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# Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract ($F_4P_1$)

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## SUMMARY

An extract of *Dermatophagoides pteronyssinus* culture has been fractionated by chromatography on Sephadex G-100 and Pevikon block electrophoresis to obtain a partially purified allergen ($F_4P_1$). This preparation has a molecular weight of between 15–25,000 Daltons, migrates slowly on electrophoresis, and is colourless in solution. The skin-test reactivity of $F_4P_1$ was comparable to that of crude *D. pteronyssinus* extract. $F_4P_1$ was radio-labelled with $^{125}I$ and used in an antigen-binding radioimmunoassay to measure IgG, IgA and IgE antibody (ab) to *D. pteronyssinus*. IgG, ab was detected in serum from 32/34 (94%) mite-allergic persons, and from 10/31 (30%) non-allergic persons. IgA ab and IgE ab were found in sera from 22/34 (65%) and 27/34 (79%) allergic persons respectively. Neither IgA nor IgE ab could be detected in sera from non-allergic persons. An excellent correlation was found between radioallergo-sorbent technique (RAST), using crude *D. pteronyssinus* extract and IgE-binding activity (BA) for $F_4P_1$, ($r = 0{\cdot}94$, $P<0{\cdot}001$). The antigen-binding assay for IgE BA was as sensitive as RAST, but less sensitive than PK testing. There was a very good quantitative correlation between IgG BA and IgE BA ($r = 0{\cdot}84$, $P<0{\cdot}001$). IgG BA was shown to rise in the serum of three patients treated with injections of *D. pteronyssinus* extract.


## INTRODUCTION

The role of mites of the Dermatophagoides species, notably *Dermatophagoides pteronyssinus* and *D. farinae* as allergens of house dust have been well established (Voorhorst, Spieksma-Boezema & Spieksma, 1964; Voorhorst *et al.*, 1967; Maunsell, Wraith & Cunnington, 1968; Holford-Strevens *et al.*, 1970; Miyamoto *et al.*, 1968). Mite sensitivity is thought to be of great importance in the aetiology of perennial rhinitis and extrinsic asthma (Morrison-Smith *et al.*, 1969; McAllen, Assem & Maunsell, 1970; Frankland, 1971). In the past, crude extracts of mite culture have been used both for skin-testing and for measuring serum IgE antibodies (ab) in the radioallergo-sorbent technique (RAST) (Wide, Bennich & Johansson, 1967; Stenius & Wide, 1969). RAST results have been shown to correlate well with other methods of assessing IgE ab, i.e. skin-testing, PK and provocation tests (Wide *et al.*, 1967; Berg, Bennich & Johansson, 1971; Bennich & Johansson, 1971). In addition, IgG ab to Dermatophagoides has been demonstrated in sera from patients by radioimmunoelectrophoresis (D'Souza *et al.*, 1973; Gabriel *et al.*, 1977).

The most sensitive method for measuring IgG ab to allergens is the double antibody or antigen-binding technique (Osler, Mulligan & Rodriguez, 1966; Newcomb & Ishizaka, 1967; Yunginger &

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Gleich, 1973; Platts-Mills *et al.*, 1976). Antigen-binding activity associated with IgE has also been demonstrated (Ishizaka, Ishizaka & Hornbrook, 1967). Recently we have reported the use of an antigen-binding technique for measuring IgE ab in parallel with IgG and IgA ab (Platts-Mills *et al.*, 1978). However, antigen-binding techniques require purified or at least partially purified allergens (Marsh, 1975; King, 1976). As yet no purified allergen has been described for either *D. farinae* or *D. pteronyssinus*. On the other hand, several estimates of the molecular weights of mite allergens have been made, based on the skin-test reactivity of fractions of mite extract separated by gel filtration (Miyamoto, Oshima & Ishizaki, 1969; Romagnani *et al.*, 1972, 1976).

The present report describes the partial purification of a *D. pteronyssinus* allergen which has good skin-test reactivity and is suitable for radiolabelling. Using this allergen an antigen-binding radioimmunoassay has been developed for measuring IgG, IgA and IgE ab to *D. pteronyssinus*.

## MATERIALS AND METHODS

*Allergen preparation. Dermatophagoides pteronyssinus* whole culture was kindly provided by Bencard (Betchworth, England). 117 g culture was extracted in 1L PBS by stirring overnight at 4°C. The extract was centrifuged at 23,000 *g* for 60 min, and the supernate concentrated down to 50 ml over an Amicon PM10 filter (Amicon, Lexington, Massachussetts, U.S.A.). The dark brown concentrate (Extract I) was then dialysed for 24 hr against two changes of borate-buffered saline pH 8·0 (BBS), and sterilized through an 0·45 $\mu$m Millipore filter. 7·5 ml Extract I was applied to an upward flow, gravity fed, 75 × 2·5 cm Sephadex G-100 column, equilibrated in BBS at 4°C. Samples of 6·3 ml were collected at a flow rate of 10 ml/hr, and the absorbance of each sample was measured at 280 nm. The eluted samples were then pooled in six fractions designated F1-6. These fractions were freeze-dried, resuspended in 4 ml BBS and centrifuged at 12,000 *g* for 30 min. The protein content of the fractions was determined by the method of Lowry *et al.* (1951) using ovalbumin as a standard. On the basis of skin-testing (see Results), fraction 4($F_4$) was used for further purification.

Preparative block electrophoresis of $F_4$ using Pevikon C-870 (Shandon Southern Ltd., Runcorn, Cheshire) was carried out as described by Müller-Eberhard (1960). Approximately 30 mg $F_4$ was applied to a 50 × 10 × 1 cm block of Pevikon equilibrated in 0·1M barbital buffer pH 8·6 at 4°C. Electrophoresis at 260V and 50 mA was continued for 26 hr. The protein concentration of 10 ml eluates of 1 cm segments of the block was then determined. The eluates were pooled and concentrated on the basis of their protein content and reactivity on immunodiffusion against rabbit anti-D. pteronyssinus serum (see Results). The $F_4P_1$ fraction was rechromatographed over Sephadex G-100 and the single, apparently homogeneous, peak was concentrated by negative pressure dialysis. After pooling, all fractions were stored at −70°C.

