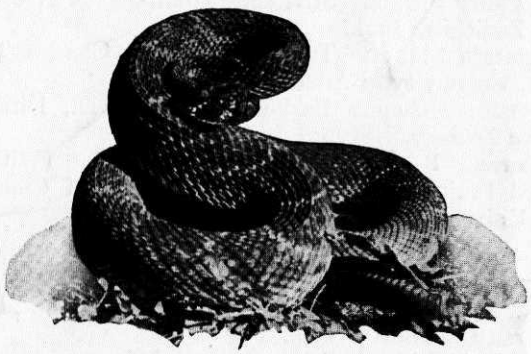
L. Stejneger
Apr. 24. 1931
QL
646
A63
Rept

Bulletin
of the
Antivenin Institute of America

Glenolden, Pa., U. S. A.

SMITHSONIAN
JUN 10 1966
LIBRARY



VOL. IV, NO. 4 MARCH, 1931

PUBLISHED QUARTERLY AT PHILADELPHIA, PA.

Bulletin
of the
Antivenin Institute of America

VOL. IV MARCH, 1931 No. 4

Edited by

THOMAS BARBOUR, R. H. HUTCHISON *and* AFRANIO DO AMARAL

WITH THE COLLABORATION OF OTHER MEMBERS OF THE STAFF OF THE INSTITUTE

The BULLETIN contains scientific contributions to, and current information on, the relation of snakes and other poisonous animals to man, with special reference to the highly venomous species of North and Central America, the West Indies, and northern South America. The subject matter covers taxonomy, biology and geographical distribution of species; the economic and public health aspects of the snake-bite problem; the collection of venoms, their composition, properties, and physiological effects; the production of antivenins; and related serological and immunological problems.

Address all communications to the BULLETIN OF THE ANTIVENIN INSTITUTE OF AMERICA, Box 1404, Philadelphia, Pa.

107. ANTIVENIN: ITS PREPARATION AND STANDARDIZATION

BY THOMAS S. GITHENS, M.D.

It has long been known that the repeated injection of minute amounts of snake venom into animals leads to the production by these of substances capable of neutralizing the poisonous effects of the venoms, and that animals which have been thus treated are protected against the poisonous action of the venom. It has also long been known that these neutralizing substances pass into the blood of the protected animals and that if the blood be drawn and allowed to clot, the neutralizing substances will be found in the serum. If venom be mixed with such serum and the mixture injected into animals, they will show no ill effects even though many times as much venom as would kill an unprotected animal be given.

Serum containing such protective substances is known as "antivenin" and is closely related in its properties to the antitoxins which neutralize bacterial toxins.

In preparing the antivenin for human use, horses are injected subcutaneously with small amounts of a dilute solution of venom and the dose repeated every few days, being slightly increased at each injection. The horses eventually reach a state of protection in which they will bear very large doses of venom, several hundred times as much as would kill a normal horse.

When the horses reach this stage they are bled. The serum is separated from the red blood cells and concentrated by a special process which removes a large proportion of the proteins, but leaves the antivenin in the final product. This concentrated serum constitutes the antivenin of commerce.

NATURE OF VENOMS

Snake venoms are viscid, commonly yellow liquids, which dry rapidly on exposure to the air, leaving a yellow, glistening mass, not unlike dried gelatine. The solid matter, which is readily soluble in water, constitutes from $\frac{1}{4}$ to $\frac{1}{3}$ of the fresh venom. When dried, the venom retains its poisonous and other properties for years. A freshly made watery solution resembles in all essentials the fresh venom.

Snake venom is a highly complex mixture containing proteins, mucus, fatty matters, salts, epithelial debris, and several poisons, which appear to be of protein nature. Fresh venom is slightly acid in reaction because of the presence of a volatile acid which disappears on drying, the solution of dried venom being neutral. The toxalbumins of rattlesnake venom are destroyed by heating to 70° or 80°C. They do not dialyse through parchment. They may be precipitated with little alteration by strong solutions of salts, by alcohol, or by small amounts of acid. Such precipitates contain the poisons mixed with small amounts of mucus and inert proteins.

The actions of venoms on the body are very complex and vary much from one species to another, probably owing to differences in the proportionate amounts of the several poisonous substances which they contain. It is not certain whether each of the different actions of venoms on the body depends on the presence of a distinct poisonous substance or whether a small number of such substances may exert different toxic actions on various tissues and organs.

Among the more important effects of rattlesnake venoms on the body, we may distinguish effects on the tissues near the bite, effects on the blood, and effects on distant organs and tissues to which the venoms are carried by the lymph and blood.

