

Polyethylene glycol-modified ragweed extract: Comparison of two treatment regimens

E. F. Juniper, M.C.S.P., J. O'Connor, R.N., R. S. Roberts, M.Tech.,
S. Evans, B.Sc., F. E. Hargreave, M.D., and J. Dolovich, M.D.
Hamilton, Ontario, Canada

Fifty ragweed-sensitive subjects were each administered a course of 12 injections of polyethylene glycol-modified ragweed extract. To investigate the effect of two treatment regimens, subjects were paired according to their skin sensitivity to ragweed extract and the severity of their rhinoconjunctivitis the previous year. One of each pair was randomly allocated to receive injections at weekly intervals, and the other subject was to receive injections daily until a local or systemic adverse reaction occurred and then to switch to a weekly schedule for the rest of the course. Blood was obtained immediately before each injection and at the end of the study to examine the rates of change of ragweed-specific IgE and IgG antibody levels and to evaluate whether these or other variables could be used to predict the occurrence of adverse reactions. Immediate reactions were minimal in both groups, but more late reactions occurred in subjects receiving the modified rush regimen; therefore, although the total number of injections received by the two groups was similar, the total dose of protein injected in the rush group was only half that achieved in the weekly group. Treatment induced increases in ragweed-specific IgE and IgG antibodies in both groups; the increase in IgE was similar in the two groups, but the increase in IgG was higher in the weekly group. There was a marked decrease in skin sensitivity to ragweed extract after treatment that was directly related to the IgG antibody response, and therefore, these changes were greater in the weekly group. Initial skin sensitivity to ragweed and a previous adverse reaction appeared to be the best determinants of the occurrence of an adverse reaction, but the relationships were too weak for these indices to be useful as predictors in individual cases. (J ALLERGY CLIN IMMUNOL 78:851-6, 1986.)

PEG-modified ragweed extract can reduce symptoms of ragweed pollen-induced rhinoconjunctivitis when it is administered in a sufficiently high dose.¹ A series of injections delivering a total dose >400 µg of protein, calculated to be equivalent to 28.9 µg of AgE, induces a rise in ragweed-specific IgG antibody

Abbreviations used

PEG: Polyethylene glycol
AgE: Antigen E

From the Departments of Medicine, St. Joseph's Hospital and McMaster University, Department of Clinical Epidemiology and Biostatistics, and Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada.

Supported by a grant from Pharmacia Diagnostics, AB, Uppsala, Sweden.

Received for publication Dec. 3, 1985.

Accepted for publication April 2, 1986.

Reprint requests: E. F. Juniper, M.C.S.P., Firestone Regional Chest and Allergy Unit, St. Joseph's Hospital, 50 Charlton Ave., East, Hamilton, Ontario, Canada L8N 4A6.

ies and reduces nasal and eye symptoms and the amount of medications required during the pollen season.¹ In previous studies injections were administered at weekly intervals during a period of 10 to 12 weeks. This study was designed to investigate the effectiveness of administering the injections by a modified rush regimen in which the dose is increased daily until there is an adverse reaction. Such a regimen would be more convenient and allow initiation of treatment shortly before the onset of seasonal pollen exposure. At the same time we recorded the doses that produced ad-

TABLE I. Subject characteristics

	Weekly	Rush
Skin sensitivity to ragweed (μg)*		
0.2	2	4
2.0	20	17
20.0	2	1
200.0	1	3
Skin sensitivity to PEG-modified ragweed (μg)†		
2.0	1	0
20	2	1
200	14	13
2000	4	6
>2000	4	5
Severity of ragweed-induced rhinoconjunctivitis the previous year		
Mild	11	9
Moderate	11	13
Severe	3	3
Gender		
M	18	13
F	7	12

*Lowest concentration of ragweed causing a 3 mm wheal.

†Lowest concentration of PEG-modified ragweed causing a 3 mm wheal.

verse reactions and attempted to identify variables that could be used to predict their occurrence. This allowed us to investigate the potential for initiating treatment at higher doses in the future.

SUBJECTS AND METHODS

Subjects

Fifty ragweed-sensitive adults, with a history of seasonal rhinoconjunctivitis, volunteered to participate in the study (Table I). None had received treatment with PEG-modified ragweed extract previously or any other immunotherapy within the previous 12 months. There were 31 male and 19 female subjects, aged from 19 to 63 years. Each had a history obtained by questionnaire, a physical examination, an ECG, and blood and urine obtained for routine laboratory screening. Subjects with illnesses other than allergic rhinoconjunctivitis and asthma, pregnant women, and those at risk of pregnancy were not included in the study. The protocol was approved by the Research Committee of St. Joseph's Hospital. All subjects gave informed written consent.

