

IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS

III. Clinical Aspects: The Relationship of Reagin and Hemagglutinating Antibody Titers to Results of Hyposensitization Therapy

Carl E. Arbesman, M.D., S. Zwi Kantor, M.D., Doris Rapp, M.D.,** and Noel R. Rose, Ph.D.,
Buffalo, N. Y.*

THE first two articles of this series¹⁻² demonstrated that about 90 per cent of the sera from ragweed-sensitive patients had positive hemagglutination tests against ragweed and that in at least two-thirds of the patients the titer of this antibody increased fourfold or more following specific hyposensitization therapy. It was also shown that there was no relationship between the age of the patient, duration of previous symptoms, amount of treatment given, or number of injections and the increase in hemagglutination titer.

The purpose of this article is to demonstrate the relationship of the change in hemagglutination titer to the change in titer of skin-sensitizing antibody (reagin) and the degree of relief from specific hyposensitization therapy. In addition, the effect of the amount of pollen extract injected and the number of injections on the change in reagin titer and the degree of relief obtained will be reported.

Gordon and associates³ have shown that there is a close relationship between the hemagglutinating and reagin titers. Others⁴ have reported that there is an initial rise in reagin titer following specific hyposensitization therapy and then a return to the same or lower levels with continued treatment. There has been no evidence of actual desensitization (disappearance of reagin) following intensive specific hyposensitization pollen therapy, and no relationship has been demonstrated between the change in reagin titer and the results of specific therapy.⁵

METHODS AND MATERIALS

The hemagglutination titers were determined by both the tanned-cell and the bis-diazotized benzidine methods previously described.¹

Reagin Titers.—At least two, and sometimes three, samples of serum from each of twenty-seven patients were studied. Serial twofold dilutions in normal

From the Allergy Research Laboratory, Buffalo General Hospital, Departments of Medicine and of Bacteriology and Immunology, University of Buffalo School of Medicine.

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*Research Fellow in Allergy, now in Tel Aviv, Israel.

**Research Fellow in Allergy.

saline of the pretreatment and posttreatment sera were injected into each of two nonallergic recipients (skin-test negative to ragweed). These sites were marked, and twenty-four to forty-eight hours later each site was tested with a constant amount of ragweed pollen extract (1,000 U. PTAN strength). Fifteen minutes after the challenging injections, the degree of wheal and erythema for each site was recorded. The highest dilution of serum that gave a definite positive reaction was called the reagin titer.

Results of Treatment.—Treatment results were arbitrarily classified as excellent, good, fair, or poor on the basis of the patient's subjective evaluation and the investigators' objective findings. Determining factors included physical examination of the nasal mucosa, the number of complaints, and the amount of symptomatic medication taken.

RESULTS

*Comparison of Reagin and Hemagglutination Titers of Twenty-seven Pretreatment Sera.**—The titers of both the hemagglutinating and the passive transfer (reagin) antibodies are graphically shown in Figs. 1 and 2. Fig. 1 demonstrates the lack of correlation between the reagin titer and the hemagglutination titer as determined by the tanned-cell method. It can be seen that six of the twenty-seven sera tested had no demonstrable hemagglutination titer but did have reagin titers. Moreover, one serum had a high hemagglutination titer but a relatively low reagin titer (320 to 8, respectively). Contrawise, three sera had reagin titers of 512 or greater and hemagglutination titers of 1 to 10 or less.

Fig. 2 shows the same lack of correlation between reagin titer and hemagglutination titer as determined by the bis-diazotized-benzidine (BDB) method.

Comparison of Relative Change in Reagin and Hemagglutination Titers Following Specific Hyposensitization Therapy.—The actual change in titers by twofold dilutions is shown in Figs. 3 and 4. The reagin titer remained unchanged in fifteen of the twenty-seven sera tested, it was decreased in one, and it was increased in eleven. As will be noted, however, sera from all but one of the patients showed an increase in hemagglutination titer by the tanned-cell method. Similar results were obtained when the BDB method of hemagglutination was used (Fig. 4). Two sera showed a decrease in BDB hemagglutination titer. In one there was no change in reagin titer, whereas the other showed a fourfold increase. These results are summarized in Table I.

