

The isolation of an allergen from extracts of *Dermatophagoides pteronyssinus* using a murine IgA myeloma with a specificity for phosphorylcholine

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Summary

The presence of a high molecular weight glycoprotein component bearing the hapten phosphorylcholine (Pc) has been demonstrated in some but not all extracts of house dust mite using a phosphorylcholine specific IgA myeloma protein (S107). The Pc bearing component was isolated from house dust mite extracts by precipitation at equivalence using whole myeloma serum and the isolated fraction was found to be allergenic as judged by a direct RAST assay using a highly house dust mite allergic human serum pool. RAST inhibition studies using free hapten and direct RAST studies using pneumococcal polysaccharide C-substance indicated that the Pc moiety did not contribute significantly to the immunodominant portion of the isolated allergen. Further studies indicated that the S107 isolated material was immunochemically different from the tridacnin reactive material and the con A reactive material previously isolated from extracts of house dust mite.

Introduction

It is now well established that the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* represent extremely important sources of environmental allergens (Voorhorst *et al.*, 1967; Miyamoto *et al.*, 1968; Morita *et al.*, 1975) which so far have been poorly characterized in terms of heterogeneity, structure and chemical composition.

The isolation and characterization of purified allergens from *D. pteronyssinus* and *D. farinae* by conventional physico-chemical techniques has so far met with little success. However, several partially purified allergenic fractions based on criteria such as mol. wt and charge have been isolated from extracts of both species of mite (Ishii *et al.*, 1973; Biliotti, Romagnani & Ricci, 1975; Nakagawa *et al.*, 1977). In summary, these studies indicated that the most potent allergenic fractions in both species of mite

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were glycoproteins with mol. wts in the range 12,500–30,000 daltons and that the allergenic activities were pronase sensitive but heat stable.

In contrast to these studies other workers have employed a selective approach to the isolation of allergens from mite extracts. Based on observations of Ishii *et al.*, (1973) that such extracts contain significant amounts of carbohydrate, Baldo, Turner & Uhlenbruck (1976) and Baldo & Uhlenbruck (1977) isolated high mol. wt allergens in relatively pure form from *D. farinae* using the carbohydrate specific lectins, tridacnin and con A. These authors suggest that this approach can, in certain circumstances, circumvent or greatly simplify conventional but time-consuming physicochemical isolation procedures and yield allergens in pure form.

In this regard, we report here the selective isolation of an allergen bearing the hapten phosphorylcholine from extracts of *D. pteronyssinus*, using the phosphorylcholine specific murine IgA myeloma S107.

Materials and methods

Preparation of mite extracts

Crude, freeze dried preparations of the house dust mite, *D. pteronyssinus*, and the mite culture medium, were kindly provided by two different manufacturers (1, 2) as were commercially prepared extracts. The water soluble components of the mites were extracted by grinding the mites in 0.1 M Tris-HCl buffer, pH 7.4, as described by Ishii *et al.* (1973) for the isolation of allergens from *D. farinae*. The resultant extracts were dialysed against four changes (each of 5 litres) of distilled water at 4°C and freeze dried. Commercial extracts from the two manufacturers were designated 1 and 2 and extracts prepared in our laboratory with mites obtained from the same source were designated 1.1 and 2.1 respectively.

Fractionation of mite extracts

Mite extracts were fractionated on Sephadex G-150 and Sepharose CL-6B. All columns were equilibrated with 0.1 M Tris HCl pH 8.0 containing 0.15 M NaCl, and the column effluents were monitored at 280 nm using a Varian Superscan 1. The mol. wt markers apoferritin and bovine serum albumin were purchased from Sigma Chemical Co. Ltd. The rabbit IgG marker was prepared as described by Stewart, Smith & Stanworth (1973).

Allergenicity of mite extracts

Sera from highly mite allergic and control patients who were allergic only to grass pollens, were obtained from the RAST diagnostic service of the Routine Immunology Unit, Princess Margaret Hospital, and a suitable pool of each was prepared. The serum pools were filtered and stored frozen at -20°C until required. The total IgE levels of the mite allergic and grass pollen only allergic pools were 1250 and 1340 u/ml respectively.

