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## NEMATOCYST VENOMS AND TOXINS

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### SUMMARY

Although many people are stung each year by marine cnidarians, proportionally few stingings are fatal. Nonetheless, nematocyst venoms are among the most potent of all known venoms. Detailed information about nematocyst venoms and toxins is presently confined to only a few species owing in part to the use of less than optimum procedures for collecting and preparing the venom. The relative merits and liabilities of isolating nematocyst venoms and toxins by four different procedures are discussed. Currently the venoms from the sea anemone (Aiptasia pallida) and the Portuguese Man-of-War (Physalia physalis) are the most completely characterized biochemically and in terms of their cellular and molecular mechanisms of action. Unusually steep mortality curves, very short lethality times, and similar pharmacological effects for venoms from at least five species representing all three classes of cnidarians suggest similar overall mechanisms of action. Hydrozoan and scyphozoan venoms, in particular, seem to share common features, with each characterized primarily by a major, single-component stoichiometric type of cytolysin. Anthozoan venoms, on the other hand, are considerably different and characterized by a multi-component catalytic cytolysin plus neurotoxins. Evidence is presented suggesting that the full potency of the physiological effects of nematocyst venoms are the result of cooperative and synergistic interactions between various components of the venom.

# I. INTRODUCTION

In this paper I evaluate the findings most relevant to understanding the nature and mechanism of action of nematocyst venoms and toxins at the cellular and molecular levels. I especially attempt to identify unresolved questions and underlying mechanisms, and at the risk of grossly over-simplifying, to propose how nematocyst venoms differ among different cnidarian taxa. The first section of this review is introductory and deals with aspects of nematocyst structure and function, public health of nematocyst stings, and the terminology and general principles of toxinology. The second section reviews selected aspects of the literature on nematocyst venoms and toxins, including methods of obtaining venoms and toxins, their physiological effects, physico-chemical and enzymic properties, lethality and cellular mechanisms of action. The third section attempts to distill current knowledge into general principles and mechanisms of action, and proposes characteristics of nematocyst venoms from different taxa.

## A. Structure and Function of Venomous Nematocysts

The cnidarians, which include the jellyfish, sea anemones, corals, and hydroids, are the most venomous phylum. Each of the approximately 10,000 species in this phylum possess tiny eversible and venomous organelles called nematocysts, which are mainly used to capture prey. There are more than 24 different morphological types of nematocysts (Mariscal, 1974). The morphological differences that distinguish different types of nematocysts imply specific functions related to capture of prey or defense, such as penetrating, holding, adhering, and entangling (Purcell and Mills, 1988). It is generally assumed that only the penetrant nematocysts are venomous and capable of stinging. For example, the fully everted tubules of holotrichous isorhizas from Physalia physalis (Portuguese Man-of-War) extend more than fifty times the diameter of the nematocyst capsule (Cormier and Hessinger, 1980a) and these nematocysts (Ionnides and Davis,1965) and those from the "sea wasp" (Chironex fleckeri; Kingston and Southcott, 1960) can penetrate into vascularized tissues of human flesh.

It is not yet known in which intranematocyst compartment of the undischarged nematocyst the venom is stored or by which route the venom is released upon discharge. Isolated Man-of-War nematocysts discharged into 1% glutaraldehyde and immunohistochemically stained with polyclonal antibodies to

the Man-of-War venom show antigenic reactivity along the length of the everted tubule (Jewell and Hessinger, in preparation) and a similar pattern of release is indicated by discharging nematocysts of other species into red blood cell suspensions (Klug et al., 1988). In addition, the barbs on the predominant holotrichous isorhizas of Man-of-War fishing tentacles appear to be hollow (Hessinger and Ford, 1988). On the other hand, when isolated mastigophores from the acontia tissue of the sea anemone Aiptasia pallida are osmotically discharged into acidic medium the soluble contents of discharging nematocysts are observed to precipitate at the tip of the everted tubule (Blanquet, 1968). Hence, both modes of venom delivery may occur depending upon type of nematocyst.

## B. Public Health Aspects

Our knowledge of cnidarian stinging incidents on humans on a global scale is meager and is based on scattered reports provided by lifeguards or published as clinical case studies. Most of these reports are from American and Australian workers dealing with the hydrozoan, the Portuguese Man-of-War, and certain scyphozoan "jellyfish," particularly the sea wasp. The information coming from the Southeastern Atlantic and Gulf coasts of the United States is extremely deficient owing to the fact that such stings are non-reportable public health illnesses.

On the basis of published reports Halstead (1965) notes that there have been 502 reported stingings with 46 fatalities world-wide from the mid-1800's to 1960. This gives a crude fatality rate of approximately 9%. However, the results of Southcott (1952, 1959) and Barnes (1960) indicate that only a small fraction of the stingings are ever published in the medical science literature. Many deaths listed as drownings may in fact be due to stings from either the Man-of-War or the sea wasp. To my knowledge there have been no more than six reported fatalities by cnidarians on U.S. coasts since 1950, and two of these accounts were only reported in local newspapers. All of these fatalities have occurred in the southeastern U.S. and have been caused by Physalia, believed to be the most harmful cnidarian in the western hemisphere.

The stings of Physalia are not usually fatal; nonetheless, many hundreds of people are stung annually on U.S. Gulf of Mexico and Atlantic beaches. Such non-fatal encounters produce excruciating pain for periods lasting from 15 minutes to several hours with pain rapidly becoming more generalized. In severe cases the acute symptoms include difficulty in breathing, apparently due to tetany of

abdominal muscles and diaphragm, convulsions and cardiac irregularities (Russell, 1966). Acute renal failure along with systemic hemolysis and attendent hyperkalemia have been reported (Spielman et al., 1982; Guess et al., 1982). In addition, delalyed cutaneous eruptions both at (M'ansson et al., 1985) and remote from (Matosow, 1980) the site of envenomation have been reported. Consequently, individuals previously exposed to the Physalia sting, those with histories of cardiovascular disorders and those who are generally immunologically hypersensitive (e.g., asthmatics) are likely to be at the greatest risk.

Current therapy for victims is entirely supportive; no antidotes or antisera are available. Neither are there adequate preventative methods other than avoidance for individuals likely to be exposed to a high risk of Physalia encounter, such as professional divers, environmental scientists, lifeguards and commercial fishermen.

While the sting from the Pacific "bluebottle" (Physalia utriculus) is considered less dangerous than the larger Atlantic Man-of-War (P. physalis), the Indo-Pacific "sea wasp" (C. fleckerii) is considered to inflict the most dangerous cnidarian sting (Halstead, 1978). At least 60 deaths are attributable to the sea wasp near Brisbane, Australia during a recent 25 year period (Cleland and Southcott, 1965). As with Man-of-War most stings from the sea wasp painful but minor injuries. Such stings produce a "weal and flare" cutaneous reaction. There is considerable edema and subsequent subcutaneous necrosis, slow healing and long-term discoloration or permanent scarring, with susceptibility to infection for years (Barnes, 1966). With fatal stings the victim collapses within 20 minutes and death appears to be due to cardiac arrest. Maintainance of respiratory exchange and circulation by artificial means have resusitated some severly stung victoms (Barnes, 1966). A commercial sea wasp antivenin (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) is available and is indicated whenever a victim has received first aid and continues to have difficulty breathing, swallowing or continues in severe pain (Halstead, 1978).

