Snake Venoms, Their Biochemistry and Mode of Action[1]

Nandor Porges[2]

1301 East Barringer Street, Philadelphia, Pennsylvania

THE VENOM APPARATUS reaches the highest development in poisonous snakes. Many other species throughout the animal kingdom also have the capacity to elaborate poison, and this capacity is manifest in a variety of forms. It is a passive defensive function in frogs, toads, and salamanders, in each of which the venom glands are distributed in the skin. In other animals the venom apparatus is a weapon of offense and defense. The male platypus has a canalized spur on each of his limbs; the spurs are connected to a venom gland on the back of the thigh. Many poisonous fishes have a venom gland at the base of the spine, and in some species the apparatus is important for the capture of food. Corals, jellyfish, and hydra have their stinging organs. Spiders and myriapods have the glands in the mouth, and scorpions and wasps have the venom in the hinder parts. In snakes, the venom glands are actually specialized salivary organs, and inoculation is by the canalized or grooved teeth. The venom not only immobilizes the prey but aids subsequent digestion of the animal tissues.

Snake venoms provide an exciting and absorbing subject for investigation. In the United States 2000–3000 snakebite accidents occur annually, of which 10–35 per cent are fatal. In Europe about 14 deaths are reported yearly, whereas approximately 25,000 deaths occur each year in India. Much study has been devoted to the mechanism by which snake venom causes death of the victim (*1–4*), and more than 6000 papers have been published on the subject of venoms.

Snake venom is a mixture, chiefly of proteins, varying in composition from species to species. Over 300 species are distributed throughout the world—a fertile field for scientific investigation (Table 1). The poisonous snakes of Europe are the "true" vipers; the Americas have no snakes of this family. Nearly all the poisonous snakes of North and South America are "pit" vipers, of which the rattlesnake is a representative species. Africa has none of the rattler type, and Australia's poisonous snakes are limited to those of the proteroglypha, characterized by permanently erect fangs. Members of all groups are found in Asia.

The venom may vary in a single species, depending on the habitat and on the physical condition of the snake. The amount ejected may be only a few drops, or as much as 2 ml in a single strike. The dry weights of solids contained in an average ejection of venom have been reported for a few species (Table 2).

TABLE 1

GEOGRAPHICAL DISTRIBUTION OF VENOMOUS SNAKES

	Viperidae		Colubridae
	Viperinae (true vipers)	Crotalidae (pit vipers)	Proteroglypha
Europe	1 genus (7 species)	0	0
Americas	0	4 genera (50 species)	2 genera (43 species)
Africa	7 genera (46 species)	0	8 genera (30 species)
Asia	7 genera (16 species)	2 genera (27 species)	27 genera (11 species)
Australia	0	0	19 genera (76 species)

In a review of animal poisons, Kellaway (*3*) recognized in snake venoms the presence of at least two toxic principles and possibly more. These are proteins or substances of protein nature, some having enzymatic properties. Thus the toxic actions of venoms were attributed to proteolytic enzymes, phosphatidases, and neurotoxins.

The proteolytic activity of venoms of the bothropic species (for example, *Bothrops atrox,* or fer-de-lance) was described as early as 1881, but it had been suggested even earlier that reptile poisons might contain agents similar to those of the digestive juices. These substances, in crotaline venoms, produce severe hemorrhagic and destructive effects at the site of the bite, and hemorrhages in the viscera; they give to the venoms their coagulating properties. Explanation of these phenomena was not forthcoming until work on the coagulation of blood led to some knowledge of venom proteases.

Venoms with coagulant properties fall into two groups. The first includes the most powerful proteolytic venoms, which coagulate pure fibrinogen *in vitro* without calcium ions, tissue extracts, or prothrombin. This group has an optimum pH of 6.5, the same as thrombin, but coagulation is unhindered by specific antithrombin. The proteolytic enzymes in these venoms behave like papain, converting fibrinogen into fibrillar gel.

