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ALLERGENS AND ANTIGENS OF *DERMATOPHAGOIDES FARINAE*

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SUMMARY

Extracts of *Dermatophagoides farinae* cultured on Gaines dog-meal plus yeast, and in particular the 'protein' fraction precipitated by ammonium sulphate, give stronger skin test reactions in house dust sensitive patients than extracts of *Dermatophagoides pteronyssinus* and of house dust. Specific IgE was found against the *D. farinae* extract in eighteen out of twenty house dust sensitive subjects. Precipitins were found in human sera against extracts of *D. farinae*, *D. pteronyssinus* and house dust and also against antigens in cereal and vegetable dusts. These precipitin tests provided evidence of related antigens in extracts of house dust and *D. farinae* and in extracts of house dust and *D. pteronyssinus*, but not in *D. farinae* and *D. pteronyssinus*.

INTRODUCTION

The discovery by Voorhorst, Spieksma-Boezeman & Spieksma (1964) that *Dermatophagoides pteronyssinus* is a major source of house dust allergen is an important contribution to this important clinical problem. The slow growth of *D. pteronyssinus* in culture on human dander and its even slower growth on other media makes it difficult to obtain adequate supplies for investigation. The finding that *Dermatophagoides farinae*, which grows more readily on a medium of dog-meal, contains allergens giving comparable skin test reactions to *D. pteronyssinus* in house dust sensitive patients (Ishizaki, Miyamoto & Oshima 1967; Miyamoto *et al.*, 1968; Maunsell, Wraith & Cunnington, 1968; Pepys, Chan & Hargreave, 1968a; Stenius, 1969, 1970) suggests that it may serve as an alternative source of allergen, giving a statistically significant correlation between skin and inhalation tests and a history of house dust allergy in patients in the United Kingdom (Pepys *et al.*, 1968a; Maunsell *et al.*, 1968).

Extracts of cultures of *D. farinae* have been examined in man by skin tests, and by serological tests for IgE antibody and for precipitating antibody. The part played by the culture medium itself in the antigenic content of the mite culture extracts has also been studied.

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MATERIALS AND METHODS

The following extracts were used for skin and serological tests:

1. *D. farinae culture extracts.* (a) Mr A. M. Cunnington of the Pest Infestation Laboratories, Slough, provided cultures of *D. farinae* on Gaines dog-meal, to which a pinch of yeast had been added. The whole cultures were defatted in ether for 24 hr and then extracted in carbol-saline for 7 days. The extracts were Seitz-filtered, dialysed in Visking tubing against running tap water overnight and then freeze dried. The culture extracts were fractionated by 100% ammonium sulphate saturation and yielded the following two main fractions.

(b) A predominantly 'protein' precipitate obtained after dialysis, passage through Sephadex G-25 and freeze-drying. Such 'protein' precipitates contained about 12·1% N and 32·4% hexose estimated by the orcinol method and expressed as galactose.

(c) A predominantly 'polysaccharide' component present in the supernatant. This contained about 1·7% N and 70·3% hexose.

(d) The low molecular weight dialysates obtained after passage through the dialysis bag and through Sephadex G-10 and the already dialysed material passing through a 10,000 molecular size Amicon-Diaflo membrane filter were freeze-dried and tested.

2. *Culture medium extracts* were prepared as in 1(a) from: (a) Gaines dog-meal, the medium for the mite culture, which contains wheat, maize, soya bean, bone and fish meal, meat by-products, animal fats and added vitamins.

(b) Yeast powder, *Saccharomycetis cervisiae.*

3. *D. pteronyssinus.* Freeze-dried extracts were prepared from a culture on human dander, provided by Dr R. Voorhorst of Leiden who also provided an already freeze-dried extract.

4. *House dust extracts.* (a) House dust extract (Ex 8, Bencards) was provided in freeze-dried form.

(b) House dust extract ('Dome/Meda') was used for the IgE tests.

