STUDIES ON THE LOCALIZATION OF CIRCULATING ANTIGEN-ANTIBODY COMPLEXES AND OTHER MACROMOLECULES IN VESSELS

I. Structural Studies*, ‡, §

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Plates 49 to 52

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In certain experimental diseases (1, 2) as well as in various human diseases such as rheumatoid arthritis (3, 4), soluble complexes of antibodies and antigens or complexes of gamma globulins have been detected in the circulation. In the experimental diseases, the observed lesions were apparently produced after these soluble complexes left the circulation and became deposited in glomeruli, arteries, endocardium, small pulmonary vessels, etc. While a similar sequence may occur in the human conditions, definite evidence for this assumption is lacking. In any case, in order for the circulating complexes or macromolecules to leave the circulation and become deposited in tissues, they must have encountered vascular structures, including endothelial cells and a basement membrane. Little is known about the passage of macromolecules, including antigen-antibody complexes, across vascular structures and, hence, experiments were devised to obtain information about the dynamic relationships of soluble complexes and other macromolecules and vascular membranes. Advantage was taken of short term experiments in which secondary factors probably played little role. In this way, studies could be made of not only the earliest anatomical location of deposited macromolecules in vessel walls, but also of the various factors that influence their localization.

The results will be presented in three parts. The first, reported here, will deal with the morphologic aspects of the vessels after localization of macromolecules and the relationship of this to certain known experimental vascular conditions. The second part, in the accompanying article, will be concerned

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with various methods of causing localization and their pharmacologic mediators, while the third part (5) will be concerned with properties of the macro molecules that influence the degree of localization.

Materials and Methods

Animals.—Male guinea pigs, weighing 350 ± 50 gm were used as test animals throughout. New Zealand white rabbits weighing 2.5 to 3.5 kg were used for the procurement of antisera.

Antigens.—5 times crystallized bovine serum albumin (BSA) was obtained from Armour Pharmaceutical Company, Kankakee, Illinois; 5 times crystallized ovalbumin (Ea) and rabbit gamma globulin (RGG) fraction II, were obtained from Pentex, Inc., Kankakee, Illinois, and human gamma globulin (HGG) fraction II, through the courtesy of E. R. Squibb and Sons, New York, and Dr. J. H. Pert of the American Red Cross.

Antibodies.—Antibodies against BSA and HGG were obtained from rabbits as reported previously (6). Antibodies to Ea were obtained from rabbits following multiple intravenous injections of alum-precipitated Ea (7). Antibodies to RGG were obtained from sheep following several monthly injections of RGG in incomplete Freund's adjuvant (7). All sera containing high concentrations of antibody were pooled. In the case of anti-BSA from which soluble antigen-antibody complexes were prepared, large pools were collected to obtain uniform results. The antibody concentrations of each antiserum were obtained using the quantitative precipitin test (7) with total reaction volumes of less than 1.0 ml. Antibodies to guinea pig complement (C′) were prepared by first precipitating heat-decomplemented rabbit anti-Ea with Ea at equivalence in the presence of fresh guinea pig serum (using acid-cleaned glassware at all times). The amount in weight of Ea–anti-Ea used was 5 times the amount of guinea pig C′ expected to precipitate with the complex (about 50 μg C′N per ml of serum). The precipitate was incubated at 37°C 1 hour, then maintained at 0°C for 24 hours with frequent stirring, and finally washed 5 times in veronal buffer containing 0.00015 M Ca^{++} and 0.0005 M Mg^{++}, brought into a fine suspension with 0.15 M NaCl, and incorporated into incomplete Freund's adjuvant (7). This is similar to the technique of McKee and Jeter (8). Rabbits were injected in the foot-pads and subcutaneously in multiple sites at weekly intervals for either 2 or 3 weeks using a total of about 2 mg C′ protein at each interval. The rabbits were bled 3 weeks after the last injections and the sera were individually tested in immunoelectrophoresis (9, 10) using fresh guinea pig serum as antigen. Only those containing a single strong band in the β-1 region were pooled. Frequently antibody against guinea pig gamma globulin was found. This was removed by absorption with purified guinea pig gamma globulin. Antibody against Ea was also present and was absorbed whenever necessary. The possibility existed that antibodies against rabbit globulin allotypes were also present, but a search for these was unrewarding.

Experiments were designed to find if the β-1 globulin in guinea pig serum, against which the antibody was directed, exhibited properties of one or more components of complement. These studies, performed with Dr. William D. Linscott, will be reported in full separately (11). In brief, however, it was found that the β-1 globulin, to which the antibody was directed, was identical with serum complement in its behavior under varying circumstances. This was found by treating the serum with agents that inactivated one or more components of complement as determined by quantitative hemolytic assay, and by (*a*) showing an alteration of the β-1 globulin in immunoelectrophoresis and (*b*) a loss in ability of the serum to bind to a preformed antigen-antibody precipitate as determined by testing with fluorescent anti-β-1 globulin. The β-1 globulin exhibits properties of the third component of complement, and,

pursuing this experimentally, it was found that absorption of the antibody with sheep cells sensitized with antibody and the first, fourth, second, and C'3c components of complement (EAC'1, 4, 2, 3c), made by exposing EAC'1, 4, 2 to purified C'3c, removed the precipitating antibody directed against the β-1 globulin. As control, absorption of the anti-β-1 globulin with EAC'1, 4, 2 had no effect on the precipitating antibody. Hence, the antibody has been designated as anti-guinea pig C'3c. It must be emphasized that non-precipitating or undetectably small amounts of precipitating antibody to other C' components may have existed as contaminants in the antiserum, and indeed in occasional immunoelectrophoretic slides, a single faint precipitin band in the α2 region was found. However, complete absorption of the antibody with a partially purified C'3b preparation that contained the α2 globulin antigen failed to diminish the fluorescent staining reaction of antigen–antibody–guinea pig complement preparations.

Purification of Antisera.—To identify contaminating antibodies in sera to be used in fluorescence studies, other than those noted in the section on antibodies to C', the antisera were subjected to analysis using double diffusion precipitation techniques in agar (12) and immunoelectrophoresis (9, 10). After identification of contaminating antibodies, their removal was accomplished by absorption with specific antigen.

