

Quantification of pollen solute release using pollen grain column chromatography

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The impact of pollen on the respiratory mucosa was modeled by studying the process by which solutes are eluted from pollen grains. Rye grass (*Lolium perenne*), short ragweed (*Ambrosia artemisiifolia*), and white oak (*Quercus alba*) pollens were packed between glass wool plugs in small columns. Water was pumped through the columns and the eluate solute yield was determined by measurement of the dry solute weight. Solute separation was rapid, and concentrations and osmolalities of the eluate decreased exponentially. Theoretical initial solute concentrations were 179 g/l for rye grass, 55 g/l for short ragweed and 349 g/l for oak pollen eluates. Theoretical initial osmolalities of the same eluates were 321 mOsm/kg for rye grass, 196 mOsm/kg for ragweed and 424 mOsm/kg for oak pollen. Sequential separation of allergens (*Lol p I*, *Amb a I*, *Amb a V*), enzymes and proteins was demonstrated by specific assays. These observations suggest that the complex stimulus produced immediately after pollen grain hydration at the respiratory mucosal fluid interface is much more intense than previously envisioned. Sequential separation of pollen components has important implications for the production of improved allergenic extracts.

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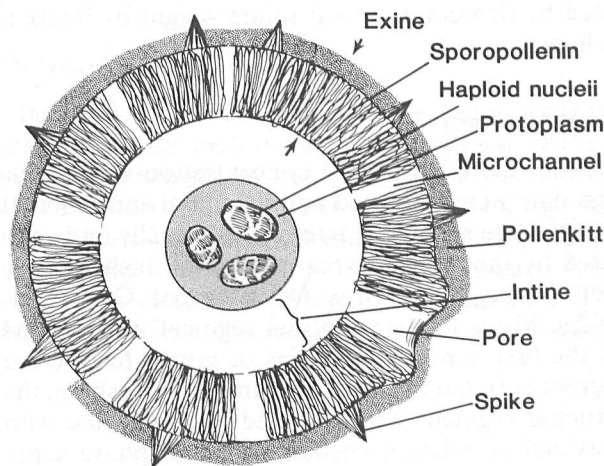
Key words: pollen; pollen solute separation; pollen grain chromatography; rye grass; ragweed; oak; *Lol p I*; *Amb a I*; *Amb a V*; allergenic extract.

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Although pollen exposure can provoke airway symptoms in susceptible allergic subjects (41), very little is known about the characteristics of the pollen stimulus that triggers rhinitis and bronchospasm. This report describes the physical attributes of the pollen solute release process (3-6) and the magnitude of this stimulus. By applying physical observations of the solute release phenomenon to the anatomy and biology of pollen grains (Fig. 1) important insights into the effects of pollen impaction on mucosal surfaces, the pathogenesis of pollenosis, and the contents of allergenic extracts can be inferred.

The pollens of *Lolium perenne* (rye grass, (1, 12)), *Ambrosia artemisiifolia* (short ragweed, (23, 30-32)) and *Quercus alba* (white oak, (11)) were studied since they are representative of the diversity of pollens implicated in allergic disease (29). Quantitative kinetic studies of pollen solute release were obtained by pollen grain column chromatography (3-6). The allergen content, enzyme activity, osmolality, solute dry weight and concentration of the fractions were determined. These observations are interpreted in terms of the potential physical interactions of the pollen grain with the respiratory mucosa and the intensity of the solute and allergen stimuli in pollenosis.



Prototypic Grass Pollen in Cross-section

Fig. 1. The structure of a grass pollen grain is shown schematically in cross section. Water soluble allergenic substances are readily eluted from the exine and intine (see text).

