

Trials of alum-precipitated pollen extracts in the treatment of hay fever

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Immunotherapy (desensitization) of hay fever with aqueous extracts of pollen is limited to rather small immunizing doses because of the risk of allergic or anaphylactic reactions. Alum-precipitated pollen extracts are absorbed more slowly, and larger doses may be given in fewer injections with a reduced rate of untoward systemic reactions. In a controlled study, alum-precipitated ragweed extract gave therapeutic results in fall hay fever equal to those obtained with aqueous ragweed extract. Immunologic responses to the two types of extract (measured by the technique of histamine release from isolated washed leukocytes) were also similar. Safer antigen preparations which permit higher doses need to be sought further.

Personnel from our laboratory and clinic have reported a series of controlled studies of immunotherapy (desensitization) of ragweed hay fever designed to elucidate some of the questions which have existed since the technique's inception in 1911. Five years of study with gradually increasing doses† of ragweed pollen extracts each year gave evidence that clinical results were dose dependent and that within some upper limit of dose (still undefined) greater amelioration of symptoms results from larger doses.^{1, 2} Evidence that improvement was not due to a cumulative effect of annual repetition of pre-seasonal treatment was found in a second study, in which the largest dose yet employed by us was given in a single intensive preseasonal course to previously untreated patients, with a result which was as good as that seen previously after several years of treatment.³

Studies in children have also given evidence of genuine efficacy when the dose is large enough. In almost none of the patients, either adults or children, have "cures" been achieved. Nearly all continue to have symptoms during the season, but these are reduced in severity.^{4, 5}

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Good Samaritan Hospital, Publication No. 21.

Supported by Grants AI 04866 and AI 08270 from the National Institutes of Allergy and Infectious Diseases and by a grant from Center Laboratories, Inc.

Received for publication Jan. 4, 1972.

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*Recipients of Research Career Development Awards from the National Institute of Allergy and Infectious Diseases during the performance of this work.

†Throughout this paper, when dosage in therapy is referred to, it means the total accumulated dose in a preseasonal series of injections.

Laboratory studies on specimens from these patients have indicated a significant although imperfect correlation with two immunologic events: (1) reduction or loss of the ability of peripheral leukocytes to release histamine when exposed to ragweed antigen E (AgE) *in vitro*; and (2) increases in titer of blocking antibodies of the IgG class which specifically inhibit ragweed antigen histamine release. Both of these phenomena seem to be dose related, blocking antibody rises being directly related to dose given in any course of injections. There has been no evidence of anamnestic responses in second and third courses. Antibodies declined slowly whenever injections were discontinued for several months or more. The frequency with which patients' leukocytes "turned off," i.e., became reduced in their ability to release histamine, also became greater as the dose increased, although some patients' leukocytes turned off at relatively modest doses whereas others receiving the highest doses still retained full ability to release histamine.^{3, 6, 7}

There also appears to be some reduction in titer of specific reaginic antibodies after several years of treatment, but we have been unable to correlate this event with the clinical response to immunotherapy.⁸

Dosage in these studies has been expressed by the now traditional method of protein nitrogen units (PNU). Nitrogenous materials are precipitated by phosphotungstic acid and a nitrogen determination is performed on the precipitate by the micro-Kjeldahl method (one PNU = 0.00001 mg. of N). Recent evidence indicates that such determinations have little to do with the biologically active antigens or allergens present in an extract.⁹ Hence, it appears, that dosage has not hitherto been expressed in any meaningful absolute terms. Nevertheless, we had begun to reach a practical limit to dosage in our patients from the untoward allergic reactions to the pollen extracts. When a certain limit is reached, which varies from patient to patient, either local swellings or evidences of mild to moderate generalized anaphylactic reactions begin to be frequent and have resulted in a very slow pace of dosage increase or, in some patients, no increase at all. Excessive numbers of injections have been required to go beyond this limit.

This difficulty, of course, has been observed clinically for years, and a variety of attempts to slow absorption of antigens from an injection site by admixing them with vegetable or mineral oils has been tried. This procedure won acceptance by some but failed to attain approval for general usage by physicians or allergists. One method, tried for the first time in 1938, has received little attention, namely, alum precipitation of pollen extract.^{10, 11} Extracts of pollen made by the use of the organic solvent pyridine instead of buffered water are produced and sold in the alum-precipitated form, but these have been criticized not because of the alum precipitation but because the high pH of pyridine (about 11) appears to denature protein antigens of ragweed extract. There is little or no immunologic response in ragweed-allergic patients to immunotherapy with this preparation.¹²

Because of the simplicity of alum precipitation as a means of preparing a slowly absorbed protein material and its long successful use for several vaccines without untoward effects, we have turned to alum precipitation of buffered-

water pollen extracts for a further exploration of the immunologic and clinical responses to such preparations in patients with hay fever.

