

STUDIES ON THE BIOLOGIC RELATIONSHIP OF ENDOTOXIN  
AND OTHER TOXIC PROTEINS\*

I. COMPARISON OF THE PROPERTIES OF SNAKE VENOM AND ENDOTOXIN

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Several biologic interrelationships of Gram-negative endotoxins and snake venom, particularly that of *Agkistrodon piscivorus*, have been suggested by scattered evidence: cross-protection between the venom of *A. piscivorus* and the endotoxin of *Salmonella* in mice (1); protection against the Shwartzman reaction by prior intradermal injection of snake venom in rabbits (2); and production of hemorrhagic necrosis in tumors by both snake venom (3) and endotoxin (4, 5). The events in shock and death after snake venom infusion in dogs (6, 7) resemble those occurring after injection of endotoxin (8). References 8 to 13 are reviews on endotoxin, and references 14 to 17 reviews on snake venoms.

The problems raised by this earlier work prompted our study of the capacity of snake venom to reproduce the well characterized biologic responses to endotoxins, as well as an immunologic investigation of the two antigens. Among the aspects of the problem considered in this first paper are:

1. Whether antigens are common to snake venom and endotoxin;
2. Whether snake venom produces a pyrogenic effect, particularly the bimodal fever curve which follows injection of endotoxin (13);
3. Whether the initial leukopenia and subsequent polymorphonuclear leukocytosis produced by endotoxin also occurs after intravenous injection of snake venom (9);

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4. Whether snake venom can either prepare for or provoke the local or generalized Shwartzman reaction (17);
5. Whether prior intradermal or intravenous injections of snake venom produce refractoriness to endotoxin (2); whether animals hyperimmune to snake venom are resistant to production of the Shwartzman reaction; and whether repeated injections of snake venom produce refractoriness to snake venom (1);
6. Whether tolerance to repeated injections of endotoxin (10) produces refractoriness to snake venom (1);
7. Whether injections of snake venom enhance the production of antibody to purified protein antigens (10); and
8. Whether injections of zymosan (18–20) or thorotrast (11), or exposure of the toxin to ferrous iron (21, 22), all of which alter the response to endotoxin, have similar effects on the toxicity of snake venom.

#### *Materials and Methods*

*Materials.*—*Escherichia coli* endotoxin (Difco Laboratories, Detroit, lot 0127.B8); dehydrated *Aghistrodon piscivorus* (water moccasin) venom (Ross Allen Reptile Institute, Silver Springs, Florida); pyrogen-free saline solution from Cutter Laboratories, Berkeley; zymosan from the laboratories of the late Dr. Louis Pillemer (Western Reserve University, Cleveland); thorotrast (colloidal thorium dioxide) from Testagar & Company, Detroit; and reagent grade ferrous sulfate were used in these studies.

*Experimental Animals.*—Rabbits were albino hybrids of both sexes, weighing 1 to 4 kg, obtained from a single local breeder. Mice used were weanling, male, C<sub>57</sub>Bl mice of subline<sub>1</sub>, weighing 18 gm, from Roscoe B. Jackson Laboratories, Bar Harbor, Maine. Animals were fed a balanced diet and offered water *ad libitum*.

*Methods.*—Immunologic comparisons of snake venom and endotoxin were made using a micro modification of the double gel diffusion method of Ouchterlony (23, 24) in which the bands were stained according to the method of Scheidegger (25); the double diffusion agar precipitin method of Preer (26); micro immunoelectrophoresis, as described by Scheidegger (25); and the qualitative precipitin method of Swift *et al.* (27).

*Preparation of Antisera.*—Snake venom antisera were prepared by immunizing 3 to 4 kg rabbits by intradermal injection of 10 mg of snake venom suspended in 0.2 cc of Freund's adjuvant. The adjuvant was prepared by adding 300 mg of killed, dried, ground *Mycobacterium butyricum* (Difco Laboratories, Detroit) to 10 cc of incomplete Freund's adjuvant, prepared with 1.5 ml arlancel A (generously supplied by Atlas Powder Company, Wilmington, Delaware) and 8.5 ml bayol F (Esso Standard Oil Company, New York). 1 volume of the adjuvant was emulsified with 1 volume of a snake venom solution containing 50 mg of snake venom per ml in 0.85 per cent sodium chloride. Each animal received 0.1 cc of this mixture into each hind foot-pad. Three weeks following this injection, 1 cc containing 1.0 mg of alum-precipitated snake venom was given intravenously every other day for three injections. 7 to 10 days following the last injection of venom, the rabbits were bled, serum pooled, and merthiolate added to a final concentration of 1-10,000.

