OBSERVATIONS ON THE DEVELOPMENT AND MIGRATION OF THE URTICATING ORGANS OF SEA NETTLES, CNIDARIA.[1]

By Louis Murbach, Ph. D.

Visitors to the seashore have frequently had opportunity for becoming more or less acquainted with Sea Anemones, Jelly-Fishes, or even the large Portuguese Man-of-War, and other Siphonophora.

If the arms (tentacles) of the former, or the long capturing filaments of the latter, have been touched, inadvertently or through a more careful examination, a burning stinging sensation was experienced where the tentacles came into contact with the more delicate skin of the hands or other parts of the body.

These animals not only can make it unpleasant for their enemies, but by the same means can also overcome their prey. The ability to exercise this offensive and defensive warfare is due to the possession of very minute weapons called *nettling organs*. The observer has now become familiar with a most important function in the economy of these animals—that of nettling—which serves both as a means for gaining their livelihood and for their protection.

It is a suitable recognition of this power, that those groups of the Cœlenterata possessing it have been called Cnidaria.

The nettling organs can be studied satisfactorily only with the microscope, as they are single-celled organs and consequently very minute. They are situated in the outer cell layer, the ectoderm of the tentacles, or on special filaments—the acontia of the Actiniæ in the gastric cavity—the pertinent tissues of which are derived from the ectoderm.[2]

[1] This paper is to constitute a brief report of those results, obtained during my occupancy of the Smithsonian table at the Naples Zoological Station, which it was thought would prove of interest to the public. The table was occupied from April 25 to June 25, 1894.

[2] I believe it is still generally held that the nettling organs of the mesenterial filaments of the *Actiniæ* are of endodermic origin. But at the time this work was done I came across a paper of Boveri's (Ueber Entwickelung und Verwandschaftsbeziehungen der Actinien; Zeit. f. wiss. Zool., XLIX, Pt. 3, 1890) confirming the view formerly held by Heider (Ein Beitrag zur Anatomie der Actinien; Sitz'ber. d. Acad. d. Wiss., Wien; LXXIX, 1879) that the mesenterial filaments are of ectodermic origin, being derived from the lining of the gullet. This would place the origin of the nettling organs of all Cnidaria from the ectoderm. I can not find that this application has before been made, yet it can not, I should suppose, so long have been overlooked.

Their position on the surface of the tentacles is generally marked by a hair-like projection—*the cnidocil*—where nettling organs stand singly. In some cases the organs are collected at the tips of the tentacles in the form of *nettling knobs*, and in others they are grouped on smaller branches of the capturing filaments in the form of *nettling batteries*. In the latter case cnidocils are seldom present.

The *nettling organs* consist essentially of a nucleated, more or less *modified, cell*, the *cnidoblast*, which contains a capsule, *the nematocyst*, inclosing a much coiled hollow thread—*the nettling thread*.

The cell-body is somewhat cup or goblet shaped, having, however, only a small aperture at the top, for the discharge of the little weapon. At the side of this opening the cnidocil stands, and at the opposite end, the cell-body is drawn out in the form of a foot or *stalk*. In the lower Cnidaria this stalk is simple, but in some of the more specialized forms of the Siphonophora the stalk and the lower portions of the cell-body contain *spiral contractile fibers*.[1]

The nucleus of the cell is almost always in a mass of granular protoplasm—the base of the goblet-shaped part—just at the side or under the capsule, which is contained in the hollow of the goblet.

The nematocysts may be spherical, oval, or cylindrical. Each is a *double-walled capsule*, transparent enough to enable one to see the fluid contents and the tortuous thread within. The outer wall is very thick, and similar to chitin. The opening in its end comes just under the opening of the cell-body. The very thin inner wall is closely applied to the outer wall, and, passing through the opening in this, becomes insensibly continuous with the nettling thread.