Preparation of Soluble Antigen-Antibody Complexes.—BSA–anti-BSA soluble complexes were prepared by precipitating antibody from the antiserum at the point of equivalence, then washing the precipitate 3 times with 0.15 M NaCl at 0–4°C and dissolving the washed precipitate in an excess of BSA. The amount of BSA used to dissolve the precipitate was 20 times that needed for precipitation at equivalence. By washing the precipitates, the complexes subsequently formed, contained in greatest part, antigen and rabbit antibody. Removal of complement prior to preparing the complexes was found not to alter the results. The antibody of the complex could be visualized in experimental tissues by using fluorescent anti-RGG, since the only rabbit gamma globulin remaining after washing of the precipitates was that bound to antigen.

Fluorescent Antibody Technique.—The technique used was that of Coons and Kaplan (13) with minor modifications as mentioned in previous publications (6).

Controls in the fluorescent antibody studies included: (*a*) removal of specific antibody by absorption with the purified antigen in order to lose specific fluorescence; (*b*) use of unrelated fluorescent antisera; (*c*) use of fluorescent antisera on normal tissue, and tissue treated in a similar but unrelated manner to the experimental condition; and (*d*) blocking of the fluorescent antibody binding by prior application of non-fluorescent antibody. The intensity of fluorescence from such a section was always compared to that of a section treated with normal, non-fluorescent globulin followed by specific fluorescent antibody. Owing to the cross-reactivity between serum proteins of different mammalian species, the various fluorescent antisera were absorbed with pooled guinea pig serum prior to treatment of tissues.

Electron Microscopy.—Tissues taken for electron microscopy were fixed in OsO_4 and embedded in vestopal W. After cutting, the sections were stained in lead hydroxide, and observations were made with a model HU-11 Hitachi electron microscope.

General Method of Obtaining Localization of Circulating Antigen-Antibody Complexes.—Circulating complexes were made to localize in guinea pig vessels if the animals were subjected to systemic anaphylaxis. An outline of this method is seen in Table I. Guinea pigs were sensitized with either 150 μg anti-HGG N or anti-Ea N and after 24 hours, injected intravenously with preformed, purified soluble BSA–anti-BSA complexes. Generally, complexes containing 500 μg anti-BSA N dissolved in an excess of antigen amounting to 25 times that needed at equivalence, a dose which had no observable clinical effects, were used per animal. Within 1

TABLE I

Localization of Circulating Antigen-Antibody Complexes by Passive Systemic Anaphylaxis

Time	Intravenous injections
0	Sensitizing antibody, 150 μg N anti-HGG or 150 μg N anti-Ea
24 hrs.	Soluble BSA, anti-BSA complexes
24 hrs., 30 sec.	Challenging antigen, 1 mg N HGG or Ea

minute, a lethal (1.0 mg N) intravenous challenge with the antigen, HGG or Ea, was given and after anaphylactic death, organs were removed for analysis. As controls, to test the specificity of the localization, guinea pigs were injected with normal rabbit serum and antigen, or unrelated complexes before the anaphylactic challenge.

RESULTS

A. Localization of Circulating Antigen-Antibody Complexes and Their Detection.—

TABLE II

Localization of Circulating Antigen-Antibody Complexes by Classical Passive Systemic Anaphylactic Shock in Guinea Pigs

No. of guinea pigs	Sensitized antibody (N)*	BSA-anti-BSA soluble complexes (Ab N)	Challenging antigen (N)	Anaphylactic signs	Vascular localization of complexes
	μg	μg	mg		
10	150 anti-Ea	500	1.0 Ea	Death	Marked
10	150 anti-HGG	500	1.0 HGG	Death	Marked
6	—	500	—	Neg.	Neg.
12	150 anti-Ea	—	1.0 Ea	Death	Neg.

* See Table I for order, route, and times of injections.

Localization by classical systemic passive anaphylaxis: Guinea pigs were treated as outlined in the final paragraph of Materials and Methods and their tissues examined by fluorescent and routine histologic techniques. Hematoxylin and eosin sections revealed evidence of pulmonary alveolar distension and marked congestion of small vessels generally (Fig. 1). No abnormalities of vessel walls could be seen. However, by fluorescent microscopy, as noted in Table II, both the BSA antigen and rabbit antibody (see section on preparation of complexes in Materials and Methods) could be found as granular deposit lying along the intima of small blood vessels, generally venules, as shown in Figs. 2 and 3. Since in studying adjacent sections for antigen and antibody, an identical appearance was noted for each reactant in the same vessels, it was assumed that these represented complexes. Host complement was also found in abundant

amounts in the walls of vessels in which complexes had localized (Fig. 4), suggesting again that the reactants visualized in the vessel walls represented complexes and not free antigen and antibody. At no time were immunologic deposits and host complement found outside of the vessels in the interstitial spaces, nor were complexes found concentrated within the vascular lumina. Of interest, frequently polymorphonuclear leukocytes and occasionally mononuclear cells were noted to contain granules of antigen and antibody even though their exposure to the complexes was for as short a time as 2 minutes (Fig. 5). These leukocytes were most apparent in the vessels of the lungs. Animals injected with complexes alone failed to show localization, as noted in Table II. Tests for the presence of the challenging antigen (Ea or HGG) failed to demonstrate its localization even though injected in high dose.

Organs involved: Studies were carried out to determine the extent of localization in different organs. In the lungs it was noted that periarterial and peribronchial venules were affected in all cases. In very few instances, however, were veins or venules in the alveolar parenchyma involved. Otherwise, in the atrial and valvular supporting regions of the heart and in the connective tissue structures of the stomach, bowel, liver, skin, abdominal wall, and renal pelvic connective tissue, venules with localized complexes could be found. Of note, the glomerular capillaries in the kidney were unaffected.

