

Allergenic activity of soluble fractions of *Dermatophagoides farinae*

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D. farinae mite fluids were separated by polyacrylamide gel electrophoresis. Four prominent bands were selected from stained gels, and corresponding gel regions eluted from unstained gels. Eluates and controls were used to scratch test 17 patients, all of whom had demonstrated positive wheal-flare immediate responses to house dust and *D. farinae* extracts, but negative to other known, non-mite dust components. Intensity and number of patient reactions varied with the eluates. Our data suggest that there may be as few as 2, and as many as 5, allergens in or on *D. farinae* mite, and capable of producing immediate wheal-flare responses in humans.

Allergenic substances from house dust are associated with dust mites as shown by the work of Kern,¹ Cooke,² and van Leeuwen.³ More recently, investigators⁴⁻¹² have used skin testing to study whole mite extracts and fractions or extracts isolated by Sephadex column chromatography.⁹⁻¹³ The present work was an attempt to identify the major allergens of *D. farinae*, using mite fractions as separated by polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Dust mites were collected* and *D. farinae* mites separated from vacuum cleaner bag material. Laboratory colonies of mites were maintained at 28° C. and 80 percent relative humidity. The rearing medium was a dry mixture of Knox gelatin crystals (40 parts), Fleischmann's active dry yeast (4 parts), and the fine particles of Gaines-burger dog food that had passed through a 325 mesh sieve (one part). After the culture was mature, it was placed in a warm detergent solution (Joy, Tween 20) to liquefy the medium and suspend particulate matter. Mites were isolated by passing the solution through the 325 mesh sieve; those retained on the sieve were washed with tap water and then air-dried at 20° C. on Whatman No. 1 filter paper.

Body fluid was extracted after grinding mites in a Virtis 45 blender, 0.2 ml. of 70 percent sucrose solution being added to the grinding vial. After blending, the mixture was centrifuged for 15 minutes at 10,000 r.p.m. to remove exoskeleton debris.†

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Received for publication July 9, 1973.

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*Detailed methodology may be found in: Shamiyeh, N. B., et al.: Isolation of mites from house dust, *J. Econ. Entomol.* 64: 53-55, 1971.

†Promptly after grinding, centrifuging, and decanting, a small precipitate developed that, on examination, proved to consist of exoskeleton and mycelium. *Penicillium* grew promptly upon transfer to appropriate culture plates, held at 20° C.

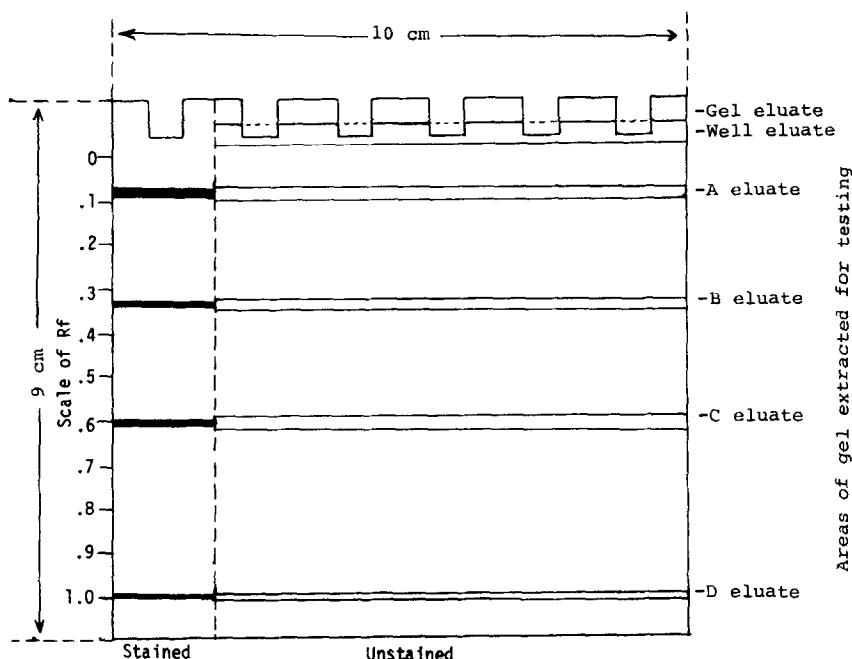


Fig. 1. Areas of ORTEC electrophoretic separation of soluble fractions of *D. farinae* selected for elution and skin testing (on the right) and Rf scale (on the left).

