

An immunochemical method to measure atmospheric allergens

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*Because particulate aeroallergens may exist in amorphous form as well as in pollen grains and fungal spores and because symptoms of allergic diseases presumably correlate with the total amount of allergen exposure, an immunochemical method of assay of aeroallergens would be useful. We report such a method based on (1) capture of airborne pollen, fungal spores, and amorphous particles 0.3 μm in diameter on fiberglass sheets with a high-volume air sampler; (2) elution of the sheets with buffered saline; and (3) analysis of eluate allergen content by radioallergosorbent test (RAST) inhibition assays. In preliminary indoor experiments we applied various quantities of short ragweed (SRW) pollen or dry *Alternaria* powder to the sheets while airflow was maintained at 1.19 m^3/min . We compared techniques for extraction of allergen from the sheets, including homogenization, cutting and soaking, and descending elution of sheets. Although all three methods successfully extracted allergen from the sheets, an 8-hr descending elution procedure was optimal from the standpoint of yield and convenience. Eluates from filters exposed to as little as 4 mg of SRW pollen or *Alternaria* powder produced satisfactory RAST inhibition curves. When the sampler was operated outdoors continuously we could measure the atmospheric allergenic activity for both *Alternaria* and SRW from July to September. This allergenic activity was highly correlated with the traditional morphologic counts of airborne ragweed pollen and *Alternaria* spores. (J ALLERGY CLIN IMMUNOL 68:194, 1981.)*

The etiologic role of airborne pollen and fungal spores in human allergic diseases is well established. Various quantitative sampling techniques to survey airborne pollen and spores have led to important advances in our knowledge of the distribution and relative importance of naturally occurring airborne allergens.¹ Although it has been a common practice to correlate the onset and severity of symptoms of respiratory allergic disorders with the appearance and prevalence of various pollen and spores, we believe that a more relevant parameter may be the actual amount of airborne allergen rather than number of pollen grains and spores. Aerodynamic considerations and experimental evidence indicate that particles of the size of pol-

len grains (15 to 40 μm) or typical *Alternaria* spores (20 to 60 by 9 to 18 μm) may be too large to penetrate to the bronchi and cause symptoms.² Moreover, preliminary data suggest that ragweed allergen may be carried in amorphous particles smaller than intact pollen.³ In view of these observations it seems likely that the pollen and spore count may not adequately reflect actual total airborne allergen content. Hence, the present investigations were undertaken to develop a specific immunochemical method for quantitation of airborne allergens.

MATERIALS AND METHODS

Sampler

We used a high-volume air sampler (GMWS 2310 Accu-Vol; General Metal Works, Inc., Cleves, Ohio) for the present studies (Fig. 1). This device collects airborne particles on a filter sheet through which a constant air flow of 1.19 m^3/min is maintained. The speed of the motor is automatically adjusted to correct for variations in flow rate caused by filter loading. During these studies we used filter sheets (fiberglass sheets, 20 by 25 cm, Grade GMS-810; General Metal Works), stipulated by the manufacturer to retain particles larger than 0.3 μm diameter at 95% efficiency.

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Abbreviations used

SRW:	Short ragweed
BCS:	Borate-citrate-saline buffer
RAST:	Radioallergosorbent test
RIU:	RAST inhibition unit

Allergens

SRW pollen (*Ambrosia elatior*) and *Alternaria alternata* powder (source strain No. 16086; American Type Culture Collection, Rockville, Md.) were purchased from Greer Laboratories, Inc., Lenoir, N.C. In preliminary indoor experiments, various amounts of *Alternaria* powder or SRW pollen were sprinkled on the filters with the sheets mounted on the sampler. The sampler was run for 10 min while the allergens were applied to simulate actual air sampling. All filter sheets were subsequently processed the same day for elution of the allergens.

Elution of allergens

Allergens were extracted from the filter sheets with BCS (0.0325M sodium borate and 0.034M sodium citrate in 0.16M sodium chloride, pH 8.2). Three different extraction techniques were compared.

Homogenization. Filter sheets were cut into small pieces and homogenized in 250 ml BCS for 2 min in a Waring blender. The resulting thick slurry was transferred to a beaker and extracted overnight on a magnetic stirrer at 4° C. The allergen extract was isolated by centrifugation or by filtration through Whatman No. 4 filter paper over a Buchner funnel; the residue was washed twice with 25 ml of BCS. A filter sheet without any allergen was processed similarly for control studies.

