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Aluminium Hydroxide Adsorbed Allergens Used in Modified RAST

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A method is described in which the conventional radioallergosorbent test (RAST) for determination of allergen-specific IgE in serum has been modified by displacing the solid-phase sorbent by a gel of aluminium hydroxide ($Al(OH)_3$). The optimum conditions for this new analysis (AlRAST) are determined, taking into account speed, costs and the ratio between responses of patients and normal individuals. The AlRAST fullfils the criteria for validity: it is specific and shows parallelism between serum dilution curves. The sensitivity and reproducibility are slightly lower than in the conventional RAST, but sufficient for most routine and screening procedures. The preparation of allergosorbents in AlRAST can be accomplished within 1 h, thus making it convenient for fast screening of patients against unusual allergen extracts, as well as for allergen standardization from dose-response curves. Further, it is possible to standardize aluminium hydroxide adsorbed extracts used for immunotherapy by using the products directly as allergosorbents in the AlRAST.

Key words: allergen standardization; aluminium hydroxide; atopy; IgE antibodies; radioimmunoassay; RAST.

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CLINICAL ASPECTS

The use of RAST (radioallergosorbent test) in the diagnosis of IgE-mediated allergy is confirmed, and widely used as a routine test. The advantage of the new AlRAST technique is the easy way of producing allergosorbent by coupling the unknown allergen extract to aluminium hydroxide. By means of this principle the same allergen extract can be used for *in vivo* and *in vitro* diagnosis including the RAST technique. Further, the commercial allergen extract coupled to aluminium hydroxide can be standardized and used for direct comparison with a known patient serum in order to standardize the extract, or select the best extract for immunotherapy. The AlRAST principle is convenient for routine tests as well as for research.

The radioallergorsorbent test (RAST) is well established in the diagnosis of atopy, as well as for standardization of allergen extracts. Since Wide and coworkers in 1967 published their first study presenting the RAST, an immunoradiometric assay for detection of allergen-specific IgE in serum (16), numerous improvements and developments of the test have been proposed. The studies are grouped along two lines: the first expanding the range of analytes from allergen-specific IgE to IgG and IgA and their subclasses (7, 8), while the second comprises technical improvements increasing the sensitivity, reproducibility, speed, etc. of the test (5, 10).

The present study concentrates on the allergo-

Abbreviations: HEC: Histamine equivalent cutaneous (1 HEC corresponds to a response in intracutaneous skin test equal to 0.1 mg/ml histamine chloride); PRIST: Paper radioimmunosorbent test (for IgE determinations); RAST: Radioallergosorbent test (paper discs used as sorbents).

sorbent with the purpose of making its preparation faster and more gentle on the allergens, which are often labile proteins. In doing this, we have taken advantage of the protein-adsorbing properties of aluminium hydroxide ($Al(OH)_3$) gel, and as allergosorbent employ a gel on to which allergen is adsorbed. Apart from this modification and the technical conditions imposed hereby, the aluminium hydroxide RAST (AlRAST) is in principle similar to the conventional RAST as described by Wide et al. (16). Furthermore, aluminium hydroxide adsorbed allergens for immunotherapy can be used as allergosorbents, thus providing an opportunity for testing patients against exactly the same allergen preparation commercially available for treatment of their disease.

The aim of the present study was to demonstrate that aluminium hydroxide adsorbed allergens can be easily prepared and are suitable for the determination of allergen-specific IgE, i.e. meets the same requirements as the conventional RAST.

MATERIAL AND METHODS

Allergen extracts

All allergen extracts were obtained from Allergologisk Laboratorium A/S, Copenhagen, Denmark, as freeze dried powder and were reconstituted with 0.15 M NaCl. The freeze dried extracts were stored at -20° C up to 1 year, and the reconstituted extracts were stored at the same temperature for up to 4 months. The allergen extracts were standardized from the supplier using RAST, crossed immunoelectrophoresis and skin prick test according to the Nordic guidelines (1). Allpyral® aluminium hydroxide adsorbed horse hair and dandruff allergens were obtained from Dome Ltd., England (No. 604, 10,000 PNU/ml).

Sera

All patient sera used were from the Allergy Clinic, Rigshospitalet, Copenhagen, and were stored at -20° C until use. Serum could be thawed and frozen several times without losing IgE-activity.

Used as serum positive for horse allergy was serum from a 21-year-old woman (M.B.H.) with rhinoconjunctivitis due to horse hair and dandruff. The patient had been hyposensitized for 3 years with a commercial extract of horse hair and dandruff (Allergologisk Laboratorium A/S, Copenhagen, Denmark), but she still had symptoms after exposure to horse. When tested intracutaneously she had equal reactions against a histamine solution of 0.1 mg/ml (= 1 HEC) and allergen extract in a dilution of 1:100,000 (w/v). When tested in RAST (Pharmacia Phadebas RAST®) against horse, serum showed a class 4 reaction (highly allergic). The patient had a total serum IgE concentration of 630 U/ml (as determined by Pharmacia Phadebas IgE-PRIST®), and antibodies against three major allergens in the horse hair and dandruff extract were demonstrated for the immunoglobulin classes IgG, IgA and IgM (12).

