PHARMACOLOGY OF PEPTIDES AND PROTEINS IN SNAKE VENOMS[1,2]

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Snake venoms are a specialized, complex saliva that may contain many proteins, mainly particular enzymes, and strongly basic polypeptides. The latter, usually called polypeptide toxins or neurotoxins, account for the whole high toxicity of krait, sea snake, and a few other venoms. Most snake venoms, however, cannot be classified simply as neurotoxic, or shock-producing, or hemotoxic and hemorrhagic, since they exert toxic and lethal effects on the blood, the cardiovascular, respiratory, and nervous systems.

Almost 6000 papers on snake venoms and venomous snakes were published up to 1963 (226), over 1000 of them on physiopharmacological and toxicological effects (17). The study of the mode of action of snake venoms has led not only to a rational treatment of snakebite and to several laboratory and therapeutic uses of venoms, but also to advances in our knowledge about some physiological processes such as blood clotting.

Actions on Neuromuscular Junction, Muscle, and Nerve

Venoms of snakes belonging to families Elapidae (numerous cobra species, kraits, corals, mambas, tiger snake, death adder, taipan, etc.) and Hydropheidae (many species of sea snakes), are characterized by their neurotoxicity, producing flaccid paralysis and death by respiratory failure. Extensive research, reviewed recently (1–3), has been carried out since, from the last century until now, mostly with venom of the Indian cobra, *Naja naja*, to determine the precise mode of action of the neurotoxins. Although neurotoxic venoms were early classified as curare-like, some experiments apparently showed an action on the respiratory center. However, uncertainty regarding

[1] The survey of the literature pertaining to this review was concluded in June 1967.

[2] The following abbreviations will be used: *d*TC (*d*-tubocurarine); 5-HT (5-hydroxytryptamine); PhA (phospholipase A); PLP (phospholipids); DLF (direct lytic factor); ACh (acetylcholine).

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the origin of the respiratory paralysis was dispelled by the proof of its peripheral nature. It was demonstrated, in paralyzed, artificially respired animals, that the indirect stimulation of the diaphragm was blocked, whereas respiratory center activity, phrenic nerve conduction, and direct muscle excitability were not affected (2–5). These, and additional data from many investigations with a large number of elapid and hydropheid venoms on *in situ* and isolated preparations (6–14), are the basis of the current consensus that snake venoms induce a neuromuscular block of nondepolarizing type. It differs from that of *d*-tubocurarine (*d*TC) in its very slow development after a long latency, in the absence or slowness of reversibility, in the imperfect or transient antagonism by anticholinesterases, and in eliciting a nontypical Wedensky inhibition. As detected by the pioneer investigators, high concentrations of some venoms, particularly those from several cobra species, also depress the contraction of directly stimulated muscles and evoke a contracture on isolated preparations (8, 10, 12, 15–17). *Naja* venoms alter conduction in amphibian and crustacean nerves only after affecting the junction and the muscle, but do not alter conduction in mammalian nerves (12, 17).

Some elapid and hydropheid neurotoxins have been isolated as nonenzymatic, purified, or crystalline preparations, homogeneous for three or more criteria of purity (18–22). They are, as a group: (*a*) dialyzable polypeptides whose mol wts range between 5500 and 7000 (18, 19, 21, 23–29), although a much smaller particle size has also been found (17, 30); (*b*) very basic, with isoelectric points at pH above 9.0 (18, 21, 23, 25, 26–32), a property consistent with the predominately electropositive nature of elapid venoms, in contradistinction to crotalid and viperid materials (33–36); (*c*) remarkably heat-stable (20, 21, 32, 37); (*d*) rich in disulfide bonds (24, 26, 27, 29, 31, 38) which are essential for their pharmacological effects (38); (*e*) very similar in their amino acid composition (28, 29); and (*f*) highly lethal, accounting for most of the venom toxicity (18, 19, 20, 27). However, it was recently reported that the major toxin in *N. naja* venom is a glycoprotein, whose biological action depends on the intactness of its carbohydrate moiety (39). The small molecular size and strong positive charge of neurotoxins are probably reflected in their low antigenicity (25, 40), as well as in their rapid, lethal absorption when applied to the mammalian conjunctiva (41). Although the only report on impairment of oxidative phosphorylation by polypeptide toxins concerns bee venom mellitin (42), the strongly basic snake venom neurotoxins might compete for iron-porphyrin groups in the respiratory chain, thus interrupting respiration and oxidative metabolism of the nerve cell (43).

