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An Ultrastructure Study of Strobilation in Chrysaora quinquecirrha with Special Reference to Neurosecretion

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AN ULTRASTRUCTURAL STUDY OF STROBILATION
IN Chrysaora quinquecirrha WITH SPECIAL
REFERENCE TO NEUROSECRETION

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

By
Marsha A. Dietz
1971
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

MASTER OF ARTS

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<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-a.</td>
<td>Stages in the life cycle of a jellyfish</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>b. Scyphistoma showing region where sections were cut</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td>A typical scyphistoma</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>A polyp during neck formation</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>A segmented polyp</td>
<td>15</td>
</tr>
<tr>
<td>5.</td>
<td>A metamorphosing polyp</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>A x-sect. through the epidermal layer of a Chrysaora scyphistoma</td>
<td>16</td>
</tr>
<tr>
<td>7.</td>
<td>Lower border of the epidermis of a scyphistoma</td>
<td>16</td>
</tr>
<tr>
<td>8.</td>
<td>A x-sect. through the epidermal layer of a strobila during segmentation</td>
<td>17</td>
</tr>
<tr>
<td>9.</td>
<td>A section through the constriction of a polyp during neck formation</td>
<td>17</td>
</tr>
<tr>
<td>10.</td>
<td>A typical neurosecretory cell</td>
<td>18</td>
</tr>
<tr>
<td>11.</td>
<td>A section through a segmented polyp</td>
<td>19</td>
</tr>
<tr>
<td>12.</td>
<td>Neurosecretory granules in the axons of a segmented polyp</td>
<td>19</td>
</tr>
<tr>
<td>13.</td>
<td>A section through the lower epidermis of a metamorphosing polyp</td>
<td>20</td>
</tr>
<tr>
<td>14.</td>
<td>Same as above</td>
<td>20</td>
</tr>
</tbody>
</table>
Scyphistomae and strobilae of the scyphozoan Chrysaora quinquecirrha were fixed and sectioned for electron microscopy. The polyps were divided into four classes on the basis of their stage of development: non-strobilating (scyphistomae), neck-formation, segmentation, and metamorphosis. Ultrastructural observations revealed the presence of neurosecretory cells containing numerous membrane-bounded granules in scyphistomae and necked polyps. Between the neck-formation and segmentation the neurosecretory granules moved from the cell body to the axons of the neurites. By the time of metamorphosis most of the neurosecretory product had disappeared from the axons. Other changes which appear to accompany strobilation included both a decrease in glycogen and the contraction of muscles along the inner edge of the epidermis.
AN ULTRASTRUCTURAL STUDY OF STROBILATION
IN *Chrysaora quinquecirrha* WITH SPECIAL
REFERENCE TO NEUROSECRETION
INTRODUCTION

The coelenterate nervous system controls a number of morphogenetic processes. Burnett, Diehl, and Diehl (1964) found growth and regeneration in *Hydra* to be controlled by neurosecretory cells. The initiation of sexuality in *Hydra* is also related to neurosecretory activity (Burnett and Diehl, 1964). Lesh and Burnett (1966) extracted an inducer from the hypostomal region of *Hydra*. This inducer appears to control the direction of cell differentiation of interstitial cells along the body. Destruction of nerve cells reduces inducer activity. This fact and the previous work with *Hydra* suggest that the inducer is a product of neurosecretory activity.

Strobilation is a unique metamorphic process by which jellyfish scyphistomae produce ephyrae that later develop into medusae. It is by this process that a change from the asexual to the sexual generation is accomplished. The changes in gross morphology which occur during strobilation have been described by a number of investigators, including Thiel (1938), Hyman (1940), Spangenberg (1968), and Loeb (1970).

