

Immunotherapy with antigen E

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Ragweed-sensitive patients were treated for 4 years with antigen E, a purified ragweed fraction. The over-all results, including a double blind study and crossover studies, suggested that antigen E was not as clinically effective as whole ragweed pollen extract. Antigen E was well tolerated, allowing administration of relatively large doses with few local and systemic reactions. In vivo reagin neutralization studies also suggested that some allergens had been lost in the preparation of Antigen E. Antigen E was highly antigenic, stimulating high titers of hemagglutinating antibodies, and precipitins could be demonstrated in some sera. Antigen E appears to be an important component of ragweed pollen. However, other allergenic fractions may also be necessary in order to provide optimal clinical results.

Most extracts used in clinical practice are rather crude substances containing much extraneous material. Isolation and identification of the active allergenic components are critically needed information which could lead to elaboration of the fundamental nature of the atopic reaction and availability of more effective clinical therapeutic agents.

Ragweed pollinosis has provided an excellent model for investigation because of its high prevalence and ready availability of large sources of allergen. In recent years, numerous investigators have fractionated ragweed pollen extract by various immunochemical techniques.¹⁻⁴ In 1962, King and Norman² described the preparation of a potent fraction of ragweed which has been termed antigen E* (AgE). Initial immunological studies⁵ indicated that this fraction, while not entirely "pure," did retain many of the allergenic properties of whole ragweed extract (WREX), and much of the extraneous material in the pollen

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*In the original paper, this fraction was termed IV_B.

had been eliminated in its preparation. Thus, AgE had characteristics suggesting it was suitable for clinical studies. Under the auspices of the Allergen Standardization Committee of the National Institutes of Health, AgE was made available to a number of investigators. This paper will report our 4 year clinical experience with AgE and some appropriately related immunological studies.

MATERIALS AND METHODS

Selection of patients

During 1965, 1966, and 1967, previously untreated ragweed-sensitive patients attending the Allergy Clinics of the Buffalo Children's Hospital and the Buffalo General Hospital were given preseasonal therapy with AgE. An additional group of previously untreated patients was given whole ragweed extract as part of a double blind study during 1965 and 1966. Following one season's therapy in this double blind study, patients were switched to either preseasonal AgE or WREX injections and observed in the subsequent year.

Seventeen patients who received preseasonal AgE therapy in 1967 were continued on perennial treatment through the 1968 ragweed pollen season.

All patients had definite ragweed sensitivity, as determined by reliable history and corroborative positive intradermal skin tests.

Materials

Antigen E was originally prepared by Dr. T. P. King of the Rockefeller Institute. In 1965, AgE was supplied in 5 ml. bottles in a concentration of 0.17 mg. per milliliter (roughly equivalent to 50,000 PNU per milliliter of concentrated ragweed extract). It was supplied through the Standardization Committee of the NIH and subsequently by a pharmaceutical laboratory.* In 1966, 1967, and 1968, this material was supplied in sterile vials in 2 different strengths. The weaker solution contained 0.5 mg. of AgE in 0.5 ml. of diluent,[†] and the stronger concentration contained 5 mg. in 0.5 ml. of diluent. The addition of 4.5 ml. of diluent gave concentrations of AgE suggested by Dr. King to be equivalent to about 14,700 PNU per milliliter and 147,000 PNU per milliliter of WREX, respectively. Antigen E was stored at 4° C. Fresh dilutions were prepared each week.

Whole ragweed extract, 50,000 PNU per milliliter, was supplied in 10 ml. vials. The same diluent was used for both ragweed extract and antigen E.

Administration of allergens

Antigen E was administered weekly or biweekly from May 1 until August 15. The dosage schedule originally planned called for 15 to 20 injections with a proposed maximum dosage of 170 μ g, which was equivalent to 50,000 PNU of WREX. In the second and third years of the study, higher doses of AgE were administered to some patients.

In 1965 and 1966, patients were divided at random into 2 groups. In the

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[†]Phosphate-buffered saline with 0.4 per cent phenol. In 1966, 1967, and 1968, 50 per cent glycerol was added.

double blind study, AgE was given to one group and WREX to the other group. Equivalent dosages of these antigens were used as much as clinically feasible.

The patients receiving perennial AgE therapy were given monthly injections of maintenance doses from October, 1967, through mid-August, 1968. In some cases, increased doses of AgE were also given for several weeks just prior to the ragweed season.

