THE TOXIN OF *PHYSALIA* NEMATOCYSTS*

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It is basic to any discussion of the pharmacology and biochemistry of the nematocyst toxin of *Physalia* to review the structure of the animal and of the organoids within which this toxin is contained. The function of these structures in the normal life of the parent organism should also be considered.

Dependent from the pneumatophore of *Physalia* is an assemblage of polyps of different kinds. Outstanding among these are the dactylozooids, or fishing tentacles. In an adult *Physalia* the fishing tentacles may extend 100 feet in length. Coaxial with the dactylozooid and running along its entire length is a band of specialized tissue that covers diverticulae of the gastrovascular cavity of the tentacle. When the tentacle contracts it shortens more completely than this superficial structure, which causes the latter to be thrown into loops and folds referred to as "batteries." The nematocysts are contained in cnidoblasts located in the superficial epithelium of the battery. The toxin to be described in this paper is contained as a structureless fluid within the nematocyst capsule. In life it fills the globular cavity and bathes the surface of the nematocyst tubule.

There are two general sizes of nematocysts: the smaller is about 9 μ in diameter, and the larger is approximately 3 times as large. There may be functional differences between these classes of nematocysts, but our studies have not revealed them. Nematocysts are distributed throughout the thickness of the battery epithelium: the larger ones occupying the entire thickness of the epithelium and the smaller ones being symmetrically disposed between the larger ones both horizontally and vertically.

The mature nematocyst nearly fills the cnidoblast within which it is produced, pushing the now-pyknotic nucleus to one side of the almost structureless cytoplasm. The globular capsule wall appears to be invaginated at one point. The highly coiled internal tubule retains its connection with the capsule wall at this point. What would have been an open connection between the lumen of the internal tubule and the cytoplasm of the cnidoblast is closed by the operculum. A cnidocil or trigger protrudes a few microns into the ambient water.

In nature the fishing tentacles are in continuous restless motion, alternately relaxing and contracting, constantly sampling the water beneath the pneumatophore. When the tentacle brushes against a prey organism, whether it is planktonic crustacean or larger fish, the cnidocil of superficial nematocysts are stimulated and they trigger the immediate release of the coiled nematocyst thread. When fully uncoiled, this may be several hundred times as long as the diameter of the parent capsule. The tubule is armed thoughout its length with chitinous barbs and spines and constitutes an extremely effective "tangle." If the tip of the nematocyst thread should penetrate the prey organism, this hol-

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low threadlike tube conducts the nematocyst toxin directly into the body of the prey. In our laboratory it has been observed frequently that the nematocyst thread can penetrate even a surgical glove. It should be emphasized that the magnitude of the response to contact with the prey organism is proportional to the area of contact between tentacle and prey. It is conceivable that a single copepod might elicit the discharge of 20 to 50 adjacent nematocysts, while contact between the tentacle and the side of a fish would discharge hundreds of

