

Failure of Cobra Venom Factor to Prevent the Generalized Shwartzman Reaction and Loss of Renal Cortical Fibrinolytic Activity

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Previous studies have shown that renal cortical fibrinolytic activity is absent in rabbits given one or two injections of endotoxin. Using a fibrin slide technic, we have studied the effect of endotoxin on cortical fibrinolytic activity in rabbits depleted of terminal complement components (C3-C9) by cobra venom factor. The loss of cortical fibrinolytic activity induced by endotoxin is not prevented by pretreatment with cobra venom factor. Additionally, this complement depletion did not prevent development of glomerular fibrin deposition, cortical necrosis or thrombocytopenia in the generalized Shwartzman reaction (Am J Pathol 74:19-30, 1974).

RENAL CORTICAL FIBRINOLYTIC ACTIVITY is absent in rabbits developing the generalized Shwartzman reaction and disappears following a single injection of endotoxin.¹ That cortical fibrinolytic activity is not inhibited following incubation of tissue sections in solutions containing high concentrations of endotoxin suggests that endotoxin-induced inhibition of kidney plasminogen activator is mediated through humoral factors.

As the complement system may play a role in the development of the generalized Shwartzman reaction,²⁻⁴ we have studied the effect of endotoxin on cortical fibrinolytic activity in rabbits depleted of terminal complement components (C3-C9) with cobra venom factor.

Materials and Methods

Endotoxin

Endotoxin (lipopolysaccharide B from *Escherichia coli* 026:B6) was obtained from Difco Laboratories, Detroit, Mich. The preparatory and provocative doses of endotoxin ranged between 0.35 to 0.40 mg.

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Supported by Grants AM 12375, HE 05662 and HE 06313 from the National Institutes of Health, The Minnesota Heart Association, The Cardiovascular Program Project Grant HE 06314 and the Graduate School of the University of Minnesota.

Accepted for publication September 19, 1973.

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Cobra Venom Factor

Lyophilized cobra venom (*Naja naja*) was obtained from the Ross Allen Reptile Institute, Silver Springs, Fla. Cobra venom anticomplementary factor was isolated by the method of Muller-Eberhard and Fjellstrom.⁵ Our anticomplementary factor contained 1600 µg of protein and 500 units of anticomplementary activity/ml, where 1 unit of anticomplementary activity is defined as the reciprocal of the dilution of 0.1 ml of cobra venom factor which reduces the hemolytic activity of 0.5 ml of a 1:20 dilution of normal human serum by 50% after incubation at 37 C for 20 minutes. The cobra venom factor was also shown to cause conversion of human C3 proactivator to C3 activator and to change the electrophoretic migration of properdin *in vitro*.⁶

Complement Determinations

Hemolytic complement titers ($C'H_{50}$) were determined by the method of Fong *et al.*⁷ The lowest detectable level was 10 units/ml. β 1C Globulin levels were assayed by the radial immunodiffusion method of Mancini *et al.*⁸ The antiserum to rabbit β 1C globulin was prepared according to the method of Mardiney and Muller-Eberhard.⁹ The lowest detectable level was 2% of a normal adult rabbit pool.

Platelet and White Cell Counts

Platelet (phase) and white blood cell counts were performed according to standard laboratory technics.¹⁰

Histology

Renal cortical tissue was obtained within 1 minute of death in all sacrificed animals or at necropsy in animals which expired and was placed into buffered formalin and isopentane cooled in liquid nitrogen. The preparation of fibrin slides and the histologic technics for light microscopy were performed according to methods previously described.¹¹ Fibrin slides (performed only on sacrificed animals) containing two renal cortical sections from each animal were fixed in 10% formalin after 40, 60, 80 and 100 minutes incubation. Tissues for light microscopy were stained with hematoxylin and eosin and phosphotungstic acid-hematoxylin stains. Rabbit fibrinogen was isolated by the method of Blomback and Blomback.¹² Fluorescein-isothiocyanate-conjugated goat anti-rabbit fibrinogen serum was prepared by methods previously described.^{13,14}

