RABBIT ANAPHYLACTIC ANTIBODY*

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PLATE 85

(Received for publication 17 January 1966)

An antibody which is produced in a species and gives passive cutaneous anaphylaxis in that same species is called a homologous anaphylactic antibody. Of all the species studied there has only been one in which a homologous anaphylactic antibody has not been identified. This exception has been the rabbit. It is the purpose of the present work to describe passive cutaneous anaphylaxis in the rabbit and some characteristics of the antibody responsible for this reaction.

It was assumed that all guinea pig 7S antibody has the ability to mediate anaphylactic reactions in the guinea pig until Benacerraf, Ovary, and their collaborators, and White and his coworkers demonstrated that only the faster migrating 7S γ_1-antibodies had this capacity (1–3). The slower migrating 7S γ_2-antibodies lacked this property but were able to sensitize the skin of another species, such as the rat, to give passive cutaneous anaphylaxis (PCA). Mota, independently, discovered that the rat when stimulated with antigen and *Hemophilus pertussis* as an adjuvant produces an unusual antibody capable of producing PCA in rats and degranulating isolated rat mast cells (4). This antibody was also different from the rat antibody responsible for giving PCA reactions in other species (5).

In all species studied to date, guinea pig, mouse, rat, dog, and man, the portion of the antibody population responsible for passive cutaneous anaphylaxis in the same (homologous) species has a faster electrophoretic mobility than the antibody which sensitizes the skin of a different (heterologous) species (1–8). In other properties, the homologous PCA antibodies fall into two distinct classes. The PCA antibodies of the guinea pig and mouse are heat stable, 7S γ_1-globulins (1–3, 6). The latent period for sensitization of the homologous species is 3 to 6 hr and the antibody persists in the skin for only 48 to 72 hr (9). When the mouse or guinea pig is immunized with antigen in Freund's adjuvant the antibody appears following repeated stimulation and persists in the circulation for a long time (1, 6).

* Supported in part (N.J.Z.) by funds from the United States Public Health Service AM-5042-09.

In contrast the homologous PCA antibodies of the rat, dog, and human are heat labile, with an electrophoretic migration faster than gamma globulins and a sedimentation constant somewhat larger than 7S (7, 8, 10). The latent period for skin sensitization varies in these species but tends to be distinctly longer than in the mouse or guinea pig. The antibody from animals of this second group persists in the skin of the homologous species for weeks or months (4, 7, 8, 10). The antibody of the rat appears during the 1st wk following immunization, remains in the circulation for only a short time, and does not reappear with subsequent antigenic challenge (4, 10). Human reagin, at least in allergic individuals, persists in the circulation for relatively long periods of time and can be restimulated (8).

Attempts to produce passive cutaneous anaphylaxis in the rabbit with rabbit antiserum have been unsuccessful (11). This is noteworthy in view of the fact that the rabbit is an excellent antibody producer. The present study was prompted by the thought that the rabbit, like the rat, might form homologous PCA antibody as an early primary immune response. Thus it might have escaped detection previously because the hyperimmune sera no longer contained the antibody. This proved to be the case.

Materials and Methods

Animals.—Albino rabbits weighing 2500 to 3000 g were used for immunization and PCA reactions. Female guinea pigs (Hartley strain) weighing 200 to 300 g were used for guinea pig PCA reactions.

Antigens.—Bovine gamma globulin (BGG), Armour Pharmaceutical Company, Kankakee, Illinois (Lot T 30203). Crystalline bovine serum albumin (BSA) Pentex, Inc., Kankakee, Illinois. The antigens were conjugated with dinitrophenyl sulfonic acid sodium salt (DNP), Eastman Organic Chemicals, Rochester, New York, according to the method of Eisen and contained approximately 35 moles of DNP per mole BGG, and 15 moles DNP per mole BSA (12).

Pontamine sky blue (6BX) was obtained from E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware.

Phosphate-Buffered Saline pH 7.2.—1 liter of water was added to 6.8 g NaCl, 1.483 g $Na_2HPO_4 \cdot 7H_2O$, and 0.433 g $KH_2PO_4 \cdot H_2O$. In passive hemagglutination and passive lysis studies 1 g of gelatin was added to a liter of phosphate-buffered saline (gelatin-buffered saline).

Immunization.—The antigen (DNP-BGG 3 mg N/ml) in phosphate-buffered saline was emulsified with an equal amount of Freund's complete adjuvant (Difco Laboratories, Inc., Detroit) and injected into each of the rabbit's foot-pads. Total dose ranged from 0.5 to 1.0 ml per animal (0.75 to 1.5 mg antigen N). On the 5th to 7th day after immunization and every 2nd or 3rd day thereafter the animals were bled from the heart or central ear artery. One group of 12 animals was bled daily from the marginal ear vein from the 5th to 13th day following immunization. The blood collected was allowed to clot for 1 hr at room temperature, separated by centrifugation at 4°C, and then stored frozen in aliquots without preservatives at −20°C until used.

Passive Cutaneous Anaphylaxis (PCA).—Rabbit PCA was performed in albino rabbits of either sex weighing 2000 to 3000 g. Intradermal injections of 0.2 ml of whole serum, serum fractions, or dilutions of serum in phosphate-buffered saline were made into the back of freshly

shaven rabbits. Although as many as 36 spots could be injected, the usual practice was to employ 12 to 24. All tests were performed in duplicate. After a latent period of 60 to 84 hr, the animals were challenged intravenously with 1.5 ml of DNP-BSA (3 mg N/ml) and 1 ml of 5% pontamine sky blue dye in 0.15 M saline. The resulting lesions were recorded and measured after 30 to 60 min.

