Transmission electron microscopical investigations of the heart muscle cells of *lamellidens marginalis*.
The past several decades have witnessed a revolution in biological science as our understanding has been brought to the molecular level of organization. The advances that attended these developments came largely from the clever and ingenious biological application of instrumentation and methodology that originated in the laboratory of the physical scientist (Paul F. Agris, 1978). The introduction of the transmission electron microscope in biology in the early fifties of this century has completely revolutionized the structure of biological information. With time the preparative schedules for the biological fine structural studies have been perfected by the use of newer ultramicrotomes, fixation techniques, staining procedures and embedding methods. Associated with this progress in biological ultrastructural analysis were the intrusions of several newer designs in the microscopic electron-optical column itself. Among the animals without backbones, the members of the phylum Mollusca have not been adequately or properly investigated from the point of detailed submicroscopic analysis. However, quite a number of important cell biological thesis have been formulated with molluscan samples, like, the ciliated epithelia of bivalve forms (Sedar, et al., 1952; Fawcett and Porter, 1954),
muscles (Lacy and Horne, 1956), squid cartilage (Person and Philpott, 1962), cilia of the tentacle of Planorbis sp. (Millonig, 1957), 'neurosecretory elementary particles' in molluscan nerves (Berm, et al., 1961), etc. Further, the fine structures of the statocyst, optic gland of Octopus, and style-sac, etc. were becoming gradually apparent. Even in the field of molecular biology the biological materials from the molluscs (like, β-haemocyanine of Helix pomatia) have been ideal to decipher the intra-atomic/intra-molecular substructure of proteins (Van Bruggen, et al. 1962a and b).

The origin of true striated muscles in molluscs is a complex but sequential phenomenon (Nisbet and Plummer, 1963). The fibrillar organization of the molluscan muscles was established early by Philpott and his workers (1960). Among the early electron microscopists, North (1963) was a pioneer in elucidating the heart muscle structure of a mollusc. It was extremely difficult to prove during these years the existence of striations or periodic segmentations in the myofilaments in molluscan muscles, because of poor ultrastructural survey of the muscles from a wide variety of genera and species. It remains to be seen whether one can approach the problem either by electron microscopy (Pal and Bess, 1978; 1979) or by biochemistry to reach the probable phases of evolution in the differentiation of striated muscles in this phylum which includes a diverse forms of organisms.

"Most molluscan hearts studied have been reported to be covered by an epithelial epicardium" (Hill and Welsh, 1936). According
to the review of Hill and Walsh (1966), most malacologists agree on the point that there exists a 'typical molluscan heart cell'. This requires immediate confirmation by means of electron microscopy and associated techniques. It is also not clear whether the epicardial or myocardial muscle fibres are fundamentally similar or dissimilar with the noncardiac muscle fibres in this group of organisms. According to Motley (1933) and Esser (1934), the fresh-water bivalves possess non-striated myocardial fibres. There is no endocardium.

So far similar studies are performed with the members of the class Gastropoda. Species of Helix seems to be considerably well-studied. According to North (1963), the myofibrils of the cardiac muscle cells of *H. aspersa* consist of both 250 Å - thick and 50-70 Å - thick filaments. Baxter and Misbet (1963) studied under the electron microscope the myofibrillar arrangement in the heart of *Archachatina marginata*. This species shows the existence of striated muscle fibres containing both 180 Å - thick and 50-60 Å - thick myofilaments. Spindle-like dense bodies are also distributed around these myofilaments.

It is doubtful whether the molluscan muscle cells should be regarded as muscle fibres, because syncitium is absent in most of the cases. However after the use of previous workers it can be said that the muscle cells or fibres of the bivalve molluscs are extremely interesting cell models from the point of crystallinity of the paramyosin molecules and low energy.
expenditure. The molluscan muscles are biochemically unique in that these possess a high percentage of paramyosin. Further, the capability of these muscles to maintain prolonged contraction for hours/days without relaxation and with very little of energy expenditure, is according to Jordon (1918), known as 'Sperrung' or 'Catch' mechanism. Muscular relaxation is due to the unlocking of the catch. There are several highly specialized anatomical sites in bivalve molluscan organisation where the musculature is patterned differently. These include powerful adductor muscles, byssus musculature, siphonal muscles, intestinal wall as well as the heart tissues. Cardiac muscles portray an unique assembly of various contractile elements in these organisms (Hayes and Kelly, 1969; Kelly and Hayes, 1969; Rutherford, 1972; Nagi and Salamati, 1975). The biological investigations of the cardiac muscle cells of these molluscs have been sadly neglected. This is partly true for the molluscan nervous system (Gerschenfeld, 1962). Recently several authorities critically examined the fine structural details of the heart muscle cells of both European and New World species. Here the heart muscle cells and fibres of a fresh-water Indian unionid bivalve mollusc, _Lamellidens marginalis_ has for the first time been examined under the transmission electron microscope (TEM) (Pal and Dass, 1978; 1979). The ultrastructural configurations of both epicardial and myocardial muscle fibres have been outlined in considerable details.

From the survey of the earlier literature on the microscopic anatomy of the heart in this phylum it is not clear whether the
connective tissue or elastic tissue elements are universally present in this organ. Pretter and Graham (1962) described the occurrence of muscle fibres in the connective tissues of main arteries of a number of gastropods. They further demonstrated the presence of deposits of fats or calcium in the connective tissues. The present thesis examines thoroughly the existence of the connective tissue elements in the epicardium and the myocardium of the heart of *L. marginalis*. The results would be interpreted in terms of cardiac function.

The molecular mechanism of muscular contraction and relaxation in bivalve molluscs is unclear. Apart from the roles of the various excitatory and inhibitory substances, there remains the question of 'bridge' formation and its degradation between the actomyosin and the role of paramyosin components of the myofibrillar system in these animals. According to Rhagg (1971), muscular contraction falls into four sub-processes: (a) the excitation of the plasma membrane, (b) the 'excitation contraction coupling', (c) conversion of ATP to chemomechanical energy and (d) biosynthesis of ATP within the cell. In an excellent study on the innervation of bivalve hearts, Carroll and Cobbin (1972) presented evidence on the existence of several suspected chemical mediators of cardiac responses in *Tapes maenlingi*. The recent ultrahistochemical studies on the membranes of the myocardium of Modiolus demissus demissus by Watts and Pierce (1976 and 1978) demonstrate the activities of acetylcholinesterase along the cell surface. A purified fraction of cell surface membranes of the ventricle shows an eight-fold
increase in the activity of acetylcholinesterase in the bivalve. These results, and studies of similar nature do indicate the importance of the regulatory mechanisms in the excitation and inhibition for cardiac output in bivalves. With regard to the unusual importance placed on the characteristics of the plasmalemma of the cardiac muscle cells or fibres in bivalves, the recent studies on the 'nexus' complexes by Brink and his workers (1979) need documentation. The distribution and varying alternations in the structure of the nexus in the cell periphery are clearly indicative of their role in electrophysiological phenomena related to cellular coupling and uncoupling. The present thesis gives fine structural evidence in support of the occurrence of 'nexus' - complexes on the muscle fibres of myocardium of *Lamellidens marginalis*.

In the present chapter it is attempted to give a description of the submicroscopic morphology of the cardiac tissues of *L. marginalis*. The various fine structural features of the muscle cells have been mapped out to offer concrete suggestions or to forward logical explanations towards the existing problems on the cardiac activity of the bivalve molluscs. It would also indicate the newer information or data on the fine structure of the cardiac muscle cell of an invertebrate.
The initial steps were similar to those as outlined in the chapter one.

