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

II. Enhancement of Susceptibility to Snake Venom by Endotoxin

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Endotoxin-induced changes in nonspecific resistance to bacteria and viruses have received widespread attention in recent years. Animals pretreated with Gram-negative endotoxins are more susceptible to viruses and both Gram-negative and Gram-positive bacteria for a short period, and many of them later develop increased resistance to such organisms and their destructive effects (1–6). The spectrum of increased resistance was recently reviewed by Shilo (7), Gledhill (8), and Bennett and Cluff (9).

In an investigation of the biologic interrelationships of *Escherichia coli* endotoxin and *Agkistrodon piscivorus* venom, another instance of endotoxin-induced hypersusceptibility came to our attention (10). Rabbits given a single injection of endotoxin intradermally or intravenously showed greatly increased susceptibility to *A. piscivorus* venom administered 18 to 24 hours later; and those given a 7-day course of endotoxin injections were highly susceptible to venom challenge as long as 3 days after completion of the endotoxin regimen.

To define these biologic interrelationships between endotoxin and snake venom further, studies were undertaken to determine: (*a*) doses of endotoxin and venom needed to establish and demonstrate hypersusceptibility; (*b*) the latency of the endotoxin-induced hypersusceptibility state (EIHS), if any; (*c*) the duration of EIHS following a single injection of endotoxin; (*d*) the role of route of injection in inducing and demonstrating EIHS; (*e*) the effect of

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prior administration of venom on susceptibility to endotoxin; (*f*) the existence and nature of the EIHS demonstrated with venoms other than *Agkistrodon piscivorus*.

Materials and Methods

Endotoxin used in all of these studies was *Escherichia coli* endotoxin (Difco Laboratories, Detroit, lot 0127.B8). Venoms obtained in dehydrated form from the Ross Allen Reptile Institute, Silver Springs, Florida were: water moccasin (*Agkistrodon piscivorus*), Florida diamondback rattlesnake (*Crotalus adamanteus*), yellow cobra (*Naja flava*), Russell's viper (*Vipera russellii*), common krait (*Bungarus candidus*), and South American rattlesnake (*Crotalus durissus terrificus*). The tiger snake (*Notechis scutatus*) venom used was kindly furnished to us by Dr. Findlay E. Russell of Los Angeles. All solutions were prepared just prior to injection with pyrogen-free saline from Cutter Laboratories, Berkeley.

The experimental animals in this entire series were 1 kg white rabbits of both sexes, obtained from a single local breeder, fed Purina rabbit pellets and offered water *ad libitum*.

Routes of Toxin Administration.—In all experiments precautions were taken to insure complete dissemination of the material injected by each of the routes studied.

Intradermal injections were made with tuberculin syringes, using new, ½ inch, 27-gauge hypodermic needles. A standard volume of 0.20 ml was injected at each site on the shaved skin of the abdomen. When larger quantities of material were injected, multiple skin sites were used so that the volume of 0.20 ml at a single location was not exceeded. Rabbits were discarded from the experiments when the intradermal injection was not into the dermis, as judged by the raising of a superficial, blanched bleb.

Extreme care was taken with *intraperitoneal injections* to assure that all material was introduced into the peritoneal cavity. Short, ½ inch, 25-gauge hypodermic needles were used. The injections were made in the lower half of the abdomen through the vertical midline.

Intramuscular injections were all made into the quadriceps muscle group with a 1 inch, 25-gauge hypodermic needle.

Intravenous injections were made into the marginal ear vein with 1½ inch, 23-gauge needles.

RESULTS

Lethal Dose Ranges of Endotoxin and Snake Venom, with Comparison of Time of Death.—To establish the lethal ranges of *E. coli* endotoxin and *A. piscivorus* venom, the toxins used in most of the experiments to follow, the studies summarized in Table I were performed. Although a few rabbits died following intravenous administration of smaller doses of endotoxin, only the 10 mg dose approached an LD_{100}. An LD_{100} is difficult to establish with endotoxin; in every group of rabbits injected with massive doses of endotoxin, occasional rabbits survive, a variability attributed to age differences (11), daily cyclic phenomena (12), nutritional state (13), changes in intestinal flora (14), and even seasonal variations (15).

The results of the venom studies established 8 mg of *A. piscivorus* venom as the LD_{100} and 5 mg as the LD_{50}, as shown in Table I.

