

Ragweed hay fever: treatment by local passive administration of IgG antibody

GERALD J. GLEICH and JOHN W. YUNGINGER

Departments of Medicine and Pediatrics, and the Allergic Diseases Research Laboratory, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Summary

To determine whether the local administration of IgG antibodies might reduce the symptoms of ragweed hay fever, we tested the effect of this treatment in fourteen patients in a double-blind pilot trial. The patients were randomly divided into two groups, treatment and placebo, and their symptoms and medication usage were determined during the ragweed pollination season. One group used a nasal spray consisting of phosphate-buffered saline (PBS) containing human serum albumin, whereas the other used PBS containing purified IgG from a subject with a high titre of IgG antibody to partially purified ragweed antigen E. Treated and control groups used comparable quantities of nasal spray, and neither noted any ill effects. Comparison of individual symptoms of hay fever, medication usage, and total symptom scores showed no significant differences between the groups except a lessening of eye symptoms ($P < 0.05$) in the treated group. The results suggest that nasal administration of IgG antibody up to five times daily does not alter the severity of symptoms in ragweed hay fever. Furthermore, total serum IgE protein and specific IgE levels increased comparably in both groups, suggesting that passive administration of IgG antibody does not suppress the production of IgE antibody during the pollination season.

Introduction

During the past two decades, numerous investigators have demonstrated the efficacy of immunotherapy in the treatment of ragweed hay fever (Johnstone, 1957; Lowell & Franklin, 1963, 1965; Lichtenstein *et al.*, 1966; Lichtenstein, Norman & Winkler, 1968; Sadan *et al.*, 1969). Treated patients have fewer and less severe symptoms than do untreated controls: treated patients have a reduction or even a loss of histamine release from peripheral blood leukocytes, and they form IgG antibodies to the injected pollen antigens. These IgG antibodies are the blocking antibodies described originally by Cooke *et al.* (1935) and Loveless (1940) and later by Lichtenstein, Holtzman & Burnett (1968). *In vitro* IgG antibodies may reduce histamine release presumably by competing for allergen, but the levels of IgG antibodies are not highly correlated with the degree of symptomatic improvement (Lichtenstein, Norman &

Winkenwerder, 1971).

In 1970, Connell & Klein presented data suggesting that the instillation of IgG antibody into the nasal cavity reduced the degree of nasal obstruction in patients being challenged with pollen extracts. Because of this observation and because the role of IgG antibodies in mediating improvement in allergic rhinitis is not clear, we studied whether the administration of IgG-antiragweed antibody by nasal spray might alter the symptoms of patients with ragweed hay fever during the pollination season. In addition, we measured the content of IgE protein and IgE antibody to ragweed at intervals to determine whether the seasonal increases in IgE protein and IgE antibody (Levy & Osler, 1967; Sadan *et al.*, 1969; Berg & Johansson, 1971; Levy *et al.*, 1971; Yunginger & Gleich, 1973) were altered by this treatment.

Materials and methods

Patients. From thirty volunteers, we selected fourteen patients who had ragweed hay fever with typical symptoms beginning in mid-August and extending to mid-September for a pilot study. All fourteen patients had positive wheal-and-flare skin tests to crude ragweed pollen and negative or less intense reactions to extracts of *Alternaria*, a common allergen in our area. Patients were assigned to either a treatment or a placebo group (Table 1) by use of a table of random numbers. The treatment

Table 1. Patients with ragweed hay fever

Group	Males	Females	Age (years)	
			Mean	Range
Treatment	3	4	30.9	24-39
Placebo	6	1	37.0	28-49

group contained more females than did the control group, and the patients were somewhat younger. However, in prior studies of ragweed hay fever, Norman, Rhyne & Mellits (1966) showed that matching of patients on the basis of age and sex is not useful.