Neurotoxins in elapid and hydropheid venoms fall into one of three groups according to the mechanisms by which they block the neuromuscular junction. In the one, there is an antidepolarizing neuromuscular block by both post- and presynaptic actions (*N. naja atra* cobrotoxin or cobra neurotoxin). The main mechanism is a competitive decrease in the sensitivity of the end-plate to the depolarizing action of acetylcholine (ACh) (44–46). Unlike crude venoms, the pharmacological properties of cobra neurotoxin are

the same as those of *d*TC, except for the slowness of onset and the lesser reversibility of the paralysis. The latter difference, attributed by some authors to the anticholinesterase impurity in their isolated preparations (47), is probably due to the larger molecular size of basic polypeptides in comparison to *d*TC (9, 44). In common with *d*TC, a Wedensky inhibition and an inhibition of antidromic activities of the motor nerve are properties of cobra neurotoxin which are attributable to its presynaptic site of action, a mechanism suggested by work on several cobra and coral venoms (2, 10, 44–46, 48), and considered by Russell as the major mechanism of action of *Naja* venoms (17). The second group of neurotoxins shows a postsynaptic action exclusively, as exemplified by α-bungarotoxin, the most abundant polypeptide in *Bungarus multicinctus* venom (45, 46, 49, 50). Its pharmacological actions are similar to those of cobra neurotoxin or *d*TC, but it induces an irreversible neuromuscular block which is not relieved by neostigmine, and is without Wedensky inhibition. An exclusively presynaptic action is characteristic of the third class of neurotoxins and is exemplified by β- and γ-bungarotoxins, which block the neuromuscular junction by reducing the ACh output from the presynaptic terminals, as does botulinum toxin. However, unlike the latter, and like *d*TC, they elicit Wedensky inhibition and post-tetanic potentiation (46, 49).

Another basic polypeptide in cobra venom, cardiotoxin, causes contracture and inhibits direct and indirect stimulation of skeletal muscle (15, 16), decreases the ACh release from the nerve endings and blocks axon conduction and ganglionic transmission; all of these properties derive from its primary action, a depolarization of cell membranes (44–46, 51). Some of the effects of cardiotoxin are digitalis-like, including the systolic arrest produced by high doses (15, 16, 19, 52). Cardiotoxin differs from digitalis in not enhancing contractility, in depolarizating heart muscle as the cause of its cardiotoxicity, and in other pharmacological actions (51). Musculotropic and depolarizing properties were previously ascribed to cobra neurotoxin, most probably because of the coexistence of cardiotoxin in incompletely separated neurotoxic fractions (44, 48). On the other hand, binding between the basic polypeptides and acidic proteins of larger molecular size in the crude venom (17, 23, 30, 48) accounts for earlier efforts to ascribe depolarizing and neurotoxic actions to phospholipase A (PhA), and for the conflicting reports on the molecular size and dialyzability of nonenzymatic toxins. The cardiotoxin, isolated by Sarkar in 1947 (15, 16), was actually a complex of the basic polypeptide bound to large proteins (16, 51, 52).

Neurotoxicity, sometimes prevailing over the hemorrhagic and local symptoms, has been reported as a feature of clinical envenomation by rattlesnakes (53). Yet, only the South American rattler, *Crotalus durissus terrificus*, and but one of the many North American species, *C. scutulatus*, secrete markedly neurotoxic, nonhemorrhagic venoms (54–56). Thus, the crotalic envenomation familiar to South American physicians and investigators is almost unique among rattlesnake venoms, and is closely related to the elapid venoms (57, 58). In 1938, Slotta & Fraenkel-Conrat crystallized a protein,

crotoxin, from the venom of *C. d. terrificus*, that showed both neurotoxic and PhA or hemolytic properties (59). It had a mol wt of 30,000, an isoelectric point at pH 4.7, disulfide bonds, and was homogeneous by solubility, ultracentrifugal, and electrophoretic criteria (59–61). Besides the acidic crotoxin, and as a geographic intrasubspecific variation, some venoms contain crotamine, a spastic paralysis-inducing basic polypeptide, rich in lysine and in disulfide bonds. The physicochemical and biological properties of crotamine served to confirm a new subspecies, *C. d. crotaminicus*, and to distinguish between crotamine-positive and crotamine-negative venoms (10, 26, 29, 62–67). The crotamine-positive venoms evoke spasmodic crises or persistent muscular rigidity, followed by muscle hypotonia, flaccid paralysis, and death; whereas the crotamine-negative venoms elicit only the second phase of the syndrome (10, 68). Crotactin, a neurotoxin free of PhA, was found to be the lethal principle of crotoxin (69, 70). According to other investigators, crotoxin appeared to be a firm complex between a basic protein and an acidic one, separable as biologically inactive dinitrophenyl-derivatives (71). However, the basic component of crotoxin cannot be identical with crotactin, inasmuch as the latter is the only acidic, nonenzymatic neurotoxin isolated from snake venoms (pHi 4.3) (3). Another neurotoxin, called Toxin III (3, 70), was separated from *C. d. terrificus* venom, but its pharmacological properties suggest it is a complex of crotactin and crotamine.

