

A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever

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In a double-blind study, we compared the effects of the Rinkel and the current standard methods of immunotherapy with ragweed pollen extract and those of placebo on symptoms of ragweed hay fever and immunologic parameters in 43 patients highly sensitive to ragweed. Each had a skin-test end point by Rinkel serial titration at 1:312,500 w/v or greater dilution, a 2+ skin test to ragweed AgE 0.01 µg/ml, and in vitro histamine release by ragweed pollen extract. None had had immunotherapy for at least 7 yr. Patients were matched on the basis of leukocyte histamine release to ragweed pollen extract and assigned to treatment groups. Fourteen received ragweed pollen extract by the Rinkel method, 14 received placebo, and 15 received ragweed pollen extract by the current standard method weekly between February and October, 1979. Rinkel method doses were derived from skin-test end points and were advanced to 0.5 ml of the end-point dilution; current standard method doses were advanced to the highest tolerated dose. The median maintenance dose for Rinkel method patients was 0.5 ml of 1:1,562,500 w/v (0.001 µg AgE), and for current standard method patients was 0.3 ml of 1:100 w/v (11 µg AgE). An additional unmatched group of nine similar patients received Rinkel method immunotherapy in both 1978 and 1979. Under the conditions of this study, the current standard method of immunotherapy produced a significant decrease in ragweed hay fever symptom-medication scores, increase in antiragweed IgG levels, and decrease in seasonal rise in antiragweed IgE levels in comparison with the effects of either Rinkel method or placebo. The effect of the Rinkel method on these variates was not significantly different from the effects of placebo.

There are two widely used methods of immunotherapy for ragweed pollen hay fever, here called the current standard and Rinkel methods. These methods

have been described in a recent publication.¹ Briefly, they differ in that the Rinkel method employs an "optimal dose" determined by skin-test titration that is usually close to 0.5 ml of the end-point dilution, and, in highly sensitive patients, is on the average 10,000 times lower than the maximum dose used in the current standard method; the current standard method involves rapid escalation of dose to maximum levels that contain 2.7 µg or more of ragweed AgE, and that are often near the highest tolerated dose. We have reported the results of a randomized, double-blind, placebo-controlled study done in 1978 that indicated that Rinkel method immunotherapy was no more effective than placebo given in an imitation of the Rinkel method for relieving symptoms of ragweed

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TABLE I. Patients' characteristics at time of admission to study

Characteristic	Number of patients	Treatment groups			
		Ragweed standard method	Placebo	Ragweed Rinkel method 79	Ragweed Rinkel method 78/79
Patients	52	15	14	14	9
Male/female	39/13	12/3	10/4	10/4	7/2
Age (yr)					
18-35	33	9	9	9	6
36-50	19	6	5	5	3
Positive mold skin test	17	4	5	4	4
Immunotherapy with ragweed before study	9	2 (1962)* (1969)	2 (1963) (1958)	3 (1966) (1948-49) (1967)	2 (1945-55) (1971)

*Parentheses indicate years in which immunotherapy was given.

TABLE II. Schedule for therapy of patients receiving ragweed pollen extract by the current standard method

Dose No.*	Dilution No.	Concentration (w/v)	Dose (ml)	Dose No.	Dilution No.	Concentration (w/v)	Dose (ml)
1	6	1:312,500	0.15	13	2	1:500	0.15
2			0.3	14			0.2
3			0.5	15			0.3
4			0.15	16			0.5
5	5	1:62,500	0.3	17	1	1:100	0.15
6			0.5	18			0.2
7			0.15	19			0.3
8			0.3	20			0.5
9	4	1:12,500	0.5	21	conc.	1:20†	0.15
10			0.15	22			0.2
11			0.3	23			0.3
12			0.5	24			0.5

*Doses were given at approximately weekly intervals.

†1:20 w/v contained 187 µg of ragweed AgE per milliliter and when employed undiluted for administration of doses 21 to 24 was prepared by diluting lyophilized short ragweed pollen extract with isotonic saline that contained 0.4% phenol instead of 50% glycerol-saline.

hay fever or for inducing changes in antiragweed IgG and IgE levels in patients highly sensitive to ragweed.¹ On the other hand, earlier controlled studies by this and other groups have established that the current standard method of immunotherapy, with maximum maintenance doses containing the equivalent of 2.7 to 22 µg of AgE, is more effective than placebo for relieving symptoms of ragweed hay fever, for increasing antiragweed IgG levels, and for decreasing seasonal increase in antiragweed IgE levels.²⁻²⁶ The purpose of the present study was to compare in a similarly controlled study the effects of the current standard method, Rinkel method, and placebo in patients highly sensitive to ragweed pollen.

MATERIAL AND METHODS

Patients

We recruited 52 patients from three sources: (1) nine patients who had received Rinkel method immunotherapy with ragweed pollen extract in 1978¹; (2) eight patients who had received placebo in 1978¹; and (3) 35 new volunteers recruited for the study. Each had a history of seasonal rhinitis in late August and September for at least the 2 prior years, and a positive skin test to ragweed pollen extract at a concentration of 1:312,500 w/v or less and to ragweed AgE at a concentration of 0.01 µg/ml or less. Their leukocytes released histamine when incubated in vitro with ragweed pollen extract. Nine patients had had immunotherapy prior to the study, but none of these had had immunotherapy since 1971 (Table I). Patients with major concomitant rhi-

TABLE III. Examples of end-point titrations done during the ragweed pollen season on each patient in the current standard method ragweed, Rinkel method ragweed 79, Rinkel method ragweed 78/79, and placebo groups

Pa- tient	Diameter (mm) of wheal produced 10 min after intradermal test													
	Ragweed—standard dilutions							Ragweed—Rinkel 79 dilutions						
	5 ⁻¹¹	5 ⁻¹⁰	5 ⁻⁹	5 ⁻⁸	5 ⁻⁷	5 ⁻⁶	5 ⁻⁵	5 ⁻¹⁰	5 ⁻⁹	5 ⁻⁸	5 ⁻⁷	5 ⁻⁶	5 ⁻⁵	
1		4	4	4	6.5	9.5		5	5	7.5*	10	12		
2		4	4	4	7*	9			4	5	8*	11		
3			3	4.5	5	7*	10	4	4	4	7*	13		
4			5	5	7*	9		4	4	6*	8	10		
5			4	4	8*	11				5	5	8*	10	
6	5	5	7*	9	10				4	4	7*	9		
7			4	4	6.5*	11		4	4	7*	9			
8			5	5	7*	9.5			5	5	7*	9.5		
9	3	3	3.5	6*	11			4	4	6*	8	13.5		
10			5	5	8*	11			5	5	7*	10		
11			4	4	7.5*	9.5				4	4	6*	10	
12		5	5	7*	10				5	5	7.5*	10	13	
13			3	3	5	8*	9.5		5	5	6.5*	9		
14		4	4	7*	9	12		4	5	8.5*	10			
15			4	4	7*	10								

Titrations in the placebo group were done with serial dilutions of the placebo solution; titrations in the other three groups were done with serial dilutions of ragweed pollen extract. Shown are the diameters in millimeters of the wheals produced 10 min after intradermal tests with the indicated dilutions.

