IMMUNOLOGICAL INDUCTION OF INCREASED VASCULAR PERMEABILITY

I. A Rabbit Passive Cutaneous Anaphylactic Reaction Requiring Complement, Platelets, and Neutrophils*

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Increase in vascular permeability has been demonstrated in experimental inflammatory reactions of immunological origin. In serum sickness of rabbits there is some evidence that this increase in vascular permeability, which is induced by vasoactive amines, plays a pathogenetic role in the deposition of circulating immune complexes (1,2). Complexes above a certain size become trapped along the internal elastic lamina in arterial walls and the subsequent accumulation of neutrophils precipitates the arteritis which is characteristic of this condition. In glomeruli, the complexes deposit along the internal aspect of the basement membrane and this process too involves the action of vasoactive amines.

Considering the importance of immunologic permeability reactions, studies were undertaken to determine the types of antibody which could cause an increase in vascular permeability in rabbits, and the cells with which they interact in the blood. To carry out analyses in vivo, the acute passive, cutaneous anaphylactic (PCA) reaction was selected. PCA reactions have been produced in rabbits previously (3–6) and the activity has been ascribed to a heat-labile, electrophoretically fast-migrating antibody which is produced early in the immune response. This type of antibody has been named homocytotropic antibody (7). In the present studies, in addition to homocytotropic antibody, a permeability-inducing antibody was found, the action of which is apparently dependent upon the participation of complement, platelets, and neutrophils (8). The properties and the apparently novel mechanisms of action in vivo of this second type of antibody are described in this paper. The following paper (9) examines the activity of the antibody in vitro.

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Materials and Methods

Production of Antibody.—New Zealand white rabbits weighing 2.0–2.5 kg were immunized with bovine serum albumin (BSA). Three injection schedules were employed. (*a*) BSA (12 mg N) in isotonic saline was injected intravenously as one dose; (*b*) BSA (5 mg N) was injected into the footpads with incomplete Freund's adjuvant; and (*c*) BSA (1.5 mg N) was injected into the footpads with complete Freund's adjuvant according to the method of Zvaifler and Becker (3) for inducing homocytotropic antibody formation. The rabbits were bled at intervals. Because the antibodies produced were also to be examined in an in vitro system (9), plasma (0.01 M EDTA as anticoagulant), rather than serum, was generally separated. It was ascertained that PCA reactions were induced equally well by serum or plasma. The plasma was stored at −20°C.

Passive Cutaneous Anaphylactic (PCA) Reactions.—New Zealand rabbits (2.0–2.5 kg) were shaved along the back, and 0.1 or 0.2 ml of the antibody-containing plasma, plasma fraction, or dilution of plasma in phosphate buffered saline (PBS) was injected intradermally. After a sensitization period of 48 hr (except when this parameter was deliberately varied, as described later), 1.5 mg BSA N/kg was injected intravenously along with 2 ml of a 2.5% solution of Evans blue dye in PBS. The size of the blueing in the skin was measured after 30 min and the area calculated from the mean diameter. All preparations were examined in two or more rabbits, on different parts of the back, and the mean values taken.

Reversed Passive Arthus Reactions.—Rabbits were injected intradermally with hyperimmune rabbit anti-BSA antiserum (100 μg antibody N) and challenged immediately with intravenous antigen. The reaction was performed on the same rabbits that were used for the PCA tests.

Depletion of Platelets and Neutrophils.—Sheep anti-rabbit platelet and sheep anti-rabbit neutrophil antisera were prepared and exhaustively absorbed with plasma and formed blood elements as described previously (2, 10). Globulin fractions of these antisera and of normal sheep serum were injected intraperitoneally into rabbits 18 hr before the PCA reaction was induced by the intravenous injection of antigen.

The numbers of platelets in the blood in rabbits given antiplatelet antibody fell to below $1 \times 10^4/mm^3$, whereas neutrophil counts were slightly raised. With this degree of thrombocytopenia, trauma to the skin led to ecchymoses, so that great care was employed in shaving the back before reading cutaneous reactions. Complement (CH 50) levels were never reduced more than 40% by any of these treatments.