These studies have been divided into 3 phases: (1) an initial unblinded trial of alum-precipitated mixed ragweed extract in a small number of patients to test safety and immunologic responses; (2) a similar trial of alum-precipitated mixed timothy and orchard grass extract; and (3) a complete controlled trial of alum-precipitated ragweed extract comparing matched groups of patients treated with placebos. The results indicate that: (1) Alum precipitates are adequate immunizing agents, resulting in good blocking antibody responses. (2) Based on comparisons with placebo-treated patients, amelioration of symptoms with alum precipitate is similar to that attained with whole extract. (3) With alum precipitate, fewer injections will give adequate clinical results and antibody levels. (4) Fewer generalized reactions occur with alum precipitate than occur with whole extract.

METHODS AND MATERIALS

Evaluation of hay fever symptoms

A daily symptom diary card was kept by each patient from August 12, 1968, to September 27, 1969. Each patient was requested to evaluate his symptoms for the previous 12 hours at noon and again at bedtime. Separate ratings were given for sneezing, stuffy and runny nose, red itchy eyes, and coughing. Each symptom was scored as no symptoms (0), symptoms for less than 30 minutes (1), 30 minutes to 2 hours (2), or more than 2 hours (3). Antihistamine tablets (chlorpheniramine maleate, 4 mg.) were furnished to each patient, and the number of tablets taken daily was added to the symptom score. The numeric scores were totaled to derive a daily symptom score for each patient day. Analysis of results from these diaries has shown that the interpretation of the group average data was the same whether the use of antihistaminics was included or not. A mean score for the season was obtained for each patient, and these in turn were averaged to obtain the mean symptom score for each treatment group.

Each patient was interviewed 2 times at 14 day intervals during the hay fever season by a physician who was not aware of the treatment received by the patient. At each interview the patient was asked to rate each symptom (sneezing, stuffy nose, rhinorrhea, and red, itchy eyes) as severe, moderate, slight, or none. The eyes were examined and the severity of conjunctivitis was rated as none, slight, moderate, or severe. The nasal mucous membranes were examined for edema, change in color, and amount of secretion. The ratings for symptoms and physical signs were scored for each patient.⁶ When the scores of the physicians agreed closely with the symptom indexes derived from daily diaries, the former were not reported further.

Pollen counts

Quantitative ragweed pollen counts were obtained from a rotoslide sampler placed at a suburban location in Baltimore. Results are reported as grains per square centimeter on the sampling slide.

Histamine-release studies

The basic methodology employed in these studies has been reported previously,^{6, 13, 14} although we have subsequently made some technical modifications in our histamine-release techniques. We now essentially follow the microtechnique of May and associates,¹⁵ the only exception being the addition of 0.2 ml. of 8 per cent HClO₄ to the "completes," i.e., the cell aliquot which defines the total histamine. Blocking antibody assays are made by the dilution

technique described previously.¹⁰ This involves the use of one or more standard cell donors of blood type O. The control tubes contain these cells suspended in 5 per cent normal human serum (NHS) (blood type AB) together with an antigen concentration sufficient to cause 60 to 80 per cent histamine release. Each serum from an allergic donor (AHS) is added in 4 to 5 dilutions to other tubes containing aliquots of the same cell preparation. The serum concentration is kept constant at 5 per cent through the addition of the normal serum. Per cent inhibition relative to the control is calculated, a plot constructed, and the point of 50 per cent inhibition interpolated. The reciprocal of the number is the antibody titer. In each experiment a standard allergic serum is run and the results from the various experiments normalized, if necessary. This technique yields antibody titers which show a correlation coefficient of 0.94 with our previously described assay.⁷ It is used for convenience.

Alum precipitates

Alum-precipitated mixed ragweed extract was furnished in 1968 by Center Laboratories as a 40,000 PNU per milliliter concentrate with appropriate tenfold dilutions down to 10 PNU per milliliter. In 1969, both alum-precipitated mixed timothy and orchard grass extract and alum-precipitated ragweed extract were furnished as a 20,000 PNU per milliliter concentrate. Subsequently, the ragweed extract was found to contain 0.019 mg. of antigen E. (AgE) per 10,000 PNU by double diffusion in gel of the redissolved alum precipitate.

