

Mites and house dust allergy. IV. Antigen and allergen(s) of *Dermatophagoides pteronyssinus* extract

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Summary

The antigenic composition of *D. pteronyssinus* extract was investigated by using antisera raised in rabbits by injection of the mite extract in Freund's adjuvant. Immunodiffusion analysis revealed the presence in the mite extract of many antigenic constituents, most of which were also common to human dander and house dust extracts. Four antigenic components not shared by human dander appeared to be common to *D. farinae* extract.

D. pteronyssinus extract was then fractionated on Sephadex G-75 and DEAE-Sephadex. Immunodiffusion analysis of the most active fraction revealed, after heat-treatment, the presence of three precipitin lines, one of which appeared not to be shared by nutrient medium or by *D. farinae* extract constituents.

When the anti-mite rabbit serum was fixed to an immunosorbent and incubated with a house dust extract it was constantly able to induce a strong decrease or a complete loss of the allergenicity of the dust extract in man. These results suggest that mite allergen(s) are immunogenic in the rabbit and provide more direct evidence for identity of the allergens in house dust and mite.

Introduction

The epidemiological and immunological evidence associating the house dust mite *Dermatophagoides pteronyssinus* and related species with house dust allergy is well documented (Voorhorst *et al.*, 1967; Maunsell, Wright & Cunningham, 1968; Miyamoto *et al.*, 1968; Pepys, Chan & Hargreave, 1968; Holford-Strevens *et al.*, 1970). In previous papers we ourselves studied the relationship between dust and mite allergens by comparison of skin reactions (Biliotti *et al.*, 1972) and by fractionation methods (Romagnani *et al.*, 1972). Cell-mediated and humoral immune responses to house dust and mite constituents in atopic and non-atopic individuals were also investigated by us (Romagnani *et al.*, 1973).

However, up to now a direct demonstration of an allergenic identity between

dust and mite extracts has not been furnished. Moreover, the physicochemical characteristics and immunologic properties of mite allergen(s) have been little investigated.

This paper concerns the study of the antigenic composition of mite extracts by the use of antisera raised in rabbits against these extracts. Attempts to separate and characterize the allergenic components of *D. pteronyssinus* and to furnish more direct evidence for an allergenic identity between mite and dust are also reported.

Materials and methods

Allergenic extracts. *D. pteronyssinus*, house dust and human dander extracts were furnished by Diephuis Laboratorium (Groningen). *D. farinae* extract was purchased by Bencard (Brentford, Middlesex). A *Saccharomyces cerevisiae* extract was kindly supplied to us by Dr Dermer, Diephuis Laboratorium. All these extracts were repeatedly dialysed against 0.002 M NH_4HCO_3 at pH 7.8 and finally against distilled water; they were then freeze-dried and weighed.

Antisera. Anti-*D. pteronyssinus* extract sera were prepared by immunizing two rabbits with dialysed mite extract emulsified in complete Freund's adjuvant. The rabbits each received one injection of 10 mg of antigen followed, at fortnightly intervals, by five separate injections of 10 mg of antigen. They were bled out 10 days after the last injection and their sera pooled.

Immunodiffusion and immunoelectrophoresis. Dust, mite (*D. pteronyssinus* and *D. farinae*) and mite culture medium (human dander and powdered yeast) extracts were compared against rabbit anti-mite sera by micro-Ouchterlony (Crowle, 1958) and immunoelectrophoresis techniques. Different concentrations (dry weight) of each extract were always used. Taking into account that the reactions of micro-Ouchterlony may vary, presumably for technical reasons, only undoubted precipitin patterns were considered.

Heat-treatment. Before analysis some samples were subjected to heat-treatment at 100°C for 20 min in glass tubes closed with screw caps. In one experiment heating at 100°C was extended to 40 min and 1 hr.

Fractionation procedures. Gel-filtration of *D. pteronyssinus* extract was performed on a column (2.5 × 100 cm) of Sephadex G-75 (Pharmacia, Uppsala) using phosphate buffered saline, pH 7.2. Bovine serum albumin and cytochrome C were run through the column as molecular weight marker proteins. One hundred and fifty milligrams of lyophilized mite extract were added to the same column and eluted at a flow of 30 ml/hr with a peristaltic pump. The optical density of the eluate was measured continuously at 280 mμ by a Fracto-Scan absorptiometer with potenziometer recorder and the eluate was collected with a Fractomat fraction collector (Buchler Instruments) in 10-ml fractions. Sugar and protein content of the fractions was evaluated by the orcinol and Lowry methods, respectively.