Experimental Design

Thirty 1-kg albino rabbits were studied in 8 groups. Group 1 (Table 1) contained 11 animals given three ear vein injections (100 units/injection) of cobra venom factor at 4-hour intervals. Twenty-four and 48 hours following the initial dose of cobra venom factor, all animals received ear vein injections of endotoxin in 2.0 ml of isotonic saline. Four animals were sacrificed at 6 hours and 4 at 48 hours following the second injection of endotoxin. Three animals died during this interval. Group 2 contained 8 animals given three injections of isotonic saline in place of cobra venom factor, followed by two injections of endotoxin. Two animals were sacrificed at 6 hours and 3 at 48 hours following the second injection of endotoxin. Three animals died during this interval. Group 3 contained 2 animals given three injections of cobra venom factor followed by two injections of isotonic saline in place of endotoxin. Animals were sacrificed 6 hours following the second injection of saline. Group 4 contained 3 animals given three injections of isotonic

Table 1—The Effect of Complement Depletion by Cobra Venom Factor on the Generalized Shwartzman Reaction and Renal Cortical Fibrinolytic Activity (CFA)

Group	Protocol*	No. of animals with glomerular fibrin		No. of animals with cortical necrosis	No. of animals with CFA†	
		Present	Absent		Absent	Present
1	CoF × 3 → E ₁ → E ₂	8	—	3	6	0
		—	3	1‡	0	2
2	S ₀ × 3 → E ₁ → E ₂	7	—	3	5	0
		—	1	1‡	—	—
3	CoF × 3 → S ₁ → S ₂	0	2	0	0	2
4	S ₀ × 3 → S ₁ → S ₂	0	3	0	0	3

* CoF indicates cobra venom factor; E₁ and E₂ indicated priming and provocative injections of endotoxin; S₀, S₁ and S₂ indicate isotonic saline in place of CoF, E₁ and E₂ respectively

† Only sacrificed animals studied

‡ Died overnight and not studied by fibrin slide test

saline in place of cobra venom factor and two injections of saline in place of endotoxin. Animals were sacrificed 6 hours following the final injection of saline. Blood samples were collected from the ear artery of animals in groups 1–4 immediately before the first dose of cobra venom factor or saline, before the first dose of endotoxin or saline, before or 4 hours following the second dose of endotoxin or saline, and immediately prior to sacrifice.

Group 5 (Table 2) contained 2 animals given three injections of cobra venom factor. Twenty-four hours following the initial injection of cobra venom factor, the animals were given a single injection of endotoxin. Group 6 contained 1 animal given isotonic saline in place of cobra venom factor. Group 7 contained 1 animal given isotonic saline in place of endotoxin and group 8 contained 2 animals given saline in place of both cobra venom factor and endotoxin. All animals in groups 5–8 were sacrificed 2 hours following the final injection of endotoxin or saline. Blood samples were obtained from animals in groups 5–8 prior to the first dose of cobra venom factor or saline, before endotoxin or saline, and immediately prior to sacrifice.

To evaluate the effect of endotoxin on the platelet and white blood cell counts in complement-depleted animals, 9 rabbits (4 from group 1) were given three injections (100 units) of cobra venom factor at 4-hour intervals, followed by injections of endotoxin 24 and 48 hours after the initial injection of cobra venom fac-

Table 2—The Effect of Complement Depletion by Cobra Venom Factor on the Loss of Renal Cortical Fibrinolytic Activity Following a Single Injection of Endotoxin

Group	Protocol	No. of animals	No. of animals		No. of animals with absent CFA
			with glomerular fibrin deposition	with cortical necrosis	
5	CoF × 3 → E ₁	2	0	0	2
6	S ₀ × 3 → E ₁	1	0	0	1
7	CoF × 3 → S ₁	1	0	0	0
8	S ₀ × 3 → S ₁	2	0	0	0

tor. As controls, 6 animals (4 from group 2) were given three injections of isotonic saline followed by two injections of endotoxin. Blood samples were obtained immediately before the first dose of cobra venom factor or saline, before the first dose of endotoxin, and 4 and 48 hours following the second dose of endotoxin.

Results

Effect of Complement Depletion on Development of the Generalized Shwartzman Reaction and Renal Cortical Fibrinolytic Activity (Groups 1-4)

Serum levels of hemolytic complement and β 1C globulin were not detected or markedly reduced following the administration of cobra venom factor to animals in groups 1 and 3 (Text-figures 1 and 2). No uniform changes in these parameters were observed in animals from groups 2 and 4.