PCA in guinea pigs was as described (11). All tests were done in 2 animals. Intradermal injections of 0.05 or 0.1 ml of antibody diluted in phosphate-buffered saline were made into the back of freshly shaved female guinea pigs. After a latent period varying from 6 to 18 hr the animals were challenged intravenously with 0.25 ml of a mixture containing equal parts of antigen (DNP-BSA, 3 mg N/ml) and 5% pontamine sky blue dye. The reactions were recorded and measured 30 min after challenge.

Agar Preparative Electrophoresis with Immunoelectrophoresis.—A modification of the technique of Ovary et al. for combined preparative and immunoelectrophoresis in agar gel was used (2). Heated agar, 1% in barbital buffer pH 8.6, 0.05 ionic strength, was placed on 3¾ by 4 in. lantern slides and allowed to cool. Then a series of wells, usually 10 to 14, were placed down the center of the long dimension of the slide. Troughs were cut at right angles to the outer two wells. The wells were filled with a total of 0.05 to 0.1 ml of serum. Following electrophoresis at 45 v as measured across the slide for 60 min, strips measuring 5 mm were cut from the agar and each was eluted into phosphate-buffered saline (ten times the volume of the serum applied to the slide). The agar strips were macerated and then run overnight at 4°C on a shaker. Sheep antiwhole rabbit serum antiserum was added to the troughs at the ends of the slide to demonstrate the serum components contained in each of the eluted fractions.

Zone Electrophoresis.—A Polyvinyl chloride (B. F. Goodrich Chemical Co., Cleveland) block was prepared as described by Müller-Eberhard and Kunkel (13). Electrophoretic separation of 2-ml serum samples was performed in barbital buffer (pH 8.6 and 0.05 ionic strength) at 250 v for 16 hr at 4°C. Following separation 1 cm strips were cut from the block and eluted into 5 ml of phosphate-buffered saline. Protein concentration was determined by measurement of absorption at 280 mμ. When necessary, protein solutions were concentrated by ultrafiltration.

Gel Filtration Chromatography.—Sephadex G-200 (A. B. Pharmacia, Upsala, Sweden) was soaked in phosphate saline buffer for 3 to 6 days to insure adequate swelling. Following repeated decanting to remove the turbid supernatant, and evacuation of air bubbles by suction, the gel was applied to a vertical column measuring 50 × 2.5 cm as described by Flodin and Killander (14). 1 ml of whole rabbit serum was applied and samples were eluted from the column under gravity at 4°C using phosphate-buffered saline pH 7.2. Aliquots of 2.0 ml were collected at a flow rate of 8 to 12 ml/hr.

Density Gradient Ultracentrifugation.—0.25 ml of whole serum, diluted with an equal volume of isotonic saline, was layered over a continuous 10 to 40% sucrose gradient (15). Following centrifugation at 35,000 RPM for 16 hr in a Spinco model L ultracentrifuge with an SW 39 swinging bucket rotor, the bottoms of the lusteroid tubes were pierced with a sharp needle and fractions of 10 to 12 drops each (approximately 0.5 ml) were collected. Sucrose was removed from the fractions by dialysis in the cold against isotonic saline for 12 to 24 hr.

Treatment with Mercaptoethanol.—Samples of serum (0.5 ml) were dialyzed against 250 ml of 0.1 M 2-mercaptoethanol for 3 hr at room temperature. Following this they were transfered to 500 ml of 0.02 M iodocetamide for 4 hr at room temperature. The reduced and alkylated sample was then dialyzed against several changes of phosphate-buffered saline for 18 to 24 hr at 4°C. Control samples were dialyzed against phosphate-buffered saline in place of the 2-mercaptoethanol, they were then treated with iodocetamide and subsequently dialyzed against phosphate-buffered saline in an identical manner to the test samples.

Passive Hemagglutination.—To a 10% suspension of freshly tanned sheep red blood cells in

phosphate-buffered saline was added an equal volume of a solution containing dinitrophenyl bovine serum albumin (DNP-BSA) with an average concentration of 2.9 mg N/ml, 15 haptenic groups per mole of protein. Following incubation at 37°C for 15 min, cold phosphate-buffered saline was added and the cells were centrifuged at 4°C and washed twice with gelatin-buffered saline. The antigen-coated cells were adjusted to a final concentration of $5 \times 10^7/mm^3$ in gelatin-buffered saline. Control cells were treated with an equal volume of gelatin-buffered saline and then handled in a similar manner to the antigen-coated cells. All sera were inactivated at 56°C for 30 min and absorbed twice with sheep erythrocytes prior to use. Serial twofold dilutions of antisera were made in gelatin-buffered saline. Titration was performed by the microtiter method. To 0.025 ml of serially diluted antibody was added 0.050 ml of antigen-coated sheep erythrocytes, the mixture was agitated with a Vortex mixer and incubated at 37°C. Appropriate antigen and antisera controls were included in all experiments. Hemagglutination patterns were read as described by Stavitsky after 2 to 3 hr of incubation and the highest dilution giving a 2+ reaction was chosen as the end point (16).

Passive Hemolysis.—Fresh guinea pig serum (2.0 ml) was absorbed four times in the cold with 0.2 ml of packed sheep erythrocytes. The absorbed guinea pig serum was diluted one to thirty with gelatin-buffered saline. Sufficient Ca^{++} and Mg^{++} were added to insure that when the complement was diluted with antibody and antigen solutions the mixture would contain a final concentration of 0.001 M Mg^{++} and 0.00015 M Ca^{++}. The tests were performed in microtiter plates with serial twofold dilutions of the antibody in gelatin-buffered saline. Each well contained 0.025 ml of antibody dilutions, 0.05 ml of $5 \times 10^7/mm^3$ antigen-coated tanned sheep erythrocytes and 0.025 ml of 1:30 guinea pig serum. The plates were agitated with a Vortex mixer, incubated at 37°C and read at 90 min. The reactions were read as complete, incomplete or no lysis, the last dilution of antibody still showing incomplete lysis was taken as the titer.