Antisera against *E. coli* endotoxin was prepared by the same method as that used for the venom, except that 200 gamma of endotoxin was used in the initial intradermal injection and 1 mg of alum-precipitated endotoxin was injected on each of the 3 days by intravenous injection.

*Hyperimmune Rabbits.* Hyperimmunity to snake venom was produced in a group of

rabbits by immunizing them with an intradermal injection of 10 mg of snake venom in 0.2 cc of Freund's adjuvant and, 3 weeks later, giving a course of intravenous snake venom injections extended over a 3 week period. During the first week, 1.0 mg of venom was given every other day, for three injections, followed, on the same schedule, by three injections of 10 mg of venom the 2nd week, and three injections of 20 mg of venom the 3rd week. At the end of this period rabbits were able to tolerate 2 total lethal doses ( $LD_{100}$ ) of venom (20 mg intravenously) with no apparent ill effects.

*Local and Generalized Shwartzman Reactions.*—The local Shwartzman phenomenon was produced in 1 kg rabbits by two appropriately spaced injections of *E. coli* endotoxin. The intradermal or preparatory injection (500 gamma in 0.25 ml saline) was followed in 18 to 24 hours by an intravenous injection of the same material (300 gamma in 2.0 ml saline). The local injection site was examined 4 to 6 hours after the intravenous injection for the characteristic hemorrhagic and necrotic processes characteristic of this phenomenon.

The general Shwartzman reaction was produced by two intravenous injections of endotoxin, 18 hours apart. Preparation with 300 gamma of endotoxin and challenge with 200 gamma were found to produce the highest incidence of bilateral renal cortical necrosis. The animals were killed 24 hours after the last endotoxin injection. Sections of kidney stained with hematoxylin and eosin and periodic acid-Schiff reagents were examined for evidence of hemorrhage and necrosis, as well as for fibrinoid deposition in the glomerular capillary loops.

*Quantitative Antibody Determinations.*—Antibody production to bovine serum albumin (BSA) (Armour Laboratories, Chicago) was measured by the quantitative precipitin technique of Heidelberger and MacPherson (28) on sera collected 9, 14, 19, and 30 days after injection of BSA.

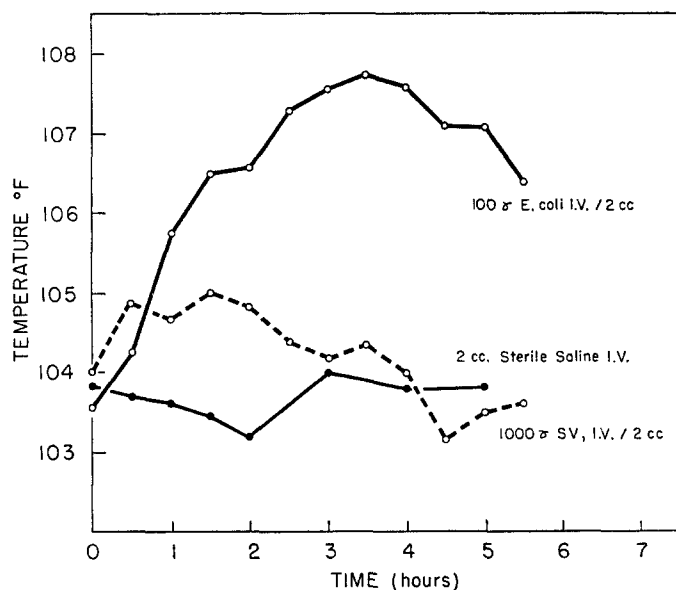
## RESULTS

*Immunochemical Comparison of Endotoxin and Snake Venom.*—Spink (6) was unable to show cross-reactivity between water moccasin venom and endotoxins of *Salmonella typhi*, *S. typhimurium*, and *E. coli*. Our results confirm these observations. No evidence of identical or cross-reacting components was obtained by capillary tube precipitin reactions, agar diffusion techniques of Ouchterlony and Preer, or immunoelectrophoretic characterization. In both the endotoxin-anti-endotoxin system and the snake venom-anti-snake venom system, multiple antigen-antibody complexes were shown to exist, but at no time could we demonstrate correspondence of lines or spur formation with a micro modification of the Ouchterlony technique (Figs. 1 to 6) or identity of bands by the Preer and immunoelectrophoretic methods.

