THE PATHOLOGIC EFFECTS OF INTRAVENOUSLY ADMINISTERED SOLUBLE ANTIGEN-ANTIBODY COMPLEXES*

I. Passive Serum Sickness in Mice

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The pathogenesis of experimental serum sickness in the rabbit has been extensively investigated since the description of the characteristic lesions, glomerulonephritis, arteritis, and endocarditis, by Rich and Gregory (1). It has been established by several investigators that the lesions appear during the immune phase of antigen elimination from the blood, at a time when antigen-antibody complexes are present in the circulation (2, 3). The disease is of short duration and the lesions regress after antigen has been completely eliminated and free antibody appears in the blood (2). Dixon and coworkers demonstrated antigen and probably antibody in the lesions of serum sickness by means of the fluorescent antibody technique (3), thus supporting Germuth's hypothesis (2) that the pathological changes are the result of localization of antigen-antibody complexes as such at the sites where the lesions appear. The validity of this interpretation has been strengthened by observations on the biological properties of antigen-antibody complexes. The important role of these complexes in the pathogenesis of certain hypersensitivity states has only recently been recognized. It has been shown that they can produce anaphylaxis in guinea pigs (4) and mice (5, 6), contraction of isolated guinea pig smooth muscle (7) and inflammatory changes in the skin (8), whose severity is proportional to the amount injected.

More direct evidence for the role of soluble antigen-antibody complexes in the pathogenesis of serum sickness was provided by observations previously reported from this laboratory showing that the intravenous injection of large amounts of soluble antigen-antibody complexes produced the characteristic lesions of serum sickness in normal mice within 36 hours (6, 9).

In the present study these observations have been extended. The incidence of the various lesions has been studied with several antigen-antibody systems. The evolution and duration of the lesions, the possible role of anaphylaxis in their pathogenesis, the effect of cortisone, and of an antihistamine have also been investigated. The extent to which the complexes used were dissociated *in vivo* was explored.

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Materials and Methods

Animals.—Swiss Webster male and female mice ranging from 20 to 35 gm. were used.

Antigens.—Hen ovalbumin, 3 times recrystallized, was obtained from Worthington Biochemical Co., Freehold, New Jersey, and bovine serum albumin (BSA) was obtained from the Armour Laboratories, Chicago.

Antisera.—Antisera were prepared in rabbits against ovalbumin and BSA and in chickens against BSA. The rabbits were immunized as follows: 10 mg. of antigen in 1 ml. of saline were emulsified with an equal volume of Difco complete adjuvant containing 2 mg. *Mycobacterium butyricum* per ml. This was injected into the muscles of the back of the neck, and again 1 week later. Seven days later intravenous injections of alum-precipitated antigens (10) were initiated and were administered 3 times weekly for 4 weeks, 1.5 mg. being given for the first 3 injections, and on each subsequent week the amount of antigen was increased by 0.5 mg. per injection. The sera were pooled according to their antibody content, each pool representing the sera of 4 or more rabbits.

The chickens were immunized with alum-precipitated BSA intravenously 3 times weekly and were given 1.5 mg. per injection the 1st week, then 2 mg. and 2.5 mg. per injection the 2nd and 3rd weeks, respectively. The chickens were bled by cardiac puncture 5 days after the last injection. Two pools of antisera were prepared, and each pool contained sera of 4 chickens. All sera were stored at −20°C. With chicken serum, freezing and thawing was associated with formation of non-specific precipitate, which was removed by centrifugation prior to analysis and use.

The various pools of antiserum were analyzed for antibody content and antigen-antibody ratio at equivalence by the quantitative precipitin technique (10). Chicken antisera were analyzed at a salt concentration of 0.9 per cent rather than the higher salt concentration used by Goodman (11). The precipitates were analyzed for antibody protein by ultraviolet absorption at 280 mμ in the case of rabbit antibody (12), and by the micro-Kjeldahl technique in the case of chicken antibody.

The pools of antiserum used in the experiments were as follows:—

Rabbit antiovalbumin	Pool	I	which contained	4.6	mg.	antibody	protein/ml.
	"	II	"	"	5.2	"	"	"	"
	"	III	"	"	3.5	"	"	"	"
	"	IV	"	"	7.8	"	"	"	"
	"	V	"	"	4.0	"	"	"	"
	"	VI	"	"	2.0	"	"	"	"
Rabbit anti-BSA	Pool	I	which contained	2.3	mg.	antibody	protein/ml.
	"	II	"	"	4.3	"	"	"	"
	"	III	"	"	3.0	"	"	"	"
Chicken anti-BSA	Pool	I	which contained	0.93	mg.	antibody	protein/ml.
	"	II	"	"	1.42	"	"	"	"

Preparation and Injection of Soluble Complexes.—All the experiments to be described were carried out with antigen-antibody complexes redissolved in antigen excess. The antibody was precipitated from the antiserum by the addition of the amount of antigen required to produce optimal precipitation of antibody at equivalence. The precipitate was allowed to form overnight at 4°C. and was then washed twice with cold saline before being redissolved by the addition of 15 to 20 times the amount of antigen used to precipitate at equivalence. The solubilization of the complexes was performed at room temperature and usually occurred within 1 hour, but sometimes, especially with the chicken anti-BSA system, required up to 2 hours.

The soluble complexes were injected as soon as the precipitate had redissolved. The solutions were prepared in such a way that 0.5 ml. contained the amount to be injected. The complexes were injected into the tail vein.

An attempt was made to diminish the incidence of anaphylactic death often seen following the first injection of ovalbumin rabbit antiovalbumin complexes by the prior administration of the antihistamine promethazine hydrochloride (phenergan) in the dose 25 mg./kilo, intraperitoneally.

