

IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS TREATED BY A SINGLE REPOSITORY ANTIGEN INJECTION

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THE treatment of allergic rhinitis and asthma due to pollens by the injection of allergens emulsified in oil has been attempted in the past on several occasions. However, the results were not very encouraging.^{1, 2} It is only recently^{3, 4, 5} that practical and useful methods of treatment, based on the emulsion principle, have been devised. Although this mode of treatment is now widely used, several pertinent questions remain unanswered, such as the utilization of objective methods which might demonstrate the efficiency or superiority of such treatment over the conventional method.⁶ This paper is primarily concerned with immunologic studies (SSA† titers, blocking antibody titers, hemagglutinin titers) on the sera of 35 ragweed-sensitive patients who were all treated by a single repository ragweed injection in June, 1960, at the Royal Victoria Hospital Allergy Clinic.

METHODS AND MATERIALS

1. *Selection of Patients.*—Only patients who had clear-cut histories of ragweed sensitivity with positive skin tests to ragweed were included in this study. Most of these patients had been treated in 1959 by the conventional method (multiple injections of aqueous solutions of WSR‡) but all immunization injections had been terminated by August, 1959.

2. *Repository Treatment.*—The material used for preparing the emulsions was provided by the American Academy of Allergy as part of a large scale survey on this form of therapy. The mixture contains, in its oil phase, 9 parts of Drakeol 6 VR white mineral oil to 1 part of specially treated Arlacel A. The aqueous phase contains equal amounts of the water soluble extracts of defatted short and long ragweed pollen. Merthiolate (1:10,000) was incorporated as a

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†SSA = Skin sensitizing antibody referred to generally as reagin.

‡WSR = Water soluble extract of ragweed pollen.

preservative. Two parts of the oil phase were used for each part of the aqueous phase. The emulsion was prepared by hand, using two Luer-Lock syringes with a double hub needle. The dosage used in each case was governed by the clinical sensitivity of the patient and by the titration of his skin reactivity with varying concentrations of a saline solution of WSR. Details of the method used in this study are outlined in a recent article by Feinberg and associates.⁷ All injections were given by the intramuscular route in the latter part of June and in the early part of July. In those cases in which a local or a systemic reaction was observed following the repository injection, the reactions were graded as follows: local reactions at the site of injection were graded from 1+ to 4+, according to the degree of erythema, edema, and amount of discomfort present. Systemic reactions were also graded from 1+ to 4+. Mild symptoms of allergic rhinitis or generalized pruritus after the injection were classified as a 1+ reaction. Reactions characterized by either generalized urticaria and/or angioedema and/or asthma and/or severe gastrointestinal disturbances were classified as 2+, 3+, or 4+, according to the severity of those symptoms. After the ragweed season, the degree of subjective clinical improvement obtained by each patient was recorded as either complete, marked, moderate, fair, or none.

3. *Immunologic Studies.*—Blood was drawn from the patient before the repository injection was administered and 2½ months later when the ragweed season had ended. The blood was allowed to clot at room temperature for a few hours and then in the cold room at 4° C. overnight. The next day the serum, obtained by centrifugation, was frozen and stored in a deep freeze. Wassermann tests were performed on each post-treatment serum and a careful inquiry as to the possibility of recent or past history of hepatitis was done in each case. Pre- and post-treatment sera were always analyzed simultaneously. In each determination the end point titer is expressed as the reciprocal of the highest dilution of serum still giving a definite positive reaction. All ragweed or serum solutions used for passive transfer tests were made up in saline (0.9 per cent NaCl).

A. *Hemagglutinin titer determination:* The BDB (bis-diazotized benzidine) technique, as described by Gordon, Rose, and Schon,⁸ was used with the following modification: Neither the normal rabbit serum (used in the preparation of the "diluent" at a concentration of 1:100 in phosphate buffer) nor the human serum to be tested was de complemented. The human serum was absorbed by the addition of an equal volume of packed rabbit erythrocytes and permitted to stand for 15 minutes at 4° C. The probability of obtaining hemolysis of the red cells because of the presence of complement precluded the use of room temperature for absorption. The red cells were then removed by centrifugation and discarded. The same amount of packed washed rabbit red cells was then added to the human serum again and absorption was allowed to proceed for another 15 minutes at room temperature. The red cells were then centrifuged off and discarded. With his modification of the absorption procedure we had no or only slight hemolysis, and nonspecific agglutination of the rabbit red cells by the human serum never occurred in the subsequent steps of the technique. We

also found that the slight hemolysis present in certain cases did not interfere with the normal course of the experiment. A single control serum of known titer was always run with each experiment.