RESULTS

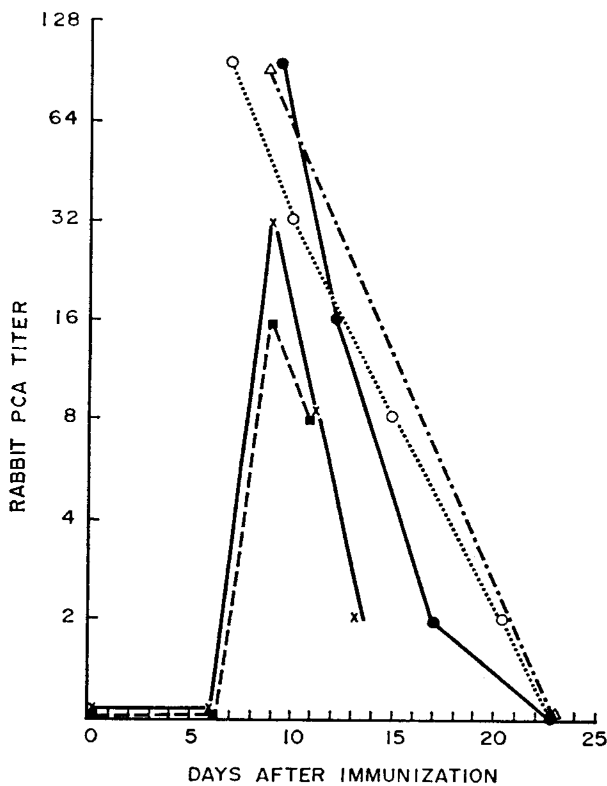
Passive Cutaneous Anaphylaxis.—The reactions produced in the skin of the rabbit can be seen in Fig. 1. Serial twofold dilutions of antibody result in proportionally smaller lesions. High titered antisera caused the appearance of a speckled blueing about the periphery of the lesion within 5 to 10 min, the completely blued lesions evolved in 20 to 30 min. The lesions from less active antisera developed more slowly but were complete by 45 min. Active antisera frequently produced slightly elevated edematous lesions, but none became hemorrhagic or necrotic. Various areas of the rabbit's back were checked for reactivity and comparable lesions were produced in the area from the shoulder blades to the pelvic brim. The skin over the vertebral column and the flanks were not used for testing. Twenty-four tests, 4 lines each with six tests, could be easily placed on the rabbit's back. The reactivity of different rabbits varied considerably; the reasons for these differences were not analyzed systematically but did not appear to be due to the age, sex, or weight of the rabbit, nor were significant seasonal variations noted.

Latent Period and Persistence of Skin Fixation.—The rabbit anaphylactic antibody must remain in the skin a finite period of time prior to antigenic challenge before positive PCA reactions can be obtained. As is customary, this interval will be referred to as the latent period. Intravenous antigen administration 2, 6, 12, and 24 hr after the intradermal injection of 0.2 ml of anaphylactic

TABLE I

Proportion of Rabbits Developing PCA Antibody of a Given Titer

PCA titer	No. rabbits
No activity	25
Undiluted	2
1:2	4
1:4	3
1:8	4
1:16	3
1:32	2
1:81	4
Total	47



TEXT-FIG. 1. Effect of time following immunization of rabbit PCA titer. The time of appearance and duration of detectable rabbit anaphylactic antibody from 5 rabbits which produced the highest titers of anti-DNP-BGG anaphylactic antibody.

antibody produced no skin lesions. Positive reactions appeared after a latent period of 36 to 48 hr, but did not reach their maximum intensity until 60 to 84 hr. While no further enhancement of reactivity was obtained by prolonging the latent period it is noteworthy that PCA reactions were still elicited after latent periods of from 7 to 17 days.

