PROPERTIES OF GUINEA PIG 7S ANTIBODIES

III. Identification of Antibodies Involved in Complement Fixation and Hemolysis*

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In the preceding papers (1, 2) it was demonstrated that guinea pigs immunized with single proteins or protein-hapten conjugates produced two major types of antibodies differing in their electrophoretic mobility. These antibodies were identified as γ_1 and γ_2; both had a sedimentation coefficient of approximately 7S. Gamma-1 or "fast" migrating antibodies conferred passive cutaneous and systemic anaphylactic reactions in guinea pigs; gamma-2 or "slow" migrating antibodies did not mediate these activities.

The present report is concerned with the ability of slow and fast guinea pig antibodies to fix complement *in vitro* in the presence of antigen and to participate in certain *in vivo* reactions, which apparently involve the fixation of complement. It has been found that γ_2 antibodies bind complement in the presence of antigen and that γ_1 antibodies fail to do so. These differences may depend on the presence of a complement-fixing site on piece III of γ_2 antibodies and the absence of this site from piece III of γ_1 antibodies (3). The slow antibody components of guinea pig antisera were found to be very effective in conferring passive Arthus reactivity (in the guinea pig), whereas fast components were relatively ineffective. These data suggest that complement activity is involved in the mechanism of the Arthus reaction.

Studies of guinea pig anti-sheep erythrocyte sera demonstrated, in addition to the usual slow and fast antibodies, a highly efficient hemolysin of intermediate electrophoretic mobility. Whether this activity is a function of a third type of antibody, which is perhaps produced in response to the particulate properties of the antigen, or to antigenic heterogeneity of the red cell membrane, remains to be determined.

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Methods

Procedures used to prepare antigens, immunize guinea pigs, process serum, and prepare purified antibodies have previously been described (1). The following technique was used to immunize guinea pigs with sheep erythrocytes: Sheep erythrocytes were washed 4 times in cold saline. A 50 per cent suspension of erythrocytes was emulsified with an equal volume of complete adjuvant (Difco Laboratories, Inc., Detroit). Initially, 0.1 ml of emulsion were injected in each of the four foot-pads of guinea pigs weighing 350 to 400 gm. Fourteen days later, erythrocytes were injected in 5 per cent suspension in 0.15 M saline intradermally at four sites, 0.1 ml/site. These intradermal injections were repeated 3 times at 7 day intervals. Thereafter, moderately severe Arthus reactivity had been attained. The animals were exsanguinated under nembutal® anesthesia 7 days after the last skin test.

Serum proteins were separated on starch blocks as described (1). The protein concentration of eluates obtained from ½ inch strips was determined using the Folin technique (4). Eluates were dialyzed for 18 hours at 5°C against distilled water, containing 0.005 M phosphate buffer, lyophilized, and subsequently reconstituted in 1.0 ml of saline (effecting an approximate 15 × concentration of eluate). Initial PCA titrations of the fractions (2), as well as other serologic tests, were performed with reconstituted fractions diluted 1:10 with saline. Fractions yielding positive results were titrated to their end-point.

Fractionation of Gamma Globulin.—Gamma globulin was isolated by the addition of saturated ammonium sulfate to guinea pig serum to a final concentration of 40 per cent. The resulting suspension was centrifuged at 3500 RPM for 30 minutes, the precipitate was dissolved in distilled water and dialyzed as above.

Agglutination Techniques.—(*a*) Direct: guinea pig anti-sheep erythrocyte agglutinins were titrated as described (4). Serial dilutions in two-fold steps were made using isotonic saline containing 200 mg bovine serum albumin (BSA) per 100 ml saline. To 0.5 ml aliquots were added 0.1 ml of 0.5 per cent cell suspensions. After incubation at 5°C for 18 hours, patterns of agglutination were read and scored according to the method of Stavitsky (5). (*b*) Indirect: tannic acid–treated sheep erythrocytes were prepared as described (4). To 0.8 ml of packed, tanned cells suspended in 5 ml phosphate-buffered saline, pH 6.4, was added 0.8 ml of 2,4-dinitrophenyl-bovine serum albumin (DNP-BSA) solution (12.12 mg protein/ml, 30 haptenic groups per mole of protein). Reactants were mixed and held at 20°C for 30 minutes. Cells were similarly coated with picryl–guinea pig albumin (Pic-GPA). To 0.25 ml of packed tanned cells suspended in 4 ml of buffered saline was added 0.5 ml of Pic-GPA (2.26 mg protein/ml, 44.5 haptenic groups per mole of protein). Coated cells were washed once in phosphate-buffered saline, pH 7.2, and suspended in this buffer as a 0.5 per cent cell suspension. 0.1 ml of cell suspension was added to 0.5 ml of serially diluted antibody solutions (made in 0.2 per cent BSA-saline), and patterns of agglutination observed after 18 hours. It should be noted that antibody solutions were inactivated at 56°C for 30 minutes and those used for passive agglutination or lysis were twice absorbed with sheep erythrocytes prior to use.