Study design

Each subject had duplicate skin prick tests with tenfold serial dilutions of common ragweed extract, *Ambrosia elatior* (0.002 to 200 $\mu\text{g}/\text{ml}$), PEG-modified common ragweed extract (0.002 to 2000 $\mu\text{g}/\text{ml}$), and histamine (1mg/ml). Extracts were prepared from the same raw material and, as

TABLE II. Percentage of injections producing reactions

Reactions	Weekly	Rush
None or local <4 cm	77	69
Local 4 to 10 cm	18	22
Local >10 cm	5	9
Systemic	4	8

in our previous study,¹ were supplied by Pharmacia Diagnostics AB, Uppsala, Sweden. Subjects were paired according to their skin sensitivity to ragweed and PEG-modified ragweed, the severity of ragweed-induced hay fever the previous year, and gender. One subject of each pair was assigned randomly to receive all the PEG-modified ragweed extract injections at weekly intervals, and the other subject was to receive the injections by a modified rush regimen. In the latter, the injections were administered daily (except at weekends) until there was a local reaction >4 cm in diameter or systemic symptoms occurred; all subsequent injections were administered at weekly intervals. In both groups all subjects were scheduled to receive 12 injections starting at a dose of 0.2 μg of protein (0.015 μg of AgE). This was doubled at each clinic visit up to a maximum of 600 μg (45 μg of AgE). This was the same dosing regimen that was used in our previous study.¹ Immediate reactions were recorded in the clinic 20 minutes after each injection, and then the subjects recorded the size and characteristics of local and systemic reactions at 8 to 12 hours, 24 hours, 48 hours, and 72 hours with a standard questionnaire. If a local reaction 4 to 10 cm in diameter or mild systemic symptoms occurred, the dose of the next injection was not increased. If the local reaction was >10 cm in diameter or if there were moderate systemic symptoms that did not require treatment, the dose of the next injection was reduced. If a local reaction extended from elbow to shoulder or if systemic symptoms required treatment, no further injections were administered.

Blood was obtained before each injection and 1 week after the last injection for the measurement of ragweed-specific IgE and IgG antibody levels. The duplicate skin prick tests with serial dilutions of ragweed extract and PEG-modified extract were repeated after the last injection. During the injection period, the skin test solutions, made up in 50% glycol, were stored at 4° C. At the end of the study, each subject had duplicate skin prick tests with a solution of PEG, 8000 molecular weight (Ayerst Laboratories, Montreal, Canada), 10 mg/ml in 50% glycol. Any response with a wheal 3 mm or larger was considered positive.

Ragweed-specific IgE serum antibody levels were measured by RAST,² and IgG antibody levels were measured by a technique based on the binding of the IgG of test serum to a Sepharose protein A complex followed by the addition of ¹²⁵I-labeled ragweed.³ Results were derived by reference to standard curves produced with separate IgE and IgG antiragweed antibody-containing serum pools with undiluted reference serum arbitrarily designated 100 units.

Analysis

Group differences were examined with unpaired *t* tests and within subject changes with paired *t* tests. Linear regression analysis was used to examine relationships between doses of PEG-modified ragweed extract and immunologic and skin-sensitivity changes with treatment. Discriminant function analysis was used to examine variables believed to be important in predicting adverse reactions. Differences were considered significant at $p < 0.05$ (two-tailed).

RESULTS

Fifty subjects started the study, and 38 of these subjects received the full course of 12 injections. Six subjects in the weekly group and five in the rush group were withdrawn because of large local reactions. One subject, in the rush group, withdrew after two injections despite no adverse reaction.