*End-point dilution.

nitis symptoms in the mold-dominated seasons in July and late October and November were excluded. Where possible, we recruited patients with negative skin tests to mold extracts (*Alternaria*, *Hormodendrum*, *Helminthosporium*, *Botrytis*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*) at a concentration of 1,000 protein nitrogen units (PNU)/ml. Seventeen patients with positive skin tests to molds were included in the study (Table I). Few patients fulfilling all of these criteria were present in the Johns Hopkins Allergy Clinic or in the authors' allergy practices and most had to be recruited by news media releases.

Reagents

The short ragweed pollen allergenic extract and the histamine caramelized glucose placebo solution were the same as those used in the 1978 study.¹ Serial fivefold dilutions were prepared as described previously.¹

Initial screening evaluation

Initial evaluation of new prospective patients was done as described previously.¹

Comprehensive initial evaluation

Patients meeting the criteria for entry into the study were evaluated by methods described previously for hematocrit, total and differential white blood cell counts, total eosinophil counts, ragweed extract-induced in vitro leukocyte

histamine release, serum levels of antiragweed IgE, total IgE, and antiragweed IgG, and skin-test end-point dilutions with ragweed pollen extract.¹

Treatment

Patients from sources (2) and (3) were divided into three comparable groups on the basis of cell sensitivity to short ragweed pollen extract as determined by in vitro leukocyte histamine release. Histamine-release values were divided equally into four ascending categories of values (low, medium low, medium high, and high), and patients in each category were assigned in a random manner to the three treatment groups. Treatment groups were balanced with regard to patients with positive skin tests to molds as shown in Table I. By random selection, one group of 15 patients received ragweed pollen extract by the current standard method; a second group of 14 patients received ragweed pollen extract by the Rinkel method; and a third group of 15 patients received placebo given in an imitation of the Rinkel method. An additional group of nine patients who had received Rinkel method immunotherapy with ragweed pollen extract in 1978 received ragweed pollen extract by the Rinkel method for the second consecutive year.

Treatment of Rinkel method ragweed and placebo groups commenced on February 27, 1979, with administration of 0.1 ml of the end-point dilution of extract (or placebo). Further treatment was given at approximately weekly inter-

Pa- tient	Diameter (mm) of wheal produced 10 min after intradermal test											
	Placebo dilutions						Ragweed—Rinkel 78/79 dilutions					
	5 ⁻¹⁰	5 ⁻⁹	5 ⁻⁸	5 ⁻⁷	5 ⁻⁶	5 ⁻⁵	5 ⁻¹⁰	5 ⁻⁹	5 ⁻⁸	5 ⁻⁷	5 ⁻⁶	5 ⁻⁵
1	4	4	6.5*	9.5			5	5	8*	9.5	13	
2		4	4	6*	8	10.5		4	4	4	6.5*	9
3	5	4	7*	9	11.5			5	5	4	7.5*	9.5
4		5	5	7*	9	12	5	5	7*	9		
5			5	5	7*	9		4	4	7*	9	12.5
6		4	4	7*	9			4.5	4	6.5*	8.5	
7		4	4	7.5*	10			4	4	7*	9	
8		5	5	7*	9			4	4	6.5*	8	
9		4	4	7*	9			4	4	7*	9	
10		4	4	7*	9							
11		4	4	7.5*	9.5							
12		4	4	7*	10							
13		4	4	7*	8.5							
14	4	4	4	7.5*	10.5							

vals, usually increasing consecutive doses by 0.05 ml. Since the optimal dose has been reported to be, on the average, 0.5 ml of the end-point dilution,^{28, 29} an attempt was made to reach this dose in the treatment program by the onset of the pollen season. The optimal dose was administered at approximately weekly intervals during the ragweed pollination season. All patients received an optimal dose of ragweed pollen extract or placebo on, or as close as possible to, Friday, August 31, just before the Labor Day weekend, the traditional peak of the ragweed pollen count and hay fever symptom scores. This allowed assessment of the ability of an optimal dose of ragweed pollen extract and placebo to relieve hay fever symptoms due to inhalation of ragweed pollen. Repeat determinations of the end-point dilution were done just before giving immunotherapy to each patient on 3 to 9 (median 5) separate visits between August 1 and October 6 to pick up changes in end point due to pollen exposure, and the dose was adjusted so that patients received 0.5 ml of the new end-point dilution. During the course of treatment, redeterminations of end-point titration were accomplished with short ragweed pollen extract or placebo, as appropriate, so that the placebo-treated patients were not receiving ragweed extract during the period from February 27 to October 8, 1979, when the effects of short ragweed pollen extract and placebo were being compared.

Patients who received ragweed pollen extract by the current standard method commenced treatment on February 27, 1979, and received at approximately weekly intervals the doses shown in Table II or the nearest dose to the scheduled dose which was tolerated.

Treatment of all groups was continued until the end of ragweed pollination on October 8, 1979. The patients were

provided with tablets containing chlorpheniramine maleate 4 mg and with tablets containing carbinoxamine maleate 4 mg and pseudoephedrine 60 mg (Rondec) for relief of hay fever symptoms not controlled by the subcutaneous injections, and instructed to take one tablet every 4 hr if necessary for relief of these symptoms.

Blinding methods

Personnel who conducted the study were divided into an evaluating team and an injecting team. The evaluating team interviewed and examined the patients, checked diaries, and monitored symptom-medication scores. They checked end-point titrations at suitable intervals, using syringes containing known concentrations of unknown solution (either ragweed pollen extract or placebo as appropriate) supplied by the injecting team. The evaluating team remained completely blinded with reference to the treatment given to any patient.

The injecting team evaluated the results of end-point titrations and the individual treatment schedules and injected the appropriate dose of ragweed pollen extract or placebo into each patient. They prepared appropriate dilutions for end-point titrations. They were not blinded with reference to the treatment given to the patients, and had no role in evaluating the results of therapy.

The patients did not know what treatment they were receiving.

