

IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS

II. Effect of Specific Hyposensitization Therapy on Hemagglutinating Antibody Titers

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IN A previous article¹ we studied the specificity and sensitivity of the hemagglutination test as carried out by the tanned-cell and the bis-diazotized-benzidine techniques. The effect of specific hyposensitization with ragweed pollen extracts on the hemagglutinating titers of sera from patients suffering from ragweed hay fever after treatment but before the onset of the ragweed pollen season and after the ragweed pollen season will be presented here. Sera were taken prior to the specific therapy (pretreatment). The effect of the inhalation of ragweed pollen during the hay fever season, the amount of treatment (amount of pollen extract injected), and/or the number of injections on these hemagglutination titers will also be reported.

Feinberg and colleagues² demonstrated that there was a higher hemagglutination titer following hyposensitization therapy (by the tanned-cell technique). Gordon and associates,³ using the bis-diazotized-benzidine (BDB) technique, likewise showed an increase in titer in four patients from whom they had both pretreatment and posttreatment blood samples. They and other investigators⁴⁻⁶ showed that serum from treated patients had higher titers than those from patients who were not given specific hyposensitization therapy. We extended this work by comparing both techniques in a larger number of patients and observing the titer changes in the same patient.

METHODS AND MATERIALS

Sera were collected, with sterile precautions, from 113 patients with ragweed hay fever who had not had any specific hyposensitization therapy for at least four months prior to the initial bleeding. These sera were stored in the freezer until ready for testing. Of these 113 patients, only sixty-nine were available for bleeding prior to the onset of the ragweed pollen season (post-treatment I). Forty-five of these patients also were bled and sera were collected

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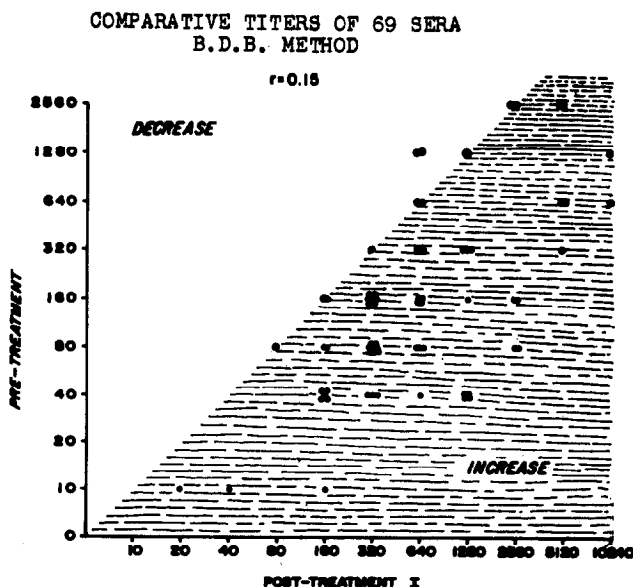
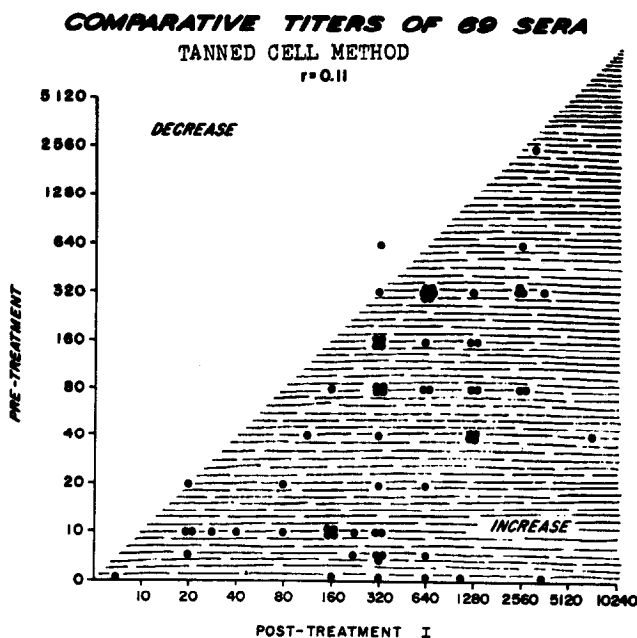
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after the ragweed pollen season. Preseason (posttreatment I) and postseason (posttreatment II) sera were obtained from an additional eight ragweed-sensitive patients who received no specific hyposensitization therapy. These "controls" were used to determine whether the inhalation of pollens had any effect on their hemagglutinating titers.