Following two injections of endotoxin, 8 of 11 cobra venom factor-treated animals (group 1) and 7 of 8 saline controls (group 2) developed the generalized Shwartzman reaction, characterized by massive glomerular fibrin deposition (Table 1 and Figure 1). Kidneys from 3 of the animals with glomerular fibrin deposition in each group showed gross bilateral renal cortical necrosis, as did kidneys from 1 animal in each group without glomerular fibrin deposition (Figure 2). The intensity of immunofluorescence for fibrin was similar in animals with the generalized Shwartzman reaction who did or did not receive cobra venom factor (Figure 3A and 3B). No gross or histologic abnormalities were detected in kidneys from animals in Groups 3 and 4 (Table 1).

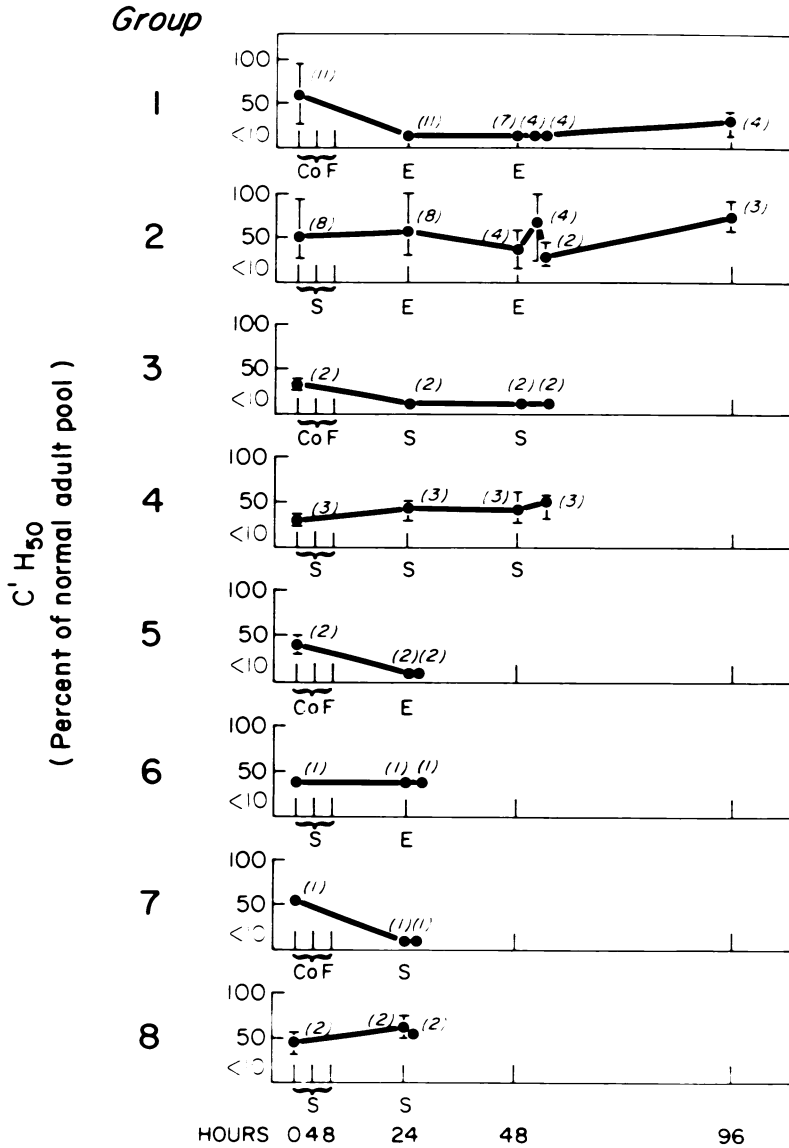
Tissue from 6 cobra venom factor-treated and 5 saline animals who developed the generalized Shwartzman reaction was studied by the fibrin slide technic. Cortical fibrinolytic activity was absent in renal cortex from all of these animals (Table 1). Lytic activity was present in cortex from 2 animals in group 1 which did not develop the generalized Shwartzman reaction and from all animals who received saline with or without cobra venom factor (groups 3 and 4).