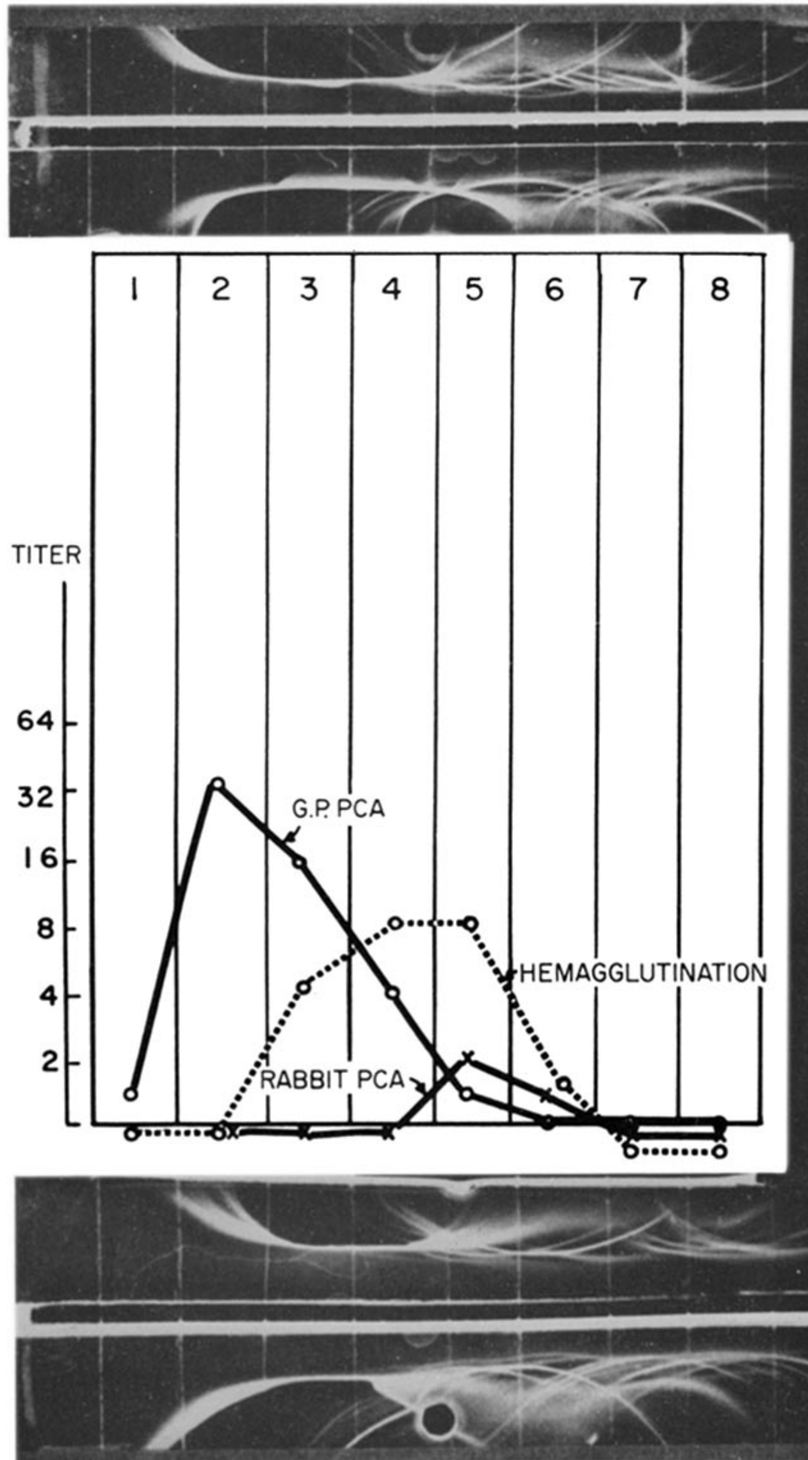
Frequency, Time of Appearance, and Duration of Skin-Sensitizing Antibody.—Forty-seven rabbits were immunized with DNP-bovine gamma globulin and bled at frequent intervals to follow the appearance and development of anaphylactic antibody. The results are shown in Table I. Twenty-two of the 47 rabbits (46.8%) produced detectable amounts of skin-sensitizing antibody. The earliest appearance was on the 5th day following immunization, the majority had demonstrated antibody by the 7th to 9th day; only 1 animal began to produce antibody later than the 9th day. The pattern of antibody development was quite consistent, the highest titers appeared initially and declined thereafter, the rate of fall appeared proportional to the heighth of the initial antibody titer. The results for the 5 animals which produced the most anaphylactic antibody are shown in Text-fig. 1. Skin-fixing antibody appeared at the end of the 1st wk following immunization and then rapidly declined over

TABLE II

Effect of Heating on Rabbit PCA Antibody

Sera	Temperature	Time	PCA titer
	°C	*min*	
DNP 6	22	120	1:16
	56	30	1:16
	56	60	1:4
	56	120	1:2
DNP 11	22	90	1:32
	56	90	1:8
DNP 15	22	240	1:8
	56	60	1:2
	56	120	Undil.
	56	240	0

TEXT-FIG. 2. The distribution of hemagglutinating and anaphylactic antibody activities in the eluates from agar strips obtained after electrophoretic separation of rabbit anti-DNP serum (14-1) taken 9 days after immunization. The wells contained a total of 0.08 ml of rabbit antiserum. Precipitin arcs were developed with sheep antiwhole rabbit antiserum placed in troughs at each end of the slide. The origin is between strips 4 and 5, the cathode to the left, the anode to the right.


1
2
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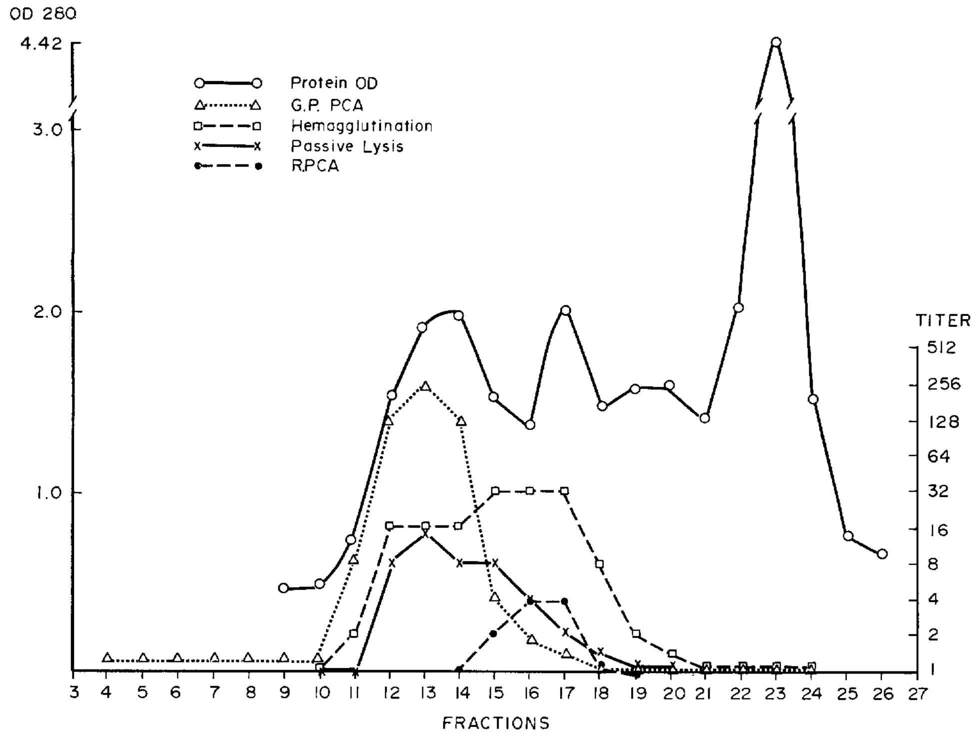
G.P. PCA
HEMAGGLUTINATION
RABBIT PCA

the next 2 to 3 wk. In low titered antisera (less than 1:8) activity was demonstrable for only 2 to 4 days. Three of the 22 rabbits deviated from this overall pattern; in these low titers of skin-fixing antibody appeared at the expected time but remained at these levels (1:8 or less) for 38 days; no explanation for this aberrant behavior was found.