Neutrophils were depleted to below $200/mm^3$ by the antineutrophil antiserum, which was without appreciable effect on platelet or lymphocyte counts. Neutrophils were also depleted by administration of nitrogen mustard (Mechlorethamine HCl, Merck, Sharp & Dohme, West Point, Pa.) 1.75 mg/kg, intravenously 3 days before the PCA challenge. To prevent bacterial infection, penicillin (3000 units) and streptomycin (50 mg) were also injected.

Depletion of C3 with Cobra Venom Factor (CoF).—The C3-inactivating factor in the venom of the cobra *Naja naja* (Ross Allen Reptile Institute, Silver Springs, Fla.) was prepared by elution from DEAE cellulose to remove the neurotoxin (11). The factor was assayed on human complement, one unit being that amount necessary to reduce to 50% the hemolysis of 2×10^8 EA produced by 1/20 dilution of normal human serum. Most preparations had protein concentrations of approximately 4 μg/unit. The preparations were sterilized by passage through a Micropore filter (0.45 μ) and kept at 4°C.

To deplete the levels of C3, rabbits were injected with a total of 400 units/kg body weight divided into six intravenous injections spaced over a 48 hr period before the PCA challenge. These animals remained healthy, had normal platelet, and slightly increased neutrophil counts. CH 50 levels, however, were reduced to 10–18% of the pretreatment levels.

In all the depletion studies, the rabbits were also injected intradermally with dilutions of histamine (Eli Lilly & Co., Indianapolis, Ind.), synthetic bradykinin (Sandoz Pharmaceuticals

Hanover, N. J.), and of compound 48/80 (Burroughs Wellcome & Co., Tuckahoe, N. Y.) to demonstrate normal reactivity to these substances.

Treatment with Antihistamine and Antiserotonin.—Chlorpheniramine maleate (Chlor-Trimeton, Schering Corp., Bloomfield, N. J.) was administered intramuscularly in a dose of 20 mg/kg, 2–4 hr before PCA challenge. The effectiveness of the treatment was ascertained by intradermal injections of up to 1 μg histamine, which no longer produced a blueing reaction. The permeability due to bradykinin (0.1 μg) injections was not affected by this dosage, but that due to compound 48/80 was inhibited. An antagonist of serotonin, methysergide maleate (Sansert, Sandoz Pharmaceuticals, Hanover, N. J.), was injected intramuscularly (0.5 mg/kg) at the same time interval as for the antihistamine.

Fractionation of PCA Antibody.—Pevikon block electrophoresis was performed in barbital buffer pH 8.6, ionic strength 0.05, for 40 hr at 8.5 v/cm. 8 ml of the serum was applied and the eluates from 1 cm strips were concentrated by negative pressure dialysis. The fractions were examined for their content of precipitating antibody, and for their ability to fix rabbit complement in the presence of antigen. They were also tested for PCA activity.

Diethylaminoethyl (DEAE) cellulose chromatography was carried out in 0.01 M sodium phosphate buffer at pH 8.0. The serum was dialyzed against this starting buffer and applied to the column already equilibrated with it. After the nonadherent proteins had been removed, a gradient of NaCl in the 0.01 M phosphate buffer was applied and the adherent proteins were eluted. Immunoelectrophoretic analysis showed IgG globulin in the first two peaks.

Density gradient ultracentrifugation was carried out in a linear sucrose gradient from 10 to 37% sucrose in PBS. 0.3 ml of serum, diluted 1:2 with PBS, was applied to the top of the gradient and the tube centrifuged at 50,000 rpm for 5 hr in a Spinco Model L ultracentrifuge. IgG and IgM preparations were run together at the same time to provide markers. Fractions were removed from the bottom of the tube and tested for protein by the Folin method, and for PCA activity.

Complement Assays.—Hemolytic complement levels were determined by the method described previously for rabbit complement (10). The fixation of C3 in the PCA lesions was demonstrated by the use of fluorescent anti-C3 antibody (12).

Quantitative Precipitation.—The method described by Kabat and Mayer (13) was employed.