*Antisera.* Rabbit anti-*D. pteronyssinus* serum was prepared by injecting two NZW rabbits with 1 mg *D. pteronyssinus* Extract I in FCA (Difco Labs, Detroit, Michigan, U.S.A.), at fortnightly intervals. The rabbits were bled 11 days after the fifth injection, and the serum stored at −20°C. The preparation of mono-specific anti-IgG, anti-IgA and anti-IgE has been described previously (Platts-Mills & Ishizaka, 1975; Platts-Mills *et al.*, 1976, 1978). The antisera were raised in goats, by repeated immunization with Fc fragment of IgG, IgA myeloma protein or Fc fragment of IgE (PS) myeloma protein (Takatsu, Ishizaka & Ishizaka, 1975). Each antiserum was made monospecific by repeated passage over immunosorbent columns of Sepharose 2B, linked to human proteins by the cyanogen bromide technique (Axen, Porath & Ernbach, 1967). Anti-IgG was absorbed with Fab, IgA and IgE. Anti-IgA was absorbed with IgG and serum from an IgA-deficient patient, and anti-IgE was absorbed with IgG.

*Skin testing.* Intradermal skin-testing with *D. pteronyssinus* Extract I, Sephadex G-100 fractions, $F_4P_1$, and the Group I protein of Rye grass pollen, Rye I (Marsh, 1975) was carried out on the arms of twenty-one volunteers, who had been informed of the risks involved. The preparations were sterilized through an 0·45 $\mu$m Millipore filter. Serial ten-fold dilutions of allergens were made from 10 $\mu$g/ml down to $10^{-6}$ $\mu$g/ml in 0·05% human serum albumin (HSA) in phenol saline. Skin tests were performed by successive intradermal injection of 0·05 ml of increasing concentrations of allergen. The diameter of the wheal and flare was recorded 20 min after injection.

PK testing was performed with five sera from healthy individuals (Australia antigen negative) using only two recipients, M.C. and T.P.M. whose total serum IgE was 31 ng/ml and 175 ng/ml respectively. The sera were fractionated with saturated ammonium sulphate (SAS) and dialysed exhaustively against BBS. The 33–50% SAS fraction, which has been shown to be enriched for IgE (Carson, Metzger & Bazin, 1975), was diluted from 1:10 to 1:1600 in 0·05% HSA phenol saline, and sterilized through an 0·45 $\mu$m Millipore filter. 0·05 ml aliquots of suitable dilutions were injected intradermally. After 24 hr each site was challenged with 0·05 ml of a 1 $\mu$g/ml solution of *D. pteronyssinus* Extract I, and the wheal and flare reactions recorded.

*$^{125}I$ labelling of $F_4P_1$.* Iodination was carried out using the Chloramine-T technique in a modification of the method of Klinman & Taylor (1969). To 40 $\mu$g $F_4P_1$ was added 50 $\mu$l 0·3M phosphate buffer pH 7·5, 1·0 mCi $^{125}$NaI and 40 $\mu$g Chloramine T. The reaction was stopped after 65 sec by adding 40 $\mu$g sodium metabisulphite and 3 min later the reaction mixture was diluted with 2 ml 0·4% BSA BBS containing 50 $\mu$L 1% potassium iodide. After dialysis against three changes of BBS the

$^{125}$I-labelled $F_4P_1$ was 85% precipitable by trichloracetic acid, and 68% could be bound by hyperimmune human serum (see below).

*Measurements of antibodies to D. pteronyssinus.* Specific IgG, IgA and IgE ab to *D. pteronyssinus* were measured by a double antibody antigen-binding radioimmunoassay, analogous to those used for measuring IgG and IgA ab to Ragweed antigen E (antigen E), and IgG, IgA and IgE antibody to Rye I (Platts-Mills *et al.*, 1976, 1978). Patient's serum was incubated with 7 ng $^{125}$I-labelled $F_4P_1$ for 4 hr at room temperature. Specific antibody was precipitated overnight at 4°C by adding monospecific goat anti-IgG, anti-IgA or anti-IgE. The quantity of antiserum necessary was estimated by a semi-quantitative precipitation technique (Campbell *et al.*, 1970).The precipitates were washed four times in BBS and radioactivity counted in a 16-channel $\gamma$ counter (NE 1600, Nuclear Enterprises Ltd., Edinburgh). All assays were performed in plastic tubes coated with 2% BSA to reduce non-specific binding. Carrier immunoglobulins were added to samples containing insufficient immunoglobulin to form a precipitate. The carrier protein for IgE assays was 0·1 ml of a 1:100 dilution of PS myeloma serum (19·5 mg/ml IgE). For the IgA assays 0·1 ml of a 1:50 dilution of IgA myeloma was used. In general, IgG carrier (0·1 ml of a 1:100 dilution of IgG myeloma serum) was necessary only for the control curve and for sera diluted more than 1:50. The radioactivity bound by precipitates of carrier immunoglobulins was < 0·4% added radioactivity. A pool of sera from three patients who had been desensitized with aqueous extracts of *D. pteronyssinus* for several years was arbitrarily defined as containing 13,000 units IgG BA/ml. Serial two-fold dilutions of this pool from 1:100 to 1:51,200 were assayed for IgG BA and the results (control curve) were plotted on a log linear scale. The radioactivity bound by patient's serum was converted to values for IgG-, IgA- or IgE-binding activity (BA) by reference to this control curve set up in parallel with each assay. Assays were performed routinely using 0·1 ml allergic serum diluted 1:12·5, 1:25 and 1:50 for IgG BA, and 1:6, 1:12·5 and 1:25 for IgA and IgE BA. Results were calculated as the mean of the three values. Sera containing high levels of BA were diluted further so that at least 80% of the added radioactivity was not bound by any class of ab, i.e. the antigen excess was maintained at four-fold. Four sera from non-allergic persons were assayed at 1:12·5 and 1:25 in each assay to define the negative background. A result was regarded as positive if it was at least two standard deviations above this background.