## C. Terminology and General Principles

Mariscal, in his 1974 review, noted that most of the reports on nematocyst venoms or toxins are difficult to harmonize with others in this field and often it is not certain whether the toxins are of the nematocyst origin. Even in those cases claiming that the toxins were extracted from pure nematocysts, there is some doubt concerning the

purity and stability of these preparations. Perhaps this ambiguity would be resolved if (i) a definition of the nematocyst venom and toxins were agreed upon, and (ii) reliable methods were available to obtain and to study these venoms.

## 1. Definitions and Terms

Classically, venoms are defined as toxic secretions intended for injection into other animals for use in either capturing food, aggression, or defense. In the case of cnidarians the venom refers to soluble material originally contained within undischarged penetrant nematocysts. It does not include or refer to cytoplasmic substances that might also be toxic. Neither does the term, nematocyst venom, necessarily refer to any single toxic substance within the nematocysts, but to all the soluble materials extruded from the nematocyst at discharge regardless of whether the individual components are toxic alone or not.

The term, toxin, must also be used precisely in order to distinquish it from poison. In general, toxins are defined as poisonous substances of biological origin. Toxins confer adaptive advantage to the organisms employing them by exerting adverse biological effects on other organisms. In the context of this review, the term "toxin" implies a specific anatomical site of origin, a unique mode of deployment, and a specific biological function. With respect to studying cnidarian toxins it is essential that care be taken to distinguish between those toxins that originate within nematocysts as constituents of the nematocyst venom, and those poisonous substances that originate outside the nematocysts as constituents of the tissues. Whether poisonous substances of extra-nematocyst origin should be termed "toxins" or simply "poisons" may be difficult to determine since it depends upon whether these substances assist in capture prey or defense or whether they exist as normal tissue components which are only incidentally harmful to other organisms.

Finally, it is helpful to use such terms as potency and specificity when comparing different nematocyst toxins with each other or with toxins of other origins. Potency is a quantitative term that refers to effective toxicity or lethality. Lethality is commonly expressed in terms of $LD_{50}$ values in standardized animals, such as white mice, and is expressed in units of $\mu g/kg$ body weight. Analogous units may be used to express toxicity in terms of physiological or biochemical effects. Specificity is a qualitative term that is not expressed in terms of defined units but refers to relative restriction of function and/or site of action and

is, therefore, difficult to objectively measure.

## 2. General Principles

Before proceeding with an evaluation of nematocyst toxins some additional generalizations regarding toxins may be of use.

a. Most Toxins Target Plasma Membranes. There are several reasons why most toxins have as their primary target sites the plasma membranes of susceptible cells. (i) The plasma membrane is the most accessible part of a target cell, especially if the toxin is macromolecular and/or hydrophilic. (ii) Relatively less toxin is needed to saturate binding sites at a given density on a two-dimensional cellular surface than at the same concentration within the three-dimensional cytoplasmic space enclosed by that cellular surface. Thus, the amount of toxin needed to exert its effect is theoretically reduced when it acts on the surface of the target cell. (iii) Many vital functions which are mediated by plasma membranes serve as potential targets. Such functions include excitability, regulation of metabolism, maintenance of cell integrity, transport, and recognition. Of these functions excitability, regulation, and cell integrity are most often targeted by toxins since inhibition or disruption of these functions is most likely to have immediate deleterious or lethal effects.

b. Most Toxins are Proteinaceous. This is true for several reasons. (In cases where the toxin is not a protein the target site is likely to be a protein.) (i) Protein toxins are two to five orders of magnitude more lethal than non-proteinaceous poisons. (ii) Proteins can be designed to interact specifically with any other kind of molecular site. (iii) Some protein toxins may possess enzymatic activity in order to amplify the extent of their biological effects due to a turnover of target-derived substrates. (iv) Some protein toxins, due to their large size and three-dimensionsal structure, may be able to span the plasma membrane to affect membrane permeability and/or cytoplasmic processes. (v) Some proteins are capable of undergoing functional and conformational changes from inactive to active states in response to changes in extrinsic conditions. Thus, a toxin could be inactive but stable within the nematocyst, and become active and labile upon release or upon contact with a target membrane. Venom toxins, in particular, are almost always proteins. Furthermore, venom toxins are not likely to harm the predator when ingested with envenomated

prey since protein toxins are not likely to survive the action of digestive proteases.

c. Venom Toxins Act Rapidly. They are designed either to have an immediate defensive effect to stun or induce pain in a potential predator or to have an immediate offensive effect to kill or paralyse prey.

## II. NEMATOCYST VENOMS AND TOXINS

The following evaluation is presented in terms of the two difficulties that have most consistently hampered research of nematocyst venoms: (i) development of methods for obtaining nematocyst venom free of cytoplasmic components, and (ii) development of suitable assays from which a systematic study of the mechanism of toxicity could be directed.

### A. Methods of Obtaining Nematocyst Venom

Four general methods have been used in attempts to obtain nematocyst venoms and toxins.

#### 1. Extracts of Whole Animals and Tentacles

Possibly because mature nematocysts are fragile structures and very prone to discharge by slight mechanical shock workers have often attempted to study nematocyst venoms by extracting toxic substances from the whole cnidarian tissue. Richet (1902; 1903) and Richet and Portier (1936) isolated three pharmacologically active extracts from whole tissues of cnidarians: (i) an aqueous extract termed "hypnotoxin," which caused cardiac arrest; (ii) an alcohol extract termed "thalassin," which caused the release of histamine; and (iii) a glycerin extract termed "congestin," which induced anaphylaxis. None of the active components of these extracts have been chemically identified.

Several quarternary ammonium bases have been identified in extracts of whole sea anemone (Actinia equina; Ackermann et al., 1923; 1924; 1953). Welsh and Prock (1958) listed many identifiable quaternary amines in tissue extracts from a variety of cnidarians, but found tetramine, which has toxic properties, only in hydra. In addition, Welsh (1956; 1960) reported finding 5-hydroxy-tryptamine (5-HT or serotonin), a powerful pain producer, histamine releaser, and neuro-

transmitter, in all cnidarians tested. The highest levels of 5-HT were found in so-called nematocyst-rich tissues (acontia and tentacles). Mathias et al., (1960), however, found that the levels of 5-HT in the tissues devoid of nematocysts, in Calliactis parasitica, were as high as that found in the tentacles. These workers also found a histamine-releasing substance from a sea anemone while Unvas (1960) described a similar substance from a jellyfish (Cyanea).

In addition to the many low molecular weight chemicals which have been identified in cnidarian extracts, researchers have more recently found toxic activity associated with various protein fractions. The earliest work of this type (Kline and Waravdekar, 1960; Shapiro, 1968a) followed the growing conviction now held by most workers in the field that the nematocyst venoms are proteinaceous whereas the low molecular weight pharmacologically active compounds of the tissue extracts represent materials from outside the nematocysts. By far, most of the work with proteinaceous extracts of whole tissues has involved sea anemones. This body of work is reviewed elsewhere in this volume by Kem (1988).

