

Cross-antigenicity among six species of dust mites and house dust antigens

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Body and excreta of Dermatophagoides farinae were found to be an important allergen in house dust. However, house dust contained more than 35 species of mites. The major mites in house dust are Glycyphaginae and D. pteronyssinus, which make up 31.8 per cent and 27.2 per cent, respectively. D. farinae comprises only 4 per cent of the total mites, as an average, in house dust in Japan. Consequently antigenic relationships between house dust and 6 species of mites, including D. farinae, were studied by the method of the skin testing and the in vitro neutralization of skin-sensitizing antibody. It was found that D. farinae was most closely related to house dust antigen in comparison with 5 other species of mites: Chortoglyphus arcuatus, Blomia sp., Aleuroglyphus ovatus, Suidasia nesbitti, and Tyrophagus putrescentiae. Cross-antigenicity was found among these mites although this was not complete, and it was considered that each species of mite had its own characteristic antigen. Moreover, the antigenicity of D. farinae was quite potent, and D. farinae appeared to possess the major common antigen among these species of mites. Consequently the existence of identical antigenicity between house dust and D. farinae was not surprising even though D. farinae constituted only a few per cent of the mites in house dust in number. D. farinae and C. arcuatus were more potent in skin reactivity in comparison with other species of mites, but cross-antigenicity between these 2 species of mites was not as close as that between the other species. The bodies of mites had identical antigenicity with the excreta.

It has been found that close antigenic similarity exists between house dust and a dust mite, *Dermatophagoides* farinae* Hughes, 1961, as reported previously.^{1, 2} House dust collected from various parts of Japan was found to contain more than 35 species of mites. The number and percentage of each species of mite are variable, however.⁴ Since *D. farinae* makes up only 4 per cent of the total number of mites, it was thought that cross-antigenicity exists between the various species of mites. Since 5 other species of mites, *Chortoglyphus arcuatus*

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*It was proposed recently by one of our authors (S. O.) that the scientific name of *Dermatophagoides* Bogdanoff, 1864, should be changed to *Mealia* Trouessart, 1897, for the reason of taxonomy.³

