

Uncovering the "fine details" of pollen allergen transport

Aerosols, including familiar airborne allergens, comprise solids (dusts) and liquids (mists) small enough to be passively suspended in air at least temporarily. A broad range of aerosol dimensions is recognized with many types, sized well below 1 μm , serving as nuclei for moisture condensation and often affecting outdoor visibility. As increasingly smaller units are considered, the distinction between single molecules or molecular aggregates and the most minute aerosols becomes difficult. Similarly, since solid particulates may have substantial associated moisture, the physical characteristics of smaller dust and mist units often overlap.

Pollen grains, with diameters typically from 12 to 60 μm , have been cited repeatedly as examples of the largest aerosols enjoying extensive atmospheric transport. Their relatively generous dimensions ensure that pollens will have limited trajectories,¹ predominant upper airway deposition,² and efficient capture by rapidly moving, narrow collection surfaces (e.g., rotorods).¹ These characteristics differ sharply from those of the most prevalent aerosol fractions in urban air that are sized below 1 μm .³ Particles, of *defined morphology*, in this diminutive range are essentially unknown. However, there are indications that certain effectively "amorphous" biogenic agents may exert important health effects, at least in part, as submicronic or paucimicronic (one to a few micrometer) units. Simple chemicals (e.g., phthalic anhydride and chloroplatinates) are readily divisible and would appear to deserve the highest interest in this respect; however, minute fragments of seed pomaces, arthropod emanations, and the dried residues of animal secretion and urine may also carry important activity. Whatever their nature, suspended pauci- and submicronic units are expected to follow entraining air cur-

rents faithfully, achieve substantial lower airway penetration, and be most amenable to collection by suction samplers (e.g., wind-oriented filters).^{1, 2}

Assayable particulate allergens, of cat⁴ and guinea pig⁵ origin, sized well below 5 μm , have been demonstrated recently in naturally occurring indoor aerosols. In addition, fractions with typical diameters of 5 to 10 μm (and probably smaller) have been demonstrated to carry rat urinary allergens.⁶ Mite allergens, specifically PI, have been associated in part with the same (i.e., <10 μm) aerosols, despite their presumed origin in fecal pellets with aerodynamic diameters above 10 μm .⁶ These studies have required collection modalities offering not only high efficiency for small particles but also recoveries in homogeneous size fractions with limited overlap. A variety of cascade impactor devices have served these purposes well in adaptations processing small⁷ or quite large⁸ volumes of air, although size separations have not been absolute. In addition, where suction devices are exposed upright (i.e., at a right angle to ambient flow), predictable particle losses occur, these deficits rising with wind speed and effective aerosol size.¹

As animal allergens often appear to originate in residues of urine^{5, 9, 10} and external secretion¹¹ that easily fragment, their dispersion as minute aerosols is to be expected. Similarly, the comminution of fecal pellets and other divisible aggregates to form ultrafine particles may be explained readily. However, the operation of these same processes would not predict that pollen allergens should be associated with pauci- and submicronic aerosols. Since just this finding has been confirmed, the processes potentially responsible have been sought with increasing interest.

The occurrence of ragweed pollen allergens in aerosol fractions smaller than intact ragweed grains was reported by Busse et al.¹² in 1972. Their assay methods (including P-K neutralization) left little doubt that pollen allergens were present in the particle deposits they secured on Andersen sampler plates (Andersen 2000, Inc., Atlanta, Ga.). Furthermore, direct examination of impingement points was

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attempted to exclude the presence of intact pollen grains from collections ostensibly limited to pauci- or submicronic units. Unfortunately, particle overloading was substantial in the long exposure used by these investigators. This effect made microscopy difficult and increased the risk of "slippage" (viz., that intact grains might pass between sampler stages), allowing allergen elution at interim stops on moist particle clumps. In theory, such migratory grains ultimately might be lost despite activity remaining on residual deposits. However, efforts to invoke this scenario alone in explaining the findings of Busse et al.¹² recently have become untenable. Studies by our own group have used the passage of air through molecular membrane filters to achieve an "absolute" removal of intact pollen grains.^{13, 14} Although free of visible pollen and pollen exine fragments, aerosol passing filters of 5 μm pore size has demonstrated both crude ragweed and antigen E activity when the material was assessed by direct skin testing and an IgG-dependent, ELISA-inhibition assay. To our surprise, material passing a 0.8 μm filter and obtained by "freeze-out" of entrained moisture has demonstrated the same allergen specificities by in vivo and in vitro test criteria. Rigorous efforts to document, by direct microscopy, the exclusion of stainable exine from these stained and cleared, pauci- and submicronic deposits have characterized our studies. Care has been taken to maintain a closed system and *unidirectional* flow of all air and liquids through collection filters during air sampling, extraction, and staining. This precaution should virtually exclude the risk that pollen grains, originally contaminating microaerosol deposits, might go unnoticed (by microscopy) because of being washed from filters and later sequestered in other system components.

On a day-to-day basis, levels of ragweed grains and of ragweed allergen activity passing 5 and 0.8 μm pore size filters have demonstrated a positive relationship but also some notable discrepancies. These findings suggest that different sets of prevalence determinants may affect the several allergen-bearing aerosol fractions. However, the effects even of fundamental variables such as date, diurnal period, humidity, rainfall, and air speed on allergen in minute microaerosols are unknown.

