SEROLOGICAL EVIDENCE OF IMMUNITY WITH COEXISTING SENSITIZATION IN A TYPE OF HUMAN ALLERGY (HAY FEVER)*

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PLATE 31

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Using ragweed hay fever as an example of a type of allergy we record serological studies which were undertaken to explain the protection resulting from pollen injections. The results have indicated to us the production of an inhibiting or immune type of substance that prevented the allergen from reacting with the sensitized cell, and they have also demonstrated the coexistence of both sensitizing and immune antibodies in the specifically treated patients.

The term allergy is used today in a general sense to designate a number of specific reactions that have important clinical, pathological and immunological differences.

It seems possible now to differentiate one group occurring spontaneously in man; that is, without artificial parenteral stimulation, subject to hereditary influence and evidenced clinically by an edematous type of reaction that quickly follows contact of the allergen with the sensitized cell. Immunologically this group is characterized by skin and mucous membrane sensitization with the sensitizing antibody demonstrable in the blood serum as well as by the entire absence from the blood of precipitins and smooth muscle sensitizing antibody so regularly found in artificially induced allergy (anaphylaxis). A review of the literature on these points together with additional studies is contained in an article by Cooke and Spain (1). De Besche (2) has confirmed this work.

With the discovery of the transferable skin sensitizing antibodies in the serum

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of allergies of this group, Prausnitz and Küstner (3) explained, in part at least, the mechanism of this type of allergy.

The pollen sensitization of man (hay fever) is representative of this group. The beneficial result of specific therapy in pollen allergy has rested upon clinical observation. As a result of many independent studies for more than 20 years, it is now accepted as a fact that such injections afford satisfactory clinical immunity, but there has not yet been offered any solution of the protective mechanism. On account of its frequency, ragweed hay fever afforded the best opportunity for such a study.

EXPERIMENTAL

On the theory that it might be possible to demonstrate the existence of an immune substance in the blood of treated hay fever cases, we first proceeded to note the clinical effects of transfusion, using treated patients as blood donors for untreated patients with the same sensitization having active hay fever, some with asthma, in the beginning of the pollen season.

In 1931, 1932, 1933 and 1934 twenty cases were transfused and the effects carefully noted. The donors used had all been actively and continuously treated for at least a year, but in no case had a donor received a pollen injection within 2 weeks of the transfusion. At the time of transfusion all donors were positive by intradermal test to ragweed pollen extract and their serum transferred the sensitization to normal human skin. The donors themselves had satisfactory clinical results through the balance of the pollen season.

The recipients of this theoretically immune blood received no treatment other than transfusion and lived their usual lives in the usual pollen atmosphere. Sixteen of the twenty cases had satisfactory results which lasted through the remaining 4 to 5 weeks of their hay fever season. 96 other patients received repeated 10 cc. doses of the supposedly immune serum subcutaneously with clinical improvement in 60 per cent.

These results, in some cases striking, indicated to us the presence of a transferable protecting substance which we then sought to demonstrate by serological studies.

In this work the method of passive transfer was employed, using the skin of normal non-allergic people as test subjects to provide test sites made with serum of patients sensitive clinically and by test to ragweed pollen. Bleedings were made and serum obtained from the patient group before and after treatment with ragweed extract. Hereafter in this paper the term test subject denotes the non-

sensitive group whose skin was used for sites and tests. The ante-treatment serum is called Serum A, and the post-treatment serum called Serum P. The pollen extracts used throughout this work were prepared from ethyl ether extracted pollen in alkaline saline fluid and were standardized on the protein nitrogen basis (4) (100 units = 0.001 mg. protein nitrogen). Dilutions were made in physiologic salt solution. As near as we can estimate 60,000 of our units equalled 100,000 Noon units (5) calculated on a weight by volume basis as used by Harley (5).

The synopsis of the histories and essential facts regarding the eight cases whose serums in special were studied for this paper is as follows:

St. Case 2025. Male. Age 40. Ragweed hay fever with asthma of 12 years duration. Some ragweed injections in 1929. Intradermal test with ragweed 1,000 units marked (++++). Tests after treatment were approximately the same. Serum A taken Aug. 10, 1933. He was transfused on Aug. 15, 1933, and was one of the sixteen cases referred to with satisfactory results in 1933. Treated at weekly intervals with giant ragweed from Oct. 24, 1933, with moderate constitutional reactions when dosage exceeded 10,000 units. Dose reduced to 5,000 units. The last injection was given Oct. 2, 1934. Serum P taken Oct. 24, 1934, when total dosage had been 230,000 units. 90 per cent relief in 1934 (3 days of hay fever).

Sc. Case 2105. Male. Age 46. Ragweed hay fever since 1931. Never previously treated. Intradermal test with ragweed 1,000 units marked (++++). Test after treatment showed very slight reduction in activity. Serum A taken Sept. 6, 1933. Treated at weekly intervals with giant ragweed until 10,000 units had been reached, when injections were given every 2 weeks and finally every month. Last five injections 60,000 units each with dosage totalling 585,535 units. Last dose given Nov. 17, 1934. Serum P was taken Jan. 18, 1935. 80 per cent relief (7 days hay fever).

