

Immunochemical estimations of allergenic activities from outdoor aero-allergens, collected by a high-volume air sampler

J. JENSEN,^{1,3} L. K. POULSEN,^{1,3} K. MYGIND,^{1,3} E. R. WEEKE^{2,3} & B. WEEKE^{1,3}

¹Laboratory of Medical Allergology, Medical Department TTA 7542, State University Hospital, Copenhagen, ²Roskilde County Hospital, Medical Department, and ³Aerobiological Group, Danish Asthma and Allergy Association, Denmark

To quantify airborne allergens in amorphous and morphological particles, a survey with collection of aero-allergens on glass fibre filters by means of a high-volume air-sampler (HIVOL) was conducted. In preliminary laboratory experiments we compared various filter elution techniques, and the pulverizing elution technique was found to be optimal with regard to yield and convenience. When a surfactant, Tween 20 (0.5% v/v), was added to the elution buffer, a recovery of 80% could be obtained. Allergens in eluates were analysed by means of an IgG-subclass RAST inhibition assay. This immunochemical method for quantification of airborne allergens was validated, as a high recovery of timothy grass pollen allergens was eluted from air filters, and eluates were shown specific by RAST inhibition. The amount of immunochemically measured airborne timothy and birch allergens collected by means of the HIVOL sampler was highly correlated with pollen counts obtained with a Burkard sampler (pollen trap) situated in the same place.

Key words: aerobiology; allergens; *Betula verrucosa*; high-volume air-sampler; IgG-subclass RAST; *Phleum pratense*.

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CLINICAL ASPECTS

The quantification of prevailing airborne allergens existing in pollen grains as well as amorphous particles of respirable size, is valuable for making accurate associations between symptoms in patients suffering from allergic asthma and exposure. In the present study we describe an immunochemical method for measuring atmospheric allergens collected by means of high-volume air samplers, and a technique for elution of allergens from glass fibre filters is developed.

Grains of pollen and other biological particles in respirable sizes are known to be the causative agents in allergic asthma and hay fever (18). Thus it is of interest to quantify airborne allergens.

Various quantitative sampling techniques, e.g. the roto-slide (9) and the Andersen sampler (4), have been used to survey pollen and fungal spores in the atmosphere. Analysis has been performed by microscopic identification and enumeration, and the results have led to important advances in our knowledge of the distribu-

tion and relative importance of naturally occurring airborne allergens (13). However, the recording of symptoms in allergic patients before and after the pollination season may not be related to traditional methods for quantification of allergenic activity (16). Thus determination of sub-pollen-size allergenic particles would be valuable.

Recently, a method for quantification of atmospheric airborne allergens in amorphous particles has been introduced (3). Airborne particles are captured on glass fibre filters by high-

volume air-samplers (flow 1.1 m³/min) and allergens are eluted by means of the descending elution technique. Eluates are analysed for allergenic activity by means of immunochemical assays. Using these principles, the aim of this study is to validate the method with respect to quantification of atmospheric timothy and birch allergens.

MATERIAL AND METHODS

To collect atmospheric allergens we used an upward facing high-volume air-sampler (Accu-Vol, General Metal Workers, Cleves, Ohio USA) mounted with glass fibre sheets 20 × 25 cm, grade GMS-810, which retained particles larger than 0.3 µm in diameter with a 95 % efficacy.

Lyophilised timothy allergen extract (*Phleum pratense* No. 225) and birch allergen extract (*Betula verrucosa* No. 108) were purchased from ALK, Denmark. When reconstituted in 1 ml of glycerol-NaCl solution 1:1 (v/v), the concentration was 10⁶ SQ-E/ml. The extracts were stored at 4°C according to the manufacturer's instructions. Throughout the study, the solution was used within 1 month of reconstruction.

Elution of allergens

In preliminary laboratory experiments 16,000 SQ-E of timothy allergens diluted in 100 µl of buffer was sprinkled uniformly on filter sheets. After evaporation of buffer the filters were processed the same day by various elution techniques. A phosphate buffer 1/15 mol/l (Sørensen) pH 7.0 was selected as elution buffer. Three different elution techniques were compared, and experiments were carried out at room temperature:

Descending elution. Performed according to prior descriptions (3). Allergens were eluted from filter sheets analogously to paper chromatography. The upper end of the filter sheet was soaked in a buffer reservoir containing 150 ml of buffer. The lower end was folded symmetrically to form a trapped tip, which worked as an outlet for the elution buffer. The eluate

was collected in a beaker. The equipment was encapsulated in plastic to reduce buffer evaporation.