5. *Vegetable dust extract* was prepared from the 'kiboko' dust, the dried outer flesh of the East African coffee berry (Pepys, Longbottom & Jenkins, 1964). This extract was included because the supernatant obtained by ammonium sulphate precipitation contains an antigen, so-called 'D' antigen, which was first found in extracts of certain mouldy hays (Pepys & Jenkins, 1965). This gave reactions in a zone additional to the main A, B and C zones, observed when mouldy hay extracts were tested by immunoelectrophoresis against farmers' lung sera (Jenkins, 1964; Pepys & Jenkins, 1965). It was present in some extracts of mouldy bagasse (Hearn & Holford-Strevens, 1968), barley house dust, other vegetable materials and in extracts of *D. farinae* cultured in dog-meal medium, but was not demonstrated in the particular original dog-meal used for the culture.

This electrophoretically fast-moving, negatively charged, antigen gave precipitation reactions in Ouchterlony double-diffusion tests (Pepys *et al.*, 1964), with about 70% of sera from apparently healthy subjects not exposed to mouldy hay, and also with sera of sixteen out of twenty-five asthmatics, fifteen out of thirty-four bronchitics and eleven out of twenty-two patients with pulmonary fibrosis. The 'D' antigen is of importance in analysing extracts of *D. farinae* and other mites cultured on dog-meal and possibly on other cereal or vegetable media.

(b) An extract of *Cladosporium herbarum* was also tested because it contains yet another

'polysaccharide' antigen present in extracts of house dust (Pepys *et al.*, 1964), of *D. farinae* and of the kiboko dust.

All the freeze dried extracts were prepared for testing in milligrams dry weight per millilitre.

Skin test

The prick method was used for skin testing and the mean weal diameter of the reactions was measured in millimetres after 15–20 min. Reactions were regarded as unequivocally positive if larger than 1 mm mean diameter and were roughly graded as follows: $+\pm$ to $++$ = 4–7 mm diameter, $+++$ = 7–10 mm diameter, $++++$ = 10 mm diameter or more.

Serological tests

Tests for IgE antibody. Extracts from unfractionated *D. farinae*, Gaines dog-meal, yeast and house dust (Dome), were tested against the sera of patients for the presence of specific IgE as described by Stenius & Wide (1969).

The reaginic antibodies (IgE) in serum were semi-quantitatively estimated by the radio-allergosorbent technique (RAST) as previously described by Wide, Bennich & Johansson (1967), with a few modifications. For the preparation of the polymer–allergen conjugates, 100 mg of the dried extracts of the mites and the medium constituents were dissolved in 1 ml of 0·075 M phosphate buffered saline of pH 7·4 and incubated with 100 mg of CNBr-activated Sephadex at +4°C for 3 days. The 0·1 M 'Tris' buffer of pH 7·4 previously used in the assay procedure was changed for a 0·075 M phosphate buffered saline of the same pH. For the calibration of the second step of the RAST reaction, where the labelled anti-IgE antibodies were added, the uptake of radioactivity on known amounts of an immunosorbent consisting of IgE coupled to Sephadex was determined each time. The degree of positive reactivity in the RAST was expressed in 'sorbent units' (SU) where 1 SU is the radioactivity uptake on the immunosorbent to which 1 pg of IgE was coupled. The basis for this method of expressing the reactivity in the RAST will be discussed in detail elsewhere (Wide, to be published).

The RAST reaction was considered positive when the radioactivity uptake was twice that of the negative controls and negative when the uptake was less than 1·5 times the control values. The values between positive and negative reactions were regarded as 'doubtful'.

Agar-gel precipitin tests

Preliminary tests made by the Ouchterlony method in 3-mm agar gel layers showed the presence of precipitins to some of the test extracts. The subsequent tests for reasons of economy were made by a modification of the micro-method of Crowle (1958), using 1·5% agar in a mixture of McIlvaine's phosphate–citrate buffer and saline. After 48 hr the test plates were washed in saline, dried and stained with naphthalene black. The inclusion of citrate in the agar is necessary because extracts of vegetable dust and their fungal flora may contain glycopeptides which behave like pneumococcal C-substance and give precipitation reactions with C-reactive protein (Pepys *et al.*, 1964). These reactions are prevented by the sodium citrate. The reactions in the micro-method may vary, presumably for technical reasons, and where there was doubt the result was not included. In some cases it was less

sensitive for demonstrating precipitins than the Ouchterlony test performed in 3-mm agar layers.