Tissue cubes of 0.5 cm³ were pre-fixed in 6% ice-cold glutaraldehyde (Sabatini, et al., 1963) in 0.1 M phosphate buffer (pH 7.2 to 7.4), and were post-fixed in 1% O₃O₄ in distilled water for one hour in each fixative at 4°C, and dehydrated through the grades of ethanol. These were subsequently embedded in Epon through propylene oxide. L.K.B. I and Reichert ultracut UM0 4 were used to cut thin (600 Å to 700 Å) sections. Ultrathin sections were stained for 1 hour in a saturated filtered aqueous solution of Uranyl acetate and subsequently in lead citrate for 5 minutes (Reynolds, 1963).

The initial electron microscopic studies were done on Siemens EM502. However, the great bulk of the work was carried out on Philips 200 transmission electron microscope operated at 60 Kv, with 50 µ objective aperture. The electron microscopic observations were recorded on Kodak 35 mm. films. Negatives were developed in Kodak microdol fine grain developer and enlarged optically whenever required.
Under the transmission electron microscope the thin sections of the epicardial cells of the heart of \textit{L. marginalis} show extremely irregular outlines. Usually these cells are slender, elongated and uni-nucleated, showing various surface processes and architectures, and intracytoplasmic bodies, like, mitochondria, golgi apparatus, etc., together with numerous defined and undefined membrane-bound structures.

A. **Typical Epicardial Cell**

A typical epicardial cell is characterised by the absence of intracytoplasmic contractile elements. The cell surface is composed of the plasma membrane (100 Å thick) which is irregularly thrown into numerous but less prominent microvilli. A greater part of the cell periphery of these cells is bathed with the pericardial fluid. The microvilli are further devoid of the usual extraneous fuzzy coat observed elsewhere in diverse animal cells (Fig. 1). Laterally the plasma membranes of the neighbouring cells take highly sinuous course and demonstrate several features, like, desmosome, followed by septate desmosome and tight junctions (Figs. 1 and 2). In some preparations the plasma membranes show the typical tri-lamellar 'unit membrane'
characteristics (Figs. 3 and 4). The connective tissue lying below the epicardial cells is highly extensive and full of several interesting subcellular features (Figs. 5 and 6). In general such areas show moderate electron-density and are filled with: (a) fine granular materials and (b) fibrillar elements (Figs. 6 and 7). Infrequently, some preparations show the appearance of connective tissue cells containing a dense nucleus (Fig. 8), several profiles of mitochondria and numerous membranous configurations (Fig. 9). The connective tissue matrix, apart from possessing arrays of fine fibrillar elements (Fig. 9), also contains scant glycogen, large but irregular electron dense bodies (Fig. 9) and a variety of membrane-bound vesicles (Fig. 3).

The following is the description of the different fine structural peculiarities associated with the epicardial cells of *L. marginalis*:

(1) Cell periphery: The plasma membranes of the neighbouring epicardial cells often show circular invaginations in sections within the cytoplasm of another cell (Fig. 6). Both small and large types of clear lake-like areas presumably belonging to intercellular space are frequently encountered along the lateral plasma membranes and interrupt both tight junctions and septate desmosome (Fig. 10). In general, the highly extensive septate desmosomes appear in horse-shoe shaped configurations (Figs. 1 and 2). On occasions, the cell surface of the epicardial cells demonstrates features which may be called pinocytotic channels. These channels could be relatively
wide (0.2 μ) (Fig. 7) and narrow (0.1 μ) as well (Fig. 11). Further, these channels either result in closely bound small subplasmalemmal vesicles (0.2 μ) (Fig. 7) or remain as bulb-like entities (0.15 μ) (Fig. 12) on the surface of the cell. It is not difficult to record the abundance of membrane-bound pinocytotic vesicles within the cytoplasm of these cells (Fig. 12). In sub-cellular loci where the cell periphery makes contact with the connective tissue matrix it usually extends in bizarre fashions (Figs. 5, 9, 11 and 12).

Additionally, there occur in between neighbouring cells small vesicular bodies containing an electron-dense body (Fig. 1). Such extracellular entities presumably arise due to dehiscence of the cell periphery.

(2) Nucleus: The epicardial cells are mononucleates (Figs. 1 and 5); the nucleus is strikingly large, ovoid in shape and is composed of nuclear envelope (0.03 μ in thickness), chromatin clumps and nucleoli (0.7 μ in diameter). The margin of the nucleus, on occasions, shows irregular outline (Fig. 13). The outer membrane of the nuclear envelope is studded with ribonucleoprotein particles (Figs. 1 and 14a). The perinuclear space is uniform in width and the distance between the two membranes of the nuclear envelope is 300 Å. The membrane associated hetero-chromatin clumps usually portray an wavy outline, while the others are evenly distributed within the nucleus. Besides, there occurs another set of dense chromatin substances, the size of which varies from
180 Å to 250 Å in diameter; these entities are loosely oriented within the nucleus and these are corpuscular or granular in nature (Figs. 13 and 14a). The interchromatinic space is meagre in these nuclei. Although there are apparent impressions of the pores and interchromatinic spaces (several in numbers) varying from 0.18 μ to 0.27 μ, on the nuclear envelope, the true features of the pore-complex are hardly met with in these preparations. The nucleolus of the epicardial cells is usually single (Fig. 1) but is large, 0.65 μ (on average) in diameter, and compact in nature. However, there are cases where two nucleoli are observed (Figs. 1 and 5). The outer rim or cortex of the nucleolus is filamentous, while the core is granular. These two regions are highly differentiated in epicardial cell nuclei (Fig. 1). Although the nucleolus is spherical in outline, it may show irregular features only when it is associated with some chromatin and they make occasional contacts with the peripheral heterochromatin (Figs. 1 and 5).

(3) Mitochondrion : There is an abundance of well-formed mitochondria in these cells. These mitochondria are small in size and elongated to oval in shape. The cytoplasm of the epicardial cells is generously populated by the mitochondria. Usually the mitochondrial cristae are haphazardly oriented, those are large in number and appear in sharp negative contrast (Fig. 1). The matrix of the mitochondrion is highly granular and electron-dense.
Apparently there is no indication either of balloonning of the outer membrane of the mitochondrion or the presence of any intramitochondrial inclusions. In general mitochondria show a topographic distribution along the cell periphery and nuclear envelope within the epicardial cells.

(4) Golgi bodies: The epicardial cells possess characteristic golgi apparatus (Figs. 6 and 8). This cell organelle is formed of several concentrically arranged smooth surface flattened saccules (250 Å in thickness) with terminal portions containing electron-dense materials. Usually it maintains a horse-shoe shaped appearance (Fig. 8). The golgi apparatus also contains multivesicular body (0.36 μ in diameter) with its usual peculiarities and small vesicles (Fig. 1) (each 0.05 μ in diameter).

(5) Cytosomes: These important cell organelles with multiple functions occur in the cytoplasm of epicardial cells (Figs. 6, 10 and 13). These are pleomorphic in nature and are usually rounded in appearance. These are delimited by a membrane. Large varieties (0.5 μ in diameter) with electron-dense particles and conspicuous lipid inclusions are seen together with smaller ones (0.16 μ in diameter) with electron opaque materials. These organelles possess highly electron-dense granular materials in which lie lipid-like bodies and small dense microstructures.
Infrequently, a cluster of varying forms of cytoaomes occurs within the cytoplasm (Fig. 15). These are single membrane delimited structure of variable sizes with electron dense matrices loaded with discrete particles.

