

Effect of reagins and allergen extracts on radioallergosorbent assays for mite allergen

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Summary

The reproducibility of the radioallergosorbent (RAST) inhibition and direct binding assays with mite allergen were investigated in the presence of heterogeneous extracts and non-mite-sensitive atopic sera. Both contain components similar to potential contaminants which would occur in the assay of mite allergen and dust allergen extracts. The standardized inhibition and direct binding assays employed had a day to day ($n = 4$) coefficient of variation $[(s.d. \times 100)/\text{mean}]$ of 15% and 24% respectively. The inhibition assay for mite allergen was reproducible in the presence of protein concentrations of added plant, fungal, arthropod and animal extracts in excess of the protein concentrations that occur under the operational mite assay conditions. The mite inhibition assay was also reproducible in the presence of non-mite allergen extracts, with and without additional sera containing IgE specific for the non-mite allergens. The binding of a constant quantity of mite allergen to the activated solid phase in the direct binding assay was reproducible in the presence of added bovine serum albumin, and of a fungal or arthropod extract, representing the heterogeneous components of an allergen extract at the concentrations of total protein known to occur in the direct binding assay of mite extracts.

Introduction

Radioallergosorbent (RAST) assays of both the inhibition (Gleich *et al.*, 1974) and direct binding (Ceska, Eriksson & Varga, 1972) types are becoming the accepted method of allergen assay. Whilst the general mechanism of their operation is understood, quantitative data on factors influencing their accuracy, reproducibility and specificity have not been detailed. Only one publication (Yman, Ponterius & Brandt, 1975) has demonstrated the reproducibility of the assay, but in this case one assay

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component especially activated paper discs was not commercially available at the time of writing and details of the mode of preparation were not described.

The direct binding RAST allergen assay involves the binding of the unknown allergen(s) to an activated solid phase (commonly paper discs), followed by recognition of the allergen by atopic serum immunoglobulin E (IgE). The antibody binds in proportion to the quantity of allergen and is quantified using radioactively labelled anti-IgE. The direct binding assay is similar to a skin test, in that it measures the response to all the allergens in an unknown sample, and the spectrum of IgE present in the serum. It is therefore best suited to extracts containing one allergen, or where sera of individual specificities are available. From theoretical considerations another problem is apparent. Allergens have been demonstrated to be a minor and variable component of the total protein in an extract (Yuninger, Jones & Gleich, 1976). Brighton Lampard & Fordham (1975) have shown that the paper discs used in an allergen assay, when activated by the accepted cyanogen bromide technique, only bound between 2.5 and 6.5 μg of chicken ovalbumin protein per disc, depending on the type of paper used. However, quantities of protein similar to this are often present in allergen assay systems, independent of the amount of allergen present, and so competition for binding sites between allergen and non-allergen protein could occur. As the non-allergenic portion of the total protein varies between extracts, it could be the source of unrecognized variation. The blocking effect of increasing quantities of both allergen and non-allergen proteins on the assay of a fixed quantity of mite allergen was therefore studied.

The inhibition RAST allergen assay determines the ability of an unknown allergen to compete with, or block, the binding of specific serum IgE to a fixed quantity of solid phase bound allergen. The assay requires that the atopic serum contains IgE specific for the solid phase allergen, both of which then act as a standard against which the unknown is compared. The IgE bound to the solid phase is inversely proportional to the capacity of the unknown to function as an inhibitor, and is quantified using radiolabelled anti-IgE, as in the direct binding assay. From the model it would be expected that the unknown allergen(s) could be quantified relative to a solid phase standard and that other soluble allergens, proteins and immunoglobulins present in the assay system would not interfere. Such an assay system capable of quantifying one allergen in a mixture of allergens would be capable of quantifying mite allergen in house dust extracts (a mixture of mite, animal dander, fungal and other allergens) or specific allergen fractions in a complex allergen extract, providing suitable reference allergens for binding to the solid phase and atopic sera containing IgE specific for those allergens were available. This theoretical ability of the inhibition type assay to function in the presence of allergens and proteins not under direct investigation was tested by assessing the interference with a standardized inhibition assay for mite allergen produced by the addition of heterogeneous extracts containing non-mite allergens, sera containing IgE specific for those non-mite allergens and other non-allergenic extracts.

