

Inhalation challenge with ragweed pollen in ragweed-sensitive asthmatics

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We reexamined the ability of inhaled ragweed pollen to induce bronchoconstriction in ragweed-sensitive asthmatic patients using a turbo-inhaler to administer pollen quantitatively. Adult subjects were selected for study on the basis of fall season asthmatic attacks, positive skin test, histamine release, RAST, and bronchial challenge responses to ragweed extract. Not one of 12 such subjects had any bronchial response to oral inhalation of whole pollen grains even when the dose was increased to 7640 pollen grains (more than the estimated maximum daily exposure in season), whereas nasal challenge by the same method produced brisk hay fever responses without bronchospasm. On the other hand, when the pollen was ground to fragments with a size range of 1 to 8 μm , oral inhalation produced a 35% fall in airways conductance in six of seven subjects in doses ranging from 59 to 20,000 pollen grain equivalents. Atropine pretreatment did not modify the response to pollen fragments, making an irritant response unlikely. These data, coupled with earlier observations that no more than a few pollen grains penetrate further than the larynx, raise further questions about the role of whole ragweed pollen in fall asthma in allergic patients. In addition, ragweed-allergic asthmatics appear not to have their symptoms at the time of maximum pollen load in the air. We believe that small-particle allergens other than ragweed pollen should be considered in most cases of fall seasonal asthma. (J ALLERGY CLIN IMMUNOL 71:302, 1983.)

The clinical impression that plant pollens are often a cause of seasonal asthma has been generally accepted for many years. Several studies of immunotherapy with extracts of grass pollens and ragweed pollen indicate that asthmatic symptoms are relieved, perhaps more readily than hay fever symptoms.¹⁻³ At the same time it has long been known that particles the size of common airborne pollens (ca. 20 μm) are not only effectively filtered out by the nose but even during mouth breathing are incapable of passing the larynx in any number.⁴ It has been necessary to postulate either that exposure to pollen in the nose or pharynx activates bronchoconstrictor reflexes or that some pollen antigen is naturally present in the air during

pollination in particles small enough to be drawn into the lower airways.

Evidence for both theories has been presented. Hoehne and Reed⁵ found that ragweed pollen placed on the nasal mucosa of subjects with "ragweed" asthma did not result in a drop in FEV₁, whereas pollen placed on the posterior third of the tongue or inhaled did result in a drop in FEV₁ in at least some subjects. On the other hand, Fontana et al.⁶ challenged 13 ragweed-sensitive children to ragweed pollen grains in an environmental chamber, and no patient developed significant changes in spirometric values or symptoms of asthma, while seven showed symptoms of allergic rhinitis and conjunctivitis; however, it is not clear how many of the ragweed-sensitive subjects had histories of presumed pollen asthma. Wilson et al.⁷ radiolabeled grass pollen and showed that human subjects inhaling pollen grains suffered essentially no deposition of the radiolabel in the trachea, bronchi, or lungs, whereas an aerosolized water extract of the pollen was readily deposited in the lung. Although Michel et al.⁸ have identified by anatomic study pollens of grasses and trees in the lungs and mucous secretions from autopsy specimens and lung tissue after pneumonectomy, the pollens

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Abbreviations used

S_{GAW} :	Specific airway conductance
PD_{35} :	Provocation dose resulting in 35% fall in S_{GAW}
FEV_1 :	Forced expiratory volume in 1 second
RAST:	Radioallergosorbent test
PNU:	Protein nitrogen unit

were present in small number, and the possibility of postmortem contamination of the specimens has been raised.⁹ After finding only modest numbers of ragweed pollen grains in the trachea and bronchi of guinea pigs exposed to air containing 10^6 times the amount of ragweed pollen in ambient air during the average ragweed season, Hobday and Townley¹⁰ conclude that the absolute number of pollen grains that might penetrate to the lower human respiratory tract on natural exposure is likely to be very small.

Because they realized that whole pollen has little chance to enter the bronchial airways, Busse et al.¹¹ sampled outdoor air during the ragweed season and showed that ragweed antigen could be found in the stages of an Andersen sampler collecting particles less than $5 \mu\text{m}$ in size even though microscopic examination revealed no pollen grains. The concentration of antigen of small particle size in the air was not estimated.