TABLE I. COMPARISON OF RELATIVE CHANGE IN HEMAGGLUTINATION AND REAGIN TITERS FOLLOWING SPECIFIC HYPOSENSITIZATION THERAPY

TECHNIQUE	NO. OF SERA WITH INCREASED HEMAGGLUTINATION TITER	CHANGE IN REAGIN TITER		
		INCREASE	SAME	DECREASE
Bis-diazotized-benzidine	23	8	14	1
Tanned-cell	26	11	14	1

*These sera were obtained from five patients who never received any specific hyposensitization therapy with ragweed pollen extract and twenty-two patients who had undergone treatment in previous years but had received no treatment for at least one year.

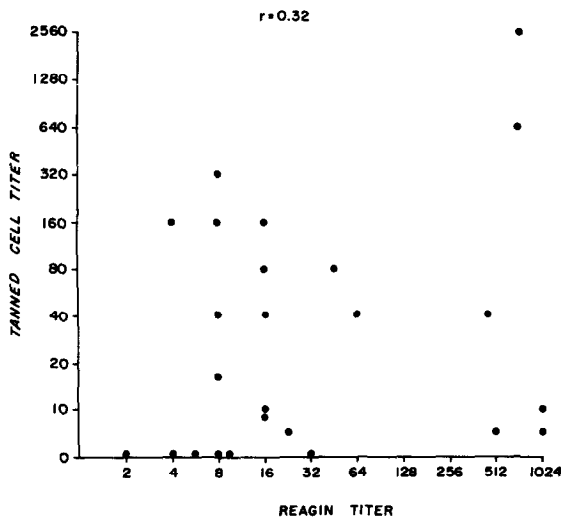
**ABSOLUTE TITERS IN 27 PRE-TREATMENT
SERA**

Fig. 1.—Black dots represent the tanned-cell titer and the reagin titer of each patient.

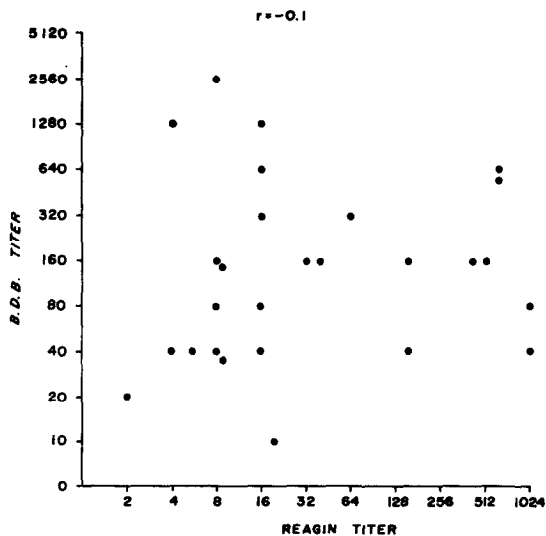
**ABSOLUTE TITERS IN 27 PRE-TREATMENT
SERA**

Fig. 2.—Black dots represent the BDB titer and the reagin titer of each patient.

**RELATIVE TITERS IN 27 SERA
PRE-TREATMENT TO POST-TREATMENT II**

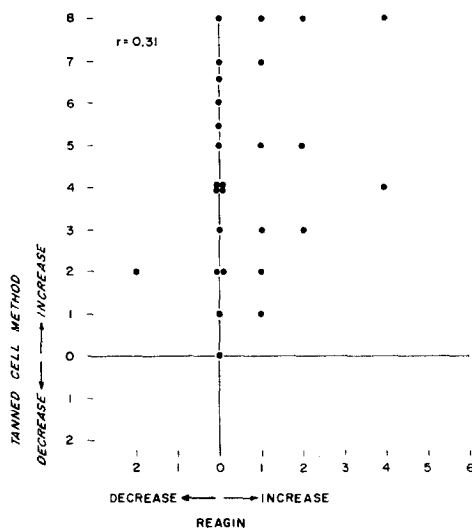


Fig. 3.—Comparison of the relative increase or decrease in tanned-cell titer with the change in reagin titer from the beginning to the end of the observation period. The numbers indicate the number of twofold dilutions increased or decreased.

