ULTRASTRUCTURAL CHARACTERIZATION OF *DAPHNIA* HEART MUSCLE

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The hearts of vertebrates are myogenic whereas those of most arthropods are neurogenic. However, the heart of *Daphnia* is an exception, as indicated by Bekker and Krijgsman (1) whose studies led to the conclusion that *Daphnia* heart is myogenic with apparent inhibition by extracardiac cholinergic nerves. Since *Daphnia* have hearts similar to vertebrate hearts and are transparent and easy to handle, this species is a useful one for the study of cardiac response to drugs. Viehoever and Cohen (2), and Sollman and Webb (3) have utilized *Daphnia* for this purpose.

Although cardiac function studies have been conducted for *Daphnia*, the lack of knowledge of the histology or ultrastructure of its heart has precluded any correlation of structure and function for this tissue. Cardiac musculature is reported to be striated in Arthropoda (4) but has not been characterized for *Daphnia*. Ultrastructural studies on the heart have not been carried out for any of the arthropods. Therefore, we set out to determine the ultrastructure of *Daphnia pulex* heart and specifically to determine whether it is striated.

MATERIALS AND METHODS

Daphnia pulex adults were fixed in 1% osmium tetroxide buffered in veronal-acetate with sucrose added. They were fixed intact, dehydrated, and embedded in Epon 812. Sections of the entire organism were cut at approximately 1 μ, stained with toluidine blue, and examined by light microscopy. In this way, it was possible to identify the heart and dissect or trim away all other tissue. Ultrathin sections were then cut for electron microscopy and adjacent thick sections were utilized for orientation.

RESULTS

The *Daphnia* heart is located dorsally, posterior to the brain, and is slightly flattened and elongated. It ranges from 0.2 to 0.4 mm in diameter in the fixed state and is very thin walled. By light microscopy, its wall appeared to be no more than one to three cell layers thick, and over much of its circumference it is only a single cell layer thick. It was not possible to characterize the cells in detail morphologically even when 1-μ sections were used. Nuclei are rare, most sections being devoid of them, while mitochondria are numerous and visible as small dense bodies.

Electron microscopy revealed the cells of the heart wall as long and thin and clearly established their subcellular structure. They are characterized by long striated myofibrils, an abundance of sarcoplasm, an irregular and often indented cell surface, and giant irregular mitochondria (Fig. 1). The heart wall varies in thickness from 2 to 50 μ, most of it being a single cell layer about 5 to 10 μ in thickness. At a few points, it was found to be four to five cells thick. There are openings in the heart wall where no cells or membranes exist, and these may represent ostia.

Striated myofibrils, 1 to 2 μ in diameter, were found in all cells but are not the most abundant cellular component, the mitochondria being the most striking feature. Myofibrils are all parallel within a single cell, and when cut longitudinally only one or two of them are present in the plane of section. When cut in cross-section, there are never more than four to five myofibrils randomly arranged across the width of the cell. Although they occasionally appeared to fuse or divide, it was rare for them to be closely packed and the usual arrangement was that of individual myofibrils separated by cytoplasm, including mitochondria and sarcoplasmic reticulum.

The myofibrils are cross-striated with a sarcomere length generally being 1.5 to 3.0 μ. The relationship of this variation to contraction stages is not evident from the material studied. The A bands and I bands are present as well as a dense Z band (Fig. 2). The I band is quite narrow under these conditions, being less than $\frac{1}{2}$ μ in most fibrils. There was no evidence of an H or M band in any of the many sections examined. When two fibrils are located side by side, the adjacent Z bands are aligned.

The myofibrils are composed of myofilaments of two different sizes. A small 50- to 60-A filament extends through the A and I bands, originating in the dense Z band and appearing to be continuous in the A band without being interupted to form an H brand. The large filaments are about 120 A in