Further questions about ragweed pollen as a cause of autumnal asthma have been raised by Bruce et al.¹² in a paper from this laboratory studying 29 patients identified clinically as having typical "ragweed" asthma by a history of a September or October exacerbation of asthma, along with a high degree of skin-test and peripheral blood basophil sensitivity to ragweed extract as well as specific ragweed antibodies measured by RAST. When these individuals kept daily diaries of asthmatic symptoms, the time of maximum symptoms failed to correlate with ragweed pollination but was more consonant with the time of appearance of mold spores such as *Alternaria* and *Hormodendrum* (*Cladosporium*). Furthermore, immunotherapy with ragweed extract appeared not to modify the seasonal asthma in comparison with placebos, although the doses achieved in this study were low.¹²

The study reported here was initiated to study more thoroughly the responses of ragweed pollen-sensitive asthmatics to quantitative exposure to ragweed pollen and particulates of smaller size containing ragweed antigens. These studies tend to confirm that intact pollen grains are unlikely to cause an allergic reaction in the lung manifested as asthma.

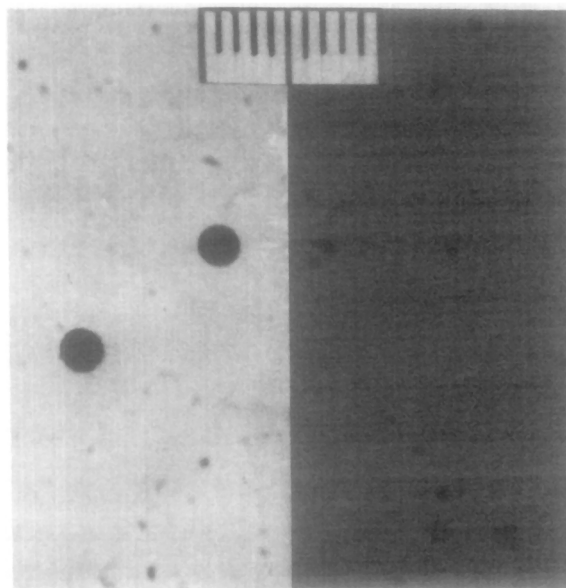


FIG. 1. Photomicrograph of ragweed pollen suspension before (left) and after (right) extensive fragmentation in a ball mill. (Scale divisions, $10 \mu\text{m}$.)

MATERIALS AND METHODS

Subjects

Subjects selected for study had a history of asthma in the ragweed season without perennial symptoms of asthma, positive intradermal skin tests to ragweed pollen extract at 100 PNU/ml, and positive bronchial challenges with aerosolized ragweed extract. Of the 15 patients taking part in these studies, six were randomly selected for bronchoprovocation with methacholine, and each had a positive response in the asthmatic range. Fourteen had symptoms of rhinitis during the ragweed pollen season.

Ragweed pollen extract

Crude short ragweed pollen extract (reconstituted from lyophilized Lot No. 16608FD; Center Laboratories, Port Washington, N. Y.) was used for intradermal skin testing¹³ and bronchoprovocation.^{14, 15} This extract assayed at $26.7 \mu\text{g AgE/ml}$ and $100 \mu\text{g protein nitrogen (10,000 PNU)/ml}$.

Ragweed pollen

Short ragweed pollen (Lot No. 18-56-72; Greer Laboratories, Lenoir, N. C.) was stored in a sealed container at -20°C . By direct counting in a hemocytometer of pollen grains well dispersed in normal saline, 1 gm of pollen was found to contain $3.18 (\text{SD } 0.20) \times 10^8$ pollen grains. Ragweed pollen fragments were prepared by Greer Laboratories by milling a portion of the same lot of whole ragweed pollen. Pollen was milled continuously for 42 hr at 75 rpm in a mill jar 6 inches in diameter and 8 inches in height, using 30 Burundum stones weighing 25 gm each and measuring $\frac{3}{4}$ by $\frac{3}{4}$ inches. Milling was performed at room temperature. At the conclusion of the milling procedure, only fragments and

