

CLINICAL EFFECTS OF RAGWEED ANTIGEN EMULSION

A Double-Blind Study

Sidney Friedlaender, M.D., and Alex S. Friedlaender, M.D., Detroit, Mich.

WITH the recent popularization of emulsified antigen therapy as an approach to the specific treatment of allergic disease^{1, 2, 3} there has come an increasing awareness of the paucity of controlled clinical studies to evaluate its effects.^{4, 5} This not only true in the case of allergens emulsified in oil but also applies to the conventional multiple-dose aqueous treatment, accepted by almost all workers in the field of allergy as an effective method of treatment. The Specific Therapy Committee of the American Academy of Allergy carried out an extensive clinical study of emulsified antigen therapy during the 1960 ragweed pollinating season which embraced 30 collaborators and 1,777 patients.⁶ Although extensive data were obtained which indicated that ragweed repository therapy was probably as effective as conventional multiple-dose aqueous antigen treatment, the study pointed out the need for a carefully controlled double-blind study of emulsified antigen, aqueous antigen, and placebo. Consequently, plans for such a double-blind study were outlined for the 1961 ragweed season. The mechanics of the study proved to be staggering enough to require additional time and financial support, so that it was necessary to delay this collaborative study to a future time. Another deterring factor in carrying out the study during 1961 was the agreement by the Committee that any new study should embrace only previously untreated, scratch-test positive patients with a clear-cut history of rhinitis or asthma coinciding with the ragweed pollen season, and who reside in areas where appreciable amounts of ragweed pollen are encountered. A preliminary survey of prospective collaborators early in 1961 indicated that very few patients present themselves in the spring of the year in time for such a study, unless some other allergic sensitivity or event brings them to the clinic or physician's office. Thus it became apparent that a suitable number of acceptable patients could only be obtained by advance preparation during the previous pollen season, when new subjects would be treated symptomatically and instructed to return the following year in time for a double-blind study. In face of this delay, the authors themselves attempted to set up a double-blind study among their own patients to evaluate the results of a single injection of emulsified ragweed antigen.

From the Department of Medicine, Allergy Clinic, Sinai Hospital of Detroit, and the Departments of Microbiology and Medicine, Wayne State University College of Medicine.

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SELECTION OF PATIENTS

Prior to July, 1961, it was possible to find in a large Allergy service only 20 patients who gave both a clear-cut history of ragweed pollinosis and a positive scratch reaction to the pollen, and who had never received specific antigen treatment for this sensitivity. In addition, there were 25 patients who had been treated by repository ragweed emulsions prior to the 1960 ragweed season, and who had received no further ragweed antigen since that time. While it was considered desirable to use patients sensitive only to ragweed, the majority had other inhalant sensitivities; to eliminate these patients would have precluded a study for the 1961 season.

TABLE I. PATIENT DATA (PREVIOUSLY UNTREATED [GROUPS A AND B]; PREVIOUSLY TREATED [GROUPS C AND D])

PATIENT	WEAKEST REACTING DILUTION (PU)	OTHER ALLERGY				
		NONE	TREE	GRASS	MOLD	DUST
Group A (Active Emulsion)						
1	206			X	X	
2	16,666		X	X		X
3	1,852	X				
4	1,852					X
5	617	X				
6	16,666			X		X
7	1,852	X				
Group B (Placebo Emulsion)						
8	5,555				X	X
9	1,852				X	X
10	5,555	X				
11	617		X	X		
12	206			X	X	X
13	16,666					X
14	617			X		X
15	555			X		
Group C (Active Emulsion)						
16	16,666				X	
17	50,000				X	X
18	16,666				X	X
19	16,666				X	
20	1,852	X				
21	617	X				
22	1,852	X				
23	1,852	X				
24	1,852	X				
25	23	X				
Group D (Placebo Emulsion)						
26	1,852			X		
27	5,555				X	X
28	16,666					X
29	5,555	X				
30	16,666					X
31	1,852	X				
32	1,852				X	
33	617	X				

Each patient was scratch tested with serial dilutions of aqueous pollen extract to determine the weakest reacting concentration. While this is not claimed as an infallible guide of the patient's antigen tolerance or degree of hypersensitivity, it does serve as one objective measure, at least for classification purposes.