*Effect of Snake Venom and Endotoxin on the Temperature Response.*—The temperature response to endotoxin is bimodal, with an initial elevation at 1 hour, a leveling off or return toward normal at 2 hours, and a final peaking to the highest level 4 to 5 hours after injection. By contrast, snake venom injected intravenously either produced no fever or produced a small initial elevation of temperature, without a tendency to a bimodal pyrogenic response. Text-fig. 1 illustrates the serial temperature response to endotoxin, snake venom, and saline, in groups of rabbits.

*Effect of Endotoxin and Snake Venom on Leukocytes.*—After administration of endotoxin, the total leukocyte count and neutrophil count showed a drop be-

gining 30 minutes after injection and continuing for 2 hours, and then a rise to a maximum at 24 hours. In contradistinction, following injection of venom, the total leukocyte count did not fall, but rather began a steady rise to a peak 8 hours after injection. A parallel rise in the number of neutrophils was noted. Composite curves of the total white blood count and total neutrophil count following injection of snake venom and endotoxin in rabbits are compared in Text-fig. 2.

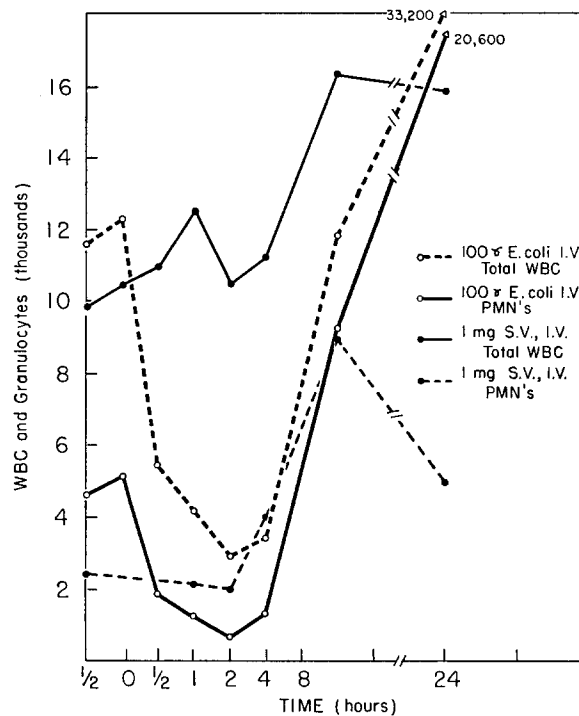


TEXT-FIG. 1. Comparison of the mean febrile response of 4 rabbits receiving intravenous endotoxin (100 gamma), 4 rabbits receiving intravenous snake venom (1 mg), and 4 rabbits receiving intravenous saline (2 cc). Intravenous injection at 0 time.

*Necrotizing Reactions Produced by Snake Venom and Endotoxin.*—Shimkin and Zon (3) showed that injection of snake venom produced hemorrhagic necrosis in mouse tumors, remindful of the response of certain tumor-bearing rats and mice to administration of endotoxin. In the gross and histologically, the necrosis is similar to that of the local Shwartzman phenomenon (17). In these experiments we attempted to use snake venom to prepare for or provoke the local Shwartzman reaction.

As shown in Table I, snake venom did not prepare for or provoke the local Shwartzman phenomenon in any instance. It is of interest, however, that the rabbits injected intradermally with 500 gamma of endotoxin, followed in 24 hours by 300 gamma of venom intravenously, often died immediately after the injection of snake venom.

*Preparation for and Provocation of the Generalized Shwartzman Reaction.*—When rabbits were prepared for the generalized Shwartzman reaction with intravenous snake venom, challenged 24 hours later with intravenous endotoxin, and sacrificed 24 hours after the provoking injection, none showed the bilateral renal cortical necrosis characteristic of the generalized Shwartzman phenomenon. Table II.



TEXT-FIG. 2. Comparison of the leukocyte response to an intravenous injection of 1 mg of water moccasin venom (4 rabbits) and 100 gamma of *E. coli* endotoxin (4 rabbits). The mean cell count in 4 rabbits is represented by each point on the graph. Intravenous injections at 0 time.