**RELATIVE TITERS IN 27 SERA
PRE-TREATMENT TO POST-TREATMENT II**

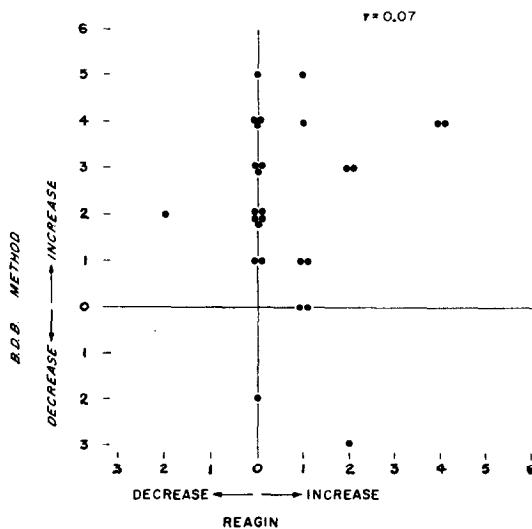


Fig. 4.—Same comparison as shown in Fig. 3, but based on the BDB instead of the tanned-cell test.

Changes in Reagin Titer During Course of Hyposensitization Therapy.—Three samples of sera from ten of the twenty-seven patients were studied for their skin-sensitizing activity. All three samples of four of these sera showed no change in reagin titer. There was a decrease in titer in one patient's post-treatment I serum,* but then a marked increase in titer over the pretreatment serum was seen in the posttreatment II serum.† There was a steady increase in the reagin titer with each successive sample during hyposensitization therapy in two patients. In the remaining three patients the reagin titer was unchanged between the first and second samples of sera, but there was an increase in titer with continued therapy in the third (posttreatment) sample.

Effect of Amount of Treatment and Number of Injections on Change in Reagin Titer.—There appeared to be no relationship between the total amount of pollen extract given a patient and the change in reagin titer. In some patients who received relatively large dosages of pollen extract there was no change in reagin titer, whereas in others who received much smaller dosages there was an increase in titer. Likewise, the total number of injections of pollen extract had no relationship to the change in reaginic titer. The titer changes, the total amount of treatment, and the number of injections are shown in Figs. 5 and 6. One can readily see that in some cases the reagin titer did not change, even when as much as 180,000 protein nitrogen units of ragweed extract was given, whereas the patient with a twelvefold increase in reagin titer received a total of only 10,000 protein nitrogen units. As to the number of injections of extract influencing the reagin titer, Fig. 6 shows that of three patients receiving twenty-four injections, one patient had no change in reagin titer, another had a twofold increase, and the third had a twelvefold increase.

Effect of Amount of Treatment and Number of injections on Results of Hyposensitization Therapy.—Seventy-four patients received specific hyposensitization therapy with ragweed pollen extract. Of this group, eighteen had excellent relief and thirty-five had satisfactory results. Those who had good or excellent results received much larger amounts of pollen extract than those who gave only a fair or poor response to treatment (Table II).

TABLE II. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	AVERAGE TOTAL AMOUNT OF EXTRACT*
Excellent	18	38,100
Good	35	34,200
Fair	8	13,500
Poor	13	7,200
Total	74	

*Expressed in PTA nitrogen units.

There is no apparent correlation between the results of treatment and the number of injections used for specific hyposensitization (Table III). However,

*Posttreatment serum but previous to ragweed season.

†Posttreatment serum but following ragweed season.

