

Immunochemical and enzymatic analyses of extracts of the house dust mite *Dermatophagoides pteronyssinus*

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The allergenicity and enzyme content of extracts of Dermatophagoides pteronyssinus whole mite, cuticle (CUT), and spent-growth medium (SGM) used in its culture have been examined. Both direct RAST binding and RAST-inhibition studies demonstrated that the whole mite extract was approximately threefold more potent than either the CUT or SGM extracts. Crossed immunoelectrophoresis and crossed radioimmunoelectrophoresis demonstrated that whole mite extracts contained 36 antigens of which nine were judged to be allergens (Dpt 1, 3, 4, 7, 8, 12, 17, 22, and 31). In contrast, extracts of CUT and SGM both contained 15 mite antigens of which five were judged to be allergens (Dpt 4, 8, 12, 17, and 22). Enzyme studies demonstrated that whole mite extracts from various sources contained esterases, phosphatases, aminopeptidases, and glycosidases. Both the CUT and SGM extracts contained a similar spectrum of enzymes, although the glycosidases were present at relatively low levels. Combined crossed immunoelectrophoresis and enzymic studies identified three antigens (Dpt 6, 13, and 24) with esterase activity, one antigen (Dpt 27) with β -glucuronidase activity, and one antigen (Dpt 14) with acid phosphatase activity. Thus far, none of these enzymes has been demonstrated to be allergenic. Both enzymic and immunochemical studies suggested that not all mite allergens are of an excretory/secretory origin, but some (e.g., Dpt 1, 3, 4, 7, and 31) are derived from the whole mite per se. (J Allergy Clin Immunol 77:14-24, 1986.)

Previous studies from this and other laboratories, by use of CIE and CRIE have demonstrated that there are several immunochemically distinct allergens in extracts of the house dust mite *Dermatophagoides pteronyssinus*.¹⁻³ Thus far, however, only two have been characterized in any detail. The major allergen Dpt 12 (identical to P₁, described by Chapman et al.,⁴ and Dp 42, described by Lind and Lowenstein² was demonstrated to have an apparent molecular weight in the region of 24 to 30,000 daltons and to be extremely heterogenous with regard to isoelectric point.⁴ The allergen Dpt 4, in contrast, was demonstrated to be a

Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
CSL:	Commonwealth Serum Laboratories
B:	Beecham Research Laboratories
HDM:	Whole mites devoid of medium
CUT:	Cuticle
SGM:	Spent-growth medium
UGM:	Unused growth medium

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very high-density lipoprotein allergen with an apparent molecular weight of 244,000 daltons.⁵

In order to characterize further these and other allergens from the mite, large quantities of material are required. To this end we have examined extracts of whole mite, CUT, and, in particular, SGM, in order to determine which of these starting materials would provide optimum yields in large scale allergen isolation procedures. The potential advantages of being able to use materials such as SGM are twofold. First, in comparison to whole mites, it is relatively easy to obtain large quantities of starting material. Second, the SGM contains large quantities of mite feces that,

in other studies, have been demonstrated to contain the mite allergen P₁.⁶

In this study we have examined the allergenicity and composition of various mite extracts with the use of RAST assay and CRIE. In addition, we have examined mite extracts for the presence of enzymes and have correlated some of the enzymatic activities detected, particularly acetylcholinesterase and butyrylcholinesterase, acid phosphatase, and β -glucuronidase with antigens identified by CIE in an attempt to determine whether such enzymes are allergenic.

MATERIAL AND METHODS

Preparation of mite extracts

Mite extracts were obtained from several sources. CSL-HDM, CUT, SGM, and UGM were kindly provided by CSL, Parkville, Australia. Unfractionated mites and medium (B-HDM) together with B UGM were also provided by Beecham Research Laboratories, Melbourne, Australia. Extracts of both CSL and B material were prepared according to methods described previously for whole mites.¹ For commercial reasons the manufacturers did not provide details either of the composition of the mite growth medium or of the method of separating mites from CUT and medium. In addition, three mite extracts prepared from unfractionated mites and medium (AF A, AF B, and AF C) were provided by Ms. Annette Ford (National Institute for Biological Standards and Control, London, England).

Antisera

The anti-HDM extract antiserum was raised in sheep. Briefly, three sheep were each immunized subcutaneously with 2 mg of CSL HDM extract. The extract was dissolved in 1 ml of 0.01 mol/L of phosphate buffer, pH 7.2, containing 0.15 mol/L NaCl (phosphate-buffer saline), and emulsified with Freund's complete adjuvant. A secondary injection of extract (2 mg) in Freund's incomplete adjuvant was administered 28 days later, and the sheep were bled 10 days after this and subsequent booster injections. Sera from each sheep were pooled and stored frozen at -20°C until the sera were required.