RAST and RAST-inhibition assays were performed essentially as described by Wide, Bennich & Johansson (1967) and Yman, Ponterius & Brandt (1975) using ¹²⁵I-labelled anti-human IgE obtained from Pharmacia (South Seas). Allergens were coupled to cyanogen bromide activated paper discs as described by Ceska, Eriksson & Varga (1972). In the RAST-inhibition studies using free phosphorylcholine as inhibitor, 25 µl of neat mite allergic serum was added to 25 µl (containing from 0–40 µm) of

phosphorylcholine in 0.1 M Tris HCl buffer, pH 7.4. Discs coupled with the isolated S107 reactive material (3.2 µg/disc) were added. Following a further 18 h incubation at room temperature, the discs were washed three times with RAST buffer (Ceska *et al.*, 1972) and 50 µl of ¹²⁵I-radiolabelled anti-IgE was added. The percentage radiation bound to the discs was determined after three washes.

Analytical methods

Protein and carbohydrate content of mite extracts and gel-filtration column effluents were determined using the Lowry modification of the Folin-assay (Lowry *et al.*, 1951) and the phenol-sulphuric acid assay (Dubois *et al.*, 1956) respectively. The primary standards used in these assays were bovine serum albumin (Sigma Chemical Co. Ltd) and D-glucose (B.D.H. Ltd).

S107 myeloma serum

The murine IgA plasmacytoma S107 was kindly provided by Professor N. L. Warner and maintained by passage in BALB/c mice. Serum was obtained from mice bearing the tumour and stored frozen at -20°C until required. The specificity of the myeloma was determined using partially purified pneumococcal polysaccharide C-substance isolated according to the method of Liu & Gotschlich (1963). The phosphorylcholine specific IgA protein was isolated from S107 serum by gel-filtration chromatography on Sepharose CL-6B equilibrated with 0.1 M Tris-HCl buffer, pH 8.0 containing 0.15 M NaCl. The active material was identified by double diffusion analysis (Ouchterlony, 1958) in 1.5% agarose (Sigma Chemical Co. Ltd.) using pneumococcal polysaccharide C-substance at a concentration of 1 mg/ml in 0.01 M phosphate buffer, pH 7.2 containing 0.15 M NaCl (PBS). Double diffusion plates were pressed and dried according to the method of Laurell (1972) and stained with Beibrich scarlet (Crowle & Cline, 1977) purchased from Searle Diagnostic.

Reactivity of S107 serum with mite extracts

The reactivity of mite components with phosphorylcholine specific IgA myeloma was determined by double diffusion analysis and quantitative precipitin analysis (Kabat, 1961). Double diffusion analysis was performed in 1.5% agarose using a 1:12 dilution of the S107 serum in PBS. In the quantitative precipitin analysis 100 µl of S107 serum diluted 1:10 with PBS was added to 100 µl of the mite extract, prepared in this laboratory, over the range 400–5000 µg/ml. The mixture was incubated at room temperature for 1 h and subsequently for 3 days at 4°C. The tubes were centrifuged and the precipitates washed twice with ice cold saline. The amount of protein in the precipitates was determined using either the ninhydrin reagent (Schiffman, Kabat & Thompson, 1964) or the Lowry modification of the Folin assay (Lowry *et al.*, 1951) and the results expressed in optical density units. Quantitative precipitin-inhibition assays using phosphorylcholine chloride (Sigma Chemical Co. Ltd) were performed essentially as described by Leon & Young (1971).

Isolation of S107 reactive material from mite extracts

The S107 reactive material was isolated from 32 mg of whole mite extract by precipitation at equivalence using the S107 serum. The experimental conditions were the same as those described for the quantitative precipitin analysis. The washed precipitate was dissociated with 0.01 M phosphorylcholine in 0.1 M Tris HCl buffer pH 7.4. The IgA

component was removed by sequential immunosorption with both glutaraldehyde crosslinked (Avrameas & Ternynck, 1969) rabbit anti-whole S107 serum and rabbit anti-mouse IgA antisera. The immuno-absorbants were removed and the reactive material dialysed against distilled water and then freeze dried. The freeze dried material was reconstituted with PBS.

Reactivity of mite components with tridacnin

The lectin tridacnin was isolated from the haemolymph of the clam *Tridacna maxima*, obtained from the north-west coast of Western Australia. The lectin was purified on acid treated Sepharose 6B as described by Baldo & Uhlenbruck (1977). The reactivity of mite fractions and gel-filtration column effluents with tridacnin was determined in a haemagglutination-inhibition assay using human O+ve red blood cells. Serial doubling dilutions of inhibitor were prepared in saline containing calcium ions (Baldo & Uhlenbruck, 1977) and an equal volume (50 μ l) of tridacnin, previously diluted to give a haemagglutination titre of 1:4 and corresponding to a tridacnin concentration of 2.5 μ g/ml, added. After incubation for 1 h at 37°C, 50 μ l of 1% human O+ve cells were added and after a further incubation for 1 h at 37°C the inhibition titres were read.