The local effects are due to a toxalbumin which is more readily destroyed by heat than most of the other constituents. This causes locally more or less severe pain due to an irritant action on the nerves and sensory endings. It exercises a peculiar action on the walls of the blood vessels, causing them to become permeable to the blood, which escapes from the vessels as clear lymph and as local hemorrhages, leading to marked swelling and discoloration of the tissues near the bite. A great deal of fluid may thus pass out from the blood-vessels, and if incisions are made in the skin near the bite, the fluid will ooze out as a yellowish or more or less blood-stained liquid. This liquid may carry with it considerable amounts of the venom.

The action of rattlesnake venom on the blood affects chiefly its clotting powers and the red blood cells. Small amounts of venom added to blood generally tend to cause clotting, larger amounts to prevent it. Evidences of each of these effects are likely to be seen after a snake bite. The red cells are dissolved by the venom, the degree of hemolysis varying markedly with different kinds of venom. The white blood cells are less affected.

Various organs are found to be susceptible to the action of the venom, which exercises a destructive effect on the functional cells. The most striking symptoms are paralytic, due to a so-called neurotoxin. The lesions include neuritis of the small nerves of the limbs. Both sensory and motor cells of the spinal cord show degenerative changes, most marked in the dorsal and lumbar cord. Changes are also found in the cells of the medulla oblongata. Paralysis results, beginning in the forelegs of animals (or wings of birds), extending to the hind legs and finally to the muscles of respiration. When these are involved, death follows quickly.

The kidneys are also markedly affected by the destructive action of the venom. The secretory cells are injured, resulting in albuminuria, and the urine is often stained red by hemoglobin or blood. The blood-vessels are dilated and may be distended by altered blood. Degenerative changes in the liver are also seen, and yellow discoloration of the eyes and skin from mild jaundice is common in severe cases when death does not occur soon after the bite.

The changes in the blood and vessels lead secondarily to hemorrhages in various parts of the body; the particular poison responsible for these effects being known as hemorrhagin. Hemorrhage may occur from the nose or in the eyes. Bleeding from the eyelids has often been reported after bites by the fer-de-lance (*Bothrops atrox*) of tropical America.

THE PROPERTIES OF ANTIVENIN

The antivenin prepared by injecting a horse with the venom of one species of snake is able to neutralize the poisonous action of all the different constituents of this venom, preventing both the

general effects on the central nerve system and the local effects on the blood-vessels and tissues. This antivenin is also effective against the venoms of poisonous snakes of closely related species but not against those of more distantly related forms. Thus, the serum of horses injected with the venom of the western diamond-back rattler (*Crotalus atrox*) is effective against the venoms of other North American rattlesnakes (genus *Crotalus*) and also against the venoms of the moccasin and copperhead (genus *Agkistrodon*), as well as against that of the tropical snake of the same genus (*A. bilineatus*). The serum is, however, almost ineffective against the unrelated venoms of the Indian and African cobras, against those of the "barba amarilla" and the bushmaster (genera *Bothrops* and *Lachesis*) of tropical America and against the various snakes of Australia. This serum is moderately effective against the powerful venom of the tropical rattlesnake or cascabel (*Crotalus terrificus*) although much less so than a specific serum obtained from horses injected with cascabel venom.

Neither venom nor antivenin is effective when administered by mouth, but develop their activity when injected under the skin, into a muscle, or into the blood stream. The action of the venom is most regular when the last method of administration is used, as the severe local reaction set up after injection into the tissues influences absorption of the poison irregularly and in a manner which differs with different individuals. For exact quantitative work the intravenous method of injection is therefore to be preferred.

By injecting pigeons intravenously with dilute venom solutions, it is possible to determine very exactly the smallest amount which will bring about the death of birds of this species. The amount can be determined to within 5 or 10 per cent and the test can be repeated and checked by persons familiar with the technic. The dose which is just sufficient to kill a pigeon weighing 350 Gm. (12 oz.) when injected into the vein at the base of the wing is called the minimum fatal dose or minimal lethal dose (commonly abbreviated as m.l.d.). Calculated as weight of dried venom this varies with different species of snakes from as little as 0.004 mgm. to as much as 0.65 mgm. (1/100 grain). The venoms of the western diamond-back rattlesnake and most other rattlesnakes have m.l.d.'s of about 0.15 mgm. (1/400 grain).