Immunoelectrophoretic analyses

CIE and CRIE were performed essentially as described previously⁷ with the use of agarose (type 5, Sigma Chemical Co., St. Louis, Mo.) and sheep anti-HDM. The choice of a sheep antiserum rather than a rabbit antiserum for use in such assays was based on the need for relatively large volumes of antisera. To overcome the suggestion that nonspecific radiostaining may occur if antisera other than rabbit are used,⁸ all sera used in CRIE studies were absorbed with insolubilized normal sheep serum⁹ before use. The ¹²⁵I-labeled antihuman IgE reagent used in CRIE was obtained from Pharmacia South Seas Pty., Ltd., Sydney, Australia. Autoradiography was performed by use of Kodak Ortho G film (Eastman Kodak Co., Rochester, N. Y.) in combination

with a Kyokko PS II (Fugimex, Perth, Australia) intensifying screen. CIE gels were stained for protein by use of Biebrich scarlet¹⁰ and for lipoprotein by use of Sudan black B.¹¹ The relative amounts of certain allergens in CSL SGM and CUT were determined by tracing around an enlarged image of the appropriate CIE peak on paper and comparing its size with that of the corresponding peak present in CSL HDM. Enzymic activities associated with unstained CIE precipitin bands were determined with published methods.^{12,13} Nonspecific protease activity was detected with the casein-agarose overlay technique.¹⁴ Esterase (acetyl and butyl), acid and alkaline phosphatases, β -glucuronidase, and β -N-acetyl-D-glucosaminidase activities were determined with α -naphthyl acetate and α -naphthyl butyrate, α -naphthyl phosphate, naphthol-AS-BI- β -D-glucuronic acid, and naphthol-AS-BI-N-acetyl- β -D-glucosaminide substrates (Sigma Chemical Co.), respectively. After development, the CIE plates were washed with distilled water and dried.

Enzyme activity

The enzyme content of the mite extracts was determined by use of the Api-Zym system¹⁵ according to the manufacturer's instructions. Nineteen enzymes were assayed: acid and alkaline phosphatase, esterase, esterase lipase, lipase, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, phosphoamidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, β -N-acetylglucosaminidase, α -mannosidase, and α -fucosidase. To each well of the Api-Zym tray containing the appropriate freeze-dried buffer and naphthol-coupled substrate was added either 66 μl of each mite extract (1 mg of dry weight per milliliter), growth medium (1 mg/ml), or saline control, and the tray was incubated at 37°C for 4 hours. After incubation the free naphthol released from the substrate was detected by coupling with equal volumes (33 μl) of fast blue BB (0.35% w/v in 2-methoxyethanol) and 2 mol/L of Tris buffer, pH 7.6, containing 10% w/v of sodium dodecyl sulfate. The amount of product formed was semiquantitatively assessed with the color chart provided with the system (see Table III).

RAST

RAST assays were performed as described previously^{1,16} with ¹²⁵I-labeled antihuman IgE (Pharmacia South Seas Pty., Ltd.). Allergens were coupled to CNBr-activated paper discs,¹⁷ and the concentration of allergen was expressed in micrograms per milliliter. Individual sera from atopic patients with both clinical and laboratory evidence of hypersensitivity to mites were obtained from the Princess Margaret Hospital. Sera from nonatopic individuals were obtained from healthy laboratory personnel. Pools of sera from 50 atopic patients (RAST score 4) and 10 nonatopic control subjects were prepared from sera obtained as described above and absorbed with glutaraldehyde⁹ cross-linked normal sheep serum.

Direct RAST binding and RAST-inhibition techniques with a pooled mite-allergic serum were used to evaluate the

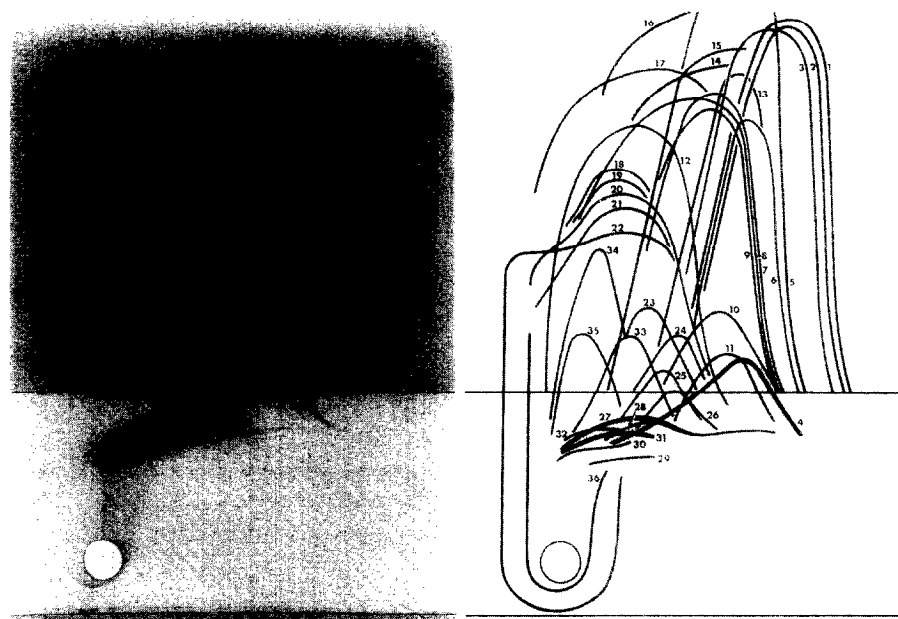


FIG. 1. CIE analysis of an extract of *D. pteronyssinus* (750 µg) with a sheep antiwhole mite extract antiserum at a concentration of 52 µl/cm² and a schematic diagram of the antigens contained therein. Anode at top and right.