Ce. Case 2055. Female. Age 31. Ragweed hay fever since 1932. Never previously treated. Intradermal test with ragweed 1,000 units marked (++++) before treatment. This was reduced during treatment, and was only slight (+) to 1,000 units after treatment. Serum A taken Oct. 20, 1933. Treated at weekly intervals with giant ragweed until 60,000 units was reached. Last injection Aug. 8, 1934, dosage totalled 301,700 units. Serum P taken Sept. 15, 1934. 90 per cent relief (3 days hay fever).

Bu. Case 2022. Male. Age 55. Ragweed hay fever of 15 years duration. Never previously treated. Intradermal test with ragweed marked (++++) to 1,000 units before treatment and approximately the same after treatment. Serum A taken Nov. 6, 1933. Treatment started at weekly intervals with giant ragweed until 60,000 units was reached on Sept. 7, 1934. Total dosage 430,480 units. Serum P taken Oct. 1, 1934. Had no hay fever in 1934.

Kr. Case 2042. Male. Age 10. First definite hay fever in 1933 was untreated. Intradermal test before treatment on Nov. 10, 1933, marked (++++) to 1,000

units and the same after treatment. Serum A taken Nov. 13, 1933. Treated with low ragweed at weekly intervals until receiving 10,000 units in June, 1934. Treatment interrupted until September, 1934, when dosage was reduced to 5,000 units. Last dose Sept. 25, 1934, 5,000 units, totalling 93,190 units. Serum P taken Nov. 2, 1934. 70 per cent relief (9 days of hay fever).

Bl. Case 2076. Male. Age 11. Ragweed hay fever since 1932. Never previously treated. Intradermal test marked (++++) to 1,000 units ragweed before treatment. Tests after treatment showed slight reduction of activity. Serum A taken Sept. 6, 1933. Treated with low ragweed at weekly intervals until 21,000 units was reached and a constitutional reaction resulted. Dosage was reduced, last injection Oct. 15, 1934, 5,000 units, totalling 192,280 units. Serum P taken Nov. 7, 1934. 70 per cent relief (9 days of hay fever).

Sp. Case 2054. Male. Age 47. Ragweed hay fever since 1932. Never previously treated. Transfused with satisfactory results on Sept. 7, 1933. Intradermal reaction marked (++++) to 1,000 units ragweed before treatment. Reactions after treatment were a trifle less in activity. Serum A taken Sept. 19, 1933. Treated at weekly intervals from October, 1933, with giant ragweed up to 10,000 units when the interval was increased to 2 weeks and dosage increased to 60,000 units. Total units 473,650. Last injection Sept. 21, 1934. Serum P taken Oct. 5, 1934. 70 per cent relief (9 days hay fever).

Bo. Case 2045. Female. Age 38. Ragweed hay fever since 1931. Never previously treated. Intradermal reactions marked (++++) to 100 units ragweed. After treatment skin reactions reduced to moderate (++) to 100 units. Serum A taken Aug. 30, 1933. Transfused on Aug. 31 with excellent results in 1933. Treatment started Oct. 20, 1933, with giant ragweed until 60,000 unit dose was given. Kept at that level for four doses until Sept. 10, 1934, the date of last injection. Dosage totalled 519,240 units. Serum P taken Sept. 20, 1934. 85 per cent relief in 1934 (5 days of hay fever).

Precipitin tests were done on both Serum A and Serum P of all these cases except Kr. and Bo. The antigens used were giant and low ragweed extracts and their pure proteins. There was no precipitation.

Our observations consisted of a comparison of the Serum A and Serum P of each of these eight cases with reference to:

1. The relative amounts of skin sensitizing antibody in the Serums A and P to determine its increase, decrease or disappearance under treatment.

2. The reactions of test subjects' skin both to injections of serum-allergen mixtures at the time the transfer sites were made and to the subsequent tests of these sites.

The normal skin test subjects vary somewhat both as to their acceptance of transfer and their reactivity to test, hence crucial comparisons must be made on

the same test subject at the same time. Extracts of pollen deteriorate. We have found this to be definite and demonstrable in extracts 2 months old but not at 1 month. The extracts used in this work were made fresh every month. The serums must be kept sterile. The question arose early as to the possible loss of skin sensitizing capacity of aging serum. Levine and Coca (6) have stated there was no apparent loss in 3 months' time. Our tests indicated none in 1 year when serum was kept sterile at 8°C. The non-specific reaction produced by heterologous serums when injected intradermally for skin sites has introduced some difficulty. Chant and Gay (7) studied this point and stated that such reactions started at once, reached a maximum in 15 minutes and began to fade in less than 30 minutes. Our observations were in entire accord. These "irritative" reactions will be discussed later under serum-allergen mixture tests as they have been a cause of erroneous interpretation by previous observers.