Elution by recycling. A system for recycling elution buffer was made by means of a chromatographic column (Bio-Rad, 13 × 2.5 cm), connected to a peristaltic pump (Pharmacia, P-3, flow 0.7 ml/min). The filter sheets were folded and rolled into a small cylinder, which was then placed in the column. Elution was performed with 60 ml of buffer, and 40 ml of eluate was collected. The filter retained 20 ml of elution buffer.

Elution of pulverized filter. Filter sheets were pressed (pressure 300 kg/cm²) and pulverized in a mortar. The powder was transferred to a tube and extracted under vertical rotation with 10 ml of elution buffer. The eluate was isolated by centrifugation.

Measurement of allergenic activity

Eluates obtained by descending elution or elution by recycling were dialysed with 3000 Daltons molecular weight cutoff (Dialyse tubes, Struers, Denmark) against deionized water, lyophilised and then reconstituted in 2 ml of RAST buffer (PBS; sodium-phosphate buffer 10 mmol/l, NaCl 100 mmol/l, azide 0.01 %, pH 7.0).

Eluates were analysed for allergenic activity by an inhibition radio-allergo-sorbent test modified from IgG subclass RAST (7, 8). Sera were selected from subjects undergoing immunotherapy and were known to contain high titres of IgG₄ specific for timothy and birch allergens. The inhibition IgG-subclass RAST was selected, because IgG developed during specific immunotherapy is known to represent a "broader" response to allergens than IgE, as IgG is directed against more allergens (Søndergaard et al., manuscript in preparation). Further, IgG-subclass RAST is sensitive, and only low amounts of sera are required.

Reference allergens were adsorbed to polystyrene tubes (Maxisorp tubes, Nunc, Roskilde, Denmark) and three layers of anti-

bodies were subsequently incubated. In a series of separate tubes, sera diluted 1:800 in PEG-buffer (4% v/v PEG 6000 in RAST buffer) were mixed with an equal volume of eluate or reference allergen in varying concentrations. The mixture was allowed to react for 24 h and was then centrifuged for 20 min at 3500 rpm (2000 *g*) was transferred to allergen-coated maxisorp tubes. The second layer included monoclonal mouse anti-human IgG-4 antibodies, and tubes were finally incubated with ¹²⁵I-labelled rabbit anti-mouse IgG. The tubes were counted for 1 min in a gamma counter. The human IgG antibodies used for the RAST inhibition assay consisted of sera containing a high titre of IgG₄ specific for timothy or birch allergens from subjects undergoing immunotherapy.

Results were run in duplicate and means were calculated as % inhibition:

$$100 - (\text{inhibited sample/uninhibited sample}) \times 100$$

The data producing between 20 and 80% inhibition was subject to linear regression analysis to produce a straight line of best fit to the approximately linear part of the sigmoid relationship between inhibition and log allergen volume. The reference allergen was run in parallel, and values of eluate concentration (SQ/ml) were extrapolated from the dilution at which they elicited 50% inhibition. If the coefficient of fit (*r*) was less than 0.9 the data was discarded, and the extrapolation principle was not used if the regression slopes of eluate and reference curves were not identical (non-parallel curves). Otherwise the data must be expressed in arbitrary units as the reciprocal of the volume in ml required for 50% inhibition.

In the present study the allergen concentration giving 10% inhibition was defined as the detection limit. For timothy and birch reference allergen extracts, the detection limit was to SQ-E/ml and 25 SQ-E/ml, respectively. The coefficient of variation for measuring timothy eluates are 15% and 25% for eluates tested repeatedly the same day and on different days, respectively.

Assessment of specificity

To investigate whether non-timothy components in eluates from air filters cause unspecific interference in RAST inhibition, the following experiment was carried out. One ml of a pool of eluates prepared by mixing eight eluates obtained during the timothy pollen season, was added to a tube containing gel-adsorbed rabbit antibodies raised against timothy allergens. This immunosorbent was prepared by swelling 1 mg of protein-A-sepharose (Pharmacia, Sweden) in 5 ml of PBS and adding 100 µl of anti-timothy-rabbit-sera (prepared according to Harboe & Ingild (11)). The pool containing timothy allergens reacted for 2 h with the immunosorbent, and the supernatant was then tested by timothy IgG RAST inhibition. The allergenic activity of the non-adsorbed pool was also measured. The procedure was repeated for a *Cladosporium* allergen pool. The specificity of the immunosorbent was checked by adding a reference timothy and reference *Cladosporium* extract to anti-*cladosporium* and anti-timothy immunosorbents respectively. The supernatants were tested for allergenic activity.

