

Allergenic and antigenic relationship between three species of storage mite and the house dust mite, *Dermatophagoides pteronyssinus*

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We have explored the antigenic and allergenic relationship between the house dust mite Dermatophagoides pteronyssinus and three species of storage mite, Glycyphagus destructor, Acarus siro, and Tyrophagus longior. Crossed immunoelectrophoresis demonstrated that all the mite extracts contained multiple antigens but that there was only limited cross-reactivity between the different species. Six sera were obtained from workers exposed to storage mites and with occupationally related lower respiratory tract symptoms. All workers had specific IgE to D. pteronyssinus and to one or more of the storage mites. The pattern of reactivity varied between the different sera, two responded primarily to D. pteronyssinus and A. siro and four sera to D. pteronyssinus and G. destructor. Only weak responses were observed to T. longior. RAST-inhibition and affinity-absorption experiments demonstrated that D. pteronyssinus had at least three groups of distinct allergenic determinants, determinants specific to D. pteronyssinus, determinants shared with A. siro, and determinants shared with G. destructor. Similarly, both A. siro and G. destructor have specific allergenic determinants and determinants shared with D. pteronyssinus. The findings demonstrate the complexity of the immunologic responses to the different mite species. (J ALLERGY CLIN IMMUNOL 1989;84:108-17.)

Voorhorst et al.¹ were the first to recognize that the pyroglyphid mite, *Dermatophagoides pteronyssinus*, was the major allergenic component of house dust. Subsequently, it was found that the nonpyroglyphid storage mites that are found in large numbers in stored grain, hay, and other foodstuffs² also cause allergic disease. Sensitization to the storage mites *Glycyphagus destructor*, *Tyrophagus longior*, *Acarus farris*, and *Acarus siro* has been reported in stock and dairy farmers,³⁻⁵ arable farmers,^{6,7} and dock workers at a grain terminal.⁸ A survey of farmers in Scotland indicated that 21% had allergic symptoms caused by storage mites.⁹ Similarly, a study of farmers in Scotland indicated that the prevalence of immediate hypersensitivity reactions to storage mites was 6.2% and

Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radio immunoelectrophoresis
IEF:	Isoelectric focusing
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
pI:	Isoelectric point

that mites were more important allergens than either pollens or animal dander.^{10,11} These studies demonstrated that storage mites are an important cause of allergic symptoms among farming communities. However, several investigators have noted that many subjects with positive skin prick tests to storage mites also react to *D. pteronyssinus*,^{7,8,12} which raises the question as to the clinical importance of storage mites compared to *D. pteronyssinus*.

Statistical analysis of skin prick test wheal areas to storage mites and *D. pteronyssinus* in 210 patients allergic to *D. pteronyssinus* indicated the house dust mite and storage mites contained distinct allergens.^{12,13} Similarly, a comparison of RAST responses demonstrated there was no correlation between re-

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sponses to *D. pteronyssinus* and four species of storage mites, whereas responses to the storage mite species, *G. destructor*, *G. domesticus*, and *T. putrescentiae*, demonstrated significant correlation.¹⁴ These findings were confirmed by RAST-inhibition studies with sera from farmers allergic to storage mites.¹⁴ In addition, Korsgaard et al.¹⁵ found no relation between *D. pteronyssinus* and storage mites. Arlian et al.^{16, 17} used CIE and CRIE to investigate the allergenic and antigenic composition of the storage mite *T. putrescentiae* and cross-reactivity with the house dust mite *D. farinae*. They found both mite species contained multiple antigens and allergens, but only two antigenic and allergenic determinants were shared.

In this study, we have used CIE to examine the cross-antigenicity between *D. pteronyssinus* and three storage mite species, *G. destructor*, *T. longior*, and *A. siro*. Cross-allergenicity has been examined by RAST inhibition and affinity absorption with sera from workers with lower respiratory tract symptoms associated with exposure to storage mites.

MATERIAL AND METHODS

Mite extracts

Mite species, *A. siro* and *T. longior*, were cultured on yeast: wheat germ (3:1 wt/wt) medium and then transferred to yeast only. After an additional three generations, the cultures were frozen in liquid nitrogen. Attempts to culture *G. destructor* by this method was unsuccessful, since the mite would not grow on the yeast medium. This mite was cultured on the yeast: wheat germ medium until the food supply was almost exhausted, and then the culture was frozen. The house dust mite, *D. pteronyssinus*, was cultured on ox-liver powder.

Mite and mite-media extracts were prepared by tumble mixing mite cultures or media (1.5 gm) in 0.02 mol/L of ammonium bicarbonate (20 ml) overnight at room temperature. Insoluble material was removed by centrifugation for 30 minutes at 48,000 g. The supernatant was dialyzed against 2 L 0.02 mol/L of ammonium bicarbonate (one change) for 24 hours at 4°C and then was lyophilized. The protein content was determined according to the method of Lowry et al.¹⁸

Serum samples

Blood samples (10 to 20 ml) were collected from either agricultural workers⁷ or dock workers⁸ with lower respiratory tract symptoms associated with occupational exposure to storage mites. Serum was separated and stored at -20°C.

Preparation of allergen disks

Paper disks (Schleicher & Schuell, Inc., Keene, N.H., No. 589/3) were activated with cyanogen bromide.¹⁹ Activated disks (600 mg), 5 ml of 20% mite extract or 2% media extract, and 15 ml of 0.1 mol/L of sodium bicarbonate were tumble mixed overnight at room temperature.

Unreacted sites on the disks were blocked with ethanolamine (50 mmol/L). The disks were washed one time with 0.1 mol/L of sodium bicarbonate, twice with 0.1 mol/L of acetate buffer, pH 4.0, three times with PBS, and stored in RAST buffer solution (Pharmacia Ltd., Uppsala, Sweden) at 4°C.