B. The Ultrastructural Localization of Macromolecules in Vessels.—The exact position of the localized complexes in the vessel walls could not be determined by fluorescence microscopy, although using 3 to 4 micron sections, it appeared that the complexes frequently deposited, not along the luminal surface, but rather beneath endothelial cell nuclei. In an attempt to find more precisely the exact position of deposition in relation to the cellular elements of the vessel wall, electron microscopy was employed. Such information was essential to an understanding of the mechanisms responsible for the localization. However, since plasma proteins could not readily be identified with this technique, electron-dense purified carbon was substituted for the complexes. The carbon particles measured between 350 and 530 A in diameter, roughly in the range of width of small antigen-antibody complexes. Thus, 5 guinea pigs, previously sensitized with 150 μg N anti-Ea, were injected with 35 mg purified carbon in 0.15 M NaCl and within 30 seconds with 500 μg N Ea. Following anaphylactic shock, cardiac and peribronchial tissues were removed for ultrastructural and light microscopic studies. By light microscopy, the carbon could be found localized in vessel walls in great abundance (Fig. 6), comparable in appearance to the antigen-antibody complexes. As was true of the complexes, no localization of carbon occurred in the absence of anaphylaxis. Although the amount was generally less, a similar picture was obtained when 0.5 mg carbon was used. However, by electron microscopy, several features became clear as demonstrated in Figs. 7 to 9: (*a*) There was a great concentration of carbon in the

vessel wall, very little in the lumen, and none in the interstitial spaces; (*b*) The carbon apparently penetrated gaps between the endothelial cells where it could still be found and passed beneath the endothelial cells as shown in the figures, coming to lie between endothelial cell and pericyte. The furthermost point of penetration through the wall appeared to be an external limiting membrane, without doubt the basement membrane. This membrane was frequently indistinct and it was difficult to tell whether it encircled the pericytes, thus lying in a position between pericyte and endothelial cell and forming at all times a limiting membrane to the passage of carbon particles. It clearly passed along the outside border of pericytes however; (*c*) The endothelial cells, despite the multitudes of surface invaginations and vacuoles, did not contain carbon particles; and (*d*) Although small amounts of carbon could be found in the vascular lumina, there was little or none adhering to the luminal surface of the endothelial cells.

C. Relationship of the Localization of Complexes to Vascular Disease.—It was of interest to determine whether an inflammatory reaction would develop in the vessel as a result of the localization of soluble complexes and complement. In particular, it was of interest to find if vascular necrosis of the Arthus type would follow the localization since antigen, antibody, and complement were present in the vessel wall. Accordingly, guinea pigs were injected with soluble complexes intravenously and subjected to severe shock. Instead of employing anaphylactic shock with antibody and antigen, histamine was administered intravenously to provoke shock since it was found (see the accompanying paper) that soluble complexes as well as carbon localized identically during histamine or antibody-antigen shock. After 20 to 25 seconds, when profound shock had occurred, antihistamine was injected intravenously and shock terminated. The guinea pigs demonstrated an abundance of complexes and complement in their vessel walls when sacrificed immediately. The vessels otherwise were morphologically normal by light microscopy (see Fig. 1). After 5 hours, guinea pigs were sacrificed and tissues examined for lesions and the presence of soluble complexes. The details of the experiments and the results are noted in Table III. It was found that while complexes were still visible in vessel walls, although to a lesser degree than immediately after injection, there were only rare polymorphs in the lumina of vessels and no vascular necrosis was observed as would be seen in Arthus reactions. In particular, polymorphs were never found aggregated in the vessel walls.

In order to find if similarly treated guinea pigs were capable of supporting an Arthus reaction, and to compare accurately the Arthus vessels and those in which circulating complexes localized, guinea pigs were given cutaneous reversed passive Arthus reactions at a time when the localization of circulating complexes was provoked by anaphylaxis. As noted in Table III, no inflammation was observed in the pulmonary or cardiac vessels where circulating complexes and complement had localized. However, the vessels in the reversed

passive Arthus site had undergone severe inflammatory changes with the influx of polymorphs and necrosis of the vessel walls and with hemorrhage and edema extending into the surrounding dermis (Fig. 10). Fluorescent antibody observation of the early Arthus sites revealed antigen and rabbit gamma globulin deposited in and around the vessel walls, as noted in Fig. 11. These reactants also were frequently found in the vascular lumina, as may be seen in Fig. 12. While the apparent amount localized in the vessels was large when 0.10 mg Ab N was injected, when only 0.02 mg Ab N was used to produce the

TABLE III

The Short Term Effect of Antigen-Antibody Complex Deposits on Vessels after (a) Localization from the Circulation and (b) Localization in Arthus Reactions

No. of guinea pigs	Soluble complexes (Ab N intravenously)	Passive Arthus reaction (Ab N intradermally)‡	Vascular reactions (5 hours after injection)	
			Cardiac and pulmonary vessels	Arthus vessels§
	mg	*mg*		
10	0.50	—	Neg.	—
10	0.50	0.02	Neg.	Pos.
		0.10	Neg.	Pos.
5	1.00	0.10	Neg.	Pos.

* Soluble complexes injected intravenously 30 seconds before 100 μg histamine (base)· After profound shock attained, 20 μg pyrilamine injected intravenously and shock was terminated.

‡ Reversed passive Arthus reaction performed at the time of injection of soluble complexes using 1.0 mg HGG N intravenously followed by antibody intradermally.

§ Each reaction developed to an intensity equal to that in animals not treated with soluble complexes.

reaction, the amount deposited appeared to be less than that in the vessels where circulating complexes had localized. Using fluorescein-labeled antibody against guinea pig complement, it was possible in each case to demonstrate concentrations of host complement, or more specifically C'3c, with the immune reactants in the vessel walls and lumina of the vessels of the Arthus reaction, as seen in Fig. 13. Hence, in the passive Arthus reaction, antigen, antibody, and C'3c were found in the vessel walls, as they had been in the vessels where circulating complexes had localized. However, in vessels of the Arthus site, the immune reactants were found extending out into the lumen and in the confines of the wall itself and were present generally in greater amounts.

DISCUSSION

These results demonstrated that circulating antigen-antibody complexes and host complement were localized in the walls of small vessels of guinea pigs

subjected to anaphylactic shock. The vessels involved were in general venules and they could be found in every organ studied.

Information regarding the mechanism involved in this localization was obtained from ultrastructural studies with the electron microscope in which electron-opaque macromolecules of purified carbon were substituted for soluble complexes. It was found that these particles penetrated the vessel walls only to the point of the basement membrane where their further passage was apparently restricted. These results suggested that during anaphylaxis in which there was a release of histamine (as noted in the accompanying article), the vessels underwent an increase in permeability. Small molecules, such as simple serum proteins, were allowed to pass through the vessel walls, while macromolecules or particles such as carbon became entrapped along what is seemingly a filtering semipermeable membrane.