The allergenic activity of different fractions was estimated by the outcome of skin tests on humans sensitive to *D. pteronyssinus*. On the basis of the results of skin tests, the fractions were pooled, repeatedly dialysed against distilled water and lyophilized. The fractions of the first and third pool were gel-filtered twice more on the same column of Sephadex G-75. The material of the second pool, which showed the highest allergenic activity, was fractionated on a DEAE-Sephadex column (2.5 × 10 cm) equilibrated with a phosphate buffer 0.05 M pH 7.8. After extensive dialysis against this buffer the sample was added on top of the column and eluted by stepwise increase of

NaCl in the starting buffer. Three fractions were collected and tested, for skin activity on sensitive patients and for antigen analysis against anti-mite rabbit serum.

Immunosorbent technique. A rabbit anti-mite and a rabbit anti-*Holcus lanatus* grass pollen extract serum were used. Gammaglobulins of these sera were obtained by precipitation with 18% Na_2SO_4 and incubated with CnBr-activated micro-crystalline cellulose, according to the method described by Wide (1969). For this purpose 3.5 ml of the gammaglobulin solution were absorbed on 3.5 g of activated polymer, which was then washed. One millilitre of a 1% house dust and 0.5% mite extract were incubated overnight with both the anti-mite and anti-*Holcus lanatus* cellulose-absorbed rabbit gammaglobulins. The supernatants were dialysed, sterilized by filtering on Millipore and injected, at ten-fold serial dilutions, in a group of ten atopic patients who showed positive skin reactions to mite and house dust extracts.

Skin testing. 0.02 ml of each sample were injected intradermally in the volar aspect of both forearms of sensitive patients. Skin reactions were evaluated as previously described (Biliotti *et al.*, 1972).

Results

When the anti-mite rabbit serum was compared against *D. pteronyssinus* extract by immunodiffusion on slides coated with 5 mm of agar, only a few precipitin lines were

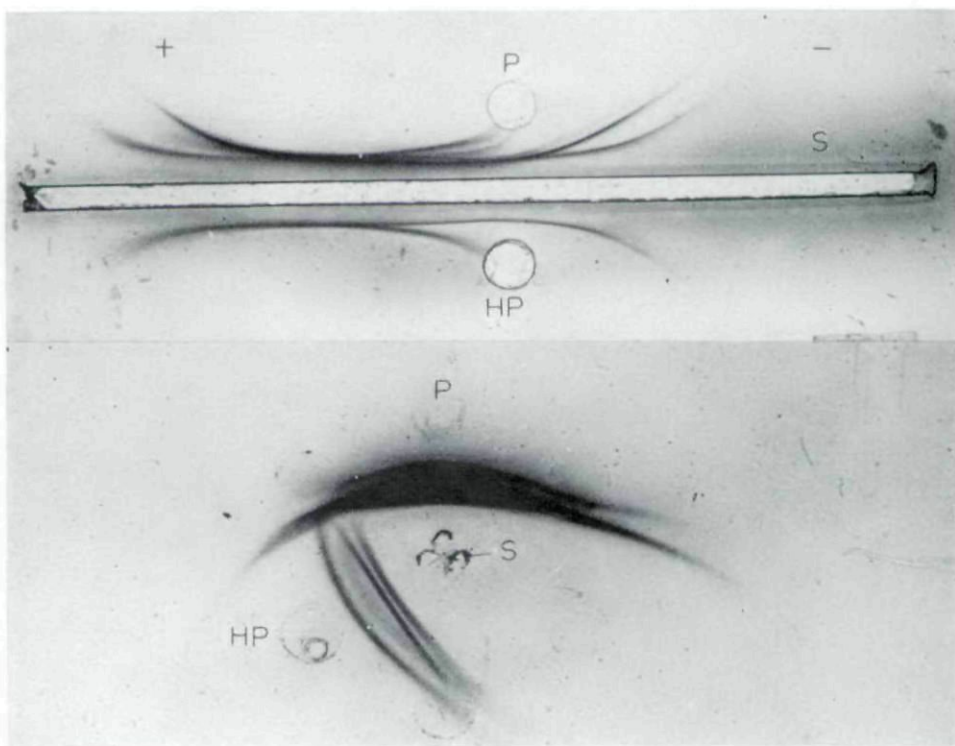


Fig. 1. Precipitation pattern of anti-*D. pteronyssinus* extract rabbit serum by immunoelectrophoresis and micro-Ouchterlony techniques. P = unheated *D. pteronyssinus* extract; HP = *D. pteronyssinus* extract heated for 20 min at 100°C; S = anti-mite rabbit serum. The mite extract was used at a concentration of 40 mg/ml (dry weight) in immunoelectrophoresis and of 25 mg/ml in micro-Ouchterlony.

observed, but by immunoelectrophoresis and micro-Ouchterlony techniques many more precipitin lines could be detected (Fig. 1).

