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# Contractile Activities of the Acontial Filaments of *Metridium senile* var. *fimbriatum* Verrill[1)]

By

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*(With 10 Text-figures and 1 Table)*

The neuromuscular structure of the sea anemone acontium has long attracted the attention of various workers (O. Hertwig and R. Hertwig, 1879; Carlgren, 1893; Parker, 1916; Yanagita and Wada, 1959), as an interesting material for morphological and physiological investigation.

O. Hertwig and R. Hertwig (1879) reported that a longitudinal muscle band is seen on the outer face of the cross arm of the T-shaped mesogloeal axis which is demonstrated in a transverse section of the acontium of *Sagartia*.

According to Parker and Titus (1916), in the acontia of the closely allied genus *Metridium marginatum*, similar longitudinal muscle bands are situated on the two sides of the stem of the T-shaped mesogloea. This observation agrees with the description of Carlgren (1893) for the acontia of *Metridium dianthus* and *Sagartia*. Carlgren suggested that the observation of O. Hertwig and R. Hertwig was probably a mistake (cited from Parker and Titus, 1916).

A thin layer of punctate substance lying just external to each row of muscle fibres was regarded by O. Hertwig and R. Hertwig (1879) as the nervous element of the acontium, but Carlgren (1893) and Parker and Titus (1916) could not confirm the statements as to the neuromuscular structure of the *Sagartia* acontium. Parker and Titus (1916) also reported that if stimulus was limited to one end of a long actonium, only that end responded with contraction into a knot. Moreover, acontia were not enough anesthetized, for anesthetized acontia would still become knot when immersed in meat juice, as unanesthetized acontia did. From these observations, Parker and Titus concluded that the acontia in *Metridium* contained no nervous element and that their muscle was stimulated directly.

Lately, Yanagita and Wada (1959) have presented a diagrammatical

1) This study was supported by a grant of the Japan Association for the Advancement of Science to Professor Tamasige, in 1968.

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reconstruction of the microscopical make-up of the *Diadumene* acontia as seen in the cross section. Their description of the structure of the muscle bands corresponds to that shown in Carlgren's (1893) drawing of the *Sagartia* acontium. The acontia of *Diadumene,* surrounded by a single-layered endodermal epithelium, are furnished with three kinds of effectors: i.e., cilia, cnidae and the muscle fibres. The last is present in the form of a pair of tape-like bundles of longitudinal fibres, which are developed along the median line of the ciliated band. Their contraction causes bending of the acontial filament toward the ciliated band, which results in either two dimensional or three dimensional coiling of the filament.

The present author found the spontaneous contraction caused the bending or coiling the acontial filaments of *Metridium.* The contractions were rather difficult to observe directly but when the pieces of 2–3 mm length were used their contractile behaviours were clear by microscorpical observation. If the acontium is nonnervous nature, as concluded by Parker and Titus, the question rises where the original site of spontaneous contraction in the acontial filament is. The present work is performed to clarify the site of the spontaneous conctractions.

**Acknowledgement**

The writer wishes to express her sincere thanks to Prof. Mituo Tamasige of Hokkaido University for his kind guidance and encouragement throughout the course of these experiments and his improvement of the manuscript. Her thanks are also due to the staff of his laboratory, where the work was done and facilities were afforded.

## Material and Methods

The acontia of the *Metridium senile* var. *fimbriatum* Verrill were used. The actinians collected at Muroran bay and on the coast of Wakkanai in Hokkaido were kept in the aquarium of the aerated sea water in the laboratory

(1) Intensity measurement of the contraction.

Intensity of the contraction is represented by the contraction ratio calculated using the formula as follows (see Fig. 1).

$$\text{Contraction ratio, } S = \frac{l_0 - l_i}{l_0}$$

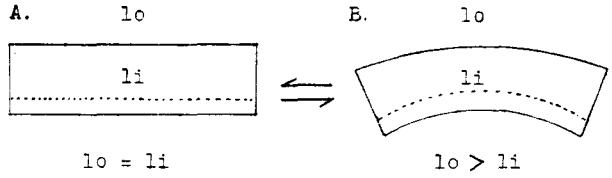


Fig. 1. Diagram of the acontial piece for measurement of contraction ratio. A shows the acontial piece in relaxed phase. B shows that in contracted phase. $l_0$ is the length of the surface of the side on which the nematocysts are situated. $l_i$ is the length comparable to that of the longitudinal muscle.

