

Physicochemical properties of reaginic antibody

II. Characteristic properties of reaginic antibody different from human γ A-isohemagglutinin and γ D-globulin

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Two reaginic sera from atopic patients were fractionated by chromatography on DEAE cellulose columns, and by gel filtration through Sephadex G-200 columns. The skin-sensitizing activity in the chromatographic fractions did not parallel the concentration of γ D-globulin (IgD). Gamma-D globulin in the reagin-containing fractions and one of the original sera were precipitated with rabbit antibody specific for γ D-globulin. Essentially all reaginic activity in the fractions and in the serum remained in the supernatants, indicating that the reaginic antibody is not γ D-globulin.

Comparisons were made between the reaginic antibody and human γ A anti-A isoagglutinins. When anti- γ A-globulin antibody was added to the mixture of the reagin-containing fraction and γ A anti-A isoagglutinin, the isoagglutinin was completely precipitated, whereas all reaginic activity remained in the supernatant. In sucrose density-gradient ultracentrifugation, the reaginic antibody sedimented intermediate between polymer and monomer forms of γ A-globulin antibodies. The average sedimentation coefficient of the reaginic antibody was estimated to be 8 S. The electrophoretic mobility of the reaginic antibody is slower than γ A isoagglutinin in Sephadex G-25 zone electrophoresis. These findings together with our previous report¹ indicate that the reaginic antibody is associated with none of the known four immunoglobulins, i.e., γ G-, γ M-, γ A-, and γ D-globulins, and suggest the presence of a fifth immunoglobulin as a carrier of reaginic activity.

In a previous paper,¹ evidence was presented that reaginic antibody in two atopic patients' sera was associated with a unique protein other than γ A- or γ G-globulin. When the sera were fractionated by chromatography on diethylaminoethyl (DEAE) cellulose columns and by gel filtration, reaginic activity

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in the fractions did not parallel either γ A- or γ G-globulin concentration. It was also found that essentially all skin-sensitizing activity in the reagin-containing fractions remained in the supernatant after the precipitation of both immunoglobulins with rabbit antibodies specific for one of these proteins. The reaginic activity in the supernatant was coprecipitated with γ G-globulin-anti-light chain antibody complexes, indicating that the skin-sensitizing antibody was associated with a protein having light chain(s) of immunoglobulins.

A fourth immunoglobulin, γ D (IgD), was demonstrated by Rowe and Fahey² in normal human sera. The present experiments were undertaken to study whether the reaginic antibody is γ D-globulin. In view of the difference of the reaginic antibody from the bulk of γ A-globulin with respect to antigenic structure, the physicochemical properties of the reaginic antibody are compared with those of human γ A-isoheamagglutinin. The results indicate that the reaginic antibody is not associated with γ D-globulin and that the antibody is different from γ A-isoheamagglutinins with respect to antigenic structure, molecular size, and electrophoretic mobility.

MATERIALS AND METHODS

Reaginic sera and their fractions

The patients' sera and their fractions used in the present experiments were the same as those described in a previous paper.¹ The serum from a ragweed-sensitive patient is a gift from Dr. Maxwell Richter and the other serum from an egg-sensitive patient was obtained at the Children's Asthma Research Institute. The globulin fraction of the ragweed-sensitive serum was obtained by precipitation with 1/2 saturation of ammonium sulfate and was chromatographed on DEAE cellulose (California Biochemical Corporation, Los Angeles, California) columns. Proteins were eluted stepwise with 0.005M, 0.01M, 0.035M, and 0.08M phosphate buffers of pH 8.0, and the fractions eluted with each buffer were pooled separately (Frs. I to IV). The protein eluted with 0.035M buffer (Fr. III) was further fractionated by gel-filtration through a Sephadex G-200 (Pharmacia, Uppsala, Sweden) column into three fractions (Frs. III₁, III₂, and III₃).¹ Fr. III₁, having the highest skin-sensitizing activity, was rechromatographed on a DEAE cellulose column. The fraction was dialyzed against 0.02M phosphate buffer (pH 8.0) and applied to the column (2 by 45 cm.) which had been equilibrated with the buffer. Protein fractions obtained with 0.02M and 0.035M phosphate buffers were pooled separately and concentrated (Frs. III₁₁ and III₁₂). The protein fractions of the egg-sensitive serum were obtained by gel filtration through Sephadex G-200 columns followed by DEAE cellulose column chromatography. The molarity of the phosphate buffers used to obtain each fraction and the protein concentration of the final preparations are shown in Table I.

