creased the arginine synthetase activity. The results with arginase were not as clear cut as they also appeared to be affected by losses in weight. All changes in activity of the 3 enzymes, arginine synthetase, arginase, and lactic acid dehydrogenase, observed with various treatments in intact rats were also observed in adrenalectomized animals.

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Biologic Relationship of Endotoxin and Other Toxic Proteins. IV. Effect of Heparin on Endotoxin-Induced Hypersusceptibility to Snake Venoms.* (29348)

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Parenteral administration of sublethal doses of endotoxin is known to alter the nonspecific resistance of mammals to bacteria and viruses, and has recently been shown to affect susceptibility of rabbits to some snake venoms(1), including those of *Agkistrodon piscivorus* (water moccasin), *Vipera russellii* (Russell's viper), and *Notechis scutatus* (tiger snake)(2). Although these are very complex toxins and their multiple activities are not well defined, it is known that Russell's viper and tiger snake venoms are powerful coagulants and that water moccasin venom has important hemorrhagic activity(3). Many venoms combine anticoagulant and coagulant activity, and this is true of all three of these venoms: *Vipera russellii* and *Notechis scutatus* also have anticoagu-

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lant activity, and *Agkistrodon piscivorus* has a coagulant property(4). Thus, coagulant activity emerges as one of the common denominators of venoms that demonstrate endotoxin induced hypersusceptibility (EIHS) suggesting that endotoxin-induced alterations in resistance to venoms might involve the coagulation mechanism.

The studies to be described involved an assessment of the effect of heparin on the vulnerability of normal and endotoxin-pretreated rabbits to these 3 venoms. Earlier workers reported that heparin offered limited(5,6) and complete(7) protection against Russell's viper venom in rabbits, as well as limited protection against tiger snake venom in guinea pigs(8). The finding by Good and Thomas(9) that heparin protects rabbits against the local and generalized Schwartzman reaction is also pertinent, in view of the similarity of some of the hemorrhagic effects of gram-negative endotoxin and water moccasin venom(10,11).

Heparin did not afford even temporary protection against the lethal effects of the venoms in endotoxin-treated animals, apparently ruling out enhanced susceptibility to coagulants as a mechanism of EIHS. In normal animals heparin minimized the coagulant activity of *Vipera russellii* and *Notechis scutatus* venoms and protected the animals against the characteristic rapid death from lethal doses of the venom. The effect was temporary, however, and all the animals succumbed within 24 hours, presumably to other toxins.

Materials and methods. The endotoxin used in these studies was *Escherichia coli* endotoxin (Difco Laboratories, Detroit, lot no. 0127.B8). The venoms of *Vipera russellii* (lyophilized and dried) and *Agkistrodon piscivorus* (lyophilized) were furnished by Ross Allen Reptile Institute, Silver Springs, Fla. The *Notechis scutatus* (lyophilized) was obtained from the Commonwealth Serum Laboratories, Dept. of Health, Melbourne, Australia. The heparin sodium was obtained from Upjohn Laboratories, Kalamazoo, Mich., and included 10,000 Toronto units per 100 mg. Solutions of sodium heparin and venoms were prepared with fresh, pyrogen-free saline (Cutter Laboratories, Berkeley, Calif.)

The experimental animals were hybrid albino rabbits of both sexes, weighing 1 kg, obtained from a single local breeder, fed Purina rabbit pellets, and given water *ad libitum*.

The EIHS (endotoxin-induced hypersusceptibility state) model involved intravenous administration of 100 γ of endotoxin, followed in 2 or 24 hours by snake venom challenge. All injections were made via the marginal ear vein. The injected vessel was milked and a paper clip placed over the site to control bleeding.

Autopsies were performed on all dead rabbits as soon after death as possible, usually within 5 minutes. The results of gross examination were recorded, and significant findings are reported here.

Results. Effect of lethal doses of Russell's viper venom. As a basis for the heparin studies with Russell's viper venom, 2 groups of animals were injected intravenously with a lethal dose of the venom, observed until they died, and autopsied. Five hundred gamma of the venom, an LD_{100}, killed all the animals within a few minutes. On autopsy large clots were found within the heart, and often within the lungs and other viscera. No other gross pathologic changes were apparent, as would be expected with rapid death. A smaller amount of venom, 300 γ, also proved to be lethal, killing the entire group of 10 rabbits. However, only 5 succumbed within the first hour, and the other 5 survived for about 4 hours. Before dying these animals were dyspneic and expelled a foamy, serous secretion from the nostrils. Autopsy revealed severe pulmonary hemorrhage and necrosis, but no evidence of large clot formation. The lungs were edematous and filled with a foamy, serous exudate.