The acontial pieces of 3 mm length were mounted on a glass slide on which surface a drop of sea water was supplied and covered with a cover slip, then the piece was photographed through a micr oscope (×20) at 30 seconds interval for 20 minutes. The obtained photographs were enlarged considerably and $l_o$ and $l_i$ were measured with curvimeter. This device is very simple to operate and the accuracy is also sufficient, the error was within 5 per cent. The $l_i$ is drawn at the position of one fourth (the diameter of the acontium was divided into four) from the surface of the ciliated band. Determination of the position was done based upon the geometrical reconstruction of the acontial structure as seen in its cross-section (see Fig. 2).

Intensity-time curve was plotted using the data obtained.

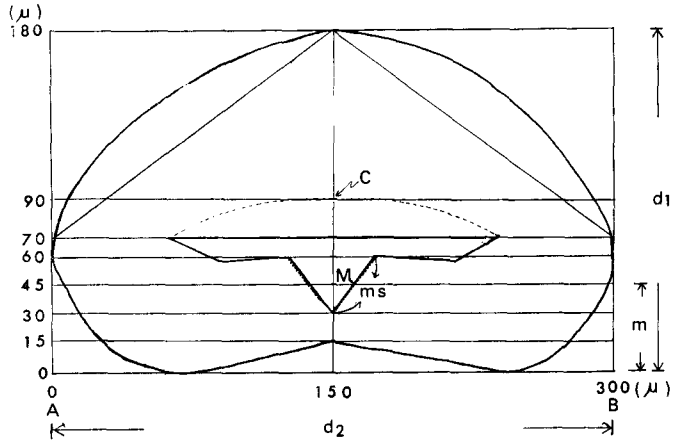


Fig. 2. Geometrical diagram of the cross section of the acontium for measurement of contraction ratio. C: the centre of the cross section of the acontium. M: the point which bisects the length of ms (the muscle sheet). $d_1$: the short diameter of the cross section of the acontium. $d_2$: the long diameter of it. m: the length of the perpendicular line from M to the line AB. $d_1:d_2=2:3$. $d_1:m=4:1$.

(2) Frequency measurement of the spontaneous contraction.

A. Contraction phase.

On the intensity-time curve, the time intervals from the time of increse in the intensity value to the next time of decreases in the intensity value were calculated. Their value was named as "contraction phase" (C).

B. Relaxation phase.

Similarly, "relaxation phase" (R), the mean value of the time intervals of relaxation phase, was obtained.

C. Frequency of contractions.

Frequency of the spontaneous contraction was expressed as the reciprocal of the time sum of "contraction phase" (C) and "relaxation phase" (R), as follows;

$$F = \frac{1}{C+R}$$

(3) Ionic experiment.

As the experimental media, the sea water which contains isotonic K-ions, Ca-ions or Mg-ions excessively in volume ratio was used. Each concentration of K-, Ca-, or Mg-ions in the sea water was four times, eight times or sixteen times respectively of the concentration of those ions in Herbst's artificial sea water. pH was adjusted to 8.2 by 0.05% $NaHCO_3$. Each solution was applied simply by the use of inlet and outlet pipette after the sea water had been drawn off so as to leave the acontium on the slide glass with a negligible amount of adhering sea water. The observation of the response was performed under the microscope ($\times 20$) for 30 minutes continuously.

(4) Electric stimulation.

An electric stimulator (MSE-20, made by Nihonkoden Co.) and a pair of Ag-AgCl electrodes were used for electric stimulation. The proximal part of the acontial filament (15 mm length) was attached to the anode and the adjacent part of the filament was attached to the cathode out of the sea water. Thus, the suspended acontia in sea water filled in a transparent small bath were directly observed for 10 minutes after application of the electric shocks.

(5) Morphological observation.

To recognize the site of muscle of the acontial filament, it was fixed with Bouin's fluid. Paraffin sections were cut 3–4 $\mu$ thick and Heidenhain's Azan stain (Pantin, 1946) and Hansen's Haematoxylin-eosin stain were used.

