REAGINIC ACTIVITY ASSOCIATED WITH IgG IMMUNOGLOBULIN*,‡

By ROBERT T. REID, M.D., PERCY MINDEN,§ M.D., and RICHARD S. FARR, M.D.

(From the Division of Allergy, Immunology, and Rheumatology, Scripps Clinic and Research Foundation, La Jolla, California)

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Attempts to characterize the proteins in serum responsible for reaginic activity began in 1931 when Sherrer, using an ammonium sulfate fractionation technique, demonstrated that reagin could reside in both the euglobulins and the pseudoglobulins but was absent in the albumin fraction (2). Subsequently, numerous investigators using different techniques found reaginic activity in both gamma and beta globulins as classified on the basis of electrophoretic mobility (3–8), and in 1959, Heimlich *et al* showed that reagin could be associated with either high or low molecular weight immunoglobulins (9). Also in 1959, Heremans and coworkers demonstrated a new class of immunoglobulins which they called B_2A (10) and Schultze (11) showed that these immunoglobulins had antibody activity.

Subsequent to 1959, most investigations concerning the nature of reagin have demonstrated that reaginic activity resides in what is referred to as IgA immunoglobulin ($B_2A, \gamma_1A, \gamma A$).[1] These investigators have used (*a*) the zinc sulfate method to partially isolate the IgA fraction (13, 14); (*b*) physical methods to isolate relatively pure IgA and immune precipitation of the IgA and IgG fractions (15); (*c*) isolation of IgA from saliva without contamination by other immunoglobulins (16); (*d*) blocking of the Prausnitz-Küstner (P-K) reaction with purified IgA (17); and (*e*) blocking of the P-K reaction with the heavy chain of IgA which had been isolated after reduction and alkylation of whole IgA (18).

Taken as a whole, the literature suggests that the capacity of an immunoglobulin to fix to skin and subsequently elicit a P-K reaction is a quality associated with the heavy chain of the IgA globulin (18). This concept is in keeping with the currently held broader principle that the biological characteristics of various immunoglobulins such as complement-fixing capacity, placental transfer, and especially skin-fixing ability is conferred by the heavy chain of the immunoglobulin involved. Recently, however, six

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[1] Throughout this report, the nomenclature proposed at the World Health Organization meeting on Nomenclature of Immunoglobulins was used (12).

reports have appeared in the literature (1, 19–23) which indicate that immunoglobulins, other than IgA, also have the capacity to elicit a P-K reaction. Loveless (19) and Rockey *et al.* (20) reported a serum devoid of detectable IgA which had P-K reactivity against ragweed pollen extract. Perelmutter *et al.* (23) described a serum with both IgA and IgG reagin directed against ragweed pollen extract and Terr and Bentz (22) described reaginic activity in IgG and IgM immunoglobulins against horse serum proteins. These latter reagins were induced by the injection of horse serum and the IgG portion was resistant to heating to 56°C for 4 hr and to treatment with 0.1 M mercaptoethanol, whereas the IgM was sensitive to both these procedures. Fireman *et al.* (21) also described a reagin in the IgG class in a patient with telangiectasia ataxia who had no IgA in his serum. This reagin also differed from the traditional descriptions of reagin in that it was heat stable and remained in the skin for only short periods of time. This laboratory (1) described an unusual immunoglobulin which was heat labile, would fix to skin for at least 14 days, and in which the bulk of reaginic activity was found outside the IgA class of immunoglobulins. The present report describes these studies in detail together with some studies on four additional sera.

The finding of reaginic activity associated with immunoglobulins other than IgA suggests the need to reexamine the currently held concepts regarding the factors that are important in determining whether a particular immunoglobulin will or will not fix to skin and confer passively transferrable immunologic reactivity.

Materials and Methods

Antigens.—Crystalline bovine serum albumin (BSA) (Armour Pharmaceutical Co., Kankakee, Illinois, lot A69702), house dust, horse dander (10% solution), *Lolium perenne* (5% solution, perennial rye grass), *Cynodon dactylon* (5% solution, Bermuda grass pollen), Hollister-Stier Laboratories, Los Angeles, were used throughout this study.

Sera.—Five patients with symptoms of allergic rhinitis and/or bronchial asthma, who had strongly positive wheal and flare skin reactions to a number of allergens, were bled and their sera separated. For this report, the sera have been designated as Nos. 1, 2, 3, 4, and 5. Serum 1 was of particular interest because of the strong reaginic activity to BSA. Consequently, this serum was examined by all the procedures listed below. Sera 2, 3, 4, and 5 were examined only by anion exchange chromatography (DEAE) and by the passive transfer (P-K) skin test.

I^{131}-Labeled BSA.—For radioimmunoelectrophoresis studies and the antigen-binding capacity tests described below, the BSA was labeled with Iodine^{131} (I*BSA) using a modification (24) of the chloramine-T method (25).