These observations are similar to those in rats of Majno and Palade (14) and the data of earlier work in mice by Alksne (15), in which localization of colloidal HgS from the circulation was induced locally by intramuscular injections of 5-hydroxytryptamine or histamine. In these studies, the basement membrane was found to block passage of the HgS. In addition, these observations, in agreement with those of other workers (14), indicate that the localization of these macromolecules from the circulation may not be explained by either rapid phagocytosis by endothelial cells or adherence to a sticky luminal surface of endothelial cells, since neither of these was found to any great extent. Peterson and Good (16) reported a similar lack of immediate uptake of thorotrast by the endothelial cells of venules in passive cutaneous anaphylaxis sites, but mention was made of the colloidal material found along the luminal surface of the endothelial cells.

While occasional small collections of carbon particles were found along the luminal surface of endothelial cells in the present studies, these were uncommon and were most often found associated with gaps between endothelial cells as if being drawn into the intercellular space. They represented but a small fraction of the total amount of carbon deposited. Hence, the term "granulopexis" of small vessels and their endothelial cells, is probably best explained by the deposition of macromolecules along the basement membrane, rather than by rapid phagocytosis by activated endothelial cells.

If one may assume that macromolecules such as soluble complexes, localize similarly to carbon, and that in order for such macromolecules to gain entrance to the tissues they must encounter a basement membrane whether it be along arteries, endocardium, veins, or glomerular capillaries, this position between endothelial cell and basement membrane may well be considered to be the earliest target of immunologic reactants localizing from the circulation. This would constitute the earliest focus from which inflammatory changes would develop. Indeed this deposition of material beneath endothelial cells along the basement membrane has been seen in acute glomerulitis in both serum sickness

(17) and acute human gomerulonephritis (18), and in the endocardial, arterial, and valvular lesions in acute experimental serum sickness (19). Currently, studies are being carried out to determine the changes that immediately follow this primary localization.

In studies of the short term pathologic effect of the deposited complexes, it was somewhat surprising to find that polymorphonuclear leukocytes were not attracted and vascular damage did not ensue. In comparing these vessels with those in the Arthus reactions on the same animals, many similarities were found to exist between the two situations: antigen-antibody complexes deposit in the vessel walls in both, host complement accumulated with the complexes in both, and the vessels involved were anatomically similar and could even be found in the same tissue, the skin. However, in the case of the Arthus reaction, after deposition of the immunologic reactants in the vessels, polymorphs accumulated in great numbers and necrosis of the walls ensued leading to massive edema and hemorrhage, as noted previously (20). This did not occur in the venules in which circulating complexes localized. This failure could not be explained by the absence of some essential host factor, since cutaneous Arthus reactions could be elicited in the same animals simultaneously. This probably could also not be explained on the basis of preformed complexes not being phlogogenic, since preformed complexes have been shown to attract polymorphs when injected directly into the skin of guinea pigs and rabbits and cause local reactions as severe as when antigen and antibody are injected into the skin separately (20). Of the various possible explanations of this finding, the one most attractive would appear to be that the position of the deposited complexes and probably the amount deposited over the 5 hour period did not favor the attraction of polymorphonuclear leukocytes and development of a necrotizing reaction. Probably as a result of a smaller quantity localized when circulating complexes became deposited in the vessels, the complexes at no time crowded all the available space in the vessel walls nor became concentrated in the lumen of an affected vessel. Instead, judging from the results of the studies with the electron microscope in which carbon was substituted for complexes, the complexes were probably sequestered beneath endothelial cells. This hidden position might well have protected the complexes from circulating polymorphs or have prevented strong chemotaxis of polymorphs which therefore would not have concentrated in the area. On the other hand, in cutaneous vessels of an Arthus site, deposits of antigen, antibody, and complement were found not only in the vessel walls, but also extending out into the lumen in accordance with earlier findings (20–22). Such a location was undoubtedly far more favorable for a chemotactic influence on polymorphs. The localization pattern reported here may be like that in the glomerular or endocardial lesions of acute serum sickness where depositions of complexes appear to form beneath endothelial cells (17) and in general do not attract enough polymorphs to produce severely

necrotic reactions. Indeed, the present observations might suggest that the relative absence of necrosis in the glomerular and endocardial lesions in serum sickness, or perhaps in acute glomerulonephritis, where deposits have also been observed beneath endothelial cells (18), may be attributed to the smaller amount of complexes sequestered beneath endothelial cells that would therefore not attract polymorphs. In the glomerulus, however, an additional factor may be the flow of filtering fluid over the capillary loops across the basement membrane which would tend to flush out any chemotactic agents released by the complexes along the basement membrane. While the afore mentioned possibility may be true of the endocardial and glomerular lesions, for reasons not yet apparent it is probably not true of the arterial lesions where relatively large deposits of complexes occur and where polymorphs and necrosis of tissue are commonly seen. Hence, in considering the avenues by which these two types of lesions are mediated, *i.e.* on the one hand the vasculonecrotic reactions in which polymorphs play an essential role in mediating injury (*e.g.* the Arthus vasculitis), and on the other, the lesions of increased permeability and endothelial cell proliferation, the mediators of which are not known (*e.g.* the glomerulitis of acute serum sickness), it may well be that the amount and position of the localized antigen-antibody complexes govern which avenue of mediators is followed, and, therefore, what type of disease pattern develops.

These studies appear to be in variance with other reported studies (23) in which hemorrhagic lesions developed in sites of passive cutaneous anaphylaxis (PCA) following either an intravenous injection of complexes or the production of a passive Arthus reaction in a different location. In attempting to explain these results, it would seem probable that the hemorrhagic lesions were produced by something other than localizing complexes. The quantities of antibody in the complexes were often far less (6 μg Ab N) than the minimum required for a hemorrhagic Arthus reaction, and probably a considerable proportion were removed by the reticuloendothelial system and did not reach the PCA site. In addition, using the fluorescent antibody technique, which easily detects localized antigen in the most minimal Arthus reactions, complexes could not be found localized in the PCA site in our hands over a wide dosage range of complexes. Hence, it would seem that the hemorrhagic lesions were produced by a mechanism other than a localization of complexes in the PCA site followed by the development of an Arthus vascular reaction.

