PROPERTIES OF GUINEA PIG 7S ANTIBODIES

II. Identification of Antibodies Involved in Passive Cutaneous and Systemic Anaphylaxis*

By ZOLTAN OVARY, M.D., BARUJ BENACERRAF, M.D., and KURT J. BLOCH,‡ M.D.

(From the Department of Pathology, New York University School of Medicine, New York)

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Passive anaphylaxis of the guinea pig has been subjected to numerous immunological studies (1). They have established for both systemic (2) and cutaneous anaphylaxis (3) the levels of specific antibody required, and the need for a latent period of sensitization (4, 5) which is generally believed to be the time required for the binding of the antibody to tissue receptors. Further evidence for antibody fixation to tissues in guinea pig anaphylaxis is derived from studies of the gamma globulins which are able to inhibit sensitization of guinea pig skin by specific antibody (6, 7) or provoke reverse passive anaphylaxis in this species (8). These effects can only be obtained with 7S gamma globulins from species, such as rabbit or man, whose antibodies can passively sensitize guinea pig skin.

Recent studies of passive cutaneous anaphylaxis (PCA) in the guinea pig carried out with papain digests of rabbit 7S gamma globulins have revealed that the ability to bind to tissue receptors is a property of Porter piece III (9) and therefore of the H chains of 7S-sensitizing antibodies.

It has been generally assumed that the ability to mediate anaphylactic reactions in the guinea pig is a property of all 7S guinea pig antibodies (1). Because of the sensitivity of these biological reactions (2, 3) and the convenience of the techniques, the ability of immunized guinea pigs to respond with anaphylactic symptoms to an intravenous injection of antigen or the ability of a serum from these animals to transfer PCA has been accepted as sole evidence of an antibody response (10). Conversely, the absence of these anaphylactic reactions has been interpreted (11, 12) as evidence for failure to produce 7S antibodies, at least in the minimal amounts required to passively transfer anaphylactic reactions.

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Identification in guinea pig sera of two types of 7S antibodies (13), γ_1 and γ_2, raised the question whether both antibody types are able to mediate anaphylactic reactions. The data presented in this paper indicate that the 7Sγ_1 antibodies are able to transfer passive systemic or cutaneous anaphylaxis in the guinea pig, while γ_2 antibodies will, in proper concentration, specifically block PCA. Guinea pig 7Sγ_2 antibodies are unable to inhibit passive sensitization by a heterologous immunological system and therefore appear to lack receptors for fixation to guinea pig tissues.

Methods

Antigens used, methods of immunization, immunoelectrophoresis technique, starch block electrophoresis technique, isolation of anti-DNP and antipicryl antibodies were all described in the preceding paper (13).

Passive Cutaneous Anaphylaxis.—PCA was performed as described (14). Intradermal injections of the suitable antibody dilutions in 0.15 M NaCl were made into the back of freshly shaved guinea pigs weighing 250 to 300 gm. Each sample dilution was injected into 3 to 4 guinea pigs. As many as 12 sites per animal were used. After a latent period of 4 to 6 hours, the animals were challenged intravenously with a mixture of the antigen and Evans blue dye. (Generally, 0.5 ml of antigen dilution mixed with 0.5 ml of 1 per cent Evans blue dye in 0.15 M NaCl was used.) The reactions were read 30 minutes after challenge and the diameters of the blue spots, which constitute positive reactions, were measured. PCA inhibition experiments were performed by mixing the antibody fractions to be investigated immediately prior to the intradermal injections. Intravenous challenge with the antigen-dye solutions was made 3 hours later. Care was exercised to use the smallest amounts of antigen capable of producing maximal reactions in control animals injected with the sensitizing antibody alone. The amounts of reactants used in individual experiments will be indicated.

RESULTS

1. Separation of PCA Activity by Immunoelectrophoresis of Antisera.—Guinea pig antisera were submitted to electrophoresis in agar gel and the location of the two populations of 7S antibodies (13) identified by the precipitin arcs formed with specific antigen. A simple method was devised to test fractions obtained from these regions of the gel for their ability to transfer anaphylactic reactivity in the guinea pig.