Reactivity of mite components with concanavalin-A (con A)

The reactivity of mite fractions and gel-filtration column effluents with con A (Calbiochem, A grade) was determined by double diffusion analysis in 1.5% agarose using con A at a concentration of 1 mg/ml in 0.15 M NaCl. In other experiments the con A reactive material present in extracts of house dust mite was adsorbed using a commercial preparation of con A coupled to Sepharose 4B (Pharmacia, South Seas) in a batch procedure. Typically, 10 mg of moist adsorbant was incubated with 200 μ l of a 10 mg/ml solution of house dust mite extract for 45 min at room temperature with gentle stirring. After incubation the mixture was centrifuged and the supernatants examined for reactivity with both con A and the S107 serum. Mannan (Sigma Chemical Co. Ltd) at a concentration of 1 mg/ml was used to monitor the activity of the con A material used in these experiments.

Other methods

The isolated S107 IgA myeloma was iodinated with ^{125} I (The Radiochemical Centre, Amersham) according to the method of Hunter & Greenwood (1962). The iodinated material had a specific activity of 25 μ Ci/ μ g IgA.

Results

Reactivity of S107 serum with mite extracts

Double diffusion analysis using the S107 serum indicated that a component bearing phosphorylcholine was present in commercially available extracts of *D. pteronyssinus* (2) and *D. farinae* (2) and in extracts of *D. pteronyssinus* prepared in our laboratory (2.1). A line of identity between these two species of mite and the S107 was seen (Fig. 1). However, the component was not present in all commercially available preparations since commercial extracts from one particular manufacturer (1) failed to react with the S107 serum (Fig. 1). Likewise extracts prepared in this laboratory from mites supplied by this manufacturer (1) failed to react with the S107 serum over the wide concentration range of 1–50 mg/ml. The sensitivity of the double diffusion system was such that

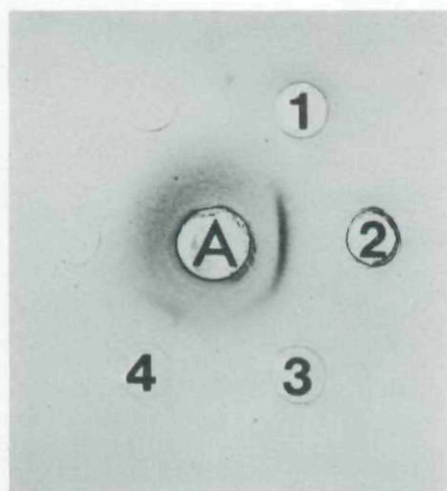


Figure 1. Agarose gel double diffusion analysis of (1) a commercial preparation of *D. pteronyssinus* (source 1) at 600 $\mu\text{g/ml}$, (2) extract of *D. pteronyssinus* prepared in our laboratory (2.1) at 10 mg/ml , (3) a commercial preparation of *D. pteronyssinus* (Source 2) at 12 mg/ml and (4) a commercial preparation of *D. farinae* (Source 2) at 12 mg/ml against S107 serum diluted 1/12 with PBS (A).

mite extracts which reacted with the S107 serum did so at concentrations as low as 300 $\mu\text{g/ml}$.

The reaction between the S107 and the mite extract (2.1) demonstrated by double diffusion analysis was confirmed in quantitative precipitin analyses and a typical precipitin curve is shown in Fig. 2. The reaction between the mite extract and the S107 serum was inhibited by free phosphorylcholine as demonstrated by quantitative precipitin inhibition studies (Fig. 3), and the final concentration of free hapten required to give 50% inhibition in these studies was 3.8×10^{-5} M. This figure is comparable to that