(6) Lipid Inclusions: One or two lipid droplets are observed in the cytoplasm of the epicardial cells (Figs. 1 and 5). These appear as spherical bodies without any membrane but with faint electron-opacity.

(7) Glycogen particles: There are numerous glycogen particles in the epicardial cells (Figs. 1, 4 and 9). These particles are moderately electron-dense and may remain single or are arranged in small or large clusters, more frequently forming chains, each generally maintaining individual outlines. Usually these occur in the cytoplasmic groundplasma in the form of clear glycogen 'lakes' (Figs. 4 and 9). Each glycogen particle measures 400 \( \mu \) in diameter. These are possibly a glycogen particles.

(8) Ribosomes and Polysomes: Discrete bodies of ribonucleo-protein particles or ribosomes are present in the cytoplasm of the epicardial cells. These are smaller (150 \( \mu \) in diameter), than the size of glycogen particles (Figs. 7 and 14b). Often these particles are arranged in rows lying close to the plasma membrane, more frequently near
the desmosomes (Figs. 1 and 9). Individual ribosomes occurring in clusters, called 'polysomes' are frequently seen in the cytoplasm (Figs. 4, 9 and 13). Often, ribosomes are observed in association with the RER system (Fig. 6).

(9) Endoplasmic Reticulum: Rough-surfaced endoplasmic reticulum is few in number and does not show any pattern in their distribution (Figs. 13). The canaliculi of RER contain poorly dense granular materials, while the membranes and the associated ribosomes are highly electron-opaque. Non-extensive smooth-surfaced membranes which are quite different from those of the Golgi apparatus are also observed in the cytoplasm of the epicardial cell (Figs. 11 and 13). Some of these RER membranes, on enlargements, show typical tri-lamellar unit membrane configuration (Fig. 15).

(10) Microtubules: Few microtubules are seen on rare occasions in the cytoplasm of the cell (Fig. 9) associated with pinocytotic vesicles and are oriented along the long axis of the epicardial cell.

(11) Undefined features: Among the undefined structures in intra- and extracellular sites the smooth membrane bound vesicles need careful observations. Usually such bodies are clear in appearance but may, on occasions, contain
microstructures of varying texture and nature (Figs. 6 and 9). Several elongated worm-like, electron-dense membranous structures are often seen in the cytoplasm close to the plasma membrane of the epicardial cells (Fig. 1). Occasionally these bodies may be ovoid in shape (Fig. 5).

B. Connective Tissue Cell

Only one type of connective tissue cell has been observed in the ultrathin sections of the epicardial tissues of *L. marginalis*. Such a cell is nucleated and the cytoplasm is generously populated by well-developed mitochondria (Figs. 8 and 9). These mitochondria are big in size in comparison to those observed in epicardial cells and are elongated to oval in shape. It is difficult to preserve the plasma membranes of these cells. Outside the cell periphery lie extensive areas of connective tissue matrices containing both granular materials and fibrillar elements. The fibrillar structures characteristically lack the periodic segmentations. The connective tissue cells may show nuclei of varying forms and electron opacity. (a) They may be spherical in shape, with electron dense chromatin materials obliterating the nuclear pore (Fig. 3), or (b) with distinct wavy peripheral chromatin and electron-lucent euchromatin (Fig. 9) allowing the nuclear pore region to appear in between perinuclear channels. The cytoplasm is characterised by the presence of
numerous mitochondria that may show divisions (Fig. 9), and swarms of membranous profiles and membrane-bound electron-dense bodies (Fig. 9) which are disperely oriented. Besides, the cytoplasm contains ribosomes, a few glycogen particles and scanty amounts of RER. Some of the profiles of RER show connection with the smaller dense inclusions, suggesting a case of biogenesis of these structures. Some aggregates of several mitochondria and membranous sacs accompanied by contractile filaments within a membrane-bound area seem to suggest their continuity with the myocardial cells (Fig. 8). In such areas the mitochondria demonstrate a close similarity with those of the myocardial cells. This cell apparently does not show the presence of the Golgi apparatus, lipid inclusions and vesicles.

C. **Myocardial cell**

The myocardial cells or muscle fibres of *Immelidene marginalis* constitute a major portion of the cardiac tissue components. There is no endocardium. The myocardial fibres are uni-nucleated and elongated spindle-shaped cells generally tapering at the two ends and are characteristically surrounded by connective tissue elements (Fig. 16). These are smooth muscle cells which are frequently observed occurring in bundles (Fig. 20). The myocardial muscle cells are characterised by the presence of numerous cortical contractile myofilaments (Figs. 16 and 17). These cells are usually seen in sections appearing in different
planes suggesting a random disposition of these contractile cells in the myocardium of this bivalve (Fig. 50). In general the cell shows a huge central area of electron-lucence where a few mitochondria and the centrally placed large nucleus are observed (Figs. 16, 17, 18 and 19). The various contractile elements usually occupy the subplasmalemmal cytoplasm of the muscle fibre (Figs. 17, 18 and 19). The various microanatomical features of these cells are described in the following order to point out the general plan of submicroscopic organization of the myocardial tissue:

1. **Cell periphery**: The muscle fibres are bounded by trilamellar unit membrane of 100 Å thickness. The plasma membranes of these cells demonstrate more than one substructural peculiarity in the form of: (a) lateral 'balloon'-like swellings, (b) cytoplasmic bridges, (c) terminal villiform microstructures, (d) nexus, (e) attachment plaques and surface modifications in the form of dense plaques, desmosomes and hemidesmosomes.

Often, in low power electron micrographs of several muscle fibres both lateral swellings and finger-like microappendages as well as the villiform terminal microstructures between the two neighbouring cells are observed (Fig. 20). In some muscle fibres regional 'cup' or 'saucer' shaped depressions are observed on the plasmalemma to make room for the counterpart in the neighbouring cells (Fig. 20).
(a) The shape, size, and the number of the above mentioned 'balloon'-like evaginations per muscle cell vary considerably. They are arranged in series along the periphery of the muscle cell and may be slender, finger-like or wide. In most of the cases these lateral processes seem to fuse with each other to increase in bulk and thereby imparting a very complicated reticular pattern (Fig. 21) on the cortical cytoplasm. Generally these lateral processes protrude out to establish cellular connections with the same of the neighbouring fibres but in some micrographs their fusion also with the terminal processes of the neighbouring cells have been observed (Fig. 22). These extensions reach the connective tissue matrices between the muscle fibres. Between the associated cells the 'balloon' of lateral plasmamembranes fuse with each other to form the cytoplasmic 'bridges' (b). These bridges may be complete (Fig. 21) or incomplete (Fig. 23). When incomplete, the fusion micro-area is followed by tight junction and intercellular space (Fig. 23). But in complete 'bridges' stout or thick cytoplasmic channels between the lateral aspects of the cells are established. These channels traverse the connective tissue matrices (Fig. 21). Apart from these subcellular peculiarities, some of these lateral processes with slender finger-like configuration may take up terminal positions and modify themselves to form the (c) terminal villiform microstructures (Fig. 22), imitating a collapsible gate (Figs. 20). These microstructures establish contacts between two or more cells involving either the terminal
end of the other or the lateral balloon-like swellings of the adjacent cells. Like the case in lateral swellings these terminal processes may also fuse together to form reticular cytoplasm and few membrane bound microvesicles (Fig. 22). A series of linearly arranged pillars of cytoplasmic entity give these terminal processes comb or collapsible gate-like configurations (Figs. 21 and 22). Few cells produce blunt balloon-like terminal processes (Fig. 47). Hence a single muscle fiber may exhibit two types of terminal bridging devices, i.e., balloon-like and comb-like. (d) Among the various membrane-interactions displayed by the myocardial muscle cells, the 'nexus'-like structures need careful elucidations. These are extensive areas where the two plasma membranes are placed in exceedingly intimate associations to demonstrate a penta-lamellar appearance and exclude the maximum of the intercellular space (Fig. 23). The average thickness of the nexus is 200 \( \AA \) (200 \( \AA \) to 210 \( \AA \)). The intercellular space close to nexus is 300 \( \AA \). The fusion region between the two cells is 50 \( \AA \) thick whereas the clear areas at two sides of the membrane is 60 \( \AA \) thick. (e) Dense bodies or attachment plaques are found on the sarcolemma associated with the contractile elements, particularly the thin filaments. Cytoplasmic invaginations are found associated with attachment plaques (Figs. 37 and 51). In a few cases, these attachment plaques are found to be withdrawn towards the cytoplasmic side of the cell along with the thin filaments (Fig. 51). Dense material may totally obscure the membrane and the extracellular space in the immediate region of
the plaque. Tight junctions may be found near these regions (Fig. 19), and fuzzy coat on the extracellular side of the plasmalemma (Figs. 31 and 39). Dense plaque-like structure may be found on the surface of the lateral baloons (Fig. 45) or on the other sites of the plasmalemma (Figs. 16 and 39), and near the desmosomes and homidesmosomes the extraneous fuzzy coats are found. Frequently the neighbouring muscle cells show varying degrees of syncitial loci (Figs. 24 and 25) where the cell periphery gradually loses its identity.

