

REAGINIC AND BLOCKING ANTIBODIES

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AT THIS point in the symposium, you may be aware of a sudden change in the nature of the reports, due to some obvious factors. So far, the discussion of antibodies has dealt with normal animals or normal persons subjected to some specific experimental procedure, usually with a highly purified antigen, and the observation of the results from it.

I would imagine that one of the worst things that could happen in an immunologic laboratory would be for all the cages to be opened during the night, and the animals become mixed up, so that the immunologist did not know what treatment any of them had received. That would be exactly the situation with which we deal in clinical allergy all the time.

Our difficulties are increased by the fact that, of all the many substances with which we test and treat patients, I am unable to find one which qualifies as a pure antigen. Finally, in studying the antibody reactions, we are forced to depend almost entirely upon very unsatisfactory biologic indicators of antigen-antibody reaction.

The term *atopic reagin* was applied to the active principle of allergic sera by Coca and Grove¹ because of doubt as to whether it could be considered a true antibody. The reagin was believed to be limited to the human species and to result largely from hereditary influences. It was not even clear at that time whether or not contact with the allergen was important in its development.

With the passage of time and the introduction of new facts, most of these concepts have been modified. In naturally occurring allergy, the hereditary factor is still considered important by most of us, but the development of specific sensitization is now generally believed to result from contact with the antigen. Particularly in regard to geographically distributed antigens, such as pollens, it is generally agreed that persons are sensitive only to antigens with which they may have had previous contact. In the case of foods and household substances, it is often impossible to prove contact or lack of contact. With intense contact with certain antigens, such as horse serum and *Ascaris*, it was soon found that the influence of contact in the production of reagins was so great that they seemed to be developed regardless of the hereditary background. Evidence of similar reaginic substances has since been found in the serum of other species, such as spontaneously sensitized dogs and cattle and experimentally sensitized guinea pigs and rabbits, so they are no longer considered limited to man.

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I believe that the reagin has come to be considered a type of antibody, more by an extension of the limits of the term *antibody* than by any more typical features that have been observed as time went on. Whether all the antibodies capable of passively sensitizing human skin should be considered reagins is a matter of opinion. For that reason, I prefer to use the simple descriptive term *skin-sensitizing antibody*, rather than *reagin* or *reaginic antibody*.

As you all know, the first evidence of antibodies in human allergy was offered by Ramirez² in 1919, when he reported the passive transfer of horse asthma by a transfusion from a patient suffering from horse asthma. This same phenomenon of transfer of sensitivity by transfusion has been more intensively studied by Loveless³ and others, and it has been shown definitely that when blood from a hay fever patient is transferred into a normal person, the active principle disappears from the blood stream in a relatively few hours, but the skin and eye test become positive and remain so for many weeks, suggesting that the antibody has a marked affinity for these tissues and that its presence in these tissues causes their reaction to the antigen.

The more familiar phenomenon of local passive transfer was described in 1921 by Prausnitz and Küstner,⁴ who showed that if serum from an allergic patient is injected intracutaneously into a normal skin, a sharply defined local sensitization was produced. This phenomenon is generally demonstrable with the sera of patients showing well-marked immediate-type urticarial skin reactions, and is the basic method of demonstrating and studying the skin-sensitizing antibody. The only way we can attempt to determine the amount of such antibody is by testing progressive dilutions of the serum and noting the end point at which reaction no longer occurs. Since this depends on the biologic reactivity of the skin of the test subject, which is quite variable, the method lends itself more to comparisons than to absolute measurements.

Concerning the mechanism of the reaction between antigen-antibody, very little can be said except that the more dilute the serum, the higher the concentration of antigen required to produce a detectable reaction.

Fig. 1 represents the results of studies on five test subjects, with a single highly potent anti-castor bean serum. The dotted line and the dots (in which there is a great deal of overlapping) represent the actual observations on five different test subjects, and I have rather arbitrarily drawn what seems to be a reasonable representation of the mean. It is fairly close to a straight line at first and then, as the end point of the serum is approached, all the lines go up off the edge of the graph and thus the mean curves up.

It is equally important to point out that in these five test subjects sensitized with one serum, we find that in one case the end point was 1:100; in two cases it was 1:1,000; and in two cases it was 1:10,000. This sort of variability in different individuals is typical of this phenomenon, so that comparison of two sera on the same subject are more significant than absolute titers.