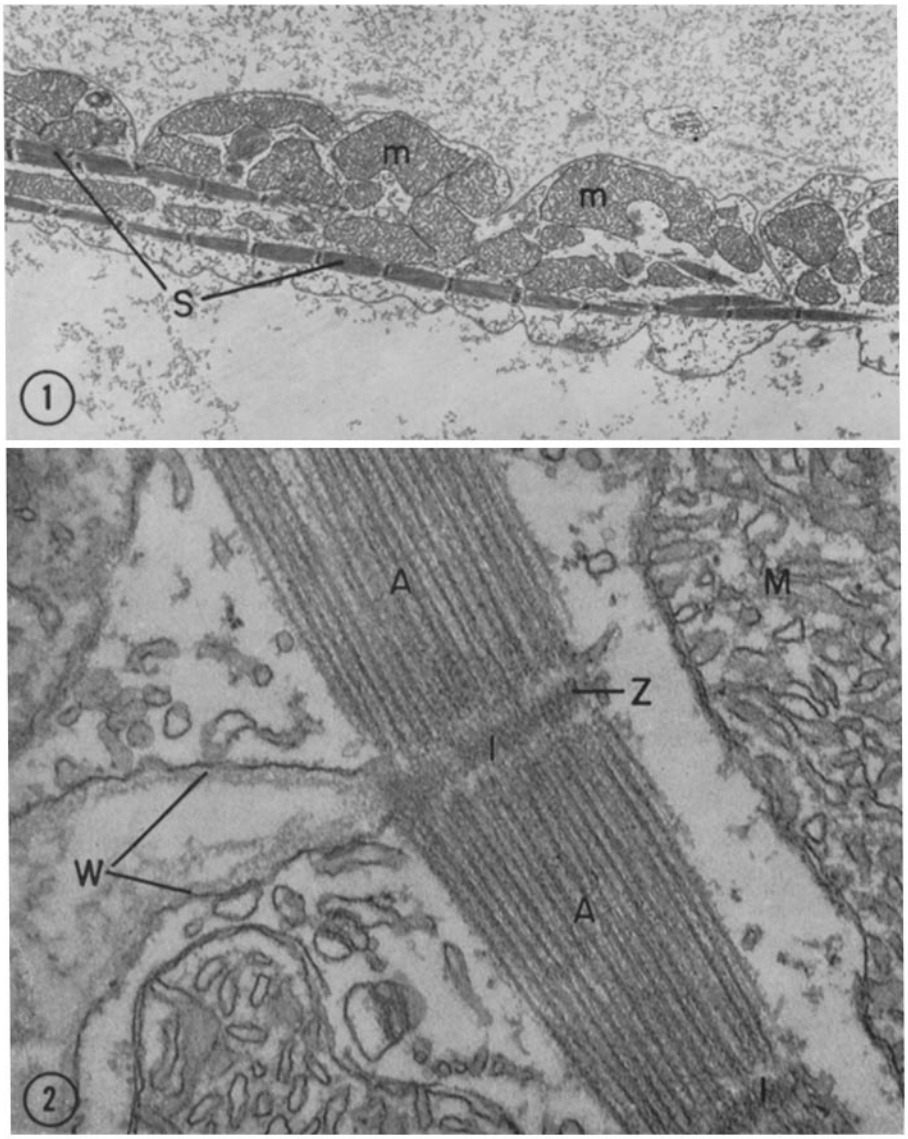

FIGURE 1 Electron micrograph of the wall of *Daphnia* heart, showing its single cell thickness. The wall contains several striated myofibrils (*S*) and numerous large mitochondria (*M*). × 3500.

FIGURE 2 Electron micrograph of striated muscle of *Daphnia* heart. Structures illustrated include A band (*A*), I band (*I*) with dense (*Z*) band, cell membrane (*W*) and mitochondria (*M*). × 43,000.

diameter and limited to the A band. Interdigitating filaments of these general sizes are reported for vertebrate and arthropod muscle (5). The filaments form a hexagonal pattern in cross-section, with interdigitation of the two filament types. The specific pattern of the hexagonal lattice has not been determined.

Giant mitochondria are the most prominent cellular component and they vary greatly in size and shape, often being 4 to 5 μ in diameter. Some single mitochondria are so large that they account for more than one-half of the width of the cell. Their shape varies greatly and most are irregular in outline with indentations and convolutions of

the surface. Because of their irregular shape, many of the smaller mitochondria may be projections from larger mitochondria in another plane of section. They are filled with tubular cristae, a variation from the situation seen in vertebrate muscle (Fig. 2). The prominence of large mitochondria with numerous cristae might be expected, in view of the rapid heart rate and the resulting energy requirements. Although the heart rate is generally reported as 120 to 450 beats per minute (6, 7) we find that a rate of 500 beats per minute is normal for adult *Daphnia* in our laboratory and for those found in fresh unpolluted water in this area. With drug stimulation, the *Daphnia* heart is capable of rates as high as 900 beats per minute.

The sarcoplasmic reticulum consists of a few scattered smooth vesicles and short tubules which are randomly located in the cytoplasm, without having any special localization near the myofibrils and any relationship to the I or Z bands as in vertebrate striated muscle. Ribosomes exist free, unattached to membrane surfaces, and are in the form of randomly scattered rosettes or clusters of rosettes. Both the vesicles of sarcoplasmic reticulum and the ribosomes are loosely scattered in a cytoplasm that is homogeneous and of low density. None of the components are tightly packed as in other types of muscle. The mitochondria and sarcoplasmic reticulum are not related to any part of the myofibrils although most of the mitochondria are located on one side of the cell and the myofibrils are near the other cell surface. This condition has no relation to the particular side of the cell—whether epicardial or endocardial—but varies from place to place along the heart wall. All of the large mitochondria are clumped in this way, although a few of the smaller mitochondria could be found any place in the cytoplasm, on either side of the myofibril or between myofibrils when several are present.

The cell wall is quite convoluted, with its contour generally following that of the masses of mitochondria or that of a single large mitochondrion. At various intervals, the cell membrane dips deeply into the cytoplasm to approach a Z band or to touch it (Fig. 2). This condition represents the only specific relationship between a cell component and a specific site along the striated fibril and may correspond to the orientation of the sarcoplasmic reticulum in vertebrate cardiac muscle and skeletal muscle. A basement membrane is present at both the epicardial and endocardial surfaces of the cell.

DISCUSSION

The structural features of the *Daphnia* heart are in many respects similar to those of striated and cardiac muscle of other species and support the belief that *Daphnia* may be a useful species for the study of cardiac function as influenced by drugs. There are some unique features that make this heart especially interesting, among them being that the heart wall is only one cell thick over most of its circumference and that this cell forms both the epicardial and endocardial surfaces of the heart. The mitochondria are large and are located near the fluids of the circulatory system, only scanty cytoplasm and one cell membrane intervening. This may prove interesting in the comparison of variations in function and structure of these mitochondria as influenced by drugs. We now have work in progress to correlate structural changes with drug activity.

SUMMARY

The structure of *Daphnia pulex* heart was studied by means of light and electron microscopy. The heart wall, for the most part, is one cell thick, with occasional regions being four or five cells in thickness. These cells are quite thin and contain many giant mitochondria, long striated myofibrils, an abundance of sarcoplasm, and an irregularly indented cell surface. The striated myofibrils are composed of filaments of two sizes, 50 to 60 A and 120 A, the former forming A and I bands with a prominent Z band but no H or M bands, and the latter being limited to the A bands.

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