

STUDIES ON THE VENOMS OF NORTH AMERICAN PIT VIPERS

THOMAS S. GITHENS

Mulford Biological Laboratories, Sharp & Dohme, Glenolden, Pennsylvania

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For several years past, in connection with the work of this laboratory, we have been gathering data on the venoms of North American pit vipers, and we present here a statistical analysis of certain phases of this work, dealing chiefly with the amount of venom which can be obtained by squeezing the venom glands and the toxicity of the venom thus gotten from each species.

The venoms of all the pit vipers of the United States (except those of two rare species, *Crotalus confluentus concolor* and *C. triseriatus*) are included, as well as those of the rattlesnake and moccasin of Central America, the Central American Cascabel (*Crotalus terrificus durissus*) and the tropical moccasin (*Agkistrodon bilineatus*). The venoms of the other varieties of *C. terrificus*, namely the Mexican diamond rattler (*C. t. basiliscus*) and the South American Cascabel (*C. t. terrificus*) are not included nor are the rattlesnakes limited to Mexico and Southern California (*C. exsul*, *C. polystictus* and *C. stejnegeri*). Venoms of the large Central and South American genus of *Bothrops* are likewise excluded.

All venoms were carefully collected, centrifugated and dried at 37°C. The venoms of *C. terrificus* were gathered and dried at the serpentarium at Tela, Honduras: all Western species were prepared by Mr. L. M. Klauber, curator of the Natural History Museum at San Diego, California. Several lots of venom of *C. atrox* were obtained from Colonel M. L. Crimmins and other experts in Texas. All other venoms were collected in our laboratory at Glenolden.

The fresh liquid venom, when properly dried, yields from 20 to 35 per cent of total solids, averaging about 30 per cent. The bulk of this is proteid in nature, consisting of peptones, proteoses and true proteins. All figures for venoms, except where the contrary is distinctly stated, are based on dry weight.

I. AMOUNT OF VENOM OBTAINED BY EXTRACTION

The amount of venom which can be extracted by squeezing or "milking" the venom glands is subject to wide variation. In general, the yield from most pit vipers is from 0.1 to 0.3 cc. of fresh venom, giving 30 to 100 mgm. when dried. The average amount obtained from a number of individuals of the same species varies almost directly with the size of the species and large individuals will commonly yield more venom than smaller ones of the same type. Our largest vipers, the Texas and Florida diamondback rattlers and the red rattler, show average yields of 0.5 cc. or more, yielding hundreds of milligrams, while small species, such as the green rattler, the tiger rattler, the massasauga, and the pigmy rattler average less than 15 mgm. of solids.

There is moreover, wide individual difference within the same species, many snakes, especially after several weeks in captivity, yielding little or no venom, while other specimens which have not been extracted or had opportunity to bite for a long time, as after hibernation, may give amounts far above the average of the species. For this reason only averages of fairly large numbers of extractions have definite value. The largest yield we ever obtained was 4 cc. of fresh venom from a large Florida diamondback. The total solids were low, only 864 mgm. or 21.6 per cent. An extraction of about the same size is reported by L. M. Klauber from a Texas diamondback.

Table 1 shows the average yield of venom for each of the 26 species studied and the number of individual extractions on which it was based. It also shows for each species the maximum yield for a single snake or small group of snakes.

II. TOXICITY OF PIT VIPER VENOMS

The venoms of the pit vipers are all complex mixtures, containing poisons which act on the blood, the blood vessel walls, the

central nervous system and other tissues. When injected subcutaneously or intramuscularly, they induce local reactions, characterized by edema and hemorrhage, and followed by weak-

