

Exposure of hay fever subjects to an indoor environmental grass pollen challenge system

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Summary

A new indoor nasal allergen challenge system has been developed which allows subjects with hay fever to be exposed to pre-determined concentration of grass pollen grains in the immediate environment. Thirteen subjects were exposed during the winter months to an atmosphere containing approximately 1000 grains/m³ for 30 min. No changes in nasal airway patency or symptoms of nasal congestion were recorded. It was necessary to increase the pollen concentration to approximately 9000 and 35 000 grains/m³ in two subjects and six subjects respectively, before a positive response was recorded. This response was reproducible in all subjects. Administration of intra-nasal sodium cromoglycate before exposure significantly inhibited the decrease in nasal airway patency and when administered 1 min before exposure totally abolished symptoms of nasal congestion. This new challenge system may therefore be used as an *in vivo* screen to assess the potential therapeutic value of drugs in hay fever under controlled conditions.

Introduction

Hay fever symptoms can be produced in sensitive subjects by instilling an extract of grass pollen into one or both nostrils or by natural exposure to atmospheric grass pollen during the summertime. The protective effects of drugs in hay fever can be assessed by measuring the characteristics of air flow through the nose in the former and by asking the patient to record the incidence and severity of hay fever symptoms each day in the latter. Both methods, however, have disadvantages.

Intra-nasal application of allergen extract is unnatural and the incidence and/or severity of nasal irritation, rhinorrhoea, sneezing and congestion is sometimes different to the incidence and severity of the nasal symptoms obtained by natural exposure. In addition, the concentration of allergen extract necessary to evoke a positive response is frequently higher than the concentration of the same extract that produces a positive weal and flare response when pricked into the skin. The risk of an

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anaphylactic reaction is therefore higher following intra-nasal allergen challenge and care must therefore be taken when allergen extracts are instilled into the nose of patients with asthma, patients with a high degree of atopy and patients with an obvious correlation between symptoms and natural exposure to specific allergen.

The assessment of the efficacy of drugs in the treatment of hay fever involves recording hay fever symptoms each day by the patient while being treated with drugs, orally or topically, under controlled conditions. The intensity of the allergen challenge is dependent on the concentration of grass pollen in the atmosphere and the duration of outdoor activity by the patient. Neither of these factors are controlled during clinical trials and it was therefore decided to build an artificial indoor challenge system which could control both factors but still mimic natural exposure.

The concentration of grass pollen in the atmosphere is normally expressed as the mean number of pollen grains/m³ over 24 h. It is considered to be high when it reaches 200 grains/m³ (gs/m³). Figure 1 illustrates the diurnal variation on such a day. Concentrations in excess of 1000 gs/m³ therefore occur during several 30 min periods and these criteria were used for the indoor environmental challenge system. This paper describes the challenge system in detail and a series of studies carried out to establish the incidence and reproducibility of the response and the identification of a positive control to see if the system was capable of detecting drug-induced inhibition of a positive response.

Subjects

All subjects were employees of Glaxo Group Research, Ware, Herts, with a history of mild hay fever. Only subjects with a positive skin test (weal > 5mm) to Bendards Mixed Grass Pollen Extract (B12) and a well-defined recent history of hay fever, requiring intermittent treatment with oral anti-histamines or topical nasal decongestants, were candidates for the study. Subjects requiring nasal steroids were not considered. No

