

Original Articles

ANTIBODIES TO RAGWEED EXTRACT

DEMONSTRATION BY COLLODION PARTICLE TECHNIQUE IN IMMUNE RABBIT
SERUM BUT NOT IN SERUM OF TREATED PATIENTS*

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NEW avenues for investigating the immunology of pollen hay fever have been opened by the recognition of the thermostable or blocking antibody.¹ Additions to the knowledge of this antibody have been retarded because present methods for analysing it are crude and cumbersome.²⁻⁹ A simple in vitro test is needed for more detailed study of this antibody. Such a test would, also, provide new approaches to the standardization and purification of pollen and other extracts, and to the identification of allergens and allergenic relationships. An attempt to devise such a test was prompted by the preliminary report of Cohen and Weller.¹⁰ They described the demonstration of precipitins in the sera of treated hay fever patients but not in the sera of untreated pollen-sensitive patients. Lowell¹¹ had demonstrated agglutinins in the sera of rabbits immunized with ragweed extracts.

This is the report of experiments which confirm the observations of Lowell but do not confirm those of Cohen and Weller. It is of interest that Lowell¹² also was unable to demonstrate specific agglutinins in the sera of treated ragweed hay fever patients, with the collodion particle technique used by Cohen and Weller.

METHODS AND RESULTS

General Methods.—Collodion particles were prepared as described by Cannon and Marshall.¹³ The stock suspension used throughout these experiments was of such a concentration that 1.0 ml., when diluted with 8.0 ml. of triply distilled water, had a turbidity equal to that of a MacFarland nephelometer No. 5. All extracts were prepared from a mixture of equal parts of giant and dwarf ragweed pollen.

Collodion particles were mixed with pollen extracts and incubated for varying periods of time at various temperatures. The sensitized particles were washed and adjusted to the standard turbidity. They were then added to serial dilutions of sera, incubated, and centrifuged. The degree of agglutination was determined in the usual manner by resuspension of the sedimented particles. Parlodion particles, similarly prepared, were used in numerous experiments instead of collodion.

No consistent difference could be shown between sera from normal and from untreated and treated ragweed-sensitive individuals, although the identical procedures described by Cohen and Weller¹⁴ were used. In an attempt to explain this discrepancy, a detailed study of the variable factors which might

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effect the results was carried out. More than 230 experiments with nearly 2,000 separate agglutination tests were performed with sera from 50 normal, 32 untreated, and 30 treated ragweed-sensitive patients. Each normal person had a negative skin reaction and a negative history of ragweed hay fever. Inasmuch as specific agglutinins were not demonstrated by any of these experiments, they will not be reported in detail. The variable factors which were investigated are listed below.

I. Variations in the Preparation of Ragweed Extracts

1. Ten per cent ragweed pollen (weight by volume) in isotonic saline.
 - A) Ph adjusted to 5.6, 6.0, 7.0, 7.9.
 - B) Precipitated by ammonium sulfate at Ph 5.0 and 3.0.
 - C) Heated to 60° C. and 37° C. for one hour at Ph 7.0.
 - D) Heated to 80° C. and 100° C. for twenty minutes at Ph 7.0.
 - E) Precipitated by HCl and redissolved in N/30 NaOH in 1 per cent NaCl.
 - F) Concentrated to 1:10 volume, then dialysed against distilled water.
 2. Ten per cent defatted ragweed pollen in isotonic saline:
 - A) Ph varied.
 - B) Concentrated and dialysed.
 - C) Precipitated with HCl and redissolved in physiologic saline and in alkali.
 - D) Precipitated with alcohol and redissolved in saline.
 3. Various other concentrations of untreated ragweed pollen in saline, e.g., 0.1 per cent, 1.0 per cent, 2.0 per cent, 5.0 per cent, 20 per cent.
 4. A similar variety of concentrations of extracts of defatted ragweed pollen in saline.
 5. Phosphate buffered saline extracts.
 6. Distilled water extracts of defatted and of untreated pollen, 5 per cent, 10 per cent, 20 per cent, 40 per cent weight by volume.
- None of these variables affected the equivocal results of the preliminary experiments.

