

Pollen grain column chromatography: Quantitation and biochemical analysis of ragweed-pollen solutes

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*The kinetics, quantitative yield, and sequence of solute release during the extraction of allergenic substances from short ragweed (*Ambrosia artemisiifolia*) pollen were compared with a conventional batch-type method and the novel technique of pollen grain column chromatography. With the batch method, 14.6 ± 1.7 mg of pollen solutes were eluted per 100 mg of dried defatted pollen in 1 minute; the 24-hour solute yield was 27.4 ± 2.7 mg. With the column method, 3.7 ± 1.3 mg of pollen solutes were eluted in 1 minute; the 24-hour solute yield was 29.3 ± 2.1 mg. The kinetics of solute release with the column method were modeled as the simultaneous first-order elution of ragweed-pollen solutes into three hypothetical compartments. The theoretical initial solute concentration was 50 gm/L. The isoelectric focusing patterns, optical properties, distributions of enzymes, Ra5, and antigen E activities were consistent with the sequential separation of ragweed-pollen solutes and the three compartment model. Enzyme activities were eluted either maximally in the first minute (phosphatases and N-acetyl- β -glucaminidase) or delayed until 10 minutes (leucine aminopeptidase). Ra5 was eluted rapidly, whereas antigen E was eluted during a more prolonged period. Pollen grain chromatography provides a simple, reproducible method for studying pollen solute release (J ALLERGY CLIN IMMUNOL 1988;81:1126-34.)*

Pollen grains contain large amounts of water-extractable solutes. Pollen solutes include the allergenic proteins,¹ lectin-binding factors and lectins,² complex carbohydrates, lipids, nucleic acids, enzymes, and the factors responsible for female flower recognition and plant fertilization.^{3, 4} Allergenic pollen extracts are complex mixtures of these components.⁵⁻⁸ Pollen extracts are conventionally prepared by suspending pollen grains in buffer for up to 24 hours. The separation of pollen solutes during extraction is rapid and ordered.^{9, 10} For example, short ragweed (*Ambrosia artemisiifolia*)-pollen cationic antigens and the Ra5 and Ra6 allergens are released within 16 minutes with the conventional, batch-type extraction,¹¹⁻¹³ whereas other antigens, such as AgE, Ra3, and the bulk of acidic components, are released in relatively low amount initially, but continue to be released for as long as 4 days.^{12, 14} Conventional rag-

Abbreviations used

AgE:	Antigen E
OD:	Optical density
OD ₂₈₀ :	OD at 280 nm
T _{1/2} :	Halftime

weed extracts contain an abundance of slowly released acidic components and a dearth of the rapidly released cationic components.^{8, 12, 14}

This article presents the results of a quantitative study of the pollen elution process. The pollen solute yield obtained by a conventional batch-type extraction method was compared with a novel chromatographic method of pollen extraction. This method uses pollen grains as the matrix of analytical and preparative chromatography columns. Chromatographic elution of pollen solutes from the columns by constant solvent flow permits the collection of separate fractions for rapid analysis. Studies of the composition and concentration of the fractions can be used to gain an understanding of the process of pollen solute elution. With first-order kinetics, the elution process can be modeled as the separation of pollen solutes into three hypothetical

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compartments. This model permits a reliable prediction of eluted solute masses and concentration. Specific pollen enzyme and allergen activities were assayed, and both rapid and delayed patterns of separation were found. The information obtained from the study of the process of pollen elution has important implications of our understanding of pollen biology, allergenic pollen extract preparation and standardization, and the pathogenesis of pollenosis.

METHODS

Conventional pollen extracts

Dried, defatted short ragweed (*Ambrosia artemisiifolia*) pollen (lot No. 18G56-90) was obtained from Greer Laboratories (Lenoir, N.C.) and stored with desiccant at 25° C in sealed containers. Conventional extracts were prepared with methods comparable to those used for the manufacture of allergenic extracts with the exception of the use of excess solvent to maximize solute recovery and facilitate concentration measurements. The methods described by Stull et al.¹⁵ were used to suspend 200 mg of pollen in 200 ml of deionized water (1/1000 wt/vol) at 25° C. The pollen suspensions were gently agitated. Aliquots were removed at timed intervals between 0.5 and 1440 minutes and centrifuged at 1000 rpm for 10 minutes before analysis.

