Light and Electron Microscopy of a Muscle from *Diplodon variabilis* Maton

BY

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(2 Plates)

INTRODUCTION

THE ULTRASTRUCTURE OF MOLLUSC MUSCLE has been described by COHEN *et al.* (1971), MCKENNA *et al.* (1973), RICHARDOT *et al.* (1971), SZENT-GYORGYI *et al.* (1971), WILSON (1969), ZS-NAGY *et al.* (1971), PLESH (1977). Although there exist many papers that study muscle ultrastructure in other invertebrates [DEWEY *et al.* (1973), JENSEN *et al.* (1975), KRYVI (1971, 1973), ROSENBLUTH (1968) and SMITH *et al.* (1973)] to our knowledge, no investigations have been made on *Diplodon variabilis*, which is an autochthonic species of Argentina.

This paper presents new findings of the structure of the muscle of two organs of the bivalve *Diplodon variabilis* at the light and electron microscopic levels. For our study we chose the musculature of the foot and the muscle tissue of the mantle, both showing the same features.

MATERIAL AND METHODS

Young and adult *Diplodon variabilis* clams were collected from the rivulet Miguelin-Punta Lara, Buenos Aires and were immediately dissected, after making a gonadal puncture in order to determine the sex. For light microscopic study, pieces of the foot and mantle were separated from the living animal and fixed in Bouin's and Carnoy's fluids. The material was dehydrated in ethanol, embedded in paraffin and sections (6μm) were stained with hematoxylin-eosin and iron-hematoxylin. For electron microscopic study, small pieces of the foot and mantle were fixed in Millonig's fluid for 120 minutes at 0°C. Specimens were dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained with uranyl-acetate and lead citrate and examined with an Elmiskop I electron microscope operated at 60 KV.

RESULTS

No structural or ultrastructural differences were observed between the foot and mantle muscular tissue. The muscular fibres are disposed in bundles. They are present in both faces of the mantle, valvar and pallial (Figure *1*) and the bundles penetrate into the "roundlet" [the distal part of the ventral border of the mantle]. In the foot, the sections of the muscle are always organized in bundles (Figure *2, 4*).

The muscular cells have large multiple peripheric nuclei (Figure *3*). With the iron-hematoxylin technique, as well as with the phase contrast microscopy, we have observed longitudinal striation (Figure *4*).

At the ultrastructural level, the muscle cells are seen as somewhat irregular in shape and show flattened peripheral nuclei which contain dense masses of chromatin condensed along the nuclear membrane. (Figure *5*). A thin basement membrane surrounds each muscle cell.

Most of the cytoplasm is occupied by myofilaments, which are arranged parallel to the axis of the cell (Figure *6*). The myofilaments are variable in diameter—thick and thin ones can be seen without any constant relationship occurring between them (Figure *7*). It should be remarked that it is possible to observe myofilaments of intermediate thickness. All the myofilaments show an oblique striation,

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due to the presence of alternating light and dark bands (Figure *8*). The clear bands are approximately twice as wide as the dark ones. Electron opaque material seems to radiate from the border of the dark bands into the cytoplasmic matrix (Figure *9*). Myofilaments are embedded in a somewhat lighter matrix, containing variable amounts of alpha and beta glycogen granules (Figure *7*).

The periphery of the fiber presents vesicles of granular endoplasmic reticulum and numerous free ribosomes. Round mitochondria are located close to the cell membrane. The mitochondrial matrix is dense and the cristae, often dilated, are irregularly arranged (Figure *5*). Collagen fibrils and unmyelinated axons are found between muscle cells (Figure *10*).

DISCUSSION

In the last several years the fine structure of the muscle of numerous invertebrates has been the focus of widespread interest. In this respect, there is a vast bibliography on the subject. DEWEY (1968; 1973), JENSEN *et al.* (1975), KRYVI (1971), ROSENBLUTH (1972).

