

Agglutination test with ragweed pollen

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An agglutination test was devised to study ragweed pollen grains. Rabbit antiragweed sera were shown to contain "complete" antibodies causing clumping and increased sedimentation of ragweed pollen and "incomplete" antibodies detectable by means of the indirect Coombs test. With human sera, only the indirect Coombs test was positive. The human antiragweed factor responsible for this reaction was present in 88 per cent of adult sera. There was no difference in the incidence of positive reactions observed in patients suffering from ragweed hypersensitivity and subjects without it. Evidence was presented that the antiragweed factor detectable in human sera is a serum globulin of IgM type. The possible natural-antibody character of this factor was discussed.

Saline extracts of ragweed pollen, as well as their fractions, prepared by several methods have been frequently employed for various serologic procedures including diagnostic tests.

On the other hand, whole ragweed pollen has hardly been used as a test-tube antigen. The only extensive investigation to be quoted was performed by Campbell and Sussdorf,¹ who used ragweed pollen in immunofluorescence studies with human and rabbit sera. In the present study, the possibility of employing ragweed pollen grains in an agglutination test was explored.

MATERIALS AND METHODS

Antigens

Pollens of short ragweed (*Ambrosia elatior*), birch (*Betula populifolia*), and timothy (*Phleum pratense*) were purchased from Greer Drug and Chemical Corporation, Lenoir, North Carolina. They were defatted by Soxhlet filtration with ether, dried, and stored in a desiccator at 4° C.

Sera and their fractions

(a) Antiragweed pollen sera were supplied by Dr. R. E. Reisman of the Buffalo General Hospital. They were produced by intradermal immunization of

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rabbits with a 5 per cent suspension of short ragweed pollen incorporated into complete Freund adjuvant. (b) Sera of patients with ragweed hypersensitivity were obtained from private practice and the Allergy Clinics of Buffalo General Hospital and of Children's Hospital. (c) Sera of individuals without ragweed hypersensitivity were obtained from Buffalo General Hospital and Children's Hospital. (d) Cord sera were supplied by the Obstetrical Departments of Buffalo General Hospital and Children's Hospital. (e) Sera of normal adult individuals living in France, Switzerland, and Israel were obtained through the courtesy of Dr. C. Langlois, Dr. H. Bonstein, and Dr. Z. Kantor, respectively. (f) Cohn's Fractions II and V of pooled normal human serum were commercial products of Pentex, Incorporated, Kankakee, Illinois. (g) The cuglobulin fraction as well as the fractions precipitable and nonprecipitable by zinc sulfate were produced from fresh human sera by the method of Vaerman and co-workers.² (h) The IgG fraction obtained by DEAE cellulose chromatography of human serum was kindly provided by Dr. Y. Yagi of Roswell Park Memorial Institute, Buffalo, N. Y.

Plain agglutination test

Ragweed pollen grains were washed 5 times in buffered saline, pH 7.2, and suspended at a 5 per cent concentration. Equal volumes of the ragweed suspension and the serum at various dilutions were mixed in test tubes. The test was performed in glass tubings with an inside diameter of 0.19 cm. and a height of 11 cm.; the lower end of each tubing was sealed with modeling clay. The tubings were filled with ragweed-serum mixtures by means of Pasteur pipettes, and they were placed vertically in a rack (Fig. 1). Readings were taken after 30 minutes of incubation at room temperature. In strong positive reactions (3+), most of the agglutinated ragweed was sedimented and only scarce agglutination was visible in the supernate. Moderately positive reactions (2+ and 1+) appeared as a definite clumping of ragweed pollen throughout the mixture and only part of the agglutinated ragweed formed a sediment. In negative tests (-), no agglutination was recognizable; the test mixture was uniformly turbid and only a trace of sediment was formed. The method described above was selected after failure in procedures using test tubes of various diameters.