Pollen grain chromatography

Analytical chromatography was carried out in 1 ml of ground-glass tuberculin syringe barrels with inner diameters of 5 mm (Popper & Sons Inc., New Hyde Park, N.Y.). The exit port of the syringe was covered by a 4 mm diameter, 0.8-micron type AA Millipore filter (Millipore Corp, Bedford, Mass.). One hundred milligrams of ragweed pollen was gently packed into the column (0.37 ml, 2 cm bed height), and the column was plugged with glass wool. In other experiments, pollen was packed into 5-mm inner diameter Pasteur pipettes between glass wool plugs. In preparative experiments, a 20-ml Filtromatic ground-glass syringe barrel (Filtromatic Co., Baltimore, Md.) was packed with 4 to 6 gm of pollen between layers of glass wool. Each column was inverted, and the eluting solvent was pumped upward through the pollen bed. Solvent flow was controlled with a 2120 Varioperpex II peristaltic pump (LKB, Bromma, Sweden). The flow rates for analytical columns were 0.03 to 3.4 ml/min/100 mg of pollen, and for preparative columns, 0.7 to 2.4 ml/min. In different experiments, analytical columns were eluted at room temperature with deionized water, with pH 7.4, 0.15 mol/L NaCl and 0.05 mol/L Tris buffer, or with the latter buffer containing 0.01% borate and 10% sucrose.⁹ Fractions were collected at 0.5- to 30-minute intervals with a 2112 Redirac fraction collector (LKB).

Solute concentrations

Eluate fraction concentrations were calculated from measurements of either solute dry weight or OD. The dry solute weights of fractions eluted with deionized water from pre-

parative columns were obtained by heating aliquots of known volume and weight in glass or porcelain crucibles in a forced convection drying oven at 107° C until a minimum weight was obtained. Solutes were weighed on an analytical balance (Mettler Instrument Corp., Hightstown, N.J.) with a precision of ± 0.0002 gm. Dry weights of fractions collected from preparative columns were 0.02 gm or greater, and the experimental errors of these gravimetric measurements varied between $\pm 2\%$ and 10% . Despite the difference in the magnitude of the experimental precision, the solute yields calculated from dry weights of fractions from analytical and preparative columns were comparable. Sample volumes and the observed weights were used to calculate the concentration and the yield of pollen eluate solutes. Solute yield was expressed as milligrams per 100 mg of pollen. The weight of volatile substances in dried defatted pollen was found by drying the pollen to minimum weight at 107° C. Pollen-packed columns were weighed before elution and reweighed after drying the eluted column to minimum weight at 107° C. The column weight difference, corrected for the weight of volatile pollen substances (6.2 ± 1.3 mg for 100 mg of pollen, six experiments), was used to estimate the mass of the eluted pollen solutes.

The OD₂₈₀ of eluate aliquots was measured with a Perkin-Elmer lambda 3B UV/VIS spectrophotometer (Perkin-Elmer Co., Oak Brook, Ill.) with a 1-cm light path. Volumetric dilutions of fractions were used to measure the OD when an initial measurement was >0.3 to 0.6 optimal range of the spectrophotometer. Extinction coefficients ($E_{1\%, 1\text{ cm}, 280\text{ nm}}$) for ragweed-pollen solutes were calculated in the conventional manner from measurements of OD and dry solute weight as the absorbance expected from a 1% (10 gm/L) solute solution in a 1-cm light path at 280 nm. In subsequent experiments, ragweed solute concentrations were calculated by dividing the observed OD by the appropriate extinction coefficient for the ragweed-pollen extract or the column eluate fraction. Solute mass was found by multiplying the concentration by the solvent flow rate and duration of the fraction collection time.

Kinetic analysis of observations

Graphs of the logarithm of eluate solute concentration were plotted against elution time and inspected. Apparent straight line segments were identified and analyzed by linear regression with the method of least squares. The slopes and Y intercepts (b) of the linear segments were analyzed algebraically and used to calculate apparent first-order elution rate coefficients (k , minutes⁻¹ = $2.303 \times \text{slope}$) and half-times of elution ($T_{1/2}$, minutes = $0.693/k$) with use of defined relationships.^{16, 17} Experimental kinetic parameter values obtained in compared experiments are presented as the mean $\pm$ standard deviation. These parameters were also used to estimate the solute mass eluted during finite-time intervals and to describe the reproducibility of the elution process. The mass of solutes in each compartment, or for any time interval, can be calculated by combining equations, integrating over the appropriate time interval, and multiplying by the flow rate.

