THE CUTANEOUS REACTION TO SOLUBLE ANTIGEN-ANTIBODY COMPLEXES

A Comparison with the Arthus Phenomenon*, ‡, §

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In previous work from this laboratory (1) it was shown that a considerable portion of an intravenously injected antigen, bovine serum albumin (BSA), circulated in a globulin-bound form during the immune phase in rabbits. This globulin-bound BSA in all likelihood represented circulating antigen-antibody complexes. In further studies (2, 3), it was shown that the lesions found in rabbits with experimental serum sickness developed during the time that the antigen-antibody complexes were circulating. Further, it was postulated by Germuth (4) and by us (2, 3), that these soluble complexes were responsible, at least in part, for the lesions of serum sickness. However, whether soluble complexes could bring about pathologic changes in tissues was not known.

An investigation was therefore undertaken to test the effect of soluble antigen-antibody complexes on tissues *in vivo*. This was performed by injecting rabbits intracutaneously at various dosage levels. The complexes were prepared in a relatively pure form by precipitating the antibody with antigen at equivalence, washing the precipitate, and finally dissolving the precipitate in antigen excess. This was done to exclude factors in serum other than antibody which might influence the reactions. Amounts, in terms of antibody, were used to equal those required to produce minimal to maximal local passive Arthus reactions (5). The lesions produced by the soluble complexes were compared quantitatively and qualitatively with (*a*) the local passive Arthus, (*b*) the reversed passive Arthus, and (*c*) the active or classical Arthus reactions.

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The *in vivo* effect of soluble antigen-antibody complexes has been studied by others. Opie (6) in 1924 reported that a combination of horse serum and anti-horse serum when injected intradermally into rabbits caused local inflammation. Germuth and McKinnon (7) studied the effect of soluble antigen-antibody complexes prepared in antigen excess following intravenous injection into guinea pigs. They found varying degrees of anaphylactic shock in 133 of 162 animals. Trapani, Garvey, and Campbell (8), and Ishizaka and Campbell (9), have recently reported that the activity of soluble complexes is greatest when prepared in antigen excess. In three sera used (9), it was shown that when complexes were made from a slow gamma component of the antisera, activity occurred in antigen excess only. Their evidence is based both on experiments utilizing the contraction of guinea pig ileum with a Schultz-Dale technique and also on experiments resulting in increased capillary permeability in guinea pig skin following intradermal injection of the soluble complexes. Tokuda and Weiser (10) have recently reported that antigen-antibody complexes prepared in antigen excess produced anaphylaxis in mice.

In 1924, Opie (6) by relating intensity of the cutaneous reaction with the precipitin titer demonstrated the antigen-antibody nature of the Arthus phenomenon. This was confirmed by Culbertson (11) and Cannon and Marshall (12). Antigen and antibody have been used in various combinations and injected *via* different routes to produce antigen-antibody reactions in the skin (5, 6, 11–15). However, the actual site of combination of antigen and antibody in the cutaneous tissue is unknown, and a part of this study was therefore undertaken to find the *in vivo* localization of these various reactants using the fluorescent antibody technique of Coons and Kaplan (16).

Materials and Methods

Preparation of Soluble Antigen-Antibody Complexes.—Antiserum to crystallized bovine serum albumin (BSA[1]) was obtained from repeated courses of injections of saline solutions of BSA in rabbits. The anti-BSA sera were pooled and analyzed for antibody nitrogen by the quantitative precipitin method (17). Various pools used contained 0.50, 1.38, and 1.48 mg. antibody nitrogen (Ab N) per ml. All sera were kept frozen at −20°C. until used. To prepare the soluble complexes, an amount of BSA antigen was added to antisera to reach the point of equivalence. The combination was then incubated at 37°C. for one-half hour and then refrigerated at 0 to 3°C. overnight. The precipitate thus formed was washed three times with 0.15 M saline at 0 to 1°C. An additional amount of BSA was then added so that the total amount of BSA in the system equalled three to four times that needed for precipitation at equivalence. After incubation at 37°C. for an hour, the precipitate dissolved in the existing antigen excess. Occasionally a longer period of time was required to dissolve the precipitate, the complexes then being stored in the cold until a solution was achieved. The soluble complexes thus formed were used for injection.

Method of Injection and Grading of Reactions.—Male albino rabbits and guinea pigs were used in each experiment. Injections were made on the flanks in all cases 24 hours after removing the hair with electric hair clippers. No differences in reaction intensity were detected when the site of injection was varied. Volume of injected materials was less than 0.3 ml. unless otherwise specified. The intensity of maximal reaction was graded as follows:—

±, mild edema and erythema equal in intensity to the reaction obtained by injection of an equal volume of antigen or antiserum.

[1] Armour Laboratories, Lot No. P67908.

+, mild edema with erythema both lasting 24 hours and measuring 1.5 cm. in greatest diameter.

++, mild to moderated edema, erythema measuring more than 3 cm. in greatest diameter; brownish discoloration measuring up to 0.5 cm.

+++, moderate edema; erythema measuring 3 to 4 cm.; hemorrhage and brownish discoloration measuring 0.5 to 1.5 cm. in diameter.

++++, severe edema; marked erythema and hemorrhage measuring more than 1.5 cm.; sloughing of reaction site.

This system of grading is similar to that of Opie (6) in which sloughing is required for the ++++ designation.

Injections of soluble complexes were performed using the quantities of antigen and antibody listed below. The reactions produced were compared in the gross and microscopically to the three other types of cutaneous antigen-antibody reactions mentioned, the BSA rabbit anti-BSA system being used throughout: (*a*) the local passive Arthus (LPA) in which antiserum of known antibody content was injected intradermally followed within 30 to 60 minutes by a superimposed intradermal injection of the antigen, 0.25 mg. BSA nitrogen (BSA N); (*b*) the reversed passive Arthus (RPA) in which, in all cases, 8 mg. BSA N was injected intravenously followed in one-half to one hour by an intradermal injection of known quantities of antiserum; (*c*) classical or active Arthus reactions in which highly sensitized rabbits were injected intradermally with 0.18 mg. BSA N.

The following control injections were performed in normal, unsensitized rabbits: (*a*) 0.3 ml. antiserum, (4 rabbits); (*b*) 180 μg. BSA N contained in either 0.3 or 0.4 ml. 0.15 M NaCl or 0.3 ml. normal rabbit serum, (4 rabbits); (*c*) a 0.15 M NaCl suspension of antigen-antibody (BSA-anti BSA) precipitate containing 2.5 mg. Ab N, which was prepared at equivalence and washed twice with 0.15 M NaCl at 0–2°C., (5 rabbits); and (*d*) a saline suspension of 2.7 mg. N of heat denatured BSA, prepared by heating BSA in saline to 100°C. for less than one minute, centrifuging off, and finally washing the insoluble, denatured protein twice with 0.15 M NaCl at 0–2°C., (5 rabbits). Biopsies of all control tissues were taken.

