

Physicochemical properties of reaginic antibody

III. Further studies on the reaginic antibody in γ A-globulin preparations

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Three sera from ragweed-sensitive patients were fractionated by chromatography on DEAE cellulose columns and gel filtration. It was confirmed that the reaginic activity in the chromatographic fractions did not parallel γ A-globulin concentration. The γ A-globulin fractions from the same sera contained reaginic activity. When the γ A-globulin preparations were further fractionated by chromatography on DEAE cellulose columns, the reaginic activity was concentrated into one of the three sub-fractions. The precipitation of γ A-globulin in the subfraction and the original γ A-globulin preparation with rabbit anti- γ A-globulin antibody was not accompanied by any detectable decrease of reaginic activity. The results indicated that the reaginic activity in the fractions was not associated with γ A-globulin, but with impurities which were not detected by immunodiffusion methods. The subfractions of the γ A-globulin preparation from a ragweed-sensitive patient blocked passive sensitization of normal human skin with reaginic antibody of a heterologous system. The blocking activity of the fractions paralleled the sensitizing activity against ragweed-pollen extract. It was also found that a fraction containing reagin but no detectable γ A-globulin had a definite blocking activity, whereas γ A-myeloma proteins did not. These findings suggest that both the blocking activity and reaginic activity are associated with the same protein other than γ A-globulin.

In previous publications,^{1, 2} evidence was presented that reaginic antibody in two atopic patients' sera was associated with a unique immunoglobulin other than γ G-, γ A-, γ M-, or γ D-globulin. When reagin-containing fractions of the sera were mixed with the antibody specific for γ G-, γ A-, or γ D-globulin to precipitate one of these proteins, essentially all reaginic activity remained in the supernatant. It was also found that the sedimentation coefficient of the reaginic

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antibody was about 8 S.² These findings indicate that the reaginic antibody is associated with a protein different from the four known immunoglobulins, but do not exclude the possibility that some of the known immunoglobulins as well may be carriers of a part of the reaginic activity. There has been a considerable body of evidence which supports the concept that reagin is associated with γ A-globulin. Fireman, Vannier, and Goodman³ as well as Augustin, Connolly, and Lloyd⁴ reported that reaginic antibody was precipitated with anti- γ A-globulin antibody. It was also found that the γ A-globulin fraction of reaginic sera had reaginic activity⁴⁻⁶ and that passive sensitization of human skin with reaginic antibody was blocked by previous treatment of the skin sites with normal γ A- but not with γ G- or γ M-globulin.⁷ The present experiments were undertaken to study whether a part of reaginic antibody is associated with γ A-globulin with sera of three patients other than those studied in previous reports.^{1, 2} The results confirm the previous findings⁴⁻⁶ that γ A-globulin fraction of the sera had high reaginic activity. However, activity in the γ A-globulin fraction is not removed by precipitation of γ A-globulin by rabbit antibody specific for γ A-globulin, suggesting that the reaginic activity is associated with impurities present in γ A-globulin preparations.

MATERIALS AND METHODS

Reaginic sera

Three serum samples from ragweed-sensitive patients were studied. Serum from one patient (Serum I) was supplied by Dr. H. Fudenberg, University of California, San Francisco, California; the other two serum pools, one from untreated patients (Serum II) and the other from treated patients (Serum III), were supplied by Dr. L. M. Lichtenstein, Johns Hopkins University. A serum sample from an egg-sensitive patient was also used. It was the same sample as that studied in previous experiments.^{1, 2}

Gamma-A-myeloma proteins

Three preparations of γ A-myeloma protein were supplied by Dr. H. Fudenberg. These were purified from patients' sera by starch-block electrophoresis.