Effect of One Injection of Endotoxin on Renal Cortical Fibrinolytic Activity in Rabbits Given Cobra Venom Factor (Groups 5-8)

The sera of the rabbits given cobra venom factor (groups 5 and 7) had no detectable hemolytic complement activity or β 1C (Text-figures 1 and 2). No uniform changes were seen in the sera of the remaining animals (groups 6 and 8).

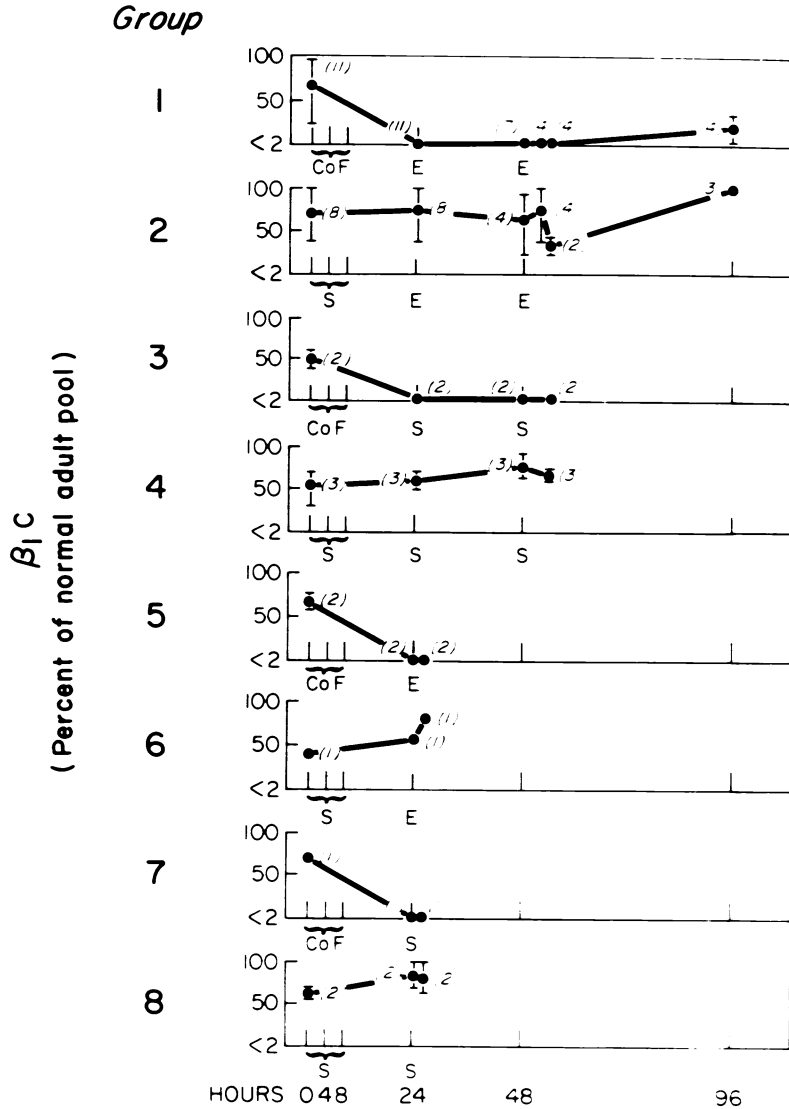
Tissue from all animals in groups 5 to 8 was normal by light and immunofluorescent microscopy (Table 2).

Renal cortical fibrinolytic activity was absent (Table 2) in tissue from animals given one injection of endotoxin preceded by cobra



TEXT-FIG 1—C₁H₅₀ levels in Groups 1-8 at varying time intervals after initial injection of either saline (S) or cobra venom factor (CoF). E = endotoxin. Numbers in parentheses indicate the number of animals studied at that time interval. Vertical lines indicate range.

venom factor (group 5) or saline (group 6). Lysis was present in tissue from animals given saline, with (group 7) or without (group 8) cobra venom factor.