TABLE 1
Yield of venom
In milligrams of dry venom per snake

SPECIES		NUMBER	TOTAL	MAXIMUM	AVERAGE
Tropical moccasin	<i>Agkistrodon bilineatus</i>	5	375		75
Copperhead	<i>Agkistrodon mokasen</i>	80	4,490	90	56
Water moccasin	<i>Agkistrodon piscivorus</i>	264	25,980	190	98
Grand Canyon rattlesnake	<i>Crotalus abyssus</i>	1	40		40
Florida diamondback rattler	<i>Crotalus adamanteus</i>	35	15,270	864	437
Texas diamondback rattler	<i>Crotalus atrox</i>	252	43,350	654	175
Sidewinder	<i>Crotalus cerastes</i>	124	2,710	67	22
Cerberus rattlesnake	<i>Crotalus cerberus</i>	10	890	150	89
Prairie rattlesnake	<i>Crotalus confluentus</i> —strong	432	13,010	36	30
Prairie rattlesnake	<i>Crotalus confluentus</i> —weak	8	680	110	85
Lower California rattler	<i>Crotalus enyo</i>	8	267	33	33
Timber rattlesnake	<i>Crotalus horridus</i> —strong	42	1,760	70	42
Timber rattlesnake	<i>Crotalus horridus</i> —weak	28	2,280	180	80
Green rattlesnake	<i>Crotalus lepidus</i>	28	162		60
San Lucan rattlesnake	<i>Crotalus lucasensis</i>	16	3,620	370	226
Great Basin rattlesnake	<i>Crotalus lutosus</i>	56	3,630	150	65
Bleached rattlesnake	<i>Crotalus mitchellii</i> —strong	15	1,200	90	80
Bleached rattlesnake	<i>Crotalus mitchellii</i> —weak	98	13,300	200	135
Blacktailed rattlesnake	<i>Crotalus molossus</i>	52	10,710	540	200
Pacific rattlesnake	<i>Crotalus oreganus</i>	405	28,740	190	70
Price's rattlesnake	<i>Crotalus pricei</i>	60	360	80	60
Red rattlesnake	<i>Crotalus ruber</i>	327	82,120	425	250
Mojave rattlesnake	<i>Crotalus scutulatus</i>	182	7,160	110	40
Panamint rattlesnake	<i>Crotalus stephensi</i>	5	360	88	72
Dog-faced rattlesnake	<i>Crotalus t. durissus</i>	120	13,890	280	116
Tiger rattlesnake	<i>Crotalus tigris</i>	14	146	17	10
Willard's rattlesnake	<i>Crotalus willardi</i>	1	37		37
Massasauga	<i>Sistrurus catenatus</i>	69	970	33	14
Pigmy rattlesnake	<i>Sistrurus miliarius</i>	7	23	10	3½

ness and paralysis. When given intravenously, especially in excessive dose, they may kill within five or ten minutes by convulsions, apparently asphyctic, and perhaps due to interruption of the circulation by intravascular clotting. After somewhat

smaller doses, paralysis is the usual manifestation. This begins, in pigeons, usually within fifteen minutes, as a weakness of the legs, the pigeon settling to the floor of the cage. As paralysis advances, the neck becomes weak, the beak, and finally the entire head resting on the floor. After this stage is reached, recovery is rare. According to the dose, paralytic death may result in from fifteen minutes to twelve or eighteen hours.

The results recorded here were obtained by the intravenous injection of pigeons, and the power of the different venoms to cause acute, paralytic death in this species was alone determined. The expression "toxicity of venom" as used in this paper must, therefore, be understood to have this limitation. No implication is made that the hemolytic, blood coagulant, or other activities of the different venoms would bear the same relationship to one another or that paralytic doses for other kinds of animals would necessarily differ in direct proportion to the fatal doses for pigeons.

Each lot assayed included, as a rule, the venom of several or many different individuals. A few studies of the venoms obtained from single individuals yielded no information of special importance, but showed that snakes kept in the same cage under identical conditions, might have venoms of widely different value.