Methods

Pollen chromatography

Ether-defatted and air-dried *Lolium perenne* (perennial rye grass, lot 51X24-6D), *Ambrosia artemisiifolia* (short ragweed, lot 18S56-9D) and *Quercus alba* (white oak, lot 8-122-71) pollen were obtained from

Abbreviations: *Lol p I*, rye grass group 1 allergen; *Amb a I*, ragweed antigen E; *Amb a V*, ragweed allergen Ra5

Greer Laboratories (Lenoir, NC). Pollen columns were made by packing 0.7 to 9.9 g of pollen between glass fiber plugs (Corning, Elmyra, New York) in 9 mm inner diameter Pharmacia K columns (Pharmacia, Uppsala, Sweden). Deionized water was pumped with a variable speed 2120 Varioperpex II peristaltic pump (LKB, Bromma, Sweden), and fractions collected in a 2112 Redirac Fraction Collector (LKB, Bromma, Sweden). The rate of separation of pollen solutes was measured as a function of solvent flux in milliliters of solvent/min/100 mg of pollen (4). Flow rate was determined by measuring the change with time of solvent volume in a graduated reservoir. Fractions were collected at 1- to 2-min intervals for the first 30 min, 10- to 30-min intervals to 120 min, and then at 30- to 90 min intervals for up to 1440 min.

Solute concentrations

A measured volume of each eluate fraction was placed in a weighed porcelain crucible and dried to minimum weight in a 107°C forced air drying oven. Weights were measured on a Mettler H20T analytical balance (Scientific Products, Evanston, IL). Sample weights had a maximum error of $\pm 1\%$. The minimum weight was taken as the soluble weight and used to calculate the total solute in each fraction. The concentration of the fraction was calculated by dividing the total solute weight by fraction volume.

Kinetic analysis

Plots of the logarithm of concentration versus elution time were inspected and the initial and terminal straight line segments determined visually and analyzed by the least squares method of linear regression (Lotus 123, Lotus Development Co., Cambridge MA). The steep initial segment was defined as the first consecutive series of points to give the highest correlation coefficient. In similar fashion, the terminal segment was analyzed to give a line with maximal correlation coefficient. For 3-phase separation, the remaining middle segment of points was also analyzed by least squares.

The y-intercept at time 0 was taken as the theoretical initial solute concentration (13, 28) and represented the local pollen solute concentration at the moment of pollen hydration. In 3 identical experiments the precision of this theoretical value was $\pm 11\%$, while the precision was $\pm 13\%$ in 7 experiments performed over a wide range of solvent fluxes.

Osmolality

The osmolality (mOsm/kg water) of duplicate samples from column fractions were measured by freez-

ing point depression using a Model 3MO Advanced MicroOsmometer (Advanced Instruments Inc., Needham Heights, MA). Graphs of the logarithm of the osmolality of fractions were plotted against elution time and the initial theoretical osmolality determined by extrapolation.

Allergen analysis

The allergen concentrations were analyzed by ELISA inhibition. Immulon II (Dynatech Inc., Alexandria, VA) microtiter plate wells were coated with 100 μ l of reference rye grass or ragweed extracts (1 μ g/ml in pH 9.6 bicarbonate buffer; Greer Laboratories, Lenoir, NC) and incubated at 4°C overnight. Two hundred μ l of a standard solution of monospecific polyclonal rabbit anti-rye (*Lol p I*), anti-ragweed antigen E (*Amb a I*), or anti-ragweed Ra5 (*Amb a V*) sera (Greer Laboratories, Lenoir, NC) and varying amounts of column eluates were diluted in pH 7.4 phosphate buffered saline containing 0.0005% Tween-20 (PBS-Tween) and 1.0 M NaCl. The eluate fractions and diluted antisera were added to duplicate wells and incubated for 4 h at room temperature. After washing with the PBS-Tween, alkaline phosphatase-labeled goat anti-rabbit IgG (Sigma, St. Louis, MO) was added. The plates were incubated 4 h at room temperature and washed with PBS-Tween. Enzyme substrate (50 μ l of 1 mg/ml p-nitrophenyl phosphate in pH 9.8 diethanolamine buffer) was added and the absorbance measured 30 min later at 405 nm with a Biotek automated ELISA reader (Biotek, Burlington, VT). The inhibition produced by different dilutions of column eluate fractions was compared by linear regression analysis and used to calculate the relative antigen content of the fractions. The relative antigen content of the ragweed pollen eluate was converted to the antigen concentration using prior measurements of the antigen concentration of the reference extract.

Enzyme analysis

The activity of eluate enzymes was screened using API-ZYM strips (21). Eluate fractions were diluted to 2 ml, 2 drops added to each of 20 microcupules, and incubated 4 to 6 h at 37°C. The production of enzymatic end products was detected by color-developing reagents. Color intensity was used as a semi-quantitative measure of enzyme activity.