Immunoelectrophoresis of a specific antiserum was performed after adding the serum to each of 4 wells on the plate. Following electrophoretic separation, the corresponding antigen was added only to the outside troughs in order to "develop" the top and bottom agar bands. The middle bands were cut into six 4 mm strips, numbered 1 to 6. Individual strips were homogenized and extracted with 1 ml of 0.15 M NaCl. The eluted fractions as such, or in a dilution of 1:10, were then investigated for PCA activity. By this technique, the PCA activity of each fraction could be correlated with the precipitin arc developed in the top and bottom agar bands.

The results of a typical experiment performed with guinea pig antiovalbumin serum Ea 48 are presented in Fig. 1. The PCA activity was found to migrate with the "fast" antibody fraction.

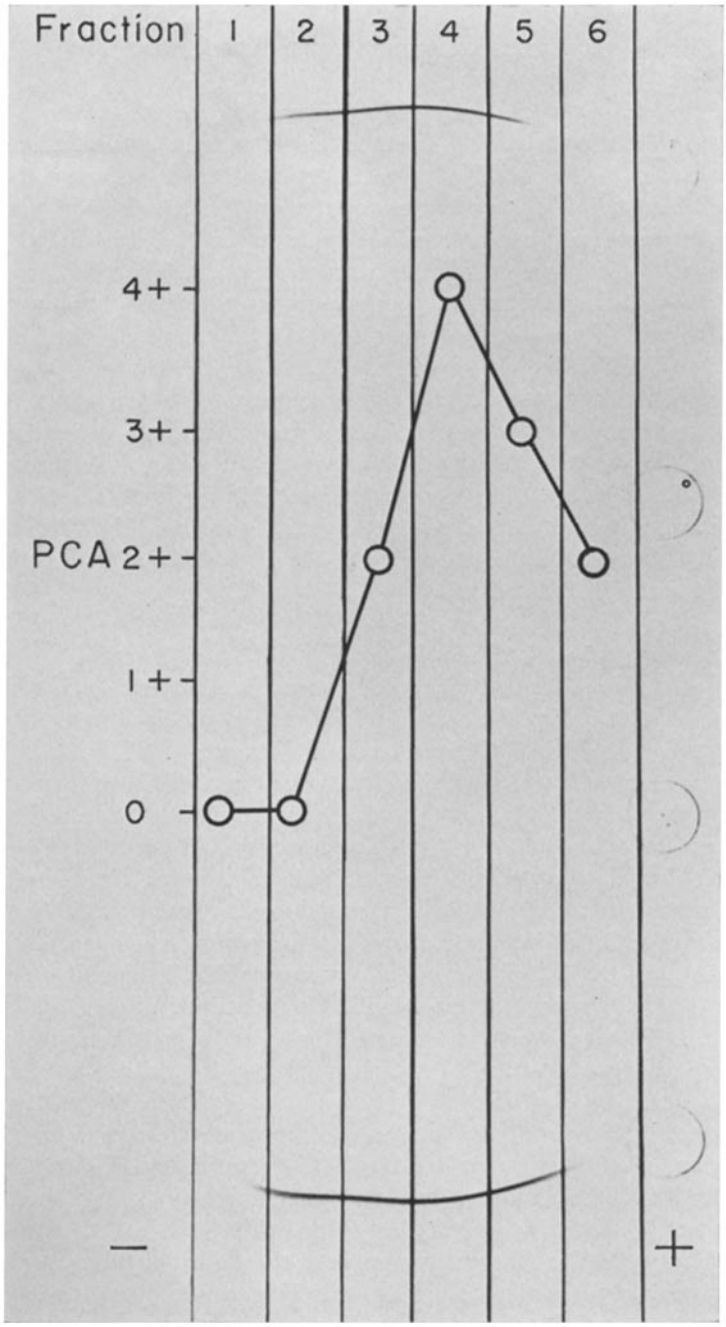

FIG. 1. Passive cutaneous anaphylactic activity of eluates from agar strips obtained after electrophoretic separation of guinea pig antiovalbumin serum EA 48. Wells were filled with 0.01 ml of guinea pig antiserum. The two middle agar bands were cut into 4 mm strips and extracted. Precipitin arcs were developed with 0.1 ml of ovalbumin, 0.2 mg/ml, placed in top and bottom trough.

