

Allergen carriage by atmospheric aerosol

II. Ragweed-pollen determinants in submicronic atmospheric fractions

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Outdoor air was drawn by a vacuum system through a 0.8 μ m molecular membrane filter and a back-up, refrigerated condensor from 8 A.M. to 5 P.M. daily during the 1982 ragweed-pollen season. Sample sets from each day were collected and stored separately. Condensate was collected in a freezing jacket, thawed, refiltered through a 0.45 μ m filter, and lyophilized. Reconstituted condensates evoked wheal-and-flare skin reactions in a ragweed-sensitive individual and produced significant inhibition in an IgG-ELISA system by use of ragweed-pollen protein or ragweed antigen E conjugated to polystyrene microtiter plates, pooled serum of patients on ragweed immunotherapy, and alkaline phosphatase-labeled anti-human IgG. Earlier, in 1983 in this JOURNAL, we reported the presence of airborne ragweed-pollen antigen in aerosol fractions below 5 μ m. The present data demonstrate similar activity in filtrates well below the micronic range. Furthermore, these antigenic properties are substantially associated with atmospheric water vapor—either naturally or as a readily-induced result of the collection procedure. These results suggest that naturally occurring mists, although free of native particulates, may yet carry allergens of clinical significance. (J ALLERGY CLIN IMMUNOL 74:64, 1984.)

For years the role of ragweed and other pollens in the provocation of seasonal bronchial asthma has been debated with increasing vigor. It has been demonstrated that particles aerodynamically larger than 10 μ m in diameter are effectively removed from the inspiratory air stream as it passes through supraglottic airways,^{1, 2} an observation that has cast some doubt on the pathophysiologic basis of ragweed asthma.³

Busse et al.,⁴ a decade ago, suggested that ragweed allergens could be airborne, apart from intact pollen grains. More recently, we have demonstrated that aerosols capable of passing through a 5 μ m filter can cause positive skin tests in a ragweed-sensitive subject and demonstrate antigenic activity in an IgG-ELISA inhibition system.⁵ This article reports an-

tigenic activity associated with substantially smaller (less than 0.45 μ m) units or vapors recovered by freeze-out of entrained moisture from volumes of filtered outdoor air.

METHODS

During 8 A.M. to 5 P.M. periods on weekdays from July 29 to October 26, air samples were collected outside a sixth floor window of the Kresge Medical Research Building in Ann Arbor, Mich., by use of the apparatus depicted in Fig. 1. Suction was provided by a GAST (Benton Harbor, Mich.) $\frac{1}{4}$ horsepower pump, calibrated to draw 1 cu ft/min through an in-line filter-condenser system.

The entry port was a Gelman (Ann Arbor, Mich.) 47 mm magnetic holder containing 0.8 μ m pore-size filter as a barrier to pollen and other micronic units. The opening faced an unobstructed (prevailing) wind fetch to the southwest.

A vertically positioned 40 cm condenser cooled by continuously circulating ethylene glycol (-15° C) was positioned down stream from the entrance filter. Condensate dripped into a flask submerged in an ethylene glycol bath (-15° C) where it remained frozen until it was changed at the end of each 9-hour collecting period.

Frozen condensates were allowed to thaw at 4° C to permit volume measurement, then filtered through a 0.45 μ m millipore (screening) filter to document any possible

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Supported by Research Grant AI-10181 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service.

Received for publication Oct. 24, 1983.

Accepted for publication Jan. 17, 1984.

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whole-pollen contamination, lyophilized, and stored at -70°C .

Both the entrance and the screening filters were air dried at 37°C , stained with basic fuchsin or phenosafranine, and clarified with mineral oil. One quarter of each $0.8\text{ }\mu\text{m}$ entrance filter was counted microscopically ($312.5\times$), and the average pollen grains per total filter were calculated. The entire surface of each screening $0.45\text{ }\mu\text{m}$ filter was scanned visually at $312.5\times$ for pollen grains.

The lyophilized samples of atmospheric condensate were examined for ragweed antigenic activity by ELISA inhibition. The unknown specimen of each day was reconstituted separately with 2 ml of distilled water. This "full strength" condensate was also diluted to $\frac{1}{2}$ and $\frac{1}{4}$ strength, and 0.45 ml portions of the three strengths incubated with 0.45 ml aliquots of a 1:500 serum pool from 15 ragweed-sensitive patients on specific aqueous immunotherapy. After 1-hour incubation at room temperature, the amount of ragweed-specific IgG available for binding to either ragweed protein or antigen E was measured by use of an IgG ELISA previously described.⁵ Briefly, $200\text{ }\mu\text{l}$ aliquots of these potentially inhibited sera were incubated for 2 hr at room temperature in microtiter plates coated with $10\text{ }\mu\text{g/ml}$, $200\text{ }\mu\text{l/well}$ of either ragweed protein (prepared by precipitating defatted short ragweed pollen with 100% ammonium sulfate and exhaustively dialyzing the redissolved precipitate against distilled water) or ragweed antigen E (National Institutes of Health Research Resources Branch lot A601-902-185). After washing with phosphate-buffered saline with 0.02% sodium azide and 0.75% Tween 20, $200\text{ }\mu\text{l}$ of goat anti-human IgG labeled with alkaline phosphatase (Tago, Burlingame, Calif., code 2490, lot 900903), diluted 1:1000, was added to each well, and the plates were incubated overnight at room temperature. Two hundred microliters (1 mg/ml) of substrate, p-nitrophenyl phosphate, (PNP, Sigma 104 phosphatase substrate lot 33F-6065; Sigma Chemical Co., St. Louis, Mo.) were added after a final washing. Absorbance at 405 nm was read at 6, 12, and 24 min by use of a Titertek Multiscan (Flow Laboratories, McLean, Va.). The reaction rate in each microtiter well was represented by the slope (m) of the regression equation $y = mx + b$. Percent inhibition was calculated by the formula:

