COMPOSITION AND MODE OF ACTION OF SOME INVERTEBRATE VENOMS[1]

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A recent, increased interest in invertebrate venoms has resulted in new knowledge concerning their compositions and modes of action. However, much remains to be done with these venoms, especially in regard to the chemical nature and mechanisms of action of the truly toxic components. Not only might this lead to more effective treatment in cases of human poisoning, but the isolated and purified toxic factors should provide valuable tools in further exploration of basic cellular processes.

In this review, no attempt will be made to cover the entire recent literature; instead, illustrative examples will be selected. We shall consider venoms as mixtures of substances produced by secretory cells and normally communicated by a process of biting, stinging, or otherwise wounding the enemy or prey. However, in at least one group to be considered, that is those gastropod molluscs whose hypobranchial glands produce a complex venom, the active constituents appear first to gain entrance to an intact victim. We shall adopt the view that venoms, generally, serve two functions: one defensive, due to pain-producing components; the other in feeding, by paralysis of the prey.

The best-known venoms of invertebrates are complex mixtures of pharmacologically active substances. Tertiary amines, such as 5-hydroxytryptamine (serotonin, 5-HT), and histamine, may occur together with choline esters or tetramethylammonium hydroxide (tetramine). Kinins and other active peptides may be present. The truly toxic, usually paralyzing, factors of some invertebrate venoms have been shown to be proteins. It would not be surprising if this turned out to be universally the case. Enzymes such as hyaluronidase and phospholipase are known to occur in some invertebrate venoms, but their roles are probably subsidiary.

In attempts to understand the mode of action of a venom too little attention has been paid to the possible synergistic and multiple actions of its several parts. Until this is done, the complexities of poisoning by venoms will not be understood.

After enumerating the principal groups of venomous invertebrates, with mention of the type of venom apparatus found in each group, we shall consider those components of venoms that may serve mainly as pain-producers and facilitators of absorption and distribution, and then deal with the little-known, but more important, paralyzing factors.

[1] The survey of the literature pertaining to this review was concluded in June 1963.

[2] I am indebted to Drs. Betty M. Twarog and Findlay E. Russell for references to some of the recent literature on invertebrate venoms.

Principal Groups of Venom-Producing Invertebrates

Coelenterates.—All members of this phylum are characterized by the possession of stinging organelles or nematocysts. While relatively few, such as the "stinging jellyfishes" and Portuguese Man-of-War, inflict distressing, but rarely fatal, reactions in humans, most species use their hypodermic-like nematocysts to immobilize their normal prey.

Nemerteans or "ribbon worms."—Some members of this marine phylum have a poison gland and stylet associated with the proboscis (e.g. *Amphipora*). The prey is stung preparatory to feeding.

Molluscs.—Among the gastropod molluscs, the cone shells (*Conus* spp.) have a venom apparatus. This is made up of a muscular bulb, a coiled venom duct, with gland cells and a highly modified radular apparatus with the teeth altered for injection of the venom [Kohn, Saunders & Wiener (1); Endean & Rudkin (2)]. Some species of *Conus* feed on worms, some on other molluscs, and others are fish eaters. Only the last are considered dangerous to man (2).

Members of the family *Muricidae* (marine snails) constitute a second group of venomous predatory molluscs. Their hypobranchial, or adrectal, gland secretes a complex mixture of substances used in relaxing the prey (e.g. barnacles and bivalve molluscs). There are no specialized structures for injecting the venom, but the habit of surrounding the prey with the foot allows an accumulation of glandular products in its immediate vicinity [Welsh (3)].

Carnivorous, marine snails of the genus *Neptunea* will be included in this review since their salivary glands have a high tetramine content that may be introduced into the prey after wounding with the radula.

The posterior salivary glands of some of the cephalopod molluscs (e.g. *Octopus*) produce a highly toxic saliva that is introduced into the prey (e.g. crabs) when they are bitten by the parrot-like beaks. Rapid paralysis ensues.

Echinoderms.—The jawed pedicellariae and hollow spines of some sea urchins have associated glands. Pain-producing and paralyzing factors are produced by these glands.

Arthropods.—Within this phylum there are many venomous species. They include the centipedes, spiders, scorpions, bees, and wasps. The centipedes and spiders have biting chelicerae or "mandibles," while the scorpions, bees, and wasps have a stinging apparatus at the posterior end of the body.

