Electron Microscopic Studies of Satellite Cells in the Cardiac Muscle of Brachyura*

By

Minoru Midsukami

Department of Anatomy, School of Medicine, University of Kanazawa, Kanazawa, Japan

(Director: Prof. Dr. Ryohei Honjin)

During the course of an electron microscopic study of the skeletal muscle fiber of frogs, Mauro (1961) discovered the presence of peculiar cells, lying between the plasma membrane and the basement membrane of the muscle fiber, and called them satellite cells. According to him, upon alerting other investigators to this finding, similar cells were found in electron micrographs of other skeletal muscle fibers. Mauro suggested that these cells might be pertinent to the regeneration of the skeletal muscle. The present paper deals with the fine structures of similar cells found in the brachyuran cardiac muscle and may help to solve some of the problems associated with the physiological phenomena of the heart.

Materials and Methods

Two species of adult edible crabs from the Japan Sea, Chionoecetes opilio O. Fabr icius (180-250 g) and Charybdis japonica A. Milne E d w ar d s (130-190 g), were used as materials. Both male and egg-bearing female animals were examined during the winter. After leaving the crabs in the aquarium for a few days, dissection and fixation were carried out as follows. The chelipeds and the first to fourth ambulatory legs were detached from the cephalo-thorax at each ischiopodite, and the dorsal carapace of the cardiac region was usually removed by cutting it off along the gastric, branchial, and intestinal region. Complete and rapid removal of the carapace is to be avoided, since the delicate pericardial membrane surrounding the

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pericardial cavity is located immediately beneath the carapace. After exposing the heart by careful incision, the beating heart was dissected free and lifted into a container of physiological solution for marine crab (25 g NaCl, 2.6 g KCl, 2.6 g CaCl₂, 1.6 g MgCl₂, 0.07 g NaHCO₃ per liter distilled water). The isolated heart was then opened longitudinally by an incision on the mid-ventral line and pinned out on a paraffin sheet. Then the cardiac muscle fibers were excised promptly and immersed in the fixative and cooled for 2 hours in a refrigerator. All samples were stirred moderately every 15 to 20 minutes during the fixing period. They were then dehydrated in a graded series of ethanol, and immersed in a mixture of equal parts of ethanol and embedding medium for 1 to 2 hours and in the pure embedding medium two or three times for 30 minutes respectively. At the final immersion the accelerator was dissolved into the medium. After being taken out from the refrigerator, the materials were embedded in gelatin capsules and polymerized in a 60°C oven for 48 hours.

The fixative used was a one per cent solution of osmium tetroxide in the physiological solution (cf. Midsukami, 1963). This gave as good preservation as when s-Collidine was employed as a basis for buffering fixative (Bennett and Luft, 1959). The embedding medium was epoxy resin, Epon 812 (Luft, 1961). In winter, the best result was obtained when solutions A and B were mixed in the ratio 3:2.

Silver and gray sections were made with glass knives in a Porter-Blum microtome and mounted on collodion coated grids, which were usually strengthened by evaporation of a thin layer of carbon. Contrast in the specimens was enhanced by staining with a saturated solution of uranyl acetate in distilled water for about 2 hours. After immersion the grids were ordinarily washed for several minutes in distilled water. Electron micrographs were made with a Hitachi HU-11 electron microscope at original magnifications of 3,000 to 50,000.

**Results**

Essentially the same condition was observed in the satellite cells of both crabs *Chionoecetes* and *Charybdis*. This cell has been overlooked hitherto, because of the striking paucity of cytoplasm relative to its nucleus. Moreover, in the multinucleated cardiac muscle cell of brachyurans, the nuclei are situated not only on the peripheral
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region but also in the central area, so that with a light microscope the nucleus of the satellite cell is not distinguishable from that of the muscle cell. The satellite cell is wedged between the plasma membrane and the basement membrane of the muscle fiber (Figs. 1 to 6). The surface of the muscle fiber is not distorted outward but instead this cell protrudes inward pushing aside the components of cardiac muscle cell. The plasma membrane of this cell is in apposition with that of the muscle fiber, being separated from the latter by a space of about 150 Å. This space is similar to that of the outer chamber of mitochondria lying in the sarcoplasm.