The possible role of complement as a mediator of the final injury in the Arthus vasculitis was supported by the finding of abundant amounts of host complement (C'3c) in the affected vessel walls of Arthus reactions. One point of mediation has been suggested by the recent *in vitro* observations of Boyden (24), in which a complement-like substance was required in addition to antigen-antibody complexes to produce a positive chemotactic action on polymorphs. If

these observations may be applied to the Arthus phenomenon, then the complement may serve, along with the antigen and antibody, to attract polymorphs to the vessel walls and thus bring about the necrotic reaction. Recent experiments, using complement depleted animals, have suggested that despite the deposition of antigen and antibody in the vessel walls, polymorphs did not enter the site and Arthus reactions did not develop (25).

SUMMARY AND CONCLUSIONS

A short term model in which circulating antigen-antibody complexes and host complement localized in vessel walls of guinea pigs was analyzed. Localization was accomplished by subjecting the animals to anaphylactic shock.

The circulating macromolecules, such as antigen-antibody complexes, appeared to localize by being trapped in the vessel wall along the basement membrane that acted as a filter during a state of increased permeability of the vessel.

It was suggested that this point of localization between the endothelial cell and the basement membrane may well represent the earliest focus of inflammation in diseases caused by the deposition of injurious macromolecules such as soluble antigen-antibody complexes from the blood stream.

Complexes localized in the vessel walls did not provoke Arthus-type vasculonecrotic reactions even though in both these vessels and in cutaneous Arthus reactions antibody, antigen, and host complement (C'3c) were deposited in the vessel walls. The possibility was presented that since circulating macromolecules and probably complexes deposited in (*a*) relatively small amounts, and (*b*) in a position beneath endothelial cells, they were not strongly chemotactic toward circulating polymorphonuclear leukocytes. Vasculonecrotic reactions, therefore, were not observed. It was brought out that this may be similar to the situation in glomerulonephritis induced by localized immune complexes, in which severe necrosis is not observed.

In the Arthus vascular reaction, host complement was found microscopically accumulated with the immune reactants in affected vessel walls.

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BIBLIOGRAPHY

1. Dixon, F. J., Vazquez, J. J., Weigle, W. O., and Cochrane, C. G., Pathogenesis of serum sickness, *Arch. Path.*, 1958, **65**, 18.
2. Dixon, F. J., Feldman, J. D., and Vazquez, J. J., Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of glomerulonephritis, *J. Exp. Med.*, 1961, **113**, 899.
3. Kunkel, H. G., Müller-Eberhard, H. J., Fudenberg, H. H., and Tomasi, T. B.,

Gamma globulin complexes in rheumatoid arthritis and certain other conditions, *J. Clin. Inv.*, 1961, **40,** 117.

4. Tomasi, T. B., Fudenberg, H. H., and Finley, N., Possible relationship of rheumatoid factors and pulmonary disease, *Am. J. Med.*, 1962, **33,** 243.
5. Cochrane, C. G., Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels. Factors influencing localization, to be published.
6. Cochrane, C. G., Weigle, W. O., and Dixon, F. J., The role of polymorphonuclear leukocytes in the initiation and cessation of the Arthus vasculitis, *J. Exp. Med.*, 1959, **110,** 481.
7. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 2nd edition, 1961.
8. McKee, A. P., and Jeter, W. S., The demonstration of an antibody against complement, *J. Immunol.*, 1955, **76,** 112.
9. Williams, C. A., Jr., and Grabar, P., Immunoelectrophoretic studies on serum proteins. 1. The antigens of human serum, J. Immunol., 1955, **74,** 158.
10. Scheidegger, J. J., Une micro-methode de l'immunoelectrophorese, *Internat. Arch. Allergy*, 1955, **7,** 103.
11. Cochrane, C. G., and Linscott, W. D., Studies of antisera directed against guinea pig β-1 globulin. Relationship to complement, to be published.
12. Oudin, J., Specific precipitation in gels and its application to immunochemical analysis, *Methods Med. Research*, 1952, **5,** 335.
13. Coons, A. H., and Kaplan, M. H., Localization of antigen in tissue cells. II. Improvements in method for the detection of antigen by means of fluorescent antibody, *J. Exp. Med.*, 1950, **91,** 1.
14. Manjo, G., and Palade, G. E., Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability; and electron microscopic study, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 571.
15. Alksne, J. F., The passage of colloidal particles across the dermal capillary wall under the influence of histamine, *Quart. J. Exp. Physiol.*, 1959, **44,** 51.
16. Peterson, R. D. A., and Good, R. A., Morphology of vascular permeability; an electron microscopic study, *Lab. Inv.*, 1962, **11,** 507.
17. Feldman, J. D., Electron microscopy of serum sickness nephritis, *J. Exp. Med.*, 1958, **108,** 957.
18. Farquhar, M. G., Vernier, R. L., and Good, R. A., An electron microscope study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus, *J. Exp. Med.* 1957, **106,** 649.
19. Kniker, W. T., and Cochrane, C. G., unpublished data.
20. Cochrane, C. G., and Weigle, W. O., The cutaneous reaction to soluble antigen-antibody complexes. A comparison with the Arthus phenomenon, *J. Exp. Med.*, 1958, **108,** 591.
21. Feldman, J. D., unpublished data.
22. Movat, H. Z., and Fernando, N. V. P., Allergic inflammation. I. The earliest fine structural changes at the blood-tissue barrier during antigen-antibody interaction, *Am. J. Path.*, 1963, **42,** 41.