Results

Kinetic analysis

Rye grass pollen solute release was rapid. Pollen solute concentrations during the first 2 min ranged from 90 to 250 g/l regardless of the flux of the aque-

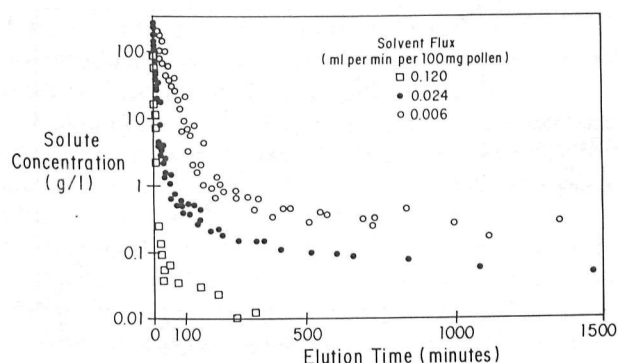


Fig. 2. Rye grass pollen. The concentrations of rye grass pollen solute are shown for 3 solvent fluxes. Although the rate of decrease in concentration is different for each solvent flux, the theoretical initial solute concentrations at zero time were similar. The elution process exhibits 2 phases for rye grass pollen.

ous solvent (Fig. 2). The mean theoretical initial solute concentration ($\pm$ SEM) for 7 experiments and 3 solvent fluxes was 179 ± 20 g/l (18% w/v). This value represents a theoretical maximum, as the eluate solute concentration decreased rapidly as flow of the solvent made more fluid available to the pollen grains. The concentration decreased exponentially to the range of 1 g/l, after which the rate of change slowed, and concentrations reached the 0.1 to 0.01 g/l range. When plotted as the logarithm of concentration versus time, the solute concentrations decreased 100-fold in linear fashion. The rate of concentration change was dependent upon the solvent flux (Fig. 2).

Solvent flux also influenced the rate of solute separation as measured by cumulative mass of eluted solutes (Fig. 3). As expected, rapid solvent flux facilitated pollen solute separation, while slow flux delayed separation and shifted the cumulative elution curve to the right. The mean ($\pm$ SEM) cumu-

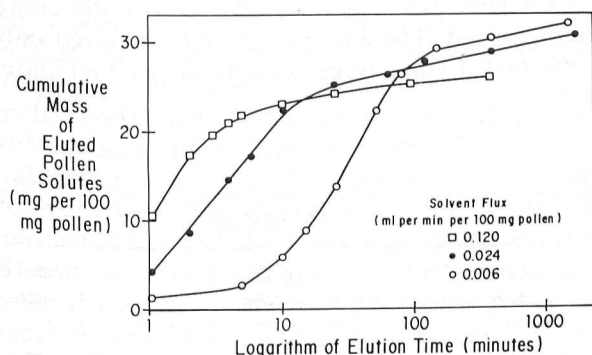


Fig. 3. Rye grass pollen. Using 3 solvent fluxes, the cumulative yield of rye grass pollen solute differed during the initial period of elution, but became comparable after prolonged elution. Although low solvent flux caused a slower separation of solutes and lower initial yield, the ultimate yield with prolonged elution appeared slightly greater.

lative solute mass at 1000 min was 31.1 ± 1.5 mg/100 mg rye grass pollen. The curvilinear plots of the cumulative mass of solute eluted against time demonstrated prominent inflections near points that corresponded to the times required for solute concentration to decrease 100-fold to 1 g/l. The mean cumulative mass of solutes separated in this early phase of rapid change in solute concentration was 27.4 ± 1.7 mg/100 mg pollen. This represented 88% of the total extractable solutes. This suggests that the bulk of the solutes eluted from rye grass pollen were rapidly released constituents of the pollen grain.

Oak pollen solute separation at a solvent flux of 0.01 ml/min/100 mg pollen was similar (Fig. 4). The initial theoretical solute concentration was 349 g/l. First order solute separation continued at a steady pace for approximately 20 min, then decreased to a slower rate of separation from 40 to 900 min. During the initial rapid separation phase, 20.2 mg/100 mg pollen (67% of the total extractable solute mass) were released. Ragweed solute separation was more complex, as 3 phases of separation were noted (Fig. 5). The theoretical initial solute concentration was 55 g/l. In the initial phase of separation, 23.5 ± 1.2 mg per 100 mg pollen (74% of the total extractable solute mass) were eluted. In the subsequent 2 phases, 4.1 ± 0.4 and 4.2 ± 0.8 mg/100 mg pollen were released.