Antibodies specific for immunoglobulins

Rabbit antisera specific for one of the three immunoglobulins, γ G, γ A and γ M, were the same preparations described in the previous papers.^{1, 2, 8} They were obtained by immunization with one of the three immunoglobulins from normal human sera, followed by absorption of cross-reacting antibodies with appropriate purified immunoglobulins at the equivalence region. In immunodiffusion, each antiserum gave a single precipitin band of identical specificity with human serum and the respective immunoglobulin, but not with the other two immunoglobulins. The γ -globulin fraction of the anti- γ A-globulin rabbit serum was purified by chromatography on diethylaminoethyl (DEAE) cellulose (Mann Research Laboratory, New York, New York) columns.^{1, 2} The goat anti- γ A-globulin was purchased from Hyland Laboratories, Los Angeles, California.

Fractionation of reaginic sera

A globulin fraction of the sera of ragweed-sensitive patients was obtained by precipitation with ammonium sulfate at half saturation at pH 7.0. The precipitate obtained from 100 to 200 ml. of each serum was dissolved in saline, dialyzed against 0.005M phosphate buffer (pH 8.0), and applied to a DEAE cellulose column (3.5 by 25 cm.) which had been equilibrated with the buffer. Proteins were eluted stepwise from the columns with phosphate buffers (pH 8.0) increasing in molarity; for example, 0.005M, 0.01M, 0.02M, 0.035M, 0.05M, and 0.08M buffers. Fractions of 5 ml. were collected and absorption at 280 $m\mu$ was determined.

The reagin-containing fractions were further fractionated by gel filtration through Sephadex G-200 (Pharmacia, Uppsala, Sweden) columns. A 5 ml. sample was applied to a Sephadex G-200 column (2 by 110 cm.) and eluted with borate-buffered saline (pH 8.0). Fractions of 2 ml. were collected and absorption at 280 $m\mu$ was determined.

The γ A-globulin fraction of reaginic sera was obtained by the zinc-precipitation method described by Vaerman, Heremans, and Vaerman⁹ with slight modifications. Details of the method were described in previous papers.^{2, 8}

Immunodiffusion

Double diffusion in agar was carried out by the method of Ouchterlony.¹⁰ Immunoelectrophoresis was performed on microscope slides by the modification of Scheidegger.¹¹ The plates were incubated at room temperature for 48 hours, washed, and the precipitin bands stained with amido-Schwartz 10B. The goat antihuman serum used in the immunodiffusion experiments was purchased from Hyland Laboratories, Los Angeles, California. The concentration of γ A-globulin in chromatographic fractions of reaginic sera was estimated by immunoplates containing goat anti- γ A-serum.¹² As standards, serial dilutions (12.5 to 200 μ g N per milliliter) of γ A-globulin were tested simultaneously. The plates were incubated at room temperature overnight, and the precipitating areas around the wells were measured under magnification. The concentration of γ A-globulin in the fractions was estimated from a standard curve.

Prausnitz-Küstner reactions

The tests were carried out on individuals who were not sensitive to the allergen tested. One-twentieth milliliter of the test samples was injected intracutaneously. Twenty-four hours later the sites received 0.02 ml. of ragweed-allergen extract (Center Laboratories, Inc., New York, New York) containing 10 PNU or 0.02 ml. of 0.01 per cent crystalline egg albumin (5 times recrystallized, Nutritional Biochemical Corp., Cleveland, Ohio). Erythema-wheal reactions, which appeared 15 to 20 minutes after the injection of allergen, were traced on a transparent plate applied to the areas. When the reaginic activity of several samples was compared, all of the samples were tested on the same individuals in order to minimize individual differences in skin responses.

