

Ragweed pollen challenges in a controlled environment

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Clinical manifestations of allergic reactions to airborne ragweed pollen have been produced by direct challenge of patients in an Environmental Control Unit. The experiment was so designed and carried out in an effort to eliminate all possible external psychological factors that might interfere with the validity of pollen provocation results. This preliminary study has verified the effectiveness of a newly devised provocation technique using ragweed pollen in quantitative manner in an Environmental Control Unit. In our study group, children with definite clinical symptomatology during the ragweed season developed symptoms on ragweed pollen provocation. The amount of clinical reactivity to pollen challenge directly correlated with the intensity of the intradermal ragweed skin test reaction.

Allergic manifestations in the atopic patient are due to environmental allergens modified by a combination of factors including irritants, infections, climatic changes, and stress. Various investigators have made attempts to correlate human sensitivity to a specific antigen such as ragweed pollen by direct provocation.¹⁻⁵

Connell⁶ recently reported some interesting relationships between the inhalation of ragweed pollen and the degree of nasal congestion produced using a system of quantitative intranasal provocation testing. In these previous studies, the environment in which the subjects were tested was not controlled prior to or during the specific provocation challenge. In addition, the patients were aware when challenge experiments were being performed. One can question the possible effect these extraneous factors may have on the experimental findings. Except for data on nasal resistance^{6,7} there has been no objective systematic attempt to measure the patient's reaction quantitatively over prolonged periods before, during, and after the pollen challenges.

Provocation testing has been proposed by some investigators as a direct and specific method of diagnosing the cause of allergic respiratory diseases. This procedure can also provide information relating antigenic stimulation to the

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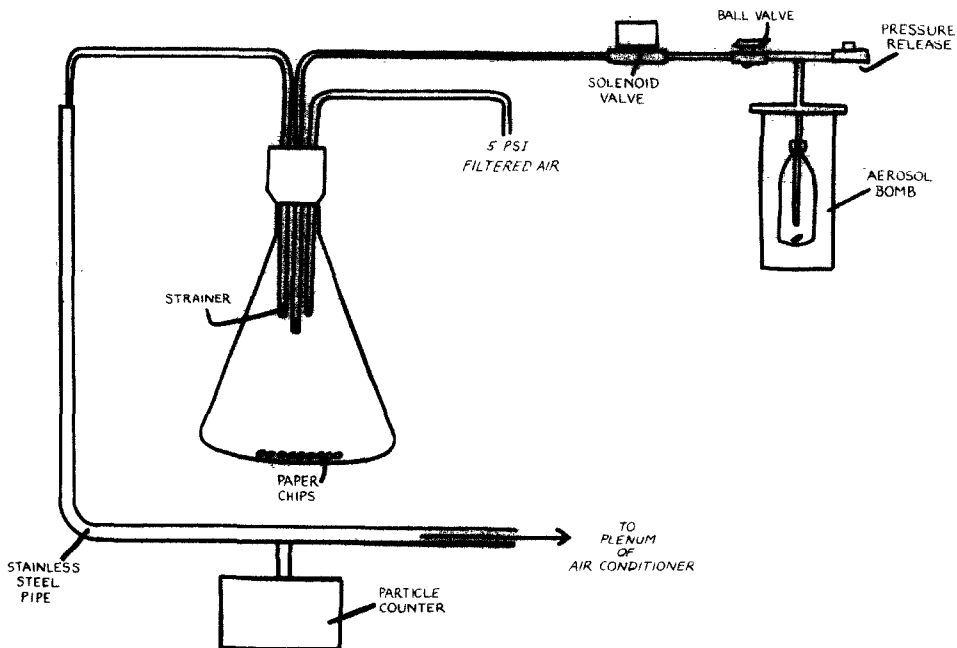


FIG. 1. The over-all pollen dispersal system.

significance of positive skin test reactions, clinical symptomatology, patient sensitivity, and the "summation" and the "priming" effects of inhaled allergens.

In our study, we have attempted to study the effects of ragweed pollen challenges with a newly devised system of provocation under controlled environmental conditions in a group of atopic children. The observations have been carried out in the John A. Hartford Environmental Control Unit at St. Vincent's Hospital and Medical Center of New York. This three-bed unit, 16 by 16 feet, has been described in detail previously and we mention here only some salient physical characteristics of the room. The air is changed at least 30 times per hour and is passed through multiple filters that remove 99.7 per cent of particles 0.3μ and larger. The average air velocity is less than 2 m.p.h. The room can also be sterilized at 250° F. to eliminate any airborne organisms.