The hollow nettling thread, in its resting condition, is turned, outside in, back into the nematocyst, lying coiled up more or less regularly in the fluid contents of the latter. In this condition its present lumen (the walls of which constitute its outer surface after discharge) is filled with a viscid fluid, which gives the discharged thread its adhesive and irritating character. The discharged thread is often twenty times longer than the longest diameter of the capsule. The thread of the spherical capsules is usually a simple slightly tapering tube, having on its outer surface three spiral rows of very fine barbs. In the case of the oval capsules, a widened cone-shaped basal portion is joined to the thread. A small intermediate piece bears some very small, backwardly directed spines, while on the basal portion near its junction with the intermediate piece there are three large spines directed backward. The thread of the cylindrical capsules differs principally from

[1] Heretofore the appearance caused by the spirals was mistaken for cross striations, and the parts in question were thought to represent cross striped muscle. But after finding the spiral filaments, I still wish to assert a muscular nature for them, as stated in a previous paper. Recently Schneider (Zool. Anz., No. 464) confirmed the presence of spiral structures in *Velella*, but he denies their contractile nature, without giving substantial reasons. Since spiral muscles are now found in Cephalopods, I hold to my interpretation.

the last in having the greater portion of its basal part covered by long slender spines. In the discharged or evaginated condition, the thread is at least partially filled by the fluid contents of the capsule.

In order to *cause* the *nematocyst* to be *discharged*, a proper stimulus—a minute crustacean, or worm, or an enemy—must come into contact with the *cnidocil*, for it is the *sensory part* of the nettling organ. The stimulus at one cnidocil may be distributed by nerve connections to the surrounding nettling organs, thus inducing explosions *en masse*. Next the cell-body and stalk contract, and this double pressure on the nematocyst, is transmitted by its fluid contents to all parts of the thread within, and it begins to be evaginated from its basal part outward to its end, with explosive rapidity.[1] The thread newly shot out unites the two very efficient conditions, namely, a large adhesive surface enhanced by very minute barbs, and by a sticky substance which also acts as a poison.[2]

The nettling poison has never been chemically analyzed, but its nature has rather been inferred from its effect on other animals. It was formerly supposed by many to be somewhat similar to formic acid. The small animals that are caught by a Cnidarian as prey make a few convulsive movements and then are apparently dead and are ingested by their captor. Anyone may experience the effect of the fluid in a more marked degree than on the hands, if he will touch his tongue to the tentacles of a sea-anemone. It is not unlike the sensation perceived on touching the tongue to a freshly cut root of Indian turnip (*Arisæma triphyllum*), and may last several hours or a day. Indeed, Professor Leuckart records a case where a lead pencil which had some weeks previously been used in manipulating a siphonophore, on being accidentally touched to the tongue, caused this nettling.[3]

The above brief review of what is held at the present time on the anatomy and physiology of the nettling organs also contains the principal points of my recent paper on this subject.[4]

In that paper I reviewed the pertinent literature, and would refer those who desire a fuller account to it. The preceding has been given to make clearer what is to follow. For the same reason it may be well to briefly give my results from alcoholic material on the development and migration of nettling organs, as presented by the same paper.

[1] In alcoholic material one can occasionally cause the thread to be slowly everted by continuous pressure on the cover glass.

[2] By all authors before me, the noxious fluid was supposed to be contained in the nematocyst. Their chief argument for the belief that fluid flows from the capsule is, that the size of the capsule and thread is less after the discharge of the latter. This does not hold when we remember that the volume of the fluid in the capsule equals the volume of the capsule minus the volume of the contained thread. How could this fluid then, after evagination of the thread, fill both capsule and thread as tensely as before? Besides this, all of the authors since Möbius, have left out of account the substance (fluid) that fills the lumen of the invaginated thread.

[3] Zur näheren Kenntniss der Siphonophoren von Nizza; Archiv f. Naturgesch., 1854.

[4] Archiv f. Naturgesch, Pt. 3, 1891 (one plate and one woodcut).