Some authors have attributed the paralysis evoked by crotamine-negative venom to a direct action on the muscle (10, 72). Yet, the paralytic effects of the crude venom and of crotoxin are very similar to those of elapid neurotoxins, and probably the only cause of the crotoxin-induced paralysis is a nondepolarizing neuromuscular block, the main mechanism of which is postsynaptic (12, 17, 73–75). The pharmacological action of crotamine, originally regarded as veratrine-like (68), was then classed as a contracture similar to that produced by substances that interfere with muscle glycolysis (76). It is currently established that the contracture-producing action of crotamine and the effects of veratrum alkaloids have several properties in common, e. g., direct action on the muscle fiber, and antagonism by Ca^{2+} and Mg^{2+}, but they also show several essential differences (10, 77).

Other Crotalidae venoms produce little or no neuromuscular block (2, 9, 12, 75). An exception is *Agkistrodon piscivorus* venom, whose neurotoxicity has been compared to that of *C. d. terrificus* (75, 78). Neurotoxicity is exhibited by venoms from snakes in several Viperidae genera (79–82), and is a result of a peripheral nerve conduction block (81, 82). An example of this mechanism of action is viperatoxin, the most important of the two neurotoxins isolated from *Vipera palestinae* venom (80, 81). The physicochemical properties of viperatoxin are similar to those of elapid polypeptides (83).

Actions on the Cardiovascular, Respiratory, and Central Nervous Systems

With few exceptions, profound cardiovascular depression is the most

common experimental pharmacological effect of snake venoms, and predominates over the paralytic action in the venom of the spitting cobra, *N. nigricollis* (10, 31, 68, 84). Circulatory shock is usually found in patients severely envenomated by vipers and pit vipers (17, 85–87). Artificial respiration prolongs survival but cannot prevent death after respiratory arrest by paralyzing venoms (4, 5, 51, 88–91). Peripheral collapse, not the slowly-evolving neuromuscular block, causes prompt death following large doses of *N. naja* and other venoms (4, 10, 15, 16, 51, 54, 90, 92). Intravenously injected snake venoms generally cause a diphasic shock in mammals: a precipitous fall of systemic arterial pressure in 15 to 30 sec, followed by a period of partial recovery; and then a gradual or rapid descent of systemic arterial pressure terminating in death (4, 5, 88, 89, 93–99). With some venoms, the sharp fall in systemic arterial pressure is irreversible, or is followed by a transient hypertension (triphasic shock) (81, 87, 94, 100, 101). A brief apnea occurs simultaneously with the acute hypotension; it may be preceded by respiratory stimulation and followed by a long period of tachypnea (4, 5, 81, 87, 94, 97, 99, 101). The respiratory stimulation after some venoms results from activation of the chemoreceptors in the aortic and carotid sinus bodies (87, 102) and a direct action on the respiratory center, while the apnea results from stimulation of vagus nerve fibers in the lungs (87). After the acute respiratory changes and a latency of variable duration, neuromuscular-blocking venoms progressively decrease the respiratory rate, and finally arrest respiration (4, 5, 94).

The precipitous fall in systemic arterial pressure was attributed by some authors to a direct venom action on the heart (16, 103, 104). Actually, decrease in myocardial contractility, as well as marked cholinergic-like bradycardia, are associated with (91, 94, 97–99, 103), but are not the cause of the initial hypotension (94, 98). Furthermore, the concomitant, irreversible suppression of the CNS electrical activity produced by rattlesnake and cobra, but not by viperid, venoms (81, 90, 94–98), is neither a cause nor a result of the sharp drop in systemic arterial pressure (95). It is currently agreed that the abrupt hypotension is attributable to a peripheral action upon the capillaries (88, 94, 98, 99, 102). It does not consist of vasodilatation or loss of vascular resistance, as believed by some authors (88, 89, 99, 102), but is initiated by an increase in the resistance within the pulmonary circuit, with a subsequent deficit in left heart output, an increase in pulmonary artery, systemic venous, and cisternal pressures, and changes in the ECG (17, 98). A marked fall in the circulating blood volume, a result of pooling of blood in the chest and in the lungs, is regarded as the cause of the cardiovascular collapse (17, 98). This conclusion is further substantiated by the rapid weight gain of venom-perfused isolated lungs (98, 105), and by the selective accumulation of venoms in the lungs *in vivo* (50, 106). Some venoms cause abrupt hypotension through pooling of blood in the pulmonary circuit or in the hepato-splanchnic bed, or both (94). The rapidity of the hypotensive response and the hepato-splanchnic pooling of blood are thus features common to the

circulatory collapse induced by snake venoms and by bacterial endotoxins (94, 95, 107). The mechanism of the abrupt hypotension evoked by *V. palestinae* venom is exceptional, and consists of a peripheral vasodilatation due to a primary action of viperatoxin on the medullary vasopressor centers (81).