EXPERIMENTAL RESULTS

The globulin fraction of the reaginic sera was obtained by precipitation with one-half saturation of ammonium sulfate, and was chromatographed on DEAE cellulose columns. Proteins were eluted stepwise with 6 phosphate buffers of increasing molarity and the fractions eluted with each buffer were pooled separately. After concentration by negative pressure filtration, each pool was tested for reaginic activity by Prausnitz-Küstner (P-K) tests. Twofold dilutions of each fraction were injected intracutaneously into normal individuals for passive sensitization to determine the minimal dose of each required to give positive P-K reactions. The results shown in Table I indicated that the reaginic activity was eluted from DEAE cellulose columns with 0.01 to 0.035M phosphate buffers. On a weight basis, Fraction III which was obtained with 0.02M phosphate buffer showed the highest reaginic activity in all cases. In view of the previous findings² that the reaginic antibody is associated with the protein larger than γ G-globulin, Fraction III was applied to Sephadex G-200 columns. A single peak was obtained, and most of the reaginic activity was present in the ascending portion of the peak. The ascending portion (Fraction III₁) and the descending portion (Fraction III₂) of the peak were pooled separately and concentrated. As shown in Table I, Fraction III₁ had much higher reaginic activity than Fraction III₂. The chromatographic fractions were tested for the presence of γ G- and γ A-globulins by immunodiffusion, and the relative concentration of γ A-globulin was estimated by immunoplates containing anti- γ A-globulin antibody. Gamma-G-globulin was detected in all fractions, whereas Fractions I and II did not contain a detectable amount of γ A-globulin. The relative concentration of γ A-globulin was highest in Fractions III and IV. It is evident in Table I that the distribution of the reaginic activity in the chromatographic fractions did not parallel the γ A-globulin concentration. These findings were essentially the same as those reported in two different reaginic sera in a previous article.¹

Table I. *Distribution of reaginic activity in chromatographic fractions of patients' sera*

Fraction	Buffer for elution (mole/L.)	Minimum dose of protein N (μ g) for a positive P-K reaction*		
		Serum I	Serum II	Serum III
I	0.005	> 4 (0)	> 2 (0)	> 8 (0)
II	0.01	2.0 (0)	0.4 (0)	1.6 (0)
III	0.02	0.5 (15)	0.1 (31)	—
III ₁	0.02†	0.1 (29)	0.05 (53)	0.4 (54)
III ₂	0.02†	1.0 (5)	0.5 (18)	1.6 (26)
IV	0.035	1.0 (63)	0.4 (32)	1.6 (32)
V	0.05	> 4 (17)	> 2 (10)	> 8 (9)
VI	0.08	> 4 (10)	> 2 (5)	> 8 (5)

*For passive sensitization, 0.05 ml. was injected. Numerals in parentheses are relative concentrations (per cent) of γ A-globulin determined in immunoplates; (0) represents less than 1 per cent in total protein.

†Frs. III₁ and III₂ were obtained by gel filtration of Fr. III through Sephadex G-200 columns.

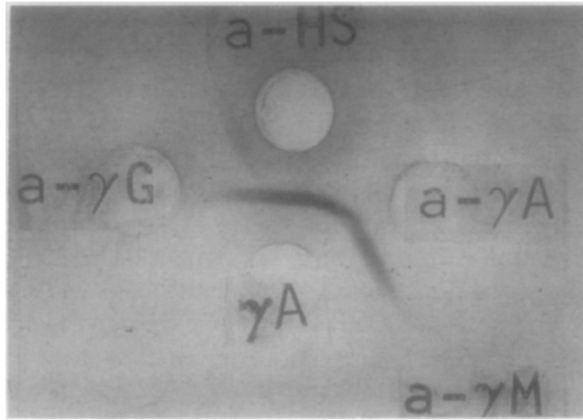


Fig. 1A

A, Immunodiffusion of a γ A-globulin preparation in a center well against antihuman serum (*a HS*), and the antisera specific for γ G-, γ A-, or γ M-globulin in peripheral wells.