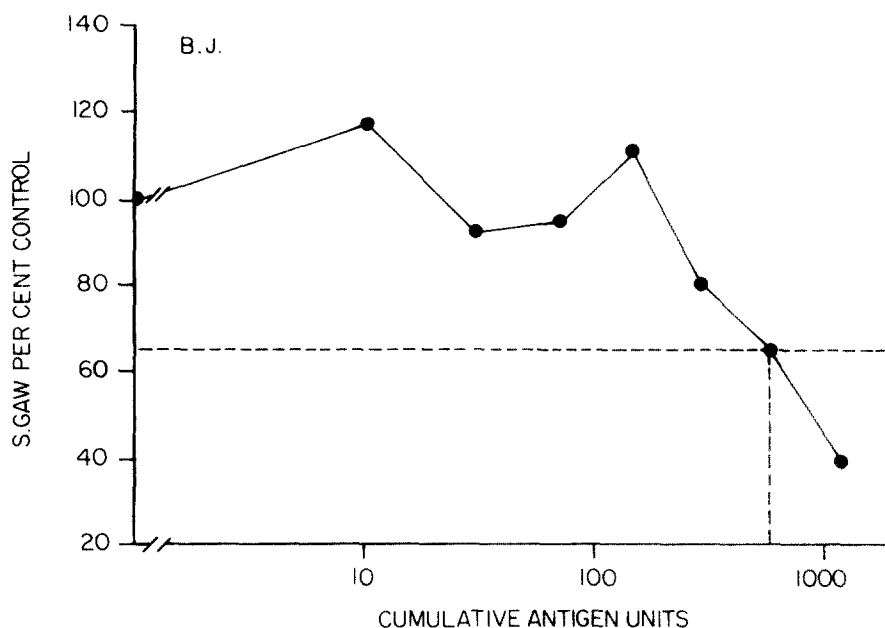


FIG. 2. Dose-response (percent control S_{GAW}) curve in a ragweed-sensitive asthmatic patient inhaling increasing doses of an aerosol of ragweed extract. Dashed line, A 35% drop from control S_{GAW} . A perpendicular dropped from the intersection of the dashed line and the dose response curve gives the PD_{35} on the abscissa.

TABLE I. Delivery of ragweed pollen grains by turbo-inhaler*

Expected pollen dose (grains)	No. of capsules tested	Pollen grains delivered		
		Mean $\pm$ SD	Range	Maximum deviation from expected dose (%)
1,280	4	1,296 $\pm$ 116.5	1,133-1,409	11.5
320	6	329 $\pm$ 42.1	264-369	17.5
40	12	39.4 $\pm$ 5.4	32-48	20.0

*Pollen grains in lactose were gently blown at a distance of 2 cm onto microscope slides lightly coated with petrolatum. After staining, pollen grains were counted by microscopic examination.

no intact pollen grains were identified on microscopic examination of fragment preparations stained with Calbera's stain. As is apparent from the photomicrographs of stained preparations of intact and milled pollen in Fig. 1, whole pollen grains are approximately 20 μ m in diameter, while the ragweed pollen fragments ranged from approximately less than 1 to 8 μ m in diameter. The dose of pollen fragments used for inhalation challenge is expressed as grain equivalents, according to the calculated weight of the ragweed pollen.

Quantitative ragweed pollen challenge

Whole or fragmented pollen was mixed with lactose (Lactose, U.S.P., Lot No. 63558; Merck and Co., Inc., West Point, Pa.) and delivered by turbo-inhaler¹⁶ (Spinhaler; Fison's Corp., Bedford, Mass.). Stock mixtures contained 10,240 and 320 grains (whole pollen) or grain equivalents (pollen fragments) per 25 mg. Serial two-fold dilutions of stock mixtures in lactose, to cover the range of 40 to 2056 grains or grain equivalents per 25 mg, were

prepared every 2 wk and dispensed into No. 2 gelatin capsules. Twenty-five milligram amounts of the mixtures were delivered by turbo-inhaler, with visually complete emptying of capsules containing 28.4 mg, since an average of 12% of the contents adheres to the capsule and turbo-inhaler during delivery. That the actual amount of pollen delivered was close to that expected from the serial dilutions of pollen in lactose, and that pollen delivery was highly reproducible are demonstrated by the data in Table I. The maximum deviation of the delivered from the expected dose was 20.0%, with the amount of deviation being progressively less with capsules containing more pollen grains. Even for the capsules containing 40 grains/25 mg, in which the amount of deviation was largest, eight of 12 capsules tested had a delivered dose within 10.0% of the expected dose, and 10 of 12 delivered doses were within 15.0% of the expected value.

Stock pollen mixtures, lactose, and filled capsules for challenge were stored at room temperature under vacuum in a desiccator jar, using P_2O_5 desiccant. These conditions of