Materials and methods

Atopic serum

Blood was collected from twelve patients with a skin prick reaction greater than a 4.0 mm diameter weal when tested with Commonwealth Serum Laboratory (CSL,

Parkville, Victoria, Australia) D strength (1000 units per ml) mite extract. All sera were screened for high mite-specific IgE by RAST assay, and nine were high (a mean of twenty-two times foetal cord serum binding) and the three lowest (a mean of two times cord serum binding) were rejected. Foetal cord serum, lacking IgE, represents background non-specific binding. One further serum was eliminated after it demonstrated strong mite precipitin lines (IgG antibodies) on counter-immunoelectrophoresis. The pooled sera was stored in 1.0 ml aliquots at -20°C .

Allergen extracts

Mite allergen, *Dermatophagoides pteronyssinus*, a laboratory extract (designated mite extract L), was prepared from lyophilized mites purchased from CSL. The mites were free of culture medium when examined microscopically, using $\times 40$ magnification. The extract was prepared by homogenizing 0.1 g of mites in a Teflon® blender in a final volume of 10 ml of a 0.01 M ammonium bicarbonate, stirred overnight at 4°C , centrifuged at 12,000 g for 10 min and millipore-filtered ($0.22\ \mu\text{m}$) prior to storage under sterile conditions at 4°C . The extract contained 1.54 mg of phosphotungstic acid (PTA) precipitable protein per ml, as measured by the Folin ciocalteau method (Williams & Chase, 1968). *Thermoactinomyces vulgaris*, *Micropolyspora faeni*, *Aspergillus* mixed species and *Sitophilus granarius* extracts were all concentrated preparations recommended for use in the diagnosis of hypersensitivity pneumonitis and were obtained from Hollister-Stier (Spokane, Washington, U.S.A.). They contained 1.48, 2.45, 2.73 and 2.84 mg of PTA precipitable protein per ml. The dry rat liver extract was prepared as described by Kawamura (1968) and an aqueous extract containing 62.5 mg of PTA precipitable protein per ml was used. Cat hair and rye grass extracts, CSL, D strength (10,000 units per ml), contained 0.34 mg and 0.24 mg of PTA precipitable protein per ml.

Anti-IgE

^{125}I -labelled anti-IgE was purchased as the isotope for the RAST specific IgE assay (Pharmacia, Uppsala, Sweden).

Preparation of allergosorbents

Cyanogen bromide activation of paper discs was carried out as described by Ceska *et al.* (1972) and in Affinity Chromatography (1976), with the incubation time increased from 3 to 10 min. For the preparation of paper discs used as allergosorbent for the solid phase allergen in the inhibition assay, 1.0 μl of mite extract L, or 20 μl of rye extract (previously dialysed for 3 hr, against coupling buffer), was used per disc. The incubation volume during binding was the minimum volume that still allowed free movement of the discs. Mixing was either by gentle mechanical stirring or vertical shaking.

Comparison of allergosorbents

Three different allergosorbents were compared in the direct binding and inhibition assays. Paper discs (6.0 mm) punched from Whatman 540 grade paper, Merck microcrystalline cellulose and Sephadex G-100 superfine were all tested. Equal dry weights of each allergosorbent were activated on the same day. When used in assays, dry weights of each, equal to the weight of a paper disc, were used in each tube and kept in

suspension by a 60° angle of rotation at 0.008 g. When the allergosorbents were compared, four-fold dilutions of 12.5 µl of mite extract L was used in the direct binding assay or as the soluble inhibitor in the inhibition assay. 10 µl of extract L was bound per disc (or equivalent) for use as the solid phase allergen in the inhibition assay.