For electrophoretic separations the body fluids of 0.1 Gm. mites (wet weight) were used. This allowed for a test sample volume (in 70 percent sucrose) of 20 μ L, of which some 5-8 μ L was mite material. Electrophoresis was by the ORTEC slab, polyacrylamide gel, pulse-power system.* The gel slab was composed of 8 per cent polyacrylamide at the well and bottom layers, 6 per cent in the middle layer, and 4½ per cent in the top layer. pH was maintained at 9.0 and voltage at 325 throughout the experiment. Pulse power rate was regulated to produce desired amperage. At the beginning of the run, pulse was set for 75 per second, and 46 mA obtained; at 5 minutes the pulse rate was stepped up to 150 per second, and 61 mA obtained; at 10 minutes the rate was adjusted to 22 per second and 63 mA obtained; at 15 minutes the rate was adjusted to 300 per second, and 59 mA obtained and held constant for the final 5 minutes of the run. The gel slab was then removed, and the longitudinal column under one of the wells cut free and stained with amido-Schwartz, while the rest of the slab was temporarily frozen. Of 17 stained bands revealed by this procedure, the 4 most dense were selected for further study (Fig. 1). Parallel strips 2 mm. wide and centered on the 4 bands chosen were sliced from the freshly thawed unstained part of the gel. Proteins were eluted from these strips with 0.9 per cent saline and excess water and salt removed using dialysis tubing and Aquacide powder. Thus concentrated, eluates were frozen at -20° C. until used for scratch testing. During the week required to test 17 subjects, the eluates were kept at 4° C. and not refrozen. Subjects tested were East Tennessee residents known (from prior scratch test screening) to be wheal-flare responders to *D. farinae*-rich dust extract from our own laboratory and to commercial house dust extract (Greer Laboratory, Lenoir, N. C.), but negative to Endo dust extract and to as many of the mite-rich dust

*Instruction Manual, 4200 Electrophoresis System, ORTEC, Inc., 100 Midland Rd., Oak Ridge, Tenn. The pulse-power system provides small pulses of current flow separated by cooling periods to keep the temperature reasonably low.

Table I. Results of scratch tests and related controls: Mite homogenate, well eluate, and moving band eluates

Test	Patient No.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Negative controls; scratch only									1								
Phenol saline; 50% glycerine																	
Sucrose 35%; 50% glycerine						1											
Aquacide supernate; 50% glycerine														1	1		
Unused gel eluate; 50% glycerine														2	1		
Positive controls; 50% glycerinated histamine 50 (mg. %)	3	3	4	2	4	3	3	4	4	3	3	2	3	4	4	3	2
<i>D. farinac</i> -rich house dust extract	2	2	2		2		3	3	2	3		3	3	3	2		
Mite homogenate					2				1	3			3	2	2		
Eluates concentrated; 50% glycerinated																	
Well (origin) eluate								1						2			
Eluate A								1									
Eluate B		2										2					
Eluate C	2			2	2		2	3		2		2	3	3	4	2	
Eluate D				1		1		2		2		2	2	2	3	2	2

Test result rated zero (blank in table) to 4+, patient 15 eluate C as to size of wheal-flare development in the skin (back or volar forearm) read at 10 to 15 minutes when the histamine-positive control had reached near-maximal size.

extract contaminants as we* could identify. (These included *Penicillium*, *Alternaria*, corn smut, pine pollen, cedar pollen.) Scratch tests were read as 0 to 4+ wheal-flare. The results, using the 4 band eluates, the whole extract, and appropriate controls, appear in Table I.

RESULTS

Histamine control produced wheals varying from 2+ to 4+ in relative size. Patient 9 gave a 1+ false positive scratch blank response; no other patient responded to the plain scratch ($\frac{1}{2}$ inch, made with tip of short bevel No. 20 needle, and no blood drawn). Three other patients (6, 14, and 15) gave a 1+ response to the 70 per cent sucrose alone. Five of the patients failed to respond to the same dust-rich mite extract to which they had responded earlier in the year; 4 of these, plus 6 other patients, failed to respond to the fresh mite homogenate. Only one, Patient 11, failed to respond to all mite fractions or mixtures.

Eluate A produced a response in only one, Patient 8. Eluate B produced somewhat stronger responses, but in only 2 patients (2, 12). Eluate C produced the more intense responses, and in 11 patients (of whom one did not appear to respond to eluate B). Eluate D produced responses in a final group of 10 patients, and seemed somewhat less potent than eluate C. However, 4 patients showed differing responses to eluates C and D; patient 17 responded only to D, and 1 and 7 responded only to C. The responses in all cases were 2+.