Ammonium Sulfate Test (ABC-33).—Antibody to BSA was measured in duplicate samples by precipitating I*BSA-antibody complexes with 50% saturated ammonium sulfate (SAS). Details of this procedure and the calculations involved were carried out as previously reported (26) except that the SAS precipitates were washed once with 3 ml of equal parts of SAS and borate buffer.

Antigen-Precipitating Capacity (P-80).—The capacity of serum 1 to precipitate BSA was measured in duplicate using a variation (26) of the technique described by Talmage and Maurer (27) in which a constant amount of I*BSA in 0.5 ml of a 1:100 normal human serum diluent was added to 1 ml of serial dilutions of antiserum.

Microimmunodiffusion.—A 1 mm layer of ionager 2 (Consolidated Laboratories, Inc.,

Chicago Heights, Illinois) was applied onto microscope slides and wells cut with a template (LKB Products, Stockholm, Sweden). Central wells were filled with whole human serum 1. Six peripheral wells were filled with BSA antigen in concentrations ranging from 1.0 mg/ml to 0.03 mg/ml. The slides were incubated in a humidified chamber at 4°C for 72 hr, washed with normal saline for 48 hr, dried, and the precipitin bands stained with Amido black.

Hemagglutination studies were performed by the micromethod and plexiglass plates described by Sever (28). Sheep cells were prepared and tanned according to Stavitsky (29).

Passive cutaneous anaphylaxis (*PCA*) was performed using a variation of the technique described by Ovary (30). 16 hr after the intradermal injection of 0.1 ml of test serum, the guinea pigs were injected intravenously with 1.0 ml of a BSA-Evans blue dye solution containing 0.2 mg BSA N/ml and 10 mg. Evans blue/ml in saline, the dosage recommended by Campbell *et al.* (31).

Immunoelectrophoresis (*IEP*) was performed by the micromethod of Scheidegger (32). Electrophoresis was done on 1 × 3 in. microscope slides covered with 2 ml of 2% agar (Difco Laboratories, Inc., Detroit) in 0.0375 ionic strength veronal buffer (pH 8.6) as the support medium. Two outer wells and a center trough were cut by an Egaton template (National Instrument Laboratories, Washington, D. C.). The antisera (Hyland Laboratories, Los Angeles) were as follows: (*a*) goat, anti-human serum, lot 3802TID, 3802U5; (*b*) goat, anti-B_2A globulin, lot 206-7ABS 8962, GP 7964F; (*c*) goat, anti-human B_2M globulin, lot G37-98 P3316; and (*d*) goat, anti-human 7S, lot GP 11-63.

Radioimmunoelectrophoresis was performed by the method of Yagi *et al.* (33) with minor modifications. IEP plates were prepared as described above and after washing, approximately 0.1 ml BSA solution containing 1 γ-BSA N/ml and approximately 1.5 μc I^{131}/γ-BSA N, was applied to the trough. After standing overnight, the slides were washed for 1 min with distilled water, dried in the open air, and the agar surface applied to the emulsion surface of undeveloped Eastman Kodak high contrast projector slide plates, Eastman Kodak Co., Rochester, New York. After 7 days, the exposed plates were developed in the normal manner and the immunoelectrophoresis slides were stained with Azocarmine-G.

Passive Transfer Test (*P-K*).—0.1 ml aliquots of the test sera or the fractions under study were injected intradermally into the recipients. At intervals of 24 hr to 14 days, the sites were challenged by the intradermal injection of 0.02 ml of the appropriate antigen. The BSA antigen contained 1 mg/ml and the other antigens were diluted 1:100 or 1:1000 with buffered saline. Skin tests were graded from 0 to 4+ as follows:

	Wheal diameter	Erythema diameter
	mm	*mm*
±	<5	0
1+	5–10	10–15
2+	10–13	20–25
3+	13–15	25–35
4+	>15 and pseudopodia	>35

Anion Exchange Chromatography (*DEAE*).—All five sera were separated on diethylaminoethylcellulose (DEAE) by stepwise elution according to the method of Sober *et al.* (34), utilizing the following buffers: (*a*) phosphate buffer pH 7.0, 0.01 M; (*b*) phosphate buffer pH 7.0, 0.025 M; (*c*) phosphate buffer pH 6.0, 0.1 M; and (*d*) phosphate buffer pH 5, 0.3 M. Before application to the column, the sera were diluted 1:2 and dialyzed overnight against 0.01 M dibasic sodium phosphate. 5 ml fractions were collected in a Rimco fraction collector and read on a Beckman DU spectrophotometer at 280 mu. The peaks were pooled, dialyzed,

lyophilized, resuspended in buffered saline, and dialyzed again. They were sterilized by passing through a Seitz filter, frozen, and stored at −20°C until tested. Examination of the peaks by IEP revealed that peak 1 contained only IgG, peak 2 contained occasional traces of IgG and IgA, and peak 3 contained IgA and occasional traces of IgG. Peak 4 contained IgM and occasional traces of IgA and IgG.

