

Cross antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*

L. G. Arlian, D. P. Geis, D. L. Vyszynski-Moher, I. L. Bernstein, and
J. S. Gallagher *Dayton and Cincinnati, Ohio*

The crossed antigenicity and allergenicity of the storage mite Tyrophagus putrescentiae (TP) and the house dust mite Dermatophagoides farinae (DF) were characterized by means of crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis. DF extracts exhibited 32 antigens and as many as eight were demonstrated to be allergens. DF feces exhibited 20 antigens and six of these were allergens. Twenty antigens and two allergens were demonstrated for TP. Two antigenic and allergenic determinants were shared by DF and TP, and two determinants were also shared by DF feces and TP feces. TP feces and DF shared two antigenic and allergenic determinants. Our results demonstrated that the two mites and their feces extracts contain multiple antigens and allergens. (J ALLERGY CLIN IMMUNOL 74:172-9, 1984.)

The mite TP on occasion may occur in house dust in significant numbers along with the common house dust mites of the genus *Dermatophagoides* spp. when relative humidity is high and cereal and flour products are available as a source of food.¹⁻⁷ More importantly, these mites are very common in grain storage and transfer facilities, barns, hay, straw, and hay and grain dust.^{5, 6, 8-14} Exposure to these mites and their products may occur in homes, and significant occupational exposure among agricultural and grain elevator workers does occur. These mites can also contaminate and destroy long term *Dermatophagoides* spp. cultures that are maintained for extract preparation.

Many studies have suggested TP is an allergenically important mite. CIE and CRIE results indicate that mite bodies and feces contain as many as 20 antigens and five allergens.¹⁵ Of 100 British bronchial

Abbreviations used

TP:	Tyrophagus putrescentiae
DF:	Dermatophagoides farinae
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmuno-electrophoresis
CM:	Culture medium

asthma patients, 94% gave positive skin tests to *D. pteronyssinus*, and 34% gave positive skin tests to TP.² Wraith et al.¹⁶ skin tested 72 London bronchial asthma or allergic rhinitis patients and found 99% were positive to *D. pteronyssinus*, 51% were positive to TP, and 72% to 79% were positive to three other stored-product mites. Extracts of six mite species provoked positive skin responses in 60 Brunei, South East Asia, asthmatic patients.⁷ *D. pteronyssinus* elicited positive skin reactions in 67% of the patients, TP elicited positive skin reactions in 50%, and three other storage mites were allergenic in 35% to 45% of the patients. In another study investigating nine mite species found in house dust, the highest incidence of skin reactions was elicited by *Euroglyphus maynei* (85%), *D. pteronyssinus* (81%), and DF (74%), whereas TP and several other species elicited small numbers of positive responses.¹⁷ RAST, leukocyte histamine release, skin, and nasal provocation testing

From the Departments of Biological Science and Physiology, College of Science and Engineering and School of Medicine, Wright State University, Dayton, Ohio, and Department of Medicine, Division of Immunology, Cincinnati Medical Center, University of Cincinnati Medical Center, Cincinnati, Ohio.

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Reprint requests: L. G. Arlian, Departments of Biological Sciences and Physiology, College of Science and Engineering and School of Medicine, Wright State University, Dayton, Ohio 45435.

of farmers and grain handlers indicated that asthma, allergic rhinitis, and respiratory symptoms can be associated with storage mites present in stored grain, straw, or hay dust.^{6, 18-23}

Although the antigenicity and allergenicity of *Dermatophagoides* spp. and TP are well documented, the extent of their cross-reactivity has not been determined. Cross-reactivity between storage and house dust mites is of considerable importance from the standpoint of both sensitization and immunotherapy. Little is known about the antigens common to both groups. However, varying degrees of reactivity to both house dust and storage mites have been reported.^{16, 17, 24-26} The purpose of this investigation was to evaluate by CIE and CRIE the cross antigenic and allergenic properties of common storage mites TP and DF.

