

RESISTANCE OF THE OPOSSUM (*DIDELPHIS VIRGINIANA*) TO ENVENOMATION BY SNAKES OF THE FAMILY CROTALIDAE

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R. M. WERNER and J. A. VICK. Resistance of the opossum (*Didelphis virginiana*) to envenomation by snakes of the family Crotalidae. *Toxicon* 15, 29-33, 1977.—Whether the North American opossum, *Didelphis virginiana*, has a natural resistance to envenomation by 12 species of snakes from the families Crotalidae, Viperidae, Elapidae, and Hydrophidae was studied. Challenge of the anesthetized opossum was by actual snakebite, and by i.m. or i.v. injection of 4-60 times the dose known to be lethal to susceptible mammals. Some animals that survived the snakebite were also given venom intravenously 40-90 min after bite challenge. Heart and respiratory rate, EKG, and blood pressure were monitored for 2 hr after envenomation, and surviving animals were observed for signs of poisoning for 30 days. The opossum survived the venom of the following snakes of the Family Crotalidae: eastern diamondback rattlesnake, western diamondback rattlesnake, copperhead, cottonmouth moccasin, Korean mamushi, and Central American moccasin. It died when challenged with the venom of the Indian cobra, Chinese cobra, coral snake, cape cobra, puff adder, and sea snake. These findings show promise of the opossum being an excellent experimental animal for venom research, particularly by identifying its protective mechanisms.

INTRODUCTION

THE OPOSSUM, *Didelphis virginiana*, has received a good deal of attention in recent years as an experimental animal, with highly varied applications to scientific investigations (JURGELSKI, 1974). From their evolutionary history, marsupials represent a specialized side branch of the mammalian class, diverging from mammalian stocks at an early stage. The opossum is considered a primitive member of the class Mammalia which developed to its present form in the Cretaceous period.

Our interest in the opossum began when Mr. Jack Kilmon, a Baltimore herpetologist, told us that the opossum is believed to eat venomous snakes and may be resistant to snake venom (KLAUBER, 1972). Certainly, this slow-moving, nocturnal animal would be an easy target for snakes and probably would have difficulty surviving if it had no protective mechanism. If the opossum did show resistance it might prove to be useful as an experimental animal for venom and therapy research.

A search of the literature revealed that the resistance of various animals to snake venom is well documented. However, most of the reports deal with the resistance of reptiles (BONNETT and GUTTMAN, 1971; CLARKE, 1969; KELLAWAY, 1937; KLAUBER, 1972; NOGUCHI, 1904; PHILPOT, 1954; PHILPOT and SMITH, 1950). As an example, PHILPOT and SMITH (1950)

*The views of the authors do not purport to reflect the position of the Department of Defense.

showed that the king snake, *Lampropeltis getulus*, has a high degree of resistance to several snake venoms. They also reported that the serum of the king snake has a greater ability to neutralize venom from the water moccasin, *Agkistrodon piscivorus*, when mixed *in vitro* and injected into mice or injected separately from the venom than does Wyeth's polyvalent antivenin. The goal of this field of research is to discover a naturally occurring antidote to venom or to develop an antiserum that can be obtained more easily than the present method of preparing antivenins by immunizing horses over a period of months.

There are some reports of the resistance of mammals to venomous snakebite (BOQUET, 1948; VELLARD, 1949, 1950). Vellard has done an extensive study of the resistance of various South American mammals, including species of *Didelphis*. Our study was undertaken to investigate whether the North American opossum is resistant to snake venoms and to single out, if possible, to what species of snakes this resistance extends.

We have established a colony of opossums, which adapt well and seem content when confined in standard cages for other animals of equal size (about 8–10 lb). They thrive on a diet of regular commercial cat food. Our colony consists of opossums that were captured south of Edgewood, Maryland, on the Gunpowder Neck, which is located on the western shore of the Maryland Coastal Plain Province. The only two poisonous snakes normally found in Maryland are of the Family Crotalidae: the northern copperhead (*Agkistrodon contortrix mokeson*) and the eastern timber rattlesnake (*Crotalus h. horridus*) (HARRIS, 1975). Only the northern copperhead has been reported on the western shore of the Maryland Coastal Plain Province, and occasional encounters with the northern copperhead in this area could be expected (HARRIS, H. S., 1976*). We were therefore, particularly interested in what effect Crotalidae venoms would have on the opossum.