When either a mite culture medium (human dander and powdered yeast, equal weighparts) or a human dander extract were tested with the same antiserum, five precipitin lines were seen. No precipitin lines were ever observed between anti-mite serum and a *Saccharomyces cerevisiae* extract, although it was tested at concentrations ranging between 10 μ g and 80 mg/ml.

At least five and probably six precipitin lines remained with the *D. pteronyssinus* extract after absorption of the rabbit antiserum with human dander. When the human dander-absorbed rabbit serum was compared against a *D. farinae* extract, four precipitin lines were detected.

After heating of the *D. pteronyssinus* extract at 100°C for 20 min only four and perhaps five precipitin lines remained (Fig. 1), three of which appeared to be shared by human dander.

Two out of four cross-reacting *D. farinae* antigens appeared to be heat-stable constituents. In this connection it should be noted that the heat-treatment of the mite extract resulted in a very slight reduction of its allergenicity in man. No further loss of allergenic activity was observed even if heating at 100°C was extended to 40 min and 1 hr.

When the rabbit serum was tested by the micro-Ouchterlony with a house dust extract, six precipitin lines were seen, five of which related to human dander constituents (Fig. 2). The sixth precipitin line appeared to be due to a heat-labile constituent of mite extract not shared by human dander.

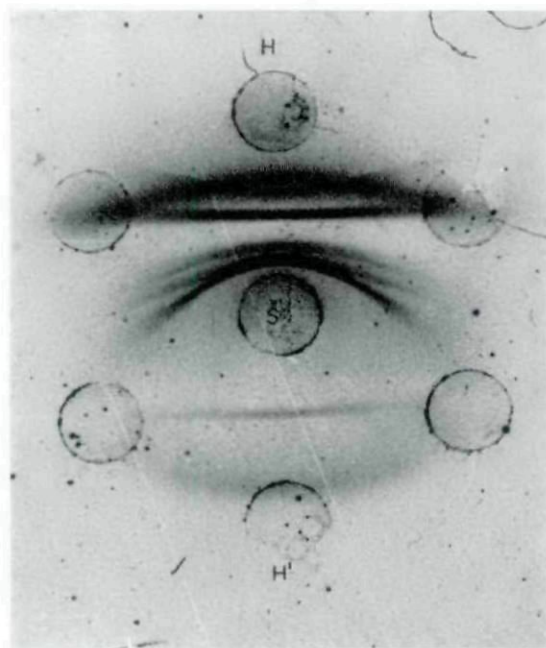


Fig. 2. Micro-Ouchterlony pattern between anti-*D. pteronyssinus* extract rabbit serum and a house dust extract. H = house dust extract (25 mg/ml); H' = house dust extract (0.25 mg/ml); S = anti-mite rabbit serum.

By gel-filtration on Sephadex G-75 of the mite extract it could be demonstrated that the highest allergenic activity was related to the material eluted between the BSA and cytochrome C molecular weight regions, which accords with other data previously reported (Romagnani *et al.*, 1972). When the most active fraction obtained by gel-filtration on Sephadex G-75 was added to a DEAE-Sephadex column and eluted stepwise by incorporating increasing concentrations of NaCl in the starting buffer, it was possible to show that the most potent activity was located in the material recovered by using 0.4 M NaCl as elution medium (Fig. 3).