1. Titration of the Relative Amounts of Skin Sensitizing Antibody in Serums before and after Treatment

Influenced by observations in experimental anaphylaxis the effects of pollen therapy are generally ascribed to desensitization. In the type of allergy represented by hay fever cellular antibody may be tested for through the skin by prick, scratch or intradermal method. Using the intradermal method, Cooke (8), Levine and Coca (9) and others have recorded no material objective change in the test in the majority of cases treated to a successful clinical result. Markin (10), Brown (11) and Harley (12) on the contrary have reported abolition of skin reaction after treatment using the scratch or prick method. These discrepancies may be due to the difference of technique and may be explainable, but if one claims cellular desensitization it should be only after use of the more delicate test, the intradermal, and not the less delicate, the scratch or prick.

In the summaries of the eight cases here presented, no significant differences could be noted in the intradermal reactions before and after treatment with the exception of Ce., No. 2055, where it was definitely reduced but not abolished. Cellular desensitization was not demonstrable in these long and excessively treated cases using the intradermal test.

The amount of skin sensitizing antibody in the serum has been determined by injecting increasing dilutions of sensitive serum into normal skin (passive transfer) and testing these sites 24–48 hours later. Levine and Coca (6) found no decrease of serum antibody in the serum

TABLE I

Titration of the Amount of Skin Sensitizing Antibody in Serums before and after Treatment

Serum dilutions*	Bu. serum†				Kr. serum†			
	(Test subject D.L.)		(Test subject M.G.)		(Test subject H.R.)		(Test subject F.S.)	
	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P
1–10	+++	++			++++	++++	++++	++++
1–25					+++	+++		
1–50					+++	++	++++	++++
1–100	++	++			+++	++	+++	+++
1–200	++	+						
1–300	+	0	++	+	++	+	+++	+++
1–400	±	0	+	±				
1–500	±	0	±	0	+	+	++	++
1–700							+	+

Serum dilutions*	Bo. serum†		St. serum†		Ce. serum†		Sc. serum†	
	(Test subject E.S.)		(Test subject G.W.)		(Test subject J.N.)		(Test subject O.C.)	
	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P
1–10	+++	++++			++	++	++	++
1–25	++	+++	+++	+++				
1–50	+	++	+++	+++	++	+	++	++
1–100	±	+	+++	+++	0	±	+	+
1–200	0	±			0	0	+	+
1–300			+	+				
1–400								
1–500			±	+				

Serum dilutions*	Sp. serum†				Bl. serum†			
	(Test subject G.W.)		(Test subject C.T.)		(Test subject C.T.)		(Test subject H.R.)	
	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P	Serum A	Serum P
1–10	++++	++++	++++	++++	++++	++++	+++	+++
1–25	+	+++					++	+++
1–50	+	+++					±	++
1–100	±	+++	±	++++	+	+++	0	++
1–200			0	+++	±	++		
1–300	0	+++	0	++	0	+	0	+
1–400			0	+	0	0		
1–500	0	+++	0	±	0	0	0	0

\+ = degree of skin reaction.

± = doubtful skin reaction.

0 = negative skin reaction.

A = serum taken before treatment with ragweed extract.

P = serum taken after treatment with ragweed extract.

* 1/10 cc. of these serum dilutions in physiologic saline was placed in each site.

† The serum dilution sites were tested with 1/40 cc. of low ragweed 100 units per cc. 48 hours after they were made.

of treated patients. In some cases it was materially increased. Gay and Chant's (13) findings were in accord. Harley (12) using the same technique stated, "In one case the serum reagin disappeared completely, in the others they were markedly reduced." Markin's (10) reports agree.

This titration of skin sensitizing capacity of serums is of importance in the interpretation of the results of the mixture experiments later described. One can reasonably assume that a serum with a greater concentration of antibody would transfer its sensitiveness in a higher dilution than a serum in which the antibody was less abundant. If this is so it is also indicated that a serum containing a greater amount of antibody would require a greater amount of allergen to effect desensitization. This is confirmed by our studies, not given here in detail, which have shown us that ante-treatment serums that did not transfer beyond a 1–10 dilution were neutralized by an equal amount of pollen extract containing about 50 units per cc., whereas a serum that transferred in a 1–1,000 dilution required an equal amount of extract containing nearly six times as many units, hence this dilution method is a relatively accurate means of determining the amount of the skin sensitizing antibody.

In the serums of our eight cases shown in Table I the comparison of Serum A with Serum P showed equality of sensitizing antibody for Kr., St., Sc. and Ce. The Serum P antibody was slightly decreased for Bu., slightly increased for Bo. and Bl. and decidedly increased for Sp. As shown in the case histories all these patients received about as much treatment as Harley's cases. Our findings support those of Levine and Coca (6) and Gay and Chant (13) and disagree with those of Markin (10) and of Harley (12).