Whole ragweed extract

Whole ragweed extract was furnished by Hollister-Steir Labs. as a lyophilized extract in vials of 500,000 PNU which, when reconstituted with 10 ml. of diluent, gave a concentrate of 50,000 PNU per milliliter. As determined by double diffusion in agar gel, the reconstituted solution contained 0.027 mg. of AgE per 10,000 PNU.

Placebos

The diluent was used for placebo injections. To duplicate local wheal and erythema reactions, histamine was added. A concentrated solution with 0.5 mg. per milliliter was prepared from which dilutions were made so that a graded increase in local reactions was attained as the dose was increased.

RESULTS

Preliminary trial with alum-precipitated ragweed extract

In 1968, 11 volunteer patients with typical histories of ragweed hay fever and positive skin tests to antigens E and K of ragweed were subjected to a preliminary study of injections of alum-precipitated ragweed extract to explore the safety of using these extracts and to determine the immunologic response. The injections were started on June 12, with an initial dose of 100 PNU, and continued at weekly intervals until the second week of August. Some patients received 2 or 3 injections a week near the end of this period. Doses were raised according to the recommended schedule of Center Laboratories, unless a local or systemic reaction interfered. Generally, allergic reactions were less of a problem than they were with conventional therapy. The initial injection of 100 PNU caused mild local swelling, redness, and itching in 10 of the patients. Subsequent injections, even with an increase of dose, caused less reaction until the highest doses were reached. Two patients had generalized allergic reactions. At 1,000 PNU one patient had generalized itching and erythema which started about 15 minutes after the injection. This condition cleared rapidly after epinephrine administration. At 16,000 PNU another patient had gener-

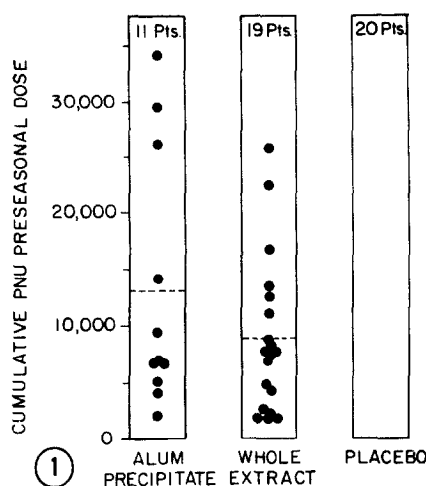


FIG. 1. Total cumulative preseasonal dose (PNU) of ragweed pollen extract administered in 1968.

alized itching which rapidly progressed to difficult breathing and hypotension. Three separate injections of epinephrine were required to bring this situation under control but the patient had recovered well in about one and one-half hours. The patient was given prednisone for 4 days to prevent recurrence and had no further trouble. In both generalized reactions, the patient had received the previous injection only 2 days before. From this experience it seems likely that the one dose per week schedule be adhered to.

Individual patients received from 7 to 11 injections which yielded a total preseasonal dose ranging from 2,060 PNU to 34,000 PNU with a mean of 13,147 (Fig. 1).

These figures may be compared with those obtained in 19 previously untreated patients receiving crude ragweed extract in aqueous solution for a first course of preseasonal immunotherapy. This treatment was started in these patients 2 months earlier and required an average of 26.7 preseasonal injections in order to reach a mean preseasonal dose of only 8,800 PNU (.023 mg. of AgII). As shown in Fig. 1, none of the larger groups of patients treated with aqueous solutions reached a dose of over 25,000 PNU, whereas 3 of the alum precipitate-treated patients did so.

Antibody responses are summarized in Fig. 2. Inspection of these data show that antibody responses compared favorably with responses of 19 patients receiving similar amounts of aqueous extract. These titers all represent antibodies to antigen E, and the presence of such good responses is a demonstration that antigenically active antigen E is present in the alum precipitate.