METHOD OF STUDY

The subjects admitted to this study were atopic children ranging in age from 8 to 13 years. These children all had positive reactions to ragweed pollen on skin testing with solutions of 100 and 500 protein nitrogen units (PNU). The studies were carried out from December through June of two consecutive years. Informed parental consent was obtained in all cases.

The patients were admitted into the Environmental Control Unit in groups of 2 or 3 and remained in the room for a period of $5\frac{1}{2}$ days. On admission, standard orders on all the children included a complete blood count, urinalysis, a nasal smear for eosinophils, nose and throat culture with sensitivity, a tine test, chest and sinus x-rays, stools for ova and parasites, serum immunoglobulins, sweat test, and pulmonary function tests. The patients were

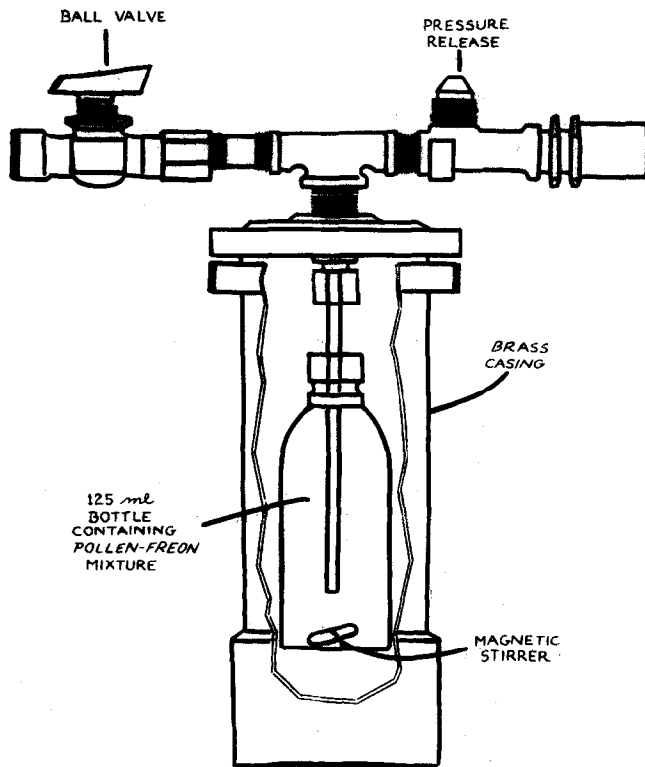


FIG. 2. Refillable brass aerosol bomb with plastic bottle liner.

also placed on a modified hypoallergic diet (Fontana)* to eliminate certain foodstuffs. The first 1½ days were used in acclimatizing the patient to the Unit. During this period, baseline pulmonary data were established with a Wright peak flowmeter and a HiFi spirometer, and baseline values for clinical status and eosinophils were also determined and recorded. During the next 3½ days, the ragweed pollen challenges were administered through the air-conditioning system in a manner to be described.

The challenges were run according to the following schedule: 3 challenges on the first testing day (Tuesday), 4 challenges on the second and third testing days, and 3 challenges on the fourth testing day. The challenges were spaced evenly during the day. Each challenge involved close observation of the children before, during, and after a 5 to 7 minute period of injection of the pollen into the room through the air-conditioning system. The physician carried out his close-up physical examination approximately 8 minutes after the end of each challenge. The pulmonary function tests were done at the same time.

A one-way mirror in the observation room of the Unit allowed the physician conducting the test a continuous observation period before, during, and after the testing periods. The patient's condition and reactions were subjectively and objectively evaluated and scored. One physician was responsible for all testing in order to ensure uniformity in interpretation of

*Modified hypoallergic diet (Fontana) avoids chocolate, cocoa, nuts and seeds, shellfish, spicy foods such as ketchup, mustard; snack foods such as potato chips, pretzels, popcorn; artificially flavored juices, soda except ginger ale and 7-Up, and milk is limited to one glass per day.

TABLE I. Clinical symptoms scoring system

	No symptoms	Symptoms less than ½ hr.	Symptoms for ½ to 2 hr.	Symptoms for more than 2 hr.
Sneezing	0	1	2	3
Coughing	0	1	2	3
Runny, stuffy nose	0	1	2	3
Red, itchy eyes	0	1	2	3
Total	0			12

results. All of the data derived from the tests were entered on standardized forms for statistical evaluation.