For some of the more recent listings and descriptions of venomous species and the components of venoms the reader may refer to references (4) to (9). However, it should be pointed out that many listings and discussions of venomous invertebrates include only those that man finds obnoxious or dangerous. To a struggling water flea, the miniature hydra is a deadly enemy and no less venomous than the deadliest reptile is to man. Hydra injects into *Daphnia* a protein with most unusual pharmacological properties. It may also inject serotonin, acetylcholine, tetramine, and other substances. Thus, in the evolution of one of the most primitive of truly venomous animals, a pattern was established that can be seen with minor modifications throughout all venomous groups, both vertebrate and invertebrate.

It is our purpose, in that which follows, to point out the importance of observing the pharmacological action of a venom on the members of the particular species on which the venomous animal normally preys. While those components that are pain-producing, and therefore generally defensive (e.g. serotonin, histamine, acetylcholine, and kinins), may protect against a wide variety of animals, those that are offensive, and used to immobilize prey (usually proteins), may have a much more restricted action, as in the case of hydra, already mentioned, or the solitary wasp that is stocking its nest with other insects or spiders that may continue to live for days, weeks, and sometimes for months, although unable to move. To test the real effectiveness and mode of action of such a venom, its actions should be observed on arthropod muscle and nervous systems, not those of white mice or some other vertebrate, which never constitute the normal prey of the wasp.

Serotonin in Venoms

Serotonin occurs in many invertebrate venoms, sometimes in extremely large amounts (10 to 14). We shall discuss its occurrence in the several groups of venomous invertebrates already mentioned.

Evidence for the presence of serotonin in the nematocysts of coelenterates is indirect and controversial. Pharmacological tests and chromatography detected what appeared to be serotonin in the tentacles of the sea anemones, *Metridium senile* and *Calliactis parasitica* [Welsh (15)]. Subsequently very large amounts (up to 600 μg/g freeze-dried tissue) were found in the coelenteric tissues of *C. parasitica* [Mathias et al. (16, 17)], where it was shown by histochemical procedures to be concentrated in certain endodermal cells [Vialli & Casati (18)]. These results led Mathias, Ross & Schachter (17) to conclude that the small quantities of serotonin found in anemone tentacles were without significance. However, in *Metridium*, more serotonin is found in the nematocyst-bearing tentacles and acontia than elsewhere in the anemone (13, 19). *Calliactis parasitica* appears to be an exception among the coelenterates thus far studied, in having a large amount of serotonin in an area that is not rich in nematocysts. It now needs to be shown whether the serotonin of tentacles and acontia is actually a constituent of their nematocysts.

Early histochemical observations on the hypobranchial glands of *Murex trunculus* (a source of Tyrian purple) revealed a characteristic amine [Vialli (20), Vialli & Erspamer (21)] that later led to the finding of serotonin (80 to 290 μg/g) in this organ (10, 11). No serotonin has been detected in the hypobranchial glands of certain other members of the family *Muricidae* (3, 13).

Posterior salivary glands of *Octopus vulgaris* from the Mediterranean contain large amounts of serotonin (300 to 500 μg/g) (10, 11) but smaller amounts (68 and 72 μg/g) were found in two specimens of the octopus of Bermuda that is generally considered to be the same species (13). Posterior salivary glands of *Eledone moschata* have a high content of serotonin, but those of *Octopus macropus* lack this amine (10, 11, 12).

Among the arthropods, serotonin is found in the venom or venom appara-

tus of many species that sting or bite. In scorpions, serotonin distribution is spotty, while in social wasps it has been found in all species thus far studied.

Among the scorpions, *Leiurus quinquestriatus* has been reported to yield up to 4 mg serotonin per gram of dry venom [Adam & Weiss (22, 23, 24)] while a much smaller amount was found in the venom of *Buthotus minax* (24). Both of these are Old World scorpions. Examination of nine species of scorpions from North and South America failed to reveal serotonin in extracts of venom or telsons (sting segments) except in two specimens of an inadequately identified species from Arizona (13, 14).

A small amount of serotonin was found in extracts of whole chelicerae of the large venomous centipede, *Scolopendra viridicornis* (14).

Serotonin appears to be commonly present in the venom of South American spiders (14). The venom of the poisonous *Phoneutria fera* gave values ranging from 0.5 to 2.5 mg/g dry venom (14).