Gel Filtration.—Serum 1 was fractionated on Sephadex G-200 (Pharmacia, Upsala, Sweden) using the technique described by Terr and Bentz (35). The column was 116 cm in length, 3.8 cm in width, and the flow rate 5 to 10 ml/hr. 5 ml aliquots were collected and the protein concentration determined by absorption at 280 mu on the Beckman DU spectrophotometer.

Pevikon Block Electrophoresis.—Serum 1 was fractionated using the method of Müller-Eberhard (36). Proteins were migrated on the support medium in veronal buffer pH 8.6, 0.05 M. The proteins were electrophoresed at 400 v for 18 hr at 4°C. The block was then cut in ½ in. fractions and the proteins eluted with buffered saline. Fractions were immediately dialyzed against phosphate-buffered saline (pH 7.0), and then concentrated by negative pressure dialysis. Protein concentrations were determined either by absorption at 280 mu on a Beckman Spectrophotometer or by the Lowry modification of the Folin-Cicolteau method (37).

Density gradient ultracentrifugation was performed as described by Kunkel (38). Serum 1 was diluted 1:2 and 1 ml layered over the top of a 4 ml sucrose density gradient of from 10 to 40% sucrose and spun for a total of 16 hr at 35,000 RPM in a Spinco model L ultracentrifuge using a SW-39 head. The fractions were collected by perforating the bottom of the plastic tube with a needle and collecting half ml fractions.

Mercaptoethanol Reduction.—Serum 1 was dialyzed against 0.1, 0.2, and 0.3 M 2-mercaptoethanol for 48 hr, then against 0.01 M iodoacetamide for 48 hr, and then against buffered saline for 48 hr. P-K studies were compared before and after mercaptoethanol treatment.

RESULTS

Throughout the study, either the whole serum or a fraction obtained by one of the fractionation methods was studied for its capacity to give a positive P-K test. The whole serum or fraction was also evaluated for the type of immunoglobulin present by immuno- and radioimmunoelectrophoresis, and then for antibody content by the ammonium sulfate test or by hemagglutination. In whole serum, precipitating ability was measured by gel diffusion and the P-80 test.

Studies of Whole Serum 1.—Some of the reaginic properties of serum 1 are presented in Tables I and II. A 1:32 dilution of serum was capable of passively transferring skin reactivity to crystalline BSA and a 1:16 serum dilution transferred reactivity to *Cynodon dactylon*. After heating the serum to 56°C for 4 hr, reactivity against both BSA and *Cynodon dactylon* was completely abolished. Tests for duration of reactivity at the passively transferred sites showed there was no reduction in the strength of reaction for at least 14 days following injection of serum into the test site. As previously reported (39, 40) treatment with 0.1 M mercaptoethanol almost completely destroyed P-K reactivity to the pollen antigen, but reactivity to BSA was much less affected. After treatment with 0.2 and 0.3 M mercaptoethanol, however, reaginic activity to both antigens was completely destroyed. To verify that 0.1 M mercaptoethanol had been

effective in splitting disulfide bonds in those immunoglobulins sensitive to disulfide splitting agents, this patient's isoagglutinin titers were compared before and after treatment with 0.1 M mercaptoethanol. Before treatment, the

TABLE I

Prausnitz-Küstner Titer to BSA and Cynodon Dactylon before and after Heating to 56°C for 4 Hr, and Treatment with Mercaptoethanol

Whole serum			Heated serum		Mercaptoethanol-treated serum					
					0.1 M		0.2 M		0.3 M	
Serum dilution	BSA 1 mg/ml	*Cyn. dact.* 1:10 dilution	BSA 1 mg/ml	*Cyn. dact.* 1:10 dilution	BSA 1 mg/ml	*Cyn. dact.* 1:10 dilution	BSA 1 mg/ml	*Cyn. dact.* 1:10 dilution	BSA 1 mg/ml	*Cyn. dact.* 1:10 dilution
1:1	4+	3+	0	0	3+	1+	0	0	0	0
1:2	4+	3+	—	—	3+	±	0	0	0	0
1:4	4+	3+	—	—	2+	±	0	0	0	0
1:8	3+	2+	—	—	2+	0	0	0	0	0
1:16	2+	1+	—	—	±	0	0	0	0	0
1:32	2+	—	—	—	±	0	0	0	0	0
1:64		0	—	—		0	0	0	0	0

TABLE II

Changes in Antibody Activity before and after Heating to 56°C for 4 hr, or Treatment with Mercaptoethanol

	Whole serum	Heated serum	Mercaptoethanol-treated serum		
			0.1 M‡	*0.2 M*	*0.3 M*
ABC-33 at 0.02γ-I*BSA N/ml	7.3	5.2	4.5	4.8	4.2
Hemagglutination titer	1:64	1:16	None	None	None
P-80 at 0.02γ-I* BSA N/ml	None	None	None	None	
Immunodiffusion band	None	Not done	Not done	Not done	Not done
PCA (guinea pig)	None	Not done	Not done	Not done	Not done

‡ 0.1 M 2-mercaptoethanol in phosphate-buffered saline pH 7.0.

anti-A and anti-B titers were 1:112 and 1:244 respectively, and after treatment, both titers fell to zero.