*This identification was greatly helped by the making of coverslip "wet mounts" examined at 400 $\times$, of non-mite dust debris remaining on the 325 mesh sieve. Identification of pollens and molds was by Dr. and Mrs. Burton Rudolph.

DISCUSSION

Elution and concentration of the bands by the method described gave us fairly potent fractions for testing. Eluate C, for example, produced a 4+ response in Patient 15, when the homogenate itself produced only a 2+ response in the same patient. But obviously the whole story is not revealed by looking only at the 4 fractions selected. There are at least 13 other bands recognizable from *D. farinae* body fluids handled in this way, but we did not test their activity. The method limited us to the use of immediate wheal-flare responses from skin surface testing, and precluded use of any but crude semiquantitative parameters. Any extremely high molecular weight or positively charged carrier molecules can be assumed not to have been retrieved for study by this particular method. Allergens present in any of 13 additional areas where staining revealed precipitated protein but too faint to suggest major quantity would likewise have been missed. By the same token, allergens in unstained gel sections would not have been revealed by this method.

Whereas different potencies of a single antigen (allergen) having different mobilities in this system might account for some of these results (as best seen with Patient 12, for example), we think it likely that our small mixed human population of dust mite-allergic patients has recognized antigenic differences between eluates C and D. The subgroup comprised of Patients 1, 5, and 7 appears not to recognize eluate D in an atopic way, but does respond to eluate C. The last patient recognizes eluate D but not eluate C. Patient 2 is also an enigma in this series, since unique in responding to eluate B. If one discards the results represented by these two exceptional patients, and by Patient 6, the remaining good evidence suggests that there is a single allergen having two mobilities. Statistically, one discards isolated, unique bits of data with impunity, perhaps. Doing this, we can easily reduce our data to the probability that a single allergen, having differing carrier molecules and also differing concentrations along the gel, has accounted for the greatest number of observations. However, in the case of Patients 8 and 14, eluates made from the well region after electrophoresis produced responses suggesting that an allergen had remained at the origin. This would have occurred in the case of insoluble or uncharged allergenic complexes. If allergenically identical to the moving bands, why did not more patients respond? In this case, it seems unreasonable to postulate a single allergen with three separate mobilities and/or affinities for carrier molecules. This leaves us with indirect evidence of at least two allergens. As to the possibility of data contamination from the non-mite, *Penicillium* allergens, we are assured: the subjects were pre-selected to exclude mold-allergic patients. (Indeed, one, Patient 17, responded to nothing commercially available in our office, and only to *D. farinae* mite. Several other subjects, including her brother, tested much later with the same battery, also appear to ignore non-mite allergens.) It would seem that as few as 2, and as many as 5 allergens have been indirectly demonstrated in the mite we have studied. If none of the data be discarded, then one must invoke 5 allergens as the maximum possible number. By no reasonable stretch of our imagination can we rule out the minimal number of two separate allergens. It is entirely possible that all bands have separate allergenicity, and that a fifth separate allergen remained in the gel at the well

region. We have no direct data to indicate this is actually the case, however, from our protocol described.

CONCLUSIONS

Extension of this method is indicated. More mite material would permit split samples and use of more gel strips (17 bands and 16 clear strips). One might well raise precipitating antisera in appropriate animals, such that Ouchterlony gel double-diffusion experiments might be carried out to reveal antigenic differences. At the same time, allergens not retrieved by our protocol as reported would be available for patient testing. A much larger patient series would be required to show statistical significance.

Finally, with larger quantities of mite fractions, quantitative measurements of specific activity could be made. Intradermal tests with known quantities offer the possibility to titrate to those dilutions best suited for distinguishing true antigenic differences in a series such as this. Further, with skin test readings made sequentially at 15 minutes, 6 hours, 24, 48, and 72 hours, one might employ even types III and IV immune responses in a selected patient population, by which a definitive look could be had at the differential allergenicity of soluble fractions of the common floor mite of North America.

We wish to thank Mr. Nabil Shamiyeh for his continued help with the house dust mite project, Mrs. Roenella Hornsby, R.N., who performed all skin testing and recording, Merrill Chase, Ph.D., who reviewed and commented upon the manuscript, and Margaret Lyon, who performed the clinical research coordinating in the allergy office.

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