B. Skin-sensitizing antibody (SSA) titer determination: The classical passive transfer method of Prausnitz and Kustner was used. On day 1, 0.05 c.c. of different concentrations of the human serum to be tested were injected into the skin of a recipient who was not sensitive to ragweed. The dilutions of the serum used were: 1:10, 1:25, 1:50, 1:125, 1:250, 1:500, 1:1000, 1:3000, and 1:6000. Twenty-four hours later, 0.05 c.c. of a saline solution of WSR containing 2,000 PNU* per cubic centimeter was injected into each site. The reaction was read after 20 minutes. The last definite positive reaction was recorded as the end point titer.

C. Blocking antibody titer determination: A "standard" serum obtained from a ragweed-sensitive subject was used as a constant source of reagin. By preliminary studies, using the passive transfer technique, it was found that the "reaginic activity" of 0.05 c.c. of a 1:100 dilution of this particular serum was completely neutralized by 0.05 c.c. of a saline solution of WSR containing 15 PNU per cubic centimeter. Each serum to be tested for blocking antibodies was heated at 56° C. for 1 to 2 hours in order to inactivate the SSA. The absence of skin-sensitizing activity after heating was demonstrated by passive transfer. It was found that some sera had to be heated for 2 hours to completely destroy the reaginic activity.

The blocking experiment was carried out in the following manner. On day 1, multiple sites on the skin of a suitable recipient were injected with 0.05 c.c. of a 1:100 dilution of the standard serum. On day 2, one cubic centimeter aliquots of serial dilutions (undiluted, 1:2.5, 1:5, 1:10, 1:15, 1:20, 1:30) of the unknown heated serum to be tested for blocking activity were incubated at room temperature for at least one hour with one cubic centimeter volumes of a solution of WSR in saline containing 30 PNU per cubic centimeter, so that the final dilutions of the serum would be 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, and 1:60, and the final concentration of WSR in the mixture would be 15 PNU per cubic centimeter. At this point, 0.05 c.c. of each of these mixtures was injected into each site. On day 3, each site was reinjected with 0.05 c.c. of a solution of WSR containing 2,000 PNU per cubic centimeter. The reactions were read after 20 minutes. The reciprocal of the dilution of the unknown serum in the site giving the last definite positive reaction was recorded as the blocking antibody titer.

RESULTS

1. Changes in Hemagglutinin Titer.—Twenty-two out of the 35 sera had an increase in hemagglutinin titer of fourfold or more. Eight of the sera exhibited a twofold increase in titer and in 5 the titers remained the same (Table I). No decrease in titer was observed. Neither the amount of ragweed given, the

*PNU = Protein nitrogen unit.

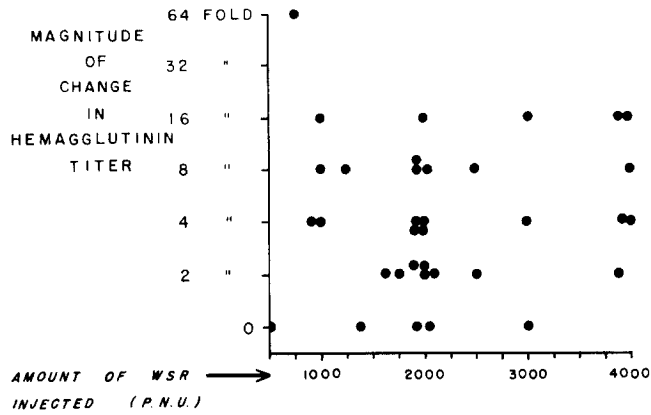


Fig. 1.—Relation between the amount of ragweed injected and the magnitude of change in hemagglutinin titer (35 cases).