Following administration of a lethal dose of the moccasin venom, the rabbits regularly succumb within an hour. The pupils contract and the ears droop; the animals became hyperactive, often cry out, develop rigidity, seizures, and die.

By contrast, animals dying of endotoxin sicken over a period of hours, develop labored respirations, and die. Seizures and hyperactivity are almost never seen in the endotoxin group.

Hypersusceptibility of Endotoxin-Treated Animals to Water Moccasin Venom.—The hypersusceptibility of rabbits pretreated with 100 gamma of endotoxin intravenously to 500 gamma of moccasin venom given intravenously 24 hours later is evident in the data of Table II. The deaths in the hypersusceptible animals were immediate, and the sequence preceding death was the

TABLE I

Lethal Doses of Endotoxin and Moccasin Venom, with a Comparison of Time of Death

Intravenous dose	Time of death					
	Endotoxin			Snake venom		
	1 hr.	24 hrs.	48 hrs.	1 hr.	24 hrs.	48 hrs.
10 gamma	0/8*	0/8	0/8	0/10	0/10	0/10
100 "	3/150	10/150	20/150	0/10	0/10	0/10
200 "	0/8	2/8	2/8	1/10	1/10	1/10
250 "	0/10	2/10	3/10	0/11	0/11	0/11
300 "	0/12	1/12	1/12	—	—	—
500 "	0/10	2/10	3/10	25/200	28/200	30/200
1 mg	1/20	1/20	1/20	9/42	10/42	11/42
2 "	—	—	—	3/29	3/29	3/29
5 "	0/5	1/5	1/5	50/110	55/110	60/110
8 "	—	—	—	40/40	—	—
10 "	2/5	3/5	4/5	58/61	58/61	58/61

* No. of deaths over the total No. of animals.

same as that described above for animals receiving lethal doses of snake venom alone.

Development of Hypersusceptibility and Its Duration.—Table III summarizes the data on hypersusceptibility as a function of the interval between endotoxin treatment and snake venom challenge. There is a latent period lasting from ½ to ¾ of an hour before hypersusceptibility is clearly demonstrable, a period of 3 days of relatively stable susceptibility, with a falling off after that time. Although not shown in the table, subsequent studies have indicated that the EIHS continues for at least 7 days, but at a reduced level. At 7 days it may be elicited by 1 mg of venom, still within the sublethal range for most normal animals.

The pattern of hypersusceptibility to venom does not coincide with that of

TABLE II

Hypersusceptibility to Water Moccasin Venom in Endotoxin-Treated Rabbits

Experimental plan	No. of rabbits	Deaths*	
		1 hr.	24 hrs.
Intravenous injection of 100 gamma of endotoxin, followed in 24 hrs. by 500 gamma of snake venom	30	23/28	23/28
Controls—100 gamma of endotoxin only	15	0/15	2/15
Controls—500 gamma of venom only	15	1/15	1/15

* No. of deaths within the period specified (after venom injection) over the No. of animals surviving the endotoxin injection.

TABLE III

Duration of Hypersusceptibility to Water-Moccasin Venom in Endotoxin-Treated Rabbits‡*

Interval between endotoxin and snake venom injections	No. of rabbits	Deaths§	
		1 hr.	24 hrs.
hrs.			
0	15	0/15	3/15
¼	10	3/10	5/10
½	15	7/15	9/15
¾	10	5/10	5/10
1	40	30/39	33/39
2	100	81/98	87/98
4	12	12/12	—
8	10	8/10	8/10
16	5	3/3	—
24	30	23/28	23/28
48	5	3/5	3/5
72	15	9/11	9/11
96	5	0/5	0/5

* 500 gamma *Agkistrodon piscivorus* venom, intravenously.

‡ 100 gamma *E. coli* endotoxin, intravenously

§ Number of deaths within the period specified (after snake venom injection) over the No. of animals surviving the endotoxin injection.

endotoxin-induced susceptibility to infectious processes. The latter lasts about 24 to 48 hours, followed by a period of increased resistance (7, 8).

EIHS to Varying Doses of Water Moccasin Venom.—Table IV is a compilation of experiments in which varying doses of venom were given intravenously to rabbits pretreated with 100 gamma of endotoxin 2 hours earlier. The smallest

amount of *A. piscivorus* venom which was consistently lethal to hypersusceptible animals was 500 gamma.