Serum from a 22-year-old woman (H.D.) highly allergic to *Phleum pratense* (timothy) was used as a serum positive for timothy allergy. For 5 years the patient had received hyposensitization treatment with Allpyral 5-grass mix® (Dome, England), before the start of the pollen season. Intracutaneous 1 HEC was produced with an extract of timothy (Allergologisk Laboratorium A/S, Copenhagen, Denmark) in a dilution of 1:100,000, corresponding to 10 times higher sensitivity than an average pollen-allergic Danish patient. RAST against timothy: class 4 (highly allergic) and total IgE 2,500 U/ml.

Used as normal human serum (NHS) was a pool of sera from blood donors from the blood bank at Rigshospitalet, negative when tested in RAST against pools of animal dandruffs, pollen and fungi.

In one experiment IgE-poor sera (total IgE concentrations < 10 U/ml as determined by PRIST) were pooled and used as diluent instead of NHS.

IgE-free horse serum was obtained from Pharmacia, Sweden.

Aluminium hydroxide RAST (AlRAST) (recommended procedure)

The AlRAST was performed as decsribed below, unless otherwise stated. All samples were tested in duplicate, and when the coefficient of variation (CV) exceeded 25 % the sample was retested.

Preparation of allergosorbent (coupling). In a 12.5 ml tube (120 × 10 mm polystyrene, Nunc, Roskilde, Denmark) 50 µl 2 % (w/v) $Al(OH)_3$ (Alhydrogel®, batch 916, supplied by Superfos A/S, Vedbæk, Denmark) is mixed with 200 µl allergen extract in a suitable concentration (the optimal concentration varies between different allergen systems and has to be individually determined) and 2 ml tris buffer 0.1 mol/l, pH 7.5 with 0.1 % sodium azide, and is allowed to react for at least 15 min. The gel is centrifuged down (2 min at 3,000 rpm = 1,500 *g*) in a Hettich centrifuge (Hettich Ltd., W. Germany) and the supernatant is removed by gentle suction. The gel is washed by adding 2.5 ml tris buffer and centrifuged as described above. The washing procedure is repeated three times. After the third wash the precipitate is resuspended in 5 ml tris buffer ("tris$^+$") including 1 % tween 20 and 0.3 % bovine serum albumin (Sigma, lot A 4503) and can be used immediately or stored at 4° C.

When making allergen pools, equal volumes of allergosorbents made from different allergens are pooled and used in the same concentration as allergosorbents containing only one allergen.

Serum incubation. 50 (or 100) µl of the well-mixed allergosorbent suspension (the allergosorbent can be held in complete suspension for at least 5 min before any precipitation occurs) is added to 50 (or 100) µl serum in a 4.5 ml tube (50 × 8 mm polystyrene, Nunc, Roskilde, Denmark). If serum is to be diluted NHS is used as diluent. The tube is placed in a 12-tube holder and covered with parafilm for at least 3 h at room temperature. After incubation 2.5 ml "tris$^+$" is added using an automated washing device (Erylavit®, supplied by Hettich Ltd., W. Germany) which is able to add or remove buffer simultaneously to or from the 12 tubes in a tube holder. The tubes are centrifuged using the tube holder as insertion of the centrifuge. This washing procedure is repeated three times.

^{125}I-anti-IgE incubation. After removal of the supernatant 50 µl ^{125}I-rabbit anti-human IgE (Pharmacia, Sweden), corresponding to an activity of 1–2 × 10^5 cp5m (counts per 5 min) is added to the gel. The tubes are covered with parafilm and left at room temperature overnight. The following day the gel is washed three times as described above and the tube is counted in a gamma-counter (Palle Medicoteknik, Rødovre, Denmark) (counting efficiency 50 %) for 5 min.

Scoring system. In the preliminary experiments the response of a test serum is expressed relative to the response of normal human serum (NHS), i.e. the C_p/C_n-ratio (counts of test (patient) serum relative to counts of NHS). This scoring system is suitable when optimizing the test, but when many sera are to be analysed and compared it is preferable to express the response of test serum relative to a reference serum. Several dilutions of the reference serum are assayed, and a standard curve can be drawn in a linear-log or a log-log plot. If the test is valid, dilutions of the test serum should produce a curve parallel to the standard curve (i.e. with the same horizontal distance between curves throughout the range of measurement). The amount of allergen-specific IgE in the test serum can thus be expressed relative to the reference serum by transforming the horizontal distance between the curves to sorbent units (SU) (see Fig. 7 for a graphic depiction):

Sorbent units of test serum =

$$100 \times \text{anti-log}\left(\frac{\text{horizontal distance between curves}}{\text{length of one decade}}\right)$$

Alternatively, a single concentration of test serum can be used, and in a similar way be expressed in sorbent units. The production of a serum dilution curve from at least two concentrations has the advantage, however, that interference by e.g. "blocking antibodies" of IgG type will be disclosed by the nonparallelism of the test serum dilution curve and the standard curve (2, 11).

