Ultrastructure of the Dog Cardiac Muscle Cell

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Observations on the cellular structure of the dog heart as seen in the electron microscope are described. Electron micrographs illustrate the ultrastructure of the dog myocardial cell, especially the relationships of the intercalated disc and the sarcolemma to form the cell boundaries and the relationships of the myofibrils and "Z" bands to the sarcolemma which result in an "accordion-like" appearance of the cell after contraction.

The intercalated disc has been the subject of much controversy in the study of the classical cardiac tissue section by optical microscopy. The primary debate was over whether or not they represented a cell boundary. The syncytial concept gained wide acceptance, primarily because it has been helpful in explaining the physiologic characteristics of heart muscle.

Little controversy now exists as to cellular nature of the mammalian heart. Several investigators offer much evidence in favor of the cellular theory. The present concept involves the interpretation of the intercalated disc as representing the transverse cardiac cell border. This report offers further evidence for the cellular nature of cardiac muscle and elaborates on the morphology of this cell in the dog.

Methods

Cardiac tissue taken from approximately 2-year-old dogs with normal electrocardiograms was cut into millimeter blocks and fixed for one half hour in buffered 2 per cent osmium tetroxide at pH 7.4. It was then dehydrated through graded isopropyl alcohols, phosphotungstic acid, 0.1 per cent, being added to the final 100 per cent alcohol. The material was embedded in a mixture of 90 per cent prepolymerized butylmethacrylate and 10 per cent prepolymerized methylmethacrylate, degassed to eliminate bubbling, polymerized in an oven at 60 C, and sectioned on a Porter-Blum ultramicrotome. All of the figures with the exception of 3 and 4 were taken with a Hitachi electron microscope, model HU-9. Figures 3 and 4 were taken with an RCA electron microscope, model EMU-3b.

Results and Discussion

The dog cardiac muscle cell has the appearance of a micro-accordion-like structure with all its cytoplasmic components geared to the compression-extension action of the cell. The cell is bordered transversely to the myofilaments by intercalated discs and longitudinally it is encased by the sarcolemma (figs. 1 and 6).

The intercalated disc is a plasma membrane, continuous with the sarcolemma. The disc forms a line of separation between muscle fibers, which were clearly demonstrated as noncontinuous across the disc (fig. 2). As observed by Selby, this phenomenon is present in the desmosomes of the epidermis. The filaments or tonofibrils of the Malpighian cells terminate at a plasma membrane similar in appearance to the intercalated disc. Inasmuch as comparable structures also occur in smooth muscle as termination points for muscle filaments, it would seem that intercalated discs represent a specialized type of cell boundary observed in conjunction with filamentous materials in various parts of the body. Contrary to earlier light microscope observations, the intercalated disc divides the cardiac tissue into cellular units in the embryo.

The contractile material is fastened along the margin of the cardiac intercalated discs by a dense substance, thus establishing a
Fig. 1 Top. Longitudinal section of myofibrils at the junction of cardiac cells. Two intercalated discs (ID) transverse the fibrils from sarcolemma to sarcolemma (SI). The filaments terminate in the dense material which compose the membrane. Note irregular path of the intercalated disc which "drops" two sarcomere levels across extreme right cell. "Z" band traverses cell from sarcolemma to sarcolemma crossing areas of sarcoplasm. Arrows, connections of "Z" bands with sarcolemma. × 10,500.

Fig. 2 Bottom. Longitudinal section of myofibrils through area of the intercalated disc (ID). The continuation of intercalated disc membrane with the plasma membrane of the sarcolemma (SI) is discernible (arrow 1). A connection between the inner plasma membrane of the sarcolemma and the "Z" band is evident (arrow 2). The plasma membrane of the intercalated disc (circled) appears to have folded areas or tube-like structures. The myofibrils do not cross the intercalated disc. × 29,800.
FIG. 3 Top. Longitudinal section through an area of the contracted cardiac muscle nucleus (N). Four fibers are observed between the nucleus and the sarcolemma. The "Z" bands traverse the cell from sarcolemma to sarcolemma and bridge the sarcoplasm between fibers (arrows). Usually one mitochondrium is observed in each sarcomere and these are compressed in the contracted cell. The compressed nucleus is surrounded by sarcoplasm with a number of mitochondria confined to corresponding sarcomere levels at one pole. X 18,750.

FIG. 4 Bottom. Longitudinal section through contracted cardiac muscle cell nucleus (N). The nucleus is surrounded by sarcoplasm and separated from the sarcolemma (SI) by 2 µm.