## Results

(1) *Spontaneous activity.*

A. Attached acontia.

When the *Metridium* is stimulated mechanically, many acontia were protruded through the cinclides or mouth and then the protruded acontia become down along the body wall. These acontia responded quickly with coiling to the external stimulation as stretching, cutting, and agitation by jet current of sea water but they were kept quiescently after 10 minutes or so, and they exhibit spontaneous contractions in rather irregular intervals. In any case, all the protruded acontia were again housed in the gastric cavity of the *Metridium* after about one hour from the beginning of the semi-rhythmic spontaneous contraction.

B. Isolated acontia.

Even when the isolated acontial filaments were immersed freely in sea water on the glass slide, they showed spontaneous contractions.

When long pieces of the filament were used to observe the spontaneous contractions, complex movement, knotting or snarling, were observed. After about 10 minutes in sea water, the strong knot or snarl became loose knot or snarl but the complex movement continued spontaneously.

The simple form of the spontaneous contraction, bending or coiling, was observed when short pieces of the filament (2–4 mm) were used which were cut from a long piece of the acontial filament.

An example of the simple form is shown in Fig. 3. In this case, rotation or forward movement of the pieces was observed which was induced by ciliary movement.

The each mean value of intensity and frequency of the spontaneous contraction obtained from five short pieces is given in Table 1 and the intensity time curve obtained from a piece is shown in Fig. 4.

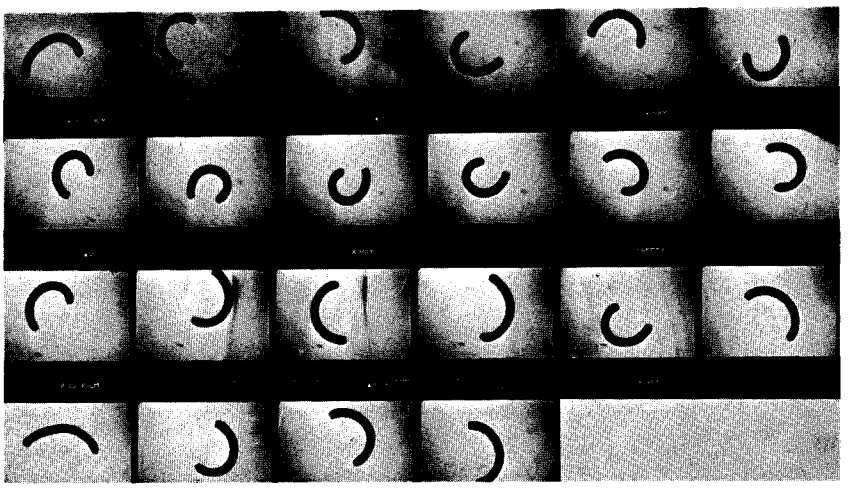

Fig. 3.

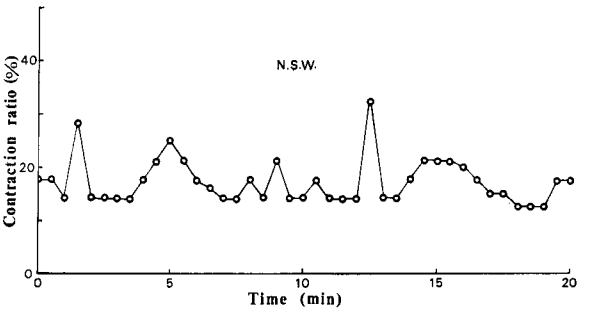


Fig. 4. The spontaneous contraction of the acontial piece (3 mm. in length) in normal sea water.

Table 1. Frequency and intensity of spontaneous contraction. Mean values were obtained from the five acontia. Temp. 16.5°C

| | Relaxation phase | Contraction phase |
|---|---|---|
| Frequency 1/119/sec | 66 sec | 53 sec |
| Intensity (contraction ratio) | 15% | 22.6% |

(2) *Ionic experiment.*

A. Effect of sea water containing excess Ca-ions.

The effects of excess calcium on the spontaneous contractions were shown in enhancement of their intensity. As is seen in Fig. 5, the intensity of contraction of the piece in the normal sea water was rather small but immersion in $16 \times CaCl_2$ sea water immediately caused strong contractions and produced a tendency to increase the frequency of contraction. $4 \times CaCl_2$ sea water did not show visible effect on the contraction but $8 \times CaCl_2$ sea water caused rather strong contractions.