Observations

We obtained daily patient symptom-medication diaries, June 18 to October 8; weekly physician records of symptoms and physical findings, February 27 to October 8; daily

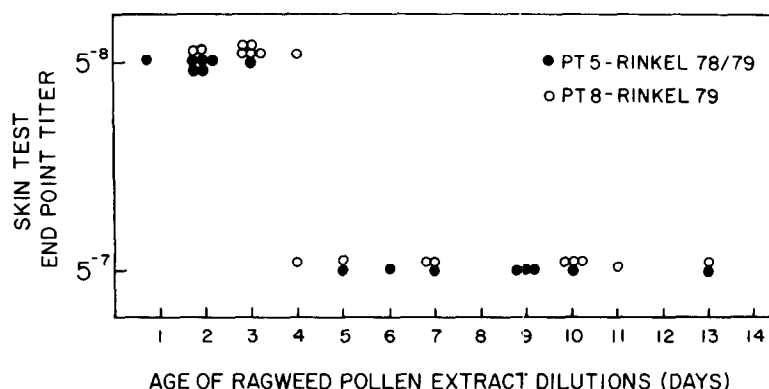


FIG. 1. Relation between potency of ragweed pollen extract (expressed as skin-test end-point titer) and age of extract (expressed as number of days between preparation of set of dilutions from freshly reconstituted lyophilized extract and application of skin test) as determined in two patients for 13 sets of dilutions of ragweed pollen extract made about weekly between July 7 and October 1. Five sets of dilutions were checked twice in patient (PT) 5 and six twice in PT 8; the checks were about 1 wk apart. Five were checked once in PT 5 and four, once in PT 8. Nine sets were checked at least once in both patients.

ragweed pollen counts, August 8 to October 12; levels of antiragweed IgG, antiragweed IgE, and total IgE before treatment (January to February), just before ragweed pollination (July 30 to August 6), and after ragweed pollination (October 8 to 15); and in vitro leukocyte histamine release by ragweed pollen extract before (January to February) and after (October to November) treatment using methods described previously.¹

Analysis of data

Data were analyzed using nonparametric statistical methods for group comparisons.³⁴ Data reduction and statistical testing was performed using the Clinfo computer system, provided by the National Institutes of Health Division of Research Resources to the Johns Hopkins University School of Medicine. For nonpaired observations, Wilcoxon's nonpaired rank-sum test was used. For paired samples (repeated observations on the same individuals), Wilcoxon's signed-ranks test was employed. The results of the sign test and Spearman rank correlation coefficients were also examined for paired data where appropriate. Since total serum IgE values are known to be normally distributed if log transformed, the *t* statistic (paired or unpaired, as appropriate) was used to test for differences for this immunologic variate.

RESULTS

Matching

All four treatment groups (current standard method, Rinkel 79, Rinkel 78/79, and placebo) proved to be well matched with reference to age, sex, incidence of positive skin tests to molds, in vitro histamine release by ragweed pollen extract, pretreatment levels of antiragweed IgG, antiragweed IgE, and total IgE in serum, and skin-test end points with ragweed pollen

extract and placebo (Tables I and III, and Figs. 4 to 8). When current standard method, Rinkel 78/79, and placebo groups were compared using nonparametric methods, there were no statistically significant pretreatment differences among the groups with regard to any of these variables.

Dosage achieved

Current standard method group. Maximum single doses during the ragweed pollen season ranged from 3.7 to 46.8 (median 11.1) μ g of ragweed AgE. The cumulative dose of AgE given from start to finish (February to October) ranged from 18.1 to 351.2 (median 84.9) μ g of AgE.

Rinkel method group. All of these patients received 0.5 ml of their end-point dilution at the time of each visit during the ragweed season. Compared with the current standard method, maximum and cumulative doses were much less (Rinkel 79 group—*Maximum*: range 0.00024 to 0.006, median 0.0012 μ g AgE; *Cumulative*, range 0.007 to 0.827, median 0.027 μ g AgE. Rinkel 78 and 79 group—*Maximum*: range 0.00024 to 0.006, median 0.0012 μ g AgE; *Cumulative*, range 0.005 to 0.078, median 0.030 μ g AgE). Multiple end-point titrations were done between February and October (Rinkel 79 group: range 11 to 25, median 13 titrations; Rinkel 78 and 79 group: range 11 to 22, median 14 titrations). The end points showed little variability when measured with dilutions of the same age (Fig. 8). Since the potency of the ragweed pollen extract as measured by end-point titration in two carefully studied patients was five times greater on days 1 to 3 than on days 5 to 12 after

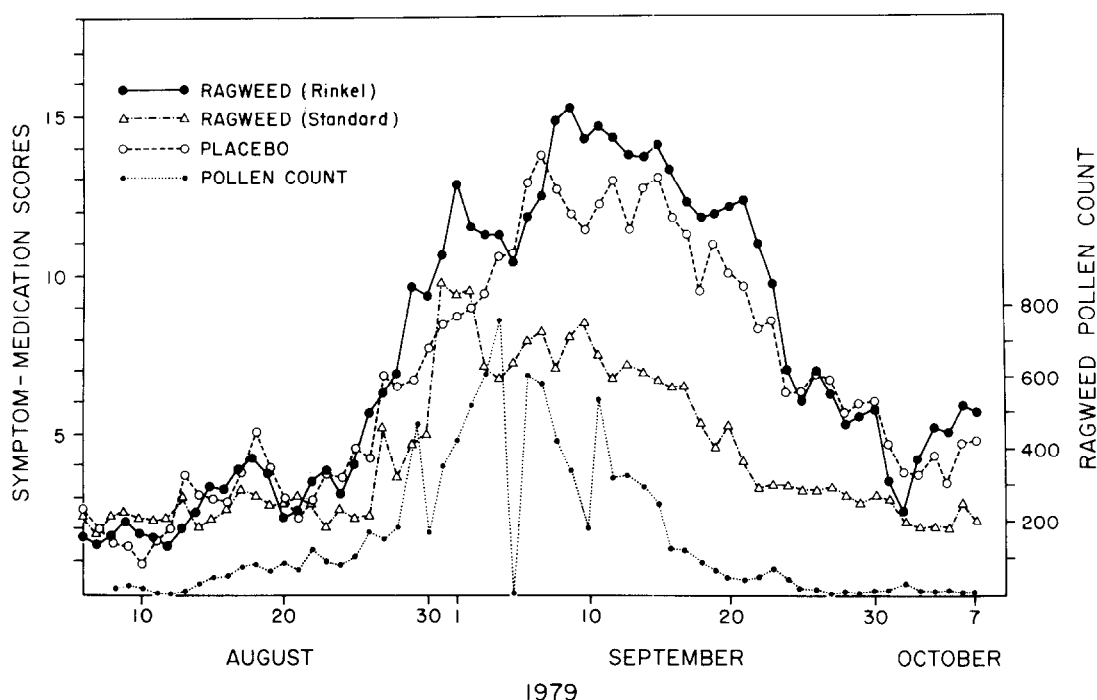


FIG. 2. Arithmetic mean of daily symptom-medication scores for the groups indicated, compared with the pollen count reported as ragweed pollen grains per square centimeter of roto-slide. Rise and fall of symptom-medication scores were significantly correlated with rise and fall of ragweed pollen counts for standard ragweed group (Spearman correlation coefficient, $r_s = 0.696$, $p < 0.01$), placebo group ($r_s = 0.626$, $p < 0.01$), Rinkel 78/79 group ($r_s = 0.718$, $p < 0.01$), and Rinkel 79 group ($r_s = 0.603$, $p < 0.01$). Symptom-medication scores of standard group were significantly less ($p < 0.01$) than those of Rinkel and placebo groups; those of Rinkel and placebo groups were not significantly different ($p = 0.30$).

