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ANTIGENIC RELATIONSHIPS IN ATOPY

II. *Cross-Reactions of Anti House Dust Sera with Inhalant Glycoprotein Allergens from Different Sources*

By

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INTRODUCTION

Antisera raised in animals against whole or against purified allergen extracts have been used by several investigators for studying the composition of such extracts or for the assessment of the immunological and chemical homogeneity of isolated allergen fractions (1, 4, 14, 15, 18, 20, 22, 23, 27). While these immunochemical data may be useful in guiding the isolation procedure of atopic allergens and for studying their antigenicity in rabbits, the use of antisera against allergen preparations for investigating the antigenic relationship among different atopic allergens has found only limited application (16, 21, 27). Yet, an immunochemical approach to cross-reactivity in allergy might well lead to a better understanding of the multiple sensitivity encountered in the majority of atopic patients.

The observation that positive skin tests to extracts of house dust, old kapok, aged cotton linters and old chicken feathers often obtain simultaneously in many atopic individuals led *Wagner & Rackemann* (26) to study the immunological relationship between these extracts. By cross-neutralization of passively sensitized and „desensitized“ skin sites in PK-tests, and by in vitro neutralisation of reaginic sera they arrived

at the conclusion that kapok, house dust and feathers do not contain any common principle; however, their results did not justify the conclusion, because the experiments lacked a quantitative basis (allergen concentrations, serum dilutions).

Coulson & Stevens (13), by anaphylaxis in guinea pigs sensitized to several house dust extracts or to cotton linters extract, demonstrated common antigens in cotton linters, egg white, and all samples of house dust; in a few cases crossed reactions to wool antigens were also observed. *Wodehouse* (27), working with rabbit antisera to crude house dust extracts, described cross-precipitation during gel-diffusion experiments with dandruff extracts of several animal species (cat, dog, cattle, horse, rabbit); also, precipitation occasionally occurred of the anti house dust serum with human reaginic sera, which led *Wodehouse* to propose that house dust must contain material of human origin. In confirmation, we have recently described the presence of (denatured) human serum albumin in extracts of house dust and of human dandruff (10, 23), together with γ A and γ G globulin (4, 24) and traces of 2.5 S α_2 -glycoproteins (4).

Having obtained antisera to a purified and specific allergen fraction from house dust (9, 23) and having studied their interaction with homologous and heterologous antigens (23), it was considered of some significance to apply the antisera further to supplement previous studies (5) on the antigenic relationship among different allergens from various sources.

MATERIALS AND METHODS

Antisera against purified house dust allergen fraction E, and against the less active fraction D, were raised in rabbits as before (23). The precipitation reactions of these antisera with homologous and heterologous dust samples have been extensively covered elsewhere (23). Analytical techniques involved gel-diffusion experiments according to *Ouchterlony* and immuno-electrophoresis in agar gel at pH 8.2. The immune sera were used undiluted; 5 per cent solutions of the antigens in saline or in veronal buffer pH 8.2 were used unless otherwise stated.

The allergens employed were all checked for skin-reactivity by intradermal assay in hypersensitive atopic individuals. The following allergens were used:

House dust allergen, fraction E, isolated from batches of house dust collected either at Liège (Belgium) or at Utrecht (Holland); preparations from both sources were immunochemically and analytically indistinguishable. Fraction D, precipitated at pH 3.1, was also employed (for isolations compare ref. 9 and 11).

Human dandruff allergen, fraction E (supernatant at pH 4.2) and fraction D (precipitated at pH 4.2) were both employed (for isolation and properties compare ref. 4 and 10).

Feather allergens (7). A dialyzed extract of aged feathers (mixed chicken and duck) from an old mattress was used (code: FE-A, analysis: 1.6 per cent N, 2.7 per cent bound hexoses, 4.7 per cent bound hexuronic acid) as well as the fraction precipitated from FE-A with $(\text{NH}_4)_2\text{SO}_4$ in the range of 35-80 per cent saturation (code: FE-C, analysis: 3.0 per cent hexose, 8.8 per cent uronic acid).

Cotton linter allergens. The fraction precipitated with $(\text{NH}_4)_2\text{SO}_4$ in the range of 35-80 per cent saturation, from a dialyzed extract of old (yellow) cotton fibres was used (CL-C); also, a fraction was isolated from a crude extract according to the procedure for house dust fraction E (9, 11); code: CL-E.

