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A CONTROLLED STUDY OF ALLERGEN PRODUCTION IN CULTURES OF *DERMATOPHAGOIDES PTERONYSSINUS*

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Numerous studies have been devoted to the possible relationship between the skin-active components in extracts of cultures of mites of the family Pyroglyphidae on the one hand, and of house dust on the other (1, 13, 19, 21, 22, 25, 29, 30, 32, 34). The evidence propounded for the allergenic relationship or identity of these has relied heavily on the results of comparative skin tests and on the correlation between the mite count in the dust and the allergenic potency of the extracts (1, 13, 19, 21, 25, 26, 27, 29, 30, 31, 32, 34).

Reports on the comparison of house dust extracts and unrefined extracts of *Dermatophagoides* spp. grown on various culture media are conflicting. Almost perfect correlations were recorded by Spieksma & Voorhorst (30, 32) and by Miyamoto et al. (25). Less good correlation was reported by Bernecker (1), Charpin et al. (19), Kawai and associates (22) and Young & Bangma (34). Several of these investigations are not strictly comparable, because of lack of detail on the mode of extract preparation, allergen and mite concentration, culture media, and proper controls. Also, individual research groups worked with different mite species, e.g. *D. farinae* and *D. pteronyssinus*. Though mite populations are pre-

sent in houses all over the world, the numbers and relative proportion of individual species vary (18).

Little work has been done to compare directly isolated allergens from dust and mite extracts, while differences of opinion and confusion have arisen regarding the properties of *antigenicity* vs. *allergenicity*. Studies on antigen composition of mite culture extracts have contributed little. Miyamoto and colleagues (27), working with extracts of *D. farinae* grown on dried *Daphnia* failed to observe precipitating antibody in the serum of rabbits immunized with either total mite culture extract or house dust extracts. Dasgupta & Cunliffe (20) found that extracts of *D. pteronyssinus* and *D. farinae* share a common antigen; only 1 out of 4 rabbit antisera to house dust contained precipitins against *Dermatophagoides* spp. No mite-specific antigen was detected by Kawai and associates in hyper-immune rabbit antisera to *D. pteronyssinus* grown on human skin scales (22).

The problem of major contamination by antigens or allergens from the culture medium was raised at an early stage, especially in relation to mites grown on dried human dandruff and yeast powder (3). A significant number of positive skin or bronchial challenge reactions to extracts of mite-free control medium were reported by Stenius & Wide (31), Young & Bangma (34) and, to a lesser extent, by Miyamoto et al. (25) and by Biliotti et al. (13). By immunodiffusion analysis, Dasgupta & Cunliffe (20), Kawai et al. (22) and Wolfenberger et al. (33) confirmed that the dominant antigen(s) in mite extracts cultured on human dandruff derives from human epithelial material, not from the mites.

Some investigators described very high allergenic potency in extracts of the fecal pellets of mites (21). Also, the frequency of positive reactions observed with extracts of mite-free culture medium, incubated under identical conditions as the mite-containing culture, led Young & Bangma to suggest that degradation reactions proceeding in the media might account for the increased allergenicity, even in the absence of mites (34). In an attempt to integrate existing evidence for

the generation of allergens by Maillard-type degradation reactions (2) and the above observations, Bronswijk suggested that the passage of house dust debris through the mite alimentary tract might create optimal conditions for augmentation of allergenicity by Maillard-type degradation (14).

The present studies were initiated in order to clarify these points and perhaps permit a re-evaluation of the role of mites in human allergy. A preliminary report was incorporated in a recent communication (17).

MATERIALS AND METHODS

1. Mite Cultures and Control Media

Wheat germ flakes (1 kg) and yeast powder (1 kg) were obtained commercially and were thoroughly mixed in a blender; the mixture was sieved to pass a 16-mesh filter. Samples of about 90 g of this mixture were transferred into each of 21 Erlenmeyer flasks (200 ml), which were plugged with cotton wool and sterilized by dry heat for 3 h at 150° C. Three groups of 7 flasks were subsequently arranged as follows.

Group A. The medium in each of 7 flasks was infected with about 50 specimens of *D. pteronyssinus*, together with a few grams of rearing medium from the stock culture.

Group B. The media were infected with a few grams of a non-sterile rearing medium from the stock culture in order to produce the proper microflora; these samples remained mite-free.