All specimens of cutaneous tissues procurred by biopsy were taken in duplicate; half of each specimen was fixed in 10 per cent formalin for 24 hours for routine histologic sections while the opposite half specimen was frozen at −70°C. in a mixture of butylalcohol and solid carbon dioxide.

Fluorescent Antibody Technique.—The frozen specimens were studied for localization of antigen and rabbit globulin using the fluorescent antibody technique of Coons and Kaplan (16), with certain modifications as noted in previous publications from this laboratory (18, 19).[2] Ethanol fractionation of the fluorescein-conjugated antiserum was found unnecessary and was dispensed with. Adsorption with acetone-dried liver powder was performed by shaking the conjugates with the powder, 150 mg. powder per 1 ml. of conjugate, for 1 hour at room temperature. The powder was then removed by centrifugation leaving a clear solution for use.

RESULTS

1. Gross Reactions.—Intradermal injections of soluble complexes were performed using doses of 0.03, 0.30, 0.43, 0.65, 1.20 and 2.66 mg. Ab N. In all instances the reaction first became apparent in the gross at about 5 hours after injection, reaching a maximum at 20 to 24 hours and decreasing in intensity thereafter. Maximal reactions are listed according to dosage in the first column

[2] Fluorescein amine was prepared through the courtesy of Dr. Klaus Hoffman of the Department of Biochemistry, University of Pittsburgh School of Medicine.

of Table I. At doses of 0.43 mg. Ab N and below, the reaction was marked by a diffuse area of erythema and edema. At the lowest dosage, 0.03 mg. Ab N, the reaction in the gross was only slightly greater than that produced by an equal

TABLE I

*Intensity of Gross Reaction as a Function of Quantity of Antibody Used**

Mg. Ab N	Soluble complexes	LPA‡§	RPA‡‖
2.66	++++		
	++++		
	++++		
	++++		
1.20	+++		
	++		
	+++		
0.65	++	++	++++
	+++	+++	++++
	±	+++	++++
	+++	+++	
	++	++	
	++	++	
0.43	++	++	++++
	++	++	++++
		++	++++
0.30	++		++++
	+		++++
	++		++++
0.03	±		
	±		
	±		

* Intensity of reaction judged by the presence and amount of edema, erythema, hemorrhage, necrosis, and tissue sloughing in the reaction site.

§ LPA produced by i.d. injection of antibody followed by superimposed injection of 0.25 mg. N BSA in each case.

‡ 0.4 ml. required at the 0.65 mg. Ab N level.

‖ RPA 8 mg. N BSA i.v. followed by antibody i.d.

volume of antiserum injected as a control although, as described below, the microscopic findings of this complex-produced reaction were similar in character to those induced by larger dosages. Using doses of 0.65 mg. Ab N and above, the erythema and edema spread to large areas with diameters measuring up to 6 cm. (Fig. 1). At these dosages, hemorrhage and necrosis occasionally oc-

curred. Sloughing of the skin was observed at the 2.66 mg. Ab N dosage level (Fig. 2).

The cutaneous reaction to soluble complexes was compared to the local passive Arthus (LPA)[3] and the reversed passive Arthus (RPA)[4] using the amount of antibody in terms of nitrogen as the common reference point. Quantities of antibody larger than 0.65 mg. Ab N could not be used in the LPA and RPA reactions owing to the large volumes of antiserum required. The comparative results are tabulated in Table I. It can be seen that the cutaneous reaction to complexes and the LPA are similar in intensity at the dosage levels shown. The LPA reactions reached their maximum at about 20 to 24 hours and were marked by diffuse erythema and edema. They were indistinguishable macroscopically from the complex-produced reactions using equivalent amounts of antibody. On the contrary, the RPA reactions, as seen in the 4th column of Table I were of maximal intensity at levels of as low as 0.30 mg. Ab N, which is in agreement with the results obtained by Fischel and Kabat (5). It should be noted, however, that this evaluation was based on the degree of hemorrhage and necrosis produced in these lesions, which was the striking feature of the RPA reaction (Fig. 3). Actually the total amount of edema formed was nearly equal in all three types of reactions, being concentrated about the injection site in the RPA reaction, while diffusely spread in the complex and LPA lesions. Another striking difference was the time required for development of maximal intensity of reaction. The RPA reached its maximum within 5 to 8 hours, while, as stated previously, the complex and LPA reactions required 20 to 24 hours for full development. As is well known, this rapid development of reaction also occurred in the classical or active Arthus reactions in which actively sensitized rabbits with circulating antibody were given intradermal injections of the homologous antigen.

Control injections were made with each protein solution used in the manner described. In all cases in which control solutions of BSA were injected, a mild erythema developed within 30 minutes, but subsided with the dissemination of injected material leaving a faint pale area about 1 cm. in diameter at 24 hours. Injection of 0.3 ml. of the antisera used showed a diffuse erythema and edema within an hour. By 24 hours only a 1 to 2 cm. area of mild erythema and edema persisted. Sites in which BSA–anti BSA precipitates were injected persisted for 48 hours as firm nodules 1 to 2 cm. in diameter with erythema localized over the injection site. The erythema was of low intensity, and necrosis was not seen. The sites into which heat-denatured BSA had been injected persisted for 48 hours as small, firm nodules with only slight erythema noted.

2. *Microscopic Studies.*—Microscopic studies were performed on biopsies of reactions taken at 1, 5, 8, 24, and 48 hours and 5, 7, 9, and 11 days after the final injection. All tissues were stained as routine with hematoxylin and eosin.

[3] LPA, local passive Arthus reaction.

[4] RPA, reversed passive Arthus reaction.

(*a*) In the *complex reactions*, at all dosage levels, the earliest changes as seen in the 1, 5, and 8 hour specimens consisted of margination of polymorphonuclear leukocytes (polymorphs) along the endothelium of vessels and the presence of polymorphs in the tissue around small vessels. However, polymorphs did not accumulate in the vessel walls. By 8 hours, polymorphs had spread throughout the dermis in large numbers, especially in the area immediately above the underlying muscle layer. Many of these leukocytes had become necrotic. No especial intramural vascular accumulation was noted. Few vessels contained thrombi, with only an occasional capillary or small vein showing leukocytic plugging. Larger vessels (over 20 microns in diameter) rarely showed thrombosis. This was true of sections at both 8 and 24 hours. An occasional small vein was necrotic at 8 hours. By 24 hours degeneration of the polymorphs was marked and many were seen containing large quantities of phagocytized material. Mononuclear cells were now conspicuous, noted especially in perivascular areas. At this time, necrosis of vessel walls was more commonly seen. This appeared as a deep pink, amorphous change in the vessel wall typical of fibrinoid change, involving either the entire circumference or just one portion of the wall (Figs. 4, 5). At times, this necrotic area was limited to the outer portion of one segment of the vessel wall. Leukocytic infiltration of the necrotic areas was not a striking feature. The endothelial cells appeared more prominent and, again, thrombosis was uncommon in the affected vessels. Edema was still present and hemorrhage from small vessels could be seen, especially in the higher dosage levels. Frequently at 24 and 48 hours after injection, large, pink, acellular zones of tissue necrosis were found in the upper dermis corresponding to the necrotic zones seen in the gross. By 48 hours polymorphs were infrequently seen, but mononuclear cells were common around vessels and throughout the dermis. Sections taken at 7 and 9 days after injection revealed a perivascular accumulation of mononuclear cells and only an occasional mature plasma cell.