Anti-A isoagglutinins

Three individuals of type 0 received two subcutaneous injections of 1 mg. of A substance (Pfizer Laboratories and Knickerbocker Inc., New York) one week apart, and were bled one week after the second immunization. Pro-

Table I. *Distribution of reaginic activity and immunoglobulins in chromatographic fractions of atopic sera*

Fraction	Phosphate buffer for elution*	Protein concentration	Presence of†		Minimum concentration for detecting	
			γA	γG	γD ‡	Reaginic antibody§
A. A ragweed-sensitive patient's serum						
	mole/L.	μg N/ml.			μg N/ml.	μg N/ml.
I	0.005	760	—	+	> 1600	100
II	0.01	760	—	+	> 760	2
III ₁₁	0.02	177	+	+	88	0.1 - 0.2
III ₁₂	0.035	790	+	+	25	2
III ₁	0.035	420	+	+	54	1
III ₂	0.035	790	+	+	790	10
B. An egg-sensitive patient's serum						
A	0.005	330	—	+	> 330	> 40
B	0.01	170	—	+	> 170	0.8
C	0.02	260	+	+	260	0.2
D	0.035	1050	+	+	130	0.8
E	0.05	140	+	+	> 140	4

*Molarity of phosphate buffers for stepwise elution from DEAE cellulose columns.

†Relative concentration of the proteins were described by Ishizaka and Ishizaka.¹‡ γD was detected by immunodiffusion. > signs represent negative reaction with the protein concentration.

§Detected by P-K reaction. Five-hundredths milliliter was injected for passive sensitization.

||Frs. III₁ and III₂ were obtained by gel filtration through a Sephadex G-200 column.

cedures for the purification of γG - and γA -globulin from the immune sera were the same as reported previously.³ Gamma-G globulin fraction was obtained by chromatography on a DEAE cellulose column. Eight milliliters of one of these sera was dialyzed against phosphate buffer (pH 8.0, 0.01M) and applied to the column (2 by 45 cm.) which had been equilibrated with the same buffer. The protein fractions eluted with the buffer were pooled and concentrated by negative pressure filtration. Gamma-A globulin was purified from 100 to 150 ml. of each serum by the method of Vaerman and co-authors.⁴ Euglobulin was precipitated by dialysis of the sera against 0.02M phosphate buffer (pH 6.0). The γG -globulin in the supernatant was removed by precipitation with 0.05M zinc sulfate at pH 6.8 at 28° C. Supernatant fluids were dialyzed against distilled water and concentrated. The γA -globulin in the supernatant was then precipitated with $\frac{1}{2}$ saturation of ammonium sulfate (pH 7.0) and further purified by blocked electrophoresis in Pevicon C-870 (Fosfatbolaget, Sweden), with barbital buffer of pH 8.6 and ionic strength of 0.1.⁵ Eluates from each 1 cm. segment were tested by immunodiffusion against the goat antiserum specific for γA -globulin and antihuman serum.³ Only eluates giving a single precipitin band of identical specificity with both antisera were pooled, dialyzed against distilled water, and concentrated in the cold by negative pressure filtration. The γA -globulin fraction from each serum was tested for purity by immunodiffusion and then pooled. The final preparation (250 μg N per milliliter) gave a single precipitin band of identical specificity

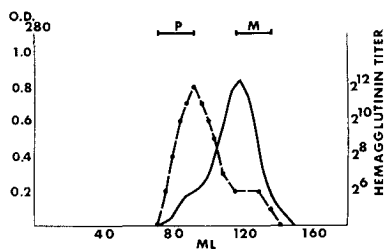


Fig. 1

Gel filtration of γ A-globulin through a Sephadex G-200 column. A solid line represents absorption at 280 m μ . A broken line represents the hemagglutinin titer of the fractions.

with the anti- γ A-globulin serum and anti-whole serum but not with either anti- γ G- or anti- γ M-globulin.

The γ A-globulin was fractionated into polymer and monomer forms by gel filtration.³ Six milliliters of γ A-globulin were dialyzed against borate buffered saline and applied to a Sephadex G-200 column (2 by 100 cm.). Two milliliter fractions were collected and absorption at 280 m μ determined. As reported previously,³ hemagglutinating activity did not parallel the protein concentration of the chromatographic fractions (Fig. 1). The ratio of hemagglutinating titer to the absorption at 280 m μ was calculated for each chromatographic fraction and the fractions with similar ratios were pooled separately (Frs. P and M in Fig. 1) and concentrated. The protein concentration of Frs. P and M were 38 μ g N per milliliter and 286 μ g N per milliliter, respectively.

