A NEW CONFIGURATION OF THE SARCOPLASMIC RETICULUM

WOLF H. FAHRENBACH. From the Oregon Regional Primate Research Center, Beaverton, Oregon

INTRODUCTION

In the course of the investigation of the cuticle of a number of lower Crustacea an extraordinary type of sarcoplasmic reticulum was encountered in the ostracod *Cypridopsis vidua* (O. F. Müller) 1776. This freshwater crustacean, about 0.5 mm in length, is enclosed by a bivalved, cuticular, and partially calcified carapace. These two valves are closed by an adductor muscle which has an array of myofilaments differing from that of other arthropodan muscles, a feature observed in a number of other crustaceans and currently the subject of further investigation. The appendicular muscles, with which the present study is concerned, radiate in various directions from cuticular infoldings of the body wall. The three-dimensional topography of the sarcoplasmic reticulum of all muscles of the animal is identical with the exception of the intestinal musculature, which contains no sarcoplasmic reticulum at all.

The literature dealing with ostracod muscles consists of three papers. Zenker (1) observed that many muscles are made up of a single fiber showing prominent transverse and very faint longitudinal striations, the latter presumably due to the sarcoplasmic reticulum. Zenker (1), as well as Daday (2) and Aronson (3), noted the great differences in sarcomere lengths of ostracod muscles, the last author making this peculiarity as well as vernier formations his principal subject of study.

MATERIALS AND METHODS

Cultures of the ostracod *C. vidua* were obtained from Powell Laboratories Division, Gladstone, Oregon. Animals were immersed for 2 hours in ice-cold 1.3 per cent osmium tetroxide buffered to pH 7.4 with *s*-collidine. Since the cuticle of the animal is unusually impervious to fixing solutions, it had to be gently broken by cutting a small portion of the carapace and body away without grossly disturbing the structure of the animal. The ostracods were then dehydrated through a series of 30, 50, 70, 85, 95 and 100 per cent cold alcohols, two changes of propylene oxide, and embedded in Araldite (4). Sagittal sections of major portions of ostracods were cut with glass knives on an LKB Ultrotome, picked up on uncoated, 400-mesh grids, doubly stained with uranyl acetate and lead citrate (5), and lightly carbon-coated. Electron micrographs were taken with the RCA EMU3-F electron microscope.