Evaluation of clinical results

All patients were interviewed and examined weekly throughout the pollen season. In addition, each patient kept a daily record card, recording the type and severity of symptoms and amount of medication needed for symptomatic relief. The clinical response, graded as satisfactory or unsatisfactory, was determined from evaluation of these data. For those patients in the double-blind study, evaluation was done by a physician unaware of the type of therapy administered.

Immunological studies*

Hemagglutination. Serum was collected from each patient prior to therapy (pre-Rx) and following therapy (post-Rx), just before the pollen season. Hemagglutinating antibodies were determined by the tanned-cell technique,^{5, 6} with WREX, AgE, and Pool C† as the coating antigens. The optimal concentrations for coating the tanned red blood cells were AgE, 0.15 mg. per milliliter; Pool C, 0.04 mg. per milliliter, and 9,000 PNU per milliliter of WREX.

Precipitation. Precipitation tests were performed by the double-diffusion method.

The pre- and posttreatment sera were tested against WREX, AgE, and Pool C. As positive controls, monkey and rabbit antisera to whole ragweed extract were used.

Reagin titration. Selected paired pre- and posttreatment sera were assayed for reagins by the standard passive transfer technique (Prausnitz-Küstner reaction). Serial dilutions of these sera (0.1 ml.) were injected intradermally in duplicate sites into each of 2 nonatopic recipients. Each site was challenged with either 0.02 ml. of WREX (60 PNU) or 0.02 ml. of AgE (0.2 µg) 24 hr. later.

In vivo neutralization studies. This technique was utilized to compare the relative "allergenicity" of AgE with WREX. A 1 to 10 dilution (0.1 ml.) of each of 6 sera from ragweed-sensitive patients was injected in duplicate sites into the upper arm of 2 nonatopic recipients. One set of sera was challenged daily with AgE. Once the sites were exhausted and no reaction occurred with AgE, each site was challenged 24 hr. later with WREX. The second set of

*Details of these results will be presented in another publication.

†Pool C, another fraction of whole ragweed, was kindly supplied by Dr. R. Spitzer of the Chicago Medical School and prepared by the method described by Callaghan and Goldfarb.¹

passively sensitized sites was challenged daily with WREX. When these sites failed to react, each was challenged 24 hr. later with AgE.

RESULTS

Clinical results

Over-all Results. For study over this 4 year period, 88 patient years of AgE therapy were available. During their first year of therapy 48 previously untreated patients received AgE, and 40 patients received AgE during their second year of therapy. A satisfactory clinical response occurred in 57 of the 88 patients. Of the 48 previously untreated patients, 31 responded satisfactorily. In the group of 40 patients receiving AgE for the second year, 26 did well (Table I). This latter group included 12 of 17 patients on perennial therapy and 14 of 23 patients who received preseasonal injections.

Double blind study. During 1965 and 1966, comparable doses of WREX and AgE were administered to previously untreated patients in a double blind study. In each group were 18 patients. A good clinical response was obtained in 16 of the 18 patients receiving ragweed and in 11 of the 18 patients treated with AgE (Table II).

Crossover studies. Although not part of a preplanned study, a number of patients received a change in therapy in successive years. Thus, 3 groups of patients were available for comparison.

GROUP I. In the first year of specific treatment, 6 patients who responded poorly following AgE therapy were switched to whole ragweed extract the following year. All responded satisfactorily in the second year (Table III).

The total dosage of antigen given these patients in both years was quite variable. The AgE dose ranged from 0.03 mg. to 0.7 mg. (9,500 PNU to 210,000 PNU) and the WREX dose varied from 50 PNU to 47,000 PNU. All of these patients except one received lower comparable doses of whole ragweed extract than of antigen E given the previous year.

