

© PHYSICOCHEMICAL AND IMMUNOCHEMICAL
CHARACTERIZATION OF THE HIGH MOLECULAR
WEIGHT ALLERGENS FROM *DERMATOPHAGOIDES*
PTERONYSSINUS WITH PARTICULAR REFERENCE TO
THE TRIDACNIN, CON-A AND S107 REACTIVE
COMPONENTS.

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(Accepted for publication April 18, 1980.)

Summary. High molecular weight allergens which react with the S107 murine IgA myeloma and with the lectins Concanavalin A (Con-A) and tridacnin have been identified and characterized in extracts of the mite *Dermatophagoides pteronyssinus*. Mite extracts from two different manufacturers were fractionated by Con-A affinity chromatography. The major antigen in both the Con-A bound and unbound fractions, which was antigenically indistinguishable, reacted with tridacnin and the S107 myeloma. This high molecular weight, multi-reactive antigen corresponded to allergen Dpt 4, suggesting that Dpt 4 exists as isoallergens, each differing in the degree of glycosylation or in the type of carbohydrate moiety comprising the glycan part of the allergen. Mite media extracts also reacted with Con-A, tridacnin and the S107 myeloma but were not allergenic *per se*.

INTRODUCTION

Baldo, Turner and Uhlenbruck (1976) and Baldo and Uhlenbruck (1977a) described the isolation of an allergen from *D. farinae* using tridacnin and Con-A. They established that the tridacnin reactive allergen was a glycoprotein of high molecular weight and heterogeneous as judged by polyacrylamide gel electrophoretic analysis. The Con-A reactive material isolated from *D. farinae* was also shown to be allergenic but not as allergenic as that isolated with tridacnin. Double diffusion analyses indicated that the Con-A isolated allergen did not react with tridacnin or vice versa. Stewart, McWilliam and Turner (1980) described the isolation from *D. pteronyssinus* of a high molecular weight glycoprotein allergen bearing phosphoryl choline (Pc). Gel diffusion studies indicated that this material was also different from that which reacted with Con-A and tridacnin. In none of these studies, however, was the relationship between the allergens examined using

an anti-mite antiserum. In this report, a study of the antigenic relationship between these allergens is described.

To facilitate such studies, a haemagglutination-inhibition assay designed to detect Pc-containing components has been developed. It is based on a previous haemagglutination assay described by Leon and Young (1970) in which it was shown that a Pc-containing substrate could be attached to red blood cells using Con-A. In the system described here, use has been made of the observation that the intestinal parasite *Ascaris suum* contains large amounts of Pc (Crandall and Crandall, 1971). This material, rather than β -lipoprotein used by Leon and Young (1970) or Pc coupled to a protein carrier (Chesebro and Metzger, 1972), has been used since it is readily available and simply prepared. This material has been coupled to sheep red blood cells with glutaraldehyde (Avrameas, Taudou and Chuilon, 1969), resulting in a stable (2-3 months) reagent.

MATERIALS AND METHODS

Except where stated, all the methods used in this study have been described in detail in a previous paper (Stewart and Turner, 1980).

S107 mouse myeloma serum and detection of Pc-containing components

The S107 serum and an ^{125}I -radiolabelled antibody preparation from this serum were prepared as previously described (Stewart *et al.*, 1980). Visualization of Pc-containing components in crossed immunoelectrophoretic (CIE) analyses of mite extracts was achieved by incubating CIE plates with radiolabelled S107 antibody analogous to crossed radioimmunoelectrophoresis (CRIE). Plates were incubated with 4×10^6 c.p.m in 17 ml of incubation buffer for 48 h at room temperature. The plates were then washed four times with 10 ml of 0.15% w/v NaCl and air-dried. Autoradiography was performed as described for CRIE (Löwenstein, Aukrust and Gravesen, 1977).

The presence of Pc-containing components in mite extracts and gel-filtration column effluents was determined using a haemagglutination inhibition assay employing sheep red blood cells coated with *Ascaris suum* body fluid extract kindly provided by Mr. A. S. McWilliam. Cells were coated with the extract using glutaraldehyde according to the method of Avrameas *et al.* (1969). Serial doubling dilutions of inhibitor were prepared in 0.01 M phosphate buffer, pH 7.2 containing 0.15 M NaCl and 1% bovine serum albumin (PBS-BSA) and an equal volume (50 μl) of S107 serum previously diluted to give a haemagglutination titre of 1:4. After incubation for 1 h at 37°, 50 μl of 1% sensitized sheep red blood cells were added and the inhibition titres read after a further incubation for 1 h at 37°. Results were expressed in haemagglutination inhibition units (HAIU) where one unit is the amount of inhibitor per well at the end point. Specific activity was expressed as HAIU per mg inhibitor protein (Smith and Walborg, 1976).