Serum desensitization was not obtained by us and post-treatment serum in all cases was demonstrably an actively sensitizing serum as shown by passive transfer tests, therefore as normally circulating in the patient's body it must keep the tissues sensitive. That this is true was shown by the direct positive intradermal test on these patients after complete treatment. The only interpretation that can be placed on these findings is that there is no evidence of protection through desensitization, cellular or humoral, nor yet any evidence that the clinical immunity from specific injections might be afforded by an increase of sensitizing antibody.

2. *The Reactions of Serum-Allergen Mixtures in Non-Sensitive Test Subjects*

Injections of serum-allergen mixtures into normal skin have been recorded, but no account seemingly has been taken as to whether the serums in these mixtures were obtained before or after treatment. There are no observations in the literature in which a study has been made of the comparative behavior of mixtures of allergen with Serum A and with Serum P such as we now record. Coca and Grove (14), Levine and Coca (6, 15), Clarke and Gallagher (16), Baldwin (17) and others have reported no reactions at the time the sites were made. Gay and Chant (13) first reported the finding of positive specific reactions. They have been supported by Foran and Lichtenstein (18) and Harley (5), and by our own findings previously (19) and now recorded. These contradictory reports may be due to the failure to discriminate between serum taken before and that taken during or after treatment, or they may be explained by the fact already mentioned that injection of heterologous serum into normal skin produced an irritative non-specific reaction with wheal and erythema readily confused with the specific reaction.

The non-specific reaction begins at once, reaches its maximum in 15 minutes, and with most serums the erythema begins to disappear before 30 minutes and is practically gone in 1 hour, leaving a pale round elevated papule. The specific reaction begins more slowly, and erythema and wheal are still active at the end of an hour at which time these tests should be read.

In making allergen-serum mixtures precautions for sterility were used. A definite amount of a serum was mixed with an equal amount of the allergen in varying strengths, in these cases ragweed pollen extract. The mixtures were allowed to stand about 15 hours. Harley (5) contends that there is a binding of allergen to antibody because of the negative skin reaction in sensitive patients tested with incubated serum-allergen mixtures. Our previous experiments which we shall not give here in detail were carried out in duplicate, one set at 8°C. and the other at 37°C. Care was taken to assure an excess of antibody over allergen. Intradermal tests on ragweed sensitive patients were made with the two mixtures in varying concentrations of extract and

controlled by the usual saline extracts of the same strength. No differences could be observed, hence Harley's assumption of inactivation through binding of antigen by antibody could not be confirmed. The serum mixtures used in this study were kept at 8°C. In order to make this presentation clear the complete protocol of the typical Experiment 26 will be given.

Experiment 26.—Bu.'s Serum A and Serum P were each mixed in test tubes with an equal volume of a low ragweed extract (LR34E) of a certain unit strength as indicated in Table II. Also dilutions with physiological saline were made of both Serums A and P. These preparations made Apr. 11, 1935, were allowed to stand at 8°C. overnight and 1/10 cc. of each mixture or dilution was injected intradermally on Apr. 12 into each site in the skin of the back of normal test subject (D. L.). Twenty-two separate sites were made and marked with an indelible pencil. The 1 hour reactions of the ten mixtures were recorded. There was of course no 1 hour reaction with the serum dilutions. The test subject returned on the 14th of April for the tests of the sites. At this time there was injected into each site 1/40 cc. of a ragweed extract (LR34E). We had chosen quite arbitrarily a reasonably strong extract containing 1,000 units per cc. to test mixture sites, and a weaker extract, 100 units per cc., for testing dilution sites. The details of the mixtures and the results are recorded in Table II, and illustrated in Figs. 1 and 2.

These results showed that at the time the sites were made on Apr. 12 the mixtures of ragweed and Serum A gave positive 1 hour reactions but the saline control was negative. (Fig. 1, Column 2.) On the contrary the Serum P ragweed mixtures gave no 1 hour reaction except for a suggestive or doubtful reaction with the 1,000 unit extract. This is significant as it seems to indicate that excess of allergen may jump the immune barrier, so to speak, and this suggests the possibility of measuring the amount of protective substance (Fig. 1, Column 3).

When these ten mixture sites were tested on Apr. 14 the Serum A mixtures that had previously reacted were now negative, whereas the Serum P mixtures previously negative or doubtful were now positive.

The comparison of the saline dilutions of Serum A and Serum P showed a slight but definitely greater amount of sensitizing antibody in Serum A. Considering the extent of treatment recorded in the synopsis of the Bu. case this cannot be interpreted as desensitization sufficient to explain the almost perfect clinical result recorded for that season.

Similar experiments with the serums of the eight cases studied have been grouped and the results recorded in Table III. They are quite strikingly uniform throughout. It is interesting to note that all Serums A (except Bl.) had been neutralized by an equal amount of

TABLE II

Experiment 26, Test Subject D.L.