The toxicity of the venom of each species commonly varied within a rather narrow range, the most active lot being not more than two or three times as potent as the weakest. The venom of the Cascabel was atypical in this respect, the assays having a range from 0.025 to 0.26 mgm., other values being scattered between these extremes. The venoms of three other species, the timber rattler, the prairie rattler and the bleached rattler, were peculiar in falling quite sharply into two groups of widely different toxicity, with no venoms of intermediate strength. No explanation of these divergences is at present available. It does not depend on differences in habitat of the individuals, on length of captivity, previous extractions, or other readily recognized features.

Although all of the rattlesnakes are closely related, belonging

to the same genus, they show wide differences in the activity of their venoms. Certain species such as the red rattler (average M.L.D. 0.54 mgm.) and the blacktailed rattler (average 0.32 mgm.) have very weak venoms, while others such as the tiger rattler (average 0.004 mgm.) and Mojave rattler (average 0.007 mgm.) have venoms of extremely high toxicity. The range thus found within this single genus is as great as that found on comparing venoms from totally unrelated species of snakes. Most cobra and mamba venoms, for example, will fall within this range. Wide differences in toxicity are noted even when closely related species are compared. Thus the Grand Canyon rattler, Cerberus rattler, prairie rattler, Great Basin rattler, bleached rattler, Pacific rattler, and Panamint rattler are now considered to be subspecies of *C. confluentus*. The toxicity of the venoms within this group varies however from 0.04 mgm. (Grand Canyon rattler) to 0.4 mgm. (weaker lots of the bleached rattler). For this reason, we have taken the liberty, in this article, to class them as distinct species.

Some light may be thrown on one factor influencing toxicity when the members of the genus *Crotalus* are arranged in the order of probable evolutionary development. It is generally accepted by herpetologists, that the poison first developed by venomous snakes was that paralyzing the nerve centers and that the actions on the blood and the blood vessel walls were evolved later. Thus in the elapine snakes, including the cobras, mambas and coral snakes, which are less highly differentiated than the vipers, the locally acting poisons are present in small amount and in the simpler types of pit viper, such as the moccasin and copperhead, the neurotoxic poisons are found in relatively high proportions. Local reactions, on the other hand, are much more severe after bites by the most highly differentiated poisonous snakes, the North American rattlesnakes. If this be true, it would seem probable that by a method of testing based solely on the paralytic effect of the venom, the more primitive snakes would show up to better advantage, and their venoms would be found more toxic. That this is apparently the case among the rattlesnakes, was shown in an earlier article (1). In this article it was

pointed out that the Mojave rattler (*C. scutulatus*) which is considered the most primitive of our rattlesnakes, had an extremely potent venom (M.L.D. 0.007 mgm.). In one line of descent we find the prairie rattler and Grand Canyon rattler (M.L.D. 0.04 and 0.05 mgm.) followed by the Pacific and Cerberus rattlers (M.L.D. 0.1 mgm.) and these by the Panamint rattler and sidewinder (M.L.D. 0.2 and 0.12 mgm.). In another line we find the Texas diamondback rattler (M.L.D. 0.14 mgm.) followed by the Florida diamondback (M.L.D. 0.28 mgm.) and by the San Lucan and red rattlers (M.L.D. 0.4 and 0.52 mgm.). There is no evidence, however, that the stronger crotalic venoms contain relatively more of the neurolytic poison.

The differences in potency assume additional interest when the average toxicity is compared with the average yield. Among the pit vipers of North America, the snakes secreting small amounts of venom have, in general, more toxic venoms than those yielding large amounts, so that the number of fatal doses per average extraction approaches more nearly a constant than either the toxic dose in milligrams of dry venom or the average amount of venom per snake. This relationship is well illustrated by comparing the green rattler, the Grand Canyon rattler, the Great Basin rattler, the San Lucan rattler and the red rattler. Although the toxic doses of these venoms are respectively 0.01, 0.06, 0.11, 0.4 and 0.54 mgm. the fatal doses per extraction vary only from 500 to 667. Although the strongest venom is more than 50 times as active as the weakest, there is only a difference of 30 per cent in the number of M.L.D. in one extraction. Only two species show a wide divergence from this general rule that low yield is associated with potent venom and vice versa. The pigmy rattler (*Sistrurus miliarius*) exhibits a very low yield associated with a very weak venom. As severe poisoning in man often follows the bite of this species, it seems probable that further assays would modify one or both of these figures. In contrast, the Mojave rattler (*C. scutulatus*) has both a high yield and a potent venom. As the only bite by this snake reported in man resulted in recovery, it would seem that this figure is also open to question.