% Inhibition =

$$\frac{\text{Slope "direct" abs.} - \text{slope "inhibited" abs.}}{\text{slope "direct" abs.}} \times 100$$

Daily temperatures and relative humidity readings were made with a Bendix Model 594 Hygrothermograph (Baltimore, Md.) located on the roof of the Kresge I Research Building. Precipitation data were collected by the Atmospheric and Oceanic Science Department of the University of Michigan at their North Campus site, approximately 1 mile away. Statistical comparisons between ELISA inhibition, pollen levels, and climatic variables used the Spearman rank-order correlation.

A ragweed-sensitive subject was prick tested with ragweed-protein antigen at concentrations of 20, 2.0, 0.20, and $0.02\text{ }\mu\text{g/ml}$ and with full strength, reconstituted,

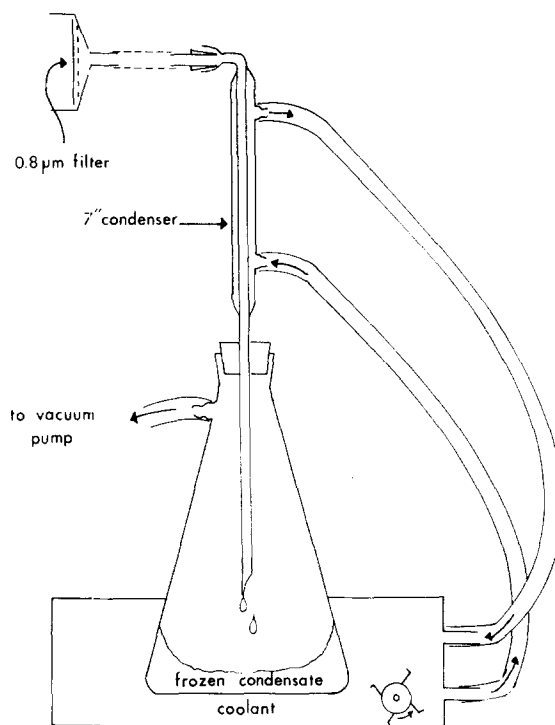


FIG. 1. Diagram of air sample collection apparatus.

filtered condensates. Skin tests were read in 15 min by use of the following criteria: 0, no erythema or induration; 1+, 5 mm erythema; 2+, 10 mm erythema; 3+, 15 mm erythema and 5 mm induration (wheal); 4+, 20 mm erythema and 10 mm induration (wheal) with pseudopods.

Student's *t* test was used to examine relationships between skin test and inhibition percentages.

RESULTS

The collecting apparatus was operated from July 29 through October 26, a total of 60 days. Total ragweed pollen calculated ($\frac{1}{4}$ of pollen on entrance filter counted $\times 4$) varied from a low of four pollen grains on the final collection day to a high of 8112 on August 30 (Fig. 2). No pollen contamination was found on screening filters on 31 days, one to five pollen grains were observed on 21 days, and more than eight on 5 days.

Ragweed-protein IgG-ELISA inhibition assays were done on 42 lyophilized condensates (all collection days were from July 29 to October 7). Results are depicted in Fig. 2. The log of the pollen counts on entrance filters and percent of ragweed-ELISA inhibition for specific dates were directly correlated ($r = 0.527$, $p < 0.0005$). Statistical analysis of relationships among pollen component recoveries and air quality variables is presented in Table I. Samples collected between October 8 to 14 and 18 to 26 were done as pooled samples and do not enter into the

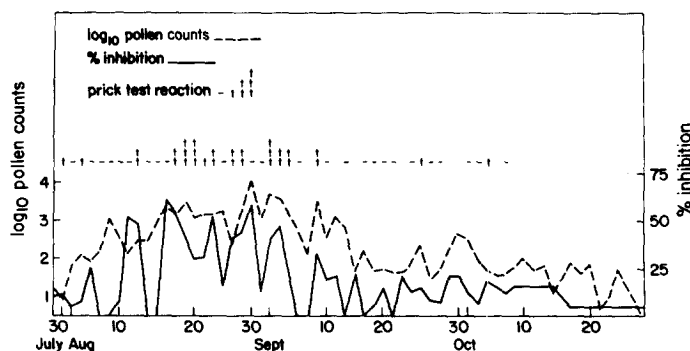


FIG. 2. Comparison of ragweed-protein ELISA inhibition with $\log_{10}$ pollen counts on entrance filters and skin test results.