Osmolarity

Column fraction osmolalities decreased in logarithmic fashion for each pollen (Fig. 6). The theoretical initial osmolalities for oak (424 mOsm/kg) and rye (321 mOsm/kg) were greater than those for ragweed (196 mOsm/kg). For ragweed, there was no difference in the osmolality of fractions obtained from dried, and dried and defatted pollen samples. The main mass of the osmotically active solutes extracted

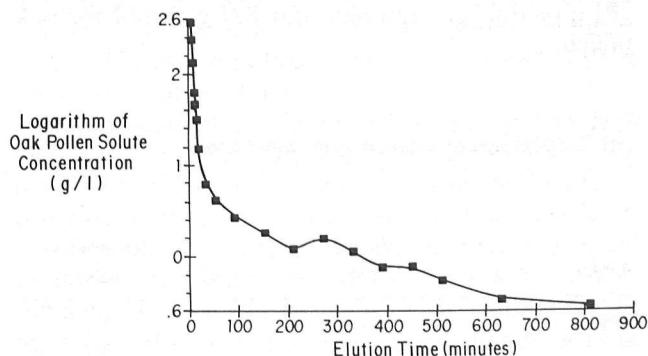


Fig. 4. White oak pollen. The eluate concentrations from white oak pollen decreased rapidly in the first 20 min at a solvent flux of 0.01 ml/min/100 mg pollen. After 20 min the eluate concentrations decreased at a slower rate. The extraction process exhibited 2 phases.

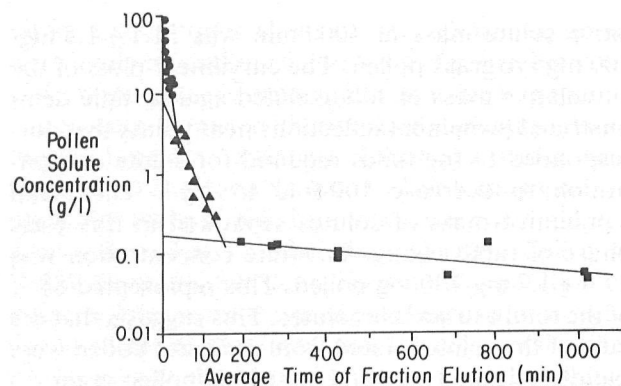


Fig. 5. Ragweed pollen. Using a solvent flux of 0.15 ml/min/100 mg pollen, ragweed pollen solute separation exhibited 3 phases (7 experiments) and differed from the elution patterns of rye grass and white oak pollens. The 3 symbols depict the 3 phases of the elution process.

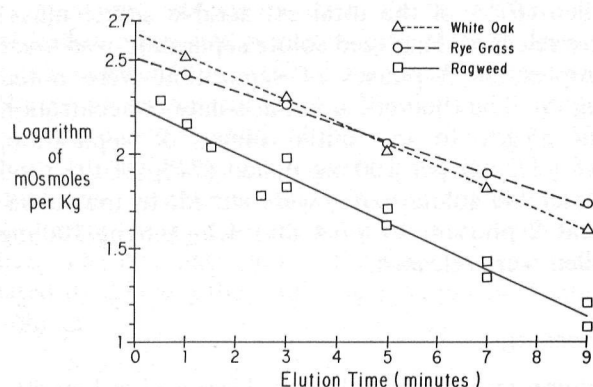


Fig. 6. Osmolarity. During the initial rapid phase of the elution process, the osmolality of the eluate fractions decreased in log-linear fashion for all 3 pollens. Note the differences in the initial theoretical osmolalities of the 3 pollens at zero time.

in water were determined by dividing the theoretical initial concentrations by the theoretical initial osmolalities. The values were 558 g/osmol for rye grass, 281 g/osmol for ragweed, and 825 g/osmol for oak pollen.