The pollen dispersal system

The pollen was dispersed through the air conditioner by feeding pollen from a mixing chamber. An air flow at about 1,000 feet per second through the mixing chamber picked up the pollen and carried it past a particle counter to the plenum of the air conditioner. The overall pollen dispersal system (Fig. 1) had to meet the following criteria: (1) The dispersal system should not physically affect the pollen. This was verified by comparing the density of chaff and broken grains before injection with that found on the sampling slides within the room. (2) The pollen should enter the room as single grains, not clumped. Thus each count registered represented only one grain.

We were able to meet these criteria by constructing a refillable brass aerosol bomb with a 125 ml. plastic bottle liner (Fig. 2). The bottle was filled with a mixture of short defatted ragweed pollen and Freon for each challenge. A magnetic stirrer within the bottle kept the mixture reasonably homogeneous. When the bomb valve was opened by a timing circuit, the Freon-pollen mixture was fed to a mixing chamber where the Freon evaporated. A 5 p.s.i. filtered airstream carried the pollen via a 40 foot stainless steel conduit to the plenum of the air conditioner. A sampling tee joint located in the conduit led to the particle counter.

The bomb was thoroughly cleaned before each challenge. It was then filled with 100 ml. of Freon and about 2 Gm. of short defatted pollen (this mixture was kept uniform throughout the experiment, independent of the dose to be administered at a particular challenge). The bomb was sealed and allowed to temperature-stabilize, which resulted in the Freon generating a pressure of 70 p.s.i. within the bomb. The bomb valve was controlled by a percentage timer with a duty cycle of 7.5 seconds. Adjusting the "open time" of the valve was our main method of controlling the pollen density within the room. Immediately prior to each challenge, the counter calibration was checked. The prescribed dose of pollen was administered evenly over a period of 5 minutes. Settling of the pollen¹⁰ was no problem in our study since it applies only to still air. The room is cleared of suspended pollen because the air in the room is removed and filtered within minutes after the pollen injections are completed.

Challenge procedure

The protocol directed the number of challenges per day, the amount of pollen to be injected during each challenge, and the timing of the challenges. The flow sheet for the first two days of challenge was generally rigid. The procedures for days 3 and 4 were varied in accordance with the responses elicited during the first two days. The only deviations from the protocol occurred when a challenge resulted in a severe asthmatic reaction. If the patient had experienced severe symptomatology, he was taken out of the room during the next two or three challenge periods.

Several minutes after the challenge a set of pulmonary function tests, including FEV₁, TVC, etc., was run on each patient. The physician then made direct examinations of ear, nose, throat, and chest and took a nasal smear for eosinophils. After the physical examination by

TABLE II. Overall patient profile and reaction to provocation on the basis of clinical symptoms score

Patient	Classification by skin test (see text)	History of clinical ragweed symptoms?	Average ENT score (maximum = 12)	Correlation of CS score with total pollen dose per test period (r value)
1. V. C.	B	Yes	1.5	0.742 (p < 0.001)
2. R. M.	B	No	0	0
3. F. M.	A	Yes	5.0	0.655 (p < 0.02)
4. R. C.	C	No	0	0
5. J. B.	A	Yes	1.6	0.56 (p < 0.01)
6. E. R.	B	No	0	0
7. E. P.	A	Yes	2.7	0.69 (p < 0.001)
8. H. H.	A	Yes	3.8	0.69 (p < 0.001)
9. M. G.	B	Yes	0.1	0.02
10. J. K.	B	No	1.7	0.74 (p < 0.001)
11. J. T.	B	Yes	2.3	0.87 (p < 0.001)
12. R. M.	C	No	0	0
13. K. G.	B	No	0.2	0.16 (p < 0.50)

the physician, observation of the children was continued for 2 hours from the observation unit to determine the severity and duration of symptomatology following the ragweed provocation challenge. These observations were used to compute the clinical symptoms (CS) score for that specific challenge. The CS scoring system used is shown in Table I.