Preparation of Histologic Sections.—The mice were sacrificed by decapitation and tissues fixed in 10 per cent buffered formalin. Sections of heart, lung, liver, spleen, and kidney were routinely stained with hematoxylin and eosin. In some cases sections were stained with phosphotungstic acid—hematoxylin (PTAH), Weigert's fibrin stain, azocarmine stain, and Masson's trichrome stain.

EXPERIMENTAL

Lesions Produced by Intravenous Administration of Soluble Complexes Prepared from Various Antigen-Antibody Systems

The following antigen-antibody systems were used: ovalbumin rabbit antiovalbumin, BSA rabbit anti-BSA, and BSA chicken anti-BSA. Each animal received 3 injections in 24 hours and each injection contained 3 mg. of antibody protein in the form of soluble complexes in antigen excess, prepared as described. Control groups consisted of mice which were untreated, and of mice given the same number of injections of equivalent doses of antigen alone, either in saline or in normal rabbit serum, and of mice given rabbit antiovalbumin serum alone. The quantity of antigen amounted to about 7 mg. of ovalbumin or 12 mg. of BSA per injection.

In the first series of experiments performed with ovalbumin rabbit antiovalbumin complexes or BSA rabbit anti-BSA complexes, anaphylactic reactions were observed, occurring almost exclusively after the first injection, with a mortality in the ovalbumin system of 25 out of 84 animals. In an attempt to reduce the severity of the anaphylaxis, the antihistamine phenergan was administered as described. The mortality in the animals subsequently used was 1 out of 41. These data concerning anaphylaxis are the total results obtained in a number of different experiments with this system, and are, therefore, not amenable to simple statistical evaluation. The mice which received BSA chicken anti-BSA showed no signs of anaphylaxis whatsoever.

The incidence of lesions found in mice after this course of injections of soluble complexes is shown in Table I. Most of the mice were sacrificed 24 hours after the last injection, but some were sacrificed as late as 8 days. In a few animals, focal necrosis was seen in the heart, liver, or spleen, but similar lesions were occasionally found in controls, including untreated mice. None of the lesions to be described below were found in animals of the control groups (Fig. 1).

There was a high incidence of glomerulonephritis in all the experimental groups amounting to 89 per cent with the ovalbumin rabbit antiovalbumin system. Arteritis or endocarditis was observed with each immunological system but with a considerably lower incidence than that of glomerulonephritis. The incidence of arteritis is difficult to determine accurately because of its focal nature.

The character of the glomerular lesions was similar in each experimental group. The glomeruli were enlarged, hypercellular, relatively bloodless and infiltrated with neutrophiles. The glomerular cells were swollen. With any given system, the severity varied within and between experimental groups. In severe cases all of the glomeruli were involved, and heavily infiltrated with neutrophiles; karyorrhexis was present in many glomeruli (Fig. 2). In moderate cases most of the glomeruli were involved and the degree of hypercellularity and leucocyte infiltration was less marked (Fig. 3). In mild cases many glomeruli were not involved and those that were showed relatively slight neutrophile infiltration and hypercellularity (Fig. 4). The majority of animals injected with ovalbumin rabbit antiovalbumin complexes showed moderate or severe glomerulonephritis while only mild to moderate lesions were seen in the mice given BSA rabbit anti-BSA complexes.

TABLE I

Incidence of Glomerulonephritis, Arteritis, and Endocarditis in Mice Given 3 Injections of Soluble Antigen-Antibody Complexes in 24 Hours and Sacrificed within 8 Days

Material injected	No. of mice	No. with glomerulonephritis	No. with arteritis	No. with endocarditis
Ovalbumin rabbit* antiovalbumin	55	49	6	3
BSA rabbit* anti-BSA	15	8	3	3
BSA chicken* anti-BSA	12	9	0	2
Ovalbumin or BSA plus normal rabbit serum	10	0	0	0
Rabbit antiovalbumin serum	5	0	0	0
Normal mice	33	0	0	0

* Each injection contained 3 mg. of antibody dissolved in 15 to 20 times antigen excess.

The arteritis involved small muscular arteries and had the following distribution: 4 instances in the lungs, 2 in the heart, 2 in the stomach, and 1 in the urinary bladder. The arteritis was characterized by focal necrosis of the vessel wall which was infiltrated with leucocytes, some of which were fragmented (Fig. 5). In some instances, there was endothelial hyperplasia.

The endocarditis usually involved the mitral valve and was characterized by thickening of the valve leaflet and infiltration with leucocytes (Fig. 6).

The Course and Duration of Glomerulonephritis Produced by Ovalbumin Antiovalbumin Complexes

The ovalbumin rabbit antiovalbumin system was used in experiments designed to study the course and duration of glomerulonephritis because the most uniformly severe glomerular lesions were observed with this system.

The soluble complexes were prepared as described above and 3 injections, each containing 3 mg. of antibody redissolved in 15 to 20 times antigen excess, were administered in 24 hours. The results of these experiments are shown in Table II.

In the mice sacrificed within the first 4 days, the incidence of glomerulonephritis was very high (90 per cent) and of these, 41 per cent were severe. In the group sacrificed between the 5th and 10th day, there was still a high incidence (86 per cent), but most of the animals showed mild nephritis (66 per cent). In contrast to the early group, there were fewer neutrophiles within glomeruli and more of them appeared normal. In many cases increased amounts of eosinophilic material were seen within glomeruli which probably represented swollen cytoplasm. Azocarmine and Masson's trichrome stains did not show any increase in basement membrane-like material.

These results indicate that the lesions rapidly regress following a single series of injections and are self-limiting in the absence of further insults. Indeed, in mice sacrificed between 14 and 30 days, no glomerular abnormalities were found.