Figs. 1 and 2.—Numerals indicate the reciprocal of the highest dilution of serum which produces a positive hemagglutination reaction. All points in the shaded areas indicate an increase in titer following specific hyposensitization therapy.

The tanned-cell and BDB hemagglutination techniques were employed in these studies as described in our previous article.¹

Hemagglutination titers of the various samples of sera from a particular patient were measured at the same time. These tests were done at least in duplicate by both methods.

RESULTS

Comparison of Pretreatment Sera and Posttreatment I (Preseason) Sera.—

Figs. 1 and 2 compare the titers of sixty-nine sera taken prior to injection treatment with ragweed pollen extract and after the patients had received injection treatment but prior to the onset of the ragweed pollen season. As can be seen from both of these charts, both the tanned-cell and the BDB methods revealed an increased titer in the sera following injection treatments. The amount of increase varies considerably from patient to patient, and there is no apparent relationship between the titer of antibody originally present and the increase following treatment.

Table I shows the extent of change in titer by the tanned-cell and BDB techniques. With the former method, sixty-four of the sixty-nine patients showed at least a twofold increase in titer, the sera of four patients were the same, and

TABLE I. COMPARATIVE TITERS OF SIXTY-NINE SERA PRETREATMENT AND POSTTREATMENT I

TITER CHANGE	TANNED-CELL METHOD		BDB METHOD	
	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	64	92.7	58	84
Fourfold	47	68.4	43	62.3
Eightfold	38	55	22	31.8
Same	4	5.8	9	13.0
Decrease	1	1.4	2	2.9

in one patient the titer was decreased. Forty-seven patients demonstrated at least a fourfold increase in titer, and thirty-eight showed at least an eightfold increase. By the BDB method fifty-eight of the sixty-nine patients showed at least a twofold increase and forty-three showed a fourfold increase, whereas only twenty-two showed an eightfold increase.

Even though the absolute titers as determined by each method differ greatly, the relative degree of increase in the serum titers measured by the two methods before and after treatment appears to be similar. A comparison of the relative increase or decrease in titers determined by the BDB and tanned-cell techniques is given in Fig. 3. These results are summarized in Table II.

TABLE II. COMPARATIVE TITERS OF PRETREATMENT AND POSTTREATMENT I* SERA BY BDB AND TANNED-CELL METHODS

BDB AND TANNED-CELL TITERS INCREASED	TANNED-CELL TITERS INCREASED, BDB TITERS SAME OR DECREASED	BDB TITERS INCREASED, TANNED-CELL TITERS SAME OR DECREASED	BOTH TANNED-CELL AND BDB TITERS DECREASED
54	10	4	1

*Sera taken after course of preseasonal hyposensitization therapy but prior to the ragweed pollen season.

Comparison of Titers of Pretreatment Sera and Posttreatment II Sera.—There were fifty-three paired sera for study by both techniques. As can be seen from Table III, the increase in titer from pretreatment to posttreatment II (pre-season) was essentially the same as that seen in a comparison of pretreatment titer to pre-season sera titer (posttreatment I) (Table I). Hence, we can assume that there was little or no effect on the titer during the pollen season, even though additional treatment was given. These data are borne out in the next series of experiments and hold true for both the BDB and the tanned-cell techniques.

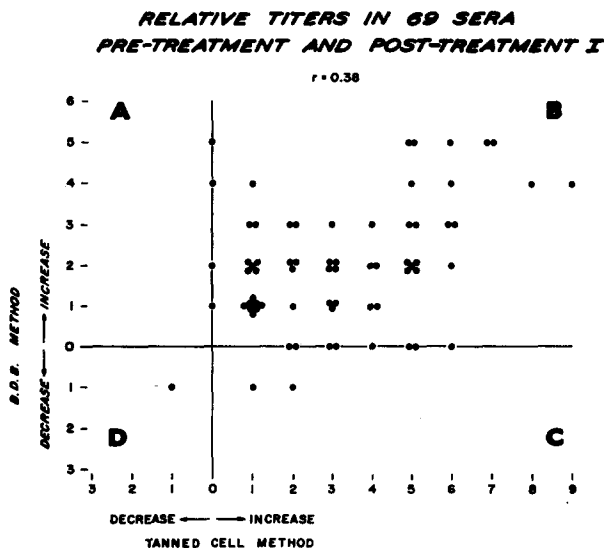


Fig. 3.—A indicates an increase in titer by the BDB method and a decrease by the tanned-cell method. B shows an increase in titers by both the BDB and the tanned-cell methods. C indicates an increase by the tanned-cell method and a decrease by the BDB method. D indicates a decrease by both the tanned-cell and the BDB methods. *Horizontal axis:* No change in titer by the BDB method. *Vertical axis:* No change in titer by the tanned-cell method. Each number represents a twofold change. r = Correlation coefficient.