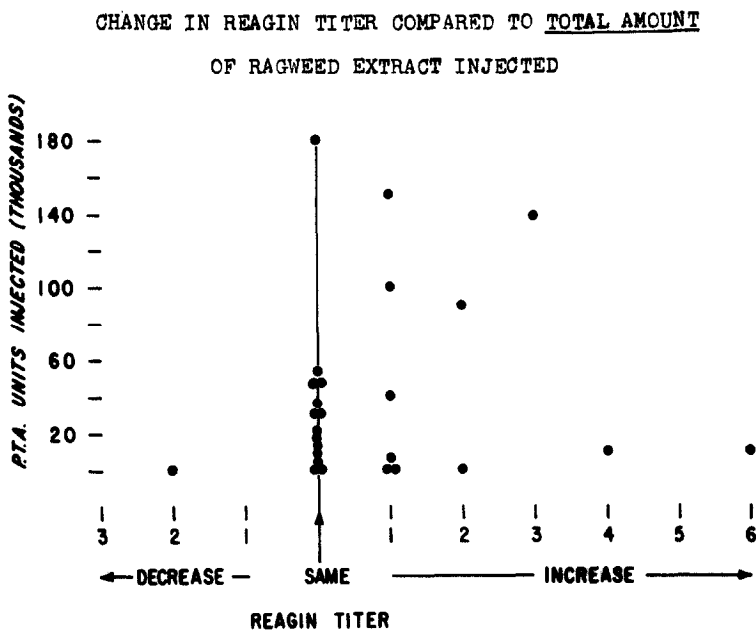


Fig. 5.

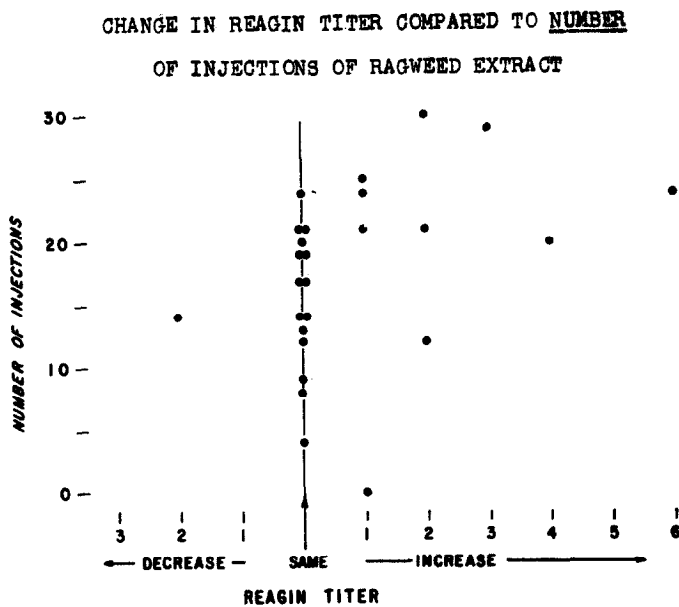


Fig. 6.

Figs. 5 and 6.—The change of reagin titer is expressed by the number of twofold dilutions increased or decreased during the observation period.

those patients who had the best results of treatment received, on the average, more injections than any other group.

TABLE III. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	AVERAGE NUMBER OF INJECTIONS
Excellent	17	20.4
Good	31	14.2
Fair	8	17.8
Poor	12	15.1
Total	68	

Response to Specific Hyposensitization Therapy and Change in Hemagglutination Titer.—Response to therapy was excellent in seventeen of the sixty-nine patients studied and good in thirty-two. The mean change in hemagglutination titer (increase), determined by both the BDB and tanned-cell methods, following pollen injection therapy is shown in Table IV. Although it appears that those patients with a satisfactory response to therapy (good or excellent) had a greater mean change in titer than those with a poor response, the difference is not statistically significant.

TABLE IV. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	MEAN CHANGE IN TITER	
		BDB	TANNED-CELL
Excellent	17	2.3	2.64
Good	32	2.0	3.6
Fair	8	2.0	2.0
Poor	12	1.4	2.4

DISCUSSION

Although an increase in the hemagglutination antibody following specific hyposensitization has been observed, its significance is as yet unknown. We found no relationship between the results of therapy and changes in these hemagglutination titers. This may or may not indicate that we are measuring some type of blocking or protective antibody, as others^{6, 7} have shown that there is not always a correlation between the blocking antibody titer and the clinical response. Experiments are now in progress to compare the so-called blocking antibody titer to the hemagglutinating antibody titer.

In our experiments, we found no correlation in either the actual reagin and hemagglutination titer or the relative changes in each following treatment. This would indicate that we are not simply measuring the skin-sensitizing antibody by these techniques. In addition to this fact, all the sera tested for hemagglutination were inactivated and titers were not affected, whereas it is well known that reagin is inactivated when heated to 56 degrees for one hour.