Thus, large clot formation is characteristic of the immediate lethal effect of Russell's viper venom, but is less evident in delayed action of smaller lethal doses in some animals.

Effect of heparin on susceptibility to Russell's viper venom. Before assessing the effect of heparin on EIHS to Russell's viper venom, a series of experiments was done to confirm earlier findings(7) that heparin offered sig-

TABLE I. Protection Against Russell's Viper Venom with Sodium Heparin.

Intravenous heparin (mg)	Intravenous* snake venom (γ)	Deaths† 1 hr	4 hr	12 hr
100	500	0/6	5/6	5/6‡
50	500	0/6	3/6	6/6
10	500	0/6	4/6	6/6
5	500	1/6	6/6	—
1	500	4/6	6/6	—
—	500	6/6	—	—

* 30 min. after heparin injection.
†No. of deaths within the period specified over total number of animals.
‡ One animal died at 24 hr.

nificant protection against the lethal effects of the venom. An LD_{100} (500 gamma) of venom was given to rabbits intravenously 30 minutes after intravenous administration of varied amounts of heparin from 1 to 100 mg.

Animals given 10 to 100 mg of heparin lived at least 2 hours after envenomation, but died within 12 hours (Table I). Varying the heparin dose from 10 to 50 to 100 mg had little effect on time of death. With the lower heparin doses, 1 and 5 mg, some deaths were also delayed, particularly in the 5 mg group, but all the rabbits died within 4 hours.†

At autopsy, the protection against immediate, fatal clot formation was evident. Although severe pulmonary hemorrhage and necrosis were present, there was no coagulation of blood. The pathologic changes resembled those described for animals that received 300 γ of Russell's viper venom without heparin and succumbed within a few hours.

Effect of heparin on the EIHS to Russell's viper venom. That a hypersusceptibility to Russell's viper venom exists in endotoxin treated rabbits was demonstrated in an earlier study(2). In the present series, rabbits were pretreated with 100 γ of endotoxin, followed in 90 minutes by either 10 or 100 mg of heparin. The challenging venom dose, given 30 minutes after heparin, varied from 50 to 100 γ, all sublethal doses in normal rabbits. The results are shown in Table II. The death rate was very similar in the heparin-treated and control EIHS groups, and there was no evidence of a delay in the lethal action of Russell's viper venom in the treated group. Clots were not observed in the rabbits receiving heparin, endotoxin and venom.

Effect of heparin on susceptibility to water moccasin venom. Studies were also undertaken to determine whether intravenous heparin (100 mg) would either increase or decrease susceptibility to water moccasin venom injected one hour later. Because of the possibility that heparin might enhance venom toxicity, 200 γ of venom, a sublethal dose in normal rabbits, and 5 mg, an LD_{50} in normal animals, were the dose levels used. The one-hour interval was based on the results of previous experiments showing that blood is rendered incoagulable 30 to 60 minutes after administration of 100 mg of heparin.

There were no significant differences in the number of deaths in the heparin-treated and control groups with either the sublethal or LD_{50} doses of *Agkistrodon* venom (Table III).

Effect of heparin on EIHS to water moccasin venom. It was suggested that endotoxin treatment might enhance susceptibility of animals to a factor which is secondary in toxic effects in normal animals but becomes primary (and lethal) in much smaller doses when the resistance level is changed. Hence,

TABLE II. Failure of Heparin to Protect Against EIHS to Russell's Viper Venom.

Intravenous endotoxin (γ)	Intravenous* heparin (mg)	Intravenous† snake venom (γ)	Deaths‡ 1 hr	24 hr
100	100	100	9/10	9/10
100	10	100	5/5	—
100	10	75	4/10	4/10
100	10	50	2/10	2/10
100	—	100	9/10	9/10
100	—	75	3/10	3/10
100	—	50	4/10	4/10
—	—	100	0/5	0/5
—	—	75	0/5	0/5
—	—	50	0/5	0/5

* Administered 90 min. after injection of endotoxin.
† Administered 30 min. after injection of heparin.
‡ No. of deaths within period specified (after injection of venom) over total No. of animals.

† Although not noted in the table, an experiment was also done with a 5 minute interval between heparin and venom administration. The results were essentially the same as those tabulated for the 30 minute interval.

TABLE III. Effect of Heparin on Susceptibility to Water Moccasin Venom.

Experimental plan	Snake venom dose	Deaths* 1 hr	Deaths* 24 hr
100 mg of heparin intravenously, followed in 1 hr by intravenous snake venom	200 γ	1/10	2/10
	5 mg	6/10	6/10
Intravenous venom alone	200 γ	1/10	1/10
	5 mg	5/10	5/10

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

although the blood of normal animals poisoned with *Agkistrodon piscivorus* venom becomes hypocoagulable, the possibility that a coagulant factor operates in susceptible animals needed consideration.