Absorption tests were made by adding the particular extract to the serum so as to give a final concentration of 10 mg/ml.

The γ-globulin of four sera was separated by the method of Levy & Sober (1960) for tests against the different extracts.

RESULTS

Skin tests

In a group of forty-three asthmatic patients who had reacted previously to house dust extract or *D. farinae*, the extracts of *D. farinae* and its fractions, *D. pteronyssinus* and house dust (Bencards Ex 8) were compared at concentrations of 0·1, 1 and 10 mg/ml, the tests being distributed randomly.

TABLE 1. Prick test—concentration of extract

	Average weal diameter (mm) for total group					
	No. tested		No. tested		No. tested	
Test extract	43–10 mg/ml		43–1 mg/ml		36–0·1 mg/ml	
	No. positive	Weal dia. (mm)	No. positive	Weal dia. (mm)	No. positive	Weal dia. (mm)
D. farinae	40	3·4	36	2·3	21	0·8
D. farinae precipitate fraction	36	6·3	36	3·7	28	1·7
D. farinae supernatant fraction	26	1·4	16	0·47	3	—
D. pteronyssinus	36	2·6	27	1·3	12	1
House dust (Bencards Ex 8)	36	1·7	19	0·5	3	—

Table 1 shows that, whilst there were comparable numbers of positive reactions, the extract of *D. farinae* was about ten times stronger than that of *D. pteronyssinus*. The precipitate ('protein') fraction of *D. farinae* was in turn ten times stronger than the unfractionated extract of *D. farinae* and 100 times stronger than the supernatant ('polysaccharide') fraction, which also gave fewer positive reactions. It was also 100 times stronger than the house dust extract which however gave comparable numbers of positive reactions.

The material removed by dialysis from the original extract of *D. farinae* was tested at 10 mg/ml and gave weak reactions in two of eight patients who reacted to the precipitate fraction. Similarly, the filtrate which passed through a 10,000 molecular size Amicon-Diaflo membrane-filter gave a weak reaction in only one subject. There seems to be little if any small molecular allergenic material in the *D. farinae* extract.

Prick tests with the ammonium sulphate supernatants of extracts of coffee and bagasse

were made with concentrations of 25–100 mg/ml and positive though weaker reactions were obtained to the coffee extract in sixteen out of eighteen and to the bagasse extract in twelve out of eighteen patients giving positive reactions to *D. farinae*.

Serological test results

RAST tests for IgE. The sera of twenty asthmatic patients giving strong prick test reactions to *D. farinae* extract 10 mg/ml were tested for specific IgE to extracts of *D. farinae*, Gaines dog-meal and yeast and to house dust extract (Meda/Dome). Table 2 shows that specific IgE

TABLE 2. RAST tests for specific IgE*

Patient No.	Clinical history house dust allergy	Early onset asthma	Late onset asthma	Prick test *D. farinae*	Prick test other	RAST, IgE, results			
						House dust ('Meda/Dome')	*Dermato-phagoides farinae* extract	Gaines dog-meal extract	Dried yeast extract
1	+		+	++++	0	1·9	12·5	1·4	1·4
2	0		+	+++	0	8·1	270	1·2	1·4
3	±		+	++	±	2·8	68	0·9	1·2
4	0		+	+++	0	1·3†	7·6	0·7	1·2
5	±		+	++++	0	4·1†	86	1·0	1·5
6	+		+	++	0	1·8†	1·9	1·0	1·4
7	0		+	++++	0	4·5	57	2·5	1·5
8	+	+		++	3	1·8†	26	1·1	0·7
9	0		+	+±	0	0·8	1·7	0·9	0·8
10	+		+	+++	0	1·4	46	1·2	1·2
11	+		+	++	0	1·1	5·6†	1·3	1·3
12	+		+	+++	0	3·8†	155	1·1	1·2
13	0		+	+±	0	0·9	4·2	1·2	0·8
14	0		+	++	0	0·9	5·6	1·4	0·9
15	±		+	+++	0	1·5†	40	1·4	0·8
16	0	+	+	+++	4	1·4	5·8	1·1	1·4
17	+	+		+++	4	2·5	24†	1·3†	1·4
18	+	+		+++	5	2·3†	37	1·0†	0·7
19	+	+		+++	3	1·6†	22	0·9	1·0
20	+	+		+++	5	7·4	110	1·2	1·3

* Values expressed in SU.
† Positive precipitin test to the relevant extract, all other tests negative.

antibodies were present in eighteen out of the twenty sera to the *D. farinae* extract, to the dog-meal extract in one, in none to the yeast and in eight to the house dust extract. There was no correlation between positive IgE and precipitin tests to extracts of *D. farinae*, Gaines dog-meal or yeast.