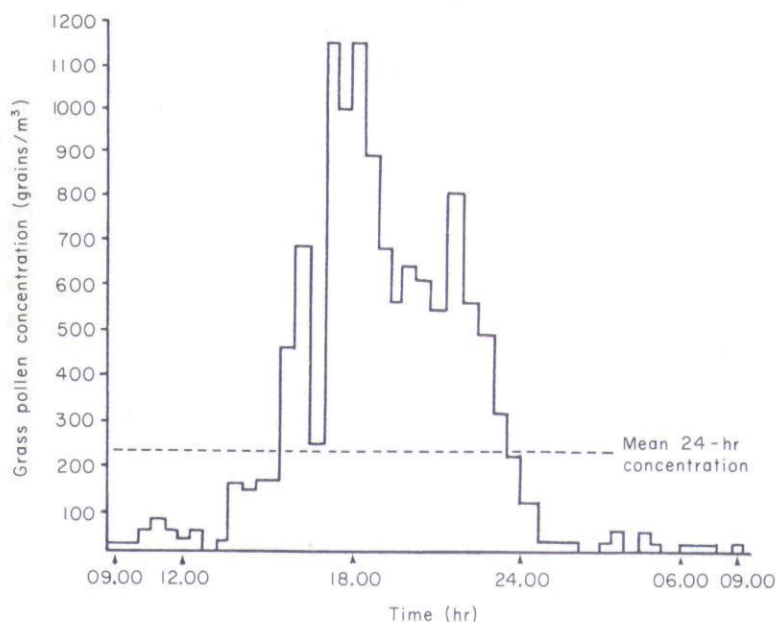


Fig. 1. Diurnal variation in grass pollen concentration 11.6.78.

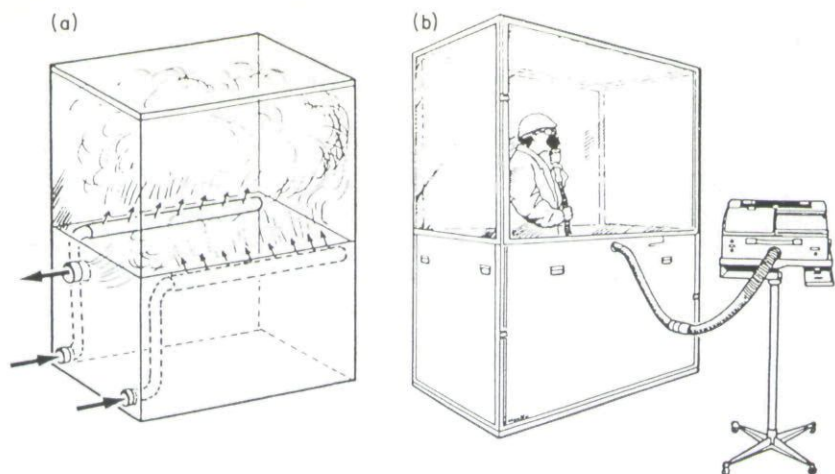


Fig. 2. Diagram of challenge system. (a) Air circulation: dimensions, height = 210 cm, length = 156 cm, width = 96 cm. (b) Use of vitalograph within system.

subject was allowed to take part on any study day if symptoms of nasal congestion were present on that day. All subjects gave informed consent, and approval from an Ethical Committee was obtained before the start of each study.

Challenge system

A box was built 190 cm high, 156 cm long and 96 cm wide (Fig. 2). The lower half was made of blockboard, 3 cm thick, and the upper half of transparent polythene, 2 mm thick. A circulation pump (Secomak Air Products, Stanmore, model 574) was used to blow air into the box through two ports connected to rigid plastic tubes, 15 cm diameter, running along both sides of the box beneath the blockboard/polythene boundary. A 2 mm slit was cut along the length of each tube so that air was forced out through these slits ten degrees upwards from the horizontal. The same pump extracted air from the box through a single port, 15 cm diameter.

Grass pollen (30 mg) was weighed into a transparent, hard, size 3, gelatin capsule and three 1 mm holes punched carefully into both ends. This capsule was then placed inside a larger gelatin capsule, size 0, which also had three 1 mm holes punched through both ends. The dual capsule was placed inside a round 500 ml flask and the grass pollen released slowly into the box by passing air at 15 l/min through the flask and into the box. This rate of air flow was found to be sufficient to cause the capsule to vibrate and shake the pollen grains slowly out of both capsules and into the stream of compressed air.

