(44.2%). For all 3 compounds less than 8% of the urinary iodine was in a solvent-extractable (presumably unchanged) form. In view of the relatively small amounts of the radiopaques excreted in the urine in an unchanged form, and their solubilities at physiological pH values, it does not appear that renal damage due to actual physical blockage should represent a significant hazard. Nevertheless, the suggestion of Nelson(14) that these compounds should be accompanied by an adequate fluid intake seems in order.

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Biologic Relationship of Endotoxin and Other Toxic Proteins. V. Alterations of Blood-Brain Barrier and Susceptibility to Snake Venom.* (30368)

EDWARD V. STAAB,† ROBERT A. GOOD,‡ AND RICHARD M. CONDIE§
Pediatric Research Laboratories, Variety Club Heart Hospital, University of Minnesota, Minneapolis

Hypersusceptibility to water moccasin venom and a variety of other snake venoms has been established in rabbits by parenteral administration of Gram-negative endotoxin(1). The physiologic locus of the endotoxin-induced hypersusceptibility state (EIHS) is unknown. The role of the central neurotoxic component of venom has been suggested by various observations in the course of these studies. First, the early death of the endotoxin-treated animals challenged with moccasin venom is preceded by labored respirations, convulsions, paresis, and a variety of autonomic symptoms, indicating profound effects on the nervous system.

Second, of the 3 major families of venomous snakes, Elapidae, Viperidae, and the Crotalidae, only venoms from the latter 2 have been associated with central neurotoxic components. The Elapidae venoms, with the exception of tiger snake venom, did not elicit the typical EIHS; 2 of 3 Crotalidae venoms and the single Viperidae venom used did(2-6).

The third factor suggesting the possible importance of central neurotoxins was the earlier demonstration(7) that parenteral administration of *E. coli* endotoxin alters the

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† Medical Fellow, Dept. of Radiology, Univ. of Minnesota.
‡ American Legion Memorial Heart Research Professor of Pediatrics and Microbiology.
§ Research Fellow, Cardiovascular Clinical Research Center, Grant 6314.

blood-brain barrier, increasing the permeability of the cerebral vessels and permitting the entry of supravital dyes which are normally excluded from the central nervous system.

Many other methods for altering the blood-brain barrier have been demonstrated (8,9), and it was suggested that these offer an opportunity for further exploration of the role of the barrier in susceptibility to some snake venoms. Two methods that alter the normal vasculature of the central nervous system were available to us: X-ray(10) and experimental allergic encephalomyelitis (EAE)(10,11).

The X-ray studies were carried out with sublethal, tissue-altering doses of irradiation to the whole body and to the head only. EAE was produced with central nervous tissue and adjuvant emulsions, with consequent clinical paralysis and characteristic central nervous system pathology. The blood-brain barrier is affected under both conditions, with penetration of supravital dyes, such as trypan blue, into the central nervous system.

Materials and methods. The experimental animals in these studies were albino hybrid rabbits of both sexes, weighing 1-3 kg, obtained from a single local breeder, fed Purina rabbit pellets and given water *ad libitum.*

The endotoxin used was derived from *Escherichia coli* (Difco Laboratories, Detroit, lot 0127.B8) and the snake venom from *Agkistrodon piscivorus* (water moccasin) (Ross Allen Reptile Inst., Silver Springs, Fla.). They were prepared for intravenous injection with pyrogen-free saline solution from Cutter Laboratories, Berkeley, Calif.

Endotoxin induced hypersusceptibility to venom (EIHS). One kg rabbits were injected with 100 γ of endotoxin intravenously, followed at varying intervals, up to 96 hours, by 500 γ of *Agkistrodon piscivorus* venom intravenously. Time of death was tabulated during the ensuing 24 hours.

X-irradiation. Whole-body X-irradiation was administered by a 220 KV General Electric X-ray machine. Rabbits (1 kg) were placed in a perforated plastic box designed to prevent forward and lateral movements of the animal. The irradiation was delivered to the lateral midline of the box with a cone-to-box distance of 65 cm. Phantom studies were made prior to each experiment to determine the quantity of scatter. Less than 2% error could be related to scatter in all experiments reported. With the machine operating at 220 KV and 15 milliamperes, using a 0.25 mm copper filter and a 1 mm aluminum filter, 37.7 r per minute was delivered as a midplane dose. Irradiation was then delivered for 5.3 minutes to each side of the animal for a total midline dose of 400 r per rabbit.

X-irradiation of the head: One kg rabbits were placed in a lead-shielded box so that the head was the only unprotected region of the body. The box was constructed to prevent any movement of the head from side to side or to different levels. Irradiation was delivered to the lateral aspect of the cranium. The machine was operated at 220 KV with a 0.25 mm copper filter and a 1 mm aluminum filter. The half value layer was 1 mm copper. With a focal skin distance of 25 cm, 222.2 r per minute was delivered for 4.5 minutes to each lateral side of the head. Each rabbit received 1000 r/air to each side of the unshielded head, for a total X-ray dose of 2000 r/air. The midplane dose was 1500 rads.

