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## THE AMAZING NEMATOCYST

Jacque Rifkin

### Summary

Granular electron-dense material is contained both within the tubule and the capsule. The matrices contained within each compartment are different chemically from one another. During discharge, the cnidocil apparatus on the nematocyte is triggered. Polymerisation of the capsular matrix occurs, water rushes into the capsule and discharge of the tubule takes place. As the tubule everts, granular matrix contained within it emerges progressively as discharge occurs. As tubules transfix capillaries in the dermis, tubular matrix (venom) passes into them. The capsular matrix emerges once the entire tubule everts. Venom obtained by disruption of nematocysts of *Chironex fleckeri* was injected into mice by the intravenous, intraperitoneal and subcutaneous routes. Mice survived injections delivered by the intraperitoneal and subcutaneous routes. This suggests that only material delivered by the intravenous route is responsible for the rapid systemic effects manifested after a serious sting.

The implications of this mode of envenomation for the first aid treatment of *C fleckeri* are discussed.

**Cnidarians**

The cnidarians are a very large group of animals comprising at least 7,000 species with a variety of forms and a diversity of habits. The group name means nettle because each species is armed with millions of minute stinging capsules or nematocysts. Some examples of cnidarians include all corals, sea anemones, sea whips, sea fans, stinging hydroids, the bluebottle and the box jellyfish.

The cnidarian which is of greatest importance to humans in the Indo-Pacific region is the box jellyfish or *Chironex fleckeri*. This animal has been responsible for at least 70 recorded fatalities in the past 100 years.

**Nematocysts**

A nematocyst consists of a capsule containing a tightly coiled and pleated inverted tubule. This tubule may or may not bear spines. The microbasal mastigophore from the box jellyfish has a cigar-shaped capsule which is slightly wider at the apical end. The noun mastigophore refers to those nematocysts in which the distal tubule continues beyond the shaft. The adjective microbasal refers to those nematocysts bearing a short tubule which is less than three times the capsule length. In *Chironex fleckeri* mastigophores, the basal shaft region bears long spines and

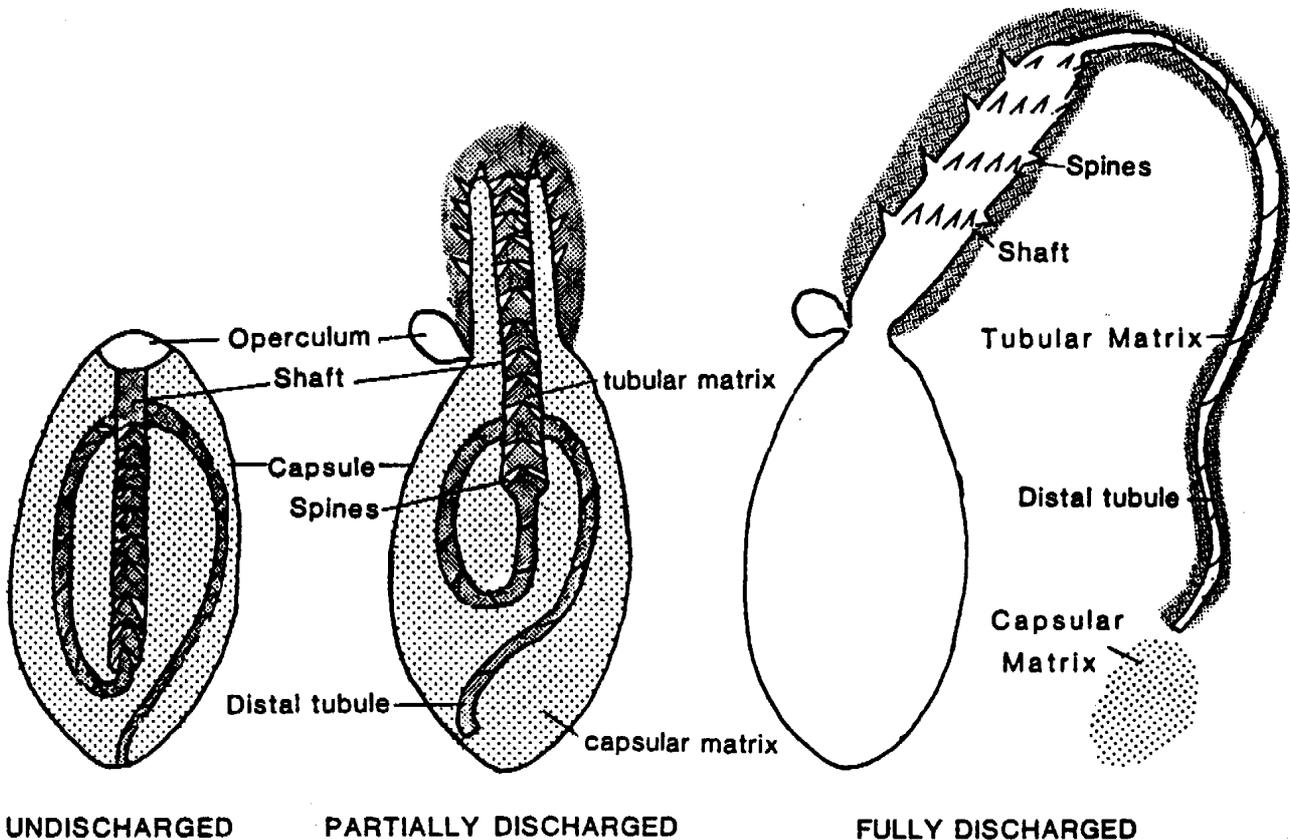
the distal tubule region bears shorter spines. Edean and Rifkin<sup>1</sup> determined that these nematocysts were important in mammalian envenomations.