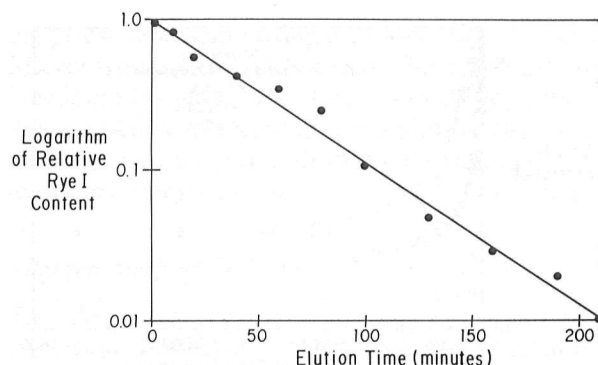


Fig. 7. *Lol p I* Rye grass pollen antigen. The relative concentration of *Lol p I* (rye group 1) allergen in eluate fractions decreased in log-linear manner and paralleled the solute concentration of the elution curve.

Allergen analysis

Lol p I (rye group 1) allergen concentration levels were highest in the initial column fractions, and decreased exponentially (Fig. 7). The rate of decrease in allergen concentration paralleled the solute concentration elution curve.

In contrast, ragweed *Amb a V* (Ra5) was released more rapidly than *Amb a I* (antigen E) from columns with a flux of 0.02 ml/min/100 mg pollen (Table 1). The *Amb a V* concentration in column fractions collected between 0 and 20 min was 71 µg/ml and the yield was highest in the 20 to 240 min fractions. In contrast, *Amb a I* was delayed in its separation, with 74% of total extractable *Amb a I* being released between 240 and 1440 min.

Enzyme analysis

Ragweed enzyme profiles show that acid phosphatase, alkaline phosphatase, phosphohydrolase, and C8 lipid esterase were released for the entire elution period. The enzyme activities detected only in the first 10 min were N-acetyl-β-glucaminidase,

Table 1. Yield of ragweed allergens in column eluate fractions

Antigen	Fraction min	Concentration µg/ml	Estimated yield		
			µg/mg solute	µg/100 mg pollen	Percent of total
<i>Amb a V</i>	0-20	71.0	3.0	14.0	22
	21-240	3.0	7.0	39.0	64
	241-1440	1.0	6.0	8.0	14
<i>Amb a I</i>	0-20	1.0	0.4	0.3	6
	21-240	0.2	0.9	1.1	20
	240-1440	0.01	5.8	4.0	74

β -galactosidase, and α -mannosidase. Leucine aminopeptidase was not detected in the first 10 min, but reached high levels after 20 min of elution.

Using rye grass pollen, only low activities of several enzymes were found. A C4 esterase activity was eluted between 0 and 20 min only. Alkaline phosphatase and acid phosphatase activities were not present in the first 5 min, but were present at 20 min. A low level of C8 esterase activity was eluted continuously.

White oak pollen acid phosphatase and phosphohydrolase were eluted in high activity and in all fractions, while low activities of alkaline phosphatase, and lipid esterases of 4, 8 and 14 carbon lipids were not released until after 20 min of solvent flow.

Discussion

The anatomy and biology of pollen grains (Fig. 1) provide important insights into the effects of pollen impaction on mucosal surfaces, the pathogenesis of pollenosis, and the contents of allergenic extracts. Pollens are the male gametophytes of flowering plants, and are complex life forms (17, 24, 25, 39). Important windborne allergenic pollens are generated in enormous numbers by inconspicuous flowers of trees, grasses and weeds. In their random flights to female flowers, pollen grains inadvertently contact the human respiratory mucosa. Pollen grains contain cytoplasm, 2 or 3 haploid nuclei and all plant organelles including proplastid precursors of chloroplasts (Fig. 1). The cytoplasm is surrounded by a series of protective concentric spheres. The innermost sphere is a protein-laden pectino-cellulose layer called the intine. The intine is enclosed by a highly structured shell of sporopollenin, a complex biopolymer of carotenoids or related compounds that resist biodegradation. The sporopollenin structures are preserved as fossils and provide the earliest evidence for the presence of flowering plants on this planet (39). Numerous proteins, lipids, flavonoids and other pigments, and non-protein plant growth regulators are packed in the arcades formed by the sporopollenin latticework. The sporopollenin with its enclosed proteins and other biochemicals make up the exine layer of the pollen. The intine and exine are so rich in enzymes that they have been considered extracellular lysosomes. These enzymes (3) and other factors have important functions in pollen defense (e.g. fungicidal RNAases), pollen germination and fertilization. The surfaces of anemophilous flowering plants' pollen are impregnated with pollenkitt, a complex sticky lipid mixture that provides an oil-like barrier which protects the pollen grain from ambient moisture and ultraviolet radiation.