Shapiro pioneered the work on extracted, protein neurotoxins. He began with an acetone extract of the tentacles of the sea anemone, Condylactis gigantea, and purified a single, homogenous, low-molecular weight, basic protein (1968a) which transformed action potentials in lobster ventral nerve cord giant fibers into cardiac-like potentials (1968a,b). The neurophysiological action of the protein on nerve membranes was later elucidated by voltage clamp experiments (Narahashi et al., 1969). In spite of the toxic and neurophysiological activity associated with the extracted protein, it has never been shown to be a component of nematocyst venom. At best, the extraction method can isolate only one nematocyst toxin at a time. At worst, the extraction method can yield pharmacologically active substances of non-nematocyst origin (Mathias et al., 1960).

2. "Milked" Venom

By this method, live isolated tentacles of Chironex (Barnes, 1967) are electrically stimulated to discharge their nematocysts into and through a specially treated amniotic membrane. Nematocyst tubules which fully penetrate the membrane emit their venom on the opposite side of the membrane from which it is collected by rinsing. This procedure simulates the stinging reaction of the cnidarian against its prey, but in this case only small quantities of emitted venom can be collected and analyzed.

The venom collected in this manner is very toxic and stable (Barnes, 1967) but it is quite conceivable that some of the biologically active, components of the venom are removed by adsorption to biological moieties on the surface of the membrane. It is widely known that toxic components of many venoms, including nematocyst venoms (Hessinger and Lenhoff, 1973b; Lim and Hessinger, 1979), bind to cell surfaces.

## 3. Extracts of Isolated Nematocysts

Some recent workers have endeavored to avoid the ambiguities associated with crude extracts of tentacles and whole animals, and have sought to isolate intact nematocysts from which the toxic contents could be extracted. In general, the nematocysts are isolated from disrupted nematocyst-bearing tissue and then disintegrated to release their venomous contents. Although these methods ensure that the nematocyst contents are included in the final nematocyst extract, the toxicites of many extracts have tended to be low and unstable. Possible reasons for this are described below.

a. Several Types of Nematocysts Are Obtained (Table I). Endean et al. (1969) reported that isolated nematocyst preparations from the sea wasp consist of: 43% microbasic mastigophores, 42% atrichous isorhizas, 13% microbasic euryteles, and 2% holotrichous isorhizas. However, histological studies of the skin of human victims indicate that only the microbasic mastigophores are likely to be of any clinical significance (Kingston and Southcott, 1960). Burnett et al. (1968) and Blanquet (1970) have also reported four different types of nematocysts in their preparations of nematocysts from the tentacles of Chrysaora quinquecirrha (the sea nettle) and Lane (1960) reported that Physalia tentacles yield two distinct sizes of holotrichous isorhizas. Recently Burnett et al. (1986) separated the two sizes of Physalia nematocysts by flow cytometry, but the method is not suitable for obtaining preparative quantities of nematocysts. It is, however, relatively easy to separate large quantities of the two sizes of isorhizas in Physalia by differential centrifugation (Tamkun and Hessinger, 1981).

b. Immature Nematocysts May Be Isolated Preferentially. Mature nematocysts are fragile and notoriously susceptible to discharge. Methods which disrupt nematocyst-bearing tissues by techniques such as homogenization (Phillips and Abbott, 1957) or pressing the tissue through mesh screens (Lane and Dodge, 1958; Burnett et al., 1968; Banquet, 1970) may

discharge mature nematocysts while yielding mostly immature nematocysts which contain "unripe" venoms (Barnes, 1967).

c. Isolated Nematocysts Are Often Contaminated. Nematocysts are often isolated with adhering cytoplasmic materials. Uncontaminated nematocysts when sedimented, however, are pure white in appearance. Lane (1960) noted that preparations of isolated Physalia nematocysts have the appearance of "grey putty." Such nematocysts, when veiwed by phase-contrast microscopy or by scanning electron microscopy (Cormier and Hessinger, 1980) are contaminated with "fibrillar baskets" which are attached to the capsules. These can be removed with proteolytic enzymes.

Lane (1961) found his extract of isolated Physalia nematocysts to contain a heterogenous mixture of small peptides. This extract also exhibited substantial proteolytic activity and showed rapid loss of toxicity (1967). Nematocysts prepared from Stomolophus are contaminated with pigmented cytoplasmic debris (Toom and Chan, 1972a) and yield extracts possessing proteolytic activity (Toom and Chan, 1972b). Bodansky and Rose (1922) have shown that both the nematocyst-bearing and nematocyst-free tissues of Stomolophus possess proteolytic activity. Cytoplasmic contaminants (Goldner et al., 1969) and proteolytic activity (Burnett and Calton, 1974a; Lal et al., 1981a; Calton and Burnett, 1982; 1983) characterize nematocysts prepared by conventional methods from both the sea nettle Chrysoara and from the Man-of-War (Lal et al., 1981b).

We have not been able to detect proteolytic activity in venom obtained from the uncontaminated nematocysts from the sea anemone, Aiptasia pallida (unpublished observations) nor in toxic extracts of nematocysts from Physalia after they have had their fibrillar baskets removed (Tamkun and Hessinger, 1981). We, therefore, believe that reports of proteolytic activity associated with venomous nematocyst contents actually reflect the presence of contaminating substances of non-nematocyst origin. As a general rule, venom preparations exhibiting proteolytic activity possess low toxicity (Table I).

d. Disintegration Techniques Solubilize Contaminants. The disintegration techniques used to release the soluble, venomous contents of the isolated nematocysts also solubilize some of the proteins from the nematocyst capsules, including any adhering contaminants. We have detected this with SDS-polyacrylamide electrophoresis (unpublished observations). Using nematocyst capsules from Aiptasia that were discharged osmotically (see next section) and washed extensively to free them of all venom, we monitored the total amount and variety

TABLE I. Lethalities of nematocyst venoms

| Genus (cnida type)[a] | $LD_{50}$ (μg/kg) | Preparations[b] | References |
|---|---|---|---|
| Stomolophus (ME) | | | Toom and Chan, 1972a |
| | 5,300 | NE | Toom and Chan, 1972a |
| Chrysaora (ME/HI/HA; 55:25:20) | | | Burnett et al., 1968 |
| | 2,000 | NE | Burnett and Goldner, 1970 |
| | 2,500 | NE | Burnett and Gould, 1971 |
| | 1,400 | NE | Burnett and Calton, 1973 |
| | 2,780 | NE | Cobbs et al., 1983 |
| Chironex (MM/ZI/ME/HI; 43:42:13:2) | | | Endean et al., 1969 |
| | 250-400 | MV | Olsen et al., 1984 |
| | 12.5 | TE | Crone and Keen, 1971 |
| | 11 | TE | Calton and Burnett, 1986 |
| Aiptasia (MM) | | | Blanquet, 1968 |
| | 136 | OD | Hessinger and Grove, 1979 |
| | 300[c] | OD | Hessinger et al., 1973 |
| Neurotoxin-II | 11[c] | purified | Hessinger et al., 1973 |
| Physalia (HI) | | | Cormier and Hessinger, 1981 |
| | 2,000 | NE | Lane, 1960 |
| | 750 | NE | Garriott and Lane, 1969 |
| | 700 | NE | Calton and Burnett, 1973 |
| | 700 | NE | Burnett et al., 1986 |
| | 145 | NE | Tamkun and Hessinger, 1981 |
| | 70 | NE | Flowers and Hessinger, 1981 |
| | 75 | NE | Loredo et al., 1985 |
| | 32 | NE | Hessinger, 1988 |
| Physalitoxin | 200 | purified | Tamkun and Hessinger, 1981 |

[a]AI, atrichous isorhiza; HA, homotrichous anisorhiza; HI, holotrichous isorhiza; ME, microbasic euryteles; MM, microbasic mastigophore.