Six subjects in the weekly group and two in the rush group received the maximum cumulative dose of 1255.4 μg of protein (94.2 μg of AgE); all other subjects had one or more reactions that necessitated holding or reducing the dose on one or more occasions. One subject developed mild nasal stuffiness immediately after one injection, but there were no other immediate systemic reactions. Immediate local reactions were mild and never exceeded 6 cm in diameter. Late local and systemic adverse reactions (more than 8 hours after injection) occurred more frequently in the rush group ($p < 0.01$) (Table II), and the first dose producing systemic symptoms or a local reaction >4 cm in diameter tended to be lower in the rush group (mean = 5.33 μg of protein, SD = 3.76; 0.40 μg of AgE, ± 0.28) than in the weekly group (mean = 7.20 μg of protein, SD = 4.12; 0.54 μg of AgE, ± 0.31). As a result, the mean cumulative dose achieved by the weekly group was 483.1 μg of protein and 36.2 μg of AgE (range 15.4 to 1255.4 μg of protein) (Fig. 1) that was significantly higher than the 264.3 μg of protein and 19.8 μg of AgE (range 9.0 to 1255.4 μg of protein) achieved by the rush group ($p < 0.05$), even though the mean number of injections received by the two groups was similar (weekly = 11.28; rush = 11.30). After the change to a weekly schedule, 40.1% of injections produced late reactions in the rush group compared with an overall reaction rate of 24.8% in the weekly group. The mean number of reactions per subject was 3.0 in the weekly group and 3.8 in the rush group. Systemic symptoms, in both groups, were mild and included rhinoconjunctivitis and asthma. These developed between 15 minutes and 24 hours of the injection and lasted up to 3 days. However, the injections were administered during the grass-pollen season, and these symptoms may have been due to natural allergen ex-

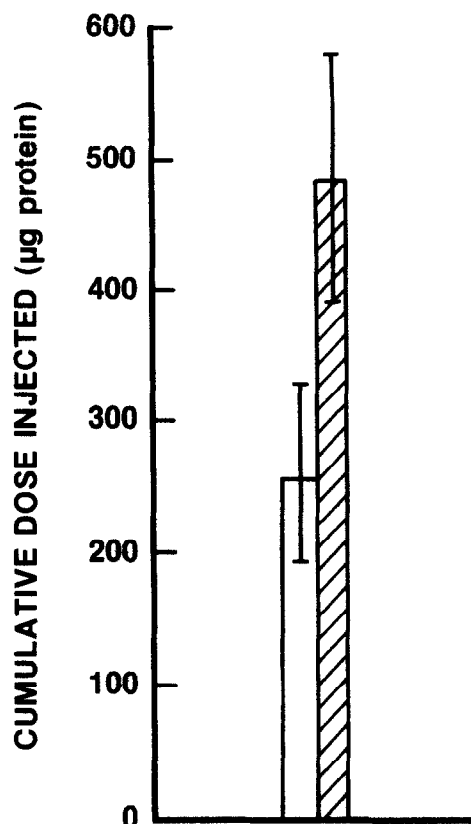


FIG. 1. Cumulative dose of PEG-modified ragweed extract achieved by the rush group (□) and the weekly group (▨); 100 μg of protein corresponds to 7.5 μg of AgE. Error bars = SEM.

posure. Similarly, subjects also recorded drowsiness, nausea, and neck tension, but it was not possible to determine whether any of these systemic symptoms were injection related. Treatment, required after only three injections in the weekly group and after six in the rush group, consisted of chlorpheniramine maleate, beclomethasone nasal spray, naphazoline eye-drops, salbutamol aerosol, and acetaminophen, and, in most cases, only one application was needed. In only one instance were systemic symptoms accompanied by a local reaction.

The initial skin sensitivity was similar in the two groups both for ragweed extract ($p = 0.17$) and PEG-modified ragweed extract ($p = 0.11$), and there was a strong linear relationship between sensitivity to the two extracts ($p < 0.0001$). Treatment decreased skin sensitivity to ragweed extract (weekly, $p < 0.0001$; rush, $p = 0.004$), the reduction being greater in the weekly group ($p < 0.05$). After treatment, five subjects demonstrated responses to duplicate PEG skin prick tests (mean wheal diameters of 4.5:2, 3:1, 5:0, 3.5:0, and 5.5:0 mm, respectively).

The mean ragweed-specific IgE and IgG antibody

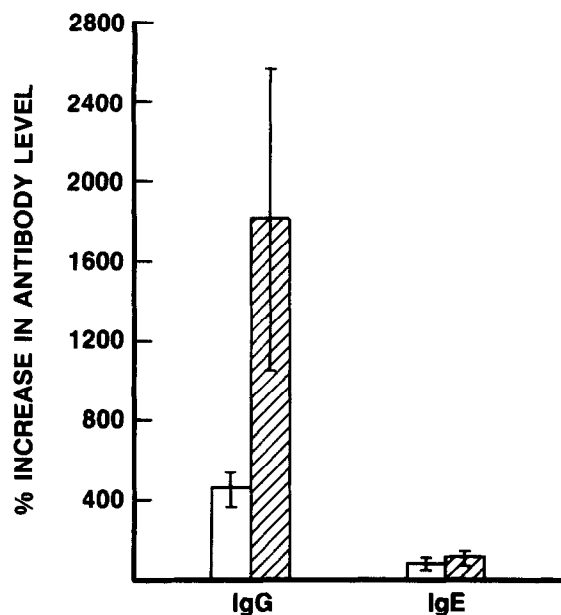


FIG. 2. Percentage increase in ragweed-specific IgG and IgE antibody levels during the injection period in the rush group (□) and the weekly group (▨). Error bars = SEM.