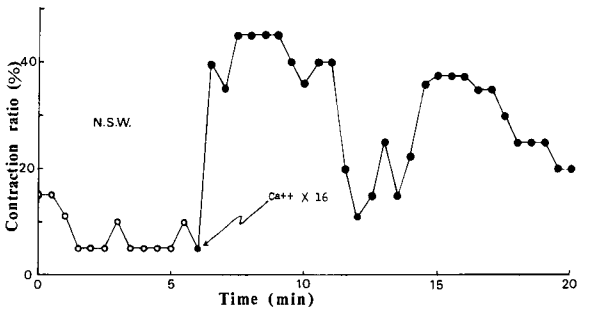


Fig. 5. The effect of Ca-ions on the spontaneous contraction of the acontial piece (3 mm).

B. Effects of sea water containing excess Mg-ions.

Both of the spontaneous contractions and the ciliary movements were completely abolished quickly (about within 20 seconds) with $16 \times$ Mg-ion sea water. These effects were reversible. When the acontial pieces were transfered in the normal sea water, after treatment in the excess Mg-ion sea water for 20 minutes, both of the spontaneous contractions and the ciliary movements recovered the normal state in 5–10 minutes. Effects of $8 \times$Mg-ions-sea water were weaker than those of $16 \times$Mg-ions-sea water, and the rotation or locomotion was kept still slightly but the intensity and frequency of the spontaneous contraction were decreased, as is shown in Fig. 6.

C. Effects of sea water containing excess K-ions.

$4 \times$K-ions sea water had no detectable effects but the effect of $8 \times$ K-ions sea water was seen as a tendency to induce relaxation of the contraction. After about 2 minutes of application of the experimental solution, the coiling or bending of the acontial pieces was enhanced, however, after several minutes, such activities decreased, and so weak local contraction was still continued.

(3) *Electric stimulation.*

The single electric square pulses of 1.0 millisecond duration were used in various degrees of the intensities (1–30 volts). In the all cases of 15 acontial

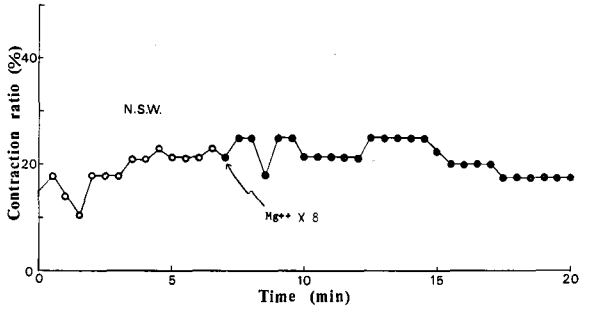


Fig. 6. The effect of Mg-ions on the spontaneous contraction of acontial piece (3 mm).

filaments examined at temperature between 23° and 15°C, the visible responses to each of the single electric stimuli were not observed at all in spite of the careful direct observation.

When the acontia were stimulated by repetitive square pulses of 1 millisecond duration at 10, 20 or 30 voltages at interval of one second for period of 30 seconds (Temp. 23°–26°C) the strong contraction was not induced. But, the slight contractile response of the acontial muscle in form of bending was observed only in 6 cases of 15 acontia examined, in 4 to 6 seconds after application of the repetitive pulses of 20 volts. The bending appeared at middle part of the filament and the form of roughly U-shaped of the acontial filaments was maintained for about 20 seconds. The relaxation of the contraction was started from the distal end and progressed to the proximal region of the filament.

(4) *Morphological observations.*

As can be seen in Fig. 7 (a, b), in the cross section of the acontium, the mesogloea is roughly T-shaped. The muscle fibres develop in the two sides of the joint of the stem of the T-shaped mesogloea (Fig. 7. b). It is confirmed that these muscle cells are of the primitive musculo-epithelial cells. Diameter of single muscle filament was calculated as 1 $\mu$ or so (Fig. 8. a, b). The striation of the muscle could not be recognized by the ordinal microscope or polarizing microscope observation.

