

Quantitative intranasal pollen challenge

II. Effect of daily pollen challenge, environmental pollen exposure, and placebo challenge on the nasal membrane

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Acute episodes of allergic rhinitis were produced in the laboratory with quantitated challenges of ragweed pollen. Nasal congestion caused by ragweed inhalation was measured objectively as often as every two minutes during an episode of hay fever. Challenges given one hour daily on four successive days decreased the nasal threshold for allergic rhinitis more than fivefold. This effect is called "priming of the end organ." The priming effect was readily reversed by stopping challenges for several days. It was shown that environmental exposure during the ragweed pollinating season also primed ragweed-sensitive individuals. Reversibility of the priming effect required weeks after seasonal pollen exposure had ceased. In four subjects challenged during the season, the pollen dose which caused an acute episode of allergic rhinitis varied according to skin-sensitizing antibody titer; the higher the titer, the greater the susceptibility. In six ragweed-sensitive subjects tested, neither hay fever symptoms nor nasal congestion followed placebo challenges.

Blackley¹ showed in 1873 that an inhalant antigen, when in contact with the nasal mucosa, can cause allergic rhinitis in susceptible individuals. Following this observation, one approach to the study of allergic rhinitis would be to produce acute clinical attacks with a quantitated, single antigen stimulus under controlled conditions. Indeed, such experiments have been attempted for years. Two major obstacles have prevented their success. The first is the quantitative measurement of the antigen itself. For example, pollen commonly associated with allergic rhinitis is microscopic and settles rapidly in air—characteristics

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which make a controlled environment difficult to maintain. Therefore, quantitation of pollen doses requires sophisticated equipment and techniques. The second major obstacle has been the lack of techniques for objectively measuring the allergic attack.

Early attempts at quantitating pollen challenges have varied from crude estimates of undiluted pollen sprayed from an atomizer to similar sprays in which the pollen was diluted with nonallergenic material, such as tale or non-indigenous pollen. Feinberg and Steinberg² applied ragweed pollen directly to the nasal mucosa and estimated that 20,000 to 30,000 grains were required to produce signs of hay fever. Since they had calculated that a patient inhaled approximately 500 ragweed pollen grains in a 24 hour period during the pollinating season, they questioned whether, in their experiments, the pollen was applied to the sensitive parts of the nose and whether a smaller dose evenly distributed by repeated inhaled exposures would be more efficient in causing reactions. In addition, they described great difficulty in evaluating and quantitating the nasal reactions that occurred.

Blumstein³ described a technique in which a toothpick was dipped in pollen and then placed under the patient's nose while he inhaled vigorously. Later Tuft, Blumstein, and Heck⁴ employed this technique but used pollen diluted with tale. Patients inhaled the most dilute pollen mixture. If no reaction occurred, the next stronger dilution was inhaled. This procedure was continued until a nasal reaction occurred or until the undiluted pollen was used. A positive reaction was indicated by sneezing, rhinorrhea, or nasal blockage, symptoms usually occurring within 5 minutes of challenge.

Mock⁵ performed pollen challenges in an air-conditioned room. Pollen was sprayed into the room, and a fan regulated pollen fallout to produce a 15 minute count of 30 pollen grains per square centimeter. The amount of pollen inhaled was estimated from the fallout. Halpern⁶ and Ryssing^{7, 8} used sprays of the antigen extract rather than the antigen itself.

In most studies using these methods, the antigen was delivered in one or two bursts, a technique generally not comparable to environmental challenge or to accurate quantitative reproducibility.

Recently, two techniques have been described with which measurable amounts of pollen can be delivered intranasally. Lowell⁹ has used an insufflator containing a mixture of pollen and lactose. The patient inhales nasally from the insufflator, which is weighed before and after challenge. The quantity of pollen administered is calculated indirectly by the change in weight. The author has recently reported a technique which uses a specially constructed instrument for quantitative pollen delivery.¹⁰

Objective evaluation of the allergic episode resulting from a challenge has been as difficult to measure as the quantitation of the challenge itself. Sneezing, rhinorrhea, and examination of the nasal membranes are, in part, objective measures of allergic rhinitis but still require some form of subjective quantitation on the part of the observer. The patient's subjective appraisal of nasal, eye, ear, or throat itching or of postnasal drip is valuable in describing the acute attack, but does not lend itself to accurate quantitation.

Methods for quantitating nasal congestion, whether it be due to hyperemia or edema, have recently been devised.^{11,12} These methods provide a means of not only evaluating nasal congestion in seconds but permit measurements to be made continuously with every respiration.

This report describes results of quantitative intranasal ragweed pollen challenges administered to allergic subjects. Hay fever reactions were evaluated objectively by changes in nasal patency.

MATERIALS AND METHODS

The technique of quantitative pollen administration is described in detail in a previous publication.¹⁰ Briefly, the equipment consists of a large flask (22 L.) supported on a stand by a hollow glass axle. The lumen of the axle is continuous with the lumen of the flask. Dry, defatted pollen is blown into the flask. The flask is continuously rotated on the glass axle so that the pollen is uniformly suspended in the air within the flask. A swim-type mask is attached to a T-tube on one axle of the flask. Pollen enters the nose when the patient inhales with the mask applied to his face. Such challenges can be given as often as every two minutes. A scanning device is in the tubing between the mask and the flask. The number of pollen grains inhaled is determined after each series of inhalations by examining the scanner microscopically and counting the pollen grains. For every grain on the scanner, one grain enters the mask. The scanning technique is accurate to within ± 15 per cent for each series of respirations. Changes in nasal patency, objectively measured, are used to evaluate the acute episode of allergic rhinitis caused by challenges. Nasal patency is measured by a previously described technique¹² after each series of inhalations of pollen from the mask. These measurements are accurate to within ± 5 per cent.