Group C. These media were kept completely sterile.

The three groups of flasks were incubated for 15½ months at room temperature (17–22° C) at 75 per cent relative humidity (in desiccators over aqueous saturated NaCl-solution). After this period, the media were examined for microorganisms by inoculation of aliquots into Sabouraud-Glucose Agar in tubes. The medium of groups A and B was found to contain bacteria and fungi, those of group C contained no viable germs.

The total number of (dead and living) mites in the samples was determined by a flotation method described by Bronswijk (16). Group A contained 54 *D. pteronyssinus* specimens per gram of medium, i.e. about 500 mites per flask. Taking into account the long incubation period, this is a very low mite concentration (15). Since mites cannot be reared in the laboratory on a sterile wheat-germ medium alone, the slow growth of the mite population most likely was caused by pre-sterilisation of the rearing medium. The growth of the micro-

organisms from the few added grams of stock-culture medium may have been the limiting factor in mite population growth. No mites were found in groups B and C.

The media of groups A, B and C were finally pooled separately into 3 large containers, which were kept at $60 \pm 10^\circ \text{C}$ for 6 h; this killed all stadia of mite growth and caused sufficient dehydration to inhibit the growth of micro-organisms. The products were coded A, B and C; the code remained unbroken to the chemical and clinical investigators of our group until after the final experiments.

2. *Extraction*

The three batches of culture medium, 800 g each, were extracted separately at room temperature for 2 h with 2 L of phosphate-phenol buffer pH 7.4, with vigorous stirring. The buffer composition was identical to that described for the extraction of house dust allergen (5, 6, 12). After high-speed centrifugation, the supernatant fluids were dialyzed extensively against distilled water at 4°C , and were then dried by lyophilization. Yields are shown in Table 2.

The overwhelming percentage of water-soluble contaminant components from the medium ingredients forced us to adopt a fractionation procedure based on the known properties of allergens (6) and on the assumption that the physico-chemistry of mite allergens is closely akin to that of house dust allergens (5, 23, 27, 28). The choice and justification of the purification techniques are discussed in the Results section.

3. *Analytical Techniques*

A highly purified house dust allergen preparation, fraction E, was prepared from a pool of vacuum-cleaner collected dust as described (5, 12). Hyperimmune rabbit antiserum against this product was raised with the aid of Freund Complete Adjuvant (Difco) as reported before (4). Human dandruff allergen, fraction E, and its rabbit antiserum were produced in similar fashion. Fresh human sera for complement assay were obtained from glass-clotted venous blood of normal and allergic donors. Methods of optical analysis and of nitrogen and carbohydrate assay have been described in previous papers from this laboratory (6, 8, 12).

Skin tests were performed in atopic individuals reactive to commercial and to purified house dust allergen. Tests were done by the intracutaneous route, applying 0.05 ml of the appropriate solution in neutral physiological phosphate-saline buffer. The mite and culture medium subfractions were assayed at 1000 $\mu\text{g}/\text{ml}$ of lyophilized, soluble material, the house dust allergen preparation at 2 $\mu\text{g}/\text{ml}$.

It was not feasible to establish the skin-reactivity of each and every subfraction in this fashion. Therefore, enrichment of the allergen fractions was founded on two rationalisations: (1) the purification procedure was based on existing

knowledge of the physicochemical behaviour of house dust and mite allergens; (2) *in vitro* complement consumption tests were relied upon for guiding the fractionation process. It has been shown for house dust extracts that the outcome of such complement assays is clinically relevant (9). Also, good correlation was observed between skin and complement tests for other allergens (10, 11). Complement tests were routinely carried out as follows: a 15 μ l sample of human allergic serum was brought into 0.5 ml veronal-saline buffer, containing Ca^{++} and Mg^{++} ions (VSB^{++}) and different quantities of lyophilized subfractions in the range of 0.5–100 μ g. The mixture was incubated for 30 min at 37° C, and then 2.5 ml of VSB^{++} and 1.0 ml of optimally sensitized sheep red blood cells was added (2.5×10^8 cells EA/ml). Incubation was prolonged for 60 min at 37° C, then the tubes were centrifuged and the colour of the supernatant was read at 541 nm. Controls included a serum sample preincubated in the absence of allergen, and a water-lysed control for 100 per cent haemolysis. The complement-inactivating capacity of the subfractions was read from dose-response graphs and was routinely expressed as the dose, in μ g lyophilized substance, required for 50 per cent inactivation of the available haemolytic complement.