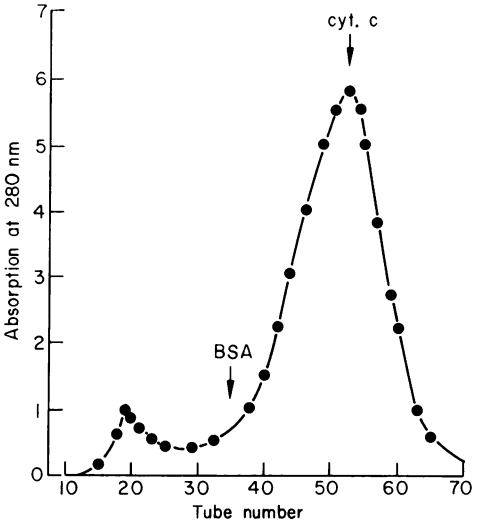
*RAST assay.*The RAST of Ceska, Eriksson &Varga (1972) was carried out using *D. pteronyssinus* Extract I coupled to filter-paper discs (Whatman Grade 541, 0·6 cm diameter). All RAST estimations were performed in duplicate and standardized by reference to a control curve. The control curve was obtained by assaying IgE ab in two-fold dilutions of an allergic serum which had been arbitrarily designated to contain 6500 IgE RAST units/ml. A positive RAST was defined as two standard deviations above the mean levels for sera from five non-allergic persons. RAST was also used to assess the allergenicity of Sephadex G-100 fractions of *D. pteronyssinus* Extract I, using 1 $\mu$g of each fraction coupled to a cyanogen bromide activated disc. The RAST was performed with each fraction using eight sera from allergic persons, and four sera from non-allergic persons.

*Total IgE.* A double antibody inhibition radioimmunoassay was used to measure total IgE as described previously (Gleich, Averbach & Svedlund, 1971; Platts-Mills *et al.*, 1978).

## RESULTS

The elution profile for Sephadex G-100 fractionation of *Dermatophagoides pteronyssinus* Extract I showed two peaks at 280 nm. A small peak eluted with the void volume, and a very broad peak eluted over the 5–65,000 Daltons molecular weight range, indicated by the positions of BSA and cytochrome c markers (arrows in Fig. 1). The fractions were pooled and tested by quantitative intradermal skin-testing; maximal skin-sensitivity was shown with fractions 3 and 4. In general, the skin-reactivity of these fractions was ten- to one-hundred-fold greater than that of the other fractions in each of the six individuals tested. When each of the fractions were coupled to RAST discs 8/8 sera from allergic persons showed maximal binding towards fraction 4 (see Materials and Methods, Fig. 1). There was no significant binding to any fraction by sera from non-allergic persons. Separation of fraction 4 by Pevikon block electrophoresis showed two protein peaks (Fig. 2). The major peak, $F_4P_2$, migrated faster than albumin, was dark brown in colour, and by immunodiffusion showed no reactivity with a rabbit antiserum to Extract I of *D. pteronyssinus*. The smaller peak, $F_4P_1$, showed only a faint brown colour, migrated slowly, and was strongly reactive on immunodiffusion (Fig. 2). This fraction, $F_4P_1$, was passed through Sephadex G-100 and eluted with a molecular weight of between 15–25,000 Daltons. This rechromatographed material was used for skin-testing and radiolabelling and is hereafter referred to as $F_4P_1$.

Radiolabelled $F_4P_1$ was used in a double antibody antigen-binding assay to measure specific IgG, IgA and IgE ab to *D. pteronyssinus*. The effect of the amount of antigen added to this assay was investigated over the range 0·5 ng–5 $\mu$g $F_4P_1$ added/tube. Five $\mu$g $^{125}$I, $F_4P_1$ was diluted with 95 $\mu$g cold $F_4P_1$, and serial ten-fold dilutions of this solution were made. One hundred $\mu$l of each dilution of antigen was used to assay IgG and IgE ab in six sera diluted 1:12·5. The results in Fig. 3a and 3b show that




| Fraction number | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Skin reactivity | + | ++ | +++ | ++++ | ++ | + |
| RAST | + | ++ | +++ | ++++ | ++ | + |

FIG. 1. Fractionation of *Dermatophagoides pteronyssinus* Extract I on Sephadex G-100. Arrows indicate the positions of BSA (MW 65,000) and cytochrome c (MW 12,500) markers. For each fraction skin-test reactivity was assessed by quantitative intradermal skin-testing. Each fraction was also coupled to cellulose discs and used for RAST. Typical results are shown on a semi-quantitative basis (+ to ++++).