Conventional pollen extracts (35, 40) are manu-

factured by isolating the supernatant obtained by suspending defatted pollen grains in buffer overnight. Pollen extracts are complex mixtures composed of pollen wall constituents including allergens (4), enzymes (8, 20), complex carbohydrates, polyphenolic flavonoids (16, 37, 39), and poorly characterized male flower recognition molecules (20, 24, 39). Although the antigenic properties of allergenic extract components have been characterized, the natural functions of these interesting molecules remain unknown. Evidence suggests that the pollen solutes that trigger respiratory mucosal reactions *in vivo* may differ from the contents of conventional allergenic extracts (22, 33). Sequential elution of pollen wall components and degradation by intrinsic enzymes may contribute to this phenomenon (8, 27). These principles may also apply to fungal spore extracts (7, 36, 38).

The novel method of pollen grain column chromatography permits quantitative study of the elution of pollen solutes. The magnitude and sequence of solute release from the dried and defatted rye grass, white oak, and short ragweed pollens (4) and fresh *Abutilon* pollen (3) has been characterized. Solute separation from these phylogenetically diverse and structurally different pollens (29) was surprisingly similar, suggesting that rapid, sequential release of pollen factors with high initial local solute concentrations and osmolalities may be characteristic features of pollen hydration in pollen columns, on mucosal surfaces, and in the natural environment.

In addition to the implications for more efficient preparation of better pollen extracts, the information in this report provides an awareness of the allergen load that could be imposed on the respiratory mucosa during seasonal acute and chronic pollen exposure. Acute pollenosis occurs rapidly after inhalation of pollen (9, 34) indicating that the pollen solutes released most rapidly and in the highest concentrations must be responsible for triggering immediate hypersensitivity responses. The solutes of rye, oak and ragweed pollens that elute rapidly constitute 20 to 27% of the pollen mass, or 67 to 88% of the total extractable solutes.

Rapid sequential release of ragweed pollen proteins has been demonstrated by isoelectric focussing (4). The rapidly released phase contained 7 cationic proteins with pI's of 9.3 to 10.7 and several faint-staining neutral proteins. In the second phase, the predominant bands were at 10.7, 9.0, 8.0 and 7.4, but neutral and acidic bands were also numerous. In the terminal phase, the predominant bands were at pH 6.0 and below pH 5.5 with no cationic material apparent. In contrast, rye grass proteins showed less variation by isoelectric focusing (4).

The rate of solute separation in pollen grain column chromatography is dependent on solvent flux.

Sequential separation of the eluted pollen solutes was maximized by using a slow solvent flux which limited the amount of fluid available for pollen solute dissolution. Pollen solute separation followed the kinetics of a first order process, showing 2 or more linear phases on a graph of the logarithm of concentration versus elution time (13, 28). First order kinetics are expected for dissolution processes. The uniformity of the several phases of solute separation suggests that pollen solutes are held respectively in the exine and intine matrices of the pollen grains by similar forces and that the dissolution of solutes from these anatomical locations were physically homogeneous processes.

Compartmentalization of pollen solutes has been suggested by Knox and Heslop Harrison (18, 26), with rapid release of exine protein from the entire surface area of the pollen grain, but delayed release of intine proteins via the pollen microchannels (Fig. 1). The sequential elution of allergens and enzyme activities, and changes in isoelectric focusing patterns seen with pollen grain column chromatography (3, 4) are consistent with the existence of hypothetical solute compartments within the pollen grain, and an ordered, time-dependent sequence of pollen solute release.