Cutting and soaking. The filter sheets were cut into small pieces and soaked overnight in 250 ml of BCS at 4°. The allergen extract was recovered by filtration and washing as above.

Descending elution. Allergens were extracted from filter sheets by descending elution in a large polyethylene tank; stainless steel troughs (30 by 8 by 2.5 cm) served as buffer reservoirs. The upper end of the filter sheet was mounted in the buffer reservoir with paper clips. The lower end of the filter was folded symmetrically and stapled to form a tapered tip for elution, and the eluate was collected in a beaker.

For comparison purposes, *Alternaria* and SRW pollen extracts were also prepared directly in BCS.

Measurement of allergenic activity

All eluates were dialyzed with 3500 molecular weight cutoff casing (Spectrapor-3; Spectrum Medical Industries, Inc., Los Angeles, Calif.) against deionized water and were then lyophilized. The lyophilized powders were reconstituted in a constant volume of distilled water or BCS for further allergen assay. We studied the effect of dialysis and lyophilization on the yield of SRW allergen and observed no

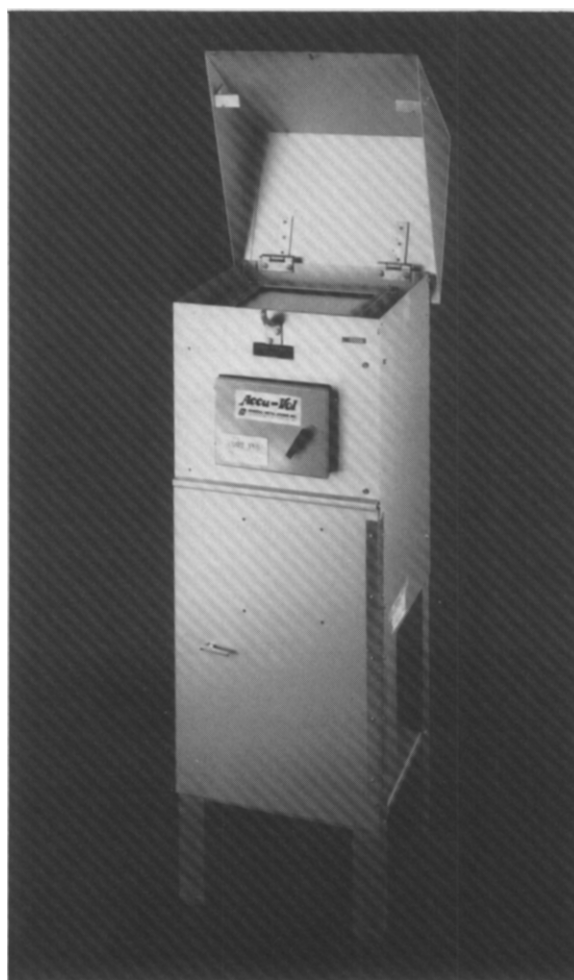


FIG. 1. High-volume air sampler (GMWS 2310, Accu-Vol) with mounted filter holder and filter sheet.

loss of allergenic activity in the extracts with these procedures.

Eluates were assayed for their allergen content by a mini- or maxi-RAST inhibition procedure.⁴ The SRW solid-phase allergen was prepared as described previously.⁵ Briefly, CNBr-activated microcrystalline cellulose (1 gm) was reacted with 400 mg of crude SRW pollen extract. The resulting polymer was mixed for 60 hr, washed repeatedly, and resuspended at 1 mg/ml in RAST diluent.⁶ The solid-phase *Alternaria* allergen was prepared similarly by reacting a 70% (NH₄)₂SO₄-precipitable fraction of crude *Alternaria* extract (150 mg) to 1 gm of CNBr-activated microcrystalline cellulose.⁷ The solid-phase ragweed antigen E (AgE) was prepared by reacting 1 mg of AgE with 450 mg of activated cellulose. Immediately after the AgE addition, human serum albumin (9 mg) was added to the activated cellulose as a filler protein.⁸

The IgE antibody pool used for the *Alternaria* RAST inhibition studies consisted of equal volumes of defibrinated plasma from seven nonimmunized subjects who were highly