The sting apparatus of two species of ants from Trinidad and three species of solitary wasps from Florida failed to yield measurable amounts of serotonin (14). On the other hand, the venom, venom sacs, or entire sting apparatus of all social wasps thus far examined have yielded relatively large amounts of serotonin (10, 13, 14, 25, 26). The largest amount of serotonin thus far found in Nature (up to 19 mg/g) occurs in the venom sacs of the European hornet, *Vespa crabro* (26). The venom of the much-feared wasp, *Synoeca surinama* of South America, may contain even larger amounts (14). In the social wasps, the amount of serotonin in the venom apparatus and venom appears to be directly related to the reported painfulness of the sting (14).

The stinging spines of one of the urticant caterpillars (*Automeris* sp.) contain large amounts of serotonin (14).

There is no evidence that serotonin contributes directly to the toxicity of arthropod venoms. Adam & Weiss (22) removed 95 per cent of the serotonin from *Leiurus* venom by acetone extraction and the lethal dose was unchanged. The most important role of serotonin in invertebrate venoms, in which it has been found, may be as a producer of pain and, therefore, defensive. However, it might, in a variety of ways (e.g. increased permeability and blood flow), facilitate the spread of the paralyzing or lethal factors of a venom.

Histamine, Histamine-releasers, and Other Tertiary Amines

Histamine and histamine-releasers have been demonstrated in a variety of invertebrate venoms and venom-producing structures. Tissues of five species of coelenterates were examined for histamine by Mathias et al. (17). Tentacles of the sea anemones, *Actinia equina* and *Anemonia sulcata*, yielded more histamine (20 to 150 μg/g dry tissue) than any other body part. Body wall, with its acontia and nematocysts, also yielded histamine. It was found to be absent from, or present in small quantities in tissues of *Calliactis parasit-*

ica, *Metridium senile*, and *Physalia*. A histamine-releasing substance, "thalassine," has been isolated from tentacles of *Actinia equina* [Jacques & Schachter (25)]. A similar principle has been obtained from tentacles of the jellyfish, *Cyanea capillata*, and shown to be highly effective in releasing histamine from isolated mast cells of the rat [Uvnäs (27, 28)].

The venom-producing, posterior salivary glands of *Octopus macropus* and *Eledone moschata* contain histamine (29).

Histamine also occurs in venoms of certain arthropods. It has been repeatedly demonstrated in the venom of the honeybee (4, 30), and occurs in the venom of the wasp, *Vespa vulgaris* (25), and the hornet, *Vespa crabro* (26). In *V. vulgaris* its average value was reported as 4.3 mg/g wet weight of venom sacs, while in *V. crabro* it ranged from 14 to 30 mg/g dry weight of venom sacs when the extraction procedure employed hot, acidified (HCl) Tyrode solution (pH 3–4).

In addition to histamine and serotonin other amines have been found in some venoms. A variety occurs in the posterior salivary glands of the cephalopods. Tyramine may be present (e.g. *Octopus vulgaris*) (30), also octopamine (*p*-hydroxyphenyl-ethanolamine) (32, 33), and dopamine (Hartman et al. 34). Ghiretti (35, 36) has shown that none of these amines contributes significantly to the toxicity of the saliva of *Octopus vulgaris* although they mimic the excitation that precedes paralysis in a poisoned crab.

Quaternary Ammonium Compounds

Several choline esters, especially acetylcholine, have been found in venoms or venom producing structures. Acetylcholine is present in hydra in relatively large amounts but it is not certain that it occurs exclusively in the nematocysts (37). It is present in the posterior salivary glands of *Octopus vulgaris* (38). A substance with acetylcholine-like properties has recently been demonstrated in extracts of the pedicellariae of *Lytechinus variegatus*, a sea urchin (39). The highest levels of acetylcholine reported from a natural source (18 to 50 mg/g dry weight) have been found in the venom sacs of the European hornet (26).

Choline esters, other than acetylcholine, that have been identified in the hypobranchial glands of gastropod molluscs (*Muricidae*) are murexine (urocanoylcholine), senecioylcholine (β-β-dimethylacryloylcholine) and acryloylcholine [Crescitelli & Geissman (40) for a summary of the literature].

Tetramine (tetramethylammonium hydroxide) is one of several quaternary ammonium bases occurring in marine coelenterates (41, 42). Since it is the only such base also found in fresh water hydra, and the only one to have a paralyzing action on crustaceans, it was suggested (42) that it may be restricted to nematocysts. Salivary glands of the marine gastropod, *Neptunea arthritica*, have yielded up to 7 to 9 mg tetramine per gram of gland [Asano & Itoh (43, 44)]. Fänge (45) found a comparable amount in the salivary glands

of *Neptunea antiqua*. Histamine and choline derivatives also are present in the salivary glands of *N. arthritica* (44).