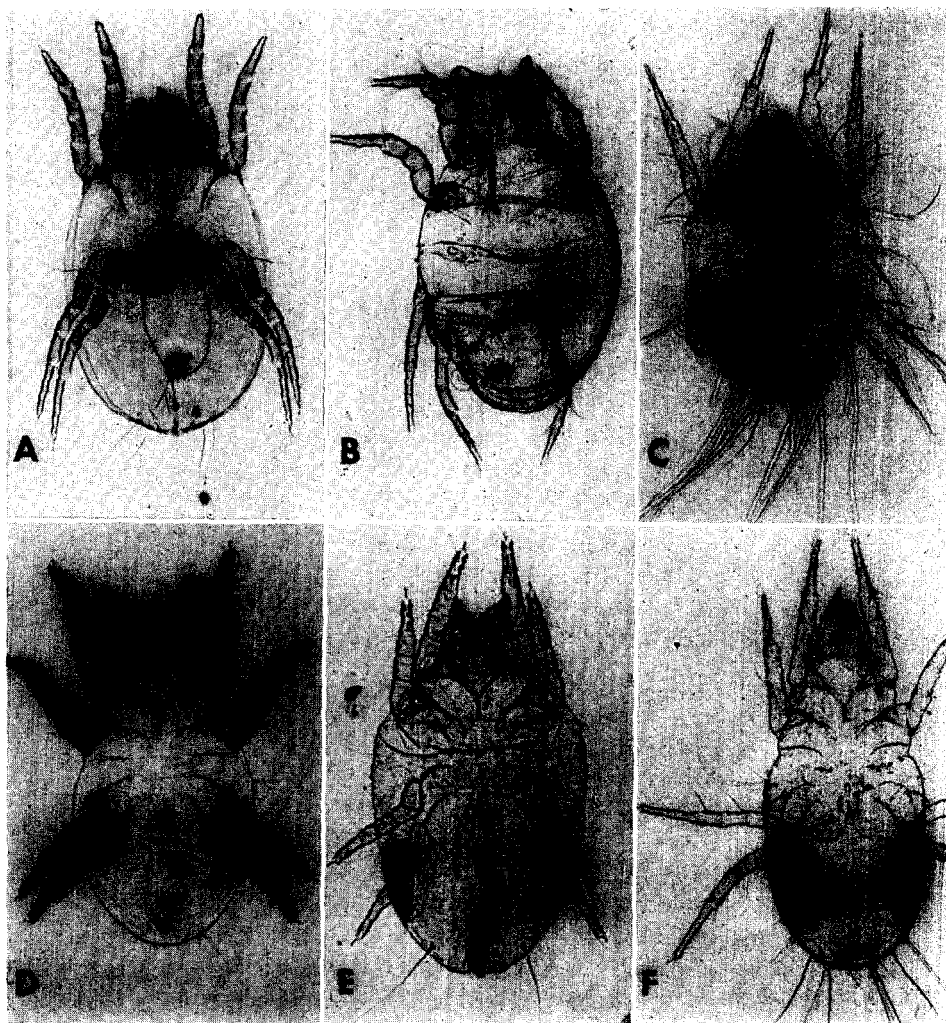


Fig. 1

Pictures of 6 species of mites studied. *A*, *Dermatophagoides farinae*, female; *B*, *Chortoglyphus arcuatus*, male; *C*, *Blomia* sp., female; *D*, *Aleuroglyphus ovatus*, male; *E*, *Suidasia nesbitti*, female; *F*, *Tyrophagus putrescentiae*, female.

Troupeau, 1879, *Blomia* sp., *Aleuroglyphus ovatus* Troupeau, 1878, *Suidasia nesbitti* Hughes 1948, and *Tyrophagus putrescentiae* Schrank, 1781, can be grown in pure culture, further studies have been carried out to clarify this point. Pictures of these mites are presented in Fig. 1. From our extensive studies, it was found that *C. arcuatus* makes up 5.5 per cent of the mites in house dust, *Blomia* 6.1, and *T. putrescentiae* 2.5, in Japan. On the basis of counting 10,000 mites in house dust, it was concluded that *A. ovatus* and *S. nesbitti* are negligible in number. More detailed information will be reported in the near future.⁴

SUBJECTS

Fifty patients (35 males and 15 females) with bronchial asthma, allergic rhinitis, or both, and positive skin reactions to house dust were subjected to skin testing. Their ages ranged from 15 to 54 years. Ten healthy volunteers with negative histories for allergies and negative intradermal skin tests to house dust and mites were chosen as the recipients of P-K tests.⁵

MATERIALS

Bodies of purely cultivated mites and excreta were extracted as reported previously,² and a 1:10,000 dilution of extract (weight by volume) was used as the standard solution for skin tests. *D. farinae*, *C. arcuatus*, and *Blomia* were cultivated with the use of dried *Daphnia* for nutrition.^{1, 2} *Aleuroglyphus ovatus*, *Suidasia nesbitti*, and *Tyrophagus putrescentiae* were cultivated with the use of laboratory animal food for mice with humidity control. These 3 mites occur normally in flour, grain, or stored food products. Excreta of *C. arcuatus* and *Blomia* were used in this study. Since excreta of *D. farinae* had already been investigated,^{1, 2} it was thought that no further studies of the 3 other species were needed. Commercially available house dust extract from Torii & Co., Ltd., was employed as house dust antigen in this study.

METHODS

Skin tests

A series of twofold dilutions of house dust extract was made from the 1:1,000 concentration and of mites from 1:10,000 material. Extract of the excreta of mites was similarly diluted from 1:10,000. At least 3 extracts of mite bodies were tested on the same day in each individual. Others were tested later but within one month. As too many skin tests annoyed the patients, all extracts were not tested in every subject. Each of the subjects was intracutaneously injected with 0.02 ml. of extract and the maximum dilution which elicited positive skin reactions was considered to be the end point. Skin tests were usually performed on the volar aspect of the forearm. Correlation coefficients of the end points were calculated among the subjects who showed positive reactions. Regression curves were calculated, and standard error of estimate (which indicates deviation from the regression line) was obtained for each pair of the end points. In the calculation, n of $2^n \times 1,000$ (or $\times 10,000$) of the end point was used as previously reported.^{1, 2} Correlations of skin reactions elicited by various antigens were compared.