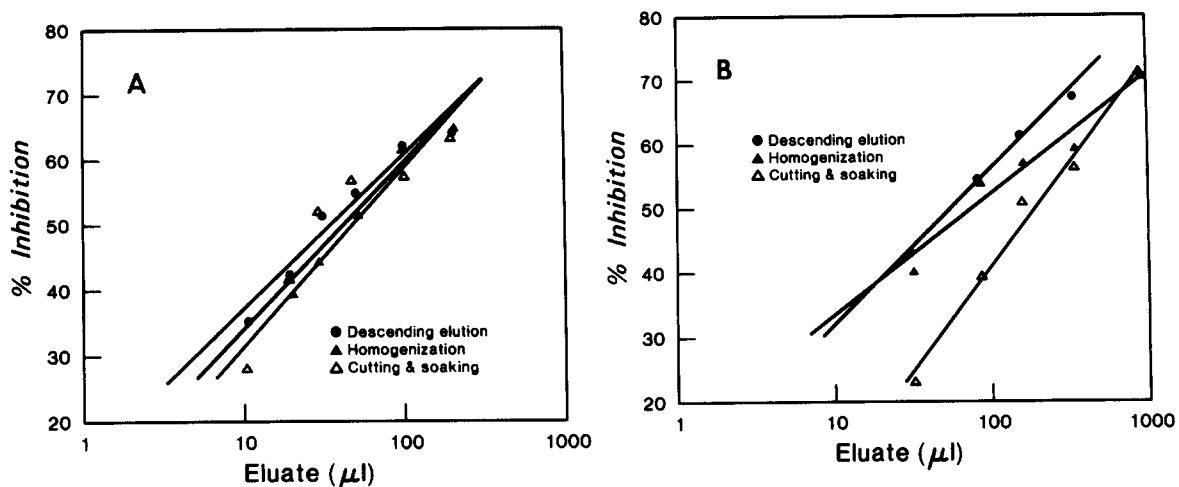


FIG. 2. Comparison of allergenic activity in extracts prepared by three different techniques, showing RAST inhibition curves with eluates of *Alternaria* powder, 100 mg (A), and SRW pollen, 100 mg (B).

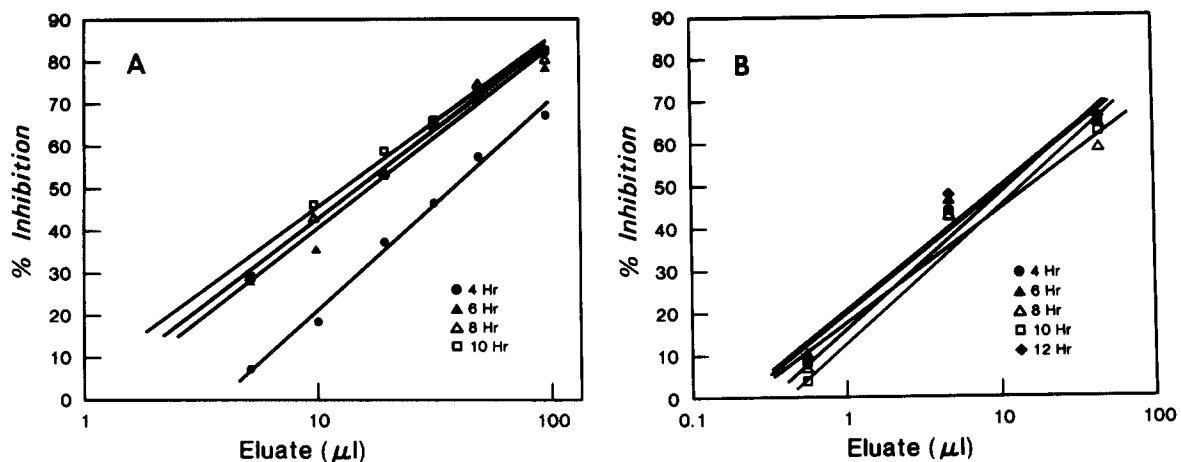


FIG. 3. Time course studies using descending elution for extraction of SRW pollen and *Alternaria* powder. RAST inhibition curves with eluates obtained by descending elution at different time intervals are shown. A, SRW pollen, 100 mg; B, *Alternaria* powder, 100 mg. The inhibition curve with 12-hr SRW eluate was similar to the curves produced by 8- and 10-hr eluates and is not shown.

allergic to *Alternaria*. The IgE antibody pool used for the SRW and AgE RAST inhibition studies was from 12 nonimmunized subjects highly allergic to SRW pollen.