Leukocyte sensitivity studies in these patients by the histamine-release method showed that 7 of the 9 patients developed a reduction in cell sensitivity or reactivity. Such reductions in cell sensitivity and/or reactivity during immunotherapy with aqueous extracts have been described elsewhere in detail.^{3, 5, 6, 7}

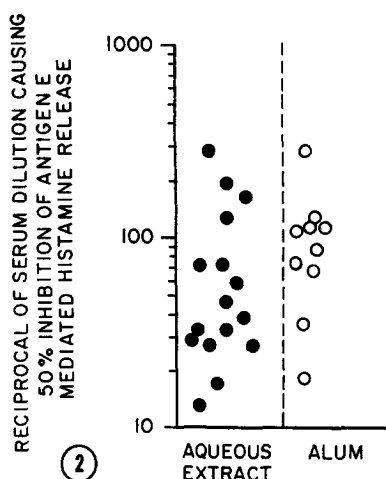


FIG. 2. Preliminary study 1968. Antibody levels in treated patients (placebo-treated patients all < 10).

In the absence of matched patients treated with placebos for comparison, no definitive clinical evaluation could be made. However, data from interviews with physicians and daily symptom diaries were collected and provide some indication of how these patients progressed. During the first 2 weeks of the season physician interview scores averaged 11.28 and during the second 2 weeks 7.77, resulting in an average for the season of 9.31. This result may be compared with those of larger groups of patients receiving either whole aqueous extract or placebos in a controlled study carried out by us during the same year.³ The average for patients receiving placebos was 15.12 and for treated patients was 9.63, thus showing that the small group receiving alum-precipitated extract had a result similar to that with aqueous extract and progressed better than those receiving placebos.

Ten of the alum precipitate-treated patients turned in symptom diaries with an average score of 6.32. For comparison, the patients receiving whole aqueous extract reported an average symptom score of 6.49, whereas placebo-treated patients had an average symptom score of 11.13.

Preliminary trial with alum-precipitated grass extract

Following the favorable experience with ragweed extract, a similar preliminary trial was undertaken with mixture of equal parts of timothy and orchard grass extract. Thirteen patients with typical histories of late May and June hay fever were selected and sensitivity to timothy and orchard grass was established by intradermal skin testing with these two antigens. Injections 2 times per week were given for a period of 5 weeks until the end of April. Local reactions to the initial doses were somewhat larger than with doses equal in PNU of ragweed extract. For this reason, rises in dosage progressed somewhat more slowly than with ragweed extract. In retrospect, injections should have been started earlier in the year and been given at weekly intervals to allow more time between injections for antibodies to develop.

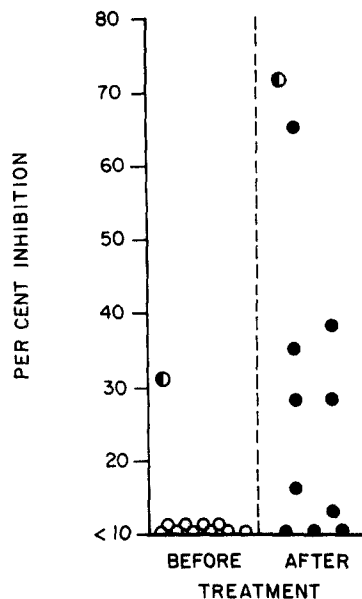


FIG. 3. Antibody levels to timothy extract in patients undergoing treatment with alum-precipitated grass extract. The half-circles indicate the levels in the one patient who had a significant titer before treatment.

Evaluation of clinical results was performed by the same methods used with ragweed extract. Twelve patients finished the course of injections and received cumulative doses ranging between 55 PNU and 1064 PNU with an average of 490 PNU. As there was no untreated group for comparison, evaluation of improvement was not possible.

Histamine-release studies indicate that 8 of these patients had detectable rises in blocking antibodies, whereas 3 did not (Fig. 3). The rises in titer are less than those observed with alum-precipitated ragweed extract. This appears to be due to 2 factors: (1) Doses were smaller with grass extracts. (2) Antibody responses to desensitization with grass extracts are generally of a lower level than with ragweed extract.*

Controlled trial of alum-precipitated ragweed extract

For the ragweed pollination season of 1969, a controlled comparison of alum-precipitated ragweed extract, whole extract of ragweed, and placebos was organized. The patients receiving whole extract were mostly individuals receiving their second course of desensitization with this extract, which was carried over from the 1968 evaluation. The same was true of the patients receiving placebos. Eight of the patients who had received alum-precipitated ragweed extract were retreated in 1969, and the remainder of the patients who received alum-precipitated extract were recruited either from individuals who

*Lichtenstein, L. M.: Unpublished data.