Indirect Coombs test

One-half milliliter of a 5 per cent ragweed suspension was mixed with an equal volume of serum at various dilutions. The mixtures were incubated in test tubes for 30 minutes in a water bath at 37° C. During the incubation, the tubes were shaken several times to prevent the sedimentation of ragweed. Thereafter the ragweed was washed 5 times and resuspended in 1 ml. of Coombs serum. For experiments with rabbit sera, goat antiserum to whole rabbit serum obtained by Proom's procedure³ was used. For the experiments with human sera, rabbit antiserum to human globulin produced by the method of Milgrom and co-workers⁴ was used. Both these Coombs sera were employed at a dilution of 1:100. Ragweed pollen suspended in Coombs serum was pipetted into the tubings and examined for agglutination in a similar way as in plain agglutination.

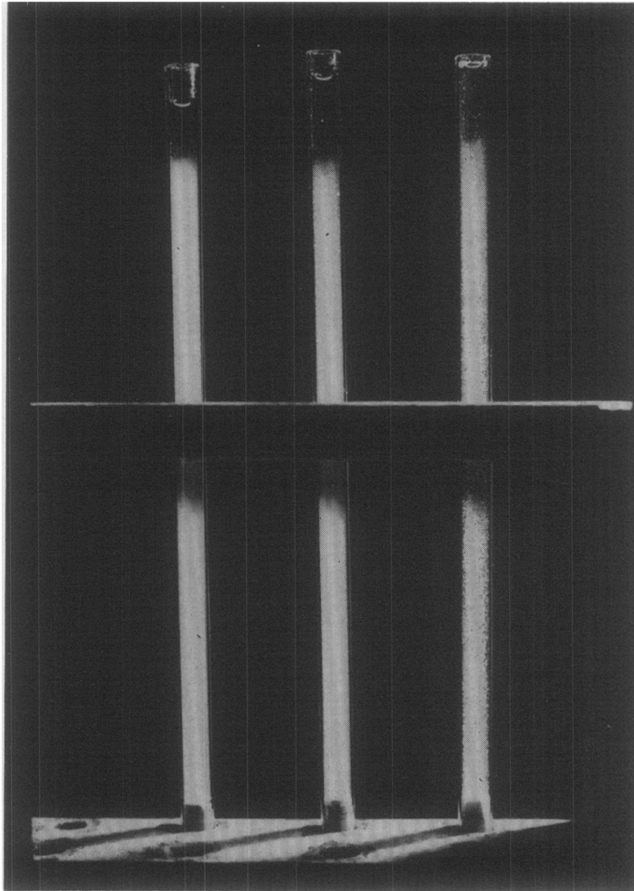


Fig. 1

Agglutination of ragweed pollen. Left, negative reaction; middle, doubtful reaction; right, positive reaction, 2+.

Absorption experiments

A 5 per cent ragweed suspension was distributed in 1 ml. portions and the grains were packed by centrifugation. The supernatant was discarded and the sediment resuspended in 1 ml. of 1:10 diluted serum to be absorbed. The tubes were shaken at room temperature for 30 minutes. The ragweed was sedimented by centrifugation and the absorbed sera were withdrawn. Absorptions with birch and timothy pollens were performed in a similar manner.

RESULTS

Rabbit sera

All of 12 rabbit antisera which were tested gave positive results in the plain agglutination test; the titers varied between 1:50 and 1:400. In several instances, positive test mixtures were expelled from tubings to test tubes and were gently

shaken. So treated, ragweed agglutinates dispersed immediately. As controls, 18 normal rabbit sera were studied and none was positive, even at a dilution of 1:10.

In order to determine whether some "incomplete" antibodies participate in the reaction between ragweed pollen and the rabbit immune sera, the indirect Coombs test was applied. A sample experiment is illustrated in Table I, in which titrations in the plain agglutination test and in the indirect Coombs test are compared. All of the 12 antisera gave considerably higher titers by the Coombs test, the highest titer observed in this test being 1:3,200. Here again, ragweed agglutinates could be easily dispersed by shaking.