RAST

Serum (200 µl of 1:4 dilution in PBS) was incubated overnight at room temperature with a mite-allergen disk. Disks were washed three times with 1.5 ml of 0.9% saline containing washing solution additive (Pharmacia), and then 50 µl of ¹²⁵I-labeled rabbit antihuman IgE (Pharmacia) and 50 µl of PBS were added. After an additional overnight incubation at room temperature, the disks were washed three times and bound ¹²⁵I was measured. All the assays were performed in duplicate. The results were expressed as a RAST percent binding that was defined as percentage of added counts per minute that was bound to the disk incubated with the test serum minus the percentage of added counts per minute bound to the disk incubated with the negative control serum.

RAST inhibition

Mite-allergen disks were incubated overnight at room temperature with 200 µl aliquots of doubling dilutions of sera. The IgE bound to the disk was measured, and log counts per minute were plotted against log serum dilution. The highest concentration of serum on the linear portion of this plot was selected for use in the RAST-inhibition experiments, except where supplies of serum were limited when a 1:15 dilution was used.

For RAST inhibition, 100 µl aliquots of serum diluted in PBS to half the dilution determined by the above procedure were mixed with 100 µl of PBS or 100 µl aliquots of inhibitors diluted in PBS. Each inhibitor concentration was tested in duplicate. A mite-allergen disk was added immediately to each tube, and the tubes were incubated overnight at room temperature. Negative controls were obtained by incubating disks with 100 µl of the same dilution of control serum plus 100 µl of PBS. IgE bound to the disks was measured as described in the RAST procedure. The mean counts bound to the disks incubated with negative control serum were subtracted from the mean counts bound to the disks with the test serum, and the results, expressed as percentage inhibition, were calculated as follows:

$$100 - \left(\frac{\text{cpm}^* \text{ bound to disk with inhibitor}}{\text{cpm}^* \text{ bound to disk without inhibitor}} \right) \times 100$$

*cpm corrected for binding to disk with negative serum.

Affinity absorption

Affinity absorbants were prepared as previously described²⁰ with 3.5 ml of reconstituted CNBr-activated Sepharose 4B and 10 mg, protein content, of mite extract.

Serum (0.25 ml) and PBS (0.75 ml) were tumble mixed with affinity absorbant (0.25 ml) for 10 minutes. The slurry was centrifuged at 1000 g for 30 seconds, the supernatant

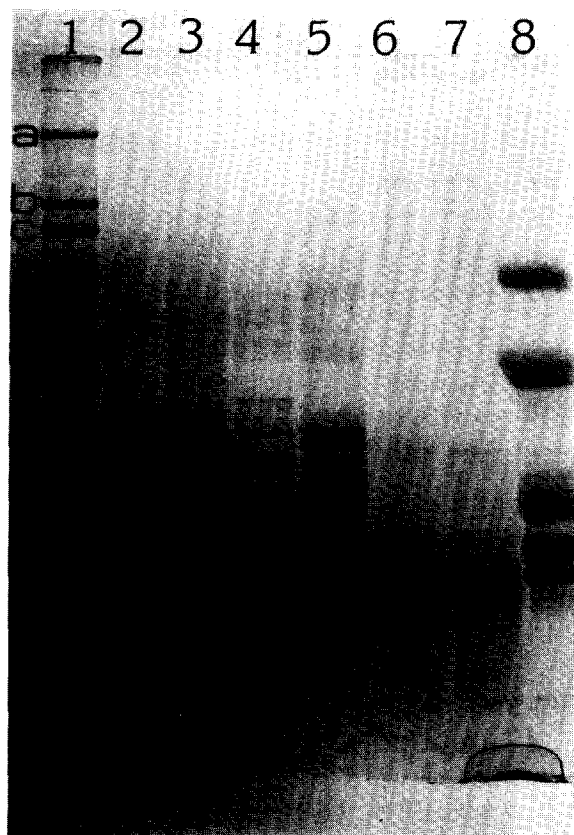


FIG. 1. SDS-PAGE in density-gradient acrylamide gel (7.5% to 17.5%) under reducing conditions. Lane 1, high molecular weight markers; lane 2, *A. siro*; lane 3, *D. pteronyssinus*; lane 4, *G. destructor*; lane 5, *T. longior*; lanes 6 and 7, mite medium; lane 8, low molecular weight markers. Molecular weight markers, daltons times 10^{-3} : a, 205; b, 116; c, 97; d, 66; e, 45; f, 36; g, 29; h, 24; i, 20.1; j, 14.2.

was aspirated, and the gel was washed three times with PBS. Bound antibody was desorbed by tumble mixing the gel with 0.1 mol/L of glycine/HCl buffer (pH 2.8) (0.9 ml) for 5 minutes. After centrifugation, the supernatant was immediately neutralized with 0.2 mol/L of phosphate buffer (pH 7.4) containing 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and then dialyzed against PBS overnight at 4° C.