Reactivity of mite components with tridacnin

The reactivity of mite fractions and gel filtration column effluents with tridacnin was determined in a haemagglutination-inhibition assay using human 0 +ve red blood cells as previously described (Stewart *et al.*, 1980). Results were expressed as described for S107 reactivity.

Reactivity of mite components with Con-A

The reactivity of mite fractions and gel filtration column effluents with Con-A was determined by double diffusion analysis using Con-A at a concentration of 5 mg/ml (Sigma Chemical Co. Ltd.) or in a haemagglutination-inhibition assay using guinea-pig red blood cells (Smith and Walborg, 1976) analogous to that described for the tridacnin and S107 assays. Results were expressed as described for S107 reactivity.

Isolation of Con-A reactive components

Con-A reactive components from mite extracts or gel filtration fractions were isolated by affinity chromatography using a commercial preparation of Con-A coupled to Sepharose 4B (Pharmacia South Seas) equilibrated with 0.1 M acetate buffer pH 6.0 containing 1 M NaCl and 1 mM CaCl_2 , MgCl_2 and MnCl_2 . Con-A reactive components were eluted with α -D-methylmannoside (Sigma Chemical Co. Ltd.). Column effluents were monitored at 280 nm and the Con-A unbound and bound fractions, together with a sample of the original material, were dialysed against four changes (each of 5 litres) of distilled water. The dialysed samples were lyophilized and reconstituted with PBS and the protein content determined.

Molecular weight distribution of S107 and tridacnin reactive components

Samples of fraction 2 obtained by chromatography on Sephacryl S-300 were electrophoresed overnight in 4-30% polyacrylamide gels as described in a previous paper (Stewart and Turner, 1980). The position of the components was determined by staining one complete section of the gel with Coomassie Blue G-250. The gel was cut into sections containing the component under consideration, macerated and incubated with 3 ml of 0.15 M w/v NaCl for 24 h on a rotating wheel at 4°. The gel was centrifuged and the supernatant dialysed against 2 litres of distilled water (three changes) and then lyophilized. The material was redissolved in 100 μ l PBS and examined for reactivity with tridacnin and S107 serum.

RESULTS

Optimum conditions for sensitizing sheep red blood cells with A. suum extract

The optimum amount of *A. suum* extract required to sensitize SRBC for agglutination by S107 serum was determined over the concentration range 1-125 mg per 0.1 ml packed SRBC. The results from this experiment are shown in Table 1 and it can be seen that this extract served particularly well as a source of Pc. The minimal optimal concentration was found to be 25 mg/0.1 ml packed SRBC, and at this concentration typical haemagglutination titres of 6×10^6 were achieved. The specificity of the reaction was determined in a haemagglutination inhibition assay using the free hapten Pc, the lowest inhibitory concentration of which was found to be 12 μ M using the assay conditions described. This assay was used to detect Pc-containing components in extracts of *D. pteronyssinus*.

Detection of Pc-containing components

In a previous paper (Stewart *et al.*, 1980), Pc-containing components were not detected in all mite extracts studied, and it was suggested that the double diffusion assay used to detect such material was insufficiently sensitive. Because of this, the

TABLE 1
Quantity of Ascaris suum body fluid extract required to optimally coat sheep red blood cells for reactivity with the mouse IgA myeloma S107.

Amount of <i>A. suum</i> extract in mg/0.1 ml packed SRBC	Haemagglutination titre (reciprocal)
2.5	192,000
12.5	3,072,000
25	6,144,000
62.5	6,144,000
125	1,536,000

Quantity of glutaraldehyde was kept constant throughout.

two mite preparations used in that study were re-examined for the presence of Pc-containing components using the haemagglutination-inhibition assay just described. Both extracts reacted with the S107 serum indicating the presence of Pc (Table 2). However, a quantitative difference, expressed in haemagglutination inhibition units per mg protein, between the two extracts was found. The mite material from source 2, which was shown previously to react with the S107 serum in double diffusion analysis, had a 7.6-fold greater amount of Pc-containing material than that from source 1, which did not react in the double diffusion assay.

TABLE 2

Reactivity of mite extracts prepared from two different manufacturers and their respective mite media with tridacnin, S107 and Con-A.

	HAIU/mg <i>D. pteronyssinus</i> extract		
	Con-A	Tridacnin	S107
HDM Source 1	260	21,040	960
Medium Source 1	150	602	19
HDM Source 2	7,600	3,120	7,360
Medium Source 2	0	0	7,816

One haemagglutination inhibition unit (HAIU) is defined as the minimum amount of material required to inhibit the agglutination of sensitized cells by the appropriate reagent. Results are expressed as the number of HAIU/mg of protein. The figures represent the average of three independent experiments.

