

EFFECT OF REPOSITORY THERAPY ON IMMUNOLOGIC RESPONSES TO POLLEN EXTRACT

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RAGWEED pollen extract emulsified in mineral oil has been used as a repository form of desensitization treatment of patients with pollinosis with apparent success by an increasing number of allergists.¹⁻⁷ The effects of such repository injections on serum antiragweed antibody levels, including skin sensitizing activity, "blocking" antibody, and hemagglutination antibody, have been recently investigated by several groups.⁸⁻¹¹ This report is concerned with a study of the immune responses to pollen extracts as measured by several *in vitro* techniques in a relatively large group of individuals given a single repository injection of ragweed shortly before the 1959 season. As a comparison, another group of individuals receiving conventional aqueous therapy throughout the year were similarly studied. The relative changes in antibodies, as measured by the classical *in vivo* blocking antibody procedure, by the passive hemagglutination procedure using the bis-diazotized benzidine method, by complement fixation and by precipitin reactions, were determined and compared to clinical observations.

METHODS AND MATERIAL

Subjects.—Five groups of individuals, all residing in the Pittsburgh area, were included in this study as follows: GROUP 1: 8 nonallergic individuals received a single injection of ragweed pollen emulsion, GROUP 2: 3 ragweed-allergic patients received a placebo emulsion without ragweed, GROUP 3: 347 ragweed-sensitive hay fever patients were treated with conventional multidose aqueous extract prior to the season, GROUP 4: 13 "new" hay fever patients who had had no prior hyposensitization therapy received a single repository injection of ragweed pollen, and GROUP 5: 110 ragweed-sensitive hay fever patients, transferred from maximal doses of multiple aqueous extract treatment, received a single ragweed emulsion injection.

All ragweed patients had unequivocal histories of ragweed pollinosis and were highly sensitive by scratch tests to ragweed extract. Most patients were clinically allergic only to ragweed pollen or, occasionally, to other allergens not of clinical importance during the ragweed season. Individuals treated with

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conventional multiple aqueous ragweed extract only were receiving the maximum tolerated dose of aqueous extract. This dose was reached as a result of multiple pre-seasonal injections of extract.

Emulsion.—Emulsions similar to those described by Brown¹² were used. These contained Drakeol and Arlacel as the emulsifying agent. Final concentrations of extract containing 1,500, 2,500, 5,000, 7,500, or 10,000 protein nitrogen units (PNU) per milliliter of emulsion were prepared. These emulsions also contained epinephrine, as suggested by Brown. Similar results as those reported here have been obtained in subsequent years (1960 and 1961) with smaller groups of individuals who have been treated with emulsions without drugs.

Emulsion injection: Six weeks prior to the onset of the 1959 hay fever season, emulsion injections totaling 1.0 ml. per patient were given subcutaneously in the deltoid area in three equally divided doses 15 minutes apart.¹² Fifteen to 20 minutes prior to these injections, 0.15 ml. of epinephrine was administered subcutaneously at a point proximal to the area in which injection was made. A long action antihistamine was given orally at the same time. Following the emulsion injection and a wait of 30 minutes, patients were discharged with antihistaminic medication to be taken for 2 days if needed.

Dose of emulsion: The dose of ragweed emulsion given to previously untreated patients (Group 4) was determined by scratch tests observing the following schedule—patients with scratch test positive for 10 PNU received 1,500 PNU emulsion, scratch test negative to 10 PNU but positive to 40 PNU received 2,500 PNU emulsion, scratch test negative to 40 PNU but positive to 160 PNU received 5,000 PNU emulsion, scratch test negative to 160 PNU but positive to 640 PNU received 7,500 PNU, scratch test negative to 640 PNU but positive to 2,560 PNU received 10,000 PNU emulsion. Patients transferred from aqueous extract to ragweed emulsion received doses equivalent to ten to fifteen times the previous aqueous extract dose.

Aqueous Injections.—Three hundred and forty-seven ragweed-sensitive patients were treated with multiple injections of conventional aqueous extract. Three hundred and three had been on aqueous therapy for a period of 2 to 15 years prior to this study and were at the maximum dosage level. Forty-four subjects had received aqueous therapy only for the treatment period prior to the 1959 season.

Serum Collection.—An initial bleeding (pre-season serum) was obtained aseptically 6 weeks prior to the season (immediately prior to the repository injection for those receiving emulsion) and 4, 6, 8, and 12 weeks thereafter (post-treatment sera). All sera were stored sterilely at -20° C. until used.

Immunologic Studies.—

Blocking antibodies: Blocking antibodies were measured by the classical method of in vitro neutralization exactly as described by Loveless.¹³ A twofold or greater change in the amount of ragweed neutralized by the blocking antibody in this procedure was considered valid. Titrations were carried out with sera obtained from 96 of the subjects receiving either emulsion or aqueous

therapy. A total of 60 volunteers, not sensitive to ragweed, from a local penal institution (Allegheny County Workhouse) were utilized as recipients for the 360 individual serum titrations.