An attempt is made to administer the pollen at a constant rate throughout the experiment. The rate of administration for different days is determined by previous experience with the patient or the purpose of the experiment.

One important consideration in determining the rate of pollen administration to be used in challenges is the rate of pollen inhalation which might occur environmentally. Feinberg and Steinberg² estimated this to be 500 grains in 24 hours from data obtained on one day's sampling. I made quantitative environmental pollen counts using a cascade impactor in the New York City area twice a day, at 8 A.M. and 6 P.M. during the 1966 ragweed pollinating season. Thirty liters of air were sampled on each occasion. The highest count was 0.66 ragweed grains per liter. Since each individual sampling period was of short duration, made only twice daily, and in only one location, this figure only represents one estimate of maximal environmental exposure. At rest, with a tidal volume of 8 L. per minute and an environmental pollen concentration of 0.66 ragweed pollen grains per liter, a patient would inhale approximately 320 pollen grains per hour. The term "maximal environmental exposure" as used hereafter refers to a pollen inhalation rate of 320 grains per hour. This rate would vary widely, depending on a variety of circumstances. Mouth breathing would preclude any pollen landing on the nasal mucosa. Likewise, indoor exposure, air conditioning, and a variety of air cleaners would decrease the amount of pollen inhaled.

Greater exposure would occur with an increase in minute volume or proximity to pollinating plants. The total amount of pollen administered to our subjects during a one-hour period varied from a fraction of the maximum environmental exposure to one of 5 times this value.

Patients

Allergic rhinitis has been precipitated in all 15 subjects tested thus far who are allergic to ragweed pollen, as determined by skin tests and a history of symptoms during the ragweed pollinating season. In one other subject, who had a strongly suggestive history of ragweed hay fever but negative ragweed skin tests, inhalation of 1,500 ragweed pollen grains in one hour produced neither symptoms nor airway change. Pollen inhalation of 10,000 to 50,000 grains in one hour in nonallergic subjects did not cause symptoms or nasal congestion.

In order to avoid the harmful effects which theoretically could be produced by excessive pollen inhalation administered in a brief period, an attempt was made to titrate each subject. This was done by challenging for approximately one hour on a number of days with gradually increasing inhalation doses. Because of the time involved in this preliminary testing and in the experiments to be described, only four subjects could be studied extensively. One subject, R. D., has had hay fever for three years and never received injection therapy. She has a skin-sensitizing antibody titer (SSAT) of 100.* A second patient, L. A., has had marked hay fever for more than five years and has been receiving hypsensitization injections for two years but in low doses because of marked local reactions. L. A. has a SSAT greater than 500. The third patient, S. F., has had hay fever for more than ten years, has a SSAT of 10, and has been receiving injection therapy for the past two years. The fourth subject, B. W., is of interest and was studied on many occasions because she had questionable hay fever symptoms during the last three ragweed pollinating seasons. She has a positive intracutaneous skin test (6 mm. wheal) to a concentration of 1,000 protein nitrogen units (PNU) per milliliter of aqueous ragweed extract and a negative test to 100 PNU per milliliter. Her SSAT is zero. She has never received injection therapy. Patients R. D., L. A., and S. F. were also allergic to other environmental antigens, such as grass, trees, etc. They were not challenged when these antigens were in the environment.

Intranasal pollen challenges

Intranasal challenges of defatted dwarf ragweed pollen were administered at various times prior to, at weekly intervals during, and following the ragweed pollinating season.

Intranasal challenges were conducted in the following manner: Nasal airway measurements were made every 5 minutes during a 15 minute control period. Variation in these measurements was no more than ± 10 per cent. After baseline nasal airway measurements had been made, the subject took 2 to 10 inhalations from the mask attached to the pollen-dispensing apparatus. The nasal

*Reciprocal of greatest serum dilution which could passively transfer sensitivity by the Prausnitz-Küstner technique.

airway was then remeasured. The amount of pollen inhaled was determined. This sequence of challenge and measurement was repeated at approximately two- to five-minute intervals. A number of end points for a challenge were considered, such as severe hay fever symptoms, a specified decrease in nasal patency, total pollen dose (TPD) administered, or a specified time period. Generally, challenges were arbitrarily stopped when a 33 per cent decrease in nasal patency had occurred. Symptoms of hay fever were always moderate to severe at this time. If a 33 per cent decrease in patency did not occur, challenge was stopped at the end of one hour of pollen administration.

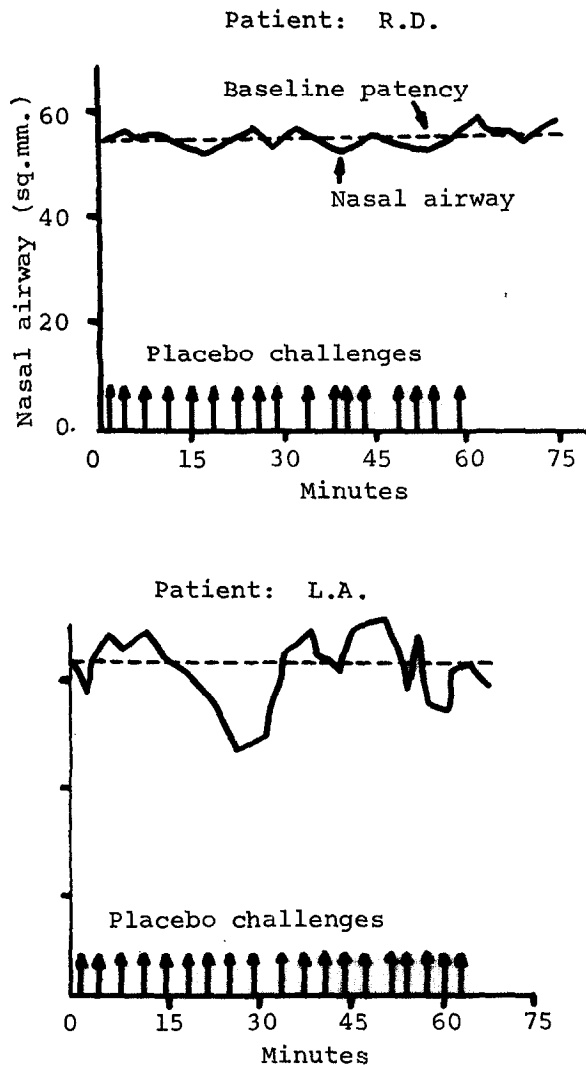


Fig. 1

Changes in nasal patency in two subjects during placebo challenges.