The amount of tridacnin and Con-A reactive material present in these extracts was also determined. It can be seen from Table 2 that, whereas the extract from source 2 contained more S107 reactive material than that from source 1, the reverse was seen for tridacnin reactivity. In this instance, extract from source 1 contained 6.7 times as much tridacnin reactive material. Extract from source 2 had a 29.2-fold greater amount of Con-A reactive material than that from source 1.

Extracts of the media upon which the mites were grown were also tested for reactivity with Con-A, tridacnin and S107 and these results are shown in Table 2. The mite medium from source 1 showed low reactivity with tridacnin and S107, 2.8% and 1.9% respectively of the reactivity shown by the mite extracts. However, it showed 58% of the reactivity of the mite extract with Con-A. The mite medium from source 2 had an amount of S107 reactivity comparable to that shown by the mite extract but, in contrast, it did not react with either Con-A or tridacnin. The two media extracts were visibly and chemically different, and the percentage protein content of media extracts 1 and 2 were 18% and 58% respectively and carbohydrate content 82% and 42% respectively.

Molecular weight distribution of Pc-containing components

The molecular size of the S107 and tridacnin reactive components in an extract of *D. pteronyssinus* from source 1 was determined by gel-filtration on Sephacryl S-300 (Fig. 1). Two major S107-reactive peaks were found, one of which eluted in the void volume. A significant amount of Pc-containing material was also apparent on the trailing edge of peak 2. A similar distribution pattern was obtained for tridacnin reactivity.

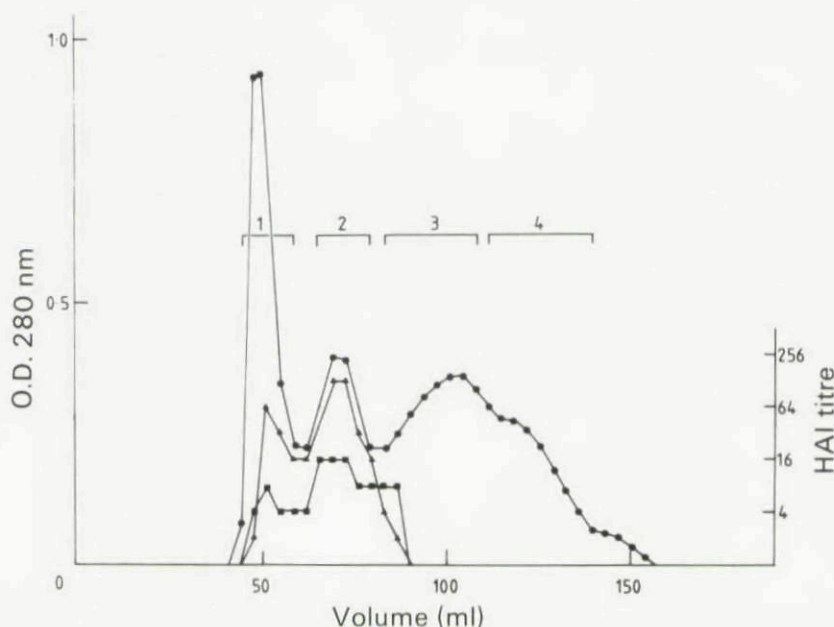


Fig. 1. Sephacryl S-300 elution profile of a water soluble extract of *D. pteronyssinus* (40 mg). The column was equilibrated with 0.1 M Tris-HCl buffer (pH 8.0) plus 0.15 M NaCl. The fractions were monitored for absorbance at 280 nm (●—●) tridacnin reactivity (▲—▲) and S107 reactivity (■—■). V_0 corresponds to an elution volume of 50 ml.

Further studies were undertaken using gradient polyacrylamide gel electrophoresis (PAGE). Sephacryl S-300 fraction 2 (Fig. 1) was subjected to gradient PAGE and the separated components were eluted and tested for reactivity with the appropriate reagent using haemagglutination inhibition assays. The results are shown in Fig. 2. Tridacnin reactivity was associated with material with apparent molecular weights above 130,000 daltons. The reactivity was particularly evident in the high molecular weight region of the gel where carbohydrate rather than protein staining was evident (Stewart and Turner, 1980). S107 reactivity paralleled that seen for tridacnin, but the very high molecular weight material was inactive. The low molecular weight component 7 did not react with tridacnin or S107.

Isolation of Con-A reactive material

Whole mite extracts from both sources, together with Sephacryl S-300 fraction 2 from source 1, were fractionated by Con-A affinity chromatography. Con-A bound material was eluted stepwise with 0.05 M and 1 M α -D-methylmannoside and the resulting fractions were examined for reactivity with either S107, tridacnin or Con-A. The results obtained with the whole extracts are shown in Table 3.