Skin-sensitizing antibody: Several sets of sera obtained from subjects before and after emulsion treatment were compared with sera obtained at the same time from aqueous-treated subjects as to skin sensitizing activity by the classical Prausnitz-Küstner procedure. Sera from 12 emulsion-treated subjects and 5 aqueous-treated subjects were so tested. Samples of 0.05 ml. of serially diluted sera (passed through a sterile Millipore filter and exposed to ultraviolet light for 30 minutes in small plates), starting at 1:10 and ranging to 1:5,120, were injected intradermally into recipients not sensitive to ragweed. Twenty-four hours later, 0.05 ml. of an aqueous solution of ragweed extract containing 1,500 PNU per milliliter was injected into each site. The reciprocal of the highest dilution of serum which resulted in a positive sensitization reaction (2 plus) was recorded as the end point titer. Fourfold or greater differences in titer were considered significant.

Hemagglutination: The indirect hemagglutination test using the bis-diazotized benzidine (BDB) method, first described by Pressman and co-workers,¹⁴ then by Stavitsky and Arquilla,¹⁵ and adapted to the ragweed pollen system by Gordon and his associates,¹⁶ was used. Short ragweed pollen extract (*Ambrosia elatior*) at a 0.1 per cent concentration was coupled to a 2 per cent suspension of washed rabbit erythrocytes with a 1:15 dilution of BDB. All sera were absorbed with nontreated rabbit erythrocytes and heated at 56° C. for 30 minutes prior to use. Controls consisted of the use of uncoated red blood cells, inhibition of positive titers with ragweed, positive serum controls, negative serum controls, and red cells treated with unrelated antigens, such as bovine serum albumin and grass pollen extracts. All titers reported were obtained from hemagglutination tests carried out simultaneously with each serum with the same preparation of ragweed-coated cells. All sera were tested in duplicate and each test repeated at least twice on separate days. Titers are recorded as the reciprocal of the highest dilution of serum which gives a completely positive hemagglutination pattern after 18 hours of incubation at room temperature. Incomplete hemagglutination patterns ("donuts" or partial pattern formation of red cells) are recorded as negative.

Complement fixation: The complement fixation (CF) activity of the various sera incubated with ragweed extract was determined by a standard complement fixation procedure.^{17, 18} A 1 per cent concentration of aqueous ragweed pollen extract and two hemolytic units of complements were used.

Precipitin tests: Qualitative precipitin tests in liquid medium were performed with all sera, using a standard procedure.¹⁸ A 0.2 ml. volume of 1:2 diluted serum was incubated with varying concentrations of ragweed extract for one hour at 37° C. and for 48 hours at 10° C., then centrifuged to obtain precipitates. Gel diffusion procedures were carried out in capillary tubes (Preer-Oudin techniques¹⁹) and on plates (Ouchterlony method²⁰) with each serum.

Clinical Observations.—

Clinical relief: Patients were seen every 2 weeks during the season and after the termination of the season. The degree of clinical relief as evaluated subjectively by the patient and as observed during examinations were graded as follows: (1) *marked relief*—practically no disturbing symptoms and no necessity to use drugs; (2) *moderate relief*—occasional mild symptoms necessitating occasional medication, and (3) *no relief*—no effective relief during the season.

Ophthalmic test: The conjunctival mucous membrane test as described by Loveless²¹ was used as a rough index of threshold sensitivity to pollen in patients with eye symptoms during the season. Nonglycerinated aqueous ragweed extract in increasing concentrations, starting at 10 PNU, was placed in the conjunctival sac. Successive additions of increasing concentrations were made at 5 minute intervals until a recognizable reddening occurred in the caruncle. Sixty-four ragweed-sensitive patients were tested prior to and following ragweed emulsion treatment. A twofold or greater change in concentration of ragweed extract needed to give a positive result was considered valid in evaluating increase or decrease of eye sensitivity to ragweed extract prior to and following therapy.

Delayed hypersensitivity: A delayed tuberculin-type sensitivity test was performed on 33 patients treated with ragweed emulsion. One tenth of a milliliter of aqueous ragweed extract at concentrations of 500 PNU and 2,500 PNU was injected 4 to 5 weeks following emulsion treatments. The injection sites were observed 24 and 48 hours later.

EXPERIMENTAL RESULTS

Antibody Responses.—

Hemagglutination: Comparison of the hemagglutinating titers of the sera of the test subjects, prior to and following treatment with either the repository, conventional aqueous, or control preparations, indicated a wide variety in both hemagglutination titers and in the degree of change in titers. Tables I and II indicate the hemagglutinating titers for various groups. The majority of those patients treated with repository ragweed injection (Groups 4 and 5) showed a fourfold or greater increase in hemagglutination antibody. Such increases in titer were found regardless of whether the patient received the emulsion injection following previous treatments with aqueous ragweed extract or had had no prior treatment. Similar increased titers following conventional therapy with aqueous ragweed extracts prior to the season were found in half of those control patients (Group 3) having been given such therapy (Fig. 1, Tables I and II). Such increased titers are similar to those reported by others.²²⁻²⁴ Fig. 1 indicates some individual hemagglutination curves obtained with sera from individuals receiving only aqueous injections.