Hypovolemia alone, or in association with loss of peripheral arteriolar resistance, or with vasodilatation and increased blood viscosity, have been regarded as the mechanisms whereby *Crotalus* venoms produce the secondary shock (89, 99. 108). The influence of the following factors must be considered in any explanation of the terminal phase of the circulatory shock by snake venoms: (*a*) Blood pooling in the peripheral vascular bed, blood loss from the vascular tree, or both, is produced by venom constituents that act on the vasculature itself as does cobra venom (88, 90, 96). (*b*) Cardiac failure results from cardiotoxic action of cobra (15, 16, 88, 91) and probably of other venoms as well (109). Cardiotoxin can elicit an abrupt, lethal fall in systemic arterial pressure (16), although its concentration in cobra venoms is too small to produce acute cardiovascular changes. (*c*) Hypoxia results from the respiratory depression produced by the paralyzing venoms (4, 5, 15, 16, 84, 90). On the other hand, respiratory failure, which seems to be the general cause of death by snake venoms, may be induced by the cardiovascular effects, through CNS ischemia resulting from prolonged hypotension (16, 90, 92–94, 97, 102). Either cardiotoxin, cobra neurotoxin, or vasculotoxic factors in cobra venom can induce circulatory and respiratory failure, as does the whole venom (16, 90).

C. d. terrificus venom (crotamine-negative) provokes clonic convulsions one hour or more after intravenous injection into unanesthesized dogs (73, 101). Neither this effect nor the acute respiratory and cardiovascular disturbances in anesthetized dogs and cats is elicited by crotoxin or crotamine intravenously (73, 87, 101). Yet, crotoxin produces lethal clonic convulsions, instead of paralysis, when injected intra-ventricularly into conscious cats (73). Several other venoms, introduced by the same route, produce either convulsions or extreme lethargy, but do not cause the typical circulatory collapse caused by their intravenous injection, although they do show late cardiovascular effects (11, 110, 111). As pointed out by some authors (73, 111), these results indicate that snake venoms can slowly break down the blood-brain barrier which prevents their entering or leaving the CNS (50), a hypothesis supported by the hypersusceptibility of rabbits to snake venoms after treatment altering this barrier (112). Thus, a *C. d. terrificus* venom constituent could break down the barrier to gain entry for crotoxin into the CNS (73). However, a protein recently isolated from *C. d. terrificus* venom seems to reproduce the convulsions, the triphasic shock, and the respiratory changes elicited by the intravenous injection of crude venom (101). Some of several reported actions of snake venoms on the CNS seem to have diagnostic or therapeutic significance (113–115).

Role of Autopharmacology in Cardiovascular and Other Effects

Snake venoms act on many organs, mainly the lungs, to release histamine as well as unsaturated hydroxy-fatty acids that elicit a slow but strong contraction of guinea pig ileum and human bronchioles (slow-reacting substance) (109, 116–125). This fact and the resemblance between snake venom-induced and anaphylactoid shocks (85, 122, 123, 126) led to the belief long ago that histamine was the mediator in the cardiovascular effects of snake venoms (1, 31). Histamine is released by venom phospholipase A through lysolecithin formation, but not by such other venom constituents as cobra neurotoxin or proteases (45, 117–120, 122, 127). Besides this cytolytic attack on the mast cell membrane, mast cell degranulating factors in snake venoms, e. g., crotamine, are able to induce release of histamine and 5-hydroxytryptamine (5-HT), by stimulating cellular secretory activity (122, 123, 125, 127). Proteins lacking PhA, proteolytic, and coagulant properties in *N. naja* and *C. d. terrificus* venoms release, respectively, the potent histamine liberator anaphylatoxin from anaphylatoxinogen, and 5-HT from platelets (127–129). Bradykinin is rapidly formed by snake venoms from a plasma globulin precursor, bradykininogen. Bradykinin belongs to the group of vasoactive peptides, among which are found the most potent vasodilating and vasoconstricting agents, showing opposite effects on blood pressure (93, 122, 126, 130–132). Kallidin, or factor DK, and a pain-producing substance, which were discovered before bradykinin, proved to be almost identical to bradykinin in their chemical and pharmacological properties (131, 132). A venom esterase that releases bradykinin from bradykininogen is the salivary kallikrein of the snake (133). Bradykinin is a more potent hypotensive agent and a more universal vasodilator than histamine, a more potent pulmonary vasoconstrictor than histamine, norepinephrine, or 5-HT, and a more potent bronchoconstrictor than histamine or ACh (126, 130–132, 134, 135). Parasympathetic centers are stimulated by bradykinin (136), in keeping with the acute generalized parasympathetic disturbances, including marked pupillary constriction seen in rattlesnake envenomation (56, 137). The stimulating effects on smooth muscle exhibited by crotamine, crotoxin, and crotactin (10, 63, 70), are likely the result of autopharmacological actions (122, 125, 127).