A serum can be classified according to the sorbent units in "allergy classes" and in this study the following classification criteria are used:

Allergy class	SU	Designation
I	0–4	negative or doubtful response
II	5–20	weak response
III	21–100	moderate response
IV	100–	strong response

Al-RAST inhibition. 250 µl serum and 250 µl allergen extract in varying concentrations are mixed and allowed to react overnight at 4° C. The following day the mixture is centrifuged (5 min at 3,000 rpm = 1,500 *g*) and 2 × 100 µl of the supernatant are tested in the standard procedure of the Al-RAST.

A RAST-procedure using paper discs as described by Ceska et al. (5) was used according to the instructions of the supplier (Pharmacia, Sweden). Total serum IgE concentrations were determined by PRIST according the instructions of the supplier (Pharmacia, Sweden).

RESULTS

Specificity

It was demonstrated that adsorption of IgE and ^{125}I-anti IgE to the sorbent itself could be kept at a reasonably low level. $Al(OH)_3$ washed in buffer without allergen extract was incubated with serum from a patient (H.D.) highly allergic to *Phleum pratense* (timothy). The $Al(OH)_3$ was then washed, incubated with ^{125}I-anti IgE as in the normal procedure and finally counted. The bound activity was in the order of 5 % of the response seen when $Al(OH)_3$-*Phleum pratense* was used as allergosorbent. The activity bound in this way on $Al(OH)_3$ without allergen was about 70 % of activity bound when using timothy allergosorbents and NHS.

As a further test of the specificity of the AlRAST a serum from a patient allergic to cat dander (total IgE 848 U/ml; RAST class 4 against hair and dandruff from cat, class 2 against dog and cow and class 1 against horse and guinea pig) was tested against a panel of animal dander allergens. As can be seen from Table 1, the serum reacted strongly when tested against cat dander allergosorbent, but only 1.5–2 times stronger than NHS, when tested against four other animal dander allergosorbents.

The specificity of the test was confirmed by changing the AlRAST into an indirect assay. When allergen and serum were mixed before incubation with the allergosorbent, it was possible to inhibit the response of former positive sera, thus producing an inhibition dose-response curve (Fig. 12).

Table 1

AlRAST-response (cp5m) to different allergosorbents. Animal dandruff was coupled to $Al(OH)_3$ as described and tested against serum from a patient allergic to cat dandruff and NHS. The samples were tested in quadruplicate; coefficient of variation in % in parenthesis

Serum	Animal dandruff				
	Cat	Dog	Guinea pig	Horse	Cow
Patient	8602 (19.1)	519 (11.0)	622 (26.4)	539 (56.4)	734 (14.6)
NHS	316 (5.1)	259 (18.5)	318 (9.4)	331 (42.6)	492 (14.0)
C_p/C_n	27.2	2.0	2.0	1.6	1.5

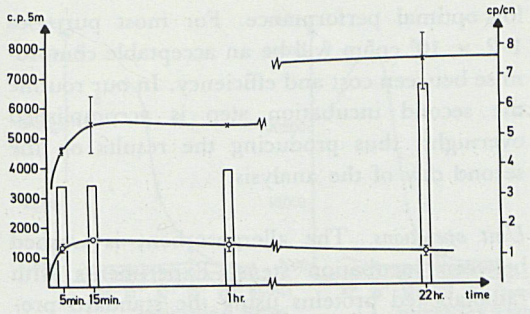

Fig. 1. Effect of coupling time on response. $Al(OH)_3$ was mixed with horse allergen and allowed to react 5 min, 15 min, 1 h and 22 h. After washing, the allergosorbent was tested against serum from a patient allergic to horse (×) and NHS (○). Bars indicate C_p/C_n ratios.

Technical aspects

Various aspects of the test were investigated in order to maximize the C_p/C_n-ratio taking account of speed and costs. The conditions described in Material and Methods were found to be optimal, and only some selected parameters will be presented here.

Coupling. It was shown that allergen extracts preserved with azide, phenol, or as 50 % glycerol extracts, could be used for coupling without interference as compared with unpreserved extracts.

Proteins adsorb to $Al(OH)_3$ within a few minutes. As can be seen from Fig. 1, increasing the the coupling time from 15 min to 22 h only slightly increased the binding. There was a decrease in the response of NHS when longer coupling times were used, indicating that the complete saturation of binding sites in $Al(OH)_3$ is a time-consuming process. Instead of using long coupling times, we tried to reduce the unspecific adsorption of proteins in the incubation steps by use of appropriate buffers.

There was a slight improvement in the C_p/C_n-ratio when the coupling was carried out at 4° C compared with 20° C and 37° C. Revolving the tubes during the coupling did not improve C_p/C_n. Thus the coupling was carried out at room temperature without mechanical stirring.

Optimal coupling was achieved when $Al(OH)_3$ was diluted 40 times, giving a final concentration in the coupling procedure of 0.05 % (data not shown).

To reduce the unspecific binding in the later sequences of the analysis, buffer with or without tween and/or bovine serum albumin was tested for resuspension and wash of allergosorbent. When 1 % tween *and* 0.3 % bovine serum albumin were included in the buffer, C_p/C_n improved due to a reduction of C_n.