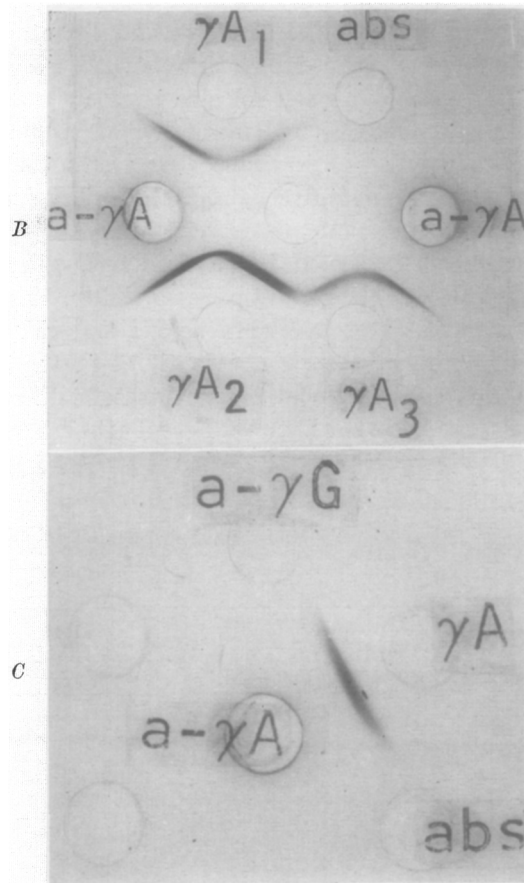


Fig. 1, B and C

Ouchterlony plates showing lack of γ A-globulin in the supernatants (*abs*) after precipitation of this protein in Fr. γA_1 (*B*) and γ A-globulin fraction from Serum II, (*C*). A center well in Fig. 1*B* was filled with the rabbit anti- γ A antibody used for the precipitation, and two peripheral wells (*a-γA*) were filled with a goat anti- γ A antiserum.

The γ A-globulin fraction of the same reaginic sera was obtained by the zinc-precipitation method⁸ and tested for purity by immunodiffusion. In all three sera, the γ A-globulin fraction gave a single precipitin band with antihuman serum and anti- γ A-globulin serum but not with the antisera specific for one of the other two immunoglobulins. A representative result is shown in Fig. 1A. The γ A-globulin fractions gave a single precipitin band with antihuman serum in immunoelectrophoresis (Fig. 2). Serial twofold dilutions of the γ A-globulin fractions were injected intracutaneously into normal individuals for passive sensitization to determine the minimal dose of the protein required for a positive P-K reaction. As reported previously,^{4,6} the γ A-globulin fraction had high reaginic activity (Table II). Since the reaginic activity in patients' sera was eluted with 0.02M phosphate buffer from DEAE cellulose columns (Table I), and since γ A-globulin is chromatographically heterogeneous, the γ A-globulin preparations of the reaginic sera were further fractionated by chromatography on DEAE cellulose columns. A 5 ml. volume of the preparation was applied to a DEAE cellulose column (2 by 50 cm.) which was equilibrated with the buffer, and the proteins were eluted stepwise with 0.02, 0.035, and 0.08M buffers. A representative chromatogram of a γ A-globulin preparation from Serum II is

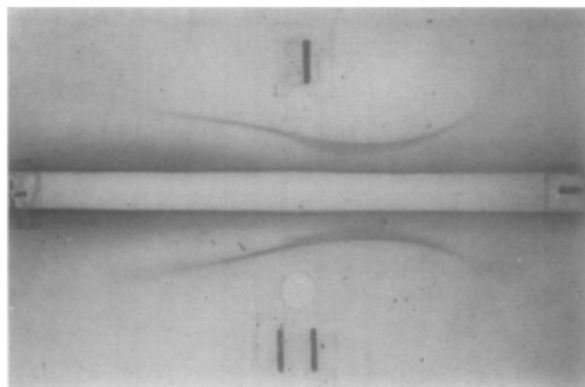


Fig. 2

Immunoelectrophoresis of γ A-globulin preparations from reaginic sera (I, II). A trough was filled with antihuman serum.

Table II. *Distribution of reaginic antibody in subfractions of γ A-globulin**

Subfraction	Buffer for elution† (mole/L.)	Minimum dose (μ g N) for P-K reaction		
		Serum I	Serum II	Serum III
Unfractionated	—	0.3	0.1	0.2
1	0.02	0.06	0.05	0.03
2	0.035	2.0	0.25	0.4
3	0.08	> 1.5	0.5	1.6

*See footnote for Table I.