Atmospheric pollen data

During the periods June to September 1986 and April to June 1987 we collected atmospheric samples for quantification of timothy and birch allergen activity by means of a high-volume air-sampler 20 m above ground level, 2 km from the centre of Copenhagen (The Danish Meteorological Institute). A constant air flow of 1.19 m³/min (1713 m³/24 h) was maintained, and filter sheets were changed every 24 h. Filter sheets were stored at -20°C. They were eluted according to the pulverizing technique and were kept at -20°C before analysing. Simultaneously, pollen grains were monitored by means of a Burkard volumetric spore-trap, placed beside the high-volume sampler. This sampler is a modified version of Hirst's spore trap (12), in which particles are caught on adhesive tape and the total air-volume per 24 h is 14.4 m³. The tape was analysed by light microscopy for pollen grains, which were determined according to species and level of genera, and counted (15).

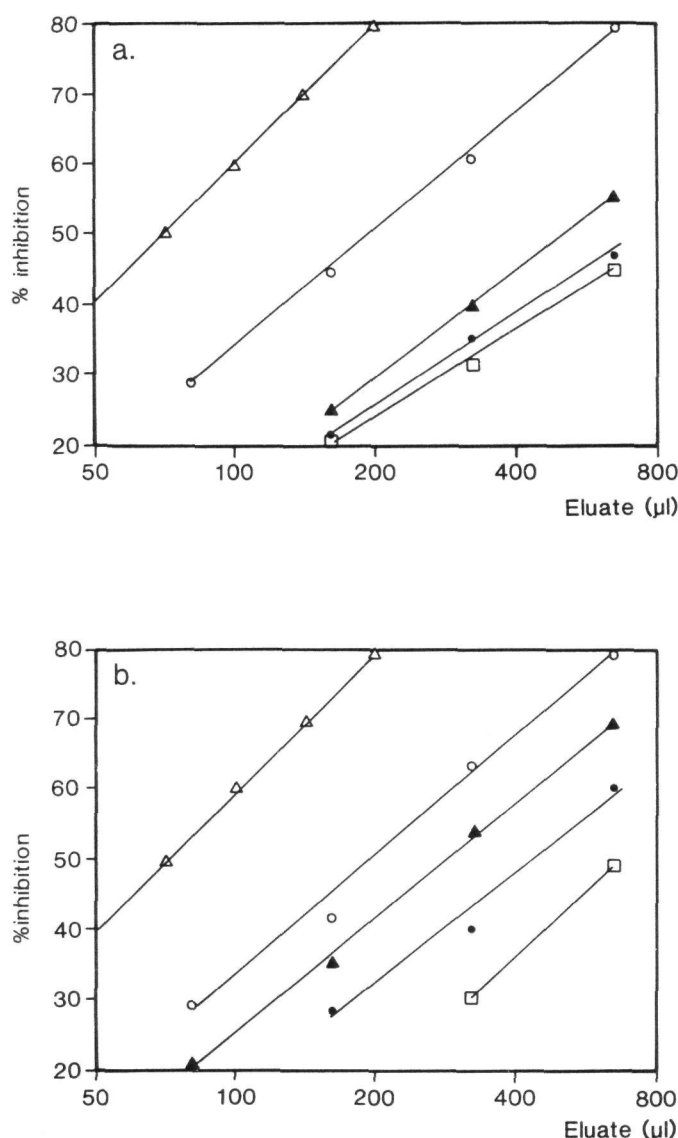


Fig. 1 a, b. Kinetic study applying descending and recycling elution of timothy allergens. Varying volumes of eluate were mixed with 600 μ l of serum (dilution:800) and tested in inhibition IgG-subclass. RAST inhibition curves for *descending eluates* (a) $\circ$: 0-4 h; $\blacktriangle$: 4-8 h; $\bullet$: 8-12 h; $\square$: 12-24 h and *recycling eluates* (b) $\circ$: 0-1.5 h; $\blacktriangle$: 1.5-3.0 h; $\bullet$: 3.0-4.5 h; $\square$: 4.5-6.0 h obtained at four time intervals are shown. $\triangle$: reference curve (reference extract dialysed and lyophilised according to the procedure for eluates). 800 μ l of eluate contained 400 SQ-E allergen.