Nematocysts are stimulated to discharge when mechanical and chemical stimuli such as those delivered by prey species are applied to the triggering apparatus or cnidocil. The cnidocil is the putative sensory receptor, or "hair trigger", found at the apical end of nematocytes from most cnidarian classes. Each consists of a flagellum surrounded by two series of microvilli.

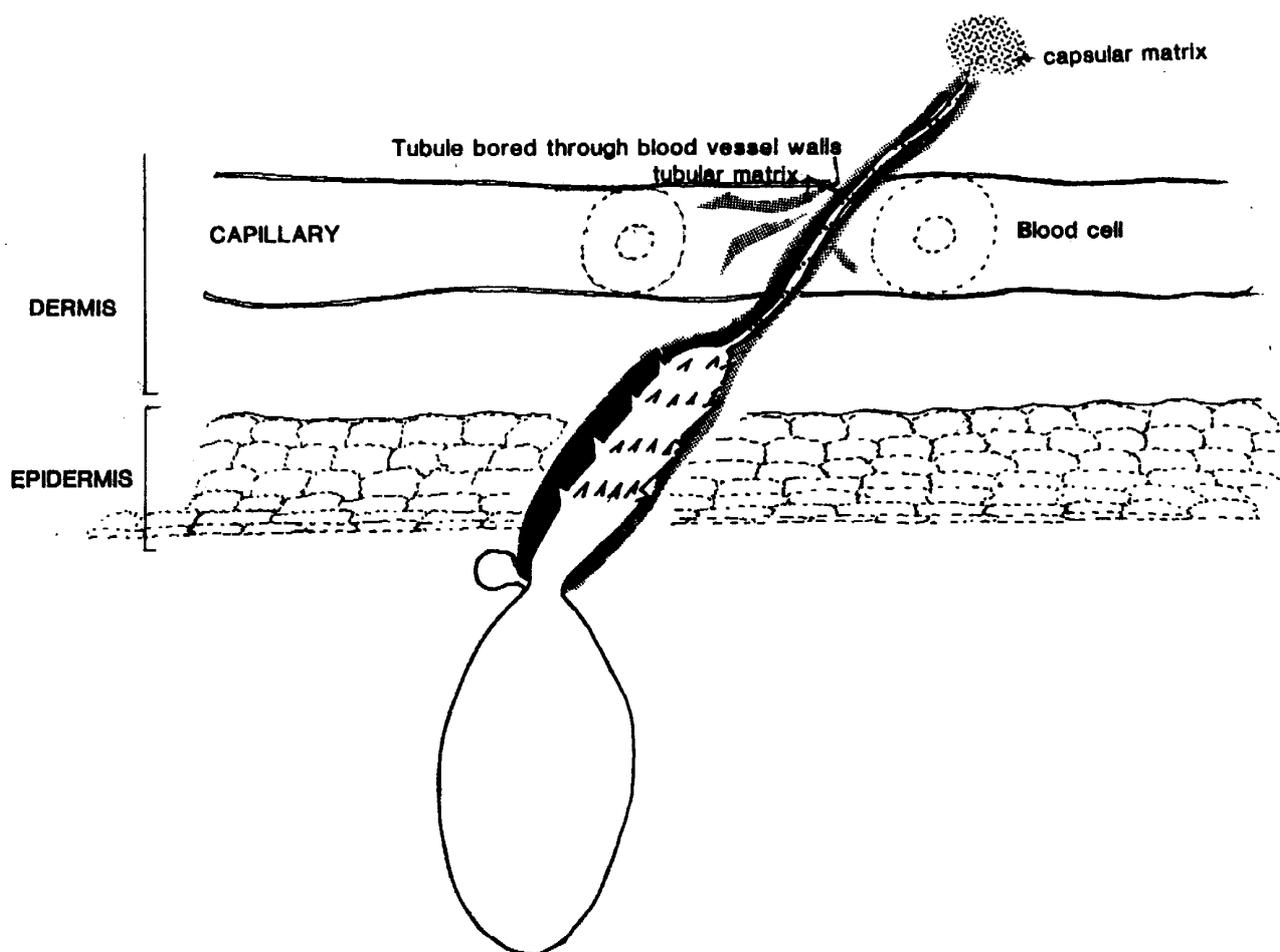
Once stimulation of the cnidocil is effected, the operculum, which is located at the apical end of the capsule, (Figure 1) is tripped, then the tubule begins to evert. Spines, which are found on the inside of the nematocyst tubule (Figure 1) emerge in a rotary fashion that assists the tubule to penetrate flesh.

Material which is present on the inside of the capsule passes out of the end of the fully discharged tubule. That material which was present within the undischarged tubule is released gradually as the tubule everts (Figure 1).

Toxic material from within disrupted nematocyst capsules was injected intravenously, intraperitoneally and subcutaneously into mice. Those mice that were injected subcutaneously and intraperitoneally with the contents of



**Figure 1.** Stages in the discharge of nematocyst tubules. Note the manner in which capsular and tubular matrices are released.



**Figure 2.** Diagram showing the fate of the capsular and tubular matrices in a nematocyst discharged into mouse skin.

up to 512,000 nematocysts survived. Those mice that were injected intravenously with the contents of more than 25,000 nematocysts died within 2 minutes of the injection. This suggests that during envenomation of mammals, only toxic material that is introduced directly into blood vessels is responsible for fatalities, at least in the short term.<sup>2</sup>

Thin sections of undischarged nematocysts show that there is granular electron-dense material present within undischarged tubules. Granular electron-dense material is also present within the capsule.<sup>2,3</sup> Scanning electron micrographs show granular tubular matrix scattered over the surface of discharged nematocysts and between the spines.