MATERIAL AND METHODS

Mite cultures

Pure DF cultures were maintained on a 1:1 ratio of Purina laboratory chow and Fleischmann's (Standard Brands, Inc., New York, N.Y.) granulated dry yeast at room temperature and 75% humidity. As all mite-life stages crawled to container rims to escape overcrowding, they were collected by aspiration, immobilized, killed by freezing, and desiccated and stored over Drierite (W.A. Hammond Dierite Co., Xenia, Ohio).¹⁵ Microscopic examination of this material revealed it contained predominantly bodies and exoskeletons of all life stages. Its active and pharate life-stage composition was as follows: 56% adults, 9% tritonymphs, 24% protonymphs, and 11% larvae. An occasional fecal pellet was visible outside the mite body, but more importantly, 1 to 2 fecal pellets were visible in the gut of 48% of the whole bodies. DF feces was collected by sieving (106 μ m) mite culture contents, which were dried and stored in a similar manner. Fecal matter consisted of predominately fecal pellets with a few body, exoskeleton, and CM fragments. The particle composition was: 96% fecal pellets, 1.6% bodies and exoskeleton fragments, and 2.4% CM fragments (most 1 to 3 times the size of fecal pellets).

TP were grown on whole wheat flour and collected as they migrated from the cultures to escape overcrowding.¹⁵ To kill and dry mites, they were warmed at 50°C overnight and then completely desiccated over Drierite. The life-stage composition of this material was 81.3% adults, 16.3% nymphs, and 2.5% larvae. Again, insignificant fecal particles were visible outside the mite bodies, but 72.7% of the bodies contained at least one visible fecal pellet. No CM was visible.

Dry TP+CM consisted of the material that remained after the contents of an entire culture was subjected to several days of Berlese-Tullgren extraction.²⁷ This extraction procedure removes most active stages. With this extraction procedure, the culture contents were placed on a screen and several layers of cheesecloth inside a Tullgren funnel, and

an incandescent light appended over it. The heat from the light bulb slowly desiccates the culture contents from the surface downwards, forcing mites to migrate progressively downward through the medium to escape desiccation. The mites eventually reach the screen and fall through. The TP+CM contained dead specimens of all life stages and mite products, such as feces, exoskeletons, and CM. The TP+CM was dried and stored over Drierite.

Extract preparations

Dried test material was defatted in anhydrous ethyl ether and then placed in a corresponding volume of 0.9% sodium chloride to provide a 1:40, 1:20, or 1:10 w/v dilution, ground in a tissue grinder, cold sonicated 30 min (sonicator operated 3 min on, 3 min off to prevent heating of extract), and extracted at least 48 hr at 4°C.²⁸ Extracts were centrifuged in the cold, filtered (0.45 μ m Acrodisc, Gelman Sciences, Inc., Ann Arbor, Mich.), and depending on their use, kept at 4°C or -15°C. Protein contents were determined by the standard Bio-Rad (Bio-Rad laboratories, Rockville Centre, N.Y.) method with the use of bovine gamma globulin as a reference protein.

Antisera

DF, DF feces, TP, and TP+CM antisera were produced by subcutaneous immunization of two New Zealand white rabbits for each extract. To stimulate DF feces antibodies, both rabbits received initial injections of 1 mg DF feces protein emulsified in complete Freund's adjuvant, followed by two 0.5 mg doses in Freund's incomplete adjuvant. For the remaining extracts, each rabbit was initially injected with extract containing 0.5 mg protein in complete Freund's adjuvant. Second and third injections containing one fifth the dose (0.1 mg protein) emulsified in incomplete Freund's adjuvant at 2 and 4 wk produced low antibody titers as determined by twofold dilutions in Ouchterlony gel-diffusion plates. These rabbits were subsequently boosted twice at 2-week intervals with 1 mg (DF) or 2 mg (TP) of protein in incomplete adjuvant. Commencing 2 wk after the last boost, 35 to 40 ml of blood was collected from the central ear artery at three successive weekly intervals. Serum was separated, pooled, and frozen.