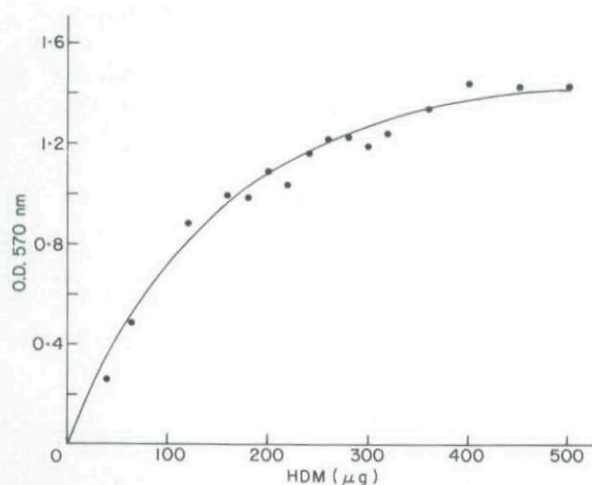


Fig. 2. Quantitative precipitin reaction between an extract of *D. pteronyssinus* prepared in our laboratory (2.1) and the S107 serum diluted 1:10 with PBS. 100 μl of S107 serum was added to an equal volume of PBS containing mite extract over the range 0-500 μg .

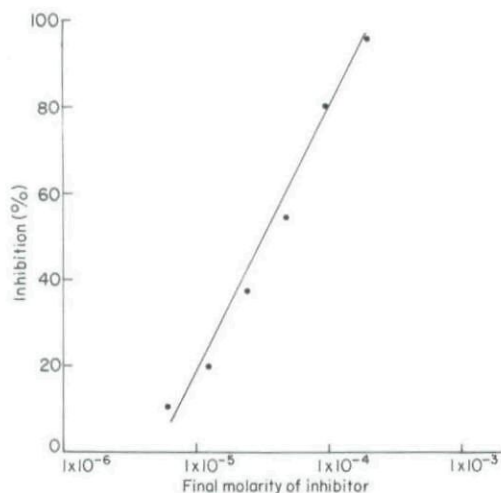


Fig. 3. Inhibition of the quantitative precipitin reaction between an extract of *D. pteronyssinus* prepared in our laboratory (2.1) and the S107 serum diluted 1:10 with PBS with the free hapten phosphorylcholine. Concentration of inhibitor expressed as the final concentration of inhibitor in the reaction mixture.

obtained by Leon & Young (1971) using the S107 serum and pneumococcal polysaccharide C-substance.

Gel-filtration studies

The mite extract (2.1) was fractionated first on Sephadex G-150 in an attempt to characterize the S107 reactive material and a typical elution profile is shown in Fig. 4. The S107 reactive material was shown by double diffusion analysis to be associated only with the high mol. wt material which eluted in the void volume. This material was concentrated and further fractionated on Sepharose CL-6B and a typical elution profile is shown in Fig. 5.

The S107 reactive material was again identified by double diffusion analysis and only one reactive fraction was identified. Using the known mol. wt markers apoferritin (430,000 daltons), rabbit IgG (150,000 daltons) and bovine serum albumin (69,000 daltons) it was shown that the reactive material eluted in a similar position to that of

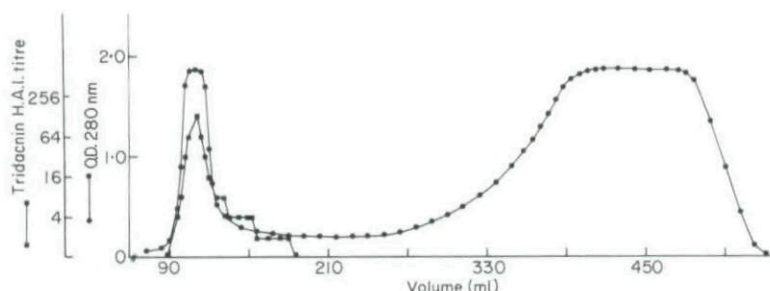


Fig. 4. Sephadex G-150 elution profile of a water soluble extract of *D. pteronyssinus* (180 mg, 2.1). The column (2.6 cm $\times$ 100 cm) was equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The fractions were monitored at 280 nm ($\bullet$) and tested for reactivity with tridacnin in a haemagglutination-inhibition assay using human O+ve red blood cells. Inhibition titres were expressed as a reciprocal ($\blacksquare$).