This method, although lacking in precision, has the advantage of not requiring previous isolation and fractionation of specific antibody. Such procedures might be suspected of denaturing or otherwise altering antibodies causing them to lose their capacity to transfer PCA. Antisera obtained immediately after bleeding were examined by this technique, thus eliminating the additional possibility of introducing storage artefacts. To avoid possible enzymatic digestion of antibodies, plasminogen activation was inhibited by the immediate addition of ϵ-aminocaproic acid to fresh guinea pig blood in a final concentration of 0.2 M (15).

Blood obtained from a guinea pig immunized with pentachlorobenzoyl-guinea pig albumin was treated in this manner, the serum was subjected to immunoelectrophoresis, and the PCA activity of the agar strips examined. The result of this experiment is presented in Fig. 2. The ability to transfer PCA in the guinea pig resided only in the fast migrating antibody fraction; the slow migrating fraction precipitated well with the pentachlorobenzoyl antigen but did not transfer PCA activity.

II. Studies with Isolated Antibodies.—

Antibodies with PCA activity: To pursue these experiments, anti-DNP antibodies were prepared from two pools of antisera obtained from guinea pigs immunized with DNP-BGG in complete adjuvants: anti-DNP pool (5531–5534) from sera of 4 guinea pigs and anti-DNP pool (3, 7, 9) from sera of 3 guinea pigs. The purified antibody pools were then subjected to electrophoresis on starch block, fractions were eluted as described and 1 μg of antibody from each fraction was assayed for PCA activity in guinea pigs. 2,4-dinitrophenyl–bovine serum albumin (DNP-BSA) was used to elicit the reactions. Confirming the findings obtained with the immunoelectrophoretic technique, only the fast migrating anti-DNP antibody fraction corresponding in mobility to that of the 7Sγ_1 globulin was able to transfer PCA in the guinea pig (Fig. 3). From the two anti-DNP preparations, the starch block fractions with or without PCA activity were separately pooled and the following experiments carried out:

Antibodies capable of transferring systemic anaphylaxis: The ability of fast and slow migrating antibody fractions to sensitize guinea pigs for systemic anaphylaxis was investigated. Results of these experiments are found in Table I. As for PCA, the ability to mediate systemic anaphylaxis is an exclusive property of the fast migrating antibody fraction.

Blocking antibodies: A study was made of the ability of the non-sensitizing slow migrating γ_2 fraction to act as a blocking antibody and to inhibit the anaphylactic reactions elicited by the fast moving antibody fraction. These experiments are presented in Table II. The slow migrating anti-DNP fraction was mixed with the sensitizing fraction in an approximate proportion of 100:1 in order to favor preferential binding of antigen. Under these experimental