Neutralization of skin-sensitizing antibody

In vitro neutralization tests of skin-sensitizing antibody⁵ were carried out with each antigen according to the previously reported method,² except different concentrations of antigen were used in the neutralization. In the test of each pair of antigens, three aliquots of allergic serum were mixed with one antigen extract, the other antigen extract, and saline, respectively. These mixtures were incubated at 37° C. for one hour, and a series of P-K sites was made on the normal recipients with twofold or fourfold dilutions of each sample. Forty-eight to 72 hours later, antigen was injected into the P-K sites, and P-K titers were

compared between a series of P-K sites made of serum mixed with saline and a series of P-K sites made of serum mixed with heterologous antigen (after ascertaining that no reaction occurred at the P-K sites made of serum mixed with homologous antigen). Reduction of P-K titers was thought to be due to cross-reactivity of antigens with each other. In the previous report, a 1:20 dilution of house dust extract and 1:200 dilution of mite extracts were employed in neutralization. From the titration of antigenic potency in relation to skin-sensitizing antibody titer thereafter, it became clear that a 1:200 dilution of house dust extract and 1:2,000 dilution of mite extracts still represented excess antigen. Consequently, a 1:200 dilution of house dust and 1:2,000 dilution of mite extracts were employed in neutralization. Six different reaginic sera were used, and at least two different sera were tested with each pair of antigens. For example, antiserum against *D. farinae* was mixed with the extract of *C. arcuatus* for neutralization, and the postneutralization P-K titer upon testing with *D. farinae* was compared with original P-K titer against *D. farinae*; the opposite procedure was carried out with different antiserum.

RESULTS

Skin tests

The number of subjects tested and the correlation coefficients of the end points between antigens are shown in Table I, and representative examples are shown in Figs. 2 and 3. Correlation coefficient against house dust was highest

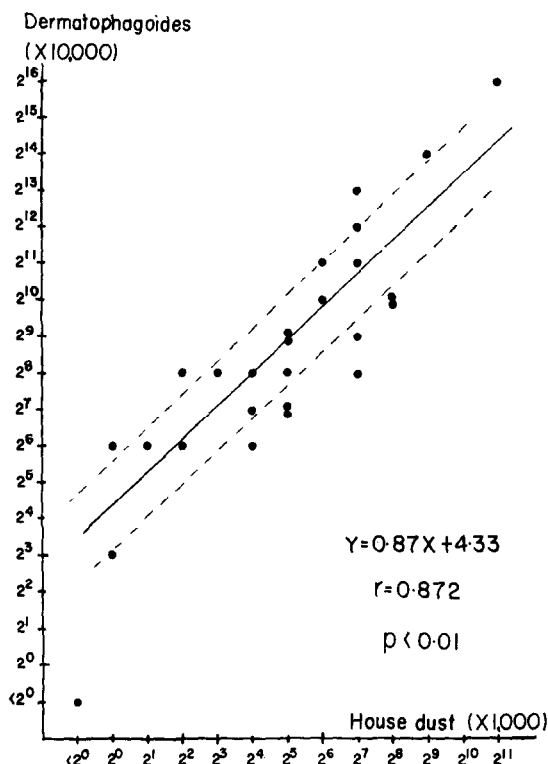
Table I. Correlation coefficients of the end points of the skin tests between each antigen

Antigen	Antigen	No. of subjects	r	S.E.
H.D.	D.f.	25	0.872	1.31
	C.a.	25	0.232*	3.67
	Blomia	26	0.498†	2.50
	A.o.	20	0.562	2.76
	S.n.	20	0.486†	3.14
	T.p.	20	0.565	3.03
D.f.	C.a.	37	0.538	3.26
	Blomia	37	0.621	2.28
	A.o.	21	0.758	2.21
	S.n.	21	0.779	2.20
	T.p.	21	0.820	2.02
C.a.	Blomia	37	0.853	1.53
	A.o.	21	0.773	2.15
	S.n.	21	0.868	1.72
	T.p.	21	0.843	1.92
Blomia	A.o.	21	0.824	1.88
	S.n.	21	0.796	2.07
	T.p.	21	0.788	2.16
A.o.	S.n.	21	0.872	1.63
	T.p.	21	0.950	1.06
S.n.	T.p.	21	0.718	2.46

r, Correlation coefficient; S.E., standard error of estimate (indicates deviation from regression line); H.D., house dust antigen; D.f., *Dermatophagoides farinae*; Blomia, specific name undertermined; C.a., *Chortoglyphus arcuatus*; A.o., *Aleuroglyphus oratus*; S.n., *Suidasia neshbitti*; T.p., *Tyrophagus putrescentia*. All p values were less than 0.01 except those indicated.

*0.2 < P < 0.3.

†0.01 < P < 0.05.

