

Antigenic components of house dust allergens

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Antigenic analysis of house dust extracts chosen for their diversities of origin shows that all have at least three antigens which give precipitin bands with antibodies of their homologous antisera. One of these precipitin bands is always more prominent than the other, regardless of the source. While a single major and two minor components appear to be common to all house dust extracts, the specific composition and possibly the concentration of the reactants in each of these components tend to vary in different dust extracts.

House dust is a ubiquitous, perennial, and extremely potent allergen. One hypothesis concerning the nature and origin of the dust allergen is that the allergen is the product of the action of microorganisms upon vegetable fibers or other substances. Innumerable other materials have also been implicated. The first published recognition of the fact that house dust "acts as antigenic substances producing a true protein sensitization" was that of Kern,¹ in 1921. He produced skin reactions in an asthmatic patient with an extract made from the dusty contents of the latter's cotton-filled mattress. In this early report, he stated that dust sensitization is the most common single cause of the allergic types of asthma seen in adults. Subsequent investigators have confirmed this hypothesis.

Shortly after Kern's discovery, Cooke,² in 1922, pointed out that in house dust there is a specific factor not the sum of its parts, and that the dust allergen may be found in nearly equal potency in house dust extracts from all parts of the world. Since then, the nature of the dust allergen has stimulated the interest of many investigators. Most of the subsequent studies have served to emphasize the importance of two discoveries: (1) that house dust is one of the most common inhalant causes of asthma, and (2) that it contains its own specific allergen.

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After the studies of Cooke, almost two decades passed before the chemical nature of house dust extracts was examined. Rimington and co-workers,³ in 1947, concluded that house dust is a combination of polysaccharides (mainly hexoses) and polypeptides. They hypothesized that the allergic nature of the house dust is due to both the carbohydrate and protein fractions. Vannier and Campbell,⁴ in 1959, determined the chemical composition of four house dust extracts, one of which was Endo house dust, a commercial house dust extract. The materials from all four extracts were found to consist of a heterogeneous mixture of acidic polysaccharides (85 to 88 per cent) with varying amounts of polypeptides (12 to 15 per cent).

While it is apparent that the chemical composition and active substance(s) responsible for the antigenicity of the house dust allergen have been well studied, comparatively little work has been done to determine the relationships among house dust extracts by serological methods. A notable exception was the excellent study of Wodehouse,⁵ who studied house dust extracts and specific antisera from several sources and pointed out several interesting relationships. Because a great deal of heterogeneity exists in the content of dust allergen in dust samples obtained from different sources, the present investigation was initiated to determine if a relationship could be established among house dust allergens obtained from different homes within a restricted geographical area with the use of serological methods. The antigenic spectra of house dust extracts from a number of different households in the metropolitan area of Phoenix, Arizona, were compared not only with each other, but also with a commercial house dust preparation. In this study, an attempt has been made to determine the qualitative relationship among house dust preparations when these extracts were tested in both homologous and heterologous systems. In addition, the serological relationships between these autogenous house dusts and a commercial house dust preparation have been investigated. These studies were made with the immuno-double-diffusion gel agar technique of Ouchterlony,⁶ as modified by Wilson and Pringle⁷ and Feinberg.^{8,9}

METHODS

Preparation of house dust allergens

Autogenous house dust extracts were made from house dust samples collected from selected locations throughout the metropolitan Phoenix area. Dust samples were collected in vacuum cleaner bags from floors, divans, beds, draperies, and other areas within cooperating homes arbitrarily selected to represent east, west, south, and central Phoenix. Extracts were prepared with Coca's solution. Following initial filtration, concentration by pervaporation, and sterile filtration with a 0.45 micron (HA) Millipore filter, an aliquot of each extract was mixed with an equal quantity of Freund's adjuvant.¹⁰ Specific antisera were prepared by intramuscular inoculation of the extract-adjuvant mixture into mature white rabbits, weighing approximately 6 to 7 pounds. Inoculations of 1.0 ml. were given at weekly intervals for a six-week period. Blood samples were collected by cardiac puncture, placed in conical centrifuge tubes, allowed to clot at room temperature for approximately six hours, and centrifuged at 3,000 r.p.m. for

fifteen minutes. The serum was collected and preserved in 1:10,000 Merthiolate. A minimum of three rabbits was used for each dust extract and the sera were pooled prior to analysis.

Immunodiffusion analysis

Special flat-bottomed heavy-walled glass Petri dishes, 6 cm. in diameter, were used for the immunodiffusion analysis. Ionagar No. 2* was used in a 0.2 per cent concentration to coat the inner surfaces of the diffusion plates to prevent the underrunning of the reactants. Eight milliliters of a 1 per cent suspension of Ionagar prepared in saline containing 1 to 10,000 concentration of Merthiolate was then added to each plate and allowed to gel.

