

Clinical and immunologic evaluation of a purified fraction of ragweed pollen (delta)

A double-blind study

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The delta fraction appeared to be as effective clinically as whole ragweed extract in the relief of symptoms of ragweed pollinosis. The immunologic response, as measured by the hemagglutinating and reagin antibody titers, is essentially the same following treatment with either delta or whole ragweed extract. This fraction can be readily reproduced. Its allergenic activity may be measured in terms of dry weight. This is a definite step toward the standardization of ragweed antigens.

The isolation and identification of the active allergen or allergens in ragweed pollen could well be of fundamental and practical importance. This information may lead to a better understanding of the mechanism of allergic sensitization, to means of more accurate diagnosis and therapy, and, finally, to a better method of standardization of allergy extracts. In addition, patients would not receive injections of extraneous materials which might possibly be potential sensitizers.

Although saline extracts of whole ragweed pollen have been used in the diagnosis and treatment of ragweed pollinosis for almost half a century, the true

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antigenic or allergenic nature of this material has not yet been determined. As long as 40 years ago, attempts were made to fractionate ragweed pollen extracts by various chemical methods available at that time.^{1, 2} About 25 years ago, Stull and co-workers³⁻⁵ separated ragweed extract into two major fractions by the use of ammonium sulfate precipitation. Extensive studies were done with these materials; however, a purified, identifiable material could not be isolated.

Within recent years, several workers⁶⁻⁹ have reinvestigated this problem using many of the modern immunochemical techniques of fractionation. In a previous article,¹⁰ we reported comparative studies of several of these fractions. One of these materials, the "delta" fraction, prepared by Lea and Sehon,⁶ was found to be very active as measured by several standards. In vivo neutralization experiments demonstrated that it was the only fraction which was able to neutralize completely the reaction of reaginic sera to a subsequent challenge with whole ragweed extract. Cece and associates¹¹ also noted the failure of these other fractions as well as their own to inhibit this reaction with whole ragweed extract. They did not study the delta fraction.

Delta also produced the highest percentage of positive hemagglutination reactions in the sera of untreated ragweed-sensitive patients. Delta was highly skin reactive by direct testing. Gel diffusion studies revealed that although much of the extraneous material of whole ragweed pollen extract had been removed in its preparation, it still contained several antigens. In these latter two respects, the other fractions were quite similar to delta.

Thus delta was somewhat "purified" (or at least the antigens were more concentrated) and seemed to contain most of the active allergens in whole ragweed extract. In view of these facts, it was deemed advisable to study the clinical response of ragweed-sensitive patients treated with this material.

METHODS

I. Selection of patients

Patients from the Allergy Clinics of the Buffalo General Hospital and Children's Hospital were carefully selected for this study. All had definite clinical and dermal ragweed sensitivity, and none had ever received previous specific hyposensitization therapy.

II. Materials

Delta was supplied as a sterile dry powder in 0.5 mg. amounts. It was dissolved in buffered saline just prior to use. Fresh solutions of delta were made up every 2 weeks. Following Dr. Samuel O. Freedman's advice,¹² on the basis of skin reactivity and response to subcutaneous administration, 0.5 mg. of delta was considered equivalent to 10,000 protein nitrogen units (PNU) of whole ragweed extract.* Throughout the remainder of this article, delta is expressed in PNU on this basis.

A 15 per cent extract of defatted short ragweed pollen in Coca's solution†

*Subsequent determinations indicated 0.5 mg. of delta contained 13,000 PNU.

†Coca's solution: 0.5 per cent NaCl, 0.275 per cent NaHCO₃, and 0.4 per cent phenol.

was prepared in our laboratory. Fresh dilutions of this extract were also prepared every 2 weeks.

III. Therapy

The patients in this study were divided at random into two groups on the basis of comparable age distribution. There were eight patients under age 16 and four patients over age 16 in each group. One group received gradually increasing doses of standard aqueous whole ragweed extract. The second group of patients received gradually increasing amounts of the delta fraction. The dosage schedule is outlined in Table I.