The venoms of the second group, unlike those of

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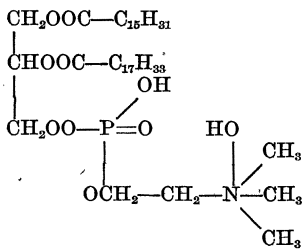
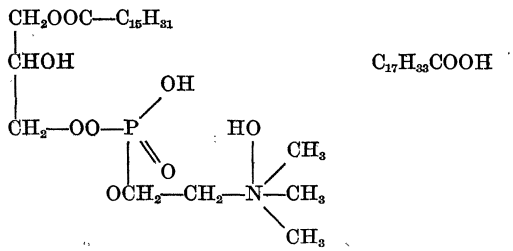
the first, are unable to coagulate pure fibrinogen. They convert prothrombin to thrombin. The activity of these substances is analogous to that of trypsin but is many times more potent. The proteolytic enzymes may contribute to the hypotensive action of snake venom through damage to vascular endothelium, with consequent escape of blood from the vessels and possibly liberation of histamine.

The phosphatidases also play a part in poisoning, causing hemolysis and most of the effects on the heart and circulation. Hemolysis by venom is accelerated by lecithin, since oleic acid splits off to form lysolecithin. Lecithin upon complete hydrolysis yields four products: glycerol, fatty acids (palmitic, stearic, oleic), phosphoric acid, and choline. When the phosphatidase, lecithinase, acts on lecithin, oleic acid and lysolecithin are formed, as follows:



A lecithin, glyceryl mono-palmitate mono-oleate, mono-phosphate ester, yields in the presence of lecithinase:



Lysolecithin + Oleic acid

Peculiarly, the lysolecithin itself is actively hemolytic, and its effects on the heart are similar to those of venom. Like some venoms, it acts on isolated muscle and causes, among other effects, contractures, fibrillation, increased inhibition of water, loss of potassium, and excitability. It attacks the capillary endothelium and causes hemorrhage of the lungs. However, because the protein of the plasma offers some protection, large doses are required to kill animals. The hemolytic action of lysolecithin is inhibited by egg albumin in solution. The hemolytic activity of the Australian snake venoms is directly related to their lecithinase content. Cobra venom is more active in dilute than in strong solution, because the lecithin is absorbed by the proteins, which coagulate in the concentrated venom.

The abrupt fall of systemic blood pressure that results when noncoagulant venoms are injected intravenously is caused in large part by the action of phosphatidase in forming lysolecithin and liberating histamine. The venoms from *Crotalus atrox* and *Denisonia*

TABLE 2

AVERAGE CONTENT OF SOLIDS IN A SINGLE EJECTION OF VENOM

Species	Milligrams
Viperidae	
Viperinae (true vipers)	
Vipera aspis (European viper)	8–10
V. russelli (Russell's viper)	200–300
Bitis gabonica (puff adder)	600–1000
Crotalidae (pit vipers)	
Crotalus atrox (Texas diamond-back or red rattlesnake)	90–175
C. terrificus (tropical rattler)	30–60
C. adamanteus (Florida diamond-back or Eastern rattlesnake)	240–437
Agkistrodon piscivorus (water moccasin or cottonmouth)	90–150
C. horridus (timber rattler)	42–90
Bothrops atrox (fer-de-lance)	100–180
B. jararaca (fer-de-lance type viper)	200–330
Colubridae	
Naja haje (Egyptian cobra)	19–48
N. naja (Indian cobra)	*
N. flava (African cobra)	*
Denisonia superba (Australian copperhead)	*
Acanthophis antarcticus (Australian death adder)	*
Bungarus fasciatus (banded krait)	*
Pseudechis porphyriacus (Australian black snake)	*

* Information not available.

superba seem to produce a histaminelike effect. *Vipera aspis* venom also increases capillary permeability. More careful study has shown that injection of the venoms of *C. atrox, D. superba,* and *Naja naja* causes the liberation of histamine and coagulable protein. The effects of intravenous injection of cobra venom in cats and dogs may be explained by the release of histamine, which exerts most of its action at the site of its liberation. It has been shown that histamine is formed if coagulation is prevented. It appears that the platelets and leucocytes aggregate and then disintegrate, liberating an intermediate substance (a plasma protease), which subsequently releases histamine from its protein-bound inactive form.