CIE

CIE was used to visualize extract antigens by use of procedures similar to that of Arlian et al.,¹⁵ Anderson and Baer,²⁹ and Weeke.³⁰ Briefly, Svendsen's buffer was used to horizontally electrophorese 8 to 12 μ l of extract on 10 by 10 cm glass slides (anode at top) at 200 V for 30 min in 1% agarose gel at 15°C. Gel strips (1 by 5 cm) containing the antigen were cut and transversely placed 1.5 cm from the bottom of 5 by 7 cm gel-bond slides. Three milliliters of agarose containing 0.15 ml of rabbit antisera was poured above and 1 ml of agarose containing 0.075 ml of rabbit antisera was poured below the antigen-containing strip. If antibody titer was low, antisera was first concentrated in a Minicon (Amicon Corp., Danvers, Mass.) concentrator (B125 or B15) two to five times before combining with the

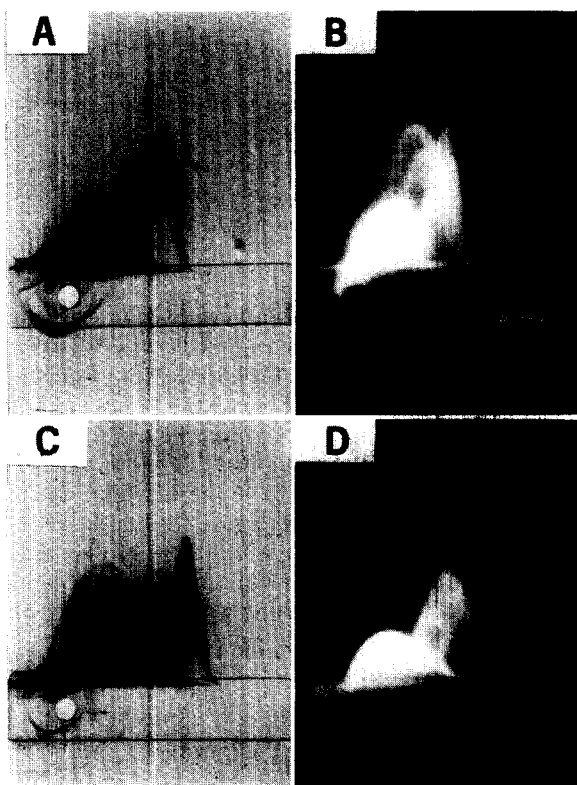


FIG. 1. A and C. CIE pattern of DF extract reacting with rabbit anti-DF serum. B, Autoradiograph (CRIE) of the CIE pattern with patient No. 1 serum. D, Autoradiograph (CRIE) of the CIE pattern with patient No. 2 serum.

TABLE I. Mite extract protein content (1:40 w/v dilution)

Extract	No. of lots tested	Protein* ($\mu\text{g/ml}$) $\pm$ SD
DF	6	760 $\pm$ 122
DF feces	3	387 $\pm$ 33
TP	5	1342 $\pm$ 360
TP + CM	2	583 $\pm$ 46
TP feces	2	758 $\pm$ 272

*Bio-Rad method.

agarose. Perpendicularly, second dimensional electrophoresis was performed with 40 V (anode at the top) at 9° C for 18 to 20 hr. Electrophoresed gels were pressed, washed, dried, stained with Coomassie brilliant blue R-250, and destained.

In CIE homologous reactions, an extract antigen was electrophoresed into antiserum produced to that antigen. Concentration and the quantity of the antiserum on the upper gel were adjusted to give both the maximum number of antigen/antibody precipitin lines and sharp resolution of these precipitates. To determine antigens common to different extracts, heterologous CIE reactions were performed in