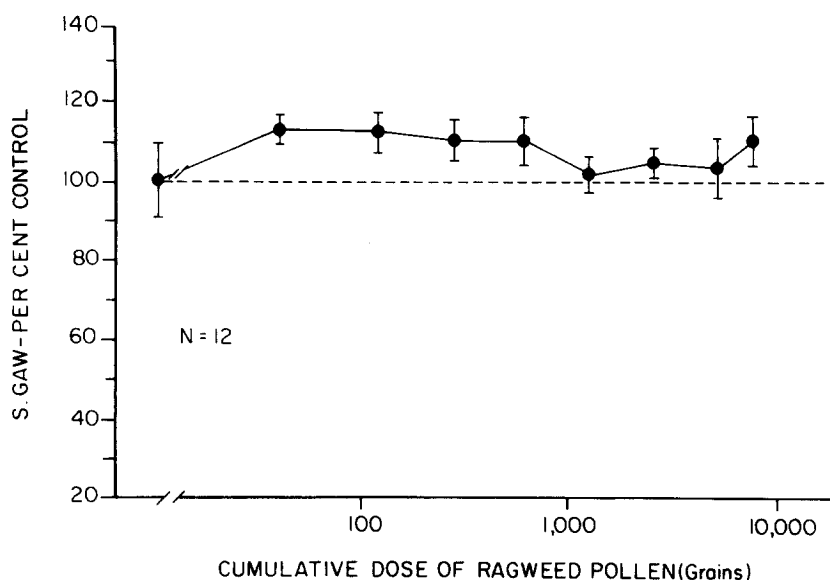


FIG. 3. Mean dose-response (percent control S_{GAW}) curve for 12 ragweed-sensitive asthmatic patients inhaling increasing numbers of whole pollen grains. Dashed line, Control S_{GAW} .

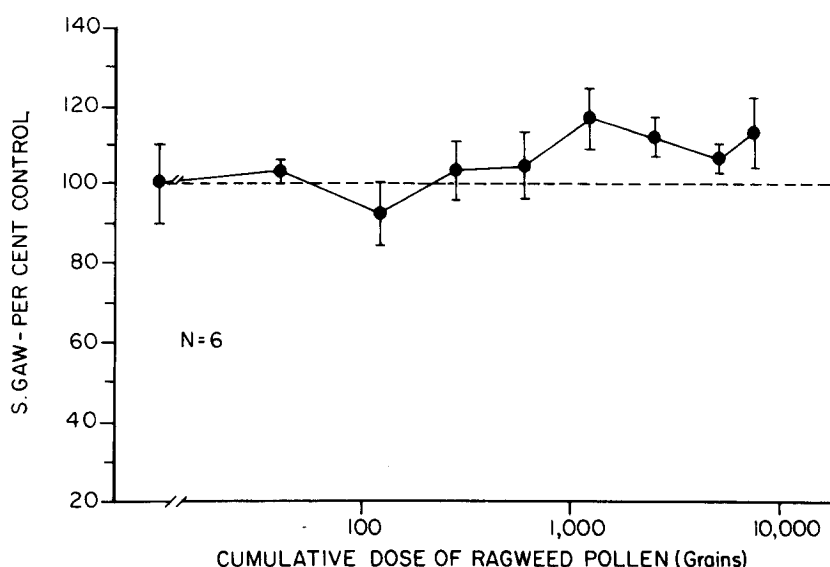


FIG. 4. Mean dose-response (percent control S_{GAW}) curve for six ragweed-sensitive asthmatic patients inhaling increasing numbers of whole pollen grains during the season of ragweed pollination. Dashed line, Control S_{GAW} .

preparation and storage were found to be essential for prevention of aggregation of pollen fragments and clumping of lactose (since it is hygroscopic), thus allowing even dispersal of pollen as well as complete and reproducible emptying of capsules during provocation testing.

Oral inhalation challenge

Subjects were free of asthmatic symptoms at the time of testing and took no medication for 48 hr before testing. Quantitative bronchial challenge with crude ragweed pollen extract was performed as previously described,^{14, 15} and

quantitative provocation with ragweed pollen was carried out by the method described above with similar timing of challenges and testing of responses. A variable-pressure body plethysmograph was used to determine the S_{GAW} by the method of Dubois et al.^{17, 18} We used S_{GAW} for measurement of airway function during bronchoprovocation because it is more sensitive than FEV_1 and is less tiring for the patients. After a baseline S_{GAW} determination, patients inhaled normal saline as a control for ragweed extract or 25 mg lactose as a control for pollen. Subjects then inhaled sequential graded doses of nebulized crude ragweed extract,