Table I. *Clinical effectiveness of antigen E therapy*

<i>Treatment</i>	<i>No. of patients</i>	<i>No. improved</i>
Previously untreated	48	31
Second-year therapy:		
Preseasonal	23	14
Perennial	17	12
Total	40	26
Total no. of patients	88	57

Table II. *Double blind study of clinical response to hyposensitization therapy with antigen E and whole ragweed extract*

<i>Antigen used</i>	<i>No. of patients</i>	<i>No. improved</i>
Antigen E	18	11
Whole ragweed extract	18	16

Table III. *Comparison of clinical effectiveness of antigen E and whole ragweed extract therapy in successive years.**

<i>Patient</i>	<i>First year treated with antigen E† (PNU)</i>	<i>First year results</i>	<i>Second year treated with ragweed extract (PNU)</i>	<i>Second year results</i>
J. B.	210,000	N. I.‡	2,100	I.‡
M. S.	100,000	N. I.	1,800	I.
D. S.	75,000	N. I.	2,000	I.
J. S.	13,500	N. I.	47,000	I.
M. A.	135,000	N. I.	300	I.
H. B.	9,500	N. I.	50	I.

*Total dose (PNU) of allergen administered to 6 patients switched from antigen E to whole ragweed extract in successive years (Group I).

†Assuming that 0.5 mg. of antigen E was equivalent to 150,000 PNU per milliliter.

‡I., improved; N.I., not improved.

Table IV. *Comparison of clinical effectiveness of whole ragweed extract and antigen E therapy in successive years**

<i>Patient</i>	<i>First year treated with ragweed extract (PNU)*</i>	<i>First year results</i>	<i>Second year treated with antigen E (PNU)†</i>	<i>Second year results</i>
V. B.	150,000	I.‡	32,500	I.
F. B.	250,000	I.	100,000	I.
J. M.	145,000	I.	75,000	I.
P. P.	70,000	I.	635,000	I.
J. E.	65,000	I.	178,000	I.
R. W.	120,000	I.	538,000	I.
C. G.	120,000	I.	6,000	I.
J. B.	14,000	I.	210,000	N. I.‡
M. E.	60,000	I.	1,256	N. I.
J. S.	20,000	I.	172,000	N. I.
J. V.	290,000	I.	518,000	N. I.
D. L.	111,000	N. I.	180,000	I.
Total improved		11		8

*Total dose (PNU) of allergen administered in 12 patients switched from ragweed to antigen E in successive years (Group II).

†Assuming that 0.5 mg. of antigen E was equivalent to 150,000 PNU per milliliter.

‡I., improved; N.I., not improved.

GROUP II. This group consisted of 12 patients treated with whole ragweed extract the first year and switched to antigen E the second year. During the first year of therapy, 11 of the 12 patients responded well. Only 7 of these 11 patients had satisfactory results when treated the second year with antigen E. It is interesting to note that 3 of the 4 patients who did not show improvement received higher comparable doses of antigen E than of whole ragweed extract in the previous year, and 4 of the 7 patients who benefited from therapy the second year received less antigen E than whole ragweed extract in the first year (Table IV).

The one patient who responded poorly to whole ragweed extract the first

Table V. Comparison of clinical effectiveness of antigen E in successive years*

Patient	First year treated with AgE (PNU)	First year results	2nd year treated with AgE (pre- seasonal) (PNU)	Second year results
K. A.	197,000	I.†	232,000§	I.
R. C.	120,000	I.	655,000§	I.
W. E.	72,000	I.	12,500§	I.
J. F.	12,500	I.	550,000§	I.
K. H.	156,000	I.	550,000§	I.
J. J.	120,000	I.	75,000§	I.
N. S.	155,000	I.	100,000§	N. I.†
M. J.	110,000	I.	40,000§	N. I.
M. K.	9,500	I.	22,500§	N. I.
D. S.	28,000	N. I.	75,000§	N. I.
J. V.	517,500	N. I.	170,000§	N. I.
K. G.	30,000	I.	23,000¶	I.
M. H.	130,000	I.	99,000¶	I.
D. L.	180,000	I.	228,000¶	I.
C. M.	75,000	I.	285,000¶	I.
S. T.	190,000	I.	300,000¶	I.
L. S.	70,000	I.	54,000¶	I.
A. L.	40,000	I.	70,000¶	I.
P. J.	75,000	I.	21,000¶	I.
P. N.	70,000	I.	30,000¶	I.
F. R.	95,000	I.	90,000¶	I.
V. B.	32,500	I.	87,000¶	N. I.
P. S.	60,000	I.	66,000¶	N. I.
J. U.	12,000	I.	135,000¶	N. I.
J. V.	170,000	N. I.	270,000¶	N. I.
B. I.	42,500	N. I.	87,000¶	N. I.
M. C.	210,000	N. I.	189,000¶	I.
L. M.	95,000	N. I.	105,000¶	I.
Total improved		22		18

*Total dose (PNU)† of antigen E administered in successive years to 28 patients (Group III).