Doses of Endotoxin Producing EIHS to Moccasin Venom.—The effect of varying the dose of endotoxin was then studied, using a uniform venom dosage of 500 gamma and a consistent 2-hour interval between toxin injections. As shown in Table V, hypersusceptibility is produced by endotoxin doses as low as 1 gamma, and is consistent through the range up to 100 gamma.

A second group of animals was pretreated with 1000 gamma of endotoxin and challenged with smaller doses of venom. The susceptibility of these rabbits to smaller quantities of venom is evident when these data (Table V) are compared to those in Table IV on groups pretreated with the routine 100 gamma of endotoxin.

TABLE IV

Susceptibility of Endotoxin-Treated Rabbits to Various Doses of Moccasin Venom

Experimental plan	No. of rabbits	Snake venom	Deaths*	
			1 hr.	24 hrs.
		gamma		
Intravenous injection of 100 gamma of endotoxin followed in 2 hrs. by varying doses of water moccasin venom	10	50	1/10	1/10
	12	100	7/12	7/12
	100	500	81/98	87/98
	17	1000	14/17	15/17
Intravenous injection of varying doses of water moccasin venom only	10	50	0/10	0/10
	10	100	0/10	0/10
	10	500	1/10	1/10
	42	1000	9/42	10/42

* No. of deaths within the period specified (after snake venom injection) over the No. of animals surviving the endotoxin injection.

Effect of Route of Injection of Endotoxin on Susceptibility to Water Moccasin Venom.—Table VI records a typical experiment on the effects of intradermal and intraperitoneal injection of endotoxin on hypersusceptibility to venom; comparable data for endotoxin treatment by the intravenous route are shown in Table III.

Hypersusceptibility was demonstrated in animals given endotoxin by all three routes. In the intradermal group, the peak of susceptibility was early, dropping off sharply between 4 and 24 hours. Following intraperitoneal administration, susceptibility increased gradually up to 48 hours, followed by a sharp decline. As noted earlier, hypersusceptibility following intravenous injection of endotoxin is evident within an hour, remains at a relatively constant level for 72 hours, and drops off thereafter.

Effect of Route of Injection of Snake Venom on Demonstration of EIHS.—A group of rabbits were prepared for snake venom administration by intravenous injection of 100 gamma of endotoxin. The interval between injections, the dose of water moccasin venom, and the route of injection were varied. Table VII records the various combinations of these factors, the experimental results, and appropriate control data.

TABLE V

Hypersusceptibility to Moccasin Venom of Rabbits Given Various Doses of Endotoxin

No. of rabbits	Endotoxin*	Snake venom‡	Deaths§	
			1 hr.	24 hrs.
	gamma	*gamma*		
6	1	500	6/6	
6	10	500	5/6	5/6
4	50	500	3/4	3/4
100	100	500	81/98	87/98
8	1000	100	7/8	7/8
10	1000	50	9/10	9/10
10	1000	10	1/10	1/10
10	1	—	0/10	0/10
10	10	—	0/10	0/10
10	50	—	0/10	0/10
10	100	—	0/10	1/10
10	—	10	0/10	0/10
10	—	50	0/10	0/10
10	—	100	1/10	1/10
10	—	500	1/10	1/10

* *E. coli* endotoxin, administered intravenously 2 hours before challenge with venom.

‡ *Agkistrodon piscivorus* venom, given intravenously.

§ No. of deaths within the period specified (after snake venom injection) over the No. of animals surviving the endotoxin injection.

When untreated rabbits were given 500 gamma to 50 mg of venom either intradermally or intraperitoneally, all the animals survived. One of two rabbits receiving 200 mg intradermally died; both animals receiving 200 mg intraperitoneally lived. As shown in Table VII, hypersusceptibility was demonstrated in endotoxin-treated rabbits with the 50 mg venom dose given either intradermally or intraperitoneally.

Twenty and 50 mg of venom were lethal for normal rabbits when injected intramuscularly, but 500 gamma and 5 mg were not. There is evidence of increased susceptibility to intramuscular venom in the endotoxin-treated group,

but it was demonstrated less uniformly than it was in the intravenous, intradermal, and intraperitoneal groups.

It is noteworthy that rabbits tolerate, with no apparent ill effects, massive quantities of snake venom administered intraperitoneally. Marked resistance to intradermal venom was also evident.

