eggs. This factor was encountered in all H.D. Am. F.s tested and may be of etiologic significance in the disease. This factor is filterable, doubtless has the capacity to reproduce itself and probably has some slight lethal effect on chicken embryos.

Received May 11, 1950. P.S.E.B.M., 1950, v74.

Neutralization of Pit Viper Venom by King Snake Serum.* (17959)

V. B. PHILPOT† AND RALPH G. SMITH

From the Department of Pharmacology, School of Medicine, Tulane University, New Orleans, La., and North Mississippi Hospital, Holly Springs, Miss.

There is considerable published evidence that certain venomous and non-venomous animals show some degree of tolerance to animal venoms(1-4). Numerous authentic incidents are recorded in the literature of snakes being bitten by other venomous snakes resulting in some instances in death and sometimes in survival(5). The fact that death may occur indicates that tolerance is limited; but, when the approximate amount of venom injected by the strike is considered on the basis of dosage per unit of body weight, the tolerated dose in the snake must be very large in comparison with that in man in order to permit any survivals. It has also been shown experimentally that some homologous and non-homologous serums exert a limited antivenin action(1,6,7).

The present work was suggested by the common belief in rural areas of this region that the king snake is immune to the bite of American vipers and by the report of Ditmars(8) of the survival of a king snake *(Lampropeltis getulus)* bitten by a water moccasin of much larger size. The investigation involved primarily a study of the degree of neutralization of viper venom by king snake serum as determined by the lethal action of the venom in mice. Experiments are also presented showing the low degree of toxicity of heat-detoxified king snake serum. With the technic used in our experiments it has been possible to show a higher degree of protection than, to our knowledge, has hitherto been reported for reptilian sera. An abstract of this work has already appeared (9).

Materials and methods. The chain king snake *(Lampropeltis getulus* Floridans) and the spotted king snake *(Lampropeltis getulus* Holbroki) obtained from Ross Allen's Institute, Fla. and from local collectors were used in these experiments. Blood was collected by severing portions of the tails following which it was allowed to clot and the serum separated by centrifugation. The serum was detoxified by heating it in test tubes in a water bath at 56°C for 30 minutes,

*. Supported in part by a special research grant from School of Medicine, Tulane University.

† Now situated at The Methodist Hospital, Brooklyn, N. Y.

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a process used for detoxication of snake serums by other workers(1,6,7). When not used immediately, it was stored at -20°C. The venoms of the Florida rattlesnake *(Crotalus adamanteus)* and of the water moccasin *(Agkistrodon piscivorus)* were obtained from Ross Allen's Reptile Institute in dry powdered form. Stock solutions of 1.0 or 0.5% were prepared in 0.9% NaCl solution and when not used immediately were preserved at -20°C. Wyeth's Antivenin *(Nearctic crotalidae)* Polyvalent was obtained on the open market.

Experimental procedure and results. a. *Neutralization of moccasin venom by king snake serum.* The toxicity of moccasin venom was determined by intraperitoneal injection into 97 white mice of varying dosages of from 3 to 15 mg/kg in volumes of from .13 to .45 cc. The LD_{50} as evaluated by the method of Miller and Tainter(10) was 6.25 ±s.e. .34 mg/kg. Most deaths occurred within 12 hours after injection, rarely after 24 hours, and in only 1 case after 48 hours. The volume of detoxified king snake serum which would save mice from an arbitrary dosage of 15 mg/kg (approximately 2½ LD_{50}'s) was then determined. Stock solutions of venom were diluted with varying proportions of 0.9% NaCl solution and serum to yield a venom concentration of 0.1%. After standing for 15 minutes, doses of the mixture containing 15 mg/kg of venom and from .37 to 3.0 cc/kg of serum were injected intraperitoneally into 114 mice. Sixty percent of the mice receiving 1.0 cc/kg of king snake serum were saved from 2½ LD_{50}'s of moccasin venom. Treatment of all data showed an ED_{50} of 1.0 ± s.e. 0.1 cc of serum per kg of mouse. This dosage of venom without serum caused death in 22 mice in from 20 minutes to 2 hours, only 3 surviving longer than 1 hour. In 53 mice essentially the same results were obtained by the intraperitoneal injection of 15 mg/kg of venom, followed immediately by king snake serum, without mixing *in vitro*. Half of the mice were saved by even less than 1 cc/kg of serum (ED_{50} = .71 ± s.e. .12 cc/kg). In an experiment in which 47 mice were used approximately 3 cc/kg of Wyeth's polyvalent antivenin was necessary to achieve the same effect (ED_{50} = 3.15 ± s.e. .49 cc/kg).

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TABLE I.
Antidotal Action of 1 cc of Detoxicated King Snake Serum Against Moccasin Venom in Mice.

Venom, mg/kg	No. of mice Used	Survived
33	8	8
45	5	4
45*	6	5
60*	4	0
67.5	5	1
100	4	0

* Venom and then serum injected separately (intraperitoneally). Others mixed before inj. Wt range of mice, 24-35 g.