TABLE III. COMPARATIVE TITERS OF FIFTY-THREE SERA PRETREATMENT AND POSTTREATMENT II

TITER CHANGE	TANNED-CELL METHOD		BDB METHOD	
	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	44	83	41	77.5
Fourfold	34	64.2	30	57
Eightfold	26	49	17	32
Same	7	13.2	5	9.4
Decrease	2	3.8	7	13.2

Comparative Titers of Posttreatment I Sera and Posttreatment II Sera.—The effects of inhalation of ragweed pollen during the pollen season plus additional injection therapy on the hemagglutination titers are illustrated in Figs. 4 and 5. As can be seen from these charts, most of these titers remained relatively the same, and there were just as many that showed an increase as there were that showed a decrease during this time interval (Table IV). The data are further borne out by the fact that of the eight untreated ragweed-sensitive patients, there was no change in titer in two, a decrease in titer in one, and an

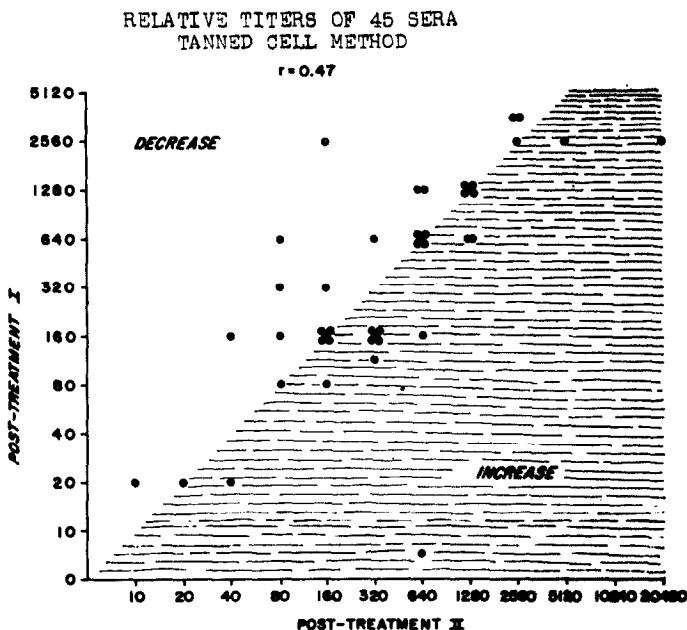


Fig. 4.

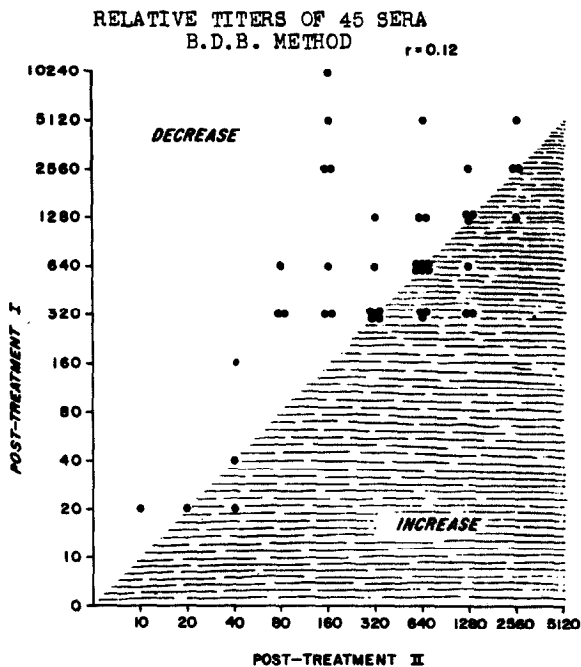


Fig. 5.