Atmospheric pollen and spore counts

Ragweed pollen grains and *Alternaria* spores were monitored outdoors during the period from July to September 1980 by means of a Rotoslide sampler.⁹ Simultaneous atmospheric samples for the quantitation of *Alternaria* and SRW allergen activity were obtained by continuously operating the high-volume air sampler side-by-side with the Rotoslide sampler from July to September, changing the slides and the filter sheets every 24 hr.

Quantitation of SRW pollen

SRW pollen (5 mg) was suspended in 25 ml of distilled water containing two drops of Contrad 70 detergent (Decon Laboratories Ltd., England). The suspension was gently rotated for a few minutes. The number of pollen grains per milligram was calculated by counting the suspended pollen with a hemocytometer; the mean value from ten such counts was 318,250 grains/mg. Considering previously published values of 50 to 100 grains/m³ of air per day for ragweed during the Rochester pollination season,¹⁰ we estimated that 85,000 to 170,000 ragweed pollen grains (0.27 to 0.54 mg) would be collected daily during the ragweed pollination season when the sampler was operated outdoors continu-

ously. Further, on the basis of published reports¹¹ we calculated that 0.27 mg of SRW pollen would contain approximately 600 ng of AgE.

Statistical tests

RAST inhibition results were evaluated by analysis of covariance¹² using a programmable Hewlett-Packard 9810A calculator.

RESULTS

Effect of temperature on elution

To determine the effect of temperature on allergen extraction 100 mg of SRW pollen was applied to each of three different filter sheets, which were then homogenized separately in BCS buffer and stirred overnight at 4°, 23°, and 37° C, respectively. The slopes of the RAST inhibition curves for the extracts prepared at 4° and 23° did not differ significantly, but the extract prepared at 37° had significantly less allergenic activity when compared with the other extracts. Hence, we conducted all further studies at 23° C.

Comparison of various elution techniques

To compare various elution techniques, 100 mg of *Alternaria* powder and SRW pollen were each applied to three separate filter sheets, which were then extracted by the three different techniques. Descending elution was accomplished with 150 ml of BCS over about 5 hr. All the eluates were dialyzed, lyophilized, and reconstituted in constant volumes of distilled water. RAST inhibition results are presented in Fig. 2 and revealed no significant difference in the slopes of the dose response curves produced by the three extracts of *Alternaria* (Fig. 2, A; $F_{2,9} = 1.43$, NS). The results obtained with SRW pollen are shown in Fig. 2, B. In this case, the slopes of the three inhibition curves differed significantly ($F_{2,9} = 5.82$, $p < 0.025$) because of the somewhat flatter slope of the curve produced by the homogenization extract. However, when a filter sheet exposed to 100 mg of SRW pollen was carried through the descending elution step and subsequently homogenized, the extract obtained showed residual allergenic activity. We felt that the efficiency of the descending elution procedure might be increased by increasing both the volume of BCS and the duration of the elution. Hence, further experiments were undertaken to study the time course of extraction of allergenic activity by the descending elution process.

Time course studies with descending elution

SRW pollen (100 mg) was applied to a filter sheet and extracted by descending elution with 300 ml of BCS in the buffer reservoir, and collection beakers

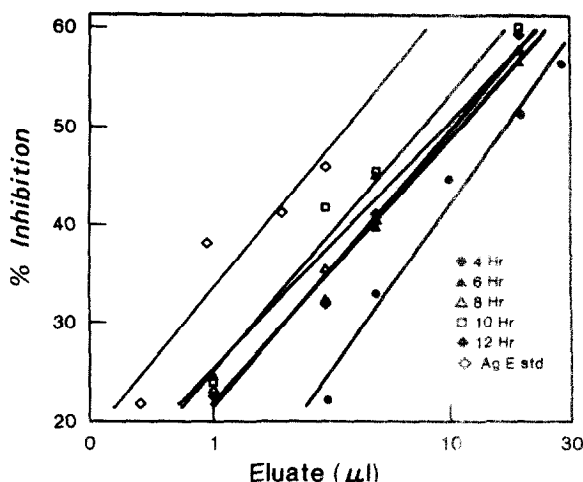


FIG. 4. AgE RAST inhibition curves with SRW eluates obtained by descending elution at different time intervals. The inhibition curve produced by a standard solution of AgE (1 µg/ml) is also shown.