A comparison of individual titers of preseason to mean peak post-treatment titers for a group of experimental subjects receiving the repository injection of ragweed extract and a group of control individuals who had received conven-

tional aqueous injections prior to the season is shown graphically in Fig. 2. Ten of the 21 individuals tested from the control subjects had increased titers, the same number had no change, while one individual showed a decrease in

TABLE I. EFFECT OF REPOSITORY AND AQUEOUS RAGWEED EXTRACT TREATMENT ON HEMAGGLUTINATION TITER CHANGES

TREATMENT GROUP		HEMAGGLUTINATION TEST (BDB METHOD)						
		SERA		PERCENTAGE OF SERA WITH TITERS IN RANGE OF:				GEO- METRIC MEAN TITER
		PRE- OR POST- TREAT- MENT	NO. TESTED	1:40 OR LESS	1:40- 1:80	1:80- 1:160	1:160- 1:320 OR HIGHER	
Ragweed-Allergic Patients	1. Nonallergic rag- weed emulsion	Pre. Post.	5 12	60 20	40 60	0 20	0 0	27 77
	2. Emulsion pla- cebo (no rag- weed)	Pre. Post.	2 4	50 50	50 50	0 0	0 0	48 76
	3. Aqueous injec- tions (no emulsion)	Pre. Post.	26 27	50 23	25 23	13 23	12 17	0 11
	4. No prior treatment	Pre. Post.	8 14	37 14	50 50	13 22	0 14	0 0
	5. Previous aqueous treatment	Pre. Post.	92 195	33 7	48 47	12 15	5 24	2 2
								120 224

Pre-treatment sera obtained within 50 days prior to ragweed season.

Post-treatment titer indicates peak geometric mean titer following therapy.

TABLE II. EFFECT OF INJECTION THERAPY ON HEMAGGLUTINATION TITER CHANGES

TREATMENT GROUP	NO. PA- TIENTS TESTED	HEMAGGLUTINATION TITERS					MEAN TITER IN- CREASE*	PER CENT SERA WITH TITER IN- CREASE
		NUMBER OF SERA WITH TITER CHANGES IN RANGE OF:						
		NO CHANGE	TWO- FOLD	FOUR- FOLD	EIGHT- FOLD	SIXTEEN- FOLD OR GREATER		
1. Nonallergic Ragweed emul- sion injected	8	4	2	2	0	0	44	50
2. Allergic Emulsion pla- cebo (no rag- weed)	3	0	2	1	0	0	28	100†
3. Allergic Aqueous injec- tions only (no emulsion)	31	14	4	8	2	3	48	52
4. Allergic Emulsion treat- ment (no prior injections)	12	2	0	5	5	0	88	83
5. Allergic Emulsion treat- ment (previ- ous aqueous injections)	106	14	24	34	22	12	104	86

*Geometric mean titer (reciprocal of highest dilution of serum producing positive hemagglutination) of post-treatment sera minus geometric mean titer of pre-season sera.

†Too few to be significant.

titer. Of the emulsion-treated group, only 6 of 84 subjects did not show a response with higher titers following therapy.

Two- or fourfold increases in titers were observed in 4 of the 8 nonallergic control individuals given a repository emulsion injection containing 2,500 PNU (Fig. 3, Table II). An initial low hemagglutination titer was found in the pre-injection sera of most of these individuals. As suggested by Arbesman,²⁵

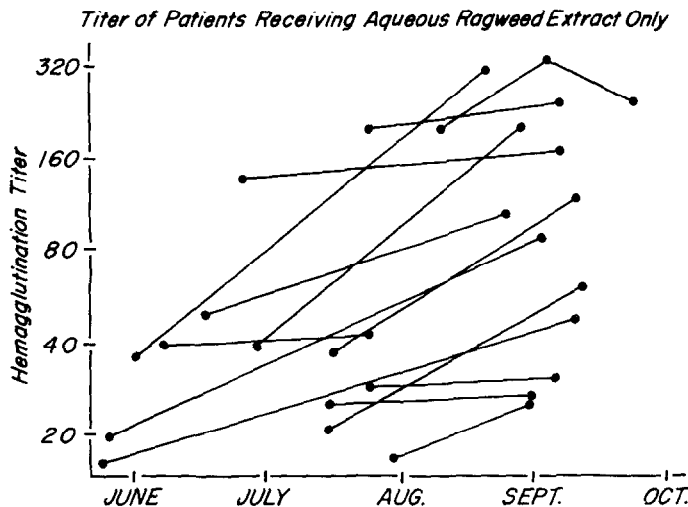


Fig. 1.—Individual hemagglutination titer curves of sera from ragweed-sensitive patients receiving aqueous extract injections but no ragweed emulsion.