RESULTS

The Stimulation of Antibodies Giving Passive Cutaneous Anaphylactic (PCA) Reactions in Rabbits.—Antisera to BSA were obtained from rabbits immunized by three different schedules. The antisera were tested in the skin of normal rabbits for their ability to produce an increase in vascular permeability, i.e., a PCA reaction, after 48 hr sensitization period. Table I shows the numbers of rabbits responding to each immunization schedule by the production of antibody capable of giving such a reaction (PCA antibody). All three immunization procedures resulted in the stimulation of PCA antibody.

In order to determine the mechanisms whereby the PCA antibodies produced the increased vascular permeability, rabbits were depleted of various blood elements before PCA reactions were performed on them. By these procedures it was possible to distinguish two types of PCA reactions.

The Separation of PCA Antibodies into Two Types by their Ability to Give PCA Reactions in Rabbits Depleted of C3.—Antisera produced by the different immunization schedules were examined for their ability to produce PCA reac-

tions in rabbits depleted of C3 by the anticomplementary factor in cobra venom (CoF). Under these conditions, a striking inhibition of the PCA reactions given by some of the antisera was noted (Table I). Antisera stimulated by injection of antigen intravenously, or into the footpads with Freund's incomplete adjuvant, only produced PCA reactions in normal rabbits. Complete inhibition was observed in the rabbits treated with CoF.

In sharp contrast, antisera from half the rabbits immunized with antigen in complete Freund's adjuvant gave PCA reactions in both normal and CoF-

TABLE I

Number of Rabbits Responding to Different Immunization Schedules by the Production of PCA Antibodies

Immunization route and vehicle	BSA N injected	Total number rabbits injected	Rabbits in which presence of PCA antibody tested*	Number of sera containing PCA Ab on day:‡					
				5	7	9	11	15	30
	mg								
Intravenous in saline	12	4	Normal	0	0	0	2	3	0
			CoF-treated	0	0	0	0	0	0
Footpads in incomplete adjuvant	5	4	Normal	0	2	3	4	4	4
			CoF-treated	0	0	0	0	0	0
Footpads in complete adjuvant	1.5	8	Normal	0	4	7	8		7
			CoF-treated	0	3	4	3		0

* Presence of antibody tested by passive cutaneous anaphylaxis: 0.2 ml serum was injected intradermally into two normal rabbits and two rabbits which were depleted of C3 with cobra venom factor prior to challenge with antigen. Antigen and Evans blue dye were injected intravenously 48 hr after the antibody, and the blueing was examined after 30 min.

‡ Days following injection of immunizing antigen.

treated animals. Rabbits immunized in this fashion have been shown to produce homocytotropic antibody (3) and as will be described below, the antibody which was still active in C3-depleted animals had many of the characteristics of homocytotropic antibody.

Since C3 depletion was inhibitory to the PCA reactions produced by some of these antisera, the term complement-dependent PCA antibody (C-dep PCA antibody) has been employed for this antibody to distinguish it from the homocytotropic antibody. It was found (Table I) that the C-dep PCA antibody stimulated by injections in incomplete adjuvant persisted for more than 30 days, in contrast to the homocytotropic variety. Moreover, C-dep PCA antibody could be detected in hyperimmune antisera produced by multiple injections of antigen.

Titrations of C-dep PCA antibody and homocytotropic antibody in normal and CoF-treated rabbits are shown in Table II. It was found that even undiluted C-dep antiserum was unable to produce a PCA reaction in CoF-treated rabbits. Homocytotropic antibody was unaffected.

In order to determine whether the CoF could be affecting the persistence of the antibody in the rabbit skin, the following experiment was performed. Eight rabbits were injected intradermally with C-dep PCA antibody and 1 day later four of them received 200 units/kg CoF as one dose. Two rabbits in each group were injected with Evan's blue dye and antigen after another 24 hr. The PCA reaction was inhibited in the CoF-treated animals. The complement levels in the remaining CoF-treated animals were monitored, and when they began to return to normal (8 days after CoF injection) these rabbits were also injected with dye and antigen. All four remaining rabbits now exhibited PCA activity, showing that the inhibitory action of CoF is not on the persistence of the antibody in the skin.