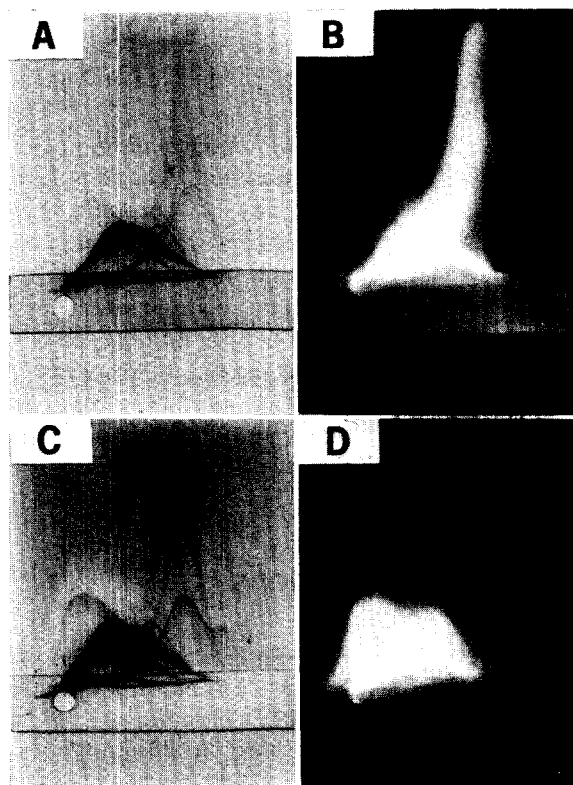


FIG. 2. A and C. CIE pattern of DF feces extract reacting with rabbit anti-DF feces serum. B, Autoradiograph (CRIE) of the CIE pattern with patient No. 1 serum. D, Autoradiograph (CRIE) of the CIE pattern with patient No. 2 serum.

which an antigen was electrophoresed into antiserum produced to a different antigen extract.

CIE with intermediate gel

CIE slide intermediate gels were also used to compare the antigen and antibody contents of the different extracts and antisera.^{15, 31-33} The previously described procedure was used except that a 1 by 5 cm intermediate gel containing a different antiserum was interposed between the first and second dimensional gels. Antibody comparison of different sera was possible by running two identical plates except one plate lacking antisera in the intermediate gel.

Tandem CIE

Common antigenicity was further tested by tandem CIE.¹⁵ Previously, referenced procedures were used except the first dimensional gel had two wells containing equal volumes of extract. Identical antigens were evidenced by fused precipitate peaks.

CRIE

CRIE methods similar to Arlian et al.,¹⁵ Anderson and Baer,²⁹ and Weeke and Lowenstein³⁴ were used to visualize extract allergen contents. Briefly, before staining, CIE plates were selected, incubated for 12 hr with gentle shaking in

TABLE II. CIE and CRIE comparisons of TP and DF extracts and antisera*

Extract (antigen)	Antiserum (antibodies)	No. of precipitates					
		CIE		CRIE			
		A	B	Patient 1		Patient 2	
				A	B	A	B
DF	DF	28	4	7	1	4	0
DF feces	DF feces	20	0	3	0	6	0
TP	TP	18	2	2	0	0	0
TP + CM	TP + CM	18	0	5	0	0	0
TP	DF	2	0	2	0	0	0
DF	TP	2	0	2	0	0	0
DF	TP + CM	2	0				
TP + CM	DF	1	0	1	0	0	0
TP feces	DF	2	0	2	0	0	0
TP feces	DF feces	2	0	2	0	0	0
DF feces	TP	0	0	0	0	0	0
DF feces	TP + CM	0	0	0	0	0	0
CM	TP	0	0	0	0	0	0
CM	DF	0	0	0	0	0	0

A, B = number of precipitin lines in the anodal or cathodal direction, respectively.

*Figures for unlike combinations of extracts and antisera indicate shared antigens and allergens.

serum from a DF-sensitive patient and RAST A buffer,* and then washed three times for 15 min with RAST B buffer.† Slides were then incubated overnight with ¹²⁵I-labeled anti-human IgE‡ and RAST C buffer,§ washed as before, pressed, air dried, and inserted into x-ray cassettes. The ¹²⁵I-labeled anti-human IgE was labeled according to the methods of Greenwood and Hunter.³⁵ The gel slides were placed under two tungsten-enhancement screens between which Kodak X-omat (XRP-1) film was located. X-ray cassettes containing the film were kept for 4, 7, 11, and 14 days at -80° C and then stained with Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.) for antigen identification.

Sera from two nondesensitized patients were used to perform CRIE. They were selected on the basis of persistent allergic symptom histories during the heating season and positive intradermal skin tests (3+ or more) to house dust mite DF and storage mite TP extract when the patients were challenged with dilutions of 10⁻³, 10⁻⁴, and 10⁻⁵ (w/v). The extracts used for skin testing were prepared as described previously and were the same as those used for CIE and antibody production in rabbits. To check for nonspecific

binding of IgE for identical precipitin lines, sera from an individual without an allergic history or clinical symptoms and also skin test negative to the two extracts were used as a control for CRIE tests. Likewise, CIE was performed by use of sera from nonimmunized rabbits to verify that all precipitin lines visualized by CIE and CRIE were the result of antibody production to antigens in the immunizing extract.