The perfect symmetry of the mammalian muscle does not exist in molluscs, although in some species of molluscs there is some degree of symmetry.

Studying the mantle ultrastructure of the pelecypod *Spisula solidissima*, WILSON (1969) describes only thick filaments in the "non striated" muscle. The figures that the author shows much resemble those we have obtained in *Diplodon variabilis*, but he presents low magnification photographs which do not reveal transversal striation.

MCKENNA & ROSENBLUTH (1973) have studied the electron microscopy of the retractor muscle of the "byssus" of two bivalve molluscs: *Mytilus edulis* and *Brachidontes comissus*. Although this work is mainly concerned with the myoneural and intermuscular joints, the pictures very clearly revealed the existence of a transversal striation in the myofilaments. COHEN *et al.* (1971), taking into account the 145 Å periodicity described in the "non striated" mollusc muscle, which is repeated at 925 Å, had fractionated the paramyosin. They found that aggregates of this substance have a 725 Å periodicity and concluded that paramyosin would form an axis covered with myosin.

JENSEN & MYKLEBUST (1975) have reported differences between the muscle of "body and vessels" from a pogonophore, *Siboglinum fiordicum*. Both muscle types have thick and thin filaments of variable diameter, but only the thick filaments of the body myocells show a clear periodicity. The authors assumed that in the muscle of the blood vessels, among the thick filaments, those which are smaller in diameter and without striation, would be similar to

Explanation of Figures *1* to *5*

Figure *1*: Light micrograph of mantle muscle fibers. Hematoxylin-eosyn × 250
v—valvar face, p—pallial face, r—roundlet

Figure *2*: Light micrograph of the foot: basal region. Hematoxylin-eosyn × 630
b—muscle bundles

Figure *3*: Light micrograph of foot muscular cells. Iron-hematoxylin × 400
n—nucleus

Figure *4*: Light micrograph of foot muscle bundles. Iron-hematoxylin × 1000
l—longitudinal striation

Figure *5*: Longitudinal section through mantle muscle cell, showing: × 10000
n—peripheral nucleus, M—mitochondria
bm—basement membrane, myofilaments (arrows)