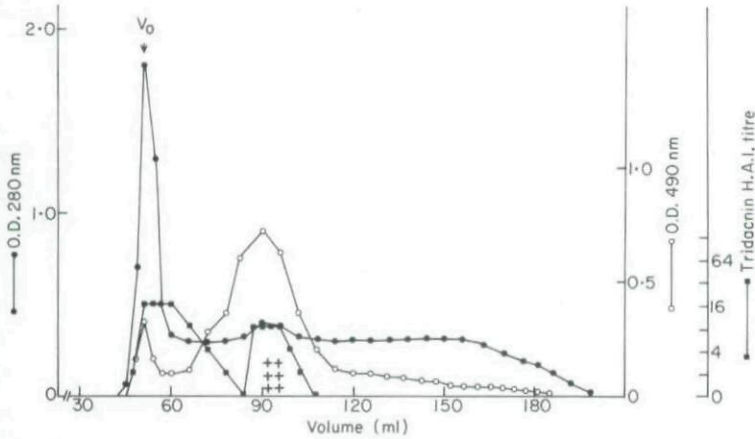


Fig. 5. Sepharose CL-6B elution profile of the high molecular weight material isolated on Sephadex G-150. The column (1.6 cm $\times$ 100 cm) was equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The fractions were monitored at 280 nm (●—●) and the carbohydrate content determined at 490 nm (○—○) using the phenol-sulphuric acid technique. The reactivity of the fractions with tridacnin was monitored in a haemagglutination inhibition assay using human O+ve red blood cells and the inhibition titres expressed as a reciprocal (■—■). The presence of the S107 reactive material in the fractions was monitored by double diffusion analysis using the S107 serum diluted 1:12 with PBS. The fractions precipitating with the S107 serum are indicated (++).

apoferritin. The majority of the carbohydrate containing material was found in this region of the elution profile.

In parallel with these studies it was shown that the tridacnin reactive material shown to be present in extracts of both *D. pteronyssinus* and *D. farinae* (Baldo *et al.*, 1976) was present in the high mol. wt material isolated by Sephadex G-150 gel-filtration (Fig. 4). Fractionation of the high mol. wt material on Sepharose CL-6B (Fig. 5) demonstrated the existence of at least two different molecular weight fractions reacting with tridacnin with the lower mol. wt tridacnin reactive fraction eluting in a similar position to that of the S107 reactive material. The high mol. wt tridacnin reactive component eluted in the void volume of the Sepharose CL-6B column.

Reactivity of S107 reactive material with tridacnin and con A

On the basis of these observations, it appeared possible that the S107 reactive material might also be the tridacnin reactive material. However, analysis of the supernatants from the S107 quantitative precipitin assay (Fig. 2) in a tridacnin haemagglutination-inhibition assay suggested that this was not the case. It can be seen from Table 1 that all of the supernatants retained the capacity to react with tridacnin. The results were expressed as the minimum amount of mite extract in mg/ml of supernatant required to inhibit the reaction between tridacnin and human O+ve red blood cells. The amount of mite extract in the supernatant required to inhibit the reaction between tridacnin and human O+ve red blood cells varied only within the experimental error associated with haemagglutination systems (i.e. within $\pm$ one haemagglutination well) throughout the entire precipitin curve suggesting that little tridacnin reactive material was lost due to co-precipitation. In similar experiments using supernatants from tridacnin precipitated mite (Figure not shown) it was demonstrated (Table 1) that all the

Table 1. Analysis of the supernatants from quantitative precipitin analyses, using either the S107 serum or tridacnin. The supernatants from the S107 precipitin analysis were analysed for reactivity with tridacnin in a haemagglutination inhibition assay using human O +ve red blood cells and the results expressed as the minimum amount ($\mu\text{g}/\text{ml}$) of supernatant, required to inhibit the agglutination of human O +ve red blood cells by tridacnin. The supernatants from the tridacnin precipitin analysis were analysed for reactivity with the S107 serum in an agarose gel double diffusion analysis. The degree of precipitation formed was empirically determined and ranged from low (+) to high (++++)

HDM ($\mu\text{g}/\text{ml}$) in supernatant	Supernatants from tridacnin precipitated HDM: Reactivity with S107 serum in Ouchterlony analysis	Supernatants from S107 precipitated HDM: Amount required ($\mu\text{g}/\text{ml}$) to inhibit agglutination of human O +ve cells by tridacnin
500	+	62.5
600	+	75
800	+	75
900	+	113
1000	+	62.5
1100	++	70
1200	++	75
1300	++	82.5
1400	++	87.5
1500	++	70
1600	++	100
1800	+++	113
2000	+++	87.5
2250	++++	105
2500	++++	115
HDM control (5000 $\mu\text{g}/\text{ml}$)	++++	70
Tridacnin control (600 $\mu\text{g}/\text{ml}$)	—	
S107 serum control (dil. 1/10)	No inhibition	

supernatants reacted with the S107 as indicated by double diffusion analysis, again suggesting that the S107 reactive material differs from that which reacted with tridacnin.