In order to make the treated and control groups more closely comparable, patients were classified and paired on the basis of scratch test reactivity, and the presence or absence of other inhalant sensitivities. Four treatment groups were formed: Groups A and B, previously untreated patients, and Groups C and D, previously treated patients. Groups A and C were to receive ragweed antigen emulsion, while Groups B and D were to be given a placebo emulsion.

Because only 15 of the previously untreated patients (Groups A and B) and 18 of the previously treated patients (Groups C and D) could be followed closely enough to obtain the necessary data for evaluation, the final groups were not as comparable in regard to scratch test reactivity and the presence of other allergy as had been hoped (Table I).

ADMINISTRATION OF EMULSIONS

The ragweed antigen and emulsification materials were supplied by Dr. Alan Feinberg, co-chairman of the Committee on Specific Therapy of the American Academy of Allergy. Ragweed was extracted with sorbitol and phosphate-phenol and diluted with sorbitol-phenol. One part of defatted ragweed pollen extract was added to two parts of 10 per cent Arlachel-A in Drakeol-VR6, making a final proportion of 1:9:5 of Arlachel-Drakeol-antigen.

The mixtures were emulsified for 30 minutes in a Brown emulsor, one or 2 days prior to use and, again, one hour before injections into patients. Each mixture was examined grossly before re-emulsification and, microscopically, after the final emulsification to insure, as far as possible, a satisfactory emulsion. Questionable emulsions were rejected. Just prior to use, 1 c.c. of the emulsions was transferred to disposable syringes labelled with the individual patients names and numbers, based on the previous classification and pairing. Just before administration, each patient was scratch tested with the treatment emulsion he was to receive, although this information did not influence a decision to give or withhold the injection. The collaborator who administered the injections was unaware of the contents of each syringe.

Injections were given on July 7 and 14, 1961. Patients in Groups A and B were scheduled to receive 2,500 pollen units or placebo in 1.0 c.c. of emulsion, while those in Groups C and D were to be given 5,000 pollen units or placebo. The dose in each case was divided into two equal parts, administered one hour apart. In the face of undue local swelling following the first portion, the second part was not given. This occurred in 2 patients included in the final evaluation; one in Group A and another in Group C.

LOCAL AND CONSTITUTIONAL REACTIONS (TABLE II)

Local swelling at the injection site, usually evident in 30 minutes and persisting for several hours to 4 or 5 days, occurred in 7 patients in Groups A and C who had been treated with antigen emulsion. One patient in placebo Group D had a mild swelling. Two patients, both receiving the active antigen, experienced urticarial reactions beginning 2 hours after injection, which lasted for 6 hours in one case and almost 12 hours in the other. In one of these (patient No. 25), only half the scheduled dose was given because of severe

immediate local swelling from the first portion. This patient also had the greatest skin test reactivity to aqueous ragweed antigen as determined by serial titrations. One patient in placebo Group B developed a mild, non-tender swelling at the injection site which began 2 days later and persisted for 5 days. A positive scratch test to the antigen emulsion before treatment predicted a local or constitutional reaction in 4 out of 7 patients. It was negative in the one case (patient No. 7) in which there was both a local and constitutional reaction.

TABLE II. LOCAL AND CONSTITUTIONAL REACTIONS (PREVIOUSLY UNTREATED [GROUPS A & B]: PREVIOUSLY TREATED [GROUPS C & D])

PATIENT	EMULSION DOSE (PU)	EMULSION SCRATCH	REACTIONS		
			IMMEDIATE LOCAL	DELAYED LOCAL	CONSTITUTIONAL
Group A (Active)					
1	2,500	—	—	—	—
2	2,500	—	Mild	—	—
3	1,250	Pos.	Severe	—	—
4	2,500	—	—	—	—
5	2,500	—	—	—	—
6	2,500	Pos.	—	—	—
7	2,500	—	Moderate	—	Hives
Group B (Placebo)					
8	—	—	—	—	—
9	—	—	—	Moderate	—
10	—	—	—	—	—
11	—	—	—	—	—
12	—	—	—	—	—
13	—	—	—	—	—
14	—	—	—	—	—
15	—	—	—	—	—
Group C (Active)					
16	5,000	Pos.	—	—	—
17	5,000	—	—	—	—
18	5,000	Pos.	Moderate	—	—
19	5,000	—	—	—	—
20	5,000	Pos.	Moderate	—	—
21	5,000	—	—	—	—
22	5,000	—	—	—	—
23	5,000	—	Moderate	—	—
24	5,000	Pos.	—	—	—
25	2,500	Pos.	Severe	—	Hives
Group D (Placebo)					
26	—	—	—	—	—
27	—	—	—	—	—
28	—	—	—	—	—
29	—	—	—	—	—
30	—	—	—	—	—
31	—	—	Mild	—	—
32	—	—	—	—	—
33	—	—	—	—	—

