dust extract

Commercially available house dust extract\* (1:10 w/v) was thoroughly dialyzed, lyophilized, and dissolved in phosphate buffer saline at pH 7.4 to yield

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\*Obtained from Torii Pharmaceutical Co., Tokyo, Japan. The mite content of the original material from which the house dust extract was prepared is unknown.

When one milligram of house dust is extracted into one milliliter of solution, this solution is defined to be 1:1,000 dilution extract in weight by volume.

a 1:1 dilution of house dust extract, since the protein and sugar contents of a 1:10 dilution of the extract were not sufficient in quantity to be measured after fractionation by the methods employed. Protein nitrogen<sup>4</sup> and glucose equivalent<sup>5</sup> of 1:1 dilution of house dust were 176.3  $\mu\text{g}$  and 934.0  $\mu\text{g}$  per milliliter, respectively.

#### Mite extract

Extract of body of purely cultivated *Dermatophagoides farinae* Hughes, 1961, was used in the concentration of 1:30 w/v. The process of extraction was reported previously.<sup>4</sup> Protein nitrogen<sup>4</sup> and glucose equivalent<sup>5</sup> of 1:30 dilution of the mite extract were 68.0  $\mu\text{g}$  and 128.0  $\mu\text{g}$  per milliliter, respectively.

#### Subjects

Six asthmatic patients with positive intracutaneous skin tests to house dust extract (1:1,000) were subjected to the skin tests with eluted fractions from Sephadex columns.

#### Experimental animals

Albino rabbits weighing approximately 3,000 grams and guinea pigs weighing about 300 grams were used for immunization against house dust and mite.

#### Immunization of animals

Experimental animals were repeatedly injected with an emulsion of equal parts of incomplete Freund's adjuvant and house dust (1:1 w/v) or mite antigen (1:30 w/v). Injections were given every 10 days for 2 months, and blood was obtained 2 weeks after the last injection.

### METHODS

#### Sephadex fractionation

House dust and mite extracts mixed with less than 0.01 ml. of a 1:1,000 dilution of I<sup>131</sup>-labeled human serum albumin (HSA)\* were subjected to gel filtration by the use of Sephadex G-50 and G-200 columns. Equilibration of the 15 mm.  $\times$  450 mm. columns and elution were done with 0.15M NaCl buffered with phosphate at pH 7.4 in a cold room. One and one-half milliliters of the extract was applied, and 2.3 ml. aliquots of the column eluate were collected. A small amount of 0.2 per cent phenol solution was passed through the same Sephadex column under the same conditions after the column was thoroughly washed with buffered solution following the elution of extract. The same column was used in the elution of house dust and mite extract. Protein content of each eluate was determined by absorbance at 280 m $\mu$ , and sugar content was determined by a slightly modified anthrone method,<sup>5</sup> in which three different concentrations of glucose solution (10  $\mu\text{g}$  per milliliter, 50  $\mu\text{g}$  per milliliter, and 100  $\mu\text{g}$  per milliliter) were used as standard solutions. For calibration purposes, eluted

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\*Activity of I<sup>131</sup> HSA was originally 0.5 mc. A minute amount of diluted solution was added, which gave sufficient counts but showed no absorbance at 280 m $\mu$ .

human serum albumin was located by radioactivity of the eluted fractions, in a well-type scintillation counter, and eluted phenol, by absorbance at 280 m $\mu$ .

### Precipitation of protein with trichloroacetic acid

House dust extract (1:1 w/v) was mixed with an equal volume of 20 per cent trichloroacetic acid and centrifuged to remove any precipitate. Supernatant fluid was dialyzed against phosphate buffer saline and was passed through the G-200 column after being mixed with I<sup>131</sup> HSA. Protein and sugar concentrations of the fractions were estimated according to the methods mentioned.

### Skin testing

Each fraction was filtered through a Millipore filter and diluted 100 times with saline to make the standard solution. Sterility was ascertained before use. For skin testing, twofold serial dilutions of the standard solution were made with sterile saline under sterile conditions. Skin testing was done with each fraction by injecting 0.02 ml. of the solution intracutaneously. The maximum dilution that elicited a positive skin reaction was considered the end point. Each patient was subjected to skin testing with house dust as well as mite fractions eluted from the same Sephadex column but not with the eluates from different Sephadex columns, since numerous skin tests annoyed the patients. Normal control subjects were tested with various column fractions. Their skin tests were entirely negative.