In the longitudinal section, the measurement of the length of the muscle fibres was difficult since the boundary of the muscle fibres is not clear but sometimes the tapered ends of the muscle fibres were observed (Fig. 9, a, b).

Heidenhain's Haematoxylin-eosin staining was not suitable to distingiuish the muscle fibre from the mesogloea. Both of the mesogloea and muscle fibres are stained in pink. Azan staining, however, is satisfactory for the present purpose. The mesogloea was stained in sharp blue and muscle fibres were stained in red.

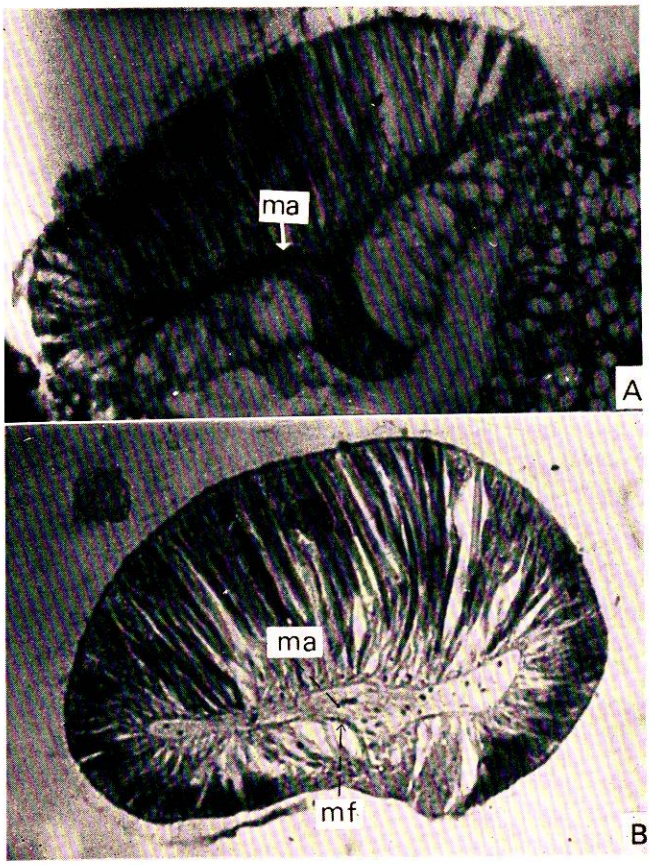


Fig. 7. The photographs representing two types of mesogloeal axis in cross section of the acontium. A: Roughly T-shaped mesogloeal axis the width of which in direction of nematocyst-ciliated band was probably decreased by shrinkage in fixation, so that muscle fibres could not be recognized clearly. B: The mesogloeal axis having short stem of the T-shaped mesogloea. Muscle fibres develop at the two side of the joint of the stem. The empty spaces under the muscle fibres are probably artifact. ma: mesogloeal axis; mf: muscle fibre. magnification: ×250.

## Discussion

(1) Site of spontaneity

The effects of Mg-ion rich sea water are recognized as to depress the spontaneous activity. In the Mg-ion excess solution, the contraction intensity gradually decreased but ciliary movements still continued though rather weakly. Ross and Pantin (1940) reported in *Metridium* mesentery that Mg-ions inhibited