Patients were skin tested with antigen E (AgE) (kindly provided by Dr Floyd McIntire, Abbott Laboratories, North Chicago, IL) to determine their relative sensitivity to this antigen, which in other studies has reflected sensitivity to whole pollen extract (Baer *et al.*, 1970). AgE was serially diluted ten-fold, at pH 7.4, in 0.01 M K_2HPO_4 - KH_2PO_4 and 0.13 M NaCl, phosphate-buffered saline (PBS), containing 0.05% human serum albumin and 0.001% thimerosal. The concentration of AgE needed to produce a 2+ (6 × 6 mm wheal) was estimated, and the patients were ranked according to skin reactivity. All of the placebo patients and six of the treatment patients were skin tested. Skin reactivity to AgE in the treatment and placebo groups did not differ significantly.

IgG antibody to ragweed AgE. The IgG antibody to ragweed pollen was purified from the serum of a patient who was receiving monthly injections of 15,000 protein nitrogen units of crude, mixed aqueous extract from both giant and short ragweed. No hepatitis-associated antigen was detectable in the patient's serum, and the Venereal Disease Research Laboratory Test was nonreactive. His serum was selected because

of its high content of IgG-antiragweed activity, as analysed by radioimmunoprecipitation (Yunginger & Gleich, 1973) (*vide infra*). IgG from the patient's serum was purified by the addition of an equal volume of 28% Na_2SO_4 (w/v) at room temperature. The precipitate was removed by centrifugation at $15,000 \times g$ for 30 min and was washed twice with 14% Na_2SO_4 . The washed precipitate was dissolved in PBS and dialysed against 0.15 M NaCl until the test for sulphate ion was negative. At a concentration of 8 mg/ml, the resulting IgG produced only a single band in immunoelectrophoresis when analysed with a potent antiserum to whole human serum. The IgG preparation contained 132 ng IgE/ml, but this was reduced to amounts not detectable by radioimmunoassay after heating at 56°C for 2 hr.

Measurement of IgG antibody by radioimmunoprecipitation. The use of radioimmunoprecipitation for measurement of IgG antibody to ragweed pollen is described in detail elsewhere (Yunginger & Gleich, 1973). Briefly, 10 μl of serum or purified IgG were incubated with radiolabelled partially purified AgE for 1 hr at 37°C and overnight at 4°C, and the resulting soluble antigen-antibody complexes were precipitated by the addition of an excess of specific goat antibody to IgG, followed by another incubation at 37°C for 1 hr and at 4°C overnight. The precipitates were washed twice with 3.0 ml of 0.1 M $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ at pH 7.5, and the radioactivity was measured in a gamma-scintillation counter.

The AgE preparation was partially purified from short ragweed pollen (Yunginger & Gleich, 1972). It contained 4.6 mg/ml of protein, of which half (2.3 mg) was AgE. When reacted with a specific rabbit antiserum to AgE, a maximum of 51% of total counts in the AgE- ^{131}I preparation was precipitated. Addition of 10% tungstic acid precipitated a mean of 98% of total counts in two experiments. Radioimmunoprecipitation demonstrated a linear relationship between the quantity of human antiragweed serum added and the amount of AgE bound (Yunginger & Gleich, 1973).

The IgG antibody to AgE was analysed by radioimmunoprecipitation to determine the quantity of AgE bound per millilitre and to estimate the intrinsic association constant (Gleich & Stankievic, 1969). The binding capacity of the IgG antibody for AgE can be measured by determining the quantity of AgE bound to IgG in the plateau region of the binding curves, and 1.0 mg of the purified IgG protein bound 0.5 μg AgE. On the assumption that the resulting antigen-antibody complexes in the plateau region of the binding curve have a molecular composition of Ag_2Ab , 0.45% of the IgG was antibody to AgE. Finally, the average intrinsic association constant, K_o , for the IgG antibody to AgE was $5 \times 10^6 \text{ M}^{-1}$. In the calculation of K_o , we assumed that only the AgE in our partially purified preparation bound to IgG. Because our partially purified AgE was prepared by gel filtration on Sephadex G-25 and G-100, the likely antigenic contaminant is AgK, which has a molecular weight similar to that of AgE. If the association constant is calculated on the assumption that both AgE and AgK are present and that they account for all of the molecules in our material, the same value for K_o is obtained as if one assumes that only AgE is present.