Production of rabbit antisera

Antibodies were produced in rabbits (New Zealand white rabbits, two per mite species) according to the method of Harboe and Ingild.²¹ Briefly, mite extracts were made up to 30 mg/ml in distilled water and mixed to an emulsion with equal volumes (characteristically 250 μ l) of Freund's incomplete adjuvant by repeated aspiration of the mixture through a 23-gauge syringe needle. Rabbits were administered a course of four intradermal injections of approximately 100 μ l each, suprascapularly, at fortnightly intervals, and approximately 45 ml of blood was obtained by bleeding 8 days after the last injection. One subsequent injection took place 6 weeks after bleeding, and 8 days later the rabbits were exsanguinated. All the serum samples were

TABLE I. Antigenic cross-reactivity between mite species: CIE of mite extracts

Rabbit antiserum to	Mite extract			
	<i>Dp</i>	<i>As</i>	<i>Gd</i>	<i>Tl</i>
	(No. of precipitin arcs)			
<i>Dp</i>	18	3	1	2
<i>As</i>	1	14	2	4
<i>Gd</i>	2	2	22	4
<i>Tl</i>	2	3	4	25

Dp, *D. pteronyssinus*; *As*, *A. siro*; *Gd*, *G. destructor*; *Tl*, *T. longior*.

tested individually by CIE for the presence of precipitating antibodies before pooling and preparing a 45% ammonium sulfate precipitated fraction for each mite species.

CIE and CRIE

CIE and CRIE were performed according to the method of Lowenstein.²² Briefly, 5 μ l of a 1% (wt/vol) of mite extract was electrophoresed into 1% agarose for 40 minutes at 10 V/cm. The 1 by 5 cm agarose gel strip containing the separated proteins was transferred to a 5 by 7 cm glassplate, and agarose containing rabbit antibodies was poured on either side to elicit a concentration on the plate of 10 μ l/cm². The proteins were then electrophoresed into the agarose at 1.5 to 2 V/cm overnight. The plates were pressed and washed successively in 0.1 mol/L of sodium chloride, and distilled water, stained in 0.5% Coomassie brilliant blue, then destained until the precipitin arcs stood out against a clear background.

In homologous CIE, the mite extract was run into rabbit antisera raised against the same species, whereas in heterologous CIE, rabbit antisera raised against one of the other mite species was used. In CIE with an intermediate gel, an additional line of agarose containing antibodies to a second species was incorporated between the first and second dimension.

To detect specific IgE binding, unstained, air-dried plates were successively incubated overnight at room temperature with 0.35 ml of serum in 8 ml of PBS, and then 8 ml of PBS-Tween containing approximately 500,000 cpm of ¹²⁵I-labeled antihuman IgE (Pharmacia Ltd.) with three washes of PBS-Tween and 0.9% sodium chloride, respectively, after the two incubations. The plates were rinsed in distilled water, dried, and placed against X-ray film (Kodak X-Omat S, Eastman Kodak Co., Rochester, N.Y.) in a cassette with an intensifying screen at -70° C for 2, 4, or 10 days or until darkening of the X-ray film was obtained. The number of precipitin arcs on the autoradiograph were noted together with the incubation time and intensity of darkening.

IEF

IEF was performed according to the manufacturer's instructions (LKB, Bromma, Sweden) with Ampholine PAG plates, pH 3.5 to 9.5, on the 2117 Multiphor II electrophoresis unit with the cooling plate maintained at 5° C by

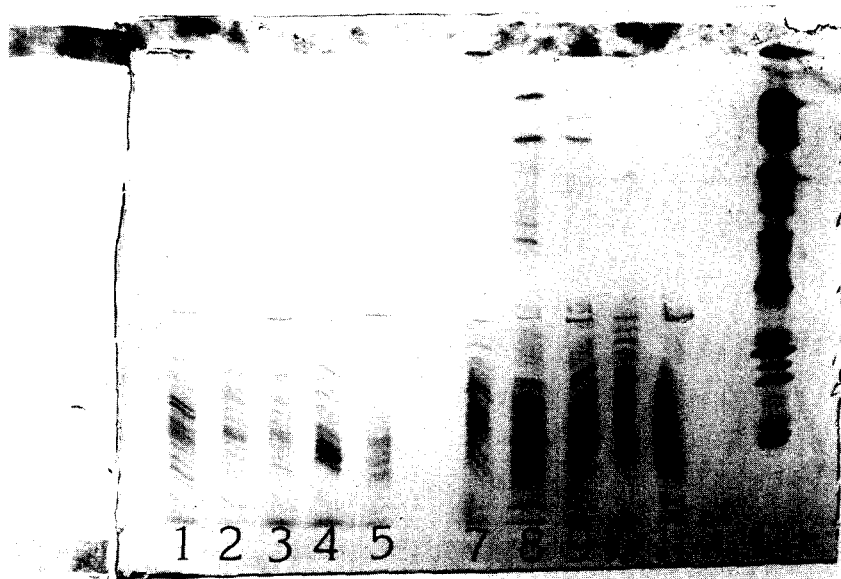


FIG. 2. IEF of mite extracts. Lanes 1-5 and lanes 7-11 contain *A. siro*, *D. pteronyssinus*, *G. destructor*, *T. longior* extracts, and storage-mite medium, respectively, at a concentration of 30 mg of protein per milliliter. Lanes 1-5, 15 μ l applied; lanes 7-11, 30 μ l applied; lane 13, pI markers.

2219 Multitemp II. Approximately 600 μ g dry weight, in a 30 mg/ml solution, of each mite extract and pI markers, pH range 4.7 to 10.6 (BDH Chemicals Ltd., Poole, England), were applied to the gel on the wicks provided.

Focusing was carried out at a maximum current of 50 mA, and a maximum voltage of 500 V at a constant power of 20 W for 2 hours with an LKB 2103 power supply. The application wicks were removed after half an hour. After focusing, the gels were fixed, stained with Coomassie brilliant blue R, and preserved according to the manufacturer's instructions.

SDS-PAGE

SDS-PAGE was performed as described by Laemmli and Favre.²¹ Gels were prepared with a density gradient of 7.5% to 17.5% acrylamide. Approximately 750 μ g of mite extracts was separated under reducing conditions along with the molecular weight markers (MW-SDS-70 and MW-SDS-200; Sigma Chemical Co.). Gels were stained in Coomassie brilliant blue R and dried onto Whatman 3 mm chromatography paper (Whatman, Inc., Clifton, N.J.).