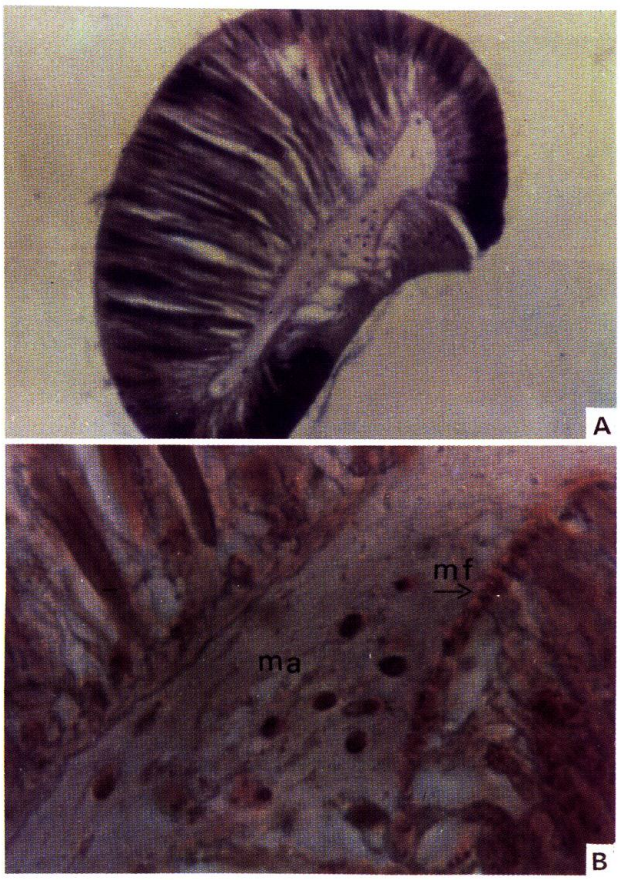


Fig. 8. Photographs representing the single layer of the muscle fibres in cross section of the acontium. A: Microscopical photograph in low power (×250). B: Enlarged photograph (×1000) of the same preparation. ma: mesogloeal axis; mf: muscle fibre. The muscle fibres form a single layer and each muscle fibre of it is the basal part of a musculo-epithelial cell.

the transmission of impulse to the mesenteric muscle from nerve net as curarization effect in the striated muscle. In the acontial filament, the nerve net is not found but the presence of the nervous elements inducing the spontaneous contraction of the acontia may be suggested.

The result that the contraction was augmented by Ca-ion excess sea water also suggests that nervous element enhanced the contractile activity. Ross and Pantin (1940) pointed out that, in the responses of *Calliactis* sphincter, calcium seems to exert its effect at the neuromuscular junction. They obtained the results that excess calcium enhanced the facilitated response. Moreover, they

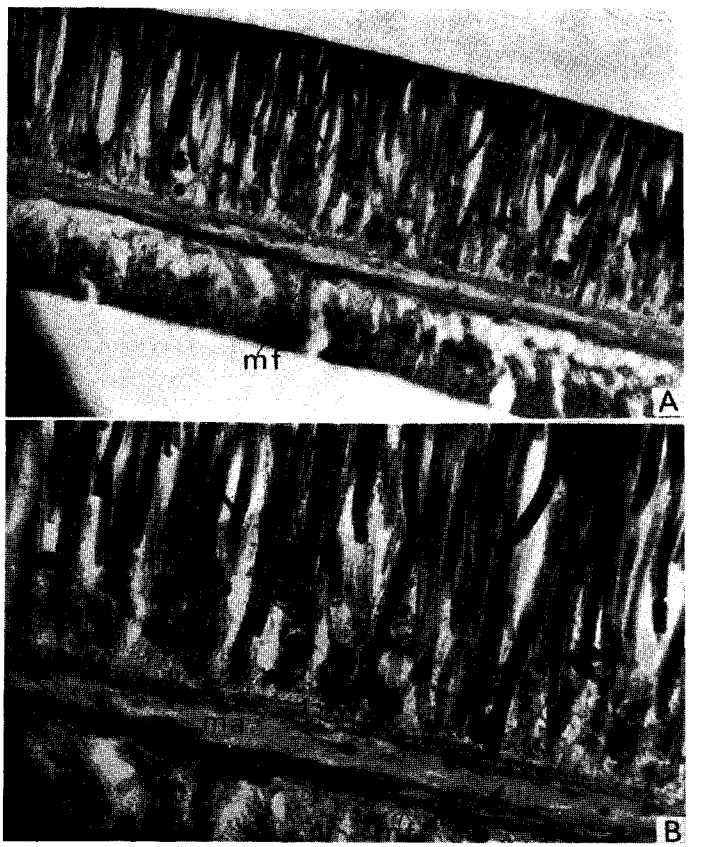


Fig. 9. The photographs representing longitudinal muscles of the acontium. The fibres are present in the form of tape-like strand. They develop on the side opposite to the array of cnidae contact with the mesogloea. A: Microscopical photograph in low power (×250). B: Enlarged photograph (×1000) of the same preparation. ma: mesogloeal axis; mf: muscle fibre.