reconstitution and dilution of the lyophilized ragweed extract (Fig. 1), 5- to 8-day-old dilutions were employed for both end-point titration and treatment whenever possible. Dilutions were made weekly from June 26 on, and end-point titrations were done often between August 1 and October 6 (range 4 to 16, median 5 times), and whenever 1- to 4-day-old dilutions were employed. Examples of end-point titrations done on each patient in the Rinkel groups with ragweed pollen extract during the ragweed season are shown in Table III. It is evident that the end points conform to the criteria described by proponents of the Rinkel method.^{27, 29}

Placebo group. All patients received 0.5 ml of their end-point dilution of placebo at the time of each visit during the ragweed season. This was the 5^{-4} dilution for two, the 5^{-3} dilution for 11, and the 5^{-2} dilution for one patients. These dilutions were labeled 5^{-8} , 5^{-7} , and 5^{-6} , respectively, in order to facilitate blinding. Examples of end-point titrations done on each patient in the placebo group with the histamine-caramelized glucose placebo are shown in Table III. It is evident that these titrations are not distinguishable

from those produced by ragweed extract in the other groups.

Symptoms

Mean daily symptom-medication scores for each group (Fig. 2) were similar and low at the beginning and end of the ragweed pollination season. During the season, symptom-medication scores rose and fell in a pattern that for each group was correlated significantly ($p < 0.01$) with the ragweed pollen count. The mean daily symptom-medication scores of the patients in the current standard ragweed were significantly lower than those in placebo ($p < 0.01$) and combined Rinkel method ragweed ($p < 0.01$) groups (Figs. 2 and 3). Twelve of 15 patients in current standard, 12 of 14 in Rinkel 79, seven of nine in Rinkel 78/79, and 11 of 14 in placebo groups had significant correlations between symptom-medication scores and ragweed pollen counts and thus appeared to be clinically sensitive. The arithmetic means of the daily symptom-medication scores between August 3 and October 7, 1979, for each patient in each treatment group were widely variable (Fig. 3). The scores of the

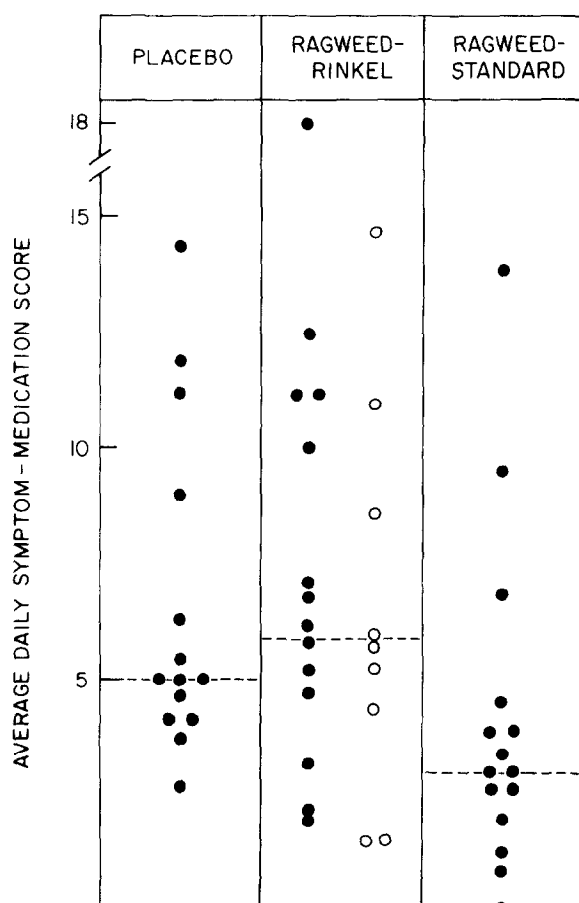


FIG. 3. Arithmetic means of the daily symptom-medication scores between August 3 and October 7, 1979, for each patient in the current standard method ragweed (CSM), Rinkel method ragweed (R 79, *closed circles*; R 78/79, *open circles*), and placebo (PLA) groups. *Dotted lines* indicate the median scores. Comparisons of scores by Mann-Whitney U test³⁴ reveal that the scores of CSM group were significantly lower than those of R and PLA groups and that those of R and PLA groups were not significantly different from each other. CSM vs PLA, $p < 0.01$; CSM vs R 79, $p = 0.05$; CSM vs R 78/79, $p = 0.10$; CSM vs R (all), $p < 0.01$; R 79 vs PLA, not significant (NS); R 78/79 vs PLA, NS; R 78/79 vs R 79, NS; R (all) vs PLA, $p = 0.30$ (NS).

current standard group were significantly lower than those in the combined Rinkel ($p < 0.01$) and in the placebo ($p < 0.01$) groups; those of the combined Rinkel and the placebo groups were not significantly different from each other (Fig. 3). There was no difference in the results when symptom scores instead of symptom-medication scores were used for the analysis.

Immunologic variates

For all variates under study, Rinkel 79 and Rinkel 78/79 groups did not differ (Figs. 4 to 8). Therefore,

in this section, the results for these two groups are combined and analyzed together.

Antiragweed IgG levels in serum are shown in Fig. 4. The levels of the current standard group rose significantly from a median of <156 arbitrary units at entry into study to a median of 4,629 units before season ($p = 0.05$) and to 19,013 units after season ($p = 0.005$). Both pre- and postseason antiragweed IgG levels of the current standard group were significantly higher ($p = 0.001$) than the corresponding levels of Rinkel and placebo groups, both of which remained at a median of <156 arbitrary units for the duration of the study. Levels of Rinkel and placebo groups did not differ significantly ($p > 0.3$) from each other. The small rise occasionally seen between pre- and postseason levels noted previously in some placebo- and Rinkel method-treated patients¹ occurred in four patients in the Rinkel group and seven in the placebo group. No detectable antiragweed IgG (<156) was found in 32 patients before treatment and in 22 after season; 21 of the 22 were in the Rinkel or placebo groups.