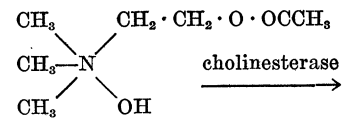
Study of the mechanism of the liberation of histamine by snake venoms has led to recognition of the role of phosphatidases. Lysolecithin is formed in perfused organs when snake venom is injected, and itself liberates histamine from the organs. Lysolecithin formation is an intermediate step in the liberation of histamine by venoms. A substance is also formed that causes subsequent changes in the reactivity of the muscle to histamine and acetylcholine. The part played by lysolecithin in snake venom hemolysis is parallel to its action in liberating histamine. The Australian black snake venom is strongly hemolytic; that of the Australian death adder, feebly so. Increasing the concentration of the latter venom increases histamine output.

The third group of toxic principles, according to Kellaway, comprises neurotoxins, which exert various actions on the nervous tissues and produce a curare-

like effect, as well as paralysis. The venom of *C. terrificus* acts directly on the bulbar centers, whereas those of *V. aspis* and *N. naja* have no such action.

Other enzyme actions have also been observed. The proteases of venoms of the Indian cobra and Russell's viper resemble trypsin. Erepsin has been found, as well as other phosphoesterases. Many of the active principles are apparently complexes of proteins. One that converts hemoglobin to methemoglobin has been isolated (1 g hemoglobin combines with 1.36 ml oxygen, which is released under reduced pressure in the body; the methemoglobin also combines with oxygen but does not release it).

The venoms are proteinaceous, and the nitrogen distribution is in the following range: total, 13.4–14.8 per cent; albuminoid, 8.8–9.6 per cent; globulin, 3.3–5.2 per cent; and nonprotein nitrogen, 4.6–8.8 per cent. The venoms of the Colubridae contain as much as 3.8–4.8 per cent sulfur; the Viperidae, 1.7–2.5 per cent. The sulfur is associated with the toxic molecule. Colubrine venoms are also high in zinc, having a content of 3.3–5.3 per cent, whereas the viperine venoms contain only 1.1–2.2 per cent. The zinc is associated with the esterase property.

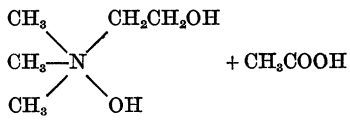


The neurotoxins of cobra venom are concentrated in a protein fraction, containing about 21 per cent of the original protein in the crude venom. One of these toxins has been found to possess a molecular weight between 2500 and 4000; analysis is as follows—C, 45.2; H, 7; N, 14.7; S, 5.5; and ash, 3. This toxin is highly potent; 0.12 μg/g has proved lethal for mice. Another, smaller, molecule has also been obtained; 1 μg is required for a lethal dose.

The neurotoxins of the crotaline venoms are somewhat larger. The neurotoxic activity of *Agkistrodon* venom is reduced by 50 per cent upon dialysis through cellophane; when the dialysate is returned, the activity is regained. Venom from *C. terrificus* has a high sulfur content, present in disulfide form. Cysteine acts on the disulfide bonds, with subsequent loss of activity as a neurotoxin. Cobra venom is not inactivated because another component reacts with cysteine. A proteinlike substance from *C. terrificus* venom has been analyzed as follows: C, 44.9; H, 6.6; N, 13.7; S, 3.6. About 10 per cent of the venom of this snake is an albuminlike, blood-coagulating principle. About 60 per cent of the whole venom contains a neurotoxic principle that is also associated with the hemolytic principle. This substance—crotoxin—has a molecular weight of about 30,000; the empirical formula is $C_{1386}H_{2086}O_{470}N_{372}S_{41}$. Crotoxin contains eighteen amino acids: arginine, histidine, lysine, leucine, isoleucine, aspartic acid, glutamic acid, proline, phenylalanine, tryptophane, tyrosine, serine, threonine, glycine, alanine, valine, cysteine, and methionine (*5*).