TABLE II

Titers of PCA Antibody in Rabbits Depleted of C3, Platelets, or Neutrophils

Recipient rabbit treated with:*	Titer of antibody giving PCA reactions‡		Arthus reactions
	C-dep PCA antibody	Homocytotropic antibody	
Untreated	32	16	+
C3 depletion (cobra factor)	0	16	±
Platelet depletion (antibody)	0	8	+
Neutrophil depletion (nitrogen mustard or antibody)	0	16	−
Normal sheep globulin	32	16	+

* The depletion techniques are described in the Materials and Methods section.

‡ The rabbits were challenged with antigen and Evans blue dye 48 hr after intradermal injections of antibody dilutions. The lesions were examined after 30 min. The end point was taken as the highest dilution of antiserum giving more than 50 mm^2 of blueing. These results are representative of four similar experiments.

Effect of Platelet Depletion.—Rabbits were depleted of platelets with sheep anti-rabbit platelet antiserum. When PCA reactions were performed on them it was found that the activity of the C-dep PCA antibody was completely inhibited (Table II). The homocytotropic antibody titer was reduced slightly in animals treated in this way, but Arthus reactions (characterized by palpable edema, hemorrhage, and neutrophil infiltration) were not inhibited. Normal sheep globulin, injected into rabbits as a control for the platelet-depleted group of animals, had no inhibitory effect on the PCA lesions.

Effect of Neutrophil Depletion.—Neutrophil depletion by sheep antineutrophil antiserum or nitrogen mustard administration was inhibitory to the PCA reactions produced by C-dep PCA antibody, but not to those produced by homocytotropic antibody. The treatments were also effective in inhibiting the Arthus reaction (Table II).

Effect of Antihistamine on the PCA Reactions as a Function of the Length of the Sensitization Period.—PCA reactions induced by homocytotropic antibody were entirely prevented in rabbits by pretreatment with antihistamine. The effect of antihistamine on the C-dep PCA reaction, however, was found to depend upon the length of the sensitization period (Fig. 1). This figure shows

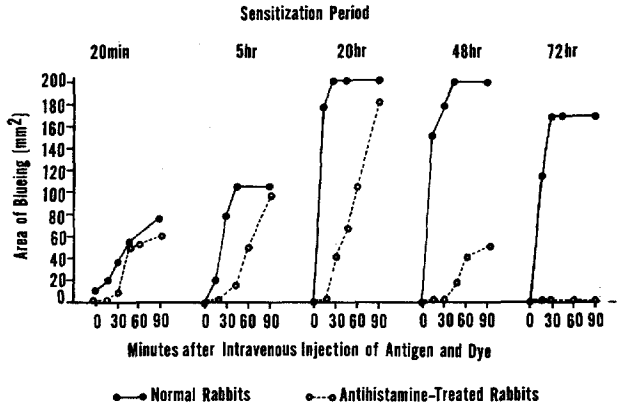

FIG. 1. The effect of antihistamine and length of sensitization period on the complement-dependent PCA reaction. The PCA reactions were produced in normal and antihistamine-treated rabbits with 15 μg antibody N. Each point represents the mean value of determinations made in three animals.

the effects when 15 μg antibody nitrogen was injected, but the results were similar using injections of 50, 5, and 2.5 μg antibody.

If the antibody remained 72 hr in the skin before intravenous challenge with antigen, the blueing lesion was completely inhibited in rabbits treated with antihistamine. Shorter sensitization periods resulted in progressively less inhibition of the final size of the blueing reaction, although the onset of permeability increase was delayed. It was also found that with the shortest sensitization period, as in antihistamine-treated animals, the lesion was still increasing in size after 60 min, whereas with longer sensitization periods in normal rabbits the lesion reached a maximal size after 30 min. Large quantities of antibody (greater than 100 μg N) were not completely inhibited by antihistamine treatment, even with a 72 hr sensitization period.