RESULTS

Biochemical characterisation of mite extracts

Biochemical analysis demonstrated that each of the four mite extracts had multiple bands of low-molecular-weight proteins (13,000 to 80,000 daltons) on SDS-PAGE and 20 to 30 Coomassie brilliant blue-staining proteins on IEF, most with pI values between 3.5 to 5.5. Typical results for the SDS-PAGE and IEF analyses are illustrated in Figs. 1 and 2, respectively. The protein content of the extracts, as measured ac-

cording to the method of Lowry et al.,¹⁸ was similar, ranging from 0.3 to 0.6 mg/mg dry weight of extract.

Antigenic analysis of mite extracts

Homologous CIE with the four mite extracts revealed 14 to 22 Coomassie brilliant blue-staining precipitin arcs (Fig. 3, A through D). The precipitins were specific for mite-derived components, since none of the antisera elicited arcs with a heterologous CIE system in which a mite-media extract was used as antigen. Antigenic cross-reactivity between the storage mite extracts and *D. pteronyssinus* was studied by heterologous CIE. The results (Table I) demonstrated that *G. destructor* and *T. longior* had two antigens and *A. siro* had one antigen that cross-reacted with *D. pteronyssinus*. In addition, it was found that there was only very limited cross-reactivity among the three species of storage mite; for example, of the 14 precipitin arcs observed with *A. siro* in a homologous CIE, in a heterologous system with *A. siro* antisera, *T. longior* extract elicited four precipitin arcs and *G. destructor* elicited two arcs. CIE experiments with two different rabbit antibodies in successive gels on the anode side of the sample demonstrated that only one antigen was common to all four mite species.

Specific IgE binding to mite allergens

The sera were tested for mite-specific IgE by RAST and CRIE. All sera had RAST binding of at least 1% to *D. pteronyssinus* and to one or more of the storage mites, but the pattern of reactivity to the storage mites

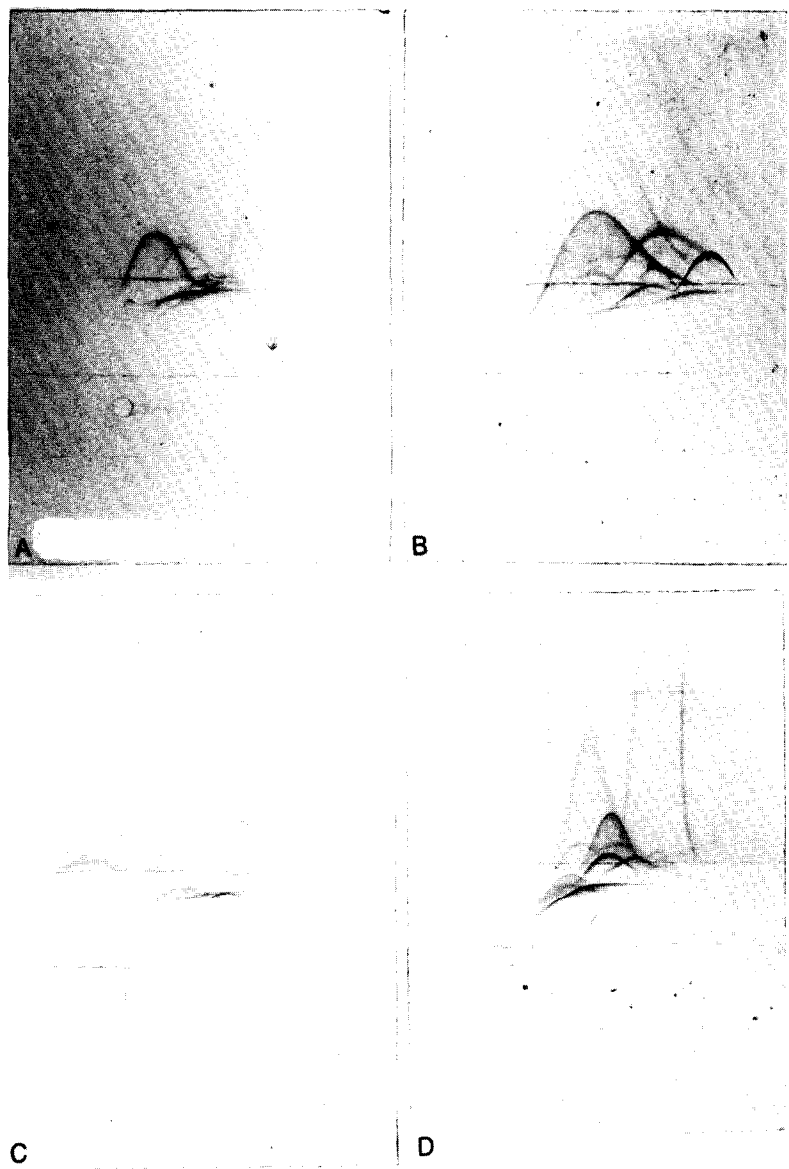


FIG. 3. CIE A, *A. siro*. B, *D. pteronyssinus*. C, *G. destructor*. D, *T. longior*.