TABLE I. RAST analysis of CSL HDM, CUT, SGM, and UGM extracts

Extract	Direct RAST binding		RAST inhibition*	
	µg/ml [†]	Potency [‡]	µg/ml	Potency
Whole mite	10.3	1	4.2	1
CUT	27	0.38	14	0.30
SGM	29	0.35	19	0.22
UGM		—		—

*RAST inhibition was performed with whole mite extract-coupled discs.

[†]Amount of material required to elicit either 50% maximum binding of ¹²⁵I-antihuman IgE or 50% inhibition in the appropriate RAST assay.

[‡]Relative potencies determined by dividing the amount of whole mite material required to elicit either 50% maximum binding or 50% inhibition by the corresponding values obtained for CUT, SGM, UGM.

allergenicity of extracts of CSL HDM, CUT, SGM, and UGM. Data from direct RAST-binding experiments were evaluated by a four parameter logistic model,¹⁸ and the potency of each extract was expressed as the concentration of extract coupled to the activated paper disc required to bind 50% of the maximum bindable ¹²⁵I-labeled anti-IgE. Data from RAST-inhibition experiments were evaluated by linear regression analysis. Experiments were performed in duplicate.

RESULTS

RAST studies

The relative potency of each of the CSL extracts was examined by both direct RAST binding and RAST-inhibition assays with a mite-allergic serum pool. CSL HDM was the most potent extract judged by the concentration of extract required to either bind 50% of the maximum bindable ¹²⁵I-labeled anti-IgE

or produce 50% inhibition of RAST, namely, 10.3 and 4.2 µg/ml, respectively (Table I). In contrast, 29 and 14 µg/ml, and 27 and 19 µg/ml of CSL SGM and CUT, respectively, were required to achieve the same degree of RAST binding and RAST inhibition. The UGM was nonallergenic in both assay systems. The RAST-binding curves obtained with CSL HDM, CUT, and SGM were similar with regard to the maximum amount of ¹²⁵I-labeled anti-IgE bound, but the slopes obtained with CSL CUT and SGM extracts were slightly shallower than that obtained with CSL HDM as judged empirically (data not presented). Similar results were obtained in RAST-inhibition studies. In both direct RAST binding and RAST inhibition, the slopes obtained with CSL CUT and SGM were similar.