†Assuming that 0.5 mg. of antigen E was equivalent to 150,000 PNU per milliliter.

‡I., improved; N.I., not improved.

§Preseasonal.

¶Perennial.

year did have a satisfactory response following antigen E therapy in the second year.

GROUP III. In 2 successive years, 28 patients were given antigen E therapy. During both years, 11 patients received preseasonal therapy, and 17 patients received perennial therapy during the second year. Of the total group, a satisfactory response occurred in 22 patients the first year and 18 patients the second year (Table V).

Perennial therapy may have been slightly more advantageous than pre-seasonal therapy. The perennial group consisted of 17 patients, of whom 13 responded well the first year and 12 the second year. Of the 13 patients responding to therapy in the first year, 10 continued to do well with perennial therapy. Of the 4 patients not responding the first year, 2 did well following the second year of therapy.

In the group of 11 patients receiving preseasonal therapy both years, 9

patients responded satisfactorily the first year. Only 6 of these 9 continued to do well the second year, and only 2 patients did poorly both years.

No specific relationship between dose and clinical response was apparent. For example, patient J. V., who received a large amount of antigen E, had a poor response, whereas patient J. F. had a good clinical result following minimal therapy. Perhaps more specifically, there was no evident relationship between dose and result in those patients whose clinical response changed in successive years.

As a sidelight of this study, there were 9 patients in the above groups who responded well in successive years. The first year 4 received ragweed and 5 received antigen E. All received antigen E in the second year. In the third year no therapy was given and all 9 patients continued to be relatively asymptomatic.

Antigen E tolerance

Antigen E was well tolerated, and moderate doses were usually readily administered. A total of 1,411 injections of antigen E were given; 7 systemic reactions and 20 local reactions were observed. This reaction rate was lower than that noted for ragweed extract (Table VI).

The total and top doses of AgE were quite high. For the 3 years the average top and total doses were 0.10 mg. and 0.46 mg. This is equivalent to about 30,000 PNU and 137,000 PNU of whole ragweed, respectively. In 1966

Table VI. *Antigen E tolerance*

	<i>Ragweed</i> 1965-66	<i>AgE</i> 1965-66 (double blind study)	<i>AgE</i> 1966 (2nd year Rx)	<i>AgE</i> 1967	<i>AgE</i> 1968 (perennial Rx)	<i>Total</i> <i>AgE</i>
No. of patients	18	18	16	37	17	88
No. of injections						
Total	350	322	278	619	202	1,411
Individual average	19	17	17	17	12	16
Range	9-24	8-23				
Systemic reactions						
Total	9	4	3	—	—	7
No. of patients with reaction	5	2	1	—	—	3
Local reactions						
Total	14	7	7	6	—	20
No. of patients with reactions	11	4	5	4	—	13
Average top dose (PNU)	24,250	7,000	61,000	25,000	16,000	30,000
Range	750- 50,000	1,000- 42,500	500- 135,000	500- 90,000	3,000- 39,000	
Average total dose (PNU)	106,000	65,000	278,000	81,500	125,000	137,000
Range	4,000- 288,500	1,250- 197,500	1,250- 653,000	1,500- 235,000	21,000- 30,000	

an attempt was made to give very large doses of AgE to previously treated patients. In this group, the average top and total doses of AgE were equivalent to about 61,000 and 278,000 PNU of whole ragweed, respectively.

Reagin titration

Reagin titers of 6 paired sera which were obtained prior to and following therapy were determined. Three patients were treated with AgE and three with ragweed.

Reagin titers were not affected significantly by therapy with either AgE or WREX. No difference was noted when reaginic sera were challenged with either antigen (Table VII).