Sites	Apr. 11		Apr. 12	Apr. 14
	Serum-ragweed mixtures			
	Equal amounts		1 hr. reactions when sites were made, Fig. 1	Reaction to test with ragweed 1,000 units per cc., Fig. 2
	Bu. serum	Ragweed extract units per cc.		
			(Column 2)	(Column 2)
1	A	50	+++	0
2	A	100	+++	0
3	A	150	+++	0
4	A	Saline (control)	0	++++
			(Column 3)	(Column 3)
5	P	150	0	++
6	P	300	0	++
7	P	500	0	++
8	P	700	0	++
9	P	1,000	±	+
10	P	Saline (control)	0	+++
Sites	Serum dilutions			
	Bu. serum	Saline dilution	1 hr. reactions when sites were made, Fig. 1	Reaction to test with ragweed 100 units per cc., Fig. 2
			(Column 1)	(Column 1)
11	A	1–10	0	+++
12	A	1–100	0	++
13	A	1–200	0	++
14	A	1–300	0	+
15	A	1–400	0	±
16	A	1–500	0	±
			(Column 4)	(Column 4)
17	P	1–10	0	++
18	P	1–100	0	++
19	P	1–200	0	+
20	P	1–300	0	0
21	P	1–400	0	0
22	P	1–500	0	0

ragweed 150 units per cc. or less on at least one of two test subjects. This means that the sensitizing antibody had been used up in the reaction at the time the mixtures were injected, and is proved by the

TABLE III

Reactions at the Time the Mixture Sites Were Tested with Ragweed 48 Hours after They Were Made

Mixture for sites*			Reaction of sites when tested 48 hrs. later with ragweed†		Mixture for sites*			Reaction of sites when tested 48 hrs. later with ragweed†	
Serum		With LR units per cc.	(Test subject M.R.)	(Test subject D. L.)	Serum		With LR units per cc.	(Test subject M.L.)	(Test subject O.C.)
	A	50	+	0		A	50	±	+
	A	100	±	0		A	100	0	+
	A	150	±	0		A	150	0	
	A	Saline	++++	++++		A	Saline	++++	++++
Bu.					Sc.				
	P	150	+++	++		P	50	++++	
	P	300	+	++		P	100	+++	+++
	P	500	+	++		P	150	+++	+++
	P	700	+	++		P	300		+++
	P	1,000	±	+		P	700		++
	P	Saline	++++	+++		P	1,000	±	++
						P	Saline	++++	++++
			(Test subject M.G.)	(Test subject T.Y.)				(Test subject D.S.)	(Test subject L.L.)
	A	50	+			A	50	++	
	A	100	+			A	100	+	
	A	150	0	++		A	150	+	
	A	Saline	+++	++++		A	300		±
St.						A	Saline	++++	+++
	P	50	++++		Bl.				
	P	100	++++			P	50	++++	+++
	P	150	+++	+++		P	100	++++	
	P	300		+++		P	150	++++	
	P	500		+++		P	300		++++
	P	700		+		P	500		++++
	P	1,000	+	+		P	700		+++
	P	Saline	++++	++++		P	1,000		+
						P	Saline	++++	++++

+ = degree of skin reaction.

± = doubtful skin reaction.

0 = negative skin reaction.

* 1/10 of these mixtures of equal volumes of serum and ragweed extract or serum and saline was placed in each site.

† Tested with 1/40 cc. low ragweed 1,000 units per cc.

TABLE III—*Concluded*

Mixture for sites*			Reaction of sites when tested 48 hrs. later with ragweed†		Mixture for sites*			Reaction of sites when tested 48 hrs. later with ragweed†	
Serum		With LR units per cc.	(Test subject N.P.)	(Test subject T.Y.)	Serum		With LR units per cc.	(Test subject R.H.)	(Test subject L.L.)
Kr.	A	50	+++		Sp.	A	50	+	
	A	100	±			A	100	0	0
	A	150	0	0		A	150	0	
	A	Saline	++++	++++		A	Saline	+++	+++
	P	50	++++			P	50	++++	
	P	100	++++			P	100	++++	
	P	150	++++	++++		P	150	++++	++++
	P	300		++++		P	300		++++
	P	500		++		P	500		++++
	P	700		++		P	700		++++
	P	1,000		0		P	1,000		+++
	P	Saline	++++	++++		P	Saline	++++	++++
			(Test subject D.H.)	(Test subject J.N.)				(Test subject R.B.)	(Test subject E.S.)
	A	50	0	0		A	50	+	0
	A	100	0	0		A	100	0	0
	A	150	0			A	150	0	0
	A	Saline	++++	++++		A	Saline	++++	++++
Ce.	P	50	+++		Bo.‡	P	50	++++	++++
	P	100	+++	+++		P	100	++++	++++
	P	150	+	++		P	150	++++	++++
	P	300		++		P	1,000	±	±
	P	500		++		P	Saline	++++	++++
	P	700		++					
	P	1,000	0	++					
	P	Saline	++++	++++					

‡ To the poorly transferring Bo. serum treated and untreated a small amount of Fe.'s untreated serum was added to increase its skin sensitizing capacity.

fact that the 48 hour tests of the sites were negative. In striking contrast is the fact that when Serum P (except Kr.) was used in the mixtures, every site was still giving positive reaction when tested 48 hours later, although a much greater amount of ragweed (1,000 units per cc.) had been used in the mixtures. In other words, Serum P had not been neutralized by a reaction when sites were made even with

this strong extract. Since we have already shown that there was no greater amount of sensitizing antibody (except Sp.) to require this additional antigen, we feel justified in assuming the presence of an inhibiting agent in the serum of patients after they had been specifically treated.