were subsequently changed at 4, 6, 8, 10, and 12 hr. An estimate of cumulative allergenic activity eluted from the filter at different periods was obtained by pooling one fifth of the eluate from a particular time period with one fifth of the volume of the eluate from each of the previous collection periods. All five pooled eluates were dialyzed, lyophilized, and reconstituted in 2.5 ml of distilled water. RAST inhibition results for each eluate are presented in Fig. 3, A. Allergen recovery with descending elution increased with time up to 6 to 8 hr. Similar time course studies with *Alternaria* powder showed similar allergen activity in the 4-, 6-, 8-, 10-, and 12-hr extracts (Fig. 3, B).

To confirm that relevant allergens were being obtained by descending elution, the SRW eluates were also assayed for their AgE content by RAST inhibition with solid-phase AgE (Fig. 4). All eluates contained AgE activity, and the AgE content of these extracts increased with elution time up to 8 hr. We conducted all further studies using an 8-hr descending elution procedure.

Sensitivity of RAST inhibition assay

To determine the minimum amount of allergen required on the filters for elution and assay by RAST inhibition, various quantities of *Alternaria* powder and SRW pollen (4, 20, 100, and 500 mg) were applied to the filter sheets. The sample filters were extracted by descending elution with 300 ml of BCS for 8 hr. After dialysis and lyophilization, filter eluates were reconstituted in 2.0 ml of BCS and tested by RAST inhibition. Satisfactory dose-related inhibi-

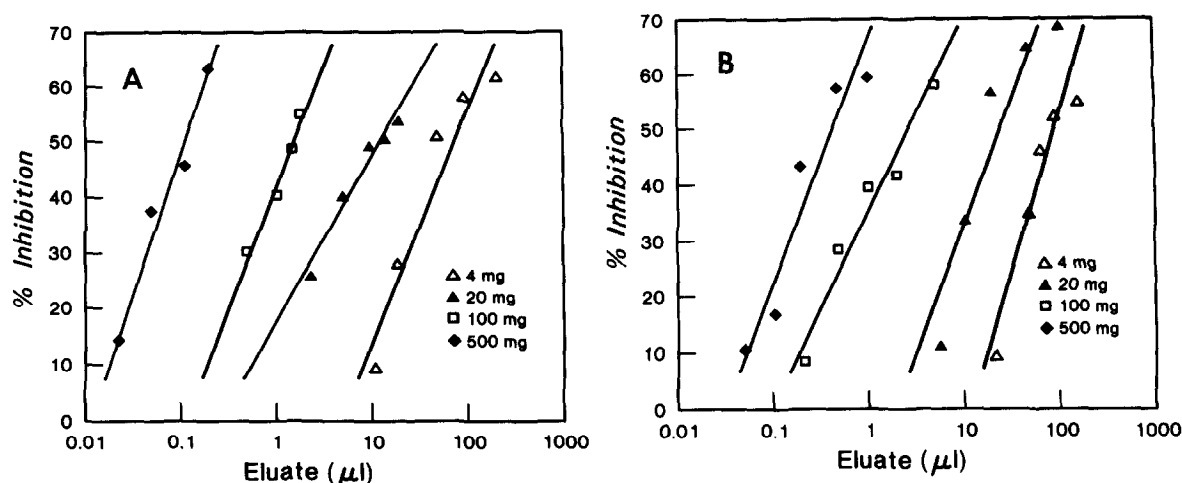


FIG. 5. Dose response curves produced by eluates obtained by descending elution from filter sheets exposed to various quantities of allergens, as measured by RAST inhibition. A, SRW pollen; B, *Alternaria* powder.

tion curves were produced in all instances (Fig. 5).