CLINICAL EVALUATION

The clinical evaluation of each patient was made during the 1961 season by one of the investigators who was unaware of the composition of the various groups. This was accomplished during weekly interviews from August 15 to September 22. If a patient missed more than one appointment during the season, he was dropped from the study. Each patient kept a daily record of

symptoms and medication used which were recorded on a specially prepared form. At the time of each weekly visit the daily record was reviewed with the patient. Medications prescribed for each patient consisted of one popular antihistaminic tablet for hay fever symptoms, an ephedrine-theophylline-barbiturate tablet for asthma, and an isopropyl-epinephrine package nebulizer for more intense asthmatic symptoms. Steroids were not used. While it was initially planned to assign a numerical value to each period of symptoms and to each tablet or inhalation used, as suggested by Lowell,⁷ this system could not be followed. Instead, a simplified daily score was assigned on the basis of the daily record reviewed at the time of each visit. The manner in which the numerical daily score was calculated is shown in Table III. The total seasonal score was

TABLE III. DAILY SCORING—SYMPTOMS AND MEDICATION

SYMPTOMS	MEDICATION	SCORE
None	None	3
Mild	1 or 2 pills of one or both types with good relief	2
Moderate	More than 2 pills of one or both types, or inhaler use with good or moderate relief	1
Severe	Poor or no relief from maximum medication	0

compiled for each patient based on 32 consecutive days from August 15 to September 15, inclusive. Complete absence of symptoms during the season without the use of medication would result in a maximum score of 96, scaling down to zero in a patient who experienced severe symptoms every day without appreciable relief from maximum medication. The total seasonal scores for each patient and the mean scores for each group are presented in Tables IV and V.*

TABLE IV. CLINICAL RESULTS (TOTAL PATIENT SCORES)

PREVIOUSLY UNTREATED PATIENTS		
PATIENT	SEASONAL SCORE	MEAN SCORE
Group A (Active Emulsion)		
1	56	
2	67	
3	61	
4	70	
5	68	
6	28	
7	79	
		59.9
Group B (Placebo Emulsion)		
8	50	
9	34	
10	34	
11	30	
12	62	
13	60	
14	19	
15	60	
		43.6
	Difference	16.3

*Statistical analysis was performed by Mary E. Patno, Ph.D., Department of Biostatistics, School of Public Health, University of Michigan.

TABLE V. CLINICAL RESULTS (TOTAL PATIENT SCORES)

PREVIOUSLY TREATED PATIENTS		
PATIENT	SEASONAL SCORE	MEAN SCORE
Group C (Active Emulsion)		
16	66	
17	51	
18	70	
19	22	
20	45	
21	68	
22	54	
23	82	
24	76	
25	72	
		60.6
Group D (Placebo Emulsion)		
26	30	
27	18	
28	59	
29	54	
30	58	
31	56	
32	68	
33	52	
		49.4
	Difference	11.2

Among the previously treated patients, Group A, treated with active emulsion, showed a greater mean score than Group B, treated with placebo; the difference was 16.3. In the previously treated patients of Groups C and D, the mean score of those treated with active emulsion exceeded by 11.2 the score of those treated with the placebo. If significance tests are applied to Groups A and B, it would appear that, if the active emulsion were in truth no more effective than placebo, the probability of a difference this large, or larger, occurring by chance is approximately equal to 8 per cent. Therefore, if one states that the active emulsion was more effective than the placebo, there is an 8 per cent chance of the statement being wrong. The limits of the 90 per cent confidence interval estimate of the difference were 1.4 and 31.1.

In Groups C and D, if the active emulsion were no better than the placebo, the probability of a difference in mean scores this large, or larger, occurring by chance is approximately equal to 22 per cent. The 90 per cent confidence estimate of the true difference is from zero to 25.

Since the mean scores of both groups who received the active emulsion are similar, one might combine the two groups, showing a difference of 13.8 between the groups treated with active emulsion and placebo. Now if one states that the experience of patients who received the active emulsion was more favorable, there is a 2 per cent chance of being wrong. The limits of the 90 per cent confidence interval estimate of the difference in scores were 4.1 and 23.5.