II. Use of Particles Other Than Collodion

1. Parlodion particles, *Chromobacterium prodigiosum* (Alperstein), red blood cells type O, and Kahn antigen were used instead of collodion particles. Agglutinations of each of these sensitized particles was nonspecific and unpredictable⁷ with human sera. Parlodion and collodion particles behaved comparably in numerous tests.

III. The Effect of Repeated Washing of Sensitized Particles

Washing sensitized particles one, two, three, and four times with isotonic saline, triply distilled water, and phosphate buffered saline had no effect on agglutination with any of the sera.

IV. Variation in Time and Temperature Used for Sensitization

1. One, four, eight, and sixteen hours at 37° C. and 4° C.
2. Four hours at room temperature.
3. One hour at 60° C.
4. Ten and thirty minutes at room temperature.

V. Variations in Treatment of sera.

Sera from normal persons and from treated and untreated ragweed patients were subjected to the following influences within a few days after collection.

1. Heated at 60° C. for one hour.
2. Heated at 37° C. for one hour.
3. Room temperature.
4. Adsorbed by untreated collodion particles at 37° C. for one hour.
5. Several accidentally contaminated sera before and after passage through Seitz filters. The contaminated sera agglutinated the particles in high dilution. Seitz filtration removed the agglutinating factor.
6. Globulin was precipitated from sera and dialysed.

Discussion of the results: A typical experiment using sensitized parlodion particles and sera from normal and from treated and untreated ragweed sensitive individuals is outlined in Table I. It is obvious that the results were not

TABLE I. AGGLUTINATION OF RAGWEED SENSITIZED* PARLODION PARTICLES BY SERA FROM NORMAL HUMANS AND FROM TREATED AND UNTREATED CASES OF RAGWEED POLLINOSIS

INITIALS OF SUBJECTS	TYPE OF SERUM	DEGREE OF AGGLUTINATION† DILUTIONS OF SERUM							
		1/4	1/8	1/16	1/32	1/64	1/128	C ¹	C ²
IR, GAS, COP NE	Normal	0	0	0	0	0	0	0	0
STE, BAI, HO	Normal	±	0	0	0	0			
PAR, DAW	Normal	±	±	0	0	0			
SIM, DEW, WRI	Normal	+	0	0	0	0			
EVA, GOO	Normal	+	+	±	0	0			
FRE	Normal	+	±	0	0	0			
HOP	Normal	1+	±	0	0	0			
HOU	Normal	+	+	+	0	0	0	0	0
BAL	Normal	1+	±	±	0	0	0	0	0
HEC	Normal	2+	1+	+	0	0			
BRU	Treated	0	0	0	0	0	0		0
BEO	Treated	0	0	0	0	0	0		0
LEI	Treated	0	0	0	0	0	0		0
HAR	Treated	±	0	0	0	0	0		0
ALL	Treated	1+	±	0	0	0	0		0
COL	Treated	1+	+	0	0	0	0		0
GJE	Treated	2+	+	±	0	0	0	0	0
KIR	Treated	1+	1+	1+	+	0	0	0	0
ALP	Untreated	0	0	0	0	0	0		0
DIL	Untreated	+	±	0	0	0	0		0
MAN	Untreated	1+	±	0	0	0	0		0
NOR	Untreated	1+	1+	+	0	0	0		0
FRA	Untreated	1+	1+	1+	±	0	0		0
CAR	Untreated	2+	1+	+	+	±	0		0
HAN	Untreated	4+	3+	3+	2+	1+	+	0	0

C¹ = unsensitized particles in serum; C² = sensitized particles in saline.

*Particles sensitized with a 20 per cent saline extract of pollen for four hours at 0° C. Incubated overnight at 4° C. before reading agglutination.