Placebo challenge

When the tube between the pollen flask and the subject is occluded, the subject breathes only room air. This adjustment could be made without the subject's knowledge. Challenge carried out in this manner, but in every other way similar to actual pollen administration, constituted a placebo challenge. Placebo challenges were given only after a subject had developed hay fever during many previous pollen challenges and had come to anticipate an acute episode of allergic rhinitis whenever challenged.

RESULTS

1. Placebo challenges

Six ragweed-sensitive subjects were challenged with placebo on one or more occasions. Fig. 1 illustrates the results generally found. Symptoms did not occur during any of these challenges. A 33 per cent or greater decrease in nasal patency was considered significant during pollen challenge. During placebo challenges, the variation in nasal airway patency was usually less than ± 10 per cent of the base-line patency. On one occasion, the airway patency in a subject decreased 30 per cent briefly but then increased to its original level even though placebo challenge was continued.

2. Reproducibility

When the same pollen dose was administered to the same patient, nasal congestion was observed to differ from one day to another. One of the conditions causing the variation in response, i.e., successive daily challenges, is described later. If the subject had not been challenged with ragweed pollen and had not had hay fever symptoms due to environmental inhalants for several days preceding challenges, the results of challenges with the same dose were quite uniform.

Fig. 2 illustrates challenges in one subject, R. D., on four different occasions. As a prerequisite, the subject was not challenged on the five days preceding each of the challenges illustrated. Only minimal congestion occurred with a fairly constant total pollen dose (TPD) of 900 to 1,300 pollen grains given over approximately one hour on each of the days. The congestion evident during these challenges may be compared to Figs. 3 and 4, which show marked congestion in the same patient and illustrate variations noted under different conditions.

3. Challenge on successive days

Challenge of the same patient on successive days showed that on each succeeding day nasal congestion was more marked and occurred earlier with administration of a smaller pollen dose. Fig. 3 is an example of daily challenges in R. D. On the first day, a relatively large pollen dose was tolerated with few symptoms and moderate decrease in airway size. On the second day, a smaller dose caused the same degree of nasal congestion. By day 4, one fifth of the dose easily tolerated on day 1 produced early and marked airway congestion.

4. Recovery period

The increase in nasal reactivity caused by daily pollen challenges is reversed by elimination of pollen exposure. In the experiments cited in Fig. 3, pollen