The specific antigen-binding capacity of this serum to BSA was measured by the ammonium sulfate test before and after heating the serum for 4 hr to 56°C, before, and after decomplementing and before and after treatment with various concentrations of mercaptoethanol (Table II). An untreated control sample had an ABC-33 of 7.3 γ. After heat treatment, or decomplementation,

the ABC-33 value fell to 5.2 γ. After mercaptoethanol treatment, it fell to 4.5 γ. This reduction in antibody-binding capacity may simply be a reflection of protein destruction as a result of the physical or chemical treatments employed. However, a reduction of specific reaginic antibody cannot be excluded. There was complete loss of hemagglutinating capacity after treatment with the various dilutions of mercaptoethanol, but after heating, this was only partial.

P-80 and microimmunodiffusion studies with this serum revealed that the antibody with capacity to bind BSA did not have the capacity to precipitate BSA. This is in keeping with previous observations that human sera containing

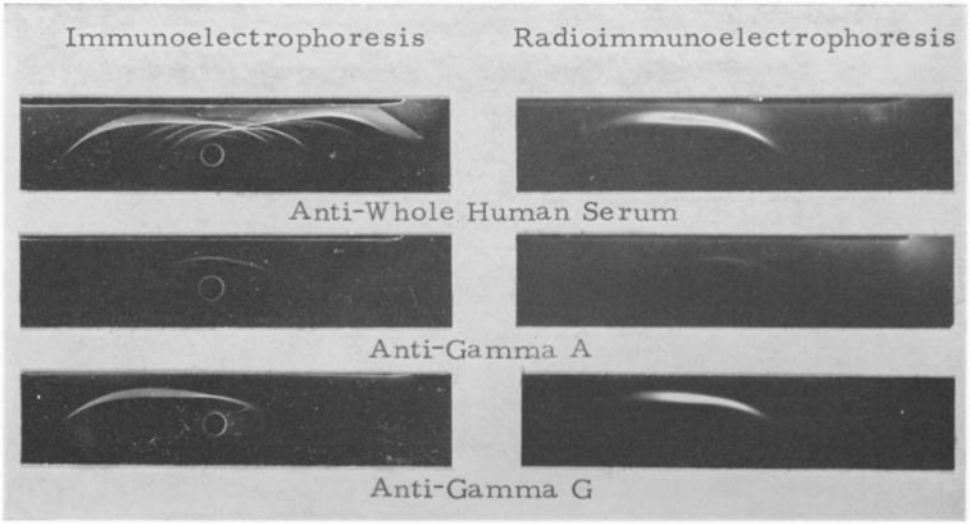

FIG. 1. Immunoelectrophoresis and radioimmunoelectrophoresis of whole serum utilizing I*BSA in the radioimmunoelectrophoresis slides.

anti-BSA with or without reagin activity tend to have very low antigen-precipitating efficiencies (41).

PCA studies showed no evidence of antibody activity in this serum although there was a strongly positive reaction with the control rabbit anti-BSA serum that had comparable antigen-binding capacity.

Radioimmunoelectrophoresis studies (see Fig. 1) clearly demonstrated that this serum contained IgG as well as IgA with capacity to bind I*BSA. Grossly at least, it appeared that the faster migrating IgG globulin bound more I*BSA than did the less highly charged IgG globulin, an observation in keeping with the Pevikon electrophoretic data. The radioimmunoelectrophoresis plates before and after treatment with heat or mercaptoethanol were grossly similar, indicating that these procedures did not affect the migrational characteristics or the I*BSA binding capacities of either IgG or IgA.

DEAE Fractionation of Serum 1.—(See Fig. 2) P-K activity against BSA was limited to peaks 1 and 2 which contained only IgG by immuno- and radioimmunoelectrophoresis arcs whereas peak 3, which was devoid of P-K activity, contained the only detectable IgA and minute amounts of IgG. It would appear, that the reagin under study was separated from the IgA and separated with immunoglobulins of the IgG class. When antigen-binding capacity and hemag-

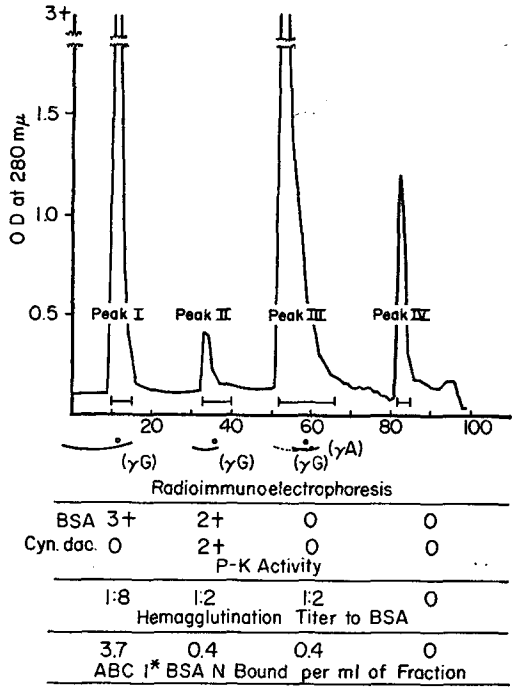

FIG. 2. DEAE fractionation of reaginic activity. Anion exchange chromatography (DEAE) of serum 1. P-K activity is limited to peaks 1 and 2 which contain IgG and are devoid of IgA. No P-K activity was noted in peak 3 which contained the bulk of IgA. The majority of antibody activity by ABC and hemagglutination is in peak 1.