† γ A-globulin was fractionated by chromatography on DEAE cellulose columns.

shown in Fig. 3. The proteins eluted with different buffers were pooled separately and concentrated by negative pressure filtration (Fractions γA_1 , γA_2 , and γA_3). The concentrations of γA -globulin in the subfractions were estimated using immunoplates containing anti- γA -globulin, and reaginic activity of the fractions was determined by P-K tests. It was found that over 50 per cent of the reaginic activity in the γA -globulin fraction was recovered by elution with 0.02M phosphate buffer (Fraction γA_1), whereas, the major part of γA -globulin was eluted with 0.035M buffer. On a weight basis, Fraction γA_1 showed the highest reaginic activity (Table II). It is evident that the reaginic activity and γA -globulin concentration in the subfractions were not parallel with each other.

The γA -globulin in Fraction γA_1 was precipitated with anti- γA -globulin antibody. Two-tenths milliliter of the fraction containing 10 to 16 μg N was incubated with an equal volume of the rabbit anti- γA -globulin preparation at 1 to 2° C. overnight. The precipitates formed were removed, and the presence of γA -globulin in the supernatant was tested by immunodiffusion and interfacial-ring tests. The supernatant fraction did not give a precipitin band against anti- γA -globulin by either method, indicating that γA -globulin in the fraction was precipitated (Fig. 1B). The supernatant and the unabsorbed fraction were compared with each other for skin-sensitizing activity in P-K reactions. Serial dilutions of the preparations were injected into normal individuals, and the skin sites were challenged with ragweed allergen. It was found that essentially all the reaginic activity remained in the supernatants (Table III). The absorption

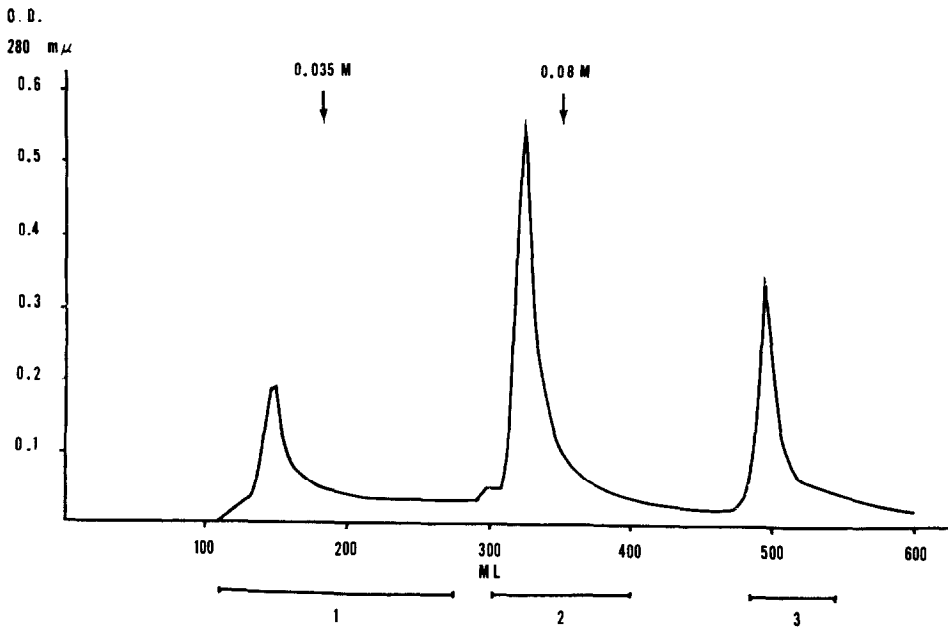


Fig. 3

Subfractionation of a γA -globulin preparation from Serum II on a DEAE cellulose column. The starting buffer was 0.02M phosphate pH 8.0. Fractions (1, 2, and 3) under the figure were pooled separately to get Frs. γA_1 , γA_2 , and γA_3 .