RESULTS

The protein contents of the extract preparations diluted 1:40 (w/v) ranged from 387 to 1342 µg/ml (Table I). Although all these extracts were prepared on the same weight/volume basis, their protein contents varied significantly with the components. DF and DF feces contained about half the amount of protein as comparable TP extracts (significantly different at the $p < 0.01$). DF and TP extracts contained significantly more ($p < 0.01$) protein than their respective feces.

The CIE for DF extract run against its corresponding antiserum, detected 28 anodically moving and four cathodically moving antigens (Table II, Fig. 1). Twenty anodic antigens were demonstrated in DF feces extract by use of DF feces antiserum (Table II, Fig. 2). Many of the precipitin lines were identical in shape and position when CIE slides of the two extracts and their corresponding antisera were compared. CIE experiments of TP extract and anti-TP serum exhibited 18 anodic and two cathodic antigens in TP extract (Table II). Likewise, the homologous

*RAST A buffer: 500 ml of 0.1M phosphate buffer (pH 7.5), 1 gm BSA, 5 ml Tween 20, 0.5 ml of 10% sodium azide (NaN₃).

†RAST B buffer: 1000 ml of 0.1M phosphate buffer (pH 7.5), 10 ml Tween 20.

‡Anti-human IgE provided by D. R. Hoffman, Department of Pathology and Laboratory Medicine, East Carolina University, School of Medicine, Greenville, N. C.

§RAST C buffer: 20 ml of calf serum, 5 ml Tween 20, 5 ml of 10% NaN₃, 470 ml of 0.1M phosphate buffer (pH 7.5).

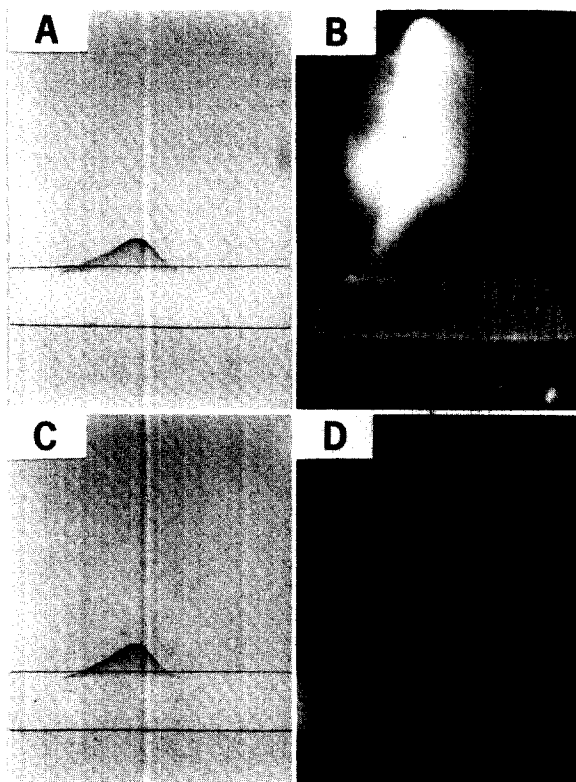


FIG. 3. A and C. CIE pattern of TP extract reacting with rabbit anti-DF serum. B, Autoradiograph (CIE) of the CIE pattern with patient No. 1 serum. D, Autoradiograph (CIE) of the CIE pattern with patient No. 2 serum.

CIE of TP+CM extract (primarily feces) and TP+CM antisera exhibited 18 precipitin lines.