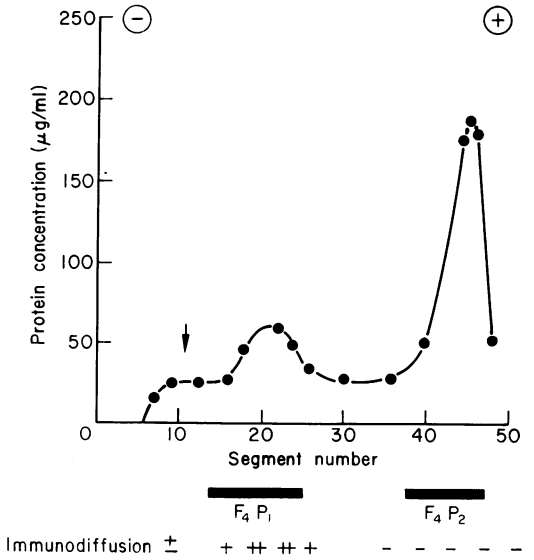
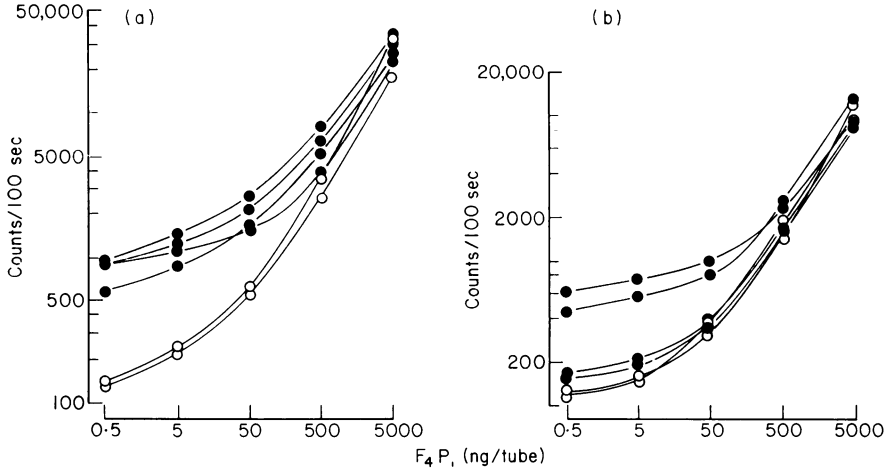



FIG. 2. Pevikon block electrophoresis of Sephadex G-100 Fraction 4. Arrow indicates the position at which 30 mg $F_4$ was applied to block.




| | Percentage radioactivity bound by IgG | | | | | Percentage radioactivity bound by IgE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Allergic | 28·80 | 6·06 | 2·13 | 1·00 | 0·44 | 18·10 | 3·19 | 0·78 | 0·29 | 0·16 |
| Non-allergic | 3·74 | 1·01 | 0·43 | 0·34 | 0·23 | 3·65 | 0·66 | 0·26 | 0·18 | 0·16 |

FIG. 3. Radioactivity bound by serum from skin-test-positive (●——●) and skin-test-negative (○——○) individuals. Sera were incubated with various quantities of $^{125}I$ $F_4P_1$ over the range 0·5–5000 ng/tube and then precipitated with anti-IgG (Fig. 3a) or anti-IgE (Fig. 3b). Also shown for each quantity of $F_4P_1$ is the percentage of radioactivity bound by allergic sera (top line in Fig.) and by non-allergic sera (bottom line in Fig.)

maximum differences between allergic and non-allergic sera were achieved by adding 0·5–5 ng radiolabelled antigen for both IgG and IgE ab. With increasing quantities of antigen (over 50 ng $F_4P_1$/tube) although the percentage radioactivity bound by non-allergic sera decreased, the absolute levels of radioactivity (counts/100 sec, Fig. 3a and 3b) increased dramatically. This resulted in a progressive loss of sensitivity because the sensitivity of the assay depends on the absolute background radioactivity with serum from non-allergic persons. Assays for IgG, IgA and IgE BA were carried out routinely using 7 ng $^{125}I$, $F_4P_1$/tube. The other details of this assay were very similar to those used previously for antigen E and Rye I (see Materials and Methods). Typical dilution curves for IgG and IgE BA are shown in Fig. 4. The IgE BA curves could be superimposed on those for IgG BA, and therefore all quantitative results were obtained by extrapolation from a single IgG BA control curve.

Specific IgG, IgA and IgE ab to *D. pteronyssinus* were measured by antigen-binding assay in the serum of thirty-four persons who had positive prick tests with *D. pteronyssinus* extract (Bencards Ltd.) Fig. 5. Sera were also studied from thirty-one non-allergic persons with negative skin tests. The results show that IgG BA was detectable in 32/34 sera from prick-test-positive persons, IgE BA was detectable in 27/34 and 22/34 sera contained IgA BA. In sera from prick-test-negative persons IgE and IgA BA were not detectable, but in 10/31 sera IgG BA could be detected. However, the levels of IgG BA were much lower than those in most prick test positive persons (Fig. 5). Twenty-eight of the prick-test-positive persons had perennial rhinitis suggestive of house dust sensitivity, and the remaining six persons had rhinitis, but their symptoms did not seem to be related to house dust. In sera from these six 'asymptomatic' persons IgE BA and IgA BA were not detectable, but 4/6 contained low levels of IgG BA. In Table 1 the clinical symptoms, intradermal skin-test reactivity and serum ab levels of fifteen prick-test-positive volunteers are shown. The results of intradermal skin-testing with *D. pteronyssinus* Extract I and $F_4P_1$ showed that all fourteen persons tested were sensitive to both preparations. In general, the minimum quantity of $F_4P_1$ necessary to give a positive skin-test was about one-fifth the quantity of Extract I. To check the specificity of the skin-tests non-allergic persons were skin-tested with 10 $\mu$g $F_4P_1$: none of these five gave a positive reaction. In addition, two persons with grass-pollen hay fever who gave positive skin-tests of $10^{-6}$ $\mu$g Rye I failed to react to 10 $\mu$g $F_4P_1$. Using the IgE-rich fractions of five sera from skin-test-positive persons, there was a good correlation between PK titre tested with *D. pteronyssinus*