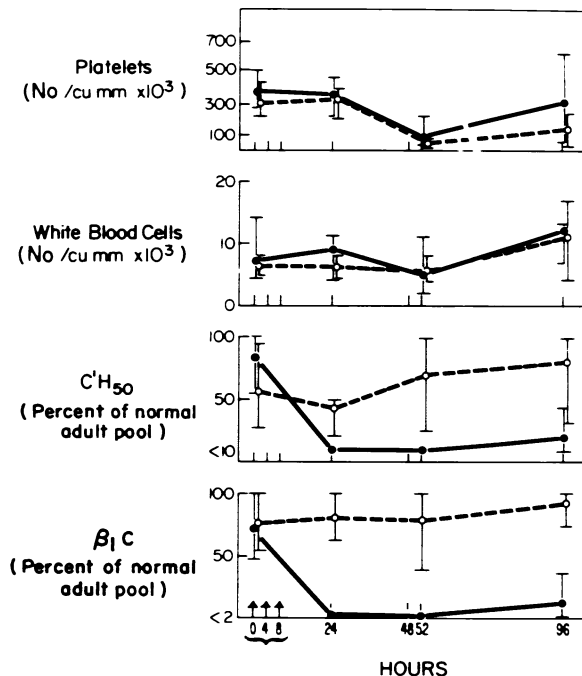


TEXT-FIG 2— β_{1C} globulin levels in groups 1-8. See Text-figure 1 for explanation.

Effect of Complement Depletion on Platelet and White Blood Cell Counts Following Two Injections of Endotoxin

Hemolytic complement and β_{1C} globulin levels were not detected or markedly reduced in sera from the 9 animals receiving cobra venom factor, whereas no uniform changes were seen in sera from the 6 saline controls (Text-figure 3).

TEXT-FIG 3—Platelet and white blood cell counts and complement levels in animals given two injections of endotoxin (at 24 and 48 hours) preceded by either cobra venom factor (solid lines) or saline (dotted lines) at 0, 4 and 8 hours. Vertical lines indicate ranges.



Four hours following the second injection of endotoxin, platelet counts were markedly reduced in both groups of animals (Text-figure 3). Parallel changes in white blood cell counts were observed in both groups.

Discussion

Although the role of complement in the generalized Shwartzman reaction has not been defined, several relationships between complement and endotoxin are clear. Endotoxin activates the terminal components of the complement system (C3–C9) with minimal consumption of the early components.^{2,15,16} This activation may occur by means of the properdin system.¹⁷ Endotoxin–complement interactions produce alterations in the coagulation system, vessel wall reactivity and permeability, platelet aggregation, and neutrophil chemotaxis.²

We have previously suggested that the initial injection of endotoxin prepares for the generalized Shwartzman reaction by inducing endothelial cell injury and depletion of plasminogen activator.¹ Further endothelial damage by the provocative dose of endotoxin, in the presence of diminished lytic capacity, may then allow thrombosis upon exposed glomerular basement membrane. As endotoxin did not inhibit cortical fibrinolytic activity *in vitro*, it seemed likely that products released following *in vivo* activation of the complement system by

endotoxin could produce endothelial cell damage and loss of renal cortical fibrinolytic activity.

Our results suggest that depletion of the terminal components of complement by cobra venom factor does not prevent loss of cortical fibrinolytic activity following development of the generalized Shwartzman reaction or following a single dose of endotoxin. Cobra venom factor alone had no effect on lytic activity. Thus it appears that the terminal components of complement are not involved in the mechanism by which lysis is lost.

The development of thrombocytopenia and the generalized Shwartzman reaction in decomplemented animals is at variance with other reports. Polak and Turk were able to prevent the local Shwartzman reaction in 8 of 9 guinea pigs by administration of rabbit anti-guinea pig C3 serum prior to the provoking dose of endotoxin.³ However, the administration of anti-thymocyte serum, which produced a marked fall in hemolytic complement titers sufficient to inhibit development of the Arthus reaction failed to prevent the local Shwartzman reaction in 8 of 9 guinea pigs. They suggested that C3 is necessary for the development of the local Shwartzman reaction, whereas the Arthus reaction requires other complement components.

Fong and Good prevented the local and generalized Shwartzman reactions by administering cobra venom factor prior to endotoxin.⁴ Although we have been unable to resolve the differences between the studies, it is evident that Fong and Good used a different method to prepare cobra venom factor,¹⁸ as well as a different preparation of endotoxin. Previous studies have indicated that our endotoxin preparation activates the complement and properdin system *in vitro*.^{6,19} That 4 rabbits given cobra venom factor and endotoxin developed frank cortical necrosis suggests that depletion of the terminal complement components does not prevent the occurrence of necrosis.