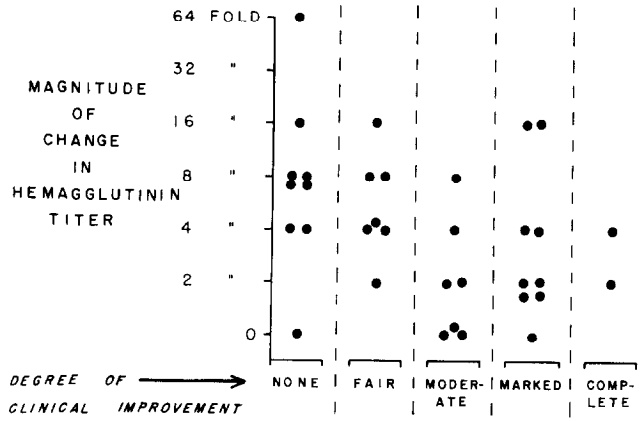


Fig. 2.—Relation between the degree of clinical improvement and the magnitude of change in hemagglutinin titer. (34 cases).

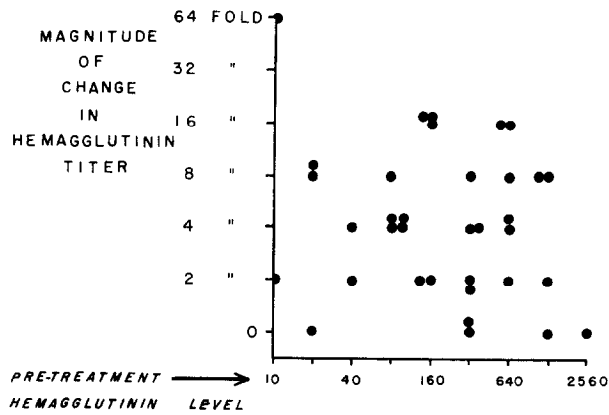


Fig. 3.—Relation between the magnitude of change in hemagglutinin titer and the pre-treatment hemagglutinin level (35 cases).

degree of subjective clinical improvement, nor the pre-treatment hemagglutinin level could be correlated with the magnitude of change in titer (Figs. 1, 2, and 3).

2. *Changes in Skin Sensitizing Antibody (SSA) Titer.*—SSA titers were determined in 31 cases, the remaining 4 patients having had a history of past or recent hepatitis. An increase in titer was considered significant only when there was more than a twofold difference between the pre- and post-treatment sera. Eight patients had an increase in SSA titer after repository therapy, which was accompanied in all by an increase in hemagglutinin titer. In 12

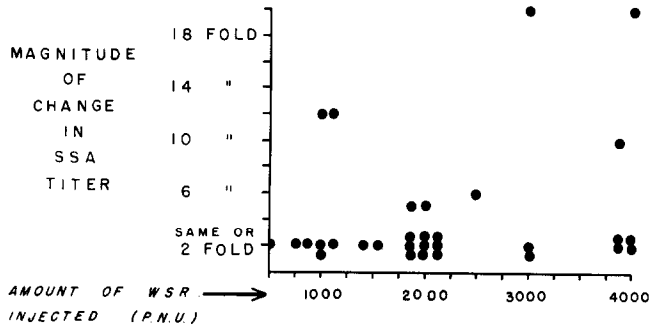


Fig. 4.—Relation between the amount of ragweed injected and the change in SSA titer (31 cases).

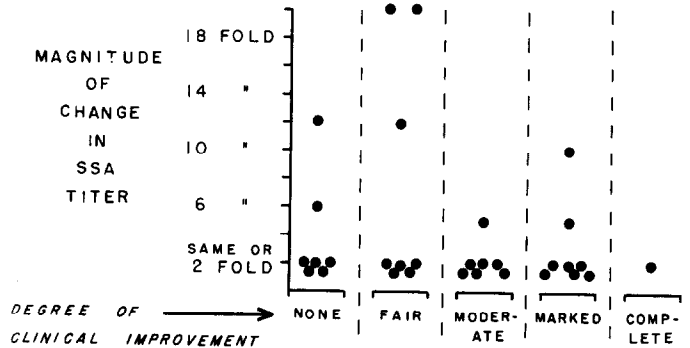


Fig. 5.—Relation between the degree of clinical improvement and the change in SSA titer (31 cases).

cases, however, in spite of a rise in hemagglutinin level, the SSA titer remained unchanged. In 11 sera, no variation in SSA titers was observed and there was either no change or a twofold increase in hemagglutinin titer. A twofold increase in hemagglutinin titer is not considered significant (Table II.) A decrease in SSA titer after treatment was not observed in any serum. No relationship could be established between the variations in SSA titer and the degree of subjective clinical improvement or the amount of ragweed injected (Figs. 4 and 5).