Similarly, it was also possible that the S107 reactive material might be the con A reactive material present in mite extracts (Baldo & Uhlenbruck, 1977). Initial double diffusion analyses indicated that house dust mite extracts from both manufacturers precipitated with con A in contrast to their reactivity with the S107 serum. Further studies indicated that the con A adsorbed mite extract still retained its capacity to react with the S107 serum but not with con A (Fig. 6), suggesting that the S107 reactive material is a different component to that which reacts with con A.

Isolation of S107 reactive material

The S107 reactive material present in the mite extract (2.1) was isolated by precipitation at equivalence based upon experimental conditions determined in the analytical

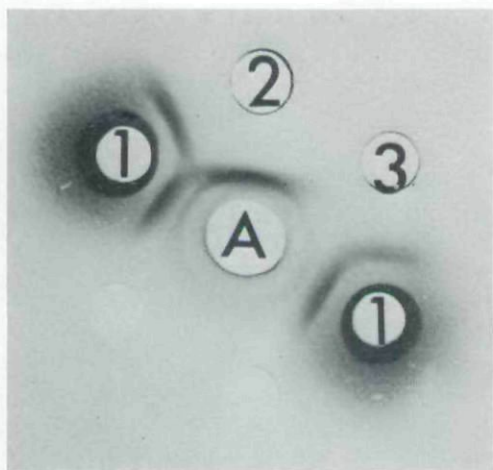


Fig. 6. Agarose gel double diffusion analysis of (1) S107 serum diluted 1:10, (2) *D. pteronyssinus* extract (source 2) at 1 mg/ml, (3) con A adsorbed *D. pteronyssinus* extract at 1 mg/ml against con A at 1 mg/ml (A).

quantitative precipitin studies. The washed precipitate was dissociated with 0.01 M phosphorylcholine and the IgA component removed by sequential immunoabsorption with both insolubilized rabbit anti-S107 serum and rabbit anti-mouse IgA antisera. Protein and carbohydrate estimations indicated that the material was glycoprotein in nature with 16% carbohydrate. The percentage yield of this material was found to be 1.6 of the water soluble extract and 0.08 of the whole mite starting material.

When the isolated material was examined by double diffusion analysis it reacted strongly with the S107 serum and produced a line of identity with pneumococcal polysaccharide C-substance and the whole mite extract (Fig. 7). It was also demonstrated in this study that an extract of the mite medium failed to react with the S107

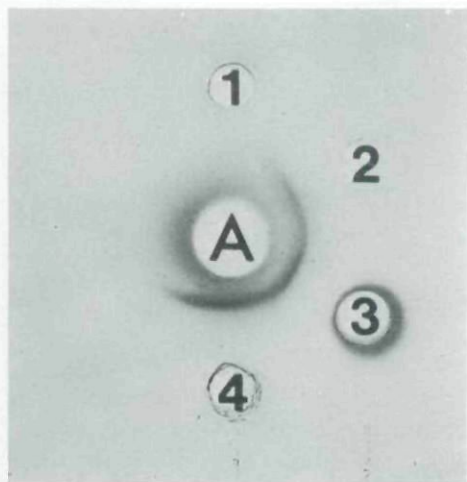


Fig. 7. Agarose gel double diffusion analysis of (1) mite growth medium (source 2) at 5 mg/ml; (2) original *D. pteronyssinus* extract (2.1) at 4 mg/ml; (3) isolated S107 reactive material (2.1) at 500 µg/ml; and (4) pneumococcal polysaccharide C-substance at 5 mg/ml against S107 serum diluted 1:12 with PBS (A).

serum (Fig. 7). The S107 reactive material at a concentration of 500 $\mu\text{g/ml}$ did not react with either rabbit anti whole mite extract antiserum or a rabbit anti S107 antiserum. Similarly, it did not react with either tridacnin or con A.