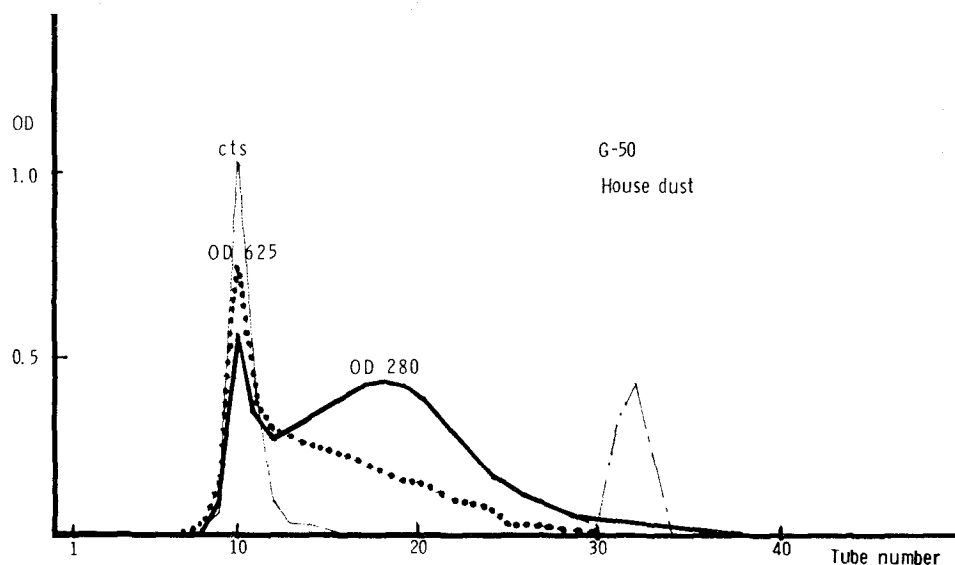


Fig. 1

Fractionation of house dust extract on Sephadex G-50. *cts*: Counts of I<sup>131</sup> human serum albumin. —: *O.D.*<sub>280</sub> indicating protein concentration. ....: *O.D.*<sub>625</sub> according to the anthrone method, indicating sugar content; 10  $\mu$ g per milliliter of glucose corresponds to 0.181 of *O.D.*<sub>625</sub>, 50  $\mu$ g per milliliter to 0.955, and 100  $\mu$ g per milliliter to 1.753. — · — · —: Eluate of phenol determined by *O.D.*<sub>280</sub>.

### Gel diffusion and immunoelectrophoresis

House dust (1:1 w/v) and mite extract (1:30 w/v) were tested by gel diffusion and immunoelectrophoresis against rabbit and guinea pig antisera. Each fraction was also examined by gel diffusion to see the location of antigenicity of both extracts for the experimental animals and to demonstrate the relation between antigenicity for the animals and the allergic human subjects.

## RESULTS

### Fractionation and skin test results

Fractionation of house dust extract on Sephadex G-50 resulted in two peaks of protein, and the sharp peaks of protein and sugar coincided with the peak of  $I^{131}$ -labeled HSA (Fig. 1). Mite fractionation on the same column presented the sharp peak of sugar but a flat curve of protein (Fig. 2). The most potent antigenic fraction detected by skin reactivity was contained in those fractions with the peak of  $I^{131}$ -labeled HSA in mite as well as house dust fractions, as shown in Figs. 3 and 4.

Fractionation on Sephadex G-200 resulted in large peaks of protein and sugar in house dust (Fig. 5). These peaks corresponded well. Fractionation of the mite extract on the same column resulted in similar curves (Fig. 6). End