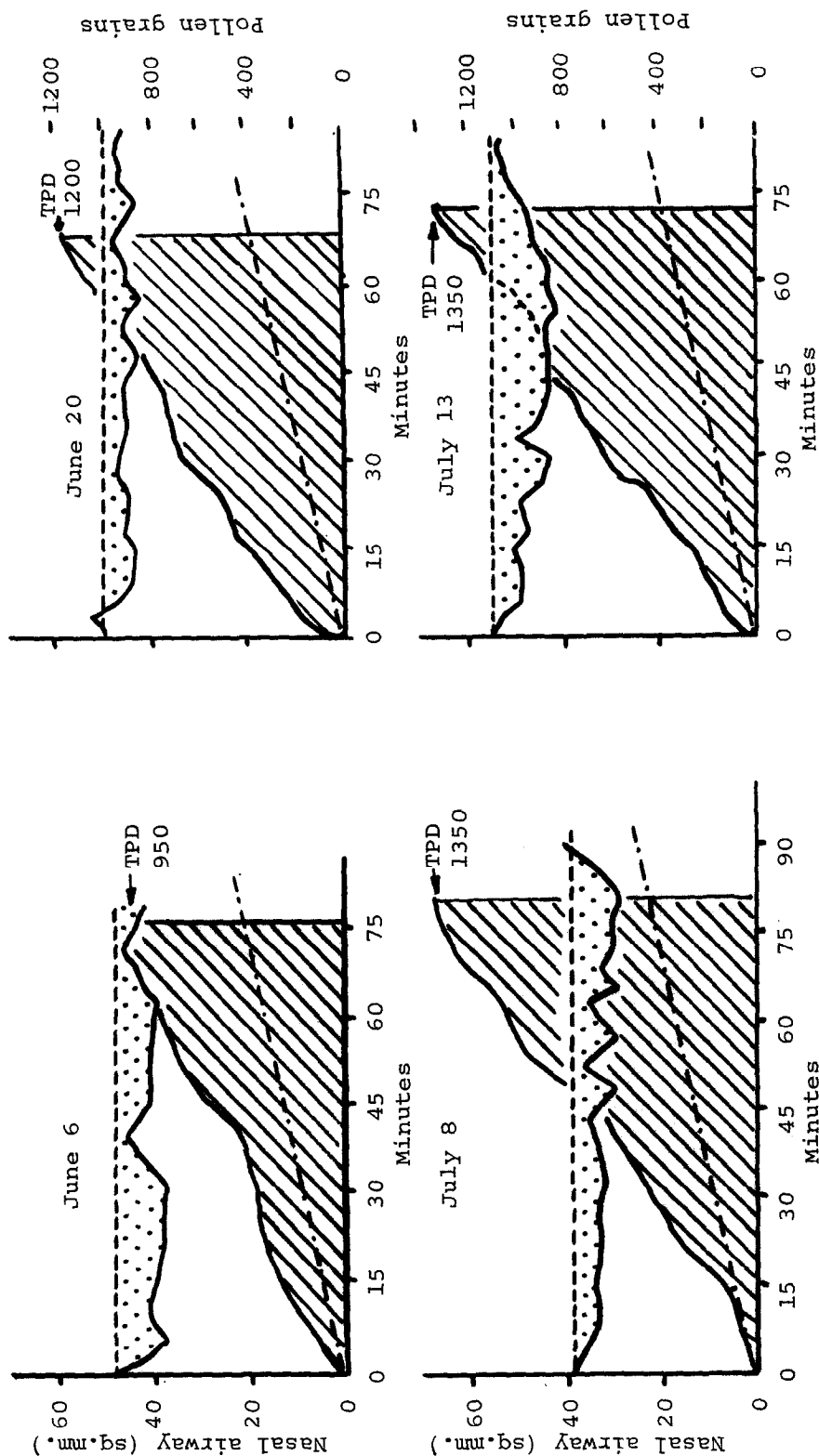


Fig. 2 Same subject challenged on 4 days. Total pollen dose (TPD) varied from 950 to 1350 pollen grains. No pollen exposure for 5 days preceding each challenge day. The dot-dash line (— · — · —) indicates the theoretical rate of pollen inhalation on a "severe hay fever day." (For Figs. 2 to 6, the stippled area indicates decrease in nasal patency. Hatched area shows cumulative pollen dose.)

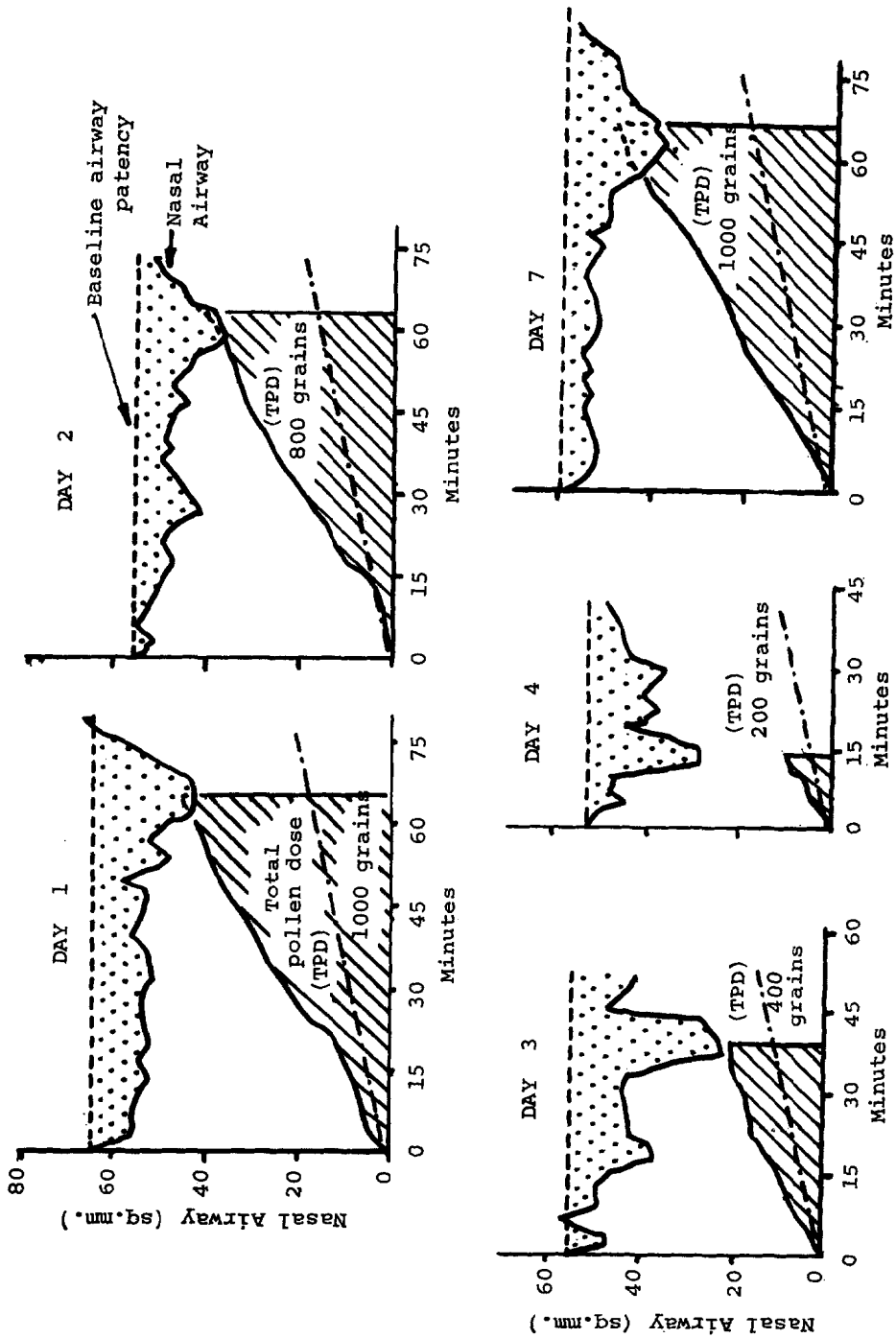


Fig. 3 Decrease in nasal threshold during 4 successive days of pollen challenge ("priming of the end organ"). Day 7 illustrates the increase in nasal threshold ("recovery of end organ") caused by absence of pollen challenge on the fifth and sixth days.

was not given on days 5 or 6. On day 7, a challenge dose as large as that given on day 1 was required to produce significant nasal congestion, indicating a decrease in nasal reactivity from day 4 to day 7.