Figure 1

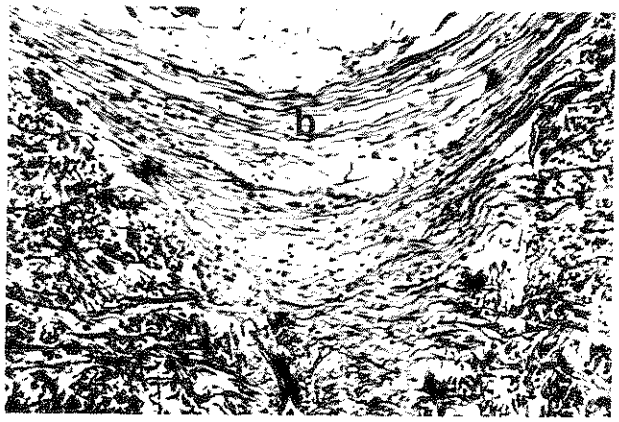

Figure 2

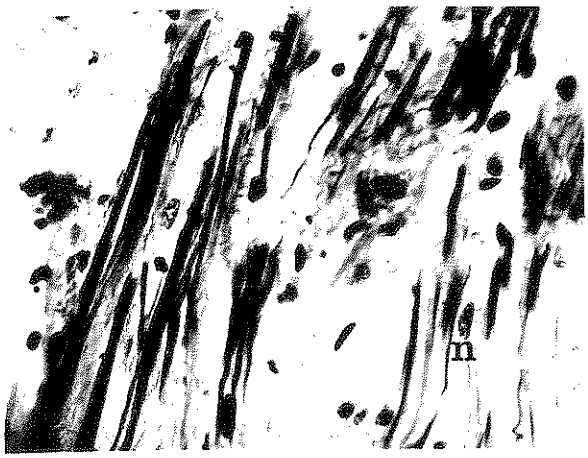

Figure 3

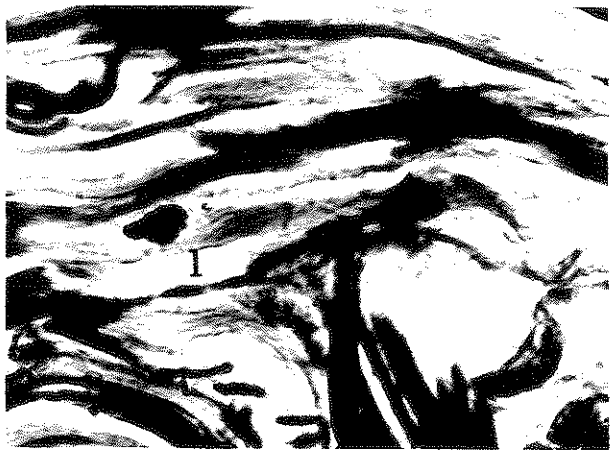

Figure 4

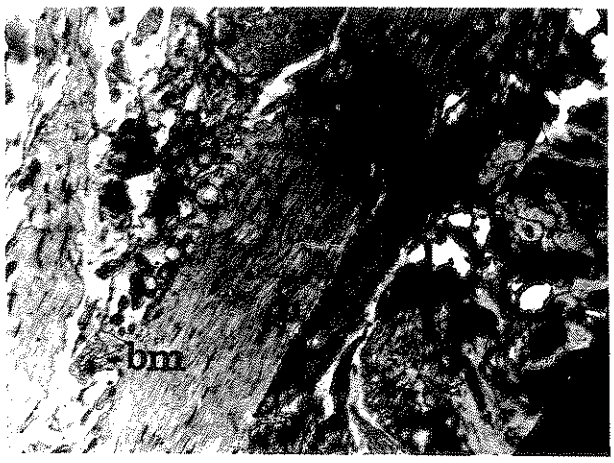

Figure 5

those of the heart and blood vessels from molluscs and annelids.

In the buccal bulb muscle of the mollusc *Ferrisia wantieri* (Richardot *et al.*, 1971) fibers have been described which are intermediate between striated and smooth type. These fibers resemble those of the embryonic myocardium of vertebrates. The figures that the authors show are similar in several aspects to the ones obtained by us from *Diplodon variabilis*. However, in *Ferrisia wantieri* discontinuous Z bands connecting myofibrils are present.

Paramyosin, thick striated myofilaments have been found in epithelial cells from annelids, where muscle could be considered as striated (Smith *et al.*, 1973). Other authors have described an "obliquely striated" muscle in annelids (Kryvi, 1971; Rosenbluth, 1968) and crab (Dewey, 1973; Rhea *et al.*, 1973) which seems to be a complex muscular tissue with a particular oblique striation, showing light and dark disks, presence of Z bands, and a well developed sarcoplasmic reticulum. It is interesting to note that in annelids (Kryvi, 1971) with "obliquely striated" muscle, images are shown where the filaments present an irregular distribution, similar to the one we have observed in some muscular cells of *Diplodon variabilis*.

Whether the sliding theory is acceptable or not for mollusc muscle has been a matter of discussion. Zs-Nagy *et al.* (1971) thoroughly discussed this subject and termed "polymorphous" the muscle that they consider to be neither striated nor smooth. This author studied the adductor muscle of the mollusc *Anodonta cygnea*, which is similar in many aspects to the *Diplodon variabilis* muscle, and accepts the independent contraction theory for this kind of tissue.

It can be concluded that the muscle from the *Diplodon variabilis* foot and mantle represents a particular muscular tissue which is rather similar to the one described in the adductor muscle of the *Anodonta cygnea*. As in that case, the denominations "striated" or "smooth" would not be suitable. In accordance with Zs-Nagy, (1971) we propose to name this type of muscle "polymorphous" and suggest that it, too, may contract by the mechanism of independent contraction.