Hemolysis.—(*a*) Direct: the scaled-down procedure for titration of hemolytic antibody was used (4). Results were expressed in H_{50} units (6). (*b*) Indirect: the method used was similar to the above described indirect agglutination procedure. Serial dilutions were performed in veronal buffer (4). After incubation at 5°C for 18 hours, 0.1 ml guinea pig complement (containing 12 C′ H_{50} units per ml) was added to each tube, reactants mixed, and incubated at 37°C for 15 minutes. Degree of lysis was estimated visually on a scale of + to ++++. The last dilution yielding approximately 50 per cent hemolysis was considered end-point of the solution tested.

Complement Fixation.—Complement fixation tests were performed as described (4). Optimum antigen concentrations were determined by checker-board titration. The following antigen concentrations yielded the highest antiserum titer: 0.5 per cent sheep erythrocytes,

3 μg DNP-BSA per ml, 2 μg ovalbumin per ml. (5 times recrystallized, Pentex Inc., Kankakee, Illinois.) The test system consisted of 0.5 ml of antibody dilution, 0.5 ml of guinea pig complement containing 2.5 C′ H_{50} units and 0.5 ml of antigen dilution. Reactants were mixed, held at 0°C for 18 hours followed by addition of 0.2 ml of sensitized sheep erythrocytes, and incubation at 37°C for 30 to 60 minutes. In tests using sheep erythrocytes as antigen, residual complement activity was determined on carefully decanted supernates obtained by centrifugation. Degree of hemolysis was determined visually and/or spectrophotometrically. Results were expressed in C′ F_{50} units (determined as in reference 6) or as that antibody dilution which, in the presence of antigen, fixed approximately 1.5 units of C′ H_{50}. (Sheep erythrocytes were obtained from Probio, Inc. New York; guinea pig complement, lyophilized, was obtained from Certified Blood Donor Service, Incorporated, Jamaica, New York; amboceptor was obtained from Baltimore Biological Laboratory, Incorporated, Baltimore.

Anticomplementary activity was encountered in eluates obtained from the "slow" gamma globulin region of starch blocks prepared with 8 to 10 ml of most whole sera, and also in the "fast" gamma globulin region of starch blocks prepared with 8 to 10 ml of the globulin fraction prepared by salt fractionation of 30 to 40 ml of serum. Anticomplementary activity could be overcome by including 0.5 ml of inactivated pooled normal guinea pig serum in the initial test dilution (7).

Passive Cutaneous Anaphylaxis (PCA) in Guinea Pigs and Rats.—PCA was performed in guinea pigs as previously described (2). The following amounts of antigen were used to challenge test animals: 0.25 ml of packed sheep erythrocytes, 250 μg DNP-BSA, 500 μg ovalbumin, 200 μg Pic-GPA.

PCA reactions were induced in male and female albino rats weighing between 150 and 180 gm (8). Rats were injected intradermally with designated quantities of antibody in a volume of 0.1 ml in six sites on the shaved dorsal surface. After a latent period of 90 minutes, challenging dose of antigen (1 mg ovalbumin) and dye contained in 1 ml volume was injected into the tail vein. Animals were killed after 30 minutes, skinned, and the reactions read by measuring the diameter of the blue area on the inner surface of the skin.

Reverse Passive Arthus Reactions.—Reverse passive Arthus reactions were performed as described (9). Guinea pigs weighing 350 to 400 gm were injected intravenously with 1 ml of ovalbumin solution (2 mg/ml). Antibody solutions were immediately injected intradermally in 0.1 ml volumes. Reactions were observed after 2 hours on both outer and inner surface of the skin and the extent of hemorrhage recorded.

RESULTS

I. Separation of Complement-Fixing Activity of Guinea Pig Immune Sera by Starch Block Electrophoresis.—

Experiments with antihapten sera: The data in Fig. 1 summarize results of tests performed with one of several electrophoretically separated individual guinea pig anti-DNP–BGG[1] sera. It may be seen that the protein content of the gamma globulin region is distributed about two peaks. With the serum tested, eluates obtained from the slow region appeared to have a greater protein concentration than eluates obtained from the fast region. However, antibodies capable of agglutinating, tannic acid–treated sheep erythrocytes coated with DNP-BSA were distributed throughout the gamma globulin region. Guinea pig skin-sensitizing antibodies were demonstrated by passive

[1] DNP-BGG = 2,4-dinitrophenyl–bovine gamma globulin.

cutaneous anaphylaxis in eluates obtained from the "fast" region of the starch block only (fractions 7 to 15). Despite the presence of agglutinating antibodies, eluates obtained from the "slow" region (fractions 1 to 6) failed to sensitize guinea pig skin for PCA at the protein concentration tested.