In view of these findings, it was decided to attempt to intensify the glomerulonephritis by increasing the number of injections of complexes to see whether this would modify the course of the disease.

TABLE II

Duration and Severity of Glomerulonephritis Produced in Mice by Redissolved Ovalbumin Rabbit Antiovalbumin Complexes

Days after last injection	No. of mice	No. of mice with glomerulonephritis			
		Mild	Moderate	Severe	Total
1–4	46	5	19	17	41
5–10	14	8	4	0	12
14–30	15	0	0	0	0

Mice were given 3 injections in 24 hours, containing 3 mg. of antibody per injection dissolved in 15 to 20 times antigen excess.

Accordingly, 24 mice were injected twice daily for 3 days with 3 mg. of antibody redissolved in 15 times antigen excess. Groups of mice were sacrificed at weekly intervals up to 4 weeks starting 24 hours after the last injection.

The glomerulonephritis seen in the group sacrificed at 1 day was somewhat more severe than observed previously (Fig. 7). Minimal inflammatory changes were seen in glomeruli as late as 20 days, but by 28 days the kidneys appeared completely normal.

The Effect of Cortisone on the Development of Glomerulonephritis Produced by Soluble Antigen-Antibody Complexes

Because of its known anti-inflammatory effect, an attempt was made to modify the severity of the glomerulonephritis by treatment with high doses of cortisone.

The experiment was performed on 27 mice which were treated as follows: 19 mice received cortisone injections for 5 days; 1 mg. on days 1 and 2, 2.5 mg. on day 3, and 5 mg. on days 4 and 5; of these mice, 11 were given a course of 3 injections of ovalbumin rabbit antiovalbumin complexes containing 3 mg. of antibody, prepared as described above, on the 4th and 5th day of cortisone treatment; 8 additional mice received soluble complexes alone in the same dosage and schedule. The mice in each group were sacrificed 1 and 4 days after treatment. The results are shown in Table III.

The cortisone treatment resulted in diminished severity of the nephritis but failed to protect completely. Histologically, there were fewer neutrophiles in the cortisone treated group. In addition, in 4 of the 5 mice which received cortisone and complexes and were killed 1 day later, there was striking accumulation

TABLE III

Effect of Cortisone on Nephritis Produced by Soluble Antigen-Antibody Complexes

Treatment	Days after last injection of complexes	No. of mice	No. of mice with glomerulonephritis			
			Mild	Moderate	Severe	Total
Soluble complexes*	1	5	4	1	0	5
and cortisone‡	4	6	6	0	0	6
Soluble complexes	1	4	0	1	3	4
" "	4	4	0	1	3	4
Cortisone‡	1	4	0	0	0	0
"	4	4	0	0	0	0

* Soluble antigen-antibody complexes: 2 injections of ovalbumin rabbit antiovalbumin 3 mg. in 15 times antigen excess on 4th day and 1 on 5th day of cortisone treatment.

‡ Cortisone acetate treatment 5 days: 1 mg. every day for 2 days, next day 2.5 mg., then 5 mg. daily for 2 days.

of amorphous, eosinophilic material filling many glomerular capillary loops (Fig. 8). This material stained positive with Weigert's fibrin stain and blue with Mallory's PTAH. Smaller amounts were found in 2 of the mice from the same group killed 4 days after treatment. In our experience, similar material was occasionally found in small amounts in animals given complexes alone and killed shortly after the last injection. The mice given cortisone alone showed no glomerulonephritis, but did show slight dilatation of glomerular capillaries, a change which has been described in rabbits given cortisone (13, 14).

Study of Dissociation In Vivo of Soluble Complexes Prepared from Rabbit Antibody

The lesions of serum sickness in the rabbit and those seen following the intravenous injection of soluble antigen-antibody complexes into mice result from the localization of antigen and antibody at the sites where tissue damage occurs

(15). The question arises as to whether the localization of complexes occurs as such or whether localization is a result of recombination *in situ* following dis-

TABLE IV

Study of Dissociation of Soluble Antigen-Rabbit Antibody Complexes by Passive Cutaneous Anaphylaxis in Guinea Pigs

(A) Latent Period 5 Hours

Guinea pig No.	Antibody nitrogen, μg.								
	Ova anti-ova in 20 × antigen excess				Antiovalbumin				
	16.0	5.0	1.0	0.2	0.2	0.05	0.02	0.005	0.002
1	−	−	−		+++	++	±		
2		−	−	−		+	−	−	
3	−	−	−	−					
4							+++	+	−
	BSA anti-BSA in 20 × antigen excess				Anti-BSA				
5	−	−	−		++	++	+		
6		−	−	−		+	−	−	
7	−	−	−	−					
8							+++	++	−

(B) Latent Period 18 Hours

Guinea pig No.	Antibody nitrogen, μg.												
	Ova anti-ova in 20 × antigen excess								Antiovalbumin				
	100	10	5.0	3.3	1.0	0.2	0.1	0.02	0.2	0.05	0.02	0.005	0.002
9	−	−		−									
10											+++	+	−
11			−		−	−			++++	++++	+		
12			−		−	−			++++	++++	+		
13						−	−	−		++++	+++	±	
14						−	−	−		++	±	−	

Animals challenged with 2 mg. of antigen plus 0.5 ml. 1 per cent Evans blue intravenously.

sociation. A further question is whether dissociation and recombination *in situ* is required for the development of the lesions. That antigen and antibody are constantly dissociating and recombining when in the form of soluble complexes in antigen excess is evidenced by the fact that antigen-antibody precipitates redissolve in antigen excess. Furthermore, with certain antigen-antibody complexes of guinea pig origin of presumably high dissociation constants, Rosen-

berg, Chandler, and Fischel (16) have demonstrated considerable dissociation of antibody from complexes *in vivo*, by showing that such systems in considerable antigen excess were able to sensitize guinea pig skin for passive cutaneous anaphylaxis. However, not all complexes show this property (17). It seemed to be desirable to investigate the antigen-antibody complexes employed in these experiments with respect to their capacity to dissociate *in vivo* using the technique of Rosenberg, Chandler, and Fischel (16).