5. Changes in nasal reactivity observed during and after the ragweed season

Two of the subjects were challenged at weekly intervals during the ragweed pollinating season; the other two were challenged less frequently. Fig. 4 illustrates challenges in R. D. A typical preseasonal day, June 20, is used as a reference. On this day, a TPD of 1,200 grains caused no significant congestion. Early in the season, August 18, the nasal membrane was already less resistant, and 800 grains produced a definite decrease in patency. Thereafter, progressively smaller doses produced greater decreases in patency. On September 9, 150 grains caused severe and prolonged congestion. Environmental pollen exposure was markedly decreased after September 8 and minimal or absent after September 15. Repetition of weekly challenges through the remainder of September and in October showed that an increase in nasal threshold was occurring, but it was not until November 29 that a pollen dose as large as that given during pre-seasonal challenges could once again be tolerated.

6. Comparison of four patients challenged on same day during pollinating season

Four subjects varying in their degree of sensitivity to ragweed were challenged on the same day during the pollinating season.

On August 25 (Fig. 5), B. W., the least-sensitive subject, had neither symptoms nor airway change during administration of a TPD of 950 grains, approximately three times the highest estimated environmental pollen inhalation rate. S. F., who had a low SSAT, inhaled 1,200 grains and experienced a moderate decrease in nasal patency. R. D. and L. A., who had higher SSAT's, showed decreases in nasal patency similar to S. F., but at one fourth to one eighth of the pollen dose.

Challenges were done on September 9 (Fig. 6) after two additional weeks of environmental exposure. B. W. had mild nasal congestion but no symptoms at a TPD of 1,000 grains. S. F. had approximately the same decrease in airway patency, but to one half of the pollen dose given August 25. R. D. had a greater and more prolonged episode of nasal congestion on September 9 with less than one half the pollen dose given August 25. These findings indicate again the increase in nasal reactivity following longer periods of environmental exposures and the variation in individual patients.

DISCUSSION

Two recent innovations in technology were applied to the study of allergic rhinitis.^{10, 12} With the previously described nasal airway measuring device,¹⁰ nasal congestion was rapidly and accurately assessed during acute episodes of allergic rhinitis caused by pollen inhalation. The pollen challenges described demonstrate the necessity for such a method of rapidly determining nasal air-

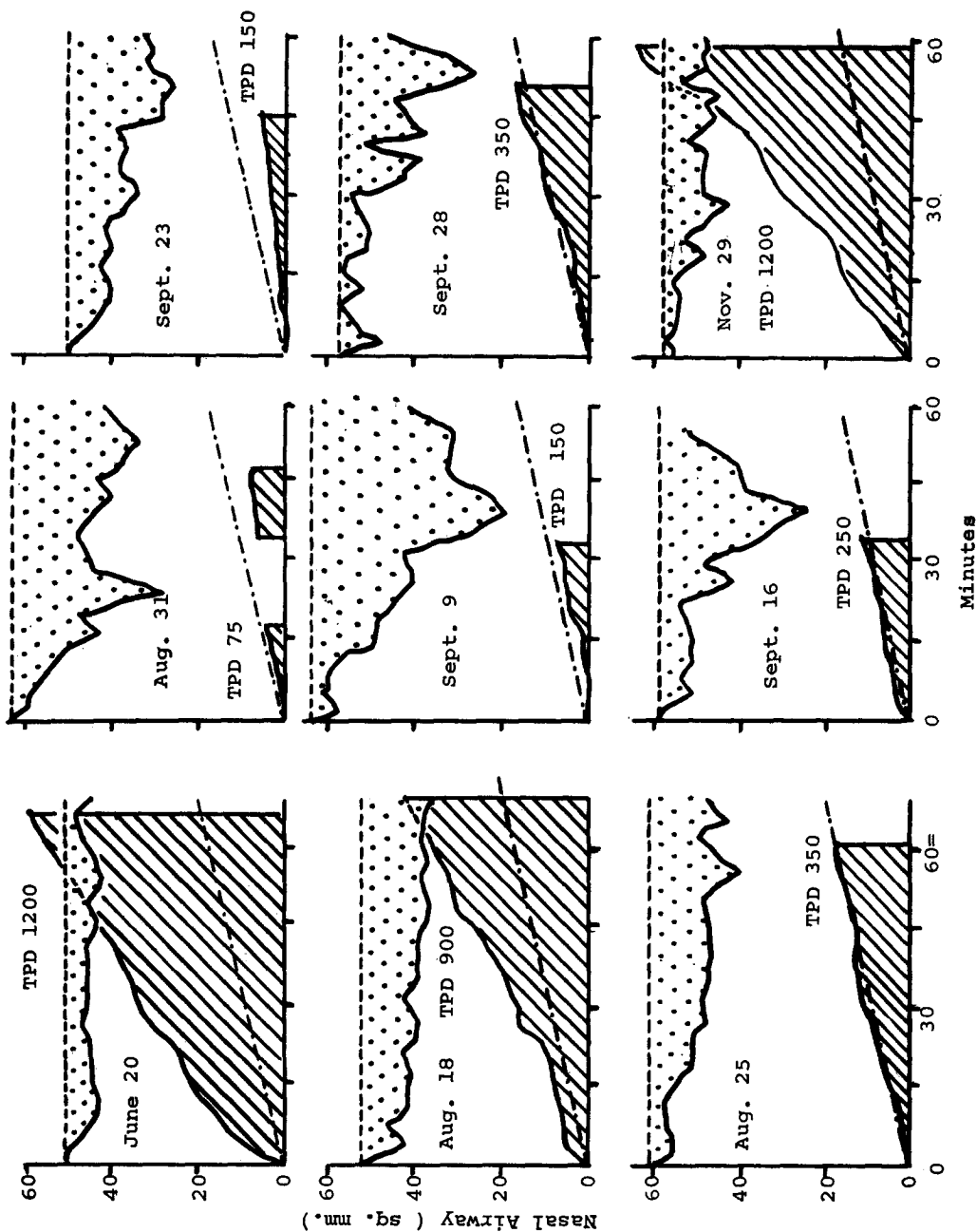


Fig. 4
Decrease in nasal threshold during ragweed pollinating season and subsequent recovery after the season.