Batches of discs optimally coated with either CSL

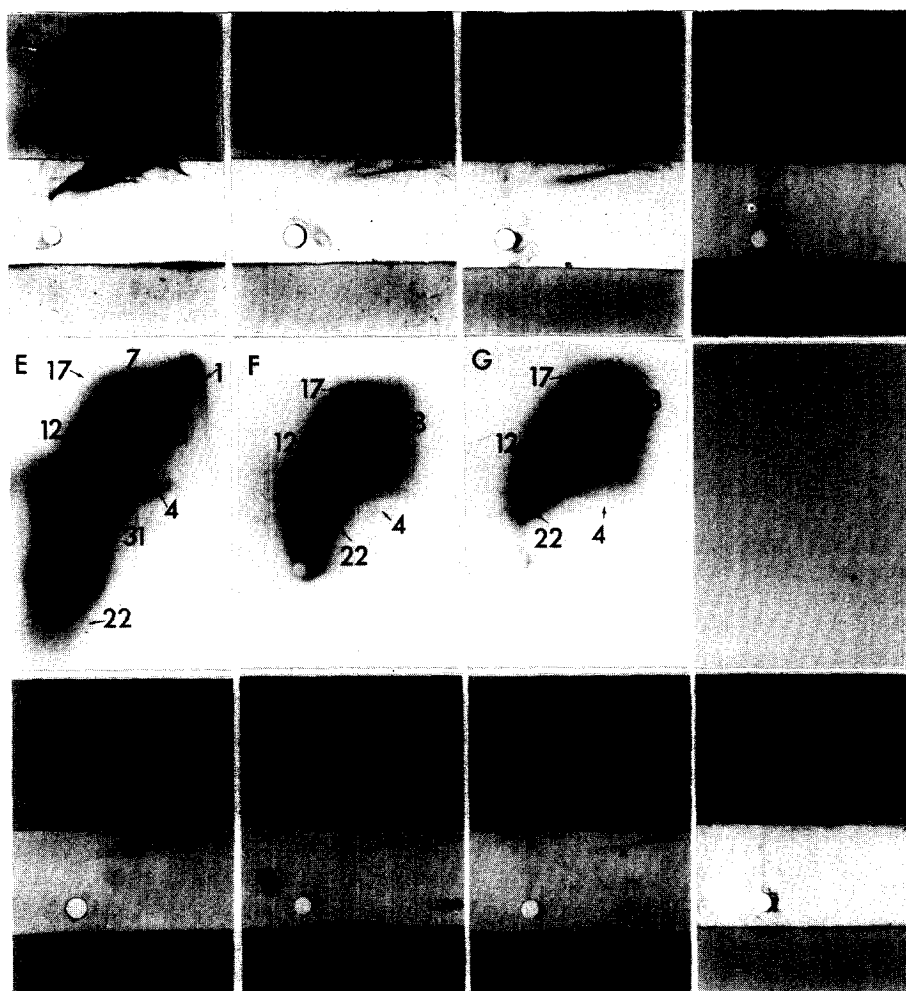


FIG. 2. A-D, CIE. E-H, CRIE. I-L, Lipoprotein. A, E, I, Analyses of *D. pteronyssinus*. B, F, J, Spent growth medium. C, G, K, Cuticle. D, H, L, Unused growth medium. CIE was performed with a sheep antiwhole mite extract antiserum at a concentration of $52 \mu\text{l}/\text{cm}^2$ and $750 \mu\text{g}$ of each mite extract. CRIE was performed by use of a mite-allergic serum pool and ^{125}I -anti-IgE (5×10^5 cpm per plate). Autoradiography was performed for 11 days. CIE plates were stained for lipid with Sudan black B, and the arrows (A-C and I-K) indicate antigens judged to be lipoproteins. Anode at top and right.

HDM, SGM, or CUT were prepared, and individual sera from a panel of mite-allergic patients with varying RAST scores were reacted with each type of disc. The mean percentage radiation bound and standard deviation for HDM, SGM, and CUT discs were 12.5 ± 9.8 , 13.7 ± 10.9 , and 13.4 ± 10.4 , respectively. One-way analysis of variance indicated that there were no significant differences between means.

CIE and CRIE studies

CIE and CRIE analyses of CSL HDM, CUT, SGM, and UGM were undertaken to determine their antigenic and allergenic composition and, specifically, to determine which extracts contained the allergens Dpt 4 and Dpt 12. Thirty-six antigens were detected in extracts of CSL HDM (Fig. 1), nine of which were

allergens as judged by radiostaining on CRIE (Fig. 2, A) by use of a mite-allergic serum pool. The allergens corresponded to antigens 1, 3, 4, 7, 8, 12, 17, 22, and 31. Both CSL SGM and CUT contained 15 mite-specific antigens (Fig. 2, B and C) of which five were allergens (Fig. 2, F and G). These corresponded to mite antigens 4, 8, 12, 17, and 22. The relative amounts of these five allergens in CSL SGM and CUT were determined (Table II). The relative amounts of Dpt 8, 12, and 17 in these two extracts were similar to that found in CSL HDM, but Dpt 4 and 22 were present in lower amounts.

Control CRIE studies were performed by use of a nonmite-allergic serum pool and a serum containing high levels of IgE (3600 U/ml) but not with the mite-allergy (RAST) negative serum. Radiostaining, fainter than that observed with the mite-allergic pool was



FIG. 3. **A,** CRIE analysis of the supernatant (750 µg). **B,** Precipitate (300 µg) from a 50% saturated ammonium sulfate fractionation of the spent growth medium used in the culture of *D. pteronyssinus*. CIE was performed with a sheep antiwhole mite extract antiserum at a concentration of 52 µl/cm². CRIE was performed with a mite-allergic serum pool and ¹²⁵I-anti-IgE (5 × 10⁵ cpm per plate). Autoradiography was performed for 5 days. Anode at top and right.

TABLE II. Relative* amounts of allergens common to extracts of CSL HDM, SGM, and CUT

Antigen	HDM	SGM	CUT
4	100	21	16
8	100	120	66
12	100	77	99
17	100	97	131
22	100	49	15

*The relative amount of allergen is expressed as a percentage of that found in the whole mite extract.

found with mite antigen 4 by use of both the nonallergic serum and the serum containing high levels of IgE. In addition, very faint radiostaining was found with antigen 5 and 12 with the serum containing high levels of IgE but not with the mite-allergy negative serum pool (data not presented). CSL UGM was demonstrated not to contain either mite-specific antigens or allergens (Fig. 2, *D* and *H*).

The lipoprotein nature of mite allergen 4 in extracts of CSL HDM, CUT, and SGM was confirmed by staining appropriate CIE plates with Sudan black B (Fig. 2, *I* through *L*). The latter two extracts contained an extra lipid staining component more anodal than allergen 4 that was not evident in the CSL HDM extract. (This component was not assigned an antigen number and is thus not marked in Fig. 1.)

A common approach used in the isolation of aller-

gens is an initial ammonium sulfate precipitation step, and such a step, by use of 50% saturation, has been used in the isolation of Dpt 12 from SGM.¹⁸ It was of interest to determine whether any mite allergens remained after precipitation of various mite extracts. The precipitate and supernatant from CSL SGM were examined by CRIE by use of a mite-allergic serum pool. The results demonstrated (Fig. 3) that allergens were present in both the precipitate and supernatant. The mite allergens Dpt 4, 8, 17, and 22 together with relatively small amounts of Dpt 12 were found in the supernatant. Dpt 12 was found to be the major allergen present in the precipitate, although relatively small amounts of Dpt 8 and 22 were also found.