Some authors deny the role of histamine in the snake venom-induced acute shock, mainly because antihistaminics fail to modify the response (16, 99, 105). They attribute the cardiovascular effects of cobra and other venoms exclusively to cardiotoxin or to the release of adenosine (16, 116). That the release of either histamine or 5-HT is of minor importance in the general toxicity of snake venoms has been shown with isolated venom proteins causing those effects (117, 129), but there is no doubt that lysolecithin evokes acute hypotension, and that the combined effects of 5-HT, histamine, slow-reacting substances, bradykinin, and other must be significant (117, 123). Bradykinin is regarded as the main or only mediator in the venom-induced circulatory collapse but not in the persistent shock (99, 130, 131, 138, 139).

The abrupt fall and subsequent recovery of systemic arterial pressure may be explained by the immediate release and prompt destruction of bradykinin, and by the rapid plasma bradykininogen depletion found in experimental and clinical poisoning (99, 138, 140). However, histamine and 5-HT are probably more important than bradykinin as mediators of the increased capillary permeability caused by snake venoms. This action might account for the edema and hemoconcentration concomitant with the venom-induced shock, and for the breakdown of the blood-brain barrier, facilitating accessibility into the CNS (99, 124, 139, 141–143).

Actions on Blood Coagulation

In addition to neurotoxicity, interference with blood clotting by snake venoms has been most extensively studied (1, 144–147). Since the classification of snake venoms into coagulants and anticoagulants in 1767, many contradictory conclusions have been drawn about their actions on blood clotting. Many venoms were regarded as coagulant by some workers, and anticoagulant by others. Coagulant effects are exhibited in the three families of land venomous snakes, according to the following mechanisms: (*a*) Thrombin-like action accounts for the marked coagulant nature of many crotalid venoms and is possibly restricted to this family, with the exception of the potent coagulants secreted by Australian elapid snakes (33, 139, 148–155). *V. palestinae* venom has only a cothrombic activity (147, 156, 157). (*b*) The activation of Factor X (incomplete thromboplastic action), is followed by conversion of prothrombin into thrombin in the presence of Factor V, phospholipids and Ca^{++}. This is a mechanism typical of some viperid venoms, the prototype of which is Russell's viper venom (147, 150, 158, 159), but which is probably also exhibited by many crotalid venoms, although masked by their potent thrombin-like effects (150). Since the coagulant action of Russell's viper venom is entirely due to this very specific mechanism, this venom is useful in the diagnosis of the Stewart-Prower defect (147). (*c*) Prothrombinase action or direct conversion of prothrombin into thrombin is a property of a few elapid and viperid venoms (150, 156, 160, 161).

A larger number of mechanisms account for the anticoagulant effects of snake venoms: (*a*) Antithromboplastic action or destruction of thromboplastin is found widely among elapid and viperid venoms, and in the only sea snake venom whose anticoagulant mechanism has been elucidated, but frequently less in the crotalids (147, 149, 151, 152, 156, 162–164). This action is exerted by some venoms through several specific mechanisms such as inhibition of Factor VIII and Factor IX, and interference with platelet action (147, 155, 163, 165, 166). (*b*) Inactivation of Factor V is found in the three families of land venomous snakes (145, 147, 149, 151, 152, 162). (*c*) Destruction of prothrombin, claimed for some elapid and crotalid venoms, is considered by some authors as a universal mechanism of anticoagulant venoms. This idea probably arises out of confusion with the above actions (149, 151,

152, 162, 167). (*d*) Fibrinogenolysis and fibrinolysis are characteristic of many crotalid venoms, although found in other venoms (33, 93, 144, 146, 147, 149, 151–154, 156, 162, 167, 168). (*e*) Direct plasminogen activation, without interaction with the serum plasminogen activator, is shown by some crotalid and viperid venoms (159, 160). (*f*) The heparin content of blood can be increased through an autopharmacological mechanism (147, 164). This was previously deemed a result of antithrombic, heparin-like factors contained in cobra and Russell's viper venoms (169, 170).