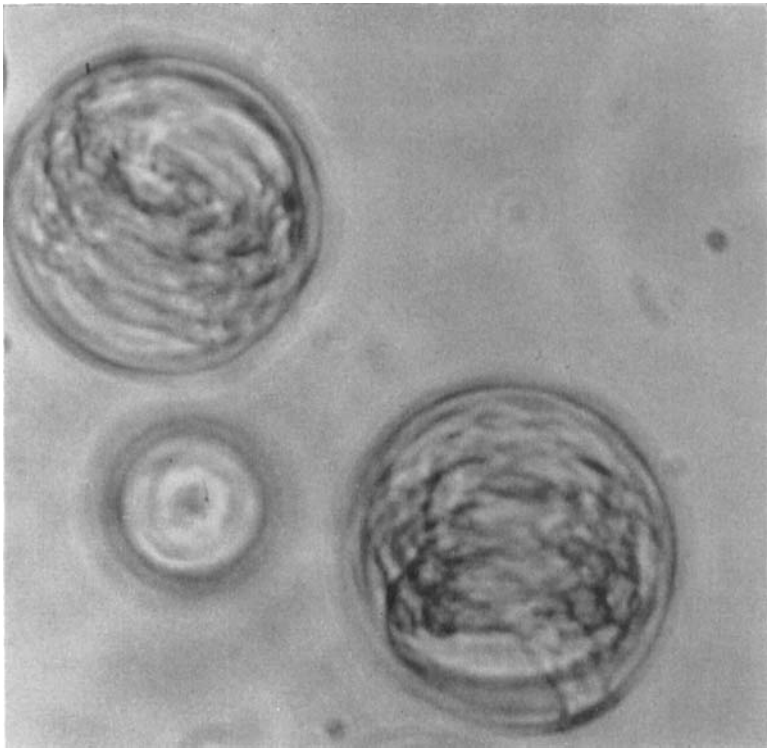

FIGURE 1. Undischarged nematocysts separated from tentacular tissue. The coiled internal tubule and the operculum of the larger nematocysts are noteworthy. Examples of both size groups of nematocysts are shown. ×1600.

thousands of nematocysts. Initial gentle stimulation of the nematocyst probably evokes rapid release of the nematocyst thread but does not dislodge the parent capsule from its position within the epithelium. Vigorous resistance of the unsubdued prey greatly increases the numbers of nematocyst involved in the reaction and dislodges many from the epithelium. These are replaced by cnidoblasts that differentiate outside the battery and, secondarily, come to occupy a definitive position in the battery epithelium.

The mechanism by which the internal capsular thread uncoils and propels itself into the ambient water is still obscure. Indeed, even the nature of the adequate stimulus for discharge is uncertain. By whatever means the process

is initiated its course is fairly clear: there is a rapid eversion of the tubule that, in its essence, resembles the eversion of the finger of a glove. The process is fairly rapid but could hardly be accurately described as "projectile" since it requires several seconds to reach completion *in vitro*. There is a suggestion, from field observation and experience, that this reaction may be more rapid *in vivo*.

The tissues of the tentacle contain biologically active materials in addition

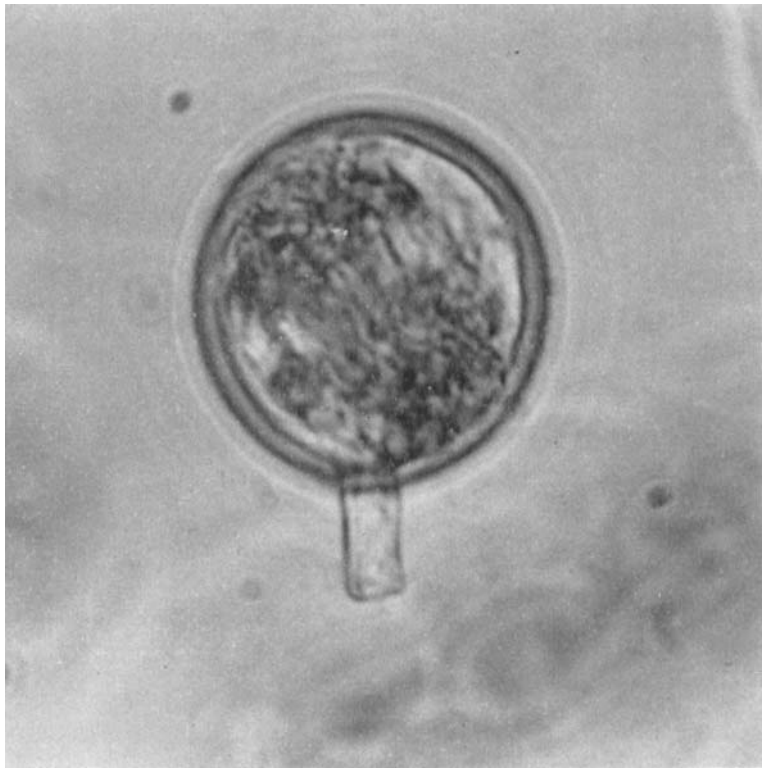

FIGURE 2. The beginning of discharge of isolated nematocyst. The hollow tubule is clearly visible. ×1600.

to the toxin that will be described here. Thalassin and congestin (Richet, 1903) almost certainly originate from general tentacular tissues outside the nematocyst. It appears unlikely that brief grinding of the whole tentacle with sand would produce a significant breakdown of nematocysts. The "*Cyanea* principle," effective in causing release of histamine from mast cells, described by Uvnäs elsewhere in this monograph, is apparently also of tentacular origin. In this connection it may be of interest that samples of *Physalia* toxin from this laboratory have been inactive in eliciting histamine release from mast cells in Sweden. To clarify the pharmacology of the nematocyst and to insure that the toxin to be described could have originated only from the nematocysts, the

following method was employed to separate still-reactive nematocysts from other tentacular materials (Lane and Dodge, 1958).