Order of Injection of Endotoxin and Snake Venom.—The effect of reversing the order of administration of endotoxin and *Agkistrodon piscivorus* venom is

TABLE VI

Hypersusceptibility to Moccasin Venom of Rabbits Given Endotoxin by Various Routes

Experimental plan	Route* of endotoxin administration	Interval between injections	Deaths‡	
			1 hr.	24 hrs.
		hrs.		
100 gamma endotoxin intradermally, followed at different intervals by 500 gamma of snake venom intravenously	ID	4	8/12	8/12
	ID	24	2/18	2/18
	ID	48	2/8	3/8
	ID	72	1/6	1/6
As above, but with endotoxin injected intraperitoneally	IP	4	2/14	2/14
	IP	24	3/16	7/16
	IP	48	5/8	5/8
	IP	72	0/7	0/7
Endotoxin controls: 100 gamma endotoxin only	ID	—	0/10	0/10
	IP	—	0/10	0/10
Snake venom controls: 500 gamma venom only, intravenously	—	—	1/10	1/10

* Routes of administration: ID = intradermal, IP = intraperitoneal.

‡ No. of deaths within the period specified (after snake venom injection) over the total No. of animals.

summarized in Table VIII. Prior injection of 500 gamma of venom enhanced susceptibility to lethal effects of 100 gamma of endotoxin when the interval between injections was an hour. None of the deaths occurred within the 1st hour. The effect was not demonstrated when venom was given 4 hours before endotoxin. When 1 mg of venom was given 1 hour before 100 gamma of endotoxin, all the animals died immediately after endotoxin administration.

Thus, prior injection of snake venom increases susceptibility to endotoxin when the interval between injections is short. The data also indicate that delayed deaths are more significant when venom is given before the endotoxin.

Effect of Endotoxin-Pretreatment on Susceptibility to Other Venoms.—The early EIHS studies were conducted with a single venom, *Agkistrodon piscivorus*;

TABLE VII

Hypersusceptibility of Endotoxin-Treated Rabbits to Moccasin Venom Given by Various Routes

Intravenous endotoxin	Interval between injections	Snake venom	Route* of snake venom administration	Deaths‡		
				1 hr.	12 hrs.	24 hrs.
gamma	*hrs.*	*mg.*				
100	1	0.5	ID	1/8	1/8	2/8
100	1	5.0	ID	0/8	2/8	4/8
100	2	50.0	ID	2/4	4/4	
100	24	50.0	ID	2/3	3/3	
—	—	0.5	ID	0/8	0/8	0/8
—	—	5.0	ID	0/8	0/8	0/8
—	—	50.0	ID	0/6	0/6	0/6
—	—	200.0	ID	1/2	1/2	1/2
100	1	0.5	IP	0/8	0/8	3/8
100	1	5.0	IP	0/7	0/7	0/7
100	2	50.0	IP	3/4	3/4	3/4
100	24	50.0	IP	1/3	1/3	1/3
—	—	0.5	IP	0/8	0/8	0/8
—	—	5.0	IP	0/8	0/8	1/8
—	—	50.0	IP	0/6	0/6	0/6
—	—	200.0	IP	0/2	0/2	0/2
100	2	0.5	IM	0/8	0/8	1/8
100	2	1.0	IM	0/9	3/9	3/9
100	2	5.0	IM	1/4	2/4	3/4
100	24	5.0	IM	1/3	1/3	1/3
—	—	0.5	IM	0/8	0/8	0/8
—	—	1.0	IM	0/8	0/8	1/8
—	—	5.0	IM	0/4	0/4	0/4
—	—	20.0	IM	0/2	2/2	
—	—	50.0	IM	0/2	2/2	

* Routes of administration: ID = intradermal, IP = intraperitoneal, IM = intramuscular.

‡ No. of deaths within the period specified (after snake venom injection) over the total No. of animals.

the studies were later extended to other venoms of the three major families of poisonous snakes: *Crotalus adamanteus* and *Crotalus durissus terrificus*, both of the Crotalidae; *Naja flava*, *Notechis scutatus*, and *Bungarus candidus*, all of the family Elapidae; and *Vipera russellii* representing the Viperidae.