Buffers

The incubation reaction and binding of IgE to the allergosorbent was found to be stable over a range of pH 5.0 to pH 9.0 in 0.067 M phosphate and 0.1 M carbonate-bicarbonate buffer systems. The buffers used were adjusted with NaCl to be isotonic to 0.9% NaCl. The buffer system supplied with the RAST isotope unit, PDT buffer (Pharmacia, Uppsala, Sweden), was found to be suitable and was used as the incubation medium. Washing of the solid phase was carried out using 0.9% NaCl with 0.2% Tween 80 added.

Standardization of assay components

The quantities of the three interrelated variables—allergen, atopic serum and radio-labelled anti-IgE used for the standardized assays—were obtained from the appropriate titration curves. To determine the volume of allergen bound to the solid phase in the inhibition assay, four-fold dilutions of 25 µl of mite extract L bound to paper discs were assayed for bound counts after incubation with 12.5 µl of atopic serum and 2.0 ng of ¹²⁵I-labelled anti-IgE per tube. Bound counts increased in proportion with the increase in volume of allergen, until a plateau was reached. The volume chosen to be bound to the disk was half of the volume of allergen at the start of the plateau region—this was 1.0 µl of extract L. For the volume of atopic serum to be used in both assays, four-fold dilutions of 50 µl of serum per tube were incubated with discs prepared with 1.0 µl of extract L per disc, followed by incubation with 2.0 ng of anti-IgE per tube. Again the volume chosen was half the volume of serum immediately before the start of the plateau region—this was 12.5 µl of atopic serum. For the optimum quantity of anti-IgE, three assays utilizing 12.5 µl of atopic sera and dilutions of 1.5 µl of extract L per disc had their bound IgE quantified, using 0.5, 2.0 and 8.0 ng of anti-IgE per tube. The volume of allergen was plotted against the percentage of the added anti-IgE bound by the disc. No plateau regions were established and the percentage of anti-IgE bound, for a fixed quantity of allergen, decreased slightly for an increase in quantity of anti-IgE. For each of the quantities of anti-IgE there was a proportional relationship between counts and the volume of extract L bound. The optimum quantity of anti-IgE (2.0 ng) was established as a compromise between sufficient count rate (1500 per min for the higher levels of mite extract L), to minimize random error, and the demands of economy. The incubation time for the serum with the solid phase bound allergen in the direct binding assay (9–15 hr) was derived by experiment, while the incubation time for the allergen with the activated solid phase (3 hr), the serum with solid phase and soluble allergen in the inhibition assay (12–18 hr) and anti-IgE in both assays (6–8 hr) were similar to those commonly used for this type of assay. Variation in incubation times between assays was not critical; for example, a reduction in the incubation time of the serum with solid phase allergen from 14 to 2 hr reduced the final bound counts by only 15%.

Direct binding assay

A standardized direct binding assay was performed as follows. Allergens in four-fold

dilutions were bound, in duplicate, to the activated allergosorbent (paper discs unless otherwise stated), as described for the preparation of allergosorbent. The greatest quantity of extract L employed was 12.5 μ l per tube. After binding and washing, each tube was incubated with 12.5 μ l of pooled atopic sera, diluted to 50 μ l with PDT buffer, followed by three 10 min washes and then incubation with anti-IgE, in 50 μ l of PDT buffer. After three washings to remove the unbound anti-IgE, the bound ^{125} I-labelled anti-IgE was counted in a gamma counter. The number of bound counts, expressed as a percentage of the radioactivity added when plotted against the log of the allergen volume, approximated a sigmoid curve. A straight line of best fit was calculated by linear regression analysis of the allergen volumes, producing 15–80% of maximum binding for the diagonal portion of the curve. Data was rejected if the coefficient of fit (r) was less than 0.90. Background binding, with either no allergen present, or with cord serum replacing the atopic serum, was less than 2% of the total added activity. The maximum bound counts rarely exceeded 40% of the total added activity. Volumes of allergen extracts producing 50% of the maximum bound counts for any assay were considered to contain equivalent quantities of allergen and were converted to arbitrary allergen units by taking the reciprocal of the volume in ml.

