1. VENOMOUS COELENTERATES

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An important aspect of the position held by COELENTERATES as poisonous animals should be emphasized immediately. All COELENTERATES, including parasitic species, produce organelles with an elaborate structure filled with a toxic substance. It is precisely this fact that makes them specially interesting as poisonous animals since their tissues are not poisonous in themselves when ingested by other animal species including man, there being a single doubtful exception (*Physalia physalis*). They are venomous in the sense that, as a phylogenetic unit they have developed an unique and very specialized system by which the venoms they produce are injected into the tissues of the prey or any offensive body that comes into contact with the coelenterate's tissues, noticeably the epidermis of the tentacles, mouth rim and of the acontia of anemones. This very specialized system is represented by the organelles mentioned above which have a complex genesis and structure. They are typical of the COELENTERATES (*CNIDARIA*) and they are called nematocysts, cnidocysts or nettle cells.

They are ovoid or elongated capsules, filled with toxin. Before being discharged they contain the butt and the tube usually coiled spirally around the butt. Upon discharge, the hollow thread and butt are evaginated inside out and function as a hypodermic syringe, emptying the contents of the capsule into the foreign tissues which have been traversed by the tube upon explosion. Discharge is abrupt, usually explosive.

Nematocysts are usually contained in small groups of a few in an epidermal cell provided with a cnidocil that plays a part in the discharge of the nematocysts themselves. The nematocyst carrying epidermal cell is frequently erroneously called a cnidoblast, but it does not usually produce the nettle cells, it just houses them after they have been completely formed by the deeper laying nematoblasts which derive directly from interstitial undifferentiated cells. Discharge may be caused by either mechanical or chemical stimuli or both. Neither agent can be said to be the universal discharge-causing factor. Nematocysts are independent effectors (1) and their discharge is not controlled by the nervous system nor is the stimulus for discharge transmitted by the nervous net. Structure and ultrastructure of the nematocysts and of the cnidocil (2) have been described. Nematocysts are very important systematic and phylogenetic characters to be taken into account (3). The degree of intraspecific variation was shown by Ito (4) and Vannucci (5) for a hydroid and a medusa respectively, their geographic variation by Ito (6) and their importance to differentiate hydroids superficially identical belonging to different species of different families was shown by Brinckmann & Petersen (7).

Actually COELENTERATES are not the first animals in the animal kingdom who have evolved organelles specialized in defense, offense and capture of prey. The trichocysts of ciliates have certain similarities to nematocysts and the polar capsules of **CNIDOSPORIDIA** are extraordinarily similar to them. **CNIDOSPORIDIA** however, on the grounds of their morphology, life cycles and other affinities must be placed among the **SPOROZOA**. The polar capsules also have a spirally coiled thread which is evaginated upon discharge and is used to attach the spore to the inner surface of the host's gut. As far as I know, the polar capsule of the **CNIDOSPORIDIA** have no toxic action. This leaves us with COELENTERATES as the only nematocyst producers in the animal kingdom.

Summing up, the toxic effect of COELENTERATES is due to their specialized organelles which inject toxic substances in other animals, when they come in contact with them. Any animal producing nematocysts is a COELENTERATE and inversely, all COELENTERATES have nematocysts As far as I know there is a single exception to this rule which on the other hand is still open to discussion: this is the ctenophore *Euchlora rubra* which carries nematocysts apparently produced by the animal itself and are not introduced as food (8). A small diversion is pertinent at this stage. I have emphasized the fact that COELENTERATES are animals that *produce* their own nematocysts, because there actually are some gastropod molluscs, the NUDIBRANCHATA or sea-slugs that feed on hydroids or siphonophores and are unable to digest undischarged nematocysts, they rather store them away in chambers at the tip of papillae the lumen of which is in continuity with the digestive tract of the slug. The mechanism by which the nematocysts are not digested, the migration from the lumen of the gut along the diverticula and finally into the chamber, which is provided with an external pore, is yet unexplained.. It is however true that the nematocysts, stored in the apical chamber of the papilla are still functional and they are used for defense by the slugs that erect the papillae when pursued and use the nematocysts effectively. They have been aptly called CLEPTOCNIDAE since they are stolen cnidocysts.

As mentioned earlier, I ignore instances in which the tissues of COELENTERATES have been found to be poisonous when ingested. There are some large species of jelly fishes which are normally eaten by man. We may refer to Kuragê in the Far East (Japan, also China) which indicates large **SEMAEOSTOMEAE** and **RHIZOSTOMEAE** as well as the Te-Baitari of Gilbert Islands which indicates *Carybdea alata* (CARYBDEIDAE), all SCYPHOMEDUSAE. When considering the toxins injected by the nematocysts, we must draw the difference between their effect on man and on small aquatic animals. The toxins are undoubtedly active on small animals, they have a paralyzing and finally a lethal effect and on this property is based the normal feeding behaviour of COELENTERATES which are all carnivores and predators. Only a few species are lethal to man or other large animals. About 30 cases of death by stinging from jelly fishes are known with certainty, almost all of them are from the Indo-Malayan area between northern Australia and the Asian mainland (recent review by Southcott (9).