Enzyme content of CSL HDM, CUT, and SGM

Extracts of CSL HDM, CUT, SGM, and UGM were analyzed, semiquantitatively, for the presence of enzymes by use of the Api-Zym¹⁵ system. These studies demonstrated (Table III) that the CSL HDM extract contained a wide variety of enzymes. Particularly prominent were acid and alkaline phosphatases, leucine aminopeptidase, phosphoamidase, β-galactosidase, esterase lipase, β-glucuronidase, β-glucosidase, and β-N-acetylglucosaminidase. Lesser amounts of esterase, valine aminopeptidase, α-galactosidase, α-glucosidase, α-mannosidase, and α-fucosidase were also demonstrated. Examination of CSL CUT demonstrated that its enzyme profile was similar to that of CSL HDM except that activities were, with the

TABLE III. Enzyme content of CSL HDM, SGM, CUT, and UGM

Enzyme	HDM	CUT	SGM	UGM	Saline
Alkaline phosphatase	5	3	5	0	0
Esterase	2	1	2	0	0
Esterase lipase	5	3	2	0	0
Lipase	0	0	0	0	0
Leucine aminopeptidase	5	3	5	0	tr
Valine aminopeptidase	1	1	1	1	0
Cystine aminopeptidase	tr	0	tr	0	0
Trypsin	0	0	0	0	0
Chymotrypsin	0	0	0	0	0
Acid phosphatase	5	5	5	1	1
Phosphoamidase	5	2	3	1	0
α -Galactosidase	3	1	tr	0	0
β -Galactosidase	5	5	3	0	0
β -Glucuronidase	5	1	0	0	0
α -Glucosidase	1	tr	tr	0	0
β -Glucosidase	5	2	tr	0	0
β -N-Acetylglucosaminidase	5	3	tr	tr	0
α -Mannosidase	4	3	1	0	0
α -Fucosidase	3	tr	tr	0	0

5 = >40 Nanomoles of hydrolyzed substrate; 4 = >20 <40 nanomoles of hydrolyzed substrate; 3 = 20 nanomoles of hydrolyzed substrate; 2 = >10 <20 nanomoles of hydrolyzed substrate; 1 = 5 nanomoles of hydrolyzed substrate; tr = >0 <5 nanomoles of hydrolyzed substrate. Each extract was analyzed at a concentration of 1 mg of dry weight per milliliter of saline.

exception of acid phosphatase, lower. Similar results were obtained with CSL SGM, although levels of acid and alkaline phosphatase, leucine aminopeptidase, and esterase were comparable with those of CSL HDM. Lipase, trypsin, and chymotrypsin were not detected in any of the extracts. CSL UGM was demonstrated to have only low amounts of valine aminopeptidase and acid phosphatase.

Enzyme activity and CIE

Attempts were made to correlate some of the predominant enzymic activities found in the CSL HDM with precipitin bands detected by CIE. Precipitin bands were detected with acetylerase and butyl esterase activity, acid phosphatase activity, and β -glucuronidase activity but not with either alkaline phosphatase or β -N-acetylglucosaminidase activities (Table IV). Three precipitin bands with both acetyl-esterase and butyl esterase activity were detected and corresponded to mite antigens 6, 13, and 24, the latter two antigens only being definitively identified after enzymic analysis (Fig. 4, A and B; Table IV); β -glucuronidase activity was found to correlate with antigen 27 (Fig. 4, C; Table IV). Because of the complexity and intensity of the precipitin bands in the area concerned, acid phosphatase activity was tentatively assigned to antigen 14 (Fig. 4, D). No protease ac-

TABLE IV. Correlation between enzymic activity and CIE antigen number

Enzyme	Antigen
Esterase	6, 13, 24
Acid phosphatase	14
β -Glucuronidase	27
Alkaline phosphatase	ND
β -N-Acetylglucosaminidase	ND

ND = no enzymic activity detected.

tivity was found with any precipitin band with the use of the casein-agarose gel overlay technique.

Enzyme content of different mite extracts

The enzyme content of five mite extracts obtained from different sources was determined by use of the Api-Zym system (Table V). These studies demonstrated that the enzyme profiles varied only slightly. Each of the five extracts was demonstrated to contain, albeit in varying amounts, acid and alkaline phosphatase, esterase, esterase lipase, leucine aminopeptidase, phosphoamidase, and β -galactosidase. The profiles of CSL HDM and B HDM were very similar, but the extracts AF A, AF B, and AF C demonstrated some variability in the presence or absence of the