At the initial stage (Fig. 19), the cell periphery establishes contact areas with another cell and there one can notice two features in these sites: (a) A minimum amount of intercellular materials or space and (b) membrane-thickening on the cytoplasmic sides. These points or loci ultimately collapse or degenerate leaving traces of membranes or their assemblies (Fig. 24) in the form of dark sinuous lines between the cytoplasm of two cells. The final configuration of confluence of 2-3 cells at a particular point emerges (Fig. 25) when a true syncitium is established. In a typical site where the cells meet and intercommunicate with one another, the cells are disposed in a radiating manner from the region of greater associations (Fig. 25). Such syncitia may form secondary or minor contact areas with another participating cell from a different zone of the cell periphery (Fig. 25). On higher magnifications areas of confluence (Fig. 25) show clear looking small vesicles and membranous or tubular profiles. The diameter of a single micro-tubule is approximately 200 Å.
(2) Nucleus: Generally the centrally placed nucleus of the myocardial cell of L. marginalis lies embedded in the clear glycogen 'lakes' of the cytoplasm (Figs. 16, 17, 18 and 19). However, it may, on occasions, also lie in the glycogen particulate zone of the cytoplasm (Fig. 25). In general, the nucleus is oval to elongated and spindle (Figs. 18, 19 and 26) in shape. However, depending on the plane of sections the nuclei may look rounded (Fig. 16) or irregular in outline (Figs. 18). The nucleus is large and possesses either one or two nucleoli (Figs. 16, 17 and 19). The nucleoli may be unequal in size (Fig. 18). The distribution and the texture of the chromatin materials within the nuclei vary greatly in these cells. There is very little dense chromatin materials lying close to the inner nuclear envelope internally imparting an wavy outline (Fig. 44). The more common pattern is very scanty amount of chromatin dispersed uniformly within the nucleus (Figs. 16, 17, 18 and 19). The chromatin materials frequently show both finely granular and corpuscular appearance (20 to 150 Å) and occasionally the electron-opacity of chromatin substances is sharply different (Fig. 26).

Some myocardial cells may show exceedingly higher amounts of non-chromatin materials within the nucleus (Fig. 44), which display maximum electron-lucence. The reasons for such appearance are not obvious.
Generally, the nucleolus of such cells is a rounded compact body (0.9 μ x 0.4 μ). The dual substructural organization of the nucleolus is apparent from the ultra-thin preparations of the myocardial cells (Figs. 16 and 18). Smaller nucleolus shows spongy appearance. However, there occurs a small, loose electron-dense body near the smaller nucleolus, while the larger ones have both outer filamentous cortex and dense core. The nuclear envelope is characteristically double-layered structure (Fig. 17), with perinuclear space 600 Å wide, but the pores are not clearly visible. In a few cases only nuclear pore at the end of interchromatinic channels are recognisable (Fig. 26). The outer membrane is generously populated by the ribonucleoprotein particles (Fig. 19). In some preparations infoldings of the nuclear membrane are observed (Figs. 16 and 18). In some rare cases the outer membrane of the nuclear envelope develop folds forming perinuclear cisterna measuring upto 2000 Å (Fig. 44).

(3) Mitochondrion: There is an abundance of mitochondria in the cytoplasm of the myocardial cells (Figs. 16, 17, 18, 19, 21, 23 and 25). In some cells the areas filled with glycogen there are fewer mitochondria compared to other subcellular foci of the cell (Fig. 18). Chondriosomes in the myocardial cells are elongated to elliptical in shape and the width varies depending on the sectional plane. In a few micrographs (Fig. 18) they show striking structural variations, and 'ball and socket' like association of mitochondria (Fig. 17) was observed. The matrix of the mitochondrion is
highly granular in most of the cases but both electron-dense and electron-lucent matrices are observed (Figs. 21, 23, 24 and 35). The cristae mitochondrialis are elongated processes which are 150 Å to 250 Å wide and are in plenty in a single mitochondrion often ending in bulbs (Figs. 28, 29, 36 and 41) and arranged haphazardly within the lumen of mitochondria (Figs. 27, 29 and 36), where the mitochondrial outer chamber is 300 Å in width. The cristae which are longitudinally, transversely and radially oriented are found tightly packed in the matrix of the mitochondria and these appear in characteristic sharp negative contrast (Fig. 36). Within the matrix of mitochondrion one can observed small electron-dense particles of 150 Å diameter as well as large dense inclusions varying from 500 Å to 1000 Å in diameter (Figs. 24, 30, 33 and 44). These electron-dense microstructures (Fig. 28) the medium-sized and moderately electron-dense spherical microstructures (Fig. 29), intramitochondrial glycogen particles (Fig. 30) and ribbon-like opaque body (Fig. 31) appear frequently in the preparations. In a few forms diffuse type (700 Å) of inclusions are also observed (Fig. 23). Clear vacuolar spaces are noted in few forms (Fig. 29) and mitochondrial fission is evident (Figs. 17, 19 and 28). There is a crowding effect of mitochondria in the nexus complex (Fig. 23) and in the balloon-like evaginations of the cell periphery (Fig. 27). Chondriosomes both with electron opaque and electron lucent matrices are found in the (a) lateral microstructures (Fig. 23), (b) bridges (Fig. 21), and in the (c) cytoplasmic channels (Figs. 21 and 23). Topographically the mitochondria are found closely associated with the nucleus (Figs. 16, 17 and 19) and intermingled with
the rows of the contractile elements (Figs. 18 and 19) or in between the cortical rows of contractile elements (Figs. 31 and 36). In a few cases (Fig. 35) mitochondria are found at one side of the muscle fibre and lie opposite to the contractile filaments and the nucleus rests in the middle (Fig. 16). In the myocardial muscle these cell-organelles are often found in intimate association with the cytosomes (Figs. 17 and 19) especially in the lateral evaginations (Fig. 33) and are found tightly opposed with the membrane bound vesicular structure deep in the cortical region amongst the contractile filaments (Figs. 21, 25, 31 and 35).