By coupling with radiolabelled allergens it could be demonstrated that about 60–75 % of the allergen was bound to $Al(OH)_3$ within 1 h, while the remainder could be removed by washing three times.

Stability of the allergosorbents. Storage experiments showed that $Al(OH)_3$ coupled with horse hair and dandruff or *Phleum pratense* allergens could be stored for 3 months at 4° C as 0.1 % and 0.01 % solutions in an azide-containing buffer leaving the C_p/C_n-ratio essentially unaltered. The C_p decreased up to 30 % as compared with freshly prepared allergosorbents, but as the unspecific binding of NHS, C_n, also fell to a somewhat lower level, it did not cause a decrease in sensitivity.

Serum incubation. The binding of the allergen-specific IgE to the allergosorbent essentially takes place within 3 h, and increasing the incubation time to 24 h only caused a minor increase in the C_p/C_n-ratio as can be seen from Fig. 2.

From a theoretical point of view the total

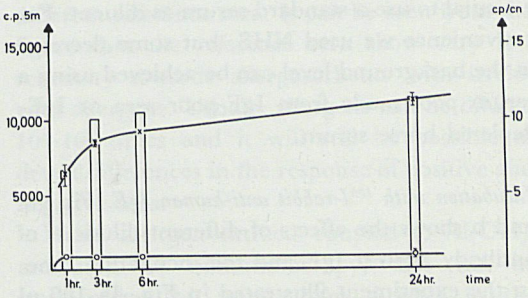

Fig. 2. Effect of incubation time on response. Allergosorbents with horse allergen were incubated with serum from a patient allergic to horse (×) and NHS (○) for 1, 3, 6 and 24 h. Bars indicate C_p/C_n ratios.

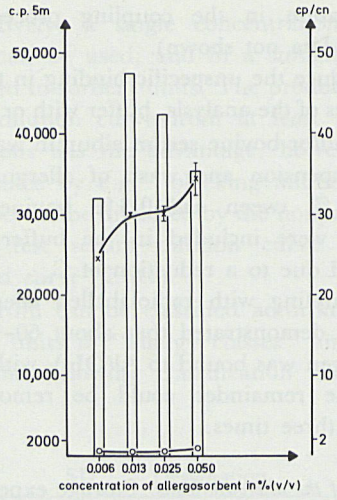

Fig. 3. Effect of sorbent concentration on response. 100 µl serum from a patient allergic to horse (×) and NHS (○) were tested against 100 µl of increasing concentrations of $Al(OH)_3$, to which the allergen was coupled. Bars indicate C_p/C_n ratios.

volume in the incubation step should be made as small as possible in order to reduce the amount of free IgE. 50 µl of serum was chosen as suitable and 50 µl of allergosorbent was the smallest volume that could be pipetted off with the desirable precision. The sensitivity of the AlRAST could be increased by doubling these volumes, but for most purposes this was unnecessary. The amount of allergen should be optimized individually for each allergen system (described later), while the concentration of $Al(OH)_3$ was found to be optimal at 0.02 % as suggested from Fig. 3.

When making dilutions of serum it proved essential to use a standard serum as diluent. For convenience we used NHS, but some decrease in the background level can be achieved using a serum pool made from IgE-poor sera or IgE-depleted horse serum.

Incubation with ^{125}I-rabbit anti-human IgE. Fig. 4a and b shows the effects of different dilutions of antibody against IgE and the incubation time. In the experiment illustrated in Fig. 4a 100 µl of 0.005 % $Al(OH)_3$ is used, and it should be pointed out that higher amounts of allergosorbent demand higher concentrations of antibody for optimal performance. For most purposes $1-2 \times 10^5$ cp5m will be an acceptable compromise between cost and efficiency. In our routine the second incubation step is accomplished overnight, thus producing the results on the second day of the analysis.

Unit operations. The allergosorbent is washed between incubation steps. Experiments with radiolabelled proteins using the standard procedure described earlier showed that the wash liquid was free of activity after 2–3 washing procedures, indicating that all unbound protein was effectively removed. Although centrifugation between the washings could be excluded, it proved safer to include a centrifugation of 2–10 min duration. Increasing the centrifugation time from 2 to 10 min increased the C_p/C_n-ratio from 12 to 16.