The disadvantage of this method is that high-molecular weight heteropolysaccharides, agglutinins, and RNA—undoubtedly present in wheat germ extracts in the early phases of purification—may also cause C-inactivation under these conditions.

RESULTS

1. Fractionation of the Extracts

Considerable technical problems were encountered in attempting the enrichment of possible atopic allergens from the total nondialysable extracts of pools A, B and C. The bulk of the extracted material consisted of very high-molecular weight polysaccharides, proteins and nucleic acids derived from the wheat germ flakes and yeast cells. The following procedure was finally adopted for each batch, A, B and C.

The total yield of nondialysable material was dissolved in 500 ml of distilled water; ethanol was added to bring the final alcohol concentration to 20 per cent (v/v). After 18 h at 4° C, the mixture was centrifuged and the precipitate was discarded. It is known that house dust allergens do not precipitate in these conditions (5, 6, 12, 23). Ethanol was removed from the supernatant by dialysis, and solid ammonium sulphate was added to 25 per cent saturation at 4° C. The precipitate settled after 2 days, and was removed by centrifugation. At

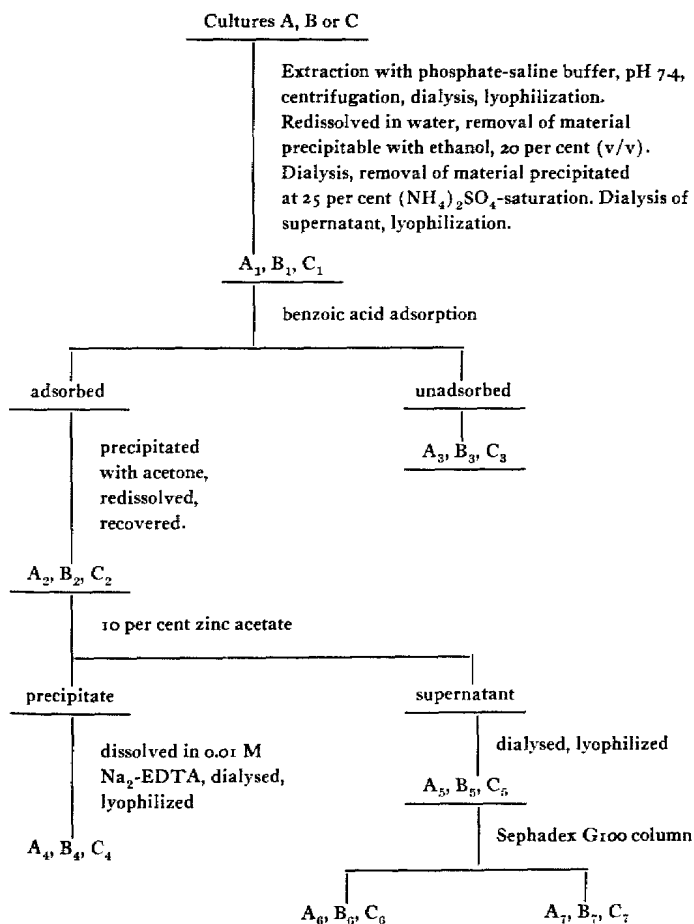


Fig. 1.

Flow chart illustrating the purification process.

this salt concentration, allergens remain soluble (5, 6). The material in the supernatant was recovered by lyophilization after extensive dialysis. Further purification then followed the

TABLE 1

Nitrogen and Carbohydrate Composition of Subfractions 4 and 5 Isolated from Mite-Infected (A), Mite-Free (B) and Sterile Media (C).

Subfraction	Per cent nitrogen*	Per cent carbohydrate**
A ₄	11.5	14.5
B ₄	11.4	16.6
C ₄	9.9	10.8
A ₅	0.9	2.5
B ₅	5.6	17.6
C ₅	2.2	7.5

* By microKjeldahl.