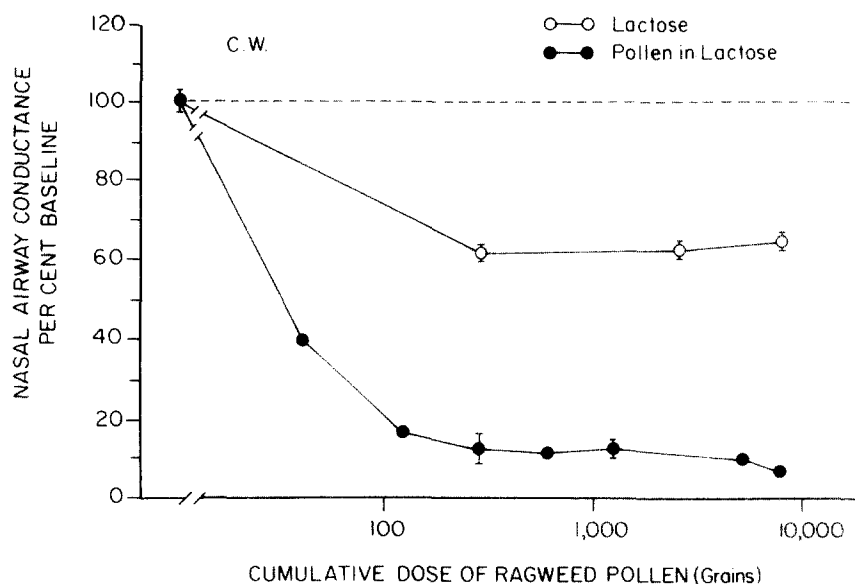


FIG. 5. Dose-response (percent baseline nasal conductance) curve in a ragweed-sensitive asthmatic patient inhaling increasing numbers of whole pollen grains and lactose into one nostril on separate occasions. *Dashed line*, Baseline nasal airway conductance.

whole ragweed pollen, or pollen fragments. S_{GAW} was determined 10 min after each inhaled dose. Dose-response curves were constructed, plotting the cumulative antigen dose on a logarithmic scale against S_{GAW} as a percentage of the control value. The cumulative dose of inhaled antigen required to produce a 35% decrease in S_{GAW} was designated the PD_{35} . The antigen dose for extract is expressed in units, with 1 U defined as the amount of antigen contained in one breath of a ragweed extract solution containing 1 μ g of protein nitrogen per milliliter. Antigen doses for whole pollen and pollen fragments are expressed in grains and grain equivalents, respectively. Changes in S_{GAW} of less than 20% or in PD_{35} of less than 10-fold are not considered significant.^{14, 15}

Nasal challenge

Intranasal delivery of pollen mixtures was carried out by the use of a turbo-inhaler designed for nasal use (Fison's Corp.). Because complete emptying of the capsules was difficult by voluntary nasal inhalation, sufficient compressed air was delivered to the air intake portion of the turbo-inhaler with the use of the Nebulization Dosimeter (Laboratory for Applied Immunology)¹⁴ to insufflate pollen mixtures gently into the nose, emptying the capsules with three or four puffs of air. Nasal airway conductance (reciprocal of resistance) was determined by the method of Solomon et al.¹⁹

RESULTS

All subjects had a positive bronchial challenge to crude ragweed extract as one criterion for entry into the study. A dose-response curve from a single bronchial challenge test in one of the patients is shown in

Fig. 2, in which the PD_{35} for pollen extract is 600 U. The PD_{35} values for the group studied varied from 5 to 15,500 U, with a geometric mean value of 426 U. PD_{35} values for patients on repeat determinations were found to vary by less than 10-fold as previously reported.¹⁴

To determine whether oral inhalation of ragweed pollen causes bronchospasm, bronchoprovocation with whole pollen was performed in 12 patients. Challenge began with a dose of 40 pollen grains delivered by turbo-inhaler, and doubling doses were sequentially inhaled. As shown in Fig. 3, there was no significant change in S_{GAW} with inhalation of up to 7640 grains, a dose many times greater than that inhaled daily during the peak of the ragweed season in the Baltimore area. In this regard, it has been estimated that an average 11-yr-old child breathing normally out of doors for 24 hr a day during a typical ragweed season in Baltimore would inhale an average of 960 pollen grains per day, and an adult would inhale 1536 grains per day (Marsh DG, personal communication). None of the subjects reported delayed asthmatic symptoms after challenge.

Bronchial challenge with whole pollen grains was repeated at the end of ragweed pollen season to see whether priming—that is, increased sensitivity to antigen after repeated exposure, as described by Connell²⁰ for allergic rhinitis—occurs and is necessary for a bronchial response to inhaled whole pollen. The graph in Fig. 4 summarizes data from bronchial challenge studies in six patients. None showed a significant decrease in S_{GAW} on inhalation challenge with