Buffers. The buffers used for the different steps in the test were examined, and it was found that ionic strengths lower than 0.1 increased the response of both patient sera and NHS, with an overall decrease in the C_p/C_n-ratio. Values of pH $>$ 8 or $<$ 5.5 reduced some of the unspecific response, but as the same results could be achieved by adding 1 % tween and 0.3 % BSA, this was preferred. Due to the convenience of using the same buffer

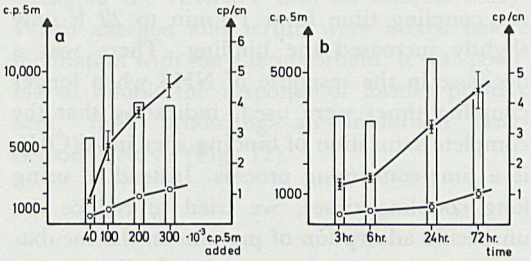

Fig. 4. a: ^{125}I-rabbit anti-human IgE incubation. Amount of tracer: 50 µl antibody in different dilutions were added to allergosorbents coupled with horse allergen and incubated with serum from a patient allergic to horse (×) and NHS (○). Incubation time labelled antibody was 18 h. Bars indicate C_p/C_n ratios.
b: ^{125}I-rabbit anti-human IgE incubation. Incubation time: 50 µl antibody in a concentration of 150,000 cp5m were added to allergosorbents coupled with horse allergen and incubated with serum from a patient allergic to horse (×) and NHS (○), and were allowed to react for 3, 6, 24 and 72 h. Bars indicate C_p/C_n ratios.

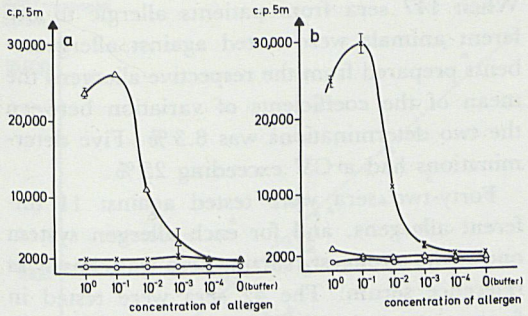

Fig. 5. Dose-response curves. Allergen extracts (*Phleum pratense* (timothy) in Fig. a, horse in Fig. b) in different concentrations were coupled to $Al(OH)_3$. 100 µl allergosorbent in a concentration of 0.05 % were incubated with sera from a patient allergic to *Phleum pratense* (▲), a patient allergic to horse (×) and NHS (○) respectively.

throughout the analysis, several buffers were tried in all steps of the AlRAST. The tris-buffer proved to be rather more effective than acetate, phosphate and sodium chloride. Thus, a tris-buffer 0.1 mol/l, pH 7.5 with 0.1 % sodium azide was used throughout the test. In the redilution and washing steps tween 20 and BSA were added to the buffer.

Quantitative aspects

Allergen. Allergen extracts display a wide variation in potency as measured in RAST and skin prick test. The AlRAST can be used for

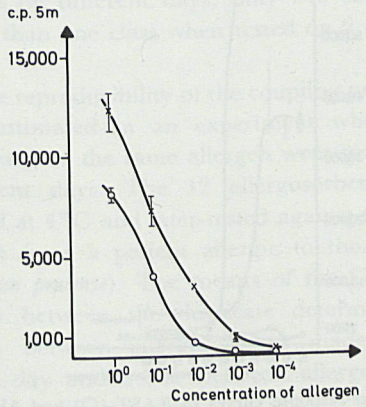

Fig. 6. Comparison of an Allpyral® extract (horse 604) (×) with an aqueous extract of horse allergen (○) in dose-response curves, prepared with serum from a patient allergic to horse.

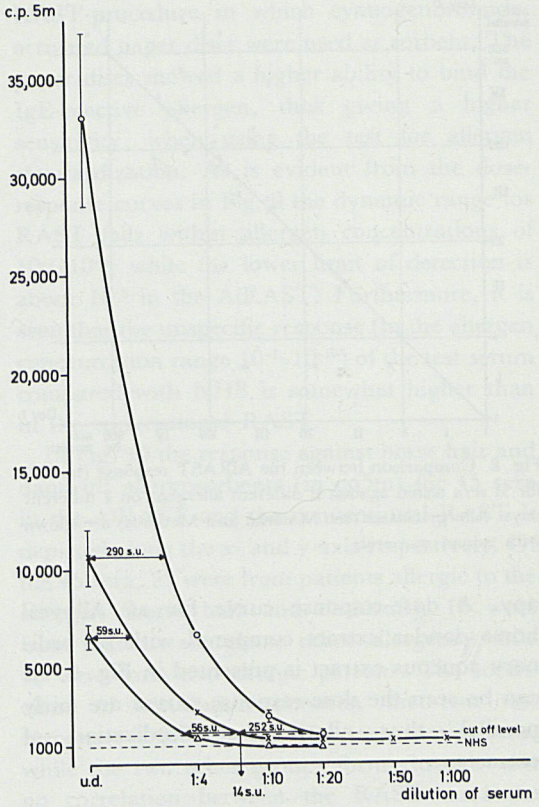

Fig. 7. Serum dilution curves. Three sera from patients allergic to horse were tested in varying concentrations. The two sera (○ and ▲) were expressed relative to the third (×) according to the procedure described in the text. The response of NHS: (-----). Sensitivity when using (×) as reference serum was 14 sorbent units.

standardization of allergens against a reference serum. Fig. 5a and b illustrates dose-response relationship when varying dilutions of allergen are coupled to $Al(OH)_3$ and tested against different reference sera. It can be seen from the figure that the reference sera show very low reactivity towards allergosorbents with irrelevant allergens. Usually allergens can be diluted 10^4–10^5 times and it will still be possible to detect differences in the response of positive and negative sera.