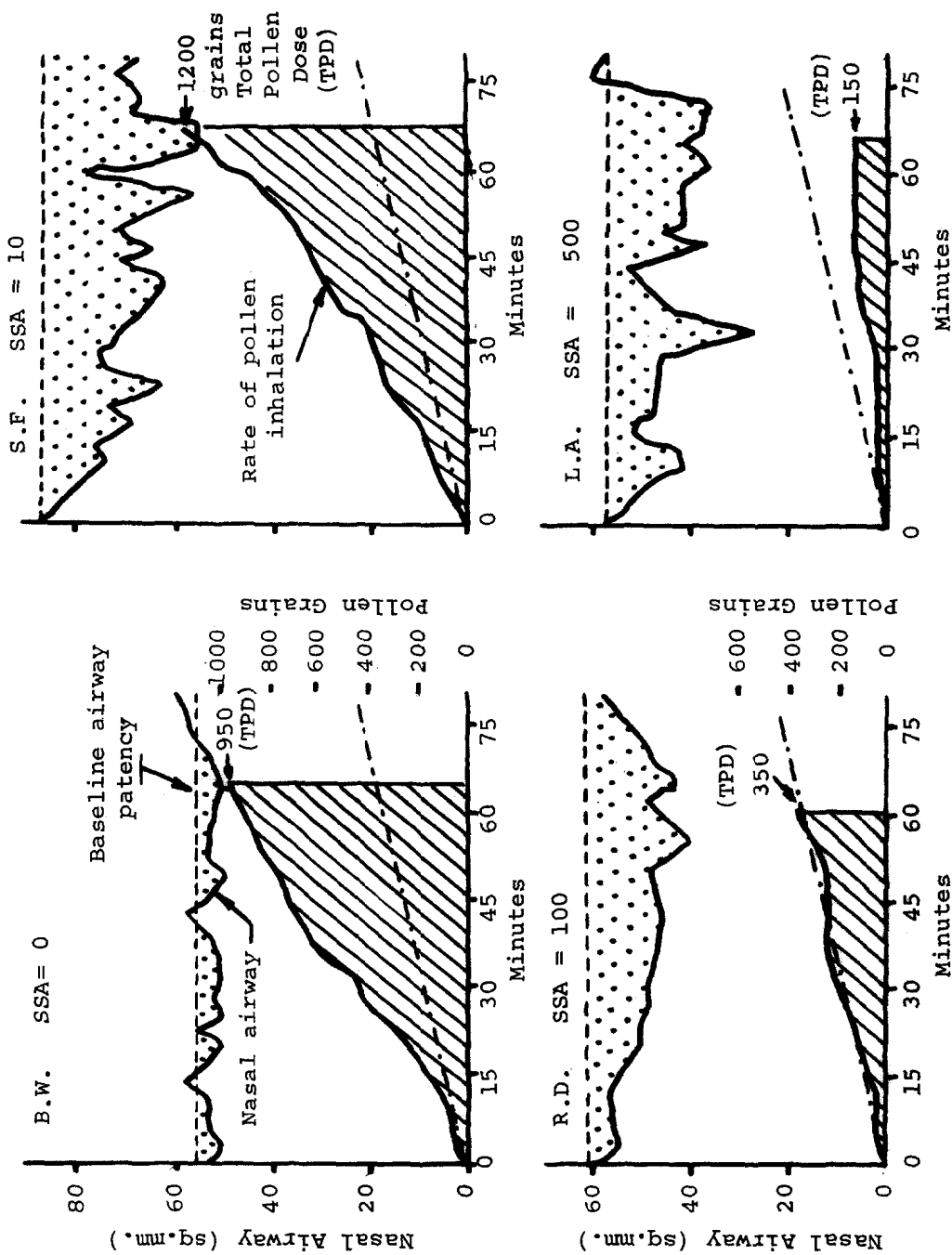


Fig. 5 Variation of nasal reactivity on August 25 during the ragweed pollinating season in four subjects who have different serological levels of skin-sensitizing antibody.