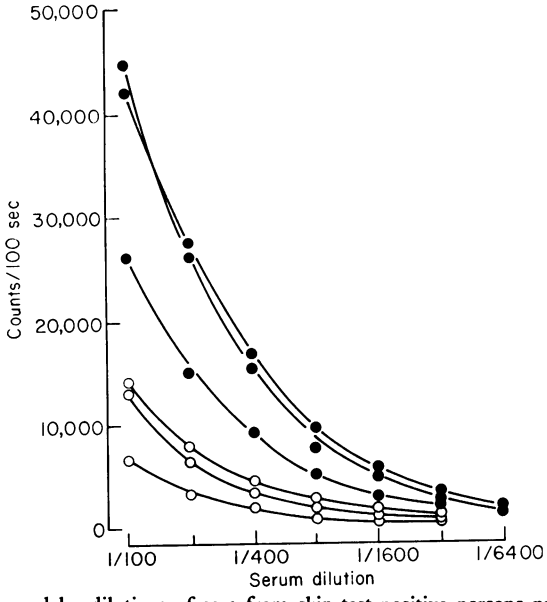



FIG. 4. Radioactivity bound by dilutions of sera from skin-test-positive persons precipitated by anti-IgG (●——●) or anti-IgE (○——○). 7 ng $^{125}$I $F_4P_1$ were added to each tube.

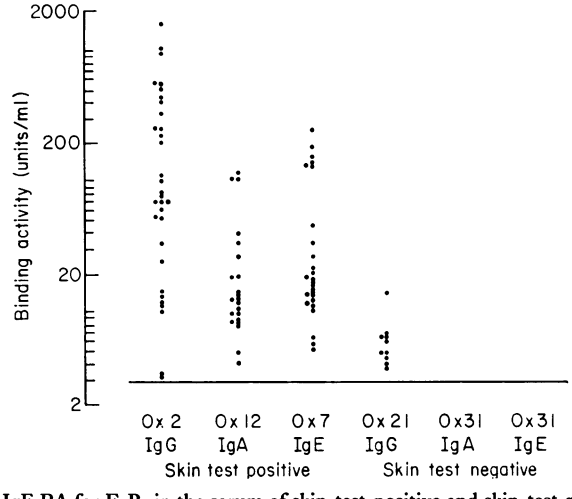



FIG. 5. IgG, IgA and IgE BA for $F_4P_1$ in the serum of skin-test-positive and skin-test-negative persons measured by antigen binding radioimmunoassay. The minimum detectable level of BA (units/ml) is indicated by the solid line. The number of sera without detectable BA is shown below this line.

Extract I and IgE BA for $F_4P_1$ (Table 1). PK tests were completely negative using two sera from skin-test-negative persons, and sera from the two persons with grass-pollen hay fever described above.

IgE ab was measured by both antigen-binding assay and RAST in thirty-three of the sera from skin-test-positive persons. An excellent quantitative correlation between the results of the two methods was found by linear regression analysis ($r = 0{\cdot}94$, $P<0{\cdot}001$, Fig. 6). Analysis of the results in Fig. 5 shows an excellent correlation between IgE BA and IgG BA ($r = 0{\cdot}87$, $P<0{\cdot}001$). The mean ratio of IgG BA: IgE BA was 7·4:1. We also found a good correlation between IgE BA and total IgE ($r = 0{\cdot}63$, $P<0{\cdot}001$), and a modest correlation between IgG BA and total IgE ($r = 0{\cdot}38$, $P<0{\cdot}02$). IgG BA was assayed in pre- and post-treatment sera from three patients who had received at least six graded injections of aqueous

TABLE 1. Antibody levels in fifteen prick-test-positive subjects assessed by intradermal skin-testing, antigen-binding assay, RAST, and P.K. testing

| Subject (Age) | Symptoms attributable* to house dust allergy* | Intradermal skin-test† Extract I 'μg/ml' | Intradermal skin-test† $F_4P_1$ 'μg/ml' | RAST‡ Rast units/ml | BA for $F_4P_1$ IgG units/ml | BA for $F_4P_1$ IgE units/ml | BA for $F_4P_1$ IgA units/ml | Ratio IgG BA:IgE BA | P.K. titre§ | Total IgE (ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| J.S. (17) | Rhinitis (grass, egg) | n.d. | n.d. | 2125 | 563 | 184 | 27 | 3.1:1 | 1600 (666) | 8850 |
| J.L. (19) | Rhinitis, asthma | $10^{-5}$ | $10^{-5.5}$ | 775 | 1040 | 135 | 119 | 7·7:1 | 400 (136) | 2059 |
| M.B. (18) | Rhinitis, facial swelling | $10^{-5}$ | $10^{-5.5}$ | 91 | 330 | 28 | < 4 | 11·8:1 | 200 (43) | 1728 |
| S.J. (39) | Rhinitis, asthma (grass) | $10^{-4.5}$ | $10^{-5}$ | < 8 | 34 | 6 | < 4 | 5·9:1 | 25 (17) | 146 |
| R.G. (30) | Asymptomatic (grass) | $10^{-3}$ | $10^{-3}$ | 10 | < 4 | < 4 | < 4 | — | 10 (< 4) | 1620 |
| M.K. (24) | Rhinitis | $10^{-5}$ | $10^{-6}$ | 325 | 443 | 130 | 13 | 3·4:1 | | 1824 |
| S.B. (16) | Asthma | $10^{-5}$ | $10^{-6}$ | 425 | 201 | 143 | 104 | 1·4:1 | | 1162 |
| S.M. (27) | Rhinitis | $10^{-4}$ | $10^{-5}$ | 53 | 258 | 20 | 19 | 13·6:1 | | 192 |
| W.M. (50) | Rhinitic (grass) | $10^{-5}$ | $10^{-5.5}$ | n.d. | 81 | 21 | 14 | 3·85:1 | | 1483 |
| P.J. (19) | Mild rhinitis (grass) | $10^{-4}$ | $10^{-5}$ | 66 | 113 | 14 | < 4 | 8·1:1 | | 2880 |
| D.T.R.(45) | Rhinitis | $10^{-4}$ | $10^{-5}$ | 25 | 72 | 14 | 8 | 5·1:1 | | 643 |
| K.B. (20) | Asymptomatic (grass) | $10^{-2.5}$ | $10^{-3}$ | < 8 | 10 | < 4 | < 4 | — | | 1248 |
| P.M. (40) | Asymptomatic (cat) | $10^{-3}$ | $10^{-3}$ | < 8 | < 4 | < 4 | < 4 | — | | 1474 |
| S.S. (39) | Rhinitis (grass) | $10^{-5}$ | $10^{-5.5}$ | 10 | 236 | 12 | 9·8 | 19·6:1 | | 377 |
| K.M. (26) | Rhinitis (grass) | $10^{-6}$ | $10^{-6}$ | 144 | 55 | 47 | 11 | 1·2:1 | | 655 |