(*b*) The *LPA* biopsy sections revealed a histological sequence of events similar in all respects to the soluble complex reactions.

(*c*) However, the *RPA and active Arthus* reactions showed distinct differences from those in which both antigen and antibody were injected locally (the complex and LPA reactions). In the RPA and active Arthus reactions, which will be described together, the characteristic microscopic feature was the marked, immediate vascular inflammation. This appeared at 1 hour as a margination of polymorphs along the endothelial surface and in the vessel wall. Also seen in occasional vessel walls was a light, pink staining, amorphous material that either replaced or masked the normal cellular structures in the wall. This material was sometimes seen on the luminal side of the intact endothelial lining indicating that it might not be derived from the vessel wall. This is of note in the fluorescence description below. By 5 hours after injection,

masses of polymorphs were seen in the vessel walls along with this pink material forming the typical picture of the active Arthus or RPA lesion (Fig. 6). Thrombosis in these reactions was frequent, especially in affected vessels, as opposed to the complex and LPA reactions in which thrombosis was infrequent. Hemorrhage and edema were also abundant by 5 hours as opposed to the complex and LPA sections. Polymorphs had spread out into the surrounding tissue and some had undergone degeneration. At 8 hours, the reactions appeared much the same although more intense, and by 24 hours, areas of acellular tissue necrosis were found in the upper dermis. At this time, perivascular and intravascular mononuclear cells were conspicuous while polymorphs were fewer in number than at 8 hours. By 48 hours, almost all the inflammatory cells were mononuclear in type, and from then on to 11 days after injection, a difference between the RPA and active Arthus was noted. In the active Arthus, mature plasma cells were seen in abundance at 5 days and 11 days; while, in the RPA as in the complex and LPA reactions, only occasional plasma cells were to be found. These results compare favorably with those of Gell and Hinde (20). These workers found that Arthus reactions in rabbits in which antibody had been passively transferred did not show plasma cell concentration by day 5, while an Arthus reaction produced in actively sensitized rabbits showed marked plasma cell accumulation at day 5 and later.

Microscopic studies of control sections in which antigen or antiserum were injected alone as described above revealed a mild to moderate diffuse infiltration of polymorphs in the dermis at 8 and 24 hours. Edema was minimal at 8 hours and not observable at 24 hours and vascular necrosis and hemorrhage were absent. Histologic sections of the sites in which BSA–anti-BSA precipitates had been injected revealed clumps of precipitated protein limited to the immediate area of injection. This precipitate was surrounded and partially invaded by a lining of polymorphs. Vessels and other structures immediately adjacent to the precipitates were unaffected. Microscopic sections of the sites in which heat denatured BSA had been injected were similar except that less surrounding polymorph aggregation was seen.

3. Fluorescence Studies.—The frozen biopsied reaction sites, as noted before, were sectioned at 6 to 7 microns on a freezing microtome, fixed in an ethyl alcohol–ether mixture and then 95 per cent ethyl alcohol and after a saline washing were overlaid with a fluorescein-labelled antibody solution. The sections were then washed and observed using the fluorescence microscope. Using fluorescent anti-BSA, *i.e.* testing for the presence of BSA, the soluble complex reaction showed under low magnification diffuse, bright green fluorescence in the area of severe reaction. The fluorescence or BSA under high power magnification was of a granular nature, the protein being diffusely scattered through the dermis and subcutaneous tissue. A concentration of the BSA was seen at the center of the lesion, in which the predominance of inflammatory cells was

seen. A study of the relationship of the BSA to vessels was made by comparing localization of the fluorescence under ultraviolet light with the position of vessels using phase microscopy of the same section or by studying the adjacent section stained with hematoxylin and eosin. It was found that while the BSA frequently came to lie in the vicinity of vessels, little, if any, could be seen in the vessel walls. This lack of vascular concentration of the antigen was noted not only in the case of normal vessels, but of special note, little or no BSA was found in the walls of vessels showing margination and diapedesis of polymorphs and in vessels in which necrosis in the wall was seen on the next section which was stained with hematoxylin and eosin (Fig. 7). In each case, however, the granular fluorescence marking the BSA could be seen in adjacent areas. Sections of the LPA reactions studied under identical conditions were indistinguishable from the complex reactions.

This lack of concentration of BSA in the vessel walls of the complex and LPA reactions is in sharp contradistinction to the localization seen in both the RPA and classical or active Arthus reactions. In the latter two, the affected vessels stand out on fluorescence microscopy as bright, granular, green rings due to their contained BSA (Figs. 8 to 11). Actually, in many sections, little BSA was found other than that fixed in vessel walls. In sections of RPA or active Arthus reactions taken 1 hour after injection, BSA was found in small vessels while at 8 and 24 hours, the larger vessels also contained BSA. This correlated with the size of the vessel affected as seen on hematoxylin and eosin–stained sections. Vessels were occasionally seen similar to those mentioned in the microscopic description above in which pink, amorphous material was seen within the lumen. This pink material was found to contain an abundance of BSA indicating that antigen and antibody have combined in an area independent of cells. BSA could be found in sections taken through 24 hours after injection in all four types of reactions studied, but in sections taken at 48 hours, BSA was rarely visualized.

Attempts to study the localization of rabbit globulin in similar sections of rabbit tissue were difficult to interpret, since the presence of an abundance of rabbit proteins in the edema fluid of the reaction resulted in a large amount of generalized tissue fluorescence. Instead, guinea pigs were studied using the BSA-rabbit anti-BSA system. RPA and soluble complex lesions were produced using 3 guinea pigs in each case. Sections of these lesions treated with fluorescent anti-BSA showed a distribution of the antigen similar to that seen in the rabbit sections. Using fluorescent sheep anti-rabbit globulin, a distribution of fluorescence corresponding to the BSA localization was observed in both the complex and RPA reactions. It is to be noted that in the case of the complex reactions, the localized globulin represented antibody, since it was that globulin only that had been bound to BSA in the washed precipitates. The results indicate that, in the case of the complex reaction, antibody is present along with the antigen

in the tissue. The localized globulin in the RPA sections may include some non-antibody globulin as well as specific antibody.