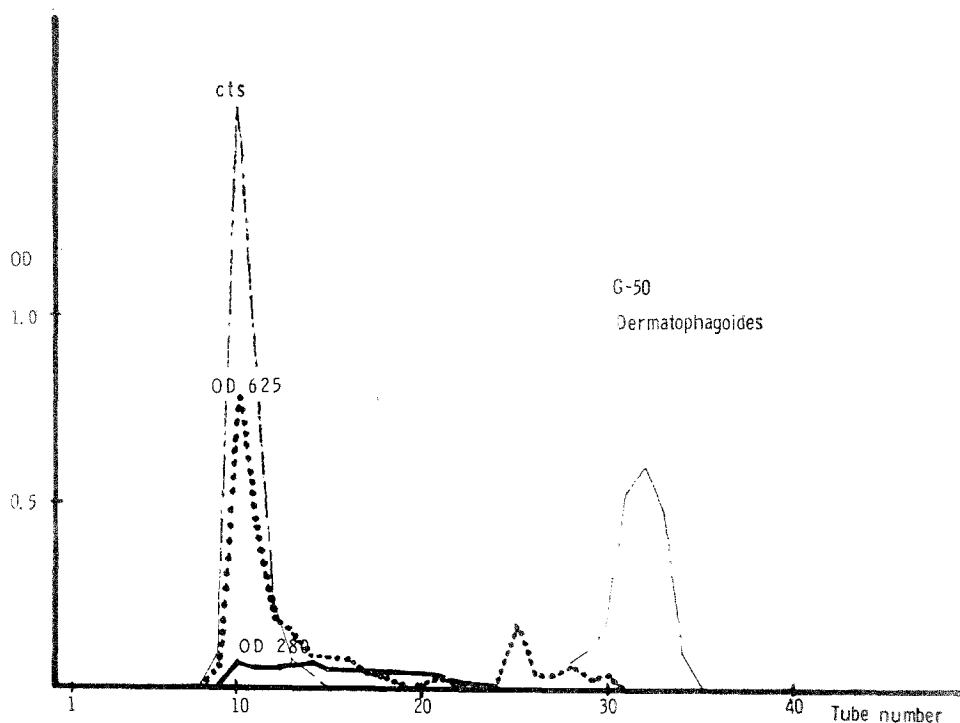


**Fig. 2**

Fraction of *D. farinae* extract on Sephadex G-50. Refer to Fig. 1 for explanation of abbreviations.

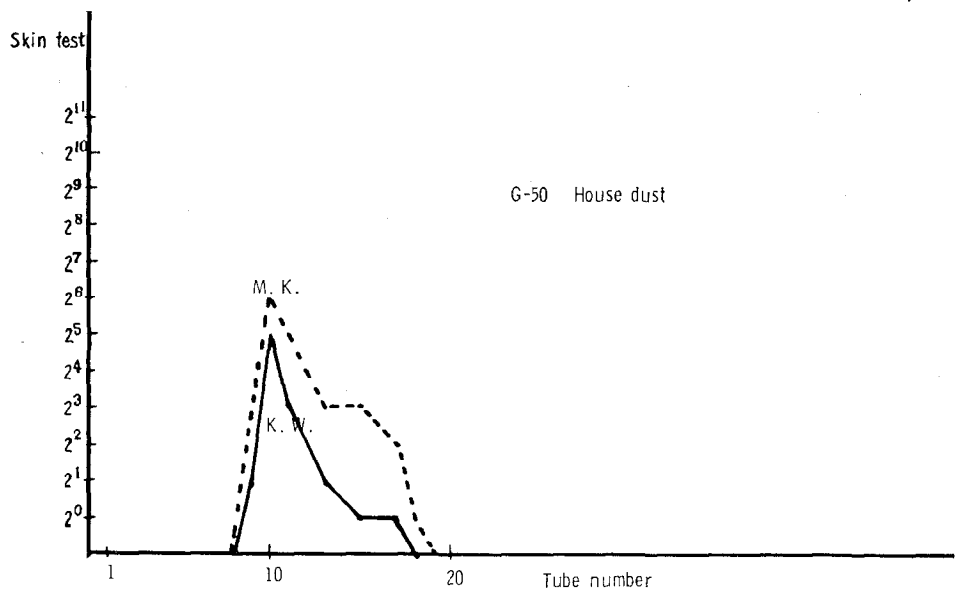


**Fig. 3**  
End points of skin tests of eluates of house dust extract from a Sephadex G-50 column. M. K. and K. W. indicate initials of patients.