Although several Feinberg agar gel cutters* were evaluated during the course of this investigation, the five-well concentric pattern appeared to give the most consistent results. Serial dilutions of the allergen extracts were made in Coca's solution. For the homologous systems, the undiluted antigen, as well as dilutions 1 to 2, 1 to 4, 1 to 8, and 1 to 16, were placed in the peripheral wells of the five-well concentric patterns. For the heterospecific systems, the peripheral wells of the five-well concentric pattern were filled with the undiluted heterologous allergens, using Coca's solution as a control. In all experiments, undiluted antiserum was placed in the central wells. The plates were incubated at room temperature ($26 \pm 2^\circ\text{C}.$) and observed daily until the bands of precipitate appeared.

Table 1. *The number of precipitating fractions observed in gel diffusion for each of the homologous allergen-antiallergen systems*

<i>Antigen-antibody system</i>	<i>Number of fractions observed with homologous antiserum at 48 hours</i>		
	<i>Major fractions</i>	<i>Minor fractions</i>	<i>Total fractions</i>
West Phoenix house dust extract	1	2	3
South Phoenix house dust extract	1	2	3
Central Phoenix house dust extract	1	2	3
East Phoenix house dust extract	1	2	3
Endo house dust extract	1	2	3

RESULTS

Although house dust is reported to be highly antigenic, strongly reacting antisera were prepared to certain of the extracts only after repeated failures. When tested, the homologous allergen-antiallergen systems did not reveal a visible precipitate in most cases until the end of a 48 hour period. The intensity of the bands of precipitate were classified as 4+ (sharp, well defined), 3+ (clear, but somewhat diffused), 2+ (faint, but clear), and 1+ (faint, but diffused.)

In all of the homologous house dust allergen-rabbit antiserum systems tested, including the Endo dust preparations, three precipitin bands were observed

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Table II. *The number of precipitating fractions shared by the heterospecific allergen-antiallergen systems as measured by gel diffusion*

<i>Rabbit antiserum</i>	<i>House dust allergen</i>				
	<i>WPHD</i>	<i>SPHD</i>	<i>CPHD</i>	<i>EPHD</i>	<i>EHD</i>
Anti-west Phoenix house dust (WPHD)		1	1	1	1
Anti-south Phoenix house dust (SPHD)	1		1	1	1
Anti-central Phoenix house dust (CPHD)	1	1		1	1
Anti-east Phoenix house dust (EPHD)	1	1	1		1
Anti-Endo house dust (EHD)	1	1	1	1	

with the five-well concentric pattern, one of 4+ intensity and two of 2+ intensity. These findings indicate that all of the dust extracts tested, irrespective of their source, consisted of a major and two minor antigen-antibody systems. However, when each of the house dust allergen extracts was tested against heterospecific antisera prepared against extracts made from the other homes as well as the commercial dust preparation, a single band of precipitate of 1+ intensity developed after 96 hours of incubation. The results of these tests are shown in Tables I and II.

DISCUSSION

The introduction of the immuno-double-diffusion gel agar technique by Ouchterlony⁶ has provided the necessary means for studying the antigenic relationships of complicated biological extracts, such as house dust. An antigenic analysis of the various house dust extracts tested shows that all have at least three antigens which produce precipitin bands with their homologous antisera. One of these bands is generally much more prominent than the others, indicating that these dust extracts all have one major antigen and two or more minor antigens. This is in accord with the findings of Wodehouse,⁵ who has reported "these dust preparations all have one major antigen and two or more minor antigens." In the former study, however, antisera to the dust preparations tested were from more than one source, including rabbits and humans, and also included sera from persons with known sensitivities to cat and rabbit danders, as well as to feathers. Rabbit antisera was prepared against two of the dust preparations. All of the dust preparations were then tested to determine their relationship to these two antisera and to the antisera from sensitive human subjects.

The most significant finding of the present investigation is not that the extracts used have in common a typical antigenic spectrum under the conditions utilized for analysis, but that when each of the antisera was tested against the heterologous antigens, i.e., house dust preparations from other sources, only *one* band of precipitate was found. The finding of a single antigen in common between each of the extracts would indicate that the major antigen noted in the homologous systems is common to all house dust extracts, in a concentration sufficient to elicit antibody formation. However, the minor components may vary in composition and/or concentration to the extent that they cannot be readily measured by precipitation in gels. If the major and minor components recognized in each of the different house dust extracts consisted of *identical* major and

minor antigens or were in approximately the same concentrations, the hetero-specific systems should have yielded essentially similar results, i.e., one major and two minor antigens. While the antiserum prepared against commercial house dust extracts has the typical major and minor reacting homologous antigen-antibody systems in gel diffusion, this preparation also had a similar precipitin pattern, i.e., one precipitin arc, when tested with the other house dust antigens. These findings in the *in vitro* system indicate that, whereas all house dust extracts apparently contain a typical antigenic spectrum in terms of visible bands of precipitate, the composition of each zone of precipitate may vary significantly from the others and may represent similar antigenic "species" but in different concentrations. If a similar relationship exists between the antigens responsible for the production of skin sensitizing antibody and the antigens measured here by gel diffusion techniques, it may then be fruitful to investigate further the relationship between the *concentration* of the antigens found in autogenous dust preparations, as opposed to commercial dust preparations, using the passive transfer neutralization technique.

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