Inhibition assay (one step)

Standard paper discs were incubated simultaneously with four-fold dilutions, in duplicate, of the inhibitor (either an unknown or standard mite extract L at a maximum quantity of 12.5 μ l per tube) and 12.5 μ l of atopic serum diluted to 50 μ l with PDT buffer. The total volume was 150 μ l. Incubation was for 12–18 hr with angle rotation. The discs were washed three times and the bound IgE was quantified with anti-IgE, as described for the direct binding assay. The relationship between the counts and the log of the allergen extract volume was sigmoid-shaped, and after log transformation of the allergen volumes, producing 15 to 80% of maximum counts with no inhibitor (Aronson & Wide, 1974), it became suitable for linear regression analysis. Data were rejected if the coefficient of fit (r) was less than 0.90. Volumes of added inhibitor, producing 50% inhibition of the tubes with no inhibitor, were considered to contain equivalent quantities of the allergosorbent–allergen and were converted to arbitrary allergen units by taking the reciprocal of the volume in ml.

Inhibition assay (two step)

Allergen, added as a soluble inhibitor, was incubated with atopic serum for 6 hr prior to the addition of the solid phase bound allergen. The incubation was then continued for a further 8 hr. The remainder of the procedure was the same as for the one step assay. A single step inhibition assay with an incubation time of 14 hr was run in parallel for comparison. The percentage inhibition was graphed against the log of the inhibitor volume.

The effect of the addition of non-mite allergen or protein on the direct binding assay

Dilutions of the following extracts: bovine serum albumin, *Thermoactinomyces vulgaris* and *Sitophilus granarius* (1000, 148 and 284 μ g protein per tube, respectively) in coupling buffer were added to a constant quantity of mite extract L (sufficient to produce 36% and 27% binding), and the preparation was used as in the direct binding assay, as previously described. Controls, with no mite allergen, were run simultaneously.

The effect of non-mite extract on the inhibition assay

In addition to the extracts used above, serial dilutions of *Micropolyspora faeni*, *Aspergillus* mixed species, rat liver extract and foetal cord serum at maximum quantities of 122, 136, 1562 and 3500 µg protein per tube, respectively, in coupling buffer were all tested as inhibitors of the mite RAST inhibition assays. Soluble mite allergen, to produce inhibition in the working range of the assay (70% and 40% inhibition), was also added with dilutions of two of the possible inhibitors (bovine serum albumin and *Micropolyspora faeni*), to test if any effect was apparent under the working conditions of the assay.

The effect of the addition of specific non-mite allergens with and without their respective IgEs on the mite inhibition assay

A standard one step inhibition assay was set up, as described previously, with and without soluble mite extract L added to produce approximately 50% inhibition. Cat hair and rye grass allergens were added to the tubes at 17 µg and 1.7 µg protein per tube. In addition, 12.5 µl of serum for donors with a strong prick test reaction to cat or rye allergens (6.0 and 10 mm respectively) was added to the tubes. All assays were performed in quadruplicate, and the significance of the differences between the assays with the additional allergens and/or serum present and the standard assays were assessed by the Wilcoxon rank sum test.

The effect of mite extract L on RAST inhibition assay for rye grass

To test the ability of mite allergen to interfere with other RAST assay systems, 0.5 µl of mite extract L was added to tubes containing paper discs bearing rye allergen plus 10 µl of rye atopic sera (from a donor with a 10 mm weal on prick testing with the allergen) in a total volume of 200 µl adjusted with PDT buffer. Rye extract (8.0 µl), to produce 60% inhibition of the rye RAST assay, was added to a similar set of tubes to test whether any effect was evident under the working conditions of the assay. All assays were performed in triplicate, with incubation procedure, times, washing and quantification of bound IgE as for the mite RAST assay.