In Table VI are calculated the *t* values for the differences between the group mean, and the *p* values indicating the probabilities that the differences could occur by chance alone. It is seen that combining actively treated Groups

A and C, as opposed to placebo Groups B and D, the difference is significant at the 5 per cent level. When the larger groups are used, these data suggest that the active emulsion was more effective than the placebo.

More graphic representations of the results are shown in Figs. 1, 2, and 3 which compare active emulsion-treated and placebo-treated patients. Another way of presenting this data is shown in Figs. 4 and 5, where mean daily scores for each group are charted for the period of study. In the previously untreated group (Fig. 4), the difference between active emulsion-treated Group A and placebo-treated Group B is more striking. Again in the previously treated patient group (Fig. 5), the experience of the patients treated with active emulsion is slightly more favorable than those who received placebo.

The possibilities that scores were influenced by the presence of other inhalant sensitivities or treatment in the previous year is, of course, recognized. Unfortunately, it is not possible to assess the extent of influence of this factor.

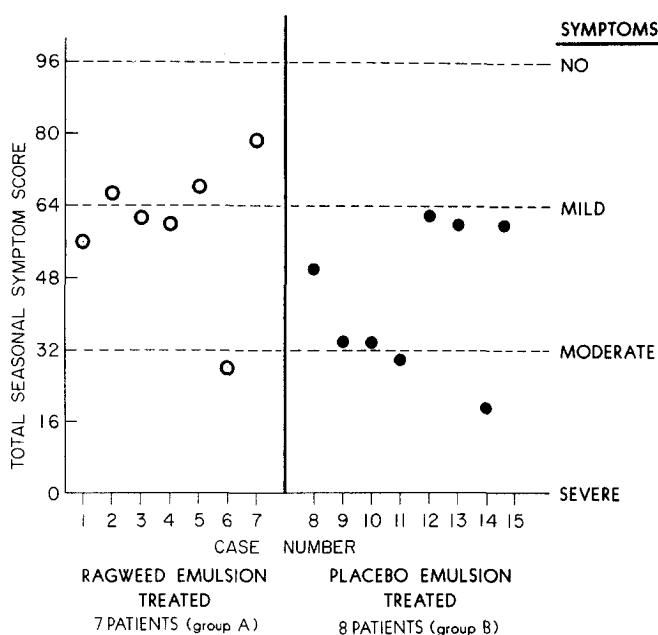


Fig. 1.—Total seasonal scores of previously untreated patients.

TABLE VI. COMPARISON OF MEAN SCORES FOR ACTIVE EMULSION- AND PLACEBO-TREATED PATIENTS

PATIENTS	ACTIVE EMULSION	PLACEBO EMULSION	DIFFERENCE	T	P
Previously untreated (15)	59.9 (Group A)	43.6 (Group B)	16.3	1.94 13 d.f.	Between 0.05 and 0.10
Previously treated (18)	60.6 (Group C)	49.4 (Group D)	11.2	1.36 16 d.f.	Between 0.10 and 0.25
Previously treated and untreated (33)	60.3 (Groups A and C)	46.5 (Groups B and D)	13.8	2.42 31 d.f.	Between 0.02 and 0.05

Similarly, the influence of 1960 treatment in Groups C and D, with what might in effect be a booster dose in 1961, cannot be assessed.

DISCUSSION

While the value of double-blind techniques in studying the subjective response is acknowledged by most careful observers, its use in allergy has been limited to a great extent by the large number of variables which are difficult to control adequately. Although it may be possible to reduce the influence of