Spangenberg, working with *Aurelia aurita*, uses the term 'strobilation' to refer to two separate processes: segmentation and metamorphosis. Loeb (1970), working with *Chrysaora quinquecirrha*, recognizes an additional process which she refers to as neck formation. Previous ultrastructural studies involving *Chrysaora quinquecirrha* have been concerned primarily with the nematocysts (Burnett and Sutton, 1969; Sutton and Burnett, 1969) and the tentacle muscles of the medusa.
(Perkins, et al., 1971). The ultrastructure of scyphistomae and strobilae of Chrysaora quinquecirrha representing each of these stages of strobilation is examined in this study. On the basis of the findings in Hydra mentioned previously, attention is focused on the nerve elements, particularly the neurosecretory cells.
MATERIALS AND METHODS

Mature medusae of the scyphozoan, *Chrysaora quinquecirrha*, were induced to spawn in the laboratory. This procedure involved holding mature males and females crowded in plastic buckets overnight. The following day fertilized eggs could be removed by pipet to subsequently develop into planulae and polyps (Fig. 1-a, 1-b). The polyps were cultured in filtered York River water collected from the VIMS dock. The cultures were fed three times a week with *Artemia* nauplii and were kept in the dark to retard algal growth.

Strobilating polyps were randomly picked from the cultures and were divided into three classes on the basis of their stage of development:

1. Neck Formation: polyps with a distinct constriction beneath the base of the tentacles (Fig. 3).

2. Segmentation: polyps with a series of constrictions which divide the body into a number of segments. The number of segments is variable, and may range from one to 16. The tentacles are still present at this stage, and ephyrae are not yet being released (Fig. 4).

3. Metamorphosis: polyps with mature ephyrae. The tentacles, septal muscles, and atrichous polyspiras nematocysts have been destroyed, and a number of new structures, including
lappets, rhopalia, manubria, etc., have
developed (Fig. 5).

Scyphistomae and strobilae were fixed in 3% glutaraldehyde
and post-fixed in 1% osmium tetroxide according to the rapid method of
Hayat and Giaquinta (1970). The osmolality of the fixatives were cor­
rected to that of the culture medium with sodium chloride.

The polyps were cut through the hypostomal region, slightly
below the bases of the tentacles (Fig. 1-b). All of the sectioning
was done with a Porter-Blum Mt-2B ultramicrotome. The sections were
stained with uranyl acetate and Reynold's lead citrate and were exam­
ined with a Zeiss EM 9S-2 electron microscope. A Zeiss Photoscope II
was used for macro-photography. Sample sizes of 5-10 polyps were exam­
ined at each stage; results are based on the examination of 15-25 sec­
tions per polyp.
RESULTS

Examination of thin sections of *Chrysaora* scyphistomae reveals the typical coelenterate body plan with a loosely arranged epidermis and a dense, glandular gastrodermis separated by a collagenous mesoglea (Chapman, 1966). Large intracellular spaces are common in the epidermal layer. The epidermis is composed of epitheliomuscular cells, cnidoblasts, interstitial cells, and nerve cells. The nerve cells may be classified as neurosensory, ganglionic, or neurosecretory. Neurosensory cells are easily distinguished by the presence of an apical sensory hair. They are generally found between the epitheliomuscular cells with the apical flagellum projecting from the surface. Both ganglionic and neurosecretory cells occur at the bases of epitheliomuscular cells. They are similar in structure and are characterized by an irregular nucleus with several nucleoli, free ribosomes with little or no endoplasmic reticulum, glycogen granules in the perikaryon, microtubules, and complex Golgi. However, neurosecretory cells may be distinguished by the presence of electron-dense, membrane-bounded granules. These granules vary in size from 100-160 nm and may be seen in association with the Golgi or scattered throughout the perikaryon.

Neurites of neurosecretory and ganglionic cells in the scyphistoma often occur in groups and may be seen lying adjacent to the mesoglea (Fig. 9). Microtubules are frequently seen in the neurites, but neurosecretory granules are seldom present at this stage.

In the scyphistoma, glycogen granules are abundantly scatter-
ed through many of the cells of the epidermis (Fig. 6). Although some glycogen is always present, there appears to be a distinct decrease in the number of granules during strobilation (Fig. 8).