Table VII. *Effect of therapy upon reagin titer*

Patient	Therapy	Serum	Reagin Titer	
			Ragweed*	Antigen E*
D. B.	Antigen E	Pre-Rx	200	100
		Post-Rx	100	100
D. K.	Antigen E	Pre-Rx	50	50
		Post-Rx	50	50
R. W.	Antigen E	Pre-Rx	200	200
		Post-Rx	100	100
J. V.	Ragweed	Pre-Rx	100	100
		Post-Rx	100	100
J. S.	Ragweed	Pre-Rx	3,200	3,200
		Post-Rx	3,200	3,200
C. G.	Ragweed	Pre-Rx	< 10	< 10
		Post-Rx	< 10	< 10

*Sites challenged with either 60 PNU of whole ragweed or 0.2 μ g of Antigen E in 0.02 ml. volume.

Table VIII. *In vivo neutralization: Exhaustion with whole ragweed extract followed by challenge with antigen E*

Patients' serum*	Challenging antigen†				
	Ragweed				Antigen E
	Day 1	Day 2	Day 3	Day 4	Day 5
W. M.	++++‡	++++	++	0	0
A. G.	++++	+++	+	0	0
E. S.	++++	+	0	0	0
A. F.	++++	+	0	0	0
L. B.	++++	+++	++	0	0
R. B.	++++	++	+	0	0

*Serum diluted 1 to 10.

†Dose of challenging antigen was 60 PNU of whole ragweed and 0.2 μ g of antigen E in 0.02 ml. volume.

‡Skin reactions graded from negative (0) to strongly positive (++++).

Table IX. *In vivo neutralization: Exhaustion with antigen E followed by challenge with whole ragweed extract*

<i>Patients' serum*</i>	<i>Challenging antigen†</i>				
	<i>Antigen E</i>				<i>Ragweed</i>
	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Day 4</i>	<i>Day 5</i>
W. N.	++++‡	++	+	0	++++
A. G.	++++	++	+	0	++++
E. S.	++++	++	0	0	++++
A. F.	++	++	+	0	++++
L. B.	+++	++	+	0	++++
R. B.	++	++	+	0	++++

*Serum diluted 1 to 10.

†Dose of challenging antigen was 60 PNU of whole ragweed and 0.2 μ g of antigen E in 0.02 ml. volume.

‡Skin reactions graded from negative (0) to strongly positive (++++).

In vivo neutralization

One set of sensitized sites was challenged daily with WREX until no further reaction could be elicited. When tested 24 hours later, with AgE, each site was still unresponsive (Table VIII).

Another set of sensitized sites were challenged daily with AgE until no further reaction could be demonstrated. A strong positive reaction developed at each site when subsequently challenged with WREX (Table IX).

Serological findings

Hemagglutination. All patients developed an increased hemagglutinating antibody titer following preseasonal therapy. WREX, AgE, and Pool C were used as coating antigens for the hemagglutination test. The results with Pool C-coated RBC are summarized in Fig. 1. Some correlation between the antigen dose and the hemagglutination titer may be observed. AgE was perhaps slightly more antigenic than WREX. Further studies were done using WREX and AgE as coating antigens. The hemagglutination titers were generally lower but no other trends or conclusions were noted.

Precipitation. The purity of the AgE was examined by using rabbit and monkey antiserum to whole ragweed extract.

Fig. 2 shows the reactions of AgE (0.1 mg. per milliliter) and WREX (90,000 PNU per milliliter) with rabbit and monkey antisera. It will be noted that there are much fewer precipitation lines with AgE than ragweed extract. This would indicate that most of the antigens in WREX have been removed in the preparation of AgE. However, the reactions of various concentrations of AgE ranging from 0.06 mg. to 1.0 mg. with rabbit and monkey antisera reveal the presence of several remaining antigenic components. These are particularly noted in the reactions with the highest concentrations of AgE (Fig. 3).

Following AgE therapy, numerous post-treatment sera contained antibodies in sufficient quantity to be demonstrated by direct precipitation (Fig. 4). There

Total dose (PNU)	Post Rx HA Titer								
	500	1,000	2,500	5,000	10,000	20,000	40,000	80,000	100,000
< 10,000	XXX*	XXX		X	X				
10 - 20,000		OO†	XXX X	XX O	X				
20 - 50,000	X	XO	XX	XX		XX	X		
50 - 100,000	XXX	XOO			XXXX XO	X	X	XX	
100 - 200,000	XX OOO	XX	OXXX	X	OO	X	XXX XX	X	X
200 - 400,000			XO		XX	X	XO		
400 - 600,000						X	X		XX
> 600,000							XX		

*X = Antigen E treated.

†O = Ragweed extract treated.