Although pollen grains are tiny and can deliver only a small mass of rapidly released solutes, the theoretical initial solute concentrations and osmolalities at the moment of pollen-mucosal contact are high. If the osmolality of mucosal fluid is assumed to be similar to serum, then the added osmolality of pollen solutes in mucosal fluid at the moment of pollen grain hydration would approach the hypertonic osmolalities that have been shown to induce epithelial damage, basophil mediator release, and bronchospasm (2, 10, 15). These physical factors of pollen grain solute release may damage the mucosal surface and act as additive or irritant triggers in concert with IgE-mast cell mechanisms to induce respiratory tract symptoms in atopy (14, 19).

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References

- BALDO BA, SUTTON R, WRIGLEY CW. Grass allergens with particular reference to cereals. *Prog Allergy* 1982; 30: 1-66.
- BANKS JR, KAGEY-SOBOTKA A, LICHTENSTEIN LM, EGGLESTON PA. Spontaneous histamine release after exposure to hyperosmolar solutions. *J Allergy Clin Immunol* 1986; 78: 1-7.
- BARANIUK JN, BOLICK M, BUCKLEY III CE. Pollen grain chromatography: a novel method for separation of pollen wall solutes. *Ann Botany* 1990; 66: 321-9.
- BARANIUK JN, ESCH RE, BUCKLEY III CE. Pollen grain chromatography: quantitation and biochemical analysis of ragweed pollen solutes. *J Allergy Clin Immunol* 1988; 81: 1126-34.
- BARANIUK JN, ESCH R, BUCKLEY III CE. Effect of extraction technique and incubation on the size of ragweed pollen solutes. *J Allergy Clin Immunol* 1987; 79: 213A.
- BARANIUK JN, ESCH R, BUCKLEY III CE. How does pollen deliver an allergenic stimulus? *Am Rev Respir Dis* 1987; 135: 220A.
- BARNES C, PACHECO F, PORTNOY J. Time of elution-dependent electrophoretic patterns of *Alternaria* (ALT) glycoproteins. *J Allergy Clin Immunol* 1991; 87: 185A.
- CHAO NT, BUCKLEY III CE. Inhibitors of grass pollen neutral aminopeptidases. *J Allergy Clin Immunol* 1991; 87: 186.
- CRETICOS PS, PETERS SP, ADKINSON NF, LICHTENSTEIN LM. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984; 310: 1626-30.
- EGGLESTON PA, KAGEY-SOBOTKA A, SCHLEIMER RP, LICHTENSTEIN LM. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 1984; 130: 86-91.
- FAIRLY D, BATCHELDER GL. A study of oak-pollen production and phenology in northern California: Prediction of annual variation in pollen counts based on geographic and meteorologic factors. *J Allergy Clin Immunol* 1986; 78: 300-7.
- FREIDHOFF LR, EHRLICH-KAUTZKY E, GRANT JH, MEYERS DA, MARSH DG. A study of the human response to *Lolium perenne* (Rye) pollen and its components, Lol pI and Lol pII (Rye I and Rye II). *J Allergy Clin Immunol* 1986; 78: 1190-201.
- GLADTKE E, VON HATTINGBERG HM, eds. *Pharmacokinetics*. Berlin: Springer-Verlag, 1979: 11-21.
- GODFREY S. Nonimmunologic triggers in asthma. In: REED CE, ed. *Proceedings of XII International Congress of Allergy and Clinical Immunology*. St. Louis, MO: CV Mosby, 1986: 220-4.
- HAHN A, ANDERSON SD, MORTON AR, BLACK JL, FITCH KD. Reinterpretation of the effect of temperature and water content on the inspired air in exercise-induced asthma. *Am Rev Respir Dis* 1984; 130: 575-9.
- HARBORNE JB. Flavonoid profiles in compositae. In: HEYWOOD VH, HARBORNE JB, TURNER BL, eds. *The biology and chemistry of the compositae*. London: Academic, 1978: 359-84.
- HESLOP-HARRISON J. The physiology of the pollen grain surface. *Proc R Soc Lond (Biol)* 1975; 190: 275-99.
- HESLOP-HARRISON J, HESLOP-HARRISON Y. Surfaces and secretions in the pollen stigma interaction: a brief review. *J Cell Sci Suppl* 1985; 2: 287-300.
- HOLGATE ST. Contribution of inflammatory mediators to the immediate asthmatic reaction. *Am Rev Respir Dis* 1987; 135: S57-S62.
- HOWLETT BJ, VITHANAGE HIMV, KNOX RB. Pollen antigens, allergens and enzymes. In: SMITH H, ed. *Commentaries in plant science*, Vol. 2 Oxford: Pergamon Press, 1981: 191-207.
- HUMBLE MW, KING A, PHILLIPS I. API ZYM: A simple rapid system for the detection of bacterial enzymes. *J Clin Pathol* 1977; 30: 275-7.
- HUSSAIN R, NORMAN PS, MARSH DG. Rapidly released allergens from short ragweed. II: Identification and partial purification. *J Allergy Clin Immunol* 1981; 67: 217-22.
- KING TP. Immunological studies of ragweed pollen antigens. *Allergy* 1980; 35: 187-211.
- KNOX RB. Pollen and allergy. *Studies Biol* 1979; 107: 1-60.
- KNOX RB. The pollen grain. In: JOHRI BM, ed. *Embryology of angiosperms*. Berlin: Springer-Verlag, 1984: 197-271.