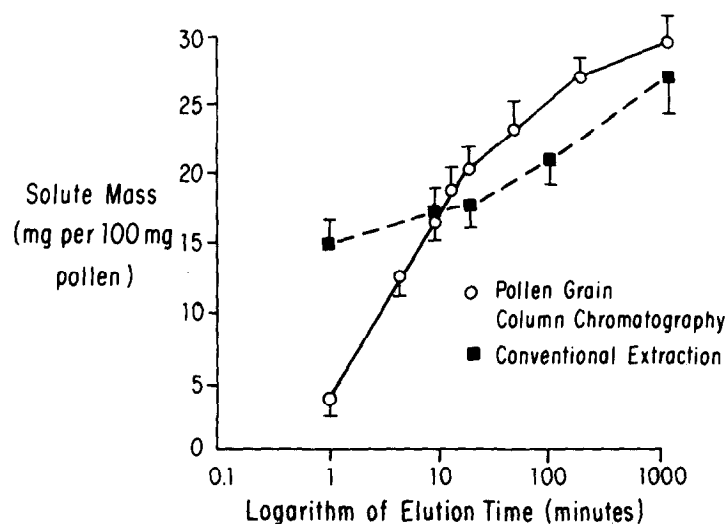


FIG. 1. The graph compares the cumulative mass (mean $\pm$ SD) of short ragweed-pollen solutes separated during conventional extraction in six experiments and by pollen grain column chromatography at a solvent flux of 0.13 ml/min/100 mg of pollen in eight experiments. The ordinate was plotted as the logarithm of elution time to demonstrate the large early differences in solute yield. The column method retarded early rapid solute elution but yielded an equivalent solute mass with continued solvent flow.

Isoelectric focusing

Isoelectric focusing was performed on preformed pH 3 to 10 polyacrylamide gels (LKB) run at 1500 V for 90 minutes. The eluate fractions were compared with a batch-type ragweed extract that is similar to generally available ragweed-pollen extracts and a series of standard proteins (pI Markers, FMC Bioproducts, Rockland, Mass.). Gels were stained with R-250 Coomassie blue according to the manufacturer's instructions.

Allergen measurement

Allergens AgE and Ra5 were measured by ELISA inhibition. Immulon II (Dynatech Industries, Alexandria, Va.) microtiter plate wells were coated with a reference short ragweed-pollen extract (1 μ g/ml in pH 9.6 carbonate-coating buffer). Two hundred microliters of a standard solution of monospecific polyclonal rabbit anti-AgE or goat anti-Ra5 antisera and varying concentrations of column eluates or reference extract were added to duplicate wells and incubated for 4 hours at room temperature. After washing the wells with PBS-Tween, an appropriate dilution of alkaline phosphatase-labeled second antibody (either anti-rabbit IgG or anti-goat IgG from Sigma Chemical Co., St. Louis, Mo.) was added to the wells. After 4 hours of incubation at room temperature and washing with PBS-Tween, the enzyme substrate, 1 mg/ml of *p*-nitrophenol phosphate in diethylamine buffer at pH 9.8, was added. Absorbance was measured at 405 nm with a Bio-Tek automated ELISA reader (Bio-TEK Instruments, Burlington, Vt.).

Inhibition of antibody binding was determined by measuring the ODs of reference-coated wells plus appropriate

antiserum, and the ODs of wells plus antisera after the addition of dilutions of standard AgE or Ra5 solutions. The percent inhibition of antibody binding produced by the standards was calculated and plotted against the logarithm of the standard allergen concentration to yield an allergen-antibody binding inhibition curve. Allergen concentrations in column eluate fractions were determined by measuring the percent inhibition of antibody binding produced by adding dilutions of the column fractions. Comparisons of the percent inhibition produced by the column eluate fractions with the standard curve at the point of 50% inhibition were used to calculate the AgE or Ra5 activity of column fractions.

Enzyme activity

The activities of eluate enzymes were screened with APIZYM (Analytab Products, Plainsview, N.Y.) enzyme substrate strips¹⁸ as described in the manufacturer's instructions. Briefly, two drops of eluate sample were applied to each of 20 microcupules that contained specific enzyme substrates. The strips were incubated at 37° C for 4 hours. Enzymatic reaction products were detected by the addition of color-producing reagents. The intensity of the color reaction was used to identify enzyme activity.

The activities of four enzymes were investigated further. Leucine aminopeptidase activity was assayed with 0.5 mmol/L leucine-*p*-nitroanilide (Sigma Chemical Co.) in 0.15 mol/L NaCl and 0.05 mol/L Tris at pH 7.4.¹⁹ The extinction coefficient of the substrate product, *p*-nitroaniline, was 9.9 M⁻¹ cm⁻¹. *N*-acetyl- β -glucaminidase activity²⁰ was assayed with 3 mmol/L 3 *p*-nitrophenyl- β -glucaminide in 50 mmol/L citrate buffer at pH 5.0. Acid