Kinins and Eledoisin

A peptide with some of the properties of bradykinin is present in the venom of the wasp, *Vespa vulgaris* (25, 46, 47). This peptide has been called "wasp kinin" and shown to differ from bradykinin in its susceptibility to inactivation by trypsin and by its chromatographic behavior. Partially purified wasp kinin contracts the rat uterus at 5×10^{-9} g/ml. It also increases capillary permeability, produces a cutaneous flare reaction, and causes pain when placed on a blister area (47). It appears that more than one such peptide may be present in the venom of *V. vulgaris* (48).

A different peptide occurs in the venom of the European hornet, *Vespa crabro*, and has been called "hornet kinin" (26). It differs from wasp kinin in that it is destroyed by chymotrypsin but not by trypsin. It differs from bradykinin in being approximately 10 times less active on the isolated guinea-pig ileum. The pharmacological properties of wasp and hornet kinins have been compared with those of other known kinins by Schachter (49, 50).

Eledoisin is one of the more fully studied, pharmacologically active peptides deriving from a venom producing organ. It has been found only in the posterior salivary glands of the cephalopods, *Eledone moschata* and *Eledone aldrovandi*. It was isolated, purified, and shown to be an endecapeptide by Erspamer & Anastasi (51, 52). Its structure, H–Pyr–Pro–Ser–Lys–Asp (OH)–Ala–Phe–Ileu–Gly–Leu–Met (NH_2), has been confirmed by synthesis (53).

Eledoisin has a potent stimulating action on all preparations of vertebrate gastrointestinal muscule that have been examined. Other smooth muscles are less sensitive. It is easily distinguished by parallel assays from all biogenic amines and other naturally occurring polypeptides with hypotensive action (54). In the dog its action is clearly hypotensive; likewise, in the rabbit, guinea pig and cat, although these three are less responsive than the dog. In the rat, eledoisin may produce hypertension after ganglionic blockade or pithing, probably through the release of catechol amines (55). The functional significance of this active product of the posterior salivary glands of *Eledone* is not yet clear (52).

Roles of Non-protein Constituents of Venoms

The compounds thus far considered appear not to be the truly toxic components of invertebrate venoms. No one of them can account for the effective paralyzing action of the venoms with which this review is mainly concerned. Although tetramethylammonium chloride mimics the action of crude coelenterate tentacle extracts when injected into crabs, and the actions of both are blocked by tetraethylammonium chloride (15), it was concluded that tetramine alone could not account for the paralysis produced by coelenterate toxin (42, 56). Likewise, while murexine (urocanoylcholine) has a neuromuscular blocking action in mammals (57), as does β-β-dimethylacrylolyl-

choline (58), it seems unlikely that these substances can account for the long-lasting paralysis of the prey produced by muricid snails, or by injected extracts of their hypobranchial glands (3).

As already suggested in this review, and by other authors, the most likely roles of the tertiary amines, quaternary bases, and kinins of invertebrate venoms are as pain producers (defensive) and as facilitators of absorption and distribution of the toxic components. In discussing the pain produced by insect stings, Keele (59) said, "If I had been asked to concoct a really potent pain-producing brew consisting of substances of low molecular weight and of animal origin the mixtures found in wasp and hornet venom would have been at the top of my list." These include serotonin, histamine, acetylcholine and kinins.

Recent evidence points toward proteins as the components of invertebrate venoms that are responsible for the peculiarly effective property of subduing and relaxing the prey preparatory to feeding, or to storing as food for the young (e.g. wasps). Some of the evidence in support of this view will now be considered.

Protein Constituents of Venoms

Since there is no conclusive evidence that the several enzymes such as hyaluronidases, phospholipases, proteinases and oxidases, often found in venoms, are directly responsible for their toxicity, they will not be given further consideration in this review.