Table IX summarizes further experimentation with Crotalidae venoms in

normal and endotoxin-treated rabbits. Hypersusceptibility to *Crotalus adamanteus* venom in endotoxin-treated animals was comparable to that noted with *Agkistrodon piscivorus* venom. The lethal effect was immediate, and the results

TABLE VIII

Susceptibility to Endotoxin in Rabbits Given Prior Injections of Moccasin Venom

No. of rabbits	Intravenous snake venom	Interval between injections	Intravenous endotoxin	Deaths*	
				1 hr.	24 hrs.
	gamma	*hrs.*	*gamma*		
4	1000	1	100	4/4	
8	500	1	100	0/8	4/8
13	500	4	100	0/13	1/13
6	1000	—	—	0/6	0/6
6	500	—	—	0/6	0/6
6	—	—	100	0/6	0/6

* No. of deaths within the period specified (after endotoxin) over the total No. of animals.

TABLE IX

Effect of Prior Injection of Endotoxin on Susceptibility of Rabbits to Crotalidae Venoms

Venom	Intravenous endotoxin	Interval between injections	Intravenous snake venom	Deaths*	
				1 hr.	24 hrs.
	gamma	*hrs.*	*gamma*		
Crotalus adamanteus (Florida diamondback rattlesnake)	100	24	500	6/6	
	100	48	500	7/7	
	—	—	500	2/6	2/6
Crotalus durissus terrificus (South American rattlesnake)	100	2	100	0/5	0/5
	100	24	100	0/5	0/5
	—	—	100	0/6	0/6
	—	—	200	3/7	7/7
	—	—	500	3/5	5/5

* No. of deaths within the period specified (after injection of snake venom) over the total No. of animals.

were the same with intervals of 24 and 48 hours between endotoxin and venom administration. With *Crotalus durissus terrificus* venom, however, increased susceptibility of endotoxin-treated animals was not demonstrated, as shown in Table IX. The studies showed that delayed deaths are a significant factor in untreated animals receiving this venom, in contrast to the immediate lethal effect of *Agkistrodon piscivorus* and *Crotalus adamanteus* venoms.

TABLE X

Effect of Prior Injection of Endotoxin on Susceptibility of Rabbits to Elapidae Venoms

Venom	Intravenous endotoxin	Interval between injections	Intravenous venom	Deaths*		
				1 hr.	12 hrs.	24 hrs.
	gamma	*hrs.*	*gamma*			
Bungarus candidus (common krait)	100	2	1	0/4	0/4	0/4
	100	2	5	0/8	8/8	—
	—	—	1	0/4	0/4	0/4
	—	—	5	0/8	8/8	—
Naja flava (yellow cobra)	100	4	100	0/4	0/4	0/4
	100	24	100	0/4	0/4	0/4
	—	—	100	0/10	0/10	0/10
	—	—	200	0/4	0/4	4/4
	—	—	500	0/4	1/4	4/4
Notechis scutatus (tiger snake)	100	2	1	0/9	3/9	5/9
	—	—	1	0/6	0/6	0/6
	—	—	2.5	3/4	3/4	3/4
	—	—	5	5/6	5/6	5/6
	—	—	10	4/4	—	—

* Deaths within the period specified (after administration of venom) over the total No. of animals.

TABLE XI

Effect of Prior Injection of Endotoxin on Susceptibility of Rabbits to Russell's Viper Venom

Experimental plan	Deaths*	
	1 hr.	24 hrs.
100 gamma of endotoxin intravenously, followed in 2 hrs. by 100 gamma of Russell's viper venom intravenously	5/5	—
Endotoxin only—100 gamma intravenously	0/5	0/5
Snake venom only, intravenously		
100 gamma	0/7	0/7
200 "	3/4	3/4
500 "	3/3	3/3

* No. of deaths within the period specified (after administration of venom) over the total No. of animals.

Table X includes data on the susceptibility of rabbits to Elapidae venoms following endotoxin injection. Pretreatment with 100 gamma of endotoxin did not seem to increase vulnerability to either *Naja flava* (yellow cobra) or *Bungarus candidus* (common krait) venoms. Such treatment did affect the response to

sublethal doses of *Notechis scutatus* (tiger snake) venom, however; 1 gamma of the venom, which resulted in no deaths in an untreated group, killed 5 of 9 endotoxin-treated rabbits within 24 hours. Deaths were delayed: none died within the 1st hour, three between 1 and 12 hours, and 2 more between 12 and 24 hours after venom challenge.

A total lethal dose of Russell's viper venom lies somewhere between 200 and 500 gamma, as shown in Table XI. One hundred gamma, a sublethal dose in normal rabbits, was given to an endotoxin-injected group, resulting in immediate death of all the animals.