Fig. 1

Hemagglutination titers of sera from ragweed-sensitive patients treated with antigen E or whole ragweed extract.

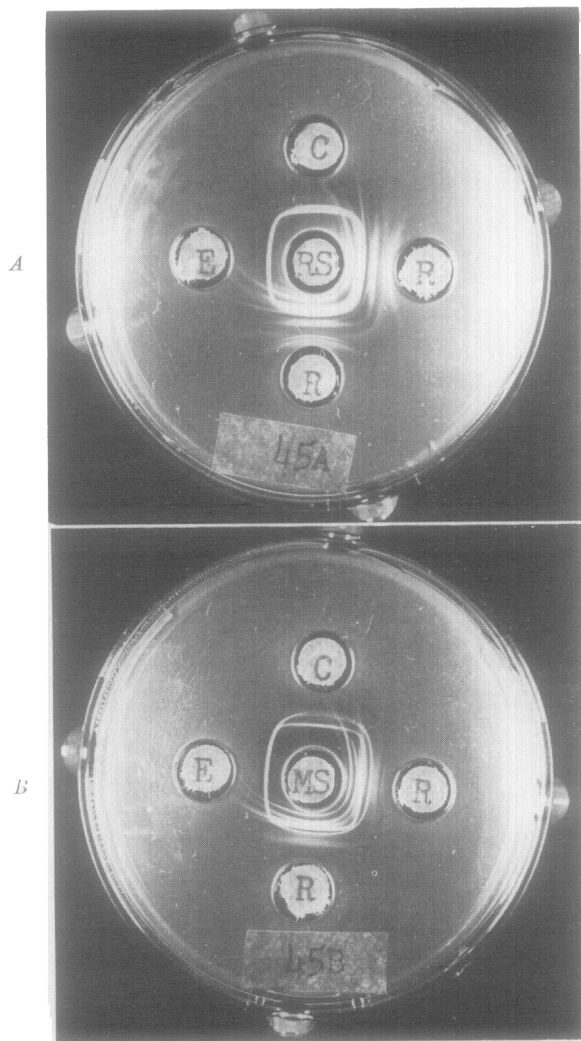
was some correlation between the level of the hemagglutination titer and the demonstration of precipitins.*

DISCUSSION

The numerous problems and variables affecting the critical judgment of therapeutic success of injection therapy have been thoroughly discussed.⁸ The most significant factors are patient selection, dosage of allergens, and clinical evaluation of therapy. Our total patient number was relatively small. Selection of patients for the study was done most carefully to insure unquestionable ragweed sensitivity. All new patients in this study had not previously received specific hyposensitization therapy in order to eliminate any possible carryover effects. It has been suggested that higher doses of extract generally lead to better relief of symptoms.⁹ For the most part, the patients treated in this study received doses of allergen even somewhat higher than is customary. Judgment of clinical response was based upon the physician's evaluation of daily symptom records and repeated weekly examinations during the pollen season. As noted, a double blind technique was used for part of this study. The examining physician was unaware of which allergen, AgE or WREX, the patient had received. We have not found a "point" system, based upon such criteria as number of pills taken or subjective estimation of symptom severity, to be of any additional value. While it would have been desirable to have treated more patients in a double blind manner, we do believe this has been a valid clinical study.

The clinical response from antigen E therapy was not quite as good as that obtained with whole ragweed extract, either in previously conducted studies

*Details in subsequent publications.

**Fig. 2**

A, Gel precipitation reaction of antiragweed rabbit serum (*RS*) and antiragweed monkey serum. *B*, (*MS*) with whole ragweed extract (*R*), antigen E (*E*), and Pool C (*C*). Antigen E and Pool C used in concentration of 0.1 mg. per milliliter. Ragweed used in concentration of 90,000 PNU per milliliter.

or in a concomitant double blind study described in this report. The crossover studies in successive years also suggested whole ragweed is a better therapeutic agent. However, in a recent publication, Norman and associates,¹⁰ found antigen E to be as clinically effective as whole ragweed extract. These investigators administered much higher doses of AgE than WREX.