In 1948 Zeller (*4*) reviewed the extensive studies published during the previous decade. Zeller believes snake venoms are potent sources of biological catalysts that are not accidental components of the venoms. Although knowledge is still limited, the enzymes are now apparently considered the real toxic principles of venoms, either through their own activity or through their influence on other reactions.

An acetylcholine-inactivating enzyme found in cobra venom by Indian workers aroused great interest because of the possible relationship to the neurotoxic principle of the cobra poison. This enzyme acts on acetylcholine to produce choline and acetic acid as follows:



Apparently there are different types of cholinesterase: one obtained from erythrocytes; another from serum; and the *c*-type, from colubrine venoms. This enzyme is related to the mechanism of nerve action and is located on the neuron surfaces. On the basis of the cholinesterase content, snake venoms seem to fall into two groups, the venoms of the Colubridae being characterized by the presence, and the venoms of the Viperidae by the absence, of cholinesterase. These, the most active cholinesterases known, act also on noncholine esters. The Q_{ChE}[3] of certain of these venoms are shown in Table 3. For comparison, values obtained with red blood cells and a purified product from the cells are given.

TABLE 3

CHOLINESTERASE ACTIVITY OF SNAKE VENOMS (Q_{ChE})

Naja melonoleuca	27,900
N. naja	13,000
Bungarus fasciatus	18,700
Denisonia superba	11,000
Pseudechis australis	90
N. nigricollis	40
Red blood cells (human)	48
Red blood cells (purified product)	8,400

In 1936 an agent was found in snake venoms that enhances the spreading of dyestuffs and infectious agents in the skin of animals. This agent is also present in invasive bacteria, other types of venom, mammalian testes, and autolysates of pneumococci. The "spreading factor" was identified with the substance that depolymerizes hyaluronic acid, the mucopolysaccharide that binds water in the interstitial spaces and holds the cells together in a gel. Hyaluronic acid has the important function of resistance to penetration by foreign matter. This resistance breaks down under the action of hyaluronidase. Hyaluronic acid consists of glucosamine, glucuronic acid, and

[3] Q_{ChE}—μl CO_2 liberated per hour from a bicarbonate solution by a milligram of dried venom in the presence of excess acetylcholine.

acetic acid, and has a molecular weight between 200,000 and 500,000. It is found free or in combination with proteins. Venoms of various species differ in hyaluronidase activity as measured by dye-spreading and reduction of viscosity. Other diffusing principles are probably present also.

In 1944, a nonhydrolytic enzyme, L-amino acid oxidase, was demonstrated in *V. aspis* venom. The interaction between this enzyme and its substrates follows two pathways. If catylase is present, the amino acid is changed to an alpha keto acid and ammonia is liberated thus: $RCH(NH_2)COOH + \frac{1}{2}O_2 \rightarrow RCOCOOH + NH_3$. If catylase is absent, the reaction takes place in two steps, the final products being ammonia, fatty acid, and carbon dioxide:

$$RCH(NH_2)COOH + O_2 + H_2O \rightarrow RCOCOOH + NH_3 + H_2O_2$$
$$RCOCOOH + H_2O_2 \rightarrow RCOOH + CO_2$$

This enzyme oxidizes all monoaminomonocarboxylic acids except glycine and threonine. Alanine and serine are oxidized with low velocity; leucine and methionine, with high velocity. The configuration of the aliphatic chain has an effect on the interaction between the substrate and the enzyme, as shown by the action of *Bitis gabonica* venom on α-aminocaproic acids. The Q_{O_2}, or microliters of oxygen used per milligram of venom, is shown in Table 4.