The activity or potency of antivenins is determined quantitatively by injecting into the veins of pigeons, mixtures of venom and antivenin prepared in the following way. Immediately after its collection, venom to be used for test purposes is purified by centrifugation, which removes epithelial cells, some mucus and other accidental contaminations. It is then carefully dried and preserved in a dry place. An accurately weighed portion is dissolved in a measured amount of physiological salt solution (0.85 percent) so as to obtain a solution containing exactly one part of venom in five hundred or one thousand parts of solution. Carefully measured amounts of this solution differing from one another by about 10 percent are mixed with a fixed volume of antivenin, usually 1 cc. (about $\frac{1}{4}$ of a teaspoonful), and the mixture kept a few minutes at body temperature to permit union of the venom and antivenin to be complete. Each mixture is then injected into the wing vein of a normal pigeon weighing about 350 Gm. (12 oz.). The pigeons are kept under observation and if one recovers it is certain that the amount of venom injected was neutralized by the antivenin. When antivenin is tested in this way, it is found that 1 cc. will prevent the fatal effects of from 3 to 4.5 mgm. of the dried venom of *C. atrox*, that is, from 20 to 30 pigeon m.l.d.'s and of similar amounts of other rattlesnake venoms.

Several related venoms, such as those of the copperhead and moccasin and prairie rattler (*C. confluentus*), have venoms the fatal dose of which for the pigeon is much smaller,—from 0.04 to 0.1 mgm.—and although the antivenin will neutralize as large a number of fatal doses, the total amount of dried venom neutralized may be considerably less. In general, the more deadly a venom, the less is ejected during a bite, and statistics show that serious results are not any more frequent after the bites of snakes whose venom is unusually toxic than after bites of larger snakes with less poisonous venoms. For example, *Crotalus confluentus confluentus*, though widespread and possessing a more deadly venom, holds third place as a cause of death, while the larger Western diamond-back (*C. atrox*) ranks first.

If similar mixtures of venom and antivenin be injected subcutaneously or intramuscularly, it is seen that the local destructive effects of the venom are also prevented. The antivenin not only neutralizes the neurotoxins which cause acute death in the pigeon, but also neutralizes the various hemolysins, coagulins and cytolysins.

The antivenin is not only effective when it is mixed with the venom before injection, but is able to prevent the poisonous effects of venom previously administered. If, for example, a rabbit be injected with 4.5 mgm. of dried venom of *Crotalus atrox* per kilogram body weight (which is more than twice the amount required to kill a normal rabbit) and 15 minutes later is given 10 cc. of antivenin, it will recover without showing signs of poisoning.

As already explained, the serum of horses immunized only against *C. atrox* venom is effective also against the venoms of those poisonous snakes of North America which figure prominently in snakebite statistics (See Bulletin A.I.A. Vol. IV, p. 43), but in practise, horses treated for the production of antivenin are injected with a mixture containing the venoms of several species of rattlesnake as well as those of the copperhead and moccasin.

Table 1 shows how effective such an antivenin is against the poisons of most of the important species of poisonous snakes of North America. The venoms were mixed with 1 cc. of antivenin and injected after standing 30 minutes at body temperature (37°C.). The first column shows the least amount (m.l.d.) that would regularly kill a 12 ounce pigeon. The second column shows the quantity of venom neutralized by 1 cc. of antivenin. In the third column the neutralizing value of antivenin is expressed in terms of the number of pigeon m.l.d.'s. neutralized.

Table 2 shows how the m.l.d. of a given venom is determined by giving a series of graduated doses and observing the point below which all survive and above which all die.

Table 3 is a protocol of a test made to determine the potency of a given antivenin.

TABLE 1

SHOWING THE AMOUNT OF VENOM NEUTRALIZED BY 1 CC. OF AVERAGE ANTIVENIN

	Pigeon m.l.d.	Venom neutralized by 1 cc.	Number of pigeon m.l.d.'s neutralized by 1 cc.
	mgm.	*mgm.*	
A. mokasen	0.12	1.5	12.5
A. piscivorus	0.10	1.3	13.0
A. bilineatus	0.07	1.0	14.0
C. adamanteus	0.14	3.7	26.4
C. atrox	0.4	3.7	9.2
C. exsul-ruber	0.6	3.7	6.1
C. horridus	0.35	5.5	15.7
C. mitchellii	0.35	4.0	11.4
C. oreganus	0.14	4.3	30.7
C. abyssus	0.06	0.4	6.6
C. confluentus	0.04	0.6	15.0
C. stephensi	0.2	2.0	10.0
C. enyo	0.1	0.5	5.0
C. terrificus	0.025	0.6	24.0

TABLE 2

PROTOCOL OF TEST TO DETERMINE MINIMAL FATAL DOSE OF CROTALUS ATROX VENOM ON PIGEONS

Pigeon weight	Venom	Venom	Venom dilution	Volume venom	Time injected	Results	
grams		*mgm.*		*cc.*			
350	*C. atrox*	0.12	1:5000	0.6	8:21	Recovery	
350	No. 8175	0.13	1:5000	0.65	8:24	Recovery	
340	No. 8175	0.14	1:5000	0.7	8:30	Recovery	
350	No. 8175	0.15	1:5000	0.75	8:40	Death at 10:04.	m.l.d. =
350	No. 8175	0.16	1:5000	0.8	8.55	Death at 9:22	0.15 mgm.