The tentacles are removed from living, freshly stranded *Physalia* in the field. They are freed of adherent debris and seawater by decantation. The tentacle tissue is returned to the laboratory and autolyzed for 24 hours at refrigerator

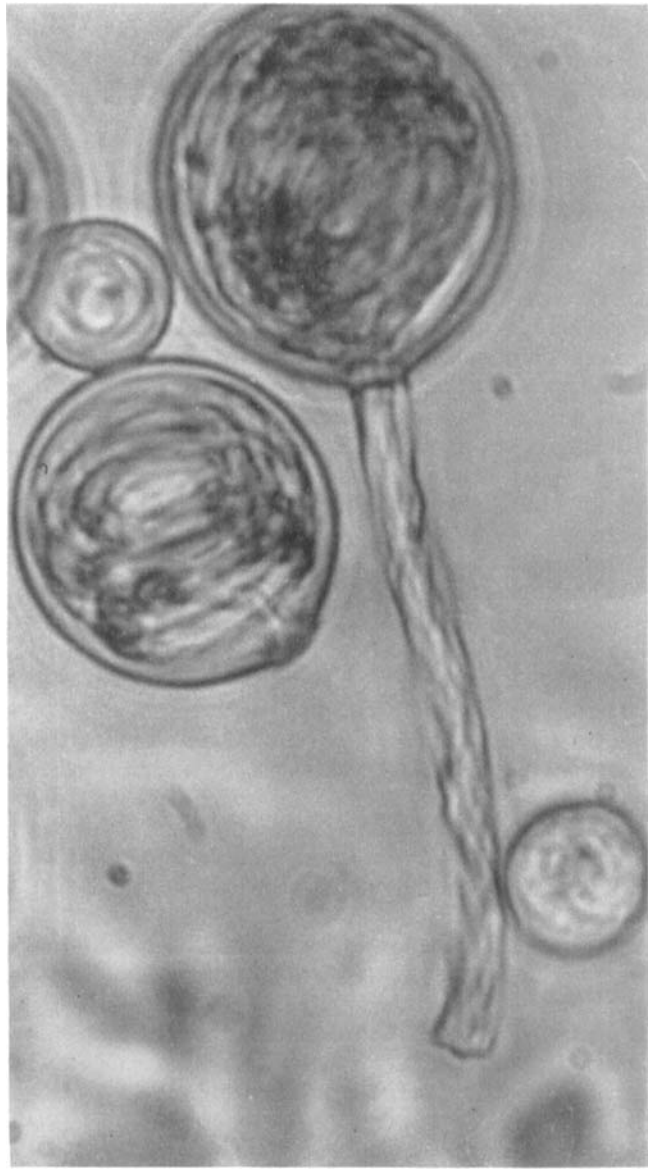

FIGURE 3. Later stage in the discharge of isolated nematocyst. The connection between the internal tubule and operculum appears in the undischarged large nematocyst in this figure. ×1600.

temperatures. After this period has elapsed, the tentacle material is forced through graded sieves of up to 40 mesh/inch to hasten the breakdown of the tissues and to accelerate the liberation of the nematocysts. The sieved brei is suspended in clean sea water and returned to the refrigerator. Autolysis proceeds rapidly and, within 36 hours, the nematocysts begin to separate from other components in the container. They do not tolerate centrifugation and are allowed to settle out by gravity. Contaminating autolyzed tissue components and other soluble materials extraneous to the nematocysts are decanted from the nematocyst layer, settled nematocysts are resuspended in additional clean sea water, and are again permitted to settle. This process of alternate suspension and settling is continued until the wash water, which initially contains considerable biological activity, is no longer toxic to *Uca* when injected into the hemocele. The washed nematocyst concentrate assumes the appearance and the consistency of freshly ground putty. Each gallon of tentaculur tissue yields 60 to 75 gm. of pure nematocysts. Representative preparations have averaged about 55 million nematocysts/gm. The washed and concentrated nematocysts may be frozen at −20° C. and stored in this condition for up to 3 years with no significant loss of reactivity to human skin.