†++ = marked agglutination; ± = weak agglutination of particles.

consistent. Four of 19 normal human sera did not agglutinate sensitized particles, five agglutinated them very weakly, and 10 caused definite agglutination. Agglutination was caused by four of the eight treated sera and by six of the seven untreated sera. Similarly, inconsistent results were obtained in all of the experiments in which human sera was used.

Experiments With Immune Rabbit Serum.—Rabbits were immunized by repeated injections of ragweed pollen extract and bled for serum. Preliminary tests showed the presence of weak agglutinating factors in normal rabbit serum. Table II shows that unsensitized collodion particles adsorbed the nonspecific agglutinating factors from normal sera but did not affect the specific agglutinin titer of the immune sera.

This demonstration of specific agglutination of particles by immune rabbit sera shows that the ragweed antigen is actually adsorbed on the particles and that the pollen extract is antigenic. It should be mentioned that whereas Lowell¹¹ did not report nonspecific agglutination of sensitized particles by normal rabbit serum, he stated later¹²: "The normal rabbit sera I used did show

TABLE II. THE EFFECT OF ADSORPTION WITH UNSENSITIZED COLLODION PARTICLES ON THE POLLEN AGGLUTININ TITERS OF IMMUNIZED AND NORMAL RABBIT SERA

RABBIT NO.	TYPE OF SERUM	ADSORPTION* WITH UNSENSITIZED COLLODION	DEGREE OF AGGLUTINATION† DILUTIONS OF SERUM				
			1/8	1/16	1/32	1/64	1/128
5	Immune	Unadsorbed	3+	3+	4+	3+	1
5	Immune	Adsorbed	4+	4+	3+	3+	2+
4	Immune	Unadsorbed	4+	4+	3+	3+	2
4	Immune	Adsorbed	4+	4+	3+	3+	2
8	Immune	Unadsorbed	4+	4+	3+	3+	2+
8	Immune	Adsorbed	4+	4+	3+	3+	3+
3	Normal	Unadsorbed	2+	1+	1+	+	1
3	Normal	Adsorbed	0	0	0	0	0
N	Normal	Unadsorbed	1+	1+	±	+	0
N	Normal	Adsorbed	0	0	0	0	0
N	Normal	Unadsorbed	1+	±	±	±	0
N	Normal	Adsorbed	1+	0	0	0	0

*0.5 c.c. of sera adsorbed one hour at 37° C. with particles sedimented from 1 c.c. of stock collodion suspension.

†Collodion suspension sensitized overnight at 4° C. with equal amount of 20 per cent aqueous extract of defatted pollen.

TABLE III. AGGLUTINATION OF POLLEN-SENSITIZED COLLODION PARTICLES BY ADSORBED* HUMAN SERA

TYPE 1 PER CENT POLLEN EXTRACT	TYPE OF SERUM	NUMBER TESTS	NUMBER SERA	NUMBER OF SERA WITH TITERS AT DILUTIONS OF SERUM							
				1/8	1/8	1/16	1/32	1/64	1/128	1/256	1/512
5 per cent Saline	Normal	15	15				1		8	6	
defatted	Treated	13	13					4	7	1	1
	Untreated	6	6			1		3		2	
10 per cent Saline†	Normal	43	41	15		2	3	9	6	8	0
	Treated	47	34	11		1	4	15	8	8	0
	Untreated	15	13	6		1	1	5		2	
20 per cent Saline	Normal	13	13	5		1	2	2	2	1	
defatted	Treated	15	14	4		3	3	3	1	1	
	Untreated	14	14	5				5	4	2	
10 per cent defatted H ₂ O conc. dialyzed	Normal	5	5	1				2		2	
	Treated	9	9	1		1		5	2		
	Untreated	2	2	1				1			
20 per cent H ₂ O	Normal	15	12	1	1	3	4	4	2	0	0
	Treated	3	3	1	1		1				
	Untreated	2	2			1		1			
40 per cent H ₂ O	Normal	7	7			1	2	4			
	Treated	4	4					3	1		
	Untreated	2	2				1		1		

*Sera adsorbed with unsensitized collodion particles one hour at 37° C. Collodion particles sensitized at 4° C. overnight. Incubation time of test—two hours at 37° C.