TABLE II. Specific IgE to mite allergens: Direct RAST, and CRIE results

Sera	<i>Dp</i>		<i>As</i>		<i>Gd</i>		<i>Tl</i>	
	RAST	CRIE	RAST	CRIE	RAST	CRIE	RAST	CRIE
1	18.1	4	6.4	2	0.8	0	3.1	1
2	3.3	NT	1.4	NT	0.4	NT	0.6	NT
3	11.2	2	1.1	0	7.5	1	1.0	0
4	27.5	6	1.1	3	11.2	2	2.1	0
5	3.1	6	0.3	0	7.6	0	1.0	0
6	19.1	4	0.6	0	1.0	0	0.1	0

Dp, *D. pteronyssinus*; *As*, *A. siro*; *Gd*, *G. destructor*; *Tl*, *T. longior*; NT, not tested.
RAST results expressed as net percent binding to allergen disk; CRIE results, number of precipitin arcs observed on x-ray film.

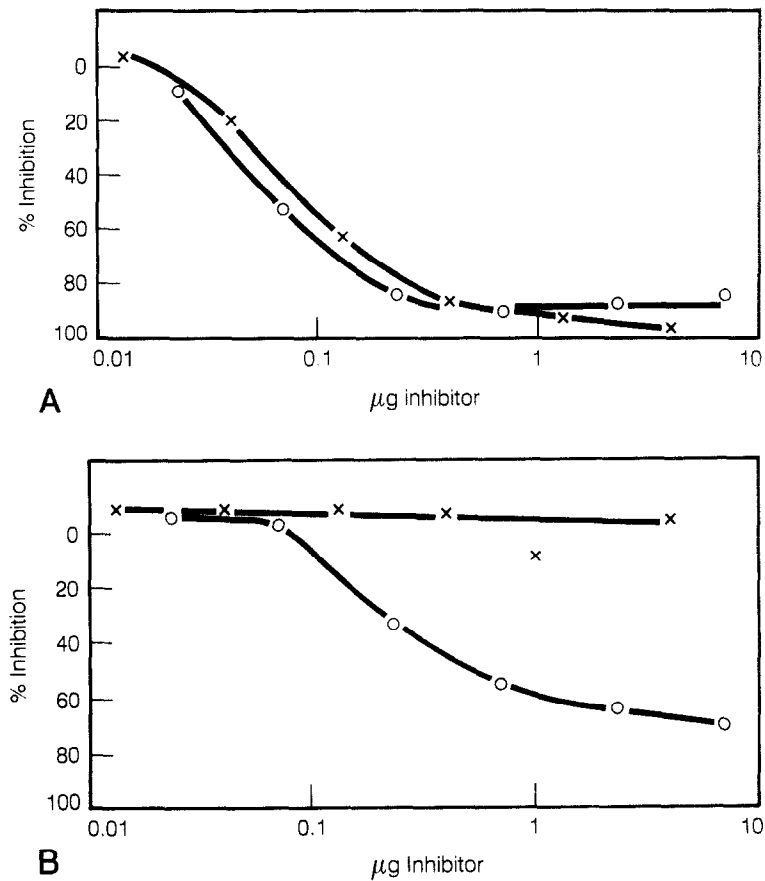


FIG. 4. RAST inhibition of mite-specific IgE, serum No. 1, by *D. pteronyssinus* (o) and *A. siro* (x). **A**, *A. siro* disks. Percentage binding to uninhibited disks, 6.1%. **B**, *D. pteronyssinus* disks. Percentage binding to uninhibited disks, 15.9%.

TABLE III. Affinity absorption of mite-specific IgE

	Material not bound to absorbant		Material eluted from absorbant	
	RAST % binding, serum 1			
	<i>Dp</i> disks	<i>As</i> disks	<i>Dp</i> disks	<i>As</i> disks
CNBr-Sepharose				
Blocked with ethanolamine	11.1	3.7	0.2	0.1
Coupled to <i>As</i> extract	10.2	0.3	0.2	0.1
Coupled to <i>Dp</i> extract	1.2	0.2	2.1	1.1
	RAST % binding, serum 5			
	<i>Dp</i> disks	<i>Gd</i> disks	<i>Dp</i> disks	<i>Gd</i> disks
CNBr-Sepharose				
Blocked with ethanolamine	3.5	5.1	0.1	0
Coupled to <i>Gd</i> extract	0.6	0.5	0.9	1.3
Coupled to <i>Dp</i> extract	0.8	3.1	0.7	0.2

Dp, *D. pteronyssinus*; *Gd*, *G. destructor*; *As*, *A. siro*.