The study was conducted in a double-blind fashion. Neither the patients nor the physicians knew which material was administered. Dosage was prescribed on the basis of the predetermined schedule. Individual adjustments were made as necessary. Extracts for the two groups were simply labeled *A* and *B*. These were administered by nurses in a separate room.

IV. Evaluation of results

All patients were questioned and examined weekly throughout the pollen season. In addition, each patient kept a daily record of symptoms and the amount of medication taken.

Evaluation of the clinical response was based upon the attending physician's judgment following weekly examination of the patients, review of their symptoms cards, and notation of any required symptomatic medication. The clinical evaluation of therapy was done without knowledge of the type of treatment which had been administered.

V. Serologic studies

Samples of sera were obtained from most patients prior to the beginning of therapy, and before and after the pollen season.

Reagin titers were determined by the standard passive transfer technique with fivefold serial dilutions of sera. All titers were determined in duplicate using two recipients. Sensitized sites were challenged after 24 hours with whole ragweed extract (3,000 PNU per cubic centimeter).

Table I. *Dosage schedule guide for whole ragweed extract and delta therapy*

<i>Dose number</i>	<i>PNU of extract</i>	<i>Dose number</i>	<i>PNU of extract</i>
1	2	11	1,000
	5		1,500
	10		2,000
	20		3,000
	40		4,000
6	70	16	5,000
	100		6,000
	200		7,000
	400		8,000
	700		10,000

Extracts were administered weekly and dose is expressed in protein nitrogen units. Five-tenths milligram of delta was considered equivalent to 10,000 PNU of whole ragweed extract.

Tanned cell hemagglutinating antibody titers were determined with pool C, another "purified" fraction of ragweed pollen, prepared by Goldfarb and Callaghan,⁷ as the coating antigen. Previous studies¹³ have indicated the potent hemagglutinating properties of pool C, and we have used this material routinely in hemagglutination studies.

Hemagglutinating antibody titers of a group of postseason sera were compared with both delta and pool C as hemagglutinating antigens. Previous studies¹³ of sera obtained from patients treated with whole ragweed extract indicated that higher hemagglutination titers were obtained with pool C as the coating antigen than with the homologous antigen. We now wished to compare delta-treated patients in this respect.

RESULTS

I. Clinical response

According to the criteria previously outlined, nine of twelve patients in each group had a satisfactory clinical response to therapy.

The highest single dose administered to patients receiving aqueous ragweed extract ranged from 100 PNU to 6,000 PNU. The majority of patients received over 2,000 PNU. The three patients who had unsatisfactory clinical responses received highest doses of 100, 3,000, and 4,000 PNU, respectively (Table II).

The total dosage administered in this group ranged from 490 PNU to 24,000 PNU. The majority of the patients received over 5,000 PNU. The three patients who responded poorly received total doses of 490, 11,000, and 20,000 PNU (Table III).

In the group receiving delta, the highest individual dose ranged from 2 PNU (0.0001 mg.) to 8,000 PNU (0.4 mg.) (Table II). Two of three patients receiving less than a highest single dose of 500 PNU (0.025 mg.) responded poorly, as did one patient who received 5,000 PNU (0.25 mg.). The total dose of delta admin-

Table II. *Range of the maximum single administered dose of ragweed and delta*

<i>Protein nitrogen units</i>	<i>Ragweed</i>	<i>Delta</i>
<500	1 (1)*	3 (2)
500-999	0	0
1,000-1,999	2	2
2,000-3,999	5 (1)	1
>4,000	4 (1)	6 (1)

*Indicates patients who responded unsatisfactorily.