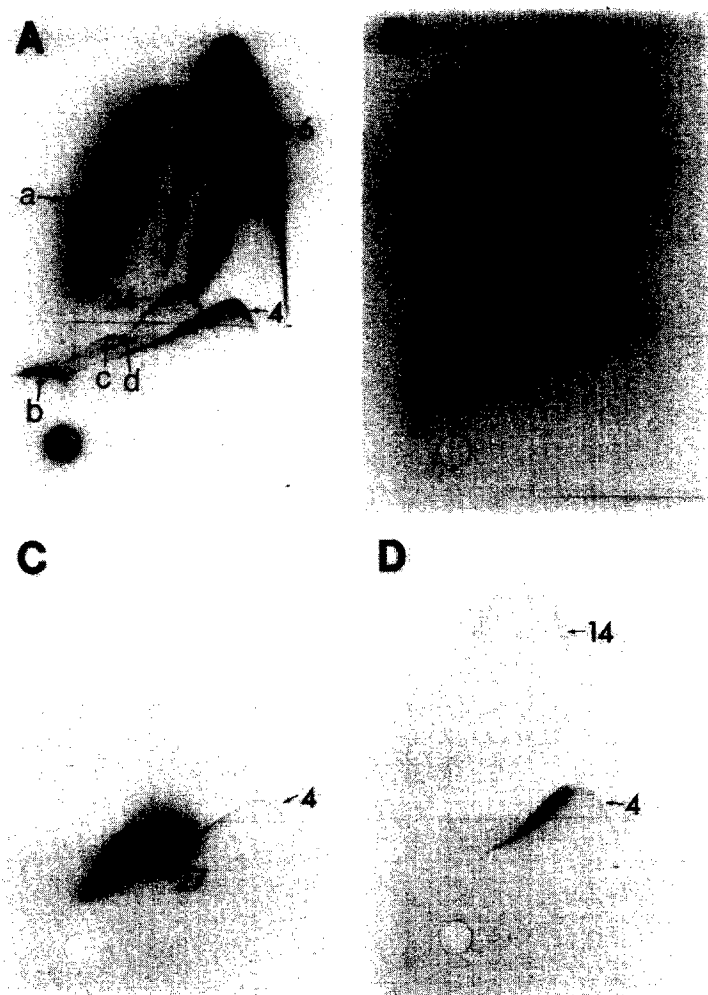


FIG. 4. Combined enzymatic and CIE analysis of *D. pteronyssinus* extract (750 μ g) with a sheep antiwhole mite extract antiserum at a concentration of 52 μ l/cm². Enzymes assayed: **A**, Esterase with α -naphthyl acetate and **B**, α -naphthyl butyrate substrates. **C**, β -Glucuronidase with naphthol-AS-BI- β -D-glucuronic acid substrate. **D**, Acid phosphatase with α -naphthyl phosphate substrate. The *numbers* refer to the antigens demonstrating enzymic activity with the exception of antigen 4 that absorbed the product of the enzyme reaction caused by its lipoprotein nature rather than any enzyme activity per se. Esterase activity was also detected (*a* and *b*) that could not be ascribed to any antigen defined by CIE. It is not clear whether faint esterase activity detected with antigens marked *c* and *d* is nonspecific or specific and is thus not described in the text.

carbohydrate-specific enzymes. Extract AF A, unlike any of the other extracts, contained a trypsinlike enzyme.

DISCUSSION

We have studied the allergenic composition of extracts prepared from whole mites, CUT, and SGM devoid of whole mites by use of both RAST and CRIE techniques. RAST studies demonstrated that extracts of CSL HDM, SGM, and CUT were allergenic and that the CSL HDM extract was approximately three-fold more potent than either CSL CUT or SGM extracts. However, when discs were optimally coated with any of the extracts, the percentage radiation

bound obtained with each type of disc was similar. Similar results have been reported with whole culture, mite body, and mite-excretion fractions.¹⁹ CIE and CRIE studies demonstrated that the mite extracts each contained several allergens. Nine allergens, designated Dpt 1, 3, 4, 7, 8, 12, 17, 22, and 31 were found in CSL HDM, and five allergens designated Dpt 4, 8, 12, 17, and 22 were found in extracts of both CSL CUT and SGM. It is not known whether the CUTs per se are allergenic, but it is unlikely. It is possible that the observed allergenicity is due to the fecal particles nonspecifically attached to the CUT, since such particles were observed microscopically (data not presented).

TABLE V. Enzyme content or mite extracts from different sources

Enzyme	CSL HDM	CSL UGM	B HDM	B UGM	AF A	AF B	AF C	Saline
Alkaline phosphatase	5	0	3	0	tr	2	1	0
Esterase	2	0	2	0	2	2	2	0
Esterase Lipase	5	0	3	tr	1	5	1	0
Lipase	0	0	tr	tr	0	0	0	0
Leucine aminopeptidase	5	0	5	0	1	4	1	tr
Valine aminopeptidase	1	1	2	0	0	1	0	0
Cystine aminopeptidase	tr	0	tr	0	0	0	0	0
Trypsin	0	0	0	0	2	0	0	0
Chymotrypsin	0	0	0	0	0	0	0	0
Acid phosphatase	5	1	5	tr	5	5	3	1
Phosphoamidase	5	1	5	tr	2	5	2	0
α -Galactosidase	3	0	4	0	0	1	1	0
β -Galactosidase	5	0	5	0	1	3	2	0
β -Glucuronidase	5	0	4	0	0	1	1	0
α -Glucosidase	1	0	tr	0	0	0	0	0
β -Glucosidase	5	0	5	0	0	0	5	0
β -N-Acetylglucosaminidase	5	tr	5	0	0	1	1	0
α -Mannosidase	4	0	2	0	2	2	0	0
α -Fucosidase	3	0	tr	0	0	1	0	0

AF = mite extracts provided by Ms. Annette Ford; 5 = >40 nanomoles of hydrolyzed substrate; 4 = >20 <40 nanomoles of hydrolyzed substrate; 3 = 20 nanomoles of hydrolyzed substrate; 2 = >5 <20 nanomoles of hydrolyzed substrate; 1 = 5 nanomoles of hydrolyzed substrate; tr = >0 <5 nanomoles of hydrolyzed substrate. Each extract was analyzed at a concentration of 1 mg of dry weight per milliliter of saline.