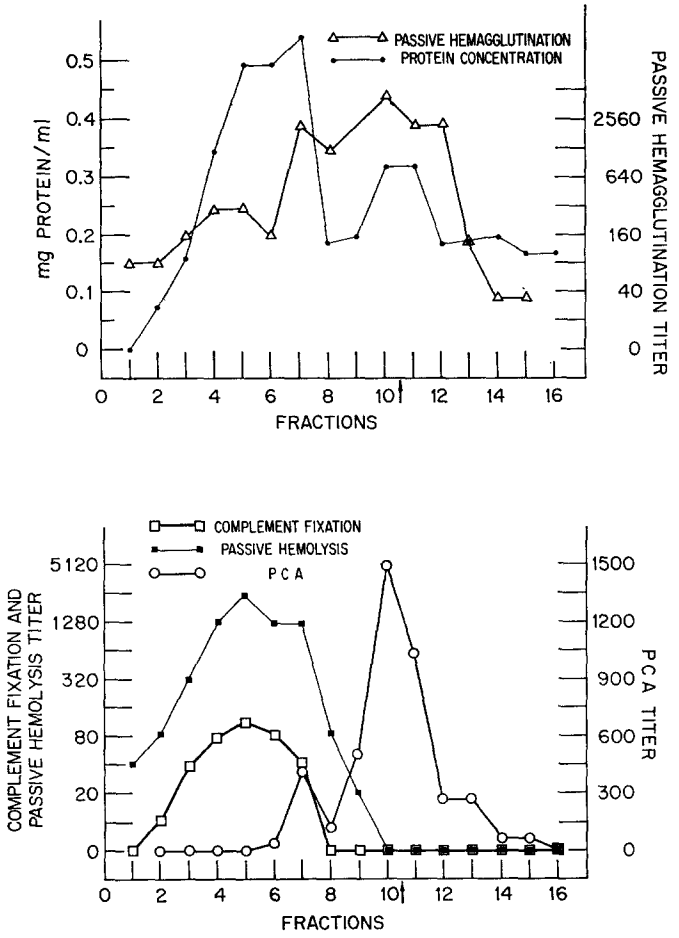

FIG. 1. Titration of anti-DNP antibodies by various serologic techniques in fractions from starch block electrophoresis of a guinea pig anti-DNP–BGG serum obtained after immunization with adjuvants. Arrow indicates point of application of serum on starch block.

Antibodies capable of fixing complement in the presence of antigen, DNP-BSA, were present in eluates obtained from the slow region (fractions 2 to 7) but not in eluates obtained from the fast region (fractions 8 to 16). Parallel results were observed in a second test involving *in vitro* fixation of complement, *i.e.* passive hemolysis: eluates from the slow region were able to sensitize DNP-

BSA-coated, tanned erythrocytes for lysis in the presence of complement; eluates from the fast region failed to do so. In each fraction passive hemolysis paralleled complement fixation and both activities peaked in fraction 5. It would therefore appear that complement fixation was a function of the γ_2 but not of the γ_1 type of guinea pig antibodies.

Experiments with antiprotein sera: The globulin fraction was prepared by $(NH_4)_2SO_4$ fractionation of a 40 ml pool of guinea pig antiovalbumin serum,

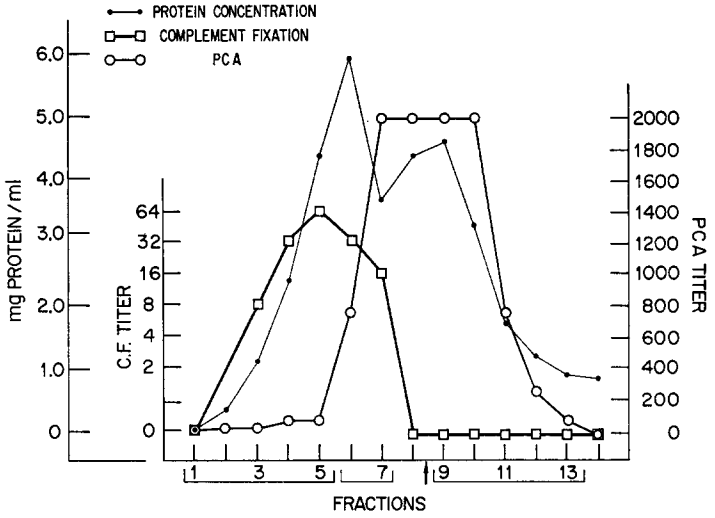

FIG. 2. Titration of complement-fixing (C.F.) and passive cutaneous anaphylactic activities in eluates from starch block electrophoresis of guinea pig antiovalbumin gamma globulin pool 1. Fractions 1 to 5, 6 and 7, and 9 to 13 were pooled for subsequent studies of reverse passive Arthus experiments (Table I). Arrow indicates point of application of gamma globulin solution on starch block.

pool I. A 10 ml solution of these globulins was separated by electrophoresis on starch block and eluates prepared as usual, Fig. 2. Again, it may be seen that the protein content of the gamma globulin region is distributed about two peaks. In this instance, eluates obtained from the slow and fast region appeared to have approximately equal protein concentrations. Guinea pig skin-sensitizing antiovalbumin antibodies were present in fractions 4 to 13 with the peak of this activity in fractions 7 to 10. Complement-fixing antibodies were demonstrated in fractions 2 to 7 but not in faster migrating fractions.