The atmosphere in the box was sampled continuously for grass pollen grains by drawing air through a slit sampler at 15 l/min for 30 min. During this time a glass slide (7.5×2.5 cm) covered with a film of white soft paraffin, 0.5 mm thick, was drawn up behind the slit (1.5×0.2 cm). At the end of the 30-min period the slide, with grass pollen grains impinged on the paraffin film, was prepared for microscopic examination by covering the film with warm glycerine jelly, sealing with a coverslip and allowing it to cool. The concentration of grass pollen grains at 3 min intervals and the mean concentration over the 30-min period was calculated. Preliminary experiments had established that 30 mg grass pollen introduced by this method maintained a grass pollen concentration of 1000 grs/m³ over 30 min.

Method

Before any subject entered the box their garments, hair and eyes were covered with overalls, cap and swimming goggles. The maximum volume of air exhaled in 1 sec through the mouth ($M.FEV_1$) and the maximum volume of air inhaled through the nose in 0.5 sec ($N.FIV_{0.5}$) was measured using a Vitalograph. The Vitalograph stood outside the box but connected to the inside atmosphere using flexible tubing. $M.FEV_1$ was measured to determine if exposure to grass pollen affected lung function and $N.FIV_{0.5}$ was measured as an index of nasal congestion. The sensitivity and reproducibility of $N.FIV_{0.5}$ of nasal airway potency has been described elsewhere [1]. Pre-exposure $M.FEV_1$ and $N.FIV_{0.5}$ were measured every 5 min for 15 min. The highest $M.FEV_1$ and the mean $N.FIV_{0.5}$ were recorded.

At zero time the subject entered the box and the door was closed. Air was circulated and grass pollen introduced into the box for 30 min. During this time the air was sampled continuously for grass pollen grains and at pre-defined time intervals, i.e. zero + 2.5 min, + 7.5 min, + 12.5 min, + 17.5 min, + 22.5 min, + 27.5 min, the subject was asked to complete a visual analogue scale marked 0–10 to represent the severity of nasal irritation, rhinorrhoea and congestion. (0 = no symptoms, 10 = maximum severity). The highest score for any individual symptom was therefore 60, and the highest total symptom score 180. The incidence and time of sneezing were also recorded. After completion of the visual analogue scale, $M.FEV_1$ (highest of two manoeuvres) and $N.FIV_{0.5}$ (mean of two manoeuvres) were measured and recorded.

Results

Thirteen subjects (nine male, mean age = 38, range: 23–50 years) took part in a series of studies. No hay fever symptoms were recorded by any subject despite the fact that 30 mg grass pollen produced a mean ($\pm$ s.d.) 30-min grass pollen concentration of 2136 ($\pm$ 710) grs/m³. The amount of grass pollen in the inner capsule was increased to 60 mg. This produced a mean ($\pm$ s.d.) 30-min grass pollen concentration of 4252 ($\pm$ 1030) grs/m³. Again no subject recorded hay fever symptoms.

Increasing the amount of grass pollen to 90 mg produced a positive response in two subjects on three occasions when the mean ($\pm$ s.d.) 30-min concentration was 9147 ($\pm$ 2660) grs/m³. A positive response was also obtained in a further six subjects on eight occasions when 180 mg grass pollen produced a mean (s.d.) 30-min concentration of 35352 ($\pm$ 8724) grs/m³. No response was obtained in the remaining five subjects using 90 mg grass pollen which produced a mean ($\pm$ s.d.) 30-min concentration of (7014 $\pm$ 4835) grs/m³ or when using 180 mg grass pollen which produced a mean ($\pm$ s.d.) 30-min concentration of 34267 ($\pm$ 8068) grs/m³.