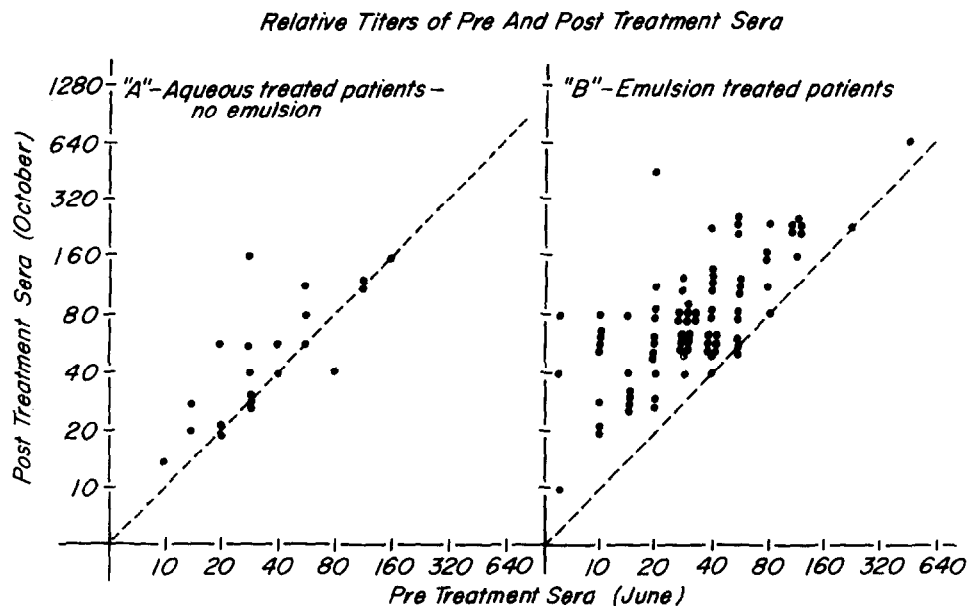


Fig. 2.—Comparison of the relative hemagglutination titers of pre- and post-treatment sera of (A) allergic patients treated with standard multiple aqueous extracts of ragweed pollen and (B) allergic patients treated with a single repository injection of ragweed extract. All points in upper segment indicate an increase in titer for post-treatment sera versus pre-treatment sera; points in area to the right of the diagonal line indicate decreased titers; points on the diagonal line indicate that titer remained unchanged.

this is thought to be an indication of the excessive sensitivity of the BDB method, thus a lower specificity than originally expected by others. However, such low levels in nonallergic individuals have not been observed by all investigators.^{23, 24}

Fig. 4 indicates the hemagglutination responses of experimental and control groups treated with either aqueous or emulsion therapy and tested for hemagglutinating antibodies prior to, during, and following the ragweed season. Each curve represents the geometric mean antibody response for each group. The hemagglutinating activities of sera from ragweed-allergic individuals following aqueous or emulsion therapy are comparable and within the same ranges.

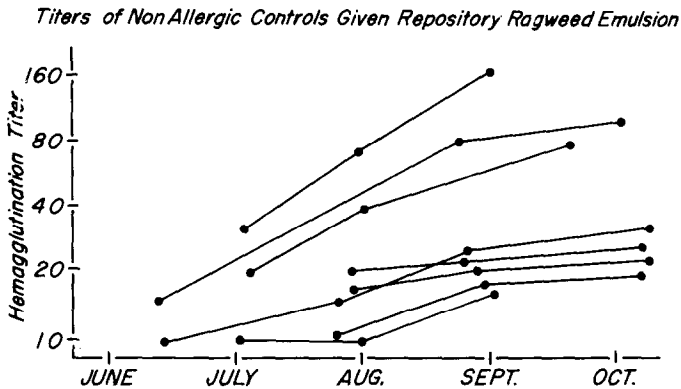


Fig. 3.—Individual hemagglutination titer curves of the sera from 8 nonallergic control individuals injected with ragweed emulsion.

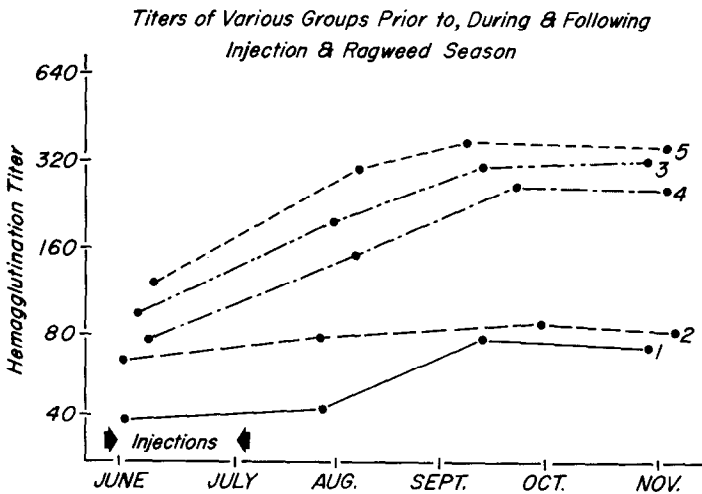


Fig. 4.—Hemagglutination titer responses in various groups following either repository or aqueous injections of ragweed extract. Geometric mean titers for each group are indicated by curves: Group 1—Nonallergic individuals treated with ragweed emulsion. Group 2—Hay fever patients receiving placebo emulsion (no ragweed). Group 3—Hay fever patients treated only with aqueous ragweed. Group 4—"New" hay fever cases treated with ragweed emulsion (no prior ragweed treatment). Group 5—Hay fever patients transferred from aqueous ragweed to emulsion ragweed.

Note: *Injections* indicate period of time over which repository injections were given to various subjects in Groups 1, 2, 4, and 5. Aqueous patients received injections during previous 10 months.