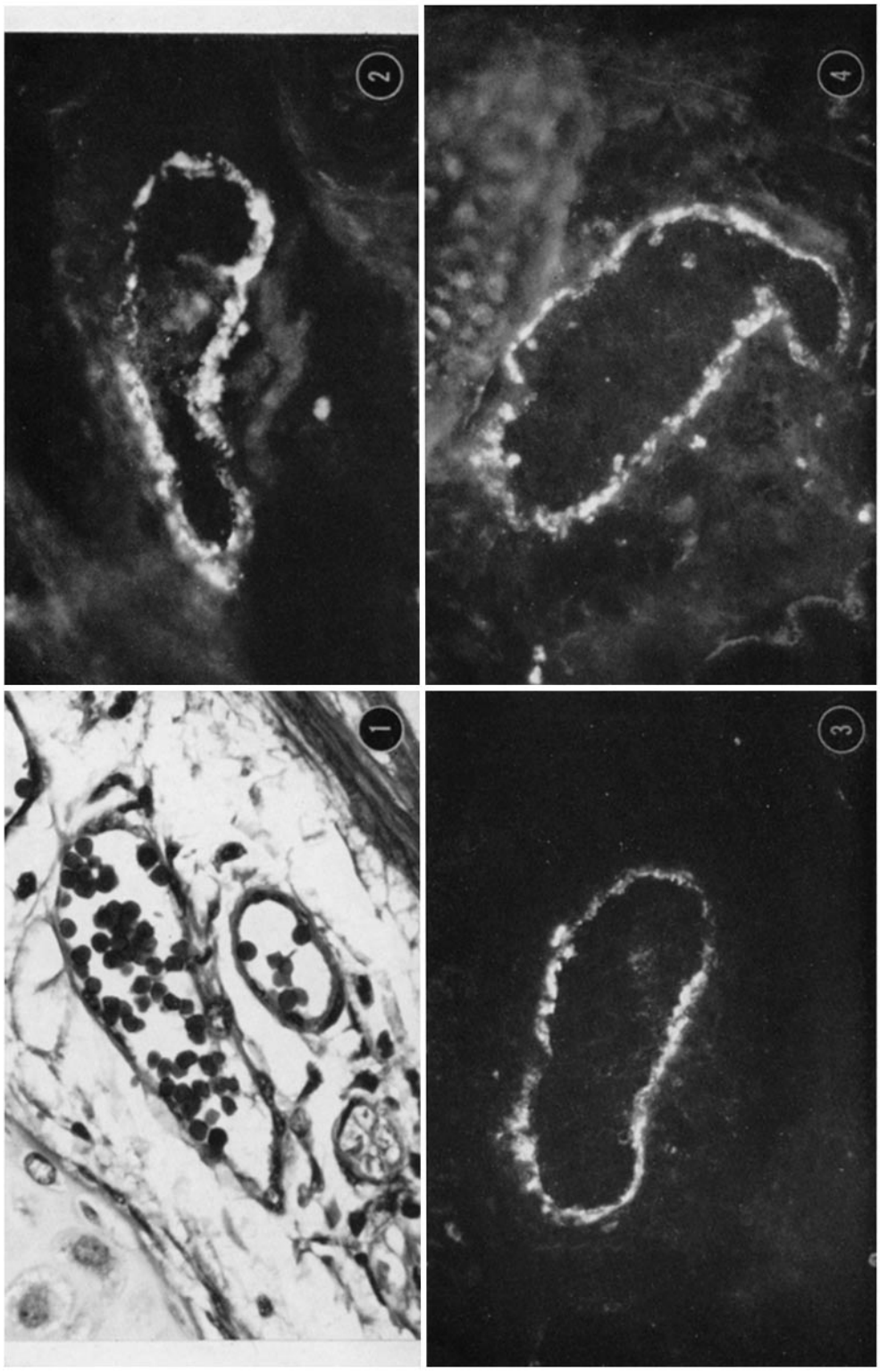
23. Siqueira, M., and Bier, O. G., Hemorrhagic reactions at sites of passive cutaneous anaphylaxis in guinea pigs after intravenous inoculation of unrelated immune complex, *Proc. Soc. Exp. Biol. and Med.*, 1961, **107**, 779.
24. Boyden, S., The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes, *J. Exp. Med.*, 1962, **115**, 453.
25. Cochrane, C. G., unpublished data.

EXPLANATION OF PLATES

PLATE 49

FIG. 1. Pulmonary vessels adjacent to a bronchus taken from a guinea pig in which circulating antigen-antibody complexes were made to localize in vessel walls during systemic anaphylaxis. Although the vessels are dilated and enlarged, little damage to the wall may be visualized. Tissue was removed several minutes after localization occurred. Hematoxylin and eosin stain. × 350.

FIGS. 2 to 4. Vessels similar to those seen in Fig. 1, but treated with fluorescent antibodies as follows: Fig. 2, fluorescent anti-BSA; Fig. 3, fluorescent anti-RGG (antibody globulin); Fig. 4, fluorescent anti–guinea pig complement. Note the similarity in appearance of all three materials, antigen, antibody, and host complement. With such a similar appearance and since complement is bound, the positive fluorescence most probably represents localized complexes. × 250.