DISCUSSION

Endotoxin-induced alterations in non-specific resistance to bacteria and viruses have been the subject of many extensive reviews in recent years (7, 8, 16). Our earlier studies (10) suggested that such alterations also affect resistance to *Agkistrodon piscivorus* venom, and the present study sought to establish and define the endotoxin-induced hypersusceptibility state (EIHS) to venom in terms of the significant variables: the doses of the two toxins and their routes of injection, and the time factors, including latency and total duration of increased susceptibility.

Hypersusceptibility to *A. piscivorus* venom was induced by intradermal, intravenous, and intraperitoneal injection of endotoxin. For 30 to 45 minutes following intravenous administration, EIHS was not clearly demonstrable with intravenous venom; hypersusceptibilty continued at a relatively constant level for 3 days, followed by at least 4 days of reduced, but still significant susceptibility to venom. With both the intradermal and intraperitoneal routes of endotoxin injection, the EIHS was of shorter duration; with the intraperitoneal route the latent period was prolonged.

The studies on the influence of route of injection of endotoxin on the EIHS are provocative. It has been reasoned that intradermal injection of endotoxin does little more than produce local inflammation. Indeed, quantities of endotoxin which are lethal by the intravenous route are not pyrogenic when injected intradermally (17). The production of EIHS to venom by intradermal administration of endotoxin (in the same doses as those used intravenously) suggests separation of the pyrogenic and resistance-altering properties of endotoxin. The results of intraperitoneal preparation add further support to this separation. Intraperitoneal endotoxin is highly pyrogenic during the early period (17), while hypersusceptibility to venom is most marked at 48 hours after intraperitoneal administration.

The degree of hypersusceptibility to venom did not appear to be dose-related, judging from the consistent demonstration of the phenomenon as the endotoxin dose varied from 1 to 100 gamma. That this is true for only a limited range is indicated by the susceptibility to lower venom doses in rabbits pretreated with 1 mg of endotoxin. Because this series aimed at a broad definition of EIHS to

venom, the relationship of endotoxin dose to both degree and duration of hypersusceptibility was not pursued beyond the studies already reported.

EIHS was demonstrated with venom injected intradermally and intraperitoneally, although untreated animals were very resistant to massive doses of venom administered by these routes. There was less resistance to intramuscular venom by normal animals, but only a moderate increase in susceptibility to sublethal doses by this route in the endotoxin-treated group.

The fact that normal rabbits tolerated large doses of *Agkistrodon* venom (25 times the intravenous LD_{100}) given by careful intraperitoneal injection, without signs of toxicity, suggests the possibility, among others, of an active detoxifying process.

The studies defining the EIHS to venom suggest that endotoxin breaks down some of the natural resistance usually afforded to such toxins as snake venom. On the surface, the simplest explanation of the observed phenomenon is that endotoxin and snake venom, already known to have some properties in common, have an additive effect in these animals. However, when the same doses of the toxins were administered in reverse order, the results were very different. There was some evidence of reduced resistance to endotoxin in venom-pretreated animals; however, it was demonstrated less consistently (half the animals died) and in a different way. The deaths were delayed and preceded by progressive signs of toxicity, as in animals dying of lethal doses of endotoxin, and were not the rapid deaths characteristic of EIHS.[1] In addition, the enhanced susceptibility to endotoxin was of short duration, not demonstrable 4 hours after injection, in contrast to the 72 hours of high susceptibility to venom following endotoxin administration. We believe, then that EIHS is a one-way phenomenon, and that the animals die immediately of toxins in the venoms to which, for unknown reasons, they are inordinately susceptible because of the preceding endotoxin injection. The latent period following the administration of endotoxin, before the EIHS is demonstrable, points to alteration of a mechanism in the body that would normally protect against such a toxin as snake venom.

Among the more prominent systems studied in relation to endotoxin-induced alteration of non-specific resistance have been the reticuloendothelial system (RES) (18, 19), the properdin system (1, 6), the blood-brain barrier (20), the hemodynamic mechanisms leading to shock (16), and iron metabolism (21, 22). The studies of endotoxin-induced hypersusceptibility to venoms bear directly on some of these problems.

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[1] When 1 mg. of venom was used in the preparatory injection, immediate deaths followed endotoxin administration. This was not pursued, but it probably reflects persistence of a substantial amount of venom in the circulation at the time of endotoxin injection. Venom circulates for long periods, and the persistence of venom in the blood may account for the endotoxin deaths following the lower venom doses as well.