**Fig. 2**

Results of skin tests with house dust and *Dermatophagoides farinae*. The $< 2^0$ indicates negative skin reaction with 1:1,000 dilution of house dust extract or 1:10,000 dilution of mite extract.

with *D. farinae* in comparison with other species of mites, thus indicating house dust antigen is most closely related to *D. farinae*. Correlation coefficient between house dust and *C. arcuatus* was 0.232, the lowest coefficient. The correlation coefficient was 0.538 between *D. farinae* and *C. arcuatus* and 0.621 between *D. farinae* and *Blomia*, their relations appearing to be less close than among other species of mites. Nevertheless, close correlations were found between all species of mites from the skin test results. The extracts of *Blomia*, *T. putrescentiae*, *S. nesbitti*, and *A. ovatus* were not as potent as those of *D. farinae* or *C. arcuatus* as antigens in eliciting skin reactions in house dust-sensitive patients, although they were extracted in the identical manner.

A very close antigenic relationship was found between body and excreta of *C. arcuatus* and *Blomia*. The correlation coefficient was 0.944 for *C. arcuatus* and 0.974 for *Blomia* (Fig. 4).

Neutralization of skin-sensitizing antibody

The P-K titer against *D. farinae* was reduced from 1,024 to 128 by *C. arcuatus* and the P-K titer against *C. arcuatus* from 16 to 4 by *D. farinae*. The

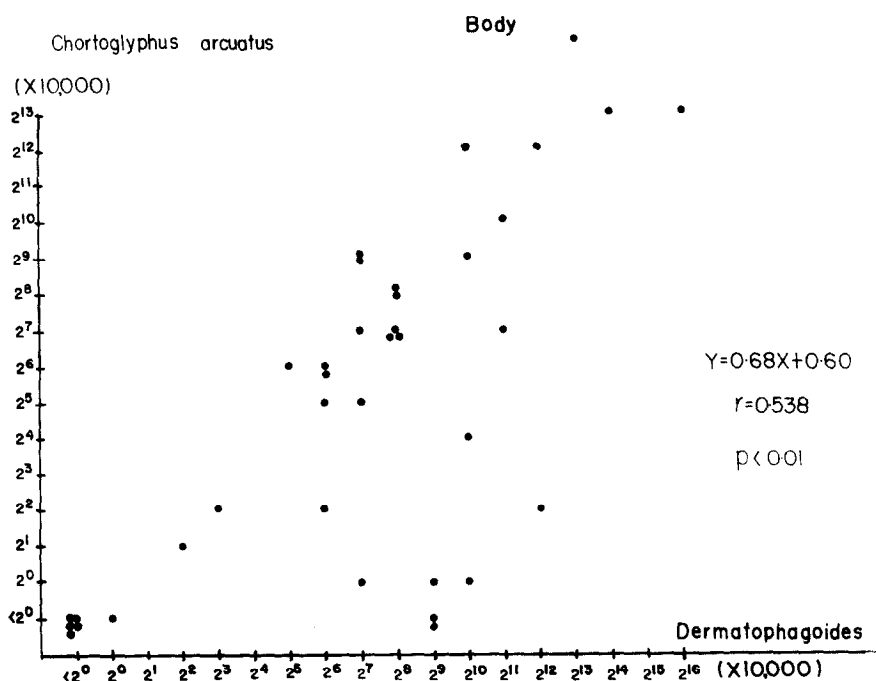


Fig. 3

Results of skin tests with *Dermatophagoides farinae* and *Chortoglyphus arcuatus*.