Experiments with purified antihapten antibody: In previous experiments with a preparation (B) of purified guinea pig antipicryl-GPA antibodies described in the second paper of this series (reference 2, Fig. 4), skin-sensitizing activity was demonstrated only in starch block fractions yielding a γ_1 precipitin line

with rabbit anti–whole guinea pig serum in immunoelectrophoresis. All eluates obtained by starch block electrophoresis of this purified antibody were presently tested for their ability to sensitize picryl-GPA–coated, tanned erythrocytes for lysis in the presence of complement. In Fig. 3 it may be seen that lytic activity was obtained only with the fractions containing γ_2 antibodies. Since there is no lytic activity demonstrated in fractions 9 and 10, which nevertheless appear to contain some γ_2 antibodies (reference 2, Fig. 4), the possibility exists that γ_1 antibodies may under suitable conditions inhibit the lytic action of γ_2 antibodies.

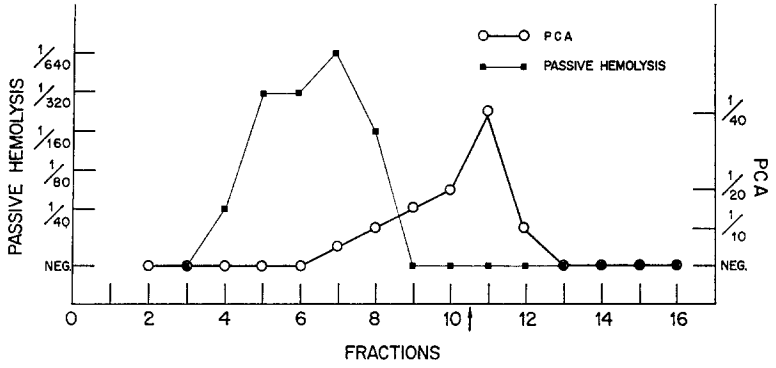

FIG. 3. Titration of passive hemolytic and passive cutaneous anaphylactic activities of eluates from starch block electrophoresis of purified guinea pig antipicryl-GPA antibodies (preparation B). Arrow indicates point of application of antibodies on starch block.

A similar study was made with purified anti-DNP antibody preparations I. P. 3 isolated from the serum of a guinea pig immunized intraperitoneally with DNP-BGG without adjuvants. This type of immunization was found to result primarily in the production of the γ_1 type of guinea pig antibodies. The result of this experiment is found in Fig. 4. This antibody preparation lacked demonstrable complement-fixing or passive hemolytic activity and appeared to consist almost entirely of the skin-sensitizing type of antibodies.

II. Biologic Activities of γ_2 Guinea Pig Antibodies.—

Reverse passive Arthus experiments with slow and fast guinea pig antiovalbumin antibodies: Fundamental differences have been demonstrated between the pathogenic mechanism of the passive Arthus and passive anaphylactic reactions; e.g., larger quantities of antibody required, absence of latent period, inhibition of the latter by antihistamines (4, 9). The sequence of events leading to hemorrhage in the Arthus phenomenon is probably initiated by the reaction of precipitating antibody with antigen within small vessel walls (10). Adherence of platelets to damaged vascular endothelium and infiltration with

polymorphonuclear leukocytes are necessary steps for the hemorrhagic necrosis to occur (11). A possible role of complement in the mechanism of the Arthus reaction is suggested by the observations of Nelson (12), who found that platelets could adhere to antigen-antibody complexes *in vitro* only in the presence of complement, and the experiments of Boyden (13) who reported the leukotactic properties of antigen-antibody complexes containing complement. The availability of two types of guinea pig antibodies, one of which, $7S\gamma_1$, appears

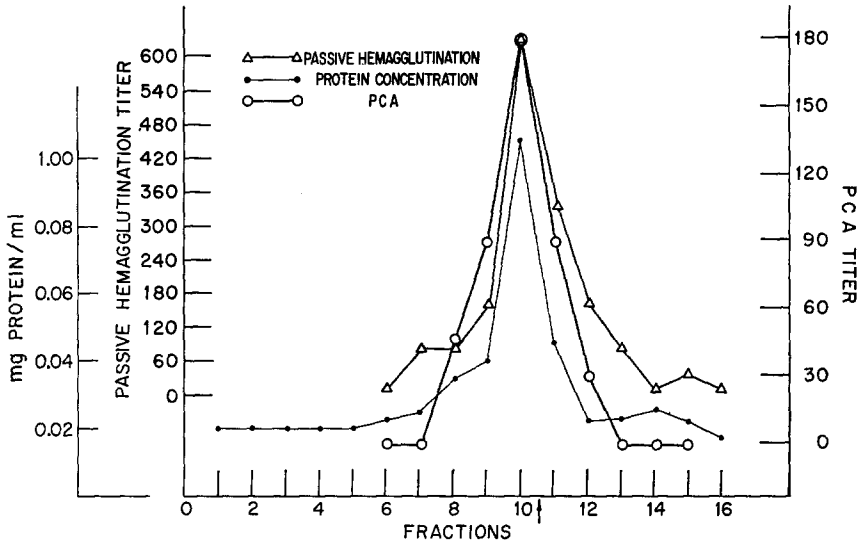

FIG. 4. Titration of anti-DNP antibodies by various serologic techniques in fractions from starch block electrophoresis of purified guinea pig anti-DNP–BGG antibodies (I.P. 3) obtained from an animal immunized intraperitoneally without adjuvants. All fractions were tested and found to be negative for complement-fixing and passive hemolytic activity at a dilution of 1:10. Arrow indicates point of application of antibodies on starch block.

to lack the site for complement fixation, led us to test the ability of these antibodies to confer reverse passive Arthus reactivity in the guinea pig.