Fourteen guinea pigs weighing between 300 and 400 gm. were used. The guinea pigs were injected intradermally on one flank with varying amounts of antigen-antibody complexes and on the other side with antibody alone. After a latent period of sensitization of 5 or 18

TABLE V

Hemolysis of Mouse Erythrocytes by Rabbit and Chicken Specific Antisera with Mouse Complement*

Antiserum‡	Complement§	Dilution of antiserum							
		1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Rabbit.....	Mouse					+++	++	+	±
Chicken A...	"	+	±	−					
" B...	"	+	+	−	−				
" B...	Mouse (heat inactivated)	−	−	−	−	−			
" B...	Mouse and versene‖	±	−	−	−	−			

Controls consisting of (*a*) antiserum 1/4 and red cells without complement, (*b*) complement and red cells without antiserum, showed no hemolysis.

* Mouse erythrocytes. 0.4 ml. of 2 per cent RBC suspension.
‡ Antiserum (Heat-inactivated at 56°) 0.2 ml. of suitable dilution.
§ Mouse complement 0.2 ml. fresh mouse serum.
‖ Versene 0.1 ml. of 5 per cent solution.

hours, the animals were challenged intravenously with 2 mg. of antigen and 0.5 ml. of 1 per cent Evans blue dye. The reactions at the sites of intradermal injection were recorded 20 minutes later. Three pools of antiserum were investigated, 2 rabbit antiovalbumin and one rabbit anti-BSA. The results of these experiments are presented in Table IV.

The data show that the rabbit antigen-antibody complexes used did not release a detectable amount of antibody, after allowing time for diffusion of excess antigen from the skin sites. In order to show that the sites where the antigen-antibody complexes were injected were capable of reacting with release of histamine and permeability changes, control experiments were performed with 3 guinea pigs, sensitizing these sites concurrently with another antibody, rabbit anti-bovine gamma globulin, and showing that passive cutaneous anaphylaxis could be demonstrated at the usual level of sensitivity following challenge with the corresponding antigen.

Study of the Ability of Chicken Antibody to React with Mouse Complement

In the experiments reported in this study, complexes prepared from both rabbit and chicken antibody were found to produce glomerulonephritis, whereas anaphylaxis was seen only with the rabbit system and not with the chicken system. Chicken antibody is generally stated not to fix mammalian complement. Information about the extent to which chicken antibody can bind and activate mouse complement is relevant to an evaluation of the role of complement in the pathogenesis of the lesions. Complement has been shown to be required for some types of hypersensitivity reactions (18).

The ability of chicken antibody to hemolyze red cells in the presence of mouse complement was investigated. Antisera against mouse erythrocytes were prepared in rabbits and chickens and showed agglutination titers ranging from 1–6,000 to 1–12,000. The results of the hemolysis experiments (Table V) showed that in the presence of mouse complement a strong chicken anti-mouse erythrocyte serum in high concentration caused slight hemolysis. Hemolysis was inhibited by heat inactivation of mouse serum or by the addition of versene to the mixture. There was definite hemolysis, but this effect was very slight and not comparable to the effect observed with rabbit anti-mouse erythrocyte serum.

DISCUSSION

The intravenous administration of soluble antigen-antibody complexes to mice, using either rabbit or chicken antibody, results in a high incidence of acute glomerulonephritis, and in some instances, in arteritis or endocarditis. The glomerulonephritis is most severe 2 to 3 days after the first injection and gradually regresses in the 2 weeks following injection. This course of events indicates that the lesions are the result of the injected antigen-antibody complexes and that an immune response on the part of the mouse does not play a pathogenetic role. This interpretation is supported by the demonstration, by means of the fluorescent antibody technique, of both antigen and antibody in involved glomeruli (15). The glomerulonephritis is generally characterized by infiltration with neutrophiles, hypercellularity, swelling of glomerular cells and relative bloodlessness of glomeruli. The arterial lesions generally show necrosis of the media, infiltration of all layers of the vessel with leucocytes, and in some cases endothelial proliferation. The number of instances of arteritis found may not reflect its true incidence in view of its focal character.

Anaphylactic shock was seen following the first and rarely the second injection of complexes prepared from rabbit antibody. This was more severe with the ovalbumin than with the BSA system. The animals injected with complexes prepared from chicken antibody showed no signs of anaphylaxis and yet showed glomerulonephritis and endocarditis. This indicates that, contrary to an earlier hypothesis (19), the release of vasoactive amines by an anaphylactic reaction is not essential for the production of lesions. Furthermore, the administration

of the antihistamine phenergan, which appeared to protect against anaphylaxis, did not prevent the development of glomerulonephritis.

An important question is why the antigen-antibody complexes localize in glomeruli and focally in heart valves and arteries, where lesions develop. It has been shown that antigen-antibody complexes, especially those of large size, are cleared by the reticulo-endothelial system (RES) (20). Colloidal preparations such as carbon, which are ordinarily cleared by the RES, may also be deposited in endothelium in other sites under certain conditions, including prolonged high blood concentrations of these colloids or damage to the endothelium by a variety of inflammatory agents (19). In such situations, glomeruli generally show the most striking accumulation seen outside the RES. The most consistently severe lesions observed in the present study have been in glomeruli, characteristically with widespread involvement of these structures, reflecting deposition of complexes. While the mechanism which leads to such localization in glomeruli is not known, it may be related to the function of glomerular filtration. In any case, it would appear to be an important factor in the pathogenesis of glomerular abnormalities. Glomerular damage as a consequence of localization of circulating colloidal material, which is retained in glomeruli, has been reported following administration of methyl cellulose (21) and saccharated iron oxide (22).