A summary of the differences between the complex and LPA reactions on the one hand and the RPA and active Arthus lesions on the other are listed in Table II. It is seen that in the former two reactions, the antigen, and as is shown in the guinea pig studies, the antibody, were found diffusely scattered in the dermis and subcutaneous tissues, with little, if any, in the vessel walls; while the RPA and active Arthus reactions showed a predominant vessel wall accumulation. The complex and LPA lesions demonstrated less severity in the gross at the lower dosage levels and less hemorrhage, tissue necrosis, vascular reaction and thrombosis and fewer leukocytes in the damaged vessels. More-

TABLE II

Comparison of Cutaneous Reactions in Which Both Ag and Ab Are Injected Intradermally with Reactions in Which Ag or Ab is Present Intravenously

	Soluble complexes or LPA	RPA or active Arthus
1. Localization of antigen	Diffusely scattered	Predominantly vascular
2. Gross reaction at low dosages*	± to ++	++++
3. Time for maximal reaction (hrs.)	20–24	5–8
4. Degree of vascular reaction	Mild to moderate	Marked
5. Leukocytes in vascular necrosis	Few	Marked
6. Thrombosis of vessels	Occasional	Frequent
7. Hemorrhage	Mild	Marked

* Less than 0.43 mg. Ab N.

over, the time required to reach the height of these two reactions was greater than that required in the case of the active Arthus and RPA reactions.

4. Controls of Fluorescence Studies.—Observations were made in the active Arthus and RPA reactions to find if unaffected vessels contained BSA. In each case in which the vessel was not affected, application of fluorescent anti-BSA failed to produce fluorescence; *i.e.*, BSA, in the vessel wall. Further, sites of injection of BSA into normal, unsensitized rabbit skin showed only pale, diffuse fluorescence at the most without specific localization. However, the possibility existed that the localization of BSA in the vessel walls of the active Arthus lesions did not represent specifically fixed antigen, but rather represented what one would expect to find when any protein contacted vessels damaged in a similar fashion. To study such a possibility, two BSA-sensitized rabbits were injected intradermally with a saline solution of 0.32 mg. N BSA with 0.32 mg. N human gamma globulin (HGG) added as an unrelated protein; and conversely 4 rabbits sensitized to HGG were injected with 0.30 mg. N HGG with 0.30 mg. N BSA added as the unrelated protein. In each case, fluorescence observation

of the homologous antigen demonstrated the typical granular fluorescence in affected vessel walls, but showed only the faint, diffuse presence of the unrelated proteins. These results indicate that the localization of homologous antigen represents specific fixation of the antigen at the site of reaction.

In all instances, sections treated with non-fluorescent anti-BSA before the fluorescent anti-BSA showed a marked decrease in intensity of fluorescence as compared to an adjacent section stained first with an unrelated non-fluorescent antibody, *e.g.* non-fluorescent anti-HGG, and then fluorescent anti-BSA. This indicated that the fixation of the fluorescent antibody could be specifically inhibited.

DISCUSSION

The data presented in this report demonstrate that a single intracutaneous injection of soluble antigen-antibody complexes is capable of producing an inflammatory reaction. With large doses, necrosis and sloughing of the tissue may be produced. On microscopic examination a diffuse infiltration of the tissue with leukocytes is seen and necrosis of vessel walls is not infrequent. Further, these results are in keeping with the supposition (2–4) that antigen-antibody complexes are responsible for the lesions that develop in serum sickness. The findings of Germuth and McKinnon (7), Trapani, Garvey, and Campbell (8), and Ishizaka and Campbell (9), and Tokuda and Weiser (10) add support to this contention. However, the cutaneous response to the injection of soluble antigen-antibody complexes need not necessarily be elicited by the complex of antigen and antibody as formed *in vitro*. To be sure, following injection, dissociation and reassociation of antigen and antibody perhaps takes place and this process may be responsible for part or all of the tissue changes that occur.

The comparison of the cutaneous reaction to soluble complexes with the local passive Arthus (LPA), reversed passive Arthus (RPA), and the active or classical Arthus reactions indicated that two general types of reactions occurred: (*a*) those resulting from the injection of both antigen and antibody locally and (*b*) those in which either antigen or antibody were present in the blood stream, and the other reactant was injected locally. The dissimilarities between these two types of reactions are summarized in Table II. In brief, when either reactant is found circulating, the antigen, and in all likelihood the antibody, become localized in vessel walls; while when both are injected locally, the antigen and antibody are found diffusely scattered in the dermis and subcutaneous tissue, but vessels contain little, if any, of these components. Further, the reactions in which both reactants are injected locally show less frequent vascular necrosis, little vascular thrombosis, only mild hemorrhage in most instances, and absence of tissue necrosis at lower dosage levels. On the other hand, with these lower dosage levels of antibody, the reactions in which antigen

is present intravenously exhibit extreme vascular, thrombotic, hemorrhagic, and necrotic changes. It would appear that when one of the reactants is present intravenously and the other is injected intradermally, as is the case in the RPA and active Arthus, the locally injected material diffuses through the tissue contacting sizeable amounts of the other reactant first at the site of vessels. The localization of antigen and in all probability antibody (as was demonstrated in the RPA in guinea pigs) in vessel walls was found to be the characteristic feature of these reactions. Some of the reaction also takes place in the interstitial space, but as demonstrated in the fluorescent antibody studies, this is of not great proportion. While the antigen and antibody combine in the smallest vessels at first (1 to 2 hours), soon larger ones are involved as noted in sections taken 5 hours after injection. It is believed that this predominant vessel wall locus of combination is responsible for the marked vascular changes that then take place as described in the earliest stages *in vivo* by Abell and Schenck (21) and others (22, 23), and which end in marked intravascular accumulation of leukocytes, thrombosis, necrosis of vessel walls, hemorrhage, and tissue death. In contrast to this, when both reactants are injected intradermally, the antigen-antibody combination is present in the interstitial space predominantly. While capillaries are probably infiltrated by the antigen and antibody in the central portion of the reaction, larger vessels are conspicuously devoid of the localization of the antigen and antibody, possibly by virtue of increased tissue resistance of the normal vessel wall. However, damage to the larger vessel walls apparently takes place, with little or no concentration of the antigen-antibody combination in the vessel walls but with the reactants present in the adjacent interstitial space. Following this vascular damage, the secondary events, *i.e.* the thrombosis, hemorrhage, and tissue necrosis, may take place. However, neither the primary vascular damage nor the secondary events are as severe as those seen in reactions in which antigen or antibody is present intravenously.

As noted above, in both the complex-produced and LPA reactions, the antigen was found diffusely scattered in granular clumps throughout the dermis and subcutaneous tissue. In particular, damaged vessels contained little, if any, BSA. This suggested that the antigen-antibody combination, while present in tissue adjacent to a vessel was capable of inducing reaction and necrosis of the vessel wall; or perhaps that very small amounts of antigen and antibody within the vessel wall, but not detectable by our technique, were capable of inducing vascular damage. That such vascular damage might be mediated by factors liberated as a result of the presence of antigen and antibody in the vicinity of vessels seems possible in view of the increasing body of evidence indicating that physiologically active substances are released from tissues and body fluids when antibody and antigen combine.