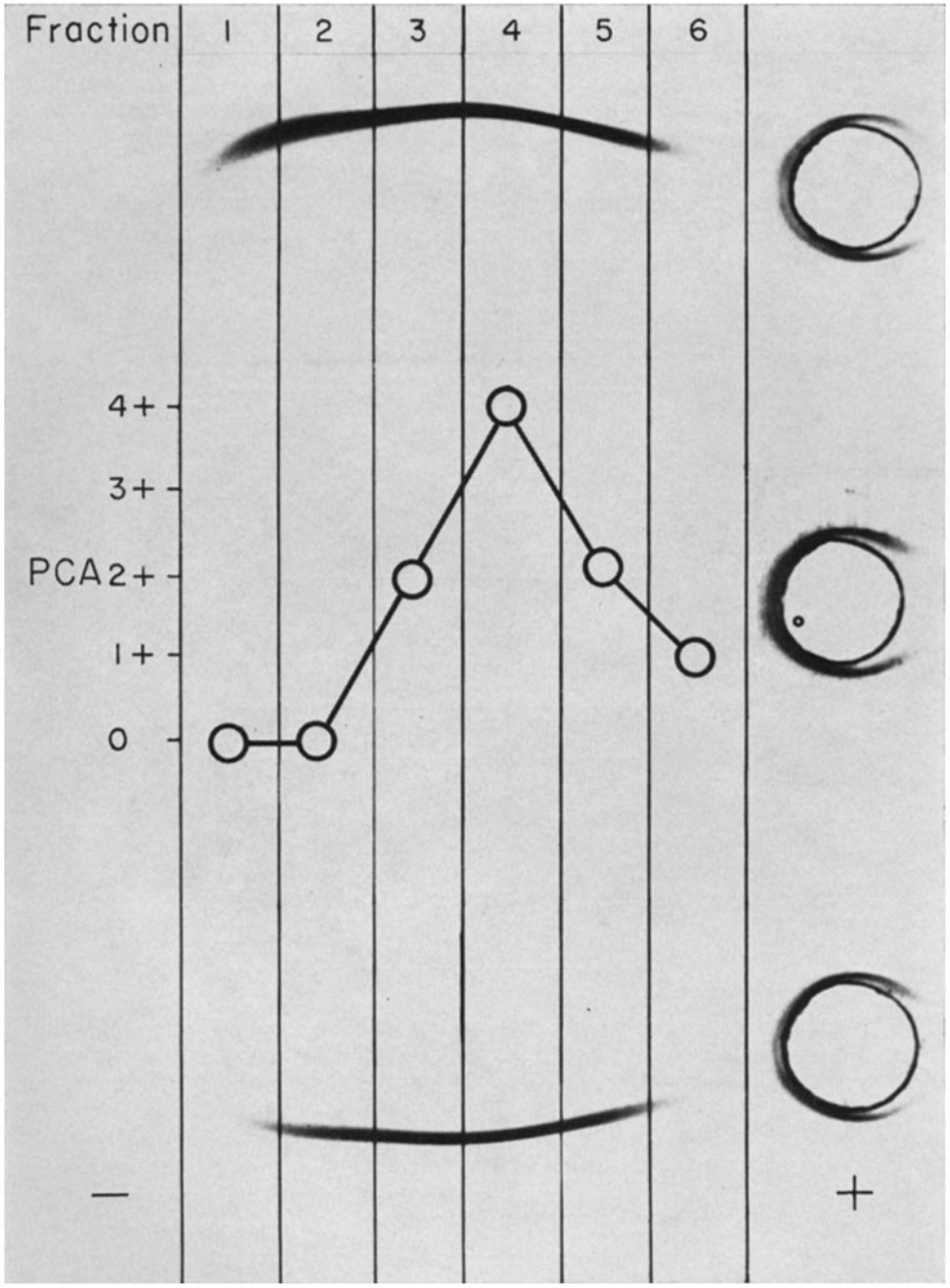

FIG. 2. Passive cutaneous anaphylactic activity of eluates from agar strips obtained after electrophoretic separation of guinea pig antipentachlorobenzoyl-GPA serum made 0.2 M with ε-aminocaproic acid immediately after bleeding. Wells were filled with guinea pig antisera. Middle band was cut into 4 mm strips and eluted. Precipitin arcs were developed with pentachlorobenzoyl-GPA, 0.2 mg/ml.

conditions, the specific blocking action of the slow migrating γ_2 fraction was easily demonstrated.

Non-specific competition: The ability of slow and fast anti-DNP antibodies to compete with sensitizing guinea pig antiovalbumin antibodies for skin fixation sites was investigated. Results presented in Table III show that

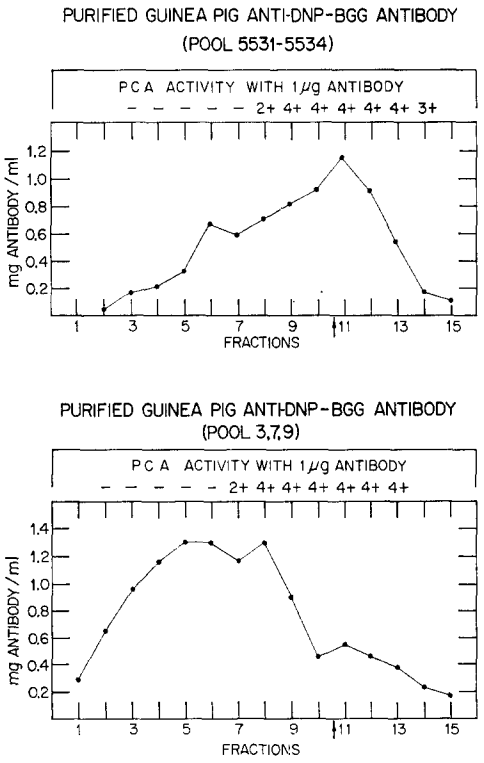

FIG. 3. Passive cutaneous anaphylactic activity of fractions obtained by starch block electrophoresis of two pools of purified guinea pig anti-DNP–BGG antibodies. Fractions correspond to ½ inch cuts of starch block. Arrows indicate point of application of antibody preparations on starch block.

adequate amounts of fast anti-DNP antibodies inhibit passive sensitization of guinea pig skin by antiovalbumin antibodies. However, an equal amount of the slow anti-DNP antibody was unable to affect this reaction. It should be noted (Table II) that under similar experimental conditions this same amount of slow antibodies specifically inhibited PCA by anti-DNP γ_1 antibodies. In the guinea pig, only the 7Sγ_1 antibody fraction possesses the ability to fix to guinea pig tissues.