Kapok allergens. The fractions KP-C (35-80 per cent $(\text{NH}_4)_2\text{SO}_4$ -precipitate) and KP-E (supernatant at pH 2.2 from a solution of KP-C) were prepared as described in a separate paper (3). Analysis: 2.0 per cent N, 34.4 per cent bound hexoses, 4.4 per cent bound uronic acid (KP-C); 2.6 per cent N, 21.3 per cent hexoses, 4.3 per cent uronic acid (KP-E).

Hay allergens. A dialyzed extract of stored hay (HH-A, 1.9 per cent N, 8.4 per cent hexoses, 2.8 per cent uronic acid) and the 35-80 per cent $(\text{NH}_4)_2\text{SO}_4$ -precipitate prepared from it (HH-C, 3.8 per cent N, 14.6 per cent hexoses, 5.4 per cent uronic acid) were employed.

Wool fractions. Fractions D and E were isolated from the 35-80 per cent $(\text{NH}_4)_2\text{SO}_4$ -fraction precipitated from a dialyzed extract of old wool; these two preparations were separated according to the procedure outlined for the corresponding fractions of house dust (9, 11).

The synthesis of the compound N-(1-deoxy-1-D-fructosyl)- ϵ -amino-caproic acid (EACAF) has been described elsewhere (6).

RESULTS

a. *Precipitation with Human Components*

House dust extracts, even in advanced stages of purification, contain proteins of human origin. It has been reported (9, 11) that the isoelectric precipitation of a purified fraction allows the separation of two groups of substances, *viz.*: the D-fraction (precipitating at pH 3.1) and the E-fraction (re-

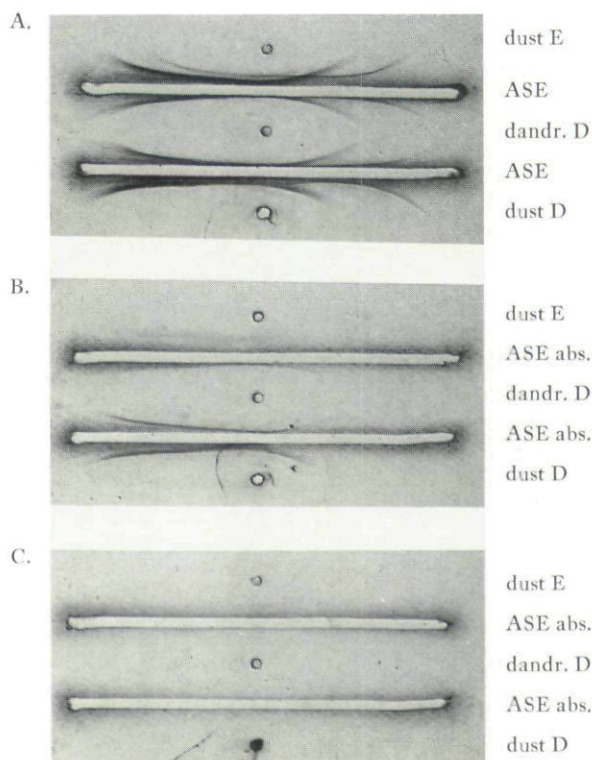


Fig. 1.

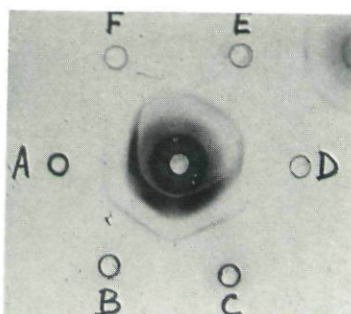
Immunoelectrophoresis in agar gel of fractions from house dust and human dandruff against rabbit antiserum (serum ASE 5) to house dust fraction E.

- A: (homologous) house dust fraction E (from dust collected at Utrecht), human dandruff fraction D and dust fraction D against undiluted ASE 5.
 B: Same antigen fractions against ASE 5 absorbed with dandruff fraction D (75 µg/0.3 ml serum).
 C: Same antigen fractions against ASE 5 absorbed with dandruff fraction D (200 µg/0.3 ml serum).

maining in solution and subsequently precipitated with acetone). Analysis shows the E-fraction to consist mainly of a heterogeneous and polydisperse mixture of plant glycopeptides (2, 9, 17), presumably of different origin, while the D-fraction encompasses precipitable (plant and animal) pro-

Fig. 2.