We mentioned earlier that the toxin contained in the capsule of the nematocysts is injected in the prey as if by a hypodermic syringe. The capsule is usually about 10-20 μ long or less, it is exceptionally large in SCYPHONOPHORA as for instance in *Agalma* where they may reach a length of some 200 μ

or more. Their numbers however are so great in the epidermis of the COELENTERATES, mainly in the tentacles and mouth rim or lips, that is has been estimated that a large medusa may inject some 3 cc of toxin in a single discharge. The nature of the toxin or toxins is not known with certainty. The principal difficulties involved are two: 1) — it is difficult to isolate nematocysts in sufficient quantity uncontaminated by proteins and indols from other tissues; 2) — the presence of different nematocysts in the same and in different species each with a different function and their different effect on the prey suggests that they probably contain different toxins. Lane & Dodge (10) have elaborated a method by which nematocysts may be isolated and the toxin extracted and tested separately from other tissue extracts.

The venom always has a paralyzing effect on other animals and when it is sufficiently strong to act on man specially when contact has occurred at places where the skin is finer or at mucous membranes, it causes a strong pain that feels like stinging fire. The paralyzing effect may be due to the toxin's action on the respiratory system. This is attributed to a glycerin soluble fraction called congestin, which would also cause a congestion of the gut and respiratory mucose. The alcoholic extract called thalassin causes itching, erythema and general irritation of the affected area allied to nausea, prostration and death in guinea-pigs. A fraction called hypnotoxin has also been described which is water soluble and was extracted from Siphonophores; it causes anesthesia, somnolence and death. All these toxins, whether they have been isolated in a pure state or not, are proteins, either mucoproteins with or without an indol absorbed on them, or compounds of tetramethylammonia associated to indols or to 5-hydroxytryptamine. Solutions of tetramine chemically pure when injected, cause responses similar to those of the extract of tentacles of different Coelenterates such as to reduce the tendency to autotomy of the fiddler-crab, *Uca mordax*, and to stimulate the excised moluscan heart. According to some authors the intense pain is caused by tetramine and paralysis to quarternary bases associated to it. Lane & Dodge (10) obtained similar results by injecting the extract of nematocysts freed and isolated from other tissues by a method they devised. The lethal dose of isolated toxin in mice is of 0.037 ml/kg of a solution containing 0.201% of total N.

As mentioned above, nematocysts are independent effectors uncontrolled by the nervous net, thus discharge may be highly localized, as for instance from a fragment of a tentacle or a small portion of the lips and also occurs in anesthetized animals. Peterson & Vannucci (11) while working out of the cycle of *Koellikerina fasciculata* observed that this species starves to death when kept at temperatures lower than 11°C because at such temperatures the nematocysts fail to discharge while all other functions appear to be maintained normally. It is interesting to note that nowhere, even at greater depths and in winter, the temperature in the Mediterranean Sea falls below 13.5 or 13.8°C at most. We don't know why low temperatures inhibit nematocysts discharge in this case and the matter requires further attention since preliminary observations suggest that an enzymatic mechanism may be at play.

Southcott (9) gives a detailed list of species of medical importance. Based on my own observations and experience I may add the following one for Brazilian waters:

HYDROZOA

Macrorhynchia philippina, PLUMULARIIDAE, colonial hydroid lacking a medusa free-living stage. It looks like inoffensive extremely delicate pens growing on algae and rocks below low tide mark. They produce an intense itching on human skin, irritation with erythema and the formation of little papulae, lasting several days. Southcott records the same effects from this species in the Indo-Pacific area; he calls it *Lytocarpus philippinus*.

Olindias sambaquiensis, LIMNOMEDUSAE, is called "relojinho" or little watch by local fishermen. It has a definite nettling action, the skin is reddened and later becomes vesiculated; stings may cause dizziness, muscle pain and dyspnoea. It is especially disagreable when hands soiled by the contact are taken to the eyes, a serious conjuntivitis may ensue.

The most disagreable and painful species in Brazil is *Physalia physalis*, popularly known as "Caravela" (Portuguese man-of-war).