When allergen extracts coupled to $Al(OH)_3$ are used for immunotherapy, they can be standardized by using them directly in the AlRAST. Alternatively, the response of a patient to a given $Al(OH)_3$-extract can be checked *in vitro* before starting an immunother-

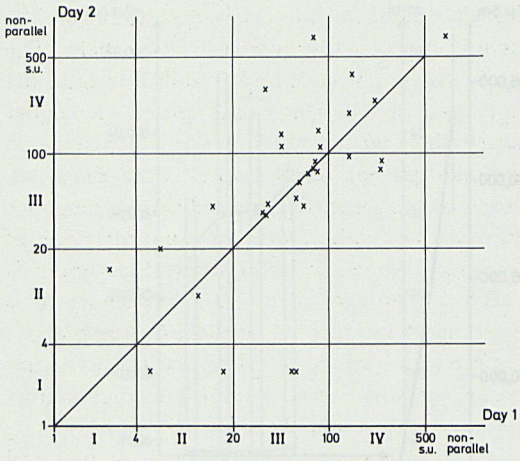

Fig. 8. Comparison between the AlRAST response (in SU) for 31 sera tested against 11 different allergens on 2 different days. Allergy classes (see Material and Methods) are shown with roman numerals.

apy. A dose-response curve for an Allpyral horse dander extract compared with an ordinary aqueous extract is presented in Fig. 6. As can be seen the dose-response curves are fairly parallel, thus allowing standardization of extracts.

Serum. When plotting the AlRAST-response (in cp5m) of decreasing concentrations of a strong reacting serum diluted with NHS, a standard curve can be obtained (Fig. 7). The use of a linear-log plot will produce a curve. Although a certain linearization of the curve is obtained when using log-log or logit-log plots, curves could be drawn and read more precisely when using a linear-log plot.

When the results of the AlRAST are to be expressed in sorbent units, the standard curve and the serum dilution curve must be parallel. As a rule-of-thumb we consider curves as parallel if the coefficient of variation between the largest and smallest number of sorbent units read from the plot does not exceeed 33 %. In Fig. 7 dilution curves from three different sera are shown and the sorbent units of the two sera are expressed relative to the third.

Reproducibility

All serum samples are tested in duplicate. When 147 sera from patients allergic to different animals were tested against allergosorbents prepared from the respective allergens the mean of the coefficients of variation between the two determinations was 8.3 %. Five determinations had a CV exceeding 25 %.

Forty-two sera were tested against 11 different allergens, and for each allergen system one of the strongest reacting sera was chosen as reference serum. The 42 sera were tested in four concentrations and the content of allergen-specific IgE was expressed in sorbent units relative to the reference serum as described earlier. This procedure was repeated with the same sera and allergens 14 days later with freshly prepared allergosorbents, thus making it possible to estimate the day-to-day variation. In Fig. 8 the results from different days are plotted against each other. It can be seen that although some large deviations from the diagonal, x = y, do occur there is no systematic deviation from this line. When calculating the CVs one must take into account that the results have been transformed by an antilog-function. Thus the CV should be calculated on the basis of the

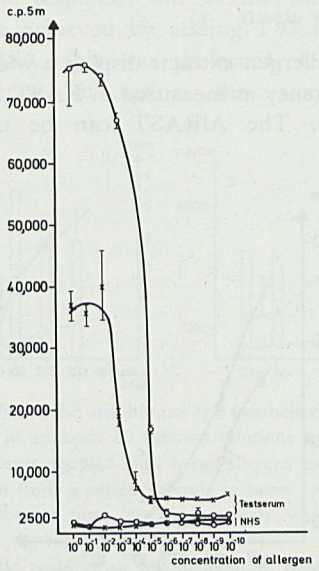

Fig. 9. Dose-response curves in RAST (○) and AlRAST (×). Horse allergen in concentrations 10^{0}–10^{-10} was coupled to cyanogenbromide-activated paper discs and $Al(OH)_3$ and tested against 50 µl serum from a patient allergic to horse and NHS.

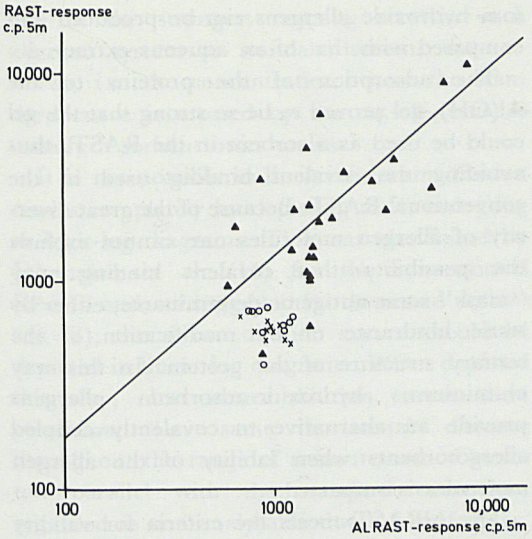

Fig. 10. Comparison between AlRAST (abscissa) and RAST (ordinate) responses (in cp5m) of 45 sera. Horse allergen was coupled to cyanogenbromide-activated paper discs and $Al(OH)_3$ and tested against 50 µl serum from 25 patients allergic to horse (▲), 10 patients allergic to other allergens (○) and 10 healthy individuals (×). Coefficient of correlation, $r_{\text{horse allergics}} = 0.76$, $Y = 645 + 0.91 \times X$.

logarithm of the values expressed in sorbent units, which are equal to the mean of the horizontal distance between serum dilution curves from serum and reference. The mean CV thus calculated is 14.3 %.