Ouchterlony plate of rabbit antiserum to house dust fraction D (from dust collected at Liège) diffused against dust antigen D (from dust collected at Utrecht), in A, B and C (1.5, 4.5 and 7.5 μ l respectively) and diffused against human dandruff fraction D (in wells D, E and F; 7.5, 4.5 and 1.5 μ l respectively). Antiserum (7.5 μ l) in centre well; time of diffusion 48 h.



teins, allergens from human dandruff (9), human serum albumin (9, 10, 23), γ A and γ G globulin (24). The latter products no doubt derive from an admixture of epithelial skin flakes in house dust, because extracts of dandruff were demonstrated to contain serum albumin, pre-albumin, α_2 -glycoproteins (other than α_2 -macroglobulin) and γ -globulins (4). The relationship between house dust and human dandruff is further illustrated in Fig. 1, which gives the immunoelectrophoretic patterns of dust fractions D and E and of dandruff fraction D against anti house dust (fraction E) serum absorbed with increasing amounts of dandruff fraction D.

It follows from Fig. 1 that precipitin formation with the D-fraction of house dust can be completely abolished by absorption of the antiserum with the D-fraction of dandruff. This is a somewhat surprising result because, chemically, the respective antigen mixtures are not strictly identical; except for some common serum protein contaminants the dandruff fraction D, constituting only a minor portion of the (glyco)-proteins in dust fraction D, shares no full antigens with fraction D of house dust (9, 10, 23). This is also illustrated by the immunodiffusion pattern (Fig. 2) of an anti house dust (fraction D) serum run against homologous dust fraction D and heterologous dandruff fraction D: although antigenic identity between individual components does exist, the antigen composition of the two preparations is dissimilar. Moreover, both fractions encompass compounds chemically distinct from those in the E-fraction of house dust, against which the anti-

serum (Fig. 1) was raised. Consequently, it may be inferred that the antiserum against house dust fraction E contains antibodies directed against one (or more) determinants shared by the different components in both D-fractions as well as by those in the E-fraction of house dust.

The occurrence of human serum proteins in house dust extracts and in extracts of human dandruff has given rise to the observation (4) that prolonged hyposensitization courses with these allergen preparations may not be without consequences to the recipient, especially because the physicochemical evidence points at a considerable degree of denaturation of the proteins in these extracts.

b. Interaction with Kapok and Cotton Allergens

Extracts of aged kapok and of old cotton fibres were chosen for studying their interaction with anti house dust sera because of the old controversy over the origin of the "house dust allergen". In fact, these materials are the most likely sources of the allergenic plant glycopeptides in dust fraction E, even though in the D-fraction protein allergens derived from animal sources (human, 9, 23, 24, possibly also from arthropods, 19) may occur besides precipitable plant proteins. *Coulson & Stevens* (13) have reported that an antigen common to house dust and cotton linters can usually be demonstrated. In accordance with these authors we have recently indicated that the antiserum against dust fraction E precipitates a similar fraction from brown cotton linters (9). Fig. 3 shows the cross-precipitation observed during the immunoelectrophoretic assay of such a preparation, CL-E, (isolated according to the procedure for house dust fraction E and not precipitable at pH 3.1) against the anti dust serum.

The preparation CL-E (physicochemically a very heterogeneous mixture of glycopeptides) consists of at least two groups of components related by common antigenic determinants (Fig. 3: end to end fusion of precipitin arcs).

Similarly, the anti house dust serum precipitated glycopro-

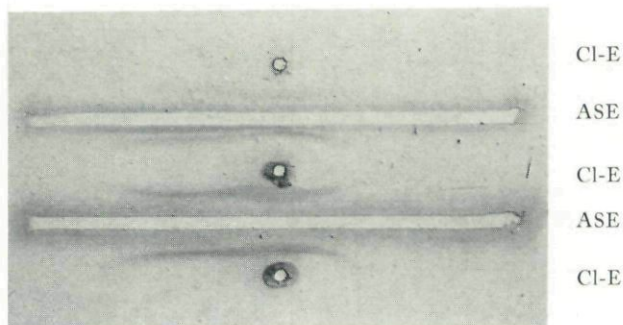


Fig. 3.

Immunoelectrophoresis of heterologous cotton fraction CL-E against antiserum (serum ASE 5) to purified house dust fraction E: CL-E (3 μ l), ASE 5 (20 μ l), CL-E (7.5 μ l), ASE 5 (50 μ l) and CL-E (10 μ l). Anode on the left.

tein fractions isolated from aged kapok fibres. Despite the fact that kapok and house dust allergens may be distinguished as distinct entities, analytically and by their specificity in intra-dermal assay (3), the anti dust sera did contain precipitating antibody for kapok fraction KP-C (Fig. 4).

In this system partial inhibition of precipitin formation was observed with N-(1-deoxy-1-D-fructosyl)- ϵ -aminocaproic acid as a hapten. Moreover, antigenic identity was found to exist between kapok and cotton fractions.