** Total hydrolysable reducing sugar, orcinol assay, galactose as reference.

schedule reported for house dust allergen, *viz.*: benzoic acid adsorption and acetone precipitation. In order to free the material at this stage from contaminant protein and glycoprotein, zinc acetate precipitation was adopted: house dust allergens react only very slowly with zinc ions. Finally, the enriched allergen fractions were passed through columns (2.5×90 cm) of Sephadex® G-100 for separation into the major subfractions, 6 and 7. The fractionation process has been reproduced in schematic outline in Fig. 1.

2. Analysis

When dealing with the problem of concentrating a minor active constituent from an overwhelming percentage of contaminant components, it is to be expected that chemical analyses of the early fractions in the procedure are not representative of the active material. This is reflected in the nitrogen and carbohydrate contents of fractions 4 and 5 listed in Table 1, which clearly fail to provide evidence for the location of the allergenic constituent among the A, B or C extracts.

A further complication, moreover, arises from the fact that the yields of purified material diminish with progressive purification, precluding extensive chemical analysis. This is shown in Table 2. One interesting feature should be pointed

TABLE 2

Yields of Lyophilized Subfractions, Starting from 800 g of Dry Culture; A = Mite-Infected; B = Mite-Free, Non-Sterile; C = Sterile Control. For Codes of Subfractions see Flow-Chart, Fig. 1.

Fraction	Yield (g)	Fraction	Yield (g)	Fraction	Yield (g)
A	38	B	49	C	51
A ₁	22	B ₁	22	C ₁	23.5
A ₂	2.2	B ₂	1.6	C ₂	0.8
A ₃	9.4	B ₃	15.2	C ₃	18.0
A ₄	1.07	B ₄	2.10	C ₄	0.57
A ₅	0.210	B ₅	0.519	C ₅	0.180
A ₆ *	0.012	B ₆ *	0.021	C ₆ *	0.009
A ₇ *	0.034	B ₇ *	0.015	C ₇ *	0.039

* Per 100 mg of fractions 5 on Sephadex Column.

out, however. The yields of fractions A₂, B₂ and C₂ are in reverse order as compared to the A₃, B₃ and C₃ subfractions. Since benzoic acid preferentially adsorbs glycoproteins, the observation that adsorption occurred in the sequence A>B>C might be interpreted to indicate that degradation reactions of proteins and polysaccharides to form unnatural glycoproteins proceeded more readily in the mite, fungi and bacteria containing culture medium than in the medium containing only fungi and bacteria, and even less easily in the sterile nutrient medium.

The total recovery of fractions 7, the products finally examined for skin-reactivity, amounted to 0.002–0.005 per cent from the dry starting material. This figure is by no means conclusive, because the lyophilized materials were exceedingly hygroscopic, which precluded exact weighing. Hygroscopicity as a problem has been frequently noted with other allergens as well (6, 7).

Rather than chemical analysis, observation of the optical properties of the various subfractions was preferred for the identification of possible allergenic material. As discussed in detail in previous publications, atopic allergens—especially those found in house dust—display peculiar absorption spectra

TABLE 3

Extinction Coefficients in the Ultraviolet at the Protein (280 nm) and Nucleotide (260 nm) Peaks of some Subfractions. Solvent: 0.1 M Phosphate Buffer pH 7.0.

Subfraction	$E_{1\text{cm}}^{1\%}$ at 260 nm	$E_{1\text{cm}}^{1\%}$ at 280 nm	Remarks
A ₄	143	obscured	turbid
B ₄	1000	obscured	turbid
C ₄	990	obscured	turbid
A ₅	no peak	0.7	
B ₅	12.0	obscured	
C ₅	16.0	obscured	
A ₇	no peak	3.35	clear
B ₇	id.	2.70	clear
C ₇	id.	66.5	polysaccharide contamination

in the ultraviolet, distinct from those observed with pure proteins or polysaccharides (6, 7, 8). Again, the fractions isolated early in the purification process contained such massive amounts of contaminants, that possible allergen-specific spectra were obscured. As shown in Table 3, optical analysis at this stage nonetheless provided some valuable indications of the nature of the major contaminants.

For example, fractions B₄ and C₄, less so A₄, produced very turbid solutions and impressive absorption at 260 nm, indicating the presence of very high-molecular weight polysaccharides and of nucleic acids, respectively. Such contaminants are, of course, to be expected in solutions which are predominantly very unspecific wheat germ extracts. Interestingly, the A₅ fraction was the first to feature a detectable 280 nm (glyco)protein shoulder, indicating that this subfraction contained the lowest percentage of nondialysable nucleic acid and carbohydrate relative to B₅ and C₅; nutrient breakdown, therefore, proceeded faster in A.