In rabbits prepared with Thorotrast®, antigen-antibody complexes have been substituted for endotoxin in the induction of the generalized Shwartzman reaction, suggesting that endotoxin may act through an immunologic mechanism.²⁰ However recent evidence suggests that the complement system may not be involved in the pathogenesis of certain forms of experimental immune nephritis. Salmon *et al* by administering a large dose of antigen to rabbits previously sensitized to ovalbumin, were able to produce a disease similar to the generalized Shwartzman reaction.²¹ Administration of heat-aggregated human fraction II, depleting the serum complement to undetectable levels prior to injection of the provoking antigen, failed to block either thrombocytopenia or

intravascular coagulation. In addition, depletion of the terminal components of complement with cobra venom factor fails to alter the character or severity of the glomerulonephritis in acute experimental immune complex disease.²² Indeed, a protective effect of the complement system has been demonstrated, since C5 and C6 are involved in the detoxification of bacterial endotoxin by serum.²³ It is also of interest that cobra venom factor will not prevent endotoxin shock in animals.²⁴

These experiments do not prove with certainty that complement plays no role in the genesis of the generalized Shwartzman reaction since it is possible that undetectable minute quantities of terminal complement components may be present in the cobra venom-treated rabbit. However, it seems clear that loss of renal cortical fibrinolytic activity and the deposition of fibrin within glomeruli was not inhibited by the striking complement depletion observed in these experiments.

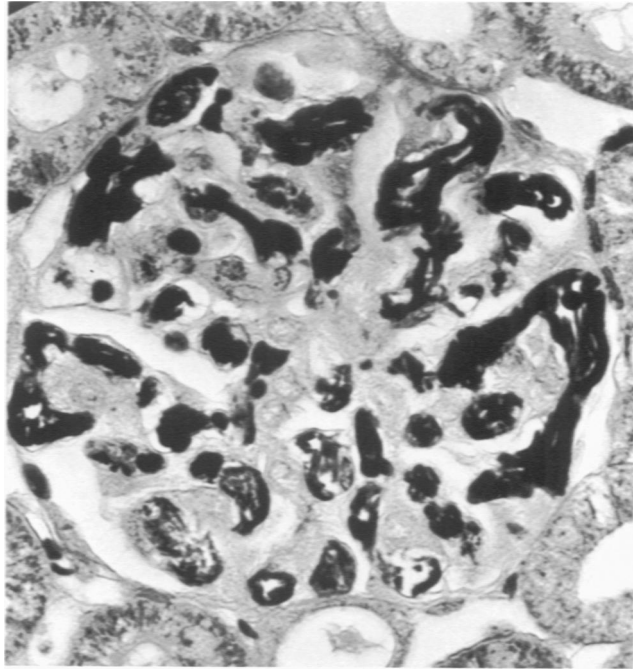
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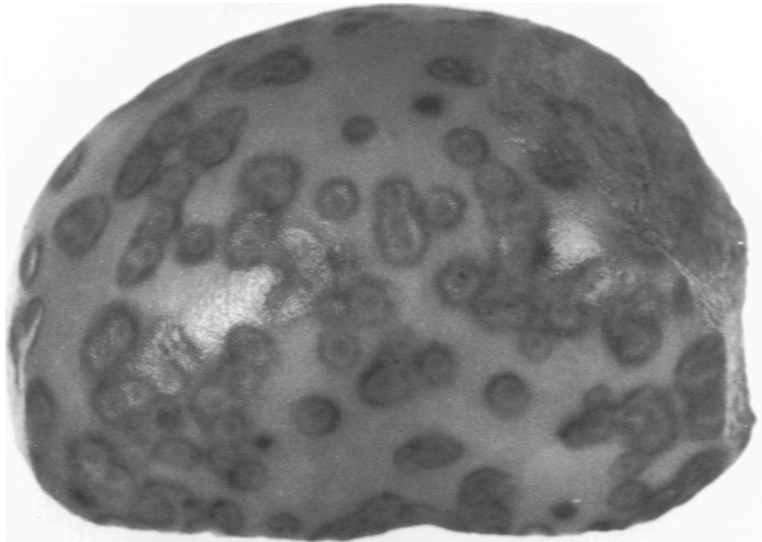
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Acknowledgments

Dr. Bergstein was the recipient of Postdoctoral Fellowship 5T01 HD 00053-12 from the US Public Health Service; he is presently with the Department of Pediatrics, UCLA Center for the Health Sciences, Los Angeles, CA 90024. The authors thank Mrs. Fern Knudson and Mr. Richard Schrader for their technical assistance.