In most instances, patients with pollinosis who had been previously treated prior to the single repository injection responded with a titer change greater than those still on conventional therapy or those who had not had previous therapy.

Complement fixation: A number of sera available for the hemagglutination assay were also tested for complement fixation activity, using ragweed extract as the antigen (Table III). In most instances, there were no significant titers, although occasionally there was fixation in low dilution such as 1:8 or 1:16. No increase in these titers was noted in any set of sera following any form of treatment, although many of the same sera had shown a marked increase in hemagglutination activities.

TABLE III. COMPLEMENT FIXATION TESTS WITH SERA FROM REPOSITORY- AND AQUEOUS-TREATED PATIENTS

SUBJECT GROUP	NO. SUB- JECTS	NO. NEGA- TIVE*	COMPLEMENT FIXATION TEST				
			NO. WITH TITER			MEAN TITERS	
			INCREASE	DECREASE	SAME	PRE.	POST.
Emulsion	22	13	0	1	8	8.2	8.5
Aqueous extract	12	6	0	0	6	7.8	7.4

*Less than 1:2 in both pre- and post-treatment sera.

Precipitin tests: No precipitating activity was noted in any serum with the use of standard liquid precipitin tests or gel diffusion tests (Table IV). Attempts to inhibit precipitates formed by rabbit antiragweed serum and ragweed extract by the preliminary incubation of extract with test human sera were equivocal and inconsistent. Likewise, no consistent increase or decrease in the amount, density, or relative position of the precipitin bands formed with the rabbit antiragweed system in gel diffusion tests could be obtained using the human sera as an addition. (Precipitins, however, were obtained in several instances with gamma globulin fractions obtained by separation of high titered

TABLE IV. PRECIPITIN ACTIVITY OF HUMAN AND RABBIT SERA WITH RAGWEED EXTRACT

TREAT- MENT GROUP	NO. INDI- VIDUALS TESTED	SERA		GEL DIF- FUSION (NO. BANDS)	PRECIPITIN μG Ab N/ML. (MEAN)	CO-PRECIPITATION†		
		PRE- OR POST- INJEC- TION†	NO.			NO. OF SERA RESULTING IN μG Ab N/ML. RANGE OF:		
						100-140	140-160	160-240
Emulsion	16	Pre.	15	0	0.1	3	7	5
		Post.	16	0	0.1	4	10	2
Aqueous	9	Pre.	4	0	0.1	1	3	0
		Post.	9	0	0.1	3	3	3
Rabbit controls*	6	Pre.	6	0	0.1	0	6	0
		Post.	6	12	148.5	0	0	6

*Control rabbits injected with emulsified extract in Freund's adjuvant (10,000 PNU/ml. as 1.0 ml. injections subcutaneously over period of 6 months).

†Pre-treatment refers to serum sample obtained prior to season or emulsion injection. Post-treatment indicates serum with maximum hemagglutinin titer obtained 2 to 3 months following injection.

‡Co-precipitin test indicates precipitin results following addition of 0.5 ml. of test antiserum to 0.5 ml. of pooled high titered rabbit antiserum containing 148 μ g antiragweed Ab N/ml. plus 0.5 ml. of 1,500 PNU per milliliter of ragweed extract.

[hemagglutination] human sera on DEAE cellulose columns. Precipitin activity was found only in gamma globulin concentrates having high hemagglutinating activity [1:5,000 or greater] and could be demonstrated both by gel diffusion and ring precipitin tests.)

Blocking antibody titers: Titration for blocking antibodies were carried out with sera from 80 subjects given ragweed emulsion therapy (Table V, Fig. 5). Of the 8 patients who had not had prior aqueous treatment (Group 4), 6 showed increased blocking antibodies in the post-injection sera as compared to pre-injection sera. The other 2 individuals showed a decrease in titer. Of

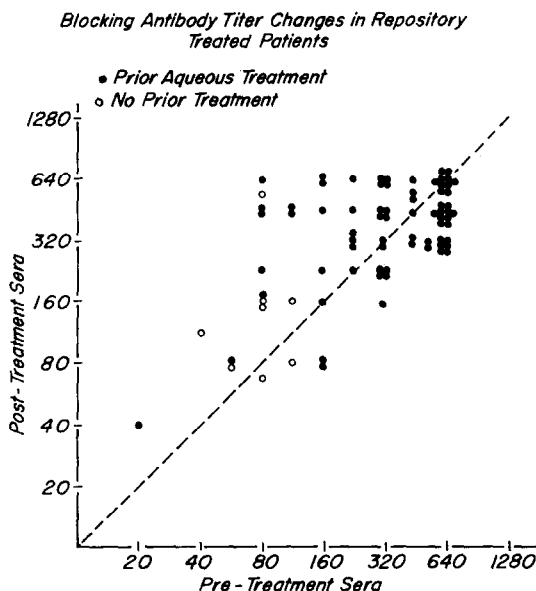


Fig. 5.—Comparative blocking antibody titers of pre-treatment sera to mean peak post-treatment sera of patients with pollinosis receiving a single repository injection of ragweed pollen emulsion.