When intravenous snake venom was used to provoke the generalized Shwartzman reaction, following preparation with intravenous endotoxin, all of the rabbits died following the injection. The increased susceptibility of the endotoxin-treated animals to venom proved to be consistent.

*Effect of Snake Venom in Producing Refractoriness to Tissue-Necrotizing Effects of Endotoxin.*—Peck and Sobotka (2) reported that prior intradermal injection of water moccasin venom, in rabbits, produced a refractory state toward the local Shwartzman reaction when preparation and provocation were attempted

at 2 weeks, 3 to 4 weeks, and 5 weeks after injection of venom. Attempting to confirm these findings, we injected rabbits with 500 gamma of snake venom intradermally. Fifteen days later, the animals were prepared for the local

TABLE I  
*Failure of Snake Venom to Prepare for or Provoke the Local Shwartzman Reaction*

No. of rabbits	Preparatory intradermal injection*	Provoking intravenous injection†	Local Shwartzman reactions‡
	<i>gamma</i>	<i>gamma</i>	
8	500 endotoxin	300 snake venom	0/3
8	500 endotoxin	300 endotoxin	8/8
10	500 snake venom	300 endotoxin	0/10
10		300 snake venom	0/10

\* Preparatory injections were made intradermally over the abdomen.

† Challenge injections were made *via* the marginal ear vein 18 to 24 hours later.

‡ No. of positive reactions over the No. of alive animals at 24 to 48 hours.

|| Five of these animals died immediately following intravenous injection of snake venom.

TABLE II  
*Failure of Snake Venom to Prepare for or Provoke the Generalized Shwartzman Reaction*

No. of rabbits	Preparatory intravenous injection	Provoking intravenous injection	Controls	Generalized Shwartzman reaction*
	<i>gamma</i>	<i>gamma</i>	<i>gamma</i>	
11	500 snake venom	200 endotoxin		0/11
9	2000 snake venom	200 endotoxin		0/9
9	300 endotoxin	500 snake venom		‡
9	300 endotoxin	200 endotoxin		6/9
10			500 snake venom	0/10
10			2000 snake venom	1/10
10			300 endotoxin	2/10
10			200 endotoxin	3/10

\* Animals were sacrificed 24 hours after provoking injection. Bilateral renal cortical necrosis required for positive results. No. of positive reactions over the No. of rabbits challenged.

‡ All animals died immediately after the provoking injection of snake venom.

Shwartzman reaction by intradermal injection of 500 gamma of endotoxin at a site 7 to 10 cm from the site of the venom injection. Provocation of the Shwartzman reaction was accomplished 24 hours later with 300 gamma of endotoxin intravenously. In a second group a 25 day interval between the venom injection and the preparing endotoxin injection was used. The results are summarized in Table III. No evidence of protection was observed, since in almost every

instance the rabbits developed the local Shwartzman reaction at the site of the intradermal injection of endotoxin.

Attempts to protect against the local and generalized Shwartzman reactions by a single *intravenous* injection of 1 mg of venom 15 days prior to preparation were also unsuccessful.

TABLE III

*Failure of Intradermal Snake Venom to Protect against Shwartzman Reaction in the Rabbit*

Experimental plan	Results* of Shwartzman reaction at:			
	15 days‡		25 days‡	
	Local§	General	Local§	General
500 gamma of snake venom intradermally¶ prior to Shwartzman reaction . . . . .	9/10	4/5	8/10	4/7
Controls—no prior intradermal snake venom . . .	10/10	3/6	10/10	5/8

\* No. of positive reactions 18 to 24 hours after provoking injection.

‡ No. of days after intradermal snake venom injection.

§ Local preparatory injection of 500 gamma endotoxin intradermally, with provoking injection of 300 gamma endotoxin 18 hours later.

|| General provoking injection of 200 gamma endotoxin intravenously 18 hours after provoking injection for local reaction in the same animals.

¶ Intradermal injections 7 to 10 cm away from sites of local Shwartzman.

TABLE IV

*Production of the Local Shwartzman Reaction in Rabbits Hyperimmunized with Water Moccasin Venom*

Experimental plan	Local Shwartzman reaction
Local Shwartzman reaction* in rabbits hyperimmune to 2 LD <sub>100</sub> of snake venom . . . . .	8/8
Local Shwartzman reaction* in normal rabbits . . . . .	8/8

\* Local preparatory injection of 500 gamma endotoxin intradermally, with provoking injection of 300 gamma endotoxin intravenously 18 hours later.