For these experiments, the following fractions obtained from guinea pig antiovalbumin γ-globulin pool 1 (see above) were combined: fractions 1 to 5 containing primarily slow components (fraction Ia), fractions 6 and 7 containing a mixture of slow and fast components (fraction Ib), and fractions 9 to 13 containing only fast components (fraction Ic). These three fractions were dialyzed against dilute buffer, lyophilized, and reconstituted. The concentration of antiovalbumin antibodies in these pools was determined by quantitative precipitin analysis (4).

Aliquots of these 3 fractions, containing 100 μg and 20 μg antibody N (AbN) per 0.1 ml, were injected intradermally into experimental and control animals. Table I presents the results of these experiments. At the higher dose level

TABLE I

Reverse Passive Arthus Reactions in Guinea Pigs Sensitized Intradermally with Slow and Fast Components of Guinea Pig Antiovalbumin Antibodies (Pool I)

Treatment	Test material	100 μg AbN		20 μg AbN		100 μg AbN		20 μg AbN		100 μg AbN		20 μg AbN	
Animal No.		1				2				3			
Antigen 2 mg ovalbumin, i.v.	Fraction Ia (slow)	++++*	17 × 18	++++	14 × 12	++++	12 × 12	++	9 × 7	++++	10 × 10	++	10 × 10‡
	Fraction Ib (mixture)	+++	11 × 10	+++	12 × 12	++++	10 × 11	+	7 × 7	+++	10 × 7	±	6 × 5‡
	Fraction Ic (fast)	++	11 × 10	++	5 × 5	++	7 × 8	±	tr	+	6 × 5	0	—
Animal No.		4				5				6			
Controls	Fraction Ia (slow)	+	5 × 5	0	—	+	5 × 5	0	—	0	—	0	—
	Fraction Ib (mixture)	0	—	0	—	0	—	0	—	0	—	0	—
	Fraction Ic (fast)	+	5 × 6	±	5 × 5	±	5 × 6	±	tr	+	5 × 5	±	tr

tr, trace amounts

* Severity of cutaneous hemorrhage and diameter of hemorrhagic reaction in millimeters; (marked edema was also observed especially with fraction Ic).

‡ Petechial hemorrhage only.

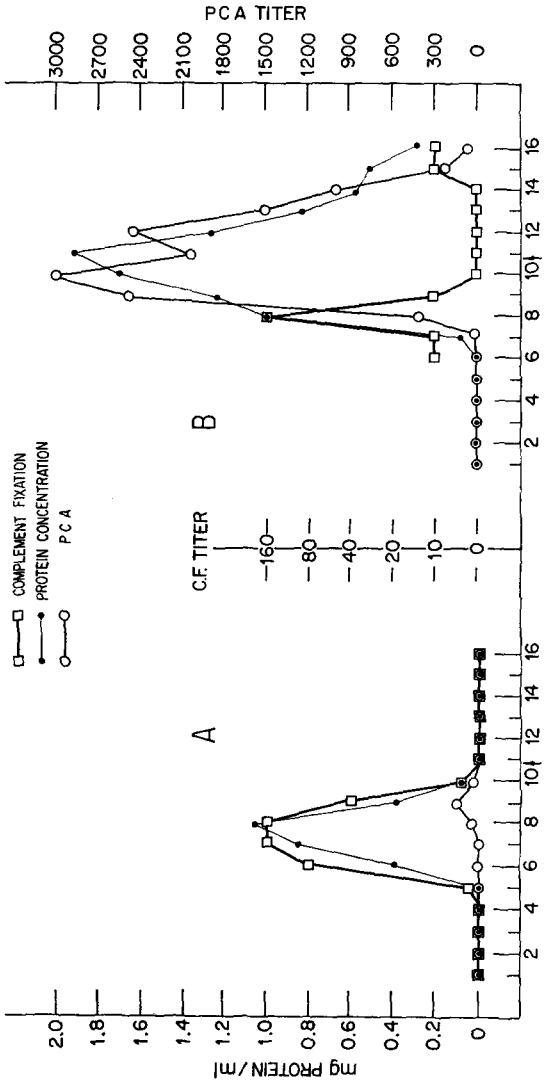

FIG. 5. Titration of complement-fixing (C.F.) and passive cutaneous anaphylactic activity of eluates from separate starch block electrophoresis of slow (*A*) and fast (*B*) components of guinea pig antiovalbumin gamma globulin pool 2. Initial slow and fast components of guinea pig antiovalbumin gamma globulin were also prepared by starch block electrophoresis. Arrows indicate points of application of slow and fast components on starch blocks.

(100 μg AbN), the antibody fractions caused a very mild reaction in control animals. In the animals who received antigen, hemorrhage produced by fraction Ia was more severe than that produced by fraction Ib. Still smaller reactions were obtained with fraction Ic at the 100 μg AbN level. These differences were more clearly demonstrated at the 20 μg antibody nitrogen level. At this antibody level, hemorrhagic reactions were observed primarily with fraction Ia (slow components). Although cutaneous edema was produced by all three pools, it was most marked with fraction Ic (fast components).