3. *Changes in Blocking Antibody Titer.*—Blocking antibody titer determinations were performed in 12 cases. Four typical samples were chosen from each group outlined in Table II. As can be seen in Table III, changes in

TABLE I. CHANGES IN HEMAGGLUTININ TITER AFTER REPOSITORY THERAPY (35 CASES)

MAGNITUDE OF CHANGE	NO. OF PATIENTS
0	5
2-fold	8
4-fold	9
8-fold	6
16-fold	6
32-fold	0
64-fold	1

TABLE II. VARIATIONS IN SSA AND HEMAGGLUTININ TITERS (31 CASES)

	NO. OF CASES
Group I Hemagglutinins increased—SSA increased	12
Group II Hemagglutinins increased—SSA no change	8
Group III Hemagglutinins no change—SSA no change	11

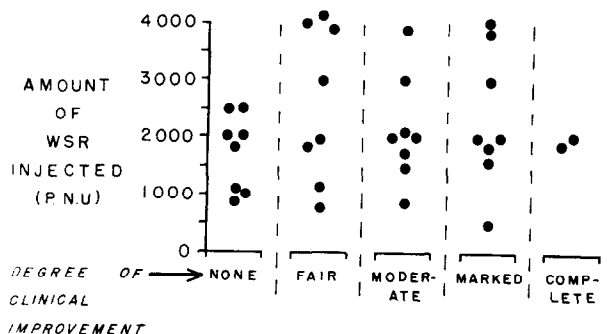


Fig. 6.—Relation between the degree of clinical improvement and the amount of ragweed injected (34 cases).

blocking antibody titers bear no relationship to either the variations in hemagglutinin levels, the changes in SSA titers, the amounts of ragweed given, or the degree of subjective clinical improvement.

TABLE III. BLOCKING ANTIBODY, HEMAGGLUTININ AND SSA TITERS, AMOUNT OF RAGWEED GIVEN, AND DEGREE OF CLINICAL IMPROVEMENT (12 CASES)

GROUP AND PATIENT	HEMAGGLUTININ TITER		SKIN-SENSITIZING ANTIBODY TITER		BLOCKING ANTI-BODY TITER		AMOUNT OF RAGWEED GIVEN (PNU)	DEGREE OF IMPROVEMENT
	PRE	POST	PRE	POST	PRE	POST		
Group I								
1	640	10,240	50	1,000	20	30	3,000	Fair
2	1,280	10,240	50	1,000	30	40	4,000	Fair
3	80	640	500	6,000	0	10	1,100	Fair
4	20	320	10	50	0	10	1,935	Marked
Group II								
5	640	10,240	1,000	1,000	5	20	3,870	Fair
6	1,280	10,240	500	1,000	0	0	1,935	None
7	40	160	50	25	10	30	3,000	Marked
8	80	320	1,000	1,000	0	20	1,935	None
Group III								
9	20	20	1,000	1,000	2	10	500	Marked
10	320	320	1,000	1,000	0	0	1,400	Moderate
11	2,560	2,560	500	1,000	0	0	1,935	Moderate
12	1,280	1,280	500	500	30	30	3,000	Moderate

4. *Relationship Between the Amount of Ragweed Given and the Degree of Subjective Clinical Improvement.*—No correlation appears to exist between the amount of ragweed given and the degree of subjective clinical improvement obtained (Fig. 6).

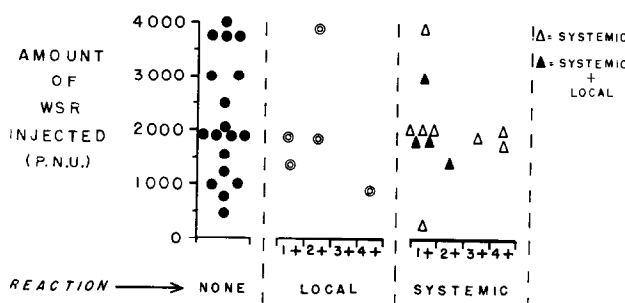


Fig. 7.—Relation between the amount of ragweed given and the degree of local or systemic reaction following repository injection (35 cases).