Con-A affinity chromatography removed all of the Con-A reactive material in the extracts of mites from both sources. However, only 16.6% and 5.2% of the bound material from sources 1 and 2 respectively were recovered by elution with α -D-methylmannoside, in marked contrast to the almost complete recovery of

TABLE 3
Reactivity of Con-A bound and unbound fractions from extracts of *D. pteronyssinus* from two different sources.*

Fraction	Total protein, mg	% Carbohydrate	Con-A		Tridacnin		S107	
			Specific activity HAU/mg	Total activity	Specific activity HAU/mg	Total activity	Specific activity HAU/mg	Total activity
<i>HDM, Source 1</i>								
Original extract	15.7	5.1	240	3,840	15,612	245,795	972	15,303
Con-A—unbound	9.34	3.7	0	0	12,549	117,207	276	2,578
Con-A—bound (0.05 M α -D-methylmannoside)	2.43	22.2	132	320	33,896	82,500	2,104	5,121
Con-A—bound (1 M α -D-methylmannoside)	0.566	32.7	564	320	4,520	2,556	9,040	5,117
% Recovery	78.5	—	—	16.6	—	82.3	—	83.7
<i>HDM, Source 2</i>								
Original extract	9.96	17.9	6,248	61,439	3,084	30,716	8,827	87,924
Con-A—unbound	6.528	11.3	0	0	1,568	10,236	3,137	20,479
Con-A—bound (0.05 M α -D-methylmannoside)	0.321	59.1	1,992	640	32,000	10,272	127,796	41,022
Con-A—bound (1 M α -D-methylmannoside)	0.022	86	116,600	2,560	235,280	5,176	465,454	10,240
% Recovery	69	—	—	5.2	—	83.6	—	81.5

* 30 mg (dry weight) of each extract was applied to a Sepharose 4B—Con-A affinity column (5 ml) and unbound material eluted with 0.1 M acetate buffer (pH 6.0) containing 1 M NaCl and 0.001 M each of $MgCl_2$, $MnCl_2$ and $CaCl_2$. The Con-A bound material was eluted stepwise with 1% and 20% α -D-methylmannoside at 25°. The fractions were exhaustively dialysed, freeze-dried and reconstituted in PBS and tested for reactivity with S107, Con-A and tridacnin by haemagglutination inhibition. Results are expressed as specific activities (HAU/mg protein) and total activities recovered. All activities were based on protein concentrations determined by Folin assay and represent the average of 2 experiments. Percentage recoveries were determined by reference to a sample of the original extract treated similarly to the Con-A bound and unbound fractions. The percentage carbohydrate was determined by dividing the carbohydrate concentration by the sum of the carbohydrate and protein concentrations.

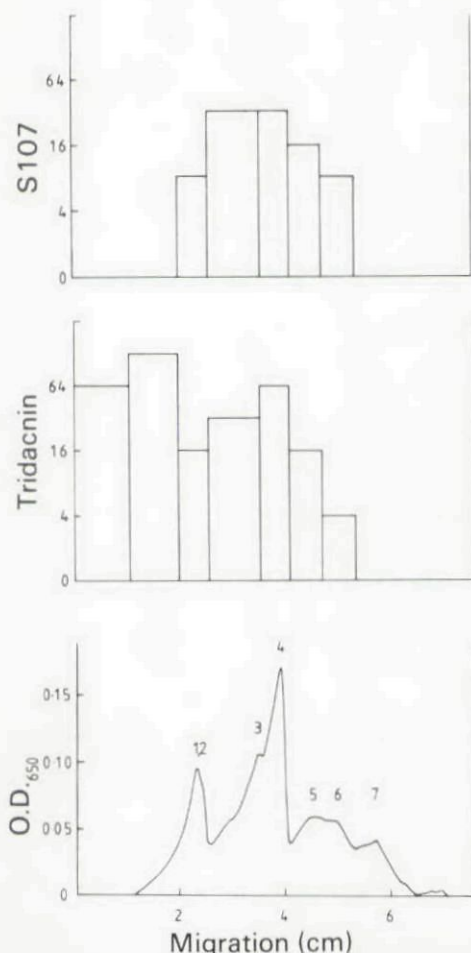


Fig. 2. Gradient (4-30%) PAGE analysis of Sephacryl S-300 fraction 2 (280 μ g). One section of the gel was stained with Coomassie Blue G-250 and scanned using a densitometer (lower panel). Components were eluted from the unstained section of the gel and tested for their reactivity with tridacnin (centre panel) or S107 serum (upper panel). Reactivity with these two reagents is expressed as a reciprocal.

tridacnin and S107 reactive material. The low yield of Con-A bound material was further indicated by the poor reactivity of the Con-A bound materials with Con-A in double diffusion analysis in contrast to the original extracts. Quantitative evidence of low yields was obtained by the Con-A affinity chromatographic separation of ^{125} I-labelled Sephacryl S-300 fraction 2 from source 1 in which it was shown that of the 50% radiolabel binding to the Con-A column, only 10% could be eluted by 1 M α -D-methylmannoside with a further 3% being eluted after shutting down the column in the presence of 1 M α -D-methylmannoside for 2 days.