The lesions produced after shorter sensitization periods became palpably

edematous and sometimes hemorrhagic after 2–4 hr, and histologic sections showed a massive perivascular infiltration of neutrophils. The lesions thus resemble those produced in the Arthus reaction. If the antibody was left in the skin for 48 or 72 hr before challenge, however, the macroscopic lesion was one of blueing (permeability increase) without palpable edema. Although histo-

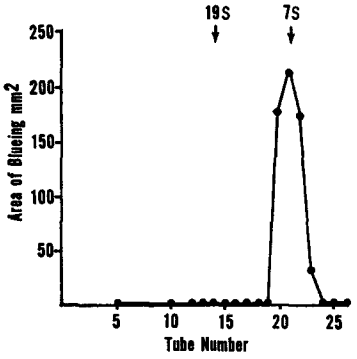

FIG. 2. Sucrose density ultracentrifugation of complement-dependent PCA antiserum. The arrows indicate the positions at which 19S and 7S markers were found.

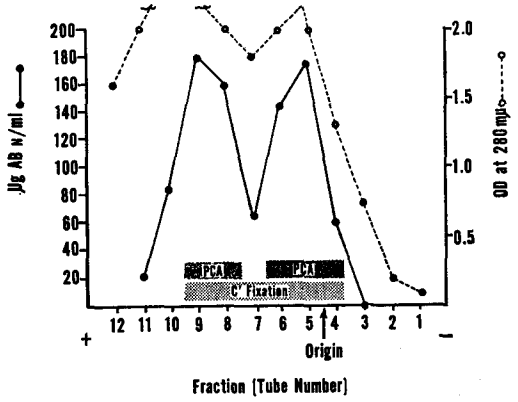

FIG. 3. Pevikon block electrophoresis of complement-dependent PCA antiserum.

logic sections did show mild neutrophil infiltration, the numbers were much smaller than with the shorter periods. Sections were examined for the presence of platelets, but only occasional clumps were observed within the blood vessels. It was not possible to determine whether platelet depletion had less inhibitory effect on permeability reaction produced after a short sensitization period, because the intradermal injections themselves produced hematomata

in animals which were already thrombocytopenic. Neutrophil accumulation was not affected by platelet depletion under these circumstances.

Treatment of rabbits with an antagonist of serotonin (methysergide maleate) did not inhibit either type of PCA reaction.

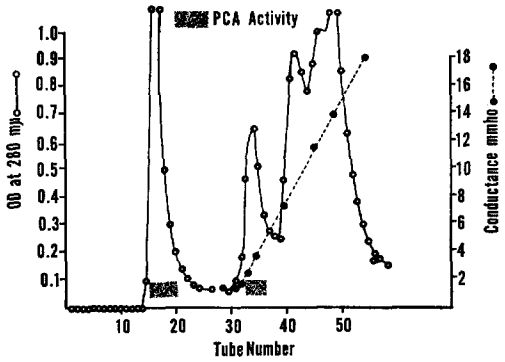

FIG. 4. Diethylaminoethyl (DEAE) cellulose chromatography of complement-dependent PCA antiserum.

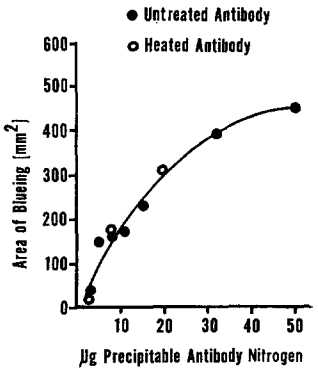

FIG. 5. Dose response curve of complement-dependent PCA antibody. The heated antibody was incubated at 56°C for 2 hr. The points represent mean values from at least four rabbits. The rabbits were challenged with antigen and dye, 48 hr after intradermal injection of antibody. The lesions were measured after 30 min.

Physicochemical Properties of the Complement-Dependent PCA Antibody.

Antisera containing C-dep PCA antibody were fractionated by electrophoresis, column chromatography, and sucrose density gradient ultracentrifugation. The PCA activity was found with the IgG (7S) peak upon density gradient centrifugation (Fig. 2). However, it appeared in both the fast and slow γ-regions from Pevikon block electrophoresis (Fig. 3) or DEAE cellulose chro-

matography (Fig. 4). The fractions containing PCA antibody fixed rabbit complement in vitro (greater than 5 CH 50 units/μg antibody N at equivalence). By the use of fluorescent antibody techniques, C3 fixation to antigen-antibody deposits in vivo was also observed. The PCA, complement-fixing, and precipitating activities of the C-dep antisera were found together at all times in the fractionation procedures employed.