Two considerations determined the choice of the subfractions to be assayed by skin-testing after the final Sephadex G-100 gel filtration step. First, high molecular weight con-

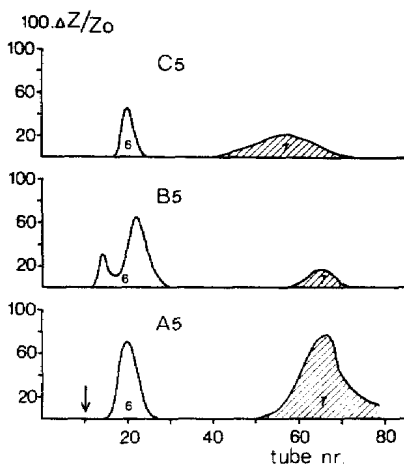


Fig. 2.

Molecular exclusion chromatography of 100 mg samples of subfractions A_5 , B_5 and C_5 on a 90×2.5 cm column of Sephadex G100. Elution was done with 0.9 per cent NaCl by gravity flow; fraction size was 5 ml. Complement inactivation by the eluates (50 μ l aliquots, to which was added 5 μ l 1:10 diluted stock VSB⁺⁺ buffer) was assessed using human serum sample 73166 (15 μ l 1:30, preincubated for 30 min at 37° C prior to residual complement haemolytic assay). Complement consumption expressed in per cent decrease of Z-values, where $Z = -\ln(1-y)$ and y represents per cent haemolysis (7, 9, 10). Active fractions were pooled as indicated to give subfractions 6 and 7, which were recovered by dialysis and lyophilization.

taminant nucleic acids and polysaccharides would be excluded in the first peak (subfractions 6). Second, the mean molecular weight of atopic allergens has generally been found to be in the 15,000–35,000 range (6); such compounds would be eluted in the position of subfractions 7 (Fig. 2).

The ultraviolet absorption spectra of these subfractions 7 are quite enlightening, in that preparations A_7 and B_7 feature the pattern typical of atopic allergens of the house dust group, whereas C_7 does not (6, 7, 8). These spectra have been reproduced in Fig. 3, which would permit the prediction that the

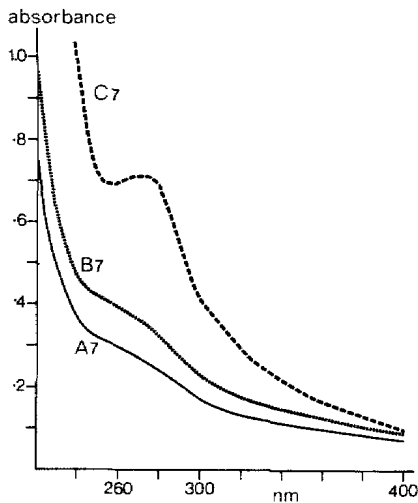


Fig. 3.

Ultraviolet absorption spectra of subfractions A₇ (0.1 per cent), B₇ (0.1 per cent) and C₇ (0.01 per cent) in 0.1 M phosphate buffer, pH 7.0; 1 cm light path.

allergenicity is mainly associated with subfractions A₇, less so with B₇ and to a considerably lower extent with C₇.

3. Assay of Activity

As outlined in the Methods Section, *in vitro* complement inactivation tests were employed for estimating biological activity during fractionation so as to reduce the number of skin tests. For the reasons given above, the (pooled) Sephadex fractions 7 were chosen for skin-testing, being the most likely candidate carriers of allergenic activity. As shown in Table 4, the complement-inactivating capacity did increase in the course of purifications, fractions 6 and 7 displaying the highest potential in this respect. Compared to purified house dust or human dandruff allergen, however, the activity remained unimpressive (Table 4).