Elution with 1 M α -D-methylmannoside at a slightly higher temperature than that used with 0.05 M α -D-methylmannoside was chosen, since studies showed that this procedure resulted in a 2-3-fold higher yield of S107 reactive material. This procedure did not significantly increase the yields of either tridacnin or

Con-A reactive materials. The influence of higher temperatures on the yield of Con-A reactive material was not investigated.

The haemagglutination inhibition studies showed that the Con-A bound material from both mite extracts reacted with both S107 and tridacnin with higher specific activities than the original extracts. Further, in each mite extract, there was material which reacted with S107 and tridacnin but not Con-A. For the mite extract from source 2, 33% of the tridacnin reactive material and 23% of the S107 reactive material did not react with Con-A. In the mite extract from source 1, 48% of the tridacnin reactive material and 17% of the S107 reactive material did not react with Con-A. The percentage carbohydrate in each of the fractions was determined (Table 3). These studies showed that the original mite extract from source 2 contained about 3-4 times as much carbohydrate as did that from source 1. Increasing amounts of carbohydrate were seen in the 0.05 M and 1 M α -D-methylmannoside eluates from both sources of mite, but the eluates from the source 2 extract contained significantly more carbohydrate than the corresponding eluates using the extract from source 1.

Immunochemical identity of Con-A, S107 and tridacnin reactive components

Double diffusion analysis (Fig. 3) indicated that the major immunoprecipitin band seen in the Con-A unbound material was antigenically identical to that seen in the Con-A bound material, notwithstanding their different reactivity with Con-A. CIE analysis (Fig. 4) confirmed this observation and, further, showed that the major antigen in both Con-A bound and unbound fractions corresponded to allergen Dpt 4 described in a previous paper (Stewart and Turner, 1980). In addition, three other antigens were detected in the Con-A bound fraction.

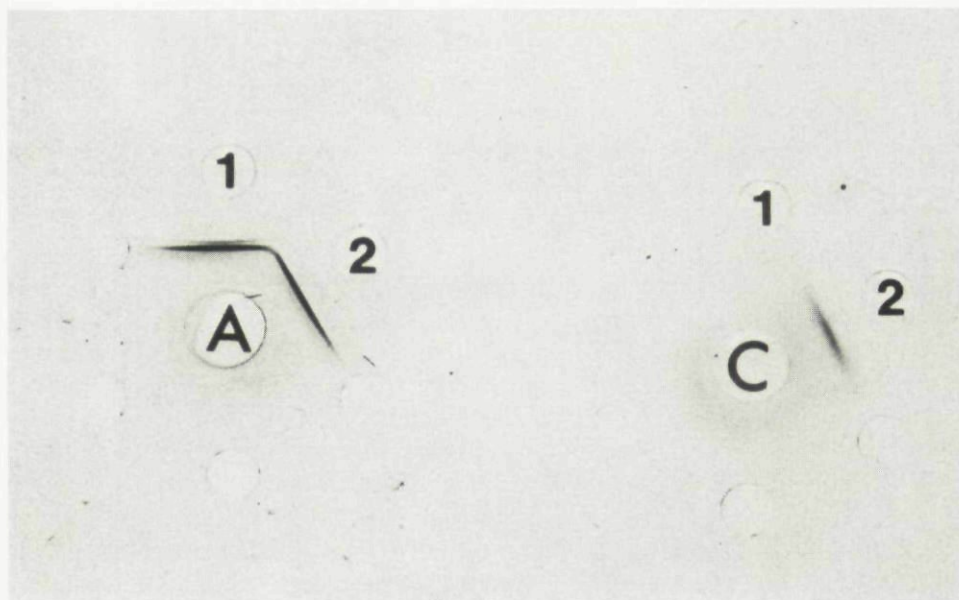


Fig. 3. Agarose gel double diffusion analysis of Con-A unbound material (1) at 3 mg/ml and Con-A bound material (2) at 1.5 mg/ml, against (A) rabbit anti-HDM antiserum and (C) Con-A at 5 mg/ml.

Studies were undertaken to determine which of the antigen(s) identified by CIE contained the hapten Pc. Unstained CIE plates of Sephacryl S-300 fraction 2 were incubated with ^{125}I -radiolabelled S107 antibody and the results from this study are shown in Fig. 5. Only one Pc-containing antigen was detected and this corresponded to allergen Dpt 4.