SUMMARY

The histomorphology of the muscle of the mantle and foot of the nacreous clam *Diplodon variabilis* was studied by means of light and electron microscopy. The musculature of these organs shows a special muscle tissue, which is intermediate between the smooth and the striated type. Ultrastructural observation reveals two kinds of myofilaments, thick and thin, which show an irregular distribution. Both types of filaments have an oblique striation and present a regular periodicity. The results of our observation led us to consider this muscle as a "polymorphous" type.

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Literature Cited

Cohen, C., & A. G. Szent-Gyorgyi
1971. Paramyosin and the filaments of molluscan "catch" muscle. I. Paramyosin: structure and assembly. Journ. Mol. Biol. 56: 223-237

Dewey, M. M., R. S. C. Levine & D. E. Colflesh
1973. Structure of *Limulus* striated muscle. Journ. Cell Biol. 58: 574-593

Jensen, H. & R. Myklebust
1975 Ultrastructure of muscle cells in *Siboglinum fiordicum* (Pogonophora). Cell. Tiss. Res. 163(2): 185-197

Kryvi, H.
1971. The structure of the longitudinal muscles of *Sabella penicillum* (Annelida, Polychaeta), with a note on the contraction properties. Norw. Journ. Zool. 19: 149-167
1973. On the presence of sarcoplasmic reticulum in obliquely striated muscles from *Sabella penicillum* (Polychaeta). Journ. Submicr. Cytol. 5: 23-28

McKenna, O. C. & J. Rosenbluth
1973. Myoneural and Intermuscular junctions in a molluscan smooth muscle. Journ. Ultrastruct. Res. 42: 434-450

Plesh, B.
1977. An ultrastructural study of the musculature of the pond snail *Lymnaea stagnalis* (B). Cell. Tis. Res. 180: 317-340

Rhea, J. C. *et al.*
1973. Unusual striated thick filaments in *Limulus* skeletal muscle. Journ. Cell. Biol. 57: 591-593

Richardot, M. & J. Wautier
1971. Une structure intermédiare entre muscle lisse et muscle strié. Zeitschr. Zellforsch. 115: 100-109; 8 figs.

Rosenbluth, J.
1968. Obliquely striated muscle. IV. Sarcoplasmic reticulum, contractile apparatus, and endomysium of the body muscle of a Polichaete, *Glycera*, in relation to its speed. Journ. Cell. Biol. 36: 245-259

Smith, D. S. *et al.*
1973. Fine structure and innervation of an annelid muscle with the longest recorded sarcomere. Tissue & Cell. 5: 281-302

SZENT-GYORGYI, A. G., C. COHEN & J. KENDRICK-JONES
1971. Paramyosin and the filaments of molluscan "catch" muscles. II. Native filaments: isolation and characterization. Journ. Mol. Biol. 56: 239-258
WILSON, D. F.
1969. The basis for double contractions and slow relaxations of non-striated muscle in a pelecypod mantle. Comp. Biochem. Physiol. 29: 703-715
ZS-NAGY, I., *et al.*
1971. The contractile apparatus of the adductor muscles in *Anodonta cygnea* L. (Mollusca, Pelecypoda). Journ. Ultrastruct. Res. 37: 1-16

Explanation of Figures *6* to *10*

Figure *6:* Foot muscle cell showing numerous myofilaments (mf) × 15000

Figure *7:* Mantle cell muscle, showing thick myofilaments (T), thin myofilaments (t), and glycogen granules (G) × 45000

Figure *8:* Myofilaments showing an oblique striation × 80000

Figure *9:* Cross-section through thick (T) and thin (t) myofilaments × 80000

Figure *10:* Interstitial space between muscle cells (m) × 13000
c—collagen, a—unmyelinated axon