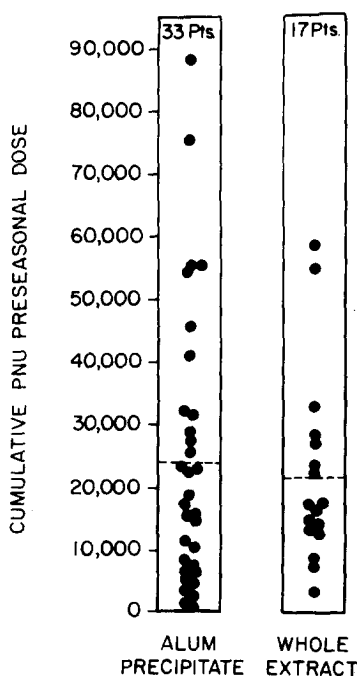


FIG. 4. Total cumulative preseasonal dose (PNU) of ragweed pollen extract administered in 1969.

had received antigens E and K in the previous year or new patients. It was established by determinations of leukocyte sensitivity with the use of histamine release with antigen E that the patients in the 3 groups were similar in their sensitivity to ragweed. Two important changes in our usual procedure were instituted in this study. Several of our studies have indicated that there is little carry-over of previous treatment effects from one year to the next. Additional evidence, as yet unpublished, indicates that relapse is complete one year after treatment is discontinued. Therefore, we were willing to mix previously treated patients with untreated ones in organizing our groups. Furthermore, as leukocyte sensitivity to antigen E has rather accurately predicted the severity of symptomatology in untreated patients year after year,^{3, 6, 7} patients were not assigned to treatment groups randomly. Rather the alum precipitate treatment group was rounded out with enough new patients to make the 3 groups comparable in leukocyte sensitivity.

There were 21 patients in the placebo group, 17 patients in the whole extract group, and 33 patients in the alum precipitate group. Injections were begun in early February and continued until the first week of August at weekly intervals. Patients in whom reactions limited dosage, were asked to come twice in some weeks in order to receive additional doses. In the alum precipitate-treated group, there were no severe systemic reactions under this schedule, and the average preseasonal cumulative dose was 23,501 PNU (.045 mg. of AgE) with an average of 16 injections (Fig. 4). Results in patients receiving treatment for the first time confirmed that fairly high doses could be attained in the

TABLE I. Reactions to immunotherapy, 1969

	Whole extract	Alum precipitate
No. patients	17	33
No. injections	300	530
Local reactions		
Mild	59	162
Moderate	1	11
Severe	0	3
No. per patient	3.5	5.3
No. per injection	0.20	0.33
Systemic reactions		
Mild	10	3
Moderate	0	3
No. per patient	0.58	0.19
No. per injection	0.033	0.011

first year of treatment with this number of injections. One patient, after an injection of 2,000 PNU, developed a firm 1 cm. subcutaneous nodule which regressed and disappeared entirely in about 4 weeks. He subsequently took larger doses without developing more nodules.

As patients in the whole extract group were carried over from 1968 and hence received their second course of immunotherapy, they required fewer injections in 1969 to reach larger doses. These patients received an average of 17.6 injections (range, 16 to 20) for an average cumulative dose of 21,260 PNU 0.57 mg. of AgE).

Table I compares local and systemic reactions in the groups. The rate of local reactions was somewhat lower with aqueous extract (0.2 per injection) than with alum-precipitated extract (0.33 per injection), but the rate of systemic reactions was noticeably higher with aqueous extract (0.033 versus 0.011 per injection). The latter difference is probably important in practical terms and not unexpected in view of the ability of alum-precipitated extracts to persist at the site of injection. Systemic reactions present the most serious danger in desensitizing injections, and a reduction in the rate of their occurrence represents a useful therapeutic advantage.

Insofar as clinical evaluations are concerned, physician scores in the alum-precipitated group ranged from 0 to 12.5 with an average of 7.5. In aqueous extract-treated patients the range was 2.0 to 13.0 and the average 6.65; whereas, in the placebo patients, the range was 3.0 to 24.5 with an average of 13.2. The differences between the scores of either of the treated groups or the placebo group is significant at the one per cent level (Mann-Whitney U test), whereas there is no significant difference in scores between aqueous extract and alum-precipitated extract.

Symptom scores from daily diaries showed a similar trend. Fig. 5 shows daily averages of symptom scores in the alum-precipitated, whole extract, and placebo groups. That symptoms were regularly less severe in the treated groups than in the placebo group is well illustrated. Furthermore, clinical results with the alum-precipitated extract were quite similar to those with whole unprecipitated extract.