It was assumed that the coagulant and bradykinin-releasing activities of snake venoms were of proteolytic nature (31, 126, 130, 144, 147, 170, 171) until recent demonstrations showed that they are due to heat-stable, nonproteolytic esterases (arginine-ester hydrolases) separable from each other (133, 139, 148, 171–173). The coagulant action of Russell's viper venom has been placed at the molecular level. The purified esterase, similar in its action to Factor VII but not identical with any coagulation factor of the human blood, splits off from Factor X a derivative which is itself an esterase (174). The crotalid, thrombin-like esterase differs from thrombin in several respects. It is only slightly inactivated by heparin (133, 139, 144–147, 155), whereas heparin inhibits the coagulant effects of Russell's viper venom (146, 159). Venom proteases, different from trypsin or plasmin, account for the fibrinolytic and fibrinogenolytic effects (33, 153, 154, 160, 167, 168), and are probably involved also in the antithromboplastic actions. Nevertheless, recent investigations (145, 147) indicate that PhA, through PLP destruction, is probably the main anticoagulant in those venoms that inhibit blood clotting at stages prior to the prothrombinase formation.

Some venoms owe their coagulant or anticoagulant effects to only one of the above mechanisms (158, 159, 161), but combined effects have been found in most venoms (147, 150, 155). The whole action of a venom on blood clotting becomes even more complex since most viperid, many crotalid, and some elapid venoms contain both coagulant and anticoagulant factors (1, 33, 139, 144–149, 151–156, 159, 161, 170). Their effects represent many different combinations of single or compound coagulant actions with single or compound anticoagulant ones. It is, thus, impossible to classify snake venoms as coagulant or anticoagulant, with the exception of a small number of elapid venoms which exhibit only coagulant properties (147), and a larger group, comprising the four families of venomous snakes whose effects are only anticoagulant (1, 146, 147, 149, 154, 167, 168). There are a few venoms showing neither coagulant nor anticoagulant effects (147, 149). At any rate, it would be illusory, as Meaume (147) pointed out, to try to classify snake venoms on the basis of the multiple mechanisms which cause their coagulant and anticoagulant effects. Knowledge concerning effects of snake venoms on blood clotting has been further complicated by the diverse reports from several investigators; e. g., at least six different combinations of mechanisms inhibiting blood clotting have been postulated as the cause of the anticoagulant effects of *N. naja* venom (147, 149, 162, 164, 165).

Venom-Induced Defibrination Syndrome

Even very small amounts of *in vitro* coagulant venoms rapidly elicit blood incoagulability in experimental animals and in patients who have been bitten (56, 84, 86, 98, 113, 139, 140, 157, 175–177), preceded by a transient increase in blood coagulability and, with high doses, by intravascular coagulation (58, 86, 126, 130, 139, 145, 146, 178–183). Although marked thrombocytopenia and destruction of several coagulation factors are also produced (85, 126, 139, 140, 177, 180, 181, 183), snake venom-induced blood incoagulability is primarily a defibrination syndrome due to intravascular clotting caused by the thrombin- or the thromboplastin-like venom esterases. This hypothesis is substantiated by several instances, as in the remarkably severe afibrinogenemia seen in patients bitten by pit vipers that produce the most coagulant venoms (86, 139, 177), and the reproducibility of the same effects with the purified coagulant enzymes (180–184). On the other hand, potent fibrinolysins, either purified or in crude venoms that are solely *in vitro* anticoagulant, do not provoke the defibrination syndrome and probably lack clinical significance (167, 178, 180). In spite of its incoagulability, blood exhibits coagulant action because it contains venom which continuously removes the inflowing fibrinogen (139, 175, 178–180, 182, 184), whose synthesis seems to be unimpaired (177, 180). This explains the persistence of the fibrinogenopenia, a clinically useful and sensitive indicator of systemic poisoning (86), the futility of the treatment with fibrinogen, and the dramatic rise in fibrinogen level after antivenin administration (139, 175, 177, 182). Activation of the endogenous fibrinolytic system, which accounts for the circulating fibrinolysin (130, 179–182), is most probably a consequence of intravascular clotting (139, 177, 181, 184, 185). However, bradykinin may also release plasminogen activators, and since plasmin can liberate bradykinin, a vicious cycle would reciprocally strengthen the fibrinolytic and the bradykinin-releasing systems (186, 187). Interference by fibrinogen- and fibrin-split products with the formation of fibrin, as well as inactivation of Factor XIII, perhaps contribute to the coagulation defect (175–177, 180, 183).

The pathogenesis of the snake venom-induced defibrination syndrome is consistent with the *in vitro* behavior of venoms endowed with both coagulant and anticoagulant enzymes. Complete inhibition of clotting is produced only by very high venom concentrations which are not attained in *in vivo* conditions (33, 144, 153, 154). Hence, the experimentally observed intravascular clotting with high concentrations, and blood incoagulability with lower concentrations of these venoms were not so contradictory to the *in vitro* results as many early reports claimed (144, 145).