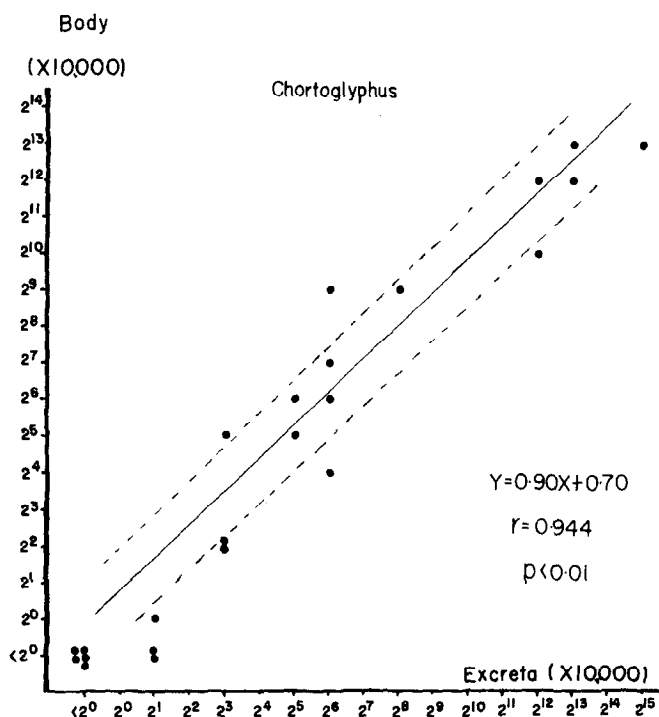
P-K titer against *D. farinae* was reduced from 521 to zero by *A. ovatus*, and the P-K titer against *A. ovatus* was diminished from 64 to 2 by *D. farinae*. Similarly, the P-K titer against one species of mite was reduced by other species as shown in Table II. Thus cross-antigenicity was found among different species of mites. A typical example is presented in Fig. 5.

DISCUSSION

As previously reported,^{1,2} *D. farinae* has a close antigenic identity to house dust, which is one of the most important causative antigens in bronchial asthma and allergic rhinitis. Since *D. farinae* makes up only about 4 per cent of the total number of mites in house dust, it was thought that the mites most likely have cross-antigenicity with each other. Further investigation of this possibility was carried out and cross-antigenicity was found from skin testing and in vitro neutralization of skin-sensitizing antibody among 6 species of mites studied. This is shown in Tables I and II.

It is desirable to test at least several sera with each pair of antigens in order to obtain mean values of the reduction of the P-K titers following neutralization. Due to the many recipients required, this is not always feasible. From the results presented, however, the existence of cross-antigenicity between mites is obvious.

Among the purely cultured 6 species of mites studied in our investigations, *D. farinae* related most closely to commercially available house dust from the

**Fig. 4**

Results of skin test with extracts of excreta and body of Chortoglyphus.

skin testing results. From the results of the in vitro neutralization of skin-sensitizing antibody, it was shown that *D. farinae* had an antigen common to all other species of mites and appeared to possess the major antigen of each species. Consequently, it was not surprising that the *D. farinae* had a high correlation with house dust.

A very close antigenic relationship was recently observed between excreta and body of *D. farinae*; the correlation coefficient was 0.957. Identical results were obtained in *C. arcuatus* and *Blomia* with correlation coefficients of 0.944 and 0.974, respectively. Consequently it was reasonably concluded that the excreta of any species of mites is almost identical with body of the same species of mites in antigenicity.

More than 35 species of mites were found in house dusts collected from 9 different parts of Japan, and 1 Gm. of house dust contained from several hundred to over 2,000 mites. When extracts of these house dusts were skin tested in about 50 allergic patients, skin reactions of most of them showed a similar pattern, although significant differences were found in some cases. The mean value of the end point of the skin test, however, closely related to the total number of mites, with a correlation coefficient of 0.912.⁶ This indicates that the potency of house dust antigen is dominated by the total number of mites contained in the dust. This is explainable due to the fact that the mites have cross-antigenicity with each other, and the amount of excreta of mites, which has

Table II. Result of the in vitro neutralization of skin-sensitizing antibody

Challenging antigen	P-K titer against challenging antigen	Neutralizing antigen	Postneutralization P-K titer against challenging antigen	Ratio between post- and preneutralization P-K titers
H.D.	32	D.f.	0	0 ~ 1/32
	8	A.o.	0	0 ~ 1/8
D.f.	64	H.D.	0	0 ~ 1/64
	1,024	C.a.	128	1/8
	1,024	Blo.	128	1/8
	512	A.o.	0	0 ~ 1/512
C.a.	512	S.n.	0	0 ~ 1/512
	64	T.p.	2	1/32
	16	D.f.	4	1/4
	16	Blo.	0	0 ~ 1/16
	4	A.o.	2	1/2
	8	A.o.	2	1/4
	16	S.n.	16	1
	32	S.n.	8	1/4
	16	T.p.	4	1/4
	16	T.p.	8	1/2
Blomia	8	D.f.	0	0 ~ 1/8
	8	C.a.	0	0 ~ 1/8
A.o.	64	D.f.	2	1/32
	16	C.a.	4	1/4
	32	S.n.	16	1/2
	8	S.n.	0	0 ~ 1/8
	32	T.p.	8	1/4
S.n.	32	C.a.	16	1/2
	16	C.a.	2	1/8
	32	A.o.	8	1/4
T.p.	32	D.f.	2	1/16
	16	C.a.	8	1/2
	16	C.a.	4	1/4
	8	A.o.	2	1/4