Identification of the antibody type which mediates PCA activity: To Precisely

TABLE I

Systemic Anaphylaxis with Fractions from Starch Block Electrophoresis of Isolated Guinea Pig Anti-DNP Antibodies (See Fig. 3)

Isolated antibody pool	Pooled starch block fractions	Sensitizing dose antibody N	Latent period	Antigen	No. of animals	Anaphylaxis
		μg	*hrs.*	*mg* DNP-BSA		
Anti-DNP–BGG (5531–5534)	4–7	60	40	0.5	5	All negative
" "	9–13	60	40	0.5	5	4 dead, 1 very severe
Anti-DNP–BGG (3, 7, 9)	2–6	50	18	1.0	5	All negative
" "	8–12	50	18	1.0	5	1 dead, 2 severe, 2 moderate
				DNP-GPA		
" "	2–6	100	40	1.0	4	All negative
" "	8–12	100	40	1.0	4	2 dead, 2 severe

TABLE II

Immunologically Specific Inhibition of Passive Cutaneous Anaphylactic Activity of the 7Sγ_1 Antibody Fractions by an Excess of the 7Sγ_2 Fraction Isolated from the Same Anti-DNP–BGG Guinea Pig Antibody Pools (See Fig. 3)

Isolated antibody pool	Starch block fractions	Dose injected antibody	Passive cutaneous anaphylaxis diameter of reactions, *mm*			
			Guinea pig No.			
			1	2	3	4
		μg				
Anti-DNP–BGG (5531–5534)	4–7	66.0	0	0	0	0
" "	9–13	0.55	15	15	15	20
" "	4–7 + 9–13	66.0 + 0.55	0	0	0	0
			Guinea pig No.			
			5	6	7	8
Anti-DNP–BGG (3, 7, 9)	2–6	69.0	0	0	0	0
" "	8–12	0.6	12	12	10	8
" "	2–6 + 8–12	69.0 + 0.6	0	0	0	0

Latent period: 3 hours; antigen: DNP–BGG (30 haptenic groups per mole protein, 120 μg per guinea pig).

TABLE III

Non-Specific Inhibition of Passive Sensitization for Cutaneous Anaphylaxis by the 7Sγ_1 Antibody Fraction of an Immunologically Unrelated System (See Fig. 3)

Antibody-provoking PCA	Competing antibody system	Pooled starch block fractions	Dose of anti-DNP antibody injected	Passive cutaneous anaphylaxis diameter of reaction, *mm*		
				Guinea pig No.		
				1	2	3
			μg			
Guinea pig antiovalbumin 47, dilution: 1/2000	None	—	—	15	15	12
	Anti-DNP–BGG pool (5531–5534)	4–7	66	15	15	12
	Anti-DNP–BGG pool (5531–5534)	9–13	68	0	0	0
				Guinea pig No.		
				4	5	6
Guinea pig antiovalbumin 47, dilution: 1/1000	None	—	—	15	15	15
	Anti-DNP–BGG pool (3, 7, 9)	2–6	69	15	15	13
	Anti-DNP–BGG pool (3, 7, 9)	8–12	69	0	0	0

Latent period: 3 hours; antigen: ovalbumin 60 μg.