* Other allergens giving clinical symptoms and positive skin-tests are indicated in parentheses. Grass = grass pollen.

† For both intradermal skin-testing and PK-testing a wheal of 6 × 6 mm was regarded as positive. Log dilutions of allergen solutions were used—intradermal skin-tests were assessed to the nearest ½ log to distinguish strongly positive results.

‡ RAST units were obtained by reference to a control curve (see Materials and Methods).

§ PK titres are the mean values obtained from tests on two recipients. The IgE BA (units/ml) of the IgE-rich serum fractions used in PK-testing is given in parentheses.

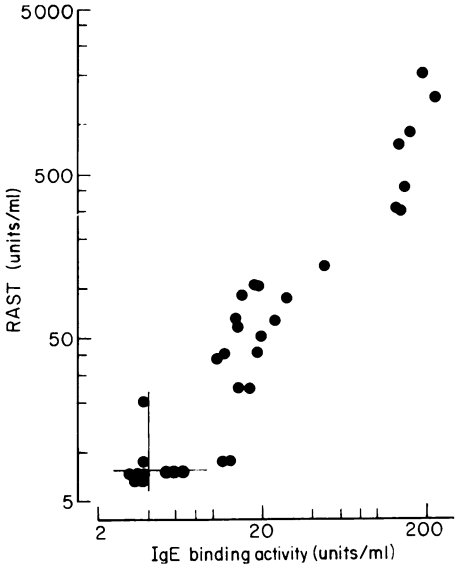



FIG. 6. Correlation between the antigen binding assay (using $^{125}I$ $F_4P_1$) and RAST (using *Dermatophagoides pteronyssinus* Extract I) for measuring IgE ab to *D. pteronyssinus* in the serum of prick-test-positive persons. The minimal level of sensitivity of each assay is indicated (———).

*D. pteronyssinus* extract between January and July. The levels of IgG BA rose from 41, 204 and 750 units/ml in pre-treatment sera, to 562, 2600 and 8000 units/ml in post-treatment sera, respectively.

## DISCUSSION

The results of gel filtration of mite allergens reported here suggest a molecular weight of between 15–25,000 Daltons, and are similar to those reported by previous workers (Miyamoto *et al.*, 1969; Romagnani *et al.*, 1972, 1976; Holford-Strevens, 1972). Attempts to further purify the allergens in mite extracts have previously been unsuccessful. Neither isoelectric-focusing (Ricci, Biliotti & Romagnani, 1975), chromatography on DEAE-Sephadex (Romagnani *et al.*, 1976) or affinity chromatography on lectins (Baldo & Uhlenbruck, 1977) have yielded an allergen with skin-test reactivity comparable to that of the crude extract. The electrophoresis of mite extract in Pevikon reported here has given very good yields of allergenic material. The large peak of rapidly migrating material, $F_4P_2$, was not found to react with rabbit antisera. This highly coloured material was assumed to be made up predominantly of the culture medium in which *Dermatophagoides pteronyssinus* was grown. The culture medium was described by the manufacturer as an animal protein of non-dermal origin which was only very weakly immunogenic in rabbits. The slowly migrating fraction $F_4P_1$ was compared with crude mite extract by skin-testing, reactivity with rabbit antisera, and by its properties when radiolabelled with $^{125}I$. There was an excellent correlation between the two preparations when compared by quantitative intradermal skin-testing (Table 1). It was disappointing that the increased skin-reactivity of $F_4P_1$ was only about five-fold. However, our estimates of the protein concentration of crude mite extract are only approximate because of the complex mixture of the proteins present. When tested by immunodiffusion and Laurell electrophoresis $F_4P_1$ gave one major line, and two feint lines. By contrast, *D. pteronyssinus* Extract I gave six lines on immunodiffusion, and at least fifteen on Laurell electrophoresis (data not shown). $F_4P_1$ could be radiolabelled with $^{125}I$ approximately three times more efficiently than Extract I. Furthermore, using serum from patients who had been treated with mite extract a large proportion (68%) of the radioactivity in $^{125}I$-labelled $F_4P_1$, could be bound by IgG ab. The properties of $F_4P_1$ were as good as those we have obtained in antigen-binding assays with other inhalant allergens, e.g. antigen E and Rye I (Platts-Mills

*et al.*, 1976, 1978). The allergen preparation of $F_4P_1$ is clearly not fully purified. However, two lines of evidence suggest that this fraction includes the major allergen or allergens present in *D. pteronyssinus* Extract I. Firstly, no patient gave greater skin-reactivity with the crude material than with $F_4P_1$. Secondly, there was an excellent correlation between estimates of IgE BA for $F_4P_1$ and IgE ab against Extract I measured by RAST (Fig. 6). Further purification and characterization of the proteins in $F_4P_1$ is in progress.