Table III. *Range of the total administered dose of ragweed and delta*

<i>Protein nitrogen units</i>	<i>Ragweed</i>	<i>Delta</i>
<500	1 (1)*	1 (1)
500-5,000	1	4 (1)
5,000-10,000	3	1
10,000-20,000	5 (1)	3 (1)
>20,000	2 (1)	3

*Indicates patients who responded unsatisfactorily.

istered ranged from 8 PNU (0.0004 mg.) to over 31,000 PNU (1.55 mg.). The patients who responded poorly received total doses of 8, 2,000, and 18,000 PNU (0.0004 mg., 0.1 mg., and 0.9 mg.) (Table III).

All patients had been tested intradermally with 30 PNU per milliliter of whole ragweed extract prior to therapy. Of the twelve patients receiving whole ragweed extract, six had 4 plus reactions and six had 3 plus reactions. Three of the patients who had 4 plus skin reactions responded poorly.

In the group of patients receiving delta, four had 4 plus reactions, four had 3 plus reactions, and four had lesser degrees of reactivity. One patient from each of these three groups responded poorly.

Three patients had systemic reactions to whole ragweed extract during the course of therapy. One patient reacted to 1,000 PNU, but subsequently was able to reach a maximum dose of 3,000 PNU. The second patient reacted to 700 PNU, but was able to reach a maximum dose of 3,000 PNU. The third patient reacted to 4,000 PNU, which was the maximum dose administered.

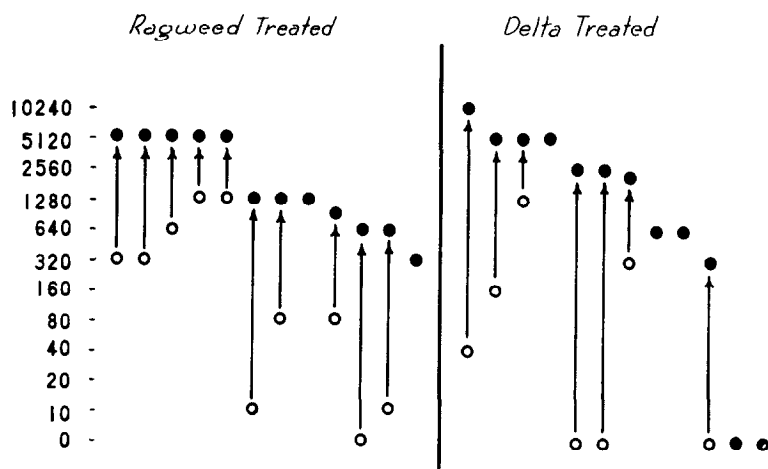
Five patients had constitutional reactions (none severe) from the delta fraction. One patient reacted to 70 PNU (0.0035 mg.), but was able to attain a maximum dose of 6,000 PNU (0.3 mg.). The second patient reacted to a maximum dose of 400 PNU (0.02 mg.). The other three patients reacted to maximum doses of 1,000 (0.05 mg.), 1,500 (0.075 mg.), and 4,000 PNU (0.2 mg.), respectively. It was of some interest that of these eight patients who had systemic reactions, three had no demonstrable reagin when sites sensitized with their sera were challenged with whole ragweed extract. One of these three patients subsequently developed a reagin titer of 5.

II. Serologic response

A. *Hemagglutination antibody titers.* Routine hemagglutinating antibody titers were determined with pool C as the coating antigen on tanned red blood cells. All but two patients, of those for whom data was available, had a four-fold or greater rise in hemagglutination titer following therapy (pretreatment to preseason). This included ten patients who received whole ragweed extract and seven patients who received delta (Fig. 1). One patient who did not have demonstrable antibodies prior to the ragweed season did have a postseason hemagglutination titer of 160. Two patients had negative preseason hemag-

Table IV. *Relation of the preseason hemagglutination titer to clinical response in ragweed- and delta-treated patients*

HA titer	Clinical response			
	Satisfactory		Unsatisfactory	
	Ragweed	Delta	Ragweed	Delta
0-100		1		1
101-500	1	1		
501-1,000	3	1		1
1,001-2,000	1	1	1	
2,001-4,000		1	1	1
>4,000	4	4	1	

**Fig. 1**

Hemagglutinating antibody response following therapy. Open circles indicate pretreatment titers; closed circles indicate preseason posttreatment titers. Half-filled circles indicate no change between the two serum samples. Titer is expressed as reciprocal of highest serum dilution giving a positive reaction.

glutination titers following delta therapy. They received total doses of 900 and 1,000 PNU (0.045 and 0.05 mg.).