TABLE 4

*Complement-Inactivating Power (CIP) of Subfractions, Expressed in Dosage of Lyophilized Material, in μg , Required for 50 per cent Diminution of Haemolytic Complement from 15 μl of Serum from Allergic Donor 73166.**

Fraction	CIP, μg	Fraction	CIP, μg	Fraction	CIP, μg
A ₁	82	B ₁	75	C ₁	31
A ₂	53	B ₂	23	C ₂	31
A ₄	25	B ₄	24	C ₄	50
A ₅	5.1	B ₅	12	C ₅	20
A ₆	6.5	B ₆	6.2	C ₆	3.2
A ₇	5.8	B ₇	5.2	C ₇	2.7

* Fractions 6 and 7 unreliable because of hygroscopicity. A factor 10 increase in CIP represents a 100–1000 factor rise in skin-reactivity (9, 10). Highly purified house dust allergen 72/E in this serum had a CIP of 0.8 μg .

Though sequential analysis of the eluted fractions from the Sephadex columns clearly indicated the highest recovery of complement-consuming substance to be associated with the originally mite-infected medium (Fig. 2), this could not be confirmed by comparing the recoveries and activities of the lyophilized pooled column fractions 7 (Tables 2, 4 and 5), due to the hygroscopicity of these final products. It was concluded at this stage that all extracts contained allergenic activity, but that extract A quantitatively outnumbered B and C in this respect (Fig. 2).

The conclusion from these *in vitro* experiments, namely that extracts of mite-infected, mite-free and sterile media all contained—qualitatively identical, but quantitatively different—allergenic activities, is corroborated by the results of intradermal skin testing. Table 5 lists the cutaneous response reactions of fractions 7 in 15 atopic individuals positive to house dust. The A, B and C products were almost equally active on a weight basis, though each fraction was considerably less potent than the purified house dust preparation 72/E injected for comparison.

TABLE 5

Results of Intradermal Skin Tests in House-Dust Sensitive Atopic Patients with Allergenic Subfractions A₇, B₇ and C₇ Obtained by Sephadex G100 Molecular Sieving (Figs. 1 and 2).

Patient	Dermatophagoides and controls			House dust, purified
	A ₇ 1000 µg/ml	B ₇ 1000 µg/ml	C ₇ 1000 µg/ml	72/E 2 µg/ml
K.	1+	0.5+	1+	1+
v.E.	2+	1.5+	1.5+	1+
T.	+	+	+	1.5+
S.	+	+	1.5+	2.5+
D.	0.5+	+	+	+
L.	+	+	1.5+	+
B.	2+	+	2+	3+
v.B.	+	+	+	1.5+
F.	+	+	+	1.5+
P.	2+	1.5+	1.5+	2.5+
Sa.	1.5+	2+	2+	1+
C.	2+	3+	2+	2+
A.	+	+	+	2+
M.	—	1.5+	—	+
v.d.P.	+	+	1.5+	3+

Reference: highly purified house dust fraction 72/E, isolated from pooled house dust obtained by vacuum cleaning. Key: 1+ = wheal no more than 5 mm diameter with associated erythema; 2+ = wheal 5-8 mm diameter with surrounding erythema; 3+ = wheal 8-12 mm, with erythema and pseudopods.

4. Immunochemical Evidence of Allergen Formation

It has been claimed that mites produce "the" house dust allergen (29, 32). Because, in current allergological theory, atopic allergens are considered to act as whole molecular antigens, antisera raised against house dust allergen would have to recognize the mite allergen immunologically as being physically identical. No pure mite allergen has been isolated so far to verify this point. Moreover, attempts to prepare pure allergen from house dust as a singular molecular entity have led to the conclusion that no such thing exists (5). In contrast to current concepts, house dust allergens were considered to be

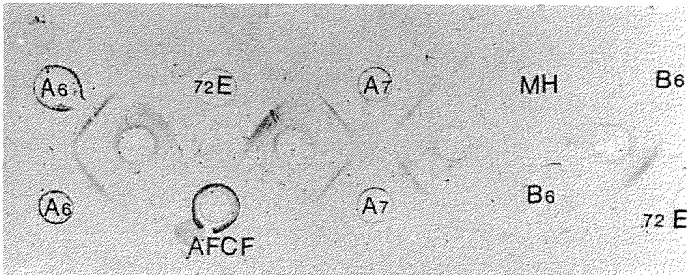


Fig. 4.