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relatively fixed position within the cell for the myofilaments. The plasma membrane of the intercalated disc has folded areas, or tube-like structures, the significance of which is at present unknown (fig. 2). The intercalated disc is formed in the area of the "Z" band, traversing the cell in a step-like manner, from one sarcomere level to another9 (fig. 1).

The longitudinal cell wall, the sarcolemma, is a double membrane, the inner layer of which has much greater density than the outer layer. Draper and Hodge,10 have proposed that the sarcolemma is composed of fibrils in layers over a basal membrane. There is no indication in our photographs that the external membrane of the dog cardiac muscle is fibrillar.

The more dense inner membrane folds into the sarcoplasm at the level of the "Z" band and comes into intimate contact with the "Z" band components, apparently as part of the tubular system of the endoplasmic reticulum (see Moore,9 Krauss and Van Antwerp,11 and figs. 1, 2, and 3). Figure 5 (lower portion) is a cross-section through the material that connects the sarcolemma to the "Z" band and it is evident that the connective system is tubular. Some micrographs suggest that the "Z" bands are comprised partially of a network of tubules. This tubular appearance is apparent in many areas where the "Z" bands serve as a functional unit, anchorage for the filaments, and consequently for the fibrils; and, because of their attachment to the fibrils, are pulled toward each other by contraction of the fibrils. The "Z" band attachment to the sarcolemma also maintains a certain rigidity at this level.

Since cell volume must remain constant, contraction of the cell forces the sarcoplasm against the sarcolemma between the "Z" band zones. The sarcolemma is slackened by contraction and can be forced outward by the pressures of the displaced sarcoplasm. The combination of these factors gives the contracted cell an accordion-like appearance.

A rather regular arrangement of tubules which are probably part of the abundant endoplasmic reticulum system,12,13 can be observed along the intracellular surface of the sarcolemma (figs. 2 and 5). The orientation of these tubules is such that they lie in close proximity to the inner membrane, and at right angles to it. The significance of the position of these tubules is uncertain, but some higher magnification micrographs show evidence of a connecting tubular system in the intercellular substance (fig. 2).

The dog cardiac muscle cell nucleus is usually displaced to one side of the cell and isolated from the sarcolemma by at least 1 myofibril (figs. 3, 4 and 6). The arrangement differs from skeletal muscle in which the nucleus is in contact with the sarcolemma. The nuclear membrane is double, the outer being less dense than the inner. The nucleolus of the dog cardiac cell is not homogeneous (fig. 4).

Consistent with the action of the muscle cell, the nucleus also folds in an accordion-like manner during contraction (figs. 3, 4 and 6), as evidenced by the fairly regular invaginations of the nuclear membrane.

Optical microscope studies of cardiac tissue, stained with Champy's procedure, indicate the presence of nerve tissue in areas of muscle fibers. The homogeneous nucleoplasm is contained within a nuclear membrane (N.M.). The nucleolus is not homogeneous. A large number of mitochondria are seen at the poles of the nucleus. A study of this electron micrograph indicates that the "Z" bands pass through the areas of sarcoplasm, between the mitochondria. At this level of magnification, the "Z" bands suggest a membranous-type structure although a vesicular or tubular arrangement is seen in certain areas, similar to the endoplasmic reticulum demonstrated by Palade and Porter (arrow). "M" and "H" bands are visible but the "Z" band has disappeared due to the contracted state of the cell. A capillary is visible in close proximity to the sarcoplasm. The dark bodies at one end of the nucleus are probably lipid in nature. X 18,750.
Fig. 5. Longitudinal mosaic of probable nonmyelinated nerve. The nerve parallels the muscle cell and appears to interdigitate with an invagination of the sarcolemma (asterisks). The extremely close proximity and the regularity with which it follows the configuration of the muscle cell indicates that it may be a neuromuscular junction. Large numbers of synaptic vesicles are visible in the cytoplasm of the fiber, some arranged in a peculiar rosette pattern (VR). A number of cross sections of relatively large tubules are visible in the area of the sarcolemma invaginations. The area between triangles is a cross section through the material connecting the "Z" bands to sarcolemma. The connection consists of a number of tubules of varying sizes. × 24,600.
Fig. 6. A longitudinal section through the cardiac muscle cell of the dog. The contracted cell is bounded transversely by an intercalated disc (ID) and longitudinally by the scalloped sarcolemma (SI). Irreversible contraction waves (GW) can be seen along both intercalated discs. The fibers frequently branch and anastomose with each other so that the fibril content of the muscle cell constitutes a continuous mass. The amount of sarcoplasm in the intrafibrillar space is small, with considerably more in the intrafiber spaces and beneath the sarcolemma. Mitochondria are especially numerous and tend to form columns between the fibers and between the fibers and sarcolemma. The nucleus (N) is separated from the sarcolemma by a number of fibers on each side and is surrounded by sarcoplasm with large numbers of mitochondria at its poles. The nucleolus is not visible. The "Z" bands appear to be firmly attached to the sarcolemma. A capillary is seen on the left of the muscle cell. X 7,500.
cardiac muscle, particularly adjacent to the capillaries. Demonstrations of nerve tissue with the electron microscope has proved more difficult. Fawcett and Selby,\textsuperscript{14} demonstrated nerve tissue in the atrium of the turtle, but a similar observation has not been made in the mammalian heart. Longitudinal sections of fibers appear in figure 5 and may possibly be unmyelinated nerve. The nerve runs in close proximity to the muscle cell. In fact, the close proximity and the apparent interdigitation with the invaginated sarcolemma of the muscle cell suggest that this may be a neuromuscular junction. A large number of vesicles, similar to those described by Fawcett and Selby\textsuperscript{14} and referred to by them as synaptic vesicles, is present. At irregular intervals such vesicles are observed and appear to be arranged in rosette patterns which occur too frequently to be attributed to chance orientation. The majority of the vesicles are arranged in a regular manner and lie in close proximity to the inner surface of the cell membrane, perpendicular to it. This arrangement and the appearance of the vesicles is similar to the endoplasmic reticulum of the muscle cells of the heart.