MATERIALS AND METHODS

The opossums† were anesthetized by one of two methods. In the initial studies the animals were given 37 mg per kg of pentobarbital sodium intraperitoneally. Additional doses of anesthetic were administered through a catheter placed in the jugular or femoral vein. As it was difficult to maintain a constant level of anesthesia with this method, an alternate method was used in subsequent studies. Ketamine HCl was administered intramuscularly at a dose of 100 mg per kg. This allowed intubation and subsequent administration of methoxyflurane with a Foregger small-animal anesthetic machine.

The opossums were envenomated either by actual snakebite into the biceps femoris muscle of the left hind leg or by i.v. or i.m. administration of 4–60 times the dose of venom known to be lethal to susceptible mammals (VICK, 1972). In some instances, when an animal survived a snakebite, it was also given venom intravenously 40–90 min after the bite. The live snakes were obtained from Biologicals Unlimited, Baltimore, Maryland. A lyophilized form of the venoms given intravenously was obtained from the same supplier and reconstituted with 0.9% physiological saline before use. In our studies all of the venoms utilized were titrated for the approximate lethal dose by i.p. injection into mice. In all cases the lethal doses in mg per kg body weight were similar to those published by other authors (RUSSELL, 1967; VICK, 1972). Rabbits, anesthetized with ketamine HCl and methoxyflurane, were given the venoms intravenously and served as additional venom controls as well as anesthesia controls. Here, again lethal doses in the rabbits were approximately the same as those published for other species of animals (RUSSELL, 1967; VICK, 1972). Approximate lethal doses for susceptible animals are listed in Tables 1 and 2. The doses of venom administered to test opossums were multiples of approximate lethal doses for dogs and monkeys (VICK, 1972).

In the opossum, either the femoral or carotid artery was catheterized for recording arterial blood pressure with a Statham pressure transducer. Electrocardiogram, heart rate, and respiration were monitored using needle-tipped electrodes placed in either side of the chest wall, and recordings made on a Narco Physiograph. Physiological functions were monitored for 2–8 hr after envenomation.

The opossums were given no drugs or other treatment after envenomation. Survivors were observed

*Harris, H. S. (1976). Curator of Herpetology, Natural Historical Society of Maryland. Personal communication.

†In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

closely for clinical signs of toxicity for 30 days. At the end of that period, they were either released into the wild or kept as a part of the colony but were not used for further envenomation studies.

RESULTS

Mortality

Family Crotalidae. Table 1 shows the crotalid species used, the approximate lethal doses normally expected for susceptible animals, the methods of challenge, the doses administered intravenously to the opossum and the outcome. Two opossums were challenged with the venom of the cottonmouth moccasin, two with Western diamondback rattlesnake and two with Eastern diamondback rattlesnake; one opossum per venom was challenged with the other three venoms. None of the animals challenged showed local signs of hemorrhage, swelling, or tissue necrosis. None of the animals died nor were there any irreversible changes in blood pressure, heart rate, respiration, or EKG.

Family Elapidae. Table 2 shows the elapid species used, the approximate lethal doses normally expected for susceptible animals, the methods of challenge, the doses administered intravenously, and the outcome. Two opossums were challenged with the venom of the Indian cobra; one opossum per venom was challenged with the other three venoms. All animals died within $\frac{1}{2}$ –24 hr after envenomation, and they all showed severe and irreversible changes in the physiological functions monitored.

Family Hydrophidae. The one opossum given sea snake venom (i.v. dose of 0.34 mg per kg) died 45 min after envenomation (Table 2). There were no significant changes in blood pressure, EKG, or heart rate, however, there was a slow, progressive decrease in respiratory rate and volume which led to complete respiratory paralysis and death.

Family Viperidae. The one opossum bitten by a puff adder showed early changes in blood pressure and heart rate that returned toward control values at 8 hr; however, the animal died within 24 hr (Table 2). Tissue edema and cutaneous hemorrhage at the site of the bite were evident, indicating envenomation was the cause of death.