Statistical tests

RAST inhibition results were evaluated according to analysis of parallelism using Student's *t*-test. When a *P*-value was found to be < 0.1 we defined that curves were parallel. Correlation between estimations of pollen counts and immunochemical estimations was analysed by Spearman's ranked correlation coefficient, $r_s(10)$.

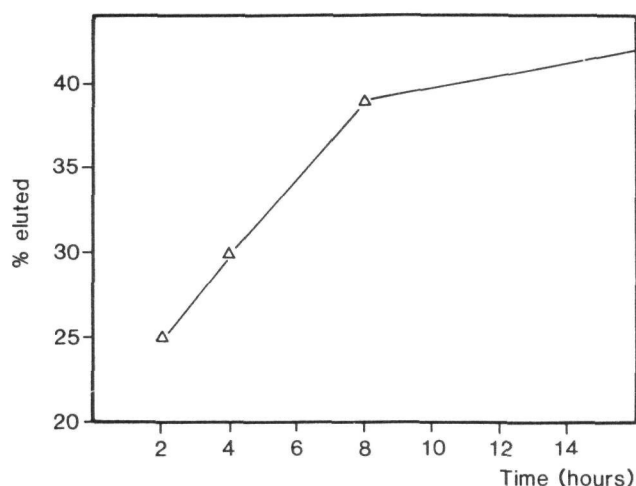


Fig. 2. Time course study using elution of pulverized filter exposed to timothy allergens. Results calculated as % eluted are shown for four different time intervals.

RESULTS

Kinetic studies of various elution techniques

We compared and performed kinetic studies of three elution techniques. The same amount of allergen was applied to each of three separate filter sheets which were then processed according to the three different techniques: descending elution, elution by recycling, and elution of pulverized filter.

Four fractions, each containing 20 ml of eluate, were collected within 24 h by descending elution. Four fractions were obtained by the recycling technique as 40 ml of eluate were collected every 90 min and replaced by a new portion of elution buffer. All eluates were dialysed, lyophilised and reconstituted in 2.0 ml RAST buffer. A reference allergen extract diluted in 30 ml of elution buffer underwent the same procedures for concentrating, and was used to produce a standard curve. RAST inhibition results are presented in Fig. 1 a, b.

Using only 10 ml buffer, elution of pulverized filter was performed, and it was possible to detect allergen activity without applying concentration procedures before analysing by RAST inhibition. In this way we obtained strictly parallel eluate and standard inhibition curves (data not shown). Results from a time

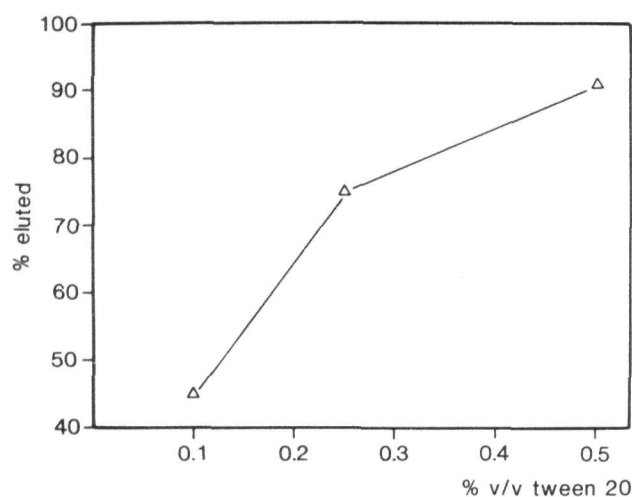


Fig. 3. Elution of timothy allergens by pulverized filter elution using various concentrations of Tween 20 in elution buffer. Results calculated as % eluted are shown.

course study were evaluated in percent of eluted allergens as a function of time (Fig. 2).

The recoveries found for the three techniques differed only slightly, but the elution of pulverized filter was very efficient and offers the great advantage of allowing eluates to be analysed without preceding concentrating procedures. Therefore further experiments were undertaken to optimize the elution of pulverized filter.