In these studies we observed that an eluate equivalent to 0.1 mg of SRW pollen (31,481 pollen grains) produced 40% to 50% inhibition in the SRW RAST. Similarly, an eluate equivalent to 0.2 mg of *Alternaria* powder produced more than 40% inhibition in *Alternaria* RAST. Further, an eluate equivalent to 24 µg of SRW pollen (7368 pollen grains) produced greater than 40% inhibition in the AgE RAST.

Recovery

SRW pollen and *Alternaria* powder (100 mg each) were extracted from filters by descending elution for 8 hr (total volume collected, 65 ml) as well as directly in 65 ml of BCS on a magnetic stirrer. The resultant extracts were dialyzed, lyophilized, reconstituted in 2 ml of BCS, and assayed by RAST inhibition. For calculating the recovery we expressed the allergenic potency of the eluate in terms of RIU per ml, as calculated by:

$$\text{RIU/ml} = \frac{1}{\text{ml eluate for 50\% inhibition}}$$

The *Alternaria* descending elution extract contained 359 RIU/ml as compared with 519 RIU/ml in the direct extract, thus giving a recovery of about 69%.

In the case of SRW pollen the descending elution extract showed greater RIU than the SRW direct extract (1312 RIU/ml and 811 RIU/ml, respectively). The increased recovery shown with the descending elution technique was thought to be produced by (1) continual exposure of pollen grains to fresh extracting fluid, and/or (2) the presence of lipids in the direct extract, which may have been retained on the filter sheet in the case of descending elution extract. Re-

peat experiments with defatted pollen again showed greater allergenic activity in the descending elution extract than that in the direct extract. However, when the 100-mg non-defatted SRW pollen was packed in a thin column (5 mm diameter) and eluted for 8 hr with BCS using a peristaltic pump, the eluate showed allergenic activity almost equal to the descending elution extract. These results indicate that the increased recovery in descending elution extract was probably caused by the continual exposure of pollen grains to fresh extracting fluid.

Correlation between morphologic and immunochemical estimations of airborne allergens

Outdoor atmospheric filter sheets were eluted by descending elution for 8 hr. The eluates were dialyzed, lyophilized, and reconstituted in 1 ml of phosphate-buffered saline. The allergenic activity for SRW and *Alternaria* was assayed in RAST inhibition assays. Ragweed pollen counts (56 days) and *Alternaria* spore counts (71 days) were compared with the atmospheric allergenic activity as shown in Fig. 6. Significant correlation was observed in both the SRW ($r = 0.59$, $p < 0.0005$) and *Alternaria* ($r = 0.68$, $p < 0.0005$) allergen systems.

DISCUSSION

Most methods used until now for quantitation of airborne allergens have involved morphologic identification and enumeration of individual pollen grains or mold spores collected by gravitational or volumetric techniques. Gravitational pollen and spore counts reflect contributions of an unknown volume of air; in addition, gravitational counts contain a relative