Hyperimmune animals, able to tolerate 2 LD<sub>100</sub> of venom without apparent ill effect, were prepared and challenged for the local Shwartzman reaction. They showed no resistance to development of the Shwartzman phenomenon. Table IV.

*Refractoriness to Snake Venom after Repeated Injections of Venom.*—Refractoriness to endotoxin is produced by serial intravenous administration of sublethal doses, so that quantities of endotoxin originally productive of vigorous

systemic adjustments have greatly diminished effects. The febrile response, hemorrhagic and necrotizing phenomena, and lethal action are either eliminated or markedly modified.

Table V summarizes experimental efforts to produce refractoriness to venom in rabbits, starting with 100 gamma of venom and increasing the dosage to 5 mg by the 8th day. As the table indicates, there was no evidence of increasing tolerance to the lethal action of the venom as a consequence of this regimen; indeed, it seemed to result in increased susceptibility. Three animals died when 3 mg was given, and the remaining seven died following the 5 mg dose.

TABLE V  
*Failure to Produce Refractory State by Repeated Injections of Moccasin Venom*

Experimental plan	Day	Snake venom	Deaths*
		<i>gamma</i>	
Daily intravenous injections of increasing doses of snake venom, 100 to 5000 gamma, over an 8 day period	1	100	0
	2	200	0
	3	400	0
	4	800	0
	5	1000	0
	6	2000	0
	7	3000	3
	8	5000	7
Control rabbits, given 1 intravenous injection of snake venom		5000	6/10

\* No. of rabbits of the initial group of 10 that died within 24 hours following the injection of snake venom.

*Refractoriness to Snake Venom after Repeated Injections of Endotoxin.*—Zahl and Hutner (1) reported that repeated intraperitoneal injections of endotoxin, in mice, resulted in increased resistance to challenge with an LD<sub>50</sub> of *Agkistrodon piscivorus* venom. Experiments attempting to confirm this early report were undertaken. C<sub>57</sub>B1 mice were given four injections of endotoxin: 10 gamma on the 1st and 3rd days, 25 gamma on the 5th and 50 gamma on the 7th day. A week later the animals were injected intraperitoneally with snake venom, 14 of them with 250 gamma and 10 with 125 gamma. The results are summarized in Table VI. Although 250 gamma of venom killed all 10 control mice, 2 of the 14 endotoxin-treated animals survived. The 125 gamma dose killed 6 of 10 control animals, but killed none in the endotoxin-treated group.

Similar studies were done with rabbits. 1 kg animals received daily increasing doses of endotoxin intravenously over a 7 day period; all were highly refractory to the pyrogenic effect of endotoxin by the 7th day. Ten days later each rabbit



TABLE VI  
*Refractoriness to Snake Venom Following Repeated Intraperitoneal Injections of Endotoxin in Mice*

Experimental plan	Time interval*	Intravenous injection of snake venom	Deaths†
Endotoxin (intraperitoneally) every 2nd day in succeeding quantities of 10, 10, 25, and 50 gamma, for a total of four injections	1 week	250	12/14
		125	0/10
Snake venom controls		250	10/10
		125	6/10

\* From last injection of endotoxin to challenge of snake venom.

† Within 24 hours.

TABLE VII  
*Refractoriness to Snake Venom Following Repeated Intravenous Injections of Endotoxin in the Rabbit*

Experimental plan	Day* challenged	Snake venom mg.	Deaths‡		Controls§	
			1 hr	24 hrs.	Deaths‡	
					1 hr.	24 hrs.
7 daily intravenous injections of 10 to 1000 gamma endotoxin in 1 kg rabbits	10	10	10/10		10/10	
	3	5	5/10	9/10	10/17	10/17
	7	5	1/6	1/6	3/8	3/8
	10	5	0/12	4/12	4/9	5/9
	15	5	2/12	4/12	8/14	11/14
Total—controls, 5 mg.					25/48	29/48

\* No. of days between last injection of endotoxin series and administration of intravenous snake venom.

‡ No. of deaths within the period specified over the total No. of animals.

§ Rabbits which received snake venom alone.

was given 10 mg of snake venom, an LD<sub>100</sub>. None of the animals survived. Table VII.