Whole antisera and IgG fractions which had C-dep PCA activity were titrated for their ability to induce PCA reactions. The dose response curve obtained is shown in Fig. 5. As little as 2.5 μg antibody nitrogen injected intradermally was sufficient to produce a blueing reaction when antigen and dye were injected 48 hr later. Increasing quantities of antibody resulted in larger areas of permeability increase. The C-dep PCA antibody (100 μg) was found to persist in the rabbit skin for at least 8 days, although the area of blueing was reduced after this time interval. The quantity of antigen which, when injected intravenously, would produce maximal lesions was found to fall into the range of 1–3 mg BSA N/kg body weight. These quantities were also suitable for PCA lesions produced by homocytotropic antibody.

Homocytotropic antibody was heat labile. However, when C-dep PCA antibody was heated at 56°C for 2 hr it was still capable of producing PCA reactions, and had the same activity as the unheated preparations (Fig. 5).

DISCUSSION

Rabbit PCA reactions have been shown to result from two mechanisms involving two types of antibody. One of these is an antibody which apparently requires an action of complement, platelets and neutrophils for its PCA reactivity. The other type of reaction did not require these participants and was brought about by homocytotropic antibody.

Characteristics of the Complement-Dependent PCA Reaction.—With a sensitization period of 48–72 hr the lesions could be inhibited by antihistaminics, suggesting that the increase in vascular permeability was caused by a release of histamine. Since depletion of platelets was also inhibitory, platelets were the most likely source of histamine. The supposition that the permeability increase was due to release of histamine from platelets was supported by the finding, presented in the accompanying article (9), that the antibody preparations could also induce histamine release from platelets in vitro. The in vitro release, like the in vivo PCA reaction, was prevented if the available complement was inhibited by inactivation of the C3 with cobra venom factor. Rabbit platelets have been shown to adhere to antigen-antibody complexes which have fixed C3, and under suitable conditions are aggregated by them (9, 14). A possible reaction sequence may therefore be postulated. Antibody disseminated in the injection site would react with the circulating antigen and fix complement (both antigen and C3 were detected in the lesions). These complexes could

then entrap passing platelets by the process of immune adherence and thus initiate platelet histamine release (9).

Neutrophil depletion was also inhibitory to the PCA reactions. Histologically, all the lesions had at least a few neutrophils present even when only 15 min was allowed to elapse between antigen injection and skin biopsy. Although their role in the PCA reactions is unclear at present, it is known that rabbit neutrophils, as well as platelets, undergo an adherence reaction with complement (15). Furthermore, recent studies in this laboratory have shown that the presence of neutrophils can augment the histamine release from platelets in vitro.[1] In addition, it may be that the neutrophils play a part in allowing the platelets to come into better contact with the complexes.

With shorter sensitization periods, i.e., less than 24 hr, the process appears to be different, and more closely resembles the Arthus reaction. Thus antihistamine treatment only delays the onset of the increased permeability and platelet histamine release may therefore only potentiate the reaction. The lesions become palpably edematous and eventually hemorrhagic and exhibit a massive neutrophil infiltration. Antibody quantity also affects the type of reaction, more antibody resulting in more neutrophil accumulation and a more Arthus-like lesion. This is particularly true of the short sensitization periods where more antibody may be present and where the injection process itself results in a neutrophil accumulation over a period of 5 hr. The clearest differentiation between the two reactions (complement–dependent PCA or Arthus) was thus obtained with a sensitization period of 48–72 hr using less than 100 μg antibody N. The dependence of the PCA reaction on platelets and histamine, as well as the small amounts of antibody required, clearly distinguish the PCA reaction from the neutrophil-dominated Arthus reaction. Many immunological inflammatory reactions including the Arthus reaction may, however, have elements of both processes participating in the production of the observed lesions. On the other hand, the PCA reaction in rats described by Lovett and Movat (16) appears to have all the characteristics of the Arthus reaction and was unaffected by antihistamine or serotonin antagonists.