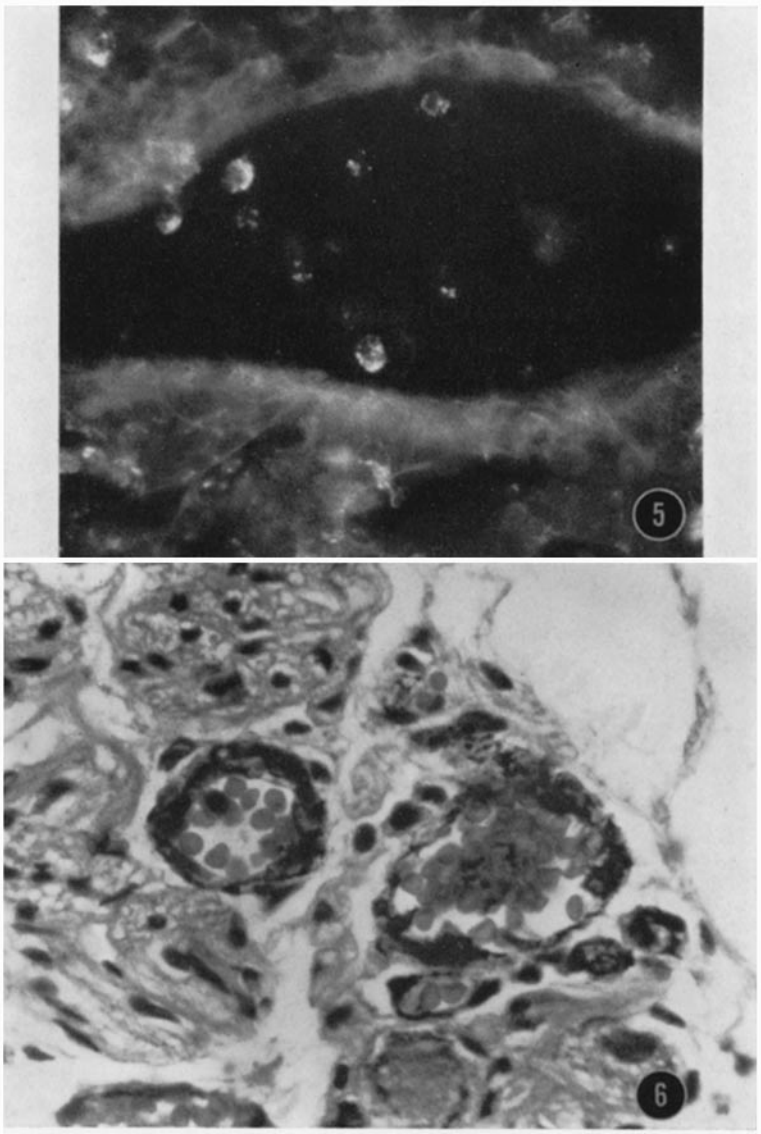


(Cochrane: Circulating antigen-antibody complexes. I)

PLATE 50

FIG. 5. A pulmonary vessel taken from a guinea pig 2 minutes after the anaphylactic deposition of circulating complexes. Note the complexes with the cytoplasm of approximately seven polymorphs within the vascular lumen. Fluorescent anti-RGG (antibody globulin). × 400.

FIG. 6. Other vessels similar to those seen in the previous figures but in which carbon was substituted for antigen-antibody complexes. Note the extensive deposition of carbon in a position similar to that of the complexes. While it would appear that the carbon is localized in all parts of the vessel wall in this 5 μ thick tissue section, it actually is deposited in greatest part between endothelial cells and basement membrane as noted in the following figures. Hematoxylin and eosin. × 400.



(Cochrane: Circulating antigen-antibody complexes. I)

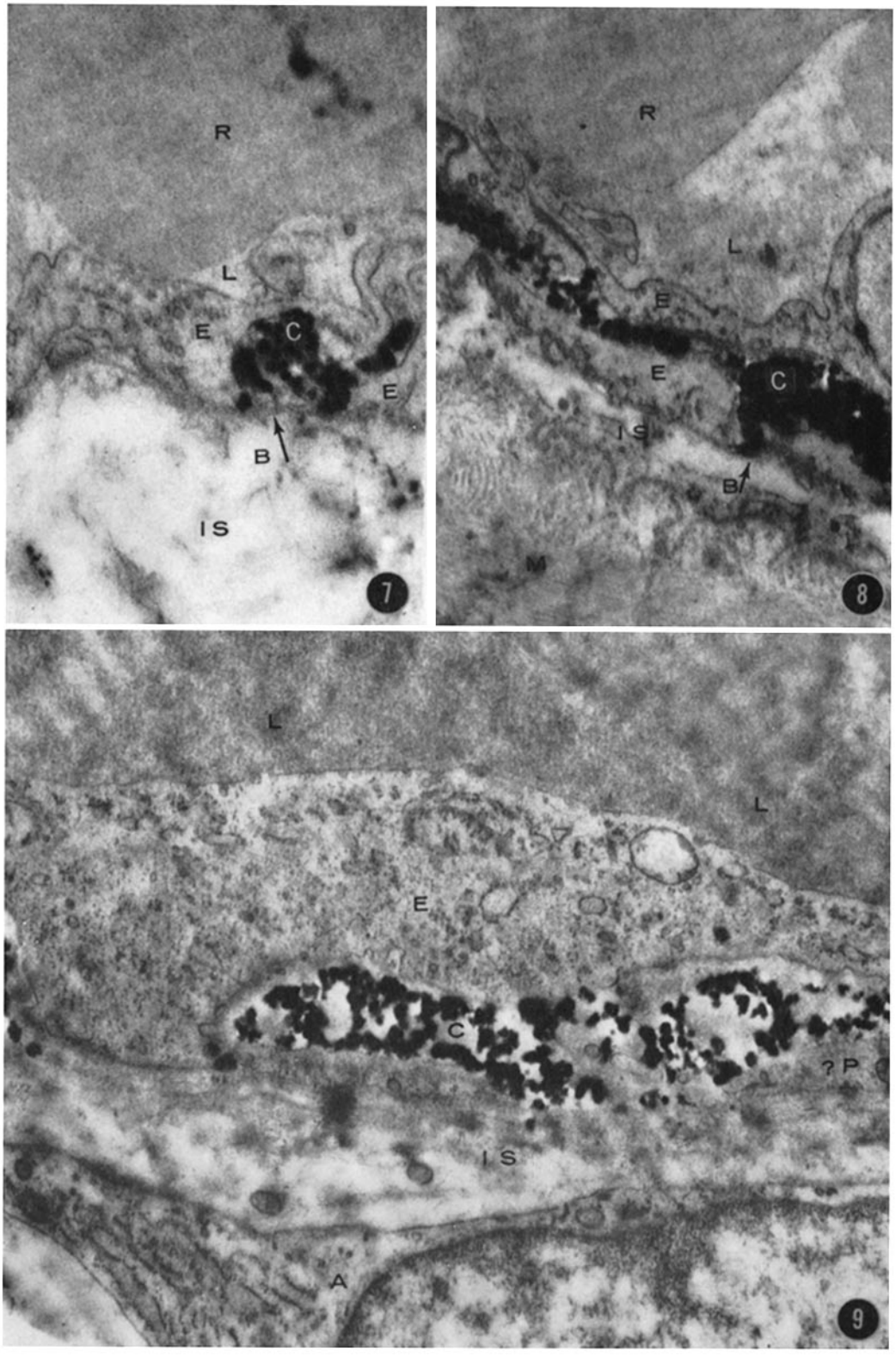
PLATE 51

FIGS. 7 to 9. All vessels shown obtained from guinea pigs 3 to 5 minutes after an intravenous injection of 35 mg purified carbon and systemic anaphylactic challenge. In each case, the flow of plasma and carbon would be from the top of the photo (lumen) toward the bottom (interstitial space). Key to abbreviations: *A*, adventitial cell; *B*, basement membrane; *C*, carbon; *E*, endothelial cells; *IS*, interstitial space; *L*, lumen; *M*, cardiac muscle fiber; *P*, pericyte; *R*, red blood cell.