Composition of the IgG nasal spray. The purified IgG was diluted seven-fold to a final concentration of 1.14 mg/ml, and thimerosal was added to a final concentration of 0.001%. Each millilitre of nasal spray contained sufficient antibody to combine with approximately 0.57 μg AgE. In 1970, we estimated that an adult with a tidal volume of 6.4 l/min inhaled approximately 0.08 μg AgE during the ragweed season. This estimate is based on roto-slide counts of ragweed pollen during the season (Ogden & Raynor, 1967), a specific gravity of 1.05 for ragweed pollen (Harrington & Metzger,

1963), the quantity of ragweed AgE of 440 mg/200 g of pollen, found by King, Norman & Connell (1964), and the assumption that all pollen inhaled was spherical and was trapped in the airway. During 1971, the roto-slide apparatus was damaged in a windstorm so that results are incomplete. However, comparison of gravity pollen counts and roto-slide counts permits an estimate of the total 1971 AgE dose, which was 0.2 μ g.

Plastic nasal spray containers were filled with 15 ml of IgG or placebo solution, and stored at -20°C . Filled containers were weighed before being dispensed and again after being returned by the patient, and the quantity of IgG administered was calculated from the difference in weight on the assumption that the density for the solution was 1.0 g/ml. Combining activity was detectable in the IgG remaining in these containers after exposure to environmental temperatures for 1 week; in several samples tested by radioimmunoprecipitation after their return, only slight losses of activity (a maximum of 16%) were noted.

Placebo nasal spray. As a control material for administration to patients, a solution of 0.05% human serum albumin in PBS and containing 0.001% thimerosal was prepared and bottled in plastic nasal spray bottles.

Measurement of IgE serum protein and IgE antibody to ragweed pollen. IgE levels in serum were measured by a double-antibody radioimmunoassay as described previously (Gleich, Averbek & Swedlund, 1971). All of the patients' serum samples were tested in the same assay to minimize variation in results. IgE antibodies were measured by the radioallergosorbent test (RAST) essentially as described elsewhere (Yunginger & Gleich, 1973), except that partially purified ragweed antigen passed through a column of Sephadex G-25 was coupled to microcrystalline cellulose.

Conduct of the study. Bottles containing either IgG or placebo nasal spray were labelled with the patient's name. Patients were given chlorpheniramine maleate, 4 mg tablets, to be taken as needed for relief of symptoms. Symptoms and treatment diaries similar to those used by Norman *et al.* (1966) were kept by the patients, beginning in late July and extending to mid-September. Patients were seen weekly during the study period, and the symptom sheets were collected. Nasal spray containers were dispensed to patients on 9 August 1971, and weekly thereafter through 13 September 1971. Patients were instructed to spray each nostril at least three times every 2-4 hr during their waking hours.

Analysis of results. Symptom scores were analysed as described by Norman *et al.* (1966), and an overall score for each patient for the season was compiled. Only one patient experienced symptoms of asthma during the study period, and these symptoms were not included in his scores. Scores were analysed by the rank-sum and rank-correlation tests and by the analysis of covariance (Dixon & Massey, 1957).