Results

Typical RAST direct binding and inhibition assay curves, and their analysis, are shown in Figs 1 and 2 respectively.

The accuracy of the assays was determined by performing four measurements on two mite extracts on different days, by both direct binding and inhibition assays. The coefficient of variation of extract L [(s.d. × 100)/mean] for the direct binding assay was 30%. If the second mite extract was assayed simultaneously, the coefficient of variation for the second extract, considering L as a constant, was 24%. For the inhibition assay, the coefficient of variation for extract L was 51.5% but, with simultaneous assay of both extracts and considering L as a constant, the coefficient of variation declined to 15.4%. The sensitivity of the direct binding and inhibition standardized assays (for 50% maximum binding or 50% inhibition) was 350 and 260 ng of the total mite extract protein. The lower limit of detection was 3.0–4.0 ng.

The design of the standardized assays was justified by the following results. Paper discs were found to be the most convenient solid phase to use. The maximum antigen-

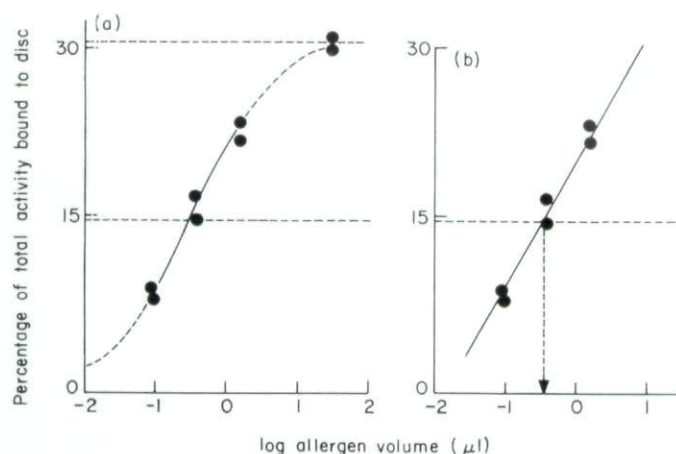


Fig. 1. Direct binding assay curve (a) illustrating the use of linear regression analysis (b) to obtain a line of best fit in the form $y = ax + b$, and a correlation of fit (r) for the approximately linear portion of the curve indicated by the solid line. In this case $y = 10.78x + 20.10$, $r = 0.99$. When $y = 15.03$ (50% maximum binding), the allergen volume equals 0.34μ l or 2941 arbitrary allergen units. Horizontal dashed lines show 50% binding, horizontal dotted one 100% binding.

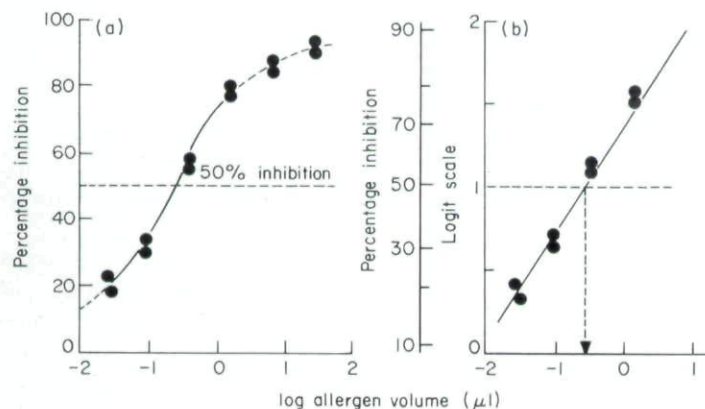


Fig. 2. Inhibition assay curve (a) of sigmoid shape illustrating the use of logit transformation and linear regression analysis (b) to obtain a line of best fit in the form $y = ax + b$, and a correlation of fit (r), for the portion of the curve indicated by the solid line. In this case $y = 0.6361x + 1.3560$, $r = 0.98$. When $y = 1.0$ (50% inhibition), the allergen volume equals 0.28μ l or 3571 arbitrary allergen units. Horizontal dashed lines show 50% inhibition.