[b]MV, milked venom; NE, nematocyst extract; MV, milked venom; OD, osmotically discharged venom; TE, tissue extracts

[c]Minimum lethal dose ($LD_{100}$) in Uca.

of materials released from the capsules following disintegration by different techniques. We found that brief sonication solubilized the capsular materials most extensively; less effective in declining order were French pressing at 16,000 psi, grinding with mortar and pestle, and motor-driven homogenization. Hence, caution should be used in evaluating venoms prepared in these ways.

4. Venom Obtained by Osmotic Discharge

The acontiate sea anemones possess acontia threads which contain high densities of nematocysts. Exposure of excised acontia threads to 1 M glycerol or sodium citrate causes the undischarged nematocysts to be extruded from the threads (Yanagita, 1959; Blanquet, 1968). The threads are removed by filtration through fine nylon netting and the remaining suspension of nematocysts are washed repeatedly in the cold by centrifugation. These can then be caused to discharge by placing them in a low ionic strength medium. The released venom is collected as the supernatant phase by sedimenting the discharged capsules. The venom collected in this manner is quite toxic (Table I) and stable (Blanquet, 1968; Hessinger et al., 1973). Unfortunately, this method can only be used to purify nematocysts from acontia.

As a model system for characterizing all of the components of a nematocyst venom the nematocysts from the acontia offer several advantages over other sources of nematocysts. (i) Acontia threads from Aiptasia possess only the large microbasic mastigophores, which contain the venom, and the much smaller holotrichous isorhizas, which contain no detectable venom (unpublished observations). These nematocysts are easily separated by either differential (Hessinger and Lenhoff, 1973a) or sucrose density gradient centrifugation. (ii) Nematocysts extruded from the acontia are free of adhering cytoplasmic materials. (iii) All of the isolated nematocysts can be discharged by hypotonic solutions indicating that only functionally mature nematocysts are extruded. This method of releasing the venom is gentle and closely simulates the natural mechanism of discharge, thereby ensuring that no capsular components are solubilized. (iv) Many acontiate anemones, such as A. pallida, can be cultivated and asexually cloned en masse in the laboratory under optimal and controlled conditions thereby ensuring a convenient and continual supply of fresh venom (Hessinger and Hessinger, 1981).

## B. Physiological Effects

A wide range of physiological and pharmacological effects have been reported for various nematocyst venoms. Because so many different physiological systems, experimental animals and methods of preparing the toxic materials and venoms have been used it is difficult to identify generalized underlying mechanisms of action common to any taxonomic group of cnidarians. To simplify comparisons the physiological effects reported here will be grouped under general categories: cardiovascular effects, neuromuscular effects, and effects on transport. Most of these effects, however, if not all, can be explained in terms of molecular effects on membrane or function (Section II. F.).

### 1. Cardiovascular Effects

Chrysaora venom depolarizes frog ventricles (Shyrock and Bianchi,1983) and dog Perkinje fibers (Kleinhaus et al., 1973). Burnett and Goldner (1969) have not only observed irregularities in cardiac conduction, but also an increase in coronary vascular resistance and arterial blood pressure. In addition, Burnett et al. (1985) have demonstrated that the venom can increase contractile force of guinea pig atria. Reports on Chironex venom, however, are conflicting. Whereas Endean (1987) has observed a venom-induced increase in contractile force of guinea pig atria, others have shown that the venom can decrease contractile force of whole rat (Endean and Noble, 1971) and guinea pig hearts (Turner and Freeman, 1969). Other parasympathomimetic effects, such as a decrease in heart rate, coronary vasoconstriction (Turner and Freeman, 1969), progressive heart failure (Endean and Henderson, 1969) and atrio-ventricular block (Endean and Noble, 1971), have also been seen. Illustrating its dual effect on cardio-vascular function, Endean and Noble (1971) reported that the venom initially increases blood pressure but later profoundly decreases it. Freeman (1974) suggested that the atrial depolarization and reduction in transmembrane action potentials observed by her is linked to an alteration of ion permeability.

Physalia venom has also produced conflicting results. Hastings et al. (1967) observed an increase in cardiac output and a strong increase in blood pressure in the dog, both sympathomimetic effects. However, mixed responses, such as an increase in ventricular repolarization and subsequent atrioventricular block in the rat (Larsen and Lane, 1966), and an early depression of atrial strip contractile force followed by later enhancement (Burnett et al., 1985), have

also been shown. Sympatholytic effects include a decrease in arterial pressure in rats (Garriott and Lane, 1969), occasional generalized hypotension and accompanying respiratory distress (Burnett et al., 1975) as well as skeletal muscle vasodilation in the dog (Loredo et al., 1985). This latter effect is due to venom-induced relaxation of rabbit arterial rings precontracted with norepinephrine (Loredo et al., 1986).

### 2. Neuromuscular Effects

Chrysaora venom decreases the action potentials of rat ventral caudal nerve and skeletal muscle (Burnett and Goldner, 1970) as well as of frog sartorius muscle (Shryock and Bianchi, 1983). Chironex venom blocks nerve conduction on sciatic nerve (Endean and Noble, 1971) and semi-purified, high molecular weight fractions of the venom elicit contraction in rat diaphragm and guinea pig ileum and vas deferens smooth muscle (Endean, 1987). Physalia venom also blocks nerve conduction on frog sciatic nerve (Larsen and Lane, 1970) and contracts isolated guinea pig ileum (Garriott and Lane, 1969).

### 3. Effects on Membrane Transport

Chrysaora venom non-competitively inhibits glucose transport in brushborder membranes from guinea pig small intestine (Watrous and Walsh, 1976) and decreases uptake and binding of calcium into isolated mitochondria (Calton et al., 1973) and into skeletal muscle sarcoplasmic reticulum (Calton and Burnett, 1973a). Chironex venom also inhibites calcium uptake in sacroplasmic reticulum from mouse striated muscle (Endean and Henderson, 1974) as does Physalia venom (Calton and Burnett, 1973a). Physalia venom also increases the rate of sodium influx into cells of frog skin (Larsen and Lane, 1970b).

## C. Physical and Chemical Properties of Purified Toxins

There is fairly uniform agreement in the literature since 1960 that the toxins of nematocyst venoms are proteinaceous (Mariscal, 1974). Of the several published attempts to fractionate and characterize nematocyst venoms only a few have been successful.