By both technics a total dosage of approximately .1 and .03 cc of king snake serum protected practically all mice and about half of the mice, respectively, from 2½ lethal doses of venom. Since these amounts of serum were far below the range of toxic dosage, the protective action of a much larger but still non-toxic dose of serum was evaluated. The results are presented in Table I.

All mice were protected against approximately 5 LD_{50}'s (33 mg/kg) of venom by 1 cc of serum per mouse, while 9 of 11 mice were protected against approximately 7 LD_{50}'s (45 mg/kg).

b. *Neutralization of rattlesnake venom by king snake serum.* Rattlesnake venom proved to be more toxic in mice by intraperitoneal injection than moccasin venom. The LD_{50} determined on 50 mice was evaluated at 1.7 ± s.e. .1 mg/kg of body weight. When the administration of 4.0 mg/kg of venom (approximately 2½ LD_{50}'s) was followed immediately by detoxicated king snake serum, both by intraperitoneal injection, .4 cc/kg of serum saved approximately half the mice while twice this dose saved 93.5% of the mice. The latter dose of Wyeth's polyvalent antivenin, however, saved only 8 of 30 mice. The results are presented in Table II.

c. *Toxicity of king snake serum.* Of equal interest to the antidotal action of detoxicated king snake serum against venoms is the relatively low toxicity of the serum itself.

TABLE II.
Antidotal Action of Detoxicated King Snake Serum and Wyeth's Antivenin Against 2½ LD_{50}'s of Rattlesnake Venom in Mice.
Separate intraperitoneal injections.

King snake serum, cc/kg	Mice saved	
	No.	%
.4	14/30	47
.8	29/31	93
1.3	5/5	100
Antivenin		
.8	8/30	27
1.3	5/5	100

The untreated serum is definitely toxic. Its lethal dose was not determined by us, but in a few mice 1 cc injected intraperitoneally produced death within 2 hours, and .25 cc by this route has been reported as a surely lethal dose(7). It is also well known that the toxicity is decreased by heating(1,6,7). The few experiments described here show that the antidotal dosages of the detoxicated serum are well below the range of toxicity. The largest antidotal dosage used was 1 cc (approximately 30 cc/kg) in mice weighing from 24 to 35 g. This dosage, injected intraperitoneally into 7 normal mice, produced no observable effect. A much larger dosage of detoxicated serum (150 cc/kg—total dosages of 2.25-4.35 cc) was tolerated intraperitoneally by 5 mice without incident. The following intravenous doses were administered to other animals without obvious effect: guinea pig (wt. 339 g) 5 cc; rabbit (wt. 1.55 kg) 5 cc; dog (wt. 13 kg) 20 cc. An intravenous injection of 16 cc of the detoxicated serum over a period of 13 minutes was well tolerated by one of us (V.P.).

Discussion. It is evident from previous isolated observations(8) and from the results reported here that detoxicated king snake serum contains a factor with high neutralizing potency against moccasin and rattlesnake venoms. We are unable to explain why Rosenfeld and Glass(7) were unable to demonstrate protection against the lethal effects of venoms by the serum of this snake although they observed an inhibition of the hemorrhagic action. A high degree of natural immunity of the king snake was incidentally confirmed by the intramuscular injection into one snake weighing 470 g of 140 mg of moccasin venom in 4 doses within 5 days. A second snake weighing 770 g similarly received 1035 mg of venom in 5 doses within 21 days, the largest single injection being 320 mg. The only adverse effect observed was slight swelling at the site of injection with the larger doses. Such doses are of a definitely higher order than lethal doses in higher animals and in man. Natural immunity is, of course, not confined to the king snake as has been shown by the authors cited above. In this connection we were able to demonstrate neutralization of moccasin venom by serums of the blue racer snake and water snake which was of the same general order as that by king snake serum. A possible therapeutic value of king snake serum can only be determined by further investigation of its potency by other technics and on other animals and by more extensive testing of its toxicity, but the results obtained in these experiments are suggestive in this regard. From the standpoint of producing the serum, an adequate number of king snakes could probably be obtained as easily as the rattlesnakes which serve as a source of venom for the immunization of horses over a period of several months in the preparation of therapeutic antivenins.

Summary. King snake serum, detoxicated by heating, and in doses well below toxic levels protected white mice against as high as 7 LD_{50}'s of moccasin venom injected intraperitoneally, when serum and venom were mixed *in vitro* or injected separately A protective action against rattlesnake venom was also demonstrated. Wyeth's polyvalent antivenin used by the same technic showed a definitely lower degree of neutralizing potency against both moccasin and rattlesnake venoms.

Dr. Max Littman and Dr. Foster N. Martin, Jr., have contributed many useful suggestions and criticisms. Mr. Bennett Elisberg informed us of the technic of collecting blood from the tail of the snake. Some of the mice were contributed by Frank and Jimmy Pittari, Phyllis Warden and Joy Daray.

Received May 12, 1950. P.S.E.B.M., 1950, v74.