Antisera specific for human immunoglobulins

Rabbit antisera specific for γ G-, γ A-, or γ M-globulin were the same preparations described in previous papers.^{1, 3} The γ -globulin fraction of the antisera, which were obtained by the immunization of rabbits with one of the three immunoglobulins, was precipitated with ammonium sulfate at $\frac{1}{3}$ saturation at pH 7.8. Cross-reacting antibody in the rabbit γ -globulin fractions was removed by absorption with appropriate purified human immunoglobulin at the equivalence region. The γ -globulin fractions of anti- γ A and anti- γ M sera were absorbed with γ G and that of anti- γ G serum was absorbed with F_{ab} fragment of the γ G-globulin. The γ -globulin fractions of the anti- γ A antisera were further purified by chromatography on a DEAE cellulose column. The protein eluted from the column with 0.01M phosphate buffer (pH 8.0) was concentrated. The specificity of the anti- γ G, anti- γ A, and anti- γ M antibody preparations was tested by immunodiffusion with human serum and purified immunoglobulins. Each antibody preparation gave a single precipitin band of identical specificity against human serum and homologous immunoglobulin but not with the other two immunoglobulins.^{1, 3} The goat antiserum specific for γ A-globulin was purchased from Hyland Laboratories, Los Angeles, California. The γ -globulin fraction of the antiserum was obtained by chromatography on a DEAE cellulose column using 0.02M phosphate buffer (pH 8.0).

The rabbit antiserum specific for γ D-globulin was supplied by Drs. J. Fahey and D. Rowe, National Cancer Institute, Bethesda, Maryland. It was prepared by immunization of rabbits with γ D myeloma protein followed by absorption of cross-reacting antibodies. The antiserum gave a single precipitin band with the standard serum supplied by Dr. Fahey but not with either γ G-, γ A-, or γ M-globulin by immunodiffusion.

Density gradient ultracentrifugation

The method of Martin and Ames⁶ was applied. A continuous sucrose density gradient of 5 to 20 per cent in borate-buffered saline (pH 7.0) was made in a total volume of 4.6 ml. A sample of 0.2 ml. was layered on top of the sucrose density gradient. In some experiments, a sucrose gradient of 12 to 36 per cent with a volume of 4.2 ml. was used,⁷ and a 0.4 ml. sample was layered on top. Centrifugation was carried out in a Spinco Model L ultracentrifuge with an SW 39 swinging bucket rotor at 35,000 r.p.m. for 18 hours. Fractions were collected by piercing the bottom of the centrifuge tubes.

Zone electrophoresis

Sephadex G-25 (Pharmacia, Uppsala, Sweden) in barbital buffer of pH 8.6 and ionic strength 0.1 was used for supporting medium. The block used was 2.0 by 1.5 by 44 cm. Electrophoresis was carried out at 380 volts for 24 hours at 1 to 2° C. Each 1 cm. segment was eluted with 3 ml. saline and the protein concentration in the eluates were determined by the Folin-Ciocalteu method.⁸

Immunological techniques

Immunodiffusion. Double diffusion in agar plates was carried out by the method of Ouchterlony.⁹ The plates were incubated at room temperature for 48 hours, washed, and the precipitin bands stained with Amido schwarz 10B. Goat antihuman serum used in immunodiffusion was purchased from Hyland Laboratories, Los Angeles, California.

Hemagglutination. Fresh human erythrocytes from an A₁ individual were used. Twofold dilutions of samples were made in barbital-buffered saline containing 0.05 per cent gelatin¹⁰ and an equal volume of a 2 per cent suspension of erythrocytes was added to each tube. The tubes were incubated at 37° C. for 1 hour and centrifuged at 1,000 r.p.m. for 3 minutes. Hemagglutination reactions were graded macroscopically from 1 plus to 4 plus.

Prausnitz-Küstner reactions. The tests were carried out on individuals insensitive to the allergens tested. One-twentieth milliliter of test samples was injected, intracutaneously. Twenty-four hours later, the sites received an intradermal injection of 0.02 ml. of ragweed allergen extract (Center Lab., New York, New York) containing 10 PN units or of 0.01 per cent crystalline ovalbumin (5 times recrystallized, Nutritional Biochem., Cleveland, Ohio). Erythema-wheel reactions which appeared 15 to 20 minutes after the injection of allergen were traced on a transparent plate which was applied to the area.

When the reaginic activity of several samples was compared, the samples were tested in the same individuals. The maximal dilution of the samples giving a positive reaction was determined in duplicate in two different individuals.

EXPERIMENTAL RESULTS

A. Reaginic antibody and γ D-globulin

The chromatographic fractions of the two reaginic sera were tested for the presence of γ D-globulin by immunodiffusion. As shown in Table I, γ D-globulin was detected in the fractions which were eluted from DEAE cellulose columns with 0.02M and 0.035M buffers, but not in the other fractions. In order to estimate the concentrations of γ D-globulin in the two fractions, twofold dilutions of the fractions were placed in peripheral wells of Ouchterlony plates against the anti- γ D-globulin serum in the center well. The minimum concentration of the fractions required to give a positive precipitin band with the anti- γ D-globulin serum are shown in Table I. Skin-sensitizing activity of the chromatographic fractions were determined by P-K reactions. Serial twofold dilutions of the reagin-containing fractions were injected intracutaneously into normal individuals and the sites were challenged with homologous allergen 24 hours later. The minimum protein concentration in each fraction required to give a positive erythema-wheal reaction is shown in Table I. In the fractions from ragweed-sensitive serum (Table I, A), Fr. II and Fr. III₁₂ were comparable on a weight basis with respect to skin-sensitizing activity. The γ D-globulin was detected in 25 μ g N per milliliter of Fr. III₁₂, whereas 30 times as high concentration of Fr. II did not contain a detectable amount of γ D-globulin. It was also found that Fr. III₁₁ having the highest sensitizing activity contained less γ D-globulin than Fr. III₁₂. Similar findings were obtained with the fractions from the egg-sensitive patient's serum (Table I, B). It is evident that the skin-sensitizing activity of the fractions does not parallel γ D-globulin concentration.