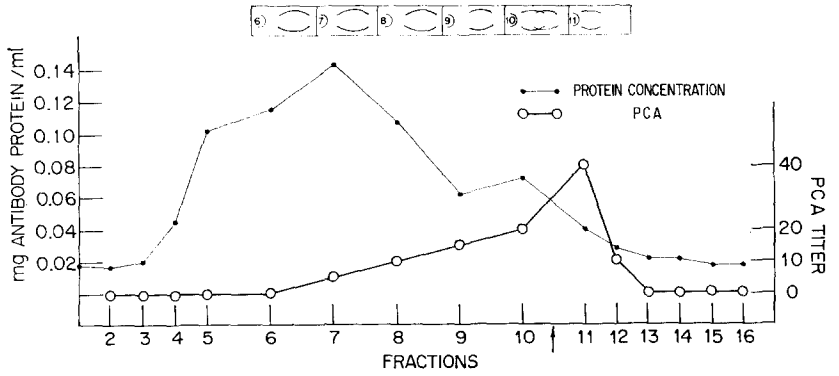

FIG. 4. Correlation of passive cutaneous anaphylactic activity of fractions obtained by starch block electrophoresis of purified guinea pig antipicryl-GPA (preparation B) with their immunoelectrophoretic pattern developed by reaction with rabbit anti-whole guinea pig serum, R1. (Schematically represented at top of figure.) Arrow indicates point of application of antibody preparation on starch block.

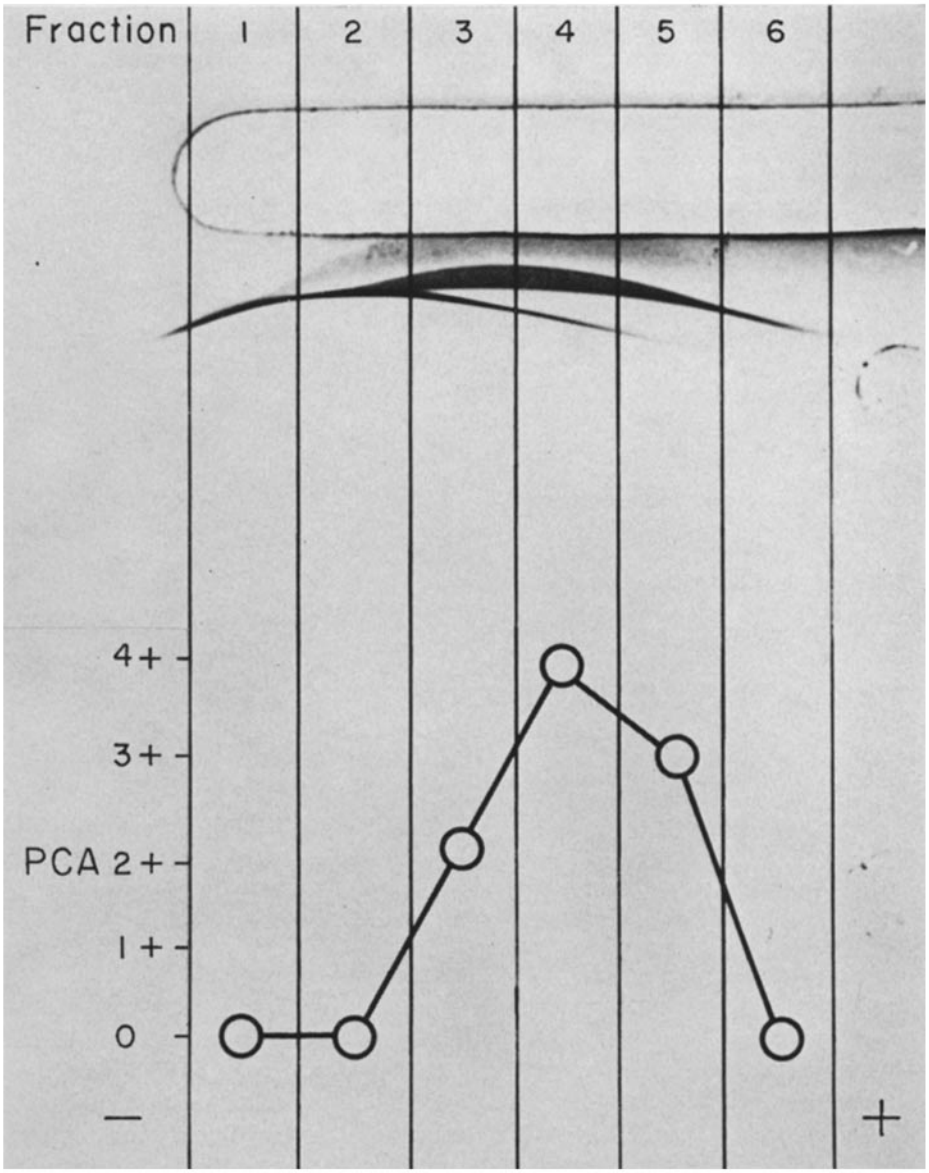

FIG. 5. Passive cutaneous anaphylactic activity of eluates obtained after electrophoretic separation of purified guinea pig anti-DNP–BGG antibody (DNP 68) Wells were filled with 0.01 ml of purified antibody. The lower band was cut into 4 mm strips and extracted. Trough was filled with rabbit anti–whole guinea pig serum, R2.

identify the antibody type capable of mediating anaphylactic sensitivity in the guinea pig as the electrophoretic γ_1 fraction, the following experiments were carried out with isolated antibody preparations.