Certain properties of the antigen-antibody complexes used in these experiments might tend to favor their deposition in glomerular endothelium. Soluble complexes were injected with large amounts of antigen excess. Ovalbumin is known to be excreted by the kidney in normal mice and this would modify the ratio of antigen to antibody in favor of antibody on passage through glomeruli, resulting in the formation of larger aggregates which are more likely to be arrested. An analogous though slower process may operate with BSA, especially after glomerular damage has been initiated (23). The difference in excretion of these two antigens may be a contributory factor in the greater severity of the glomerulonephritis seen with the ovalbumin rabbit antiovalbumin system. When complexes are initially deposited in glomeruli, the resulting endothelial damage would certainly favor further deposition. While in active serum sickness the formation of larger antigen-antibody aggregates is a result of the increasing production of antibody (2), in the experiments reported here the reverse situation exists and the formation of larger complexes is a consequence of antigen loss.

It appears that antigen and antibody are localized as complexes. While antigen is present in excess in the circulation, dissociation and recombination can occur. However, the rabbit antibody used was of such a nature that it can be considered to remain nearly completely neutralized after elimination of the excess antigen. This is shown by the failure of such soluble complexes to sensitize guinea pig skin to PCA, even when sufficient time is allowed for diffusion of the excess antigen from the skin sites.

The fact that glomerulonephritis can be produced by complexes prepared from chicken antibody raises the question as to whether complement is involved in the pathogenesis of the lesions (24). Chicken antibody is generally believed to be unable to fix mammalian complement. However, the observations on hemolysis with chicken antibody and mouse complement indicate that chicken antibody can in fact bind and activate mouse complement, although very inefficiently. This conclusion is supported by observations concerning the ability of chicken antibody and mouse complement to opsonize bacteria (25). Therefore, it is not possible to exclude completely the participation of complement in the pathogenesis of the lesions, although on the basis of the small amount fixed, it would not appear to play an important role in the production of this kind of tissue damage.

The administration of cortisone diminished the severity of the glomerulonephritis produced by intravenously administered soluble antigen-antibody complexes. However, in these animals, glomerular capillary loops were often filled with amorphous, eosinophilic material which was shown to contain both antigen and antibody by the fluorescent antibody technique (15). This material may also contain fibrin. In view of the minimal inflammatory reaction within glomeruli, the presence of large amounts of this material may be due to the paucity of neutrophiles, which have been shown to phagocytize and metabolize antigen and antibody in the Arthus reaction (26). Furthermore, it has been shown that cortisone inhibits regeneration of the RES following blockade (27). In this way blockade produced by the initial injection of antigen-antibody complexes could persist and favor the deposition in glomeruli of material subsequently injected. It is possible that the deposition of the eosinophilic material in glomeruli in these mice may occur as a result of mechanisms analogous to those operating in the generalized Shwartzman reaction. In this situation also, eosinophilic material accumulates in glomeruli and animals may be prepared for this reaction by pretreatment with cortisone (28).

SUMMARY

The intravenous administration to mice of soluble antigen-antibody complexes in antigen excess resulted in a high incidence of glomerulonephritis and less frequently in endocarditis or arteritis. These lesions are present within 48 hours of the first of 3 injections and disappear within 2 weeks.

The same pathological changes were produced with complexes prepared from either rabbit or chicken antibody. In the case of rabbit antibody, the severity of the glomerulonephritis was greater with the ovalbumin antiovalbumin system than with the BSA system.

Anaphylaxis regularly occurred in mice given complexes prepared from rabbit antibody, but was not seen following administration of complexes prepared from chicken antibody.

Pretreatment with cortisone diminished the severity of the glomerulo-ne-

phritis and resulted in accumulation of amorphous, eosinophilic material within glomerular capillaries in mice injected with antigen-antibody complexes.

The rabbit antibody used in these experiments failed to sensitize guinea pig skin to passive cutaneous anaphylaxis when injected in the form of soluble complexes. This indicates that these complexes do not dissociate to a detectable extent *in vivo* and thus favors the interpretation that complexes localize as such in the sites where tissue damage occurs.

Chicken anti-mouse erythrocyte antibody produced hemolysis of mouse red cells in the presence of mouse complement. In contrast to a similar rabbit antiserum, the hemolytic activity of the chicken antibody with mouse complement was very slight. This suggests that complement does not play an important role in the pathogenesis of these experimental lesions.