Physalia-derived toxins have been studied longer than other cnidarian toxins beginning with toxic tissue extracts

(Portier and Richet, 1902) from which the discovery of anaphylaxis was made. Lane et al. (1961) first attempted to fractionate Physalia nematocyst extracts by ascending paper chromatography and reported the presence of eight peptides. The presence of peptides is not surprising since he later found his venom extracts to have high proteolytic activity (Lane, 1967a). Attempts to fractionate Physalia venom by gel-filtration on G-200 (Calton and Burnett, 1973a) and by preparative gel electrophoresis (Burnett and Calton, 1974b) yielded eighteen and nine lethal fractions, respectively; none of which were hemolytic. More recently, a single, potently hemolytic toxin in Physalia venom was identified, purified and characterized although other, apparently non-toxic, components were also present (Tamkun and Hessinger, 1981). A monoclonal antibody to the lethal activity of Physalia venom has been prepared (Gaur et al., 1982). While it has been used to purify toxic fractions from Chironex venom (Olsen et al., 1984), from the venoms of Chrysaora and from two other species of scyphozoans, from brown recluse spider venom, and from cholera toxin (Olsen et al., 1985), it apparently has not yet been used to purify toxic components of Physalia venom.

Extracts of Chrysoara nematocysts yielded 25 lethal fractions by gel-filtration (Burnett and Calton, 1973), while immunochromatography yielded a fraction containing two major proteins having molecular weights of 100,000 and 190,000 exhibiting cardiotoxic, neurotoxic and enhanced lethal activities (Cobbs et al., 1983).

Attempts to isolate toxins from Chironex have started with "milked" venom, venom extracted from isolated nematocysts, and extracts of tentacles. Fractionations using "milked" venom have toxic activities associated with lower molecular weight fractions (10,000-30,000, Baxter and Marr, 1969; 20,000-70,000, Olson et al., 1984), while extracts of isolated nematocysts (50,000 and 150,000, Calton and Burnett, 1986; 150,000 and 600,000, Endean, 1987) and extracts of tentacles (a hemolytic fraction of 70,000 and a toxic fraction of 140,000, Crone and Keen, 1969; 1971) have toxicity associated with higher molecular weight fractions. These results strongly suggest that the biologically active, high molecular weight components found in tentacle and nematocyst extracts may be lost during the "milking" procedure.

Nearly 80% of the constituent amino acids of Aiptasia venom are glutamic and aspartic acid (Blanquet, 1968; Phelan and Blanquet, 1985). Blanquet (1968) reported finding two proteins in Aiptasia venom; one toxic to fiddler crabs (Uca) with a molecular weight of 130,000, and the other not toxic with a molecular weight of 30,000. More recent studies reveal that Aiptasia venom is more complex. At the present

we have been able to identify five toxic constituents of Aiptasia venom of which four have been isolated and partially characterized. These five proteins account for all of the known toxic effects of the venom. Currently, Aiptasia venom is physically and chemically the best characterized nematocyst venom and the venoms from Aiptasia and Physalia are the only two from which individual toxins have been purified and characterized. These are listed along with some of their physical and chemical properties on Table II.

D. Non-toxic Proteins and Enzymic Activities

Several non-toxic proteins have been found in association with venoms extracted from isolated nematocysts while most of these non-toxic proteins have been identified on the basis of enzymatic activity. One protein has been identified on the basis of the absence of aromatic amino acids (Weber et al., 1988). Although several non-toxic enzymatic activities have been listed only a few of these enzymes have been isolated, and these all constitute small portions of the venoms from which they were obtained. This low occurrance coupled with the fact that they were all extracted from nematocysts by ultrasonic disintegration and were isolated from a heterogeneous population of more than one type of nematocyst raises questions as to their origin and biological role.

Neeman et al. (1980) isolated an endonuclease with a molecular weight of 75,000 that constituted 0.8% of the protein of Physalia venom. Lal et al. purified a collagenase both from extracted Physalia (1981b) and Chrysaora (1981a) nematocysts having molecular weights of 25,000 and 34,000, and constituting 0.6% and 0.4% of the total venom protein, respectively. In addition, acid and alkaline proteases have been found in association with Chrysaora venom having molecular weights of approximately 120,000-150,000 (Calton and Burnett, 1982) and 100,000 (Calton and Burnett, 1983), respectively. Although the acidic protease constitutes an estimated 1.6% of the total venom protein it was reported that the specific activity of the enzyme is ten-fold higher in the float, where there are few nematocysts, than with the isolated nematocysts (Calton and Burnett, 1982). For the alkaline protease it is not possible to calculate the percent of the total protein from the published data.

Is it possible that these enzymes are of extranematocyst origin or components of non-penetrant nematocysts that have been isolated along with penetrants? If they originate from penetrant nematocysts do they contribute to the toxic action of the venom, or might they be "left-overs" from nematocyst assembly that impliment post-ribosomal modifications

TABLE II. Physical and chemical properties of toxic proteins purified from nematocyst venoms.

| Genus/Protein | Mol. Wt. | Charge/pI | Sed. Coef. | Quat. Struct. | CHO | Shape | % Venom |
|---|---|---|---|---|---|---|---|
| A. Aiptasia: | | | | | | | |
| Co-lytic factor[a] | 98,000 | 4.5 | 4.5 S | monomer | + | prolate | 1.3 |
| Phospholipase $A_2$[a,b] | | | | | | | |
| $\alpha$ | 45,000 | - | - | monomer | ? | - | 1.9 |
| $\beta$ | 43,000 | 8.8 | - | monomer | + | - | 3.0 |
| Direct lytic fraction[b] | 30,000 | basic | - | - | ? | - | - |
| Neurotoxin:[a,c] | | | | | | | |
| I | 15,000 | basic | - | monomer | ? | - | 12 |
| II | 12,500 | basic | - | monomer | ? | - | 10 |
| B. Physalia: | | | | | | | |
| physalitoxin[d] | 240,000 | subunits: 5.5, 8.2, 8.3 | 7.8 S | heterotrimer | + | prolate | 28 |

References: (a) Grotendorst and Hessinger, 1988; (b) Hessinger and Lenhoff, 1976; (c) Hessinger, Lenhoff and Kahan, 1973; (d) Tamkun and Hessinger, 1981.

of nematocyst precursors during development as suggested by Blanquet (1988) or might they be components of a system of nematocyst proteins that function to cause eversion of the tubule. Until proof of origin and evidence of function can be determined it is best if proposed roles for these materials be viewed with caution.

## E. Lethality

Nematocyst extracts and venom are commonly assayed for lethality on mice and/or crabs (e.g. fiddler crab, Uca pugilator). Toxicities are usually reported as the amount of venom per unit body weight needed to kill 50% of the animals within a specified period and are expressed as $LD_{50}$ values. Such $LD_{50}$ values for the more extensively studied nematocyst venoms are listed on Table I.