The standard EIHS dosages of 100 γ of endotoxin and 500 γ of venom were used, with EIHS intervals of 2 and 24 hours. Two dose levels of heparin were employed, 10 mg and 100 mg, and they were administered 15 minutes before endotoxin, 15 minutes after endotoxin, and 15 minutes before venom. Table IV summarizes these results. Neither dose of heparin seemed to offer significant protection against the lethal effects of moccasin venom, regardless of the sequence and intervals used.

TABLE IV. Effect of Heparin on EIHS* to Water Moccasin Venom.

Experimental plan	Interval between EIHS injections (hr)	Deaths† 1 hr	Deaths† 24 hr
EIHS with 10 mg heparin	2	6/8	6/8
15 min. before endotoxin	24	5/8	5/8
EIHS with 100 mg heparin	2	6/8	6/8
15 min. before endotoxin	24	8/8	8/8
EIHS with 10 mg heparin 15 min. after endotoxin	2	7/8	7/8
EIHS with 100 mg heparin 15 min. after endotoxin	2	6/8	7/8
EIHS with 10 mg heparin 30 min. before snake venom	2	9/10	9/10
EIHS, without heparin	2	6/8	6/8
	24	5/8	5/8
100 γ endotoxin only	—	0/8	1/8
500 γ snake venom only	—	0/8	0/8

* EIHS = endotoxin-induced-hypersusceptibility-state, produced by 100 γ endotoxin intravenously, and elicited after 2 or 24 hr by 500 γ of water moccasin venom intravenously.

† No. of deaths within period specified (after snake venom challenge) over total No. of animals.

Effect of heparin on susceptibility to tiger snake venom. In earlier experiments(2), tiger snake venom resulted in rapid death of normal rabbits (in doses of 2.5 to 5 γ) and in delayed death of some endotoxin-treated animals (in doses of 1 γ, not lethal to the untreated group). The present studies were undertaken to determine whether prior administration of heparin would affect susceptibility of rabbits to *Notechis* venom.

As shown in Table V, 100 mg was the heparin dose used, followed in 30 minutes by a massive dose of tiger snake venom, 100 γ, a quantity that killed all untreated animals within 90 seconds. In the heparin-pretreated group, all the animals lived for at least 3 hours, and the last died about 6 hours after venom administration.

TABLE V. Protection Against Tiger Snake Venom with Sodium Heparin.

Intravenous heparin (mg)	Intravenous snake venom† (γ)	Deaths* 10 min.	Deaths* 1 hr	Deaths* 8 hr
—	1	3/20	3/20	3/20
—	5	10/10	—	—
—	10	18/20	18/20	18/20
—	100	10/10	—	—
—	1000	10/10	—	—
—	10000	7/7	—	—
100	5	0/10	0/10	0/10
100	10	0/20	0/20	0/20
100	100	0/10	0/10	10/10
100	1000	0/10	10/10	—
100	10000	8/8	—	—

* All animals were observed longer than 48 hr.
† 30 min. after heparin injection.

As in the Russell's viper studies, autopsy confirmed the protection against the coagulant activity of the venom in the heparin-treated rabbits. The descriptions of the animals dying within minutes of *Notechis* venom injection were virtually identical to those recorded in the *Vipera russellii* series; however, the findings in the 2 heparin-treated groups differed. The *Notechis* group showed neither the foaming from the nostrils of the Russell's viper group, nor the pulmonary hemorrhage and necrosis at autopsy.

Effect of heparin on EIHS to tiger snake venom. Based on the results summarized in the preceding sections, we postulated that the EIHS to *Notechis scutatus* venom would not be altered by prior administration of

TABLE VI. Effect of Heparin on the EIHS with *Notechis scutatus*.

Intravenous endotoxin (γ)	Intravenous* heparin (mg)	Intravenous† snake venom (γ)	Deaths	
			Immediate	6 hr
—	—	100	10/10	—
—	100	100	0/10	10/10
100	100	100	8/10	10/10

* Administered 90 min. after injection of endotoxin.
† Administered 30 min. after injection of heparin.

heparin. Thus, rabbits were pretreated with 100 mg of heparin, followed in 30 minutes by 100 γ of endotoxin, followed in 2 hours by 100 γ *Notechis scutatus* venom. As shown in Table VI, there was little evidence of a protective effect.

Discussion. Earlier work on the endotoxin-induced hypersusceptibility state (EIHS) to certain snake venoms raised the possibility that this state involved altered susceptibility to the coagulant-anticoagulant activity of venom(2). In the present studies, heparin counteracted the immediate lethal effects of 500 γ, an LD_{100}, of Russell's viper venom in normal rabbits, but did not alter the eventual outcome, the death of all the animals within 24 hours. Heparin had a similar limited protective effect against large doses of *Notechis scutatus* venom, but again did not save the animals. In the endotoxin-pretreated rabbits, heparin afforded no protection against the lethal effects of the venoms.