Physiological effects

The effect of a copperhead snakebite on the physiological functions of the anesthetized opossum is shown in Fig. 1. Immediately after bite challenge there were subtle changes in respiration and blood pressure; however, within 5 min these functions had returned to

TABLE 1. LACK OF EFFECT OF CROTALID VENOMS ON THE OPOSSUM

Snake	Approximate lethal dose for susceptible animals* (mg/kg)	Challenge to opossum†
<i>Crotalus adamanteus</i> Eastern diamondback rattlesnake	1.58	15.5 mg/kg, i.v.; 105.3 mg/kg i.m.
<i>Crotalus atrox</i> Western diamondback rattlesnake	3.03	Snakebite followed by 13.7 mg/kg, i.v. 63.7 mg/kg, i.v.
<i>Agkistrodon c. contortrix</i> Copperhead	5.36	Snakebite followed by 43.4 mg/kg, i.v.
<i>Agkistrodon piscivorus</i> Cottonmouth moccasin	2.46	Snakebite Snakebite followed by 12.5 mg/kg, i.v.
<i>Agkistrodon h. brevicaudata</i> Korean mamushi		Snakebite
<i>Agkistrodon bilineatus</i> Central American moccasin		Snakebite

*VICK (1972) (approximate lethal doses for dogs and monkeys).

†All challenged opossums survived.

TABLE 2. EFFECTS OF ELAPID, VIPERID, AND HYDROPHID VENOMS ON THE OPOSSUM

Snake	Approximate lethal dose for susceptible animals* (mg/kg)	Challenge to opossum	Death time†
ELAPIDAE			
<i>Naja naja</i>	0.28	Snakebite	45 min
Indian cobra		1.07 mg/kg, i.v.	30 min
<i>Naja naja atra</i>		Snakebite	45 min
Chinese cobra			
<i>Naja nivea</i>	0.5	1.38 mg/kg, iv.	1 hr
Cape cobra			
<i>Micrurus fulvius</i>	0.34	1.70 mg/kg, i.v.	6 hr
Coral snake			
VIPERIDAE			
<i>Bitis arietans</i>		Snakebite	24 hr
Puff adder			
HYDROPHIDAE			
<i>Laticaudata semifasciata</i>	0.08	0.34 mg/kg, i.v.	45 min
Sea snake			

*VICK (1972) (approximate lethal doses for dogs and monkeys).

†All challenged opossums died.

control levels. Subsequent alterations in the physiological parameters did not occur until the animal was challenged intravenously with 8 lethal doses of reconstituted whole venom from the copperhead 40 min after the original envenomation. Again, changes in blood pressure and respiration were noted, as well as a decrease in heart rate. Yet, as had occurred after the snakebite, these changes reverted to control or near control values within 15–30 min.

Figure 2 demonstrates the effect of four lethal doses of Indian cobra venom given intravenously to the anesthetized opossum. Little or no change was noted for 15 min in heart rate, EKG, respiration, or blood pressure. Then blood pressure became very unstable, heart rate decreased, and respiration progressively deteriorated into complete apnea. The opossum died 30 min after envenomation.

DISCUSSION

The North American opossum, *D. virginiana*, shows a resistance to crotalid venoms that is in striking contrast to the effect of these venoms on other laboratory animals (VICK, 1972). Yet the opossum appears to be susceptible to elapid venoms, the sea snake (Hydrophidae), and puff adder (Viperidae). These results are consistent with work published by VELLARD (1949, 1950), which showed that the South American opossum is resistant to crotalid venoms and susceptible to elapid venoms. Vellard also showed that South American opossums are resistant to *Vipera russelli* (Viperidae). He did not conduct experiments with any representatives of the Hydrophidae family. VELLARD's work (1949, 1950) indicated that there are 'naturally occurring antibodies' in South American opossums which appear to protect them from crotalid venoms. We have done some preliminary serum neutralization studies that indicate a similar serum protective substance in the North American opossum. More work needs to be done to prove the existence of such a substance.