RESULTS AND DISCUSSION

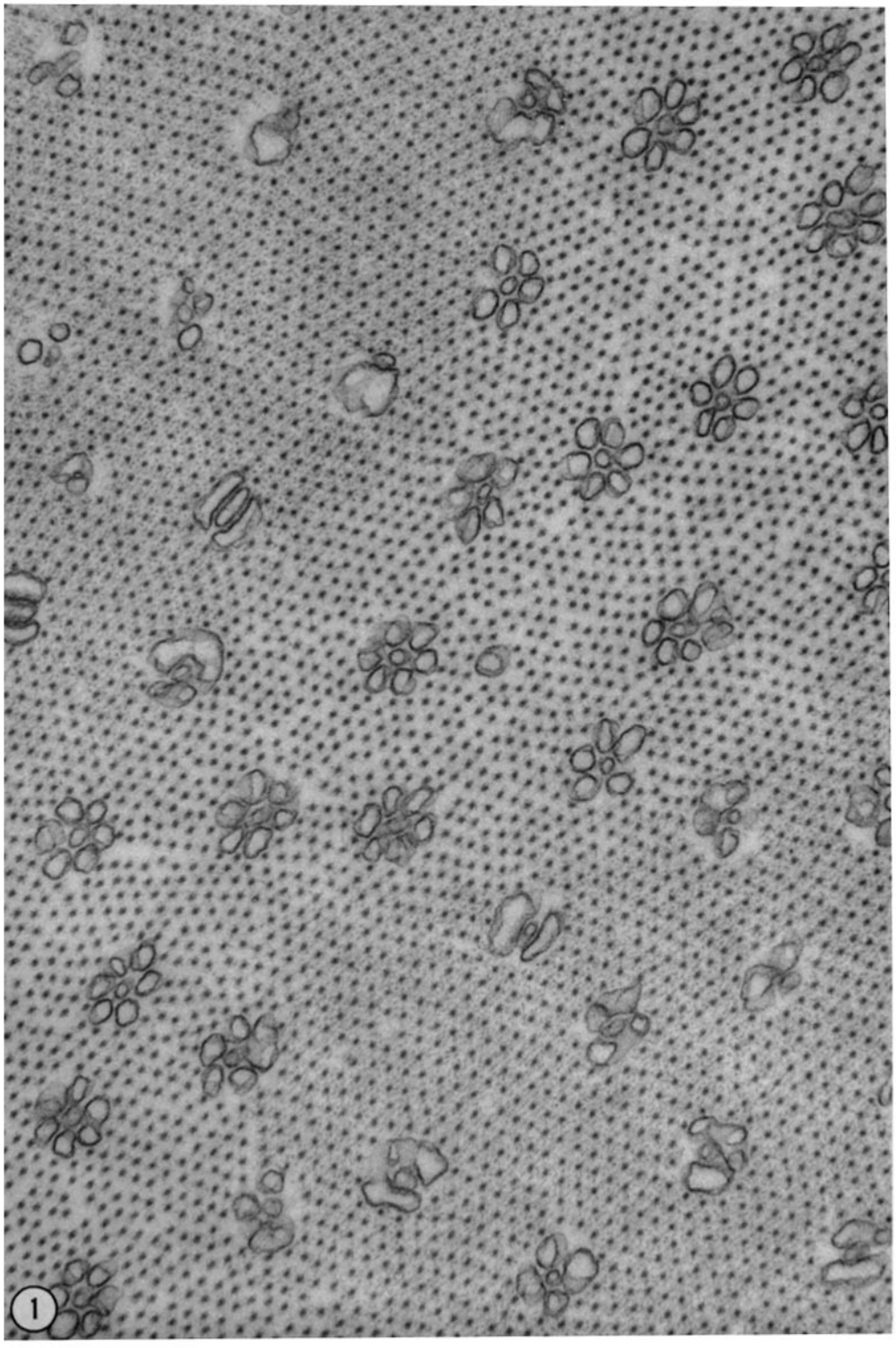
The major muscles of the appendages measure between 10 μ and 50 μ in cross-section, each muscle generally being made up of a single fiber. Furthermore, each fiber constitutes the equivalent of a single myofibril, since the usual internal subdivision into myofibrils is absent (Fig. 1). Small mitochondria, about 1 μ in diameter, commonly lie in register with adjacent sarcomeres of similar small dimensions, while muscles with longer sarcomeres have mitochondria of 5 μ or greater length in irregular array in the peripheral sarcoplasm. Glycogen granules are sparsely distributed between the mitochondria. Sarcomere lengths vary through a wide range, as observed by Aronson (3), the extremes of the A band (*i.e.* myosin filament length) extending from just under 1 μ to about 8 μ. Vernier dislocations are frequent and intramyofibrillar as a consequence of the muscle

fine structure. Nuclei are situated in the peripheral sarcoplasm. No neuromuscular junctions have been encountered.