No correlation could be found between the absolute preseason hemagglutination titer and the clinical response in either group of patients (Table IV).

Hemagglutination titers of postseason sera, obtained from both groups of patients, were determined by use of pool C and delta as coating antigens. In almost all instances higher titers were obtained with pool C (Fig. 2).

B. Reagin antibody titers. Pre- and posttreatment reagin titers of the sera of nine patients receiving whole ragweed extract were determined. The titer increased in two, decreased in two, and remained the same in five patients. The two patients in this group who responded unsatisfactorily had a decrease and an unchanged titer, respectively (Fig. 3).

Similar data were available for nine patients who received delta. Of these patients, three had an increase in titer, one had a decrease, and five remained the same. The two patients of this group who responded unsatisfactorily both had an unchanged reagin titer.

Table V. Relationship of the preseason reagin titer and clinical response in ragweed- and delta-treated patients

Reagin titer	Clinical response			
	Satisfactory		Unsatisfactory	
	Ragweed	Delta	Ragweed	Delta
0		3	1	2
5	2	1		
25	3	3	1	
125	3	1	1	1
625	1	1		

The absolute preseason reagin titer of the sera of all twenty-four patients was determined. There seemed to be no correlation of titer with the clinical response (Table V).

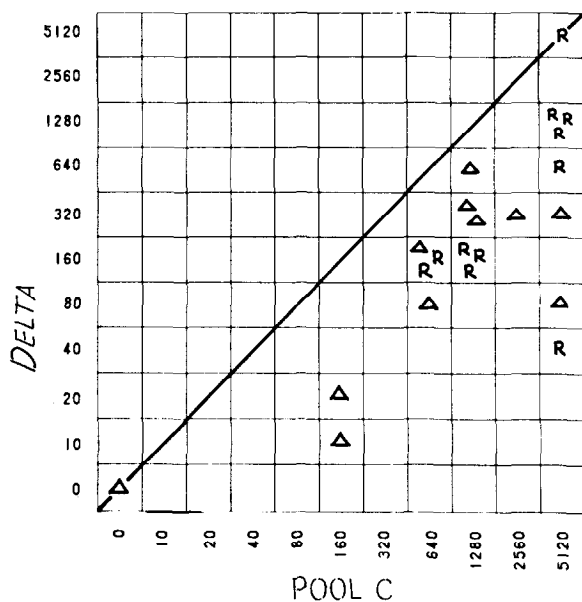


Fig. 2

Comparison of hemagglutination titers of postseason sera with pool C and delta as the coating antigens. Delta-treated patients are indicated by Δ ; ragweed-treated patients are indicated by *R*. All symbols below diagonal line indicate higher hemagglutination titers with pool C as the coating antigen.



Fig. 3

Reagin titer response following therapy. Open circles indicate pretreatment titers. Closed circles indicate preseason posttreatment titers. Half-closed circles indicate no change between serum samples. Titers are expressed as reciprocal of highest serum dilution giving a positive reaction.

DISCUSSION

The accurate evaluation of the efficacy of specific injection therapy in the treatment of ragweed pollinosis is difficult, even under the most controlled conditions. Furthermore, good results have been found in a surprisingly high number of patients receiving placebo injections.¹⁴ Some of these problems have been thoroughly discussed by Lowell and Franklin.¹⁵ In our studies we have attempted to eliminate many of the potential obstacles. First, only previously untreated patients were chosen. Thus we had a pool of "fresh" patients who had had symptoms the previous year and were likely to have further symptoms during their next exposure. Any benefit derived from previous specific injection therapy would not be "carried over" and the chance of dealing with "burned-out" ragweed sensitivity was diminished.