TABLE V. COMPARISON OF BLOCKING ANTIBODY TITER CHANGES OF PRE-SEASON SERA TO MEAN PEAK POST-TREATMENT SERA FOLLOWING AQUEOUS OR EMULSION TREATMENT

GROUP NO.	TREATMENT	NO. SUB-JECTS	BLOCKING ANTIBODY TITER					
			INCREASE		DECREASE		NO CHANGE	
			NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
1	Non allergic controls given ragweed emulsion	4	2	50	0	0	2	50
3	Ragweed patients given aqueous injections	12	4	33	6	50	2	17
4	Ragweed patients (no prior treatment) given emulsion	8	6	75	2	25	0	0
5	Ragweed patients (prior aqueous treatment) given emulsion	72	29	40	26	36	17	24

the 72 individuals who had had previous aqueous therapy at the maximum dosage (Group 5), 40 per cent showed an increase in blocking antibodies, 36 per cent a decrease, and the remainder showed no change.

Comparative titrations of the sera from 12 individuals who had received multiple aqueous injections (Group 3) indicated an increased titer in 4 subjects, a decrease in 6 subjects, and no increase in 2 (Table V). Of the 4 control non-allergic subjects injected with emulsion (Group 1), 2 had blocking antibody titers after injection, while 2 had no demonstrable antibodies.

Skin sensitizing antibody: Of the 12 patients treated with repository injection and tested for skin sensitizing antibody change, 8 post-treatment sera showed no change in titer from pre-treatment sera (Table VI). One patient had a decreased titer, 2 a twofold increase, and one an eightfold increase in reagin titer. Two of the sera from aqueous-treated patients showed no change in titer from the post-treatment sera (October) to the pre-seasonal titer (June). Two aqueous-treated patients had a two- or fourfold decrease in titer while one responded with an eightfold increase.

TABLE VI. SKIN SENSITIZING ANTIBODY CHANGE FOLLOWING REPOSITORY OR AQUEOUS THERAPY

	TREATMENT GROUP	
	EMULSION	AQUEOUS
Same	8	2
Twofold increase	1	0
Eightfold increase	2	1
Decrease	1	2

Clinical Observations.—

Clinical relief: Response to therapy appeared to be similar between patients receiving either emulsion therapy or conventional multiple aqueous injections. As can be seen by Table VII, there is comparative degree of relief between either group of patients if one takes into consideration prior treatment or lack of prior treatment.

TABLE VII. COMPARISON OF CLINICAL RESULTS OF AQUEOUS- AND EMULSION-TREATED HAY FEVER PATIENTS DURING 1959 RAGWEED POLLEN SEASON

RAGWEED TREATMENT	PRIOR TREATMENT	NO. PATIENTS	DEGREE OF RELIEF					
			MARKED		MODERATE		NONE	
			NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Conventional aqueous	None	44	29	66	12	27	3	7
	Aqueous	303	115	38	170	56	18	6
Repository emulsion	None	13	10	77	3	23	0	0
	Aqueous	110	40	36	54	49	16	15

Conjunctival sensitivity: Changes in conjunctival sensitivities were noted in most patients injected with ragweed emulsion (Fig. 6). Decrease in eye sensitivity was noted in 47 subjects out of 64 examined by the conjunctival test prior to, during, and following the hay fever season. Fifteen patients showed no changes in sensitivity, whereas 2 subjects had an increase in sensitivity to pollen extracts.

Relationship between clinical response and circulating antibody: No correlation could be made between any of the in vitro or in vivo immunologic results, either among themselves or with the degree of clinical relief observed during the pollinosis season (Table VIII). In those cases in which sufficient sera were available from patients prior to treatment and following the season, comparative assay of each set of sera in the Prausnitz-Küstner test, blocking

Changes in Ophthalmic Sensitivity to Ragweed Following Repository Emulsion Injection