binding capacity of all three was approximately equal, but paper and Sephadex G-100 consistently gave lower levels of non-specific binding than the microcrystalline cellulose. Although four times less sensitive, the single step inhibition assay was preferred to the two step assay, as the relationship between the volume of soluble mite allergen inhibitor and percentage inhibition more closely approximated a sigmoid curve, and was thus more amenable to further statistical analysis.

The effect of the non-mite extracts on the direct binding assay is shown in Fig. 3. The direct binding assay was not sensitive to the quantities of protein normally

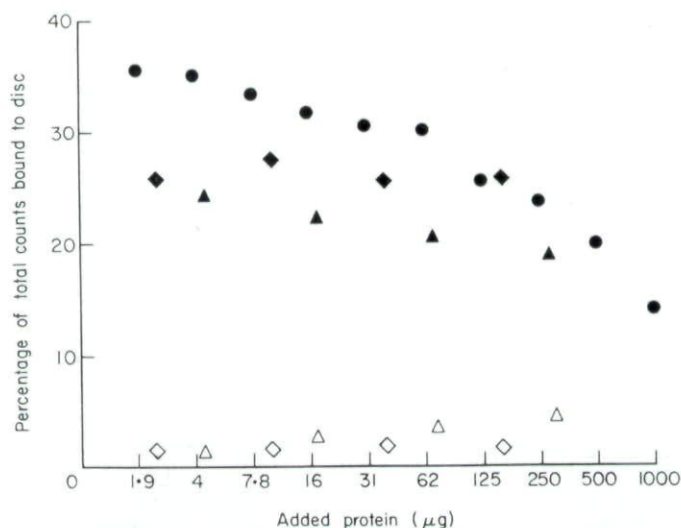


Fig. 3. The effect of increasing quantities of added extracts on the RAST direct binding assay for a fixed quantity of mite allergen. Added extracts: (●) bovine serum albumin with constant quantity of mite allergen producing 36% binding, (◆) *Thermoactinomyces vulgaris* with constant quantity of mite allergen producing 27% binding, (▲) *Sitophilus granarius* with constant similar quantity of mite allergen, (△) *Sitophilus granarius* only, and (◇) *Thermoactinomyces vulgaris* only.

encountered in the assay of commercial mite extracts by the direct binding assay (1.0–10 μg/tube). Quantities of up to 125 μg protein per tube of the *Thermoactinomyces vulgaris* extract did not interfere with the assay of a constant quantity of mite allergen, although smaller quantities of both bovine serum albumin and *Sitophilus granarius* extract did produce a lower uptake of anti-IgE.

When *Sitophilus granarius* extract was added in the absence of mite antigen, small amounts of antibody were taken up. If the counts obtained with increasing amounts of *Sitophilus granarius* in the absence of mite antigen were subtracted from the counts obtained in the tubes with the same quantity of *Sitophilus granarius* plus mite allergen, then the degree of interference occurring with *Sitophilus granarius* is similar to that occurring with bovine serum albumin.

The effect on the RAST inhibition assay of the heterogeneous extracts is shown in Fig. 4. Generally, there was little interference to the binding of specific atopic serum to the disc-bound mite allergen. There was a slight reduction in binding at high levels of foetal cord serum and a slight increase when quantities of *Sitophilus granarius* extract greater than 60 μg/tube were present. This atypical behaviour of the *Sitophilus granarius* extract continued when it was added in the presence of a constant amount of the soluble mite inhibitor. No interference of the inhibition by soluble mite allergen was observed, with either bovine serum albumin or the *Micropolyspora faeni* extract, in the presence of mite inhibitor.