The γ D-globulin in the fractions having the highest skin-sensitizing activity was precipitated with anti- γ D-globulin serum. As preliminary experiments, aliquots of Fr. III₁₁ were mixed with increasing amounts of anti- γ D serum and incubated at 0° C. for 24 hours. The tubes were centrifuged to remove specific precipitates and the supernatants were tested for the presence of either γ D or anti- γ D antibody by interfacial ring test using Fr. III₁₂ as antigen. When $\frac{1}{5}$ volume of the antiserum was added to one volume of Fr. III₁₁, neither γ D nor anti- γ D was detected in the supernatant. In order to absorb γ D-globulin in antibody excess, 0.06 ml. volume of the antiserum was added to 0.2 ml. of Fr. III₁₁. Another 0.2 ml. aliquot of Fr. III₁₁ was mixed with 0.06 ml. of saline. After 24 hours at 0° C. the specific precipitate was removed by centrifugation. The anti- γ D antibody but no γ D-globulin was detected in the supernatant. Serial dilutions of the supernatant and the control were injected intracutaneously in the same individuals and the skin sites were challenged with ragweed pollen extract 24 hours later. No significant difference was observed between the skin reactions with the supernatant and with

the control, indicating that the skin-sensitizing antibody in the fraction was not precipitated with the γ D-globulin-anti- γ D-globulin complexes (Table II). The P-K reactions were duplicated in two different individuals with comparable results. Representative reactions are shown in Fig. 2. The experiments were repeated using Fr. C of the egg-sensitive serum with similar results. Four-hundredth milliliter of anti- γ D serum was added to 0.2 ml. of Fr. C to precipitate γ D-globulin. After removal of the specific precipitate, the supernatant contained anti- γ D antibody but no γ D-globulin. The results of the P-K reactions are shown in Table II.

The effect of the precipitation of γ D-globulin in a whole reaginic serum was studied. The ragweed-sensitive serum gave a precipitin band with anti- γ D serum in immunodiffusion. The γ D-globulin in the serum was completely precipitated by the addition of one-half volume of the anti- γ D serum leaving excess antibody in the supernatant. The skin-sensitizing activity of the supernatant was comparable to that of unabsorbed serum (Table II). These results indicate that the reaginic antibody in the serum is not γ D-globulin.

B. Comparisons between reaginic antibody and γ A-isohemagglutinin

It was found in previous studies¹ that essentially all skin-sensitizing activity in the reagin-containing fractions, such as Fr. III₁ and Fr. C (Table I), remained in the supernatants after precipitation of γ A- or γ G-globulin. Since Fr. III₁₁ had the highest skin-sensitizing activity, the absorption experiments were repeated with the fraction to confirm the previous findings. Two-tenth milliliter of Fr. III₁₁ was mixed with 0.04 ml. of γ A-isohemagglutinin and an equal volume of the rabbit anti- γ A preparation was added to the mixture. After 24 hours, the specific precipitates were removed by centrifugation in the cold. A control mixture consisted of Fr. III₁₁, γ A-isohemag-

Table II. *Effect of precipitation of γ D-globulin on reaginic activity**

Preparation†	Absorption with anti- γ D	Dilutions of the preparations injected			
		220×‡	440×	880×	1760×
Fr. III ₁₁	+§	43 × 50 (12)	30 × 44 (10.5)	31 × 35 (7.5)	22 × 24 (6.5)
	-	36 × 40 (11.5)	27 × 28 (9.5)	27 × 34 (8)	13 × 17 (5.5)
Fr. C		160×	320×	640×	1280×
	+§	38 × 41 (14)	38 × 40 (12)	35 × 38 (10)	30 × 33 (8)
	-	38 × 39 (15)	33 × 41 (12)	35 × 37 (11)	25 × 36 (7)
Ragweed-sensi- tive serum		250×	500×	1000×	2000×
	+§	35 × 37 (11)	28 × 32 (9)	23 × 28 (8)	13 × 20 (7)
	-	37 × 42 (11)	28 × 35 (8.5)	20 × 25 (7)	10 × 16 (6)

*Numerals in this table represent the diameters of erythema in millimeters; the numerals in parentheses represent the average diameter of the wheal in P-K reactions.

†Fractions in Table I.

‡Unabsorbed fraction contained 0.8 μ g N per milliliter.