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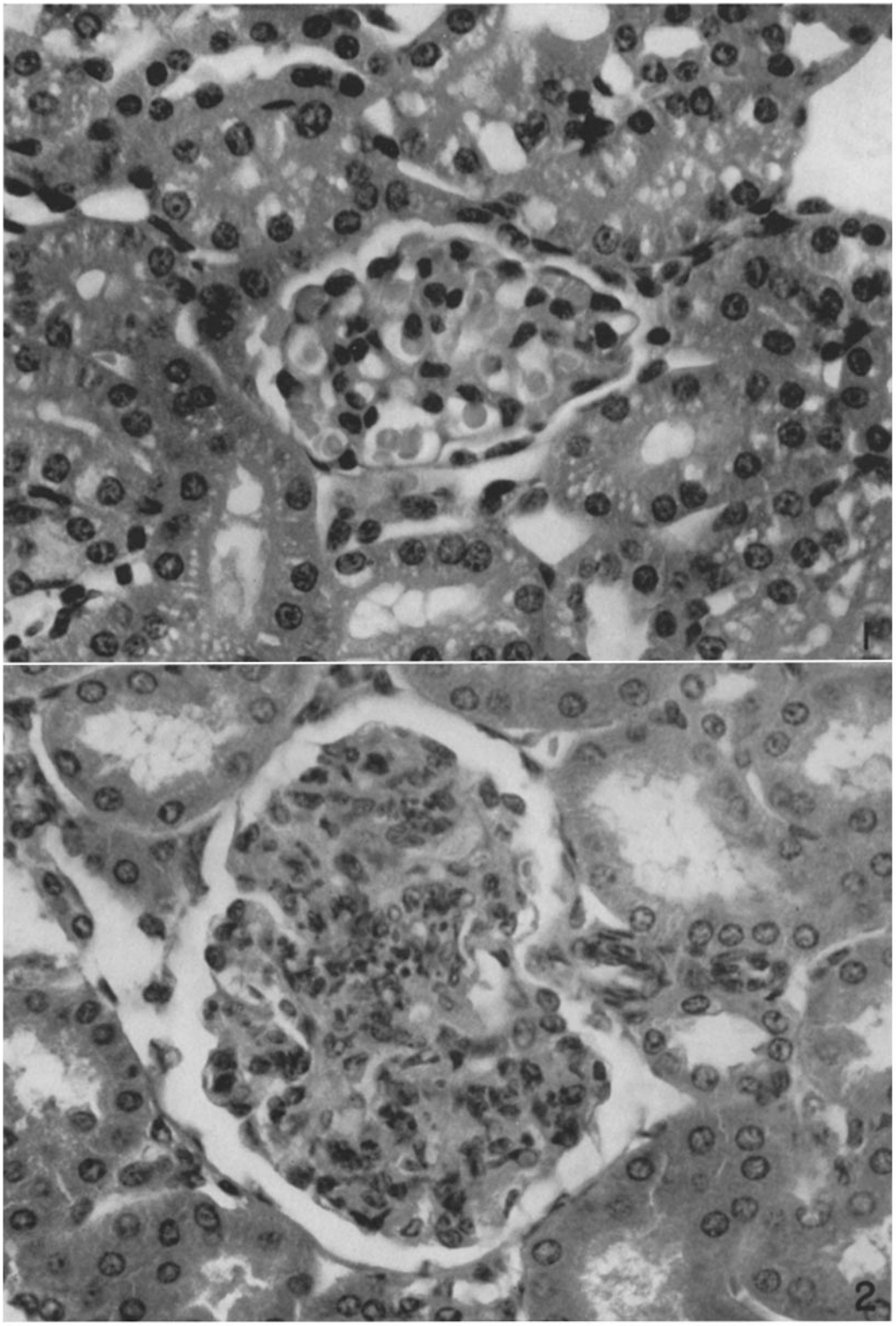
EXPLANATION OF PLATES

PLATE 11

FIG. 1. Kidney of normal mouse. Hematoxylin and eosin stain. × 455.

FIG. 2. Kidney of a mouse given 3 injections of ovalbumin-antiovalbumin complexes and sacrificed 24 hours after the last injection. The glomerulus is swollen, hypercellular, relatively bloodless and infiltrated with leucocytes, many of which are fragmented. All of the glomeruli in this kidney were involved. Hematoxylin and eosin stain. × 455.