FIG. 7. Electron photomicrograph of a venule in the lung similar to those seen in Fig. 6. Carbon (*C*) is found deposited outside the lumen (*L*), lying in a position beneath and between endothelial cells (*E*). The apparent external limiting membrane that prevents outflow of the carbon particles into the interstitial space (*IS*) is the basement membrane (*B*) (arrow). Note that carbon particles are not found along the luminal portion of the endothelial cell or within the endothelial cell vacuoles. The few dark objects in the interstitial space (*IS*) were found not to be carbon. × 28,100.

FIG. 8. A vessel in the heart, similar to that in Figs. 6 and 7. Again, the carbon is found deposited up against the basement membrane (*B*) (arrow), beneath and between the endothelial cells (*E*). Note the absence of carbon along the luminal surface and inside endothelial cells. × 19,000.

FIG. 9. Another vessel in the lung, similar to that in Figs. 6 to 8, showing a large deposit of carbon beneath the endothelial cell which separates the carbon from the lumen. Note the amorphous clumps of material lying with the carbon in the subendothelial space. This was infrequently found in these vessels, but appeared to be similar to what Majno and Palade termed chylomicra (14). × 20,000.



(Cochrane: Circulating antigen-antibody complexes. I)

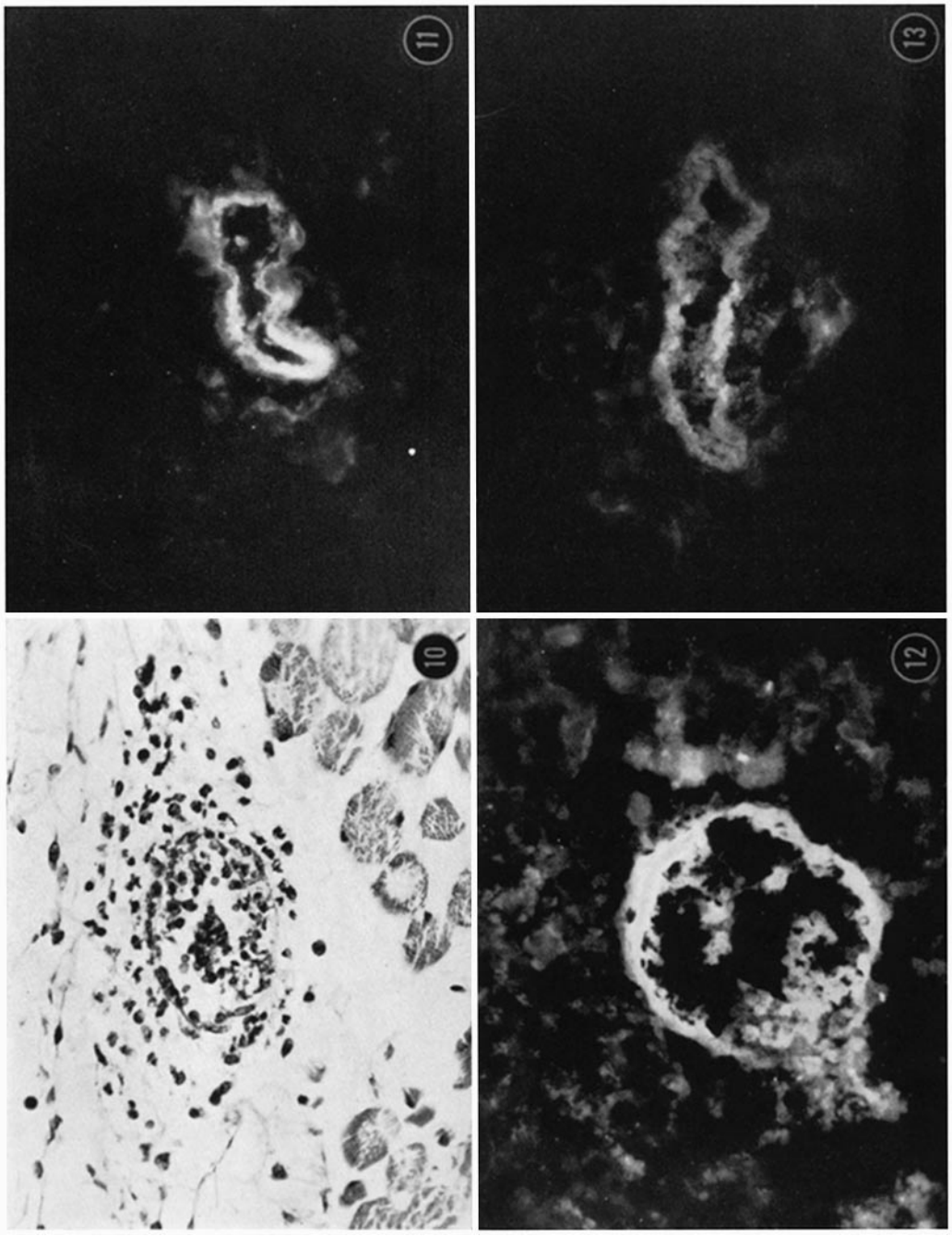
PLATE 52

FIG. 10. Vessel taken from a reversed passive Arthus site of a guinea pig 5 hours after injection. 20 μg anti-HGG N injected intradermally and 1.0 mg HGG intravenously to provoke the reaction. This animal had been subjected to anaphylactic deposition of circulating complexes at the time the Arthus reaction was begun. Note typical inflammation and necrosis marked by the influx and agglomeration of polymorphs. Hematoxylin and eosin. × 200.

FIG. 11. A similar vessel to that seen in Fig. 10, but treated with fluorescent anti-HGG to show the presence of the antigen in the vessel wall. Similar fluorescence was noted when fluorescent and RGG was used, which indicated in greatest part the location of antibody. × 250.

FIG. 12. Similar to Fig. 11, but showing deposition of antigen within the lumen of the vessel, extending out from the walls. × 250.

FIG. 13. Another vessel similar to those in Figs. 11 and 12, but treated with fluorescent anti-guinea pig (host) complement (C'3c). Hence, antigen, gamma globulin of the antibody donor species, and host complement were localized.



(Cochrane: Circulating antigen-antibody complexes. I)