Injection of small quantities of endotoxin produce marked increases in RES activity. Various colloidal agents blockade the RES and increase susceptibility to a variety of infections, paralleling the effects of endotoxin injection (19, 23). In earlier experiments we showed that injection of thorotrast, one of these colloids, increased susceptibility of rabbits to late death from the venom of *Agkistrodon piscivorus*, with toxic signs resembling those of animals dying of endotoxin administration, a very different pattern from the rapid death of normal and endotoxin-treated animals given the venom. We believe that RES blockade is probably not the cause of hypersusceptibility of endotoxin-treated rabbits to moccasin venom, although it may play a role in endotoxin-induced susceptibility to *Notechis scutatus* venom which results in late death.

The role of properdin in the non-specific defense of animals against a variety of invading micro-organisms has been disputed (23, 24). Intravenous injections of zymosan, which lower perperdin levels, apparently decrease the normal tolerance of hosts to injected bacteria and endotoxin (2, 25, 26). Zymosan had no effect on susceptibility to *Agkistrodon piscivorus* venom in our studies (10); thus, whatever the role of properdin levels is in susceptibility to endotoxin and bacteria, it does not appear to be significant for susceptibility to venom.

Although other blood-tissue barriers may play a role in the effects of endotoxin on non-specific resistance, the blood-brain barrier has been prominent in prior speculation. Parenteral injection of endotoxin alters the blood-brain barrier and permits entry of colloidal dyes from the bloodstream (20). Hypersusceptibility to sublethal quantities of certain venoms in endotoxin-treated animals may be related to this phenomenon, especially in view of the importance of neurotoxic components in some venoms (27–29).

Hemodynamic alterations following endotoxin injection, resulting in shock and death, have been studied extensively (16, 30). The deaths of endotoxin-treated rabbits following the administration of venom do not resemble those of animals in hypotensive shock, but rather occur in a very short time, preceded by hyperactivity and seizures. In addition, the shock hypothesis implies that the vulnerability of the endotoxin-treated animal is non-specific. The susceptibility to venom was demonstrated with only about half of the venoms tested.

The role of iron metabolism in EIHS and protection against venoms has been a subject of continuing study in our laboratory and will be the subject of a later paper. Ferrous iron incubated with water moccasin venom protects animals from the lethal effects of the venom, just as incubation of ferrous iron with endotoxin protects against the lethal and tissue-necrotizing effects of endotoxin (10). The latency and duration of EIHS in our studies, and the serum iron data of others (21, 22), suggest that endotoxin may lower serum iron levels, the latent period representing the time before the iron depletion reaches significant levels, and the period of hypersusceptibility coinciding with

the period of lowered levels and replenishment of iron which would protect against venom and other toxins.

EIHS was first observed with *Agkistrodon piscivorus* venom, and the additional studies to establish and define this phenomenon also used it. Experiments were later extended to other venoms to see how consistent EIHS was with venoms of other Crotalidae and whether it extended to venoms of the Elapidae and Viperidae. Of the six additional venoms used, two demonstrated the typical EIHS: *Crotalus adamanteus* and *Vipera russellii*. *Notechis scutatus* venom also revealed hypersusceptibility of endotoxin-treated rabbits, but reflected in late rather than typical early death. In three instances endotoxin-pretreatment seemed to have no effect on response to venom administration. Thus, EIHS was demonstrated with the single Viperidae venom used, with one of the additional Crotalidae venoms—the one most similar to *Agkistrodon piscivorus*—and in none of the Elapidae, in its typical form at least. Some endotoxin-treated animals were hypersusceptible to a slow-acting toxin in *Notechis scutatus* venom, however.

Two additional characteristics seem to differentiate the venoms that did and did not demonstrate EIHS in this series. The venoms that elicited it tended to be those causing consistent early death of normal animals and those with a relatively high LD_{100} for normal rabbits (at least in the range of 300 to 500 gamma). Again *Notechis scutatus* was an exception, involving as it does immediate death after injection of a very small amount of venom in normal rabbits, and delayed death as a result of an even smaller dosage in the endotoxin-treated group. This quantitative characteristic of venoms that do not demonstrate EIHS indicates that their primary lethal action depends on very minute amounts of a component(s) whose action is unaffected by prior endotoxin administration, and suggests that the lethal factor(s) which elicit the EIHS, if present at all, do not reach effective levels in the small sublethal doses; hence, the animals die as a result of the action of the primary lethal component as venom doses are increased.