(4) Cytosomes: Cytosomes are membrane-delimited cytoplasmic organelles which are poorly represented in the myocardial muscle fibre of *L. marginalia* (Figs. 17 and 19). These organelles are variable in submicroscopic organizations and contents (Figs. 19 and 33). The larger varieties possess highly electron-dense granular materials (Fig. 33) or dense microstructures (Fig. 19). In one preparation it is seen to consist of several small cytosomes coalescing with one another (Fig. 19). These cytosomes are found in the lateral (Fig. 33) and terminal processes of the muscle cell lying occasionally in association with the mitochondria (Fig. 33).

(5) Glycogen particles: There are two important characteristics of myocardial muscle fibres of *L. marginalia*. These are:
(a) the astronomical figures for the glycogen particles and
(b) the contractile fibrillar assemblies. In most of the
muscle cells glycogen appears in the form of huge glycogenic areas and clusters of glycogen particles (700 Å) between the contractile elements (Fig. 34). In all micrographs there are enumerable electron lucent spaces amongst the contractile filaments assumed to be occupied by glycogen rosettes (Figs. 35, 37 and 52). These areas measure 4200 Å to 6000 Å in length and the breadth is 2000 Å. In glycogenic areas the mitochondria are also found (Fig. 21). Individual glycogen particles stand as electron-opaque entity which usually interlaces with one another in the groundplasm of the cell. In fact, many parts of the cytoplasm of the cell appear to be formed of mosaic of glycogen particles (Figs. 20 and 30). Both rosettes of α-glycogen and chains of β-glycogen are found here (Figs. 23, 30 and 52). Apart from their presence in the cortical region with the contractile elements (Figs. 28, 36 and 51) they are also found in lateral processes (Fig. 34), bridges (Fig. 23), terminal microstructures (Figs. 22 and 47) and cytoplasmic channels (Fig. 25), and also with 'diad'-like membrane bound structures (Fig. 32).

(6) Lipid Inclusions: Moderately electron-dense spherical entities, 0.27 μ to 0.51 μ in diameter belonging to the lipid inclusions are seen in the myocardial cells (Fig. 35). These may show stratifications of electron-density. Lipid inclusions may lie close to the plasma membrane of the cell (Fig. 35). The cases of lipid droplets appearing in close association with the mitochondria are not rare (Fig. 35).
Myofibrillar assemblies: The myocardial muscle cell of *L. marginalis* is non-striated smooth cell type. The cell shows a variety of contractile myofibrillar elements which appear in different profiles according to the plane of ultramicrotomy. In general, the sub-plasmalemmal cytoplasm of the cell accommodates the greatest numbers of myofibrillar elements (Figs. 17, 18, 19 and 36). Although general observations suggest that dense assembly of myofibrillar elements is found in the cortical region of the muscle cell along the contour of the same (Fig. 20), but there are few other alternative arrangements for them, where these contractile elements are found at the core of the muscle cell (Fig. 16), or are oriented opposite to the group of the mitochondria (Fig. 35), or even in the terminal microstructures (Fig. 22) of the myocardial cells. In the near ideal longitudinal sections of the myocardial cells there appear three classes of contractile elements which maintain their characteristic thicknesses throughout (Fig. 19). These are: (1) thick filaments, (2) thin filaments and (3) 'dense bodies'. In cross sections of the cells the dense bodies could also be spotted (Fig. 41). The myofibrils are generally linearly arranged contractile microstructures oriented along the long axis of the muscle cell (Fig. 19), but oblique arrangements are also found (Fig. 17), and quite haphazard orientation of the contractile elements are not uncommon (Fig. 37).
Frequently the profiles of elongated and electron-dense assemblies of myofibrils are observed to lie in the form of stacks or piles (Fig. 38). On average these thick filaments are 1.82 µ long and 166 Å in diameter. But the range of diameter is from 110 Å to 222 Å. This electron-dense thick variety of myofilaments often displays tapering ends and usually the middle zone of a myofilament shows highest electron-density (Figs. 37 and 42). Besides, the usual linear orientation of the thick filaments (Fig. 36), divergent displays (Fig. 32), or swinging orientation of the thick filaments towards the dense bodies and attachment plaques (Fig. 37) are evident. In many cases the thick filaments may even extend up to the dense bodies and literally cross the latter or disc-like attachment sites harbouring the thin filaments (Figs. 37 and 42).

In cross sections the thick filaments on average show 270 Å thick diameter and in few cross sections aggregation of thick filaments is evident. Two or three thick filaments can take part in each aggregate. In case of linearly arranged participants the aggregate may form 1500 Å long beaded structure, but in other cases, the three participants on three different planes may be attached to each other with the aid of thin filaments. Sometimes aggregates of two or more thick filaments are found surrounded by a complete or incomplete circle of thin filaments, and these structures are evident in cross sections of the myocardial cells (Fig. 40). The thick filament units alternate with much thinner components of the contractile system of the cell (Figs. 38 and 39).

Admittedly, the thin filaments occur surrounding the thicker
fibrils (Fig. 40). Between the thick myofilaments occur a number of fine fibrils which characteristically make a gossamer-like appearance of the cytoplasm of the myocardial cell (Fig. 31). The thin myofilaments can run conceivably in all directions and often terminate or originate from the surface of thicker myofilaments (Fig. 43), or 'dense bodies' (Fig. 38), and thus display an interesting topographic association with the terminal ends of denser components (Figs. 17, 19, 36, 37, 38 and 42). On average the thin myofilaments are 1.15 μ long and 60 Å thick, and are found arranged usually parallelly along the thick filaments (Fig. 38), generally several rows of thin filaments are found around each thick filaments (Figs. 28, 39 and 49). Few micrographs give an impression that thin filaments aggregate to form the thick filaments (Fig. 43), but usually (1) thick and (2) thin filaments are found quite distinct from each other (Figs. 28, 32, 37, 40, 42 and 49). From the micrographs it is evident that the dense bodies are the attachment sites for the thin filaments (Figs. 19, 36, 37, 38, 39, 42 and 51), but the substructural details of the attachment sites including (a) the number of the thin filaments per dense body, (b) nature of attachment (c) and the number of thin filaments of the two sides of the dense disc could not be made out, because of the intense electron-opacity of the attachment sites. The thin filaments in bundles are also associated to the attachment plaques on the plasmalemma or sarcolemma (Figs. 19, 31, 34, 37, 39, 42 and 51). One of the outstanding features of the myofibrillar assemblies is the presence of numerous highly electron-dense, disc or plaque-like entities among the
different classes of fibrils (Figs. 36 and 37). On average these are 0.3 to 0.5 μ long and 0.1 μ in thickness. These appear as regular features oriented in a rather haphazard manner, and these plaque-like structures may lie at a short distance from the 'nexus' complex (Fig. 18). Generally these 'dense bodies' are found amongst the thick and thin filaments stacked at the cortical region of the cell (Fig. 19), but they even lie in juxtaposition with the plasma membrane (Fig. 37) to be known as attachment plaque (Fig. 34). In both the cases they act as anchors for the thin filaments. Sometimes the thin filaments show beaded appearance at the two sides of the dense bodies (Fig. 39). There is no patternised orientation of the dense bodies and the attachment plaques in the myocardial cells, though numerically they are significantly great in number (Figs. 19, 20, 21, 32 and 37). But, their regular and systematic orientation has been observed in apparently contracted muscle cells (Figs. 26 and 51).