### 1. Mortality Curves

The $LD_{50}$ of Aiptasia venom on mice is similar to the more toxic species of cobras (family Elapidae) such as the kraits (e.g. Bungarus; Fischer and Kabara, 1967) and the mambas (e.g. Dendroaspis; Schwick and Dickgeisser, 1963) and the most toxic of the pit vipers (family Crotalidae) including the hundred pace snake (Arcistrodon acutus; Ouyang, 1957). Consequently, the venoms from Physalia and Chironex, which are the more potent nematocyst venoms, are among the most toxic of all known venoms. Several features of the mortality curves and of the lethal effects in injected test animals are common to nematocyst venoms from all three classes of cnidarians.

The mortality curves of nematocyst venoms from Physalia (Tamkun and Hessinger, 1981), Aiptasia (Hessinger and Grove, 1979), Chironex (Baxter and Marr, 1975) and Chrysaora (Cobbs et al., 1983) display extreme vertical slopes. This is due to the narrow dose range between the maximum non-lethal doses and minimal lethal doses. Such steep mortality curves are in contrast to the broad mortality curves that have been published for various microbial toxins and reptilian venoms (Ipsen, 1951). I suggest that broad mortality curves are characteristic of toxins which act on widely distributed sites having a broad range of binding affinities whereas the narrow and steep mortality curves of the nematocyst venoms are due to toxic effects on limited or specialized target sites having a narrow range of binding affinities.

The mortality curves of nematocyst venoms also exhibit a threshold effect in which a minimal dose must be exceeded

before any lethal effect occurs. This suggests that either cooperativity occurs between the toxin molecules on the target cells to form lethal lesions or a critical number of lesions must be formed per target cell in order to produce the lethal effect.

## 2. Rate of Lethality

The time required to produce lethality by lethal doses of intravenously injected venom is fairly short for nematocyst venoms. Mice injected with $2LD_{50}$ doses of Aiptasia venom die within minutes and at five or more $LD_{50}$ doses they die within seconds (Hessinger and Grove, 1979). Multilethal doses of Chironex venom kill within minutes (Baxter and Marr, 1975). On the other hand, while Burnett et al. (1985) have reported that $3LD_{50}$ doses of Physalia venom requires an average of almost two hours to kill, we (Flowers and Hessinger, unpublished observations) find that one $LD_{50}$ dose of Physalia venom kills half the injected mice in an average time of approximately 30 minutes and $2LD_{50}$ doses kill the mice within seconds. Microbial exotoxins, in contrast, require on the order of days to kill mice injected with minimal lethal doses (Carpenter, 1965). The rapidity of the lethal effects of intravenously injected nematocyst venoms along with the observation that mice can tolerate up to $100LD_{50}$ doses of intradermally or subcutaneously injected Chironex venom without ill effect (Keen, 1970) suggests that the preferred target sites for the lethal factor(s) are on the cell surfaces of cellular components of the circulatory system.

## 3. Symptoms

Many of the symptoms exhibited by lethally injected mice are common to the different nematocyst venoms. Mice injected with $2LD_{50}$ or less of Aiptasia venom become lethargic and breathe irregularly. This is followed by partial paralysis of limb, neck and jaw muscles. Immediately before death violent convulsions and spasms of major skeletal muscles occur, sometimes causing animals to leap or jump vertically (Hessinger and Grove, 1979). Mice injected with Chironex venom also become lethargic and develop a progressive impairment of breathing movements followed by spasmodic jerking of the legs before death (Endean et al., 1969). They also show alterations in rate and depth of breathing and die in respiratory arrest (Keen, 1970) after showing terminal jumping and leaping (Baxter et al., 1972). At low doses of Physalia venom mice show no immediate symptoms but develop

respiratory distress and abdominal convulsions within minutes. Mice then become quiescent for a dose-dependent period of time ranging from a few minutes to an hour. Following this interval, the mice either completely recover or expire beginning with a series of spasmodic convulsions involving the hind legs, causing them to hop uncontrollably. A bloody urine and bloody nasal discharge is often observed (Jewell and Hessinger, in preparation).

In the fiddler crab (Uca) Aiptasia venom induces quivering of the legs, violent motor activity with subsequent rigid paralysis, extensive autotomy (loss of legs), and progressive insensitivity to external stimuli (Blanquet, 1968; Hessinger et al., 1973). With Chrysaora venom there is also a severe quivering of the legs with legs becoming hyperextended, but no autotomy (Blanquet, 1972). With Physalia venom there is a vigorous contraction of the leg extensors leading to paralysis, but no autotomy (Lane and Dodge, 1958). Common to these three venoms is the quivering of the legs whereas violent motor activity and autotomy are only seen with the sea anemone venom. The anemone venom is unique among these venoms in that it contains at least two neuro-active proteins; one responsible for causing increased motor activity, and one for autotomy (Hessinger et al., 1973). The toxin causing increased motor activity is responsible for for than two-thirds of the lethality of the venom in fiddler crabs (Table II) and is representative of the typical sea anemone neurotoxin that delays "sodium" inactivation (Kem, 1988). The higher molecular weight cytolytic proteins in Aiptasia venom, on the other hand, only produce quivering of the legs and a subsequent slow, "sleep-like" death in crabs (Hessinger et al., 1973), whereas they are responsible for most of the lethality in mice (Hessinger and Grove, 1979).

4. Synergistic Actions

Some of the constituent nematocyst proteins from Aiptasia and Physalia venoms act synergistically to produce lethal effects. For Aiptasia venom synergy is discussed in detail in the next section (II. F. 1. a.) with regard to the mechanism of venom-induced hemolysis. For Physalia venom only one lethal toxin, physalitoxin PTX), has been detected and purified (Tamkun and Hessinger, 1981) and this toxin is both a major glycoprotein of the venom and an extremely potent hemolysin. During purification of PTX it was noted that while the specific hemolytic activity was increased 3.6-fold the specific toxicity (i.e. reciprocal of $LD_{50}$) was decreased by 28% (Table II). This discrepency suggests that the venom possesses a non-lethal component, in addition to

PTX, that somehow enhances the lethality of PTX. This possibility is supported further by the observation that the lethality of the venom is largely lost upon incubating the venom at 30°C for 30 minutes while the hemolytic activity is not affected until the incubation temperature is increased to 40°C (Flowers and Hessinger, unpublished). From the examples of these two venoms, each of which originate from different classes of cnidarians (Anthozoa and Hydrozoa) and different morphological types of nematocysts (Table I), I suggest that the constituent proteins of most nematocyst venoms exhibit an economy of action based upon synergistic interactions. In such an economy the nematocyst venom, rather than being merely a simple mixture of independently acting proteins, some of which are toxic, consists instead of a system of interacting proteins. On the one hand, these interacting proteins would stabilize each other while contained within the capsule and, on the other hand, enhance, and possibly diversify, the actions of the toxins to a variety of target tissues when released from the capsule.

In summary, nematocyst venoms are lethal and very potent, having steep mortality curves that exhibit threshold effects. The onset of symptoms and lethality is rapid. Common symptoms shared by the different nematocyst venoms in mice and crabs suggest that different nematocyst venoms act via generally similar mechanisms. Lethality is likely to involve synergistic actions of different venom components acting primarily on elements of the circulatory system.