The fact that the endotoxin-induced changes in resistance are significant for the toxic action of some venoms and of no discernible consequence for the toxic action of others offers a basis for hypotheses regarding the locus of the changes. There is a severe limitation, however, in the data on snake venoms: few venoms have been well characterized, either in terms of their toxic activities, invariably multiple, or their very complex constituents. We are therefore limited to broad inferences regarding the nature of EIHS to venom.

The major toxic activities attributed to snake venoms include the coagulant, anticoagulant, proteolytic, hemolytic, hemorrhagic, neurotoxic (peripheral and central), and hypotensive (31–35). In each species, a number of different constituents may be capable of producing any of the major effects. Thus, for example, two neurotoxic and three hemorrhagic principles have been isolated from the venom of *Vipera xanthina palestinae* (36). The venoms used in this study

were chosen with major toxic principles in view: Elapidae venoms for their predominant peripheral neurotoxic activity (37); Russell's viper venom for its coagulant principle (34, 38); *Crotalus durissus terrificus* for its central neurotoxic components and lack of proteolytic effects (28); and *Crotalus adamanteus* for its similarities to *Agkistrodon piscivorus* as a relatively typical member of the Crotalidae, whose venoms are thought to involve hemorrhagic and central neurotoxic components (27, 33, 39, 40).

It seems clear that all venoms have multiple toxic effects, however overriding a single one may be. Russell's viper venom is a good example; its dominant coagulant activity is evident in the massive intravascular clotting observed in animals dying immediately from lethal doses of the venom, and the possible role of enhanced susceptibility to the coagulant action following endotoxin administration needs to be considered. However, one or more of the less dominant toxins may kill the endotoxin-pretreated animals. Some investigators have attributed residual lethal action of Russell's viper venom, following neutralization of the coagulant and hemorrhagic properties with antivenin, to a central neurotoxin (41); others (42) have questioned the existence of a Russell's viper neurotoxin.

At least one central neurotoxin seems to be present in *Agkistrodon piscivorus* venom, although the evidence for its existence is also controversial (27, 33). The early deaths of animals from this venom, preceded by pupillary contraction, rigidity, and seizures, suggest a brain stem involvement in both normal and endotoxin-treated rabbits. It is interesting that another of the Crotalidae venoms, that of *Crotalus durissus terrificus*, did not elicit the EIHS, although it has been considered to have dominant central neurotoxic activity (28).

Some of the unresolved questions regarding the possible role of the blood-brain barrier and the coagulation mechanism in EIHS are being approached in further studies, as is the question of the detoxifying effect of ferrous iron on venom. These are, however, essentially peripheral approaches, valuable in narrowing the problem down but limited in getting at its core: the specific toxin or group of toxins in venoms that elicit EIHS. A major need is a fractionation method yielding venom components with greater physical and chemical homogeneity and a narrowed range of toxic action.

SUMMARY

1. Injections of sublethal quantities of *Agkistrodon piscivorus* venom into endotoxin-treated rabbits produces a consistent early death.

2. The endotoxin-induced hypersusceptibility state (EIHS) to venom is produced by intravenous, intradermal, and intraperitoneal administration of endotoxin. The latency and duration of the EIHS vary with the route of administration.

3. EIHS is induced by as little as 1 gamma of endotoxin administered intra-

venously. Although the degree of susceptibility was no greater with a 100 gamma dose than with 1 gamma, 1 mg of endotoxin made the rabbits susceptible to smaller venom doses.

4. EIHS was demonstrated with intravenous, intradermal and intraperitoneal injection of *Agkistrodon piscivorus* venom. Endotoxin-pretreated animals were not as susceptible to venom given intramuscularly.

5. Normal rabbits are very resistant to venom given intradermally and intraperitoneally.

6. Enhanced susceptibility to intravenous endotoxin was demonstrated in animals pretreated with sublethal doses of moccasin venom. It differed from EIHS in its short duration and its outcome: a late, slowly progressive death.

7. EIHS was demonstrated with other venoms: *Crotalus adamanteus* and *Vipera russellii*, and in a modified form (resulting in late death) with *Notechis scutatus*. Endotoxin pretreatment had no effect on susceptibility to *Naja flava*, *Bungarus candidus*, or *Crotalus durissus terrificus* venoms.

8. Major hypotheses regarding the nature of endotoxin-induced alterations in non-specific resistance were considered in relation to EIHS to venom.

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