Autoradiograms (CIE), after incubation of these immunoplates with serum from the two skin test positive patients and ^{125}I -labeled anti-human IgE, indicated that both DF and its feces exhibited multiple allergen precipitin lines (Figs. 1 and 2). The serum of patient no. 1 exhibited IgE binding to seven anodic and one cathodic DF antigens and to three anodic DF feces antigens (Table II, Figs. 1 and 2). The serum of patient no. 2 exhibited IgE binding to four anodic DF antigens and to six anodic DF feces antigens (Table II, Fig. 2). Three anodically active allergens in DF extract bound IgE from both patients in identically corresponding patterns that could be superimposed with the same antigens in the CIE gels. Intense radiolabeled immunoprecipitin lines 4 days after incubation indicated a high specific IgE titer for the three common allergens in both patients. Sera from both patients contained two IgE antibodies that bound to similar determinants of two allergens in DF feces. One of these radiostaining precipitin lines was intense by comparison to the others. The x-ray films were exposed for varying durations in order to resolve the

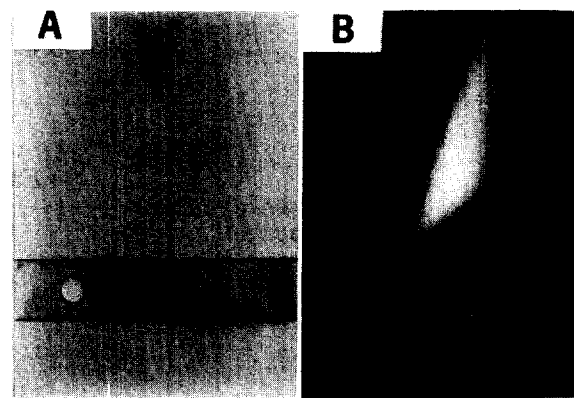


FIG. 4. A. CIE pattern of TP+CM extract reacting with rabbit anti-DF serum. B, Autoradiograph (CIE) of the CIE pattern with patient No. 1 serum.

weak and strong allergens and avoid the masking effects of the intense allergens. Appearance of radiolabeling to several allergens as early as 3 days of incubation indicated a high titer of specific-IgE antibodies in the patients' serum. None of the DF or DF feces antigens on the cathodic (−) side exhibited specific-IgE binding in patient no. 2, whereas one DF cathodic allergen was visible in patient no. 1.

Autoradiograms with the use of the nonallergic control serum exhibited three weak immunoprecipitates to DF allergens. Two of these weak immunoprecipitin lines corresponded with two strong ones observed for both patients by use of nonallergic control serum. Likewise, one weak radiostaining immunoprecipitate to DF feces by use of nonallergic control serum corresponded with the most intense allergen for the two patients. Therefore, either the asymptomatic control sera contained a low titer of specific IgE for antigens in the two extracts or some nonspecific binding occurred. Regardless, the radiostaining exhibited with use of patient sera was sufficiently more intense than that with use of control serum to clearly identify these as allergens.

CIE precipitin lines demonstrated that anti-DF serum contained antibodies against two antigens in TP and TP+CM extracts (Table II, Fig. 3). An antigen in TP+CM that was common to DF was weak and did not stain with Coomassie blue; however, it did radiostain (Fig. 4). TP+CM contains predominately feces antigens.¹⁵ However, the antigens of TP and TP+CM extracts that reacted with DF antisera were not the same since the position and profile on the gels were different. Autoradiograms of these heterologous immunoplates exhibited two allergens in TP extract and one in TP+CM actively bound IgE in the serum of patient no. 1 (Figs. 3 and 4). These probably do not represent immunochemically similar allergens com-