## F. Cellular Mechanisms of Action

Most toxins, and in particular protein toxins, act by disrupting either the structural or functional features of biological membranes. For reasons discussed previously (section I. C.) membrane-acting toxins affect primarily cell integrity, cell excitability, or membrane-mediated regulation of cell metabolism. I believe that the major physiological and lethal effects of nematocyst venoms can be explained in terms of one or more of these three classes of toxic effects.

### 1. The Cytolysins

The cytolysins alter cell membrane permeability and/or disrupt cell integrity. Cytolysis, or in the case of red blood cells, hemolysis, is one of the most commonly reported actions by nematocyst venoms since first reported in Aiptasia venom (Hessinger and Lenhoff, 1968). Hemolytic activity has been reported for nematocyst venoms from Physalia (Garriott

and Lane, 1969), the sea wasp, Chironex (Crone and Keen, 1969), the sea nettle, Chrysaora (Burnett and Goldner, 1971), and hydra (Weber et al., 1988).

In general, there are two types of cytolysins based upon differences in mechanisms of action: catalytic and stoichiometric. Both catalytic and stoichiometric cytolytic actions have been reported for nematocyst venoms, but in no case are both known to occur in the same venom.

a. Catalytic Cytolysins. The catalytic cytolysins (or "catalysins") alter membrane permeability and/or structure by chemically changing membrane structure. These cytolysins are enzymes or, more specifically, phospholipases that are capable of hydrolysing many times their number of phospholipid substrates. They act as either direct or indirect catalysins. The direct catalysins alter cell membranes by hydrolysing membrane phospholipids involved in maintaining the integrity of the plasma membrane and are typified by the phospholipases from insect and elapid snake venoms. The indirect catalysins hydrolyse extracellular phospholipids into detergent and membrane-altering products and are typified by the phosplipase $A_2$ of viperid snake venom. Lysis induced by direct catalysins is characterized by (i) time-courses of lysis that reach 100% regardless of venom dose, (ii) rectangularly hyperbolic dose-response curves of the rate of lysis (Hessinger and Lenhoff, 1973; Grotendorst and Hessinger, submitted), and (iii) the presence of phospholipase activity (Hessinger and Lenhoff, 1976; Tu, 1977).

At the present the cytolytic agents of Aiptasia, Physalia and Chironex venoms are the best characterized cytolysins of nematocyst origin. Of these only the nematocyst venom from the sea anemone A. pallida is known to possess a catalytic cytolysin, while the others possess stoichiometric cytolysins. Although it has been reported that Physalia venom possesses phospholipase activity (Stillway and Lane, 1971) the data is unconvincing and others have been unable to confirm this finding (Burnett and Calton, 1974a; Tamkun and Hessinger, 1981).

The phospholipase from Aiptasia venom, is of the $A_2$ type (Hessinger and Lenhoff, 1974) and can hydrolyse the phospholipids of osmotically ruptured red cell "ghosts" but cannot act on the phospholipids of an intact red cell membrane unless a second venom protein termed direct lytic factor (DLF) is present (Hessinger and Lenhoff, 1976). The mechanism of Aiptasia venom-induced hemolysis is rather complicated, involving the sequential interaction of three venom proteins and calcium with the red cell membrane as follows: (i) Initially, the DLF and phospholipase (Table II) bind tightly but independently to the red cell surface (Hessinger

and Lenhoff, 1973b) via trypsin- and neuraminidase-insensitive sites (unpublished).
(ii) The DLF alters the cell membrane so that phospholipids of the membrane become available to enzymatic attack by the phospholipase (Hessinger and Lenhoff, 1976).
(iii) Because the Km values for enzyme substrate and calcium are similar (Grotendorst and Hessinger, in preparation) we believe that the true substrate for the venom phospholipase is a phospholipid/calcium complex.
(iv) The co-lytic factor (CLF; Table II) interacts reversibly with the cell membrane (Ka = $3.9 \times 10^{-9}$ M) to remove lyso-phospholipids from the membrane as products of phospholipid hydrolysis ($4 \times 10^4$ molecules per molecule of CLF) following the action of the venom phospholipase (Grotendorst and Hessinger, submitted).
(v) It is believed that removal of the products of phospholipid hydrolysis produces local destabilization of the membrane around each DLF/phospholipase complex and causes the altered locus of membrane to become permeable to hydrated ions.

Aiptasia venom is the only known direct lytic venom whose phospholipase is regulated; that is, requires an accessary protein (DLF) in order to hydrolyse phospholipids of intact membranes. Furthermore, other known venom phospholipases are notoriously stable, have broad pH optima for activity, and are fairly low molecular weight, basic proteins, whereas, the Aiptasia venom phospholipase, is labile, has a narrow pH optimum, and is an acidic protein with a molecular weight of 43-45 kd (Table II). Such functional and physical differences characterize the differences between the exocrine phospholipases of pancreatic and snake and insect venom origins, on the one hand, and the regulated phospholipases that regulate endogenous ecosinoid biosynthesis in many tissues and also occur in Aiptasia venom (Brockenhoff and Jensen, 1974).

b. Stoichiometric Cytolysins. The stoichiometric cytolysins interact with membranes and physically change membrane structure so as to alter membrane permeability. Such cytolysins may form size- and/or charge-specific channels, or less specific and larger membrane pores, or may disrupt membrane integrity by micellizing membrane components. Stoichiometric cytolysins characteristically exhibit (i) graded (i.e. dose-dependent) final extents of lysis and (ii) sigmoidal dose-response curves that exhibit threshold effects at low concentrations of lysin (Tamkun and Hessinger, 1981).

Stoichiometric cytolysins have been found in several nematocyst venoms, all of which seem to lack phospholipase

activity, including venom from Physalia (Burnett and Calton, 1974a; Tamkun and Hessinger, 1981), Chironex (Keen and Crone, 1969; Crone, 1976), and hydra (Weber et al., 1988). In contrast, very little is known about the hemolytic activity of Chrysaora venom other than it is reported to be "very weak" (Burnett and Calton, 1977) if not negligible (Kelman et al., 1984). It has been shown, however, that the depolarizing effect of Chrysaora venom on excitable tissues can be explained by the demonstrated ability to form monovalent cation channels in black lipid membranes (Shryock and Bianchi, 1983). Thus, the best studied of these stoichiometric cytolysins are from Physalia and Chironex, but only the hemolysin from Physalia has been purified and characterized (Tamkun and Hessinger, 1981; Table II).

Physalitoxin (PTX) is an heterotrimeric glycoprotein and hemolytic toxin that constitutes about 25% of the Man-of-War venom protein. It is labile to heat and dithiothreitol (DTT). The susceptibility of PTX to DTT is one reason why Klug et al. (1988) did not find hemolytic activity in Physalia nematocysts discharged in DTT (another being that Physalia fishing tentacles do not possess eurytele nematocysts; Cormier and Hessinger, 1981; Hessinger and Ford, 1988). PTX is able to lyse 50% of a washed rat red cell suspension at $10^{-11}$ M, making it one of the most potent hemolysins known. Several lines of evidence point to PTX interacting with red cell membrane integral proteins (Lin and Hessinger, 1979). While PTX will not directly interact with membrane phospholipids it does bind free fatty acids, lysophospholipids and various nonionic detergents at sub-critical micellar concentrations (unpublished) indicating that PTX is a lipophilic protein (Tanford, 1980). In terms of its affinity for small amphiphiles and its hydrodynamic shape PTX resembles CLF from Aiptasia venom (see previous section), yet CLF is not lytic by itself and PTX is not able to substitute for CLF in combination with Aiptasia DLF and phospholipase (Grotendorst and Hessinger, in preparation).