§Anti- γ D but no γ D-globulin was detected in supernatants by interfacial ring tests.

||Unabsorbed fraction contained 1.6 μ g N per milliliter.

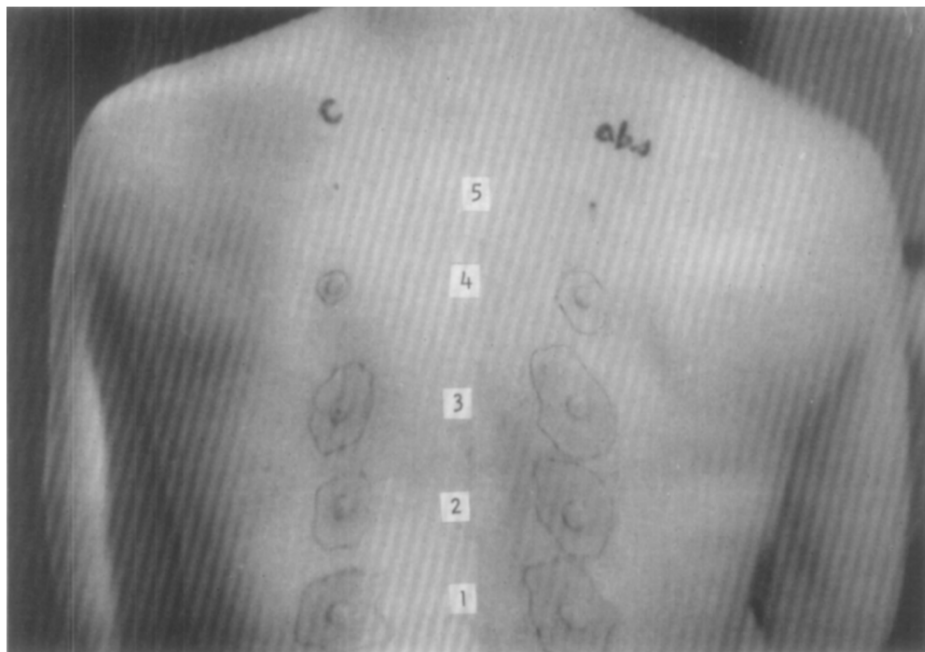
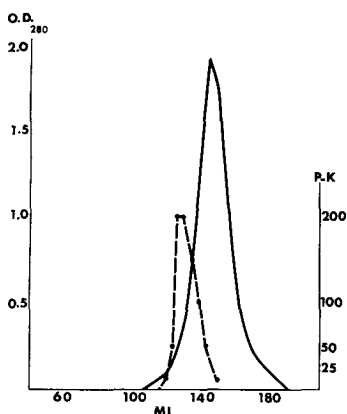


Fig. 2

Prausnitz-Küstner reactions with a reagin-containing fraction (Fr. III₁₁) before (left) and after (right) the precipitation of γ D-globulin with specific antibody. Skin sites 1-5 (bottom to top) received twofold dilutions of the control (C) and supernatant (abs) from 1/170 to 1/2720. Dilutions 1/170 of the control contained 0.8 μ g N per milliliter.

glutinin and saline, in place of rabbit anti- γ A antibody. The skin-sensitizing activity and hemagglutinin titer of the supernatant and control were determined. The isohemagglutinin was not detected in the supernatant, whereas the hemagglutinin titer of the control was 1:128. With respect to the skin-sensitizing activity, the supernatant and control showed comparable P-K titers. The maximal dilution of both solutions giving a positive P-K reaction was 1:800. These findings indicate that the reaginic antibody does not have the major antigenic determinant of γ A-globulin which is present in the isohemagglutinin. The experiments were repeated, using a goat anti- γ A-globulin preparation, with comparable results. An aliquot of Fr. III₁₁ was incubated with an equal volume of the γ -globulin fraction of the goat anti- γ A serum to precipitate γ A-globulin. Although no γ A-globulin was detected in the supernatant by immunodiffusion, essentially all reaginic activity remained in it.

In order to study molecular size of the reaginic antibody, 3.9 ml. of Fr. II was applied to Sephadex G-200 column (2 by 100 cm.) and protein eluted with borate buffered saline (Fig. 3). Serial dilutions of each 2 ml. fractions were injected intracutaneously into normal individuals to determine the maximal dilution of each fraction giving a positive P-K reaction. It was found that the skin-sensitizing antibody was present in the ascending portion of the peak,

**Fig. 3**

Gel filtration of Fr. II through a Sephadex G-200 column. A solid line represents absorption at 280 mμ; a broken line represents P-K titer of the fractions.

indicating that the reaginic antibody is larger than γ G-globulin. These findings are in agreement with previous findings¹¹⁻¹³ that the skin-sensitizing antibody in reaginic sera comes off Sephadex G-200 columns earlier than the bulk of γ G-globulin.