Results

In preliminary studies, normal subjects nebulized the IgG nasal spray solutions, up to three squeezes every 2-4 hr, without producing any evident ill effects. Also during the peak of ragweed pollination, use of the IgG nasal sprays did not result in any nasal symptoms in two normal subjects, except for a slight increase in secretions during the period immediately after use of the spray. The quantities of nasal spray used by the patients are shown in Fig. 1. Patients in both treatment and placebo groups used a mean of 48 ml of nasal spray during the 6-week study period, although there was greater variation in use by the treatment group. Because each spray contained an

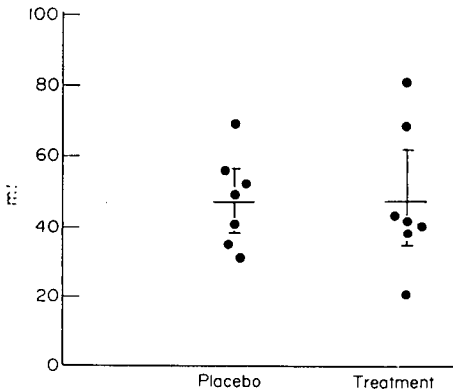


Fig. 1. Volumes of nasal spray used by placebo and treatment groups. Heavy horizontal bar denotes mean, while short bars denote limits of 1 s.d.

average of 41 μl , our patients sprayed each nostril fourteen times per day or approximately three sprays five times per day. During the study period, no patients reported any ill effects related to use of nasal spray. Because of nasal obstruction, some had difficulty nebulizing the spray at various times. Finally, we urged patients to use the spray as frequently as possible, and we doubt whether they could be motivated to increase their usage beyond that actually observed.

At the conclusion of the study, patients were queried concerning their evaluation of the effects of treatment. Three patients in each group expressed a strong preference for the nasal spray over oral antihistamine medications. Comparison of individual symptoms between the groups did not reveal any difference except for the significant ($P < 0.05$) reduction in the severity of red itchy eyes for the treated group. However, in terms of overall severity of disease, the two groups were similar (Fig. 2). Analysis

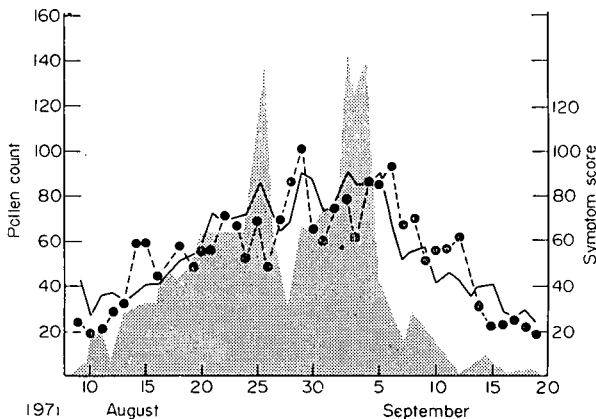


Fig. 2. Symptom scores and pollen (shaded area) count (in grains per cm^2) for 1971 ragweed pollination season. Solid line, treatment; broken line, control.

of symptoms and of drug use, both separately and together, did not reveal any differences between the groups for the entire 6-week period nor for the peak pollination weeks (August 24 to September 5). We calculate that the maximal quantity of AgE

that a person in the Rochester, Minnesota, area might inhale was 0.2 μg . The smallest quantity of nasal spray used by any patient (20 ml) contained an amount of IgG antibody to AgE, 11.4 μg , far in excess of the total AgE for the pollination season. The average amount of nasal spray used by the treatment group contained greater than 23 μg IgG antibody to ragweed AgE or about 100 times the largest quantity of AgE that a patient might inhale. Moreover, the estimate of AgE, 0.2 μg , assumed that the patient was outside at all times, whereas in reality, most patients were in air-conditioned environments during considerable portions of the season. The data of Spiegelman & Friedman (1968) indicate that pollen counts in August and September are reduced by at least 100-fold in an air-conditioned house. Therefore, the quantities of AgE that many of our patients actually inhaled were probably of the order of 2 ng for the pollination season. If so, we administered an amount of IgG antibody to AgE that was 4500- to 9000-fold greater than AgE on a weight basis or 1000- to 2000-fold greater on a molar basis.