It was noted in these experiments that the injected antigen, in each type of

reaction, was rarely observable in sections taken 48 hours after injection. Studies by Korngold, Stahly, Dodd, and Myers (24) following the loss of I^{131} BSA from an intradermal site in a sensitized rabbit, demonstrated that between 20 and 30 per cent of the antigen persisted at 24 hours. In similar studies (25), we have confirmed this data and also found that approximately half of this amount persisted at 48 hours. However, as shown in this report using the fluorescent antibody technique, the BSA does not persist for 48 hours in detectable amounts or detectable form in the specific site of the vessel wall in the active Arthus lesion.

SUMMARY

The *in vivo* activity of soluble antigen-antibody complexes was tested by a single intradermal injection in rabbits. Skin reactions were obtained marked by erythema, induration, and occasionally hemorrhage and necrosis. Microscopically, diffuse inflammation and occasional vascular necrosis could be found at all dosages. This indicates that soluble antigen-antibody complexes are phlogogenic and provides support for the suggestion that complexes are responsible for the lesions seen in serum sickness.

The reactions were similar in severity to local passive Arthus (LPA) reactions at equal dosages of antibody in the dosage range studied.

BSA antigen could be found in large concentrations in affected vessel walls of both reverse passive Arthus (RPA) and active or classical Arthus reactions. It is suggested that this predominantly vascular localization of antigen might bring about the relative severity of the RPA and active Arthus reactions, as contrasted to the complex and LPA reactions.

The finding of affected vessels in the complex and LPA reactions containing little or no antigen and antibody, while these components were present in adjacent areas, suggests that the antigen-antibody combination may cause vascular reaction and damage by the release of physiologically active mediators from the tissue or tissue fluid.

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BIBLIOGRAPHY

1. Weigle, W. O., The nature of antigen-antibody complexes formed in rabbits during an immune response to bovine serum albumin, *J. Exp. Med.*, 1958, **107**, 653.
2. Dixon, F. J., Vazquez, J. J., Weigle, W. O., Cochrane, C. G., Pathogenesis of serum sickness, *A. M. A. Arch. Path.*, 1958, **65**, 18.
3. Dixon, F. J., Vazquez, J. J., Weigle, W. O., Cochrane, C. G., The cellular and humoral aspects of the hypersensitive states, (H. S. Lawrence, editor), New York, Paul R. Hoeber, Inc., in press.

4. Germuth, F. G., Jr., Comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type, *J. Exp. Med.*, 1953, **97,** 257.
5. Fischel, E. E., and Kabat, E. A., A quantitative study of the Arthus phenomenon induced passively in the rabbit, *J. Immunol.*, 1947, **55,** 337.
6. Opie, E. L., Inflammatory reaction of the immune animal to antigen (Arthus phenomenon) and its relation to antibodies, *J. Immunol.*, 1924, **9,** 231.
7. Germuth, F. G., Jr., and McKinnon, G. E., Studies of the biological properties of antigen-antibody complexes in unsensitized normal guinea pigs, *Bull. Johns Hopkins Hosp.*, 1957, **101,** 13.
8. Trapani, I. L., Garvey, J. S., and Campbell, D. H., Stimulating action of soluble antigen-antibody complexes on normal guinea pig smooth muscle, *Science*, 1958, **27,** 700.
9. Ishizaka, K., and Campbell, D. H., Biological activity of soluble antigen-antibody complexes. I. Skin reaction properties, *Proc. Soc. Exp. Biol. and Med.*, 1958, **97,** 639.
10. Tokuda, G., and Weiser, R. S., Production of anaphylaxis in the white mouse with soluble antigen-antibody complexes, *Science*, 1958, **127,** 1237.
11. Culbertson, J. T., Relationship of circulating antibody to the local inflammatory reaction to antigen (the Arthus phenomenon), *J. Immunol.*, 1935, **29,** 29.
12. Cannon, P. R., and Marshall, C. E., Studies on the mechanism of the Arthus phenomenon, *J. Immunol.*, 1941, **40,** 127.
13. Benacerraf, B., and Kabat, E. A., A quantitative study of the Arthus phenomenon induced passively in the guinea pig. *J. Immunol.*, 1950, **64,** 1.
14. Ovary, Z., and Bier, O. G., A quantitative study of Arthus reaction and cutaneous anaphylaxis induced passively in the rat, *Proc. Soc. Exp. Biol. and Med.*, 1952, **81,** 584.
15. Ovary, Z., and Bier, O. G., Quantitative studies on passive cutaneous anaphylaxis and its relationship to the Arthus phenomenon, *J. Immunol.*, 1953, **71,** 6.
16. 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.
17. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 1948.
18. Vazquez, J. J., and Dixon, F. J., Immunohistochemical analysis of amyloid by the fluorescence technique, *J. Exp. Med.*, 1956, **104,** 727.
19. Vazquez, J. J., and Dixon, F. J., Immunohistochemical study of lesions in rheumatic fever, systemic lupus erythematosus, and rheumatoid arthritis, *Lab. Invest.*, 1957, **6,** 205.
20. Gell, P. G. H., and Hinde, I. T., Observations on the histology of the Arthus reaction and its relation to other known types of skin hypersensitivity, *Int. Arch. Allergy and Appl. Immunol.*, 1954, **5,** 23.
21. Abell, R. G. and Schenck, H. P., Microscopic observations on the behavior of living blood vessels of the rabbit during the reaction of anaphylaxis, *J. Immunol.*, 1938, **34,** 195.
22. Ebert, R. H., Barclay, W. R., and Ahom, J. J., The comparison of tuberculin and

Arthus types of hypersensitivity; *in vivo* observation in the rabbit ear chamber, *J. Lab. and Clin. Med.*, 1949, **34**, 1596.

23. Benacerraf, B., and Kabat, E. A., A quantitative study of the Arthus phenomenon induced passively in the guinea pig, *J. Immunol.*, 1950, **64**, 1.
24. Korngold, L., Stahly, G. L., Dodd, M. C., and Myers, W. G., The comparative retention of antigen in the skin of immune and normal rabbits as determined with egg albumin, *J. Immunol.*, 1953, **70**, 345.
25. Cochrane, C. G., and Weigle, W. O., unpublished observations.