Allergenicity of S107 reactive material

The allergenicity of the isolated material was determined by direct RAST binding studies in which the material was coupled to cyanogen bromide activated paper discs and reacted with a highly mite allergic serum pool. The results of this study are shown in Fig. 8. An IgE binding curve was obtained for both the isolated S107 reactive material and the whole mite extract. The maximum radiation bound by discs coupled with the S107 reactive material was 36% in comparison with discs coupled with whole mite extract which bound 42%. In contrast, the maximum radiation bound using a highly grass pollen allergic but mite negative serum pool was 2% and 3% for S107 reactive material coupled discs and whole mite extract coupled discs respectively. RAST and RAST-inhibition studies indicated that the mite medium extract did not bind IgE.

In control experiments the presence of the S107 reactive material on the activated discs was directly indicated using ^{125}I radiolabelled S107 IgA. To conserve material pneumococcal polysaccharide C-substance to activated paper discs and perform direct or the whole mite. Discs coupled with the S107 reactive material (3.2 $\mu\text{g/disc}$) bound 50% radiation and discs coupled with whole mite extract (0.8 $\mu\text{g/disc}$) bound 27% radiation. Control discs coupled with human serum albumin bound 3% radiation.

Role of phosphorylcholine moiety

It was decided to determine whether the phosphorylcholine moiety formed part of any

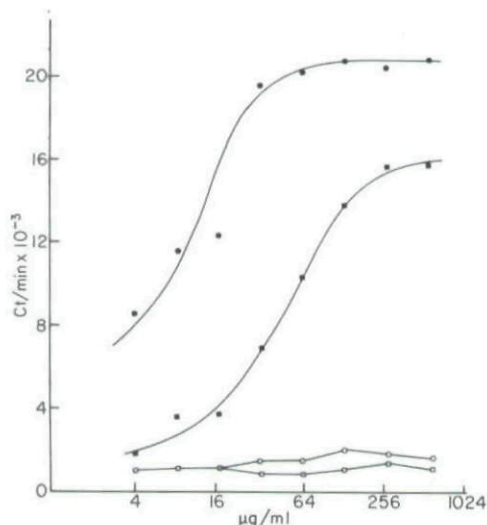


Fig. 8. Direct RAST binding curves obtained for the original *D. pteronyssinus* extract (source 2, ●—●) and the isolated S107 reactive material (■—■) using pooled mite allergic sera. Control binding curves obtained with the original extract (○—○) and the isolated S107 reactive material (□—□) using a grass pollen only allergic serum pool are also shown. Coupling of the allergens to individual cyanogen bromide activated paper discs was achieved using 50 μl of allergen over the concentration range 4–1024 $\mu\text{g/ml}$. The amount of allergen specific IgE bound to the discs was expressed as the uptake of radiolabelled anti-IgE in ct/min.

Table 2. RAST studies illustrating the capacity of free phosphorylcholine to inhibit the binding of allergen specific IgE to cyanogen bromide paper discs coupled with the isolated S107 reactive material (3.2 µg/disc). 25 µl of a mite allergic serum pool were incubated with 25 µl of phosphorylcholine in 0.1 M Tris-HCl buffer, pH 7.4, for 3 h at room temperature before the addition of the allergen coupled disc. The concentration of phosphorylcholine was expressed in µmol added to the reaction mixture

Amount of phosphorylcholine, added to mite allergic serum (µmol)	RAST (% radiation bound)
0	100
5	100
10	104
20	84
30	89
40	79

allergenic determinant present in the isolated material and this problem was approached in two ways. Firstly, RAST-inhibition studies using the free hapten were performed and the results are illustrated in Table 2. It can be seen that significant levels of inhibition were not attained over this range. The second approach was to couple pneumococcal polysaccharide-C substance to activated paper discs and perform direct RAST binding studies using mite allergic serum. The maximum percentage radiation bound over the range of 1–500 µg/ml was 1% with mite allergic serum and 2.4% with grass pollen allergic serum. The presence of pneumococcal polysaccharide C-substance on the activated discs was confirmed using the ^{125}I S107 IgA.

Discussion

The hapten phosphorylcholine has been shown to be associated with components present in extracts of parasites (Capron, Biguet, Van Ky & Rorse, 1964; Potter, 1972), bacteria (Potter, 1972) and fungi (Longbottom & Pepys, 1964). In this study the presence of a glycoprotein component containing the hapten phosphorylcholine in extracts of *D. pteronyssinus* and *D. farinae* using the S107 murine IgA myeloma is described. Such observations confirm and extend those of Baldo, Fletcher & Uhlenbruck (1977) who reported the presence of a phosphorylcholine containing component in extracts of *D. farinae* using the murine IgA myeloma HOPC 8. In their study, however, the reactive material was neither isolated nor its allergenicity assessed.