IgE protein and IgE antibody levels to ragweed antigen were measured throughout the pollen season (Tables 2 and 3). In both groups, IgE protein levels increased

Table 2. Seasonal changes* in serum IgE during 1971

Patient	July 15	August 20	September 2	September 17	October 20
Control group					
1	76	64	146	195	162
2	965	...	1532	2682	1652
3	207	314	556	925	1067
4	407	367	453	510	515
5	336	524	512	490	448
6	93	100	91	134	127
7	458	542	580	587	490
Treatment group					
1	27	31	35	49	62
2	266	238	269	324	197
3	224	261	266	292	215
4	195	186	174	202	121
5	55	41	55	66	176
6	378	425	334	386	488
7	76	69	66	65	72

* Results are expressed in nanograms per millilitre.

significantly in serum during the pollen season ($P < 0.01$, sign test), reaching maximal values generally by mid-September to mid-October. The initial levels of IgE protein tended to be somewhat less in the treated group, but no difference was found between the groups by the rank-sum test. Comparison of increases in IgE protein value, expressed as a percentage of the initial value, also did not reveal a difference (rank-sum test) between the groups. Similarly, no difference was found between the groups by analysis of covariance. No relationship was found between the initial levels and the subsequent increase in IgE protein levels.

The levels of IgE antibody also increased significantly during the pollination season ($P < 0.001$, sign test). There was no difference between the initial levels of IgE

Table 3. Seasonal changes* in IgE antibodies during 1971

Patient	July 15	August 20	September 2	September 17	October 20
Control group					
1	3.60	3.64	4.75	5.98	5.41
2	7.15	...	8.22	9.59	9.42
3	5.68	5.78	6.77	9.13	9.29
4	8.12	8.04	8.25	8.84	9.77
5	3.71	3.79	3.78	4.07	4.15
6	2.09	2.21	2.19	2.60	2.80
7	2.47	2.18	2.27	2.41	2.48
Treatment group					
1	1.00	1.09	0.99	1.19	1.21
2	5.24	5.38	5.41	5.79	5.59
3	3.48	3.57	3.52	3.53	3.87
4	3.57	3.43	3.70	3.44	3.47
5	1.18	1.00	1.13	1.60	2.10
6	0.56	0.70	0.65	0.74	0.66
7	2.86	3.01	3.01	3.33	3.28

* Results are expressed as counts bound, divided by counts added, multiplied by 100. When nonallergic serum was analysed, less than 0.8% of added counts was bound.

antibody between the groups (rank-sum test), although as with IgE protein the levels in the treated patients tended to be lower. As we have reported previously (Yunginger & Gleich, 1973), the initial levels of the IgE antibody were related to the subsequent increases ($r_s = +0.59$, $P < 0.05$). Because of this finding, we compared the increase in IgE antibody levels between the groups by analysis of covariance, a procedure that permits one to assess the increase as a function of the initial level. Analysis revealed no differences between the groups. Finally, the maximal changes in IgE protein and IgE antibody levels for both groups were positively related ($r_s = +0.57$, $P < 0.05$); this relationship was greater for the untreated patients ($r_s = +0.86$, $P < 0.001$).

Discussion

In this double-blind study, we attempted to extend the controlled laboratory observations of Connell & Klein (1970) on intranasal IgG blocking antibody to the clinical treatment of ragweed hay fever patients. The two study groups were comparable in terms of skin reactivity to AgE. Also, their levels of IgE antibody to ragweed antigens did not differ, although the control group tended to be somewhat higher. The quantities of nasal spray used by the patients were similar. Analysis of the results did not show a difference between the placebo and treatment groups either in the severity of the hay fever symptoms or in the increase in IgE protein and IgE antibody levels during the pollination season. Thus we were not able to demonstrate a reduction in the symptoms of hay fever by local passive administration of IgG antibody, at least in the dosages used here, as suggested by the report of Connell & Klein (1970). No trends to the reduction of symptoms were noted in these small pilot groups.