Double diffusion in 1.2 per cent agar gel (veronal-buffer pH 8.5) of 8 μ l 3 per cent solutions of the indicated subfractions $A_{6,7}$ and B_6 against rabbit hyperimmune serum to house dust fraction 72/E (central holes). Also shown are reactions with homologous house dust 72/E preparation and with culture filtrate antigens of *Aspergillus fumigatus* (AFCE) and an extract of mouldy hay (MH).

composed of suitable carrier molecules from various substrates, which exhibit allergenic activity by virtue of common structural sites of lysine-sugar conjugation. Such sites are introduced into the carrier molecules during Maillard-type degradation reactions (5, 7). In proof, early immunochemical studies showed that synthetic protein-sugar model compounds precipitated anti house dust and anti human dandruff sera, in

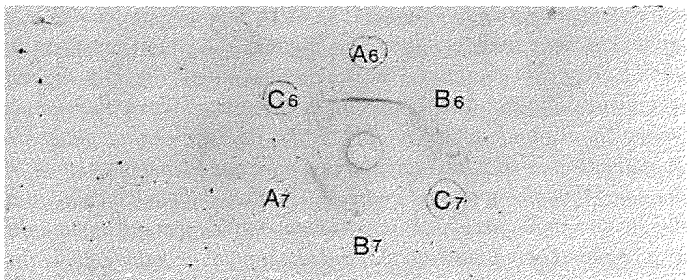


Fig. 5.

Double diffusion in agar gel of subfractions 6 and 7 from cultures A, B and C against rabbit anti house dust (fraction 72/E) serum. Undiluted antiserum; 3 per cent antigen solutions. Stain: Coomassie Brilliant Blue.

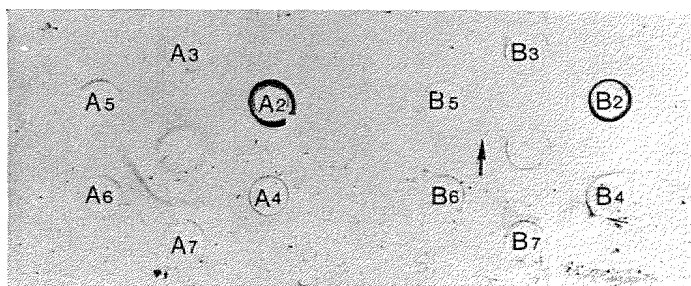


Fig. 6.

Ouchterlony plate of various A and B subfractions (3 per cent) against undiluted rabbit antiserum against purified human dandruff allergen, fraction E, in centre holes (4).

contrast to the native unreacted parent protein (4). Our present results with the mite extracts confirm these findings.

Fig. 4 presents the results of an experiment using rabbit antiserum against the highly purified 72/E preparation from house dust diffused against fractions 6 and 7 from the mite-infected medium A and the non-sterile control B, respectively. Though precipitation did occur with the A-fractions, no cross-reaction with homologous dust 72/E was apparent, nor was there any indication of identity with antigens from mouldy hay or *Aspergillus fumigatus*. As shown in Fig. 5, the A-precipitation lines merged over the B-containing wells, indicating weak antibody reactivity—and identity—with the B-antigens; precipitins against the C-products (derived from the sterile control medium) were never observed, indicating that the precipitation with the A-products *could not be attributed to an antigen from the nutrient medium*. Rather, the substance in A, and less so in B, involved in anti house dust precipitation must be a newly-formed antigenic determinant, presumably introduced into nutrient carrier molecules during degradation.

Purified house dust allergen preparations have been shown to be free of human dandruff allergens; the two allergens are chemically, and antigenically, unrelated, except for common

structural sites of lysine-sugar conjugation (6). The immunodiffusion pattern of Fig. 6 demonstrates that the allergenic products 6 and 7 from mite-infected medium A produced precipitation lines against anti human dandruff allergen (fraction E) serum; precipitations with the B-fractions were barely discernable, no reaction occurred with the C-products.

We interpret these results as indicating the presence of structures of lysine-sugar conjugation on carrier antigens derived from the medium; since these sites were detected to a lesser extent on the B-, but not on the C-products, their formation by degradation reactions must have been favoured by mite as well as bacterial and fungal activities.