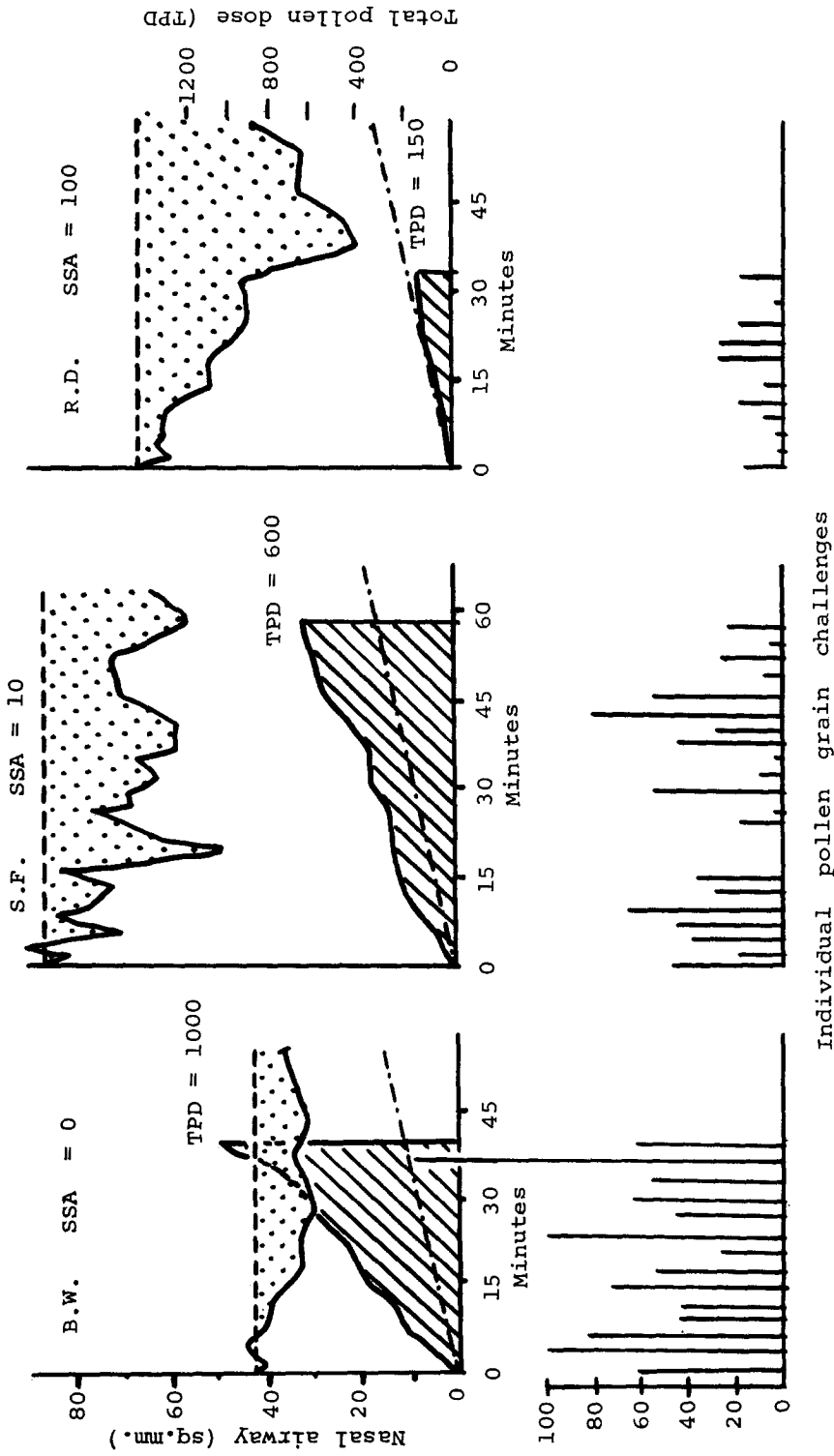


Fig. 6 Three of subjects shown in Fig. 6 challenged on September 9 illustrating increased nasal reactivity due to prolonged environmental exposure.

way, since changes in patency occurred much more quickly during and after challenge than had been expected. The pollen apparatus¹² provided a reliable instrument with which a quantitated allergic stimulus could be administered.

The pollen challenges described differed from environmental challenge in that defatted pollen was used as the challenging antigen. Several pollen samples obtained in the years 1961, 1963, and 1965 were used in preliminary experiments. All were found equally potent in causing allergic rhinitis in susceptible individuals, although subtle differences in potency would probably not be apparent.

The methods used made it possible to assess the effect of some psychic stimuli in allergic rhinitis. Placebo challenges were administered to six subjects. Neither nasal congestion nor symptoms occurred during these challenges. Each of the subjects had been challenged with pollen and developed symptoms and congestion on previous occasions. From their remarks made prior to placebo exposure, it was quite apparent that they expected to experience an episode of hay fever. These experiments illustrate that conditioning stimuli did not precipitate symptoms of allergic rhinitis during placebo challenge.

The greatest environmental pollen concentration we measured during the 1966 ragweed pollinating season was 0.66 grains per liter of air, which would indicate an inhalation rate on the order of 320 grains per hour at rest. We were surprised then, during early experiments, when a pollen inhalation rate of two to four times this amount caused only mild subjective symptoms, such as slight itching of the eyes and/or nose. Acute nasal congestion did not develop. In these early trials, subjects were only challenged every third day. Results differed markedly in later experiments in which subjects were challenged daily. An increase in nasal congestion and symptoms occurred on each succeeding day of challenge even though the total pollen dose administered was less on successive days. Thus the subjects reacted more and more severely to a constantly decreasing challenge. These findings suggest that repetitive challenges over a period of days altered the subjects in some manner. This phenomenon has been titled by the author "the priming effect on the end organ."

The priming effect also occurred spontaneously because of environmental pollen exposure during the ragweed pollinating season. The nasal threshold for pollen was high early in the season, whereas later in the season a much smaller pollen dose caused greater nasal congestion. Tuft, Blumstein, and Heck⁴ described similar findings. Their patients reacted to a more dilute pollen mixture during the season as compared to a higher pollen concentration required prior to the season. Feinberg and Steinberg² also showed that a smaller pollen dose was required to produce symptoms during the season.

The usual course of events in our subjects was one in which symptoms occurred during pollen challenges but stopped within one or two hours after exposure ceased. Occasionally symptoms recurred briefly on the evening of the challenge day or the next day if the patient had been challenged for several successive days. Symptoms rarely recurred when challenges were given for only one or two days. Lowell⁹ also reported that subjects he challenged experienced delayed nasal symptoms hours afterward or even on the day following challenge. He interpreted these as delayed symptoms and related them to the exposure

itself. A second and, we feel, more likely possibility is that late symptoms are due to environmental exposure to other antigens to which the patient is minimally sensitive or even to nonallergic stimuli. These antigens or stimuli generally do not cause symptoms in the subject but, if encountered after "priming" has occurred, may well produce overt rhinitis. This latter hypothesis requires that the priming effect be nonspecific; our preliminary experiments suggest that this is so.

The finding of a decrease in resistance, the priming effect, is not consistent with several previously published reports.¹³⁻¹⁵ Swineford and colleagues¹³ claimed hyposensitization of a number of subjects following daily inhalations of aerosolized ragweed pollen extract during the ragweed pollinating season. Treatment was started with doses of 0.25 ml. of a 1:41,000,000 to 1:400,000 dilution. The dose was increased on each succeeding day. These treatments were usually begun after seasonal symptoms had been present for a number of days. If these inhalations of aqueous extract cause desensitization even at the height of the season, it is difficult to understand why seasonal pollen exposure itself does not hyposensitize since it is present in low doses for 7 to 10 days even before most patients become symptomatic. Serious criticisms of the experiments of Swineford and colleagues¹³ are that evaluators knew what each patient received, and neither controls nor placebo-treated patients were followed.