Table III. *Effect of absorption with anti- γ A-globulin on reaginic activity**

Fraction	Absorption with anti- γ A†	Dilutions of the fractions injected		
Serum I		10 × [0.24]‡	20 × [0.12]	40 × [0.06]
γ A ₁ †	+	24 × 34 (8.5)	28 × 28 (8.5)	20 × 16 (7)
	–	22 × 36 (9)	29 × 34 (9)	18 × 25 (7.5)
Serum II		36 × [0.1]	72 × [0.05]	144 × [0.025]
γ A ₁ †	+	33 × 41 (10)	26 × 34 (9)	0
	–	35 × 45 (10)	26 × 37 (8.5)	0
Serum II		36 × [0.4]	72 × [0.2]	144 × [0.1]
γ A	+	32 × 33 (9.5)	28 × 40 (8.5)	22 × 32 (6)
	–	35 × 44 (9)	29 × 35 (8)	23 × 26 (6)
Serum III		34 × [0.1]	68 × [0.05]	136 × [0.025]
γ A ₁ †	+	31 × 40 (10.5)	30 × 32 (8.5)	18 × 20 (7)
	–	32 × 40 (10)	25 × 32 (9)	16 × 17 (6)

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

†After absorption, no γ A-globulin was detected in the supernatants by either interfacial ring tests or immunodiffusion.

‡ γ A₁ represents a subfraction of γ A globulin fraction eluted from a DEAE cellulose column with 0.02M phosphate buffer (see Table II).

§[] Indicate total N (μ g) injected in the unabsorbed fraction.

experiments were repeated with a γ A-globulin fraction of Serum II. One-tenth milliliter of the γ A-globulin fraction containing 29 μ g N was mixed with 0.3 ml. of rabbit anti- γ A-antibody for precipitating γ A-globulin. As shown in Table III, essentially all reaginic activity remained in the supernatant, which did not contain a detectable amount of γ A-globulin (Fig. 1C). These findings indicate that reaginic antibody in the γ A-globulin fraction of the atopic patients' sera is not γ A-globulin itself, but is associated with impurities which are not detected by immunodiffusion.

Our previous studies showed that the normal human γ A-globulin preparation blocked passive sensitization of human skin with reagin.⁷ Since the data described above indicated that reagin was associated with impurities in the γ A-globulin preparation, the possibility was considered that the blocking activity of the γ A-globulin preparation may be due to the same protein with which the reagin is associated. In order to study this possibility, the blocking effect of the γ A subfractions from Serum I was studied with the use of anti-egg reaginic antibody as the sensitizing antibody. The reagin-containing fractions of a serum from an egg-sensitive patient were purified by chromatography on a DEAE cellulose

Table IV. *Blocking activity of γA subfractions from a reaginic serum on passive sensitization with heterologous antibody**

Fraction	μg N injected for pretreatment				
	5	2.5	1.0	0.5	0.25
γA_1	0	0	0	14.5 (0)	19.5 (6)
γA_2	0	22 (7)	28 (8)	31 (8)	—
γA_3	0	21 (6.5)	26 (8)	30 (8)	—

*The γA subfractions from a ragweed-sensitive serum (Serum I) were used for pretreatment of skin sites. A reagin-containing fraction from an egg-sensitive serum was injected for passive sensitization into the sites, followed by challenge with egg albumin. The numerals in the table represent average diameters (millimeters) of erythema; numerals in parentheses represent diameters of wheal in millimeters. Control sites showed erythema of 28 to 33 mm. diameters with 7 to 9 mm. wheals.