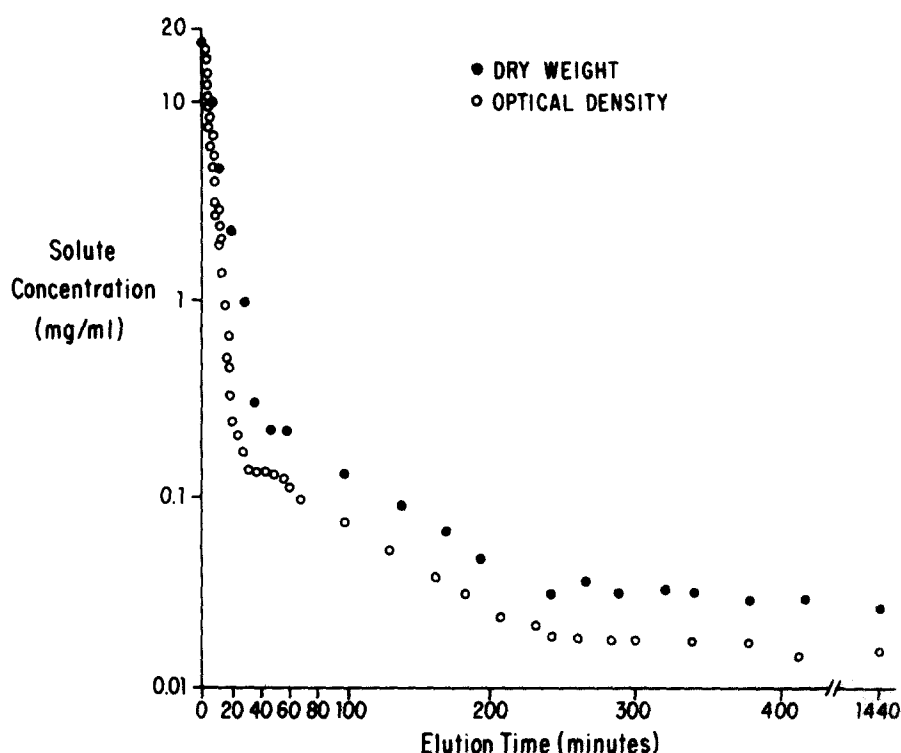


FIG. 2. The illustration presents the results of two experiments that are representative of >50 chromatographic studies of ragweed pollen. Note the logarithmic scale of solute concentration. The dry weight measures were derived from an analytical column with a deionized water flux of 0.15 ml/min/100 mg of pollen. OD measurements were converted to solute concentrations with the three extinction coefficients for fractions collected from an analytical column with pH 7.4, 0.05 mol/L of Tris, 0.15 mol/L of NaCl, 10% sucrose, 0.01% of borate buffer flowing at 0.26 ml/min/100 mg of pollen. The differences between the two curves are due to solvent flux.

phosphatase activity²¹ was assayed with 3 mmol/L *p*-nitrophenyl phosphate in 50 mmol/L citrate buffer at pH 5.0. Neutral phosphatase was assayed with 3 mmol/L *p*-nitrophenyl phosphate in 0.15 mol/L NaCl and 0.05 mol/L Tris at pH 7.4. The extinction coefficient of the product of these three reactions, *p*-nitrophenol, is 17 M⁻¹ cm⁻¹. Enzyme activity was measured by mixing an initial 0.1 ml volume (V_i) of eluate with 3 mol of the appropriate enzyme substrate to yield a final volume (V_f) of 3.1 ml. The initial OD (OD_i) of the substrate was measured at 405 nm immediately after mixing, and the final OD (OD_f) was measured after 90 minutes incubation (T_{inc}) at 25° C. Enzyme activity was calculated and expressed in milliunits per milliliter with the relation:

$$\text{Activity} = (OD_i - OD_f) V_f 1000 / T_{inc} V_i E$$

where E was the appropriate extinction coefficient.

RESULTS

Ragweed-pollen solutes were rapidly extracted with the conventional, batch-type extraction method (Fig. 1). In the first minute of extraction, the dry weight of solutes eluted was 14.6 ± 1.7 mg/100 mg of pollen. During the next 19 minutes, only 3.2 ± 1.4 mg/100

mg of pollen of additional solute was extracted. The total yield of solutes extracted in 24 hours was 27.4 ± 2.7 mg/100 mg of pollen. In contrast, ragweed-pollen grain column chromatography with a solvent flow of 0.13 ml/min/100 mg of pollen yielded an average of 3.7 ± 1.3 mg of pollen solute per 100 mg of pollen in the first minute. By 20 minutes, an average of 20.4 ± 4.7 mg of pollen solute was eluted. The total 24-hour yield by column method was 29.3 ± 2.1 mg/100 mg of ragweed pollen. The total quantity of solute eluted by the two methods was similar. However, with the column method and a low flow rate, solute elution was slower in the first 20 minutes. This comparison suggested that the column method limited the volume of fluid available for pollen solute dissolution and permitted the gradual separation of rapidly released pollen solutes.