In these crabs the nuclei of the satellite cells, which are usually from 5 to 7 μ in length and spindly in shape, are rather sparsely scattered through the fiber, occurring only on the periphery of the fiber, whereas the nuclei of the cardiac muscle cells are from 7 to 9 μ in diameter and oval or spherical in shape, occurring at all depths within the fiber. The usual double nuclear envelope is present, the two components of which are separated by a gap of 250 to 350 Å. Pores are frequently seen in the nuclear envelope. Particles about 150 Å in diameter, presumably of RNP, are profusely spread over the outer membrane and are also found in the cytoplasm, some in the free state and others attached to the surface of isolated vesicles (Figs. 5 and 6).

On the outside surface of the satellite cell, the plasma membrane wholly lies immediately beneath the basement membrane of the sarcolemma, and is not reflexed into the cytoplasm. On the inside surface which abuts on the cardiac muscle cell, some desmosomal structures are occasionally observed between the plasma membrane of the satellite cell and that of the cardiac muscle cell (Figs. 5 and 6), where they appear as specialized regions, resembling the desmosomes or adhesion plates found in cells of the other types. This structure consists of three parallel dense layers separated by two less dense ones (Fig. 7). The total width of this region is about 70 mμ. The two outer layers are not defined by local thickening of the plasma membranes and appear to be much thinner than the attachment plaques described by Odland (1958) and Tamarkin and Sreebny (1963). The middle dense layer is similar in appearance to the intercellular contact layers described in human epidermal cells (Odland, 1958; Tamarkin and Sreebny, 1963), in frog mesothelial cells (Hama, 1960) and in mouse intestinal villous epithelium (Honjin et al., 1961). However, none of the more complex profiles, such as intermediate dense layer, light zone
in the center of the attachment plaque, and tonofilament striated layer, has been observed. Thus the desmosome in the present specimen appears to be homologous to the quintuple-layered cell interconnection reported by Karrer (1960) in human cervical epithelial cells and to the adhesion plates described by Smith (1963) in the photocytes of fireflies.

Moreover, two types of continuous structure between the plasma membrane of the cardiac muscle cell and the myofibril Z band were found on the inside surface of the satellite cell (Text-fig. and Fig. 4). One type was best seen at the place where a small aggregation of dense material was present. Such an aggregation of material has also been noted in sheep, rabbit and human myocardial cells (Simpson and Oertelis, 1961, 1962; Nelson and Benson, 1963). The other type was usually discerned in the region where some sarcoplasmic components were located between the plasma membrane and the myofibril. The latter element consists of delicate small tubule, identical in thickness of membrane and in diameter to the sarcoplasmic reticulum.

In addition to these, some spherical, very simply constructed profiles of mitochondria were observed in satellite cells (Figs. 1, 2 and 4). These mitochondria show a close structural similarity to those scattering throughout the sarcoplasm. However, the former is smaller in size and includes fewer cristae than the latter. On the other hand, isolated circular or somewhat elongated vesicles were also observed in the satellite cell (Figs. 5 and 6). The vesicle is bounded by a continuous membrane which appears to be appreciably thinner than that of the sarcoplasmic reticulum. The profiles of these vesicles vary greatly in size and form, and their distribution is not always uniform.

Discussion

It is noteworthy that the satellite cell of brachyuran cardiac muscle shows close structural similarity to that of frog skeletal muscle (tibialis anticus) described by Mauro (1961). According to him, although similar cells have been found by several investigators in other skeletal muscles of the frog, namely sartorius and ileofibularis, and in the sartorius and tongue muscle of the white rat, neither peripheral nucleus nor satellite cell has been demonstrated in the cardiac muscle. Thus Mauro states that the observation on cardiac muscle is consistent with the histological fact that in
cardiac cells the nuclei are located only centrally, and suggests that the apparent inability of cardiac muscle cells to regenerate may be due to the absence of satellite cells.