Experimental allergic encephalomyelitis (EAE). EAE was produced in 2-3 kg rabbits. The nervous tissue was whole bovine spinal cord, freed of meninges and large blood vessels, cut into small pieces and forced through a stainless steel tissue press with a #80 copper wire screen inserted above the heavy steel grating. Tissue prepared in this manner was essentially free of connective tissue fragments and blood vessels, and could be forced through a 27-gauge hypodermic needle. A modified Freund's adjuvant was prepared containing 8.5 ml Bayol F (Esso Standard Oil Co., New York) and 1.5 ml of Arlacel A (Atlas Powder Co., Wilmington, Del.). To this was added 300 mg of killed, dried, ground *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.).

Ten grams of strained nervous tissue was emulsified with 10 ml of the adjuvant. Each rabbit received 0.1 ml of the material, in-

TABLE I. Duration of Endotoxin Induced Hypersusceptibility to Venom.*

Endotoxin-venom interval (hr)	Deaths† 1 hr	Deaths† 24 hr
0	0/15	3/15
¼	3/10	5/10
½	7/15	9/15
¾	5/10	5/10
1	10/13	11/13
2	8/10	9/10
24	11/14	11/14
48	3/5	3/5
96	0/5	0/5

* 100 μg of *E. coli* endotoxin, intravenously, followed at varying intervals, by 500 μg of *Agkistrodon piscivorus* venom, intravenously.

† Deaths within period specified (after venom administration) over number of rabbits surviving the endotoxin injection.

jected intradermally into each hind foot pad with a 1.0 ml tuberculin syringe with a ½-inch 27-gauge needle. Thus, each rabbit received 100 mg of bovine nervous tissue.

Results. Duration of EIHS. Table I summarizes the data on duration of EIHS to water moccasin venom. The data suggest a period of latency of 30 to 45 minutes before hypersusceptibility is demonstrable. Increased susceptibility was still evident 3 days after endotoxin treatment, but dropped off sharply thereafter.

Effect of whole body irradiation on susceptibility of rabbits to moccasin venom. 400 r/air total body radiation was delivered to a group of rabbits to test the hypothesis that the EIHS is a result of increased permeability of the so-called blood-brain barrier. Table II summarizes these experiments. Immediate challenge with venom had no lethal effect. However, animals injected at 2 and 24 hours showed a definite increase in susceptibility,

TABLE II. Effect of Whole Body Irradiation on Susceptibility to Moccasin Venom.

Experimental plan	Interval (hr)	Deaths*
400 r of total body x-ray only	—	0/10
500 μg of water moccasin venom only	—	1/10
400 r of total body x-ray, followed at varying intervals by 500 μg of water moccasin venom intravenously	0	0/10
	2	6/10
	24	5/10
	48	1/5

* No. of deaths within 1 hr of snake venom administration, over total No. of animals.

with 6 of 10 dead within one hour in the 2-hour group, and 5 of 10 in the 24-hour group. By 48 hours, susceptibility was reduced, with one death among 5 animals.

These experiments support the hypothesis that whole body X-ray would decrease the resistance of rabbits to moccasin venom, presumably because of its effect on the blood-brain barrier. The sequence roughly parallels that of increased susceptibility following intravenous endotoxin, *i.e.*, a short latent period followed by at least 2 days of hypersusceptibility.

Effect of X-ray to head on susceptibility to moccasin venom. If the demonstrated increase in susceptibility to sublethal doses of snake venom following whole body irradiation results from alteration of the blood-brain barrier, X-ray to the head alone would be expected to show a similar effect.

Two thousand roentgens/air, used in this series of experiments, did not kill any rabbits. Again, 500 gamma/kg of snake venom was given intravenously at various intervals after irradiation. As shown in Table III, there was

TABLE III. Effect of X-ray to Head on Susceptibility to Moccasin Venom.

Experimental plan	Interval (hr)	Deaths*
2000 r of x-ray to skull only	—	0/10
500 μg of moccasin venom only	—	1/10
2000 r of x-ray to skull, followed at varying intervals by 500 μg of water moccasin venom intrav.	1	3/10
	2	7/10
	24	4/5

* No. of deaths within 1 hr of snake venom administration, over total No. of animals.

some evidence of latency, since 3 of 10 animals died with a one-hour interval, 7 of 10 with a 2-hour interval, and 4 of 5 with a 24-hour interval. The deaths occurred within an hour after venom administration, and hyperactivity, labored respirations, cries, and convulsions preceded death as they do in endotoxin-treated and normal animals dying of moccasin venom.

Thus, X-ray to the head of rabbits produces hypersusceptibility to water moccasin venom, paralleling the effects of endotoxin-pretreatment and whole body irradiation.

Effect of EAE on susceptibility to moccasin

TABLE IV. Effect of EAE* on Susceptibility to Water Moccasin Venom.