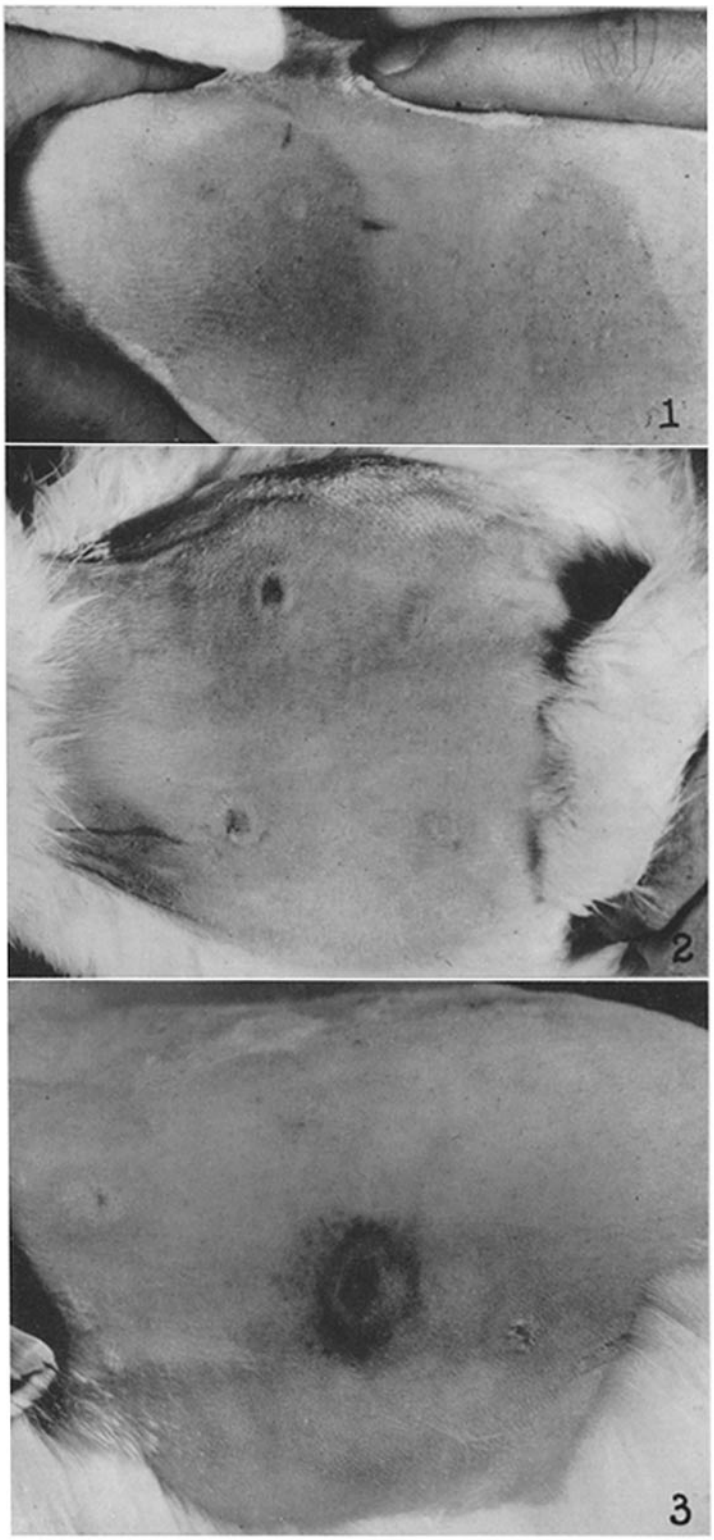
EXPLANATION OF PLATES

PLATE 37

FIG. 1. Cutaneous reaction to soluble antigen-antibody complexes (BSA-anti BSA) 24 hours after intradermal (i.d.) injection of 0.65 mg. Ab N dosage. Note edema and erythema. × ⅔.

FIG. 2. Cutaneous reaction to soluble BSA-anti BSA complexes, 24 hours after i.d. injection of dosage 2.66 mg. Ab N. The two sites at the left are the areas of injection of complexes showing necrosis and sloughing, while the two sites on the right are antiserum control injections. × ⅓.

FIG. 3. Reversed passive Arthus reaction (RPA). Photomicrograph taken 24 hours after i.d. injection of 0.30 mg. anti-BSA N into a rabbit with circulating BSA. Severe hemorrhage and necrosis are present at this dosage of antibody. × ⅔.

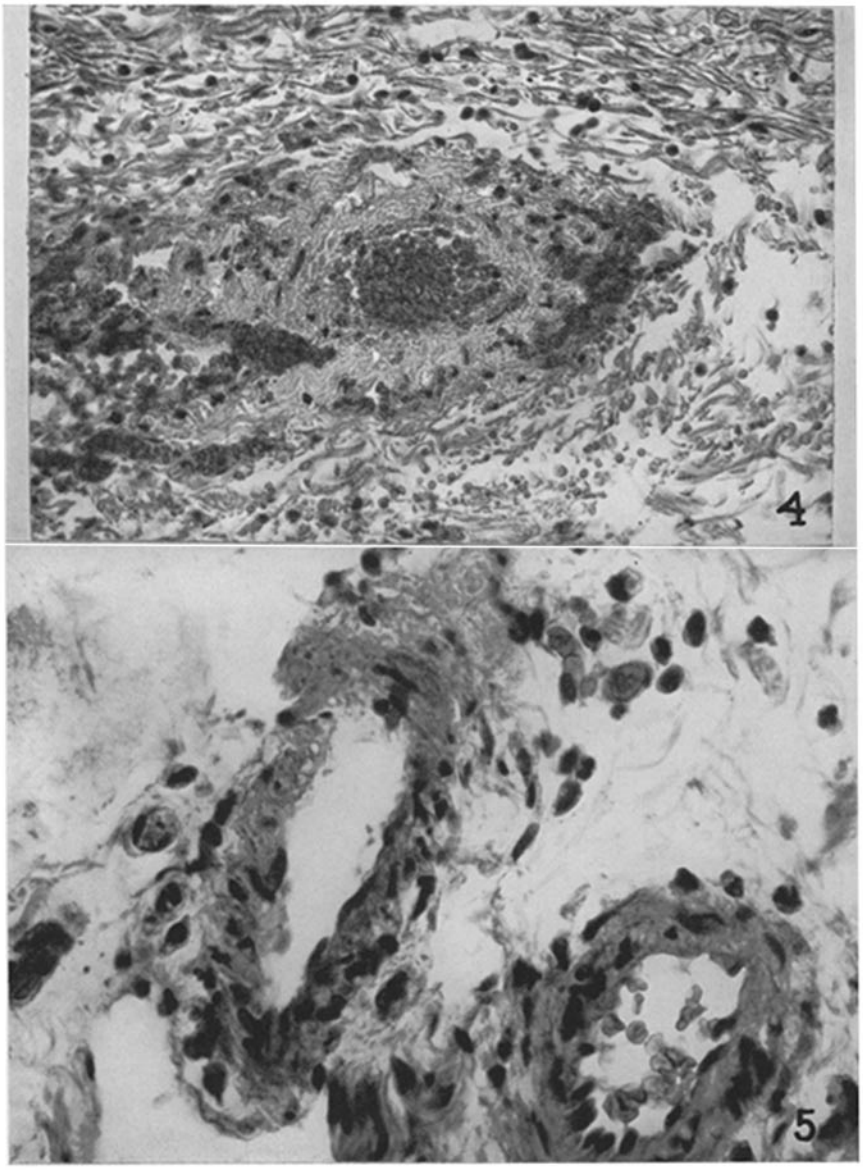


(Cochrane and Weigle: Soluble antigen-antibody complexes)

PLATE 38

FIG. 4. Necrotic vessel seen in cutaneous lesion of soluble complexes. Note hemorrhage and relatively few inflammatory cells in the necrotic vessel wall. Hematoxylin and eosin stain. × 240.