glutination tests were performed, the majority of antibody activity was in peak 1 with lesser activity in peaks 2 and 3. Peaks 2 and 3 both bound 0.4 γ-I*BSA N/ml but peak 2 had 2+ reaginic activity and peak 3 had none. Precipitating activity was not present in any fraction.

Pevikon Electrophoretic Fractionation of Serum 1.—(See Fig. 3) P-K activity closely followed the distribution of the faster migrating IgG globulin as identified by immuno- and radioimmunoelectrophoresis. Fractions 4 through 7 which contained the bulk of P-K activity did not contain demonstrable IgA as

measured by immuno- or radioimmunoelectrophoresis. P-K activity was also found in fractions 2 and 3 which were on the cathode side of the point of application (fraction 4). Fractions 8, 9, and 10 were not helpful in determining whether P-K reactivity was associated with IgA or IgG because these fractions contained both classes of immunoglobulin which bound I*BSA as detected with radioimmunoelectrophoresis. ABC-33 values closely correlated to the areas where reaginic activity was isolated although other methods of fractionation

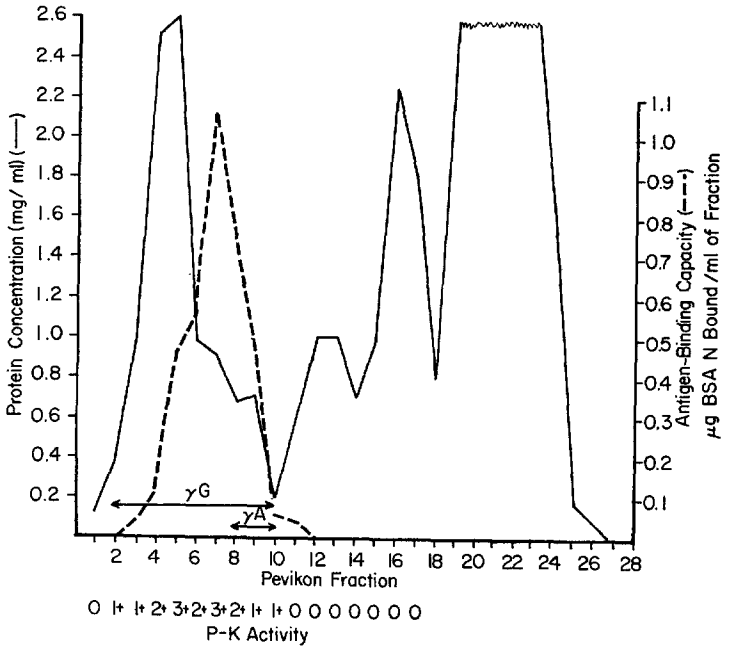

FIG. 3. Pevikon fractionation of reaginic activity. Pevikon block fractionation of whole serum 1. P-K activity closely follows the faster migrating IgG globulin. Fractions with the bulk of P-K activity do not contain IgA by IEP.

were able to dissociate much, but not all, of the I*BSA-binding capacity from P-K activity.

Sephadex G-200 Fractionation of Serum 1.—(See Fig. 4) P-K activity was present in fraction pools H, I, and J. Most of the reaginic activity was detected in pool J which contained IgG and no IgA as detected by radioimmunoelectrophoresis. Pool I with less P-K reactivity contained IgG but no detectable IgA. Although pool H did not contain detectable IgG, it did have some P-K reactivity and some IgA. Pool G was of interest because it contained the bulk of the IgA by radioimmunoelectrophoresis yet contained no P-K reactivity. Neither P-K nor antibody activity was detected in fraction pools A through D, which

would have contained any macroimmunoglobulin directed against BSA. Thus, it would appear that P-K activity was again more closely associated with IgG than with IgA. It was of interest to note that fractionation by gel