There are records in the older literature of lethal cases in animals who were fed on *Physalia* tissues, toxicity in this case is presumably due to the stinging of the gut by the Siphonophore's nematocysts. Any contact with *Physalia* tentacles, even the slightest are extremely painful, produce erythema, papulae similar to urticaria, pain and swelling of lymphatic system, dizziness, nausea and muscle pains lasting several days. The reaction to the stinging of *Physalia* is instant and violent, it diminishes gradually with time while the effect of the stinging of Hydroids is slower, growing gradually during the first hours.

SCYPHOZOA

Chiropsalmus quadrumanus (CARYBDEIDAE) from coastal water cause an intense dermatitis, erythema, oedema and muscular pains along with swelling of lmyphatic ganglia. Areas stung by the jelly-fish develop local inflammation and ulcerations, in severe cases this is followed by fever, dizziness and cold tremors.

In comparison, *Chiropsalmus quadrigatus* a closely related species from the Indo-Pacific, may be lethal through pulmonar oedema, weakening of the pulse, respiratory difficulties, cardial blocking and death accompanied by strong pains shortly after contact with the jelly-fish.

Accidents caused by jelly-fishes are frequently accompanied by nervous and psychic phenomena such as screaming, exaltation, depression, suicidal tendencies, dyspnoea and cardiovascular disorders.

It is interesting to point out that all the dangerous species mentioned from Brazil have closely related representatives in the Indo-Pacific area belonging to the same genus or to a closely related genus. They are all mentioned by Southcott (l. c.). It appears that all the Atlantic counterparts are much milder than their Indo-Pacific relatives.

Summing up we may say that the number of species dangerous to man is small in the Atlantic Ocean, while their numbers are large in the Orient, especially in the Malayan region, in Japan and in the Philippines. Jelly-fishes are specially dangerous because they frequently occur in very dense and sometimes large patches, they are frequently transparent and rather difficult to see in the water and further their long tentacles drift about with the current, sometimes quite far away from the medusa and are quite invisible.

Among the sea-anemones or **ANTHOZOA**, although many will produce stinging sensible to men, there are no really dangerous species. We may conclude by saying that among the **HYDROZOA**, the **MILLEPORINA** and other hydroids may affect men but not as acutely as the HYDROMEDUSAE; among the **SCYPHOZOA**, the SCYPHOMEDUSAE include the species most dangerous and some may have a lethal effect. The **ANTHOZOA** are not really dangerous and the disagreable effect produced by corals is primarily due to lacerations caused by their skeleton and the breakage of small fragments which become incrusted in the victim's wound. Their action through nematocysts is only secondary.

It is known that in relation to certain species a certain degree of immunity may be developed with time, as may be observed from fishermen that are frequently exposed to certain abundant species when handling the nets soiled by the jelly-fishes. This has been shown in relation to *Cyanea* a species of the order **SEMAEOSTOMEAE** abundant in the northern seas. Probably the same happens here when there are large patches of *Olindias sambaquiensis*.

Acquired immunity may be an explanation of certain curious cases of symbiosis, difficult to explain otherwise, except perhaps by natural immunity or selection. The association between different species of **PAGURIDAE**, the hermit crabs, with certain sea-anemones has been famous for centuries, specially that between *Eupagurus bernhardus* and *Adamsia palliata*. Also the fact that young fishes, mainly cod find refuge between the hanging tentacles of large medusae, as for instance *Cyanea*, is well known and equally not fully understood. The symbiosis between certain coral fishes and sea-anemones of the family STOICHACTIDAE is less widely known but has in recent years been studied experimentally. In European aquaria, noticeably at Monaco and in American aquaria, specially at Marineland of the Pacific, one may observe fishes like *Amphiprion percula* some 5-10 cm long that live in small groups of two or three in the tentacular crown of large sea-anemones of the genus *Stoichactis*. They never swim very far from their host, they defend their territory most actively and over and over again return to the sea-anemones' tentacles, lying and rubbing against them. Neither the fish appears to be stung by the coelenterate's nematocysts nor does the sea-anemone show any attempt to feed on the fishes. A feeding behaviour would immediately be induced by any other animal behaving as that particular fish. Davenport (12) studied this behaviour experimentally. It is well ascertained that both the sea-anemone may live in symbiosis with other fishes of closely related species, and viceversa, the fish may live in symbiosis with closely related sea-anemones, either may be found isolated. *Amphiprion* which have been kept in isolation for months require several days to acclimatize to the anemone, during the process they began contact slowly and gradually with the deadly tentacles. It is believed that this is the means by which the bonds between the two species are established as well as the physiological barrier that protects the fish from the nematocysts and from being swallowed by the COELENTERATE. Davenport's experiments suggest that the mucus secreted by the fish contains an active principle that raises the threshold required for nematocyst discharge in this species of sea-anemone. This active principle is inactive at the threshold of electrically induced discharge, it acts fast, is specific and thermolabile. The same principle is not contained in the fish's muscles.