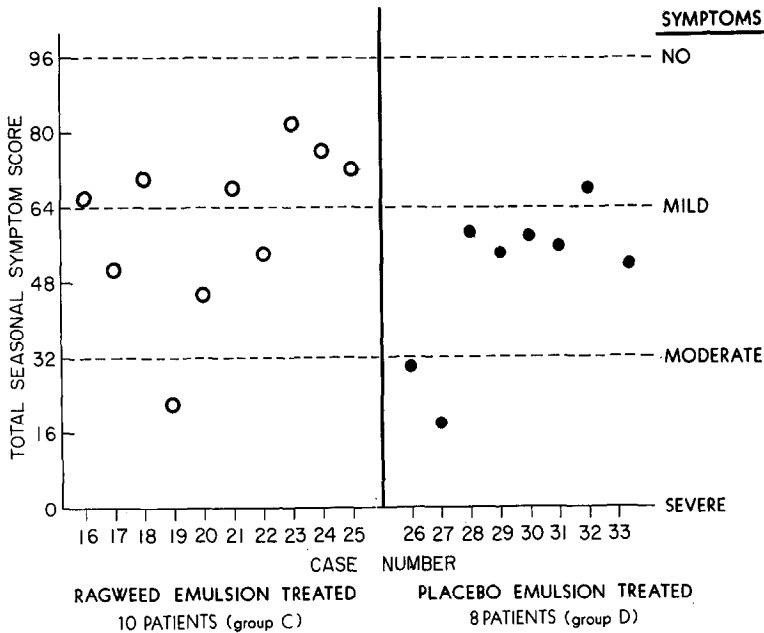


Fig. 2.—Total seasonal scores of treated patients.

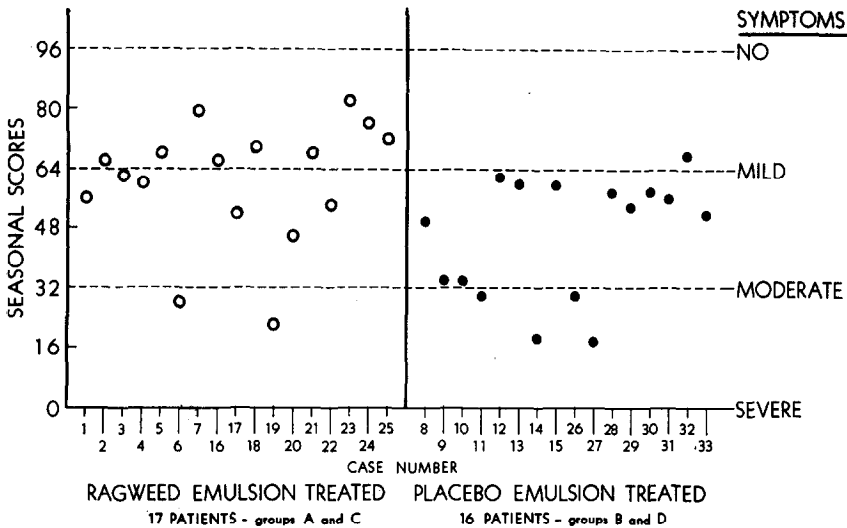


Fig. 3.—Total seasonal scores of treated and untreated patients.

patient and physician bias, there still remain variations based on differences in individual sensitivity and exposure, multiple sensitizations, and differences in immunologic response from the same antigenic stimulus. In studying the effects of emulsion antigen injection, Brown⁸ questions the value of double-blind tech-

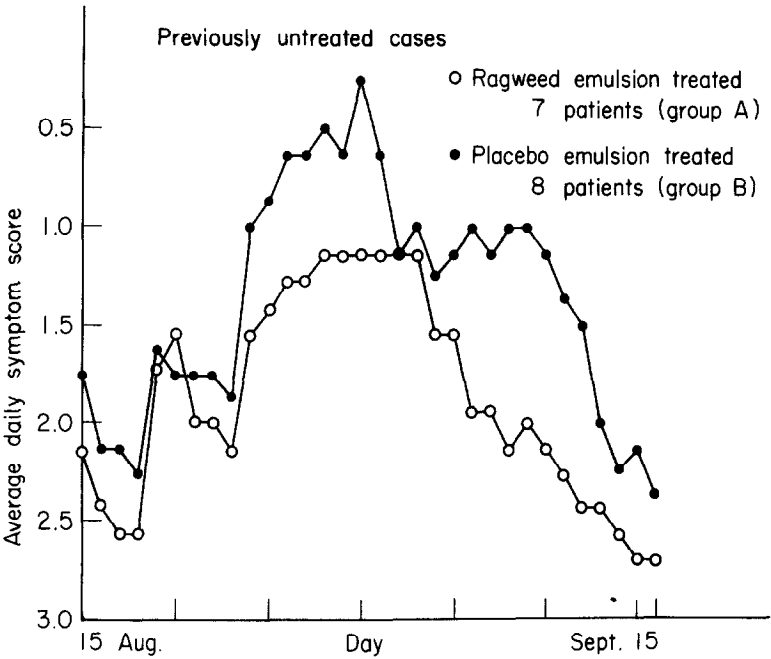


Fig. 4.