Serum antiragweed IgE levels are shown in Fig. 5. The levels of the current standard group rose significantly ($p = 0.03$) from a median of 185 ng/ml at entry to 229 ng/ml before season, and fell slightly, possibly insignificantly ($p = 0.095$), to 216 ng/ml after season. The distribution of IgE antiragweed levels in Rinkel and placebo groups was similar and both were quite different from those of the current standard group. Antiragweed IgE levels in the Rinkel group fell significantly ($p = 0.005$) from a median of 166 ng/ml at entry to 97 ng/ml before season, and rose significantly ($p = 0.001$) to 148 ng/ml after season. Levels of the placebo group fell significantly ($p = 0.002$) from a median of 114 ng/ml at entry to 45 ng/ml before season, and rose significantly ($p = 0.01$) to 116 ng/ml after season.

Total IgE levels in serum for the patient groups are shown in Fig. 6. Levels of the current standard group rose slightly and possibly significantly ($p = 0.06$) from a median of 578 ng/ml at entry to 643 ng/ml before season, and fell slightly and insignificantly ($p > 0.3$) to 626 ng/ml after season. Levels of Rinkel and placebo groups behaved similarly and quite differently from those of the current standard group. Levels of the Rinkel group fell significantly ($p = 0.001$) from a median of 579 ng/ml at entry to 327 ng/ml before season, and rose significantly ($p = 0.01$) to 406 ng/ml after season. Levels of the placebo group fell significantly ($p = 0.003$) from a median of 523 ng/ml at entry to 400 ng/ml before season, and rose significantly ($p = 0.001$) to 617 ng/ml after season.

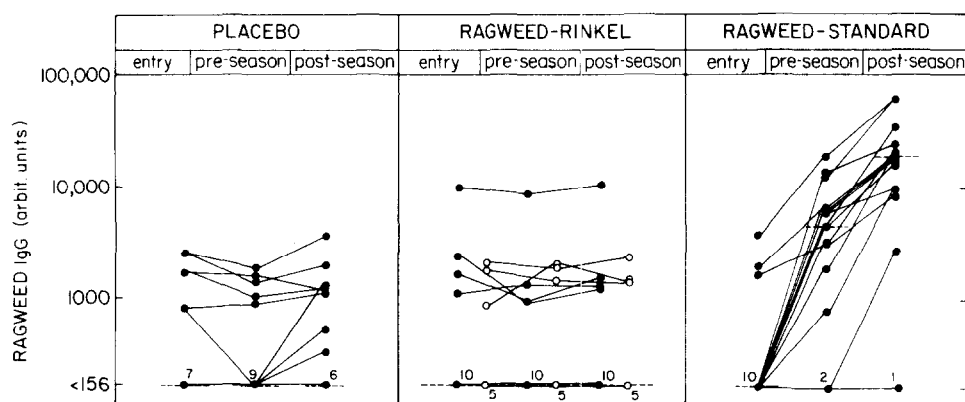


FIG. 4. Serum antiragweed IgG levels in arbitrary units at entry into study (January to February), before season (July 30 to August 6), and after season (October 8 to 15) for patients in the current standard method ragweed (CSM), Rinkel method ragweed (R 79, *closed circles*; R 78/79, *open circles*), and placebo (PLA) groups. *Dotted lines* indicate the median levels. Values less than 156 arbitrary units are considered equal to 156. CSM group median levels rose from <156 arbitrary units at entry to 4,629 before season, and to 19,013 after season; R 79, R 78/79, and PLA group median levels remained at <156 for the duration of the study. Significance of differences in levels within and between groups at indicated times by Wilcoxon's test: CSM entry vs CSM before, $p = 0.05$; CSM before vs CSM after, $p = 0.005$; CSM entry vs CSM after, $p = 0.01$; CSM before vs PLA before, $p < 0.001$; CSM after vs PLA after, $p = 0.001$; CSM before vs R before, $p < 0.001$; CSM after vs R after, $p = 0.001$. R 79, R 78/79, and PLA levels did not rise significantly ($p > 0.3$) and did not differ from each other significantly at entry or before or after season ($p > 0.3$).

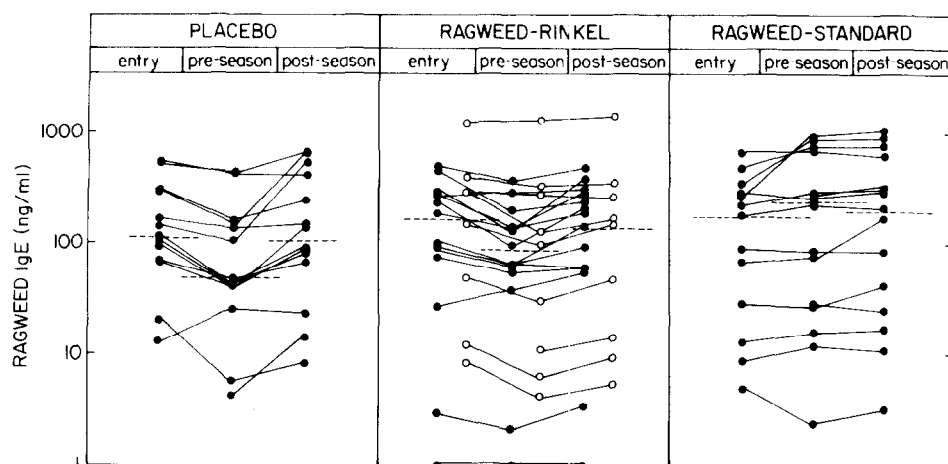


FIG. 5. Serum antiragweed IgE levels in nanograms per milliliter at entry into study (January to February), before season (July 30 to August 6), and after season (October 8 to 15), for each patient in the current standard method (CSM), Rinkel (R 79, *closed circles*; R 78/79, *open circles*), and placebo (PLA) groups. *Dotted lines* indicate the median levels. Median levels of CSM group rose from 185 ng/ml at entry to 229 ng/ml before season, and fell to 216 ng/ml after season. Significance of differences in levels at indicated times by Wilcoxon's test: entry vs before, $p = 0.03$; before vs after, $p = 0.095$. Median levels of R groups fell from 166 ng/ml (R 79: 142; R 78/79: 215) at entry to 97 ng/ml (R 79: 81, R 78/79: 99) before season, and rose to 148 ng/ml (R 79: 168, R 78/79: 148) after season. Significance of differences in levels at indicated times by Wilcoxon's test: R 79: entry vs before, $p = 0.007$; before vs after, $p = 0.002$; R 78/79: entry vs before, $p = 0.16$; before vs after, $p = 0.03$; R (all): entry vs before, $p = 0.005$; before vs after, $p = 0.001$. Median levels of placebo groups fell from 114 ng/ml at entry to 45 ng/ml before season, and rose to 116 ng/ml after season. Significance of differences in levels at indicated time by Wilcoxon's test: entry vs before, $p = 0.002$; before vs after, $p = 0.01$.