Since a *Stoichactis* acclimated to *Amphiprion* does not exhibit the feeding reactions it shows to other fishes or to a non acclimated *Amphiprion*, it may be presumed that the active principle contained in the epidermal mucus of *Amphiprion* acts on the anemone's nervous tissue as well, inhibiting its feeding reactions.

Pantin (1) studied the mechanism by which nematocysts are discharged. He showed among other points that: a) nematocysts are independent effectors (recently Ross, unpublished, has expressed the view that he has evidences to show that in one case at least the nervous system may interfere with nematocyst discharge, see Hand (13), b) discharge of nematocysts may be stimulated by mechanical and by chemical means, among the latter specially by surface active substances, c) solutions of usual foods make nematocysts more sensitive to discharge, by mechanical means.

Thus, if the discharge threshold of nematocysts is lowered by means of adequate food stuffs, inversely one must admit that the threshold for discharge must be raised by disagreable substances. If the mucus secreted by *Amphiprion percula* is disagreable to *Stoichactis*, its nematocysts will not be discharged and further the sea-anemone will have no feeding reactions, similar to what happens with *Anemonia sulcata* that does not react to and therefore does not ingest and does not feed upon fragments of flesh of the same species. As far as I can see it, this may be a tentative explanation of the behaviour of *Stoichactis* and *Amphiprion* when acclimated one to the other. Further Parker & Van Alstyne (14) showed that extract of the epidermis of the fish *Fundulus* causes the discharge of a certain number of nematocysts of the sea-anemone *Metridium* and of *Physalia* and Davenport has shown that wounded *Amphiprion* or fishes from which even a single scale is missing are no longer immune to the stinging by *Stoichactis*. Analysing all these facts the following tentative hypothesis may be drawn: *Stoichactis'* nematocysts normally react only feebly to the mucus of the epidermis of *Amphiprion*, the anemone does not react to this fish with its normal feeding behaviour, probably due to inhibition of the feeding behaviour by the mucus. Further, Davenport has shown that fishes not acclimated to the sea-anemone only gradually develop more intimate contacts with the sea-anemone. This suggests that the fish acquire immunity gradually and that the stinging and intoxications which it receives at first are not very effective due to the small amount of discharged nematocysts. It seems to me that experiments should be carried out along these lines that may take to the explanation of one of the most interesting and obscure cases of symbiosis yet described. Information available suggests an acquired immunity of the fish to the nematocysts and a feeding behaviour of the sea-anemone inhibited by the fish's mucus.

Before closing I wish to remind the audience that the present extremely modest communication was purposely just a short reminder of the venomous aspect of beautiful COELENTERATES, in a symposium that is mainly concerned with such terrific animals as serpents, spiders and scorpions.

References

1. PANTIN, C. F. A., *J. exp. Biol.,* **19** (3), 294-310, 1942.

2. BOUILLON, G., CASTIAUX, P., and VANDERMEERSSCHE, G., *Bull. Micr. appl.,* **8** (3), 61-63, 1958.

3. WERNER, B., *Helgoland wiss. Meeresunters.,* **12** (1-2), 1965.

4. ITO, T., *Rep. Tohoku Univ.,* Ser. 4, **18** (2), 211-215, 1948.

5. VANNUCCI, M., *Pubbl. Staz. Zool.* (Napoli), **31** (3), 393-420, 1960.

6. ITO, T., *Mem. Ehime Univ.*, Sec. II, **1** (2), 69-80, 1951.

7. BRINCKMANN, A., and PETERSEN, K. W., *Pubbl. Staz, Zool.* (Napoli), **31** (3), 386-392, 1960.

8. PICARD, J., *Rec. Trav. Stat. Mar. Fac. Marseille,* **15**, 99-103, 1955.

9. SOUTHCOTT, R. V., in H. L. KEEGAN, and W. V. MAC FARLANE (Editors), *Venomous and Poisonous Animals and Noxious Plants of the Pacific Region,* 1963.

10. LANE, C. E., and DODGE, E., *Biol. Bull.,* **115** (2), 216-219, 1958.

11. PETERSEN, K. W., and VANNUCCI, M., *Pubbl. Staz. Zool.* (Napoli), **31**, 473-492, 1960.

12. DAVENPORT, D., and NORRIS, K. S., *Biol. Bull.,* **115** (3), 397-410, 1958.

13. HAND, C., *Biology of Hydra,* 1961, pp. 187-202.

14. PARKER, G. H., and VAN ALSTYNE, M. A., *J. exp. Zool.,* **63**, 329, 1932.