Hemorrhagins and Hemorrhagic Effects of Snake Venoms

In contrast with the majority of elapid venoms, most of the viperid and crotalid venoms contain hemorrhagins that cause extensive extravasation of blood, leading to marked systemic hemorrhages and acute, rapid hemorrhagic

edema starting at the site of the bite (56, 79, 84, 93, 113, 139, 140, 157, 188). Blood incoagulability, per se, is a relatively benign state that may exist without causing hemorrhage, as shown by the nonhemorrhagic, defibrinating venom of *V. aspis*, in contrast to the hemorrhagic, nondefibrinating venom of *V. palestinae* (146, 157). However, the coagulation defect can aggravate the spontaneous bleeding produced by hemorrhagins (86, 139, 156, 179–181). The presence of proteases in hemorrhagic venoms was the only evidence in the past for ascribing hemorrhagic effects to proteolytic activities (1, 171). Yet, although a hemorrhagic factor has been reported as associated with most of the proteolytic activity of some venoms (79, 133, 157, 188), the main or only hemorrhagin is another protein, either of low proteolytic activity (188), or devoid of enzymatic action (79, 133, 189, 190).

Snake venom hemorrhagins are vasculotoxic agents whose target organ seems to be the lung (33, 78, 79, 93, 139, 154), and which, according to some authors (56, 157), are destructive to the vascular system. Yet, the venom-induced hemorrhage seems to occur through a morphologically intact vessel wall, by an action on the intercellular cement substance (157). This mechanism is consistent with the lack of cytocidal effects of both the proteolytic and the main hemorrhagins, the latter of which also lacks cytopathic activity (188, 191). Some of the degenerative myotoxic local effects of hemorrhagic venoms also do not parallel either the proteolytic or the hemorrhagic activities (188, 192). This finding is in agreement with the causation of marked myolysis, leading to myoglobinuria and acute renal failure, by nonhemorrhagic, nonproteolytic sea snake venoms (11, 193).

Hemorrhagins play a minor role in the lethality of most North American crotalid (55, 78), and of some predominately neurotoxic, venoms (55, 79), but they seem to be the major lethal components in other neurotoxic and nonneurotoxic venoms (188, 189), causing death in severe envenomation through bleeding into the brain, lungs, kidneys, or heart (53, 56, 81, 85, 86, 139, 157, 188, 189).

Hemolysins and Hemolytic Effects

Phospholipase A, present in all snake venoms, has been called indirect hemolysin because it can lyse washed red blood cells only via the formation of lysophosphatides, generated from added PLP (31, 157). However, elapid and a few crotalid and viperid venoms, containing a so-called direct lytic factor (DLF), lyse washed red blood cells of several species (31, 78, 117, 139, 154, 157, 194). The direct lytic factor is a strongly basic polypeptide of mol wt 2000, in contrast with the acidic PhA, whose mol wt is 20,000 (78, 157, 194–196). Snake venom PhA, like lipoprotein-lipase, breaks down PLP bound in plasma or egg yolk lipoproteins more easily than uncomplexed PLP (197). Purified PhA, venoms lacking DLF, or heparin-treated cobra venom do not lyse washed red blood cells due to the incapability of PhA to attack the PLP of the intact red blood cell membrane. The direct lytic factor is, by itself,

very weakly hemolytic (117, 198, 199). The strong hemolysis caused by the simultaneous presence of both factors, as in crude cobra venom, is concomitant with the conversion of PLP in the red blood cell membrane to lysophosphatides (198–200). These facts, plus the finding that the different sensitivity of red blood cells of several species to cobra venom-induced hemolysis reflects different susceptibility to DLF (199), are the basis for the current hypothesis that PhA and DLF must act synergistically in order to cause effective hemolysis. This is an autocatalytic process continually accelerated by the accumulating lysophosphatides, which are strongly hemolytic per se (198–200).

The physicochemical and biological properties of DLF closely resemble those of the highly electro-positive cardiotoxin (46, 51, 196), and strongly support the suggested identity of both factors (3).

