

Human dander in house dust allergy

W. D. BRIGHTON and M. D. TOPPING

*Laboratory for Allergens, National Institute for Biological Standards and Control,
Holly Hill, London*

Summary

The possible role of human dander in house dust allergy was investigated. Naturally shed human mite-free skin squames were collected from bedding and used to prepare a human dander extract. When the extract was coupled to cyanogen bromide-activated paper discs, and used in the RAST assay, IgE titres to the skin extract were observed in the sera from several patients with house dust allergy. The sera with IgE to the skin extract also had high IgE titres to either house dust, *D. pteronyssinus* or cat fur. RAST inhibition studies revealed cross-reaction between the human skin extract and both a *D. pteronyssinus* extract and a cat fur extract.

Introduction

Since house dust allergy was first recognized over 50 years ago, research has been directed towards gaining an understanding of the nature and origin of the allergens in the dust. Although it is known that house dust may contain a complex mixture of potentially allergenic components, including feathers, human and animal dander, moulds and house dust mites, the problem remains as to how many of these compounds are responsible for house dust allergy. Since the role of the house dust mite (*Dermatophagoides* sp.) in house dust allergy was first recognized by Voorhorst *et al.* (1967), many reports have been published (Maunsell, Wraith & Cunningham, 1968; Pepys, Chan & Hargreave, 1968; Miyamoto *et al.*, 1968; McAllen, Assem & Maunsell, 1970) showing a correlation between house dust sensitivity and house dust mite sensitivity, and also cross-reactivity between the two allergen preparations. These reports led to the concept that the mite was the most important allergen in house dust sensitivity (Voorhorst, 1970; Halmi & Alexander, 1971; Morita *et al.*, 1975). However, it was soon recognized that some skin test reactions given by mite extracts were due, not to the mites, but to components in the culture media used to culture the mites (Stenius & Wide, 1969). Further analysis by other workers revealed that these reactions were, in some cases, due to the human epithelial material in the culture media (Dasguta & Cunliffe, 1970; Kawai *et al.*, 1972; Wolfenberger *et al.*, 1974). Earlier studies by Berrens, Morris & Young (1966) demonstrated that a dandruff extract elicited skin

Correspondence: Dr W. D. Brighton, Laboratory for Allergens, National Institute for Biological Standards and Control, Holly Hill, London N.W.3.

test reactions in some house dust sensitive patients. Both these observations, and the finding by Lees (1973) that sieved house dust contains as much as 90% human skin squames, prompted us to prepare skin extracts, examine sera by RAST for IgE antibodies to the extracts, and to study cross-reactivity between mite extracts and the skin extracts.

Materials and methods

Collection of mixed human dander

Naturally shed human skin was collected by asking volunteers to sleep naked between clean sheets and then to vacuum-clean their sheets just before changing them. A new, previously unused, hand-held vacuum cleaner was provided specially for this purpose. After each use the material which had collected in the cleaner was brushed out, and the hairs and fibres removed with tweezers. The samples of material were examined by both differential interference microscopy and the flotation method of Maunsell *et al.* (1968), and found to be free from house dust mites. The material was stored at 4°C until used.

Preparation of mite-free skin

Although no evidence could be found that the mixed skin extract contained mites, there was still the possibility of contamination by mite faeces, etc. A preparation of mite-free human skin squames was therefore made by the following method, which was impractical for the production of a large amount of extract, but by which process a small quantity of material could be produced for reference purposes.

A male volunteer bathed and showered under running water, and then dried himself with a sterile, freshly laundered towel. He let his skin dry thoroughly without allowing contact with chairs, etc., and then scraped his skin with a stainless steel knife to liberate skin squames. The liberated squames were collected on the knife, and transferred to a sterile bottle. The yield was 60 mg of creamy white particles.