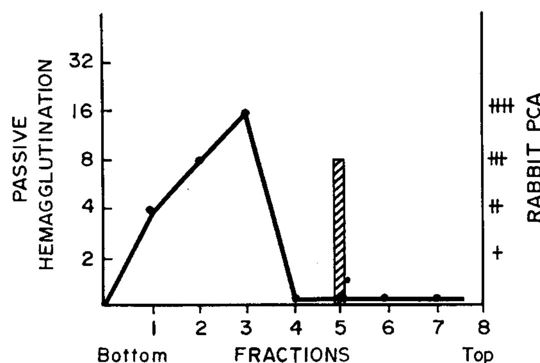
TEXT-FIG. 3. Distribution of hemagglutinating, complement fixing, and passive cutaneous anaphylactic activities in fractions from a polyvinyl block of a rabbit anti-DNP (16-1) serum obtained 9 days after immunization. Point of application of serum to the block was at fraction 17.

Hemagglutinating antibody determinations were performed on the majority of the sera tested for anaphylactic activity. No correlation was found between the hemaggluting and skin-fixing properties. Some sera showed high titers for hemagglutinating antibody and no anaphylactic antibody, in others with the highest titers of skin activity, the hemagglutinating antibody was not unusually elevated. The appearance of hemagglutinating activity paralleled the appearance of anaphylactic antibody but usually persisted after skin-sensitizing reactivity could no longer be demonstrated.

In an attempt to define the various properties of the rabbit antibody which produced PCA in rabbit skin a variety of studies were performed. The rabbit anaphylactic antibody was analyzed for heat lability, stability upon reduction and alkylation, its electrophoretic migration, and sedimentation properties. All of these characteristics were compared with the species of rabbit antibody which produced PCA in the skin of the guinea pig.

Characteristics of the PCA Antibody

Heat Lability.—The effect of heat on the rabbit PCA antibody of three individual sera is shown in Table II. A threefold fall in titer from control levels was observed when the antibody was heated at 56°C for 2 hr; in one serum com-



TEXT-FIG. 4. Titration of hemagglutinating and rabbit passive cutaneous anaphylactic activity in fractions from a sucrose density gradient separation of pooled fractions 16 and 17 from the polyvinyl block shown in Fig. 4. Anaphylactic activity is shown by the bar and passive hemagglutination by a solid line.

plete loss of activity was produced at 4 hr. The introduction of the same serum into the skin of the guinea pig demonstrated no loss of heterologous PCA activity even with the sample which had been heated for 4 hr.

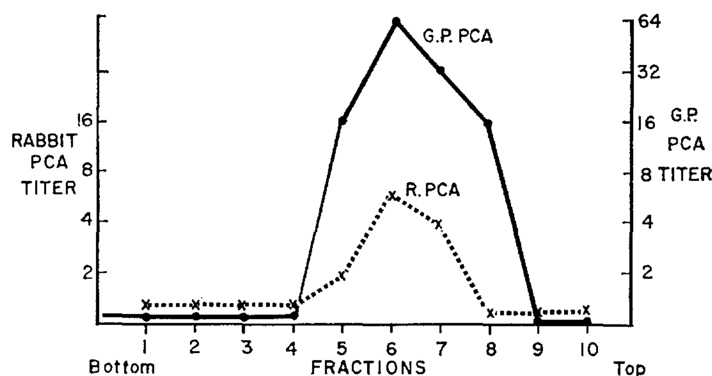
Reduction and Alkylation.—Treatment of rabbit serum with 0.1 M 2-mercaptoethanol followed by alkylation with 0.02 M iodoacetamide completely destroyed the rabbit PCA antibody. Similar treatment resulted in a 50 to 75% decrease (two- to threefold fall in titer) in activity of the rabbit antibody producing PCA in the guinea pig skin.

Electrophoretic Mobility.—Agar was employed for combined preparative and immunoelectrophoresis. The rabbit PCA antibody was never demonstrated by a precipitin reaction in gel using DNP albumin as antigen. Strips were cut from the agar after electrophoresis, eluted, and the hemagglutinating and skin-fixing activity or each segment was determined. The relative mobility of the antibodies responsible for the activity detected could be assessed by localizing

it with sheep anti-rabbit antiserum. The results of a typical experiment are shown in Text-fig. 2. The serum was obtained 9 days after immunization. The origin is between segments 4 and 5; segment 1 is the most cathodal, 8 the most anodal. The immunoelectrophoretic patterns on each end of the lantern slide identify the serum proteins present in the eluates from the various strips.

The rabbit antibody which sensitizes the rabbit skin migrated faster in an electric field at pH 8.6 than the rabbit antibody which sensitizes the guinea pig.