Detailed CRIE studies, similar to those described previously with mites^{2,3} and other allergens²⁰ in which a large panel of sera from atopic patients is examined, were not undertaken, and thus, the results reported here represent the mean response of individual sera comprising the pool.⁸ It is therefore possible that in the use of a mite-allergic serum pool, some minor allergens may not have been identified. Thus, on the basis of our data, the status, whether major or minor, of individual allergens cannot be deduced, although previous studies from our laboratory demonstrated that Dpt 12 was a major allergen.²¹ However, by use of the criterion of the intensity of radiostaining, the data suggest that, in addition to Dpt 12, Dpt 8 and 22 may represent major allergens. These allergens are now being isolated to test this hypothesis.

We have not performed immunoabsorption studies with our anti-HDM antiserum to determine whether the CUT and SGM extracts contained mite allergens/antigens not present in the HDM extract. It is possible, but considered unlikely, since the antiserum was raised against HDM, including feces, that such allergens exist. What is more feasible, however, is the possibility that the anti-HDM antiserum used failed to recognize every mite component in any of the extracts, thereby increasing the chance of missing potential allergens. Such a conclusion is supported by

our failure to correlate CIE precipitin bands with either alkaline phosphatase or β -N-acetylglucosaminidase. Herein lies one of the major drawbacks to the technique of CIE, since it is totally dependent on the antiserum used. Differences in antibody spectrum probably explains the varied CIE results obtained by different groups (including ours) with regard to the number of allergens and antigens detected, problems that have been previously discussed.^{2,3} For example, although we detected nine allergens out of a total of 36 antigens, other groups detected 11 out of 51,³ 18 out of 27, and 19 out of 29,² respectively.

Thirty-six antigens and nine allergens were detected in extracts of HDM, whereas only 15 antigens and five allergens were detected in the CUT and SGM extracts. Assuming that the allergens and antigens remain stable on storage, these findings, together with those obtained by CRIE and enzymic analysis, indicate that perhaps not all allergens are of an excretory/secretory origin but that some, for example, Dpt 1, 3, 4, 7, and 31, are derived from the whole mite per se rather than from fecal particles such as Dpt 12.⁵ Similar conclusions were drawn from CIE studies of *D. farinae* and *Tyrophagus putrescentiae*.²²

Lind et al.²³ have also examined different types of HDM preparations such as whole culture, mite feces, and mite body extracts to determine their suitability

in immunotherapeutic procedures. They demonstrated that the whole culture (similar but not analogous to the SGM used here since our preparation was deficient in mite bodies and CUTs) and the mite body preparation (analogous to our HDM) were demonstrated to contain four major allergens designated Ag 42, Ag X, Ag Y, and Ag 23. Ag 42 has been demonstrated to be analogous to Dpt 12 and P₁,⁴ and on the basis of electrophoretic mobility, it is probable that Ag Y, Ag X, and Ag 23 correspond, respectively, with Dpt 22, 17, and 8 described in this study.

Lind et al.²³ demonstrated that Ag X, Y, and 23 were present in increased concentration in the whole mite body extract relative to that observed in the whole mite culture in contrast to that observed with Ag 42. In our study the relative amounts of Dpt 12 and 17 were similar in both types of extract, but Dpt 22 was present in relatively larger amounts in the HDM extract than in the SGM extract. The data obtained here for Dpt 12 are in accord with radioimmunoassay data obtained previously.²¹

Both RAST inhibition, direct RAST inhibition, and CRIE studies indicated that the HDM extract was the most potent on a weight basis. In this study there appeared to be an empirical correlation between the RAST-inhibition titer and the amount of Dpt 12 detected in CIE analyses of different mite extracts that contrasts with the inverse empirical correlation observed in other studies.²³ Previous studies from this laboratory demonstrated there was a high correlation between RAST titer and Dpt 12 concentration when extracts containing a very large ratio of Dpt 12 to protein concentration were excluded from the analysis.²⁴ Such findings might explain those reported,³ particularly in view of the inclusion of the acetone precipitated mite culture extract in such an analysis, since the relative concentration of Ag 42 was increased severalfold above that detected in either extracts of whole culture or whole mite body and, in addition, was deficient in other major allergens. These data support the concept that mite extracts contain major allergens other than Dpt 12.

With a high level of IgE, but mite negative control serum, faint radiostaining was observed with antigens 4, 5, and 12. However, this radiostaining was always fainter than that observed with mite-allergic sera. Whether such radiostaining with this serum infers allergenicity or represents an artifact is presently unknown. Several explanations have been offered for such observations, including coprecipitation and protein-protein interactions between nonallergenic and allergenic molecules together with the presence of low levels of IgE antibodies in asymptomatic serum donors.²

With regard to Dpt 4, other studies have also dem-

onstrated radiostaining with allergens that appear to be analogous on the basis of electrophoretic mobility and CIE characteristics. In one study the authors demonstrated radiostaining with their antigen number 2 but were unable to categorically exclude it from being an allergen.² In contrast to these observations, other workers^{3, 25} have concluded that their antigens (antigen numbers 36 and Ag 1, respectively) represented a major allergen in that more than 80% of patients tested produced CRIE radiostaining, and, in addition, the purified allergen was demonstrated by other techniques to be clinically important.³ The reasons for these discrepant results are not yet clear.