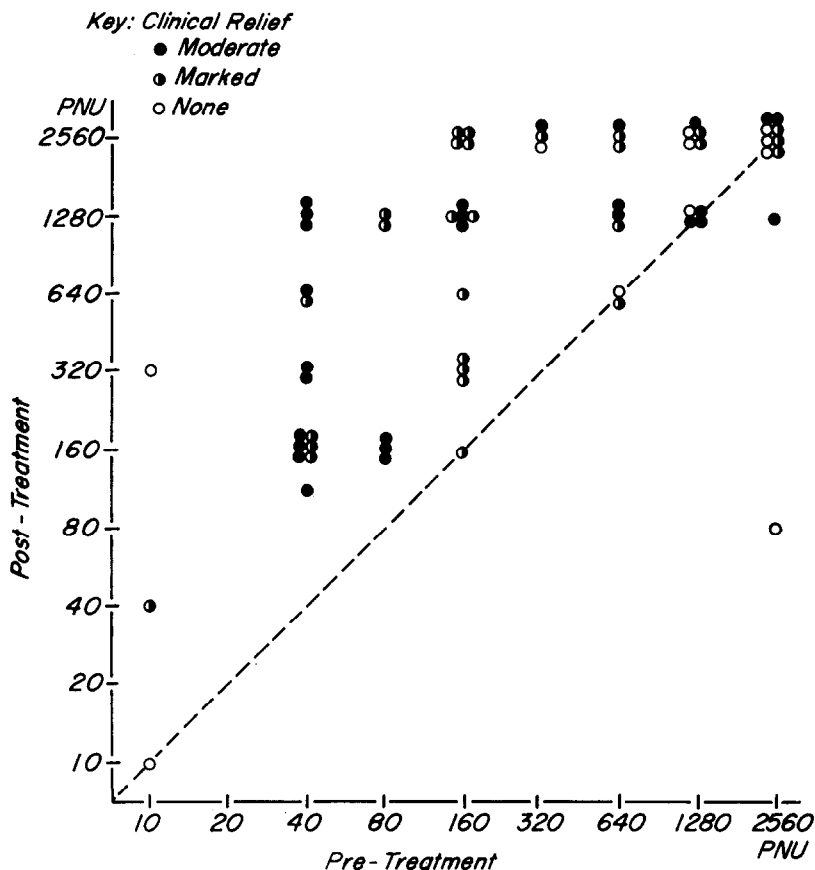


Fig. 6.—Comparative ophthalmic sensitivity to ragweed pollen extract prior to and following repository pollen injection of hay fever patients who had never received prior ragweed treatment.

antibody procedure, and hemagglutination test did not indicate any relationship between results. There were instances in which hemagglutination antibody increased many fold and blocking and skin sensitizing antibody did not change. Similar results indicating a discrepancy between skin sensitizing and other antibodies have been observed in more extensive comparisons in other studies with sera obtained from either repository- or aqueous-treated subjects.^{10, 11, 28}

Delayed reactions: There was no delayed tuberculin-type reactions observed following intracutaneous injection of test doses of aqueous ragweed

TABLE VIII. COMPARISON OF CLINICAL RELIEF TO INCREASES IN ANTIBODY RESPONSES (FOURFOLD OR GREATER) AND OPHTHALMIC SENSITIVITY TOLERANCE OF REPOSITORY- AND AQUEOUS-TREATED PATIENTS

DEGREE OF CLINICAL RELIEF	TREATMENT GROUP	NO. SUB-JECTS	PER CENT OF TOTAL SUBJECTS	HEMAGGLUTININATING ANTIBODY		BLOCKING ANTIBODY		SKIN SENSITIZING ANTIBODY		OPHTHALMIC SENSITIVITY	
				NO. TESTED	NO. WITH INCREASE	NO. TESTED	NO. WITH INCREASE	NO. TESTED	NO. WITH INCREASE	NO. TESTED	NO. WITH DECREASE
Marked	Repository	50	41	35	21 (60%)	37	9 (24%)	2	1 (50%)	27	21 (78%)
	Aqueous	144	42	11	3 (27%)	6	2 (33%)	2	0	—	—
Moderate	Repository	57	46	42	30 (71%)	29	14 (48%)	5	1 (20%)	27	20 (74%)
	Aqueous	182	52	8	3 (38%)	2	1 (80%)	2	1 (50%)	—	—
None	Repository	16	13	41	26 (63%)	14	6 (43%)	5	0	10	4 (40%)
	Aqueous	21	6	12	2 (17%)	4	1 (25%)	1	0	—	—

extract in 33 hay fever patients treated with ragweed emulsion 4 to 5 weeks prior to testing. Test sites were all negative 24 and 48 hours following the test.

Constitutional reactions: Three constitutional reactions were observed among 123 ragweed-sensitive patients given the emulsion treatment (incidence of 2.4 per cent). Two of these patients were "new" cases and had not had prior ragweed treatment. Reactions were treated by standard procedures and all patients responded within 24 hours.

DISCUSSION

This report has been concerned with the laboratory detection of measurable responses in patients with pollinosis following repository injections of ragweed emulsions. The results obtained have indicated that emulsion treatment is as effective as conventional multiple aqueous ragweed injections as far as detectable antibody responses are concerned. Similarly, reports by others, as well as from these laboratories, have indicated that such emulsion therapy is also apparently as effective as aqueous therapy as far as clinical relief is concerned.

In this study, there has been found a consistent increase in antibodies as measured by in vitro neutralization tests for blocking antibodies and by the passive hemagglutination procedure with ragweed-coated red cells. Increase in antibody responses among emulsion-treated patients was found to be as high or higher than in those subjects receiving conventional aqueous therapy prior to the season. However, it should be noted that the titer changes in the 13 "new" patients were less marked than among the 113 previously treated. Such previously treated individuals showed the most marked response as measured by the serological methods.

Contrary to these results, Arbesman¹¹ recently reported that patients receiving a single repository injection of pollen extract did not produce as good an immune response (as determined by the tanned-cell hemagglutination method) as did those receiving multiple injection therapy (as reported in his previous study²²). Only 28 per cent of the emulsion-treated individuals had a fourfold or greater increase in titer as compared to 68 per cent of those patients receiving multiple injections. Individuals receiving "priming" injections (3 to 8) of aqueous extract prior to the repository injection also showed a greater hemagglutination response than those observed following only repository injection, but not as great a response as in those patients who had received aqueous injections over a longer period of time. On the other hand, Rose's group¹⁰ found a definite increase in hemagglutination titers (bis-diazotized benzidine method) in a majority of patients following emulsion treatment. Twenty-two of 35 subjects had an increase in titer of fourfold or more, 8 had twofold increases in titer, and 5 remained the same. Most of these patients had been treated by previous multiple aqueous injections. This is similar to the cases reported here in which a majority of those persons on previous aqueous treatment prior to the repository injection responded with a higher hemagglutinin titer. Sixty-four per cent (68 of 106 patients) had a fourfold or greater increase, whereas 23 per cent (24 patients) had a twofold increase.