These studies suggest a hierarchy of toxic effects of some venoms; under certain conditions of dosage and susceptibility the animal dies as a result of the dominant toxic action (as the coagulant effect in *Vipera russellii* and *Notechis scutatus*), but under other conditions of dosage (lower doses of Russell's viper venom, for example) or under altered conditions of susceptibility (pretreatment with heparin or endotoxin), or both, a less dominant toxic effect may be lethal, sometimes after several hours or even days. That neutralization of certain toxic principles of venoms allows toxic effects of other constituents to become manifest is supported by studies on specific antivenom to *Vipera xanthina palestinae* venom. Kochwa *et al* (12,13) have demonstrated 2 neurotoxic fractions, K_1A_1 with immediate and late effects, and A_4A_5 with delayed lethal action, as well as 3 hemorrhagic fractions in this venom. They have also shown that specific antivenom does not neutralize all the toxic activity of the venom, since rabbits challenged with increased doses of neutralized venom died(14).

In the present series of studies, heparin was administered before injection of *Agkistrodon piscivorus* venom in both normal and endotoxin-treated animals. This venom causes hypocoagulability of the blood, but this is not demonstrable when the animals die immediately(15). The experiment explored the thesis that prior administration of heparin might alter susceptibility to the venom in either direction; enhance the anticoagulant effect of the venom, or inhibit it by an alteration of the coagulant-anticoagulant relationships. An additional possibility was also considered; that the additional anticoagulant would increase hemorrhage, assuming that venom damage to blood vessels as well as hypocoagulability is a factor in the overall hemorrhagic effect of *Agkistrodon* venom. Heparin had no effect on susceptibility to this venom.

A series of experiments was also done using heparin and the EIHS model with *Agkistrodon* venom. One hypothesis regarding EIHS has been that a relatively minor constituent of venoms may be lethal in the endotoxin-treated animals. Since the water moccasin venom does have a coagulant and since many aspects of coagulation are still poorly understood, it seemed wise to use heparin in the endotoxin-treated animals as well, despite the lack of effect in normal animals. Although the time intervals and heparin doses were varied, no effect on EIHS to venom was demonstrated.

How far has the type of venom or the kind of venom constituent that elicits the EIHS been defined? EIHS is generally not

elicited by *Elapidae* venoms, known particularly for their peripheral neurotoxic activity, and it is not elicited by venoms which are lethal in very small amounts(2). *Notechis scutatus* is the exception: an *Elapidae venom,* lethal to normal animals in very small doses and lethal to some endotoxin-treated animals in even smaller amounts. Doery concluded from her fractionation studies that this venom has two types of neurotoxic activity, and the description of their effects on experimental animals suggests that at least one of them may affect the central nervous system. Altered susceptibility of the central nervous system to venom toxins may be a (the) basic mechanism of the EIHS, although this is by no means established. One venom, *Crotalus durissus terrificus,* has well established neurotoxic activity, but did not elicit the EIHS(2). On the other hand, *Vipera russellii* and *Agkistrodon piscivorus,* both kill endotoxin-treated animals in very small doses, but the existence of central neurotoxins has not been unequivocally demonstrated in either(16,17). As Hadidian(3) has pointed out with reference to the action of *Agkistrodon piscivorus* venom, some of the activity labelled "neurotoxic" in earlier studies may reflect enzyme-induced changes in the permeability of nervous tissue to substances which are not ordinarily toxic but which have destructive effects when they penetrate that tissue. Such substances would not have to be neurotoxins in the pharmacologic sense to have profound effects on the function of the organism. It is known that endotoxin administration affects the so-called blood-brain barrier under some conditions, and it may well be that other blood-tissue "barriers" are involved.

Thus, the problem of the nature of EIHS to venoms remains unresolved. We believe, however, that we have ruled out a significant group of venom toxins, the coagulants, as a cause of death in endotoxin-pretreated animals.

Summary. 1. Heparin in intravenous doses of 10 to 100 mg counteracted the immediate lethal effects of Russell's viper venom and tiger snake venom in rabbits, but did not prevent the deaths of the animals from the delayed toxic effects of the venom. 2. Heparin in the same intravenous doses did not interfere with the early death that occurs in endotoxin-treated rabbits given Russell's viper venom or tiger snake venom. 3. Heparin neither increased nor decreased the toxicity of moccasin venom in rabbits. 4. Finally, heparin did not affect the EIHS to *Agkistrodon piscivorus* venom when it was administered before endotoxin, shortly after endotoxin, or just before venom challenge.

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