It may be noted in Table I that the normal rabbit serum used as a control gave a positive result in the indirect Coombs test at a dilution of 1:25. In connection with this finding, 7 normal rabbit sera were tested by indirect Coombs test, starting the titration at a dilution of 1:10. Interestingly, all but one were positive with titers of 1:20.

Human sera

Sixty sera of patients with ragweed hypersensitivity and 50 sera of patients with various other diseases and of healthy individuals were tested by plain agglutination. In no instance was a positive reaction observed. The results were also consistently negative when different concentrations of ragweed, ranging from 0.1 to 10 per cent, were employed.

Subsequently, the indirect Coombs test was employed. Of 192 human sera tested, 169 (88 per cent) were positive (Table II). The titers of positive sera varied from 1:10 to 1:80. There was no significant difference in the incidence of positive results in patients with ragweed hypersensitivity and in individuals without this disease. Also a vast majority of sera from children one half to three years old were positive. On the other hand, of 42 cord sera tested, all but two were negative. The titer of the two positive sera was 1:10. Of 31 sera from adult normal humans living in France, Switzerland, and Israel, 20 were positive. Heating the sera at 56° C. for 30 minutes did not affect the reactions.

Absorption experiments presented in Table III showed that the serum factor under study was removed completely by ragweed pollen, whereas it was hardly affected by absorption with birch and timothy pollens.

Subsequently Cohn's Fractions II and V of pooled human serum were tested for the presence of the factor detectable in the indirect Coombs test against ragweed pollen. Whereas positive reactions were produced by Fraction II, negative results were obtained with Fraction V. More specific information about the chemical nature of the factor under study was obtained from the experiment presented in Table IV. As shown, the strongest reactions were obtained with euglobulin fraction which is rich in IgM. On the other hand, the remaining serum fractions rich in IgG or IgA gave negative results or weak reactions.

DISCUSSION

The tests reported in this study could be classified as agglutination which manifested itself by the increased sedimentation rate of ragweed pollen and by

Table I. Reactions of ragweed pollen with rabbit antiragweed sera. Comparison of results obtained in (A) plain agglutination test and (B) indirect Coombs test

Antiserum dilutions 1:	R No. 32		R No. 97		R No. 70		NRS	
	A	B	A	B	A	B	A	B
25	3+	3+	3+	3+	3+	3+	—	2+
50	3+	3+	3+	3+	1+	3+	—	—
100	3+	3+	2+	3+	—	3+	—	—
200	3+	3+	1+	3+	—	3+	—	—
400	1+	3+	—	3+	—	3+	—	—
800	—	3+	—	3+	—	2+	—	—
1,600	—	2+	—	2+	—	1+	—	—
3,200	—	—	—	1+	—	—	—	—

Table II. Indirect Coombs test with human sera and ragweed pollen

Patients	Number of sera			Per cent of positive reactions
	Positive	Negative	Total	
Patients with ragweed hypersensitivity	60	10	70	85.7
Patients with various diseases and healthy individuals	70	6	76	92.1
Children with various diseases, ½ to 3 years of age	39	7	46	84.8
Total	169	23	192	88.0
Cord sera	2	40	42	4.7

Table III. Indirect Coombs test with human serum and ragweed pollen. Effect of absorption with ragweed, birch, and timothy pollens

	Dilution of serum used for sensitization of ragweed 1:			
	10	20	40	80
Unabsorbed serum	3+	3+	1+	—
Serum absorbed with pollens of:				
Ragweed	—	—	—	—
Birch	3+	2+	±	—
Timothy	3+	2+	±	—

Table IV. Indirect Coombs test with globulin fractions of human serum and ragweed pollen

Fraction	Contents of immunoglobulins as judged from immunoelectrophoresis			Concentration in mg./ml. of globulin fraction used for sensitization of ragweed pollen				
	IgG	IgA	IgM	1.0	0.5	0.25	0.12	0.06
Euglobulin	Low	No	High	3+	3+	3+	1+	±
Zn precipitate	High	Trace	Low	2+	—	—	—	—
Zn supernatant	Trace	Moderate	Trace	—	—	—	—	—
DEAE separated IgG	High	No	No	±	—	—	—	—

the appearance of clumps clearly recognizable in the reaction mixture during the sedimentation process. Since the clumps of ragweed could be easily dispersed, one should consider this reaction as a kind of pattern agglutination rather than true agglutination.