In addition to the decreases in glycogen, several other changes appear to accompany strobilation. The gross morphology of the polyp changes drastically. After the body becomes constricted into a series of segments, the tentacles are resorbed and feeding ceases until all of the segments have matured into ephyrae and are released. During this time there is frequently an increase in pigmentation, with the polyps ranging from light pink to red.

During neck-formation, segmentation, and metamorphosis, large bundles of muscle fibers appear along the inner edge of the epidermis (Figs. 9, 11). Although thin bands of muscle may be seen in this area in scyphistomae, they never occur in large bundles. It may be that the initial constrictions which occur during strobilation are the result of muscle contraction.

Several changes occur with respect to the neurosecretory material. In scyphistomae and during neck-formation, neurosecretory cells are easily recognized by the presence of neurosecretory granules (Fig. 10). Occasionally some neurosecretory granules may be seen in the neurites, but this is very infrequent. During segmentation and metamorphosis, very few cells are clearly identifiable as neurosecretory. Without the presence of the dense, membrane-bounded granules, ganglionic and neurosecretory cells are practically indistinguishable. In segmented polyps the disappearance of the dense, membrane-bounded granules from the neurosecretory cell body is accomplished by a dramatic increase in the occurrence of dense, membrane-bounded granules in
the neurites (Figs. 11, 12). These granules range in size from 90-150 nm and are similar in appearance to the neurosecretory granules seen in earlier stages. During metamorphosis, most of the neurites are again devoid of granules or contain granules that appear less full. Neurosecretory cells are again difficult to distinguish (Fig. 13, 14).
DISCUSSION

Most recent investigations of strobilation have been physiologically oriented. A number of environmental factors, including temperature, salinity, light, nutrition, oxygen, chemicals, and symbiotic organisms, have been related to the initiation of strobilation. Several investigations of *Hydra* have shown neurosecretory activity to control growth and other morphogenetic processes. On the basis of low temperature preconditioning experiments with *Aurelia* and the recent work with *Hydra*, Spangenberg (1965) and Custance (1966) have suggested a possible relationship between neurosecretion and the initiation of strobilation. Spangenberg (1968) relates any such neurosecretory activity to environmental conditioning on the basis of environmental effects on the nervous system in higher animals. Davis (1969) suggests that the cilium of neurosensory cells in *Hydra* is a type of receptor which may stimulate release of the neurosecretory material. However, experimentation with effects of various environmental factors on *Chrysaora* frequently provides inconsistent results, and the mechanisms which initiate and control strobilation are still poorly understood.

The decrease in glycogen during strobilation is not too surprising. If feeding ceases during segmentation, it seems feasible that the polyp would be forced to draw upon any stored food reserves during this period of active cell proliferation and differentiation.

Using the detailed descriptions of hydrozoan nerve elements provided by Lentz and Barrnett (1965), Jha and Mackie (1967), Lentz (1966, 1968), and Davis et al., (1968), the nerve cells of *Chrysaora*
scyphistomae are readily identifiable at the ultrastructural level. The neurosecretory cells of the scyphistomae and strobilae are particularly distinct due to their concentrated content of granules. The sudden disappearance of distinct neurosecretory cells and the increase in granule content of the neurites during segmentation suggest that the neurosecretory material is being relocalized at this time. This migration of neurosecretory material along the axons is the most frequent means of displacement and has been described in a number of other organisms (Gabe, 1966). However, there is still considerable debate as to whether or not the neurosecretory product is altered during the course of axonal migration.

At the time of metamorphosis most of the granules have disappeared from the axons, suggesting that the neurosecretory product has been released. The exact mode of release of the granule content is uncertain. However, some of the granules appear less full than others, perhaps suggesting that some of the contents have diffused out of the granules. This mode of release is in accordance with the findings of De Robertis (1962) in Hydra. He observed a decrease in size and density of the droplets in the neurites and the appearance of empty or almost empty vesicles. He suggests that the release of the neurosecretory product from the vesicles results in less dense droplets or vacated membrane-bound vesicles.