Before proceeding to our interpretation of these findings we shall record a few supplementary experiments that aid in the solution.

It was necessary to know that an inhibiting Serum P would exert the effect on the actively sensitizing Serum A of another patient. This was done by combining Bo. Serum P with Fe. Serum A controlled by Bo. Serum A and Fe. Serum A. The inhibiting effect was demonstrable (see Table III).

It was then shown that normal non-sensitive human serum combined with Fe. Serum A did not have an inhibiting effect.

The next point was to discover whether Serum P had any binding, inactivating or lytic effect on the allergen. It has already been noted that there was no discoverable precipitin in the six serums studied for precipitins. If Serum P-ragweed mixtures would give reactions equal to Serum A-ragweed mixtures when tested on sensitive cases, then binding or destruction of allergen could not be maintained. The following experiment was done.

To Bu. Serum A was added an equal volume of ragweed extract 700 units per cc. A similar mixture was made with Bu. Serum P, and Bu. Serum P with saline was used as control. These three mixtures were incubated at 37°C. for 12 hours and then were diluted with saline so that there were, of each mixture, three dilutions containing 1, 5 and 10 units of ragweed per cc. respectively. These nine dilutions were then tested on ragweed sensitive cases. The results shown in Table IV indicated no lessening in the activity of the allergen in the Serum P mixture, hence no allergen destruction.

Another question of importance concerned the specificity of the inhibiting substance. The experiments already recorded, as for example the Serum P of Bu. who was treated with giant ragweed, and Serum P of Bl. who was treated with low ragweed, had both shown the inhibiting effect against the low ragweed extract (Table III). Experiments which we will not give in detail have further shown that Serum P from these two cases also showed an inhibition of the reaction against giant ragweed. Other cases treated with low ragweed extract

showed the inhibition of reaction by Serum P against giant as well as low ragweed extract, and serum of cases treated with giant extract inhibited both giant and low ragweed extract. Thus both species of ragweed produced a common inhibiting substance, and this supports

TABLE IV

Immediate Skin Reactions on Ragweed Sensitive Cases

Ragweed sensitive cases	*Mixture 1** Bu. Serum A and low ragweed			*Mixture 2*† Bu. Serum P and low ragweed			*Mixture 3*‡ Bu. Serum P and saline (control) in similar dilutions to preceding mixtures		
	Units per cc.			Units per cc.					
	1	5	10	1	5	10			
1	+++	++++	++++	+++	++++	++++	+	+	+
2	+	++	++	+	+	+	0	0	0
3	++	+++	++++	++	+++	+++	+	+	+
4	0	0	+	0	0	+	±	+	±
5	+	++	++	+	+	+	0	0	0
6	+	+	+++	+	++	+++	0	0	0
7	++	+++	+++	++	+++	+++	0	0	0
8	++	+++	+++	++	+++	+++	0	0	0
9	++	+++	+++	++	+++	+++	±	±	±
10	+	++++	++++	+	++++	++++	0	0	0
11	++	+++	+++	++	++	+++	0	0	0
12	++	+++	++++	++	+++	+++	±	±	±

\+ = extent of skin reaction.

± = doubtful skin reaction.

0 = negative skin reaction.

* *Mixture 1.*—Bu. Serum A (taken before ragweed treatment) and low ragweed 700 units in equal parts incubated at 37°C. for 12 hours, then diluted with buffered saline to 1, 5, 10 units of ragweed per cc.

† *Mixture 2.*—Bu. Serum P (taken after ragweed treatment) and low ragweed 700 units in equal parts incubated at 37°C. for 12 hours, then diluted with buffered saline to 1, 5, 10 units of ragweed per cc.

‡ *Mixture 3 (Control).*—Bu. Serum P and saline in equal parts incubated at 37°C. for 12 hours, then diluted comparable to 1, 5 and 10 units of Mixtures 1 and 2.

our clinical experience and our previous conclusion (19) that as allergens they are qualitatively alike.

More important still is the question of whether the inhibiting substance created by the injection of ragweed pollen extract in the

specifically sensitive subject inhibited the skin reaction of timothy pollen mixed with its specific serum.

The following experiment was done.