In all sera studied, the rabbit PCA antibody was on the anodal side of the origin with peak activity in fractions 5, and occasionally in 6. In contrast, the rabbit antibody giving a PCA reaction in the guinea pig was on the cathodal

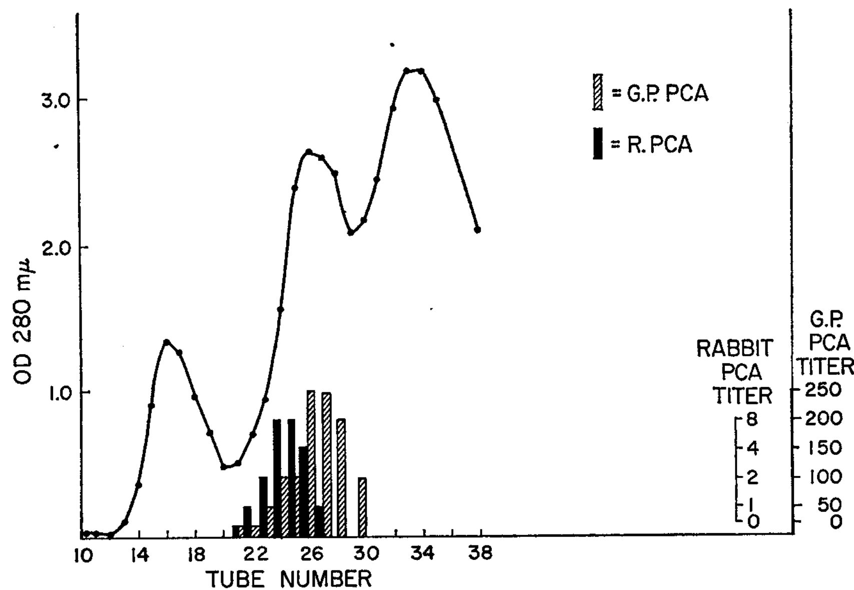


TEXT-FIG. 5. Comparison of the positions of the rabbit passive cutaneous anaphylaxis activity (broken line) and the rabbit antibody giving cutaneous anaphylaxis in the guinea pig (solid line) in the fractions from a sucrose density gradient. Rabbit anti-DNP (17-1) serum was obtained 9 days after immunization.

side of the origin, with maximum reactivity in fraction 2 or 3. Passive hemagglutination of tanned sheep erythrocytes coated with DNP-BSA could be obtained with fractions located on both sides of the origin with peak titers in segments 4 and 5.

Zone electrophoresis in polyvinyl chloride blocks provided results similar to those obtained with agar. The results from another 9th day postimmunization serum are shown in Text-fig. 3. The rabbit anaphylactic antibody migrated toward the anode, in the beta globulin fraction. The rabbit antibody which gives PCA in the guinea pig was located with the gamma globulin fraction. Its activity closely paralleled the fraction of serum which gave passive lysis of antigen-coated sheep erythrocytes. In all studies performed there was some overlapping of the antibody which fixed to guinea pig skin and that which sensitized rabbit skin, but the peak activities of the two antibodies were distinctly separable.

Differentiation between Hemagglutinating and PC Antibody.—Hemagglutinating antibody was present in both the slower and faster migrating fractions, with both polyvinyl chloride and agar electrophoresis (Text-figs. 2 and 3). It is known that in the rabbit at the end of the 1st wk following immunization, hemagglutinating activity is given by both 19S (gamma M) and 7S (gamma G) antibodies (17). The distribution noted would be consistent with a mixture of these two species of hemagglutinating antibodies. In all sera studied the electrophoretically isolated fractions with rabbit PCA activity also contained hemagglutinating antibody. It was decided therefore to attempt to dissociate these two properties of the antiserum. Fractions 16 and 17 from the zone electrophoresis shown in Text-fig. 3 were chosen because they contained the peak rabbit anaphylactic activity as well as the faster migrating hemagglutinating antibody. Aliquots of fraction 16 and 17 were combined, concentrated by ultrafiltration, and then subjected to sucrose density gradient ultracentrifugation. As seen in Text-fig. 4, the hemagglutinating activity was demonstrable in the heavier fractions at the bottom of the gradient; the lighter rabbit PCA antibody was located in fraction 5 in the region usually containing the 7S γG-antibody.



TEXT-FIG. 6. Distribution of anaphylactic activity in fractions from gel filtration with Sephadex G-200. Protein absorption at 280 mμ shown by solid line, rabbit anaphylactic activity by solid bars, and the rabbit antibody producing passive cutaneous anaphylaxis in the guinea pig by the stripped bars. Fractions of 2 ml each. The rabbit anti-DNP serum (16-1) was obtained 9 days after immunization.

Another means of differentiating these two activities was provided by the results of heating experiments. Heating at 56°C for longer than 2 hr had no effect on the hemagglutinating activity of whole serum or serum fractions but inactivated the rabbit PCA antibody.

Molecular Size.—Rabbit serum containing both rabbit anaphylactic and guinea pig skin-sensitizing antibodies were subjected to ultracentrifugation for 15 to 16 hr in a sucrose density gradient. In each of the several studies performed (Text-figs. 4 and 5) the activity of both antibodies was located in the area of the gradient where 7S antibodies are usually encountered. The peak activity of the two antibodies showed a remarkable parallelism and while it was possible to dissociate the two skin-sensitizing antibodies from the bulk of the hemagglutinating antibody, they could not be separated from each other (Text-fig. 5) by this technique.

Sephadex gel filtration is an additional method by which proteins can be separated on the basis of their molecular size and configuration. 1 ml samples of rabbit serum were applied to columns containing Sephadex G-200 gel. The fractions which were eluted from the column were analyzed for their ability to produce PCA reactions in the skin of rabbits and guinea pigs. The results from one such experiment are shown in Text-fig. 6. It can be seen that although considerable overlapping of the two antibodies occurred, the peak activity of the rabbit anaphylactic antibody was in advance of the rabbit antibody giving PCA in the guinea pig. In each of four similar studies the rabbit anaphylactic antibody activity was always on the ascending limb of the second peak eluted from the column. The maximum activity of the antibody producing PCA in the rabbit was always found 2 to 3 fractions in advance of the maximum activity of the antibody producing PCA in the guinea pig.