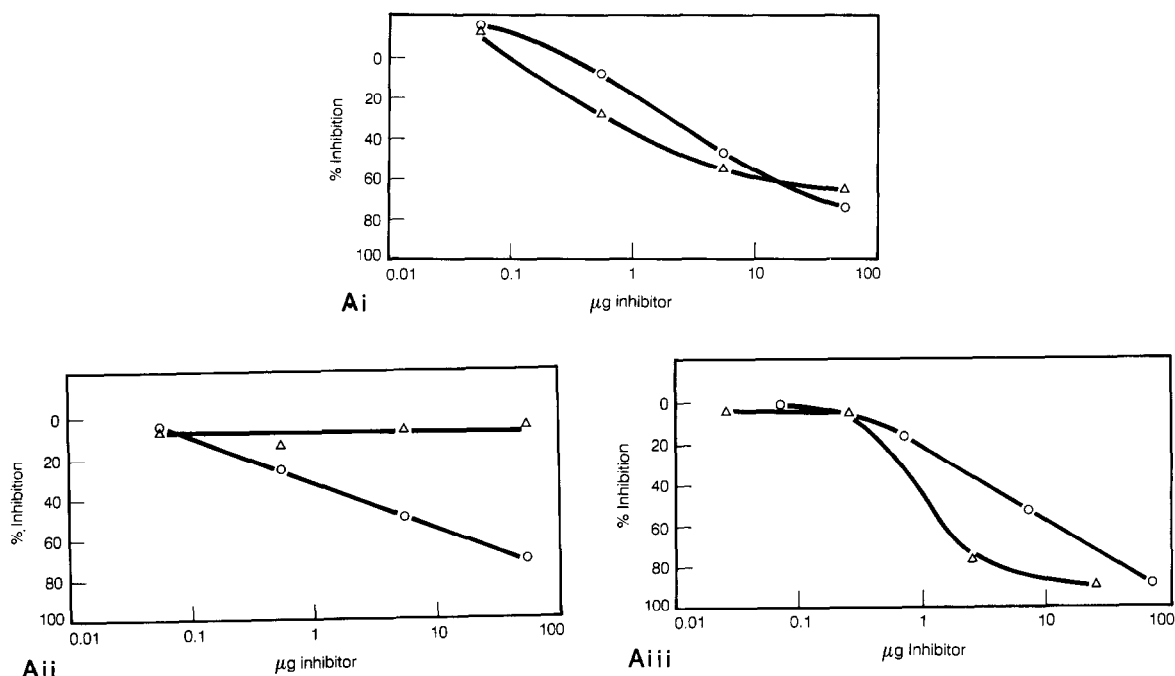


FIG. 5A. RAST inhibition of mite-specific IgE by *D. pteronyssinus* (○) and *G. destructor* (△). *D. pteronyssinus* disks. **Ai**, Serum No. 3. Percentage binding to uninhibited disks, 6.3%. **Aii**, Serum No. 4. Percentage binding to uninhibited disks, 23.4%. **Aiii**, Serum No. 5. Percentage binding to uninhibited disks, 3.9%.

varied considerably between the different sera (Table II). Sera Nos. 1 and 2 had the highest binding to *A. siro*, whereas sera Nos. 3 to 6 had the highest binding to *G. destructor*. Five of the sera had specific IgE to *T. longior*, but in each case, RAST binding was low. None of the sera had specific IgE to the mite-culture media, as all sera elicited very low RAST binding (<0.4%) to mite-culture media-extract disks.

CRIE results largely confirmed the RAST data in that of the 16 RASTs with percent binding of $\geq 1\%$, 10 RASTs were associated with precipitin arcs on CRIE. Generally, a positive CRIE result was associated with high RAST binding, whereas results with low RAST binding were negative on CRIE. In no case was CRIE positive when RAST binding was <1%. The number of precipitin arcs varied with the mite species, up to six arcs were observed with *D. pteronyssinus*, three with *A. siro*, two with *G. destructor*, and one arc with *T. longior* (Table II). A pool of human sera with high IgE concentration to major allergen of *D. pteronyssinus*, *Der p I*,²⁴ did not elicit precipitin arcs with any of the storage mite species.

Cross-reactivity between *A. siro* and *D. pteronyssinus*

Sera Nos. 1 and 2 demonstrated the highest RAST binding to *A. siro* and *D. pteronyssinus* (Table II).

RAST-inhibition studies with serum 1, with *A. siro* and *D. pteronyssinus* allergen disks, demonstrated that the *D. pteronyssinus* extract inhibited binding to *A. siro* disks as effectively as the *A. siro* extract, but *A. siro* did not inhibit binding to *D. pteronyssinus* disks (Fig. 4., A and B). Affinity-absorption experiments were carried out with activated Sepharose 4B blocked with ethanolamine and Sepharose 4B coupled to *D. pteronyssinus* and *A. siro* extracts. The *D. pteronyssinus* absorbant removed antibodies to both *D. pteronyssinus* and *A. siro*, whereas the *A. siro* absorbant only removed antibodies to *A. siro* (Table III). The use of blocked activated Sepharose 4B as a control and the elution of specific IgE from the absorbants demonstrated that incubation with the absorbant had not simply resulted in loss of antibody activity. These experiments demonstrated that the serum had antibodies to allergenic determinants common to *A. siro* and *D. pteronyssinus* and also antibodies specific to *D. pteronyssinus* allergens. Supplies of serum 2 were very limited; however, RAST-inhibition experiments with undiluted extracts demonstrated the same inhibition pattern as serum 1.

Cross-reactivity between *G. destructor* and *D. pteronyssinus*

Sera Nos. 3 to 6 demonstrated the highest RAST binding to *G. destructor* and *D. pteronyssinus* (Table