The toxin is prepared by homogenizing the concentrated purified nematocysts in an all-glass, motor-driven homogenizer. Other preparative methods have been only partially successful in breaking down the wall of the capsule. It is necessary that the nematocyst capsule actually be broken to release the liquid contents. Mere discharge of the contained tubule does not liberate all the toxin into the ambient medium. The homogenate is freed of particulate debris by centrifugation at 12,000 g at 4° C. for 20 min. Alternatively, the particulate fragments might be adsorbed on calcium phosphate gel (Wangersky and Lane, 1960). After either treatment there remains a water-clear toxin solution, which is then lyophilized. The dried toxin is stored in evacuated capsules in the dark at −20° C. Under these conditions of storage, activity is undiminished druing 3 years. The dried toxin contains 15 to 16 per cent total nitrogen by micro-Kjeldahl Assay on different preparations.

When lyophilized toxin is reconstituted with physiological saline and injected into adult male Swiss mice of about 30-gm. weight, the certainly lethal dose level for the intraperitoneal route is 2.0 to 2.5 mg./kg.

After intraperitoneal injection the onset of intoxication is immediate. Pain, increased activity, and tremors appear at once. It is thought that these symptoms are produced by local irritation rather than by direct central nervous system stimulation. After 10 min., ataxia, decreased muscle tone, flaccid paralysis of the hind limbs, and depressed rate of breathing are characteristic of injected animals. Then follows urination, defecation, aphrodisia, marked myosis, dyspnea, cyanosis, anoxic convulsions, and death due to respiratory failure. Survival time is 1 to 48 hours, depending on the dose administered. Postmortem examination shows the following gross pathology: lungs blanched; heart contracted, especially the left ventricle; hemorrhagic edema into the peritoneal cavity; and skin, nose, and ears very white.

A dose level of 16.2 μg. of dried toxin dissolved in 0.5 ml. of frog Ringer's solution was uniformly lethal when injected into the ventral lymph sac of each