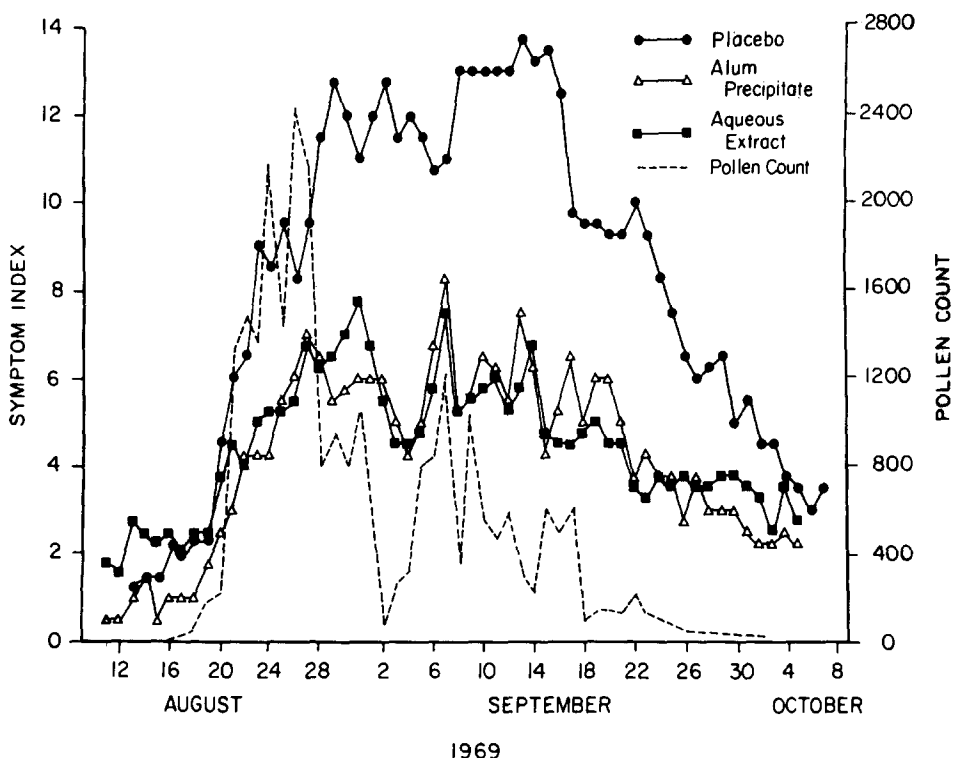


FIG. 5. Average daily symptom index correlated with pollen count in 1969.

The averages of daily symptom scores were 4.3 for the alum precipitate-treated group, 4.5 for the whole extract-treated group, and 8.5 for the placebo-treated group. Fig. 6 is a scattergram of seasonal average scores for individual patients. By the Mann-Whitney U test, the difference between the scores of alum precipitate group and the placebo group is highly significant ($p=0.00014$), as is the difference between the scores of the whole extract group and the placebo group ($p=0.0016$).

Antibody titers in the placebo patients were less than 10. Fig. 7 is a scattergram of the antibody titers in the 2 groups of treated patients and shows that the posttreatment antibody titers were nearly the same. It appears from such results that no adjuvant effect of the alum precipitate was demonstrated in human beings. The principal advantage of the alum precipitate appears to be the ability to give larger doses in fewer injections with a reduced rate of systemic reactions.

Examination of cell-sensitivity data as determined by dose-response curves of histamine release from isolated peripheral leukocytes in the presence of ragweed antigen E indicates that a proportion of treated patients sustained a reduction in cell sensitivity. Five of 17 patients treated with aqueous extract showed such a reduction, and 12 of 32 patients treated with alum precipitates showed similar reductions, whereas 3 of 22 placebo-treated patients showed spontaneous reductions. Thus it appears that alum precipitates are at least as