We believe that the antigen-antibody reaction causes a release of histamine and that the released histamine causes the actual physiologic reaction in the skin. This, obviously, introduces a number of variable factors, which are not the same in every test subject.

The reaction between the antigen and the antibody uses up a certain amount of antibody. After a sufficiently strong reaction, the site is exhausted or neutralized. This is apparent if the same site is tested on successive days with strong concentrations of the same antigen. However, this is not a very satisfactory quantitative procedure because of the difficulty of being sure that the antigen injected in the tests reaches all parts of the sensitized site.

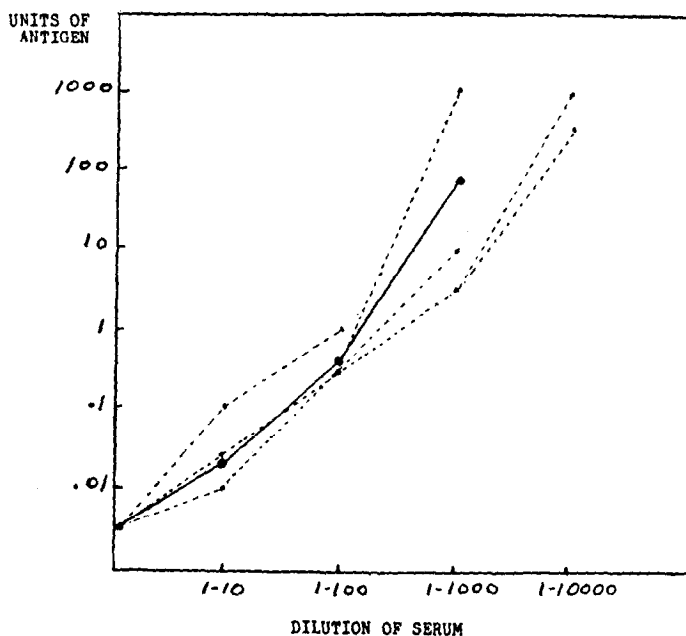


Fig. 1.—Passive transfer tests on five normal subjects with the same anti-castor bean serum. The points represent the lowest concentration of antigen producing a positive reaction.

On the other hand, if a really large excess of antigen is injected into a distant part of the body, or if the test subject eats the food to which the serum is highly reactive,⁵ one reaction may occur which completely neutralizes the antibody.

If the sensitizing antibody is mixed with varying amounts of the antigen before it is injected into the skin, subsequent testing with an excess of the antigen shows a rather sharply defined end point or neutralization point (Table I). Disregarding for the moment the dilution tests at the top of the table, it is apparent that in this particular ragweed-sensitive patient the neutralization point of the serum before treatment was 100 units; the second serum, 1,000 units; and the third sample of serum, somewhere between 300 and 1,000 units. Thus, we can measure, in a roughly quantitative way, the amount of antigen required to neutralize the antibody of a particular sample of serum.

As to the action of this component of the serum in the patient himself, we believe, from the transfusion experiments and from the fact that there is at least some correlation between the direct skin test and the content of skin-sensitizing antibody in the serum, that the same antibody which is present

TABLE I. DILUTION AND NEUTRALIZATION TESTS ON SERA OF RAGWEED-SENSITIVE PATIENT AT VARIOUS STAGES OF INJECTION TREATMENT

DILUTION OF SERUM	SERUM 1	SERUM 2	SERUM 3
	BEFORE TREATMENT	AFTER 2 MONTHS	AFTER 12 MONTHS
DILUTION TESTS			
1-100	++	++	+
1-300	+	+	+
1-1,000	0	+	0
1-3,000	0	0	0
NEUTRALIZATION TESTS			
UNITS OF ANTIGEN			
30 units	++	++	++
100 units	±	++	++
300 units	0	+	+
1,000 units	0	0	±
3,000 units		0	0
Saline control	+++	+++	+++

in the serum occurs in the skin and mucosa of the allergic person and is responsible for the occurrence of clinical symptoms or skin tests. Bradel⁶ has shown that fluid expressed from nasal polyps of allergic patients contains sensitizing antibodies in concentrations as high as 40 times that present in the serum of the same patient. On the other hand, attempts to recover skin-sensitizing antibody from skin and other tissues than nasal polyps have generally failed.