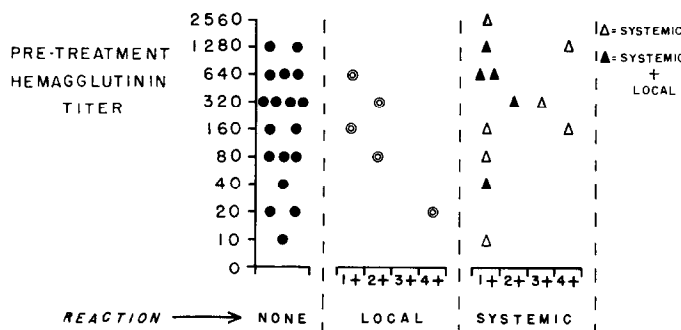


Fig. 8.—Relation between the pre-treatment hemagglutinin titer and the degree of local or systemic reaction following repository injection (35 cases).

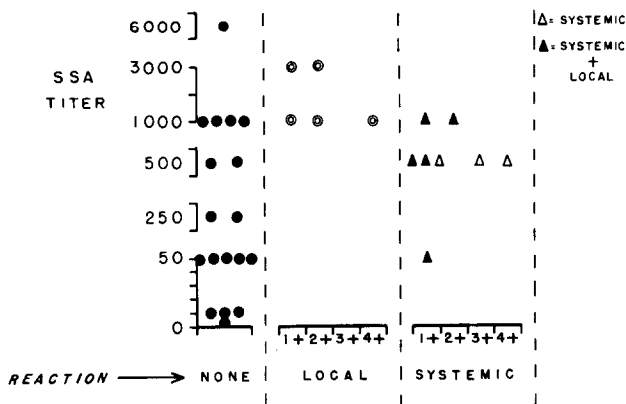


Fig. 9.—Relation between the pre-treatment SSA titer and the degree of local or systemic reaction following repository injection (31 cases).

5. *Reactions Following Repository Injection.*—A systemic or a local reaction after the repository injection was observed in 17 out of 35 cases. Twelve patients had systemic reactions but these reactions were severe in only 3. The incidence of reactions or the degree of severity of these reactions could not be related to

the amount of ragweed given (Fig. 7) or to the pre-treatment hemagglutinin level (Fig. 8). However, all but one of the patients who had reactions after the injection had pre-treatment SSA titers of 500 or more (Fig. 9). Although immunologic studies were performed in 35 cases, 46 were actually treated by ragweed repository injections, 9 of whom were asthmatics. Seven of these 46 patients had severe systemic reactions and 2 had severe local reactions. It is interesting to note that 6 of these marked reactions occurred in asthmatics, none of whom had received treatment in 1959.

DISCUSSION

The objectives of this study were twofold. First, to establish whether the presence or change in concentration of any of the immunologically identifiable constituents present in the serum of an allergic person can be related to the degree of clinical improvement observed after the immunization or "densitization" procedure with aqueous ragweed pollen extract emulsified in oil. Second, to determine whether any correlation exists between the skin sensitizing antibody (SSA), the blocking antibody, and the hemagglutinating antibody, in order to unequivocally ascertain which of the former two factors can be implicated in the agglutination reaction, if indeed either of them can.

It will be apparent from the results obtained that there was no correlation between any of the parameters measured with the exception of one. There was a tendency for reactions to occur following injection when the pre-treatment SSA titer was 500 or higher. It is notable that these results, showing a lack of correlation between the entities measured, are very similar to those described by Arbesman and his co-workers using conventional multiple injection therapy^{10, 11} as well as repository therapy.¹⁸

A number of reports have recently appeared which have implied that the hemagglutinating antibody can be viewed as existing independently from SSA.⁹⁻¹² Augustin,¹² using column chromatography, found that hemagglutinating ability could not be separated from blocking antibody activity. Nevertheless, the evidence which has been submitted, either in favor of or against SSA and/or blocking antibody possessing hemagglutinating activity, has been inconclusive.

In order to determine whether SSA could contribute to the increases in hemagglutinin titers observed with some sera after treatment, SSA titers were determined on both pre- and post-treatment sera. It was found, with the exception of 8 cases, that reagin titers did not change appreciably after treatment.

The sera displaying increases in hemagglutinin titers were divided between those which showed marked increases in SSA titers (6 cases) and those in which no significant increase in SSA titers could be detected (12 cases). In view of the fact that hemagglutinating antibody titers increased in sera where SSA concentrations remained constant, we must conclude, as did Arbesman and his group,¹¹ that reagin and hemagglutinating antibody are distinct entities.

Blocking antibody activity could be detected in only 7 of the 12 sera tested, and of these only 3 showed any increases over pre-treatment values (see Table III). The increase in hemagglutinating activity was not always accompanied by an increase in blocking antibody titer. These results, therefore, as well as those of the SSA experiments, fail to support the theory that hemagglutinating activity should be attributed solely to either SSA or blocking antibody, or to both of these constituents.