The reaginic antibody and isohemagglutinins were compared with each other with respect to sedimentation velocity in sucrose density gradient. Two-tenths milliliter aliquots of Fr. III₁₁ were mixed with 0.05 ml. of either γ G-globulin, the monomer form of γ A- or the polymer form of the γ A-globulin fraction containing isoagglutinin, and each mixture was layered on top of sucrose density gradient of 5 per cent to 20 per cent. The protein concentration of the mixtures was 0.09 to 0.4 per cent. After centrifugation at 35,000 r.p.m. for 18 hours, both hemagglutinin and skin-sensitizing activities in the gradient fractions were determined. The results shown in Fig. 4, *a* indicated that the sedimentation velocity of the reaginic antibody was intermediate between those of the monomer and polymer forms of γ A isoagglutinin. The results also showed that the sedimentation velocities of the monomer form of γ A- and the γ G-isoagglutinins were comparable (Fig. 4, *b*). With respect to γ A-globulin, Vaerman and associates¹⁴ demonstrated 10.5 S and 17 S components. In order to study whether the major component of the polymer form γ A-isoagglutinin is associated with 10.5 S or 17 S component, the γ A-isoagglutinin preparations were analyzed by sucrose density gradient ultracentrifugation. Normal human γ G- and γ M-globulins which had been previously absorbed with an equal volume of packed type A cells were used as markers. Fifty micrograms N of γ G, and 100 μ g N of γ M-globulin were mixed with 0.4 ml. of monomer fraction or polymer fraction of γ A-isoagglutinin in total volume of 0.5 ml., and 0.4 ml. of each mixture was layered on top of the sucrose density gradient of 12 per cent to 36 per cent. After centrifugation at 35,000 r.p.m. for 18 hours, the distribution of isoagglutinin was deter-

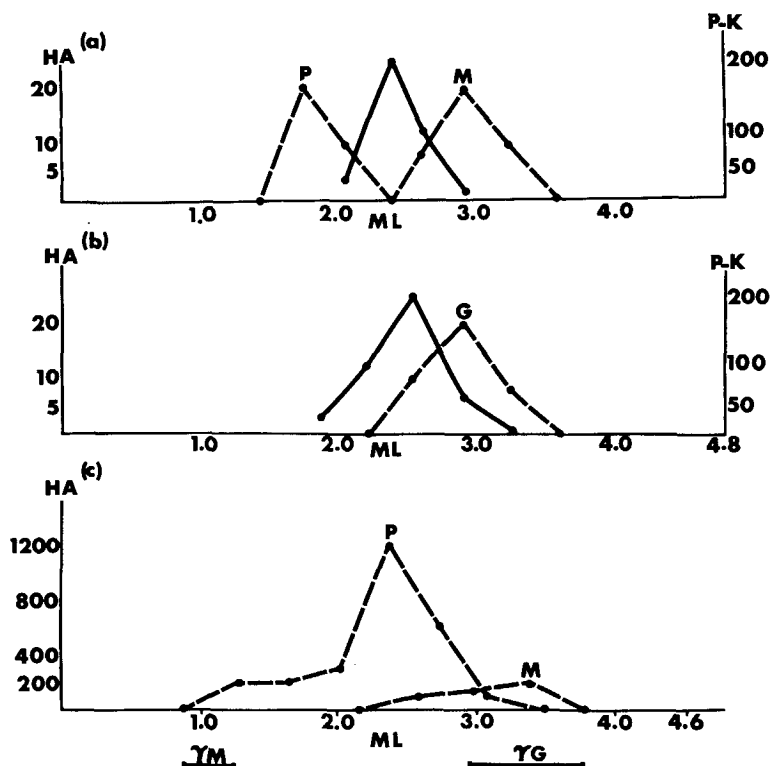


Fig. 4

Density gradient ultracentrifugation showing the distribution of hemagglutinin and reagin. Solid lines represent P-K titer and broken lines represent hemagglutinin titer of the fractions. *a*, Comparisons of reagent with polymer (*P*) and monomer (*M*) forms of γ A-isoagglutinins. *b*, Comparisons of reagent with γ G-isoagglutinin. In *a* and *b*, samples were layered on top of sucrose density gradient from 5 per cent to 20 per cent. *c*, The distributions of polymer (*P*) and monomer (*M*) forms of isoagglutinins in gradient fractions from 12 per cent on top to 36 per cent at the bottom. The presence of γ M- and γ G-globulins added as markers in *c* is shown under the figure.

mined by hemagglutination reactions and of immunoglobulins by immunodiffusion using antisera, specific for one of the three immunoglobulins. It is apparent from Fig. 4, *c* that the major component of the polymer form of γ A-isoagglutinin sedimented slightly faster than the monomer form of γ A- and γ G-globulin. In addition to the major component, the polymer fraction contained a minor component of which the sedimentation velocity was slightly slower than that of γ M-globulin. The results indicated that the major component of the polymer form of γ A-isoagglutinin is probably associated with the 10.5 S component and therefore suggest that the sedimentation velocity of the reaginic antibody is intermediate between 7 S and 10.5 S.