Whilst demonstrating the presence of a phosphorylcholine component in the commercial preparation 2 of *D. pteronyssinus* and in the extract (2.1) prepared in our laboratory the presence of this component was not detected in the commercial preparation 1 or in extracts (1.1) made from mites obtained from this source. The reasons for this difference are at present unexplained, but it is possible that the component was present in these latter extracts in insufficient quantity to be detected by the system adopted. Further, it is possible that our failure to detect the component in these extracts reflects different manufacturing techniques, which in some way influence the production of this material. In this regard, visual examination of both the mites and growth media from the two different sources revealed marked physical differences suggesting differences in manufacturing techniques.

Gel-filtration studies indicated that there was only one mol. wt species of the phosphorylcholine bearing component present in extracts of *D. pteronyssinus* with an apparent mol. wt similar to that of apoferritin, namely 430,000 daltons. However, this value may be an overestimate in view of the high carbohydrate content (Andrews, 1970) and further physico-chemical characterization studies are required.

Whereas only one mol. wt species of phosphorylcholine containing component was detected in our mite extracts the presence of at least two mol. wt species of the tridacnin reactive component were detected with mol. wts of 4×10^6 and 430,000 daltons respectively. Again, these values may be overestimates and must be regarded with caution. The presence of more than one tridacnin reactive component in extracts of *D. pteronyssinus* has also been detected by immunodiffusion studies (Baldo & Uhlenbruck, 1977). Data obtained in our studies indicate that the phosphorylcholine reactive material is different from both the tridacnin reactive components and the con A reactive component described by Baldo & Uhlenbruck (1977). In contrast to the S107 reactive material described in these studies, both the tridacnin and con A reactive components were present in mite extracts made from both sources.

The allergenicity of the phosphorylcholine containing component isolated in these studies has been established by direct RAST binding studies. We were unable to demonstrate that the phosphorylcholine moiety formed part of any major allergenic determinant present on the S107 isolated material. In other reports a variety of substances such as dermatophyte glycopeptides, pneumococcal C-substances, parasite extracts and vegetable extracts, have been shown to provoke immediate skin test reactions in patients with aspergilloma (Pepys & Longbottom, 1971). On the basis of these data it has been suggested (Baldo, Fletcher and Pepys, 1977) that, since the only known common feature between this diverse group of substances is phosphorylcholine, this moiety probably forms part of an allergenic determinant. However, there is as yet no experimental data to confirm this suggestion.

Data thus far obtained indicate that the high mol. wt allergens from mite extracts differ from the low mol. wt allergens of both species of house dust mite (Ishii *et al.*, 1973; Biliotti *et al.*, 1975; Nakagawa *et al.*, 1977). It is clear, however, that the high mol. wt allergens present in extracts of both *D. farinae* and *D. pteronyssinus* are similar. These observations are in accord with those obtained in previous studies in which it was demonstrated that antisera raised in rabbit against one species recognized common antigens in the other (Dasgupta & Cunliffe, 1970).

Our studies and those of Baldo and Uhlenbruck (1977) indicate that there exist in extracts of both *D. farinae* and *D. pteronyssinus* allergens with apparent mol. wt higher than those usually associated with other well characterized allergens (Marsh, 1974), namely < 60,000 daltons. It is generally held that this restriction in the molecular size of the allergen is associated with the capacity of low mol. wt allergens to penetrate mucosal surfaces thus eliciting IgE formation. If this is true it must be postulated that the high mol. wt mite allergens are degraded at either the alveolar or gastrointestinal mucosal surfaces and are therefore adsorbed in low mol. wt form. Evidence for such a degradation process has been described by Willoughby & Willoughby (1977). An alternative explanation to account for the allergenicity of the high molecular weight components present in mite extracts is that they result from aggregation of lower molecular weight molecules during the isolation procedures (Baldo & Uhlenbruck, 1977). However, since high molecular allergens have been demonstrated in *Ascaris suum* perienteric fluid (McWilliam, Stewart & Turner, unpublished observation) and in

extracts of *Trichophyton concentricum* (Turner & Rankin, unpublished observations), the latter hypothesis is unlikely unless aggregation is a general phenomenon.

Studies are presently under way to isolate further amounts of the S107 reactive material to both physico-chemically characterize it and establish its biological significance.

Acknowledgments

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