The amounts of IgE to the *D. farinae* extract in the different patients did not show any correlation with early or late onset of asthma, the presence of a history of house dust allergy, or whether the patient was allergic only to the house dust extract or to a number of commoner allergens. The two patients (2 and 12) with the highest values are of interest because they

developed their symptoms after coming to the United Kingdom and like the majority of such subjects were allergic only to house dust, suggesting that they had been sensitized by the mite allergen, encountered either for the first time or in large amount in Britain. In the small group of seven patients giving moderate (+ ± and + +) prick test reactions to the *D. farinae* extract, five had SU values below 10 and the mean for the group was 12·7 whereas in the thirteen giving stronger (+ + + and + + + +) reactions, the values were higher, all except two having values above 10, with a mean for the group of 67·1.

Precipitin tests

In the total number of patients with different sorts of lung disease tested (Table 3), fewer reactions were given to the *D. farinae* precipitate fraction (thirty-two out of 196) than to

TABLE 3. Agar-gel micro-double-diffusion precipitin tests

Sera	No. tested	*D. farinae*		House dust	Coffee kiboko extract	Gaines dog-meal	Yeast
		Precipitate fraction	Supernatant fraction				
Asthma (skin test positive to *D. farinae*)	89	19 21%	16 18%	42 47%	41 46%	10 11%	2 2%
Asthma-intrinsic and aspirin sensitive	27	6 22%	6 22%	14 52%	8 30%	4 15%	0 0%
Bronchitis	20	0 0%	2 10%	3 15%	NA NA	0 0%	NA NA
Cryptogenic fibrosing alveolitis	20	2 10%	6 30%	8 40%	NA NA	2 10%	NA NA
Farmers' lung	22	3 14%	5 23%	9 41%	10 42%	4 18%	2 9%
Sarcoidosis	18	2 11%	5 27%	5 27%	NA NA	1 5·5%	NA NA
Total	196	32	40	81	59/138	21	4/138

NA, Not available.

the supernatant fraction (forty out of 196). The house dust extract gave the most reactions (eighty-one out of 196); the dog-meal extract gave reactions in twenty-one out of 196 and the yeast extract the fewest (four out of 138). The coffee extract reacted in fifty-nine out of 138. There were small differences in the frequency of reactions in the different groups of patients and they did not appear to be related to particular diseases. Precipitation reactions to *D. pteronyssinus* were given by two out of twelve farmers' lung sera and three out of twelve asthmatic sera.

Absorption test with dog-meal and yeast extract

The dog-meal extract (Table 4) inhibited only one each of the reactions to *D. farinae* precipitate fraction, supernatant fraction and house dust and three of the coffee extract

TABLE 4. Absorption tests

Reactions to:	No.	Absorption with dog-meal extract		
		+	±	0
D. farinae precipitate fraction	6	0	1	5
D. farinae supernatant fraction	8	1	0	7
House dust (Bencards Ex 8)	10	1	0	9
Coffee extract	10	2	1	7

+, Complete inhibition; ±, partial inhibition; 0, no inhibition.

reactions, two completely and one partially. Absorption of the sera with yeast extract had no inhibitory effect on their reactions to the *D. farinae* extract.