TABLE 4

OXYGEN UTILIZATION BY *Bitis gabonica* VENOM ACTING ON ALPHA AMINOCAPROIC ACIDS

Amino acid	Q_{O_2}
Isoleucine, $CH_3—CH_2 \cdot CH \cdot CH_3 \cdot CH(NH_2) \cdot COOH$	158
Leucine, $CH_3—CH(CH_3) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	584
Norleucine, $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2)COOH$	284

The diaminomonocarboxylic and monoaminodicarboxylic acids are acted upon more slowly than the above acids. This is the first time that several of the amino acids have been attacked enzymatically or have been oxidatively deaminated *in vitro*. For this action to proceed, the substrate must have a free carboxyl group, an unsubstituted α-amino group, and an organic radical. A second amino or carboxyl group has an inhibitory effect. This enzyme, found in snake venom and snake tissue, has properties different from other amino acid-oxidizing enzymes and has been named L-ophio-amino acid oxidase, or ophio-oxidase. The amount of activity is specific for a genus; of the pit vipers, the genus *Bothrops* has higher activity than the genus *Crotalus* when leucine is used as a substrate. A few selected Q_{O_2} values are given in Table 5.

As mentioned previously, the rapid diffusion of venoms is prevented by the intercellular gel of the connective tissues, which forms a strong barrier. This barrier may be overcome with the aid of hyaluronidase, a solubilizing enzyme. The spreading action of hyaluronidase in turn may be inhibited by the invaded organism through the liberation of an antihyaluronidase called *anti-invasin I*. It is associated with the pseudoglobulin fraction of animal sera. It has been found that, oddly enough, the hyaluronidase of *Agkistrodon piscivorus* is not inactivated by the serum. Later it was discovered that a heat-labile agent present in the venom inactivates *anti-invasin I*, and is now termed *proinvasin I*. The proportion of hyaluronidase to proinvasin I varies. Some venoms contain large amounts of proinvasin I; hence destruction of anti-invasin I is rapid, permitting the hyaluronidase to act and favoring speedy invasion by the venom. In the presence of small amounts of proinvasin I, destruction of anti-invasin I is slight. Thus hyaluronidase is destroyed by the excess anti-invasin I, preventing invasion by the venoms.

TABLE 5

L-OPHIO AMINO ACID OXIDASE ACTIVITY OF SOME VENOMS ON L-LEUCINE (Q_{O_2})

Naja naja	100
Bungarus coeruleus	250
Agkistrodon piscivorus	380
Bothrops atrox	760
Crotalus viridis	270
C. adamanteus	50
Vipera aspis	610
V. russellii	760

The proinvasin I of snake venom may be destroyed by another agent called *anti-invasin II*. This mechanism is under study.

Investigations on snake venoms continue. Recent studies (*6*) on ribonuclease and desoxyribonuclease activity of venoms have been reported, and the data are compiled in Table 6. Venoms with the greatest neurotoxic potency exhibit highest ribonuclease and desoxyribonuclease activity. Venoms with the strongest blood-clotting and proteolytic activity have the least nuclease activity. Substances that inactivate venoms inactivate ribonuclease and desoxyribonuclease. Apparently, the hydrolyzing action of these enzymes probably implements the toxicity of venoms by supplementing the hydrolytic activity of other enzymes known to be present in venoms.

TABLE 6

NUCLEASE ACTIVITIES OF SNAKE VENOMS*

	Ribonuclease	Desoxyribonuclease
	Q_{CO_2}	Q_{CO_2}
Bothrops alternata	3.8	7.1
B. atrox	3.7	6.6
B. jararaca	4.4	8.2
B. neuwiedii	4.4	6.9
Crotalus terrificus	7.2	10.3

* Q_{CO_2} = ml CO_2 liberated/hr/mg venom from suitable substrate.