These were:

1. The nettling organs are developed in cells derived from the ectoderm.[1]

2. The inner wall of the nematocyst originates from the nucleus and grows in the protoplasm around it.[2] In consequence of this growth, a lighter area of secreted matter forms around the growing inner wall. By the abstraction of water from the secreted matter it condenses and shapes itself to the inner wall, and becomes the outer wall of the nematocyst.[3]

3. The hollow thread grows in spirals around the nucleus, as a continuation of the inner wall of the nematocyst. The growing end is nearest the nucleus. These growths are looked upon as the result of the functional activity of the nucleus in the cell.

4. When the development of the thread is complete, chemical changes are assumed to take place in the cell, causing the outer wall of the nematocyst to become firmer, and abstracting enough water from the contents of the inner wall (exosmose), so that the diminished pressure within will cause the thread to be drawn into the nematocyst. The spiral growth of the thread, during its development, favors a similar arrangement in the nematocyst.

5. When the thread is wholly within the capsule, the latter is rotated in the cell, so that the opening in the outer wall is turned away from the nucleus, and comes to lie directly under the opening in the cell-body, for the discharge of the thread.

6. The nettling organs are developed in more proximal parts of the Cnidarian body (near the bases of the tentacles in Hydromedusæ, but on the basal portion of the polyps in Siphonophora), and reach their destination on the tentacles by active amœboid migration (*Hydra*, etc.), or by displacement due to the rapid growth of the tissues (Siphonophora).

7. The stalks are probably outgrowths of the cell body, produced after the migration of the organs.

As these results were obtained from alcoholic material, save *Hydra* which was used fresh, it was very desirable to verify them on fresh and living marine animals, which I was enabled to do at Naples.

In an appendix to the paper above mentioned, I gave, as a sort of preliminary report, some of the principal results.

The presence of nettling organs in the higher Protozoa, in the Cnidaria, in the Turbellaria, and in the Gasteropoda, makes it seem desirable to compare the development, the structure, and the function of these organs in the groups named. With this in view, representatives of two of these divisions, not yet studied comparatively, have been collected.

[1] The Microtomist's Vade-Mecum, A. B. Lee, 3d ed., 1893.

[2] Eisig (Monogr. I. Capitelliden, p. 576). Perrier and Claparède hold that the nucleus is directly concerned in the origin and formation of the setæ of certain worms.

[3] I have since observed the stages of this process in *Physalia*.

Methods.—First of all, living Hydroid material was gathered so as to examine the organs in their natural condition, and also to test my conclusions in regard to migration and development. As my previous observations had not taken into account the Actiniæ, they were first examined. Fresh cerata of living *Æolidiæ* were given some attention, and many specimens were preserved for later histological work.

The methods employed were largely those already in vogue and described, only modified enough to suit the circumstances.[1]

For the examination of living tissue, a bit was placed in sea water on a slide, and a very dilute solution of aqueous methylin blue was added. The mass was then either only slightly compressed under the cover glass, or it was first teased, and then the elements were further isolated under the cover glass by lightly tapping on the latter with a pencil or other suitable object, until the desired result was obtained.

For preservation, Hydroids were killed by quickly pouring over them, placed in as little sea water as would keep them expanded, an acidified solution of corrosive sublimate, in 30 or 50 per cent alcohol. After some minutes they were removed from this mixture to the diluted pure solution, left for one-quarter hour,[2] then transferred to 70 per cent alcohol for one hour, and finally put up in 80 per cent alcohol.

Small Actiniæ were similarly treated except that the solution for killing was first heated. On some of the larger Actiniæ the narcotization process was used previous to fixing, but with indifferent success.

The few Siphonophora preserved were treated essentially like the Hydroids, except that they were killed by pouring into the least possible quantity of sea water that would keep them expanded an acidified 10 per cent solution of copper sulphate, to which was added a little corrosive sublimate solution.