Preparation of human dander extracts

(a) Mixed skin from a number of volunteers (70 g) was stirred with 700 ml of Coca's solution (0.085 M NaCl, 0.03 M NaHCO₃ and 0.043 M phenol) for 48 hr at 4°C, to extract soluble material from the skin squames. Insoluble material was removed by centrifugation at 100,000 g, and the supernatant used as a mixed skin extract. Preliminary studies showed that the concentration of the extract, as determined by dry weight, was 9.1 mg/ml; the material was not toxic in rat skin at a concentration of less than 0.5 mg/ml and gave a precipitin line with anti-human albumin in gel diffusion.

(b) The pure skin from a single volunteer (60 mg) was mixed for 48 hr at 4°C with 2.4 ml of Coca's solution, then centrifuged at 2000 g to remove insoluble material. The supernatant was used as a mite-free pure skin extract.

RAST assays

Mixed skin extract allergen discs and mite-free pure skin extract allergen discs were prepared by coupling the extracts to cyanogen bromide-activated paper discs, as described by Brighton, Lampard & Fordham (1975). Unreacted sites on the discs were blocked with EPA 33 prepared by the method of Aalberse (1974). In addition, allergen discs were made from a cat fur extract (10% saline extract), a *D. pteronyssinus*

extract (Bencard, Worthing, Sussex) and a house dust extract (a mixture of pH 3.0 and acetone fractions, prepared as described previously by Brighton & Topping, 1977).

Serum IgE concentration was determined by incubating allergen discs with 200 μ l of an appropriate dilution of serum, and labelling bound IgE with ^{125} I-labelled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden). The ^{125} I bound to the discs was counted in a Packard Tricarb Scintillation Counter.

The RAST titre for an allergen was expressed as the number of times normal by the following equation:

$$\frac{\text{ct/min bound to disc incubated with test serum}}{\text{ct/min bound to disc incubated with known negative serum}}$$

RAST inhibition

RAST inhibition experiments were carried out by the method of Brighton (1977).

Results

Sera from patients with positive skin tests to house dust, *D. pteronyssinus* or cat fur were used in the RAST assay. Dilutions of each of these sera were incubated overnight with the following allergen discs: mixed human skin extract; mite-free pure skin extract; house dust; *D. pteronyssinus*; and cat fur. In addition, negative serum, which had been shown by RAST to lack IgE to common allergens, was similarly incubated with the allergen discs. The RAST results in Table 1 showed that seven of the sera had significant RAST titres (greater than twice that of negative serum) to the mixed skin extract, and that the two sera with the highest titres to the mixed skin extract (No. 310 and 320) had RAST titres to the mite-free pure skin extract. The lower titres to the mite-free pure skin extract are probably due to a low concentration of soluble allergens in the extract used to make the discs, due to the prolonged bathing of the donor.

Table 1. RAST titres observed for human sera tested with five allergen extracts: mixed skin extract; mite-free pure skin extract; house dust extract; *D. pteronyssinus* extract; and cat fur extract

Serum no.	RAST titres				
	House dust	<i>D. pteronyssinus</i>	Cat fur	Mixed skin	Mite-free pure skin
195	2.4	1.2	12.8	5.0	0.9
264	5.2	12.4	4.4	2.6	1.1
279	4.1	8.0	1.2	1.5	0.8
283	5.7	8.4	0.9	2.4	1.0
285	2.2	9.0	1.3	1.2	1.0
297	1.6	1.6	24.9	5.5	0.9
305	3.3	3.6	1.9	1.4	0.9
309	2.5	9.6	8.8	3.0	1.7
310	12.3	26.8	46.6	9.1	2.6
320	19.5	53.5	19.8	9.7	2.4
323	1.8	6.9	14.4	1.6	0.9
329	5.0	24.3	0.9	1.5	0.8
330	2.9	12.6	1.3	0.9	0.9

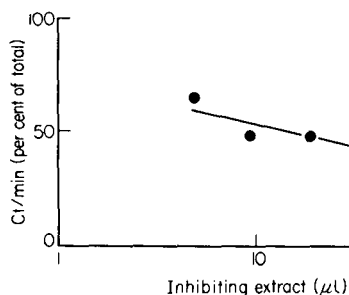


Fig. 1.