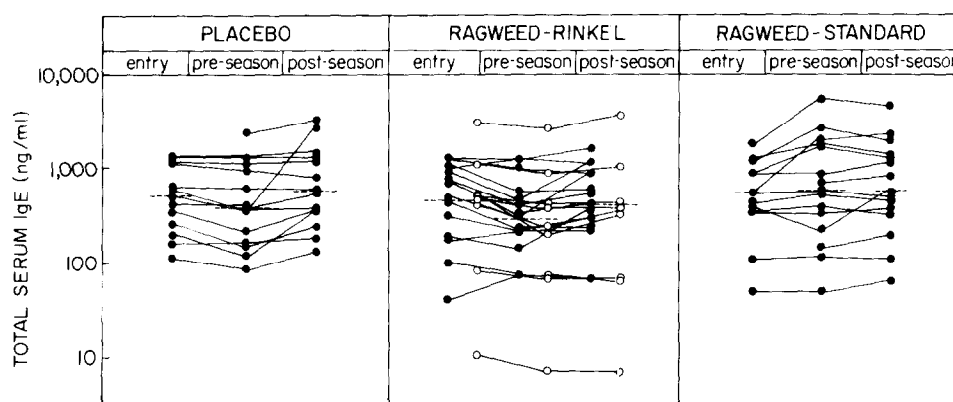


FIG. 6. Total serum IgE levels in nanograms per milliliter at entry into study (January to February), before season (July 30 to August 6), and after season (October 8 to 15) for each patient in the current standard method ragweed (CSM), Rinkel method ragweed (R 79, closed circles; R 78/79, open circles), and placebo (PLA) groups. Dotted lines indicate median levels. Median level of CSM group was 578 ng/ml at entry, rose to 643 ng/ml before season, and fell to 626 ng/ml after season. Significance of differences in levels at indicated time by Wilcoxon's test: entry vs before, $p = 0.06$; before vs after, $p > 0.3$ (NS). Median level of R groups was 579 ng/ml (R 79: 616; R 78/79: 505) at entry, fell to 327 ng/ml (R 79: 334; R 78/79: 249) before season, and rose to 406 ng/ml (R 79: 508; R 78/79: 371) after season. Significance of differences in levels at indicated times by Wilcoxon's test: R 79 entry vs before, $p = 0.02$; before vs after, $p = 0.05$; R 78/79: entry vs before, $p = 0.01$; before vs after, $p = 0.09$; R (all): entry vs before, $p = 0.001$; before vs after, $p = 0.01$. Median level of PLA group was 523 ng/ml at entry, fell to 400 ng/ml before season, and rose to 617 ng/ml after season. Significance of differences in levels at indicated times by Wilcoxon's test: entry vs before, $p = 0.003$; before vs after, $p = 0.001$.

TABLE IV. Patients' comparisons of severity of ragweed hay fever symptoms in 1979 and in year* before immunotherapy

	Less severe	Same	More severe
Current standard method	15	0	0
Placebo	9	2	1
Rinkel ragweed 79	11	2	1
Rinkel ragweed 78/79	9	0	0

*1978 for those beginning treatment in 1979; 1977 for those beginning it in 1978.

In vitro leukocyte histamine release by ragweed pollen extract is shown in Fig. 7. A few patients in each treatment group had an increase in ragweed AgE required for 50% histamine release between entry into study and after season; others in each group had a decrease. Overall, there were no significant differences among the three groups of patients with regard to changes in histamine release with therapy.

Median skin-test end-point titer (end-point dilutions) of ragweed pollen extract before season (July 2 to Aug 6) and after season (October 3 to 29) are shown in Fig. 8. These remained remarkably constant during this time frame. As might be expected fivefold dilutions did not reflect the modest rise in antiragweed

IgE (usually less than twofold) noted in the Rinkel group during the ragweed season. There was no significant difference between the end-point titers of the three groups before or after season, and no significant change in titer in any group.

Patients' appraisals of results of therapy

Notwithstanding the absence of demonstrated effect of ragweed pollen extract given by the Rinkel method or placebo on either symptom-medication scores or measurements of immunologic response, 20 of 23 patients in the Rinkel groups and 9 of 12 patients in the placebo group were of the opinion that their symptoms of hay fever were less severe in 1979 than they had been in the year before immunotherapy was started (Table IV). Ragweed pollen counts in Baltimore were lower in 1979 than in either 1978 or 1977 (Table V).

Untoward reactions

No systemic reaction occurred in Rinkel 79, Rinkel 78/79, and placebo groups. Only one local reaction exceeding 5 cm in diameter was noted in these three groups and this occurred in a patient in the Rinkel 79 group. In contrast, seven of the 15 patients in the current standard group had one or more systemic reactions (six moderate and treated with epinephrine,

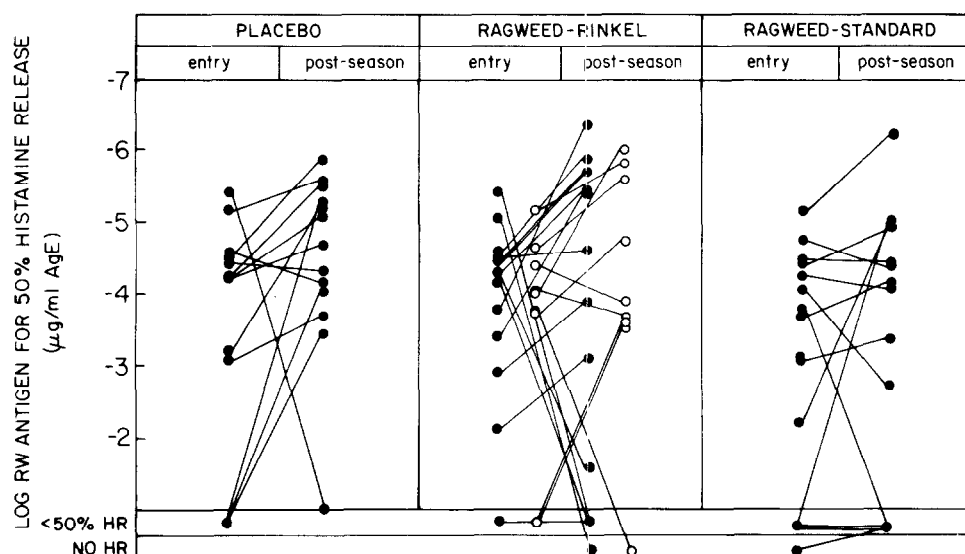


FIG. 7. Ragweed AgE content of ragweed pollen extract required for 50% leukocyte histamine release at entry into study (January to February) and after season (October to November) for each patient in the current standard method ragweed, Rinkel method ragweed (R 79, closed circles; R 78/79, open circles), and placebo groups.