Optimizing the elution of pulverized filter

16,000 SQ/ml were applied to three separate filter sheets, then underwent the pulverizing process. Three amounts of Tween 20 were added to the elution buffer to constitute 0.1, 0.25 and 0.5% (v/v) solutions, respectively. Elution was performed for 16 h. The effect of this surfactant is outlined in Fig. 3. No further experiments were undertaken to investigate the effect of higher Tween 20 concentrations as these might affect the analysing of eluates with inhibition IgE RAST.

Specificity

A test was performed to ensure that the timothy IgG inhibition RAST is specific for measuring timothy allergens in eluates obtained from outdoor filters and this test was repeated

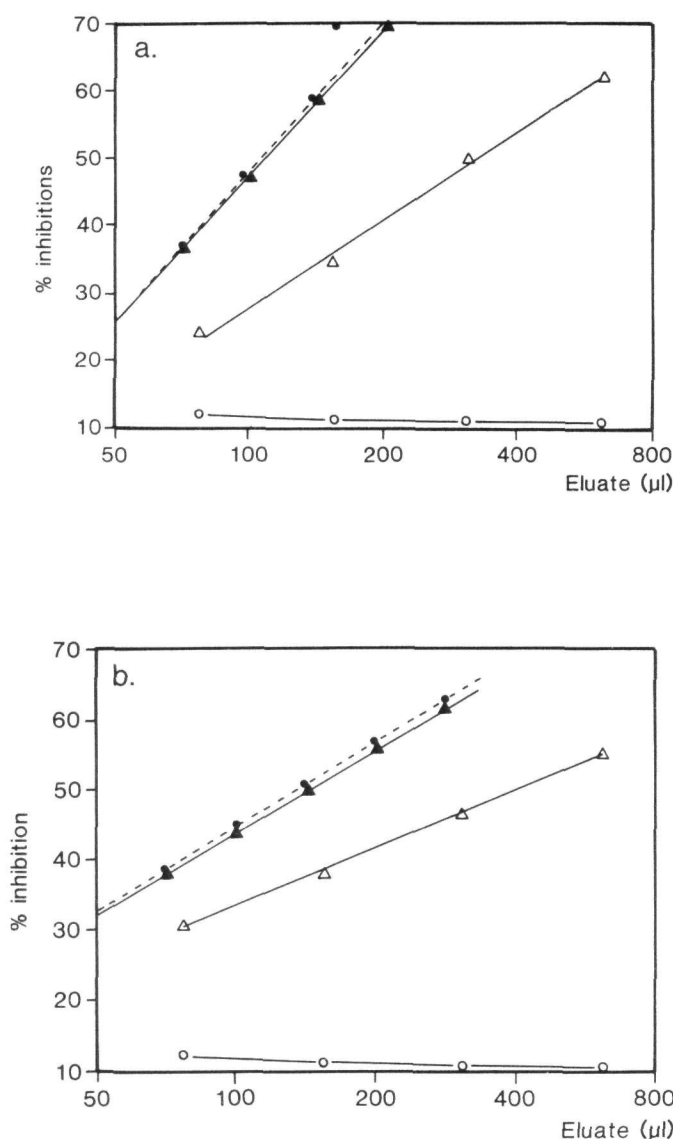


Fig. 4 a, b. Assessment of specificity. One pool of 8 eluates obtained during the timothy pollen season (a) was analysed by RAST inhibition before (Δ) and after addition of anti-timothy-immunosorbent ($\circ$). To a timothy reference extract ($\blacktriangle$) was added anti-Cladosporium-immunosorbent ($\bullet$). One pool of 8 eluates obtained during the Cladosporium pollination season (b) was tested in the same way with anti-Cladosporium and anti-timothy immunosorbent. RAST inhibition results are shown.

for cladosporium-aero-allergens. (Fig. 4 a, b). The allergenic activity of a pool of timothy eluates (a) showed maximally 10% inhibition when treated with anti-timothy-immunosorbent and the untreated pool showed a normal dose-dependent curve. The timothy reference extract treated with anti-Cladosporium-immunosorbent gave an identical dose-dependent standard curve in proportion to the untreated one.