Coelenterates.—In the long search for the toxic factor of the characteristic stinging organelles of all members of this phylum, many significant observations have been made, including the discovery of anaphylaxis (60). Much indirect evidence such as loss of activity by heat denaturation and the development of immunity by crabs that live in intimate association with sea anemones, has suggested the protein nature of the paralyzing factor, but only recently has more direct and conclusive evidence been obtained. It has been shown that the paralyzing factor of hydra, certain sea anemones (e.g. *Metridium* and *Condylactis*) and the jellyfish, *Cassiopea*, is heat labile, destroyed by trypsin and chymotrypsin, precipitated by fifty percent acetone or ethanol, non-dialyzable, and present in the soluble fraction of tentacle homogenates. When aqueous extracts of lyophilized hydra or the acetone powder of *Metridium* tentacles are injected into crayfish there is a period of increased motor activity followed by a short period of rigidity and then flaccid paralysis. When perfused through an isolated crayfish appendage the muscles contract and then relax. They fail to respond to a second dose of toxin or to electrical stimulation. With intracellular recording of muscle membrane potential the toxin produces an irreversible depolarization. When applied to the isolated nerve cord of the crayfish a brief increase in spontaneous electrical activity is followed by complete quiescence. It would appear that the toxin acts at the level of the muscle and nerve cell membrane to alter, irreversibly, the permeability barriers and the property of selective permeability (37).

Using a method [Phillips (61)] that permits the isolation of clean, undis-

charged nematocysts of *Physalia*, Lane & Dodge (62) obtained a toxic substance with similar properties to those already described; i.e., the crude toxin is heat labile, non-dialyzable, and precipitated or destroyed by a number of organic solvents [Lane (63)]. Chromatography of the toxin permits separation into nine components and elution, acid hydrolysis, and rechromatography of each of these zones has shown them to be peptides of qualitatively and quantitatively different amino acid composition (63, 64). Possibly the first chromatographic procedure causes partial breakdown of the toxic protein.

Molluscs.—The toxic principle of the poison cone shells (*Conus*) has not yet been isolated and chemically defined. Kohn et al. (1) report that the activity of the venom of *C. striatus* or *C. textile* is not lost after heating to 90 to 100 C° and that incubation with trypsin does not fully destroy the active principle, yet the toxin is non-dialyzable.

All species of *Conus* are probably predaceous. The prey is paralyzed by the venom and swallowed whole. Some species of *Conus* feed on marine worms, some on other gastropod molluscs, and others on fishes (65). The relation between toxicity of the venom of a given species and its normal prey has recently been reported (2). Muscular paralysis of the prey appears to be the biologically significant action of *Conus* venoms. Several cases of human stings have been reported, five of which were fatal (66).

In one of the most recent studies on the toxicity of the saliva deriving from the posterior salivary glands of cephalopods, Ghiretti (35, 36) has described the symptoms that follow the injection of a drop of the saliva of *Octopus vulgaris* into a crab. Excitation is succeeded by flaccid paralysis. The excitation is clearly due to acetone soluble components (amines) while the paralysis results from a component that is acetone insoluble, heat labile, non-dialyzable, and destroyed by trypsin. Ghiretti has proposed the term "cephalotoxin" for the paralyzing substance, and presents evidence that it is a protein with associated hexosamines. The mechanism of action of cephalotoxin is not yet clear although it is reported that it modifies the electrical activity of the central nervous system of crabs (36).

There appears to be only one report of a human fatality resulting from the bite of an octopus (67).

Insects.—The literature on insect toxins and venoms has recently been reviewed by Beard (68). Mention here will be made only of the paralyzing venoms of certain of the wasps. Beard's own work on the action of the venom of *Bracon hebetor* (*Habrobracon juglandis*) has contributed much to our understanding of the nature and action of a wasp venom. He has estimated that as little as one part of venom of *Bracon* in 200,000,000 parts of host blood is adequate to produce permanent paralysis in a late instar larva of the wax moth, *Galleria mellonella* (69). The venom acts primarily on body muscle while visceral muscular activity is unaffected. The heart may remain beating for long periods after the prey is completely immobilized. In a recent study of the venom of the digger wasp, *Philanthus triangulum*, it is also suggested that its action is directly on the body muscle of the honeybee, its normal prey (70, 71).

The chemistry of the paralyzing factor in wasp venoms is poorly known; however, Beard (72) concludes that braconid venom acts like a protein and can be assumed to be proteinaceous or attached to a protein.