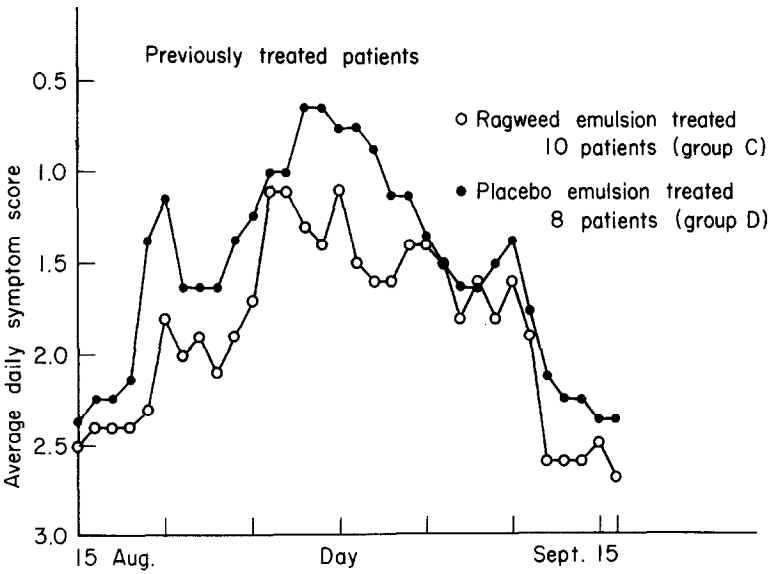


Fig. 5.

niques, claiming that the oil is an adjuvant which has been shown to produce improvement in any patient previously treated, or even skin tested, with the pollen extract.

Efforts to utilize purely objective techniques, such as the measurement of various immune responses following emulsion antigen injection, have in the past shown rather wide variations and lack of correlation with the apparent degree of clinical response.^{9, 10} A similar lack of correlation was observed in the present patients where hemagglutinating antibody against ragweed was measured before and at intervals after treatment.*

The double-blind experiment carried out in the present study is far from perfect. Any double-blind study can at best attempt to evaluate the condition of patients under a given set of circumstances and, through statistical evaluation, determine the probabilities that symptoms were influenced by the treatment administered. The possibility of error in such a study would be reduced in a larger group, which unfortunately was not available. One could also argue a number of other points, such as the preference for an undefatted rather than a defatted extract, an earlier or later time for treatment, larger doses, or multiple doses given at intervals of days or weeks. It is possible that any of these factors may well have influenced results in the antigen-treated groups. The inability to evaluate the influence of previous treatment or multiple sensitivities could also be valid criticism. One could also take issue with the method of measuring the subjective response and the manner of reducing it to a numerical value for statistical analysis.

While no apologies are made for the inability to resolve all of these variables, they are nonetheless important points to consider in any future study of this type, especially that which is planned by the Committee of Specific Therapy of the American Academy of Allergy.

SUMMARY

1. A double-blind study was carried out in 33 patients with ragweed pollinosis to evaluate the effects of a single injection of emulsified ragweed antigen. The group consisted of 18 patients sensitive to ragweed who were treated with emulsion antigen the previous year, and 15 patients who had never before been treated. Seventeen patients received from 1,250 to 5,000 pollen units of emulsified ragweed antigen in a single day. The remaining patients were given a placebo emulsion containing no ragweed.

2. Immediate local reactions occurred in 8 patients in the groups treated with ragweed emulsion and in one patient in the placebo group. Mild constitutional reactions occurred in 2 patients who received ragweed emulsion.

3. Clinical results were determined by assigning a daily score to each patient, based on the severity of symptoms and the amount of medication used each day. A total seasonal score for each patient and a mean seasonal score for each group of patients was calculated. While the total scores of groups treated

*Hemagglutination studies were performed by Lawrence Weiner, Ph.D., Department of Microbiology, Wayne State University College of Medicine.

with ragweed emulsion (both previously treated and previously untreated) were more favorable than those of the corresponding placebo-treated groups, the statistical differences between them were small and were significant at the 5 per cent level only when all antigen-treated groups were combined and compared with placebo-treated groups.

4. It is pointed out that this type of study does not allow evaluation of many variables which may influence results of treatment.

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