The antigen-binding radioimmunoassay used here was arranged to give maximum sensitivity. This was made possible by using small amounts of radiolabelled $F_4P_1$ (7 ng/tube). Results obtained with larger quantities of antigen have given very similar quantitative relationships both between classes and between different sera (data not shown). However, using higher quantities of antigen the ab levels in many of the untreated sera would not have been detectable. Because we can quantitate IgG BA in sera from untreated patients the assay is suitable for measuring the IgG ab responses to desensitizing injections. This will allow a direct evaluation of the relationship between clinical effects and the IgG ab response produced by injections of mite extract (M. D. Chapman, T. A. E. Platts-Mills, Sister M. Gabriel, H. K. Ng, W. G. L. Allan, L. E. Hill & A. J. Nunn; also M. W. Turner, J. F. Soothill, J. F. Price, M. Chapman, T. A. E. Platts-Mills, E. Hey & J. F. Mowbray—both manuscripts in preparation). The sensitivity of the assay for IgE BA was very similar to that of RAST, but approximately five-fold less sensitive than PK testing. The quantitative relationship between IgG, IgA and IgE BA to *D. pteronyssinus* in serum from untreated persons was similar to that reported previously for antibodies to Rye I in serum from patients with hay fever (Platts-Mills *et al.* 1978). Thus in most cases IgG BA > IgE BA > IgA BA and IgE BA was never found in the absence of IgG BA. The finding that a significant proportion of non-allergic persons had IgG BA to *D. pteronyssinus* in the absence of IgE or IgA BA, was not surprising, since similar results have been described for both Antigen E and Rye I (Levine, Stember & Fotino, 1972; Black *et al.*, 1976). A previous report using a modified RAST technique suggested that IgA ab to *D. farinae* were present at higher levels in sera of non-allergic individuals than in sera of allergic individuals (Stokes, Taylor & Turner, 1974). Our results show that IgA BA was present in serum from the majority of allergic persons (22/34) but was not detectable in serum from non-allergic persons. It seems possible that the previous results represent variations in the IgA ab RAST background, because several groups have found that non-specific binding of human IgA, and IgG, to RAST beads is frequently too high to allow sensitive assays (Johansson *et al.*, 1974; Soothill *et al.*, 1976). Although transient low levels of serum IgA have been shown to precede atopic disease in infancy (Taylor *et al.*, 1973; Soothill *et al.*, 1976), there is no evidence from the present investigations to suggest that poor IgA responses are involved in the aetiology of allergic illnesses of late childhood and adult life (Taylor, 1974). This may indicate that transient, low levels of serum IgA in infancy are merely a marker for some other mechanism which is related to the onset of allergy (Soothill *et al.*, 1976). The data reported here support the view that allergic patients make an immune response to inhalant allergens which includes IgG, IgA and IgE ab. By contrast, non-allergic persons make a small IgG ab response or no detectable ab response at all. The very good correlation found between IgG BA and IgE BA ($r = 0{\cdot}84$, $P < 0{\cdot}001$) adds further support to the view that the factors that control the occurrence of inhalant allergy are acting over antibody responses in general, and not over IgE ab alone.

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## REFERENCES

Axen, R., Porath, J. & Ernbach, S. (1967) Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature (Lond.)* **214**, 1302.

Baldo, B.A. & Uhlenbruck, G. (1977) Selective isolation of allergens. 1. Reaction of house dust mite extracts with Tridacnin and concanavalin A and examination of the allergenicity of the isolated components. *Clinical Allergy*, **7**, 429.

Berg, T., Bennich, H. & Johansson, S.G.O. (1971) *In vitro* diagnosis of atopic allergy. 1. A comparison between provocation tests and the radioallergosorbent test. *Int. Arch. Allergy*, **40**, 770.

Bennich, H. & Johansson, S.G.O. (1971) Structure and function of the human IgE system. *Advanc. Immunol.* **13**, 1.

Black, P.L., Marsh, D.G., Jarrett, E., Delespesse, G.J. &

BIAS, W.B. (1976) Family studies of association between HLA and specific immune responses to highly purified pollen allergens. *Immunogenetics*, **3**, 349.

CAMPBELL, D.H., GARVEY, J.S., CREMER, N.E. & SUSSDORF, D.H. (1970) *Methods in Immunology*. W.A. Benjamin Inc., New York, p. 245.

CARSON, D., METZGER, H. & BAZIN, H. (1975) A simple radioimmunoassay for the measurement of human and rat IgE levels by ammonium sulphate precipitation. *J. Immunol.* **115**, 561.

CESKA, M., ERIKSSON, R. & VARGA, J.M. (1972) Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunology*, **49**, 1.

D'SOUZA, M.F., PEPYS, J., WELLS, I.D., TAI, E., PALMER, F., OVERALL, B.G., MCGRATH, I.T. & MEGSON, M. (1973) Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: a controlled study of clinical and immunological effects. *Clinical Allergy*, **3**, 177.

FRANKLAND, A.W. (1971) Asthma and mites. *Postgraduate Medical Journal*, **47**, 178.

GABRIEL, SISTER M., NG, W.K., ALLAN, G.W.L., HILL, L.E. & NUNN, A.J. (1977) Study of prolonged hyposensitization with *D. pteronyssinus* extract in allergic rhinitis. *Clinical Allergy*, **7**, 325.

GLEICH, G.J., AVERBACH, A.K. & SVEDLUND, H.A. (1971) Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. clin. Med.* **77**, 690.

HOLFORD-STREVENS, V. (1972) Investigation of certain common inhalant allergens of animal origin. Ph.D. thesis, University of London.

HOLFORD-STREVENS, V., WIDE, L., MILNE, J.F. & PEPYS, J. (1970) Allergens and antigens of *Dermatophagoides farinae*. *Clin. exp. Immunol.* **6**, 49.

ISHIZAKA, K., ISHIZAKA, T. & HORNBROOK, M.M. (1967) Allergen binding activity of γE, γG and γA antibodies in sera from atopic patients: *in vitro* measurements of reaginic antibody. *J. Immunol.* **98**, 490.