The allergy classes are shown in Fig. 8. Although a number of sera fall into different classes on different days, only two sera differ more than one class when tested on 2 different days.

The reproducibility of the coupling procedure was estimated in an experiment where four couplings to the same allergen were made on 3 different days. The 12 allergosorbents were stored at 4° C and later tested against the same serum from a patient allergic to the allergen (*Phleum pratense*). The means of the CVs were 5.1 % between the duplicate determinations, 8.3 % between allergosorbents made on the same day and 9.8 % between allergosorbents made on different days.

Comparison with the conventional RAST. The AlRAST was compared with a conventional RAST-procedure in which cyanogenbromide-activated paper discs were used as sorbent. The paper discs showed a higher ability to bind the IgE-reactive allergen, thus giving a higher sensitivity, when using the test for allergen standardization. As is evident from the dose-response curves in Fig. 9 the dynamic range for RAST falls within allergen concentrations of 10^{-2}–10^{-6}, while the lower limit of detection is about 10^{-5} in the AlRAST. Furthermore, it is seen that the unspecific response (in the allergen concentration range 10^{-7}–10^{-10}) of the test serum compared with NHS is somewhat higher than in the conventional RAST.

In Fig. 10 the response against horse hair and dandruff allergosorbents (in cp5m) for 45 sera in the AlRAST and the conventional RAST is depicted along the x- and y-axis respectively. Of the 45 sera, 25 were from patients allergic to the allergen (horse hair and dandruff), 10 were from patients allergic to other allergens, while 10 were from non-allergic persons. The coefficient of correlation between the two test responses is 0.76 for the 25 horse-reactive sera, while the two other groups form clusters with no correlation between the RAST- and the AlRAST-response.

Allergen pooling and inhibition AlRAST. In screening procedures it is convenient to test a serum against a pool of allergens, thus reducing the number of analyses. An experiment in which two sera were tested against pools of animal dandruffs and pollen allergens as well as the individual allergens is illustrated in Fig. 11. A positive response is obtained when using a pool consisting of some of the allergens to which the patient reacts, while a pool of irrelevant allergens shows no response. It was demonstrated (data not shown) that allergen pools could be made either by pooling the allergens before coupling or by pooling the coupled allergosorbents.

Fig. 12 illustrates an experiment using the AlRAST as an inhibition test. In spite of the almost complete inhibition due to undiluted allergens, inhibition-AlRAST cannot be recommended for all allergen systems, because some of the free allergen, and eventually the allergen-

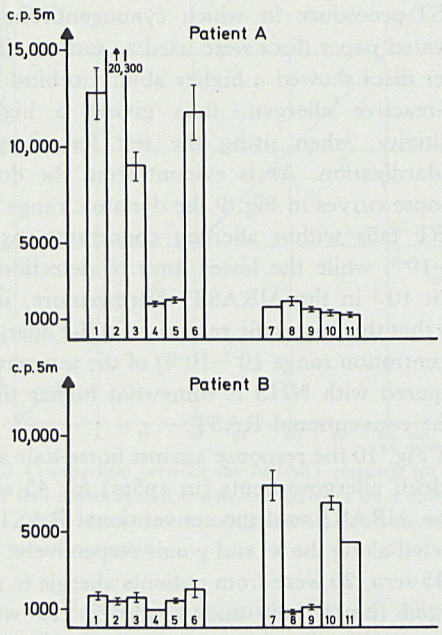

Fig. 11. Allergen pools. Two patients allergic to dog, cat and guinea pig (total IgE 707 U/ml) (A) and *Artermisia vulgaris* and *Chrysantemum lev.* (total IgE 260 U/ml) (B) were tested with allergosorbents containing:

1. Animal pool, consisting of 2, 3, 4, 5 and 6: 2. dog allergen; 3. cat allergen; 4. horse allergen; 5. cow allergen; 6. guinea pig allergen.

7. Pollen pool, consisting of 8, 9, 10 and 11: 8. *Betula pubesc.;* 9. *Phleum pratense;* 10. *Artemisia vulgaris;* 11. *Chrystantemum lev.*

IgE-complexes, will adsorb to the allergosorbent and falsely increase the response.