data are presented in Table III. The PEG-modified ragweed injections stimulated an increase in both IgE and IgG antiragweed antibodies in both groups (IgE weekly, $p = 0.0054$; rush, $p = 0.0010$; IgG weekly, $p = 0.0088$; rush, $p = 0.0092$). There was no evidence of any difference in the increase in IgE antibody between the two groups ($p = 0.40$) (Fig. 2), but the increase in IgG antibody in the weekly group was more than in the rush group ($p = 0.011$). Increases in IgG were linearly related to the total dose injected ($p = 0.005$), but a similar relationship was not found for IgE ($p = 0.16$). Increases in IgG were linearly related to reduction in skin prick sensitivity to ragweed extract ($p = 0.038$). Forty-six of the 49 subjects demonstrated a linear relationship between the cumulative dose after each injection and the IgG antibody level at that time. The slopes of these regressions demonstrated no difference between the two treatment groups ($p = 0.14$), indicating that the rate of increase in IgG is dependent on dose and not on the treatment regimen. No association was found between the initial IgE antiragweed antibody level and the first dose to cause an adverse reaction (weekly, $r = -0.16$; rush, $r = -0.18$).

Adverse reactions occurred after the first injection in three subjects in the weekly group and four in the rush group. Variables, measured before treatment, that might have been predictors of an occurrence of an adverse reaction included skin prick sensitivity to

TABLE III. Skin test sensitivity and ragweed-specific IgG and IgE antibody responses (mean $\pm$ SD)

	Injection		Difference (paired t test)
	Before	After	
Ragweed skin sensitivity*			
Weekly	16.41 $\pm$ 3.96	12.01 $\pm$ 4.85	$p < 0.001$
Rush	14.77 $\pm$ 4.27	12.50 $\pm$ 4.97	$p = 0.004$
PEG-modified ragweed skin sensitivity*			
Weekly	9.45 $\pm$ 3.32	5.91 $\pm$ 3.47	$p < 0.001$
Rush	8.25 $\pm$ 3.68	7.04 $\pm$ 3.22	$p = 0.044$
IgG			
Weekly	7.29 $\pm$ 8.14	66.9 $\pm$ 122.9	$p = 0.009$
Rush	10.43 $\pm$ 13.9	55.90 $\pm$ 98.7	$p = 0.009$
IgE			
Weekly	49.8 $\pm$ 37.5	88.5 $\pm$ 83.8	$p = 0.005$
Rush	82.7 $\pm$ 114.4	121.3 $\pm$ 146.1	$p = 0.001$

*Cumulative mean wheal diameter for the five serial dilutions.

aqueous ragweed and PEG-modified ragweed extracts and initial ragweed-specific IgE and IgG antibody levels. Aqueous ragweed skin test sensitivity was the best predictor, but the discrimination was poor with a correct predictive rate of only 63.3%. Additional variables did not significantly improve this rate. Variables, measured during the study, were also tested for their predictive value; they included the rates of increase in ragweed-specific IgE and IgG antibodies per unit dose of injection, if the subject was in the rush or weekly group, if the previous injection had produced an adverse reaction, and if the subject developed a sensitivity to PEG. The best predictor of an adverse reaction was if there had been a reaction after the previous injection, but again the correct predictive rate was poor at 69.4% with insignificant improvement with additional variables in the equation.