1



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Fig 1—Glomerulus from a rabbit given saline followed by two injections of endotoxin demonstrating massive glomerular fibrin deposition. A similar degree of fibrin deposition was observed in tissue from 8 to 11 rabbits given cobra venom factor and endotoxin (Phosphotungstic acid-hematoxylin stain, $\times 210$). **Fig 2**—Cortical necrosis in a kidney from a rabbit given cobra venom factor followed by two injections of endotoxin ($\times 3$).

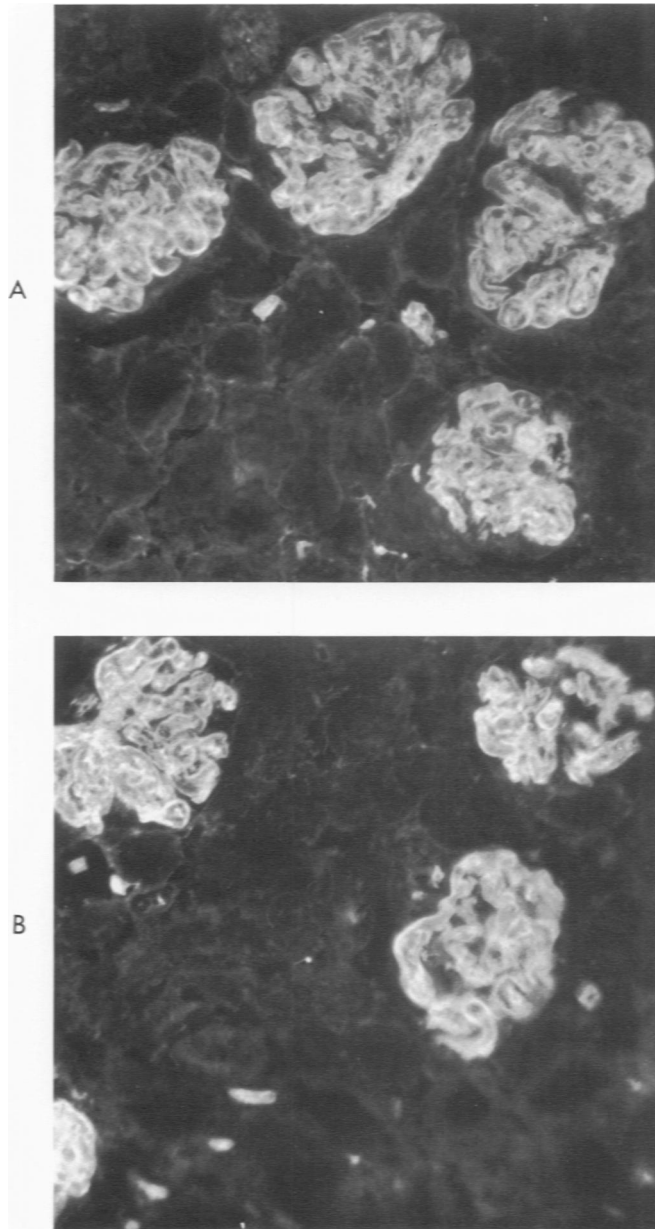


Fig 3—Immunofluorescent studies on kidneys from a rabbit given cobra venom factor (**A**) and another rabbit given saline (**B**). Both animals subsequently received two injections of endotoxin. The sections were stained with goat anti-rabbit fibrinogen serum. Massive amounts of fibrin could be detected in the glomerular capillaries of both animals. No qualitative or quantitative differences were observed in animals with the generalized Shwartzman reaction which did or did not receive cobra venom factor ($\times 72$).