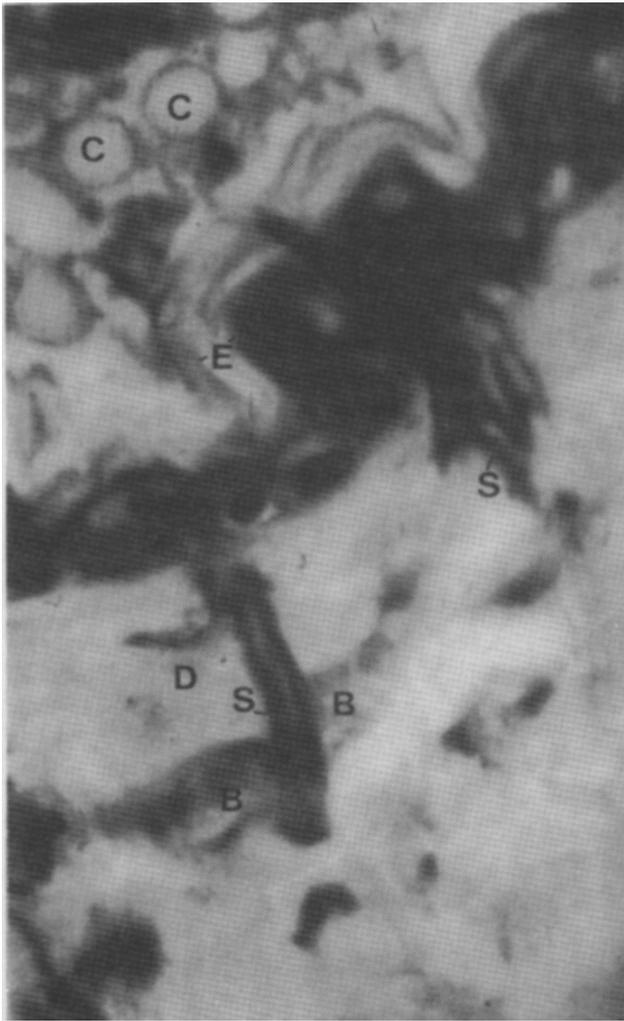
Histochemical tests and histological dyes clearly show that the material within the tubule is of a different nature to that found within the capsule.<sup>2</sup> The material within the tubules of the microbasic mastigophore nematocysts stains with basophilic dyes while that found within the capsule stains with acidophilic dyes.

Histochemical tests including the periodic acid-Schiff test for polysaccharides, the mercuric bromophenol

blue reaction for proteins and the toluidine blue for metachromatic substances show that the capsule contains protein and polysaccharide material while the intratubular material contains acid polysaccharide material.<sup>2</sup>

The skin of hairless mice stung by tentacles of *Chironex fleckeri* were sectioned, stained and examined. The numerous capsules of discharged mastigophores were found on the surface of the skin (Figures 2,3). The tubules from discharged nematocysts could be traced as deeply as 0.5 mm into the dermis. The layers of cornified regions of the skin in the region of penetrating nematocysts were also separated from one another. In the Malpighian layer cells were shrunken and the nuclei were pyknotic. Many tubules did not pursue a straight course through the skin and many were seen to transfix blood vessels (Figures 2,3).

Klug et al<sup>4</sup> showed that the tubular matrix contained venom. They discharged isolated nematocysts from a variety of cnidarians into a film of blood cells. Red blood cells that were in contact with discharged tubular matrix lysed, whereas those red blood cells in the vicinity of the capsular matrix did not.



**Figure 3.** Photomicrograph showing nematocysts discharged into mouse skin x 1,000 C=capsule, D= dermis, E=epidermis, B=blood vessel, S=shaft.

The results of the studies by Edean and Rifkin<sup>2</sup> and Klug et al<sup>4</sup> suggest that the material within the tubules is different from the material within the capsule and that the material found within nematocyst tubules of many species is toxic. The histological studies done by Edean and Rifkin<sup>2</sup> which showed tubules passing through blood vessels suggest that it is by this route that the venom reaches the heart, where the rapid cardiotoxic effects are manifested.