TABLE V. Comparison of Baltimore, Maryland, ragweed pollen counts in 1977, 1978, and 1979

Year	Pollen count (pollen grains/cm ² of roto-slide)			
	Maximum count	Date of maximum count	Cumulative count	
			Aug. 10 to Oct. 2	Aug. 24 to Sept. 13
1977	1,269	Sept. 7	16,807	11,661
1978	781	Aug. 29	13,002	8,271
1979	761	Sept. 4	9,340	7,299

three mild and treated with antihistamine, 10 very mild and not treated with pharmacologic agents). Five local reactions exceeding 5 cm in diameter occurred in four patients in the current standard group.

DISCUSSION

In this study of the Rinkel method of immunotherapy for ragweed pollen hay fever, our purpose was to compare the effects of "optimal doses" in the range of 0.5 ml of the end-point dilution of ragweed pollen extract with those of similar doses of the end-point dilution of an appropriate placebo, and with the effects of peak doses of ragweed extract containing 2.7 to 22 µg or more of ragweed AgE given by the current standard method. The dose employed for trial of the Rinkel method was chosen because it has been reported to be the average maintenance dose in patients treated by the Rinkel method. Rinkel^{28, p. 214} reported that "most patients will obtain their best relief with a 50× dose," which in his terminology is 50 times the

0.01-ml dose of extract used for the end-point skin test, or 0.5 ml of the end-point dilution. Willoughby^{29, p. 608} also stated that "the average dose for pollen is 0.5 ml" of "the end point dilution." The 2.7- to 22-µg AgE dose employed for trial of the current standard method was chosen because it has been shown to be significantly more effective than placebo for reducing hay fever symptom-medication scores,^{2, 7-10, 12-13} and, has been shown to produce striking changes in measured immunologic variates that have included increase in antiragweed IgG levels,^{7-10, 12, 14-17} decrease in the magnitude of the expected seasonal rise in antiragweed IgE levels,^{13, 16, 17, 25} and, in some patients, a decrease in ragweed-induced in vitro leukocyte histamine release.^{7-10, 13-17}

In the study reported here, immunotherapy with maximum doses of ragweed pollen extract containing 3.7 to 46.8 µg ragweed AgE in patients treated by the current standard method significantly reduced symptom-medication scores and produced significant

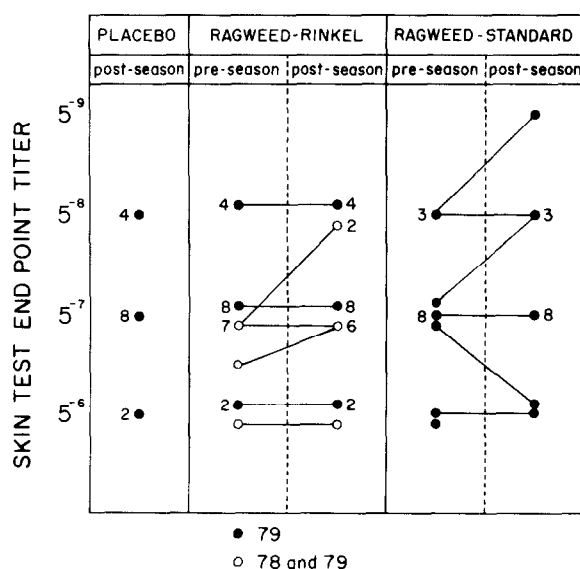


FIG. 8. Median skin-test end-point titer (end-point dilution) of ragweed pollen extract before season (July 2 to August 6) and after season (October 1 to 29) for each patient in the current standard method ragweed, Rinkel method ragweed (R 79, closed circles; R 78/79, open circles), and placebo groups. Patients in placebo group were not tested with ragweed pollen extract before season in order to avoid giving them a dose of ragweed pollen extract that might approach a significant dose (0.5 ml of end-point dilution) by the Rinkel method. Measurements were made with ragweed pollen extract diluted from freshly reconstituted lyophilized extract 5 to 12 (median 7) days before test.

changes in levels of antiragweed IgG and IgE as compared with placebo. On the other hand, as in our 1978 study,¹ treatment with 0.5 ml of end-point dilution by the Rinkel method produced effects on symptom-medication scores and on measured immunologic variates that were not significantly different from those produced by placebo.

The doses of ragweed pollen extract employed in this study differ from those received by some patients who undergo immunotherapy by the Rinkel method. Willoughby²⁹ has reported that he employed the following end-point dilutions in 100 "selected" patients who received ragweed pollen extract by this method: 5⁻⁸ in two patients (2%), 5⁻⁶ in three (3%), 5⁻⁵ in five (5%), 5⁻⁴ in 16 (16%), 5⁻³ in 15 (15%), 5⁻² in 16 (16%), and 5⁻¹ in 43 (43%). Had Willoughby's patients been given the ragweed pollen extract employed in the present study (which contained 187 μ g ragweed AgE/ml in the concentrated solution), those who received 0.5 ml of the 5⁻¹ dilution would have received a maintenance dose of 18.7 μ g AgE, and those who received 0.5 ml of the 5⁻² dilution would have received 3.7 μ g AgE. Such doses are comparable to the maintenance doses that have been shown in this and

previous studies to reduce ragweed pollen-induced symptom-medication scores and to produce immunologic responses when given by the current standard method.

The skin-test end-point dilutions in our patients were in the 5⁻⁹ to 5⁻⁶ range. The 5⁻⁶ dilution at time of dilution contains 0.01 μ g ragweed AgE/ml. A 2+ reaction (wheal, 1-cm diameter or more, plus significant erythema) to intradermal test with solution containing 0.01 μ g AgE/ml has been used by our group and others as a criteria for sufficient sensitivity to ragweed pollen for inclusion in immunotherapy studies. We selected patients with high skin sensitivity to ragweed because we have previously shown that these patients have a good correlation between the rise and fall of symptom-medication scores and the rise and fall of ragweed pollen counts.³⁰ We purposely did not select patients with low skin sensitivity to ragweed pollen because we have previously shown that many such patients have a poor correlation between symptom-medication scores and ragweed pollen counts.³⁰ Others have shown correlation between severity of ragweed hay fever symptoms and intensity of skin sensitivity to ragweed as measured by end-point titration¹⁹ and that patients who have no symptoms of hay fever in the ragweed pollen season frequently have low skin sensitivity but rarely have high skin sensitivity.^{19, 31-33}

It is possible that a group of patients can be recruited who have symptoms of hay fever only in the ragweed pollen season and end-point dilutions in the 5⁻¹ to 5⁻² range. If such patients can be recruited and are treated by this method, it will be important to prove in an appropriately controlled study that the placebo-treated patients with end-point dilutions in the 5⁻¹ to 5⁻² range have an increase in symptom-medication scores in the ragweed season such as was so clearly demonstrated by the placebo-treated patients in the present study.