Several other interesting conclusions can be drawn from these data. It has been postulated¹⁷ that reagin could be converted to blocking antibody after its reaginic activity is destroyed by heating at 56° C. If this were the case, then one would expect at least a few of the high titer reaginic sera to develop blocking activity after heating. Such was not the case, however. As can be seen in Table III, no blocking antibody activity was detected in the sera of 3 patients, Nos. 6, 10, and 11 (pre- and post-treatment), after heating. They all exhibited high reagent titers. In fact, these titers were higher than in some of the other sera in which blocking antibody activity was manifest after heating. In some of the sera which did possess blocking antibody activity after treatment, no difference could be detected in their pre- and post-treatment reagent titers. One could therefore conclude that reagin is not converted to blocking antibody after heating of the reaginic serum at 56° C. for 1 to 2 hours. This finding also implies that not only does reagin lose its capacity to elicit a triple response but that it loses all ability to react with the ragweed allergen(s).

A second interesting observation concerns the relationship of the hemagglutinating antibody and reagin. As was mentioned above, sera Nos. 6, 10, and 11 (pre- and post-treatment sera; Table III) did not manifest blocking antibody activity after heating. Since the hemagglutinin titers of 2 of these 3 sera were very high, it must also be concluded that the hemagglutinating antibody does not react with the constituent in the ragweed pollen extract to which the skin-sensitizing antibody and blocking antibody are directed. If it did, then it should be able to block the reaction between reagin and the allergen, in the same way as conventional blocking antibody. Since the blocking antibody does react with the allergen in such a way as to inhibit subsequent reaction between the allergen and reagin, it must be directed against the same component of the ragweed pollen extract, if not the same site, as is reagin.

In summary, our interpretation of the results presented above is that hemagglutinating activity is due to a factor different from reagin and blocking antibody. It is certainly not inconceivable that the ragweed pollen extract used in the hyposensitization treatment consists of a heterogeneous mixture of antigens and allergens. It has been demonstrated that ragweed extract (WSR) contains at least eleven well-defined constituents (peptides, proteins, pigments, carbohydrates).^{13, 14} Undoubtedly, not all of them are antigenic or allergenic. Moreover, it is unlikely that the only component(s) of WSR to which the affected individual reacts is the allergen. On the contrary, it is quite probable that WSR contains materials which are antigenic, but not allergenic, stimulating the formation of antibody systems unrelated to allergy in the host. The host

would therefore form three varieties of antibodies, a clinically innocuous type of antibody as well as the biologically active antibodies which we designate SSA and blocking antibody. Such a state of affairs exists today insofar as diphtheria toxoid,¹⁵ pneumococcal polysaccharides,¹⁶ and several other common immunizing materials are concerned. In the case of diphtheria toxoid, it has been shown that many of the antibody systems which are known to arise in man can be formed in response to a single complex of antigens.¹⁵

CONCLUSIONS

1. Sera from 35 patients treated with a single repository injection of ragweed pollen extract were investigated for their skin-sensitizing, blocking, and hemagglutinating titers.

2. There was a definite increase in hemagglutination titer following the desensitization treatment in the majority of patients.

3. There was no correlation between the hemagglutinating titer of any serum and its skin-sensitizing and/or blocking antibody activities.

4. There was no constant relationship between the degree of clinical improvement observed in patients and the concentration of any of the three parameters (hemagglutinin, skin-sensitizing antibody, blocking antibody) measured in the sera of these patients.

5. There was no relation between the clinical response and the quantity of ragweed administered.

6. The incidence of reactions following the repository injection could be correlated with the pre-treatment SSA titer. Most of the severe reactions occurred in asthmatics who had not been treated by hyposensitization procedures in the previous year.

7. Neither the skin-sensitizing antibody nor the blocking antibody contributes appreciably, if at all, to the hemagglutinating capacity of a serum from an allergic individual.

8. It has been demonstrated that reagin is not converted to a blocking antibody upon heating it at 56° C. to destroy its skin sensitizing activity.

9. The results obtained suggest that hemagglutinating antibody does not react with the same constituent(s) of the ragweed pollen extract to which reagin and blocking antibody are directed. These latter two antibodies must be directed to the same constituent of the allergen molecule.

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