However, another group of rabbits received the same course of endotoxin and was challenged with 5 mg of venom, an LD<sub>50</sub>. The interval between the last endotoxin injection and venom administration was 3, 7, 10, and 15 days in different groups. As shown in Table VII, a consistent low grade protection

was demonstrated, providing that the venom was administered at least 7 days after the last endotoxin injection.

*Effect of Snake Venom on Antibody Synthesis.*—Gram-negative bacterial endotoxins have been shown to enhance antibody production to unrelated simple protein antigen in the rabbit (10). To test the ability of snake venom to enhance antibody production, 1 mg of venom, a sublethal but toxic dose, was given intravenously to 3 kg rabbits with 30 mg of BSA. A control group received BSA alone. Nine, 14, 19, 30 days later the animals were bled and antibody levels determined by the quantitative precipitin method. No differences in the amount of antibody produced in the experimental and control groups were detected at any time.

TABLE VIII  
*Effect of Zymosan on Susceptibility of Rabbits to Snake Venom*

Experimental plan	Zymosan*	Snake venom	Deaths‡
	mg.	mg.	
Zymosan intravenously, followed in 1 hr. by intravenous snake venom . . . . .	50	1	1/6
Snake venom controls . . . . .	—	1	1/6
Zymosan controls . . . . .	5	—	0/7

\* The zymosan was furnished to us by the late Dr. L. Pillemer.

‡ No. dead at 48 hours over total No. of animals.

*Effects of Zymosan and Thorotrast on Toxicity of Snake Venom.*—An intravenous injection of zymosan decreases the normal tolerance of the host to subsequent injection of either viable bacteria (18, 19) or bacterial endotoxin (20). As shown in Table VIII, zymosan pretreatment did not seem to affect the susceptibility of rabbits to snake venom.

Rabbits pretreated with thorotrast are also hypersusceptible to injections of Gram-negative bacterial endotoxins (11). Refractoriness produced by repeated daily injections of increasing doses of endotoxin is abolished by intravenous injection of thorotrast (29).

To determine whether thorotrast has an effect on the normal tolerance of rabbits to snake venom, 1 kg animals were given 5 cc of thorotrast intravenously, followed in 2 hours by 1 mg of snake venom intravenously. As shown in Table IX, thorotrast-treated animals are apparently more susceptible to the venom; however, this enhanced susceptibility was reflected in late deaths (more than 1 hour after administration of venom), in contrast to the hypersusceptibility of endotoxin-treated animals (Table II) which was reflected in immediate death.

*Effect of Ferrous Iron on Toxicity of Snake Venom.*—Ferrous iron has been

shown to interfere with some of the toxic properties of Gram-negative bacterial endotoxin, the local and generalized Shwartzman reaction and the lethal effects, but not with its pyrogenicity. (21, 22).

In the present studies varying amounts of iron sulfate were incubated with a 10 mg dose of venom at 37°C for 6 hours. As shown in Table X, a protective effect was noted with 5 and 20 mg doses of ferrous sulfate. Thus, snake venom,

TABLE IX  
*Effect of Thorotrast on Susceptibility of Rabbits to Snake Venom*

Experimental plan	Thorotrast	Snake venom	Deaths*	
			1 hr.	24 hrs.
	<i>cc.</i>	<i>mg.</i>		
Thorotrast intravenously, followed in 2 hrs. by intravenous snake venom.....	5‡	1	0/8	4/8
Snake venom controls.....	—	1	1/6	1/6
Thorotrast controls.....	5	—	0/7	0/7

\* No. of deaths within the period specified over the total No. of animals.

‡ The highest dose found to be tolerated by the 1 kg rabbit.

TABLE X  
*Effect of Iron on the Toxicity of Snake Venom in the Rabbit*

Experimental plan	Iron*	Snake Venom	Deaths‡
	<i>mg.</i>	<i>mg.</i>	
Snake venom incubated at 37°C with varying concentrations of iron sulfate for 6 hrs.§	1	10	10/10
	5	10	4/8
	20	10	6/10
Snake venom incubated at 37°C for 6 hrs.	—	10	10/10

\* Iron as iron sulfate; molecular weight = 278.02, iron = 20 per cent.

‡ No. dead at 24 hours over the total No. of animals.

§ Final volumes injected = 2 cc.

like endotoxin, loses some of its toxic properties when incubated with ferrous iron.