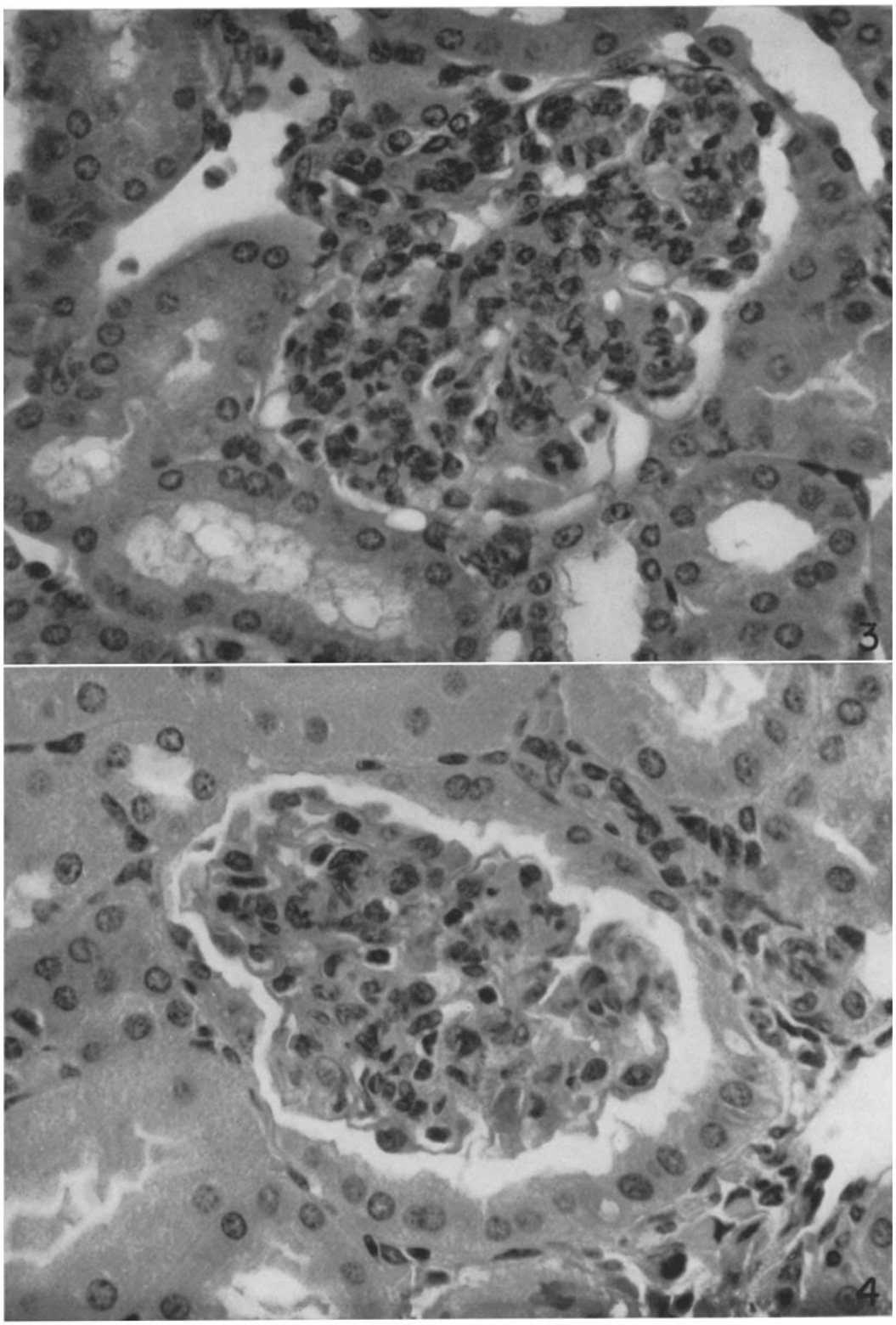


(McCluskey *et al.*: Passive serum sickness in mice)

PLATE 12

FIG. 3. Kidney of a mouse given 3 injections of ovalbumin-antiovalbumin and sacrificed 24 hours later. The glomerulus shown is swollen, hypercellular, and infiltrated with neutrophiles. A few glomeruli in this kidney showed little or no involvement. Hematoxylin and eosin stain. × 455.

FIG. 4. Kidney of a mouse given 3 injections of BSA rabbit anti-BSA complexes and sacrificed 24 hours later. This glomerulus shows hypercellularity and leucocytic infiltration, but many of the glomeruli in this kidney appeared normal. Hematoxylin and eosin stain. × 455.

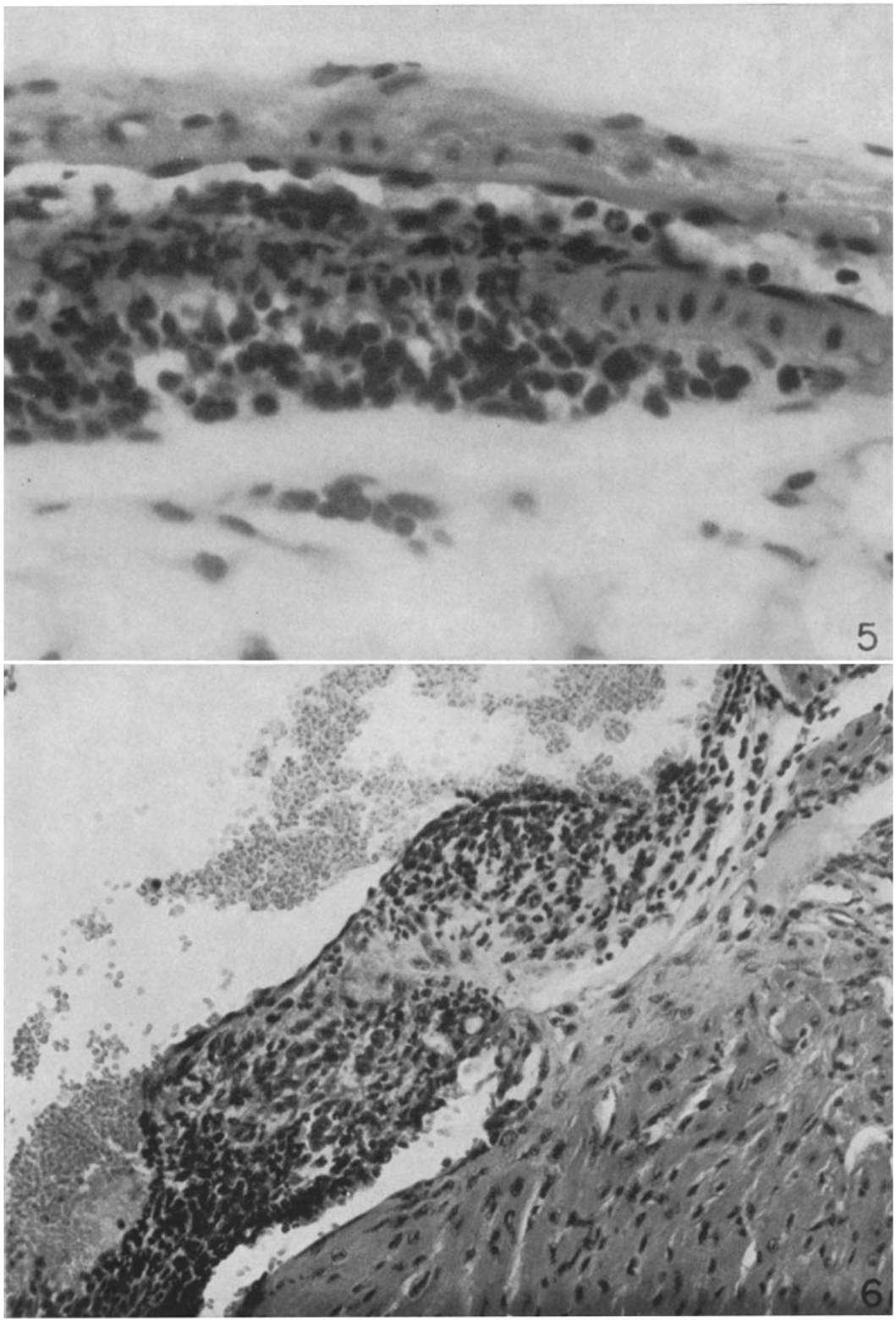


(McCluskey *et al.*: Passive serum sickness in mice)

PLATE 13

FIG. 5. Perigastric artery from a mouse given 3 injections of ovalbumin-anti-ovalbumin complexes and sacrificed 24 hours later. There is segmental, eccentric inflammation of the vessel wall with necrosis of the media. Hematoxylin and eosin stain. × 600.