In addition to the resistance shown by the opossum to the lethal effects of the crotalid family of snakes, there was a striking absence of local reaction in the case of bite challenge. No signs of hemorrhage, swelling, or tissue necrosis were observed. This is quite unusual in the case of most crotalid venoms which are rich in proteolytic enzymes. The mechanism

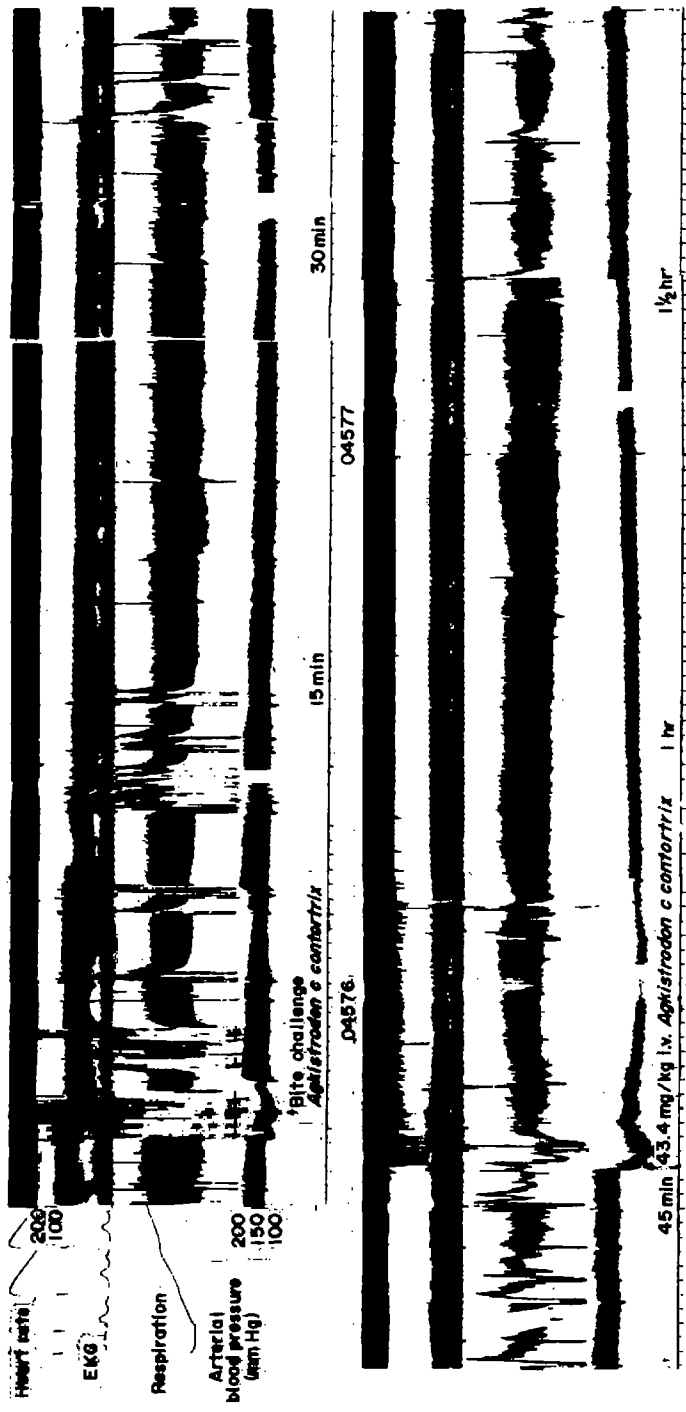


FIG. 1. PHYSIOLOGICAL EFFECTS OF COPPERHEAD VENOM ON THE OPOSSUM. Note return to control levels following bite challenge as well as i.v. challenge.