Electrophoretic mobility of the reaginic antibody was studied. In order to minimize the absorption of protein to supporting media, blocks were made with Sephadex G-25. The ragweed-sensitive serum was absorbed with one-half volume of packed type A cells, and 0.6 ml. of the serum was mixed with 0.3

ml. of γ A-globulin containing anti-A isoagglutinin. The mixture was fractionated by electrophoresis. Eluates from 1 cm. segments were tested for protein concentration, skin-sensitizing and hemagglutinating activities. The distributions of γ G- and γ A-globulins were determined by immunodiffusion, using the antisera specific for one of these immunoglobulins. As shown in Fig. 5, reaginic and hemagglutinating activities did not parallel each other; the reaginic antibody was present in the region of the starting point, whereas the hemagglutinating antibody and γ A-globulin migrated toward the anode. The experiment was repeated with Fr. III₁₁ of the serum. Six-tenths milliliter of the fraction was mixed with 0.3 ml. of the γ A-isoagglutinin preparation and the mixture was fractionated by zone electrophoresis. The results were essentially the same as those obtained with the original serum: reaginic activity was detected in Frs. 19 to 21, whereas hemagglutinin was present in Frs. 21 to 25. The peak of the hemagglutinin titer was observed in Frs. 23 and 24. It is apparent that the electrophoretic mobility of the reaginic antibody is slower than that of γ A-isoagglutinin.

DISCUSSION

The data presented in Part A clearly showed that the skin-sensitizing antibodies in two reaginic sera were not associated with γ D-globulin. The reaginic activity in chromatographic fractions of the sera did not parallel the γ D-globulin concentration. As reported by Rowe and Fahey,² γ D-globulin was not detected in the fractions which were eluted with 0.01M phosphate buffer from DEAE cellulose columns. However, the reaginic activity of these fractions (Frs. II and B) was comparable to that of Frs. III₁₂ and D which contained the highest concentration of γ D-globulin (Table I). Some γ D-globulin was detected in the fractions having the highest skin-sensitizing activity. However, the precipitation of this protein with the anti- γ D-globulin antibody was not accompanied by any decrease in the skin-sensitizing activity. It was also found that the reaginic activity in the original serum did not decrease with the absorption of γ D-globulin. These findings exclude the possibility that the reaginic antibody is γ D-globulin.

The present findings extend previous observations indicating that the reaginic antibody is not γ A-globulin. When the anti- γ A rabbit antibody was added to a mixture of γ A-isoagglutinin and a reagin-containing fraction (Fr. III₁₁), the isoagglutinin was completely precipitated, whereas essentially all reaginic activity remained in the supernatant. It is evident that the reaginic antibody does not have the major antigenic determinant of γ A-globulin which is possessed by γ A-isoagglutinin.

The differences between the reaginic antibody and γ A-isoagglutinin were also detected by zone electrophoresis and sucrose density gradient ultracentrifugation. Electrophoretic mobility of the reaginic antibody was slower than that of γ A-isoagglutinin and the sedimentation velocity of the reaginic antibody in the density gradient was intermediate between those of the polymer and monomer forms of γ A-isoagglutinins. In density gradient ultracentrifugation, Martin and Ames⁶ showed that for macromolecules of the

same partial specific volume, the ratio of the distances traveled from the meniscus was equal to the ratio of their sedimentation coefficients ($S_{20,w}$). From the results obtained in Fig. 4, *b* and assuming that the partial specific volume is the same for the reaginic antibody and γ G-isoagglutinin and that $S_{20,w}$ of γ G-isoagglutinin is 6.8, the sedimentation coefficient of reaginic antibody was estimated to be 8.0 S. The sedimentation coefficient of the polymer form of γ A-isoagglutinin (Fig. 4, *a*) was estimated to be 11.1 S from a comparison with the γ G-isoagglutinin in a separate tube (Fig. 4, *b*). This result is in satisfactory agreement with the observations of Heremans and co-authors¹⁵ and Vaerman and associates,¹⁴ who demonstrated a 10.5 S component in normal γ A-globulin. This indicates that the polymer form of γ A-isoagglutinin in Fig. 4, *a* is the 10.5 S component rather than the 17 S component demonstrated by Vaerman and co-workers.¹⁴ From a comparison of the polymer form of γ A-isoagglutinin and reaginic antibody in Fig. 4, *a*, the sedimentation coefficient of the reaginic antibody was estimated to be 8.3 by assuming that the $S_{20,w}$ of the polymer form of isoagglutinin was 10.5 S. Thus, in both cases the $S_{20,w}$ of the reaginic antibody was close to 8 S.

As reported by Anderson and Vannier,¹⁶ the distribution of the reaginic antibody in gradient fractions was homogeneous. It is unlikely that the antibodies are composed of both 7 S and 10-11 S components because the polymer and monomer forms of γ A-isoagglutinins were completely separated under the present experimental condition (Fig. 4).