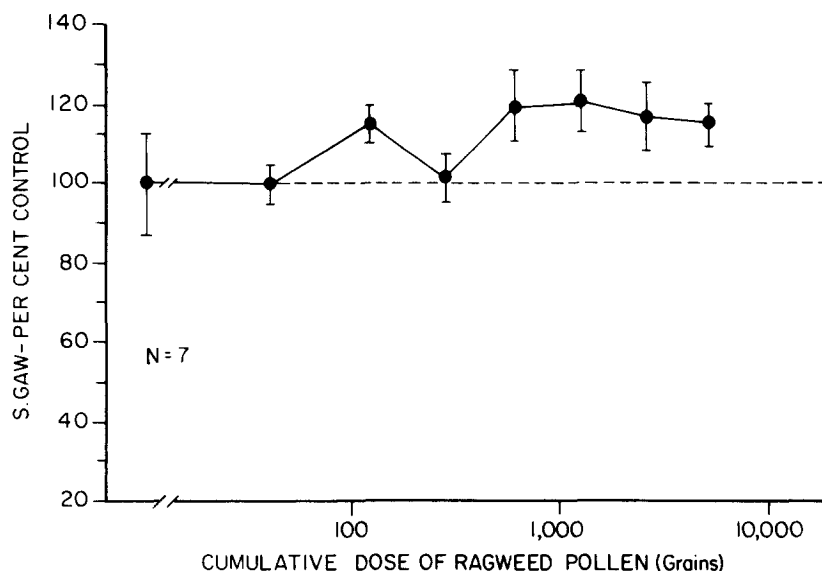


FIG. 6. Mean dose (percent control pulmonary S_{GAW}) curve for seven ragweed-sensitive asthmatic patients inhaling increasing numbers of whole pollen grains into the nose. Dashed line, Control S_{GAW} .

whole pollen after prolonged natural exposure to pollen. These results are similar to those previously published by our group,¹⁴ which failed to demonstrate a priming effect in the lung with either repeated bronchoprovocation with nebulized crude ragweed extract or seasonal exposure to ragweed pollen.

To demonstrate that our pollen preparation is biologically active, inhalation challenge with pollen into one nostril was performed. All subjects of study showed progressive ipsilateral mucosal pallor, rhinorrhea, mucosal edema, and nasal airway narrowing. Fig. 5 shows a dose-response curve of nasal airway conductance (the reciprocal of resistance) for one of the patients after nasal challenge with whole pollen. A dramatic drop in nasal airway conductance to less than 8% of the baseline value was noted, with paroxysms of sneezing and ipsilateral total airway occlusion on examination. In contrast, the response of the same patient on another day to equal amounts of inhaled lactose was only modest, without overt symptoms or signs of rhinitis. S_{GAW} was determined during nasal challenges to see whether bronchospasm was produced through reflexes originating in the nose (Fig. 6). In spite of positive nasal airway responses of each of seven subjects tested, none had significant reduction in S_{GAW} or delayed asthma after challenge with up to 10,000 pollen grains.

Because particles the diameter of whole pollen grains are excluded from the lower respiratory tract,⁴ bronchial challenge with pollen fragments that are small enough to enter the pulmonary airway was carried out. The results of one such challenge are pre-

sented in Fig. 7. A significant decrease in S_{GAW} was caused by pollen fragments, with a PD_{35} of approximately 1000 grain equivalents, in contrast to the lack of bronchial response to inhaled whole pollen grains in equivalent or greater doses. Similar results were obtained in six of seven subjects tested. Table II shows the PD_{35} values for challenge with extract and pollen fragments in seven subjects. The PD_{35} for fragments varies from 59 to 20,000 grain equivalents, and one subject did not have a bronchial response to inhaled fragments. Although the patient with the lowest PD_{35} for extract also had the lowest PD_{35} for fragments, and although the patient who showed no response to fragments had the highest PD_{35} for extract, there appears to be only a general relationship between the two values.

Premedication of subjects with inhaled nebulized atropine has been shown to prevent irritative or nonspecific bronchial reactivity of asthmatics to inhaled substances,²¹ while it does not significantly effect the PD_{35} to inhaled antigen extract.²² To show that challenges with pollen fragments were related to antigen and not nonspecific irritation, bronchial challenges with pollen fragments were repeated on another day after inhalation of 5 mg of atropine sulfate. This dose of atropine has been shown to be adequate to prevent bronchospasm after inhalation of metacholine or of citric acid, a well-recognized irritant.²¹ In Fig. 8 the bronchial response is plotted as percent baseline conductance to demonstrate the effect of atropine on resting airway conductance. After inhalation of nebulized atropine, there is an increase in