The immunodiffusion analysis of this fraction revealed the presence of five antigens, two and perhaps three of which shared by *D. farinae* and two by human dander

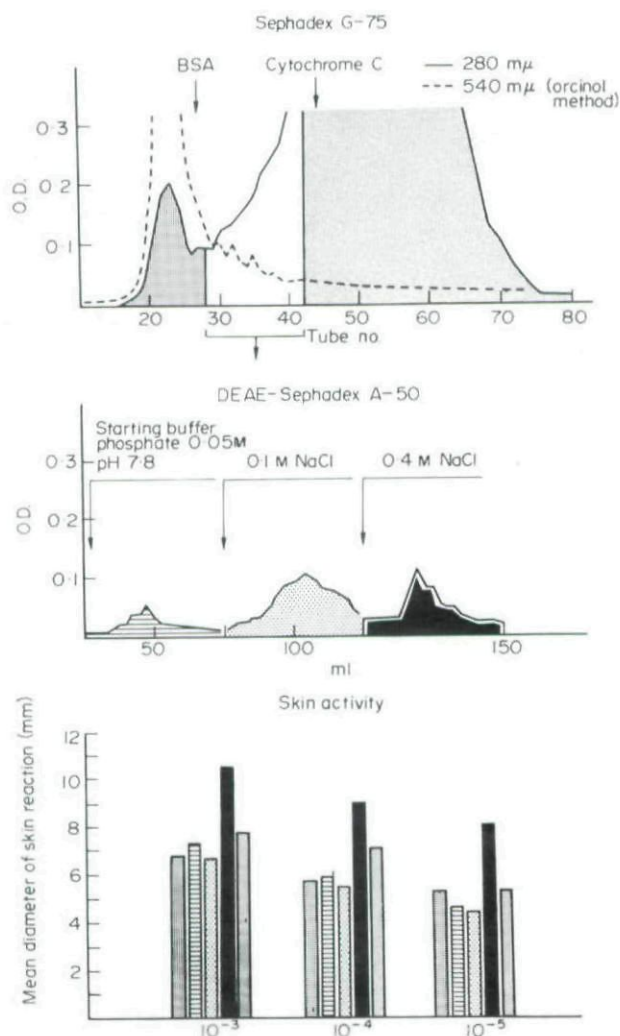


Fig. 3. Fractionation of *D. pteronyssinus* extract on Sephadex G-75 and DEAE-Sephadex. Skin activity of different fractions was evaluated by testing intradermally on sixteen mite-sensitive patients at ten-fold serial dilutions. It should be noted that the first and the third pool fractions of Sephadex G-75 were gel-filtered twice more on the same G-75 column before skin testing.

Table 1. Effect of the allergenic activity of mite and house dust extract induced by the incubation of these extracts with anti-mite rabbit gammaglobulin. Skin reactions (mean diameter in mm)

Patient no.	Mite extract preincubated with rabbit gammaglobulin against		House dust preincubated with rabbit gammaglobulin against	
	Mite extract	<i>Holcus lanatus</i> extract	Mite extract	<i>Holcus lanatus</i> extract
1	8.5 (+)	11 (+)	7 (+)	14 (+)
2	4	7.5 (+)	5.5	8
3	6	9	8	8.5 (+)
4	5	8	5	8
5	7	6	6.5	12 (+)
6	6	8 (+)	6 (+)	9.5 (+)
7	7	9	8 (+)	10 (+)
8	6	7.5 (+)	6.5	8.5 (+)
9	3	6 (+)	3	6 (+)
10	5	7 (+)	7 (+)	8 (+)
Mean value	5.75 ± 0.501	7.90 ± 0.476	6.25 ± 0.473	9.25 ± 0.723
	t paired = 5.362		t paired = 4.810	
	P < 0.001		P < 0.001	

(+) Presence of pseudopodia.

extract (Fig. 4). After heating at 100°C for 20 min three precipitin lines remained, one of which not shared by human dander, nor by *D. farinae* extract (Fig. 5).

Incubation with the anti-mite rabbit gammaglobulin constantly induced a strong decrease or a complete loss of the allergenic activity of both mite and house dust extract, whereas little or no effect was seen when the same extracts were incubated with the anti-*Holcus lanatus* rabbit serum, used as control (Table 1).

Discussion

While recognizing that mite allergen(s) are probably important in the dust allergy of many people some workers are not completely convinced by the available evidence asserting that mite constituents are by far the main allergen(s) in house dust throughout the world (Berrens, 1970; Kawai *et al.*, 1972). Much work seems still to be necessary to characterize mite allergen(s) and to study better their relationship with house dust allergy. Unfortunately *D. pteronyssinus* extracts are highly heterogenous mixtures of protein and non-protein molecules derived from mite and mite culture medium constituents.

In our experiments, immunodiffusion analysis of *D. pteronyssinus* extract revealed the presence of many constituents, five of which shared by human dander. At least five and probably more precipitin lines did not appear to be related to mite nutrient medium components and four of these were common to *D. farinae* extract, probably because of the antigenic cross-reactivity between the two species of *Acari* (Dasgupta & Cunliffe, 1970).