Serums from cases of timothy hay fever sensitive only to timothy pollen, taken before any treatment with timothy extract, were mixed with ragweed sensitive Serum A and ragweed sensitive Serum P. Equal volumes of the serum combinations with timothy extract or serum combinations with saline (control) were mixed and placed at 8° C. for 18 hours. Sites were made with 1/10 cc. of these

TABLE V

Specificity of the Ragweed Inhibiting Substance

Test subject	Mixture for sites*		Reaction to test with 1/40 cc. timothy 1,000 units per cc.
	Serum combination in equal amounts	Timothy units per cc.	
R. S.	Timothy Serum A and ragweed Serum A (Sc.)	25	+
	" " " " " " " "	50	0
	" " " " " " " "	100	0
	" " " " " " " "	150	0
	" " " " " " " "	Saline	+++
	Timothy Serum A and ragweed Serum P (Sc.)	25	+
	" " " " " " " "	50	0
	" " " " " " " "	100	0
	" " " " " " " "	150	0
	" " " " " " " "	300	0
	" " " " " " " "	500	0
	" " " " " " " "	Saline	+++

+ = extent of skin reaction.

0 = negative skin reaction.

* 1/10 cc. of these mixtures of serum combinations and timothy extract or serum combinations and saline was placed in each site.

mixtures in the skin of non-sensitive test subjects. In 48 hours the sites were tested with timothy extract. Table V is a typical example of these experiments.

This experiment yielded no evidence of any inhibiting effect by the ragweed immune substance even against as closely related a reaction as that produced by timothy pollen and its specific serum on normal skin cells. Such experiments must be widely extended with many allergens and their specific serums to establish absolute specificity of

the inhibiting or immune substance. Our results thus far indicate specificity.

The final point which we have considered in working toward an explanation of the primary inhibition of the skin reaction (at the time the mixtures are injected) when ragweed was mixed with Serum P, deals with the question of a possible neutralization of the theoretical histamine-like substance. Two points already brought out indicate that this is not the case. The H-substance of Lewis (20), if responsible for these specific reactions, must be produced as a result of the action of allergen on the sensitized cell. There was no specific reaction when the mixtures of Serum P and ragweed were injected into normal skin because there was a reaction when the sites were tested after 48 hours. If there had been a reaction as with Serum A the sites when tested 48 hours later would have been negative.

Again if these specific reactions were due to H-substance, and if absence of reactions with allergen-Serum P mixtures were due to an antihistamine effect in Serum P, then it should follow that the inhibiting effect of Serum P would be non-specific; but we have already shown that the inhibition is specific for ragweed as against timothy allergen.

A direct approach to the solution of this question has been made by testing the skin of normal persons and allergic patients with a solution of histamine in Serum A and in Serum P. The comparative reactions were so similar that there was no evidence of any antihistamine substance in Serum P.

DISCUSSION

In an interpretation of the facts brought out by these comparative studies of Serums A and Serums P the crucial point is to explain the inhibition of the reaction with the Serum P mixtures in normal skin at the time the sites are made. The cell is there, the allergen is there, and we have proven by titration that practically as much and sometimes even more of the skin sensitizing antibody is there. Then why no reaction? We have shown that there is no binding, no inactivation or lysis of the allergen. We have also explained that results cannot be interpreted as neutralization of H-like substance. What is evident is that injection of allergen-Serum P mixtures into skin produces no specific reaction at the time the sites are made, but the skin sensitizing antibody is found sensitizing the skin cells at the site when tested 48

hours later. It seems then that we must assume a block by some sort of specific inhibiting antibody. The block does not occur between the cell and the sensitizing antibody since the cells are later found sensitive. It must occur between the allergen and the sensitizing antibody, but since the test of the site is positive at the end of 2 days we must also assume that both inhibiting substance and allergen are shortly removed from the site. Also it is evident that the block is not absolute but may be overcome by the use of a sufficiently strong allergen in the mixture. In other words, the capacity to inhibit may be roughly measured.

Assuming, as we feel we have shown, the presence of a specific substance which blocks the antigen from the sensitized cell, the question may properly be raised whether this is the explanation of the clinical immunity afforded by specific treatment in this type of allergy. While it may not be the complete answer it satisfies many of the requirements. It permits one to understand the existing sensitization, shown by positive skin tests, while there is symptomatic freedom and it explains the occurrence of general reactions during treatment from dosage that will override the block. A more exacting test will be the determination that the amount of symptomatic freedom is proportional to the amount of inhibiting substance found. Studies to determine this point are being made.

SUMMARY

Using ragweed hay fever as the representative of a certain type of allergy we have made studies to determine if possible the mechanism of the protection afforded by specific injections thus far established only by clinical observation.

1. Blood transfusions and serum injections from clinically immune, treated patients stopped the clinical reaction in untreated patients, thus indicating a transferable immunity.

2. The amount of skin sensitizing antibody in the serum was found to be practically unchanged by specific injections.

3. Injection of allergen-antibody mixtures into normal skin showed an immediate (1 hour) reaction when sites were made if serum of untreated cases (Serum A) was used but none or slight reaction if serum of treated cases (Serum P) was used.