The pattern of solute elution during ragweed-pollen grain column chromatography was very reproducible (Fig. 2). Solute concentrations of early eluate fractions were very high but declined rapidly. Concentrations of column eluate fractions in the first minute were in the range of 20 to 55 gm/L. Between 15 and

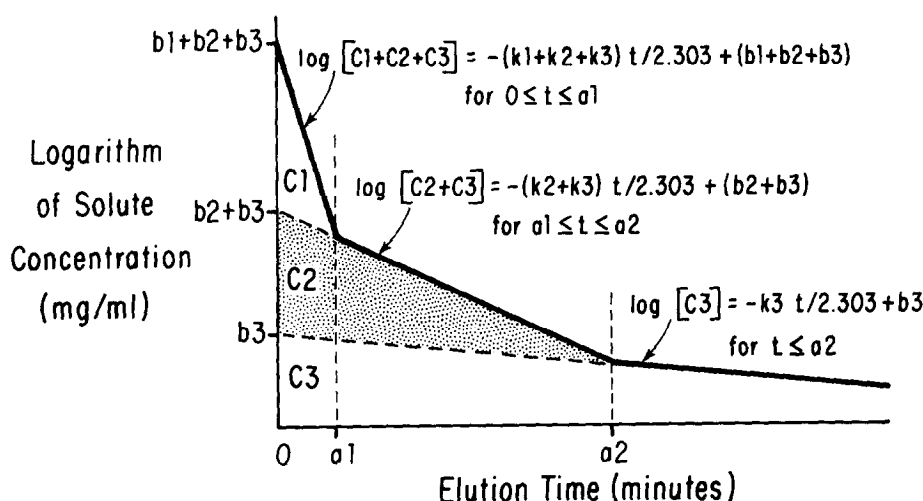


FIG. 3. The relationships between the apparent first-order elution rate coefficients (k_1 , k_2 , and k_3), zero time intercepts (b_1 , b_2 , and b_3), and the logarithms of the solute concentrations for the three hypothetical compartments of C1, C2, and C3 can be formulated in a model that permits prediction of ragweed-pollen solute elution over time.

20 minutes, the concentrations had declined to the range of 0.6 to 3.0 gm/L. When each elution curve was plotted as the logarithm of concentration versus average time of fraction collection, it was found to be consistent with three straight line segments. The correlation coefficients for straight line segments with six or more observations were ≥ 0.8 . Qualitative and quantitative comparisons of chromatographic elution curves based on OD and on dry weight measures of concentration, various columns, deionized water, NaCl-Tris, and NaCl-Tris-sucrose-borate buffer revealed few differences. The results of experiments with different pollen mass were equivalent when solvent fluxes (flow rate normalized for pollen mass; milliliter per minute per 100 mg of pollen) were the same. High solvent flux shifted the triphasic pattern to the left. Optimum resolution was obtained with small successive fractions and slow flow rates.

The mean apparent first-order rate coefficients, compartment masses, and other kinetic parameters (Table I) for 30 experiments with a solvent flux of 0.17 ± 0.08 /min/100 mg of pollen were calculated with the kinetic model depicted in Fig. 3. The rapidly released first compartment contained 74% of the extractable solutes. The three apparent first-order elution rate coefficients varied approximately tenfold from each other. The mean theoretical initial solute concentration was 55 gm/L (antilog of 1.70 ± 0.38). In nine experiments in which identical experimental conditions were used, the coefficients of variation (mean $\times 100$ /SD) of the kinetic parameters and solute yields were $< \pm 10\%$.

Extinction coefficients ($E_{1\%, 1 \text{ cm}, 280 \text{ nm}}$) varied in

relation to the mean time at which the column fractions were collected. Over selected intervals of time, the extinction coefficients of sequential eluate fractions were similar in magnitude. The mean extinction coefficient for 23 fractions with average times of collection between 0 and 20 minutes was 5.1 ± 1.9 . For the interval from 21 to 200 minutes, the mean extinction coefficient was 7.3 ± 2.5 (24 fractions). The extinction coefficient for the interval from 201 to 1440 minutes was 14.5 ± 4.8 (six fractions). This comparison suggests that the optical properties of ragweed-pollen solutes were relatively constant within the three times of collection.