From the anatomical, physiological, and biochemical findings, the biological significance of snake venoms is primarily in their digestive role. Snakes can-

TABLE 7

WEIGHT IN MG OF VENOM LETHAL INTRAVENOUSLY FOR RABBIT

Vipera aspis	0.35
V. russellii	0.05
Bitis gabonica	2.13
Crotalus terrificus	0.25
C. adamanteus	0.25
Bothrops atrox	0.01
Naja flava	0.15

not chew and mix the products of their salivary glands with the tissues of their prey. Instead, they use a highly developed injection apparatus to apply digestive agents to their food. These powerful and concentrated enzymes exert an extremely poisonous effect (Table 7). For comparison, 0.15 mg crystalline urease of plant origin, injected intravenously, will kill a rabbit through production of ammonia from urea.

In addition to the enzymes commonly found in the digestive juices of other animals, most snake venoms contain hyaluronidase and ophio-oxidase. The poisons must spread rapidly in the body of the prey to fulfill their physiological purpose. The hyaluronic acid gel and fibers of the connective tissues are invaded through destruction of anti-invasin I by the action of proinvasin I. Venoms, especially of the Viperidae, produce other local effects, such as increased permeability, through destruction of substance surrounding the blood vessels. Lecithinases attack the lipid layer of the endothelial cell surfaces, producing lysolecithins that expand the film, with consequent increase in fragility and permeability.

Powerful proteases lead to actual dissolution of blood vessels, causing spreading of erythrocytes and serum into the tissue. The spreading of venom is greater than can be accounted for by the hyaluronidase content and is explained by the action of lecithinases and proteases. Necrosis at the site of a bite is due to the primitive digestive properties of snake venoms.

Ophio-oxidase is not present in all venoms and is not required for the deleterious effects of snakebite. It is a nontoxic component that causes a protease-enhancing effect by liberating proteases and peptides bound up in the cell itself. It is a digestive agent, activating proteolytic enzymes present in the victim's body. It hastens autolysis and putrefaction. For example, the tissues of a rat injected with venom are digested about twice as rapidly as those of an untreated rat.

The biochemistry and mode of action of snake venoms are obviously complex. Boquet (*1*) concludes that a study of venoms requires knowledge and investigation of a great number of factors, among them the toxic action, the enzymatic activity, and many other diverse properties. Each of these is further divided and subdivided into smaller groups, many of which have been mentioned or discussed in this presentation.

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News and Notes

Jubilee Meeting of the South African AAS[1]

THE South African Association for the Advancement of Science was founded in Cape Town about 50 years ago; 18 months later—in 1903—Sir David Gill, His Majesty's Astronomer at the Cape, presided over the first annual congress. Since then the association has met annually, and these meetings have taken the association to all the larger towns in Southern Africa. For the Jubilee Congress it seemed fitting to return to Cape Town, where the association was born. The congress was held under the presidency of B. F. J. Schonland July 7–12, in the buildings of the University of Cape Town. The generosity of the Union government made it possible for the association to invite guests from overseas. From the United Kingdom came Sir Lawrence Bragg, director of the Cavendish Laboratory, and Sir Edward Salisbury, director of Kew Gardens; Holland sent L. E. J. Brouwer, founder of the Intuitionist School of Mathematics, and J. F. Oort, of Leiden Observatory; Canon G. le Maître and G. Magnel came from Belgium; and from France, H. Vallois, the anthropologist of the Musée de l'homme in Paris. Portugal, which played such an important role in the early development of South Africa, sent historian A. de Silva Rego. Die Suid-Afrikaanse Akademie vir Wetenskap en Kuns was represented by its chairman, T. E. W. Schumann, director of the Weather Bureau of the Union of South Africa. Sir Kerr Grant, Australian physicist, represented the Australian and New Zealand AAS, and J. D. J. Hofmeyr, head of the Department of Genetics, University of Pretoria, represented the sister association in the United States of America. R. W. James, of the University of Cape Town, was present as the representative of the Royal Society of South Africa, the oldest scientific organization in the country, which this year celebrates its seventy-fifth anniversary.

The meeting began with a formal welcome to the

[1] From an editorial in the *South African Journal of Science.*