Increased vascular permeability involving histamine may be produced by a number of other mechanisms. One of these is by the action of anaphylatoxins, generated following activation of the complement system. These substances can cause histamine release from mast cells. Neutrophils, too, contain a protein which can release histamine from mast cells and which may play a part in lesions dominated by neutrophils. Neither the neutrophilic basic protein (reference 17 and footnote 2) nor anaphylatoxin (9), however, could be shown to release histamine from platelets in vitro. Since platelet depletion was inhibitory to the PCA reaction, these substances are unlikely to play a major role in the production of the lesions.

[1] Henson, P. M., and C. G. Cochrane. Manuscript in preparation.

[2] Janoff, A. Personal communication.

Comparison of C-dep PCA Antibody and Homocytotropic Antibody.—In contrast to the complement-dependent PCA antibody, homocytotropic antibody is thought to produce increased vascular permeability by virtue of its action on mast cells or basophils which are induced to release their histamine. In accordance, antihistamine was found to inhibit the PCA reactions caused by homocytotropic antibody. Recent evidence (references 18, 9, and footnote 3) has indicated that the platelet, along with one or more of the leukocytes, may also be implicated in the release of histamine from rabbit blood by homocytotropic antibody. In spite of this, the role of histamine release from platelets in the PCA reaction induced by homocytotropic antibody appears to be small,

TABLE III

Properties of Two Anaphylactic Antibodies in the Rabbit

Properties	C-dep PCA Ab	Homocytotropic antibody*
PCA reactions	Positive	Positive
Time course of production	Early and late	Early
Electrophoretic mobility	$\gamma 1$ and $\gamma 2$	$\gamma 1$
Sedimentation	7S	7S
Heat stability	Heat stable	Heat labile
Complement fixation	Positive	Negative
PCA reactions in rabbits:		
Depleted of C3	Inhibited	Positive
Depleted of platelets	Inhibited	Positive
Depleted of neutrophils	Inhibited	Positive
Treated with antihistamine	Inhibited	Inhibited
Presence on washed blood cells‡	Not detected	Present

* Zvaifler and Becker (3).

‡ Henson and Cochrane (9).

since only slight inhibition was produced by platelet depletion. Complement has not been considered as a requirement for this reaction and indeed, C3 depletion in the present studies was not found to be inhibitory.

The complement-dependent PCA antibody may be contrasted with the homocytotropic antibody in many of its characteristics (Table III). Many different injection schedules could be employed to stimulate the C-dep PCA antibody, which persisted for a long time and was found in hyperimmune sera. The antibody was detected in both slow and fast migrating regions of the γ-globulin, sedimented in the 7S region and was heat stable. Attempts to separate the precipitating and complement-fixing properties of the antibody from the PCA reactivity have not been successful. At present, therefore, the C-dep PCA activity appears to be a property of rabbit IgG antibody. Homocytotropic

[3] Barbaro, J. F., and E. L. Becker. Personal communication.

antibody, on the other hand, probably falls into a different immunoglobulin class, analogous to IgE in man.

The PCA reaction is a useful model for examination of mechanisms of induction of vascular permeability increase. The demonstration that there are at least two such mechanisms operative in the rabbit, one due to homocytotropic antibody and one due to C-dep antibody, has implications in the study of a number of experimental disease processes in this animal. This is particularly true of experimental serum sickness where a vascular permeability increase and a role of platelets have been implicated (2), and it may now be possible to determine experimentally which antibodies are responsible.

SUMMARY

Passive cutaneous anaphylaxis (PCA) reactions were produced in rabbits by antibodies to bovine serum albumin. Two types of antibodies were found, each inducing increased vascular permeability, but by means of different mediation systems.

One of these antibodies required the presence of complement, platelets, and neutrophils for the induction of the PCA reaction, which was inhibited by antihistamine. This antibody was heat stable, sedimented in the 7S region, and was found in both fast and slow electrophoretic fractions of rabbit γ-globulin.

Homocytotropic antibody was also detected. The PCA reactions induced by this type of antibody did not require platelets or neutrophils and were not inhibited in rabbits depleted of C3 with cobra venom factor. The lesions were, however, prevented by administration of antihistamine.

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