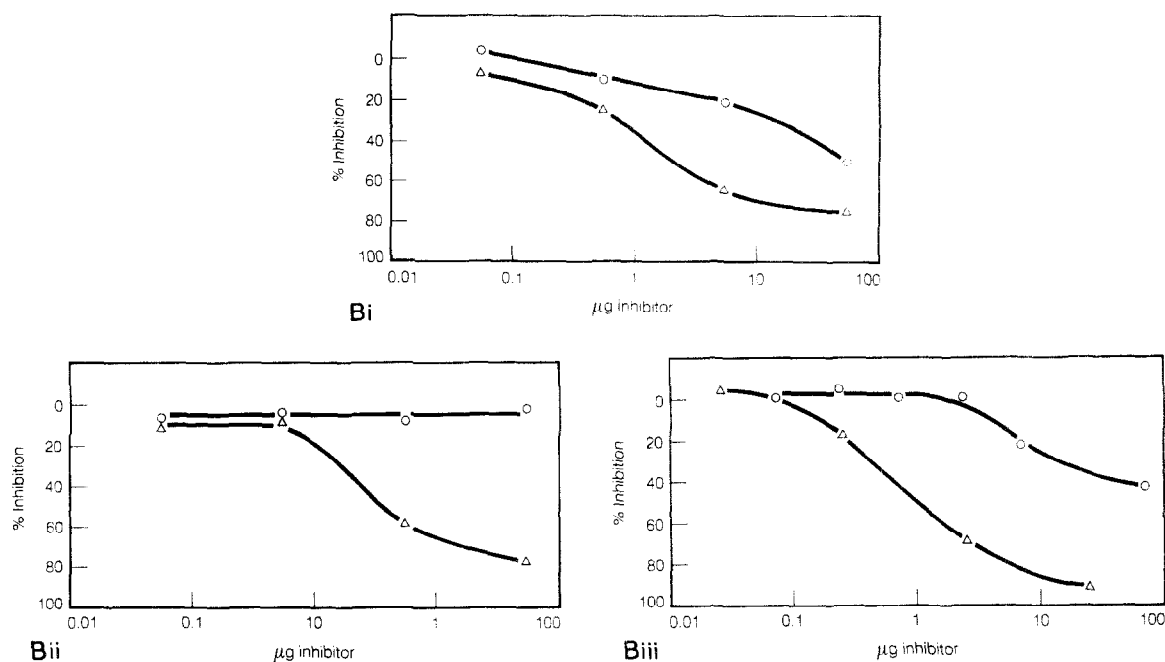


FIG. 5B. RAST inhibition of mite-specific IgE by *D. pteronyssinus* (○) and *G. destructor* (△). *G. destructor* disks. **Bi**, Serum No. 3. Percentage binding to uninhibited disks, 8.8%. **Bii**, Serum No. 4. Percentage binding to uninhibited disks, 9.4%. **Biii**, Serum No. 5. Percentage binding to uninhibited disks, 8.5%.

II). Sera 3 to 5 were used in cross-reactivity experiments, serum 6 having low binding to *G. destructor* disks. RAST-inhibition experiments were carried out with *D. pteronyssinus* and *G. destructor* allergen disks. For sera 3 and 5, the *G. destructor* extract inhibited binding to *D. pteronyssinus* disks as effectively as the *D. pteronyssinus* extract. In contrast, when *G. destructor* allergen disks were used, *D. pteronyssinus* was a weaker inhibitor than *G. destructor* (Fig. 5). Affinity-absorption experiments with serum 5 demonstrated that the *G. destructor* absorbant removed antibodies to *G. destructor* and *D. pteronyssinus*, whereas the *D. pteronyssinus* absorbant only removed antibodies to *D. pteronyssinus* (Table III). These findings corroborated the RAST-inhibition data and demonstrated that sera 3 and 5 have antibodies specific for allergenic determinants common to *D. pteronyssinus* and *G. destructor* and also antibodies specific to *G. destructor* allergens. Serum 4 demonstrated a different pattern of response. RAST inhibition clearly indicated that the serum has two populations of antibodies, one specific to *G. destructor* allergenic determinants and the other antibody population specific to *D. pteronyssinus* allergenic determinants (Fig. 5).

DISCUSSION

There is abundant evidence that the storage mites, like the house dust mite, *D. pteronyssinus*, are potent sensitizers and significant causes of allergic disease.³⁻¹¹ However, there is a limited amount of infor-

mation on the extent of cross-reactivity between occupationally encountered storage mites and the house dust mite. Clearly it is important to know if subjects allergic to house dust mites are at risk from reactions to occupationally encountered storage mites and, similarly, if subjects sensitized to storage mites are at risk of reactions to the house dust mite.

Biochemical analysis demonstrates that all the mite extracts are complex mixtures of proteins, mainly of low pI (3.5 to 5.5) and low-molecular-weight (<80,000 daltons) (Figs. 1 and 2). That these proteins are distinct antigens is evident by the finding of up to 25 precipitin arcs on CIE (Table I). Similarly, other workers have found 18 antigens in a *T. putrescentiae* extract¹⁶ and 49 in a *D. pteronyssinus* extract.²⁵ Evidence of multiple allergenic components comes from CRIE (Table III) and is in agreement with previous work on *T. putrescentiae* and *D. pteronyssinus*.^{16, 25}

The lack of correlation found by a number of investigators between *D. pteronyssinus* and storage mite skin test and RAST responses and the lack of cross-reactivity in RAST inhibition points to *D. pteronyssinus* and storage mites containing distinct allergens.¹²⁻¹⁵ In contrast, Blainey et al.^{7, 8} found close correlation between skin test responses to *D. pteronyssinus* and storage mites, but this could result from dual sensitization as well as from cross-reactivity. The most compelling evidence that the extracts contain distinct allergens is the observation that some sera have IgE to storage mites but not *D. pteronyssinus* and

*vice versa*¹⁴ and the finding (Table II) that some sera react to *A. siro* and *D. pteronyssinus* and some to *G. destructor* and *D. pteronyssinus*. However, since different subjects may recognize different allergens, there is still the possibility of cross-reactivity in subjects responding to both house dust mite and storage mites. We were interested in examining such sera to determine whether the subjects had dual sensitivities or cross-reacting antibodies.

The two sera (Nos. 1 and 2; Table II) that responded to *A. siro* and *D. pteronyssinus* had antibodies to determinants specific to *D. pteronyssinus* and to determinants common to *A. siro* and *D. pteronyssinus*. Sera Nos. 3 and 5 (Table II) that responded to *G. destructor* and *D. pteronyssinus* had antibodies to determinants common to *G. destructor* and *D. pteronyssinus* and to determinants specific to *G. destructor*, whereas serum 4 had two populations of antibodies, one specific to *G. destructor* determinants and the other antibody population specific to *D. pteronyssinus* determinants. These findings illustrate the complexity of these multiallergenic extracts. For example, *D. pteronyssinus* must contain at least three groups of allergenic determinants, determinants specific to *D. pteronyssinus*, determinants common with *A. siro*, and determinants common with *G. destructor*. In only one of the sera (No. 4) is there conclusive evidence that dual sensitization had occurred. In sera 1 and 2, although dual sensitization may have occurred, the responses could be due to sensitization to *D. pteronyssinus* determinants that cross-react with *A. siro*. Similarly, the results with sera 3 and 5 could be explained as sensitization to *G. destructor* determinants that cross-react with *D. pteronyssinus*.