Degree of clinical paralysis	Moccasin venom dose (mg/kg)	Deaths†
Complete paralysis of a front or rear extremity	1	14/16
Beginning paralysis (paresis) of a front or rear extremity	1	3/6
No paralysis‡	1	0/5
Normal animals given snake venom alone	1	2/10

* EAE = Experimental allergic encephalomyelitis, produced by intradermal injection of an emulsion of bovine central nervous tissue and modified Freund's adjuvant.

† Number of deaths within an hour after administration of snake venom.

‡ These animals received the central nervous tissue + adjuvant injection but did not develop clinical paresis or paralysis. They were considered to be a control on the effects of the injected materials.

venom. Table IV records the results of experiments with moccasin venom using the EAE model. Three groups of animals, previously treated with the nervous tissue-adjuvant emulsion, were used, classified by the degree of paralysis: complete (complete paralysis in any extremity), beginning (unquestionable paresis, but no complete paralysis in any extremity), and no paralysis.

Significantly increased susceptibility to a dose of 1 mg/kg of snake venom was evident in both groups with signs of paralysis: 17 of 22 animals died immediately, including 14 of 16 with complete paralysis and 3 of 6 with beginning paralysis. There were no deaths among the 5 animals which had received the EAE injections but had not developed signs or paresis or paralysis. In a control group of 10 rabbits given the same dose of venom without prior EAE injection, 2 animals died immediately when venom was administered.

Hypersusceptibility to venom was clearly present in rabbits that contracted EAE. It is also suggested that the degree of susceptibility parallels the extent of paralysis. Administration of the nervous tissue-adjuvant emulsion itself did not seem to enhance susceptibility to venom, since the unparalyzed animals survived the venom challenge. The disseminated characteristics of this disease process to the central nervous system again indirectly implicate this organ system in the EIHS, death with the experimental allergic encephalomyelitis-induced hypersusceptibility simulating the endotoxin-induced system.

Discussion. The existence of a blood-brain barrier was first suggested by Goldmann's finding in 1913 that the central nervous system vasculature is impermeable to trypan blue (12). The anatomic location is not known, but electron microscopy has shown that the normal architecture of the glial cell foot processes is disturbed in conditions of altered permeability(13,14).

Speculation regarding the nature and physiologic locus of the endotoxin induced hypersusceptibility state to certain snake venoms can be related to the known alterations in the permeability of the blood-brain barrier induced by endotoxin(7). This is consistent with the knowledge of the toxic actions of venoms including peripheral and particularly central neurotoxic effects(3-6).

A variety of substances gain entry to the central nervous system when the blood-brain barrier is altered by endotoxin and other means(7,8,9). This study used a venom known to elicit the endotoxin induced hypersusceptibility state, and two methods, other than endotoxin, of altering the blood-brain barrier: (1) X-ray, both to the whole body and to the head alone(15-18); and (2) experimental allergic encephalomyelitis(10-11).

It has been shown that X-ray to the head alters the blood-brain barrier so that dyes, such as trypan blue, which do not normally reach the central nervous system, do so following X-ray to the cranium(8). The smallest dose used by Clemente in these studies was 1500 roentgens/air which resulted in minimal changes in permeability.

Both series of irradiation studies produced results consistent with the hypothesis. Snake venom, in a dose which is ordinarily sublethal, killed significant numbers of the treated animals when it was given 2 or 24 hours after irradiation. The range of intervals was greater in the whole body irradiation studies, and these gave clear evidence of a latency period of 1-2 hours and a duration of 2-3 days. Thus, the irradiated rabbits revealed a susceptibility to venom which paralleled

that of endotoxin-treated rabbits. The eliciting dose was the same; and a brief latent period, continued susceptibility for 2-3 days, and immediate death from the venom were characteristic of both groups.

The characteristic pathology of experimental allergic encephalomyelitis is perivascular infiltration of lymphocytic cells, patchy in nature and located throughout the central nervous system(19). An increase of permeability into these areas is generally recognized(11).

A majority of rabbits injected intradermally with an emulsion of bovine central nervous tissue and modified Freund's adjuvant become paralyzed and eventually die (19). The disease is slowly progressive, with increasing paralysis which appears to be secondary to ascending myelitis, and ultimate involvement of the vital centers in the midbrain.

The experimental allergic encephalomyelitis model also provided support for the thesis, since the animals with complete paralysis in a front or rear extremity as a result of experimental allergic encephalomyelitis were hypersusceptible to snake venom.

Thus, increased susceptibility to snake venom following the use of X-ray or EAE has added further support to the hypothesis that a breakdown in the blood-brain barrier is a locus of abnormality. Additional information will be obtained if current attempts at isolating a component or components of snake venom that produce this EIHS are successful.

Summary. 1. The blood-brain barrier of normal rabbits was altered by the use of *E. coli* endotoxin, by X-irradiation (whole body or head alone) and by inducing experimental allergic encephalomyelitis. 2. All animals so treated were hypersusceptible to venom. 3. It is concluded that endotoxin induced hypersusceptibility to some snake venoms possibly results from alteration of the so-called blood-brain barrier.

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