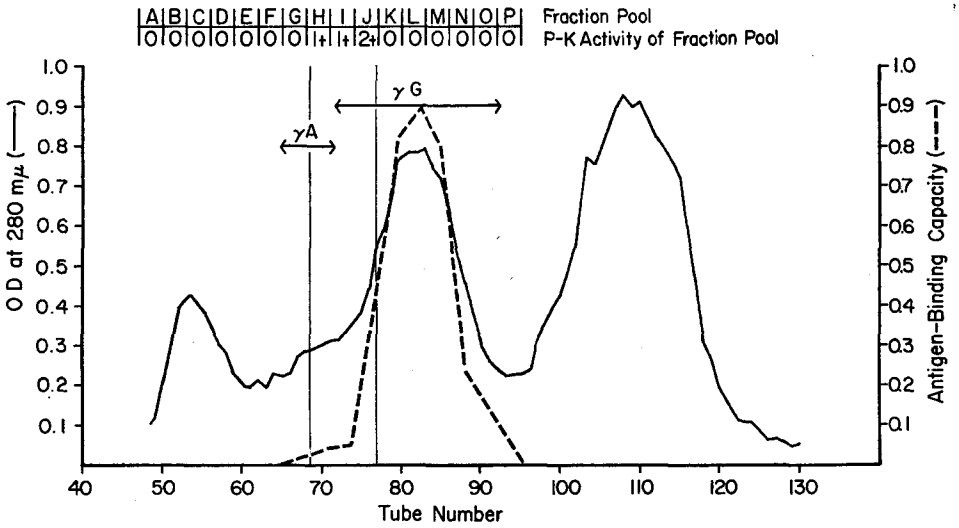

FIG. 4. Sephadex G-200 fractionation of reaginic activity. G-200 fractionation of serum 1. Most of the P-K activity is in pool J which contains IgG and no IgA by IEP. No P-K activity was detected in pool G which contains the bulk of IgA.

TABLE III

Highest Dilution of DEAE Peaks from Sera 2, 3, 4, and 5 where P-K Activity was 2+ or Higher

Serum No.....	2		3	4	5	
Antigen	*Lolium perenne*	House dust	Horse dander	*Lolium perenne*	*Lolium perenne*	House dust
DEAE peak 1.....	1:20	1:40	1:80	1:20	1:1	1:40
DEAE peak 2.....	0	0	1:80	1:40	1:10	1:40
DEAE peak 3.....	1:80	1:160	1:80	1:80	1:10	0
DEAE peak 4.....	0	0	0	0	0	0

filtration is the method of choice for separating the bulk of antigen-binding capacity from the bulk of P-K activity in this particular serum. It is stressed, however, that fractions have never been found which contained P-K activity in the absence of antigen-binding capacity.

Density Gradient Fractionation of Serum 1.—The density gradient studies revealed that the bulk of this patient's IgA had sedimentation characteristics

similar to the bulk of the IgG and P-K reactivity was associated with these fractions. This method of protein fractionation, therefore, was not useful in distinguishing between reaginic activity associated with IgG or IgA.

DEAE Fractionation of Sera 2, 3, 4, and 5.—The P-K activity in fractions obtained by DEAE chromatography of the four other sera are described in Table III. In each instance, definite P-K activity was noted in fractions from peak 1. In serum 5, there was no activity in peak 3 to house dust although there was a reaction to *Cynodon dactylon* demonstrating that heterogeneity of reagin molecules must exist in the same individual. In serum 1 (see Fig. 2), reagin activity was confined to peaks 1 and 2.

DISCUSSION

The studies described above demonstrate that the majority or all the reagin in serum 1 was not an IgA because (*a*) when whole serum was fractionated by anion exchange chromatography, activity was associated with fractions containing only IgG and those fractions that contained most of the IgA and only trace amounts of IgG were devoid of activity; (*b*) separation of serum proteins by Pevikon block electrophoresis revealed that the largest amount of skin-sensitizing antibody was in those fractions with only IgG. Some activity, however, was demonstrable in fractions containing both IgG and IgA; and (*c*) separation of serum proteins by gel filtration revealed the majority of P-K activity was associated with IgG. Only one fraction with IgA but with no demonstrable IgG, had slight reactivity.

For the same reasons, the reagin in this serum would appear to be an IgG globulin. However, the elution properties from Sephadex G-200 and the speed of migration on Pevikon block electrophoresis, suggest that this reagin does not belong to the mass of IgG but is associated with a distinct subgroup of the IgG globulins which are either larger, of a different configuration, and/or of slightly higher charge than the majority of the IgG globulins.

Using all techniques, skin reactivity was not associated with fractions containing IgM. Recently, a new class of immunoglobulin has been reported by Rowe and Fahey (42) and designated as IgD. The reagin studied in serum 1 does not seem to be IgD because it is eluted from the DEAE column before transferrin and at a lower molarity than described for IgD.

It has been well established that the immunoglobulins are complex proteins made up of two kinds of polypeptide chains each under separate genetic control and held together by disulfide bonds (43). The light chains, of either the K or L types, are present in nearly all known antibodies regardless of the type of heavy chain, and heterologous antisera directed against human light chains will react with all known classes of human immunoglobulin. The heavy chains differ in structure for each class of immunoglobulin and these structural differences confer many of the unique biological properties associated with the various