The hemolysin from "milked" Chironex venom and extracts of Chironex tentacles have molecular weights of about 70,000 (Crone and Keen, 1969). Hemolytic activity co-chromatographs with dermonecrotic and lethal activities (Keen and Crone, 1969b). As with PTX, lytic activity is labile above 30°C and below pH 6.0 (Keen and Crone, 1969a). The hemolysin is not surface-active and does not interact with monolayers of either phospholipid or cholesterol (Keen, 1972), but does interact with monolayers of gangliosides (Keen, 1973). It is sensitive to dithiothreitol and is neutralized by ganglioside and N-acetylneuraminic acid (Crone, 1976). Unfortunately, like much of the published work with Chironex these results have not been expressed in units of protein concentration,

thus it is not possible to estimate the hemolytic potency or lethality of the hemolysin.

2. The Neurotoxins

Toxins which preferentially act on excitable membranes may be referred to as neurotoxins. Since neurotoxins usually affect mechanisms associated with axonal conduction or synaptic transmission three classes of membrane-acting neurotoxins can be described: axonal; pre-synaptic; and post-synaptic. The axonal neurotoxins consist of two types: the channel blockers, as typified by tetrodotoxin and saxitoxin; and the gate inhibitors, as typified by sea anemone and scorpion neurotoxins. There are also two types of synaptic neurotoxins: pre-synaptic and post-synaptic. The pre-synaptic neurotoxins include two types: blockers of neuro-transmittor release (e.g. botulinum toxin) and stimulators of release (e.g. black widow spider venom neurotoxin). Post-synaptic neurotoxins generally block transmittor receptors as typified by the curarimimetic $\alpha$-bungarotoxin.

Several excitable tissue and organ preparations have been affected by nematocyst venoms from several species (section II. B.). In the absence of contrary data, all of these effects, with one exception, can be explained in terms of the known cytolytic or membrane permeability effects of these venoms. That one exception is a basic protein of low molecular weight (10-15,000) from Aiptasia venom. This neurotoxin is a gate inhibitor which causes violent motor activity in crabs and specifically prolongs the early, transient inward ("sodium") current on isolated crustacean ventral nerve cord (Hessinger et al., 1973). Qualitatively indistinguishable effects are produced by protein neurotoxins prepared from whole animal and tissue extracts of other sea anemones (Kem, 1988).

3. The Regulatory Toxins

Regulatory toxins either stimulate or inhibit vital metabolic processes. Classical examples of such toxins are cholera toxin, which stimulates adenyl cyclase activity via covalent alteration of transduction G-proteins in cells bearing the appropriate ganglioside surface receptors, and diphtheria toxin, which inhibites protein synthesis by covalently altering elongation factor tu.

At the present, the only reported candidate among nematocyst venoms for such an effect is the vasodilatory effect of Man-of-War venom on arterial musculature (Loredo et al., 1985). This effect appears to be due to the production

of a vasodilatory ecosinoid (Loredo et al., 1986) of endothelial origin (Gonzalez and Hessinger, in prepration) due to stimulation by the venom of an endogenous, regulatory phospholipase $A_2$ (Shier, 1980). This effect, however, has not yet been attributed to a purified venom component and, therefore, may be ascribable to a cytolytic permeabilizing effect.

## III. CONCLUDING REMARKS

Although some nematocyst venoms are among the most potent venoms known (especially Man-of-War and sea wasp venoms; Table I), proportionally few stingings of humans are fatal. This is probably because of the relatively small amounts of venom that are injected. At the present there is no known specific antidote for nematocyst stings and symptomatic treatment is all that is available in most cases. A commercial antivenin is available for life-threatening sea wasp stings.

All known nematocyst toxins are proteins that act on the plasma membranes of target cells. Common to all nematocyst venoms are cytolytic/hemolytic effects caused by either of two types of cytolysins: catalytic or stoichiometric. Hydrozoan and scyphozoan venoms possess single-component stoichiometric cytolysins. This type of cytolysin is typified by physalitoxin (PTX) from the Man-of-War venom. PTX is a high molecular weight heterotrimeric glycoprotein that is an extremely potent hemolysin. It is the only known toxic component in Man-of-War venom although evidence suggests that other venom components synergistically act with PTX to exert the full lethal potency of the venom.

Anthozoan venoms, on the other hand, possess multi-component catalytic cytolysins. The only anthozoan venom to be extensively studied is from the acontia of the sea anemone, A. pallida. The hemolytic system from this anemone consists of three synergistically interacting proteins including an unusual regulated phospholipase $A_2$. It is important to realize that although stoichiometric cytolysins have been purified from whole tissue extracts of sea anemones (Kem, 1988), these cytolysins have never been found in association with or localized in sea anemone nematocysts and, therefore, may represent proteins of extra-nematocyst origin.

In addition, anthozoans venoms are the only nematocyst venoms known to possess true neurotoxins. These neurotoxins primarily interfere with the normal inactivation of "sodium" currents in excitable membranes. Hydrozoan and scyphozoan

venoms do not possess this type or any other known type of true neurotoxin. Although the hydro-scyphozoan venoms have mainly depolarizing effects on excitable tissues these effects can at the present be explained in terms of the known cytolyic and membrane permeabilizing components of these venoms. Thus, it appears that hydro-scyphozoan venoms more generally resemble each other in that they possess potent stoichiometric cytolysins but no neurotoxin, whereas anthozoan venoms possess both multi-component catalytic cytolysins plus neurotoxins.

It is noteworthy that microbasic mastigophore nematocysts both from an anthozoan, the sea anemone (A. pallida), and from a scyphozoan, the sea wasp (C. fleckeri), possess very different kinds of venom. Thus, a single morphological type of nematocyst can contain very different types of venoms. The considerable differences in the constituent toxins of these two mastigophore venoms probably reflect the intended use of these nematocysts against dissimilar prey. The preferred prey for the sea anemone are small crustaceans, against which the anemone neurotoxins are especially potent. The preferred prey for the sea wasp, however, are fish in which the cardiovascular, myotoxic and hemolytic effects of the venom would be especially effective in disabling.

The complete biochemical and functional characterization of venom components from individual types of nematocysts is within the capablities of current technologies. This has not yet been achieved for any specific nematocyst venom. Nonetheless, the picture that emerges from the information that is currently available suggests that venom components synergistically interact in functionally specific ways. In addition to having functional significance during toxin attack on target cells following nematocyst discharge, such molecular interactions are also likely to be important before nematocyst discharge occurs. During development and maturation of the nematocyst specific molecular interactions likely guide the labile toxic components of the venom to proper intra-nematocyst compartments for future deployment while also maintaining them in stable configurations.

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