TABLE 3

PROTOCOL OF TEST TO DETERMINE VALUE OF ANTIVENIN NEARCTIC CROTALIDIC ON PIGEONS

Pigeon weight	Venom	Venom	Dose antivenin crotalidic	Venom dilution	Venom dose	Time injected	Results	
grams		*mgm.*	*cc.*		*cc.*			
350	*C. atrox*	3.1	1	1:500	1.55	2:16	Recovery	
360	No. 8175	3.3	1	1:500	1.65	2:20	Recovery	(Value 3.5
340	No. 8175	3.5	1	1:500	1.75	2:25	Recovery.	mgm. per cc.
350	No. 8175	3.7	1	1:500	1.85	2:30	Death at 3:57	antivenin.)

PRACTICAL CONSIDERATIONS

Venoms are mainly absorbed through the lymphatic spaces and pass slowly by diffusion from the point of introduction (by bite or injection) to the vital centers. Small amounts may escape into the veins giving rise at times to violent systemic symptoms soon after the bite, and if the injection be made into a vein, or a large vein near the bite be injured so that venom can pass directly into it, death may follow within an hour or even in a few minutes.

As a general rule, however, absorption is gradual and the farther from the body the injection had occurred, the slower will be the development of symptoms and the greater chance the body will have to dispose of the venom as it reaches the organs of excretion. In man, bites commonly occur on the extremities, a couple of feet away from the trunk and the vital organs. For this reason, the onset of serious systemic poisoning is longer delayed than in small animals such as rodents, in which the injection is at most only a few inches away from the body and in man there is much greater chance for the success of therapeutic measures instituted after the bite has occurred.

SUMMARY

In discussing briefly the preparation and testing of antivenins, we have in this paper dealt only with the laboratory side of the subject. An attempt has been made to outline in convenient form the facts which are already common knowledge.

The method of determining the minimum lethal dose of venom is described, and the way this is done in the laboratory is shown by the typical protocol given in Table 2.

The treatment of horses in the production of antivenin is outlined and the method of testing the serum is shown in a protocol of the potency test in Table 3.

The potency of Crotalidic antivenin against the venoms of some of the North American poisonous snakes is shown in Table 1. This table presents some new data not previously published.

From the laboratory standpoint our knowledge of the action of venoms and the value of antivenin is much more exact than that derived from clinical experience in the treatment of human beings who have been bitten. Yet the observations made in the course of extensive laboratory experience doubtless have some bearing on the clinical side. Although the subject of snakebite poisoning in man has received renewed attention within the last four or five years, there is undoubtedly much more to be learned in this field, particularly concerning the specific treatment with antivenin.

From the Antivenin Institute, Mulford Biological Laboratories, Sharp & Dohme.

108. STUDIES OF NEOTROPICAL OPHIDIA

XXIII. ADDITIONAL NOTES ON COLOMBIAN SNAKES

BY AFRANIO DO AMARAL

A second lot of snakes has recently been received at this Institute from the Rev. Brother Nicéforo Maria, of the Instituto de La Salle, who collected them also in the central section of Colombia. Upon examination this lot has proved to be still more interesting than the previous one as it contained one full new species, a second specimen of a species just described, three new races, several rare species, and a species not yet recorded from Colombia.

The localities herein recorded are the following:

Aguadita, Cundinamarca (south of Bogotá)
Guaicaramo, interior of the "llanos" (east of Bogotá)
Jericó, Jericó (southwest of Medellin)
Pacho, Cundinamarca (north of Bogotá)
Pensilvania (south of Medellin, Antioquia)
Sampedro (north of Medellin, Antioquia)
Sonsón, Sonsón (south of Medellin)
Villavicencio, Meta (east of Bogotá)

The specimens received have thus been identified:

Gen. TYPHLOPS Dm. et Bibr.

Type: *lumbricalis*

Typhlops reticulata (L.)

No. 33. Adult, collected at Villavicencio.

Black above, with light-edged scales; snout and belly white; tail white, the tip black.