FIG. 5. Another vessel (on the left) from a soluble complex cutaneous reaction showing necrosis of a portion of the wall only. Note the absence of thrombosis. Hematoxylin and eosin stain. × 360.

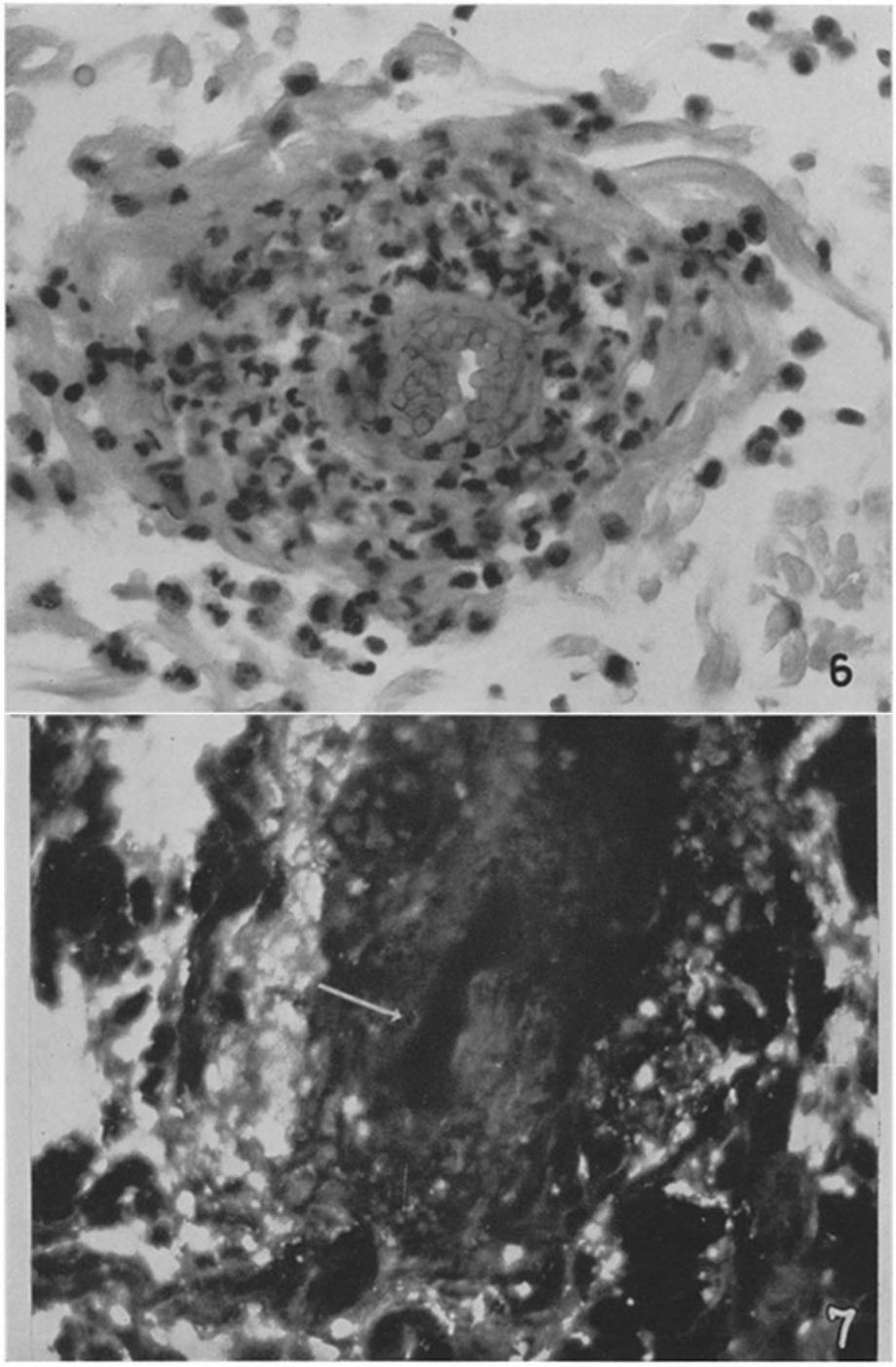


(Cochrane and Weigle: Soluble antigen-antibody complexes)

PLATE 39

FIG. 6. Vessel in a section of a reversed passive Arthus, 5 hours after i.d. injection of 0.30 mg. Ab N, showing inflammation of the vessel wall and perivascular area. Hematoxylin and eosin stain. × 360.

FIG. 7. Fluorescence photomicrograph of a damaged vessel in a soluble complex skin lesion. Granular white areas mark the presence of BSA. Arrow marks outer portion of vessel wall. The wall contains no detectable BSA while an abundance of the antigen is seen in surrounding areas. Sections taken above and below this failed to reveal BSA in the vessel wall. × 440.