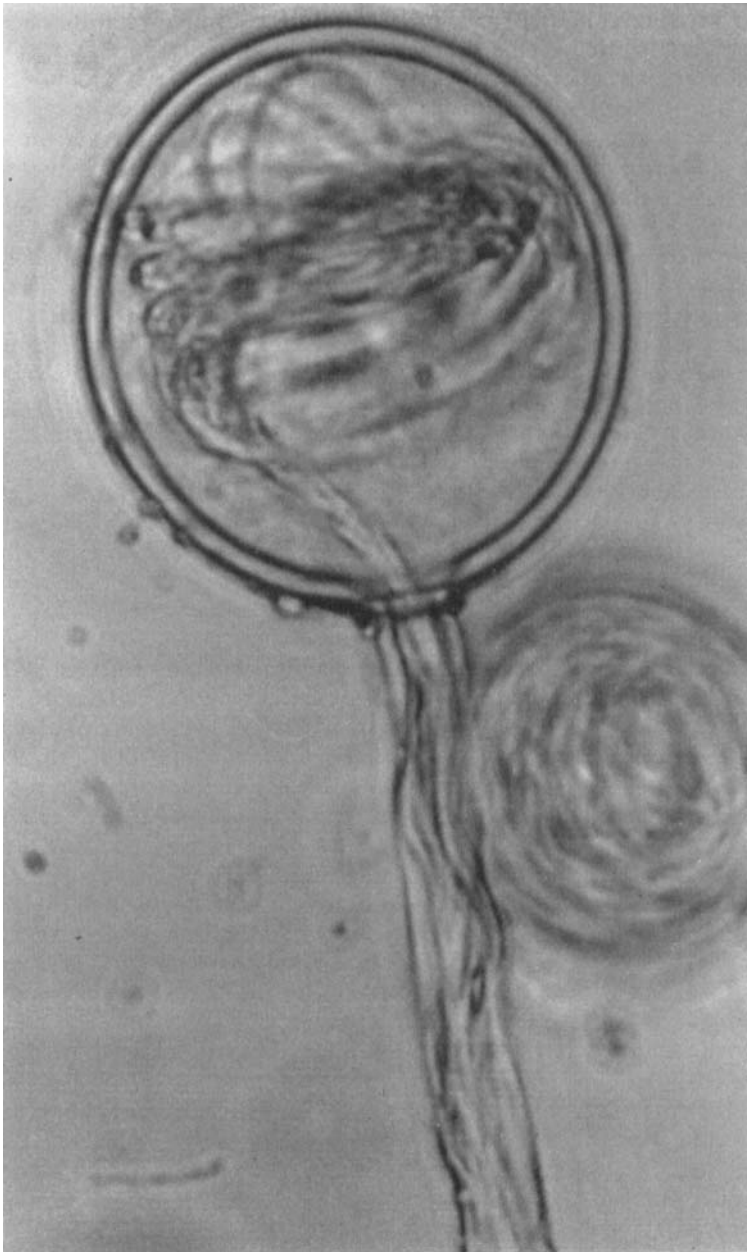

FIGURE 4. A discharging isolated nematocyst. The coiled internal tubule and the hollow nature and external architecture of the everted tubule are shown. ×2800.

of eight frogs (*R. pipiens*). There was an almost immediate appearance of localized dermal petechiae, most clearly visible on the white ventral surface. Breathing became rapid and shallow. Central nervous involvement was signalized by deterioration of the postural and righting reflexes. When spinal reflexes disappeared, electrical stimulation of the sciatic nerve produced no response, but direct stimulation of the gastrocnemius showed it to be still normally reactive. Heartbeat persisted for 12 to 24 hours. Large amounts of lymph accumulated in the subcutaneous sinuses. Viscera were hyperemic, bladder and intestine were empty, and considerable bloody peritoneal fluid was observed.

Intramuscular injection of adequate doses of crude toxin into fish is followed by immediate hyperventilation, disorientation, and spasmodic bursts of rapid swimming, alternated with prolonged periods of inactivity and death within one to four hours. Motor paralysis appears on the injected side. Sclerocorneal petechiae were frequently observed. At the site of the injection there was contraction of chromatophores, which caused a localized blanching. The general body surface away from the point of injection appeared to darken.

There was no hemolysis of erythrocytes of the mullet *Mugil cephalus* when they were incubated at 37° C. with several dilutions of crude toxin.

The toxin produced irreversible damage in the isolated heart of the clam *Mercenaria campechiensis*, which prevented duplication of response by subsequent treatment of the same preparation with similar dose levels. The initial response was reminiscent of that produced by the administration of acetylcholine, that is, diastolic arrest.

Various levels of crude toxin, when tested auxanographically with ten species of marine yeasts and seven species of marine bacteria, have been without effect. The toxin appears not to influence either survival or growth and reproduction of *Paramecium caudatus* or of *Tetrahymena pyriformis* (unpublished observations made by Bradner W. Coursen in this laboratory).

A copious precipitate is produced by mixing crude *Physalia* toxin with the plasma of the loggerhead turtle *Caretta caretta*. It was thought (Wangersky and Lane 1960) that this precipitin reaction might be invoked to explain the observed ability of the loggerhead turtle to ingest large numbers of *Physalia* without apparent damage. However, when the precipitate was removed by centrifugation and the remaining combined toxin-plasma solution was assayed on mice, it was found that the original toxicity was in no way diminished. It therefore appears that this precipitate of plasma and toxin does not explain the apparent insensitivity of the loggerhead to the toxin of the *Physalia* on which it often feeds.

Lyophilized toxin is stable for at least 3 years in evacuated capsules held at −20° C. in the dark. The toxin is thermolabile in solution, its activity being completely destroyed during five minutes at 60° C. Reaction with ethanol, acetone, diethyl ether, petroleum ether, and most other organic solvents destroys the activity of crude toxin preparations.