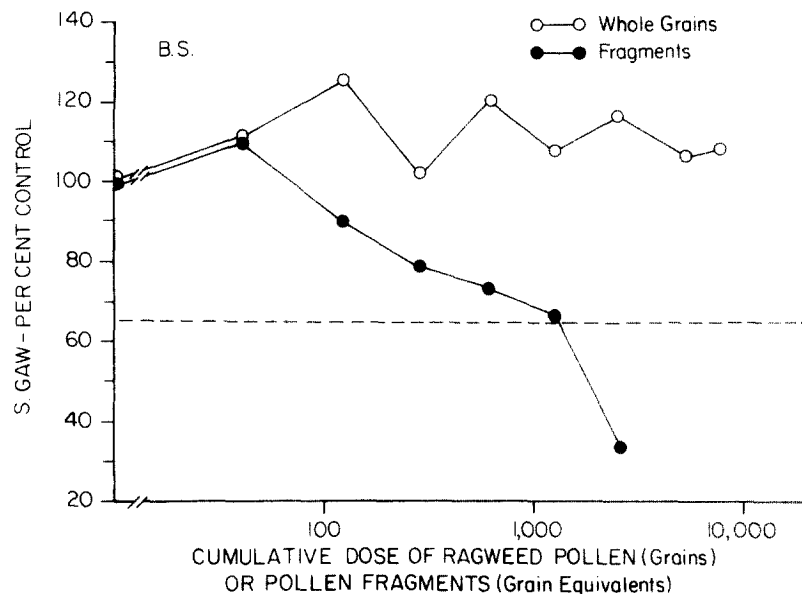


FIG. 7. Dose-response (percent control S_{GAW}) curve in a ragweed-sensitive asthmatic patient inhaling increasing numbers of whole pollen grains and pollen fragments on different days. Dashed line, 35% drop in S_{GAW} from control level.

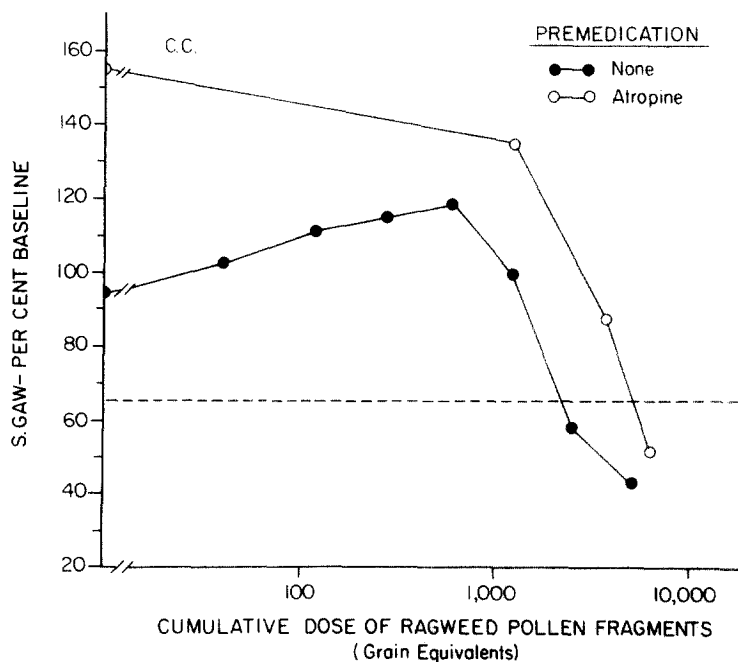


FIG. 8. Dose-response (percent baseline S_{GAW}) curve in a ragweed-sensitive asthmatic patient inhaling increasing numbers of pollen fragments after inhalation of 5 mg of atropine or after no atropine. Dashed line, 35% drop in S_{GAW} from control level.

S_{GAW} to 160% of baseline, reflecting an ablation of resting cholinergic bronchomotor tone. As pollen fragments are given, S_{GAW} drops, and PD_{35} values with and without atropine were not significantly different for any of the patients tested. The PD_{35} values in individual subjects for bronchoprovocation with

pollen fragments with and without atropine are illustrated in Fig. 9. None showed significant change after premedication with atropine. In addition to atropine premedication, two of these subjects were given five inhalations of nebulized atropine, 1.2 mg/ml, before each dose of pollen fragments. Persistence of the at-

TABLE II. PD₃₅ for pollen extract and pollen fragments

Subject	PD ₃₅	
	Pollen extract (U)	Pollen fragments (grain equivalents)
F. A.	5	59
C. C.	140	2,300
M. A.	300	20,000
J. F.	370	1,400
R. B.	460	720
B. S.	600	1,300
E. R.	2,300	NR

NR = no response with cumulative dose of 32,960 grain equivalents.

ropine effect throughout the 40 min challenge procedure was thus assured. Although premedication with atropine failed to shift the dose-response curve, premedication with isoproterenol did shift the dose-response curve to inhaled pollen fragments significantly to the right in each of three subjects tested.

