

STUDIES ON IMMUNITY IN A TYPE OF HUMAN ALLERGY
(HAY FEVER): SEROLOGIC RESPONSE OF NON-SENSITIVE INDIVIDUALS TO POLLEN INJECTIONS

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A previous publication (1) has shown that injections of ragweed extract produced certain serum changes in ragweed sensitive patients. Comparisons of serum before and after treatment were made, using the method of passive transfer. Mixtures of serum and ragweed in specified amounts were injected intradermally into sites of a normal non-sensitive test subject. The sites were observed at the time for the "immediate" reaction and were then tested at the end of 48 hours to see if the skin-sensitizing antibody had or had not been used up by the amount of ragweed used in the mixture.

Whereas ante-treatment serum was usually neutralized by equal amounts of ragweed extract of 200 P.N. units per cc.¹ or less, the post-treatment serum often required extract twenty times as strong. This occurred in spite of the fact that there was no regular increase in the amount of antibody as determined on passive transfer by the dilution test method. The explanation was offered that this phenomenon was due to a blocking type of immune body produced in the sensitive individual by the injection of the ragweed extract. The immune substance was shown to be specific and its blocking effect was also evidenced by an inhibition of the immediate reaction at the time the mixture sites were made. Ante-treatment serum-ragweed mixtures always produced an immediate reaction in the skin of the test subject while the post-treatment serum-ragweed mixtures frequently failed to show any immediate reaction.

¹ One unit equals 0.00001 mg. of protein nitrogen.

It seemed theoretically possible that these serological alterations induced by ragweed extract injected into ragweed sensitive cases might be connected with or even due to the reaction between antigen and sensitized tissue. If this were true similar serum changes might be induced by creating skin reactions in non-sensitive individuals with injections of sensitive serum and antigen while injection of ragweed extract alone in non-sensitive test subjects would not show serum changes. That this hypothesis was incorrect will be shown in the results of the study here reported.

Method

Five healthy non-allergic individuals volunteered for a course of injections over varying periods of time. Two of the volunteers (group 1) were started on injections of ragweed extract² mixed with ragweed sensitive serum. For a period of 11 weeks each subject received each week six intradermal inoculations of 0.2 cc. of a mixture of equal parts of extract and sensitive serum. The total dosage for each subject was 6600 P.N. units of ragweed and 6.6 cc. of the serum. Each of the six mixture sites produced well marked local reactions each week.

The other three volunteers (group 2) were given subcutaneous injections of ragweed extract alone (no sensitive serum) once or twice a week. The first, D., received a total dosage of 211,000 P.N. units of ragweed extract over 11 weeks; the second, G., 639,000 P.N. units over 23 weeks and the third, J., 1,055,000 P.N. units over a 56 week period.

Intradermal skin tests with strong ragweed extract (1000 and 10,000 P.N. units per cc.) were consistently negative in all five volunteers before, during and after the series of injections.

Serum specimens were obtained from blood taken before, during and after the injections. The ante-treatment serum will hereafter be designated serum A. That taken after treatment was started will be termed serum P. Precipitin tests on all serums were regularly negative before and after injection.

The five non-allergic volunteers were not sensitive to ragweed extract clinically or by skin test before or after the series of injections, hence all serums failed at all times to transfer sensitivity to the skin of other normal test subjects. It was necessary therefore to add serum from an exquisitely sensitive and hitherto untreated ragweed sensitive case in order to carry out the passive transfer studies in accordance with the technique previously described (1).

Using equal volumes of a ragweed skin-sensitizing serum and the experimental serums it was possible to make serum-ragweed mixtures for injection into other normal test subjects to determine whether the injections of ragweed and sensitive

² Low ragweed (*Ambrosia elatior*) was the only variety of ragweed used throughout this work.

serum (group 1) or ragweed alone (group 2) in our five volunteers had induced serological changes affecting the neutralization point or the immediate reaction. This was done by a comparison of the serum A with serum P of each volunteer in the same test subject at the same time.

RESULTS

Group 1 Serums.—These failed completely to show any change in the neutralization point or the immediate reaction on passive transfer. It will be observed that these subjects had received relatively small amounts of ragweed extract.

Group 2 Serums.—Numerous passive transfer experiments were performed upon many different test subjects with the serums of the three members of this group. Serum samples taken early in the course of the injections showed a trend toward an elevation of the neutralization point. This was definitely established as the large ragweed injections proceeded and is well shown in the protocol of a typical experiment.