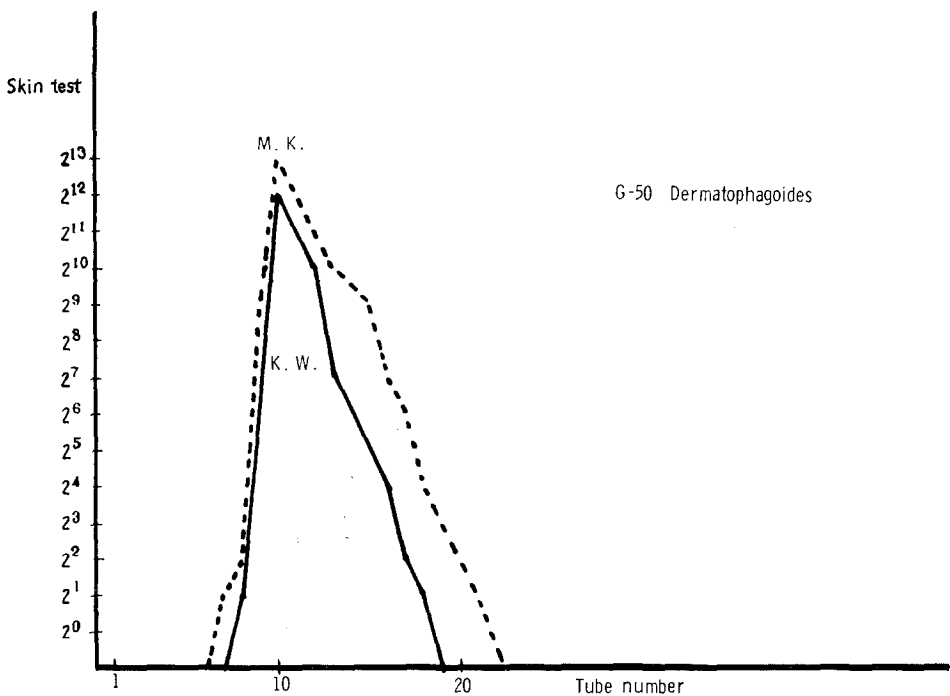


**Fig. 4**  
End points of skin tests of eluates of *D. farinae* extract from a Sephadex G-50 column. The patients (M. K. and K. W., Fig. 3) employed in skin testing with house dust eluate also were subjected to this study.

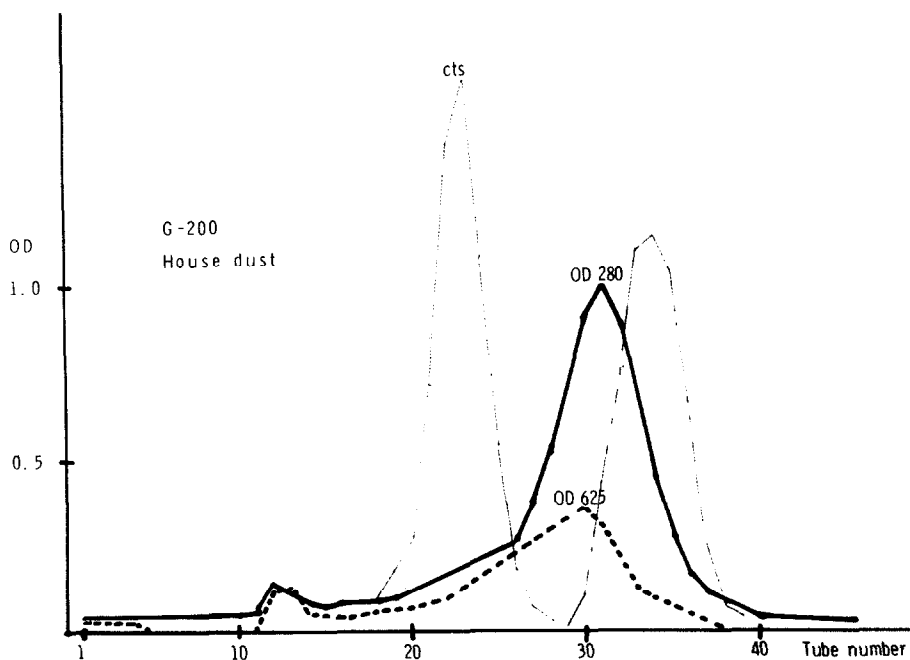


**Fig. 5**

Fractionation of house dust extract on Sephadex G-200. Refer to Fig. 1 for explanation of abbreviations.