In our prior study (Yunginger & Gleich, 1973) on the effect of hyposensitization on increases in levels of IgE protein and IgE antibody in ragweed hay fever, we were

not able to demonstrate a lessening of seasonal increases in treated patients. Because hyposensitized patients as a group had high levels of IgG antibody to ragweed antigens, we concluded that IgG antibody did not prevent the stimulation of IgE-forming cells by ragweed antigens. The findings of the present study are also consistent with this conclusion. In these two studies, we have tested the ability of IgG antibody administered via the vascular route and via nasal insufflation to prevent the seasonal elevations of IgE protein and antibody levels. We presume that the IgG antibody within the vascular system gains entry into the nasal tissues and particularly into the spaces about the IgE-producing cells in the nasal mucosa. After nebulization of IgG antibody, one could argue that the IgG antibody did not penetrate into this space. Nonetheless, this IgG antibody should have been able to combine with ragweed antigens on the pollen grain or with solubilized antigen on the mucosal surface. Our inability to demonstrate a neutralization of ragweed pollen antigens (in terms of their ability to produce symptoms and increases in IgE protein and IgE antibody levels) suggests (1) that the passively administered IgG was unable to penetrate the mucous blanket as effectively as antigens from the pollen grains, (2) that the IgG antibody was rapidly inactivated in secretions or that it was excreted, so that its combining activity was rapidly reduced *in vivo*, (3) that IgG antibody formed complexes with ragweed antigens, but these dissociated after mucosal absorption and permitted interaction with IgE antibodies, (4) that considerably larger doses or more frequent administration or both of IgG antibody are needed to achieve a therapeutic effect. We cannot decide among these possibilities, and include them as considerations for others in any future studies of the effect of local passive administration of antibodies to ragweed antigens. It is obvious that more information on IgG antibodies must be gathered before the laboratory observations of Connell & Klein (1970) can be translated into clinical practice.

Acknowledgments

We thank Mr Richard T. Jones for technical assistance with the IgE protein and IgE antibodies and Miss Gladys M. Hopkins for her assistance in the performance of the double-blind study. Dr Peter C. O'Brien aided us in the statistical analyses of the results. Dr David Marsh provided us with sample calculations of the quantity of AgE likely inhaled. This investigation was supported in part by a grant from the Mayo Foundation.

References

- BAER, H., GODFREY, H., MALONEY, C.J., NORMAN, P.S. & LICHTENSTEIN, L.M. (1970) The potency and antigen E content of commercially prepared ragweed extracts. *Journal of Allergy*, **45**, 347.
- BERG, T. & JOHANSSON, S.G.O. (1971) *In vitro* diagnosis of atopic allergy. IV. Seasonal variations of IgE antibodies in children allergic to pollens: a study of nontreated children and of children treated with inhalation of disodium cromoglycate. *International Archives of Allergy and Applied Immunology*, **41**, 452.
- CONNELL, J.T. & KLEIN, D.E. (1970) Protective effect of nasal sprays containing blocking antibody in hay fever (abstract). *Journal of Allergy*, **45**, 115.
- COOKE, R.A., BARNARD, J.H., HEBALD, S. & STULL, A. (1935) Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *Journal of Experimental Medicine*, **62**, 733.
- DIXON, W.J. & MASSEY, F.J., JR (1957) *Introduction to Statistical Analysis*, 2nd edn. McGraw-Hill Book Company, New York.