Second, patients were divided at random into two groups, on the basis of age distribution only. Other parameters of sensitivity, such as duration and severity of symptoms, skin-test reaction, and reagin titer, might possibly better equate these groups. Third, administration and evaluation of therapy was done in a double-blind fashion. This seems to be the only suitable way to obtain accurate, unbiased data. In this study, the prescribing physicians had no way of knowing which material was administered. Solutions looked alike and, furthermore, were given by nurses in a separate room. Reactions, both local and systemic, occurred from both materials.

Evaluation of ragweed symptoms was done without knowledge of type of therapy. Regular patient interviews and examinations were conducted throughout the pollen season. Symptom cards recording the daily estimated severity of symptoms and amount of medication taken were reviewed weekly. From these reports and examinations, the response to therapy was judged simply as satisfactory or unsatisfactory. Following completion of the study, the code was broken. Thus, this study was conducted in as unbiased fashion as possible and the results should be meaningful.

In this brief study, the similarity of the clinical and immunologic response to the delta fraction and whole ragweed extract was quite striking. The therapeutic response, evaluated in a double-blind fashion, was exactly the same for both groups, with nine of twelve patients responding satisfactorily. Meacock and colleagues¹⁶ have also found no marked difference in clinical response following therapy with delta or their dialyzed residue (whole ragweed extract after extensive dialysis). It appeared that patients receiving smaller doses of delta had a poorer response. In the ragweed-treated group, dosage and response seemed unrelated.

Systemic reactions occurred in both groups of patients. It was of interest that five reactions to delta did occur in five different patients. Furthermore, three of these five reactions occurred following relatively small doses of delta.

Immunologic response to therapy was also quite similar in both groups of patients. Almost all patients studied had a rise in hemagglutinating antibody titer following therapy. The rise in both groups was quite similar and the absolute preseason hemagglutination titers in both groups were also comparable.

Sera obtained from delta-treated patients also reacted in higher titers with

pool C-coated tanned red blood cells than with the homologous antigen. Thus, in this respect, antibody response to delta and whole ragweed extract is similar. The effect of therapy with each of these two substances upon change in reagin titers and the absolute reagin titers was also quite similar.

Of some interest was the lack of correlation between absolute reagin titers and clinical response to therapy. Two of the eight patients with reagin titers over 100 and four of the sixteen patients with titers below 100 responded poorly. Previous studies¹⁴ from this laboratory had suggested some relation of the absolute reagin titer to clinical response. In a much larger study, Connell and Sherman¹⁷ found a striking correlation between hay fever symptoms and skin-sensitizing antibody titer. However, the occurrence of systemic reactions during hypsensitization in three patients who had no demonstrable reagins further suggests that other factors may also affect clinical symptoms.

Pool C, the "purified" fraction prepared by Goldfarb and Callaghan, has also been used to treat ragweed-sensitive patients. Kaplan and associates¹⁸ reported that nine of thirteen patients treated with this material had good to excellent relief. Systemic reactions were encountered in seven of these patients. Hemagglutinating antibody titers rose in all but one patient. No correlation between hemagglutination titer and clinical relief was found.

It would thus appear from this study of a relatively small number of patients that the delta fraction is clinically and immunologically similar to whole ragweed extract in the treatment of ragweed pollinosis. This material may serve as an important stepping-stone in the standardization of allergy extracts. It can be prepared and reproduced in the same manner and with the same activity. It can be supplied in a dry powdered form and remain stable for long periods of time. Further studies may reveal important information regarding the chemical nature of the important allergen(s) of ragweed pollen.

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