A longitudinal section of a complete muscle cell is seen in figure 6. From this micrograph it can be seen that the contracted cell is bound transversely by the intercalated discs and longitudinally by the scalloped sarcolemma. Irreversible contraction waves, similar to those reported by Van Breeman,\textsuperscript{8} appear near both intercalated discs. The outer surface of the sarcolemma is adjacent to sarcoplasm and an oblique section of a capillary can be observed in the sarcoplasm. Unlike skeletal muscle, the fibers frequently branch and anastomose with other fibers so that the fibril content of the cardiac muscle cell constitutes a continuous mass. The amount of sarcoplasm in the interfibrillar spaces is small with considerably more sarcoplasm in the perinuclear space and between the fibers and the sarcolemma. There is a tendency toward limitation of one mitochondrion per sarcomere.

The syncytial concept of cardiac muscle has correlated well with the physiologic observations regarding the propagation of excitation through the cardiac muscle. These physiologic observations may still hold true for the cellular tissue if we assume that the conduction from cell to cell would be due to the action of local currents and these currents could be conducted intracellularly by means of the endoplasmic reticulum.\textsuperscript{15,16}

**SUMMARY**

The cardiac muscle of the dog is cellular. The intercalated disc together with the plasma membrane of the sarcolemma form the cell boundary of the dog cardiac muscle cell.

The intercalated disc is a plasma membrane continuous with the sarcolemma. It forms a transverse line of separation between muscle fibers. The "Z" bands are connected to the sarcolemma by the tubular system of the endoplasmic reticulum which serves to anchor the "Z" bands to the sarcolemma.

The nucleus of the dog cardiac muscle cell is usually observed to be displaced to one side of the cell and isolated from the sarcolemma by at least one myofibril. The homogeneous nucleoplasm is contained by a double nuclear membrane. The nucleolus is non-homogeneous. Mitochondria are especially numerous. Structures in close proximity to the muscle cell have been tentatively identified as nonmyelinated nerve.

**Summario in Interlingua**

Le musculo cardiac del can es cellular. Le disco intercalate, insimul con le membrana de plasma del sarcolemma, forma le limite cellular.

Le disco intercalate es un membrana de plasma in continuitate con le sarcolemma. Illo forma un transverse line de separation inter le fibras muscular. Le si-appellate bandas Z es connectite con le sarcolemma per le sistema tubular del reticulo endoplasmic que servi a ancorar le bandas Z in le sarcolemma.
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Le nucleo del cellula cardio-muscular del can se trova usualmente displaciate a un latere e es isolate ab le sarcolemma per al minus un myofibrilla. Le homogene nucleo-plasma es continite in un duple membrana nuclear. Le nucleolo es non-homogene. Le mitochondrios es excepcionally numerose. Structuras in le directe vicinitate del cellula muscular es identificate tentativeraente como nervo non-myelinate.

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