The human serum capable of passively sensitizing skin does not contain precipitins and does not produce passive anaphylaxis in the guinea pig. Evidence as to whether this skin-sensitizing antibody coprecipitates with antigen and a precipitating serum is confused and inconclusive. Complement fixation with such serum has been reported by Gyorgy, Moro, and Witebsky⁷ and, more recently, by Cavelti⁸ but the amounts of complement fixed have been too small for measurement and the reaction, in general, is not sufficiently constant for practical use.

It formerly was said that skin-sensitizing antibody was not demonstrable outside the body. In view of the work of Katz and Cohen,⁹ this statement obviously must be modified to say that it is not demonstrable in the absence of living cells, for Katz and Cohen showed that the addition of specific antigen to human allergic blood causes a release of histamine from the blood cells into the plasma. This phenomenon has also been studied by Noah and Brand.¹¹ Spain and his colleagues¹⁰ performed similar experiments, using human allergic serum with rabbit blood as a source of bound histamine.

One of the best-known characteristics of the skin-sensitizing antibody is its thermolability. Activity of serum is destroyed by heating at 56° C. for one-half to four hours and is not restored by the addition of complement. This is of great importance in separating the skin-sensitizing and blocking antibodies; otherwise, I am not sure how characteristic a feature it is.

Eight years ago at the meeting of this Academy, we reported a comparison of the effects of heating for four hours at 56° C. on human and rabbit anti-horse sera which contained both skin-sensitizing antibodies and precipitins.

After heating, the human serum lost both its skin-sensitizing activity and its precipitin activity as measured by the antigen-dilution method. The rabbit serum showed no change in either of these activities. Thermolability, therefore, appeared to be a species characteristic rather than a unique property of the skin-sensitizing antibody or reagin.

I have already mentioned the skin-sensitizing antibody's rather marked affinity for skin and other tissues. In local passive transfer, antibody injected intracutaneously will remain fixed for a period of several weeks without spreading more than 1 cm. in any direction, as anyone who attempts to do these experiments will find out. If he does not mark the site of the sensitizing injection very carefully, he can miss it completely when he makes the test. Unlike most human antibodies and unlike the anaphylactic antibody of guinea pigs, the skin-sensitizing antibody does not pass through the placenta from the maternal to the fetal circulation. This was shown by Bell and Erickson.¹³ In later experiments at Roosevelt Hospital,¹⁴ we also found that the placental barrier effectively withheld the skin-sensitizing antibody in forty-seven out of fifty cases, while hemagglutinins, typhoid agglutinins, and diphtheria antitoxin passed through freely. The three exceptions were apparently due to defects in the placenta. In the vast majority of cases, the skin-sensitizing antibody did not, to any extent, pass through from the maternal to the fetal circulation. It was natural to try to relate this impermeability of the placenta to the well-recognized affinity of the skin-sensitizing antibody for tissues, but actually it was impossible to recover any of the skin-sensitizing antibody from the placental tissue.

Concerning the chemical nature of the skin-sensitizing antibody, there is very little to tell. By the older methods of salting out proteins, it was found to occur in the pseudoglobulin. Vaughan and co-workers¹⁵ applied Cohn's method of fractionation with alcohol and found that, while skin-sensitizing activity was not entirely limited to any one fraction, it occurred chiefly in Fraction III_d, which consists mostly of beta globulin.

Numerous attempts have been made to identify the skin-sensitizing antibody with one of the main protein components by various methods of electrophoresis. Most workers have found that activity is not limited to any single component, but in various cases it has been found to be present in alpha₂, beta, or gamma globulin, depending on the particular procedures used.¹⁶ It is apparent that the skin-sensitizing antibody differs in this respect from most other human antibodies, which occur only in the gamma globulin.

I have already mentioned that it is now generally believed that the natural development of skin-sensitizing antibodies depends upon exposure of the susceptible individual to antigen. Further exposure to the antigen in the injection treatment of hay fever usually produces a rise in the antibody titer during the first two or three months. The changes, in most cases, are from threefold to tenfold, as shown in Table I.

In general, the injection of pollen antigens into nonallergic persons does not produce skin-sensitizing antibody. However, such antibodies are readily produced by the injection of heterologous antisera and *Ascaris* antigen. Kuhns and Pappenheimer¹⁷ have shown that the injection of diphtheria toxoid may produce a skin-sensitizing type of diphtheria antitoxin, particularly in patients with a hereditary tendency toward allergic disease.