On some Turbellaria and on *Æolidiæ*, Kleinenberg's fluid worked well for killing and fixing. After thoroughly washing in 70 per cent alcohol they were placed into 80 per cent. Other *Æolidiæ* were treated quite like the Hydroids. On still others, dilute Flemming's fluid was used as a fixative, and with good success. For preserving the external form, killing with glacial acetic acid, added in abundance, and immediately removing to weak alcohol, proved most effective. Yet much depends on the proper manipulation of the animal while the tissues are fixing.[3]

The material to be sectioned was stained with Mayer's hæmalum, picro-carmine, or with borax-carmine.

Historical.—The question of the *transposition* of *nettling organs*, for they are rarely ever used at the point where they develop, has long been an interesting one. For the Siphonophora, bearing capturing filaments, it was long ago settled by Professor Leuckart,[4] that the

[1] The Microtomist's Vade-Mecum, A. B. Lee, 3d ed., 1893.

[2] This time varied, of course, depending on the size and nature of the object.

[3] One such well-preserved specimen is due to the skillful hand of Signor Lo Bianco.

[4] Zoologische Untersuchungen; I, Die Siphonophoren, 1853.

used-up batteries and ends of the filaments, were replaced by the extensive growth (Nachschub) of the latter.

In *Velella*, Bedot,[1] later found canals filled with nettling organs extending from the great mass of developing nematocysts, under the so-called liver, to the outer layer of cells on the under side of the animal. But he did not consider the question of the manner of their transposition.

In *Hydra*, Nussbaum[2] believed that the movement of nettling organs, along the tentacles, was facilitated by the slightly twisted condition of the latter.

The reasons for my conclusion, previously stated, that the nettling organs propel themselves from place to place (excepting in the Siphonophora), were that I also found the canals which Bedot had seen in *Velella;*[3] but more than this, I found that the nettling organs are always turned with the basal, i. e., with the larger mass of protoplasm and nucleus, in the direction of motion, while the discharge pole points to the rear. Furthermore, that the fixed cell-body shows the amœboid form. Finally, that in many Hydroids one can frequently observe nettling organs lying parallel to the surface of the tentacles, their orientation as before described, showing that they are proceeding upward on the latter. This was further confirmed by the more careful drawings of two of the works consulted, one by F. E. Schulze[4] and the other by O. and R. Hertwig.[5]

Statement of results.—Now the *observations* of *living material* bring the most conclusive proof. From specimens of *Velella* to be examined, small pieces containing nettling organs were teased a little and lightly flattened under the cover glass. Many nettling organs showed amœboid changes of form. The movements were slow but definite. One case, however, which was observed for fifteen minutes, made such pronounced and rapid amœboid movements, that it might well have been taken for an *Amœba* which had swallowed a nematocyst.

As *Pennaria carolinii* was easily obtainable, it was used as a representative of the Hydromedusæ. At first a hydranth was teased and placed with some sea water under a cover glass. The protoplasm of the nettling cells was in many cases passing through changes of form, but no definite locomotion was observable. In order not to mistake any rotation of the nettling organ for change of form, in this and all subsequent cases, the spines in the base of the thread were carefully observed simultaneously with the contours of the cell-body. For other

[1] Recherches sur l'organ central et le sytsème vaseulaire de Velelles; Recueil zool. suisse, I, 1884.

[2] Ueber die Theilbarkeit d. leb. Materie, II; Archiv f. Micros. Anat., XXVII, 1887.

[3] According to his preliminary report (Zool. Anzeig., No. 464) Schneider has observed the same for *Porpita*.

[4] Ueber den Bau und die Entwickelung von Syncoryne Sarsii, 1873.

[5] Das Nervensystem und die Sinnesorgane der Medusen, 1878. Pl. V; fig. 26.

observations the hydranths were simply placed in a little sea water under cover glass and gently flattened. A point was selected where a nettling organ, not far from the base of a tentacle, was slowly changing its form. It was observed for nearly one-half hour, the ectoderm cell boundaries being used as the nearest fixed points obtainable. The large mass of protoplasm containing the nucleus of the nematoblast was turned toward the tentacle. At the end of the stated time of observation, the organ had passed through a distance equal to its own diameter. In another case a nettling organ traveled toward a tentacle a distance equal to three times its diameter; meanwhile it twice turned up endwise. Another case particularly drew attention; the cell-body was changing its form quite rapidly, progressing at the same time between the ectoderm cells, keeping close to the mesoglœa. Many cases were observed where the nettling organs were lying parallel to the surface of the tentacles.