DISCUSSION

The studies presented in this paper show that the rabbit is capable of producing an antibody which gives anaphylactic reactions in the skin of another rabbit. So far as is known, this specialized antibody has not been heretofore described for this species. In fact, the rabbit has always been considered unusual because homologous PCA reactions could not be elicited despite the presence in the rabbit antiserum of high levels of precipitating antibody. Ovary was unable to obtain passive cutaneous anaphylaxis in rabbits with homologous antisera containing as much as 5 mg antibody nitrogen per ml. The antibody used presumably belonged to the IgG class (11). Ramsdell produced occasional skin reactions with rabbit antisera if the antigen was administered within 1 hr of the placement of undiluted antibody into the skin of the rabbit's ear (18). This was probably the cutaneous analogue of systemic anaphylaxis of the rabbit engendered by the rabbit IgG antibody which requires no latent period.

From the present work, it is now obvious that the previous lack of success in

demonstrating the presence of rabbit anaphylactic antibody in immune sera stems from an unusual set of circumstances. The rabbit skin-sensitizing antibody is produced early in immunization in less than half of the animals immunized and then only in low concentrations. In addition, a long latent period of 48 to 72 hr is required between the intradermal injection of the antiserum and the eliciting of the reaction by the intravenous injection of dye and antigen. This latent period might be shortened if more active antisera were available, or, as in the case of the rat, if purified antibody was employed.

The rabbit PCA antibody persists in the skin for prolonged periods of time. We have not defined the duration of such persistence but it is at least 17 days.

The skin-sensitizing activity of the rabbit anaphylactic antibody is destroyed by treatment with 2-mercaptoethanol, as is that of the homologous PCA antibody of the rat, mouse, and human (4, 6, 8). Such inactivation is not necessarily an absolute distinguishing feature between the homologous and heterologous anaphylactic antibodies, however, because the skin-sensitizing capacity of both mouse and rabbit heterologous antibody is reduced about 75% by 2-mercaptoethanol treatment (6, 20). The fact that the skin-sensitizing activity is affected in both types of antibodies is in keeping with the view that reduction and alkylation damages a discrete area on the heavy chain responsible for binding to an appropriate receptor site in tissue (19).

The results of the experiments with Sephadex G-200 and sucrose density gradients demonstrate that the rabbit skin-sensitizing antibody presumably has a molecular weight close to that of 7S gamma globulin. On Sephadex G-200 the homologous skin-sensitizing antibody elutes consistently in advance of the heterologous antibody. Our studies of early, 9th to 12th day, heterologous antibody are the same as those for the heterologous antibody obtained by hyperimmunization which has been shown to be a 7S globulin (20). The Sephadex G-200 data therefore suggest that the homologous PCA antibody of the rabbit may be somewhat larger than a 7S. This possibility is not precluded by our inability to distinguish between the two skin-sensitizing antibodies in sucrose density gradients for the centrifugation was only carried out for 16 hr and it has been shown that 20 to 22 hr are required to separate human reaginic antibody from human 7S globulin under similar conditions (21). The problem of separating the rabbit antibodies is increased by the disproportionally greater activity of the 7S heterologous PCA antibody when compared with the homologous anaphylactic antibody. The peak activity of the two antibodies can be analyzed but considerable overlapping results because the heterologous antibody can be demonstrated over a much greater area in both the Sephadex G-200 and density gradient experiments.

Thus, the homologous skin-sensitizing antibody of the rabbit is heat labile, mercaptoethanol sensitive, nonprecipitating, and does not fix complement. It requires a latent period of 48 to 72 hr before it can be demonstrated, but once

"fixed" to an as yet unidentified skin receptor, it remains for long periods of time. It migrates faster than a γG-globulin in electrophoresis and may be somewhat larger than a 7S gamma globulin which is heat stabile, only partially inactivated by reduction and alkylation, migrates in electrophoresis as a slow gamma globulin, and fixes complement. The major reactivity of this antibody is present at the summit of the second peak on Sephadex G-200 gel chromatography. These properties of the heterologous anaphylactic antibody obtained 9 to 11 days following a single immunization are identical to those described by Ovary and coworkers for a purified rabbit gamma 2 globulin obtained after repeated immunizations (20).

Systemic anaphylaxis as it has been elicited in rabbits differs in a number of particulars from anaphylaxis in guinea pigs. Heretofore, these differences have been attributed to species variations. Little attention has been directed to the possibility that they might be ascribed to differences in the type of antibody required to produce the observed reactions. Considerable evidence suggests that rabbit anaphylaxis is not the result of the homologous anaphylatic antibody described in this paper, but is due to precipitating antibody, presumably of the 7S gamma 2 variety. Systemic anaphylaxis appears to be mediated by the union of large amounts of antibody and antigen in the circulation (22, 23). Jackson found that severe anaphylaxis did not occur until more than 3.5 mg of antibody protein per ml was present in the rabbit's serum (24). The discovery of an anaphylactic antibody of the rabbit which is analogous to the homologous anaphylactic antibody of the guinea pig should eventually permit a comparison of the same type of anaphylaxis in these two species. Better definition of the biologic role of the rabbit homologous anaphylactic antibody and its more precise physicochemical characterization awaits the availability of methods for enhancing its production.