Spiders.—Much of the voluminous literature on venomous spiders is of a clinical nature. This is not surprising in view of the frequency of painful and often fatal spider bites in the warmer parts of the world. For example, Bücherl (73) reports that several hundred persons are bitten annually in the state of São Paulo, Brazil, with occasional fatalities among children, and Bettini & Toschi-Frontali (74) record that during the years between 1938–1958 the number of cases of lactrodectismus, resulting from the bite of *Lactrodectus tredecimguttatus*, in four provinces of central Italy amounted to about 1000. Much of the work on spider venoms deals with effects on mammals and says little concerning the active principles and their modes of action on the normal prey such as other spiders and insects. An exception is found in the work of Bettini and collaborators of the Instituto Superiore de Sanità, Rome. A concise summary of earlier work on *Latrodectus* venom by themselves and others is available in which they point out the importance of dealing with venom uncontaminated with digestive enzymes (74). This earlier work indicated that the toxic properties of this spider venom resided in a single protein fraction. More recently it has been found that extracts of the poison glands of *Latrodectus tredecimguttatus*, when subjected to electrophoresis on a refrigerated column of cellulose powder, yield two active protein fractions which, when tested on the housefly, together account for 74 percent of the toxicity of the whole extract. For one of these the LD_{50} dose per housefly is 0.0011 μg of protein and for the other 0.0008 μg of protein. The two active fractions produce different symptoms in the housefly (75). In a personal communication (76) Toschi Frontali & Grosso report that they are now obtaining the two toxic fractions (venoms A and B) by a final separation with Sephadex. Venom A causes an immediate paralysis of the housefly which is slowly but spontaneously reversible at low doses, while venom B has a more retarded, but irreversible, effect. The biological activity of the purified venoms is highly labile.

Scorpions.—As examples of recent work on the protein nature of the toxins of scorpions, the results of Adam & Weiss (77) and of Miranda et al. (78, 79, 80) will be cited. The venom of *Leiurus quinquestriatus* when applied to the sartorius muscle of a toad or the rat diaphragm has an action resembling that of citrate, veratrine or low calcium. An increase in calcium raises the threshold to the venom. The active material of the venom is non-dialyzable, destroyed by heating for 30 minutes at 100 C°, and by incubation with trypsin or chymotypsin (77). While such evidence indicates that the toxic principle of *Leiurus* venom is a protein or proteins, more complete evidence now exists for the toxins of two other North African scorpions, *Androctonus australis* and *Buthus occitanus* (78, 79, 80). When the venoms of these two species were fractionated by aqueous extraction, acetone precipitation in the cold, chromatography on Amberlite C G 50, and filtration on Sephadex G-25, two active basic proteins were obtained from each species (80). The two proteins

share almost equally the total toxic activity of each venom. A tentative molecular weight of 12,000 has been assigned the proteins of *B. occitanus*. The generic name "scorpamins" has been proposed for the toxic proteins of scorpions.

According to Miranda et al. these proteins have a purely neurotoxic action. Among the symptoms they produce in mice are agitation, salivation, tonic and clonic movements, posterior paralysis, and respiratory paralysis (78). Little is known concerning the mechanism of action of scorpamins at cellular and organ level and more attention should be paid to the actions of scorpion venoms on their normal prey.

These selected examples of some of the more recent studies on the nature of the truly toxic (usually paralyzing) components of invertebrate venoms indicate, or strongly suggest that specific proteins are generally the most active principles to be found in these venoms. Furthermore, in some instances (e.g., coelenterate and some wasp toxins) there is growing evidence that paralysis of arthropods results largely from a direct action on the muscle membrane and not necessarily only in the region of neuromuscular junctions. This point may be difficult to settle because of the multiple nerve endings on each arthropod muscle fiber and the difficulty of applying venoms to nerve free regions. For those toxins that also act on vertebrate skeletal muscle this is not a problem. That a paralyzing venom or toxin may act on the central nervous system as well as on muscle is clear from the studies of Munro (37) on coelenterate toxin. While this material produces a preliminary increase in the spontaneous electrical activity of an isolated crayfish nerve cord, this is quickly followed by complete electrical silence.

The isolation, chemical identification, and pharmacological study of highly purified toxic proteins of some invertebrate venoms (e.g., coelenterates and some spiders) are made difficult by the increased "instability" of the purified material (37, 75). It is conceivable that the purification process removes unknown but necessary cofactors, and that subsidiary substances (e.g., amines and enzymes) play important roles in the action of the toxic protein(s).

It may be expected that future studies of invertebrate venoms will tell us how chemically diverse are their truly toxic components and how specific their actions relative to the normal prey and the various organ systems of the prey. Furthermore, they should clarify these mechanisms of action at cellular levels and provide new pharmacological tools for the study of basic cellular processes. Hopefully, such studies may provide new approaches to the chemical control of undesirable species.

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