Determination of pollen grains inhaled

The pollen counter (Royco) was situated on-line and thus gave us instantaneous readings of the number of grains injected into the room. It was set to register all particles 12 μ in diameter and larger. This covered the normal range of ragweed pollen and did not count dust, chaff, and broken pollen grains. The actual number density of pollen grains within the room was determined independently by use of a Rotoslide apparatus.¹¹ In this device a pair of glass slides spins for a preset time interval. A microscopic count of the grains caught on each slide allows us to calculate the pollen density (in grains/volume/time) during the spin time. The Rotoslide was first used to sample the density at different points within the room to determine uniformity of dispersion. This was done with the room empty, and with two children and a nurse in the room. We then calibrated the counter vs. actual pollen density to obtain a scaling factor to apply to our counter readings. This had been done originally by the room manufacturer 6 years previously, and we found quite good agreement between his results and the present measurements. The scaling factor was rechecked several times during the course of the experiment and remained constant.

The pollen density generated during each challenge was combined with each patient's minute volume to determine the number of grains he had inhaled. The maximum number of grains inhaled during a provocation was about 110 particles. The minimum number of ragweed grains inhaled that produced a reaction was estimated at about 15. Our minimal number of 15 compared closely with the minimal number found by Connell in his provocative studies. Even though the pollen was uniformly dispersed throughout the room, we attempted to have the children in about the same part of the room for each challenge. This was accomplished by synchronizing their play activity and rest time with the challenge time.

Throughout the experiment, the administration of the pollen was done by someone other than the physician in charge. Throughout the experiment we maintained the temperature at $72^{\circ} \pm 2^{\circ}$ F. and the relative humidity at 40 ± 2 per cent, respectively.

Data collection

The data collected included the results of the initial baseline studies, the results of each physical examination after challenge, the laboratory results plus the CS score determined after

each provocation. In addition, all statements and comments by the children indicating any measure of discomfort or irritation plus any actions by the children indicating same were also recorded. The dosage of pollen injected into the room and the resultant symptom scores were recorded by two different physicians. The actual analysis of the respiratory data and the correlations were completed after termination of the study.

CLINICAL RESULTS

The patients

A total of 13 atopic children with positive intradermal skin tests to ragweed were admitted during the study. Their skin reactivity to ragweed extract was graded according to the following criteria:

The slight or 1+ reaction consists of a wheal, in diameter slightly increased over the size of the original injected material, unaccompanied by erythema or itching.

The moderate or 2+ reaction shows an increase in size over the original wheal by 0.5 to 1.0 cm. in diameter, but remains perfectly round, with smooth outline. There is a surrounding zone of erythema and itching.

The marked positive or 3+ reaction shows a definite increase in size, often being from 2 to 2.5 cm. in diameter, with a ragged, irregular periphery and a pseudopod reaction surrounded by a zone of erythema often 3 to 4 cm. wide.

A marked active or 4+ reaction resembles the marked reaction but is larger, and there may be constitutional reactions consisting of weakness, tremors, and palpitations.

Three patients showed moderate reactions to 500 PNU.

Five patients showed marked reactions to 500 PNU.

Two patients showed marked active reactions to 500 PNU.

Two patients showed marked active reactions to 100 PNU.

One patient showed marked reaction to 10 PNU.

Seven of the patients had a history of definite seasonal clinical symptoms due to ragweed sensitization, and 6 of the patients did not. Five children had been on hyposensitization therapy for ragweed for 3 to 6 months. The remaining children were not on immunotherapy for ragweed (Table II).

The duration of their illness ranged from 8 months to 10 years. The children were asymptomatic at the time of admission, except for one patient who came in with wheezing. One of the latter patients (R. C.) was admitted with moderate wheezing and improved after a 3 day stay in the Environmental Control Unit. These children were not on any medication such as antihistamines, bronchodilators, etc., except the patient (R. C.) who came in with moderate wheezing and was on Marax.

Physical examination and CS scores

Seven children showed significant reactions to provocation with marked changes of nasal mucosa, engorgement of turbinates, sneezing, and/or coughing. Accordingly, these children had high CS scores. Of this group only one had not previously shown clinical symptoms of ragweed allergy.

Six children had no significant reactions to provocation and had correspondingly low or zero CS scores. Of this group, only one had reported having a

past history of clinical symptoms of ragweed allergy. It is to be noted that each week the study group included at least one "reactor" and one "nonreactor." This grouping was fortuitous since the patient chosen for each group was selected according to age and sex and the parents' consent to allow their children to participate in the study.

No patient developed any wheezing during challenges and no attacks of asthma were experienced. It is possible that the duration of provocations may not have been long enough to cause severe symptomatology in this preliminary study.