Figs. 4 and 5.—*Posttreatment I*: The reciprocal of titers obtained from sera after pre-seasonal specific hyposensitization therapy prior to the onset of the ragweed pollen season. *Posttreatment II*: The reciprocal of titers obtained from sera after pre-seasonal specific hyposensitization therapy, additional therapy during the pollen season, and exposure to the inhalation of pollen during the season.

Figures in shaded area show an increase in titer; those above indicate a decrease; and those on the line indicate titers that remain unchanged.

TABLE IV. COMPARATIVE TITERS OF FORTY-FIVE SERA POSTTREATMENT I AND POSTTREATMENT II

TITER CHANGE	TANNED-CELL METHOD		BDB METHOD	
	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	15	33.3	11	24.4
Fourfold	4	8.9	4	8.9
Eightfold	2	4.5	0	—
Same	21	47	18	40
Decrease	9	20	16	35.6

increase in titer in five by the tanned-cell method, whereas by the BDB method there was no change in titer in three, a decrease in titer in three, and an increase in titer in two (Table V).

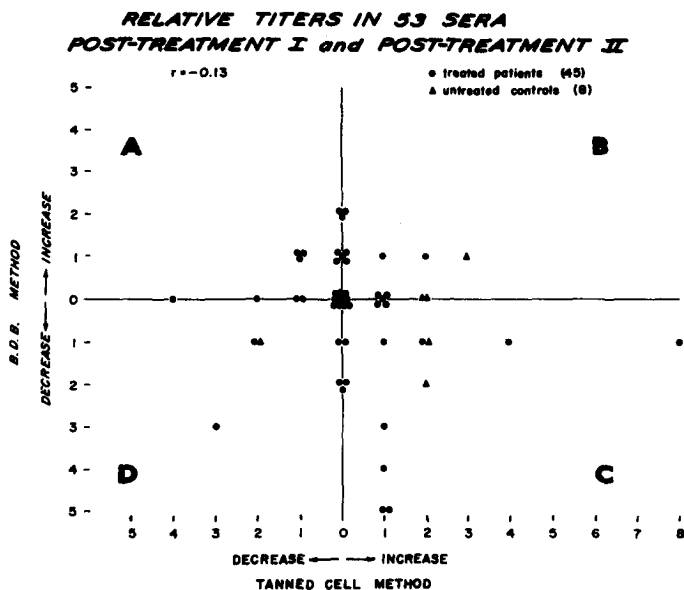


Fig. 6.—*A* indicates a relative increase in BDB titer but a decrease in tanned-cell titer. *B* indicates a relative increase in both BDB and tanned-cell titers. *C* shows a relative increase in tanned-cell titer and a decrease in BDB titer. *D* indicates a decrease in both BDB and tanned-cell titers.

Posttreatment I: Sera obtained after preseasonal specific hyposensitization therapy but prior to the ragweed pollen season. *Posttreatment II:* Sera obtained after preseasonal specific therapy, additional therapy during the pollen season, and inhalation of pollen during the ragweed season.

Horizontal axis: No change in BDB titer. *Vertical axis:* No change in tanned-cell titer.

TABLE V. COMPARATIVE HEMAGGLUTINATION TITERS OF EIGHT SERA (CONTROLS),* PRESEASON TO POSTSEASON

TITER CHANGE	TANNED-CELL METHOD	BDB METHOD
Increase	5	2
Same	2	3
Decrease	1	3

*Untreated ragweed-sensitive patients.

The relative change in titers of sera collected prior to and after the ragweed pollen season (comparison of posttreatment I and posttreatment II) is illustrated in Fig. 6. These results include forty-five treated patients and eight

untreated control patients. Once again, we note that there is no correlation between the two techniques (BDB and tanned-cell), with either the controls or the treated patients, and there was no appreciable change in titers. These results indicate that neither the inhalation of ragweed pollen during the hay fever season nor the additional therapy given during this season, once the patients had already reached their maximum dosage prior to the season, had any effect on the hemagglutination titer.

Effect of Amount of Treatment and Number of Injections on Hemagglutination Titers.—The total amount of extract which each patient received from the start of treatment was recorded. This included all material injected from the pretreatment bleeding until the time the posttreatment I (preseason) specimen was obtained. As can be seen from Table VI, we found no correlation between the amount of treatment that the patient received and the change in hemagglutination titers by either the BDB or the tanned-cell method. These data were carefully evaluated statistically.