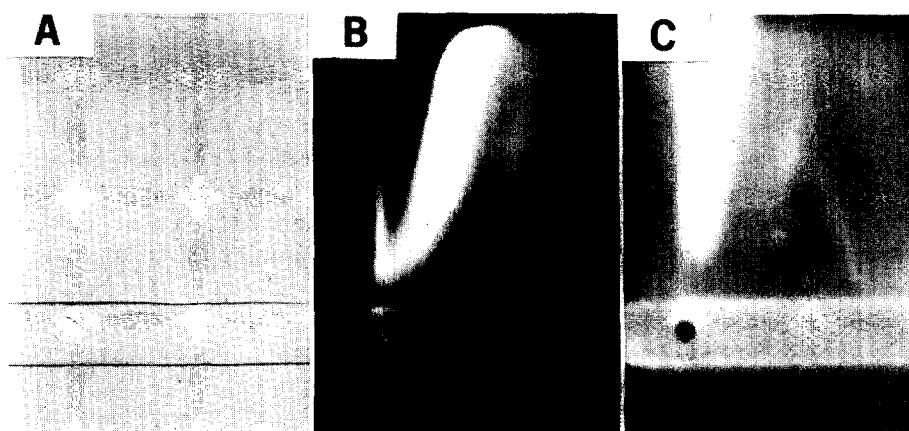


FIG. 5. A, CIE pattern of TP feces extract reacting with rabbit anti-DF serum. B, Autoradiograph (CRIE) of the CIE pattern with patient No. 1 serum. C, Autoradiograph (CRIE) of the CIE pattern of TP feces extract reacting with rabbit anti-DF feces serum with patient No. 1 serum.

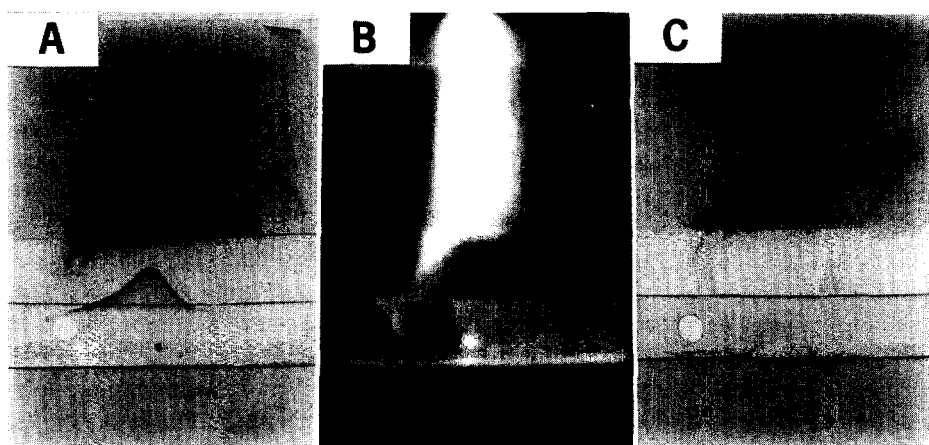


FIG. 6. A, CIE pattern of TP extract reacting with rabbit anti-TP serum in top gel and rabbit anti-DF serum in the intermediate gel. B, Autoradiograph (CRIE) of the CIE pattern with patient No. 1 serum. C, CIE pattern of TP extract reacting with rabbit anti-TP serum in top gel and control rabbit serum in the intermediate gel.

mon to both extracts because the profiles were different, but it does indicate cross allergenicity between the two storage-mite extracts and the house dust-mite extract.

Two common, weak antigens were demonstrated when TP feces extract was electrophoresed into anti-DF antisera (Table II, Fig. 5). CRIE of TP feces extract and DF or DF feces antisera also revealed that each contained two common allergens when serum from patient no. 1 was used (Fig. 5).

The CIE system by use of intermediate gels was used to help corroborate that corresponding antibodies were present or absent in different antisera and correspondingly similar antigens were present or absent in different extracts. Use of intermediate gels also indicated that DF and TP extracts contain at least two common antigens and allergens and confirmed the

heterologous CIE experiments (Fig. 6). However, the intermediate gel technique revealed that TP+CM and DF extract contained no common antigens. These crosses were inconsistent with straight heterologous CIE experiments (Table II) in which 1 to 2 were visualized. Use of intermediate gels demonstrated DF feces shared no common antigens with either TP or TP+CM extract and confirmed the straight heterologous CIE experiments in which no intermediate gels were used. Likewise, TP feces electrophoresed into DF feces or DF antisera also exhibited no precipitates and thus no common antigens when intermediate gels containing these antisera were interposed between the first and second dimensional gels. However, these results were inconsistent with straight heterologous CIE experiments in which two very weak antigens were detected.

A complete series of tandem CIE gels was run by use of all combinations of extracts and antisera in an attempt to directly compare the antigens in different extracts. No fused precipitin peaks in the reference antisera were observed that would further facilitate identification of common antigenicity between any of the extracts. However, the high complexity of DF extract and the weak antigenicity of TP extracts made this procedure inconclusive.