Most neural elements contain microtubules. They are particularly apparent in the axons. Although their function is uncertain, Slutterback (1963) proposes that they aid in the transport of water, ions, or small molecules. In the case of neurosecretory cells and axons, perhaps the abundance of microtubules may assist in the transport
of the neurosecretory product

Spangenberg's suggestion that neurosecretory materials may act to initiate strobilation in response to environmental conditioning is not supported by observations made during this study. If the neurosecretory product is active in initiating strobilation in response to the proper environmental stimulus, one would expect the material to be released at a much earlier time than was observed. Since the granules do not appear in the axons until segmentation, it seems unlikely that their release is responsible for initiating strobilation. However, they may function in regulating some of the processes involved in the maturation of the ephyrae.

On the basis of physiological as well as ultrastructural observations, it appears that neck-formation, as described by Loeb (1970), is not a definite indication of the onset of strobilation. Several preliminary temperature and salinity studies by the authors indicate that neck-formation is a reversible process that may or may not lead to segmentation. Aside from the muscle contraction below the base of the tentacles, polyps with necks are structurally similar to other scyphistomae. Neurosecretory cells are still very much in evidence, and few granules are apparent in the axons at this stage.
PLATE 1
EXPLANATION OF FIGURES

1-a Stages in the life cycle of a jellyfish.

1-b Scyphistomae showing region where sections were cut.
STAGES IN THE LIFE CYCLE OF A JELLYFISH

1a

MEDUSAE

EGG

PLANULA

1b

POLYPS

STROBLA

EPHYRA
2 A typical scyphistoma of *Chrysaora quinquecirrha*.

3 A polyp showing the constriction below the base of the tentacles which is referred to as neck-formation.

4 A segmented polyp.

5 A metamorphosing polyp.
PLATE 3

EXPLANATION OF FIGURES

6 A cross section through the epidermal layer of a *Chrysaora* scyphistoma. The cnidoblast (N) contains several large lipid droplets (L) and an abundance of glycogen (G1). X 18,000.

7 The epidermis of a scyphistoma bordering on the mesoglea (ME). Numerous microtubules (mt) can be seen in the axons (A) lying adjacent to the mesoglea. The nematocyte (N) above the region of axons is rich in ergastoplasm (er); lipid (L) is also present. X 42,750.
PLATE 4

EXPLANATION OF FIGURES

8 A cross section through the epidermis of a strobila during segmentation. The orientation is the same as that in Fig. 6. The muscles (Ms) now appear in thick bundles rather than as a thin band. There is much less glycogen (Gl) now than was seen previously. X 18,000. NM - cnidoblast.

9 A section through the constriction of a polyp with a neck. Large bundles of muscles (Ms) border on the mesoglea (ME). X 47,508.
A typical neurosecretory cell as seen in both scyphistomae and necked polyps. Membrane-bounded granules can be seen forming in the Golgi (G) and scattered in the perikaryon. M - Mitochondria. G1 - Glycogen. X 38,000.
PLATE 6

EXPLANATION OF FIGURES

11  A section through a segmented polyp, showing large bundles of muscle (MS) bordering on the mesoglea (ME). The group of axons (A) contain numerous microtubules (MT) and neurosecretory granules (NSG). X 18,000.

12  A higher magnification view of the neurosecretory granules (NSG) in the axons of a segmented polyp. Microtubules (MT) and mitochondria are also present in the axons (A). X 38,000.
PLATE 7

EXPLANATION OF FIGURES

13 A section through the lower epidermis of a metamorphosing polyp. Free ribosomes (r) and clear vesicles (v) are present, but no neurosecretory material or microtubules are evident. X 33,250.

14 Another view of the lower epidermis of a metamorphosing polyp. A few neurosecretory granules (NSG) may be seen, but generally the axons (A) contain only mitochondria (M) and free ribosomes (r). X 38,000.
BIBLIOGRAPHY


VITA

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