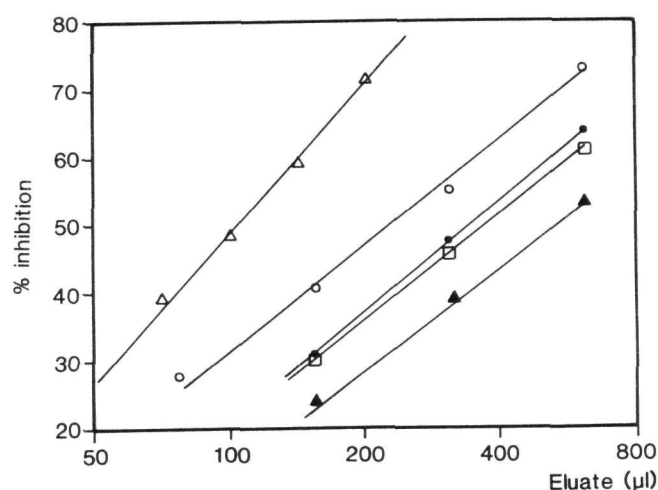


Fig. 5. RAST inhibition results from four eluates obtained during the timothy pollination season. ○: 6/10; ●: 6/25; □: 7/8; ▲: 7/22; △: standard curve.

Morphological and immunochemical estimations of atmospheric allergenicity

The high volume air-sampler was operated during the timothy and birch pollination season, and pulverized filter sheets were eluted with Sørensen buffer containing 0.5 % v/v Tween 20 and analysed for allergenic activity. Four examples of timothy filter eluates are shown in Fig. 5.

Timothy pollen counts (60 days) and birch pollen counts (20 days) were compared with atmospheric allergenic activity as shown in Fig. 6. a, b. Correlations of $r_s = 0.82$, ($P < 0.005$) and $r_s = 0.93$ ($P < 0.001$) were found for timothy and birch, respectively.

DISCUSSION

The present study demonstrates that commercially available, soluble allergens exposed to glass fibre sheets were easily eluted by means of the three tested elution techniques.

However, for the *descending elution* Fig. 1 a, b) we observed a somewhat flatter slope of the dose response curves representing eluates eluted for more than 8 h, indicating that some of the allergens may have different elution kinetics or that they have been enzymatically degraded. The inhibition curves for elution by the *recycling technique* (Fig. 1 a, b) were seen to be mutually

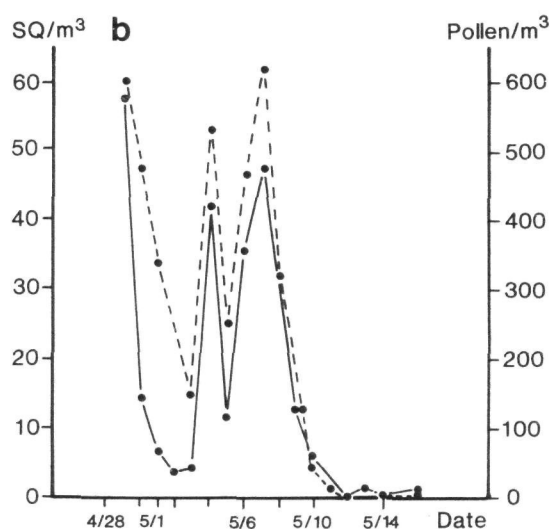
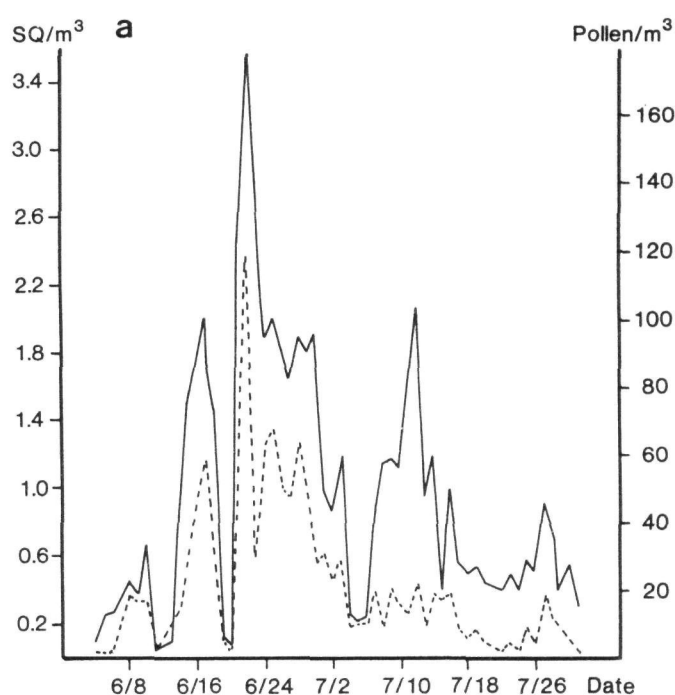