In cross sections of the myocardial cells, both thick and thin filaments show interdependence, though they hardly exhibit any regular pattern (Figs. 29 and 44). The thin filaments are observed to produce complete or incomplete circles around the thick filaments (Figs. 40 and 41). In a circle the ratio of thick filament to thin filaments may be 1:1.2±7 (Figs. 40 and 41). Sometimes, aggregates of three thick filaments are found at the centre of the circle surrounded by a group of thin filaments (Fig. 40). In a few cases two thick filaments of adjacent circles are found jointed with each other by 4 to 5 thin fibrillar
connections (Fig. 41). In the longitudinal sections 80 to 83 Å long cross bridges between thick and thin filaments are noted quite evidently (Figs. 28, 39 and 49). In cross sections these cross-bridges connecting the central thick filament to the peripheral thin components are quite revealed (Figs. 40 and 41).

(8) Microtubules : One or two isolated cases of microtubules (300 Å in diameter) are also seen in the cytoplasm of the myocardial cells (Fig. 38). These are clearly observed in regions where the myofilaments are poorly represented (Fig. 33). In a few cases lateral evaginations also exhibit microtubular structure near the plasmalemma, flanked by the linearly arranged rows of ribosomes (Fig. 42).

(9) Golgi apparatus : Golgi apparatus in the myocardial cell is not commonly seen. However, some cells show elements or components of Golgi apparatus in the juxta-nuclear position (Fig. 19). These units are non-extensive and consist of a few smooth-surfaced sacs and several membranous vesicles.

(10) Peroxisomes : One or two subcellular organelles, like, peroxisomes are observed in the myocardial cell (Fig. 44). These are membrane-delimited ovoid bodies containing granular matrical substance and a dense core. These peroxisome-like structures are found in association with polyribosomal chains and microtubules near the dense plaque on the plasmalemma.
Secretory or Excretory vesicles: Frequently cytoplasmic protuberances of the myocardial cells demonstrate unusual membranous vesicles appearing on the plasma membrane (Fig. 45). These bodies at the initial stages may have connections with the reticular and clear-looking subplasmalemmal cytoplasm of these cells. Similar membranous sac-like formations are observed lying freely in the extracellular substances around the cell periphery (Fig. 26). In the lateral balloon-like evaginating processes of the myocardial cells the excretory vesicles (on an average 0.4 μ in diameter) fuse with each other (Figs. 33 and 45) to participate ultimately in exocytotic processes. On the surface these excretory spheres may measure 0.1 μ in diameter in some cases (Fig. 45).

Intracytoplasmic vesicles: The myocardial cells often show several well-formed membranous structures which may be called as intracytoplasmic vesicles (Figs. 24, 25, 27, 33 and 44). Some of these vesicles may possess fine granular material (Fig. 44). Process similar to pinocytosis is quite evident in the myocardial muscles. Pinocytotic invaginations are found either at the tip of the lateral microstructures (Fig. 33) or beside the finger-like lateral evaginations of the cells (Figs. 37 and 42). These pinocytotic channels end in bulb-like entities (3000 Ω) in the cortical region of the cell, at the region of contractile elements (Figs. 22, 34 and 37). Enumerated vesicular
structures are found at the sub-sarcoplasmalemmal region and their diameter may vary from 1000-2000 Å (Figs. 17, 25, 31, 36 and 44). Mostly they are formed by the fusion of lateral and terminal evaginated microstructures (Figs. 21 and 22) or by the pinocytotic vesicles (Fig. 37). In the former case they may contain plaque-like structure (Figs. 24, 33 and 37) and membrane bound particles (Figs. 24 and 44) are found in the latter case. Besides these two types of vesicles, smooth membrane bound structures are found in close association with the mitochondrial surface at the cortical region (Figs. 27, 31, 44 and 45) and intermingled with contractile elements (Fig. 50). Their average diameter is 0.14 μ and the range varies from 800 Å to 2000 Å. These vesicles either remain in close apposition with the mitochondrial membrane or are 500 Å to 600 Å apart (Fig. 35) from the mitochondria. The average diameter of the vesicles in association with the contractile elements is 0.4 μ (Fig. 35).

(13) Ribosomes and Polyribosomes: Usually the ribosomes (250 Å in diameter) and polyosomes appear lying close to the myofibrils (Fig. 36). The polyosomes may be large and display cart-wheel-like appearance in some cases (Fig. 38). Individual ribosomes are observed lying in association with the rough-surfaces endoplasmic reticulum (Figs. 19 and 44). Ribosomes and polyribosomes are also found in the lateral balloon-like evaginations of the muscle cells, in association with the fibrillar and tubular structure (Fig. 42).
The myocardial muscle fibres or cells are also surrounded by an enormous amount of connective tissue matrixic substances as well as structures of defined and undefined form and functions (Figs. 16 and 17). Islets of blood or haemolymph, spherical microstructures of differing sizes, etc., are present in the matrix (Fig. 48). Usually the fine fibrillar elements are not observed in the matrix of the myocardial complexes. But, on occasions, small bundles of moderately electron-dense fibrils are seen lying in the connective tissue matrix (Fig. 42).

Between the connective tissue components float the amoebocytic cells in the haemolymph (Fig. 48). It was difficult to recognize a connective tissue cell in the myocardium of *A. marginalis*. But the characteristic features, like, the concentrically layered spheres and blood islets are soon populating the areas surrounding an individual muscle fibre (Fig. 46). The spherical microstructures (diameter varying from 2.6 to 3 μ) are apparently devoid of any limiting membrane and the central core (0.2 μ in diameter) is invariably dense and compact, while the peripheral rings are of variable densities and thickness (Figs. 46 and 48). Such regular and periodically segmented and spherical structures are indicative of their affinities with the calcite spherules described elsewhere in molluscan tissues. The islands of blood give the impression of differential electron-density; these appear as irregular bodies having no membranes and the structures
represent the precipitates of the various components of the haemolymph (Fig. 48). In cleaner zones of precipitated blood, electron-dense substructures of uniform diameter are frequently observed (Fig. 48). These units could very well represent the blood pigments of this bivalve.

E. Haemolymph cells

The haemolymph cells of _L. marginalis_ often appear in the ultrathin sections of the myocardium. These nucleated cells can readily be differentiated from myocardial muscle fibres (Figs. 16 and 48). Two important granulocytic haemolymph cells are easily characterized by the presence of dissimilar cytoplasmic granules or vesicles. The first type of cell is characterized by the presence of numerous large, electron-dense ovoid granules (0.2 \( \mu \)) and highly dense rounded nucleus, 4 \( \mu \) in diameter (Figs. 16 and 48). The second category of the granulocytic cells possesses eccentric and irregular nucleus and clear vesicles or large lucid vesicles (Fig. 16) of Cheng and Foley (1975). These cells are further characterized by the presence of several sub-plasmalemmal vesicles. In low power micrographs it is not clear whether these cells possess glycogen 'lakes' or not.
Fig. 1. Transmission electron microscopical view of the epicardial cells of *Lamellidens marginalis* showing prominent nucleus (N), desmosomes (DS), septate desmosome (SD) microvilli (MV), Glycogen particles (GLY) worm like membranous structures (arrow) and lipid inclusion (LD).
Fig. 2. Ultrastructure of the epicardial cell junction showing horse shoe shaped septate desmosome (SD).
Fig. 3. Enlarged view of the same particulating the septate nature of the desmosome (SD) in association with polyribosomes (PR).
Fig. 4. Figure detailing out the structure of the desmosome (DS) and chains of glycogen particles (GLY).