26. KNOX RB, HESLOP-HARRISON J, HESLOP-HARRISON Y. Pollen wall proteins: localization and characterization of gametophytic and sporophytic fractions. In: DUCKETT JG, RACEY PA, eds. *The biology of the male gamete*. Biol J Linn Soc Suppl 1975; 1: 177-87.
27. LARSEN LV, COPELAND TA, WEBER RW, VAUGHN TR. Allergen extraction methods: effects of time, temperature, buffer and protease inhibitors. *J Allergy Clin Immunol* 1991; 87: 186A.
28. LEHNINGER AL, ed. *Biochemistry*. New York: Worth, 1970: 150-1.
29. LEWIS HL, VINAY P, ZENGER VE. *Airborne and allergenic pollen of North America*. Baltimore: Johns Hopkins Press, 1983.
30. LOWENSTEIN H, MARSH DG. Antigens of *Ambrosia elatior* (short ragweed) pollen. I. Crossed immunoelectrophoretic analyses. *J Immunol* 1981; 126: 943-8.
31. LOWENSTEIN H, KIM TP, GOODFRIEND L, HUSSAIN R, ROEBBER M, MARSH DG. Antigens of *Ambrosia elatior* (short ragweed) pollen. II. Immunochemical identification of known allergens by quantitative immunoelectrophoresis. *J Immunol* 1981; 127: 637-42.
32. LOWENSTEIN H, MARSH DG. Antigens of *Ambrosia elatior* (short ragweed) pollen. III. Crossed radioimmunoelectrophoresis of ragweed-allergic patients' sera with special attention to quantification of IgE responses. *J Immunol* 1983; 130: 727-31.
33. MARSH DG, BELIN L, BRUCE CA, LICHTENSTEIN LM, HUSSAIN R. Rapidly released allergens from short ragweed pollen. I: Kinetics of release of known allergens in relation to biologic activity. *J Allergy Clin Immunol* 1981; 67: 206-16.
34. NACLERIO RM, CRETICOS PS, NORMAN PS, LICHTENSTEIN LM. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1986; 134: 1102A.
35. NEWELL JA. A review of the clinical properties of the allergens in pollen. *J Allergy* 1942; 13: 177-203.
36. PACHECO F, PORTNOY J, BARNES C. Enzymatic activities in *Alternaria* (ALT) preparations extracted for different time intervals. *J Allergy Clin Immunol* 1991; 87: 180A.
37. PICMAN AK. Biological activities of sesquiterpene lactones. *Biochem Systemat Ecol* 1986; 14: 255-81.
38. PORTNOY J, PACHECO F, BARNES C. Allergen activity of *Alternaria* (ALT) preparations extracted for different time intervals. *J Allergy Clin Immunol* 1991; 87: 181A.
39. STANLEY RG, LINSKENS HF. *Pollen biology biochemistry management*. Berlin: Springer-Verlag, 1974.
40. STULL A, COOKE RA. Experimental and clinical study of fresh and modified pollen extracts. *J Allergy* 1940; 11: 439-64.
41. WEEKE B, WEEKE E. Pollen allergy: environmental aspects. In: REED E, ed. *Proceedings of XII International Congress Allergology and Clinical Immunology*. St. Louis, MO; CV Mosby, 1986: 120-3.

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