To confirm the above experiments, a second pool of guinea pig globulins was prepared by $(NH_4)_2SO_4$ fractionation of 44 ml of pooled antiovalbumin serum. The resuspended globulins

TABLE II

Reverse Passive Arthus Reactions in Guinea Pigs Sensitized Intradermally with Slow and Fast Components of Guinea Pig Antiovalbumin Antibodies (Pool II)

Test material	20 μg AbN		20 μg AbN		20 μg AbN		50 μg AbN		50 μg AbN	
Animal No.	1		2		3		4		5	
	Hem.	Edema	Hem.	Edema	Hem.	Edema	Hem.	Edema	Hem.	Edema
Fraction IIa (slow)	12 × 12	20 × 20	10 × 10	20 × 20	10 × 10	20 × 20	—	—	—	—
Fraction IIc (fast)	0	25 × 20	0	25 × 25	tr	15 × 15	0	20 × 20	7 × 7	15 × 15
Guinea pig serum 25	7 × 7	20 × 15	7 × 7	20 × 20	0	7 × 7	10 × 10	20 × 20	12 × 12	15 × 15

Antigen: ovalbumin 2 mg intravenously.
Control animals injected with all test materials without antigen had no hemorrhagic reactions.
Numbers refer to diameter of edematous or hemorrhagic reactions.
Hem., hemorrhage; tr, trace amounts.

were separated by starch block electrophoresis and eluates tested for complement-fixing and skin-sensitizing activities. On the basis of these determinations, slow and fast eluates were pooled, lyophilized, reconstituted, and separately subjected to electrophoresis on starch blocks. Eluates obtained from these two starch blocks were retested, Fig. 5. Fractions 6 to 9 of starch block A (representing primarily slow components) and fractions 10 to 14 of starch block B (fast components) were combined as fraction IIa and fraction IIc. The concentration of antiovalbumin antibodies in these pools, and in guinea pig antiovalbumin serum 25, used as reference, was determined by quantitative precipitin analysis (4).

Aliquots of fractions IIa, IIc, and of anti-serum 25 containing 20 μg antibody N per 0.1 ml, and aliquots of fraction IIc and anti-serum 25 containing 50 μg antibody N/0.1 ml, were used to perform reverse Arthus reactions in guinea pigs. These antibody preparations did not provoke hemorrhagic reactions in the controls. In Table II it may be seen that slow components produced both cutaneous hemorrhage and edema while fast components (with the exception of guinea pig 5 where a slight hemorrhagic reaction was observed with 50γ AbN) produced only cutaneous edema. Aliquots of whole serum 25, con-

taining an equal amount of precipitating antiovalbumin antibodies (however, consisting of both slow and fast components) produced less severe hemorrhage than fraction IIa containing primarily slow components.

Passive cutaneous anaphylactic reactions in rats: There has been some controversy about the relationship of complement and PCA (14). Evidence presented in this series of papers suggests that in the guinea pig, PCA is provoked by antibody which, at least *in vitro*, does not fix complement in the presence of antigen.

Osler *et al.* (8) have demonstrated that in the rat PCA involves in part a

TABLE III

Passive Cutaneous Anaphylaxis in Rats Injected Intradermally with Slow and Fast Components of GuineaPig Antiovalbumin Antibodies (Pool I)

Test material	Test dose AbN	Animal No.		
		1	2	3
	μg			
Fraction Ia (slow)	5	15*	15	15
	2	10	9	tr
	1	7	0	12
Fraction Ic (fast)	5	19	19	16
	2	14	15	6
	1	12	10	0

Antigen: 1 mg ovalbumin intravenously; latent period, 90 minutes.
* Largest diameter of blue spot on inner surface of skin.

host factor resembling complement. This observation led us to investigate whether in the rat, guinea pig $7S\gamma_2$ (slow) antibodies, capable of fixing complement *in vitro* in the presence of antigen, might provoke PCA in this animal species.

Rats were injected on their dorsal surface with 0.1 ml aliquots of fraction Ia and fraction Ic of antiovalbumin pool I (Fig. 2) containing 5 μg, 2 μg, 1 μg antibody N/0.1 ml. After a 90 minute latent period, the animals were challenged intravenously with a mixture of ovalbumin and dye. Results are presented in Table III. In contrast with the behavior of the antibody fractions in anaphylaxis of the guinea pig, both slow and fast components provoked passive cutaneous anaphylaxis in the rat.

III. Experiments with Guinea Pig Anti-Sheep Erythrocyte Sera.—The data presented in Fig. 6 is representative of experiments with six individual guinea pig anti-sheep erythrocyte sera, and resembles in part the results obtained with antisera to soluble proteins or protein-hapten conjugates. Antibodies

capable of fixing complement in the presence of erythrocytes were demonstrated in fractions 4 to 9, but not in fractions 10 to 15 eluted from the starch block. Skin-sensitizing antibodies were demonstrated in fractions 8 to 15.