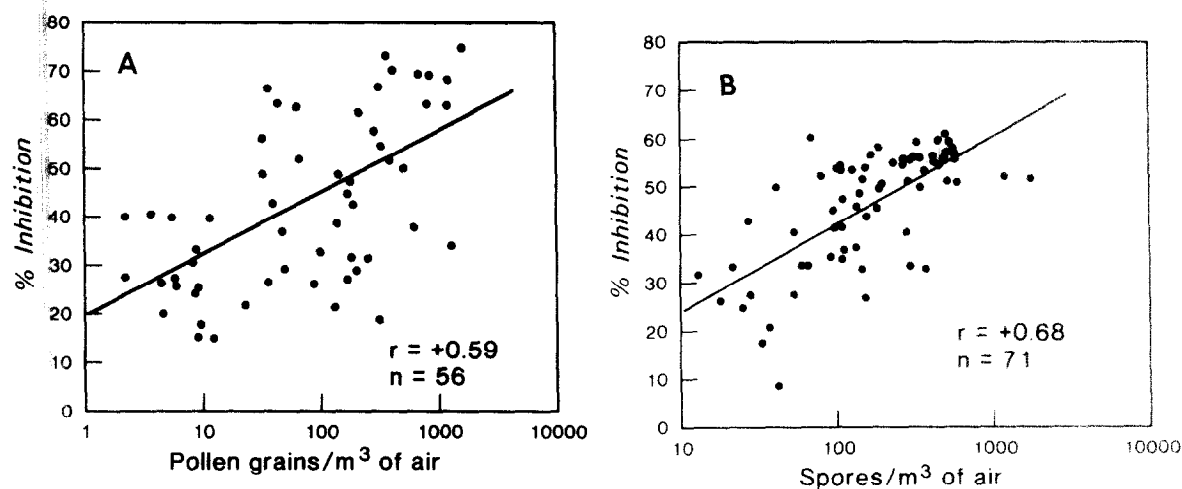


FIG. 6. Comparison of morphologic and immunochemical estimations of airborne ragweed (A) and *Alternaria* (B) allergens. Lyophilized filter eluates were reconstituted in 1.0 ml of phosphate-buffered saline. A, Ragweed data from 56 days (July 28 to September 30). Reconstituted filter eluates (0.05 ml) were tested by mini-RAST inhibition assay in duplicates (mean S.D. = 5.036%). B, *Alternaria* data from 71 days (July 1 to September 20). Reconstituted filter eluates (0.10 ml) were tested by maxi-RAST inhibition assay in duplicates (mean S.D. = 3.17%).

over-representation of spores and grains with larger diameters. Volumetric samplers provide particle counts from known volumes of air, but the collection efficiencies of impaction samplers are still low for particles smaller than $10\ \mu\text{m}$ in diameter.¹ Moreover, the adhesive properties of various substances used to coat impaction sampling devices may vary considerably,¹³ which can introduce significant variations in counts. In addition, there are several plants and fungi that liberate morphologically similar pollen or spores. Various grass pollens or fungal spores of *Aspergillus* and *Penicillium* are only two of many such examples. These particles cannot be separated by genera and are grouped together in aerobiological surveys. Such grouping of pollen and fungal spores makes the correlation of the clinical symptoms of patients with the prevalence of specific airborne pollens or fungal spores very difficult.

Various culture techniques have also been used to quantitate atmospheric fungal spores, but these methods omit nonviable spores that may nevertheless be allergenic. Moreover, exposure of culture plates is limited to short time spans; therefore, the results may not reflect the overall prevalence of various fungal spores during each 24-hr period. In addition, the media used for culture may selectively favor certain fungal genera.¹

Much evidence has accrued suggesting that atmospheric allergens may be present in particles other than pollen and mold spores. For example, ragweed allergen may be present in plant parts other than the pollen.^{14, 15} It has also been shown that ragweed al-

lergen may be carried in amorphous particles smaller than intact pollen grains.³ In the case of fungi the situation is more uncertain. Fungal allergens have been found in the culture filtrate as well as in the spores and mycelium when fungi are cultivated on laboratory media.¹⁶ A similar situation may exist when fungi grow on natural substrates. Moreover, there are airborne fungal hyphae and spore fragments that may possess allergenic activity but that cannot be identified by morphologic criteria. The contribution that amorphous dust particles coated with fungal allergens may make in the production of respiratory allergy is also unknown. In addition, fungal spores and pollen have significant variations in size and thus may possess varying amounts of allergens. Further, pollen and fungal spores in different developmental stages may contain quantitatively different amounts of allergenic activity. All these variables are inadequately addressed by morphologic quantitations of atmospheric pollen and fungal spores.

The immunochemical procedure for measurement of atmospheric allergen has been developed in an attempt to circumvent some of the above difficulties. Ninety-five percent of airborne particles larger than $0.3\ \mu\text{m}$ in diameter are collected on the fiberglass sheets used with the sampler. We have shown that allergen activity can be eluted from these sheets and quantitated by RAST inhibition procedures. By operating the sampler 24 hr daily, the volume of air sampled is over $1700\ \text{m}^3$ per day. Assuming that there is (1) an average daily ragweed pollen count of 50 to $100\ \text{grains/m}^3$ in the Rochester area,¹⁰ (2) a collection

efficiency of 95%, and (3) no appreciable allergenic activity in airborne particles other than intact pollen grains, we postulated that the method would be sufficiently sensitive for SRW allergenic activity to be detected on most days during the pollen season. Our subsequent data supported this supposition. The SRW and *Alternaria* atmospheric allergenic activity by the new immunochemical method showed a highly significant correlation when compared with either the daily ragweed pollen count or the daily *Alternaria* spore count. In future studies, the sampler will be operated with sizing heads to partition airborne particulates into different sizes to test the hypothesis that allergenic activity is associated with particles smaller than intact pollen or spores.