column and by gel filtration in a manner similar to that described for the fractionation of the ragweed-sensitive patient's sera. As described in previous papers,^{1, 2} the fraction obtained with 0.02M phosphate buffer from the DEAE cellulose column (Fraction C in Part II of this series) had the highest skin-sensitizing activity. Only 0.01 μg N of the fraction gave a positive P-K reaction. One-tenth milliliter of serial dilutions of the γA subfractions from Serum I was injected intracutaneously into normal individuals. Twenty-four hours later the skin sites received 0.02 μg N of the fraction containing anti-egg skin-sensitizing antibody, followed by challenge with egg albumin after another 24 hours. It was found that Fraction γA_1 had the highest blocking activity on a weight basis (Table IV). It is apparent that the blocking activity in the γA subfractions on passive sensitization with anti-egg antibody paralleled the skin-sensitizing activity of the fractions against ragweed-pollen extract. The blocking activity of Fractions I and II from Serum I was studied, with the use of the fraction from an egg-sensitive patient's serum as the source of reagin. As shown in Table I, neither Fraction I nor II contained a detectable amount of γA -globulin, but Fraction II had reaginic activity. It was found that even 10 μg N of Fraction I did not show any blocking effect, whereas 5 μg N of Fraction II partially blocked passive sensitization. The site which received Fraction II gave an erythema of 17 to 18 mm. in diameter without wheal; whereas the sites treated with Fraction I and the control site induced erythema of 30 to 33 mm. with a definite whealing (8 mm.). The blocking activity of three preparations of γA -myeloma proteins was also studied by the same procedure. None of the myeloma proteins blocked passive sensitization, even with as much as 10 μg N.

DISCUSSION

These data confirm previous observations that the distribution of skin-sensitizing activity in the chromatographic fractions of reaginic sera did not parallel the γA -globulin concentration in the fractions. On a weight basis, the reaginic activity of Fraction II, containing no detectable γA -globulin, was comparable to that of Fraction IV which contained a high concentration of γA -globulin. These findings were essentially the same as the previous observations with the other two reaginic sera,¹ and indicate that the reaginic antibody studied in the

previous experiments was not exceptional. The lack of parallelism between the γ A-globulin concentration and reaginic activity in the chromatographic fractions of reaginic sera was recently reported by Stanworth¹³ as well as by Perlmutter, Rose, and Goodfriend.¹⁴ It was found in a previous article² that reaginic antibody in Fraction III₁ was not precipitated with the antibody specific for γ A-globulin.

The concept that reaginic antibody is γ A-globulin is supported by the finding that pure γ A-globulin fractions of patients' sera had high reaginic activity.⁴⁻⁶ These findings were confirmed in the present experiments. No serum protein other than γ A-globulin was detected by immunodiffusion in the γ A-globulin fraction of the three patients' sera. When the γ A-globulin preparations were further fractionated by chromatography on DEAE cellulose columns, however, the reaginic activity was concentrated in a minor fraction (Fraction γ A₁) which was eluted with 0.02M phosphate buffer, and Fraction γ A₂ containing the major part of γ A-globulin did not have much activity. It was reported in a previous article⁸ that normal γ A-globulin fraction blocked passive sensitization of human skin with reaginic antibody, whereas neither γ G- nor γ M-globulin did. These findings suggest the possibility that the relatively low reaginic activity in Fraction γ A₂ might be due to nonspecific blocking of passive sensitization. However, this possibility is unlikely because Fraction γ A₁ from the ragweed-sensitive serum had higher blocking activity than Fraction γ A₂ on the passive sensitization with the anti-egg reaginic antibody. It is apparent that the relative concentration of reaginic antibody in Fraction γ A₁ is higher than that in Fraction γ A₂. Furthermore, essentially all reaginic antibody in Fraction γ A₁ remained in the supernatant after the precipitation of γ A-globulin with anti- γ A-globulin. It was also found that the reaginic activity in the original γ A-globulin fraction (Serum II) was not precipitated with anti- γ A-globulin. These findings indicate that the reaginic antibody in the γ A-globulin fraction is not γ A-globulin itself but is associated with impurities present in the fraction. Yagi and co-workers¹⁵ reported the presence of γ A anti-ragweed antibody by radioimmuno-electrophoresis, and Reisman, Arbesman, and Yagi¹⁶ detected γ A-globulin antibody in two thirds of the patients' sera studied. Our present results do not exclude the presence of γ A-globulin antibody, but highly suggest the possibility that the γ A-antibody is a blocking type of antibody rather than reaginic antibody.