TABLE 2

Toxicity of venom

In milligrams to 350-gram pigeons intravenously

SPECIES		NUMBER OF ASSAYS	MINIMUM M.L.D.	MAXIMUM M.L.D.	AVERAGE M.L.D.	AVERAGE YIELD	M.L.D. PER YIELD
			mgm.	mgm.	mgm.	mgm.	
Tropical moccasin	<i>Agkistrodon bilineatus</i>	2	0.07	0.15	0.11	75	680
Copperhead	<i>Agkistrodon mokasen</i>	13	0.05	0.20	0.12	56	470
Water moccasin	<i>Agkistrodon piscivorus</i>	16	0.06	0.15	0.11	98	890
Grand Canyon rattlesnake	<i>Crotalus abyssus</i>	1			0.06	40	667
Florida diamondback rattler	<i>Crotalus adamanteus</i>	8	0.12	0.50	0.28	437	1,600
Texas diamondback rattler	<i>Crotalus atrox</i>	29	0.09	0.30	0.14	175	1,250
Sidewinder	<i>Crotalus cerastes</i>	4	0.09	0.15	0.12	22	188
Cerberus rattlesnake	<i>Crotalus cerberus</i>	1			0.10	89	890
Prairie rattlesnake	<i>Crotalus confluentus</i> —strong	8	0.02	0.08	0.05	30	600
Prairie rattlesnake	<i>Crotalus confluentus</i> —weak	3	0.15	0.45	0.27	85	315
Lower California rattler	<i>Crotalus enyo</i>	1			0.10	33	330
Timber rattlesnake	<i>Crotalus horridus</i> —strong	7	0.02	0.08	0.047	42	890
Timber rattlesnake	<i>Crotalus horridus</i> —weak	5	0.25	0.70	0.40	80	200
Green rattlesnake	<i>Crotalus lepidus</i>	2	0.01	0.01	0.01	6	600
San Lucan rattlesnake	<i>Crotalus lucasensis</i>	1			0.40	226	565
Great Basin rattlesnake	<i>Crotalus lutosus</i>	5	0.06	0.15	0.11	65	600
Bleached rattlesnake	<i>Crotalus mitchellii</i> —strong	2	0.04	0.04	0.04	80	2,000
Bleached rattlesnake	<i>Crotalus mitchellii</i> —weak	3	0.40	0.60	0.50	130	260
Blacktailed rattlesnake	<i>Crotalus molossus</i>	6	0.12	0.40	0.32	200	600
Pacific rattlesnake	<i>Crotalus oreganus</i>	11	0.06	0.14	0.10	70	700
Price's rattlesnake	<i>Crotalus pricei</i>	1			0.20	60	300
Red rattlesnake	<i>Crotalus ruber</i>	5	0.30	0.60	0.54	250	500
Mojave rattlesnake	<i>Crotalus scutulatus</i>	4	0.005	0.009	0.007	40	5,500
Panamint rattlesnake	<i>Crotalus stephensi</i>	1			0.20	72	360
Dog-faced rattlesnake	<i>Crotalus t. durissus</i>	22	0.025	0.26	0.113	116	1,000
Tiger rattlesnake	<i>Crotalus tigris</i>	1			0.004	10	2,500
Willard's rattlesnake	<i>Crotalus willardi</i>	1			0.10	40	400
Massasauga	<i>Sistrurus catenatus</i>	6	0.006	0.04	0.024	14	540
Pigmy rattlesnake	<i>Sistrurus miliarius</i>	3	0.20	0.60	0.35	3½	10