We were aware that there may not be an absolute correlation between complement fixing and hemolysin titers of whole sera (6) because of the antigenic

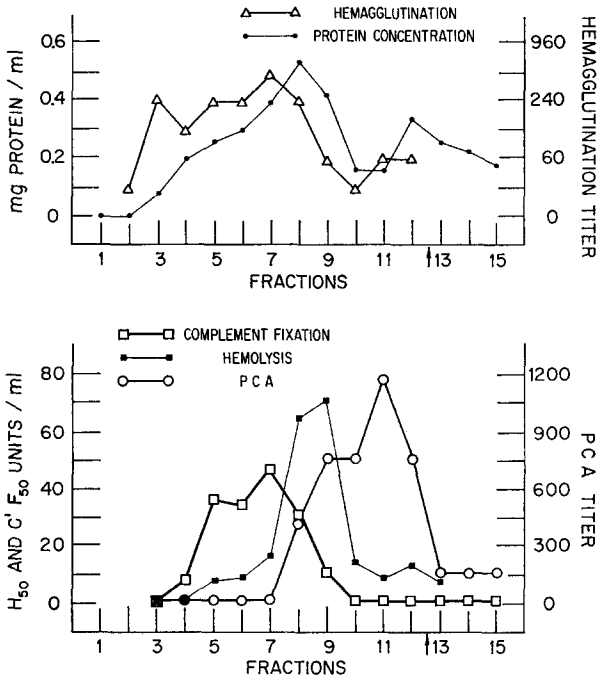

FIG. 6. Titration of hemagglutinating, complement-fixing, hemolytic, and passive cutaneous anaphylactic activities in fractions from starch block electrophoresis of serum from a guinea pig immunized with sheep erythrocytes in complete adjuvant. Arrow indicates point of application of serum on starch block.

complexity of the system. It was nevertheless expected that the hemolytic activity of electrophoretic fractions of guinea pig anti-erythrocyte sera would parallel their capacity to fix complement *in vitro*. Fig. 6 indicates that the peak of hemolysin activity was present in fractions 8 and 9; smaller amounts of hemolytic activity were present in earlier and later fraction. However, the peak of complement fixing activity was observed in fractions 5 to 7.

To exclude the possibility that this more efficient hemolysin, with a slightly faster electrophoretic mobility, was a high molecular weight antibody, pooled fractions 8, 9, and 10 of guinea pig anti-erythrocyte serum 5 were lyophilized, resuspended in 0.3 ml saline, and subjected to density gradient ultracentrifuga-

tion (15). Aliquots of solution obtained from all segments of the gradient were then tested for protein content, guinea pig skin-sensitizing, and hemolytic activity. Fig. 7 shows a close correlation among these three parameters at all levels of the density gradient. The peak protein concentration, skin-sensitizing

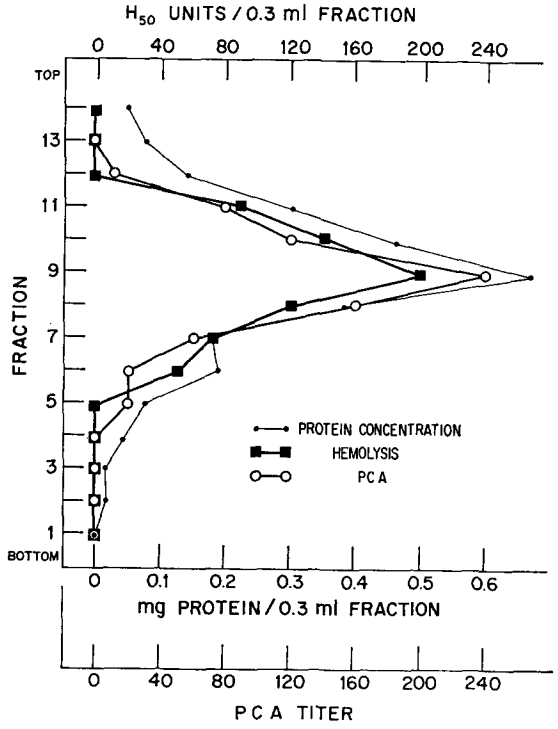

FIG. 7. Titration of hemolytic and passive cutaneous anaphylactic activity in segments from a density gradient separation of pooled fractions 8 to 10 eluted from starch block electrophoresis of guinea pig anti-sheep erythrocyte serum 5. (Serum 5 yielded a pattern of distribution of hemolytic and PCA activities in the various fractions similar to serum 7 presented in Fig. 6.)

and hemolytic activity were found in segment 9. There did not appear to be a second concentration of hemolytic activity in the lower portion of the gradient. Analytical ultracentrifugation of pooled segments 8, 9, and 10 indicated that the proteins present in these segments had a sedimentation coefficient of approximately 7S.

To exclude the possibility that efficient lysis was a function of a certain combination of the usual slow and fast antibodies, the hemolytic activity of artificial mixtures of slow and fast anti-erythrocyte antibodies were tested. For this purpose, widely separated eluates were selected (i.e., fractions 4 and

12 or 5 and 13 of guinea pig serum 23). Mixtures varying in their composition of slow antibodies from 10 to 90 per cent and of fast antibodies from 90 to 10 per cent were tested. The hemolytic activity of these mixtures did not exceed the combined activity of either slow or fast component tested alone.