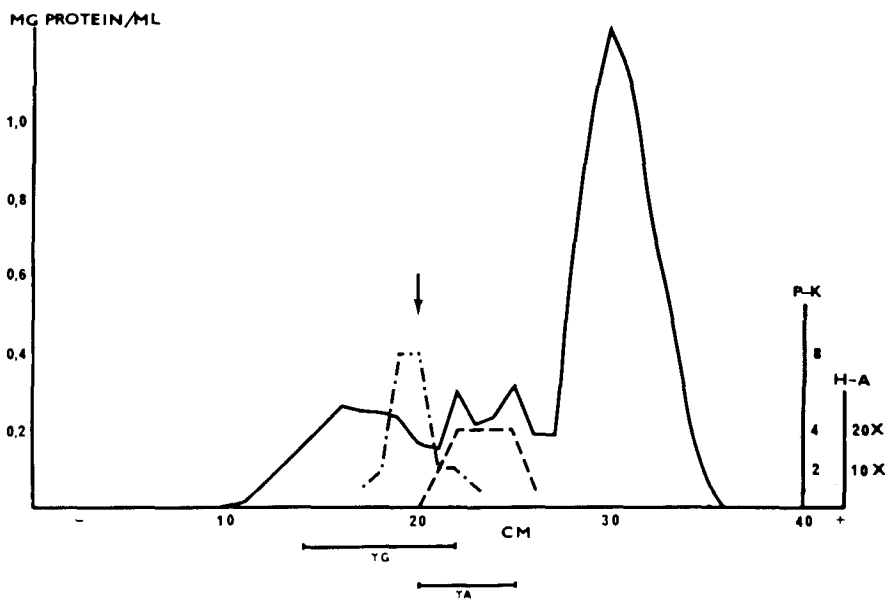


Fig. 5

Zone electrophoresis of a ragweed sensitive patient's serum. The serum sample was mixed with γ A-globulin containing isoagglutinin. A solid line represents protein concentration determined by Folin reagent. A broken line and dot-dash lines represent hemagglutinin and P-K titers, respectively. Starting point is marked by an arrow.

Gel filtration of reaginic sera through Sephadex G-200 columns was carried out by previous investigators.¹¹⁻¹³ In all experiments, a close correlation was observed between the distribution of reaginic antibody and of γ A-globulin in chromatographic fractions. However, these findings do not necessarily support the concept that the reaginic antibody is γ A-globulin. In the present experiments, the reaginic antibody in Fr. II, which lacked γ A-globulin, was eluted from a Sephadex G-200 column before the bulk of γ G-globulin and the activity in the fraction did not significantly decrease by the addition of anti- γ A-globulin.¹ It seems that the correlation between reaginic activity and γ A-globulin in the previous experiments¹¹⁻¹³ merely indicates that the reaginic antibody and γ A-globulin can not be separated from each other by gel filtration.

The physicochemical properties of the reaginic antibody studied in the present experiments are in agreement with those reported by previous investigators. Electrophoretically, the reaginic activity was generally detected in the fast γ -globulin.^{17, 18} With respect to the sedimentation velocity of reaginic antibodies in sucrose density gradient ultracentrifugation, Anderson and Vannier¹⁶ reported that the average $S_{20,w}$ of reaginic antibody in 6 patients' sera was 7.7 S and Rokey and Kunkel¹⁹ found that the reaginic antibody reacting with glucagon had a sedimentation rate in the range of 8 to 11 S. The similarities of our data to their findings suggest that the reaginic antibody studied in the present experiments is not exceptional.

As discussed above, it is apparent that the reaginic antibody is different from γ A-globulin antibody in both physicochemical properties and antigenic structure. The sedimentation velocity of the reaginic antibody also excludes the possibility that the antibody is γ M-globulin. The data presented in the present and previous papers,¹ therefore, indicate that the reaginic antibody is associated with none of the four immunoglobulins, i.e., γ G-, γ A-, γ M-, and γ D-globulins. As reported in the previous paper, the reaginic antibody was coprecipitated with γ G-globulin-anti-light chain complexes, indicating that the antibody molecules have light chain(s) of immunoglobulins. These findings strongly suggest the presence of a unique immunoglobulin as a carrier of reaginic activity.

The possibility exists that the reaginic antibody may be associated with one immunoglobulin in one group of individuals and with other immunoglobulins in another group of individuals. It has been reported that highly purified γ A-globulin from reaginic sera contained reaginic activity.^{20, 21} Fireman and associates¹¹ and Augustin and co-workers²⁰ reported that reaginic activity in patients' sera has been precipitated by the addition of anti- γ A-globulin. The present experiments do not exclude the possibility that reaginic antibody in some other individuals is associated with γ A-globulin. Further investigations are currently in progress to study whether a part of reaginic antibody is γ A-globulin.

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