Antigen E was extremely well tolerated. The reaction rate was much less than that of whole ragweed extract even though relatively higher doses of antigen E were administered. The doses of antigen E were also considerably higher

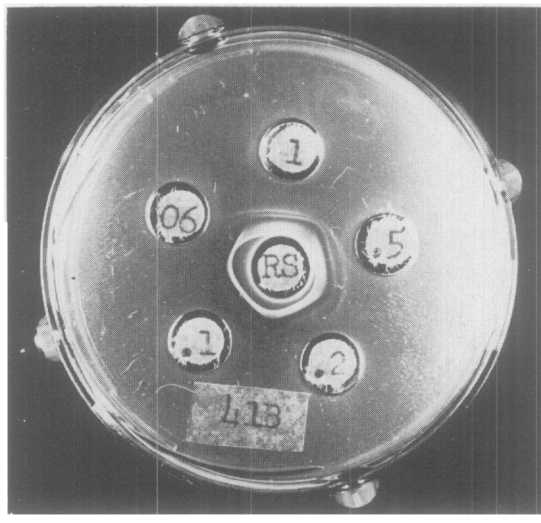


Fig. 3

Gel precipitation reaction. Rabbit antiserum to whole ragweed extract was placed in the center well (*RS*). In the peripheral wells, the numbers indicate antigen E concentration expressed in milligrams per milliliter. In the concentrations of 1 to 0.2 mg. per milliliter, several precipitation lines are visible. One precipitation line appeared only in the concentration with 0.1 mg. per milliliter.

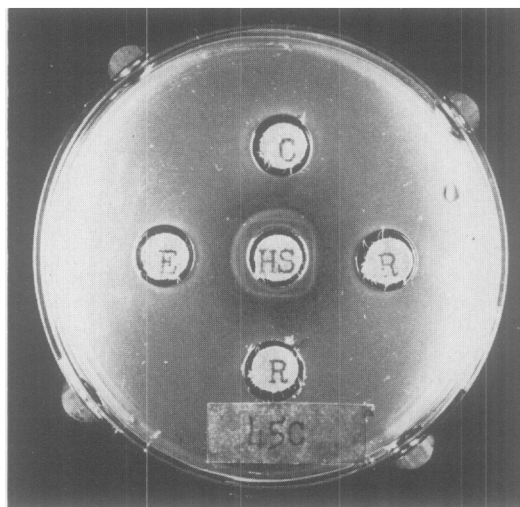


Fig. 4

Gel precipitation reaction. A patient's post-treatment serum was placed in the center well (*HS*). In the peripheral wells, the following antigens were placed: *C*—Pool C, 1 mg. per milliliter; *R*—whole ragweed extract, 90,000 PNU per milliliter; and *E*—antigen E, 1 mg. per milliliter. A precipitation line of identity with all used antigens was observed.

than those initially given by Lichtenstein, Norman, and Winkenwerder.¹¹ Subsequently, these investigators did use higher doses and also noted that in the majority of patients the dose of antigen E could be doubled each week without causing local or systemic reactions.

The allergenic properties of AgE and WREX have been compared by several methods. In a previously reported study,⁵ AgE was found to be more potent than WREX and several other ragweed fractions in eliciting direct skin test reactions in sensitive individuals. It was noted, however, that there were several patients who did have a stronger reaction with fractions other than AgE. In the present study, AgE and WREX were equally effective in titrating reagins by the classical passive transfer method. The *in vivo* neutralization of reagin technique provided a somewhat more sensitive method of evaluation of allergenic properties. Antigen E was unable to completely neutralize reaginic antibodies to subsequent challenge with whole ragweed. However, ragweed did neutralize all reaginic activity to subsequent AgE challenge. These results were found with all sera studied.

The release of histamine from leucocytes of sensitive individuals following challenge with the appropriate allergen is another measure of allergenicity.¹² Clinical ragweed sensitivity has correlated quite well with cell sensitivity to AgE. Although AgE was proved to be more potent than whole ragweed extract in its ability to release histamine, there was considerable variability among different patients' cells in the degree of this increased potency.

Antigen E is an excellent antigen. High titers of hemagglutinins were readily produced. In numerous instances, enough antibody was present to be demonstrated by direct precipitation in agar. Precipitins in human sera have rarely been produced following aqueous allergen therapy. Lichtenstein and associates,¹¹ reported that the release of histamine from sensitized leucocytes could be markedly inhibited with the serum of patients treated with AgE. The degree of this inhibition correlated with the amount of AgE therapy. It would thus appear that hemagglutination and inhibition of histamine release techniques are probably measuring the same antibody.