This immunochemical procedure is not without drawbacks, however. It is possible that the total allergenic activity measured by the method may represent the contribution of several cross-reacting genera. For example, "*Alternaria*" allergenic activity measured by this technique may represent contributions by not only *Alternaria* but also those by morphologically similar and closely related genera such as *Stemphyllium* or *Ulocladium*.¹⁷ However, the information regarding cumulative allergen activity may be helpful in the clinical correlation of symptoms of the patients. The immunochemical quantitation of atmospheric allergens will probably represent a valuable tool in monitoring inhalant allergens.

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REFERENCES

1. Solomon WR, Mathews KP: Aerobiology and inhalant allergens, in Middleton E Jr, Reed CE, Ellis EF, editors: Allergy principles and practice. St. Louis, 1978, The C. V. Mosby Co., p. 899.
2. Bates DV, Fish BR, Hatch TF, Mercer TT, Morrow PE: Deposition and retention models for internal dosimetry of the human respiratory tract. *Health Phys* 12:173, 1966.
3. Busse WW, Reed CE, Hoehne JH: Where is the allergic reaction in ragweed asthma? II. Demonstration of ragweed antigen in airborne particles smaller than pollen. *J ALLERGY CLIN IMMUNOL* 50:289, 1972.
4. Gleich GJ, Adolphson CR, Yunginger JW: The mini-RAST: comparison with other varieties of radioallergosorbent test for the measurement of immunoglobulin E antibodies. *J ALLERGY CLIN IMMUNOL* 65:20, 1980.
5. Leiferman KM, Gleich GJ, Jones RT: The cross reactivity of IgE antibodies with pollen allergens. II. Analysis of various species of ragweed and other fall weed pollens. *J ALLERGY CLIN IMMUNOL* 58:140, 1976.
6. Adolphson CR, Yunginger JW, Gleich GJ: Standardization of allergens, in Rose NR, Friedman H, editors: Manual of clinical immunology, ed. 2. Washington D.C., 1980. American Society for Microbiologists, p. 778.
7. Yunginger JW, Jones RT, Nesheim ME, Geller M: Studies on *Alternaria* allergens. III. Isolation of a major allergenic fraction (Alt-I). *J ALLERGY CLIN IMMUNOL* 66:138, 1980.
8. Adolphson CR, Goodfriend L, Gleich GJ: Reactivity of ragweed allergens with IgE antibodies. Analysis of leukocyte histamine release and the radioallergosorbent test and determination of cross reactivity. *J ALLERGY CLIN IMMUNOL* 62:197, 1978.
9. Ogden EC, Raynor GS: A new sampler for airborne pollen: the roto-slide. *J ALLERGY* 40:1, 1967.
10. Welsh PW, Yunginger JW, Tani DG, Toussaint NF Jr, Larson LA, Bourne WM, Gleich GJ: Topical ocular administration of cromolyn sodium for treatment in seasonal ragweed conjunctivitis. *J ALLERGY CLIN IMMUNOL* 64:209, 1979.
11. King TP, Norman PS: Isolation studies of allergens from ragweed pollen. *Biochemistry* 1:709, 1962.
12. Dixon WJ, Massey FJ Jr: Introduction to statistical analysis, ed. 3. New York, 1969, McGraw-Hill Book Co., Inc., p. 222.
13. Solomon WR, Burge HA, Boise JR: Performance of adhesives for rotating-arm impactors. *J ALLERGY CLIN IMMUNOL* 65:467, 1980.
14. Shafiee A, Staba EJ, Abul-Hajj YJ: Partial purification of antigen E from mixed stems and leaves of short ragweed plants. *J Pharm Sci* 62:1654, 1973.
15. Larson JB, Gleich GJ: Changes in the antigenic composition of the short ragweed plant during maturation. *J ALLERGY CLIN IMMUNOL* 56:112, 1975.
16. Prince HE, Tatge EG, Morrow MB: Mold fungi in the etiology of respiratory allergic diseases. V. Further studies with mold extracts. *Ann Allergy* 5:434, 1947.
17. Simmons EG: Typification of *Alternaria*, *Stemphyllium* and *Ulocladium*. *Mycologia* 59:67, 1967.