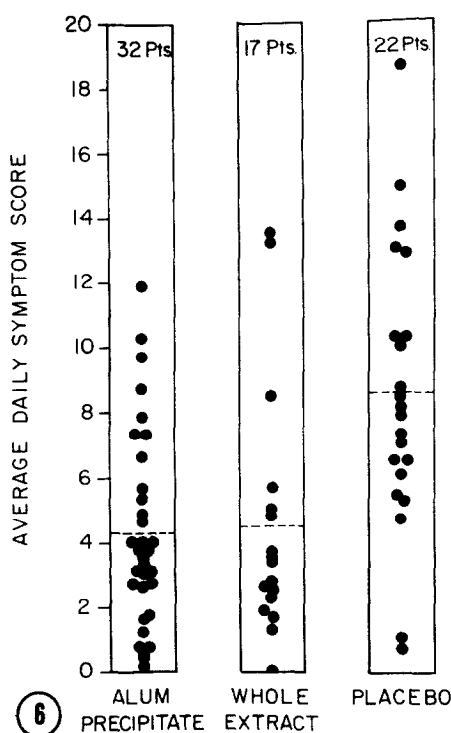


FIG. 6. Average of daily symptom score for each patient under study in 1969.

good as whole extracts in producing this particular immunologic change. As in other studies^{4, 5, 6, 7} no clear relationship between dosage and reduction in cell sensitivity was noted.

DISCUSSION

The clinical use of alum-precipitated pollen antigens was first suggested in 1934 by Garrison,¹⁶ who reported antibody responses in guinea pigs. Stull and associates,¹¹ in 1940 reported a limited uncontrolled clinical trial with such extracts in which the rate of constitutional reactions was somewhat decreased over that obtained with conventional aqueous extracts. The clinical results were encouraging. Small numbers of highly sensitive patients treated over the last 25 years have seemed to do well.¹⁷ However, the technique has been relatively neglected in the search for an effective method which has complete freedom from untoward reactions or can be given in one "shot."

In our efforts to establish whether high doses are the key to optimum results with immunotherapy, we have turned to alum precipitates as a relatively sure method of increasing doses with safety. From a strictly practical standpoint, the advantages of alum-precipitated extracts are real. It appears possible to attain therapeutically effective doses in about half the number of injections with a reduction in incidence of generalized (anaphylactic) reactions at the cost of slightly more severe local reactions. Alum-precipitated grass pollen

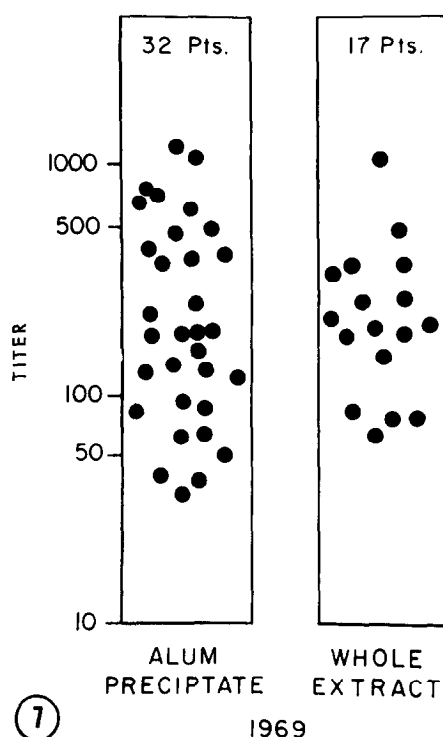


FIG. 7. Antibody levels in treated patients in 1969 (placebo-treated patients all <10).

extracts are effective in raising blocking antibody titers, and it seems likely that other pollen, mold, and inhalant antigens can also be prepared in the alum-precipitated form. Injected alumina have been used not only as alum precipitates but also as aluminum monostearate for many years with no hint of side effects from the injected aluminum.

In respect to the thesis that in immunotherapy amelioration of symptoms of hay fever depends to some extent on the dose of antigen administered, this study represents the largest doses we have yet administered to adults in a controlled study. The clinical results as judged by the difference in symptom reporting between treated and control patients are likewise the most impressive we have yet reported. As before, there are relatively few cures, and most patients report at least some symptoms. Our results in adults are similar to those reported earlier in children with doses at this level.⁴

It is clearly important that the clinical and immunologic effects of still higher doses be explored. This can be done, with some effort, with alum precipitates; but still better repository methods should also be sought. The allergoid concept (reducing the allergic toxicity of extracts while maintaining their immunologic potency) is a new approach to this problem¹⁸ currently under investigation.

As an immediate means of raising dosage while reducing the number of injections and the danger of allergic side effects, alum precipitates have utility

and can be recommended for general use employing the same principles of administration now used for aqueous extracts. We do, however, recommend that injections be given no more often than once a week in order to allow some time for antibody responses to develop between injections.