Reactions with whole ragweed monkey and rabbit antisera indicate that AgE is not a single substance. Although many of the antigens in WREX have been eliminated in its preparation, at least several easily demonstrable antigens remain. This demonstration of a multiplicity of antigens differs from previous findings¹¹ of a single precipitation line in the reaction of antigen E with anti-ragweed rabbit serum.

Although not quite as effective as Pool C, antigen E was also a reasonably good coating antigen for the tanned cell hemagglutination test.

The results of this clinical and immunological investigation of AgE suggests the following conclusions. Much of the extraneous material in whole ragweed pollen has been eliminated in the preparation of AgE, providing an excellent antigen. However, allergenic component(s) may have also been eliminated. This was demonstrated by the *in vivo* neutralization of reagin tests and also by the relative lack of local or systemic reactions to high doses of AgE. In this latter respect, there has never been firm evidence that allergenic components responsi-

ble for local skin reactions are distinct from components needed for a satisfactory therapeutic response. Our clinical result would suggest that those diminished or eliminated allergenic components are important for more effective therapy. The skin test data and the histamine release studies also suggest that the individuals may vary in their degree of sensitivity to different fractions of whole ragweed.

During the third year, 9 patients, who responded satisfactorily during 2 successive years of therapy, continued to be relatively asymptomatic without specific therapy. This observation does point out that patients successfully treated in the previous year may not be reliable subjects for further critical clinical investigation the following year. Immunologic studies in this group of patients have failed to show any distinguishing reaction patterns.

Further investigations, already underway in several laboratories,^{13, 14} may lead to evaluation of other important allergens which might be added to antigen E. The type of clinical and immunological study just described may well be used as a prototype for investigation of these future fractions.

REFERENCES

1. Callaghan, O. H., and Goldfarb, A. R.: Fractionation of the antigens from dwarf ragweed pollen, *J. Immunol.* **86**: 83, 1961.
2. King, T. P., and Norman, P. S.: Isolation studies of allergens from ragweed pollen, *Biochemistry* **1**: 709, 1962.
3. Lea, D. J., and Sehon, A. H.: The chemical characterization of nondialyzable constituents of ragweed pollen, *Int. Arch. Allergy* **20**: 203, 1962.
4. Robbins, K. C., Wu, H., Baram, P., and Mosko, M. M.: Isolation of nondialyzable allergens and antigens from low ragweed pollen (*Ambrosia Elatior*), *J. Immunol.* **91**: 354, 1953.
5. Reisman, R. E., Bonstein, H. S., Rose, N. R., and Arbesman, C. E.: Studies of purified fractions of ragweed pollen, *J. ALLERGY* **35**: 227, 1964.
6. Arbesman, C. E., Rose, N. R., Kantor, S. Z., and Beede, R. B.: Immunologic studies of ragweed-sensitive patients, *J. ALLERGY* **31**: 317, 1960.
7. Arbesman, C. E., Reisman, R. E., Bonstein, H. S., and Rose, N. R.: Allergic and immunologic studies of a "purified" fraction of ragweed pollen, *J. Immunol.* **9**: 612, 1963.
8. Lowell, F. C., and Franklin, W.: A "double blind" study of treatment with aqueous allergenic extracts in cases of allergic rhinitis, *J. ALLERGY* **34**: 165, 1963.
9. Pruzansky, J. J., Golbert, T. M., and Patterson, R.: Specific alteration in sensitivity of leukocytes of treated allergic patients, *J. ALLERGY* **41**: 114, 1968. (Abst.)
10. 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.
11. Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. W.: Clinical and *in vitro* studies on the role of immunotherapy in ragweed hay fever, *Am. J. Med.* **44**: 514, 1968.
12. Lichtenstein, L. M., King, T. P., and Osler, A. G.: *In vitro* assay of allergenic properties of ragweed pollen antigens, *J. ALLERGY* **38**: 174, 1966.
13. Goldfarb, A. R.: Separation of new skin-reactive antigens from dwarf ragweed pollens, *J. Immunol.* **100**: 902, 1968.
14. King, T. P., Norman, P. S., and Lichtenstein, L. M.: Isolation and characterization of allergens from ragweed pollen. IV. *Biochemistry* **6**: 1992, 1967.