DISCUSSION

In the guinea pig, the ability to transfer anaphylactic reactions and to fix hemolytic complement appear to be properties of two different types of 7S antibodies. Complement fixation can be demonstrated with guinea pig antibodies identified by immunoelectrophoresis as $7S\gamma_2$ (1, 2). Antibodies similarly identified as guinea pig $7S\gamma_1$ carry the receptors for fixation to tissues and sensitize for passive anaphylaxis (2). $7S\gamma_1$ antibodies apparently do not bind complement *in vitro* in the presence of antigen nor do they hemolyze antigen-coated, tanned erythrocytes in the presence of complement. Since complement fixation (3) and binding to sites in guinea pig tissues are dependent on properties of fragment III of Porter (16) and therefore of H chains (17), it must be assumed that, for the guinea pig antibody studied, a given H chain does not possess both properties.

The results of our experiments can be interpreted as evidence that the fixation of hemolytic complement is not a necessary step in anaphylaxis in the guinea pig. Our results are consistent with the findings that reverse PCA, using human gamma globulin as antigen, can be elicited in the guinea pig by antibody fragments that do not bind complement; *e.g.*, 5S fragments of pepsin-digested rabbit antibodies (18).

Unlike anaphylaxis in the guinea pig, both guinea pig $7S\gamma_1$ and $7S\gamma_2$ antibodies provoke PCA of comparable intensity in the rat. It has been previously mentioned (2) that the PCA reaction of the rat, which requires much higher levels of antibody than that of the guinea pig, probably employs different immunological mechanisms to trigger the liberation of vasoactive amines. One of these mechanisms may require the intervention of complement (8) and appears to involve the formation of anaphylatoxin (19) or similar mediators; the other mechanism appears not to require the intervention of complement but involves the ability of antibodies to bind to certain specific tissue receptors.

Experiments on reverse Arthus reactions demonstrated that guinea pig antibodies, $7S\gamma_2$, able to fix complement in the presence of antigen are much more efficient in provoking hemorrhagic lesions in reverse passive Arthus reactions than equal amounts of non–complement-fixing $7S\gamma_1$ antibodies which primarily provoke severe edema. These observations emphasize the importance of complement in the hemorrhagic necrosis of the Arthus reaction and provide additional evidence in favor of the view presented several years ago that local anaphylactic reactions and Arthus reactions should be considered as two distinct reactions with different pathogenic mechanisms (9, 20).

Experiments with guinea pig anti-sheep erythrocyte sera are discussed separately because this is the only immunological system, which we have studied, that does not exactly fit the general pattern observed with other protein or hapten antigens. The complement-fixing activity (in tests performed at 0°C) and PCA activity of these sera was distributed in starch block electrophoresis in a pattern similar to that observed with guinea pig antiprotein or antihapten sera. However, in every experiment efficient hemolytic activity, attributable to 7S antibodies, migrated faster than the peak of complement fixation, but slower than the peak of PCA activity. The difference in electrophoretic mobility of complement-fixing and hemolytic activity in this system requires explanation. In attempting to account for this discrepancy, one must first consider that, unlike well defined hapten and single protein antigens, sheep erythrocytes are a complex mosaic of largely unidentified antigens. Antibodies directed against some of these antigens may be far more efficient hemolysins than antibodies directed against other surface constituents. Such an "efficient" antibody could conceivably have a discrete electrophoretic mobility; alternatively, the possibility exists that immunization with particulate antigens such as sheep erythrocytes gives rise to a third type of 7S antibody which is able to fix complement and which is a particularly efficient hemolysin. Data available at this time do not permit a choice between these two possible explanations.

Although electrophoretic fractionation of guinea pig serum readily permits separation of two major types of biological activities, which in turn may be identified as properties of two distinct types of antibodies, an identical situation may not necessarily exist in other mammalian species. Similar studies, in man and other species, will be required to explore whether antibodies directed against a single antigenic specificity but subserving different biological activities can be found in species other than the guinea pig.

SUMMARY

Guinea pig 7Sγ_2 antibodies were demonstrated to fix complement in the presence of antigen and to sensitize antigen-coated, tanned erythrocytes for lysis in the presence of complement; guinea pig 7Sγ_1 antibodies did not participate in these reactions. Gamma-2 antibodies were more efficient in provoking hemorrhagic necrosis in reverse passive Arthus reactions than equal amounts of non–complement-fixing gamma-1 antibodies. Unlike anaphylaxis in the guinea pig, both guinea pig 7Sγ_1 and 7Sγ_2 antibodies provoked passive cutaneous anaphylactic reactions in the rat.

Efficient hemolytic activity attributable to 7S guinea pig anti-sheep erythrocyte antibodies migrated faster than the peak of complement-fixing activity, but slower than the peak of PCA activity in starch block electrophoresis. It is uncertain whether this activity is a function of a third type of antibody pro-

duced in response to the particulate property of the antigen or whether it is due to the antigenic heterogeneity of the erythrocyte cell membrane.

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