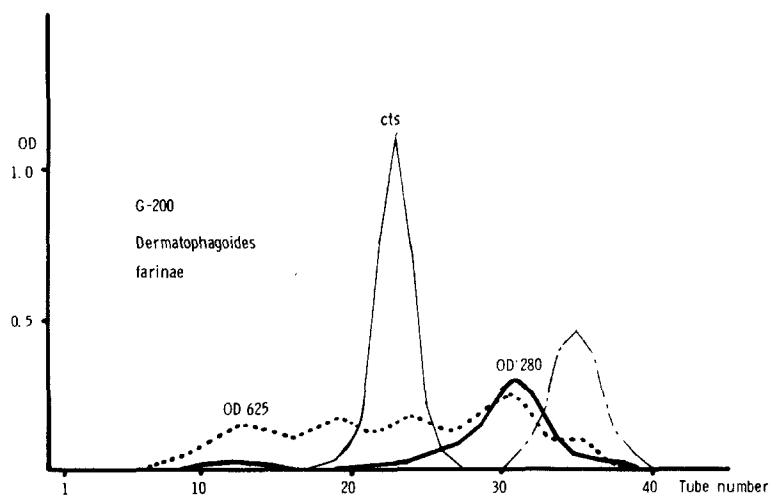


**Fig. 6**

Fractionation of *D. farinae* extract on Sephadex G-200. Refer to Fig. 1 for explanation of abbreviations.

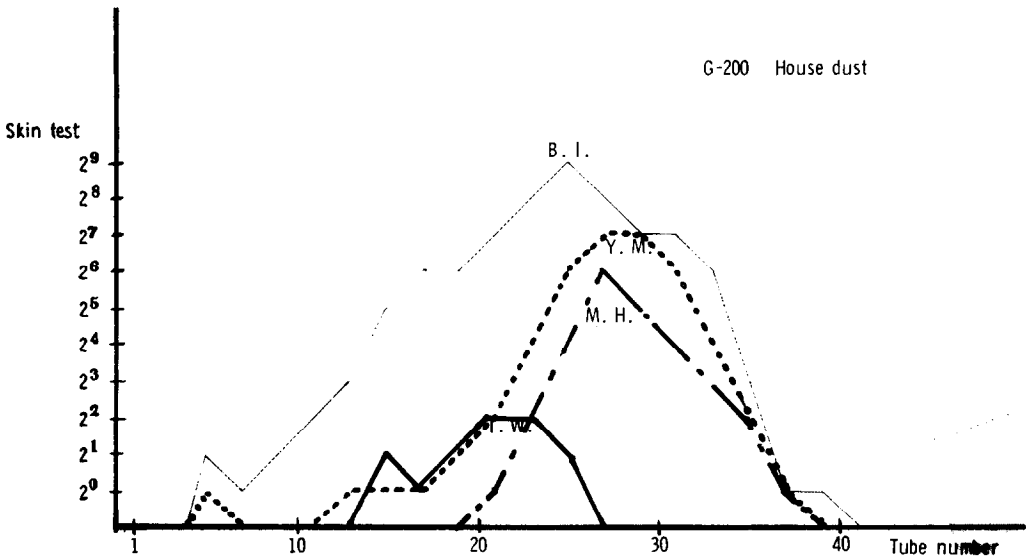


**Fig. 7**

End points of skin tests of eluates of house dust extract from a Sephadex G-200 column. Four patients (B. I., Y. M., M. H., and I. W.) were subjected to this study.