DISCUSSION

A regimen of weekly injections with PEG-modified ragweed extract had apparent advantages over a modified rush regimen. Both groups received the same number of injections, but a higher number of adverse reactions occurred in the rush group, possibly as a result of the serum half-life.⁴ As a consequence, the total cumulative dose achieved by the weekly group was nearly double that of the rush group. There was a corresponding greater increase in ragweed-specific IgG antibodies and reduction in skin sensitivity to ragweed-pollen extract in the weekly group. The direct relationship between allergen dose and IgG antibody response is in agreement with previous obser-

variations.^{1, 5, 6} However, with yellow jacket venom, a greater IgG response was elicited by an accelerated "cluster" regimen than a slow weekly regimen.⁷

Conjugation of allergen with PEG reduces the reactivity of the allergen with preformed antibody.⁸ Therefore, it was feasible to administer relatively high cumulative doses of PEG-modified ragweed extract in the course of 12 preseasonal injections. There was no assessment of the effects of injections on subsequent seasonal symptoms, but the antibody response and reduced skin sensitivity, a finding that correlates with the development of IgG antibodies,⁶ are testimony to the immunogenicity of the preparations. Systemic reactions were very mild, developed slowly, and occurred after about 8% of injections in the rush schedule and 4% of injections in the weekly schedule. This latter rate is comparable to rates for preseasonal trials with other modified antigens^{8, 9} and might well be reduced by further exploration of dosage schedules.

PEG-modified antigen has been demonstrated to prevent or abrogate IgE antibody responses in immunized mice.¹⁰ A comparable effect of this preparation in allergen-injection treatment in allergic patients would be highly beneficial. The observed rise in IgE antibodies is the type of response expected from allergen-injection treatment with conventional aqueous extracts and also has been observed previously in response to PEG-modified allergy.¹¹ It is possible that direct comparison of conventional with PEG-modified allergen in schedules that involve varying doses and varying durations of treatment would demonstrate differences between these preparations in humans.

Mildly positive skin tests with PEG after injection treatment with PEG-modified ragweed was observed in five cases. Unfortunately, no PEG skin tests for comparison were performed before the course of injections, and therefore, this can only be reported as an observation. The PEG in PEG-modified antigens has very low immunogenicity in rabbits and mice; nevertheless, the formation of anti-PEG antibodies has been stimulated by immunization of these animals.¹² In people who have received allergen-injection treatment with PEG-modified allergens, anti-PEG antibodies of the IgM class have been demonstrated.¹³ The immunoglobulin class of antibodies to PEG in patients reported here were not determined. Previously reported antibodies to PEG in injected patients tended to be transient, with a decrease or disappearance despite the continued injection of PEG-modified allergen.¹³

Although subjects were matched on ragweed skin sensitivity and severity of previous ragweed-induced rhinoconjunctivitis, the initial ragweed-specific IgE

antibody levels were not well balanced between the two groups. Five subjects in the rush group and three in the weekly group had high initial IgE levels (>100). None of these subjects had to withdraw because of large local reactions, and none had systemic symptoms. The incidence of reactions >10 cm was similar to the overall study rate in these eight subjects (rush = 6%; weekly = 5%). Therefore, it appears unlikely that the imbalance of initial IgE levels between the two groups could account for the outcome of this study. This is further supported by the fact that the discriminant function analysis did not reveal initial IgE as a good predictor of adverse reactions.

The prediction of an adverse reaction to an individual allergen injection remains imprecise, and as a consequence, adverse reactions are not entirely avoidable. The need to adhere to usual schedules of regular increments in dosage is indicated by the observation that many adverse reactions appear to be the result of human error in the choice of dose.¹⁴ The practice of dose reduction after a reaction is supported by the present study in which a preceding reaction was one of the better determinants of the occurrence of an adverse reaction. Nevertheless, the predictive value was not high, and this agrees with clinical experience insofar as adverse reactions can occur despite careful attention to the effects of serial injections and appropriate modification of the dosage administered. In the present study, 14% of subjects had an adverse reaction to the first dose of PEG-modified ragweed extract that suggests that there is little potential for introducing a higher starting dose for this extract.

We conclude from this study that a weekly regimen of preseasonal injections of PEG-modified ragweed is preferable to a modified rush regimen. Further studies are required to determine if this finding applies to other forms of allergen-injection therapy. It remains possible that a clinically acceptable daily or cluster regimen of injections could be established with PEG-modified ragweed at a lower dosage level, but this would likely be at the expense of efficacy.¹ In this study we were unable to identify indices that might be used to predict accurately the occurrence of adverse reactions.

We wish to thank all the subjects who participated in the study, and Mrs. D. Thompson and her staff at St. Joseph's Hospital Pharmacy for preparing the solutions of PEG-modified ragweed.