TABLE VI. EFFECT OF AMOUNT OF PRESEASONAL SPECIFIC THERAPY ON HEMAGGLUTINATION TITERS

TOTAL AMOUNT OF EXTRACT GIVEN*	NUMBER OF PATIENTS	HEMAGGLUTINATION TITER CHANGE					
		BDB METHOD			TANNED-CELL METHOD		
		INCREASE	SAME	DECREASE	INCREASE	SAME	DECREASE
25	2	1	1		2		
25 to 2500	23	20	1	2	21	2	
2500 to 10,000	19	16	3		17	2	
10,000 to 25,000	12	10	2		11	1	
25,000	13	12	1		13		

*Total amount of short ragweed pollen extract expressed in protein nitrogen units (P.T.A./n) given from pretreatment bleeding time to posttreatment I (preseasonal) specimen was collected.

Similar studies comparing the amount of treatment received during the pollen season and the posttreatment I and II hemagglutination titers likewise showed no correlation. Also, no relationship between the total amount of pre-seasonal and seasonal treatment and the preseason and posttreatment II titers was revealed by either method.

Sixty-nine patients were followed with pretreatment and posttreatment I sera. They received anywhere from a minimum of four injections to a maximum of thirty-two. Here again, there was no correlation between the number of injections of ragweed pollen extract and the change in hemagglutination titers as analyzed by our statistician.

DISCUSSION

In all but four of the sixty-nine patients studied, there was some increase in tanned-cell titer following injection treatments with pollen extracts. Inasmuch as the titers of the various samples of a particular patient's serum were determined at the same time, it would seem that even a twofold difference would be important. As with all serologic tests, however, if one were to consider only a fourfold or more difference as significant, there still is a high percentage (66 per cent) of increases in titer following treatment. If one were even more

critical and considered an eightfold or more increase in titer as significant, then over 50 per cent of the patients had such an increase by the tanned-cell technique.

Comparison of sera collected prior to treatment and at the end of treatment after the season had been completed showed increases similar to those of the pretreatment to the posttreatment I sera. This would indicate that the inhalation of ragweed pollen during the hay fever season and additional treatment which had been given, once the maintenance dose had been reached, did not significantly alter the hemagglutination titers. This fact is borne out very well when one compares the sera of the posttreatment I (preseason) patients to the sera of the posttreatment II (postseason) group of patients. For the most part, there was no significant change in these titers. Of those that did change, the number that increased was essentially the same as the number that showed a decrease in titers by either of these techniques. Likewise, among the eight patients who were ragweed sensitive and did not receive any injection treatments, apparently there was no significant change in the hemagglutination titers during their exposure to ragweed pollen inhalation. As a matter of fact, by the BDB method, in several instances there was a considerable decrease in the hemagglutination titer during the ragweed pollen season. This possibly could be due to a neutralization of antibodies produced by the inhalation of the ragweed antigen.

There was likewise no relationship between the amount of antibody present prior to treatment and the relative increase following the injection therapy. Apparently, there is a maximum increase, as evidenced by the fact that additional treatments during the pollen season did not materially affect an increased hemagglutination titer.

All of these data were subjected to analysis by statisticians. The only definite correlation which we could find with these various comparisons was in the experiments comparing the relative change in titers by both the BDB and the tanned-cell techniques. Here the correlation coefficient was 0.38. This would indicate that the amount of increase revealed by both methods is correlated.

Although we could find no correlation between the two techniques, the results indicated that there is an increase in hemagglutination titers after specific injection therapy and that the inhalation of ragweed pollen does not materially affect this change in hemagglutination titer.

The relationship of the increase in the hemagglutinating antibody titer to the changes in reagin titer, as well as results of therapy, will be the subject of our next report. The question naturally arises: "What are we measuring? Are we measuring the reagin content of the sera, the blocking antibody, or some other antibody or antibodies increasing as a result of injection therapy?"

SUMMARY AND CONCLUSIONS

Sera from ragweed-sensitive patients were obtained prior to and after injection treatments with specific ragweed pollen antigen. The hemagglutination

titers by the BDB and tanned-cell methods were determined, and the following conclusions can be reached:

1. There is an increase in hemagglutination titer following specific hypo-sensitization therapy.
2. There is no observable effect on the hemagglutination titer by the inhalation of ragweed pollen during the hay fever season.
3. The total amount of treatment and the number of injections of ragweed pollen extract had no direct effect on the change in hemagglutination titer.

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