Anti-Pic–GPA antibody preparation B was fractionated by starch block electrophoresis. Individual fractions were eluted from each ½ inch strip and tested for their ability to transfer PCA. In addition, each fraction was subjected to immunoelectrophoresis. Rabbit anti-guinea pig serum R1 (13) was used to detect the presence of $7S\gamma_1$ or $7S\gamma_2$ antigenic components. The results of this

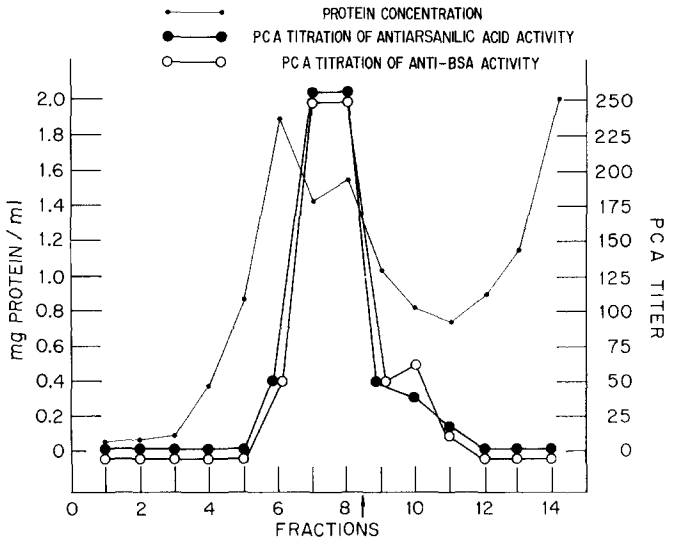

FIG. 6. Distribution of passive cutaneous anaphylactic activity specific for BSA and arsanilic acid hapten in fractions from starch block electrophoresis of a serum obtained from a guinea pig immunized with para-arsanilic diazo-BSA in complete adjuvant.

experiment are presented in Fig. 4. PCA activity is seen to begin in fraction 10 and to peak in fraction 11 corresponding with the presence of the γ_1 line.

A similar experiment was performed (Fig. 5) using the immunoelectrophoretic technique of separation described above with an isolated anti-DNP antibody from serum anti-DNP–BGG 68 prepared by immunization with complete adjuvants. The $7S\gamma_1$ and $7S\gamma_2$ lines were developed with rabbit anti-guinea pig serum R2, which has been shown to distinguish these two antibody types by a reaction of partial identity (13). PCA activity was again found in agar strips obtained from the region subtended by the γ_1 precipitin arc.

III. Electrophoretic Mobility of $7S\gamma_1$ Antibodies of Different Specificities.—The observation that, in the guinea pig, ability to provoke passive anaphylactic reactions was a property of a single homogeneous antibody type, permitted a

comparison to be made of the electrophoretic mobility in starch block of two such antibodies with different immunologic specificities. In a first experiment, an antiserum prepared by immunizing a guinea pig with bovine serum albumin conjugated with arsanilic acid hapten, in complete adjuvants (16), was separated by starch block electrophoresis. The PCA activity of each fraction was assayed using as antigen either bovine serum albumin or arsanilic acid conjugated to guinea pig albumin. The electrophoretic distribution of sensitizing antibodies directed against these two specificities are found in Fig. 6. In a