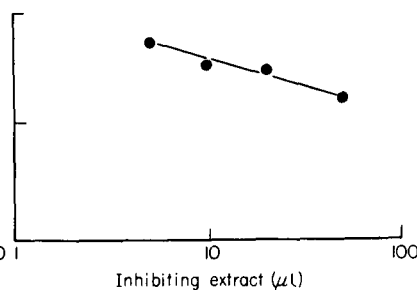


Fig. 2.

Fig. 1. RAST inhibition of cat fur allergen discs by the mixed skin extract.

Fig. 2. RAST inhibition of *D. pteronyssinus* allergen discs by the mixed skin extract.

With all the sera except one (No. 283), a RAST titre to the skin extract was associated with high RAST titres to the cat fur extract. This finding led us to look for cross-reactivity between the mixed skin extract and the cat fur extract.

An appropriate dilution of a serum which had IgE to cat fur was incubated with increasing amounts of the skin extract, then with a cat fur allergen disc. The mixed skin extract reduced the amount of IgE bound to the allergen disc, thus demonstrating cross-reactivity between the mixed skin extract and the cat fur extract (Fig. 1).

It was also observed that the RAST titres to the mixed skin extract were associated with titres to the *D. pteronyssinus* extract. Consequently, RAST inhibition was used to look for cross-reactivity between the *D. pteronyssinus* extract and the mixed skin extract. The results in Fig. 2 show partial inhibition of the *D. pteronyssinus* allergen discs by the mixed skin extract.

Discussion

The RAST results presented in this paper indicate that some patients suffering from house dust allergy have IgE antibodies to material present in the mixed skin extract, which suggests that skin allergens are important in house dust sensitivity. However, it is possible that the RAST titres observed with the mixed extract resulted from contamination of the skin with mite droppings or with mite debris. In order to see if this was the case, a mite-free pure skin extract, which could not possibly be contaminated with mites or mite droppings, was prepared from skin scraped from the body of a volunteer who had just taken a bath. The finding that two sera had RAST titres to this extract confirms that the RAST titres observed with the mixed skin extract result from antigens derived directly from skin. The lower titres observed with the mite-free pure skin extract are probably due to a low concentration of allergen in the pure skin extract.

It must now be considered as to whether the skin allergens represent an autoallergy or incompatibility reaction to genetically determined antigens. Brocteur & Versie (1967) reported the presence of blood group antigens A, B, H, Le^a and P in house dust preparations, and considered that these antigens might be partially responsible for house dust allergy. However, Berrens, Guikers & Van Rijswijk-Verbeek (1975) subsequently reported that there is no relationship between the blood group activity of a house dust preparation and its skin test reactivity, indicating that the blood group antigens are not involved in house dust allergy. Further work on the skin fractions is

planned to ascertain whether the IgE is formed against denatured skin or serum proteins, or against specific genetically determined antigens.

Since cross-reaction was observed between the mite extract and the skin extract, cross-reaction between mite extracts and house dust extracts does not prove that mites are the sole, or even the main, antigens in house dust, as the skin-derived material in the dust also cross-reacts with the mite extracts. The cross-reaction between the mite extract and the skin fraction might result from the presence in the final extract of components of the media used to culture the mites.

The cross-reaction between the human skin fractions and the cat fur extract may be due to cross-reaction between human albumin and cat albumin, since we have shown that human albumin is present in skin extracts, and Stokes & Turner (1975) have shown that cat albumin is a major allergen in cat sensitivity. However, this would imply that the human albumin is in some way antigenically different from the native albumin in the blood.

The mixed skin extract is being prepared as a standard human skin extract, and will be available from the National Institute of Biological Standards and Control to bona fide workers interested in studying the role of material derived from human skin in house dust allergy.