The results of each challenge are listed in Table 1. It appeared that a positive response correlated with a fall in $N.FIV_{0.5}$ of at least 25% at the end of the exposure period plus a total symptom score over the 30 min of at least 150. $M.FEV_1$ remained unchanged in all subjects on all occasions (data are not presented). All eight subjects in whom a positive response was produced agreed to undergo four consecutive exposures to investigate the reproducibility of the response. Each occasion was separated by at least 3 but not more than 7 days. $M.FEV_{0.5}$ was again unaffected and data not presented. The effect of successive 30-min exposure to grass pollen is illustrated in Fig. 3. There was no evidence of increased or decreased response in terms of $N.FIV_{0.5}$ or total symptom score over four exposures. In two subjects on three occasions, however, the total symptom score was less than 150 units. On these three occasions the total

Table 1. Effect of 30-min exposure of subjects with hay fever to grass pollen on N.FEV_{1.0} (l) and nasal symptoms

Subject	Mean grass pollen concentration (grs/m ³)	Total symptom score	N.FEV _{1.0} before and during exposure						
			Zero	2.5 min	7.5 min	12.5 min	17.5 min	22.5 min	27.5 min
(a) Subjects who responded to 90 mg grass pollen									
1	10 744 (1)	190	1.20	0.90	1.00	1.00	1.00	0.85	0.80*
	5391 (1)	170	1.60	1.30	1.25	1.05	0.90	0.90	0.90
2	11 308 (1)	352	1.00	0.70	0.60	0.55	0.40	0.40	0.30*
(b) Subjects who responded to 180 mg grass pollen									
3	7277 (1)	nil	2.30	2.30	2.10	1.90	2.00	2.00	2.10
	25 461 (2)	210	2.75	2.55	1.90	1.95	1.75	1.60	1.60*
4	34 429 (2)	270	2.50	2.30	2.00	1.80	1.70	1.65	1.55*
	10 711 (1)	nil	0.70	0.70	0.80	0.75	0.70	0.70	0.75
5	69 966 (2)	150	1.10	0.70	0.70	0.65	0.70	0.70	0.65*
	13 982 (1)	nil	1.20	1.15	1.15	1.25	1.10	1.05	1.05
6	20 929 (2)	180	1.25	1.10	0.75	0.85	0.85	0.80	0.70*
	7415 (1)	nil	1.20	1.15	1.15	1.25	1.10	1.05	1.05
7	50 922 (2)	170	1.30	1.40	1.15	1.20	1.05	1.05	0.80*
	10 545 (1)	35	1.35	1.40	1.20	1.05	1.00	0.90	0.85
8	50 807 (2)	255	1.10	0.95	0.95	0.50	0.55	0.50	0.50*
	8467 (1)	20	1.60	1.40	1.40	1.35	1.40	1.40	1.40
9	16 333 (2)	270	1.60	1.40	1.30	1.30	1.30	1.30	1.25*
	13 972 (2)	210	1.50	1.65	1.30	1.10	1.20	1.10	1.15*
(c) Subjects who did not respond to 180 mg grass pollen									
9	1841 (1)	nil	1.60	1.80	1.75	1.70	1.30	1.40	1.50
	7501 (1)	nil	1.70	1.60	1.30	1.30	1.40	1.65	1.65
10	49 687 (2)	45	1.55	1.45	1.30	1.30	1.35	1.40	1.30
	8722 (1)	nil	0.60	0.65	0.60	0.50	0.50	0.60	0.55
11	9735 (1)	nil	0.60	0.45	0.60	0.70	0.70	0.50	0.50
	36 948 (2)	117	0.60	0.70	0.65	0.60	0.60	0.60	0.65
12	3021 (1)	nil	1.60	1.55	1.55	1.70	1.55	1.60	1.70
	3048 (1)	nil	1.50	1.70	1.70	1.60	1.60	1.50	1.50
13	6766 (1)	20	1.60	1.60	1.60	1.70	1.75	1.60	1.50
	23 584 (2)	nil	1.70	1.70	1.60	1.40	1.35	1.45	1.50
14	26 013 (2)	27	2.10	2.10	2.00	1.95	2.05	2.25	2.05
	35 720 (2)	45	1.85	1.80	1.80	1.70	1.85	1.70	1.65
15	2026 (1)	nil	0.75	0.75	0.70	0.75	0.65	0.70	0.65
	10 140 (1)	nil	0.95	0.75	0.75	0.75	0.75	0.85	0.65
16	30 282 (2)	20	0.90	0.90	1.00	0.90	0.80	0.70	0.75
	27 235 (2)	nil	0.90	0.75	0.75	0.75	0.75	0.75	0.70
17	17 345 (1)	nil	0.95	0.90	0.85	0.80	0.75	0.70	0.80
	30 652 (2)	20	1.00	0.95	0.85	0.90	0.90	0.80	0.90
18	36 535 (2)	110	1.00	0.80	0.90	0.95	0.90	0.90	0.90
	46 019 (2)	120	0.95	1.00	0.80	0.85	0.85	0.80	0.85