Fig. 5. Epicardial cells showing bizarre nature of cellular outlines and extracellular connective tissue (arrow). Pinocytotic channels (PI) are seen in the field, together with lipid droplets (LD).

Fig. 6. Ultramicrograph detailing the structure of golgi apparatus (Ga), intracytoplasmic vesicles (arrows), microvilli (MV).
Fig. 7. Figure shows fibrillar (arrow) elements in the connective tissue compounds.

Fig. 8. Electronmicroscopical view of the nucleus (N) of connective tissue cell and an isolated group of chondriosomes (M), identical with the mitochondria observed in myocardial cells.

Fig. 9. Figure depict the structure of nucleus (N) of a connective tissue cell, with interchromatinic channels (arrow) and chains of mitochondria (M).
Fig. 10. Tight junction (TJ) and balloon-like intercellular space (ICS) between two epicardial cells.

Fig. 11. Spherical structures (arrow) at the extracellular sites of the epicardial cells.

Fig. 12. Coexistence of connective tissue elements (single arrow), epicardial cells and a part of the myocardial cell/fibre containing fibrillar elements (double arrow).
Fig. 13. Ultrastructural view of an epicardial cell showing cytosomes (CS) and smooth endoplasmic/sarcoplasmic reticulum (SER) together with glycogen particles (GLY).

Fig. 14a. An epicardial cell shows nuclear pore region (double arrows) and ribosomes associated with the nuclear envelope (small arrows).

Fig. 14b. Polyribosomes (arrow) in close association with plasma membrane (PM).
Fig. 15. Ribosomes (R) in close proximity to ER system.

Fig. 16. Transmission electron micrograph of a typical myocardial cell of *Lamellidens* in the connective tissue matrix showing nucleus (N), nucleolus (NCL), contractile elements (arrow), clear 'lake' like glycogenic (GLY) areas and granulocytes of the haemolymph fluid carrying granules (G) of varied forms and nature.
Fig. 17. A part of a myocardial cell showing cortical existence of myofilaments (arrow), clear 'lake' like glycogenic (GLY) areas and cytosome like structures (CS).
Fig. 18. Figure represents a part of the myocardial cell exhibiting a nucleus with folded nuclear envelope (arrow), a nexus complex (NX), and a couple of nucleolus (NCL).
Fig. 20. Muscle cells of *Lamellidens marginalis* in a bundle of muscles establish connections with each other.
Fig. 21. Electron micrograph showing lateral swellings of the muscle cell/fibres (arrow) to establish cellular bridges (BD) or cytoplasmic channels between two neighbouring cells. The field also displays vesicular structures (VS).
Fig. 22. Figure represents the terminal process, connecting the two cells (TP) end to end.
Fig. 23. Ultramicrograph detailing out the structure of an intercellular bridge showing intercellular space (ICS) and tight junction (TJ). Mitochondria (M) are with both electron dense and electron lucent matrices.
Fig. 24. Cytoplasmic bridge between the two cells with glycogen particles (GLY), Chondriosomes (M) and few vesicular structures (VS).
Fig. 25. Ultramicrograph showing the association of myocardial cells. Nucleus is lying in the glycogen particulate zone in one cell (arrow).
Fig. 26. A presumably contracted cardiac cell of *Lamellidens* showing nearly regular orientation of contractile elements.

Fig. 27. Lateral process of a muscle cell/fibre carrying mitochondria (M) and few vesicular structures (VS).
Fig. 28. Ultrastructure of thick (TH) and thin (TN) filaments, in association with a dividing mitochondria containing granular inclusion (arrow).
Fig. 29. Figure shows the transverse section of contractile elements (double arrows) and electron lucent inclusions in mitochondria (small arrow).
Fig. 30. Electron microscopical view of glycogen particles (GLY) and inclusions in the mitochondrial matrix (arrows).
Fig. 51. Figure demonstrates close relations of mitochondria with vesicular structures (VS), a member of the chondriosomes contains filamentous inclusion (single arrow). Attachment plaque (A) in association with extraneous fuzzy coat (double arrows) is also detectable.
Fig. 32. Ultrastructural view of contractile elements (arrow) and membrane bound 'diad' like structures (DI). Glycogen particles are also evident (GLX).
Fig. 33. Lateral processes with mitochondria (M) cytosome like structures (CS) and few vesicular bodies (VS).
Fig. 34. Figure represents, attachment plaque (A), contractile elements (arrow), glycogen particles (GLY) and dense bodies (D).

Fig. 35. Polar orientation of contractile elements (arrow) and chondriosomes (M). Close apposition of vesicular structures and mitochondria are noticeable (arrows). Lipid inclusions (LD) are observable within the cell.
Fig. 36. Figure depicts mitochondria (M), myofibrils (double arrows), and both intracellular and extracellular vesicular structures (arrows).
Fig. 37. Swinging orientation of the contractile elements (double arrows) showing attachment plaques (A), dense bodies (D), few vesicular structures (VS) are displayed in the field.
Fig. 38. Ultramicrograph represents a microtubule (MT) in association with contractile elements (double arrows) and polyribosomes (PR).
Fig. 39. Figure depicts particularly the relative position of thick filaments (TH), thin filaments (TN), dense bodies (D) and attachment plaques (A) in a transverse section of a muscle cell/fibre.
Fig. 40. Transverse section of contractile apparatus to show the thin filaments encircling the thicker component (arrow). Sectional view of dense bodies (D) and aggregated thick filaments (double arrows) are also noticeable. Thick filaments (TH) are connected with each other with thin filaments (TN).
Fig. 41. Cross section of contractile elements showing membrane bound vesicular structures (arrow). Thin filaments make complete circles around the thicker fibrils and show established connections with the latter one (double arrows).
Fig. 42. Transmission electron microscopical record showing fibrillar elements (arrows) inside and outside the lateral processes. Pinocytotic invaginations are also recorded (double arrows).
Fig. 43. Thin filaments at the two sides of the dense bodies (D) and thick elements (arrow).
Fig. 44. Figure displays the folded nature of the outer membrane of nuclear (N) envelope, vesicular structures (VS) and peroxisome like bodies (arrows).
Fig. 45. Lateral processes showing the fusion of vesicular structures (double arrows) and exocytosis (single arrow).
Fig. 46. Figure shows spherical microstructure with dense and compact central core.
Fig. 47. Balloon like lateral processes of myocardial unit carrying glycogen particles (GLY), mitochondria (M), and vesicular structures (VS).