The CSL HDM, CUT, and SGM extracts were analyzed for enzymic activity by use of the Api-Zym system, a system that has been used to study other allergenic extracts.²⁶⁻²⁹ Our studies demonstrated that the mite extracts contained several different types of enzymes, including phosphatases, esterases, aminopeptidases, and glycosidases. Both the CSL CUT and SGM extracts contained similar amounts of esterases, phosphatases, and aminopeptidases as did the whole mite extract but contained much less glycosidase activity. In general, the glycosidases are of lysosomal origin, and it is therefore perhaps not surprising to find them in association with whole mite extracts rather than with extracts rich in mite excretory/secretory products. A comparison of these data with those reported for *D. farinae*²⁷ indicated that both species of mites contained a similar spectrum of enzymes with the exception of β -glucuronidase, α -galactosidase, β -N-acetylglucosaminidase, and α -fucosidase that were missing from *D. farinae* and trypsin that was missing from *D. pteronyssinus*.

Attempts were made to correlate some of the predominant enzymic activities detected by the Api-Zym system with precipitin bands detected by CIE analysis of the whole mite extract to determine whether such enzymes were allergenic. Antigenic correlations were obtained with esterase (Dpt 6, 13, and 24), β -glucuronidase (Dpt 27), and acid phosphatase (Dpt 14), but none of these enzymes were found to be allergenic as judged by CRIE. However, such studies do not preclude allergenicity, since it is possible that they represent minor allergens that were not detected because of the use of a mite-allergic serum pool.

Physicochemical studies demonstrated that esterase was heterogeneous both antigenically and in terms of molecular weight and charge (data not presented), and the enzyme is thus similar in these respects to that found in mammalian tissues.¹² Acid phosphatase was also demonstrated to be heterogeneous. The finding of relatively large amounts of these two enzymes together with leucine aminopeptidase in SGM, a source rich in fecal particles, suggests that they are derived from

the gastrointestinal tract of the mite. This is consistent with data obtained from the study of equivalent mammalian enzymes.¹²

The enzyme content of various whole mite extracts obtained from different sources was examined. These studies demonstrated that all five extracts examined contained phosphatases, esterases, and aminopeptidases in varying amounts, but some extracts were deficient in certain glycosidases such as α - and β -glucosidase. Only one of the five extracts contained a trypsinlike enzyme. The presence of proteases or peptidases such as trypsin and leucine aminopeptidase may be one of several reasons why extracts deteriorate on storage. Recent studies²⁹ demonstrated that the incorporation of glycerol had very little effect on such enzymes, suggesting that extracts require protease inhibitors in addition to glycerol to ensure stability.

In conclusion, we have demonstrated that whole mite extracts contain several allergens, some of which are found in SGM devoid of whole mites. The data suggest that some allergens are derived from the mite per se, whereas others are found in secretions and/or excretions. In this regard it was demonstrated that the SGM represents a source suitable for the isolation and characterization of the mite allergens Dpt 4, 8, 12, 17, and 22. Certain of the mite components were demonstrated to possess enzymic activity, but, thus far, none of the enzymes studied were demonstrated to be allergenic.

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Kinetics of allergen release from house dust mite *Dermatophagoides pteronyssinus*

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*Purified bodies of Dermatophagoides pteronyssinus were extracted over different extraction times. The release kinetics of proteins, carbohydrates, and allergens were assessed by different methods. Additional attempts have been made to elucidate the extraction process also on a molecular basis. For this purpose the extracts were analyzed by isoelectric focusing, SDS gel electrophoresis, crossed radioimmunoelectrophoresis, and an immunoprint technique. Carbohydrates, proteins, and allergens were released immediately from the mite bodies but demonstrated different release kinetics. The release of carbohydrates and proteins was linear up to 48 hours but demonstrated different slopes. The major allergen P₁ demonstrated steady release up to 48 hours, whereas the overall allergenic activity as determined by RAST inhibition demonstrated typical saturation kinetics reaching a plateau after 4 to 5 hours of extraction. It was demonstrated by parallel-line assay of the RAST-inhibition curves that the allergenic determinants remained unchanged up to 48 hours. Different electrophoretic methods demonstrated that all relevant proteins and allergens were released immediately from the mite bodies. The electrophoretic patterns changed only slightly up to 4 hours. Prolonged extraction times up to 48 hours did not affect the patterns. This makes a loss of proteins or allergens by degradation unlikely. (*J ALLERGY CLIN IMMUNOL* 77:24-31, 1986.)*

The importance of the mite species *Dermatophagoides pteronyssinus* as a potent source of allergens in house dust is well established since the fundamental work of Voorhorst et al.¹ Great advances in the development of immunoelectrophoretic techniques for characterizing allergens² made it possible to elucidate physicochemical and allergenic characteristics of mite antigens. In addition, the application of modern pro-

Abbreviations used

C ₅₀ :	50% RAST inhibition
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
IEF:	Isoelectric focusing
IP:	Isoelectric point
kD:	Kilo Dalton
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
RI:	RAST inhibition

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tein separation techniques has led to an increased knowledge about the nature of major allergenic components in mite extracts.³⁻⁵