Pulmonary function tests

Pulmonary function studies did not show any significant changes in terms of reduction in FEV₁, the total vital capacity (TVC), etc., subsequent to the challenges. The one patient (R. C.) who came in with wheezing showed improved pulmonary function in this controlled environment. After a period of 48 hours, his asthma improved and he was challenged with ragweed pollen without any reaction. One other patient (J. T.) who was admitted with slight wheezing remained so even after our provocation tests. He was one of the children with no clinical history of ragweed allergy.

Nasal smear for eosinophils and other laboratory results

The nasal smears for eosinophils were repeated immediately after each challenge. Twelve of the children showed *few to many* eosinophils in the nasal smears as we continued the challenges. These patients did not have any eosinophils in their nasal smears taken before provocation. The last child showed no eosinophils after provocation and was one of those who did not subjectively or objectively respond to the challenges.

Hyperactivity

Three patients showed a state of hyperactivity and irritability after a few challenges and they seemed to be restless in bed after each challenge. At times they showed aggressive acts following the provocations. These patients were all in the group that had significant clinical reactions to the provocation test.

STATISTICAL RESULTS

Statistical analysis

Data from the 13 children studied were analyzed by comparing periods of provocation with control periods, with each child serving as his own control. The data measuring pulmonary function parameters (FEV₁, MEFR, TVC) in general gave no indication of response to provocation. This was true when the children were treated as a group, when they were classed by previous clinical history, and when they were grouped according to the severity of the skin reaction.

In contrast, the clinical symptoms (eosinophil smears and CS score) showed consistent change, with provocation in 7 children. The scoring system has been described above (Table I).

TABLE III. Correlation of clinical symptoms score with total pollen dose

Group and characteristic (see text)	No. of data points	Correlation coefficient	Confidence level (p)
Total patients (13)	306	$r = 0.24$	$(p < 0.001)$
Patients with a history of clinical ragweed hay fever	166	$r = 0.52$	$(p < 0.001)$
Patients with no history of clinical hay fever	140	$r = 0.10$	$(p < 0.30)$
"A" reactors (according to skin tests) (4)	88	$r = 0.43$	$(p < 0.001)$
"B" reactors (according to skin tests) (7)	180	$r = 0.25$	$(p < 0.01)$
"C" reactors (according to skin tests) (2)	38	$r = 0$	

On an *individual* basis, the children who did respond to the provocation showed a high correlation coefficient ($r = 0.55$ to 0.85) (Table II). As a *group*, the correlation coefficient for these children was somewhat lower ($r = 0.50$) because the degree of response varied from child to child. For the entire group of 13 children, the correlation coefficient was $r = 0.24$, and this is our benchmark value.

Since the CS score was based on observable symptomatology, to cross-check the findings we grouped the children according to the presence or lack of a history of clinical allergy symptoms. As a group, those with a previous history of allergic symptoms had a significant response to the following parameters. (1) *Total pollen count* was taken from the readings of the counter during a provocation and represented the total amount of pollen injected into the room during a provocation. (2) *Concentration* was measured in pollen grains/liter/minute and was calculated to correct for those provocations when the dose was administered over more or less than the standard 5 minute interval. (3) *Concentration inhaled* was calculated by multiplying concentration by the patient's minute volume. (4) *Total pollen inhaled* was the product of concentration inhaled and the time for each provocation. We reported here only the calculations done with *total pollen count* since the times of administration did not differ from 5 minutes frequently enough to make the values derived from use of concentration significantly different. We also have not reported those parameters involving the patient's minute volume since we did not measure each minute volume directly but took values from tables of average characteristics, and the overall trend of these calculations was the same as that exhibited by the total pollen count calculations.

Statistical summary

The significant results shown in Tables II and III may be summarized in this manner. The overall group of 13 patients had a correlation of $r = 0.24$ of the clinical symptom score with the total pollen injected (which we have found to be a valid measure of the severity of a challenge). When the children were grouped according to clinical history, those with a previous history had a much higher degree of response ($r = 0.52$) than the average, and those lacking a previous history had a much lower ($r = 0.10$) degree of response. When the

grouping was done on the basis of the severity of the reaction to skin testing, those with the most severe reaction ("A" reactors) had a high correlation ($3 = 0.43$), those with the least severe reaction ("C" reactors) had no reaction to the pollen provocation, and those with the intermediate skin response ("B" reactors) had a correlation of $r = 0.25$, which was about the average for the entire group.

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