Figures in parentheses indicate the number of 90 mg grass pollen capsules released into the C.A.B.

* Positive response.

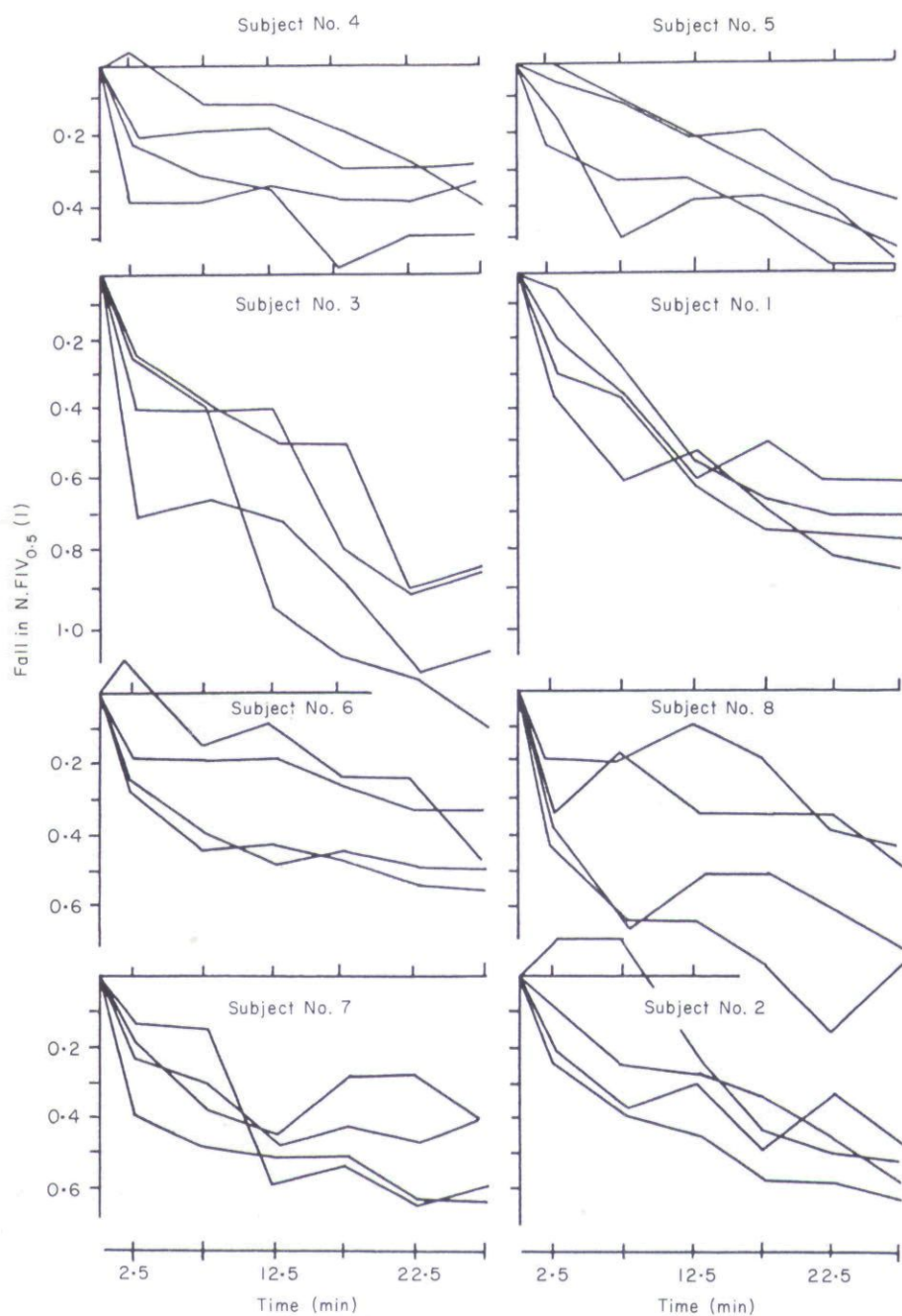


Fig. 3. Changes in $N.FIV_{0.5}$ (l) during exposure of hay fever subjects to grass pollen for 30 min.

symptom scores of 140, 120 and 145 coincided with falls in $N.FIV_{0.5}$ of 45%, 58% and 38%, respectively. It was considered that a positive response had been achieved in these circumstances and the criteria for a positive response was modified to a minimum fall in $N.FIV_{0.5}$ of 25% at the end of 30-min exposure period plus a total symptom score of at least 120.

Only five subjects were available for further investigation aimed at establishing whether or not the challenge system was capable of detecting drug-induced inhibition of a positive response. Sodium cromoglycate (0.16 ml of a 2% solution) was sprayed into each nostril 1 min before the subject was exposed to grass pollen in the box. The objective response was characterized as the area between the serial $N.FIV_{0.5}$ data and the time axis. The area following sodium cromoglycate was significantly smaller than the area following the mean response of the four consecutive exposures in the previous study (Student's t test: $P=0.05$). The mean total symptom scores are listed in Table 1. Prior administration of sodium cromoglycate markedly reduced the mean total symptom score from 201 to 29.

The preceding study was repeated under double-blind conditions. Three treatments were involved. Sodium cromoglycate 15 min before exposure, sodium cromoglycate 1 min before exposure, and placebo. The mean results are illustrated in Fig. 4. Sodium cromoglycate significantly reduced the area between the $N.FIV_{0.5}$ and time axis, irrespective of the time of administration (Student's t test: $P=0.05$). The marked reduction in symptoms following administration of sodium cromoglycate 1 min before challenge observed in the open evaluation was observed again, the mean total symptom score falling in this study from 171 to 24. In contrast there was no significant