Volunteer J., serum A (ante-treatment), taken Apr. 14, 1936. Ragweed injections were started and serum was taken at six different intervals to May 10, 1937. The fourth serum P was taken on Apr. 16, 1937, 2 weeks after a ragweed injection. Serum A and serum P were each mixed with an equal volume of serum from an actively sensitive hay fever patient who had never had ragweed injections (H. serum). To a given volume of this mixed serum in each test tube was added an equal volume of ragweed extract (or normal saline) of the strength indicated in Table I. Normal saline and another normal non-sensitive serum from an individual who had not received any injections (B. serum) were substituted for the J. serum as additional controls. In this experiment there were nineteen separate serum-ragweed (or saline) mixtures. 0.1 cc. of each mixture was injected intradermally on the back of a selected normal non-sensitive test subject thus creating nineteen separate test sites. 48 hours later each of these sites was tested by injecting 1/40 cc. of ragweed extract (1000 units per cc.). The extent of the resulting reactions is given in Table I.

The Neutralization Point.—In this experiment the sensitive H. serum, when combined with J. serum A or J. serum P or B. serum or saline with no ragweed extract added, (sites 4, 11, 15, 19), transferred sensitiveness and gave a good reaction on the 48 hour test with ragweed extract. The table further shows that the H. serum when combined with J. serum A, B. serum or saline and ragweed extract of 200 unit strength, (sites 3, 14, 18) gave no reaction on test with ragweed,

whereas these combinations with ragweed of 100 unit strength (sites 2, 13, 17) still gave a slight reaction. In other words the neutralization point lay between 100 and 200 units.

TABLE I

Skin site on test subject	Serum-ragweed mixtures for sites*			Reaction at site when tested after 48 hrs. with ragweed extract†
	Serum combinations		Ragweed units per cc.	
1	J. serum A + H. serum (ragweed sensitive)		50	++
2	"	" " + " " " "	100	+
3	"	" " + " " " "	200	0
4	"	" " + " " " "	Saline	+++
5	J. serum P + H. serum (ragweed sensitive)		200	+++
6	"	" " + " " " "	300	++
7	"	" " + " " " "	400	++
8	"	" " + " " " "	600	+
9	"	" " + " " " "	800	±
10	"	" " + " " " "	1000	0
11	"	" " + " " " "	Saline	+++
12	B. serum + H. serum (ragweed sensitive)		50	++
13	"	" " + " " " "	100	+
14	"	" " + " " " "	200	0
15	"	" " + " " " "	Saline	+++
16	Saline	+ H. serum (ragweed sensitive)	50	+
17	"	+ " " " "	100	+
18	"	+ " " " "	200	0
19	"	+ " " " "	Saline	+++

* This mixture consisted of one part of serum combination (sensitive and non-sensitive serum in equal parts) and one part of ragweed extract of designated strength. 0.1 cc. of the serum-ragweed mixture was used for creating each passive transfer site in the skin of a normal subject.

† 1/40 cc. of extract (1000 units per cc.) was injected into each site.

On the contrary the H. serum mixed with J. serum P required ragweed extract of a strength between 800 and 1000 units (sites 9 and 10) to effect neutralization.

This sixfold increase in the neutralization point seems to resemble that previously reported (1) for the treated ragweed sensitive cases.

Similar results were obtained with serums of the other two members of this group 2. It appears that in this particular the normal non-sensitive human being without developing sensitization responds to ragweed injections with immune body production as does the ragweed sensitive case. However, the degree of response was not comparable. The serum of treated hay fever patients showed an increase of neutral point from ten to twenty times that of pre-treatment serums with doses of ragweed extracts that were much smaller.

It was interesting to note, in determining the neutralization points for the group 2 serums, that the degree of change in neutralization point seemed to bear a relationship to the duration of treatment or to the amount of ragweed extract that had been injected into these non-sensitive individuals. For example the third J. serum P taken January, 1937, was just neutralized with ragweed of 400 unit strength, the fourth taken 3 months later required 800 to 1000 units, while the fifth serum P another month later was still faintly positive with 1250 units of ragweed.

By combining the J. serum A and P with an actively sensitizing serum from a case reacting to timothy pollen and making mixtures with timothy extract of varying strength, it was possible to show that the neutralization point remained identically the same. Hence it was possible to conclude that the immune substance in the J. serum P induced by injections of ragweed was not effective against timothy pollen. In other words the ragweed immune substance appears to be specific.