The experiments with rabbit antisera clearly indicated that the reaction between ragweed pollen and ragweed antibodies may be readily recognized in a plain agglutination test. The titers observed were far lower than the titers usually observed in reactions between rabbit immune sera and erythrocytes coated by ragweed extract. However, the test described in this study may have an advantage over passive hemagglutination in detecting some nonextractable surface antigens of the pollen.

It was interesting to learn that in addition to "complete" antibodies active in plain agglutination tests, rabbit antisera contained "incomplete" antibodies detectable in the indirect Coombs test. The titer of the latter antibodies was considerably higher than that of the former.

Human sera were negative by the plain agglutination test with ragweed pollen. This was the case with both normal human sera and sera of patients hypersensitive to ragweed, a great proportion of whom received desensitization therapy. On the other hand, the vast majority of human sera tested were positive by the indirect Coombs test. Not only sera of ragweed-hypersensitive patients but also sera of human beings free from this condition, including children over 6 months old, were positive. Significantly, almost all cord sera gave negative results.

The Coombs serum used in the experiments with human sera contained potent antibodies against IgG and IgM but hardly any antibodies against other serum proteins. From this, it could be deduced that the serum factor responsible for the observed reactions is an immunoglobulin. This contention was strengthened by positive reaction with Fraction II and negative result with Fraction V of human serum. Negative results obtained with cord sera played in favor of the IgM nature of the factor under investigation, since it is known that the high molecular weight IgM fail to cross the placenta.

From the serum fractions tested in the experiment presented in Table IV, the euglobulin fraction gave a strong reaction, whereas other fractions gave negative or weak results. This again plays in favor of the IgM nature of the "antiragweed factor," since the euglobulin was rich in this serum globulin whereas other fractions were rich in IgG or IgA.

In considering the nature of the "antiragweed factor" of human sera, one may contemplate the possibility that it is a normal serum globulin adhering to the surface of ragweed pollen or, alternatively, that it is a naturally occurring antibody. The ragweed specificity observed in the absorption experiment gives some evidence for the antibody character of the factor under investigation.

Other investigators also observed "nonspecific" factors in human sera which reacted with ragweed antigens. Arbesman and co-authors⁵ reported that 46 per cent of human sera originating from subjects without ragweed hypersensitivity agglutinated erythrocytes coupled with ragweed extract by means of bis-diazotized benzedine. Similarly, Campbell and Sussdorf¹ found "nonspecific"

adsorption of rabbit and human serum proteins to ragweed pollen as shown by the immunofluorescence procedure, and they discussed the possibility that "... individuals who fail to show clinical symptoms or give skin reactions may have some circulating reagins."

If the human "antiragweed factor" described in this study is really an antibody, one could have expected a higher incidence of positive reactions in subjects living on the American continent than in subjects living in countries where ragweed does not grow or is very rare. This does not seem to be the case since sera obtained from France, Switzerland, and Israel gave positive results in similar proportion to that of sera obtained from the United States. However, the validity of these data should not be overestimated since the absence of ragweed pollen in Western Europe and Israel has never been convincingly proved. On the other hand, even if ragweed were absent from these countries, one could still consider the possibility of stimulation exerted by some other substances antigenically related to ragweed.

It should also be pointed out that the naturally-occurring "antiragweed factor" does not seem to be limited to man, since positive indirect Coombs tests were also observed with normal rabbit sera.

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