Absorption test with D. farinae precipitate and supernatant fractions

Table 5 shows that in absorption tests on sera giving reactions to *D. farinae* the *D. farinae* precipitate fraction had an inhibitory effect on all of its own three reactions, one completely

TABLE 5

		Reactions to:	
		D. farinae precipitate fraction	*D. farinae* supernatant fraction
No. tested		3	4
Absorption with:			
D. farinae	+	1	1
precipitate fraction	±	2	3
	0	0	0
D. farinae	+	3	2
supernatant	±	0	1
fraction	0	0	1
House dust	+	3	3
(Bencards)	±	0	1
	0	0	0
Coffee	+	1	2
(kiboko)	±	1	1
extract	0	(1, NA)	(1, NA)
Cladosporium	+	0	0
herbarum	±	0	1
	0	3	3

+, Complete inhibition; ±, partial inhibition; 0, no inhibition.

and two partially, and in all four reactions to the supernatant fraction, one completely and three partially. The supernatant inhibited all the reactions to the precipitate fraction and either completely or partially three out of four of its own reactions. The house dust (Bencard's) extract inhibited completely all three of the reactions to the *D. farinae* precipitate fraction and three out of four of the supernatant fraction reactions and partially inhibited the fourth. The coffee extract inhibited completely one out of two of the precipitate fraction reactions and the other partially; and completely inhibited two out of three of the supernatant fraction reactions and the other partially. The *C. herbarum* extract had little or no inhibitory effect on either of the reactions to the *D. farinae*.

In absorption tests on four sera giving reactions to the house dust extract the *D. farinae* precipitate fraction inhibited one completely and two partially and the supernatant fraction inhibited two completely and one partially.

Absorption with extracts of *D. farinae* of two sera giving positive reactions to *D. pteronyssinus* had no effect on the reactions; whereas absorption with house dust extract completely inhibited the *D. pteronyssinus* reactions.

Precipitin tests on the γ-globulin fraction of the sera

Table 6 shows that precipitation reactions to extracts of *D. farinae*, house dust, coffee 'kiboko' and *D. pteronyssinus* were obtained both with the original serum and the γ-globulin fraction separated by the method of Levy & Sober (1960).

TABLE 6. Precipitation reactions of extracts of whole serum and γ-globulin

Patient	Whole serum				γ-globulin fraction			
	D. farinae	House dust	Coffee 'kiboko'	*D. pteronyssinus*	*D. farinae*	House dust	Coffee 'kiboko'	*D. pteronyssinus*
Pr.	+	+	±	0	+	+	+	0
Br.	0	0	0	+	0	0	0	+
Go.	+	±	0	NA	+	+	+	+
Wo.	+	+	+	0	+	+	+	0

DISCUSSION

The use of extracts of *D. farinae* cultured on dog-meal as an alternative to cultures of *D. pteronyssinus* cultured on human dander will be influenced by the assessment of their content: (1) of related allergens for diagnostic purposes in patients with house dust allergy, (2) of related antigens likely to be of value for hyposensitization, and (3) of allergens and antigens derived from the culture medium.

There are a number of reports showing close correlations between skin test reactions to extracts of *D. pteronyssinus* and of *D. farinae* in patients with histories of house dust allergy and giving as a rule weaker reactions to house dust extracts (Ishizaki *et al.*, 1967; Miyamoto *et al.*, 1968; Maunsell *et al.*, 1968; Pepys *et al.*, 1968a; Brown, 1968). These have been confirmed by Voorhorst, Spieksma & Varekamp (1969), and Pepys *et al.* (1968a) have also shown that inhalation tests with extracts of *D. farinae* in house dust sensitive patients,

provoke immediate asthmatic reactions. These findings suggest that the *D. farinae* extract has allergens similar or related to those of *D. pteronyssinus* and that it is acceptable for diagnostic skin tests. There is, however, suggestive evidence in small numbers of patients of other allergens in house dust besides those from the genus *Dermatophagoides* (Maunsell *et al.*, 1968; Pepys *et al.*, 1968a; Bernecker, 1968).

The demonstration that specific IgE antibodies are present against extracts of *D. farinae* in house dust sensitive patients in the United Kingdom confirms the earlier report on patients in Finland and Sweden by Stenius & Wide (1969). In our series there was no correlation of IgE titre to the *D. farinae* extract with the onset of the asthma and the degree of general atopic sensitivity of the subjects and the precipitin reactions. There was a suggestion that the titres of IgE may be related to the skin test reactions to the *D. farinae* extract. These findings confirm that *D. farinae* extracts are a source of allergens, though further studies are needed to see how close a correlation there is with *D. pteronyssinus* allergens.