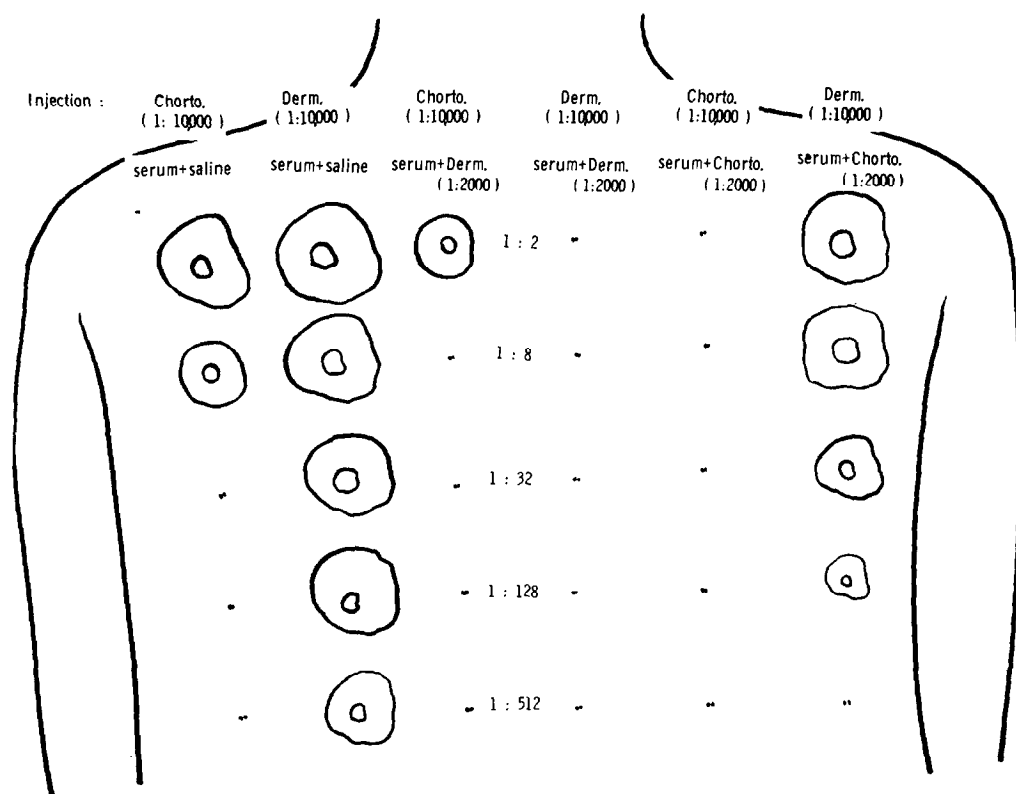
H.D., house dust antigen; D.f., *Dermatophagoides farinae*; Blomia, specific name undetermined; C.a., *Chortoglyphus arcuatus*; A.o., *Aicuroglyphus ovatus*; S.n., *Suidasia nesbitti*; T.p., *Tyrophagus putrescentiae*.

Zero postneutralization P-K titer does not necessarily mean that skin-sensitizing antibody was completely neutralized, since there is a possibility that the existence of skin-sensitizing antibody could be proved if a higher concentration of antigen were used to challenge such P-K sites.

almost complete allergenic identity with body, is influenced by the total number of mites.

From our investigation, it became clear that mites contained in house dust are the major antigen in house dust, and *D. farinae* possesses the major allergen in commercially available house dust extract in Japan. Since the cross-antigenicity between species of mites was not complete, it appeared that each species of mite also possesses its own specific antigenicity. Therefore there is the possibility that other species of mites could be the major allergen of house dust from other sources.