DISCUSSION

The turbo-inhaler was developed to deliver dry powders such as antimicrobials and cromolyn to the lower airways¹⁶ and has been used extensively for many years with the latter drug. That our system actually does deliver pollen grains into the inhaled air stream is demonstrated by the brisk attacks of hay fever induced with small numbers of pollen grains when the device was used for nasal insufflation. Although it has already been demonstrated that radio-labeled pollen grains are not inhaled in the lung in significant numbers,⁷ it seemed important to reinvestigate whether pollen delivery either orally or nasally triggers pulmonary bronchial reflexes. We could find no evidence that reflexes are activated by short-term exposure, whereas delivery of the antigen in particles small enough to reach the lung, either by aerosol or in the dry form, readily stimulates asthmatic responses in sensitive people.

This appears to leave the question whether ragweed antigen appears in the air in small-particle form in sufficient quantity to stimulate asthmatic responses. Such particles could come both from pollen fractured by being buffeted about in air currents or from broken parts of the leaves and stems of the plants, which have been shown to be rich in an antigen resembling AgE.²³ Although this idea cannot be completely discounted, there are several reasons for doubting that ragweed is the principal source of the antigen for fall seasonal asthma. Pollen-sensitive hay fever patients

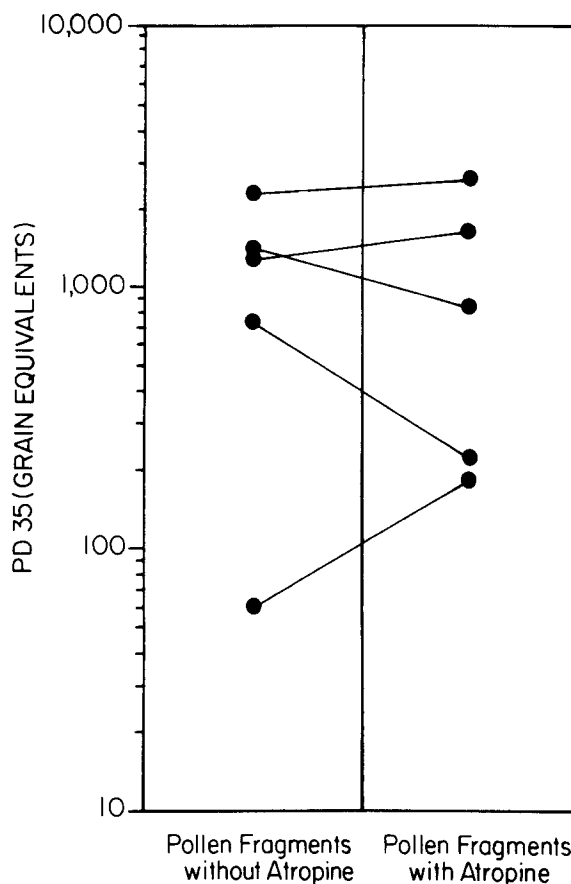


FIG. 9. PD₃₅ of fractured ragweed pollen (expressed as pollen grain equivalents) with and without premedication with 5 mg atropine by inhalation.

have their allergic symptoms in a good relationship to the appearance of pollen in the air, and most highly sensitive patients will have symptom-medication scores on daily diaries that correlate well with daily pollen counts obtained in the same area from sampling devices.¹³ Asthmatic patients, on the other hand, had peaks of asthmatic symptoms any time from August to October in the study by Bruce et al.,¹² militating against a single source of allergen being the cause of their symptoms. We pointed out in that article that allergenic spores, *Alternaria* and *Hormodendrum* (*Cladosporium*) are abundant in the air in September and October in our area and their appearance correlated with asthmatic symptoms in many of our patients.¹² Furthermore, there is abundant sporulation at this time of the year of a number of species of fungi, producing basidiospores and ascospores of a size to be readily inhaled into the lung.²⁴⁻²⁶ In addition, Lopez et al. have demonstrated positive skin test reactions to a number of species of Basidiomycetes in asthmatic patients.²⁷

We plan to investigate further the role of small

spores in seasonal asthma in atopic patients as well as the relationship of the appearance of ragweed antigen in small particles and the symptoms in asthmatic patients with known sensitivity to ragweed pollen. At this point, however, there seems to be considerable reason to doubt that whole ragweed pollen causes allergic asthma, and there is at least some question about the role that small particle ragweed antigens play in autumnal asthma.

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