DISCUSSION

Aluminium hydroxide has been used for purification of proteins (6) and as adjuvant for immunization of animals and humans (13). In the immunotherapy of allergic patients this retard principle seems to have advantages over aqueous allergen extracts, because fewer injections are necessary. A disadvantage, however, is that the biological strength of $Al(OH)_3$-adsorbed allergens cannot be estimated by *in vitro* or *in vivo* tests, but must be extrapolated from the analyses of the aqueous extracts from which they are prepared. The aluminium hydroxide RAST offers a solution to this problem, as a dose-response-curve for aluminium hydroxide allergens can be produced and compared with that of an aqueous extract.

The adsorption of the proteins to the $Al(OH)_3$-gel proved to be so strong that the gel could be used as a sorbent in the RAST, thus avoiding the covalent binding used in the conventional RAST. Because of the great diversity of allergen molecules one cannot exclude the possibility that covalent binding may "mask" some antigenic determinants, either by steric hindrance or by modification of the tertiary structure of the protein. In this way aluminium hydroxide-adsorbed allergens provide an alternative to covalently coupled allergosorbents when lability of the allergen molecules is suspected.

The AlRAST meets the criteria for validity (14). The *specificity* of the allergen-IgE binding is proven by the fact that the sorbent itself, or allergosorbents prepared from irrelevant allergens, fail to bind IgE from even IgE-rich sera.

The cut-off level of the AlRAST, i.e. the number of counts under which a test-response is regarded as negative, is defined as 1.5 times the response of NHS. This level, which is in accordance with that used by Berg et al. (4), varies with the allergen system. When using

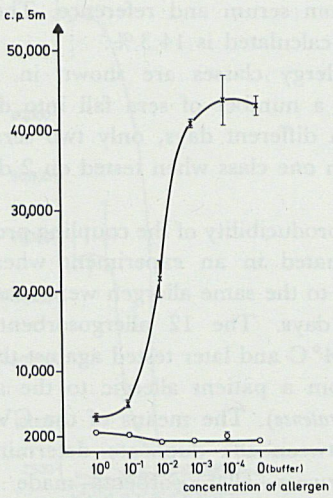

Fig. 12. Inhibition of AlRAST. 250 µl serum from a patient allergic to horse (×) and NHS (○) were incubated with 250 µl horse allergen in varying concentrations at 4° overnight. The following day the mixtures were centrifuged and the supernatants tested against allergosorbents coupled with horse allergen.

one of the strongest reacting sera in each allergen system as standard serum, the *sensitivity,* i.e. the concentration where the standard curve is crossed by the cut-off level, is usually 5 sorbent units or less.

It is not possible to linearize serum dilution curves completely by depicting them in log-log or logit-log diagrams. This was also found by Johansson et al. (11) for the conventional RAST, and, as suggested by them and Adkinson (2), the serum dilution curves should be depicted in linear-log diagrams. When depicted this way, most serum dilution curves show *parallelism*, i.e. same horizontal distance throughout the range of measurement. Curves not parallel with the standard curve are regarded as non-analysable due to interference. Experiments to determine whether this interference is caused by "blocking antibodies", circulating immune complexes containing IgE, etc., have not been conducted.

It is possible to use only one serum and one allergen as a common reference for all allergen systems. However, since standard curves and test serum dilution curves produced in the same allergen system show higher reproducibility, this is preferred.

As it is difficult to purify allergen-specific IgE, the *recovery* of the AlRAST cannot be verified. Instead the response of the AlRAST was compared with that of conventional RAST. It is seen that the responses of patient sera show a correlation of 0.76 between the two tests. Although this is not as excellent as seen in many RIAs, it is acceptable for two tests both having moderate coefficients of variation.

The *reproducibility* within the test is acceptable. The mean of the CVs of duplicate determinations is in the order of 8 % and is about the same as that reported for the conventional RAST (2). The mean of the CVs between different preparations of allergosorbent also lies within the limits reported for the conventional RAST (2, 3). The reproducibility of AlRAST-response expressed in sorbent units is low, but, taking into account the exponential nature of this expression, acceptable. When using the concept of allergy classes, which takes this in consideration, two samples of 29 (7 %) differ by more than one allergy class when tested on two different days with different reagents. In most clinical work this is acceptable, but in some situations, however, a more precise test is needed and a conventional RAST design should be used, or the AlRAST should be run in quadruplicate.

The AlRAST is a modification of the RAST-technique and has considerably reduced the time and effort required in the coupling sequences, thus making it especially suited for fast analyses with "exotic" allergen extracts or allergen pools that need individual allergosorbent preparation. Further, the test provides a new way of directly standardizing $Al(OH)_3$-adsorbed allergen extracts used for immunotherapy. Compared with conventional RAST the AlRAST requires smaller amounts of allergen, thus making it less expensive. In a conventional RAST about 50 analyses can be made with 1 ml of concentrated horse hair and dandruff allergen extract, as opposed to 2,000 analyses with AlRAST. The small amount of allergen on each allergosorbent probably accounts for the somewhat lower sensitivity seen in AlRAST compared with RAST (9).

The AlRAST is thus well suited for routine tests, and has in fact been used for 6–7 years in this country, making the total number of analyses to exceed 100,000 serum samples. In a clinical study of allergy to dog dander the AlRAST showed a somewhat lower correlation between its results and those of clinical tests than the conventional RAST (15).

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