The sarcoplasmic reticulum is predominantly longitudinally oriented, as appears to be the rule for crustaceans (Fig. 2). The course taken by the intermediate element is similar to that described for the copepod *Macrocyclops albidus* (6). From the sarcolemma, which is covered by a thin basement lamina, tubules penetrate into the fiber at the level of the Z line and branch to run longitudinally between the myofilaments. These tubules form the intermediate elements of the sarcoplasmic units to be described. In *Cypridopsis* all transverse connections run to either side of the Z line rather than in the substance of the Z line itself. On entering a sarcomere in a longitudinal direction, the intermediate element is joined by one or more cisternal elements, to be referred to as sarcotubules. Thus a dyad or, more usually, a triad is formed, although tetrads and pentads are not uncommon (Fig. 1). In all of these and more complex configurations of the sarcoplasmic reticulum, the intermediate element retains a central position, while the paralleling longitudinal sarcotubules group themselves more or less symmetrically about it (Fig. 1). The intermediate element and adjacent membranes of the sarcotubules show an increased electron opacity in the region of the A-I junction (Fig. 2). This differentiated area, which has been discussed in an earlier publication (6), is indicative of a probable physiological interaction between the structures involved. In the same area of the sarcomere, that is, near the edge of the A band, the sarcotubules branch and rebranch, often producing cisternal dilatations at the points of bifurcation. The bundle of sarcotubules and central intermediate element then traverses the H band as a pentad, hexad or heptad. The latter structure consists of the intermediate element and 6 sarcotubules and is the most frequent configuration found in the H band. Octads are not uncommon (Fig. 1), but enneads, composed of 9 profiles, are quite rare. In one instance, two adjacent sarcotubular units fused, producing a structure composed of 2 intermediate elements and 9 sarcotubules, hence a hendecad. On leaving the H band, the sarcotubules fuse with each other in the manner described above. To either side of the Z line they branch out transversely in a rather profuse manner. Fig. 2 shows only a few of such transverse branches. No pores, such as have been described by Franzini-Armstrong (7), have been observed in the cisternal elements. At the myocuticular junction the sarcotubules end blindly, while the intermediate element becomes continuous with the sarcolemma.

Two peculiarities of the muscle described here are particularly noteworthy, namely, the large myofibrillar size; *i.e.*, lack of subdivision of the muscle fiber, and the high development of the cisternal elements (sarcotubules) of the sarcoplasmic reticulum. The distribution of the intermediate element does not deviate strikingly from the pattern to be expected in crustaceans, nor is there any reason to assume particularly different physiological characteristics of the muscles of ostracods, animals which are given neither to very fast nor habitually slow motions. However, a crustacean muscle of the size described here, not exhibiting special physiological features, would be expected to be subdivided into myofibrils by partitions composed largely of cisternal components of the sarcoplasmic reticulum. Hence, a large percentage of the cisternae in a given volume of muscle would be contained in the interfibrillar partitions. In the absence of such partitions the ratio of cisternal volume to muscle volume might be maintained by an elaboration of the cisternal elements, as shown in the ostracod. Also, the spacing between the sarcotubular units averages

FIGURE 1 A cross-section through an appendicular muscle of *Cypridopsis vidua*. The section passes at a slight angle through part of one sarcomere, the H band extending diagonally across the picture from lower left to upper right, the rest of the field being occupied by the A band. The H band is principally occupied by heptads, one octad occurring near the upper right margin of the picture. At the edges of the H band transitional forms between heptad and hexad and several hexads are seen. The A band to either side of the H band is occupied by a pentad, a few tetrads, several triads, and one dyad. The intermediate element is the central and usually smallest profile of the sarcoplasmic units. × 48,500.



about 3000 A, leaving no contractile element farther than 1500 A to 1800 A distant from a sarcotubule. Such tight spacing is generally found only in very fast contracting muscles, and in the ostracod, in the probable absence of such contraction rates, it may represent a further compensation for the absence of the intermyofibrillar sarcoplasmic reticulum.

I am grateful to Dr. C. D. Evenson for identification of the species.

This study constitutes publication No. 29 from the Oregon Regional Primate Research Center, Beaverton, Oregon.

This study was supported by grant FR00163 from the National Institutes of Health, United States Public Health Service, to the Oregon Regional Primate Research Center, and partly by a grant from the Muscular Dystrophy Associations of America, Inc.

Received for publication, February 10, 1964.

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FIGURE 2 A longitudinal section through a small appendicular muscle of *Cypridopsis vidua*. The periphery of the single fiber is lined with mitochondria and some glycogen granules. Thick bundles of sarcotubules course in the muscle, appearing 3 to 4 and, occasionally, 5 tubules wide. In many places branching of the tubules both longitudinally and transversely to either side of the Z line can be seen (black arrows). Membranes of higher electron opacity (black and white arrows) are indicative of regions of apposition between the intermediate element and adjacent sarcotubules. × 19,000.