Both our collections and those of Busse et al. have been confined, essentially, to seasons of ragweed pollination in the Midwest. However, workers from the Mayo Clinic have described assayable airborne ragweed allergen in their locality substantially before as well as after local periods of pollen shedding by *Ambrosia* species¹⁵; the responsible aerosol fractions

remain to be determined. It is unclear also whether allergens of other pollen (and large spore) types are dispersed naturally in a variety of aerodynamic forms.

Limitations in descriptive data (previously cited) have scarcely discouraged speculations concerning the origin of ragweed allergens in aerosol fractions smaller than intact pollen grains; candidate theories generally center on (1) pollen or (2) nonpollen sources. Pollen is deposited extensively on moist surfaces in nature, providing widespread opportunities for elution of both "rapidly"¹⁶ and more slowly released allergens. Since minute suspended particles and large molecules tend to accumulate near air-water interfaces, they may become airborne when such films are violently disturbed.¹⁷ In fact, interface disruption by rising air bubbles, impacting droplets, or substrate motion may produce "bursts" of suspension- or solute-rich jet droplets.^{1, 17, 18} These processes could and, to an extent, probably do introduce some ragweed allergens into moving air during periods of peak pollen dispersion. However, these transfer mechanisms would not be expected to operate during periods before local ragweed populations had flowered.

Nonpollen sources also might contribute airborne ragweed allergens by primary flotation of minute plant debris or (as suggested above) by natural elution and (secondary) jet droplet dispersion. Since pollen develops in and, presumably, exchanges soluble components with a moist tissue milieu, the tapetum, male flowering parts with tapetum-lined anther sacs, offer a probably source of pollen allergens throughout the reproductive sequence. Indeed, anther tissue has demonstrated strong allergenic activity in direct tests.¹⁹ Besides the possible dispersion of tapetal cell or fluid components, *Übisch* bodies have been invoked, by at least two authors,^{20, 21} as potential vectors of allergen transport. These structures are 1 to 2 μm spherules of exine substance that are formed during pollen ontogeny in many plant species; however, they appear not to occur as such in ragweeds.

Controversy has surrounded the proposition that somatic parts of ragweeds also might be sources of pollen-associated allergens such as antigen E. After earlier negative reports, pollen-like activity was reported in the stems and leaves of both short (*A. artemisiaefolia*)²² and giant (*A. trifida*)²³ ragweeds. However, concerns that these results might reflect contamination of vegetative organs by environmental pollen were not easily dispelled. Only when allergenic fractions were demonstrated to appear in tissue cultures of ragweed leaf cells²⁴ did the allergen complement of somatic structures attract unequivocal

attention. In this issue of the JOURNAL, Agarwal et al. (p. 687) offer further evidence of allergenic similarities among extracts of ragweed pollen and of various *Ambrosia* somatic structures. Furthermore, small amounts of both crude ragweed and antigen E were present in seedlings gathered at a time (May 30) before differentiation of flowering structures should have begun. As expected, anther-bearing parts demonstrated maximum activity, substantial allergen remaining well after the plants had been frost-killed in the field; however, even roots demonstrated pollen-like components. Since ragweeds on different sites may demonstrate differences in the allergenic activity of pollen,²⁵ the authors' decision to sequentially sample plants from a single location was especially commendable.

Agarwal et al. also used an Andersen particle-sizing head⁸ and immunochemical assay to confirm the presence of ragweed allergen in paucimicronic aerosol fractions. However, mechanisms that might promote formation of small, allergen-bearing particles from normally hydrated, living plant parts remain to be defined. Ragweeds do tend to be covered by innumerable hairs (collectively termed "indument") that might fragment, yielding dispersible allergen vectors, although no positive evidence exists for such a role. Similarly, there is no reason to believe that the direct release into air of pheromones by insects and mammals or of terpenes by conifers offers an appropriate model for dispersion of molecular allergens. Unlike familiar pollen macromolecules, the former materials, on analyses, usually have proven to be relatively small, highly volatile, substituted hydrocarbons.

Conceptually, the carriage of pollen allergens by aerosols smaller than intact grains offers intriguing pathogenetic implications that transcend our manifest ignorance of the process in its most basic aspects. Opportunities for research in this field are varied and promising. However, a goal of first priority would appear to be the estimation of *relative* allergen loads borne by large and small aerosol categories and associated with defined regions, seasons, and diurnal periods. Effects of specific atmospheric variables and weather patterns on the transport of aerosol subsets comprise a second area of fundamental importance in developing clinical correlations. Later goals may do well to focus on natural determinants of small particle generation as well as the effects of other atmospheric components on the physical and immunoreactive properties of small aerosols.

Since few pollen-sized particles negotiate subcarinal airways, allergenic microaerosols may figure prominently in causing seasonal asthma among pol-

len-sensitive subjects. However, clinical interest in these agents need not be confined to their imputed role in bronchial challenge. Depending on factors of size, static charge, and moisture content, pauci- and submicronic aerosols could present highly "bioavailable" allergens to a variety of body surfaces. The ultimate relationship of such deposition to concerns such as respiratory "priming," late reactions, and effective allergen avoidance cannot be foreseen. However, it seems clear that small particles will continue to intrigue us and that, as a result, our concepts of pollinosis will never again be the same.

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