Gel diffusion pattern of antiserum to house dust fraction E (serum ASE 3) against kapok fraction KP-C. Kapok antigen in centre well (2.5 per cent solution, 10 μ l). Outer wells:

- A: 15 μ l ASE + 25 μ l NaCl (0.9 per cent);
- B: empty;
- C: 15 μ l ASE + 25 μ l ϵ -aminocaproic acid (5 per cent);
- D: empty;
- E: 15 μ l ASE + 25 μ l EACAF (5 per cent);
- F: empty.

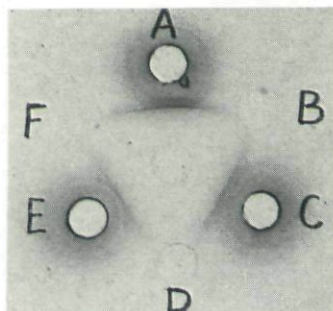


Fig. 4.

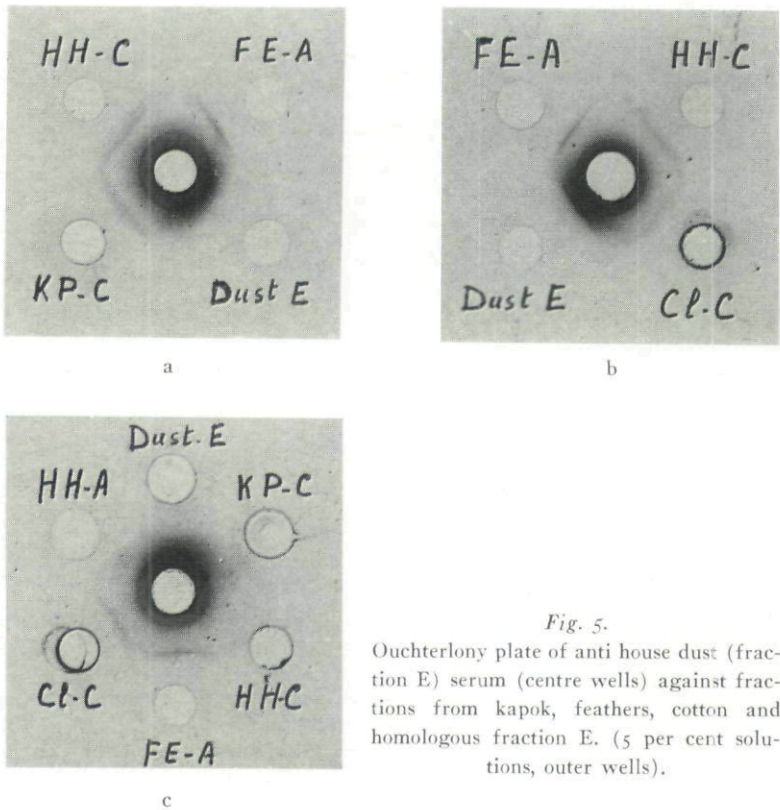


Fig. 5.

Ouchterlony plate of anti house dust (fraction E) serum (centre wells) against fractions from kapok, feathers, cotton and homologous fraction E. (5 per cent solutions, outer wells).

c. Precipitation with other Allergen Extracts

In view of the frequent finding of positive intracutaneous reactions to extracts of aged (chicken and duck) *feathers* in atopic patients sensitive to house dust, immunodiffusion experiments were performed of such extracts against anti house dust sera. Feather allergens are chemically completely different from house dust allergens (7) and may be easily distinguished from other allergenic plant glycoproteins. Some tests were also done with extracts of stored *hay*, specifically giving rise to positive wheal and flare reactions if injected into the skin of some patients with farmer's lung (20 μ g per injection).

Precipitation pattern of antiserum to house dust fraction D diffused against the corresponding D-fraction from wool (centre well, 10 μ l 2.5 per cent solution).

- A: 10 μ l antiserum ASD 9 + 30 μ l NaCl (0.9 per cent);
 B: 10 μ l ASD 9 + 30 μ l EACAF (5 per cent);
 C: 10 μ l ASD 9 + 30 μ l ϵ -aminocaproic acid (5 per cent).

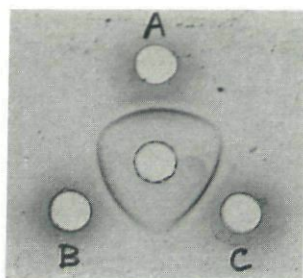


Fig. 6.