The present experiments eliminate another piece of evidence supporting the concept that reaginic antibody is γ A-globulin. It was reported in a previous paper⁸ that the γ A-globulin fraction from normal human serum blocked passive sensitization with reagin. This finding was confirmed in the present experiments; however, a fraction containing no detectable amount of γ A-globulin also blocked passive sensitization. These results are in agreement with the observations by Girard and co-workers,¹⁷ who studied passive sensitization of monkey ileum with human reaginic antibody, that normal γ A-globulin preparations blocked passive sensitization and that the γ G-globulin fraction had less, but definite, blocking activity. It was also found that¹ the distribution of reaginic activity in the sub-fractions of the γ A-globulin preparation paralleled the distribution of blocking activity on passive sensitization with reaginic antibody of a heterologous system,

and that² γ A-myeloma proteins lacked the blocking activity. The two types of data highly suggest that the blocking activity of the γ A-globulin preparation may be due to impurities, and that both reaginic and blocking activities are associated with the same protein which is different from γ A-globulin.

The present, as well as previous, results^{1, 2} are quite different from the observations of Fireman, Vannier, and Goodman³ and Augustin, Connolly, and Lloyd⁴ who demonstrated that reaginic antibody was precipitated with anti- γ A-globulin antibody. Our results were reproducible in the samples from five different reaginic sera, two of which were pooled sera from treated or untreated patients. The possibility may be considered that the precipitation of γ A-globulin was not complete in our work. However, this is unlikely because it was found that the antibody preparation completely precipitated the γ A-isoagglutinin included in a reagin-containing fraction, and that a goat anti- γ A-globulin preparation also failed to precipitate reaginic antibody.² The discrepancies between our data and those of Fireman, Vannier, and Goodman³ may be due to the difference in animal species from which the anti- γ A-antibody was obtained. They used sheep antibody, whereas rabbit antibody was employed in our experiments. The possibility exists that the major antigenic determinants in the γ A-globulin molecules may be different, depending on the animal species used for immunization. The discrepancies between the two sets of experiments may be explained if the reaginic antibody is associated with the protein which has the major γ A-antigenic determinants in sheep, but lacks the γ A determinants in either rabbit or goat. In the case of γ G-globulin, it is known that the antigenic determinants in the γ chain in monkeys are different from those in rabbits, and, using monkey antibodies, human γ G-globulin has been classified into four subclasses.¹⁸⁻²⁰ Subclasses of γ A-globulin have not been reported; however, Lichter and Dray¹⁹ found two γ A-precipitin bands in immunoelectrophoresis between some normal human sera and monkey antihuman γ -globulin sera. If γ A-globulin is composed of subclasses, it is possible that a subclass of the protein is precipitated with sheep anti- γ A-antibody but not with rabbit antibody. Another possibility is that the reaginic antibody is associated with a unique immunoglobulin, and that the anti- γ A-globulin preparations used by other investigators^{3, 4} may be contaminated with the antibody specific for the protein; whereas our anti- γ A-globulin preparation did not contain this antibody. If this is the case, the γ A-globulin preparations from reaginic sera should give two or more precipitin bands with antihuman serum in immunoelectrophoresis. An explanation for the lack of a precipitin band, other than that of γ A-globulin, must await further studies. The possibility exists that the protein is a very minor component, and that most antihuman serum antibody preparations do not contain a sufficient concentration of the antibody specific for this protein needed to give a definite precipitin band. Evidence was presented previously¹ that 0.001 to 0.002 μ g N of this protein in a reagin-containing fraction was sufficient for the passive sensitization of normal human skin for positive P-K reactions. In our more recent studies, which will be published separately, a rabbit antiserum, containing none of the antibodies specific for γ A-, γ M-, γ G-globulins and those specific for κ or λ chains in immunoglobulins, precipitated reaginic activity in atopic pa-

tients' sera. Since the previous experiments² excluded the possibility that the reaginic antibody is γ D-globulin, it is highly probable that the reaginic antibody is associated with a new type of immunoglobulin other than γ G-, γ A-, γ M-, or γ D-globulins.

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