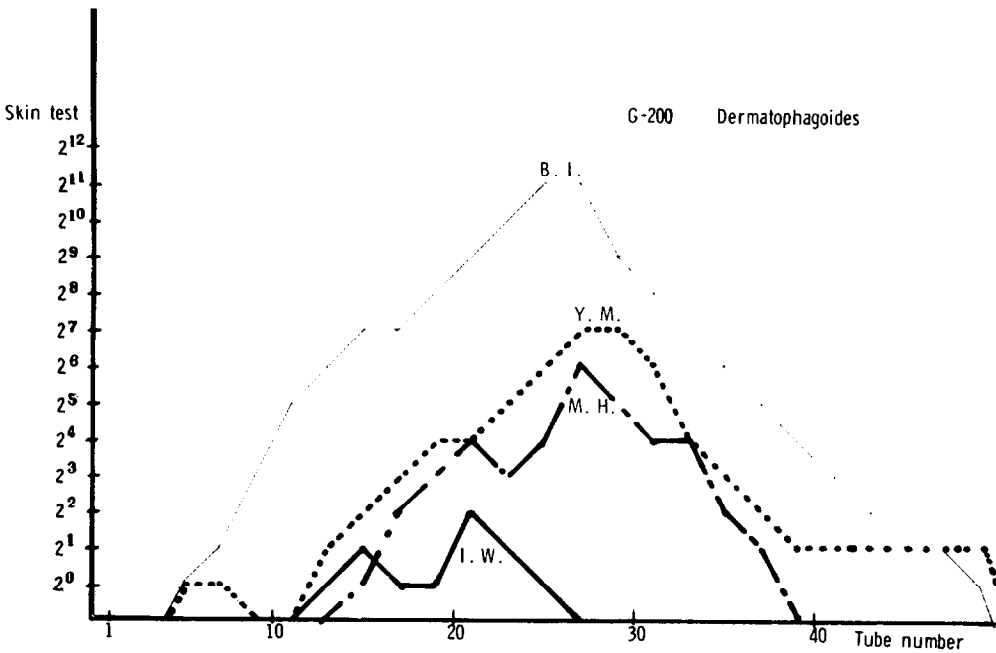


**Fig. 8**

End points of skin tests of eluates of *D. farinae* extract from a Sephadex G-200 column. The same patients (B. I., Y. M., M. H., and I. W., Fig. 7) subjected to skin testing with house dust eluates were subjected to *D. farinae*.

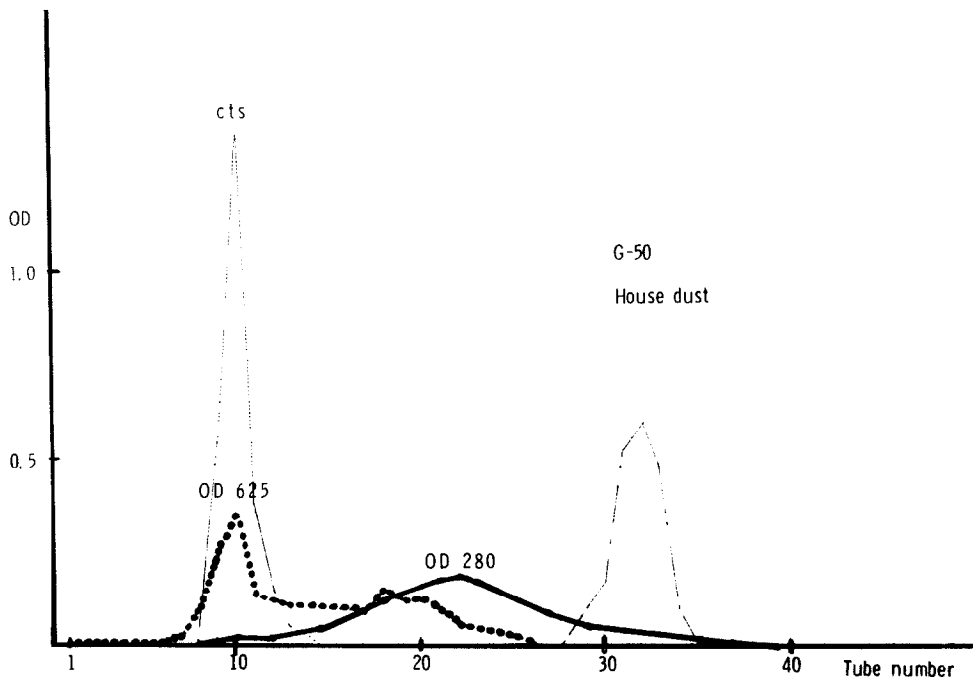


**Fig. 9**

Filtration of house dust extract on Sephadex G-50 after treatment with trichloroacetic acid. Refer to Fig. 1 for explanation of abbreviations.

points of skin tests with these fractions showed the most potent antigen located between the peaks of  $I^{131}$  HSA and phenol and at eluates earlier than at the peak of optical density at  $280\text{ m}\mu$  ( $O.D._{280}$ ), although the antigen was spread rather widely (Figs. 7 and 8). Tube numbers of fractions containing potent allergenic activity eluted either from Sephadex G-50 or G-200 matched between house dust and mite extracts in each asthmatic subject. The potent effluent peak of antigen was located after the elution of HSA. Estimating from the location of such a peak in relation to eluted fractions of HSA and phenol, the molecular weight of the most potent antigen of house dust and mite was thought to be larger than 10,000 but smaller than 69,000, most likely around 40,000 to 50,000. Antigenic fractions eliciting skin reactions in asthmatic patients, however, spread quite widely in house dust as well as mite fractions, as seen in Figs. 7 and 8. Mite fractions presented generally low optic density at  $280\text{ m}\mu$  and low sugar content in comparison with house dust fractions at corresponding tube numbers. Yet, antigenic potency of mite extract in eliciting skin reactions was higher than house dust.