FIG. 6. Mitral valve from a mouse which received 3 injections of BSA chicken anti-BSA complexes and sacrificed 24 hours later. The valve leaflet is thickened and heavily infiltrated with leucocytes. Hematoxylin and eosin stain. × 320.

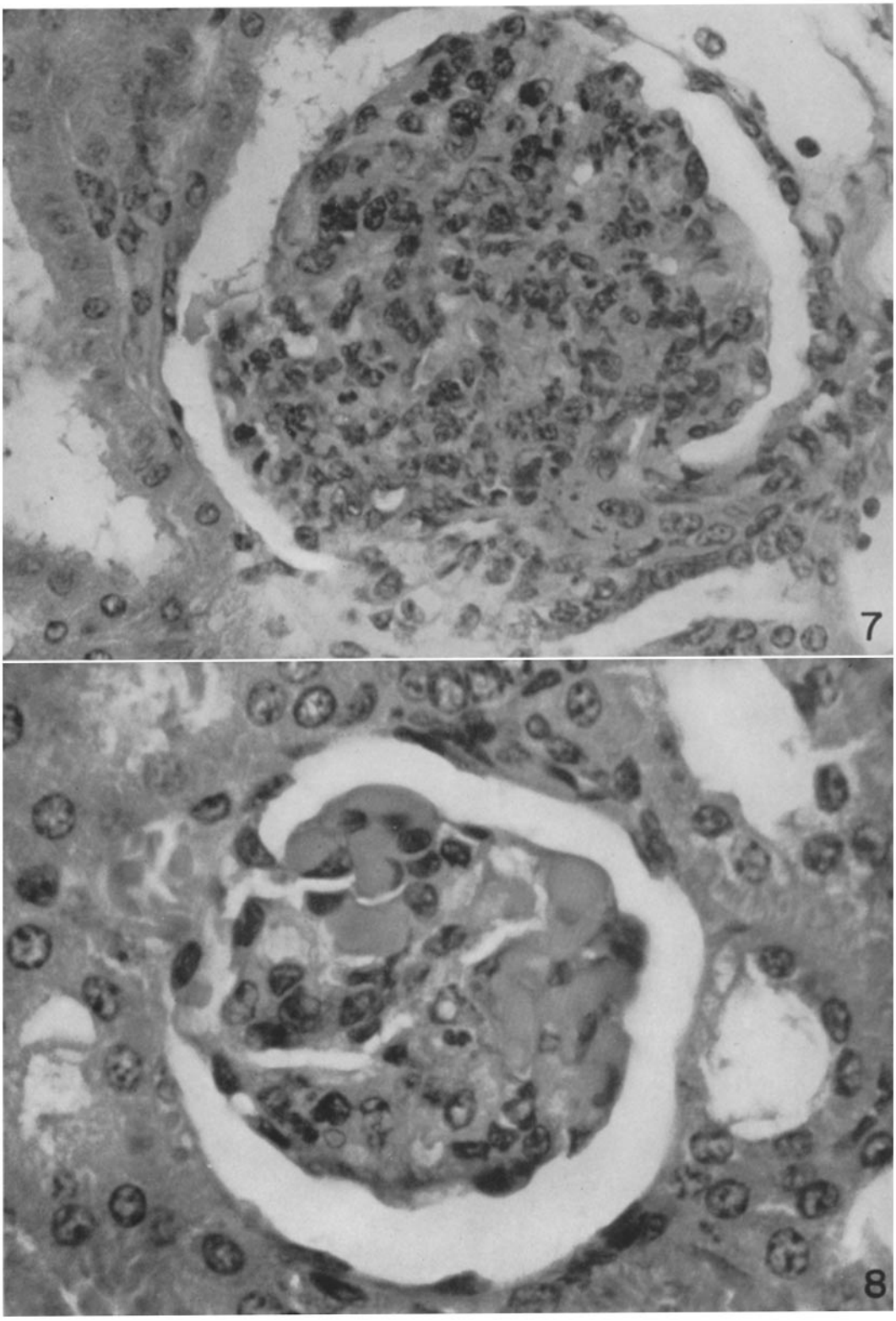


(McCluskey *et al.*: Passive serum sickness in mice)

PLATE 14

FIG. 7. Kidney of a mouse given 6 injections of ovalbumin-antiovalbumin complexes and sacrificed 24 hours after the last injection. The glomerulus is markedly enlarged, hypercellular and heavily infiltrated with neutrophiles, many of which are fragmented. Hematoxylin and eosin stain. × 500.

FIG. 8. Kidney of a mouse pretreated with cortisone, then given 3 injections of ovalbumin-antiovalbumin complexes and sacrificed 24 hours after the last injection. Some of the glomerular capillaries are filled with amorphous, eosinophilic material. Hematoxylin and eosin stain. × 800.



(McCluskey *et al.*: Passive serum sickness in mice)