The utility of the three extinction coefficients was evaluated in five additional experiments. The ODs of chromatographic fractions were converted to concentrations with the independently derived extinction coefficients. The mass of each fraction was calculated from the flow rate and time of collection. The cumulative mass (28.1 ± 6.8 mg/100 mg of pollen) was compared to the total dry weight of the eluted fractions (27.4 ± 5.5) and by indirect estimates of solute yield based on measured changes in the weight of packed pollen chromatography columns after solute elution (25.8 ± 7.1). Differences between the pollen solute weights measured by the three different methods were small and not significant evaluated with Student's paired t test. Estimates of total solute recovery based on the OD_{280} and the three extinction coefficients agreed with the gravimetric measurements of total mass. This additional series of experiments confirmed the utility of the three extinction coefficients and the differences in the optical properties of

TABLE I. Elution parameters for a three-compartment model of pollen solute separation*

Parameter	Elution compartments, mean $\pm$ SD		
	1	2	3
Rate coefficient k , min^{-1}	0.14 ± 0.08	0.0092 ± 0.0031	0.0023 ± 0.0015
Halftime $T_{1/2}$, min	5.3 ± 3.0	65.0 ± 25.0	373.6 ± 236.0
Solute mass, mg/100 mg of pollen	23.5 ± 6.7	4.1 ± 2.1	4.2 ± 2.3
Zero time intercept b , log (mg/ml)	1.70 ± 0.38	0.94 ± 0.29	-1.35 ± 0.27
Apparent intersection a , min	25.5 ± 9.1	243.0 ± 65.9	

*Average from 30 experiments.

TABLE II. Experimental evaluation of the hypothetical three-compartment model of ragweed-pollen solute elution

Time interval (min)	Yield predicted from model	Dry weight of pollen solutes*
	(mg/100 mg of pollen)	
0-20	21.5	20.0 ± 4.7
21-60	2.1	2.9 ± 1.6
61-120	1.4	1.6 ± 1.1
121-240	1.1	0.9 ± 0.4
241-600	1.1	1.1 ± 0.8
600-1440	0.7	2.8 ± 0.8
Total solute	27.9	29.3 ± 1.6

*Means and standard deviations for fractions collected from five analytical and three preparative columns. Note the departure of the 601- to 1440-minute fraction from expectations based on the elution pattern of 90% of the pollen solute.

ragweed-pollen solutes eluted during the three time intervals.

The predictive ability of this kinetic model of ragweed-pollen elution (Fig. 3) and the parameters presented in Table I were evaluated in eight additional experiments. Estimates of expected solute yield were calculated from the kinetic parameters for several time intervals with different experimental conditions (Table II). Close agreement was found between the predicted and observed dry weights except for the period from 601 to 1440 minutes. This suggests that the mechanisms responsible for generating the final 10% of total pollen solute during the 601- to 1440-minute interval of prolonged extraction differs from the process that produces the rapid elution of the bulk of pollen solutes.

Isoelectric focusing

Isoelectric focusing was done with column fractions collected with a solvent flux of 0.015 ml/min/100 mg of pollen (Fig. 4). At this slow rate, early fractions

TABLE III. Distribution of ragweed-allergen activities in column fractions

Allergen	Parameter	Column fraction (min)		
		0-20	21-240	241-1440
Ra5	Activity ($\mu\text{g}/\text{ml}$)	71	3	1
	Sp. activity ($\mu\text{g}/\text{mg}$)	3	7	6
	Yield ($\mu\text{g}/100$ mg)	14	39	8
	Yield (%)	22	64	14
AgE	Activity ($\mu\text{g}/\text{ml}$)	1.0	0.2	0.01
	Sp. activity ($\mu\text{g}/\text{mg}$)	0.4	0.9	5.8
	Yield ($\mu\text{g}/100$ mg)	0.3	1.1	4.0
	Yield (%)	6	20	74

Sp. = specific.

revealed the rapid, transient release of cationic bands followed by the delayed appearance of neutral and acidic bands. In contrast, a 24-hour batch extract of short ragweed revealed predominantly acidic bands with essentially no cationic bands. The use of more rapid solvent flux also revealed the rapid, transient release of cationic protein solutes, without the same degree of retardation of the neutral and acidic pollen solutes.