The absence of systemic and of significant local reactions to 0.1 to 0.5 ml of end-point dilution of ragweed pollen extract in this group of highly sensitive patients shows that immunotherapy by either Rinkel method or current standard method can be begun safely with 0.1 ml of the end-point dilution and that there is no need to waste time and expense by beginning with a more dilute solution. Therefore, some form of titration may be useful prior to immunotherapy to determine the starting dose.

In prior studies,^{7, 9, 14} it has been our practice to give an initial dose of 0.1 ml of a solution of ragweed pollen extract that is close to a 1:250,000 to 1:500,000 w/v dilution (dose was 0.1 to 0.2 PNU of a solution that contained either 27⁹ or 38 μ g¹⁴ AgE

per 10,000 PNU). The initial dose for the current standard group in this study was 0.15 ml of 5^{-6} dilution. This has not resulted in systemic or significant local reactions. For this reason we think it likely that highly sensitive patients with a skin-test end-point dilution of 5^{-7} (1:1,562,500 w/v) or less (5^{-8} , 5^{-9} , and so on) can safely receive a starting dose that is higher than that predicted by the Rinkel method of titration. It should be noted that 45 of 52 patients in the present study had a skin-test end point of 5^{-7} , 5^{-8} , or 5^{-9} (Table III).

It is important to emphasize that most patients in all treatment groups (current standard, 15 of 15; Rinkel 79, 11 of 14; Rinkel 78/79, 9 of 9; placebo, 9 of 12) felt that hay fever symptoms during the 1979 season were less than during the season before immunotherapy was begun and that they had been helped by the immunotherapy (Table IV). Baltimore ragweed pollen counts were lower in 1979 than in 1978, and lower in 1978 than in 1977, which may explain at least in part the improvement noted by all groups of patients. The improvement may also be due to the beneficial effects of therapeutic agents such as antihistamines and decongestants and to the well-recognized placebo effects derived from participation in a structured study, injection therapy, and desire to do well and please the physician. This state of affairs clearly points out the need for proper design and controls in such studies.

Under the conditions of the study, immunotherapy of patients highly sensitive to ragweed pollen with a maximum dose of 3.7 to 46.8 μ g of ragweed AgE given by the current standard method was significantly more effective than placebo or than Rinkel method immunotherapy with 0.5 ml of end-point dilution of ragweed pollen extract for relieving symptoms of ragweed hay fever and producing increase in serum levels of antiragweed IgG and decrease in seasonal rise in levels of antiragweed IgE. Moreover, this study and two others^{1, 35} have shown that 0.5 ml of end-point dilution of ragweed pollen extract was no more effective than placebo.

It is important to emphasize that the results of this study apply to patients highly sensitive to ragweed pollen and that the Rinkel regimen treatment was strictly limited to 0.5 ml of end-point dilution (the so-called 50 $\times$ dose). It is clear that, at least in recent years, proponents of the Rinkel method employ doses higher than 0.5 ml of end-point dilution when they recognize that the patient is achieving less than a satisfactory result and that the dose may be advanced to much higher levels and even to the highest tolerated dose.³⁶ It is also evident, as stated in the original definition of the current standard method,¹ that pro-

ponents of the current standard method may use some form of skin-test titration as a gauge of patient sensitivity^{16, 30-32} and as a guide to the starting dose.^{32, 37} Further, it is possible that some dose that lies between 0.5 ml of end-point dilution and 3.7 μ g ragweed AgE might be more favorable than either in terms of a ratio between therapeutic effectiveness and allergic side effects. We would expect such a dose to be higher than those previously shown by our group to be ineffective.^{2, 7} It is also likely that there is considerable overlap between the doses given in actual practice by the advocates of Rinkel and current standard methods. These terms, at best, are imprecise and interpreted variously by different practitioners. Hence, it would probably be useful to discard them in favor of more precise descriptions of dosage.

The physician who undertakes immunotherapy of ragweed pollen hay fever should be concerned with the degree of sensitivity of the patient. This can be measured by skin-test end-point titration, leukocyte histamine release, or antiragweed IgE levels; all these were used in the present study. Any one of the three parameters can be employed, because each gives data comparable to the others.³⁰ Skin-test end-point titration is the most available and least expensive method at this time. The results of skin-test end-point titration can be a useful guide to starting dose. For titers of 5^{-6} or more (5^{-7} , 5^{-8} , and so on), a safe starting dose is 0.1 ml of the 5^{-6} dilution. For titers below 5^{-6} (5^{-5} , 5^{-4} , and so on), a safe starting dose appears to be 0.1 ml of the end-point dilution. As a practical matter, titration with concentrations more dilute than 5^{-7} may be unnecessary for routine purposes and an added expense.

The physician should also be concerned with the need, if any, for treating the patient of low sensitivity. There are now excellent data showing that most patients highly sensitive to ragweed will have, in the ragweed season, symptoms of hay fever that correlate in severity with the rise and fall of the pollen count (Fig. 2).^{1, 2, 7, 9, 10, 12, 14-16, 30} There are also excellent data that show that some patients with low sensitivity will not have significant symptoms during the ragweed pollen season.^{30, 31, 33} There is no controlled study, however, that examines what proportion of patients of low sensitivity will have significant symptoms of hay fever during the ragweed pollen season. Moreover, while we know limits that define highly sensitive patients (limits which were used in selecting patients for this and for our earlier studies^{1, 2, 7-10, 12-17}) and limits for defining patients of low sensitivity,³⁰⁻³³ the intermediate area is much less clearly defined though of great importance to both patient and physician.

Finally, the physician should be concerned with the

relationship between measurements of the sensitivity of the patient, whether by skin-test end-point titration or by other means, and the necessary therapeutic dose. The frequency and, therefore, the cost to the patient of multiple end-point titrations will be determined by whether the result has a direct relation to the therapeutic dose. Rinkel and Willoughby have emphasized that there may be a direct relationship between end-point dilution and therapeutic dose and that the therapeutic dose is usually 0.5 ml of the end-point dilution.^{28, 29} The present study and others indicate that there is no such fixed relationship.^{1, 35, 36} There is some indication for titration or other measure of sensitivity at the onset of immunotherapy to determine the patient's sensitivity and the starting dose. We believe that thereafter there is no need for costly repetitive titrations to determine correct therapeutic dose, because no matter what the titration says, the dose will be advanced by trial and error until the patient either reports satisfactory results or can tolerate no more. In controlled studies the effective dose has equaled or exceeded 2.7 μ g ragweed AgE.^{2, 7-10, 12, 13} Our approach to dose is subject to future modification, as the evidence in future studies dictates, but seems to fit the data available at the present time.

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