Fig. 6 a, b. Comparison of morphological and immunological estimations of airborne timothy allergens (a), data from 57 days (June to August) and airborne birch allergens (b), data from 20 days (May): — SQ/m³; ---- pollen/m³.

identical. This suggests that the elution follows a first order kinetics. It is then possible to calculate the allergen concentration in eluates when at least three dilutions are tested. This is useful when accurate determinations of allergenicity in eluates are required.

For *both descending and recycling elution* we observed a difference between slopes of standard and eluate inhibition curves, and the estimation of allergenicity in eluates may be

inaccurate. In addition, the reference extract, treated according to eluates, produced a weaker slope in a % inhibition-concentration plot, and loss of allergenicity was noted (data not shown).

To evaluate the elution efficacy of the preliminary experiments we estimated the yield of every elution fraction by means of the standard curve at one point, e.g. the eluate dilution giving 50% inhibition. Nearly half of the applied allergens were eluted by descending elution within the first two fractions (40 ml, 8 h), but a higher yield, 2/3, was obtained by means of recycling elution (8: ml, 3 h). A yield of up to 90% could be obtained if the four recycled fractions were tested together.

In spite of these results, the loss of allergenicity in eluates undergoing lyophilisation presents a considerable disadvantage.

Eluates produced by the *pulverizing elution technique* (Fig. 2) gave increased allergenic activity when elution was continued to 8 h, and after 16 h nearly 50% of the applied allergens could be recovered.

The effect of adding a surfactant (Tween 20) to elution buffer is remarkable and recovery increases drastically with increasing concentrations of Tween 20. Up to 90% of the applied allergens are recovered when pulverized filters are eluted with phosphate buffer added 0.5% surfactant. However, these results confirm only the recovery of soluble allergens and do not indicate the magnitude of the elution efficacy of pollen grains.

Results of other investigators indicate that pollen grains are very efficiently extracted under the conditions applied: it has been shown that some allergens are released almost instantly from the pollen wall, and all active allergens from grass pollen are extracted completely within 15 min in PBS buffer pH = 7.0 (5). These results were confirmed under conditions simulating *in vivo* activities (14). Thus, we concluded that the pulverizing elution technique offers a useful method for elution of atmospheric allergens from glass fibre filters.

Inhibition curves representing filters collected during the timothy and birch pollination season were mutually parallel ($P < 0.05$), suggesting that no qualitative differences exist in the

atmospheric timothy allergen activity during the pollination season. But the eluate slopes differed significantly from the slope of the reference curve ($P > 0.01$) and a specificity test was performed to investigate whether eluates from outdoor filters contained non-relevant components able to influence the IgG RAST inhibition test.

Eluates with immuno-adsorbed timothy allergens gave no inhibitory activity when tested in timothy inhibition IgG RAST, indicating that no other aero-allergens, particles from outdoor air, or microscopic glass fibres are able to cause unspecific inhibitory activity. It was confirmed that the elimination of inhibitory activity of immuno-adsorbed eluates was not caused by sepharose protein A, rabbit sera, or non relevant particles, as reference timothy extract immuno-adsorbed by anti-Cladosporium-rabbit-sera gave a normal response corresponding to the unadsorbed one. These results were confirmed by the specificity test for atmospheric Cladosporium allergens. These results established the specificity of the immunochemical assay for measuring outdoor allergenic activities, and the significant differences in inhibition slopes observed for reference allergen extract and atmospheric allergens are not caused by unspecific inhibition due to non-pollen sources in eluates.

A highly significant correlation was found between pollen data and immunochemical estimations of airborne timothy and birch allergens. In spite of differences in the volumes sampled by the high-volume sampler and the Burkard sampler, we did not, in contrast to Agarwal and co-workers (2), identify allergenic activity in the filter eluates on days with no detectable pollen grains in the air. However, there was a greater response of these estimations of airborne timothy in relation to pollen counts at the end of the season than at the beginning. This indicates a possible cumulative effect of non-pollen sources or allergenic amorphous particles, not detected by the traditional method during the pollination season.