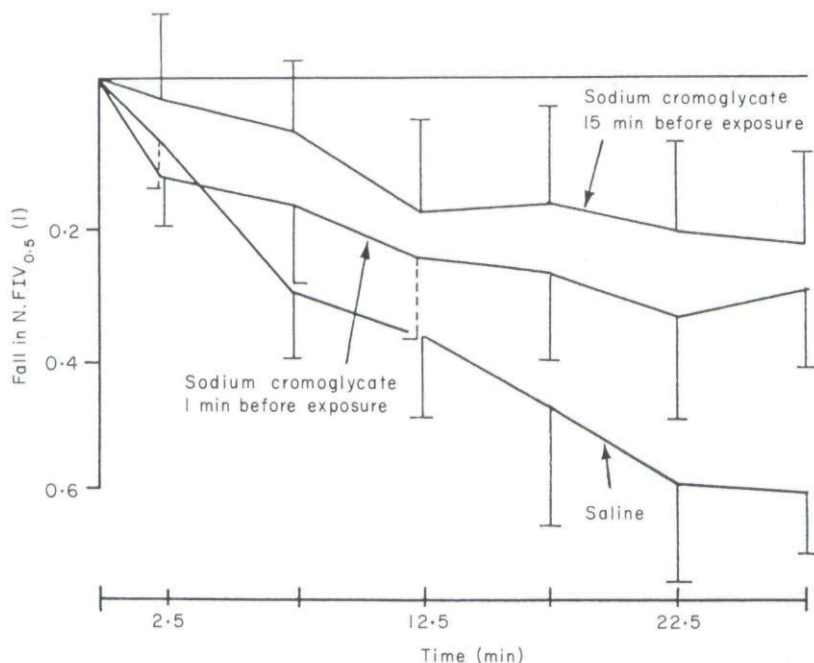


Fig. 4. Effect of sodium cromoglycate on mean $N.FIV_{0.5}$ (l) in five subjects with hay fever during exposure to grass pollen for 30 min—double-blind placebo control study. Vertical bars represent s.d. Mean symptom score after placebo = 171. Mean symptom score after sodium cromoglycate (-1 min) = 24. Mean symptom score after sodium cromoglycate (-15 min) = 212.

difference between the mean total symptom score following sodium cromoglycate 15 min before exposure and placebo.

Discussion

There are no published reports describing similar attempts to establish a controlled natural allergen challenge system, although Anderson *et al.* [2] described an environmental chamber in which they suspended polymerized plastic coated with carbon to investigate the physiological defence characteristics of the nose. It was surprisingly difficult to maintain grass pollen grains in suspension within the box for 30 min considering that thermal currents are sufficient to transport them to the top of high buildings (approximately 70 ft high), where the atmosphere is normally sampled for grass pollen grains, and that Rempé [3] found tree pollen grains present in the atmosphere 200 m above German pine tree forests. Several modifications to the challenge system were necessary and the specification (1000 gr/m³ over 30 min) was only achieved by the gradual introduction of a relatively large quantity of grass pollen into the box.

In the present system the rotation of the gelatin capsule which released the pollen grains into the box was random in nature and dependent on the turbulence produced by forcing air through the flask containing the grass pollen capsules. More sensitive control of pollen release should produce greater control of the grass pollen concentration over the 30-min exposure period. Attaching the capsule to a magnetic stirrer did not achieve greater control and perhaps more sensitive systems to establish continuous grass pollen concentration, e.g. Coulter counter techniques, may be of benefit. Such systems could then be linked to a method of increasing or decreasing the release of grass pollen from the gelatin capsules to achieve instantaneous control of grass pollen concentrations within pre-described limits.

Exposing the thirteen subjects in the challenge system produced two interesting features in that a positive response was only obtained in eight subjects, and in these subjects the concentration of pollen necessary to elicit a response was very high. No explanation can be offered for the non-response in the five subjects, even though they all suffered from hay fever symptoms in the summer preceding the artificial challenges. Similar results, however, were obtained by Pelikan [4] who subjected 600 patients with allergic rhinitis to nasal allergen challenge using allergen extracts. A positive response was only obtained in 83% of the patients. The high pollen concentrations necessary to produce a positive response in the present challenge system is also difficult to explain. The studies were carried out between October and March when the concentration of grass pollen specific IgE would be low [5] and/or the sensitivity of the end-organ response also low. Lower concentrations may have been successful in subjects with a history of more severe hay fever, or during the summer months in the original population when natural exposure would have produced the characteristic 'priming' effect [6]. It is traditional, however, to carry out nasal allergen challenge during the winter months to minimize inconvenience to the patient and to obtain pre-treatment pre-challenge data free from nasal congestion.

The positive response in the eight subjects was reproducible and subject to inhibition by pre-treatment with sodium cromoglycate. The response, however, was similar in intensity and severity to the response obtained by instilling extracts of grass pollen into the nose. The artificial challenge system however retains the benefit of mimicking natural exposure and should be useful in evaluating the potential

therapeutic properties of drugs which could prevent or inhibit the nasal symptoms present in hay fever sufferers. Ideally a positive response should be obtained with concentrations of grass pollen found in the air during the summer season. Further work is necessary to establish if this is possible with the challenge system described in this article.

Acknowledgments

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