DISCUSSION

As reviewed in the introduction to this paper, the relevancy of mites in human allergy hinges on a number of hitherto unsolved problems. First, the presumed identity of mite-derived allergens and house dust allergens depends on the isolation and comparison of both allergens in physicochemically pure form, which has not yet been achieved. Second, the real issue, of course, is whether such experiments would be at all relevant: is the allergenicity of house dust to be interpreted within an immunological framework as being due to a very special antigen, or is it to be attributed to the combined action of different antigens from various substrates sharing a common biological, non-immunological property? If, as has been convincingly demonstrated (18, 24), mite species belong to the normal habitat of house dust, like bacteria and fungi, there is every reason to suppose that they contribute soluble *antigens* to house dust extracts; but these are not necessarily *allergens*. The same holds true for material derived from the culture medium, which comprises the bulk of the solubles in the currently available mite extracts for clinical use. The search for the molecular identity and provenance of house dust allergen may be fruitless if allergenicity in man is a common biological activity exerted by quite different molecular entities.

A number of investigators have procured results which support this point of view. Extracts of mite-free culture media have regularly been found allergenic, though of lower degree of potency than the mite-infected media. This has led Young & Bangma (34) to propose that allergenicity depends on degradation reactions, which merely proceed faster in mite-inhabited media. Bronswijk has suggested that the passage of medium constituents through the mite intestinal tract might promote degradation reactions (14); more specifically, the conditions of pH and humidity were considered ideal to stimulate Maillard-reactions for the creation of lysine-sugar allergenic determinants (17). The results of the present study support these ideas.

The isolation experiments and the optical analyses clearly demonstrate that degradation reactions occur much more readily in the non-sterile media than in the sterile control. However, the difference between the mite-infected and micro-organism inoculated non-sterile media in this respect is merely quantitative. Since fractions from both extracts, A and B, exhibit allergenicity *in vivo* (skin tests) and *in vitro* (complement-inactivation tests), it appears that both mites and micro-organisms merely promote degradation reactions of the nutrient medium constituents. Even the C₇ fraction from the sterile control exhibited allergenicity; on a weight basis, the fractions 7 from all media were almost equally active, though considerably less potent than purified house dust allergen. Taken together with the recovery of activity off the Sephadex columns (Fig. 2), it is, therefore, logical to conclude that the allergenic activity derives from the medium, and that mites and micro-organisms, by promoting degradation reactions, merely *augment* the allergenicity.

The immunodiffusion experiments with anti house dust and anti human dandruff sera demonstrate that extensive degradation—as in the mite-infected medium—favours the introduction of structural sites also present on house dust and dandruff allergens. Most likely, these must be equated with

lysine-sugar structures introduced into non-allergenic carrier molecules during Maillard degradation reactions.

In conclusion, the results of these studies confirm a role for mites—and other organisms—in house dust allergen formation. By promoting the decomposition of the medium, they may contribute materially to the generation of allergenic activity, for which Maillard-type reactions between substrate constituents have long ago been held responsible (2). Consequently, it may be futile to test, or even treat, patients with specific mite-derived constituents, because allergenic activity in this sense is a common biological property shared by quite different antigenic molecules. Only in the case of long-term sensitization may mite-body antigens conceivably become recognized immunologically, e.g. by specific (IgE) antibody. In that situation, the organisms can be said to contribute specific mite-antigens to the highly divergent pool of antigens in house dust. However, almost certainly the antigenic carrier molecules derived from the food of mites and other organisms will quantitatively dominate in the dust. Since the food available to mites differs according to the social, cultural and individual status of the human inhabitants of the house, long-term immunological sensitization to any particular mite-associated antigen in house dust is a very unlikely event.

SUMMARY

Allergenic substances have been isolated from cultures of *Dermatophagoides pteronyssinus* on wheat germ flakes and powdered yeast; as controls, non-sterile nutrient medium containing no mites, as well as sterile medium, were maintained and extracted under identical conditions. Chemical purification and analyses indicated the occurrence of skin-active allergens in all three preparations. This was confirmed by the assay of skin-reactivity *in vivo* and complement-inactivating capacity *in vitro*. Quantitatively, the medium containing bacteria and fungi contained more allergen than the sterile control, but less than the mite-infected medium. The evidence indicates that

degradation reactions of nutrient components proceed faster in the non-sterile media than in the sterile control. The results of immunochemical analyses demonstrate that degradation reactions give rise to the introduction of lysine-sugar structures into high-molecular weight components from the medium. It is concluded that mites and micro-organisms have a role in allergen formation by promoting degradation reactions among nutrient constituents.

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