Busse et al. have reported the existence of non-pollen particles, e.g. ragweed allergens, in aerosol fractions smaller than intact pollen grains from plant debris (6), and later, Agarwal

et al. were able to demonstrate that ragweed plant debris could offer measureable values of atmospheric allergenicity, noted before and after the ragweed pollination season, and these observations correlated with small increases in mean symptom-score in patients with ragweed allergy (1). Solomon et al. have confirmed these observations by both clinical and *in vitro* assay criteria (17). To prove this theory it will be necessary to collect airborne allergens with size-selecting air samplers.

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REFERENCES

1. Agarwal, M. K., Swanson, M. C. & Reed, C. E.: Airborne ragweed allergens: Associated with various particle sizes and short ragweed plant parts. *J. Allergy Clin. Immunol.* 74, 687-693, 1984.
2. Agarwal, M. K., Swanson, M. C., Reed, C. E. & Yunginger, J. W.: Immunochemical quantitation of airborne short ragweed, *Alternaria*, antigen E and Alt-I allergens: a two year prospective study. *J. Allergy Clin. Immunol.* 72, 40-45, 1983.
3. Agarwal, M. K., Yunginger, J. W., Swanson, M. C. & Reed, C. E.: An immunochemical method to measure atmospheric allergens. *J. Allergy Clin. Immunol.* 68, 194-200, 1981.
4. Andersen, A. A.: A new sampler for collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* 76, 471, 1958.
5. Brown, H. M. & Thrantey, N.: Rapid extraction of grass pollen allergens and separation of their active fractions. *Acta Allergol.* 31, 22-34, 1976.
6. Busse, W. W., Reed, W. E. & Hoehne, J. H.: Where is the allergic reaction in ragweed asthma. Demonstration of ragweed antigen in airborne particles smaller than pollen. *J. Allergy Clin. Immunol.* 50, 289-293, 1972.
7. Djurup, R., Søndergaard, I., Minuva, U. & Weeke, B.: A nylon ball solid-phase radioimmunoassay for specific antibodies in human sera. Application to measurement of IgG antibodies to pollen allergens. *J. Immunol. Methods* 62, 283-296, 1983.
8. Djurup, R., Malling, H. J., Søndergaard, I. & Weeke, B.: The IgE and IgG subclass antibody response in patients allergic to Yellow Jacket venom undergoing different regimens of venom therapy. *J. Allergy Clin. Immunol.* 76, 46-55, 1985.
9. Eugene, C. O. & Raynor, G. S.: A new sampler for airborne pollen: The roto-slide. *J. Allergy* 40, 1-7, 1967.
10. Geigy, J. R.: Scientific tables, 6th ed. Diem, K. (ed.), pp. 173-182, Basel.
11. Harboe, N. & Ingild, A.: Immunization, isolation of immunoglobulins, estimations of antibody titer. In Axelsen, N. H., Krøll, J. & Weeke, B. (eds.): *Scand. J. Immunol.* 2, Suppl. 1, 160-164, 1973.
12. Hirst, J. M.: An automatic volumetric sporetrap. *Ann. Appl. Biol.* 39, 257-265, 1952.
13. Larsen, L. S.: A three-year-survey of microfungi in the air of Copenhagen 1977-1979. *Allergy* 36, 15-22, 1981.
14. Løwenstein, H. & Mygind, N.: Extraction and degradation of timothy pollen allergen during simulated in vivo conditions. *Allergy* 33, 238-240, 1978.
15. Petersen, N. P., Larsen, L., Egelund, E., Buch, H., Jurgensen, F. & Gravesen, S.: Pollen and mouldspore counts in Denmark. Description of methods, Copenhagen 1986.
16. Solomon, W. R.: Sampling airborne allergens. *Ann. Allergy*, 52, 140-147, 1984.
17. Solomon, W. R., Burger, C. E. & Muilenberg, M. L.: Allergen carriage by atmospheric aerosol. *J. Allergy Clin. Immunol.* 72, 443-447, 1983.
18. Solomon, W. R. & Mathews, K. P.: Aerobiology and inhalant allergens. In Middleton, E. Jr., Reed, C. E. & Ellis, E. F. (eds.): *Allergy principles and practice*, pp. 899. C. V. Mosby, St. Louis, 1978.

Address:

J. Jensen

Laboratory of Medical Allergology

Medical Department TTA 7542

State University Hospital

Copenhagen

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