### Mechanism of discharge

The mechanism of nematocyst discharge is not understood although various hypotheses have been proposed:

- 1 Intracapsular pressure increases by uptake of water or ions. This occurs at the moment of discharge or just before it. Uptake of water could occur either because of an altered permeability of the capsule wall or by entry of water into the capsule wall or by entry of water into the

capsule when the operculum has been dislodged. The tubule is then forced out by eversion.<sup>5,6</sup>

- 2 Energy is produced at the moment of discharge by enzymatic reactions in the cytoplasm of the cell surrounding the nematocyst.<sup>7,8</sup>
- 3 Contractile material in the capsule wall or contractile elements surrounding the capsule are activated and thus increase intracapsular pressure.<sup>9,10,11</sup>
- 4 Energy for eversion of the tubule is contained within the highly coiled and pleated tubule within the nematocyst. Once the operculum is tripped, eversion would occur.<sup>12</sup>

The latest research on the mechanism of nematocyst discharge was proposed by Edean et al.<sup>13</sup> This theory proposed that once the cnidocil apparatus is triggered by mechanical and chemical stimuli, the operculum is tripped. This applies tension to the contractile filaments surrounding the nematocyte.

The capsular matrix, which normally contains material that is not polymerised becomes polymerised and forms clusters of regularly spaced, electron-lucent granules arranged in hexagonal patterns. Immediately after polymerisation, water rushes into the capsule, increasing the intracapsular pressure. This pressure is maintained throughout the entire eversion process.

The time required for *C fleckeri* nematocysts to discharge into mammalian skin is not known, although the speed of discharge of nematocysts of *Hydra attenuata* was measured at 3 milliseconds.<sup>14</sup>

### Effects of discharge

Nematocysts that had been pulled out of the tentacle during a feeding episode or during an envenomation would normally be replaced by the migration of new nematocysts to the area.

Granular electron-dense material is present within the tubule as well as in the capsule of the mastigophore. The tubular matrix passes out of the tubule progressively as eversion takes place. Passage of tubular matrix through the skin may account for full thickness skin necrosis that has been reported after envenomation from the box jellyfish.

Some of the tubular matrix passes into blood vessels, although the bulk of material from within the tubule appears to enter the extravascular spaces. The discharged capsular matrix, emerging at the tip of the fully everted tube, would normally be deposited extravascularly as the chances of the tip of the fully everted tubule being in the lumen of a blood vessel are remote.

Two ways in which toxic material may enter the

blood stream are:

- 1 Directly intravascularly or
  - 2 Indirectly from the tissues into the lymph vessels.
- Toxic material entering the blood stream directly would reach the heart more rapidly than that entering the blood stream by an indirect route.

Sections through mouse skin reveal that adhering tentacles contained many undischarged nematocysts. In human envenomation, it is necessary to inactivate any remaining nematocysts which had the potential to discharge. Dilute acetic acid (vinegar) has been shown to inactivate *C fleckeri* nematocysts. After the application of vinegar, to inactivate nematocysts with the potential to discharge, immediate application of a pressure immobilization bandage to retard passage of injected venom from the tissues into lymph vessels is recommended. This bandage should be left in place until the sufferer is under medical care, in a hospital equipped with *C fleckeri* antivenom, and all preparations have been made to cope with collapse of the patient.

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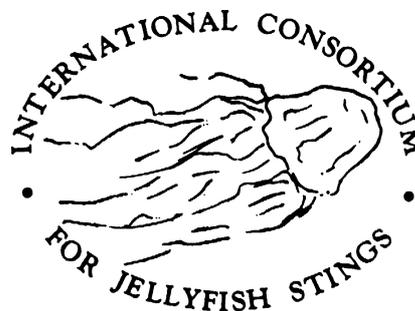
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#### THE WORK OF THE INTERNATIONAL CONSORTIUM FOR JELLYFISH STINGS

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#### Introduction

The "International consortium for Jellyfish Stings"<sup>1</sup> arose from the earlier collaborative work of a small group of clinicians, marine biologists, "in-the-field" workers and toxinology researchers scattered around North Queensland, Australia and in the U.S.A. It was conceived in particular by Professor Joseph Burnett, whose laboratory in Baltimore, Maryland, is at the forefront of jellyfish venom toxinology.<sup>2,3,4</sup> Its prime function is to create a focus for international communication between interested workers in the subject of human jellyfish envenomation. The Consortium was formed, with its letterhead symbol, in 1987.



#### Current participants

Medical, biological and marine scientists, scuba diving instructors and distinguished underwater photographers all feature in the current international mailing list. Some of these people are making original and pioneering observa-