immunoglobulins. Further, antigenic differences between the various types of heavy chain are the basis for the currently used classification of immunoglobulins. The work of Ishizaka et al. (18) suggests that the portion of antibody molecule responsible for reaginic activity in the IgA system is associated with the heavy chain of this class of antibody. It is now clear, however, that the capacities to fix to skin and elicit a wheal and erythema reaction are qualities which occur in immunoglobulins other than IgA. As demonstrated by this and other reports, a positive P-K test crosses the current immunoglobulin classification boundary based on the immunospecificity of the heavy chain and indicates that reaginic qualities are not simply a matter of the presence or absence of an IgA heavy chain. Since P-K activity is not limited to IgA heavy chain, at least four additional possibilities become plausible and deserve further consideration: (*a*) that each class of immunoglobulin has a heavy chain subgroup with a common structural moiety with capacity to confer reaginic activity. According to this notion, if the skin-fixing moiety of the different immunoglobulins were identical for each immunoglobulin class, the skin-fixing group is either not antigenic or it is not detected by the typing antisera currently available; (*b*) that each class of immunoglobulin has a heavy chain subgroup with a structural moiety with capacity to confer reaginic activity but that this moiety is distinct and unique for each major class of immunoglobulin. This skin-fixing group could be confined to the Fd or the Fc portion, or may be a property of the intact heavy chain; (*c*) since reaginic activity can no longer be considered a unique property of IgA, the skin-fixing characteristic of reagin may be determined by the light chain, regardless of its associated heavy chain; and (*d*) the skin-fixing properties are the result of the steric configuration resulting from coupling of light to heavy chains without regard to the class of light or heavy chains involved.

The relative frequency of reaginic activity in the IgG fraction, as compared to the frequency of such activity in the IgA fractions is currently not known. The finding of reaginic activity in the IgG fractions of sera from the four other patients tested (Table III) indicates that the presence of reagin in IgG is not an infrequent phenomenon. The clinical significance and relative importance of the presence of IgG type of reagin must await further study.

SUMMARY

Five human sera with reaginic activity to a number of allergens were fractionated using anion exchange chromatography. In each serum, fractions which contained detectable IgG and no detectable IgA had capacity to fix to skin and subsequently elicit a P-K reaction. Four of these sera had reaginic activity about equally distributed between fractions containing only IgG and fractions containing mixtures of IgG and IgA. A fifth serum contained reaginic activity to crystalline bovine serum albumin (BSA) and most of the activity was asso-

ciated with the fraction which contained only IgG. This serum was extensively studied using a variety of techniques and it was confirmed that most of the reagin to BSA in this serum was in those fractions containing only IgG. Since reaginic activity can no longer be considered a unique property of IgA the implications of finding antibody with reaginic qualities in immunoglobulins other than IgA are discussed.

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BIBLIOGRAPHY

1. Reid, R. T., and Farr, R. S., An unusual immunoglobulin with reagin activity, *Fed. Proc.*, 1965, **24,** 633.
2. Sherrer, E. L., The distribution of reagins in the blood plasma, *J. Allergy*, 1931, **2,** 467.
3. Newell, J. M., Sterling, A., Oxman, M. F., Burden, S. S., and Krejci, L. E., Electrophoretic separation of the antibody from human allergic serum, *J. Allergy*, 1939, **10,** 513.
4. Campbell, D. H., Cann, J. R., Friedman, T. B., and Brown, R. A., Reaginic serum fractions obtained by the electrophoresis-convection method, *J. Allergy*, 1950, **21,** 519.
5. Loveless, M. H., and Cann, J. R., Distribution of allergic and "blocking" activity in human serum proteins fractionated by electrophoresis convection, *Science*, 1953, **117,** 105.
6. Kuhns, W. J., Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid, *J. Exp. Med.*, 1954, **99,** 577.
7. Campbell, D. H., Cann, J. R., Friedman, T. B., and Brown, R., The relation of allergy reagins to electrophoretic components of serum, *Science*, 1954, **119,** 289.
8. Humphrey, J. H., and Porter, R. R., Reagin content of chromatographic fractions of human gamma-globulins, *Lancet*, 1957, **1,** 196.
9. Heimlich, E. M., Vannier, W. E., and Campbell, D. H., Sedimentation studies of skin-sensitizing antibody, *J. Allergy*, 1959, **30,** 273.
10. Heremans, J. F., Heremans, M. T., and Schultze, H. E., Isolation and description of a few properties of B_2A-globulins of human serum, *Clin. Chim. Acta.*, 1959, **4,** 96.
11. Schultze, H. E., The synthesis of antibodies and proteins, *Clin. Chim. Acta.*, 1959, **4,** 610.
12. World Health Organization meeting on nomenclature of human immunoglobulins: Nomenclature of human immunoglobulins, *Bull. World Health Organ.*, 1964, **30,** 447.
13. Heremans, J. F., and Vaerman, J. P., B_2A-globulin as a possible carrier of allergic reaginic activity, *Nature*, 1962, **193,** 1091.
14. Vaerman, J. P., Epstein, W., Fudenburg, H., and Ishizaka, K., Direct demonstration of reagin activity in purified γ_1A-globulin, *Nature*, 1964, **203,** 1046.
15. Fireman, P., Vannier, W. E., and Goodman, H. C., The association of skin-

sensitizing antibody with the B_2A-globulins in sera from ragweed-sensitive patients, *J. Exp. Med.*, 1963, **117,** 603.