The addition of 1.7 μg or 17 μg protein per tube of the cat hair or rye grass extract did not interfere with the uptake of IgE by the disc in the absence of mite allergen inhibitor, or interfere with the measurement of a fixed quantity of soluble mite allergen in a one stage inhibition assay.

The addition of a quantity of mite extract L which would be easily detected in a

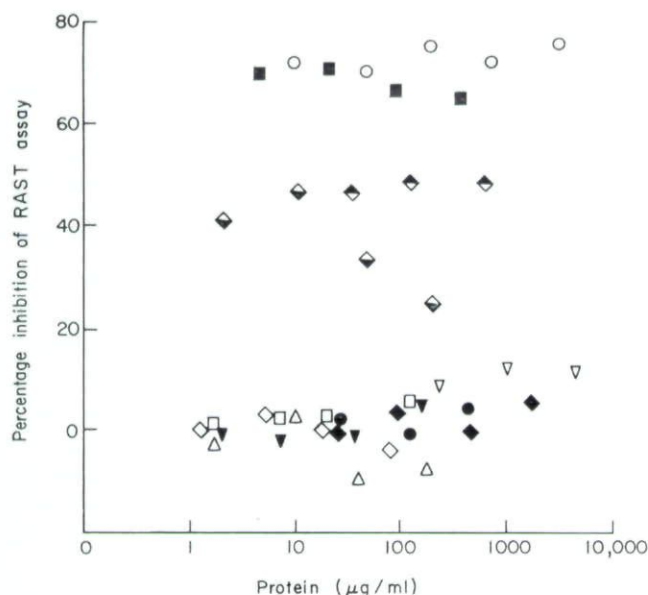


Fig. 4. The effect of increasing quantities of added extracts to the RAST inhibition assay, in the presence and absence of a fixed quantity of added mite allergen inhibitor. Added extracts: (●) bovine serum albumin, (◆) rat liver, (◇) *Thermoactinomyces vulgaris*, (▲) *Aspergillus* mixed species, (△) *Sitophilus granarius*, (□) *Micropolyspora faeni*, (▽) foetal cord serum, (◐) bovine serum albumin plus constant quantity of mite allergen producing 45% inhibition, (◑) *Sitophilus granarius* plus constant quantity of mite allergen producing 45% inhibition, (○) bovine serum albumin plus constant quantity of mite allergen producing 70% inhibition, and (■) *Micropolyspora faeni* plus constant similar quantity of mite allergen.

mite RAST inhibition assay did not effect a RAST inhibition assay for rye allergen—either when no soluble rye allergen was present or under the working conditions of the assay, when sufficient soluble rye allergen was added to produce 60% inhibition of the RAST inhibition assay for rye allergen.

Discussion

Both standardized and direct binding assays are capable of operating with a coefficient of variation of 24% or less. Utilizing the approximate relationship between log allergen strength and weal diameter, both assays are capable of distinguishing (difference of twice the standard deviation) between extracts capable of producing 3.0 and 3.5 mm weals on skin prick testing. As such, the assays are capable of discriminating between clinically significant differences in allergen extract concentrations. The coefficients of variation are comparable with those reported by Yman *et al.* (1975).

In the direct binding assay, the degree of blocking of 1.5 μg protein of mite extract by 2 to 1000 μg of non-allergen protein was surprisingly low. It may be that mite allergen, for steric or chemical reasons, bonds more avidly than any of the competing proteins. The binding capacity of activated allergosorbents may vary, depending upon the protein standard used, and might be more realistically described in terms of moles of low molecular weight protein. Despite problems with the theoretical model for the direct binding assay, in practice the pure protein and two heterogeneous extracts all failed to block the binding of mite allergen to the activated paper discs, at concentrations of total protein that would occur in the assay of mite allergens.