#### DISCUSSION

A review of the major characteristics of endotoxin and snake venom, compared in this study and investigated earlier by others, indicates that their differences are more substantial than their similarities. First, there was no evidence of immunologic cross-reactivity between these compounds, although this was sought with highly sensitive techniques. The *E. coli* endotoxin was

markedly pyrogenic; *Agkistrodon piscivorus* venom only minimally so. Endotoxin produced profound initial polymorphonuclear leukopenia followed by extreme leukocytosis, whereas venom produced no leukopenia and minimal leukocytosis. In contrast to the highly active endotoxin, snake venom had no capacity to prepare for or provoke the local Shwartzman reaction, or to prepare for the generalized Shwartzman reaction. In studies of the capacity of venom to provoke the generalized Shwartzman reaction, we found that a prior injection of endotoxin markedly enhanced susceptibility of rabbits to snake venom, a phenomenon which has proved to be consistent and which will be analyzed in a later paper (30).

In this series of experiments we were unable to confirm Peck and Sobotka's (2) finding of protection against the Shwartzman phenomenon by prior intradermal administration of snake venom to rabbits. Hyperimmune rabbits, able to tolerate 2 LD<sub>100</sub> of venom, also showed no resistance to the Shwartzman reaction. The reported protection cannot, therefore, be explained on the basis of cross-immunity resulting from antigenic determinants shared by snake venom and endotoxin. Peck and Sobotka's finding may be attributable to repeated production of the Shwartzman phenomenon in the same group of rabbits rather than to any property of the snake venom.

A parallel to the well known refractoriness to endotoxin, produced by serial intravenous administration of sublethal doses, was sought with the use of a similar series of increasing doses of snake venom up to an LD<sub>50</sub>. A refractory state was not produced; contrariwise, animals were susceptible to smaller amounts of venom after this regimen.

Attempts to increase the resistance of mice to snake venom by prior injection of endotoxin met with some success and was extended to rabbits. The enhanced resistance to venom observed in mice confirms the studies of Zahl and Hutner (1), and may be related to the capacity of endotoxin to enhance resistance of mice (31-33) and rabbits (34) to infections with organisms immunologically unrelated to the source of the endotoxin.

Results of the studies attempting to increase resistance of rabbits to snake venom by pretreatment with endotoxin are inconsistent. Animals challenged with an LD<sub>50</sub> of venom 3 days following completion of the endotoxin course showed increased susceptibility to venom; a second group challenged with the same dose of venom 7 days after the last endotoxin injection demonstrated low grade resistance. It may be that endotoxin injections just prior to administration of venom interfered with low grade protection against venom developed early in the course, consistent with prior findings that large doses of endotoxin may interfere with refractoriness to endotoxin or negate the non-specific resistance to infection produced by endotoxin (35).

Certainly, snake venom differed from endotoxin (10) in failing to alter the immunologic response to the simple protein antigen, BSA.

Although zymosan (18, 19), a compound which interferes to some extent with reticuloendothelial function, did not enhance susceptibility to snake venom, colloidal thorium dioxide, a compound which increases susceptibility to many of the effects of endotoxin (11), also made rabbits more susceptible to snake venom. It is of further interest that ferrous sulfate interferes with the toxicity of both endotoxin and venom; the basis for this action remains enigmatic (21, 22) and deserves further extensive investigation.

Although the differences in the immunochemistry and biologic effects of endotoxin and snake venom comprise the major conclusion of this study, certain similarities and interrelationships between them, particularly the interrelated resistance mechanisms of the host to both compounds, are of interest and deserving of continued study.

#### SUMMARY

1. *Agkistrodon piscivorus* venom and *E. coli* endotoxin were shown to be immunologically distinct, and to differ in certain biologic properties: effects on immune response, body temperature, and circulating leukocyte count, and capacity to prepare for and provoke the local and generalized Shwartzman reaction.
2. Neither a single prior injection of venom nor the existence of hyperimmunity to lethal doses of venom protected rabbits against the local and generalized Shwartzman reaction.
3. Serial intravenous injections of sublethal doses of venom produced enhanced susceptibility to venom rather than refractoriness.
4. Preparation for both the local and generalized Shwartzman reaction with endotoxin appeared to enhance susceptibility of rabbits to challenge with venom.
5. Tolerance to bacterial pyrogens established by repeated injections of endotoxin is paralleled by increased resistance to snake venom given at least 1 week later, in mice and rabbits.
6. Zymosan failed to enhance susceptibility of rabbits to venom, but thorostrast increased the number of late deaths from venom.
7. Exposure of venom to ferrous sulfate interferes with its toxicity.