mentioned that facilitated response of the muscles of sea anemones bears an analogy to that of vertebrate skeletal muscle during partial curarization. Tamasige (1953) reported that when a fresh frog muscle fibre without injury was immersed in a Ca-ion rich Ringer fluid, no visible effect appeared. Thus, Ca-ions have no direct effect inducing contraction of both of vertebrate and actinian muscles. Ionic effects on the spontaneous contractions of the acontial filaments suggest the nervous origin (which dose not mean the nerve net) of spontaneous contraction but morphological evidence is not completely assured because of technical difficulty in staining of nervous elements of the acontium.

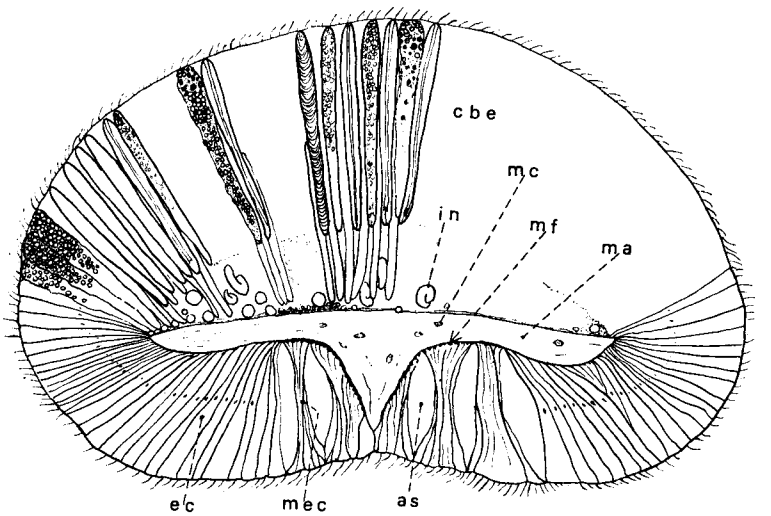


Fig. 10. The cross section of *Metridium* acontium. The muscle fibres are attached to the mesogloea, and retain connexion with epithelial cytoplasm. mf: muscle fibre; ma: mesogloeal axis; mec: musculo-epithelial cell; ec: epithelial cell; as: artifact space; mc: bipolar cell in the mesogloea; in: immature nematocyst; cbe: cnida-bearing epithelium containing two or three types of nematocysts and of gland cells.

The results that repetitive electric stimulation induced the contractions at times in spite of that a single shock was not effective, also suggest the neuromuscular facilitation in the acontial filament.

Parker (1916) could not observed the spontaneous contraction of the longitudinal muscles of the acontium and also he remarked absence of spontaneous activity in actinians. Batham and Pantin (1950), however, found that in *Metridium senile*, the muscular activity continued in the absence of external stimulation. They concluded that it was inherent. But they could not know what controled the rhythm of spontaneous muscular activity.

(2) Contractility of the acontial muscle.

Firstly, the present author was surprised to see the great ability of the acontial muscles to shorten. As in Fig. 4, the maximal contraction of the longitudinal muscle was up to 32% of its length of complete relaxation. How does the single layer of the fine muscle fibres shorten itself against the nematocysts band and mesogloea ?

Batham and Pantin (1951) showed that the contraction ratio of the body wall of *Metridium*, in either direction of it, was 80% of the maximal length and they concluded that it depended upon buckling of the circular muscle layer and of the superficial layer of mesogloea to which the muscle fibres attached. Moreover in the retractors, the permanent buckling of the muscle layer developed and the folds

of the layer in contraction of the muscle fibres could be exaggerated by temporal bucklings. They emphasized that buckling played an essential part in actinian mechanics.

On the morphological observation, the acontial muscle layer does not show the permanent buckling structure, but muscle fibres contact with the mesogloea and the appearance of the ciliated band is recognized to be a musculo-epithelial tissue (Figs. 8. B, 9. A and 10). Robson (1957) isolated musculo-epithelial cells from the mesenteries of the *Metridium senile* and pointed out the cells contribute both to the muscle-field above the mesogloea and to its overlying epithelium. Moreover, Batham (1960) showed that in *Metridium canum*, from electron microscopic study, each covering surface of a mesentery was a sheet of flagellated musculo-epithelial cells. Thus, it is suggested that the great ability to shorten the acontium also depends on the buckling of epithelial muscle and mesogloea with which the muscle fibres contact.