Most investigators used *D. pteronyssinus* in their studies and described it as the major antigen in house dust.⁷⁻¹¹ In fact, *D. pteronyssinus* is one of the major mites, making up 27.2 per cent of the total mites as an average in house dust in Japan, second to Glycyphaginae which comprises 31.8 per cent.² *D.*

**Fig. 5**

In vitro neutralization of skin-sensitizing antibody against *D. farinae* by *C. arcuatus* and against *C. arcuatus* by *D. farinae*. The numbers at P-K sites indicate dilution of serum. A 1:8 dilution was definitely positive against *C. arcuatus* but a 1:32 was completely negative. The P-K titer was arbitrarily interpreted as 1:16. Similar interpretations are made in others. The P-K titer against *D. farinae* was 1,024 which reduced to 128 against *D. farinae* after serum was mixed with *C. arcuatus*.

pteronyssinus has not been employed by us until the present time. *D. farinae* was used in our investigation instead because it can be purely cultured in our laboratory. In addition, *D. farinae* belongs to the same genus as *D. pteronyssinus*, and the antigenicity of *D. farinae* was thought to be similar to that of *D. pteronyssinus*. In fact it was recently reported that *D. culinae* (synonymous with *D. farinae*) presented a close antigenic relationship with *D. pteronyssinus*.¹² It is quite possible that *D. pteronyssinus* has almost complete antigenic identity with *D. farinae*, since *D. pteronyssinus* is the major mite in house dust; but *D. farinae* has such close antigenic identity with house dust despite the fact that *D. farinae* is a less numerous mite in house dust. The genus *Dermatophagoides* is the dominant type found in house dust universally, and it appears that different species of *Dermatophagoides* can be substituted for each other in antigenicity.

From the current investigations it has become clear that the mites living in

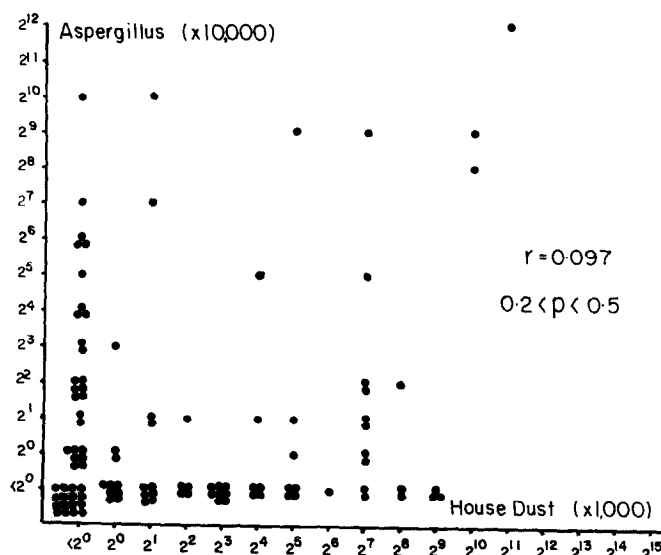


Fig. 6

Results of skin tests with house dust and *Aspergillus*. The $< 2^0$ indicates negative skin reaction with 1:1,000 dilution of house dust extract or 1:10,000 dilution of *Aspergillus* extract.

house dust are the major antigenic substances in house dust. Obviously, however, house dust is the mixture of various materials. Recently we have observed 2 patients who showed strongly positive skin reactions to house dust extract, with end points of 1:16,000 and 1:32,000, but minimal reactions against 6 species of mites. With further investigation, it was found that both of them had very strong skin reactions to silk extract, with an end point of 1:10,000,000. Consequently it is obvious that mites alone are not the only antigenic substance in house dust, and other substances contained in house dust such as molds, pollens, animal danders, fibers, etc., could be important in some cases. Molds in particular have been suspected as being major allergenic contaminants in house dust. Recently, house dust and *D. farinae* cultures were tested for growth of molds in our laboratory. *Aspergillus niger*, *Aspergillus versicolor*, *Aspergillus flavus*, *Cladosporium*, *Penicillium corylophilum*, *Penicillium nigricans*, *Curvularia*, *Trichoderma*, *Mucor*, and *Neurospora* grew from house dust used for preparing commercial extract. *Aspergillus fumigatus* and *Aspergillus versicolor* were recovered from growing mite cultures and *Aspergillus niger*, *Penicillium citrinum*, *Penicillium canescens*, *Penicillium nigricans*, *Cladosporium*, and *Paeecilomyces* from feces of mites. The end points of intracutaneous skin tests with house dust and mold extracts were obtained.¹ Correlations between them were obviously not high. Fig. 6 represents the comparative results of skin tests with house dust and *Aspergillus* extracts as an example. Consequently, molds were regarded as not being very important allergenic contaminants in house dust.

From the results obtained so far, it was concluded that the mite is the major antigenic substance in house dust.

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