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## EXPLANATION OF PLATE 51

Antigenic analysis of water moccasin venom and *E. coli* endotoxin by the micro modification of the double gel diffusion technique of Ouchterlony. All photographs  $\times 2\frac{1}{2}$ .

FIG. 1. Reaction of antivenom with: 10 mg/ml venom in wells 1 to 8; Rabbit antivenom in center well.

FIG. 2. Reaction of antivenom with: 10 mg/ml venom in wells 1, 6, 7, and 8; 100 mg/ml endotoxin in wells 2, 3, 4, and 5; Rabbit antivenom in center well.

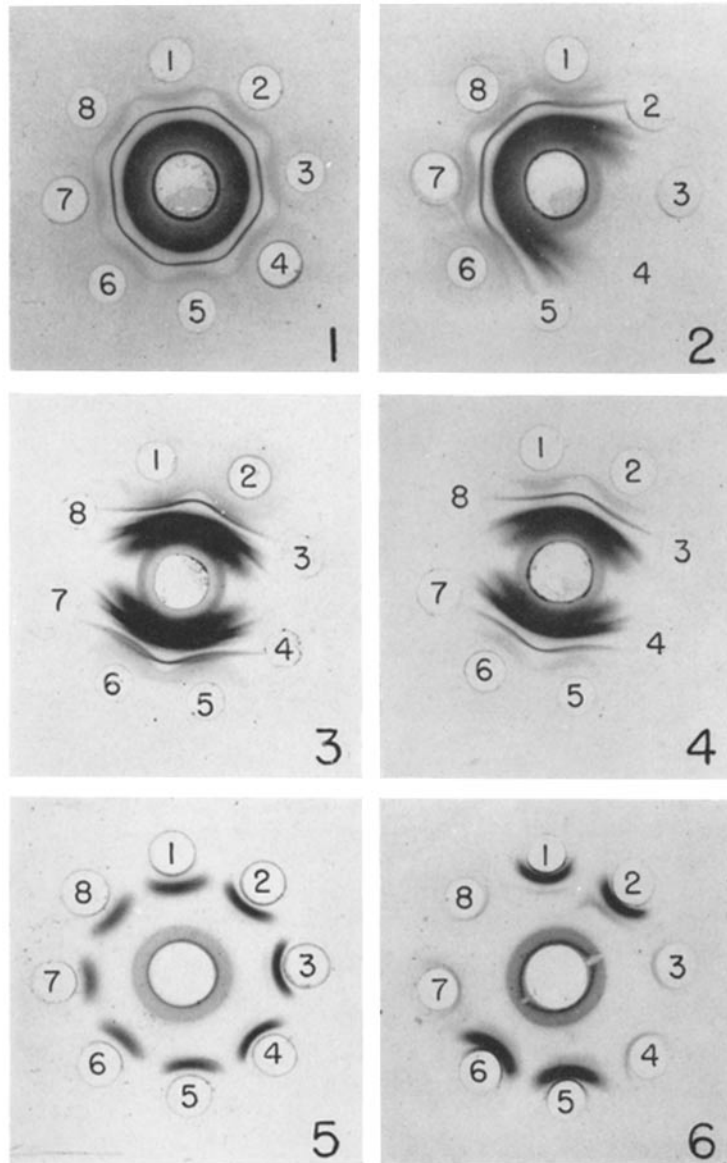
FIG. 3. Reaction of antivenom with: 10 mg/ml venom in wells 1, 2, 5, and 6; 100 mg/ml endotoxin in wells 3, 4, 7, and 8; Rabbit antivenom in center well.

FIG. 4. Reaction of antivenom with: 10 mg/ml venom in wells 1, 2, 5, and 6; 0.145 M saline in wells 3, 4, 7, and 8; Rabbit antivenom in center well.

FIG. 5. Reaction of anti-*E. coli* antibody with: 100 mg/ml endotoxin in wells 1 to 8; Rabbit anti-*E. coli* serum in center well.

FIG. 6. Reaction of anti-*E. coli* antibody with: 10 mg/ml venom in wells 1, 2, 5, and 6; 100 mg/ml endotoxin in wells 3, 4, 7, and 8; Rabbit anti-*E. coli* serum in center well.





(Condie *et al.*: Endotoxin and toxic proteins. I)