Further data were obtained by heat-treatment and fractionation of *D. pteronyssinus* extract. The allergenic activity of the extract appeared to be heat-stable and was

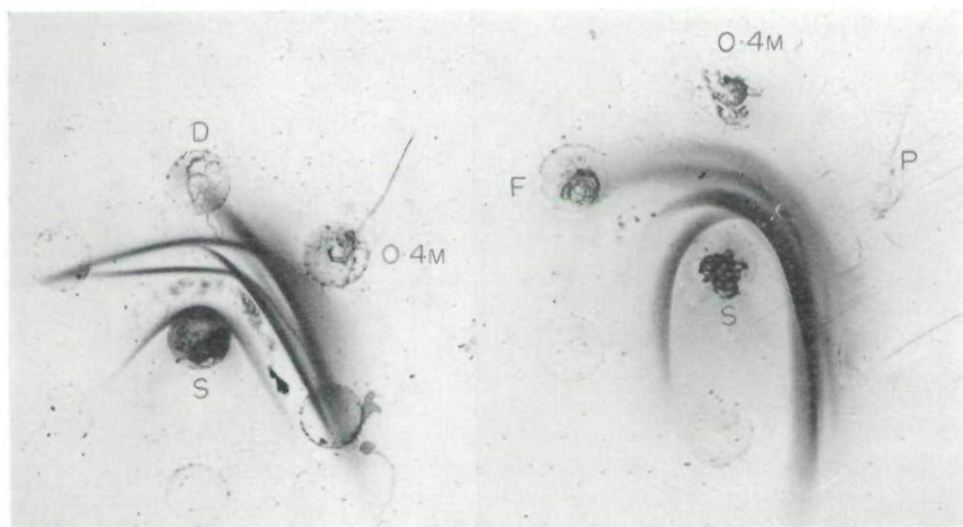


Fig. 4. Micro-Ouchterlony analysis of the most allergenic material obtained by fractionation of the *D. pteronyssinus* extract on Sephadex G-75 and DEAE-Sephadex. D = human dander extract (25 mg/ml); F = *D. farinae* extract (40 mg/ml); P = *D. pteronyssinus* extract (25 mg/ml); 0.4 M = DEAE 0.4 M NaCl fraction of *D. pteronyssinus* extract (20 mg/ml); S = anti-mite rabbit serum.

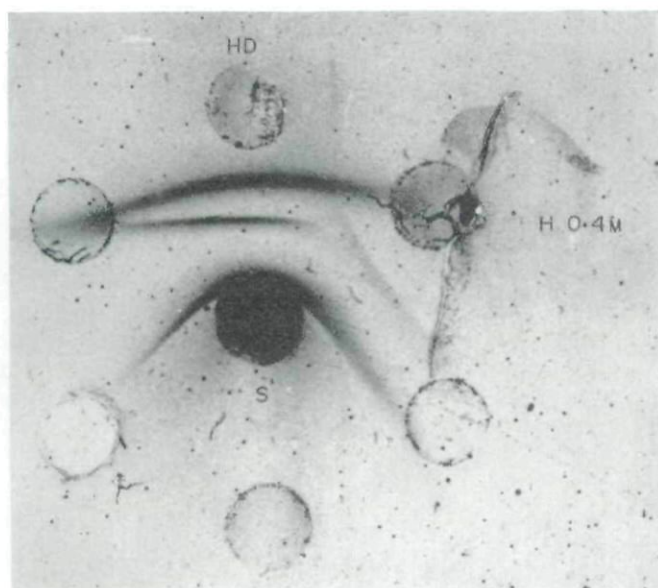


Fig. 5. Micro-Ouchterlony pattern of the DEAE-Sephadex 0.4 M NaCl fraction of *D. pteronyssinus* extract after heat-treatment (100°C for 20 min). HD = heated human dander extract (25 mg/ml); H 0.4 M = heated DEAE 0.4 M NaCl fraction (20 mg/ml); S = anti-mite rabbit serum. It should be noted that one of two dander cross-reacting lines is not clearly visible.

possessed by a fraction containing negatively charged molecules, weighing between 10,000 and 50,000. Immunodiffusion analysis of this fraction revealed the presence of five antigens, two of which cross-reacting with *D. farinae* and two with human dander constituents. After heating the fraction maintained its allergenicity in man, but only three precipitin lines remained, one of which was not shared by human dander or by *D. farinae* extract.