4. When sites made with allergen-antibody mixtures were tested

after 48 hours, reactions were absent with Serum A mixtures if enough allergen had been used, but were positive with mixtures of Serum P even though a much stronger allergen was contained in the mixture.

5. The primary inhibition of reactions with mixtures including Serum P was not due to antihistamine effect nor to binding of skin sensitizing antibody nor to binding or lysis of allergen.

6. The inhibiting antibody appears to be specific.

7. These serological studies supported by transfusion experiments have been interpreted by us as showing the development under treatment of a peculiar blocking or inhibiting type of immune antibody that prevented the action of allergen on the sensitizing antibody and hence showed in the type of human allergy under consideration (hay fever) the coexistence of sensitization and immunity.

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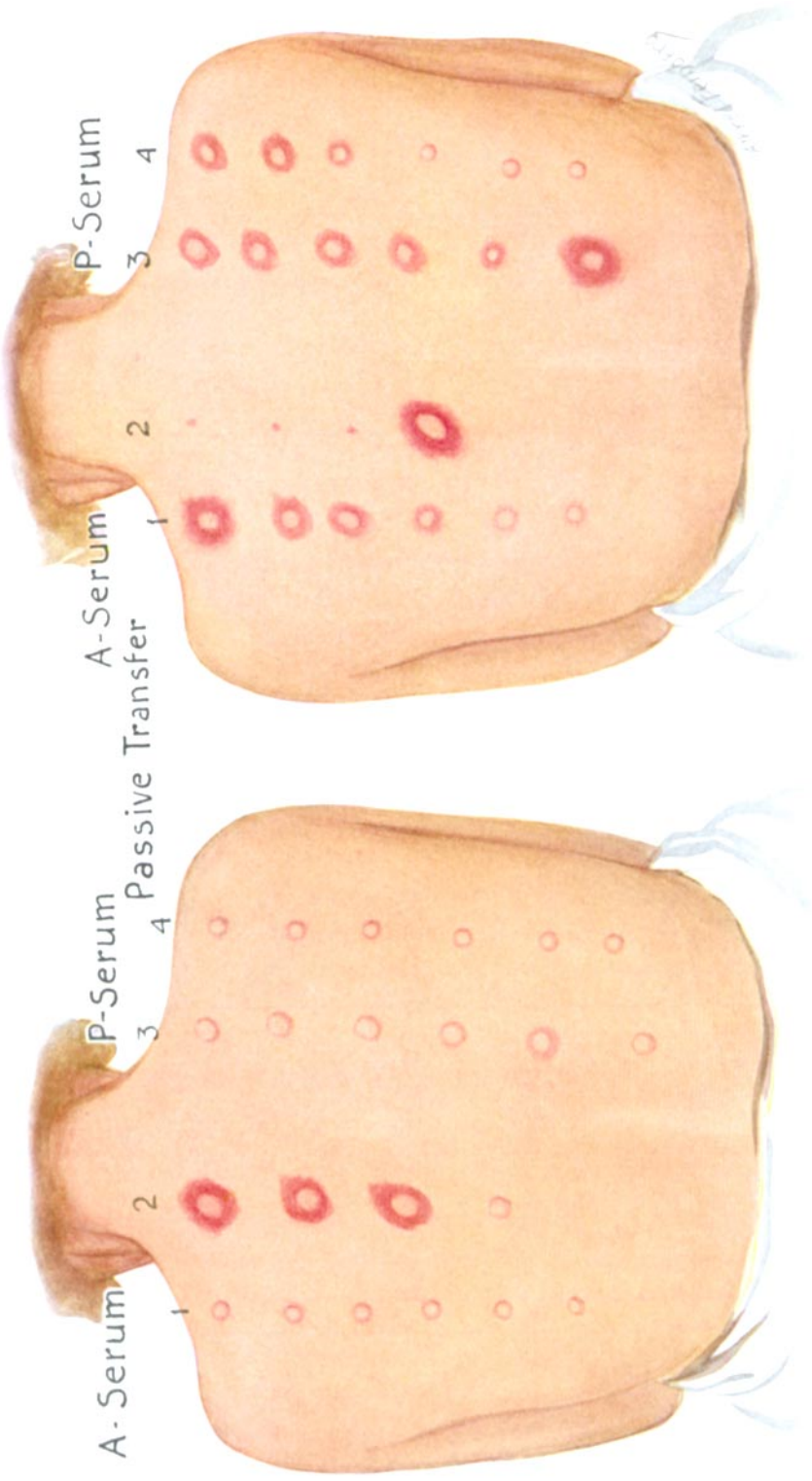
EXPLANATION OF PLATE 31

The figures illustrate Experiment 26 and the results recorded in Table II.

FIG. 1. 1 hour reactions when sites made, Apr. 12, 1935.

FIG. 2. Reactions when sites tested, Apr. 15, 1935.



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