Antibodies capable of sensitizing human skin in the same manner as human allergic antibodies may be produced in rabbits and guinea pigs by injections of pollen extract, horse serum, and egg albumin and various other antigens.

The skin-sensitizing activity of the guinea pig antisera is not correlated with the titer of anaphylactic antibody. Table II shows a number of guinea pig antiovalbumin sera, of which the anaphylactic titer in sensitizing doses per milliliter was very carefully determined by Dr. Coulson in the Department of Agriculture. These titers varied from 40 to 400. Five of these nine sera sensitized human skin very well. The serum showing a large titer of anaphylactic antibody did not, and it is obvious that there is no quantitative relationship between the anaphylactic antibody titer and the skin-sensitizing titer.¹⁸

TABLE II. SKIN-SENSITIZING AND ANAPHYLACTIC ACTIVITY OF SERA OF GUINEA PIGS SURVIVING CHALLENGING DOSES OF OVALBUMIN

GUINEA PIG NO.	SENSITIZ- ING DOSE,* MG.	INTERVAL BEFORE CHALLENGE	CHALLENG- ING DOSE,† MG.	INTERVAL BEFORE BLEEDING, DAYS	ANAPHY- LACTIC TITRE	SKIN SENSITIZ- ING TITRE
160	.02	6 wk.	.077	6	40	1-100
195	.02	6 wk.	.260	6	100	0
180	.3	6 wk.	.173	11	133	0
181	.3	6 wk.	.115	11	100	1-10
706	.3	8 wk.	.39	13	130	1-10
	s. c.‡	18 days	1.0	11	200	0
707	.3	8 days	.59	13	400	0
711	.3	8 days	.26	13	200	1-100
	s. c.	18 days	1.0	11	130	1-10

*Alum precipitated ovalbumin subcut.

†Intrav., in saline sol.

‡s. c. = second challenge.

(From Sherman and Coulson: *Proc. Soc. Exper. Biol. & Med.* 77: 245, 1951.)

The same is true of precipitin titers in rabbits sensitized with egg albumin. Table III shows a series of rabbits sensitized with alum-precipitated egg albumin and a series of rabbits sensitized with soluble egg albumin. There was not much difference in the range of precipitin content of the two groups, but all those treated with precipitated antigen sensitized human skin; none of those treated with soluble antigen did so. Without going into the possible relationship to the way in which they are sensitized, it is apparent that there is no quantitative relationship between the precipitin content and the sensitization of human skin.¹⁹

From two rabbit antiovalbumin sera, both of which sensitized human skin, the precipitin was quantitatively removed by small stepwise additions of antigen. Removing the precipitating antibody in this manner did not affect the sensitizing power of the antisera for human skin.

TABLE III. ANALYTICAL AND IMMUNOLOGICAL DATA ON RABBIT ANTISERA

SERUM NO.	SERA OF RABBITS IMMUNIZED WITH ALUM-PRECIPTATED ANTIGEN				
	PRECIPITIN N (MG. PER ML.)	VALUE OF R CALCULATED	REACTIVITY ON PASSIVE TRANSFER TO HUMAN SERUM DILUTED:		
			UNDILUTED	1:10	1:100
9	0.258	7.5	+++	++	±
10	0.381	6.1	++	+	—
11	0.358	9.0	+	+	—
12	0.595	9.3	+	+	—
13	0.531	10.2	+	+	—
14	0.604	10.0	++	++	—
15	0.338	9.0	+++	+++	+++
16*	0.553	8.9	+	—	—
130	0.170	8.4		++	±
132	0.047	7.5		+++	++
135*	0.071	10.0		++	++
164*	0.102	10.6	±	±	±
165*	0.087	7.5	++	+	+
191	0.083	9.1	+		

SERUM NO.	SERA OF RABBITS IMMUNIZED WITH DISSOLVED ANTIGEN				
	PRECIPITIN N (MG. PER ML.)	VALUE OF R CALCULATED	REACTIVITY ON PASSIVE TRANSFER TO HUMAN SERUM DILUTED:		
			UNDILUTED	1:10	1:100
1*	0.088	6.7	—		
3	0.420	7.4	—		
4	0.446	8.8	—		
5	0.193	6.2	—		
8	0.359	8.4	±		
2	0.05†		—		
6	0.05†		—		
154	0.05†		—		
155	0.05†		—		

*Analysis at two antigen levels only.