Thus, it could be presumed that the heat-stable antigens not shared by nutrient medium or by *D. farinae* were related to the allergenic activity of the *D. pteronyssinus* extract. However, on the basis of immunodiffusion analysis the inference that mite allergen(s) are immunogenic in rabbit rests without proof.

For this reason we tried to demonstrate the immunogenicity of mite allergen(s) in rabbit by an immunosorbent technique. In this way it was possible to show that incubation with mite antiserum was able to induce a strong decrease or a complete loss of the allergenic activity of the mite extract used for immunization. In this respect our results are different from those reported by Kawai *et al.* (1972), which showed that anti-mite sera presented little or no ability to block mite-mediated or dust-mediated leucocyte histamine release. This can probably be explained by the different immunization schedule we used.

With regard to the relationship between mites and house dust allergy a vast body of indirect evidence has been accumulated showing the importance of mite allergen(s) in the dust allergy of many people. However, on screening the available data no crucial experiments remain to substantiate the hypothesis that mite is the one and only producer of the 'house dust allergen'. In this respect it should be noted that when our anti-mite rabbit serum was tested against a house dust extract, six precipitin lines were observed, but five of these were common to human dander, only the sixth being related to a (heat-labile) constituent not shared by the nutrient medium.

However, when the same antiserum was fixed to an immunosorbent and incubated with a house dust extract, it was able to absorb constantly the allergenic activity from this extract, thus showing that at least in all ten patients examined by us the house dust allergy was exclusively due to allergen(s) contained in the *D. pteronyssinus* extract. We were thus able, by succeeding in producing rabbit antibodies directed against mite allergen(s) and by using an immunosorbent technique, to furnish a direct demonstration of an allergenic identity between mite and dust.

It is obviously necessary to verify the capacity of our anti-mite serum to absorb the dust allergenicity by testing a greater number of dust-sensitive patients and using dust samples of different origins. In any case, these data allow us to hope that in the near future a less contaminated material can be finally used for diagnostic and therapeutic purpose in house dust allergy.

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References

- BERRENS, L. (1970) The allergens in house dust. *Progress in Allergy*, **14**, 259.
- BILIOTTI, G., PASSALEVA, A., ROMAGNANI, S. & RICCI, M. (1972) Mites and house dust. I. Comparison between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) skin reactivity. *Clinical Allergy*, **2**, 109.

- CROWLE, A.J. (1958) A simplified micro double-diffusion agar precipitin technique. *Journal of Laboratory and Clinical Medicine*, **52**, 784.
- DASGUPTA, A. & CUNLIFFE, A.C. (1970) Common antigenic determinants in extracts of house dust and *Dermatophagoides* species. *Clinical and Experimental Immunology*, **6**, 891.
- HOLFORD-STREVE, V., WIDE, L., MILNE, J.F. & PEPYS, J. (1970) Allergens and antigens of *Dermatophagoides farinae*. *Clinical and Experimental Immunology*, **6**, 49.
- KAWAI, T., MARSH, D.G., LICHTENSTEIN, L.M. & NORMAN, P.S. (1972) The allergens responsible for house dust allergy. I. Comparison of *Dermatophagoides pteronyssinus* and house dust extract by assay of histamine release from allergic human leukocytes. *Journal of Allergy and Clinical Immunology*, **50**, 117.
- MAUNSELL, K., WRIGHT, D.G. & CUNNINGTON, A.M. (1968) Mites and house dust allergy in bronchial asthma. *Lancet*, **i**, 1267.
- 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*, **42**, 14.
- PEPYS, J., CHAN, M. & HARGREAVE, F.E. (1968) Mites and house dust allergy. *Lancet*, **i**, 1270.
- ROMAGNANI, S., BILIOTTI, G., PASSALEVA, A. & RICCI, M. (1972) Mites and house dust allergy. II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) allergens by fractionation methods. *Clinical Allergy*, **2**, 115.
- ROMAGNANI, S., BILIOTTI, G., PASSALEVA, A. & RICCI, M. (1973) Mites and house dust allergy. III. *In vitro* lymphocyte transformation and precipitating antibody to house dust and mite (*Dermatophagoides pteronyssinus*) extract in atopic and non-atopic individuals. *Clinical Allergy*, **3**, 51.
- VOORHORST, R., SPIEKMA, F.Th.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*, **39**, 325.
- WIDE, L. (1969) Radioimmunoassays employing immunosorbents. *Acta Endocrinologica*, **141** (supplement), 207.

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