The very indistinct impression of the contractile activity of the acontial filament may depend on the slow contraction of the muscle. Chapman, Pantin and Robson (1962) emphasized that the actinian muscle which has larger fibres and a richer innervation can contract rapidly than the rest. In the acontial filament, nervous elements were not yet recognized, and it is suggested that the length of muscle fibres (see Fig. 9, B) was rather shroter than that of mesenteric retractor muscle fibres (about 1 mm) measured by Chapman et al. (1962).

It is concluded that the special features of the contractilily of the acontial muscles are very slow contraction and of a great ability to shorten. Further study of the buckling mechanism of the acontial muscle is necessary to clarify contractility of the acontial muscles.

## Summary

1. Direct observation on *Metridium* revealed that the isolated acontial piece shows a spontaneous activity. The activity was bending or coiling of a more or less regular rhythm with a period of the order of 3 min.

2. A few morphological and physiological evidences that the activity consists of a kind of muscular activity were obtained.

3. In cross section of the acontium, the single layer of the fine muscle fibres (the diameter is about 1 $\mu$) develops on the side "ciliated band" and contacts with the mesogloea. The situation of the muscle layer was at the position of 1/4 of the acontium diameter from the surface of the "ciliated band". The bending or coiling of the acontium depends on the presence of the muscle fibres.

4. The rate of spontaneous contraction was very large (the mean was 22.6 per cent, the maximum was 32 per cent).

5. The spontaneous contraction was augmented by Ca-ions but Mg-ions depressed the activity.

6. Electric stimulation was not always effective for inducing the contractile

responses whereas mechanical agitation or cutting was very effective.

7. The site of spontaneity and the contractility of the acontium are discussed by the results obtained.

## References

Batham, E. J. 1960. The fine structure of epithelium and mesogloea in a sea anemone. Quart. J. Micr. Sci. **101**: 481–485.

———— and C. F. A. Pantin 1950. Inherent activity in the sea-anemone, *Metridium senile*. J. Exp. Biol. **27**: 290–301.

———— and ———— 1951. The organization of the muscular system of *Metridium senile*. Quart. J. Micro. Sci. **92**: 27–54.

Carlgren, O. 1893. Studien über nordischen Actinien. Kongl. Svenska Ventenskaps-Akademies Handlingar. **25**: 1–148.

Chapman, D. M., C. F. A. Pantin and E. A. Robson 1962. Muscle in coelenterates. Rev. Canad. Biol. **21**: 267–278.

Hertwig, O. and R. Hertwig 1879. Die Actinien anatomische und histologische Untersuchungen mit besonderer Berücksichtigung des Nervenmuskelsystems Jena. Zeit. **13**: 457–640.

Pantin, C. F. A. 1946. Notes on microscopical technique for zoologists. Cambridge.

———— 1952. The elementary nervous system. Proc. Roy. Soc. **140**: 147–168.

Parker, G. H. 1916. The effector systems of Actinians. J. Exp. Zool. **21**: 461–484.

———— and E. G. Titus 1916. The structure of *Medtridium actinoloba marginatum* Milne-Edwards, with special reference to its neuro-muscular mechanism. J. Exp. Biol. **21**: 433–458.

Robson, E. A. 1957. The structure and hydromechanics of the musculo-epithelium in Metridium. Quart. J. Micr. Sci. **98**: 265–278.

Ross, D. M. and C. F. A. Pantin 1940. Factors influencing facilitation in Actinozoa. The action of certain ions. J. Exp. Biol. **17**: 61–73.

Tamasige, M. 1953. Relation between mechanical response and membrane resistance of single muscle fibre. Jap. J. Zool. **11**: 101–115.

Yanagita, T. M. and T. Wada 1959. Physiological mechanism of nematocysts responses in sea anemone. VI. A note on the microscopical structure of acontium, with special reference to the situation of cnidae within its surface. Cytologia **24**: 81–97.