†No analysis performed on these sera; values estimated from qualitative sera.

(From Sherman, Menzel, and Seeborn: *J. Exper. Med.* 92: 191, 1950.)

As many of you know, Vaughan and Kabat²⁰ have pointed out that there is some doubt as to whether this crystalline ovalbumin is a pure enough antigen for this type of experiment and that this may not be the reaction of the pure antigen, but of a number of trace impurities. In any case, it appears that the precipitin does not carry this skin-sensitizing factor.

While the skin-sensitizing antibodies artificially induced by injections of antigens into animals are highly specific, the specificity of naturally occurring skin-sensitizing antibodies is rather vague. Perhaps this is to be expected, as they are formed as the result of casual contact with many different antigens. In passive transfer, it is common to find sera reacting with many different pollens. Do they possess a multitude of different skin-sensitizing antibodies, or does one antibody react with many different antigens? The answer is not easy.

Cross-neutralization experiments with the sera of patients with multiple allergies show many cross reactions to unrelated antigens. For example, in the case of a patient who was clinically very sensitive to dog, but also gave good passive transfer reactions to horse, the addition of dog epithelium extract neutralized the reaction both to dog and horse, whereas an attempt to neutralize with horse epithelium did not affect the reaction to either one.

TABLE IV. A COMPOSITE OF PASSIVE TRANSFER STUDIES SHOWING CROSS NEUTRALIZATION REACTIONS WITH ANTETREATMENT SERUM (WOL.)*

SERUM MIXED WITH	SERUM TESTED WITH									
	L. RAG.	SORREL	PLANT.	COCKLE.	OAK	HICK.	BIRCH	POPLAR	ASH	TIM.
Low ragweed	0	+	+	0	0	0	0	0	0	0
Sorrel	+++	0	±	+	±	0	0	0	0	0
Plantain	+++	++	++	++	++	±	±	0	±	0
Cocklebur	++	++	++	0	++	+	+	±	0	0
Oak	+++	++	++	+	±	+	0	+	0	0
Hickory	+++	++	+++	+	++	±	0	±	±	0
Birch	+++	++	+++	++	+++	++	+	+	++	+
Poplar	+++	++	+++	++	++	++	++	+	++	+
Ash	+++	++	+++	++	++	+	+	±	+	+
Timothy	+++	++	+++	++	+++	++	+	±	++	±
Control	++++	++++	++++	+++	+++	++	++	+	++	+
Dilution titer	±3000	±3000	+1000	+1000	±1000	±1000	±1000	±300	+100	+100

*This table is arranged in the order of the dilution titer; that is, the reactivity as determined previously by passive transfer tests with the same allergens in serum sites of various dilutions.

This serum diluted 3,000 times still gave slight reactions with extracts of low ragweed and sorrel, whereas with ash and timothy extracts no reaction was obtained after 100 fold dilutions.

(From Cooke: *J. Allergy* 15: 212, 1944.)

In the reverse situation, in which the patient was clinically very sensitive to horse but gave a moderate passive transfer reaction to dog, attempts to neutralize with dog produced little or no effect, whereas the horse antigen neutralized the reactions to both dog and horse.

In a third case in which the patient was equally sensitive to both dog and horse, each antigen neutralized to itself and did not neutralize to the other.²¹

To show the extent to which cross-neutralization may occur, Table IV shows a study of the serum of a patient sensitive to ten different pollens, which are arranged down and across, roughly in the order of the reactivity to each one. The serum was neutralized with each separate antigen, and then sites were tested with each one. There was a tremendous number of cross reactions and, in general, all those giving strong reactions tended to neutralize to the ones giving weaker reactions.²²

This reaches a point where it is really very hard to tell whether or not this represents a truly specific immunologic phenomenon.²³ However, there is one other approach to the problem. This has to do with the cases of patients whose serum reacts to two unrelated pollens, but in which we treat the patient