Affinity absorption on two of the sera confirmed the RAST-inhibition data. These experiments were carried out with Sepharose 4B as a solid phase, making it possible to expose the sera to a large excess of immobilized allergen. The finding that even under these conditions additional absorption of antibody did not occur further demonstrates the distinct nature of some of the allergenic determinants.

In comparison with *G. destructor* and *A. siro*, only low RAST binding was observed with the *T. longior* extract. Since these three mites occur commonly in stored cereal products, this difference is unlikely to be due to lower exposure and probably reflects lower allergenicity of this mite. Antibody to *Der p* I, the major allergen in *D. pteronyssinus*, did not elicit precipitin arcs with the storage mites, suggesting this allergen is not involved in the cross-reactions observed with some of the sera. Further work is in progress to identify the common allergens.

The finding of cross-allergenicity in four of the sera with in vitro tests does not necessarily imply that clinical cross-reactions will occur, but it certainly raises the possibility. Thus, in investigation of work-related asthma in workers exposed to storage mites, the possibility of symptoms resulting from exposure to *D. pteronyssinus* should be considered.

In conclusion, this study demonstrates both dual sensitization to antigenically distinct components of *D. pteronyssinus* and storage mites, as well as allergenic cross-reactivity, may occur in different subjects, emphasizing the complexity of the immunologic responses to the different species of storage mites and the house dust mite, *D. pteronyssinus*.

REFERENCES

1. Voorhorst RF, Spijksma ThM, Varekamp H, Leupen MJ, Lykema AW. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J ALLERGY* 1967;39:325-30.
2. Griffiths DA, Wilkin DR, Southgate BJ, Lynch SM. A survey of mites in bulk grain stored on farms in England and Wales. *Ann Appl Biol* 1971;82:180-5.
3. Cuthbert OD, Brostoff J, Wraith DG, Brighton WD. "Barn Allergy": asthma and rhinitis due to storage mites. *Clin Allergy* 1979;9:229-36.
4. Ingram CG, Jeffrey IG, Symington IS, Cuthbert OD. Bronchial provocation studies in farmers allergic to storage mites. *Lancet* 1979;2:1330-2.
5. Hillerdal G, Zetterstrom O, Johansson SGO, Engstrom B, Wiren A. Mites living in hay: an important allergen source. *Allergy* 1982;37:475-9.
6. Warren CPW, Holford-Strevens V, Sinha RN. Sensitization in a grain handler to the storage mite *Lepidoglyphus destructor*. *Ann Allergy* 1983;50:30-3.
7. Blainey AD, Topping MD, Ollier S, Davies RS. Respiratory symptoms in arable farmers: the role of storage mites. *Thorax* 1988;43:697-702.
8. Blainey AD, Topping MD, Davies RJ. Storage mite allergy in UK grainworkers [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:173.
9. Cuthbert OD, Jeffrey IG, McNeill MB, Wood J, Topping MD. Barn allergy among Scottish farmers. *Clin Allergy* 1984;14:197-206.
10. Van Hage-Hamsten M, Johansson SGO, Hoglund S, Tull P, Wiren A, Zetterstrom O. Storage-mite allergy is common in a farming population. *Clin Allergy* 1985;15:555-64.
11. Hage-Hamsten M, Johansson SGO, Zetterstrom O. Predominance of mite allergy over allergy to pollens and animal danders in a farming population. *Clin Allergy* 1987;17:417-24.
12. Wraith DG, Cunningham AM, Seymour WM. The role and allergenic importance of storage mites in house dust and other environments. *Clin Allergy* 1979;9:545-61.
13. Agha M, Gnanasakthy A. A cluster analysis of the effects of storage mites as allergens in relation to certain occupations and living conditions. *Clin Allergy* 1981;11:499-504.
14. Hage-Hamsten M, Johansson SGO, Johansson E, Wiren A. Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1987;17:23-31.

15. Korsgaard J, Dahl R, Iverson M, Hallas T. Storage mites as a cause of bronchial asthma in Denmark. *Allergol Immunopathol* 1985;13:143-9.
16. Arlain LG, Geis DP, Vyszynski-Moher DL, Bernstein IL, Gallagher JS. Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J ALLERGY CLIN IMMUNOL* 1984;74:166-71.
17. Arlain LG, Geis DP, Vyszynski-Moher DL, Bernstein IL, Gallagher JS. Cross antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*. *J ALLERGY CLIN IMMUNOL* 1984;74:172-9.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265.
19. Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J ALLERGY CLIN IMMUNOL* 1972;49:1.
20. Axen R, Porath J, Ernback S. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* 1967;214:1302.
21. Harboe N, Ingild A. Immunization isolation of immunoglobulins: estimation of antibody titre. *Scand J Immunol* 1973;2(suppl 1):161.
22. Lowenstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 1978;25:1.
23. Laemmli UK, Favre M. Maturation of the head of bacteriophage T4. I. DNA packaging events. *J Mol Biol* 1973;80:575-99.
24. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P₁. *J Immunol* 1980;125:587-92.
25. Lind P, Weeke B, Lowenstein H. A reference allergen preparation of the house dust mite *D. pteronyssinus*, produced from whole mite culture—a part of DAS 76 study: comparison with allergen preparations from other raw materials. *Allergy* 1984;39:259-74.

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