The uptake of specific IgE by the disc-bound mite allergen in the inhibition assay was highly consistent in the presence of large quantities (up to 500 µg/tube) of most added extracts (Fig. 4). The slight inhibition produced by foetal cord serum was possibly due to competition for available binding sites between mite-specific IgE and additional placentally transferred mite-specific IgG. We have previously found (unpublished observations) that most normal sera contains levels of mite-specific IgG capable of being detected by counter-immunoelectrophoresis or microdiffusion.

The assay of a fixed quantity of soluble mite allergen, producing either 40% or 70% inhibition, was unaffected by the addition of most non-mite extracts at total protein concentrations in excess of those known to occur in the assay of mite allergen. The increase in uptake of anti-IgE in the inhibition assay, which occurred when the protein content of the added *Sitophilus granarius* extract exceeded 50 µg per tube, occurred whether added mite allergen was present or not. Although there is no definite explanation for this, two possibilities exist. Firstly, uptake of IgE by paper-bound *Sitophilus granarius*. It has already been demonstrated (Fig. 3) that discs bearing *Sitophilus granarius* extract alone, after incubation with the atopic serum, will bind low levels (mean 2.5 times foetal cord serum binding) of radiolabelled anti-IgE. However, in the inhibition assays, for the *Sitophilus* extract to bind either *Sitophilus*-specific IgE or non-mite IgE cross-reacting with *Sitophilus*, the *Sitophilus* extract would be required to become bound to the paper disc. While the binding of other allergens and IgE to discs already bearing mite allergen did not occur when combinations of cat or rye allergens, with or without their specific IgE, were present, several examples of protein binding or absorption to paper are known. We have found that low levels (twice foetal cord serum binding) of uptake of anti-IgE occurred after blank discs were incubated for 2 hr with 0.5 M ethanolamine, and then subsequently incubated with mite allergen extracts and serum. We also have found that plain millipore-type filters will bind low levels of mite allergen. The binding of protein from Cocksfoot allergen extract to paper, without prior activation by cyanogen bromide, was observed by Brighton *et al.* (1975). The second possibility is both more speculative and intriguing. If a mechanism were available which lowered the level of mite IgG, but not IgE, then less IgG would be available to compete with the IgE for the allergen-binding sites and higher quantities of radiolabelled anti-IgE would be taken up. We have found (unpublished observations) that 90% of over 100 sera screened had sufficient levels of anti-*Sitophilus granarius* IgG to produce multiple, dense, precipitation lines on either counter-immunoelectrophoresis or microdiffusion in agar gel. As *Sitophilus granarius* is a grain weevil, uncommon in Australia in general and even less common in an urban environment, a large degree of cross-reactivity might exist between *Sitophilus granarius* and other environmental antigens. It may be that some of the IgG, which blocks mite allergen, binds much more avidly to *Sitophilus granarius*. It would be necessary for the same cross-reacting *Sitophilus granarius* antigen determinants not to bind mite-specific IgE. As the phenomenon lies outside the range of protein concentrations likely to be encountered in the assay of mite extracts, it is not likely to be a source of interference with such an assay.

The challenging of an inhibition assay system for a particular allergen with other soluble non-cross-reacting allergens offers a unique opportunity to see if those other allergens contain substances capable of interfering with allergen assays *per se*—and therefore capable of interfering with their own assays. It would appear that the fungal, pollen and animal dander extracts tested did not in themselves contain substances

which interfered with mite inhibition assays and, by extrapolation, with RAST inhibition assays in general. Similarly, the failure of mite extract to interfere with rye inhibition assay infers that the mite extract also does not contain substances capable of non-specific interference with RAST inhibition assays. However, extracts of the grain weevil *Sitophilus granarius* did interfere, although at concentrations of protein greater than would generally occur in an assay. The fractions and mechanism responsible are unknown. Caution would be required in interpreting the results of any RAST inhibition for this allergen.

This is the first paper to quantitatively examine the effect of the non-allergic components on RAST allergen assays. The development of internationally standardized RAST assays and their investigation by such studies would facilitate the quantification and standardization of the presently available heterogeneous allergen extracts used for research, clinical testing and treatment.

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