TABLE V. DILUTION TESTS ON WOL. SERUM BEFORE (A) AND AFTER (P) INJECTIONS OF RAGWEED POLLEN EXTRACT

DILUTIONS	REACTION FROM TEST WITH	
	LOW RAGWEED 1,000	PLANTAIN 1,000
<i>Serum A (6-13-39)</i>		
1-500	++	+
1-1000	±	0
1-2000	0	0
1-4000	0	0
<i>Serum P (8-23-39)</i>		
1-500	+++	++
1-1000	++	+
1-2000	+	±
1-4000	±	0

(From Sherman: *J. Immunol.* 40: 289, 1941.)

with only one of these pollens. Not infrequently, as shown in Table V, treatment with only one of the two pollens produces a roughly parallel increase in the amount of skin-sensitizing antibody to both pollens. The serum of this patient, before treatment with ragweed, reacted in a dilution of 1:1,000 and, after treatment, reacted to ragweed 1:4,000. The patient did not receive treatment with plantain, but the titer for plantain went from 1:500 to 1:2,000, at least suggesting that possibly the same antibody may have reacted with both pollen antigens.²⁴

When the patient with hay fever is treated with injections of antigen, the variations in the amount of skin-sensitizing antibody as determined by the dilution test and the neutralization point do not run parallel (Table I). Generally, at the early months of treatment, there is an increase in the amount of skin-sensitizing antibody by the dilution test, but the changes in the neutralization test are usually far out of proportion to those in the titer by the dilution test. Furthermore, with longer treatment, the titer in the dilution test often falls back to its original level, whereas the titer in the neutralization test continues to rise or stays at the same level.

TABLE VI. INHIBITION OF NEUTRALIZATION BY HEATED SERUM OF A TREATED HAY FEVER PATIENT

SITE	SITES PREPARED WITH MIXTURE OF	REACTION ON RETEST WITH LR 1,000
1	Nam. A serum (heated) + Hit. A serum + LR 25	+
2	Nam. A serum (heated) + Hit. A serum + LR 50	+
3	Nam. A serum (heated) + Hit. A serum + LR 100	0
4	Nam. A serum (heated) + Hit. A serum + LR 200	0
5	Nam. A serum (heated) + Hit. A serum + Saline	++
6	Nam. P serum (heated) + Hit. A serum + LR 25	++
7	Nam. P serum (heated) + Hit. A serum + LR 50	++
8	Nam. P serum (heated) + Hit. A serum + LR 100	+
9	Nam. P serum (heated) + Hit. A serum + LR 200	±
10	Nam. P serum (heated) + Hit. A serum + Saline	++
11	Normal serum (heated) + Hit. A serum + LR 25	+
12	Normal serum (heated) + Hit. A serum + LR 50	+
13	Normal serum (heated) + Hit. A serum + LR 100	0
14	Normal serum (heated) + Hit. A serum + LR 200	0
15	Normal serum (heated) + Hit. A serum + Saline	++

A serum = before treatment.

P serum = after treatment.

LR = low ragweed pollen extract.

(From Sherman: *J. Immunol.* 40: 289, 1941.)

Twenty years ago, at a meeting of this Academy, Cooke, Barnard, Hebard, and Stull²⁵ presented evidence that this difference between the changes in the dilution and neutralization tests, as a result of treatment, involved the development of a blocking or inhibiting type of antibody, distinct from the skin-sensitizing antibody and competing with it for the same antigen.

Study of the blocking antibody was greatly facilitated by the observation by Loveless²⁶ that it was thermostable and remained active when the skin-sensitizing antibody was destroyed by heat. When blocking antibody is thus separated from skin-sensitizing antibody, or the skin-sensitizing antibody is inactivated by heat, its presence may be readily demonstrated by a number of

methods. For example, if the heated serum thought to contain blocking antibody is mixed with unheated serum and ragweed in the neutralization procedure, the neutralization point is changed by the inactivation of a certain amount of the antigen by the blocking antibody.

In the middle of Table VI blocking antibody has been added to the system; above is a similar test with the serum from the same patient before treatment; and below is a test with a normal serum. It is apparent that the neutralization point is altered by the addition of the blocking antibody. Equally important is the fact that the serum taken before treatment does not contain any blocking antibody; the blocking antibody appears to be a different antibody, produced as a result of the treatment, and not an altered form of the sensitizing antibody produced by the heating of the serum.