After precipitation with trichloroacetic acid, the protein, especially that of large molecular size, was reduced markedly, and sugar content also was much reduced after this treatment (Fig. 9). The skin reactivity of each fraction was also markedly reduced. The extent of loss was estimated from the skin reactions to indicate less than one hundredth of the original potency.

### Gel diffusion and immunoelectrophoresis

Two distinct and two weak precipitin bands were observed between house dust (1:1 w/v) and anti-house dust rabbit or guinea pig serum on gel diffusion. Tube Nos. 25 through 31 of house dust fractionated on Sephadex G-200 and tube Nos. 10 through 17 of house dust on Sephadex G-50 produced precipitin bands against anti-house dust serum. The strongest bands were observed at tube No. 29 on Sephadex G-200 and No. 11 on Sephadex G-50. They did not necessarily correspond to the fraction that elicited the strongest skin reaction. Moreover it became clear that eluates with high optical density at 280 m $\mu$ , which presumably contained a high protein concentration, were not necessarily antigenic in experimental animals. This also applied to eliciting skin reactions in human subjects. Immunoelectrophoresis demonstrated two distinct precipitin bands against the 1:1 dilution of house dust, but no band was observed with any of the fractions. Precipitin bands were not observed between mite extract (1:30 w/v) and antisera of animals immunized with mite or house dust. This was probably due to an insufficient concentration of antigen in mite extract to produce a precipitin band.

### DISCUSSION

As previously reported,<sup>3, 4</sup> a close antigenic relationship was found between house dust and *D. farinae*, one of the common floor mites. In this report the antigenic relationship between house dust and *D. farinae* has been investigated further by an immunochemical method. Both house dust and the mite antigens which elicited strong skin reactions in house dust-sensitive subjects were contained in the fraction containing the I<sup>131</sup>-labeled HSA after fractionation on Sephadex G-50. After fractionation on Sephadex G-200, the most potent antigens of house dust and mite extracts were found after the I<sup>131</sup> HSA but before the protein peak and phenol fraction. The biologically potent fractions producing skin reactions did not match the peak fraction of O.D.<sub>280</sub> or sugar in the house dust or the mite extract. Moreover, these fractions did not necessarily agree with the fraction which produced precipitin bands on gel diffusion. The tube numbers of the eluted fractions yielding the skin reactions corresponded fairly well when house dust and mite extracts were fractionated on the same Sephadex. Sephadex G-50 and Sephadex G-200 exclude materials having molecular weights higher than about 10,000 and 200,000, respectively. The molecular weight of human serum albumin (HSA) is 69,000. Consequently, from the relation to the elution of I<sup>131</sup> HSA and phenol the molecular size of the most potent antigen was estimated to be larger than 10,000 and smaller than 69,000, most likely around 40,000 to 50,000.

Since the antigen that produced the precipitin band against antisera of the experimental animals did not precisely match the antigen that elicited skin reactions in allergic persons, it was thought that the major portion of the antigens in house dust did not necessarily have allergenicity in human subjects. When the extract was treated with trichloroacetic acid, not only the protein but also the sugar content was reduced markedly, and the skin reactivity decreased. Consequently it was thought that the major part of protein in house

dust antigen is in a conjugated state with sugar. Thus, the antigen eliciting skin reactions is thought to be probably a protein-sugar conjugate and most likely a protein-polysaccharide conjugate, although this statement is not exactly justified at the present time since antigenic purity is not established. Research in this line is in progress, however.

It was reported that house dust contains multiple antigens as detected by gel diffusion.<sup>6, 7</sup> In our studies, however, only four precipitin bands were obtained. This difference could be due to differences in preparation of house dust extract, differences in origin of house dust, or insufficient concentration of house dust extract as antigen for gel diffusion or immunization. Nevertheless, it became obvious that the precipitating antigen in the experimental animals is not necessarily important in human subjects in production of skin-sensitizing antibody.

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