Table 2 brings out these relationships in graphic form. It shows the average toxicity of each venom for the pigeon and the maximum and minimum toxicities observed. The table also includes the average yield, obtained from table 1 and the number of fatal doses included in such yields. This figure is obtained by dividing the average yield by the average fatal dose for each species. In the case of those venoms showing two types of varying potency, both types are given separately.

TABLE 3
Mortality of snake bite in man

SPECIES	WITHOUT ANTIVENIN			WITH ANTIVENIN			TOTAL			M.L.D. PER YIELD	LENGTH OF SNAKE
	Total	Die	%	Total	Die	%	Total	Die	%		
<i>C. adamanteus</i>	5	3	60	65	16	25	70	19	27	1,600	100
<i>C. atrox</i>	51	18	35.3	358	28	7.8	409	46	11.2	1,200	78
<i>C. horridus</i>	30	9	30	184	5	2.7	214	14	6.7	890	48
<i>C. confluentus</i>	26	5	19	128	5	4	154	10	6.5	600	44
<i>C. oreganus</i>	20	3	15	110	5	4.5	130	8	6.1	700	44
<i>C. cerastes</i>	2	1	50	15	0	0	17	1	6	183	30
<i>A. piscivorus</i>	27	4	17	167	4	2.4	194	8	4.1	890	48
<i>A. mokasen</i>	152	5	3.3	539	2	0.4	691	7	1	470	36
<i>S. catenatus</i>	3	0	0	18	0	0	21	0	0	540	24
<i>S. miliarius</i>	5	0	0	68	0	0	73	0	0	10	18

III. RELATIVE DANGER TO MAN

The statistical data on snake bite in man are derived from two sources of widely differing value. First in number and in exactness are reports sent in by physicians describing the cases in which antivenin has been used. Details concerning the kind of snake involved and the exact outcome of the case are usually to be depended on. The second source consists of newspaper clippings, the data in which are notoriously unreliable. Most of the data on cases treated by antivenin are from the first, most of those treated by other methods are from the second source.

About half the reports describe the snake simply as a "rattle-snake." When only one species occurs, or is common, in the part of the country from which the bite is reported, such cases are

ascribed to this species, otherwise they are not included, or are classed as "species unknown." Very few of the western species are included by name in these reports. Of the 24 species of pit viper occurring in the United States, data are available only on ten. These include the six eastern species and 4 of the 20 western forms. Further details on these figures will be presented in a later article.

Table 3 gives such data as are available. It will be seen by reference to this table, that in general, the larger the snake, the more dangerous it is to man. The only exception to this rule is the sidewinder (*cerastes*) the death rate from which is higher than would be expected from its size and the toxicity of its venom. There were no deaths from this snake in cases treated with antivenin, and there is little doubt that the proportion of deaths in other cases would be much lower if there were more reports. Leaving this one species aside, there is a close agreement between the death rate in man, the size of the species and the number of M.L.D. in one extraction.

SUMMARY

This study of the venoms of North American pit vipers brings out the following points:

1. The amount of venom secreted by each species varies directly with the size of the species.
2. There are great differences in the toxicity of venoms from closely related species.
3. The more primitive forms have venoms more toxic to the nerve centers.
4. Species secreting a small amount of venom tend to have more active venoms, while those yielding large amounts have weaker venoms, so that the number of fatal doses in one extraction is more constant than are other factors.
5. The larger species of viper give the largest number of fatal doses per extraction.
6. The danger to man from the bites of pit vipers, varies almost directly with the size of the species.

REFERENCE

- (1) GITHENS AND GEORGE: Bull. Antivenin Inst., 1933, 31.