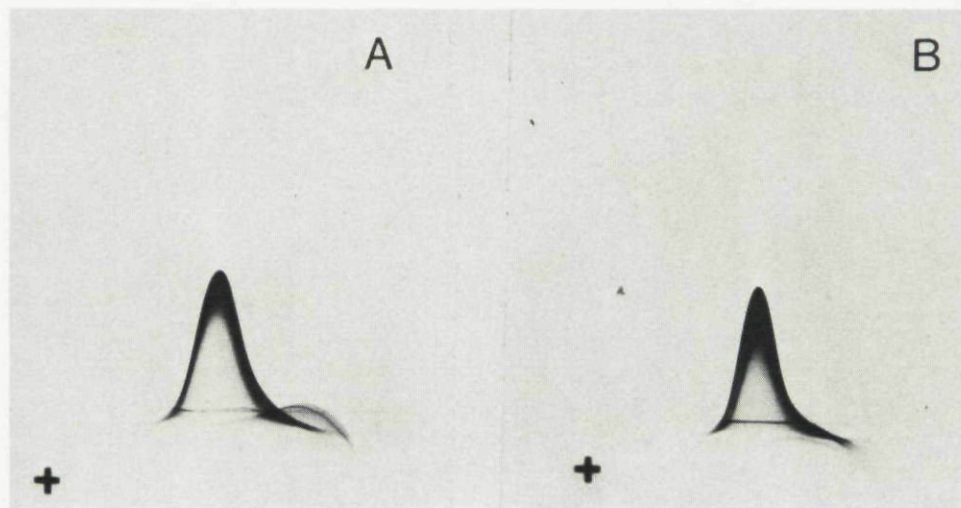


Fig. 4. Crossed immunoelectrophoresis of Con-A unbound (A) and Con-A bound (B) material from Sephacryl S-300 fraction 2. CIE was performed using 15 μg and 30 μg of bound and unbound material respectively and an antibody concentration of 20 μl per cm^2 gel area.

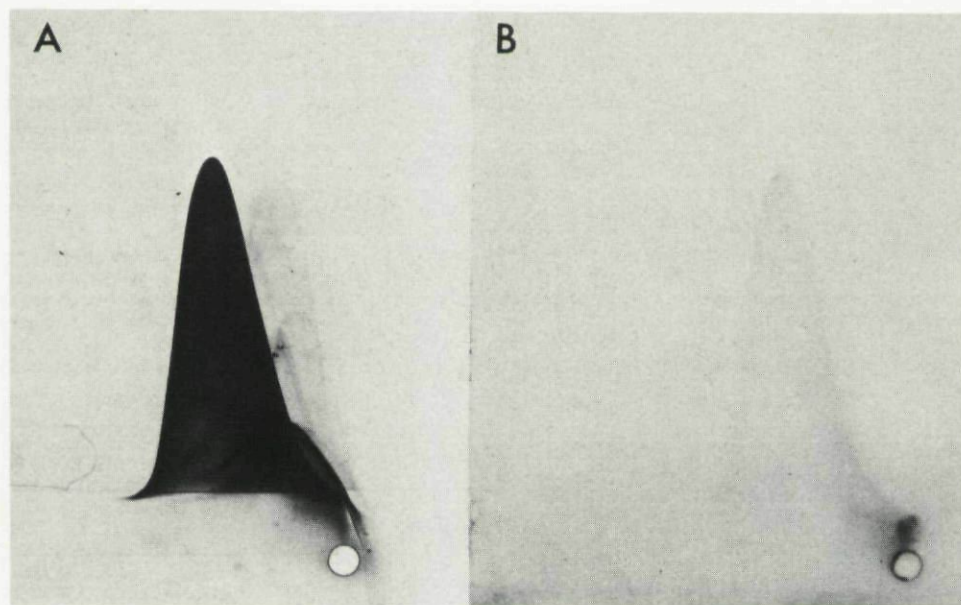


Fig. 5. Crossed radioimmuno-electrophoresis of Sephacryl S-300 fraction 2. CIE (A) was performed using 115 μg of fraction 2 and a rabbit anti-*D. pteronyssinus* antibody concentration of 10 μl per cm^2 gel area. The plate was subsequently incubated with ^{125}I -labelled S107 antibody. The autoradiograph (B) was exposed for 14 days.

Allergenicity of Con-A bound material

The allergenicity of the Con-A bound and unbound fractions from Sephacryl S-300 fraction 2 was determined by direct RAST binding studies and the results are shown in Fig. 6. The Con-A bound material eluted with 0.05 M α -D-methylmannoside was found to be allergenic, although slightly less so than either the Sephacryl S-300 fraction 2 or the Con-A unbound material.