The remainder (14 patients) exhibited no change in titers. Of the 12 individuals tested who had been on conventional therapy only, 83 per cent had an increase of fourfold or greater. However, the mean titer change was lower than with the group of patients treated with repository injection following previous conventional therapy.

The data obtained indicate that the antigenic stimulus by a repository injection is sufficient to cause a readily demonstrable increase in the hemagglutinating antibodies in patients with pollinosis. This increase is easily detectable by the bis-diazotized benzidine method, as similarly noted in Rose's report of repository-treated patients, and by the other reports of aqueous-treated individuals. The finding of a less pronounced immune response by Arbesman's group with repository-treated patients possibly indicates a different sensitivity for the tannic acid method as compared to the BDB method. Arbesman has found the use of the tanned cell method more reliable than the BDB method in measuring immune responses following conventional therapy.²⁵ A recent series of experiments,²⁶ using both the tanned cell and the BDB methods with either giant or short ragweed and rabbit erythrocytes, has emphasized the wide discrepancy in results between these methods.

Other parameters measured in the study reported here indicate that blocking antibody responses are as high or higher in repository-treated patients when compared with aqueous-treated patients. A consistent change in conjunctival sensitivity, as indicated by a decreased reactivity to pollen extract, has also been noted in emulsion-treated patients. No noteworthy change in skin sensitizing antibody has been observed using sera from a selected group of subjects. This is similar to observations noted by others.^{10, 11}

The failure to detect circulating antiragweed antibody by the complement fixation procedure or by classical precipitin reactions either in liquid or gel media is reminiscent of the difficulty encountered by others in obtaining consistent and/or reproducible results with these techniques.²⁹⁻³⁴ Several reports of the value of complement fixation or complement fixation inhibition methods as a technique for measuring antiragweed antibody have been made, but have not been used with success by others. Likewise, precipitin tests, precipitin-inhibition, and co-precipitin methods using noneconcentrated sera from allergic individuals have been of little value in detecting antibody in subjects with pollinosis. Recently, Feinberg³⁵ observed precipitins with the use of concentrated pseudoglobulin fractions from sera from allergic individuals. Preliminary data from this laboratory (to be reported in detail elsewhere) and by Sehon's group³⁶ have demonstrated precipitins with the use of concentrated gamma globulin fractions obtained by column chromatography of sera from pollen-allergic individuals. It seems likely that the precipitins are either identical or closely related to the hemagglutination activity of the sera.

As far as clinical relief is concerned, there have been many reports¹⁻⁷ of the apparent efficacy of emulsion therapy as compared to conventional treatment. In most of these studies, including this one, a similar level of clinical relief was observed following repository treatment as compared to multiple

injections. In this study, three constitutional reactions were noted. (Similar low levels of reactions have been observed in more recent series of subjects having little or no drug protection.) No delayed hypersensitivity has been observed following repository injection as compared with the results of several investigators who have obtained delayed reactions in normal volunteers following such injections.³⁷⁻³⁹

The attempt to "monitor" the response to therapy by *in vitro* procedures, as reported here and by others, has been spurred by the desire to measure immune responses to pollen by more reliable and reproducible methods than those available with *in vivo* techniques. However, no correlation has been noted between hyposensitization therapy, either repository or aqueous, and any measurable parameter.^{10, 11, 22} Several studies, including this one, have consistently shown a lack of correlation between *in vitro* titrations and therapeutic results. Similarly, there has been no correlation between dose of pollen administered and *in vitro* titers. There has also been no correlation between titers obtained by various methods, such as skin sensitizing titrations, blocking antibody assays, and hemagglutination procedures.^{10, 11, 27} Thus, it seems that, although *in vitro* techniques do indicate the antigenic stimulus in patients with pollinosis, there is little reason to suspect that these titers, obtained with techniques utilizing undefined and complex antigens, reflect anything more than another manifestation of immunologic responses to pollen extracts, without any demonstrable significance at the present time.

SUMMARY AND CONCLUSIONS

1. Repository injection of ragweed extract in emulsion form has resulted in demonstrable increases in hemagglutination titers in a majority of patients with pollinosis as measured by the bis-diazotized benzidine method.
2. These responses are comparable to those observed with patients treated with conventional multiple injections.
3. Complement fixation and precipitin tests did not detect the immune response.
4. Increases in the amount of titratable blocking antibodies following therapy were comparable between repository groups and those treated with multiple injections.
5. No consistent change in skin sensitizing antibody was observed.
6. A change in conjunctival threshold sensitivity to ragweed extract was observed, whereas no delayed hypersensitivity reactions were detected in emulsion-treated patients.
7. No correlation could be made between changes in antibody titers and degree of clinical relief.

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