This blocking activity can be demonstrated more directly by simply mixing the blocking serum with the antigen and testing the mixture in various dilutions on skin-sensitized skin sites. Table VII shows the effect of testing a series of uniform, passively sensitized skin sites with a mixture of antigen and serum taken before treatment with ragweed; serum from the same patient after treatment with ragweed; and normal serum, all of these sera being heated for four hours at 56 degrees and then mixed with the ragweed antigen. It is apparent that the blocking antibody inhibits the reaction of the sensitizing antibody and the antigen, and also that the serum of the patient taken before treatment does not contain blocking antibody.

TABLE VII. INACTIVATION OF RAGWEED POLLEN EXTRACT BY HEATED SERUM OF TREATED HAY FEVER PATIENT

UNITS	AHE. A SERUM HEATED + LR	AHE. P SERUM HEATED + LR	NORMAL SERUM HEATED + LR
1	++	0	++
1-3	+	0	+
1-9	+	0	+
1-27	+	0	+
1-81	±	0	±
1-243	0	0	0

(All sites prepared with Hyl. serum A (1-10), tested after two days with mixtures of heated serum and ragweed extract.)

A serum = before treatment.

P serum = after treatment.

(After Sherman: *J. Immunol.* 40: 289, 1941.)

The blocking antibody also does not form a precipitate with antigen. It does not produce passive anaphylaxis in guinea pigs and it does not fix measurable amounts of complement. In other respects, however, it does show a somewhat more orthodox behavior than the skin-sensitizing antibody. It can be readily produced in nonallergic patients by injection of pollen extract, although the doses required may be somewhat higher than for routine treatment of hay fever.

Unlike the skin-sensitizing antibody, but like most other human antibodies, it readily passes through the placenta from the maternal to the fetal circulation.

Menzel and others²⁷ at Roosevelt Hospital have recently completed a paper showing that, like most antibodies, it migrates with the gamma globulin in the Tiselius electrophoresis.

Its specificity apparently corresponds to that of most other antibodies produced by injections of antigen.

Bukantz and his associates²⁸ have shown that the blocking antibody of treated hay fever patients inhibits the precipitation of ragweed antigen and rabbit antiragweed precipitating antibody.

Portnoy,²⁹ in our laboratory, found that it likewise inhibited the fixation of complement by rabbit antiragweed serum.

While both of these reactions are of interest in avoiding the usual passive transfer method, they turn out to be less sensitive than the passive transfer method, so they are not a practical substitute.

The most important question about the blocking antibody is the one that is most difficult to answer. Does it protect the patient against hay fever? We have already noted the blocking antibody appears to bind and inactivate antigen whenever it is mixed with it, and the injection treatment has not been shown to produce any other immunologic change that might be expected to protect the patient against hay fever. I think that there is good evidence that the tolerance of the patient for progressively larger injections of pollen extract may be correlated with the development of blocking antibody, which protects the patient against constitutional reactions.

It is important to remember that the constitutional reaction occurs when the antigen spreads through the blood stream from the site of injection to various shock organs. During this passage through the blood stream, the blocking antibody has ample opportunity to inactivate the antigen. In clinical hay fever, on the other hand, the pollen reaches the sensitized nasal membrane directly, and the question is: does the blocking antibody have any chance to inactivate it?

This is illustrated, to some extent, by another method of demonstrating blocking antibody which was described by Maunsell.³⁰ She took serum from the patient and mixed it with pollen antigens in various dilutions, made up comparable control mixtures containing the same amount of antigen in saline, and compared these two in tests on the patient's own skin. There were differences in reaction produced by the two mixtures, indicating the presence of blocking antibody in the serum. This illustrated that the blocking antibody was capable of inactivating the antigen if it was mixed with it, but the presence of blocking antibody in the patient's blood stream did not prevent the skin reaction when the antigen-saline mixture was injected. By the same token, one might wonder whether blocking antibody in the blood stream would protect the nasal mucosa in direct contact with pollen.

You all recall many conflicting reports in previous meetings of this Academy as to the relationship of blocking antibody titers to clinical protection. I think it is safe to say that there has been no significant correlation between blocking antibody titers and clinical results. If the blocking antibody is the protective factor—and it may well be—the fact still remains to be proved.

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DISCUSSION

CHAIRMAN FEINBERG.—On one feature of Dr. Sherman's work, I think that he is correct but misinterpreted. That has to do with the exhaustion phenomenon by dissimilar antigens in a patient who is sensitive to both antigens. We can show very readily, in passive transfer or directly on a patient who is sensitive to antigens, that we can exhaust the reactivity to one antigen and to a nonantigen-releasing substance, such as Compound 48/80.