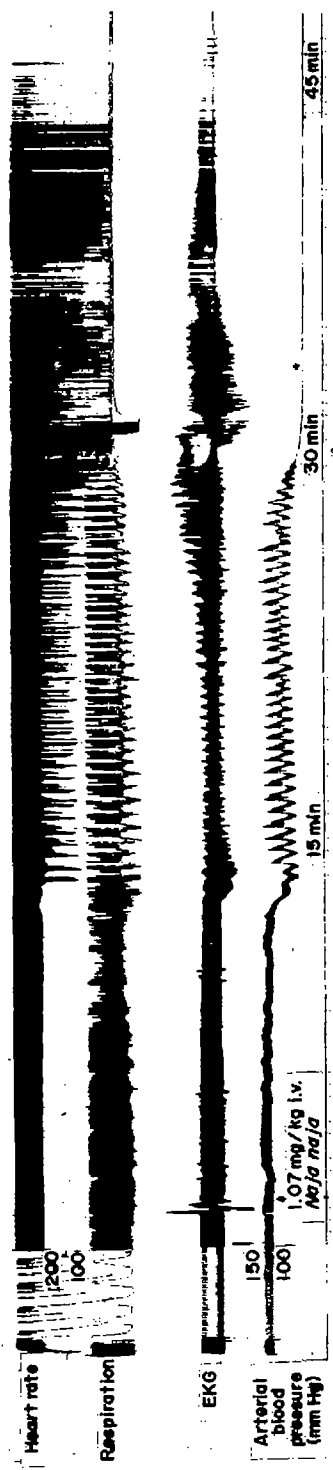


FIG. 2. PHYSIOLOGICAL EFFECTS OF INDIAN COBRA VENOM ON THE OPOSSUM.
 Note loss of vital physiological function 30 min after i.v. injection of venom.

of the opossum's resistance to tissue damage by crotalid venoms is unknown but its elucidation could have far-reaching implications in the treatment of snakebites.

Although the lethal doses of venom VICK (1972) cites were an average of those used in dogs and monkeys, and RUSSELL (1967) did not mention the animals in which he derived lethal doses, the i.v. doses used in the present study were multiples of those cited by Vick and shown to be lethal in our potency assays. We therefore have no doubt of their lethality to susceptible mammals on a weight-of-venom per kilogram-of-body weight basis. The validity of results with so few animals per venom may be questioned, but the 'all-or-none' response of the opossum to the crotalid and elapid venoms lends credence to the phenomenon of resistance. It is hoped that these preliminary findings will stimulate others in the field of venom research to pursue the study of the opossum's protective mechanism with the possible goal of application to humans.

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REFERENCES

- BONNETT, D. E. and GUTTMAN, S. I. (1971) Inhibition of moccasin (*Agkistrodon piscivorus*) venom proteolytic activity by the serum of the Florida king snake (*Lampropeltis getulus floridana*). *Toxicon* **9**, 417.
- BOQUET, P. (1948) *Venins de Serpents et Antivenins*. Paris: Flammarion.
- CLARK, W. C. (1969) Venom neutralization by rattlesnake serum albumin. *Science* **164**, 1402.
- HARRIS, H. S. (1975) Distributional survey: Maryland and the District of Columbia. *Bull. Md Herpetological Soc.* **11**, 73.
- JURGELSKI, W. (1974) The opossum (*Didelphis virginiana* Kerr) as a biomedical model. *Lab. Anim. Sci.* **24**, 375.
- KELLEY, C. H. (1937) Snake venom—III. Immunity. *Johns Hopkins Hosp. Bull.* **60**, 159.
- KLAUBER, L. M. (1972) Susceptibility, resistance and immunity to rattlesnake venom. In: *Rattlesnakes*, pp. 883–891. (KLAUBER, L. M., Ed.). Los Angeles: University of California Press.
- NOGUCHI, H. (1904) *The Action of Snake Venom upon Cold-Blooded Animals*. Washington, D.C.: Carnegie Institute.
- PHILPOT, V. B. (1954) Neutralization of snake venom *in vitro* by serum from the nonvenomous Japanese snake *Elaphe quadrivirgata*. *Herpetologica* **10**, 158.
- PHILPOT, V. B. and SMITH, R. G. (1950) Neutralization of pit viper venom by king snake serum. *Proc. Soc. exp. Biol. Med.* **74**, 521.
- RUSSELL, F. E. (1967) Pharmacology of animal venoms. *Clin. Pharmac. Ther.* **8**, 849.
- VELLARD, J. (1949) Investigations sobre inmunidad natural contra los venenos de serpientes I. *Publ. Mus. Hist. nat., Lima Ser A, Zool.* **1**, 1.
- VELLARD, J. (1950) Investigaciones sobre inmunidad natural contra los venenos de serpientes II. *Publ. Mus. Hist. nat., Lima Ser A, Zool.* **4**, 1.
- VICK, J. A. (1973) Effect of actual snakebite and venom injection on vital physiological function. In: *Toxins of Animal and Plant Origin*, Vol. 3, pp. 1001–1011, (DEVRIES, A. and KOCHVA, E., Eds.). New York: Gordon & Breach.