†Tests with four different saline extracts combined.

agglutination of ragweed coated collodion particles in low titer. This never caused confusion, however, because the immune sera gave high titers."

Adsorption of Human Sera.—Attempts to adsorb the agglutinating factor from normal human serum as had been done from normal rabbit serum were unsuccessful. Table III illustrates this point. It also emphasizes in greater detail than Table I that there was no significant difference in the ability of sera from normal, treated, and untreated ragweed-sensitive patients to agglutinate sensitized collodion particles.

In several experiments, not given in detail, some of the pollen extracts agglutinated the collodion particles suspended in saline. This emphasizes the necessity for using controls of sensitized particles in saline in addition to the obvious use of sensitized particles in normal serum.

All sera were handled aseptically after observing the high titers of contaminated sera.

DISCUSSION

The attempt to demonstrate a simple method for titrating the thermostable or blocking antibody in the serum of patients treated for ragweed pollinosis was unsuccessful. However, the experimental data suggest several comments which should be of interest.

1. The preliminary report of Cohen and Weller,¹⁰ in which they claimed to demonstrate precipitins in treated cases of grass and ragweed hay fever, could not be confirmed. Serum from one (33 per cent) of their three untreated patients had precipitins in low titer. One of their three highest titers was obtained with the serum of an untreated patient sensitive to fish and glue. This analysis of their report, plus many experiments such as those in Table I, suggest that their conclusion that specific precipitins had been demonstrated was not justified. Their single negative normal serum control was inadequate. In the absence of evidence to the contrary it is possible that their positive reactions could have been due to serum contamination.

2. The consistent specific agglutination by immune rabbit serum (Table II) showed that ragweed extract is readily adsorbed by collodion particles and that ragweed extract acts as an antigen in rabbits.

3. The agglutination of ragweed sensitized particles by some of the human immune sera may have been due to specific antibody effect, but proof of it could not be obtained by using any of the many variables mentioned above.

4. Sera from normal and untreated ragweed-sensitive persons agglutinated sensitized particles irregularly and inconsistently. Repeated efforts to eliminate these inconstant reactions were unsuccessful. Lowell¹² reports that "all normal human sera agglutinated sensitized particles to approximately the *same* titer." He stated later¹² "I have tried again to demonstrate agglutinins in ragweed treated patients without success. However, in this series, nonspecific agglutination, though present, was irregular and much less marked than formerly—I don't know why. Perhaps the first sera were contaminated."

5. It is possible, although purely conjectural, that reagin in the serum of untreated ragweed-sensitive cases was responsible for some of the stronger agglutinations. There is no known basis for an assumption that reagin and thermostable antibody should affect sensitized collodion particles differently. Agglutination by normal human sera must be eliminated before this problem can be solved.

6. Contaminated human serum, whether from normal, treated, or untreated ragweed-sensitive persons produced marked agglutinations in high titer.

7. Normal and immune rabbit sera gave positive agglutinations in the collodion particle experiments. The nonspecific agglutinins were removed by adsorption from normal rabbit sera by unsensitized collodion particles. The nonspecific agglutinins in normal human sera could not be similarly adsorbed.

8. The different effects of human and rabbit sera on ragweed-sensitized particles has not been explained. The nonspecific agglutinating factor was not demonstrated in all the normal human serum controls. This becomes more puzzling in the absence of nonspecific human serum agglutination¹³ of collodion particles sensitized by egg white, horse serum, and insulin.

SUMMARY

1. Cohen and Weller's preliminary report of the demonstration of precipitins in the sera of treated and not in untreated ragweed-sensitive patients could not be confirmed. Reasons are given which seem to show that their conclusion was not justified.

2. Neither the thermostable antibody nor reagin could be titrated when human sera were mixed with collodion or parlodion or other particles which had been sensitized by ragweed extract.

3. Antiragweed rabbit serum agglutinated ragweed-sensitized collodion and parlodion particles consistently. Normal rabbit serum controls, properly prepared, were negative.

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