TABLE I. Statistical analysis of relationships among pollen component recoveries and air quality variables

	Ragweed pollen count	Peak temperature	Average temperature	Average relative humidity	Ragweed protein inhibitions
Ragweed protein inhibition	N = 42 R = 0.527 P = <0.0005	N = 33 R = 0.462 P = <0.005	N = 33 R = 0.225 P = N.S.	N = 33 R = 0.106 P = N.S.	
Ragweed protein inhibition Per cubic centimeter	N = 42 R = 0.461 P = <0.005	N = 33 R = 0.357 P = <0.05		N = 33 R = 0.323 P = <0.05	
Antigen E inhibition	N = 12 R = 0.108 P = NS				N = 12 R = 0.402 P = NS
Ragweed pollen count		N = 42 R = 0.468 P = <0.005			

statistical analysis nor were these samples used in skin testing.

Condensate volume on each collection day varied from 31 cc to 157 cc and was directly correlated with temperature ($p < 0.0005$) and relative humidity ($p < 0.025$). Ragweed percent inhibition per milliliter of condensate was also significantly correlated with pollen recoveries ($r = 0.461$, $p < 0.005$) (Table I). When ragweed antigen E was used to coat the microtiter wells instead of ragweed protein, inhibitions with use of 12 daily samples ranged from 7.8% to 51.4%. These values, however, were not significantly correlated with daily pollen counts from the $0.8 \mu\text{m}$ entrance filters.

Ragweed-protein inhibition values correlated well with peak temperature ($n = 33$, $r = 0.462$, $p < 0.005$) (Table I). This association is almost identical to the values obtained when daily pollen counts are ranked and temperature peaks ($n = 42$, $r = 0.468$, $p < 0.005$). No correlation was found between average temperature or average relative humidity and

ELISA inhibition by use of either ragweed protein or antigen E.

Prick testing of a known ragweed-sensitive, treated volunteer revealed results also demonstrated in Fig. 2. Percent ELISA inhibition with condensates revealing negative skin tests (on skin test-negative days) was significantly lower than those from "skin test-positive" days (student t : $t = 3.76$, $p < 0.005$). Condensates producing no inhibition or low inhibition gave negative skin tests without exception.

DISCUSSION

These data further support the view that ragweed activity exists in air apart from intact pollen grains. The immunoreactive components may be dissolved in the water vapor of moist outdoor air or be associated with solid particles distinctly smaller than $0.45 \mu\text{m}$ or both. Furthermore, antigenic materials of either form may become adsorbed to the abundant submicronic aerosol present in nature, especially during peaks of plant growth cycles. A possibility exists also that

some or all of the activity recovered was leached from pollen grains intercepted by the 0.8 μm filters. The present research design does not exclude this effect as a factor influencing our results. However, if such elution does occur, then similar processes would be expected to operate generally in outdoor areas where pollen grains encounter moist surfaces and air. The possibility that antigenic components may be scavenged from solid particles by moving damp air has not been studied. However, pollen components are quickly mobilized; some release is measurable within as little as 2 sec of contact with aqueous media.^{6, 7} Release of antigen Ra5 may be complete within minutes. Furthermore, well recognized natural processes could promote the aerial dissemination of eluted substances in microdroplets.⁸ The lack of correlation between antigen E-ELISA inhibition and daily pollen counts may be due to the small number of samples studied or may be evidence that other antigens are involved in this phenomenon.

We have recently reported antigenic activity passing through 5 μm filters and producing positive skin tests and patterns of ELISA inhibition.⁵ These activity trends were not closely correlated with prevailing (day-to-day) patterns of pollen occurrence, raising the question that other sources might be active. Rebhun et al.⁹ and Shafiee et al.¹⁰ have described pollen-related antigenic activity in leaves, stems, seed, and roots of giant and short ragweed plants. However, these sources appear to be relatively weak, and the close association, in the present study, of condensate activity and pollen recoveries suggests pollen itself as the ultimate source of the microparticulate antigens demonstrated. Materials formed during pollen development, such as tapetal fluid, might also contribute extractable activity.¹¹ However, exine orbicles (Ubisch bodies)¹¹ formed within the anthers of some other species appear not to be a product of pollen ontogeny among ragweeds.¹²

Our present data imply that several aerosol classes

of quite different median size are potentially implicated as determinants of pollinosis. The relative contributions of these several atmospheric fractions to total allergen exposure remain to be estimated. Substantial interest also surrounds the possibility that submicronic particles may be important vectors of allergen challenge for subglottic airways from which intact pollens are effectively excluded.

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