From a number of electron microscopic studies on the cellular structure of cardiac muscle, especially from vertebrates and even insects, it is well known that the cardiac cells have all their sarcoplasmic components restricted to areas defined by the individual plasma membranes of the cells, and are closely associated with one another through desmosomes and terminal bars or intercalated discs (Van Breemen, 1953; Sjöstrand and Andersson, 1954; Poche and Lindner, 1955; Muir, 1957, 1963; Moore and Ruska, 1957; Fawcett and Selby, 1958; Caesar, et al., 1958; Edwards and Challis, 1960). In Brachyura, however, careful examination of electron micrographs of cardiac muscle has failed to reveal either divided appearance or such adhesion structures (Midsukami, MS). On the other hand, physiological studies of the cardiac muscles of the shore crab Sesarma haematocheir (Irisawa et al., 1957) and of the closely related species Sesarma dehaani (Ochiai, 1959) have demonstrated that the action potential of these muscle fibers consists of the spike potential components and displays two types of marked features. One is a grouping of discharges during one heart beat, the appearance of which resembles that recorded in skeletal muscle fiber. The other is the appearance of remarkable after-depolarization analogous to plateau phase. The latter is the most conspicuous characteristic of the vertebrate cardiac muscle. On the basis of these facts, it can be stated that the structural organization of the crab heart muscle is similar to that of the skeletal muscle, but this neurogenic heart possesses physiologically the characteristics of both the cardiac muscle and the skeletal muscle in vertebrates.

Recently, Venable has encountered satellite cells in the skeletal muscle of afibrillar type (levator ani muscle) of the rat and mouse (personal communication). According to him, they are morphologically identical with the undifferentiated perivascular cell (pericyte) surrounding blood vessels in the stroma, and the pericytes closely resemble the muscle fibers indenting with the intervening external lamina which are absent in some areas and very thin in others. From this evidence he concludes that the pericyte and satellite cell are either identical or are functionally associated. Venable's presumption is of great significance, because the heart is basically a single-chambered sac or tube of striated muscle, which is embryo-
Text-fig. Semidiagrammatic reconstruction illustrating the peripheral region of a cardiac muscle fiber of the crab *Charybdis japonica*, based on electron micrographs. At the upper center, a satellite cell is wedged between the plasma membrane and the basement membrane of the muscle fiber. The apposing plasma membranes are closely associated with each other by means of desmosomal structures. On the inner border of the cell, the plasma membrane of the muscle cell communicates with the myofibril Z bands by its invagination or sarcoplasmic reticulum. To simplify the diagram, other elements have been omitted.
logically a specialized portion of the blood vessel of mesenchymal cell origin. Thus it appears that the diverse though still undifferentiated brachyuran heart is a transitional form between the blood vessel and the highly organized vertebrate heart. Further, if the satellite cells are indeed related to the vexing problem of muscle regeneration, as suggested by Mauro in the case of skeletal muscle, the cardiac muscle reported here may be capable of regeneration. However, as to the origin and function of these cells, there has been no advance in knowledge except the simple speculation by Mauro (1961). Unfortunately, the frequency of occurrence of these cells in the crab heart muscle fiber and the other skeletal muscle fibers cannot be estimated at present and it is impossible to compare it with that of the pericytes. Satisfactory solution of these problems requires further studies.