The Immediate Reaction.—Mention has been made of the fact that intradermal injections in non-sensitive test subjects of mixtures of ragweed with ante-treatment serums produced a positive immediate skin reaction which frequently was not obtained with post-treatment serum-ragweed mixtures. This inhibition of the immediate reaction was also observed with the post-treatment serums of the three volunteers (group 2) who received large injections of ragweed extract. This is well shown in the results recorded in Table II. These immediate reactions were read at the end of 30 minutes. In this experiment we were making observations on pseudoglobulin fractions to be explained in detail later. The J. serums A and P were first diluted with equal amounts of saline to approximate the pseudoglobulin content of

the solutions used in the sites 9 to 16. Sites 1 and 2 showed good (+++) immediate reactions while the saline control (site 3) was essentially negative. On the contrary the J. serum P showed nothing more than a slight (+) irritative reaction (sites 4 to 7) just as did the control (site 8).

TABLE II

Skin site on test subject	Serum-ragweed mixtures for sites						Ragweed units per cc.	Immediate reactions at the time sites were made	Reaction at site when tested after 48 hrs. with ragweed extract
	Serum combinations								
1	J. serum A	+ H. serum (ragweed sensitive)					50	+++	±
2	" " "	+ " " "					100	+++	0
3	" " "	+ " " "					Saline	±	+++
4	J. serum P	+ H. serum (ragweed sensitive)					100	+	+++
5	" " "	+ " " "					300	+	+
6	" " "	+ " " "					500	+	±
7	" " "	+ " " "					700	+	0
8	" " "	+ " " "					Saline	+	++++
9	B. pseudoglobulin A + H. serum (ragweed sensitive)						50	+++	±
10	" " "	+ " " "					100	+++	0
11	" " "	+ " " "					Saline	+	++++
12	J. pseudoglobulin P + H. serum (ragweed sensitive)						100	+	++++
13	" " "	+ " " "					300	+	+++
14	" " "	+ " " "					500	+	+
15	" " "	+ " " "					700	+	0
16	" " "	+ " " "					Saline	+	+++

With the exception of the fact that J. serum A and J. serum P were diluted to half strength as explained in the text, the serum combinations and their mixtures with ragweed and the tests were carried out as were the experiments recorded in Table I and explained in the footnote to Table I.

Separation of Pseudoglobulin

It has been shown (2) that in the ragweed sensitive case the skin-sensitizing substance lies in the pseudoglobulin fraction of the serum. Further work (3) has shown that the inhibiting factor of the post-treatment serum is likewise in this same protein fraction. It was

interesting therefore to determine whether or not the inhibiting factor induced in the serum of our non-sensitive ragweed treated volunteers would also be found in the pseudoglobulin fraction. Analyses of the whole serum by Howe's method (4) showed a pseudoglobulin content of 3.63 mg. N per cc. for the J. serum A and 3.15 mg. N per cc. for the J. serum P. Purified pseudoglobulin was then obtained from J. serum P by fractional precipitation (3). Its final solution in normal salt showed a nitrogen content of 1.5 mg. N per cc. This was practically half the amount determined for the whole serum P. In the absence of sufficient J. serum A the pseudoglobulin from a normal serum B. was used as control in a solution containing 1.7 mg. N per cc.

These pseudoglobulin solutions were combined with the sensitizing H. serum and with ragweed extract exactly as has been recorded for the previous experiments with whole serum. The results recorded in Table II show that the B. pseudoglobulin A (normal) does not inhibit. The neutralization of the sensitive H. serum is practically complete with 50 units of ragweed (site 9). The J. pseudoglobulin P shows definite blocking. It required from 500 (site 14) to 700 (site 15) units of ragweed to effect neutralization.

Inhibition of the immediate reaction previously discussed was observed (Table II) with the pure pseudoglobulin fraction of the J. serum P (sites 12 to 15) as contrasted with the active (+++) reaction produced by the control mixture (sites 9 and 10) in which normal human pseudoglobulin was used.

SUMMARY

Large injections of ragweed pollen extract into normal non-sensitive volunteers did not produce a sensitization to ragweed.

Group 1 volunteers in whose skin many reactions were induced by injections of ragweed extract mixed with ragweed sensitive serum failed to show any serological changes. The theory that the immune substance found in the serum of treated ragweed sensitive cases was due to the reaction or to some substance created by it and not to the ragweed *per se* was not upheld.

On the contrary in group 2, volunteers who received larger amounts of ragweed but no sensitive serum, serological changes were induced resembling those previously observed to occur in ragweed sensitive

patients after treatment. They were demonstrable by an inhibition of the immediate reaction and by interference with the neutralization of sensitive serum by its antigen. These serological changes are therefore independent of the specific reaction characteristic of this type of allergy.

The inhibiting factor was found to be related to the pseudoglobulin fraction of the serum and was shown to be specific.

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