REFERENCES

1. Juniper EF, Roberts RS, Kennedy LK, O'Connor J, Syty-Golda M, Dolovich J, Hargreave FE: Polyethylene glycol-

- modified ragweed extract in rhinoconjunctivitis. *J ALLERGY CLIN IMMUNOL* 75:578, 1985
2. Ceska M, Eriksson R, Varga JM: Radioimmunosorbent assay of allergens. *J ALLERGY CLIN IMMUNOL* 49:1, 1972
 3. Foucard J, Johansson SGO: Immunological studies in vitro and in vivo of children with pollinosis given immunotherapy with aqueous and a glutaraldehyde-treated tyrosine adsorbed grass pollen extract. *Clin Allergy* 6:429, 1976
 4. Lee WY, Sehon AH: Suppression of reaginic antibodies. *Immunol Rev* 41:200, 1978
 5. Lichtenstein LM, Norman PS, Winkenwerder WL: Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. *Am J Med* 44:514, 1968
 6. Dantzer BS, Tipton WR, Nelson HS, O'Barr TP: Tissue threshold changes during the first months of immunotherapy. *Ann Allergy* 45:213, 1980
 7. Norman PS: An overview of immunotherapy: implications for the future. *J ALLERGY CLIN IMMUNOL* 65:87, 1980
 8. Cockcroft DW, Cuff MT, Tarlo SM, Dolovich J, Hargreave FE: Allergen injection therapy with glutaraldehyde-modified-ragweed pollen-tyrosine adsorbate: a double-blind trial. *J ALLERGY CLIN IMMUNOL* 60:56, 1977
 9. Norman PS, Winkenwerder WL, Lichtenstein LM: Trials of alum-precipitated pollen extracts in the treatment of hay fever. *J ALLERGY CLIN IMMUNOL* 50:31, 1972
 10. Akerblom E: Monomethoxypolyethylene glycol-modified allergens. *Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst* 78:231, 1983
 11. Norman PS, King TP, Alexander JF, Kagey-Sobotka A, Lichtenstein LM: Immunologic responses to conjugates of antigen E in patients with ragweed hay fever. *J ALLERGY CLIN IMMUNOL* 73:782, 1984
 12. Richter AW, Akerblom E: Antibodies against polyethylene glycol produced in animals by immunization with monomethoxy polyethylene glycol-modified proteins. *Int Arch Allergy Appl Immunol* 70:124, 1983
 13. Richter AW, Akerblom E: Polyethylene glycol-reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol-modified allergens or placebo, and in healthy blood donors. *Int Arch Allergy Appl Immunol* 74:36, 1984
 14. Vervloet D, Khairallah E, Arnaud A, Charpin J: A prospective national study of the safety of immunotherapy. *Clin Allergy* 10:59, 1980

The effects of combined H₁ and H₂ histamine antagonists on alterations in nasal airflow resistance induced by topical histamine provocation

**Thomas E. Havas, M.B., B.S., F.R.C.S.Ed., F.R.A.C.S.,
Philip Cole, M.D., F.R.C.S.(C), Larry Parker, B.D.S., D.D.S., Dip. Ortho.,
Donna Oprysk, and Anthony Ayiomamitis, M.Sc., M.H.Sc. Toronto, Canada**

*A clinical trial was undertaken to assess the effects of oral H₁ (diphenhydramine hydrochloride) and H₂ (cimetidine) histamine blockade on nasal resistance induced by topical histamine. Ten adult volunteers were tested on two separate occasions. Their noses were pretreated by oral administration of either combined H₁ and H₂ histamine antagonists or H₁ antagonist and placebo. The nasal airflow resistive response to topical histamine was then determined. Combined histamine antagonism was significantly more effective in reducing the nasal resistive response to topical histamine than H₁ antagonist alone ($p < 0.0001$). Furthermore, ingestion of the oral H₁ histamine antagonist, diphenhydramine hydrochloride, alone led to an increase in resistance of the unprovoked nose, whereas combined H₁ and H₂ antagonism did not lead to a significant change. (*J ALLERGY CLIN IMMUNOL* 78:856-60, 1986.)*

From the University of Toronto Department of Otolaryngology Air-flow Laboratories and the Gage Research Institute, Toronto, Canada.

Received for publication Nov. 25, 1985.

Accepted for publication April 2, 1986.

Reprint requests: Thomas E. Havas, M.B., 254 Old South Head Road, Bellevue Hill, 2023 Sydney, Australia.

This article is concerned with the effects of H₁ and H₂ histamine antagonists on the nasal resistive response to topical histamine provocation in healthy adult subjects.

Several previous clinical trials have demonstrated that H₁ histamine antagonists reduce sneezing and