References

- AALBERSE, R.C. (1974) *Immunoglobulin E, Allergens and their Interaction* (Ph.D. Thesis). University of Amsterdam.
- BERRENS, L., GUIKERS, C.L.H. & VAN RIJSWIJK-VERBEEK, J. (1975) Blood group activity of allergenic preparations. *Acta Allergologica*, **30**, 184.
- BERRENS, L., MORRIS, J.H. & YOUNG, E. (1966) Studies on the human dandruff allergen. *Dermatologica*, **132**, 433.
- BRIGHTON, W.D. (1977) Standardisation and control of allergen extracts. *Proceedings of the IX International Congress on Allergy*. (In press.)
- BRIGHTON, W.D., LAMPARD, J. & FORDHAM, R. (1975) Activated paper as an immunoabsorbent. International WHO-IABS Symposium on standardisation and control of allergens administered to man. Geneva 1974. *Developments in Biological Standardisation*, **29**, 166.
- BRIGHTON, W.D. & TOPPING, M.D. (1977) Standards from house dust. *Proceedings of the IX International Congress on Allergy*. (In press.)
- BROCTEUR, J. & VERSIE, R. (1967) Mise en évidence de divers facteurs de groupes sanguins dans des extraits allergéniques du poussières de maison. *Acta Allergologica*, **22**, 118.
- DASGUTA, A. & CUNLIFFE, A.C. (1970) Common antigenic determinants in extracts of house dust and *Dermatophagoides* species. *Clinical and Experimental Immunology*, **6**, 891.
- HALMI, Zs. & ALEXANDER, F.A.R. (1971) Studies on the house dust allergen. *Allergie und Immunologie Band*, **17**, 69.
- KAWAI, T., MARSH, D.G., LICHENSTEIN, L.M. & NORMAN, P.S. (1972) The allergens responsible for house dust allergy. I. Comparison of *Dermatophagoides pteronyssinus* and house dust extracts by assay of histamine release from allergic human leukocytes. *Journal of Allergy and Clinical Immunology*, **50**, 117.
- LEES, J. (1973) *Studies on the Detection, Dissemination and Immunological Interactions of Some Allergens* (Ph.D. Thesis). University of London.
- MAUNSELL, K., WRAITH, D.G. & CUNNINGTON, A.M. (1968) Mites and house dust allergy in bronchial asthma. *Lancet*, **i**, 1267.
- MCALLEN, M.K., ASSEM, E.S.K. & MAUNSELL, K. (1970) House dust mite asthma. Results of challenge tests on five criteria with *Dermatophagoides pteronyssinus*. *British Medical Journal*, **2**, 501.
- MIYAMOTO, T., OSHIMA, S., ISHIZAKI, T. & SATO, S. (1968) Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes 1961) and house dust as a causative antigen in bronchial asthma. *Journal of Allergy and Clinical Immunology*, **42**, 14.
- MORITA, Y., MIYAMOTO, T., HORIUCHI, Y., OSHIMA, S., KATSUHATA, A. & KAWAL, S. (1975). Further

- studies in allergenic identity between house dust and the house dust mite, *Dermatophagoides farinae* Hughes 1961. *Annals of Allergy*, **35**, 361.
- PEPYS, J., CHAN, M. & HARGREAVE, F.E. (1968) Mites and house dust allergy. *Lancet*, **ii**, 1270.
- STENIUS, B.G. & WIDE, L. (1969) Reaginic antibody (IgE), skin and provocation tests to *Dermatophagoides culinae* and house dust in respiratory allergy. *Lancet*, **ii**, 455.
- STOKES, C.R. & TURNER, M.W. (1975) Isolation and characterization of cat allergens. *Clinical Allergy*, **5**, 241.
- VOORHORST, R. (1970) Quantitative aspects of the problem of house dust atopy and house dust mites. *Acta Allergologica*, **25**, 237.
- VOORHORST, R., SPIEKMA, F.T.H.M., VAREKAMP, H., LEUPEN, M.J. & LYKLEMA, A.W. (1967) The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *Journal of Allergy*, **49**, 325.
- WOLFENBERGER, V., BOLEY, R.B., HALL, C.C. & ZELENZUK, L.D. (1974) The occurrence of antigens of *Dermatophagoides farinae* Hughes in allergenic extracts of house dust. *Annals of Allergy*, **32**, 252.

Copyright of Clinical Allergy: Journal of the British Allergy Society is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.