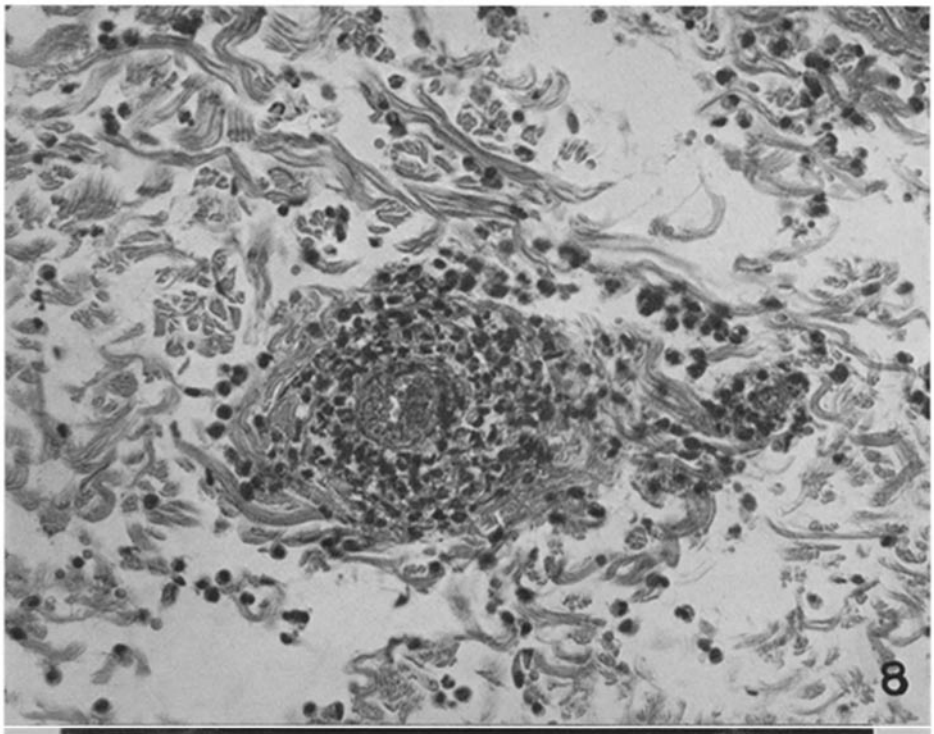


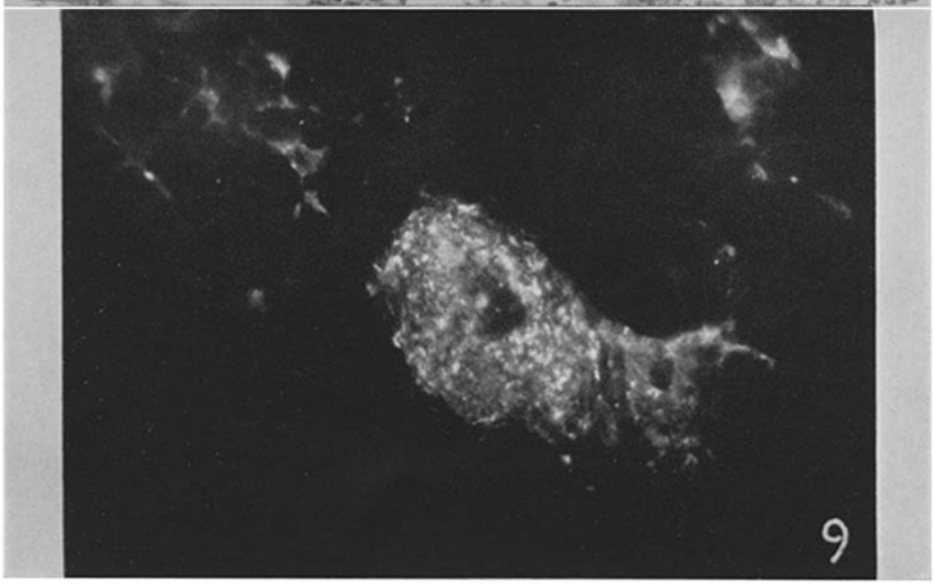
(Cochrane and Weigle: Soluble antigen-antibody complexes)

PLATE 40

FIG. 8. Vessel in an active or classical Arthus reaction, 8 hours after injection, showing inflammation of the vessel wall. Hematoxylin and eosin stain. × 280.

FIG. 9. Fluorescence photomicrograph of a similar vessel as seen in Fig. 8. Section treated with anti-BSA to detect the presence of BSA. The BSA is found concentrated in the area of vascular inflammation. A similar concentration of antigen is seen in RPA sections. × 240.



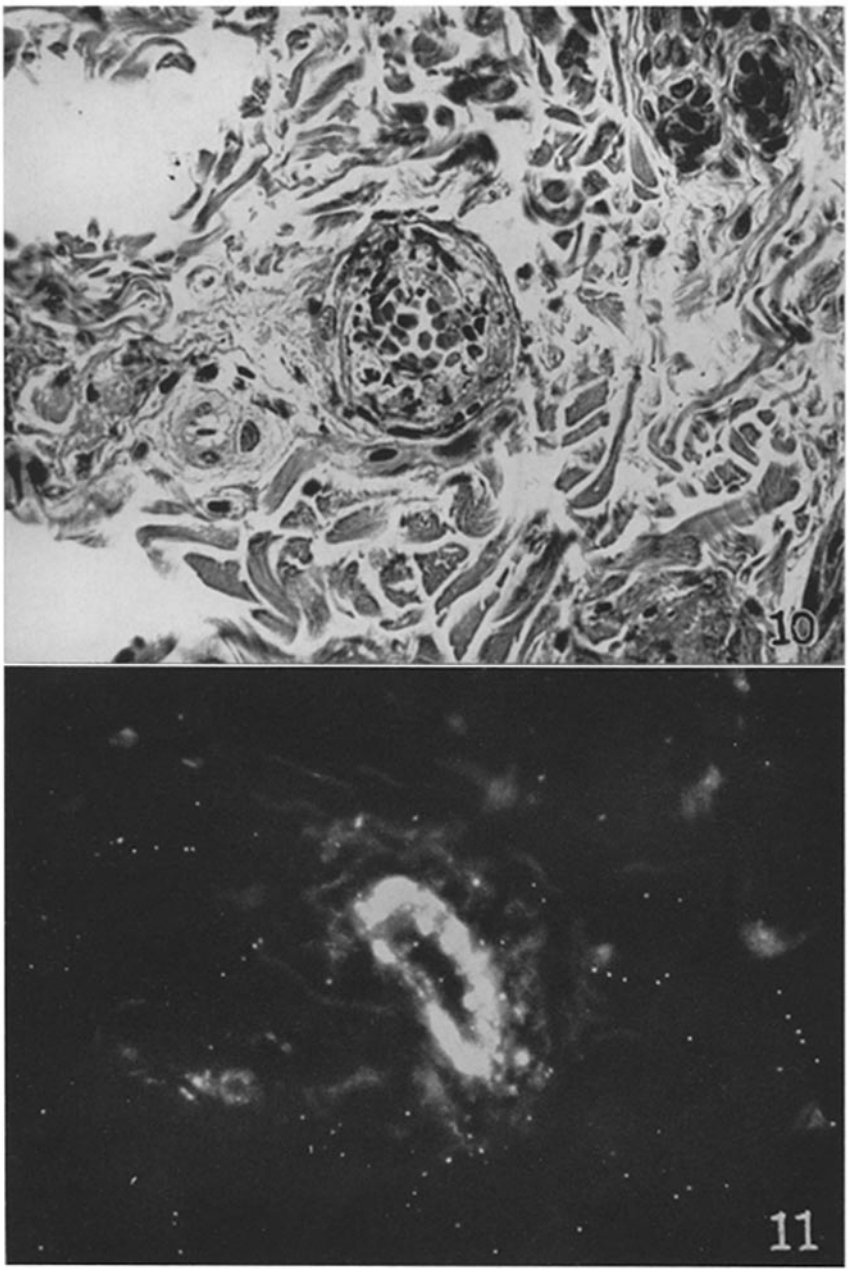


(Cochrane and Weigle: Soluble antigen-antibody complexes)

PLATE 41

FIG. 10. Microscopic section of an active Arthus reaction, 2 hours after injection. Early inflammatory changes are seen. Hematoxylin and eosin stain. × 360.

FIG. 11. Fluorescence photomicrograph of a vessel with early inflammatory changes similar to that seen in Fig. 10. The BSA is localized to the vessel wall and little spreading is seen. × 440.



(Cochrane and Weigle: Soluble antigen-antibody complexes)