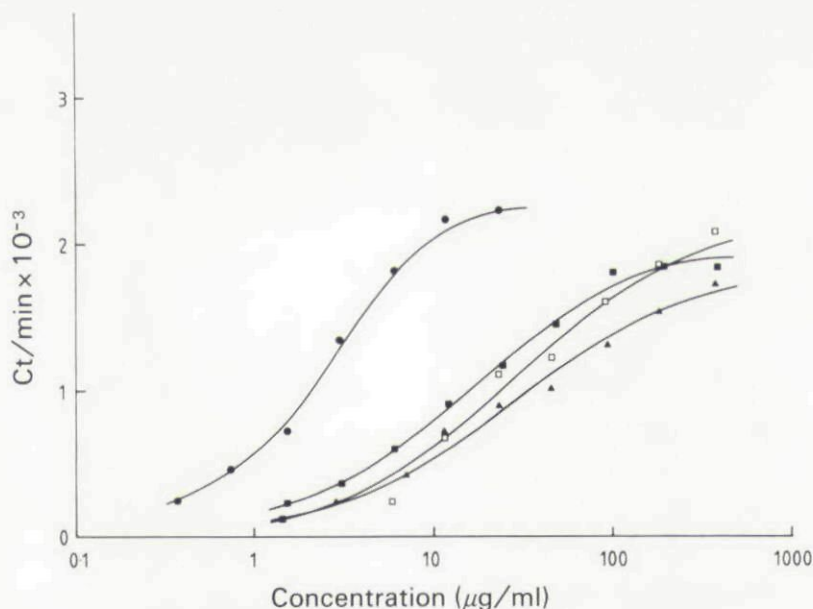


Fig. 6. Direct RAST binding curve obtained for the whole mite extract (●—●), Sephacryl S-300 fraction 2 (□—□), Con-A unbound material (■—■) and Con-A bound material (▲—▲) using pooled mite allergic serum. Coupling of the fractions to individual CNBr activated paper discs was achieved using 50 µl allergen over the concentration range 0.1–500 µg/ml (as protein).

DISCUSSION

The sensitive haemagglutination-inhibition assay described in this report has facilitated the detection of Pc-containing components in an extract of *D. pteronysinus* (source 1) which was hitherto thought to be devoid of such material (Stewart *et al.*, 1980) as judged by double diffusion. This assay has been shown to be superior to that described by Leon and Young (1970) in that *A. suum* coated cells remained stable for several months, unlike β -lipoprotein coated cells which remain stable for only 2 days. Further, the haemagglutination titres obtained with the S107 serum using *A. suum* coated cells were several orders of magnitude higher than that obtained using β -lipoprotein coated cells.

Extracts of mites from two different manufacturers were examined for reactivity with S107, tridacnin and Con-A, and each extract showed a different pattern of reactivity towards these reagents. Mite extracts from source 1 reacted strongly with tridacnin, whereas those from source 2 reacted strongly with S107.

The mite medium from source 1 reacted weakly with all the reagents but the mite medium from source 2 reacted strongly with the S107 reagent. In contrast, this latter extract did not react with S107 as judged by double diffusion (Stewart *et al.*, 1980).

The Con-A reactive material present in mite extracts from both sources was isolated by affinity chromatography but, whilst all this material appeared to bind to the matrix, recovery using α -D-methylmannoside was poor. Similarly, poor recoveries off Con-A columns, using this sugar, have been reported for other glycoproteins (Hunt, Bullis and Brown, 1975; Choi and Jensen, 1975). The Con-A reactive material which was eluted was also shown to react with S107 and tridacnin. However, Con-A affinity chromatography did not remove all of the tridacnin and S107 reactivity. The Con-A unbound fraction was shown to be devoid of Con-A reactive sugar residues as judged by both haemagglutination-inhibition and double diffusion analyses. It therefore does not represent a population of molecules which contains the Con-A reactive moiety but fails to bind to the Con-A matrix, a situation which has been reported in other studies (Lloyd, 1970; Findlay, 1974).

On the basis of the above data we cannot exclude from these reactivities any activity due to mite media components, but the nonreactivity of the media extracts, both with allergic human sera and rabbit anti-mite extract, suggest that the conclusions drawn regarding allergen Dpt 4 discussed below are valid.