The tests for IgE showed differences in the frequency of reactions to the dog-meal and yeast extracts in the United Kingdom and Scandinavian patients. Whereas in the United Kingdom patients a positive test for specific IgE was found against the dog-meal extract in one out of twenty and against the yeast extract in none, the Scandinavian sera (Stenius & Wide, 1969), gave positive reactions to dog-meal and to yeasts in eight out of thirty-three cases, of whom three reacted to both extracts. Prick tests showed similar differences, the United Kingdom subjects giving positive reactions to dog-meal in two out of twenty-seven and to yeast in five out of twenty-two (Pepys *et al.*, 1968a), and the Scandinavian subjects gave positive reactions to dog-meal and yeast extract in twelve and thirteen, respectively, out of thirty-three cases. This probably reflects allergy to different environmental and dietary factors. These findings are relevant to the assessment of specific IgE or other reactions to extracts of *D. farinae* or other mites grown on dog-meal, yeast and other media, particularly since there is evidence of differences in the patterns of allergic sensitivity in different countries.

The presence of precipitins in man against the extracts of *D. farinae* and *D. pteronyssinus* should make it possible to study the mite antigens in the subjects for whom such materials may be used for hyposensitization. Absorption experiments showed the presence of related antigen in extracts of house dust and *D. farinae*, and in house dust and *D. pteronyssinus* but so far none between *D. farinae* and *D. pteronyssinus*. In the latter respect therefore, there is still doubt as to whether *D. farinae* extracts contain antigens that might be effective in stimulating the appearance of antibodies capable of blocking reactions to the more relevant antigens in *D. pteronyssinus*. Both fractions of *D. farinae* contain identical polysaccharide or glycopeptide antigens including the 'D' region type and components related to a polysaccharide antigen of *C. herbarum*. Whilst the precipitate fraction of *D. farinae* extracts is of greater allergenic potency for skin tests than the supernatant fraction, no additional precipitins to the protein components have yet been observed in man and more tests are in progress.

The presence of precipitins to the culture medium extracts raises similar problems of interpretation of results to those of the IgE reactions to these extracts. In addition, in populations with a high incidence of allergy to the medium components such as the fish meal, care will be needed to prevent clinical reactions if such materials are likely to be present in the mite culture extracts used for diagnosis and treatment. The serological reactions to the vegetable dust extract used here, namely the kiboko dust from the flesh of the coffee berry and the skin test reactions in house dust sensitive patients to extracts of this material and

of bagasse, also show the widespread distribution of related allergens. It is not clear yet whether these particular reactions are being elicited by antigens of vegetable origin or by mites which are commonly present in these dusts. The so-called 'D' antigen present in vegetable dusts and *D. farinae* cultures on dog-meal seems to be of polysaccharide or glycopeptide nature. Much of the work on characterization of house dust allergens has been made on 'glycopeptide' (Rimington, Stillwell & Mansell, 1947) or 'glycoprotein' materials (Berrens & Young, 1961), and they are clearly of interest because of the presence of apparently similar allergens in different materials.

It was not possible to correlate the precipitin reactions to *D. farinae* with particular pulmonary disease and more work is needed. The possible role of the precipitins in allergic reactions must be considered, because there is now much evidence that in patients giving immediate reactions and with precipitins against the particular extract, the immediate reactions are followed after several hours by a second reaction, regarded as an Arthus, Type III, reaction (Pepys *et al.*, 1968b). In such subjects the clinical manifestations and provocation tests show the participation of Type III reactions in the respiratory disease, which tends to be of a more serious nature than uncomplicated immediate, Type I reactions, producing tissue reactions which may cause irreversible damage. Dual reactions to prick tests, an immediate wealing response followed several hours after its resolution by a second large oedematous reaction which resolves within about 24 hr, have been observed in repeated prick tests in some subjects. With the ubiquitous presence of potent allergenic material of this sort it is possible that Type III allergy may be found more frequently. It may limit the toleration of hyposensitization treatment, and will need to be included in the assessment of hyposensitization treatment in house dust allergy. It is also possible that this type of allergy may be playing a part in the late skin and inhalation test reactions observed by others (Herxheimer, 1952; Buffe *et al.*, 1963). If present it could determine the slow development of clinical reactions in response to everyday exposure.

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