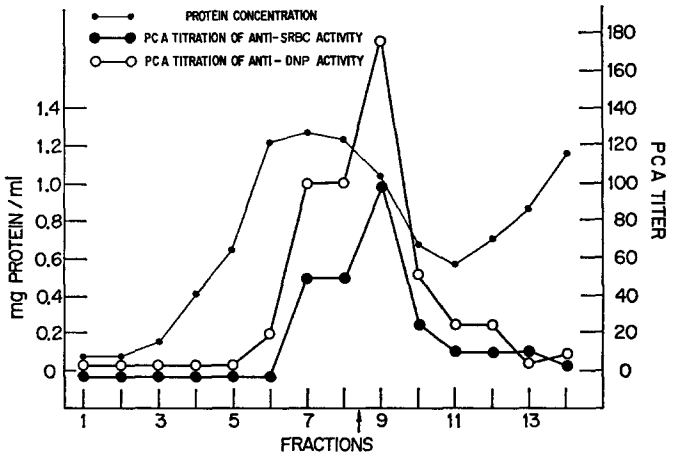

FIG. 7. Distribution of passive cutaneous anaphylactic activity specific for sheep erythrocytes and DNP hapten in fractions from starch block electrophoresis of a mixture of two sera obtained from a guinea pig immunized with sheep erythrocytes or DNP-BGG in complete adjuvant. SRBC = sheep red blood cells.

second experiment, equal volumes of a guinea pig anti-DNP–BGG serum and of a guinea pig anti-sheep erythrocyte serum were mixed. The animals had been immunized with complete adjuvants as described (13, 17). The mixture of the two sera was subjected to starch block electrophoresis and eluted fractions were examined for PCA activity using as antigen DNP-BSA or sheep erythrocytes (17). The electrophoretic distribution of these activities is recorded in Fig. 7. Both experiments show almost identical distribution of PCA activities regardless of immunological specificity.

DISCUSSION

The data presented in this paper indicate that guinea pig $7S\gamma_1$ antibodies are able to transfer passive systemic or cutaneous anaphylaxis; guinea pig $7S\gamma_2$ antibodies are unable to mediate these reactions. Gamma-2 antibodies are able to specifically inhibit PCA reactions provoked by their gamma-1

counterpart by competing for antigen. On the other hand, guinea pig $7S\gamma_2$ antibodies are unable to inhibit passive cutaneous sensitization of guinea pigs by a heterologous antibody system. $7S\gamma_2$ antibodies appear to lack receptors for fixation to guinea pig tissues. It would seem unwise however, to conclude from these findings that $7S\gamma_2$ guinea pig antibodies completely lack the ability to initiate the liberation of vasoactive amines in the guinea pig, or in other species. It is increasingly recognized that in different mammalian species, the liberation of these amines can be triggered by several mechanisms. One of the mechanisms appears to require the intervention of C′ and the formation of serum anaphylatoxin, as demonstrated in the rat (18, 19); other mechanisms, more efficient in terms of antibody requirements, best demonstrated in the guinea pig and man, involve the selective fixation of certain types of antibody globulins to specific tissue receptors. Only this last property is lacking in the guinea pig $7S\gamma_2$ globulins (6–8).

The observation that in man the antibody fraction capable of sensitizing for wheal-and-flare reactions migrates ahead of the main gamma globulin fraction in electrophoresis (20), suggests that a similar distribution of antibody activities occurs in man as in the guinea pig. It is indeed possible that, in all mammalian species, gamma globulin components of "fast" electrophoretic mobility have analogous function.

The data reported in this paper lead one to question the validity of the use of a single test of antibody activity, such as anaphylaxis or complement fixation, as the sole indication of the production of antibodies. Furthermore, these findings emphasize the need for sophistication in the interpretation of the immune response obtained under various experimental conditions.

The observation that PCA activity specific for different antigens show identical electrophoretic mobility in different guinea pigs' sera suggests, at first, that the portion of the antibody molecule concerned with specificity (S fragment) (21) does not contribute appreciably to its electrophoretic mobility at pH 8.6. But these data must be interpreted with caution because of the lack of precise quantitation of the PCA test, inherent in all biological assays, and because of the possible limitations in resolution of the starch block electrophoresis technique.

SUMMARY

Guinea pig $7S\gamma_1$ antibodies were demonstrated to mediate passive systemic or cutaneous anaphylaxis; guinea pig $7S\gamma_2$ antibodies were unable to mediate these reactions. Gamma-2 antibodies specifically inhibited passive cutaneous anaphylactic reactions provoked by gamma-1 antibodies by competing for antigen. However, gamma-2 antibodies were unable to inhibit passive cutaneous sensitization of guinea pigs by a heterologous antibody system. Guinea pig $7S\gamma_2$ antibodies appear to lack receptors for fixation to guinea pig tissues and do not compete with sensitizing antibody for receptor sites.

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