The major antigen in the Con-A bound material isolated from Sephacryl S-300 fraction 2 (source 1) was antigenically indistinguishable from that in the Con-A unbound fraction and corresponded to allergen Dpt 4 (Stewart and Turner, 1980). This allergen was shown by various techniques to react with S107, tridacnin and Con-A. Thus, it appears that allergen Dpt 4 exists as isoallergens, each one differing either in the degree of glycosylation or in the type of carbohydrate residues comprising the glycan part of the allergen. Extrapolating further, the data suggest that allergen Dpt 4 exists in a minimum of two forms; in one form as a glycoprotein containing Pc and tridacnin reactive sugar residues such as N-acetylgalactosamine (Uhlenbruck, Baldo and Steinhausen, 1975) and in the other containing Pc, tridacnin reactive sugar residues and Con-A reactive sugar residues. In this regard, it has been shown that heterogeneity in the glycan part of glycoproteins is the rule rather than the exception (reviewed by Beeley, 1974). It is not known whether allergen Dpt 4 exists without any sugar residues.

In addition to allergen Dpt 4, Con-A affinity chromatography resulted in the isolation of several other components which did not appear to be antigenically related to Dpt 4 and were not studied further. Little was deduced regarding the nature of the material which was not eluted from the Con-A matrix except that the bound material comprised protein which could be radiolabelled and, further, that this material probably contains little tridacnin or S107 reactivity since the majority of this material was accounted for in the unbound, 0.05 M and 1 M α -D-methylmannoside eluates. The high molecular weight allergen Dpt 13 shown to be present in the Sephacryl S-300 fraction 2 (Stewart and Turner, 1980) did not bind to the Con-A column.

The apparent molecular weight of the S107 and tridacnin reactive material

was studied by gradient PAGE and these studies showed that this material was heterogeneous in this regard, paralleling that determined for allergen Dpt 4 and its antigenically related material, namely 140,000-531,000 daltons (Stewart and Turner, 1980). In addition, tridacnin reactivity, in contrast to S107 reactivity, was found to be associated with carbohydrate staining material with apparent molecular weights in the range 531,000- 2×10^6 daltons.

The association of Con-A reactivity and the presence of the hapten Pc has previously been reported for pneumococcal C polysaccharide (Cifonelli *et al.*, 1966) and a C-substance like peptido-polysaccharide from *Epidermophyton floccosum* (Baldo, Fletcher and Pepys, 1977). Similarly, in our laboratory, we have characterized a high molecular weight glycoprotein from *Ascaris suum* body fluid which contains Pc, and reacts with Con-A (McWilliam, Stewart and Turner, unpublished observations). These data suggest that the association of Con-A specific sugar residues and Pc may be a general characteristic of C-substances and that Con-A affinity chromatography would provide a useful technique for their isolation.

The use of lectins has been suggested as a possible means of standardizing allergen extracts (Baldo and Uhlenbruck, 1977b) but we feel that our studies provide a cautionary note in regard to mite extracts, particularly the finding that mite media components, which often contaminate mite extracts (Dasgupta and Cunliffe, 1970; Chapman and Platts-Mills, 1978), react with lectins or similar reagents despite their being non-allergenic. Similarly, the existence of a mite allergen which exists in isoallergenic forms differentiated not only by molecular weight but also by different carbohydrate residues, notably those reacting with Con-A, suggests difficulties, particularly if Con-A were used to standardize mite extracts. Further, extracts from both sources gave comparable RAST inhibition titres (data not shown) despite their disparate reactivities with S107, tridacnin and Con-A. Failure to correlate the tridacnin reactivity of several commercially available extracts with either RAST inhibition or skin test assays has also been reported (Turner *et al.*, 1980).

In conclusion, we have confirmed our previous observations that there exists in extracts of house dust mite, *D. pteronyssinus*, an allergen(s) containing the hapten Pc. In addition, we have shown that this allergen corresponds to the allergen Dpt 4 described in a previous paper (Stewart and Turner, 1980) and, further, that it also reacts with tridacnin and Con-A. These studies contrast with those reported for *D. farinae* (Baldo and Uhlenbruck, 1977a), where it was reported that the Con-A reactive allergen did not react with tridacnin and vice versa. However, neither of the isolated allergens were tested in a haemagglutination inhibition assay which is probably a more sensitive assay than gel diffusion and, also, neither were tested for antigenic cross-reactivity with an anti-whole mite extract antiserum. Despite this, however, these studies and ours again confirm the existence of high molecular weight glycoprotein allergens in both *D. pteronyssinus* and *D. farinae* which are different from the low molecular weight allergens described by other workers (Ishii *et al.*, 1973; Biliotti, Romagnani and Ricci, 1975; Chapman and Platts-Mills, 1978; Stewart and Turner, 1980). Studies are presently under way to determine the significance of these allergens.

Acknowledgements. This study was supported in part by the Princess Margaret Children's Medical Research Foundation, Perth, and the Asthma Foundation of Western Australia. We thank Ms. Ann Butcher for excellent technical help and Mr. A. McWilliam for providing extracts of *Ascaris suum*. We are also extremely grateful to Beecham (Australia) Pty. Ltd. and Commonwealth Serum Laboratories for providing mites and growth media used in this study.

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