Since Huxley and Taylor's suggestion (1955) that in striated muscle each myofibril segment probably receives individual stimulus to contract, electron microscopic studies have indicated that the most likely pathway for such stimuli from the sarcolemma to the myofibrils is the sarcoplasmic reticulum, particularly its transverse tubular system (cf. Nelson and Benson, 1963). The tubular system has similarly been observed in the cardiac muscle of crabs, and it is particularly noteworthy that this system is also found in the place where the plasma membrane of cardiac muscle is protruded inward by the satellite cell. Accordingly, when the myofibrils are covered with the satellite cell, the intercellular space between the satellite cell and the cardiac cell seems to be a direct pathway for the transmission of activating impulses from the sarcolemma to the myofibril Z bands. The nature and rôle of the aggregation of dense material found at the point of contact of plasma membrane and Z band are not clear. Its apparent similarity to the desmosomes of other cell types suggests that one of its functions is purely mechanical adhesion: the others may include facile transmission of impulses to the Z band.

Summary

The fine structures of certain cells found in the peripheral region of the cardiac muscle fiber of two kinds of marine crabs, Chionoecetes opilio and Charybdis japonica, have been examined in detail, and these cells have been proved to be identical with the satellite cells described in vertebrate skeletal muscle fiber. The
following features have been observed.

1. The striking paucity of cytoplasm relative to its nucleus causes the cell to assume the shape of the nucleus.

2. The satellite cell is wedged between the plasma membrane and the basement membrane of the muscle fiber. The surface of the muscle fiber is not distorted outward, but instead this cell protrudes inward pushing aside the components of cardiac muscle cell.

3. On the outside surface of this cell, for most of its course, the plasma membrane lies just beneath the basement membrane of the sarcolemma, where it is not reflexed into the cytoplasm. On the inside surface, some desmosomal structures are occasionally seen between the plasma membrane of the satellite cell and that of the cardiac muscle cell.

4. Numerous particles, a few small mitochondria and some vesicles are seen in this cell.

5. Of greater interest is the evidence that the cells are identical in morphology with the undifferentiated pericytes surrounding blood vessels.

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References

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Explanation of Plates

Abbreviations

a, A band of myofibril; bm, basement membrane; ds, desmosome; mi, mitochondria; mp, plasma membrane of cardiac muscle cell; pmi, invagination of plasma membrane; sp, plasma membrane of satellite cell; sr, sarcoplasmic reticulum; z, Z band of myofibril.

Plate I

Figs. 1 and 2. Low-magnification electron micrographs of the satellite cells as they appear in the periphery of the cardiac muscle fiber of Charybdis japonica. Note that the cell is wedged between the plasma membrane of the muscle cell and the basement membrane. The plasma membrane of the satellite cell lies beneath the basement membrane, where it is not reflexed into the cytoplasm. The apposed plasma membranes can be seen on the inner border of the cell. ×7,000.

Fig. 3. An electron micrograph illustrating the satellite cell on the periphery of the cardiac muscle fiber of Chionoecetes opilio. Note the striking paucity of cytoplasm relative to its nucleus. As in Charybdis, the apposing plasma membranes of the satellite cell and the muscle cell are seen on the inner border of the satellite cell. The basement membrane is invaginated in part between the apposed plasma membranes as indicated by the arrow. ×12,500.

Fig. 4. An electron micrograph illustrating the satellite cell (Charybdis japonica). Two continuous structures between the plasma membrane of the cardiac muscle cell and the myofibril Z band are seen in this field. One type is seen at the invagination of the plasma membrane, the extremity of which is in contact with the Z band. The other type is discernible at the next Z band as indicated by the arrow. ×12,500.
Plate I

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Plate II

Figs. 5 and 6. Magnified view of the satellite cell shown in Fig. 2, indicating more clearly the cytoplasmic components and the characteristic regions occurring at the apposed plasma membranes. Numerous particles, a spherical profile of small mitochondrion and some vesicles of various sizes are seen within the cell. On the inner border of the cell, some desmosomal structures are seen. Even at this magnification, the middle dense layer is clearly distinguishable from the two outer ones. ×25,000.

Fig. 7. High-magnification micrograph of a triple-layered organization of a desmosome, such as those seen at a lower magnification in Figs. 5 and 6. This structure consists of three parallel dense layers separated by two less dense ones. Note that the two outer layers are not defined by local thickening of the plasma membranes. ×50,000.
Plate II

M. Midsukami