Other cases were observed where the nettling organs were turned in almost any direction, or again where they seemed to be reversed as if going toward the base of the tentacle, and many others in which I could detect no change of form or motion whatever. These exceptions, however, as well as the short distance traveled by the nettling organs in a given time, may find an explanation in the abnormal conditions to which the hydranths were subjected during the observations.

After the foregoing observations I feel warranted in reaffirming my previous conclusion, *that the active amœboid migration of the nettling organs is the manner in which they are transported from the point of their development to their destination.* Furthermore, I believe this will be found to apply also to all Cnidaria where similar conditions obtain as, for instance, to all except some of the Siphonophora.

In the limits of this paper it is not expedient to give a review of the literature on the origin and development of the nematocyst and thread. Suffice it to say that most of the authors heretofore agreed that the nematocyst and contents take their origin from a vacuole arising in the protoplasm of the nettle-cell. On the origin of the thread all the older authors are agreed that it arose in the nematocyst; some, from a mass of protoplasm that grew into the vacuole, and others believed it originated in the secreted contents of the nematocyst. Still another view was that both nematocyst and thread were derived from the mass of protoplasm that had grown into the vacuole. But later it was finally shown that the thread takes it origin outside the nematocyst, and consequently it must subsequently be invaginated into the capsule.

With the exception of one brief reference,[1] this fact in regard to the position of the growing thread was not applied to the Actiniæ. It was therefore desirable to learn to what extent my observations on Hydroids applied to these.

[1] Schneider, Einige hist. Befunde an Cœlenteraten; Jen. Zschr. f. Nat. 27, N. F. 20, 1892.

As already stated, I demonstrated that the *nematocyst is* of *heterogeneous origin;* the inner wall being derived from the nucleus, while the outer one results from the secretions arising around the former during its growth. Also that *the thread develops around the nucleus* of the cell, and *not* around the wall of the nematocyst, as has been heretofore held.

These points were now *reexamined in living and fresh material*, especially in Siphonophora and in Medusæ. The Actiniæ were also preliminarily examined. The same course of development as has been described from alcoholic specimens could now be most beautifully observed, the thread being slightly stained by methylin blue. With the nucleus somewhat stained the observation was very easy and decisive.

In the Actiniæ observation becomes much more difficult, because of the small size of the nettling organs of most of them. *Anemone sulcata*, then at hand and a sufficiently typical specimen, was first examined. The early stages of both capsule and thread resemble very closely those of the cylindrical ones of the Siphonophora. The inner wall of the nematocyst early takes on a curved form, the nucleus with encircling thread lying in the concavity. The spirals of the thread do not seem to be so regular as those of the Hydrozoa examined. But they could be seen in greater number. By inducing a current under the cover glass the observation may be made more certain, because different views of the nematoblast are thus obtained. Both *Adamsia rondeletii* and *Astroides calycularis* were sufficiently examined to confirm what I had observed in the other form. The latter is not a suitable form for this work, on account of the minuteness of its nettling organs.

It is my intention to subject this matter to a more thorough examination in Actiniæ; but even now I believe we are warranted in concluding *that the development of the nettling organs is the same for all the Cnidaria.*

The *Turbellaria* collected have not yet been examined for the development of their nettling organs, in the light of these newer observations; though in one form previously obtained at Leipzic, some apparently undeveloped nematocysts were found, that lead me to look for a similar plan of development to that already established for the Cnidaria.

The *Æolidia* obtained at Naples are under investigation, but so far no results are definite enough to be stated.

In conclusion I wish to thank the Director, Dr. Dorn, for courtesies while at the Naples Zoological Station, also the Secretary of the Smithsonian Institution, and the committee in charge, for the privilege of occupying the table.