It is impossible to describe the dosage of pollen extracts in terms of PNU with any real meaning. Baer and associates⁹ found ragweed pollen extracts purchased from 6 different suppliers and labeled with the same PNU content to vary 100 per cent in biological activity as measured by direct skin test and histamine release. Conversely, determinations of antigen E (the principle allergen of ragweed) by double diffusion in gel accurately predicted the activity of the extracts. In this study, determinations of antigen E concentration were available in the extracts we used in 1969. In terms of antigen E, the whole (unprecipitated) extracts and alum-precipitated extracts were found to be quite similar. From our data with both aqueous extracts and alum precipitates, annual cumulative doses of extracts containing about 0.050 mg. (50 μ g) antigen E are likely to be therapeutically effective. This is in contrast to earlier work with antigen E separated from the other constituents of ragweed pollen, in which 0.03 mg. antigen E was ineffective, and a minimum of 0.25 mg. was required to demonstrate efficacy.¹ Presumably the difference lies in antigens in whole pollen extract which cross-react immunologically with antigen E.

REFERENCES

- 1 Norman, P. S., Winkenwerder, W. L., and Lichtenstein, L. M.: Immunotherapy of hay fever with ragweed antigen E: Comparisons with whole pollen extract and placebos, *J. ALLERGY* 42: 93, 1968.
- 2 Norman, P. S., Winkenwerder, W. L., and Lichtenstein, L. M.: Maintenance immunotherapy in ragweed hay fever: Booster injections at six weeks intervals, *J. ALLERGY* 47: 273, 1971.
- 3 Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: A single year of immunotherapy of ragweed hay fever, immunologic and clinical studies, *Ann. Intern. Med.* 75: 663, 1971.
- 4 Sadan, N., Rhyne, M. B., Mellits, D., Goldstein, E. O., Levy, D. A., and Lichtenstein, L. M.: Immunotherapy of pollinosis in children: An investigation of the immunological basis of clinical improvement, *N. Engl. J. Med.*, 280: 623, 1969.
- 5 Levy, D. A., Lichtenstein, L. M., Goldstein, E. O., and Ishizaka, K.: Immunologic and cellular changes accompanying the therapy of pollen allergy, *J. Clin. Invest.* 50: 360, 1971.
- 6 Lichtenstein, L. M., Norman, P. S., Winkenwerder, W. L., and Osler, A. G.: In vitro studies of human ragweed allergy: Changes in cellular and humoral activity associated with specific desensitization, *J. Clin. Invest.* 45: 1126, 1966.
- 7 Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever, *Am. J. Med.*, 44: 514, 1968.
- 8 Levy, D. A., and Osler, A. G.: Studies on the mechanism of hypersensitivity phenomena. XVI. In vitro assays of reaginic activity in human sera: Effect of therapeutic immunization on seasonal titer changes, *J. Immunol.* 99: 1068, 1967.
- 9 Baer, H., Godfrey, H., Maloney, C. J., Norman, P. S., and Lichtenstein, L. M.: The potency and antigen E content of commercially prepared ragweed extracts, *J. ALLERGY* 45: 347, 1970.
- 10 Sledge, R. F.: Treatment of hay fever with alum-precipitated pollen extract, *U.S. Naval Med. Bull.* 36: 18, 1938.
- 11 Stull, A., Cooke, R. A., Sherman, W. B., and Hebard, S.: Experimental and clinical study of fresh and modified pollen extracts, *J. ALLERGY* 11: 439, 1940.
- 12 Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: Antibody response follow-

- ing immunotherapy in ragweed hay fever: Allpyral vs. whole ragweed extract, *J. ALLERGY* 41: 49, 1968.
- 13 Lichtenstein, L. M. and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen, *J. Exp. Med.* 120: 507, 1964.
 - 14 Lichtenstein, L. M., and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XII. An in vitro study of the reaction between ragweed pollen antigen, allergic human serum and ragweed sensitive cells, *J. Immunol.* 96: 169, 1966.
 - 15 May, D. C., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volumes of blood, *J. ALLERGY* 46: 12, 1970.
 - 16 Garrison, W. T.: Effect of alum precipitated ragweed pollen extract on guinea pigs, *Public Health Rep.* 49: 462, 1934.
 - 17 Sherman, W. B.: Personal communication.
 - 18 Marsh, D. G., Lichtenstein, L. M., and Campbell, D. H.: Studies on "allergoids" prepared from naturally-occurring allergens. 1. Assay of allergenicity and antigenicity of formalinized rye Group 1 allergen, *Immunology* 18: 703, 1970.