Allergen activity

The release of Ra5 antigen was rapid (Table III). The activity of Ra5 antigen was equivalent to 71 $\mu\text{g}/\text{ml}$ for the elute fraction collected between 0 and 20 minutes. Peak specific activity was reached in later fractions. During the time of elution of the first two compartments, 86% of Ra5 activity was extracted. In contrast, AgE activity was released more slowly. The

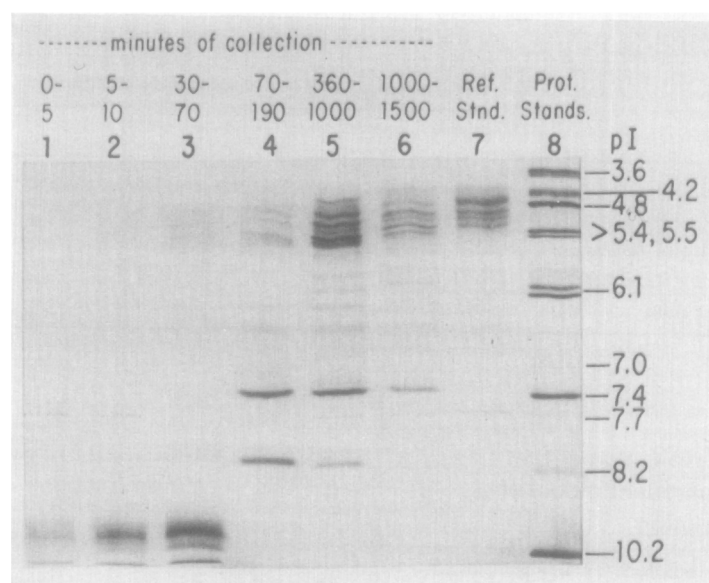


FIG. 4. Isoelectric focusing patterns are illustrated for representative column fractions collected at a solvent flux of 0.015 ml/min/100 mg of pollen. The reference standard is a batch-type extract similar to generally available ragweed extracts. The series of standard proteins are amyloglucosidase (pI = 3.6), glucose oxidase (pI = 4.2), ovalbumin (pI = 4.8), β -lactoglobulins A and B (pIs = 5.4 and 5.5), carbonic anhydrase (pI = 6.1), major and minor horse myoglobins (pIs = 7.0 and 7.4), major and minor whale myoglobins (pIs = 7.7 and 8.2), and cytochrome c (pI = 10.2).

third compartment (eluted after 240 minutes) contained 74% of the total AgE activity. Although antigen activity was maximal initially, specific activity was greatest after prolonged elution. With the column method, the yield of AgE appeared lower and the yield of Ra5 higher than those previously described by batch extraction.

Enzyme activity

With APIZYM enzyme substrate strips, four patterns of enzyme activity were detected in the ragweed-pollen grain column chromatography eluates. Enzyme activities detected with substrates for acid phosphatase, alkaline phosphatase, phosphohydrolase, and C8 lipid esterase were present in the first minute of elution and persisted for up to 600 minutes. Enzyme activities detected with substrates for *N*-acetyl- β -glucaminidase, β -galactosidase, and α -mannosidase were present only during the first 10 minutes of elution. Leucine aminopeptidase activity was not present in fractions collected during the first 10 minutes of elution but reached high levels between 20 and 40 minutes. With the conventional batch-extraction method, each of these enzyme activities was detected within 1 minute of beginning pollen extraction. No activity was detected with substrates for valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, α -galactosidase, β -glucuronidase,

α -glucosidase, β -glucosidase, α -fucosidase, C4 esterase, and C14 lipid esterase. The time course of elution of four specific enzymes is illustrated in Fig. 5. *N*-acetyl- β -glucaminidase activity was highest in the initial drops of eluate and then rapidly decreased to undetectable levels by 20 minutes. The activity of acid phosphatase and neutral phosphatase were also high initially but declined more gradually. Leucine aminopeptidase activity was not detected in the first 2 minutes, but rose rapidly to maximal levels between 20 and 40 minutes.

DISCUSSION

With pollen grain column chromatography, the process of ragweed-pollen elution exhibits distinct three phases of solute separation. These phases can be deduced from the shape of chromatographic elution curves and from differences in the distribution of solute optical properties, isoelectric banding patterns, Ra5 and AgE activity, and enzyme activities. The observed changes in solute can be modeled with first-order kinetics and three hypothetical compartments (Fig. 3) to yield highly reliable predictions of the pollen elution process (Table II). Interestingly, conventional batch-type extraction elutes pollen solutes at a much faster rate than is generally appreciated. In comparison to conventional batch-type extracts, ragweed-pollen grain column chromatography limits

the availability of water to the pollen grain and slows the early rapid separation of pollen solutes. Even with the retardation provided by the chromatographic method, solute separation was extraordinarily fast. The reproducibility of the chromatographic elution pattern over a wide range of pollen masses and flow rates and the derivation of a reliable predictive mathematical model suggest that the column method may resolve the elution events that occur at the level of the pollen grain. Although it is intuitively apparent that this new awareness of the elution process may permit development of better extraction procedures, the information currently available is not sufficient to describe optimal conditions for allergenic extract preparation. The studies presented in this article are focused on our understanding of the initial phases of allergenic solute release, such as might occur during pollen grain hydration in the limiting aqueous volume of condensed atmospheric moisture, or on the mucosal surface.