The crude toxin is nondialyzable and strongly biuret-positive. Benedict's, Molisch's, and anthrone tests are all negative, which suggests little or no polysaccharide present. One-dimensional descending paper chromatography with

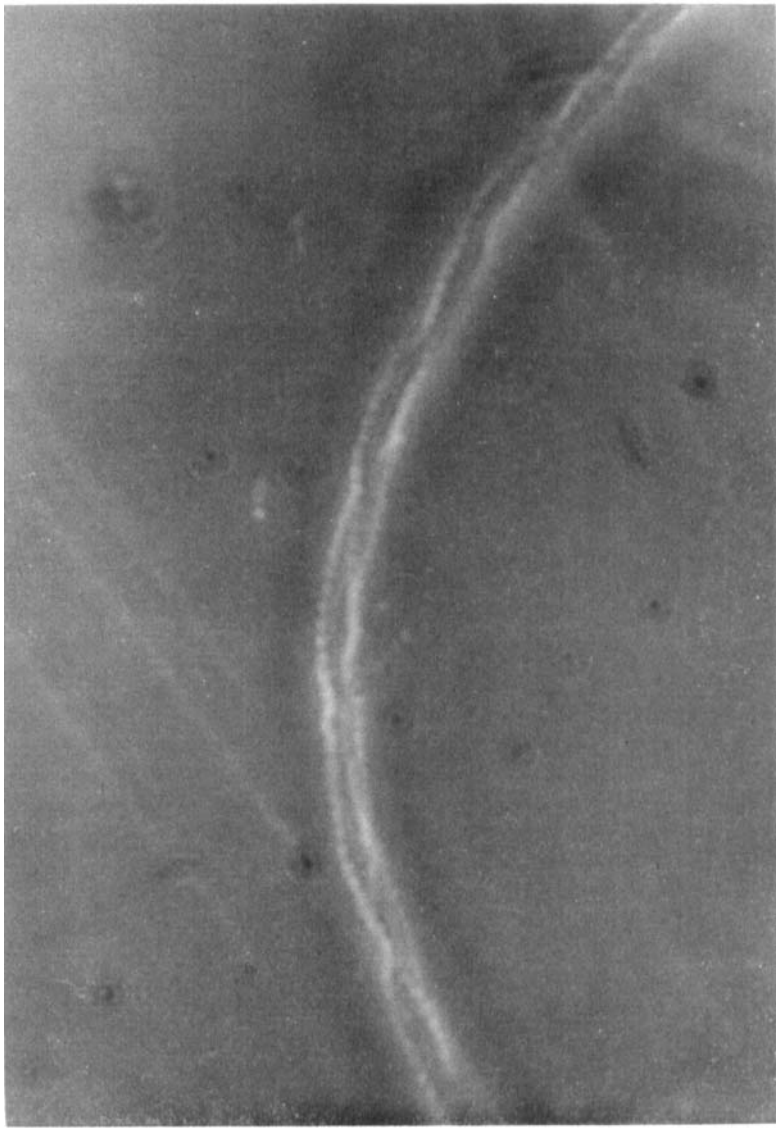

FIGURE 5. Tubule of discharged nematocyst photographed with dark field illumination. The helical bands of chitinous spines appear as rows of white dots. ×2800.

80 per cent *n*-propanol as the solvent system has clearly separated the crude lyophilized toxin into nine components. Subsequent elution, acid hydrolysis, and rechromatography of each of these zones has shown them to be peptides, of qualitatively and quantitatively different amino acid composition. Further study of the biological activity of these chromatographic fractions is one of the present objectives of this laboratory.

References

LANE, C. E. & E. DODGE. 1958. The toxicity of *Physalia* nematocysts. Biol. Bull. **115:** 219.

RICHET, C. 1903. Des poisons contenues dans les tentacules des actinies, congestine et thalassine. Compt. rend. soc. biol. **55:** 246.

WANGERSKY, E. D. & C. E. LANE. 1960. Interaction between the plasma of the loggerhead turtle and toxin of the Portugese man-of-war. Nature. **185:** 330.