DISCUSSION

In a previous study the antigenic and allergenic properties of the common storage mite TP were characterized by means of CIE and CRIE techniques.¹⁵ Results of this study clearly demonstrate a crossed antigenic and allergenic relationship between components of this mite and the common house dust mite DF.

Precipitating rabbit antibodies directed against mite antigens from both species were produced in rabbits. These antigens were well visualized by CIE. Identification of allergens by means of homologous CRIE in each extract was also achieved by use of specific IgE antisera from two mite-allergic patients and a radiolabeled anti-human IgE marker. Homologous CIE and CRIE experiments indicated that DF, DF feces, TP, and TP feces extracts were complex and contained numerous antigens and many allergens. Heterologous CIE and CRIE experiments identified some immunochemically similar antigens and allergens common to the two species of mites or mite feces. The possible origin of any antigens from CM in the mite and feces extracts was ruled out. The two mite species were cultured on different media, and therefore a common antigen was not likely to originate from culture material. More importantly, electrophoresis of CM extract into antisera produced to the mites and feces gave no precipitin lines or radio-staining. Therefore the antigens and allergens visualized originated from mites and mite products.

DF and DF feces extracts contained as many as 32 and 20 antigens, respectively. DF exhibited at least eight allergens, and DF feces exhibited at least six allergens with use of sera from the two patients. According to our immunoelectrophoresis findings, the TP extracts generally contained fewer antigens and allergens than DF. TP contained 20 antigens; two allergens could be identified by use of the same two patient's sera. Likewise, TP+CM (primarily feces) contained five allergens; three were from feces.¹⁵ Whether or not more of the TP-visualized antigens are allergens is not known. The two patients of this study did not have a history of exposure to this mite. Use of serum from a larger population of patients skin test

positive to TP, particularly grain handlers, would be necessary to determine this. In spite of the large number of antigens exhibited by each species, generally little cross antigenicity was found between them. However, CIE demonstrated that since a few rabbit antibodies evoked by immunization with either TP or DF were also directed against determinants in the other mite, there is an immunologic relationship between some TP and DF antigens. DF and TP shared two common antigens. More significant, however, was the fact that autoradiograms revealed two common allergens in DF and TP. DF feces and TP feces also demonstrated two common allergens, and TP feces shared two common allergens with DF. With the use of serum from the more allergenic patient, CRIE of TP feces extract and anti-DF feces or anti-DF sera were identical. Thus these extracts appear to contain immunochemically similar allergens.

The large number of antibodies produced in rabbits suggests these mite species provide a large antigenic pool for hypersensitization. Therefore the potential for varied sensitivity responses between patients and varied sensitivity to different mite antigens by the same patient is large. Patients with dust-mite allergy or stored food mite allergy could produce antibodies against different antigens from either or against antigens common to both stored food and dust mites. This was illustrated by the reference mite sensitive patients in this study. Patient no. 1 exhibited about twice the number of specific IgE precipitin lines to DF mites than to DF feces. On the other hand, patient no. 2 exhibited a greater number of specific-IgE precipitin lines to DF feces allergens and fewer to DF. Autoradiography also exhibited that patient no. 1, demonstrating more antibodies to DF, also had specific IgE to two TP antigens and six TP+CM antigens, whereas patient no. 2 exhibited specific-IgE responses to neither.

It is clear that each mite species and its feces contains antigens and allergens specific to each in addition to some common antigens and allergens. Since many more antigens were visualized in matched extracts and antisera for both species than with crossed extracts and antisera between species in this preliminary study, we conclude that each mite primarily possesses its own specific antigenicity. However, most of the visualized allergens of TP and TP feces demonstrated cross allergenicity with DF and DF feces. Because of the shared and specific allergenic relationship between the two mite species, the additive, synergistic, or priming antigenic effects of the presence of both mite species and mite products in an environment are unclear. Furthermore, because of the complexity of the extracts from these two mites, further

identification and characterization of specific antigens and allergens are required in order to completely understand the role these mites play in house dust- and storage-mite allergies.

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