JOHANSSON, S.G.O., FOUCARD, T., HJELM, H. & SJOKVIST, J. (1974) Determination of specific IgG antibody to soluble antigens by absorption of protein A-Sepharose. *Scand. J. Immunol.* **3**, 881.

KING, T.P. (1976) Chemical and biological properties of some atopic allergens. *Advanc. Immunol.* **23**, 77.

KLINMAN, N.R. & TAYLOR, R.B. (1969) General methods for the study of cells and serum during the immune response: the response to dinitrophenyl in mice. *Clin. exp. Immunol.* **4**, 473.

LEVINE, B.B., STEMBER, R.H. & FOTINO, M. (1972) Ragweed hay fever: genetic control and linkage to HL-A haplotypes. *Science*, **178**, 1201.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **193**, 265.

MARSH, D.G. (1975) Allergens and the Genetics of Allergy. In *The Antigens* (Ed. M. Sela) Vol. 3, pp. 271–350, Academic Press Inc., New York.

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 *Dermatophogoides pteronyssinus*. *Br. Med. J.* **ii**, 501.

MIYAMOTO, T., OSHIMA, B.S. & ISHIZAKI, T. (1969) Antigenic relationship between house dust and a dust mite, *Dermatophagoides farinae* Hughes 1961, by a fractionation method. *J. Allergy*, **44**, 282.

MIYAMOTO, T., OSHIMA, B.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. *J. Allergy*, **42**, 14.

MORRISON-SMITH, J., DISNEY, M.E., WILLIAMS, J.D. & GEOLS, Z.A. (1969) Clinical significance of skin reactions to mite extracts in children with asthma. *Br. Med. J.* **2**, 723.

MÜLLER-EBERHARD, H.J. (1960) A new supporting medium for preparative electrophoresis. *Scand. J. Clin. Lab. Invest.* **12**, 33.

NEWCOMB, R.W. & ISHIZAKA, K. (1967) Human diphtheria antitoxin in immunoglobulin classes IgG and IgA. *J. Immunol.* **99**, 40.

OSLER, A.G., MULLIGAN, J.J. & RODRIGUEZ, E. (1966) Weight estimates of rabbit anti-human serum albumin based on antigen-binding and precipitin analyses: specific haemagglutinating activities of 7S and 19S components. *J. Immunol.* **96**, 334.

PLATTS-MILLS, T.A.E. & ISHIZAKA, K. (1975) IgG and IgA diphtheria antitoxin responses from human tonsil lymphocytes. *J. Immunol.* **114**, 1058.

PLATTS-MILLS, T.A.E., VON MAUR, R.K., ISHIZAKA, K., NORMAN, P.S. & LICHTENSTEIN, L.M. (1976) IgA and IgG anti-ragweed antibodies in nasal secretions. *J. clin. Invest.* **57**, 1041.

PLATTS-MILLS, T.A.E., SNAJDR, M.J., ISHIZAKA, K. & FRANKLAND, A.W. (1978) Measurement of IgE antibody by an antigen binding assay: Correlation with PK activity and IgG and IgA antibodies to allergens. *J. Immunol* **120**, 1201.

RICCI, M., BILIOTTI, G. & ROMAGNANI, S. (1975) Antigenic and allergenic constituents of *Dermatophagoides pteronyssinus*. International WHO-IABS Symposium on Standardisation and Control of Allergens Administered to Man. Geneva 1974. *Develop. biol. Standard*, **29**, 123.

ROMAGNANI, S., BILIOTTI, G., PASSALEVA, A. & RICCI, M. (1972) Mites and house dust allergy II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) allergens by fractionation methods. *Clinical Allergy*, **2**, 115.

ROMAGNANI, S., BOCCANCCINI, P., AMADORI, A. & RICCI, M. (1976) Studies on allergens of *Dermatophagoides pteronyssinus* by Direct and Indirect RAST. *Int. Archs. Allergy. appl. Immunol.* **50**, 525.

SOOTHILL, J.F., STOKES, C.R., TURNER, M.W., NORMAN, A.P. & TAYLOR, B. (1976) Predisposing factors and the development of reaginic allergy in infancy. *Clinical Allergy*, **6**, 305.

STENIUS, B. & WIDE, L. (1969) Reaginic antibody (IgE), skin, and provocation tests to *D. culinae* and house dust in respiratory allergy. *Lancet*, **ii**, 455.

STOKES, C.K., TAYLOR, B. & TURNER, M.W. (1974) Association of house dust and grass pollen allergies with specific IgA antibody deficiency. *Lancet*, **ii**, 485.

TAKATSU, K., ISHIZAKA, T. & ISHIZAKA, K. (1975) Biological significance of disulphide bonds in human IgE molecules. *J. Immunol.* **114**, 1838.

TAYLOR, G. (1974) The nose as a model for the study of respiratory tract allergic diseases. *Ann. N.Y. Acad. Science*, **221**, 117.

TAYLOR, B., NORMAN, A.P., ORGEL, M.A., STOKES, C.R., TURNER, M.W. & SOOTHILL, J.F. (1973) IgA deficiency and the pathogenesis of infantile atopy. *Lancet*, **ii**, 111.

VOORHORST, R., SPIEKSMA-BOEZEMA, M.I.A. & SPIEKSMA, F.TH.M. (1964) Is a mite (Dermatophagoides sp.) the producer of the house dust allergen? *Allergie und Asthma*, **10**, 329.

VOORHORST, R., SPIEKSMA, F.TH.M., VAREKAMP, N., LEUPEN, M.J. & LYKLEMA, A.W. (1967) The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J. Allergy*, **39**, 325.

WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.

YUNGINGER, J.W. & GLEICH, G.J. (1973) Seasonal changes in IgE antibodies and their relationship to IgG antibodies during immunotherapy for ragweed hay fever. *J. clin. Invest.* **52**, 1286.