- GLEICH, G.J., AVERBECK, A.K. & SWEDLUND, H.A. (1971) Measurement of IgE in normal and allergic serum by radioimmunoassay. *Journal of Laboratory and Clinical Medicine*, **77**, 690.
- GLEICH, G.J. & STANKIEVIC, R.R. (1969) Measurement of penicilloyl antibodies by radioimmuno-precipitation. *Immunochemistry*, **6**, 85.
- HARRINGTON, J.B., JR & METZGER, K. (1963) Ragweed pollen density. *American Journal of Botany*, **50**, 532.
- JOHNSTONE, D.E. (1957) Study of the role of antigen dosage in the treatment of pollenosis and pollen asthma. *American Journal of Diseases of Children*, **94**, 1.
- KING, T.P., NORMAN, P.S. & CONNELL, J.T. (1964) Isolation and characterization of allergens from ragweed pollen. II. *Biochemistry*, **3**, 458.
- LEVY, D.A., LICHTENSTEIN, L.M., GOLDSTEIN, E.O. & ISHIZAKA, K. (1971) Immunologic and cellular changes accompanying the therapy of pollen allergy. *Journal of Clinical Investigation*, **50**, 360.
- LEVY, D.A. & OSLER, A.G. (1967) Studies on the mechanisms of hypersensitivity phenomena. XVI. *In vitro* assays of reaginic activity in human sera: effect of therapeutic immunization on seasonal titer changes. *Journal of Immunology*, **99**, 1068.
- LICHTENSTEIN, L.M., HOLTZMAN, N.A. & BURNETT, L.S. (1968) A quantitative *in vitro* study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *Journal of Immunology*, **101**, 317.
- LICHTENSTEIN, L.M., NORMAN, P.S. & WINKENWERDER, W.L. (1968) Clinical and *in vitro* studies on the role of immunotherapy and ragweed hay fever. *American Journal of Medicine*, **44**, 514.
- LICHTENSTEIN, L.M., NORMAN, P.S. & WINKENWERDER, W.L. (1971) A single year of immunotherapy for ragweed hay fever. *Annals of Internal Medicine*, **75**, 663.
- LICHTENSTEIN, L.M., NORMAN, P.S., WINKENWERDER, W.L. & OSLER, A.G. (1966) *In vitro* studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *Journal of Clinical Investigation*, **45**, 1126.
- LOVELESS, M.H. (1940) Immunological studies of pollinosis I. The presence of two antibodies related to the same pollen-antigen in the serum of treated hay-fever patients. *Journal of Immunology*, **38**, 25.
- LOWELL, F.C. & FRANKLIN, W. (1963) A 'double blind' study of treatment with aqueous allergenic extracts in cases of allergic rhinitis. *Journal of Allergy*, **34**, 165.
- LOWELL, F.C. & FRANKLIN, W. (1965) A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *New England Journal of Medicine*, **273**, 675.
- NORMAN, P.S., RHYNE, M.B. & MELLITS, E.D. (1966) The evaluation of agents for the treatment of seasonal respiratory allergies. *Clinical Pharmacology (International Encyclopedia of Pharmacology and Therapeutics: Section 6)* (Ed. by L. Lasagna), p. 639-652. Pergamon Press, New York.
- OGDEN, E.C. & RAYNOR, G.S. (1967) A new sampler for airborne pollen: the roto-slide. *Journal of Allergy*, **40**, 1.
- SADAN, N., RHYNE, M.B., MELLITS, E.D., GOLDSTEIN, E.O., LEVY, D.A. & LICHTENSTEIN, L.M. (1969) Immunotherapy of pollenosis in children: investigation of the immunologic basis of clinical improvement. *New England Journal of Medicine*, **280**, 623.
- SPIEGELMAN, J. & FRIEDMAN, H. (1968) The effect of central air filtration and air conditioning on pollen and microbial contamination. *Journal of Allergy*, **42**, 193.
- YUNGINGER, J.W. & GLEICH, G.J. (1972) Comparison of the protein-binding capacities of cyanogen bromide-activated polysaccharides. *Journal of Allergy and Clinical Immunology*, **50**, 109.
- YUNGINGER, J.W. & GLEICH, G.J. (1973) Seasonal changes in IgE antibodies and their relationship to IgG antibodies during immunotherapy for ragweed hay fever. *Journal of Clinical Investigation*, **52**, 1268.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.