Unlike previous studies of the rapid diffusion of solutes from pollen walls,²²⁻²⁴ the kinetic model provides a quantitative estimate of the magnitude of the antigen stimulus that might be provoked by inhaled pollen grains. The kinetic model predicts that the theoretical initial solute concentration of ragweed-pollen solutes would be 50 gm/L (5% wt/vol). This concentration is the theoretical maximum solute concentration that would be present in the immediate vicinity of a newly hydrated pollen grain. Although the total mass of solute per pollen grain may be minute, the very high local solute concentration produced by the rapid elution of pollen solutes in a limited aqueous volume could provide an intense signal to receptive elements of the mucosa. Solute concentration would rapidly decrease with further hydration and dilution by mucosal fluids. Assuming an average ragweed-pollen grain diameter of 20 μm ,²⁵ a density of 0.84 gm/ml for dried and defatted ragweed pollen,²⁶ and 0.24 as the ratio of the mass of rapidly released pollen solutes to total pollen mass (Table II), then approximately 0.8 ng of rapidly released solute would be expected per pollen grain. Nasal challenge in patients with allergic rhinitis with 10,000 to 5,000,000 ragweed-pollen grains is sufficient to produce symptoms and inflammatory mediator release.^{27, 28} This suggests that 8 to 800 μg of ragweed-pollen solutes, including 6 to 600 ng of Ra5 and 0.1 to 10 ng of AgE, would be sufficient to produce acute symptoms in a ragweed-sensitive patients.

An understanding of the process of pollen-allergen release may have implications for the pathogenesis of pollenosis. The mucosal site at which sensitization

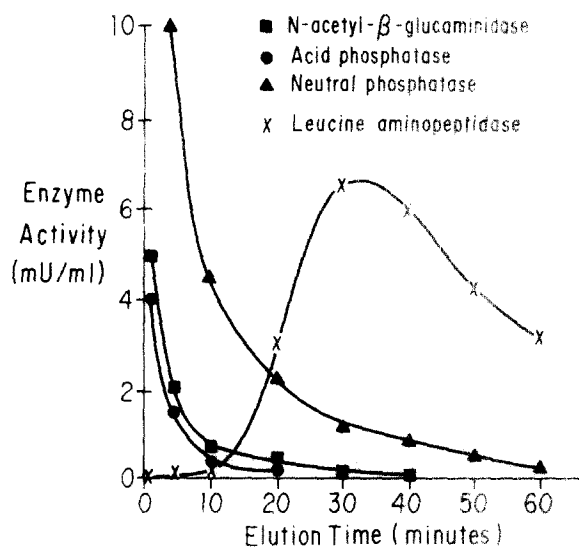


FIG. 5. Ragweed-pollen grain column chromatography elution patterns for acid phosphatase, *N*-acetyl- β -glucaminidase, neutral phosphatase, and leucine aminopeptidase activities. Note the early elution of *N*-acetyl- β -glucaminidase and acid phosphatase and the delayed elution of the leucine aminopeptidase.

occurs may determine the nature of the subsequent immune response to aeroallergens.^{29, 30} Inhaled pollen grains are deposited in the anterior and posterior nasopharynx. Within 6 to 8 minutes, the pollen is carried by mucociliary action to the hypopharynx and is swallowed within 20 minutes.³¹ The studies presented in this article clearly indicate that the nasopharynx is the site of maximum exposure to rapidly released solutes. The slowly released components could be released in the gastrointestinal tract where they would be absorbed intact or subjected to modification by digestive enzymes.³² Interestingly, entire pollen grains³³⁻³⁵ and other high-molecular-weight particles³⁶ have been isolated from the blood after intragastric administration. This "persorption" phenomenon suggests that in addition to sensitization of the nasal mucosa and tonsillar and gut-associated lymphoid tissues, the peripheral immune system may be exposed to small quantities of the late eluting components of swallowed pollen grains.

The observations presented in this article suggest that the delivery of pollen solutes to the human mucosal immune system is much more complex than previously appreciated. The methods described provide an approach toward quantitative understanding of both the sensitizing stimulus and the trigger that provokes nasal airway disease. Further studies of pollen biology will be required to fully understand the issues identified in this article. An opportunity exists

to improve our understanding of pollen allergens and the pathogenesis of allergic pollenosis.

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