SUMMARY

Antibody capable of sensitizing rabbit skin for passive cutaneous anaphylaxis is produced in the rabbit as early as 6 to 7 days following antigenic stimulation. It reaches peak activity around the 9th day and is gone by the 3rd wk. The antibody is heat labile, sensitive to treatment with mercaptoethanol, nonprecipitating and does not fix complement. In order to demonstrate PCA activity a latent period is required of from 48 to 72 hr after introduction of the antibody into the rabbit's skin; the activity can persist for at least 17 days. It has a faster electrophoretic mobility than rabbit γG-globulin, and is eluted somewhat earlier than γG-globulin from Sephadex G-200, although distinctly after γM-globulin.

No relationship was demonstrated between the rabbit PCA activity and the hemagglutinating activity found in the same sera. The rabbit anaphylactic antibody differs in almost all properties studies from the rabbit 7S antibody capable of sensitizing guinea pigs for PCA which arises at the same time. This

latter antibody found early in immunization had properties which were indistinguishable from those described for the rabbit 7S antibody giving PCA in the guinea pig found in late hyperimmune sera.

Note Added in Proof.– After the preliminary account of this work appeared (Zvaifler, N.J., and Becker, E.L., Rabbit passive cutaneous anaphylaxis *Fed. Proc.* 1965, **24,** 1965), and following submission of this paper, the study of Onoue et al., appeared (Onoue, K., Yagi, Y., and Pressman, D., Isolation of rabbit IgA antihapten antibody and demonstration of skin-sensitizing activity in homologous skin, *J. Exp. Med.* 1966, **123,** 173). The authors concluded that rabbit PCA activity resides in the 7S γA-fraction.

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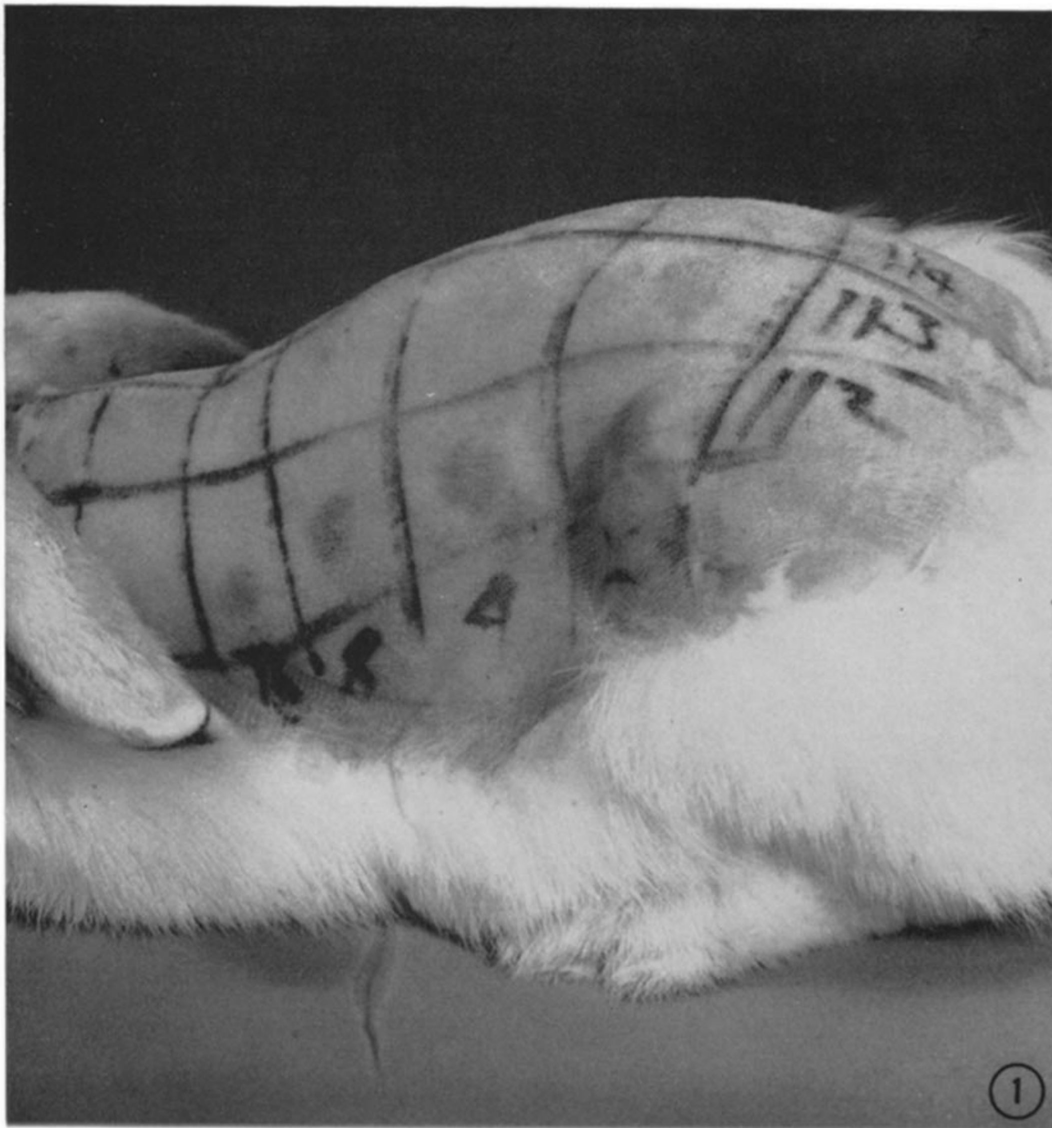
EXPLANATION OF PLATE 85

FIG. 1. Reactions produced in the skin of an albino rabbit with 0.2 ml of serial twofold dilutions of rabbit serum containing rabbit anaphylactic antibody (anti–DNP-BGG) followed 72 hr later by the intravenous injection of 1.5 ml of DNP-BSA (3 mg N/ml) and 1 ml of Pontamine sky blue dye in 0.15 M saline.



(Zvaifler and Becker: Rabbit anaphylactic antibody)