Fig. 5 a reveals antigenic similarity between kapok, hay and feather allergens, while Figs. 5 b and 5 c show a similar relationship between cotton, house dust fraction E, hay and feathers. Upon close examination of the gel-diffusion patterns, no absolute antigenic identity could be discerned; rather, the formation of spurs restricts the relationship between these various allergens to just a few antigenic determinants.

The precipitin arcs formed between cross-reacting allergens and the anti dust serum are considerably weaker than in experiments with the homologous antigen, a point also noted by *Wodehouse* (27); the heterologous antigens evidently do not exhaust the antiserum. Since the scope of the present study was restricted to the establishment of qualitative immunochemical relationships between the allergens, no attempts were made to determine antibody titers.

The anti house dust sera also contained precipitating antibody for compounds in extracts of *wool* (Fig. 6). Interestingly, the inhibition of precipitin formation by EACAF appeared to be of almost no significance if either wool fractions or feather allergens were employed as the antigens. Inhibition was more pronounced with kapok, cotton, hay and house dust as the antigen sources.

DISCUSSION

The interesting aspect of the above-described cross-precipitations of different atopic allergens with antisera to house dust

allergen lies in the fact that *purified* allergens have been employed, both as the antigens and for raising the anti house dust serum. The overall chemical composition of the active fraction E of house dust is not identical to that of the other allergens applied, while, analytically, no common contaminant has as yet been detected; also, no convincing evidence has been forwarded for a proposedly universal house dust allergen from some mysterious source (25). However, since house dust fraction E is considered to consist of a mixture of closely related glycopeptides (2, 9, 11, 17, 23), the present results do not allow the conclusion that the diverse allergen preparations do *not* share a common full antigen, because each of the allergens might in fact have been contained in the original inoculum.

Together with the chemical dissimilarity of the allergen fractions studied, a few additional observations do however favour the suggestion that antigenic determinants are shared rather than full antigens. Thus, spur formation in some of the gel diffusion patterns of fusing precipitin lines indicates antigenic similarity rather than complete identity, while the partial inhibition of precipitin formation with EACAF supports the argument that anti-lysyl-sugar antibody might be involved in the cross-precipitations observed; except for wool and feather allergens, the relevant N-glycosidic lysine-sugar units have been demonstrated chemically in the molecular structure of all the allergens employed (2, 3, 8, 10). The (a-physiological) introduction of common determinants of N-glycosidic linkage in rabbit dandruff proteins during decomposition reactions in rabbit skin, as described for the components in human dandruff (4, 10), would offer a very plausible explanation for the curious precipitation noted by *Wodehouse* (27) of rabbit dandruff extract with a rabbit antiserum for house dust extract.

In addition to the chemical evidence, the recently described immunological cross-reactions of diverse allergen preparations with antiserum to human dandruff allergen offer support to the concept that atopic allergens are indeed structurally re-

lated through common sites of N-glycosidically bound sugar (5). In contrast to these results with anti dandruff sera, however, the use of anti house dust sera does not allow of a definite choice between the alternatives of either shared full antigens between the allergen preparations or of common antigenic determinants only: the source of these allergens, according to present concepts, is not sufficiently remote from that of the "house dust allergen". It should perhaps be emphasized that a repetition of the present work with separate antisera for each of the allergens would clearly not solve the problem, nor would such antisera be less complex.

No great care has been taken to apply suitably balanced allergen-antiserum mixtures; further experiments with different antigen-antiserum ratios did not furnish essentially divergent results. The mere phenomenon of cross-precipitation, moreover, already has intriguing consequences for clinical allergy, especially for hyposensitization therapy. If full antigens are being shared by different allergen preparations, hyposensitization with house dust extracts ought to imply a diminished reactivity towards various other allergens in the course of treatment; since the relative distribution of the constituting allergens in house dust would be subject to variation among different batches, hyposensitization would also involve the risk of heightened or extra sensitivities, however. Alternatively, if atopic allergens are related by the common incorporation of lysyl-sugar units, the specificity of the determinants being dictated by the amino acid sequence around the particular lysine residue involved, hyposensitization therapy with house dust extracts would just broaden the patient's sensitivity spectrum. The latter possibility at present seems the more likely.

SUMMARY

Rabbit antisera to purified allergen preparations from house dust have been found to precipitate glycoprotein allergens from different materials (human dandruff, kapok, cotton lint, feathers, hay, wool). The precipitin patterns obtained

during immunodiffusion and immunoelectrophoresis indicate these cross-reactions to be due to antigenic determinants shared by the otherwise distinct inhalant allergens. The results are discussed in relation to the nature of house dust allergens.

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