16. Ishizaka, K., Dennis, E. G., and Hornbrook, M., Presence of reagin and γ_1A-globulin in saliva, *J. Allergy*, 1964, **35,** 143.
17. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M., Blocking of Prausnitz-Küstner sensitization with reagin by normal human B_2A-globulin, *J. Allergy*, 1963, **34,** 395.
18. Ishizaka, K., Ishizaka, T., and Hathorn, E. M., Blocking of Prausnitz-Küstner sensitization with reagin by 'A chain' of human γ_1A-globulin, *Immunochemistry*, 1964, **1,** 197.
19. Loveless, M. H., Reagin production in a healthy male who forms no detectable B_2A immunoglobulins, *Fed. Proc.*, 1964, **23,** 403.
20. Rockey, J. H., Hanson, L. A., Heremans, J. F., and Kunkel, H. G., $Beta_2A$ aglobulinemia in two healthy men, *J. Lab. and Clin. Med.*, 1964, **63,** 205.
21. Fireman, P., Boesman, M., and Gitlin, D., Association of a skin-sensitizing antibody with $7S\gamma_2$-globulins, *J. Allergy*, 1965, **36,** 194 (Abstract).
22. Terr, A., and Bentz, J., Skin-sensitizing antibodies in serum sickness, *J. Allergy*, 1965, **36,** 433.
23. Perelmutter, L., Rose, B., and Goodfriend, L., Isolation of active IgG and IgA proteins from allergic sera, *Fed. Proc.*, 1965, **24,** 632.
24. McConahey, P., and Dixon, F. J., A method of trace iodination of proteins for immunologic studies, *Intern. Arch. Allergy and Appl. Immunol.*, 1966, **29,** 185.
25. Hunter, W. M., and Greenwood, F. C., Preparation of Iodine-131 labeled human growth hormone of high specific activity, *Nature*, 1962, **194,** 495.
26. Farr, R. S., A quantitative immunochemical measure of the primary interaction between I*BSA and antibody, *J. Infect. Dis.*, 1958, **103,** 239.
27. Talmage, D. W., and Maurer, P. H., I^{131} labeled antigen precipitation as a measure of quality and quantity of antibody, *J. Infect. Dis.*, 1953, **92,** 288.
28. Sever, J. L., Application of a microtechnique to viral serological investigations, *J. Immunol.*, 1962, **88,** 320.
29. Stavitsky, A. B., Micromethods for the study of proteins and antibodies, *J. Immunol.*, 1954, **72,** 360.
30. Ovary, Z., Immediate reaction in skin of experimental animals provoked by antibody-antigen interaction, *Progr. Allergy*, 1958, **5,** 459.
31. Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H., Methods in Immunology, New York, W. A. Benjamin, Inc., 1963.
32. Scheidegger, J. J., Une micro-methode de l' immuno-electrophorese. *Intern. Arch. Allergy and Appl. Immunol.*, 1955, **7,** 103.
33. Yagi, Y., Maier, P., Pressman, D., Arbesman, C. E., and Reisman, R. E., The presence of the ragweed-binding antibodies in the B_2A-, B_2M- and γ-globulins of the sensitive individuals, *J. Immunol.*, 1963, **91,** 83.
34. Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A., Chromatography of proteins. II. Fractionation of serum proteins on anion exchange cellulose, *J. Am. Chem. Soc.*, 1956, **78,** 756.
35. Terr, A. I., and Bentz, J. D., Gel filtration of human skin-sensitizing antibody and B_2A-globulin, *J. Allergy*, 1964, **35,** 206.

36. Müller-Eberhard, H. J., A new supporting medium for preparative electrophoresis, *Scand. J. Clin. and Lab. Invest.*, 1960, **12,** 33.
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the folin phenol reagent, *J. Biol. Chem.*, 1951, **193,** 265.
38. Kunkel, H. G., Macroglobulins and high molecular weight antibodies, in The Plasma Proteins, (F. W. Putman, editor) New York, Academic Press, 1960, **1,** 279.
39. Leddy, J. P., Freeman, G. L., Luz, A., and Todd, R. H., Inactivation of the skin-sensitizing antibodies of human allergy by thiols, *Proc. Soc. Exp. Biol. and Med.*, 1962, **111,** 7.
40. Rockey, J. H., and Kunkel, H. G., Unusual sedimentation and sulfhydryl sensitivity of certain isohemagglutinins and skin-sensitizing antibody, *Proc. Soc. Exp. Biol. and Med.*, 1962, **110,** 101.
41. Minden, P., Reid, R. T., and Farr, R. S., A comparison of some commonly used methods of detecting antibodies to bovine albumin in human serum, *J. Immunol.*, 1966, **96,** 180.
42. Rowe, D. S., and Fahey, J. L., A new class of human immunoglobulins, II. Normal serum IgD, *J. Exp. Med.*, 1965, **121,** 185.
43. Fougereau, M., and Edelman, G. M., Corroboration of recent models of the γG-immunoglobulin molecule, *J. Exp. Med.*, 1965, **121,** 373.