Herxheimer¹⁴ also reported that inhalation of antigen extract caused temporary hyposensitization in some but not all patients. However, his experiments differed basically in that the subjects he challenged had asthma. He found that following daily inhalation of pollen extracts, a decrease in asthmatic symptoms occurred in 13 subjects, improvement was doubtful in 11, and in 7 symptoms were not decreased or were even worse. Two cases are of special interest because they relate directly to this report. In them, both asthma and hay fever occurred during the grass pollinating season. Asthma improved but hay fever did not after inhalation therapy.

Patterson¹⁵ reported inhalation challenges in one atopic dog. The animal clinically had allergic rhinitis and eczema associated with the ragweed season. Challenge was produced by spraying a suspension of ragweed pollen in saline into a chamber containing the animal. Hay fever symptoms occurred but could not be related to the pollen dose administered. The most likely reason for failure to produce a consistent "dose-allergic rhinitis symptom response" was that the animal could breathe through either his nose or mouth. Thus the dose administered to the chamber could not be directly related to the dose the dog inhaled through his nose. Asthma occurred when pollen challenges were excessive. However, if challenges were performed frequently, a greater pollen dose was required on each successive challenge to produce asthma. Our results are in direct conflict with those of Patterson,¹⁵ although it is doubtful that the experiments are comparable since he described this phenomenon in only one dog, the challenge was pollen in saline, and clinically, the dog had hay fever, with only excessive doses of pollen producing asthma.

Herxheimer¹⁴ and Patterson¹⁵ agree that asthma may be improved by antigen inhalation. Two hay fever patients of Herxheimer's and the hay fever patients

reported in this study were aggravated by antigen inhalation. Although both pollen asthma and allergic rhinitis are immunological diseases, it has not been shown that the same immunological mechanisms apply to both. It is possible that antigen inhalation could prove beneficial in some cases of asthma and still aggravate allergic rhinitis.

The antigen inhaled may also be a significant factor in determining whether improvement occurs. Swineford and associates¹³ used pollen extract, whereas we used defatted pollen grains. Perhaps pollen grains contain antigens or even nonallergic materials not present in the extract which could account for aggravation of symptoms.

The four subjects we studied showed a marked variation in response to the challenge dose during the ragweed pollinating season. The two patients who had the highest skin-sensitizing antibody titers exhibited more intense nasal congestion after small doses of pollen. The patient who had a titer of 10 had moderate symptoms and nasal congestion but only following a much greater pollen dose. Finally, the patient who had a sensitizing titer of zero had no symptoms and only moderate nasal congestion when challenged with high pollen doses. We have previously reported that subjective reports of symptoms made by patients correlated with skin-sensitizing antibody titer, the higher the titer the more severe the symptoms.¹⁶ The work being reported is in accord with these previous observations.

The hyperreactivity of the nasal membrane (the priming effect) produced by repeated daily challenge is reversible. In the laboratory, after priming was produced by four successive days of challenge, elimination of challenge for two days caused the nasal membrane to be once again as resistant as it had been on the first day of challenge. The priming of the nasal membranes caused by environmental exposure during the ragweed pollinating season was also reversible. However, in this case, four to eight weeks elapsed after pollination ceased before nasal reactivity returned to preseasonal levels. The findings suggest that the recovery period is related to the duration of challenge, recovery occurring rapidly after short periods of challenging but slowly when exposure to antigen has been of long duration.

The mechanism which causes the priming effect is unknown. Indeed, whether priming is due to local or systemic changes has not been established. One possible explanation for priming is that a systemic increase in the quantity or in the quality of skin-sensitizing antibody occurs. However, a recent report¹⁷ shows that the serum titer of sensitizing antibody did not change during the first few weeks of environmental exposure during the ragweed pollinating season. Therefore, it would be unusual if one hour of challenge during one or two days, such as occurred in our experiments, would be sufficient exposure to alter systemic skin-sensitizing antibody titers. It also seems unlikely that the recovery period, which can be as brief as several days, could be due to a fall in serum sensitizing titer. Local production of skin-sensitizing antibody or alterations in preformed antibody could occur rapidly enough to explain priming. Even more likely is the possibility that the first pollen challenges cause a chemotactic response so that antibody-containing cells or cells containing chemical mediators of

the allergic reaction migrate into the nasal membranes. Thus, on subsequent challenges cells required for the production of an allergic reaction are already available locally. Finally, repeated challenge may in some manner cause alterations in the tissue itself, decreasing its threshold for pollen.

The concept of the priming effect requires a reappraisal of allergic rhinitis and of possibly other immunological diseases. Generally, it has been assumed that the reaction of an individual to the same amount of antigenic exposure is the same at different times or that a greater exposure would produce more symptoms than a lesser one. In the subjects we studied, the total pollen dose, although a significant factor, was less important than the priming effect in determining the severity of any particular allergic reaction.

The priming effect may be of critical importance in interpretation of provocative inhalant testing of the type used in clinical diagnosis. It has been shown that a given quantity of antigen may not produce symptoms at one time and yet on another occasion may cause severe allergic symptoms. Thus, a provocative challenge done at a time when the nasal membrane has been free of antigen exposure may not cause symptoms, whereas at another time, when environmental exposure has been frequent, provocative challenge may produce a marked allergic reaction.

It should be stressed that the foregoing discussion applies only to the condition in which the antigen enters the body through a membrane, such as the nasal mucosa. It may not apply to situations in which the antigen is administered by injection.

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