Furthermore, we can exhaust the reactivity of the skin with a chemical histamine releaser, and we can exhaust the activity to a specific antigen to which the patient is sensitive in another area.

I believe, unless I am told differently so far as details of the experiments are concerned, that these phenomena could be explained on the basis of simple histamine depletion locally; that is, where you inject an antigen with high potency (for which there is high potency antibody), you get a marked reaction, a marked histamine release, so that the weaker antigen no longer reacts, no matter how similar it is. I would like to have you re-examine the experiments with this viewpoint in mind, if you follow me.

DR. SHERMAN.—I realize that this is a very important point in interpreting the results, and that is the reason why I showed the other approach to the method, in which you have a patient sensitive to two antigens, treat him with one, and observe the same change in the skin-sensitizing antibody titer to the one with which he is treated and the one with which he is not treated. That is not open to the same objection.

However, this does not prove that the skin-sensitizing antibody reacts with a multitude of different antigens. It may or it may not. I think it is quite obvious that the skin-sensitizing antibody, from the method of its formation in a patient exposed to many different antigens, may be quite different from antibodies which result from one or several specific stimulations with a pure, or at least a single, antigen.

DR. MARY LOVELESS.—I would just like to bring up the work of Bowman and Walzer with reference to the cross reactions that Dr. Sherman has talked about, because I repeated it and found it to be completely reproducible; in other words, if you have a strong whealing reaction in a skin area, as you would have with the so-called dominant clinical allergen, and then you cross-test this with a weaker one, that site is exhausted and will be definitely hyposensitive, so you get a much smaller wheal. I think that explains it, don't you?

DR. SHERMAN.—Yes, to a large extent.

DR. LOVELESS.—The other thing is the blocking antibody. I think the transfusion experiments which, unfortunately, I did not publish showed quite conclusively that transfusion of large amounts of blocking serum taken from a normal subject who had been immunized to pollen were promptly protective against acute hay fever, and this lasted about the expected time—ten days or so.

The blocking antibody of treated patients does accumulate in the conjunctiva and the nasal membranes, as well as the skin, and, if one does delicate threshold testing, this will definitely heighten the tolerance of all these tissues, and I believe that this is a very logical finding, to fit in with their clinical heightened tolerance.

It is true that the circulating blocking antibody would not have a chance to affect the patient's clinical behavior, but it is the tissue antibody-blocking antibody which does so.

DR. SHERMAN.—I should like to point out that, in my opinion, the type of delicate skin and eye test that Dr. Loveless is describing merely reflects the fact that the patient is not as sensitive to the pollen as he was before his treatment. As for the assumption that this is due to blocking antibody in the tissues, that is merely one good explanation. I think that it would be necessary to get the blocking antibody out of the tissues in order to consider it a definitely proved fact.

DR. LOVELESS.—Well, I mean by transfusion. He did not have it before and this definitely altered the skin and conjunctival susceptibility, which I think is good enough evidence that that is what happened. Also, you find a correlation, crudely, between the amount of increased tolerance in the eye, nose, and skin, along with the deposition of circulating antibody. This could be presumptive evidence that there is some connection.

DR. MATTHEW WALZER.—Has Dr. Sherman, from his experience with reagin, observed the phenomenon that Dr. Siegel demonstrated this morning, in which saline dilution impaired the sensitizing property of the antigen?

DR. SHERMAN.—By our method, we would not observe that, because invariably we have diluted them with saline. Therefore, we would not detect that type of change.

DR. WALTER WINKENWERDER.—In the natural history of hay fever, there are a few patients who have a clinical remission spontaneously without the history of any desensitization. That has always confused me with regard to interpreting what has occurred in terms of the blocking antibody. I wonder whether Dr. Sherman has any explanation for that.

DR. SHERMAN.—It is hard enough to say whether or not the blocking antibody is the factor when the change occurs as the result of treatment. Obviously, it is not the factor when it occurs without treatment. There are probably dozens of factors that affect the patient's reaction to pollen, and the blocking antibody is one which may. We have not yet admitted that it is conclusively proved that it does, but there are, obviously, others that definitely do.