Despite the presence of plasma PLP, whole blood is not hemolyzed *in vitro* by DLF-free venoms containing high PhA activity. However, these venoms do provoke the formation of lysolecithin which interacts with red blood cells, causing sphering and increased mechanical fragility almost instantaneously. These changes represent an arrested stage in the hemolytic process, due to a plasma PLP concentration insufficient to generate the required amount of lysolecithin, as well as to a protective action of plasma proteins (117, 157). *In vivo*, the same venoms and their purified PhA lower plasma PLP and raise plasma lysolecithin levels, but the associated sphering and crenation of red blood cells is spontaneously reversed, since the lysolecithin attachment to the cells is temporary, due to its clearance from the circulation (117, 157, 201, 202). Hence, intravascular hemolysis does not occur, or is inconstant or slight, and never appears in splenectomized animals (157, 201, 203). Defibrinating, thrombocytopenic venoms, free of DLF do cause intravascular hemolysis primarily because of the concurrent defibrination, which traumatises the red blood cells in the circulating blood. The phospholipase A plays a minor role, less important than in its similar thrombocytopenic action, by rendering red blood cells fragile (202). These results explain the apparently paradoxical findings that some DLF-free venoms, hemolytic *in vitro* but devoid of coagulant factors, do not cause hemolysis *in vivo* (93, 99, 168), whereas other venoms, with severely defibrinating properties but not hemolytic *in vitro*, are hemolytic *in vivo* (86). Intravascular hemolysis by snakebite thus shows a very complex pathogenesis. On the other hand, it is only one of several factors involved in the causation of anemia, and is probably less significant than hemorrhage, which is the origin of physio-pathological changes resulting from loss and catabolism of red blood cells into the tissues (157). However, massive intravascular hemolysis may cause nephrotoxicity (113) and cardiac failure through toxic effects of K^+ (5, 88).

Significance of Venom Enzymes in Pharmacology and Toxicity

Although for some time most of the pharmacological and toxic effects of

snake venoms were ascribed to their enzymes (204), these have now been separated from the main part of the lethality (20, 205–207). Thirty years ago, it was suggested that acetylcholinesterase was the neurotoxin of elapid venoms, which are very rich sources of this enzyme (208). However, much information has been accumulated to show clearly the dissociation of this enzymatic activity and lethality (7, 46, 49, 206), including the absence of acetylcholinesterase from coral and all nonelapid venoms, some of which are predominately neurotoxic (2, 3, 33, 204, 209). Proteases probably represent only a low percentage of the toxicity of even highly proteolytic venoms (133, 167, 188, 189); but there is no doubt that PhA, esterases, and some proteases are pharmacologically active in several respects, as shown in the preceding sections. Phosphodiesterase may contribute to the autopharmacological changes that mediate the venom-induced abrupt hypotension (3, 153, 210). Endonuclease (DNase) and phospholipase C seem to be the most important factors in the inhibitory action of cobra venom on cancer growth (211, 212).

Phospholipase A in some snake venoms facilitates penetration of ACh, *d*TC, and other substances into squid and lobster axons and, at high concentrations, blocks conduction (196, 213, 214). Inability of some venoms showing high PhA content to modify the axon permeability barriers is consistent with the existence in snake venoms of PhA's with a different ability to hydrolyze membrane-bound PLP, whether in red blood cell ghosts, platelets, or mitochondria, although all of them split PLP in homogenates. This finding may explain why some authors deem PhA as neurotoxic, whereas others do not (196, 198, 199, 215, 216). Phospholipase A decreases respiratory activity and uncouples oxidative phosphorylation by inactivating enzyme systems that depend on the integrity of mitochondria. There is a simultaneous rapid conversion of brain mitochondrial PLP to lysophosphatides (205, 217–221), and swelling and disruption of mitochondrial membranes, with release of cytochrome *c* (220, 222, 223). Inhibition of oxidative phosphorylation has also been observed in brain slices incubated with PhA and in brain mitochondria from animals injected with the enzyme (218, 223). Furthermore, crude and boiled venoms evoke ***in vitro*** the outflow of intracellular enzymes from brain and muscle (224). However, as Meldrum has pointed out (3, 48), the basic polypeptides and PhA share the property of resisting ten minute boiling at low pH, and therefore some effects of even crystalline PhA obtained from acid-heated venoms (217) are probably due to the synergistic action of both kinds of venom constituents, although they have been ascribed to PhA (217). This also explains why the depolarizing action of cobra venom cardiotoxin was attributed by some workers to PhA (44, 48, 225). The ability of PhA to penetrate intact cells in order to act on mitochondria thus remains unproved, while DLF is unable to render PLP, in the intact brain cell, available to the PhA action (196, 215). The asserted pharmacological actions of nonenzymatic neurotoxins and the accumulated reports on the non neuro-

toxicity of purified PhA (23, 33, 45, 48, 79, 153, 198, 205, 207, 215), substantiate the current belief that snake venom neurotoxicity is due to the former components (2, 3, 17, 39, 45, 46, 48, 49, 51), and not to the latter enzyme as was assumed for some time (59, 76, 217, 218). However, PhA probably contributes to the neurotoxicity of some snake venoms (39, 117, 171, 216).

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