In only one patient did we note an initial fall in reagin titer followed by a rise on continued therapy. This does not confirm the work reported by Sherman.⁴

Of the twenty-seven groups of sera tested, only eleven showed an increase in reagin titer, whereas fifteen remained unchanged and one demonstrated a decrease. One might argue that this method of determining reagin is not very reproducible. By using two recipients for each set of sera, however, it is possible to meet some of those objections.

Neither the amount of pollen extract given nor the total number of injections had any effect on the change in reagin titer. This same lack of relationship to the changes in the hemagglutinating antibody has already been reported² in a previous publication. It appears, however, that those patients who received or could tolerate the larger amounts of pollen extracts had a better therapeutic response.

The results of our three articles and the reports of others clearly show that the sera of most patients suffering from ragweed hay fever contain antibodies against ragweed which are demonstrable by indirect hemagglutination methods. In general, experience indicates that these tests measure precipitating antibody but are at least 1,000 times more sensitive than ordinary precipitin tests. We must consider the additional possibility that nonprecipitating or "incomplete" antibody may also play a role in the hemagglutination reactions, although the evidence for this is not entirely clear.

Evaluation of the significance of this hemagglutination antibody (or these antibodies) is rather difficult at this time. At first observation, one may think that the BDB reaction measures only a nonspecific serum protein that has agglutinating properties. Such a protein may indeed account for some of the agglutination observed by this method, at least in low dilutions of all human sera. On the other hand, we recognize that the reaction is quite specific for ragweed and does not even extend to the serologically related antigen, June grass. Furthermore, the titer of hemagglutination increases significantly during injection of ragweed extract. For this reason, we regard the BDB reaction as due, at least partly, to an antibody against ragweed. This antibody is present in both normal and allergic persons, conceivably due to exposure to ragweed or to a related antigen in the environment. The antibodies are generally stronger in persons allergic to ragweed and occur in a greater percentage of this group than in the general population.

The tanned-cell hemagglutination test also appears to measure a specific antibody to ragweed. This antibody is found predominantly in persons allergic to ragweed. It is not impossible that the approximately 15 per cent of "normal" persons showing reactions by this method have some clinical or subclinical allergy to ragweed. Thus, it would appear that this antibody is rather characteristic of ragweed-sensitive persons. The reason for this correlation is not known; certainly, the hemagglutinating antibodies themselves do not directly produce the symptoms of the disease, since they are not related to the severity or duration of symptoms. Perhaps their presence in the sera of allergic persons signifies a greater immunologic responsiveness of ragweed-sensitive patients to this antigen, embracing precipitating as well as skin-sensitizing varieties of antibodies.

Both forms of the hemagglutination test agree in revealing significant increases in antibody titer following hyposensitization. Essentially, this finding indicates that ragweed is antigenic in man, as it is in rabbits. In direct contrast, reagins are not consistently affected by these treatments, and not infrequently they actually decrease during hyposensitization. This finding, together with the difference in heat stability of the two antibody functions as previously described, leads us to believe that reagin does not play any significant role in the hemagglutination reactions. It is impossible, as Gordon and colleagues³ have pointed out, to exclude its participation as one of the antibodies reacting in the hemagglutination tests, but there is certainly no positive evidence of its contribution to the reaction. On the contrary, some patients with skin-sensitizing antibody had no demonstrable hemagglutination reaction. We could also find no direct relationship between the benefits of treatment and the increase in hemagglutination titer. If one chooses to attribute the effects of hyposensitization to the production of "blocking antibodies," it must again be supposed that the blocking antibody may represent only one of several antibodies producing hemagglutination. Until purified fractions of ragweed are available for this sort of testing, overemphasis on the lack of correlation will be premature.

SUMMARY AND CONCLUSIONS

1. There was no relationship between either the absolute titer or the change in hemagglutination and reagin titers following specific hyposensitization therapy.
2. There was no relationship in the clinical response to either the change in hemagglutination or reagin titers or the number of injections of pollen extract.
3. The best response to hyposensitization therapy was seen in those patients who received the largest total amount of pollen extract.

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