Fig. 47. Balloon like lateral processes of myocardial unit carrying glycogen particles (GLY), mitochondria (M), and vesicular structures (VS).
Fig. 48. Connective tissue elements around a part of a myocardial cell (MC). In the field, a spherical microstructure (arrow) and granulocytes impregnated with granules (G) are exhibited. An electron dense substructure is also observable in the field (double arrow).
Fig. 49. Thick and thin filaments showing cross bridges (arrows).
Fig. 50. Ultrastructure of connective tissue elements around the myocardial muscle cells/fibres of *Lamellidens marginalis*.
Fig. 51. A part of a presumably contracted muscle fibre showing the attachment plaque (A) pulled towards the cytoplasmic side of the cell (arrows). More or less regular orientation of contractile elements are also detectable.
Fig. 52. Electron microscopical observation of contractile filaments in a myocardial cell of *Lamellidens marginalis* showing close relation of the contractile proteins with glycogen rosettes (GLY) and diad like structure (DI) flanked with glycogen particles. Empty circular spaces (arrows) may represent the 'sockets' to hold the glycogen rosettes.
DISCUSSION

The cardiac muscle tissues of *Lamellidens marginalis* are ultrastructurally divisible into three parts: (a) epicardium, (b) myocardium and (c) connective tissue components. All these three basic histological structures of the cardiac tissues of this bivalve mollusc have been quite satisfactorily described and a host of fine structural details has been enumerated. Both epicardial cells and myocardial muscle fibres have been separated by considerable areas of connective tissues. In both the cases, the connective tissues show characteristic fibres which lack segmentations or periodicities. The epicardial cells of this bivalve do not show presence of any myofibrillar elements, while the myocardial muscle fibres possess characteristic fibrillar or contractile system.

The recent knowledge of molecular biology has indicated among other things that paramyosine or tropomyosin A constitutes about 30% of the total protein in certain muscles in Annelids and Mollusces (Threadgold, 1975). Further, it has been accepted that both thick and thin myofibrils are present in the cardiac muscles of molluscs and the ratio between thick and thin ones is 1:12 or upwards. Light microscopic study of the heart tissues of
_L. marginalis_ has clearly indicated that the muscle cells are of smooth and non-striated type. It would be worth investigating to justify that such a muscle cell either fully or partially approximates a vertebrate striated muscle cell, both from the ultrastructural and biochemical features. The fine structural details of the myocardial cells of _L. marginalis_ are essentially similar to those occurring in the smooth muscle fibres, and the topographic distribution of the various organelles and of submicroscopical structures is highly suggestive of a gross simplicity of these cells in comparison with the vertebrate striated muscles or smooth muscles.

The ultrastructural biology of the muscle cells of molluscs is still in its infancy. Among the representatives of the class Bivalvia, only a few genera and species have been utilized to obtain information on the contractile system in their muscles, in particular, the anterior byssus retractor muscle (ABRM) and the adductor muscles. The molluscan smooth muscles have been designated as 'catch' muscle because of the behaviour of paramyosin molecules (Johnson, _et al._, 1959). Paramyosin has a fast rate of crystallization and solubility. These muscles can maintain a high level of tension for long periods without fatigue. Many molecular biologists have explained the 'catch' mechanism of muscular contraction in molluscs by providing evidence for a shift or change in the state of the paramyosin system enabling it to maintain sufficient tension (Johnson, _et al._, 1959; Rüegg, 1961). An alternative hypothesis explains...
the phenomenon by the sliding filament mechanism. The tension is maintained by cross-bridges or links and their detachment at an extremely slow rate (Lowy and Millman, 1959; Hanson and Lowy, 1961). According to these workers, the cross-links in bivalve muscles are similar to those of the actin-myosin complexes and paramyosin is comparable to the myosin of vertebrate striated muscles. The supporters of Szent-Györgyi school (Philpott, et al., 1960) postulated that sufficient tension develops in an actomyosin system but is cared by paramyosin system. This is the molecular basis of 'catch' mechanism. Recently Lehman (1976) and Sanger (1979) made excellent expositions on the current status of muscular contraction both in invertebrates and vertebrates. The present thesis gives a direct evaluation of the current position in the light of the results obtained from the heart of *L. marginalis*.

Besides, this piece of investigation on the ultrastructure of the heart tissues of *L. marginalis* partially examines the fine structural details of the amoeboeytic cells or haemolymph cells which come very close to the muscle cells *in vivo*. This would be further examined under the scanning electron microscopy in a separate chapter in this thesis.

Highly informative works have been published on molluscan muscles, but mostly they are concerned with the structure and function of the ABRM (Twarog, 1967; Sobieszek, 1973) and adductor muscles of *Mytilus edulis* and of other bivalves (Philpott, Kahlbrock,
Szent-Györgyi, 1960; Hanson and Lowy, 1961; Riægg, 1963; Riægg, Straub and Twarog, 1963; Lowy, Millman and Hanson, 1964; Riægg, 1968; Elliott, et al., 1968; Elliott and Lowy, 1969; Morrison and Odense, 1974). Motley (1933, 1934) initiated the study on lamellibranch hearts and tried to report on the gross morphology and histology on the basis of observations in *Tritogonia verrucosa*, the freshwater mussel. But the ultrastructural description of the cardiac tissue in the salt water clam, *Venus (Mercenaria) mercenaria* (Kelly and Hayes, 1969; Hayes and Kelly, 1969) is a key work to understand the ultra-microscopic features of the cardiac tissue of the freshwater bivalve, *Elliptio complanatus* (Rutherford, 1972). In the present study the ultrastructure of cardiac muscle cells of an old world freshwater pelecypod, *Lamellidene marginalis* shows some convergent features with the marine and freshwater new world bivalves together with some diversities and dissimilarities which highlight some special features of its own. Unlike the pelecypod adductor muscles, which show a series of differences starting from striated to obliquely striated to smooth type (Morrison and Odense, 1974), *L. marginalis* exhibits a smooth muscle variety in its ventricular tissue like those of marine and freshwater bivalves (Hayes and Kelly, 1969) and *E. complanatus* (Rutherford, 1972) respectively and also like the ABRM of *Mytilus edulis* (Sobieszek, 1972). Esser (1934) described branching muscle fibres and anastomosing myocardial muscle cells in *Anodonta cygnea* which are also evident in *L. marginalis* in the form of trabeculae as has been described by Hill and Welsh (1966). In the present
observation no concentric ring encircling myofibres (Kinpichnekova, 1955) has been observed, but the comment of Esser (1934) that in A. cygnea the cardiac muscle cells resemble those of intestine and aorta, seems applicable basically in the present case also, though some special features have also been found. Rutherford (1972) described in the freshwater mussel, Elliptio loosely oriented cardiac muscle cells which establish only weak intercellular connections. No specialized intercellular attachment in the form of tight junction or desmosome has been described in V. mercenaria (Hayes and Kelly, 1969). But on the contrary, the cardiac cells of L. marginalis are more organized and show an unique assembly of cells some of which may maintain individual entity though others are seen to establish very precise intracellular connections via the lateral and terminal processes with a definite trend to produce syncitium and hence forming true muscle fibres. Therefore, it is evident that the ventricular tissue of L. marginalis is composed both the of muscle cells and muscle fibres and the muscle cells are of smooth type.

L. marginalis is provided with cardiac tissues composed of spindle shaped cells with central nucleus just like a typical molluscan heart cell, as described by Esser (1934) in Anodonta cygnea. After surveying a number of literatures on molluscan cardiology Hill and Welsh (1966) reported that the heart is covered by epicardium, but neither in the ultrastructural study of the marine Venus (Kelly and Hayes, 1969) nor in the freshwater Elliptio (Rutherford, 1972) the ultrastructural
features of the epicardial cells have been described. In this study on the fresh water Indian lamellibranch, _L. marginalis_, the ultrastructural details of both the epicardial and myocardial cells have been compared. They show some similarities regarding the structural details of most of the major cellular organelles, but also exhibit striking dissimilarities on the surface modifications, and also regarding the presence or absence of the contractile elements. Both the convergent and divergent characters of epi- and myocardial cells have been discussed with attempts to elucidate the possible significance.

Epicardial cells are bathed in pericardial fluid whereas myocardial cells are directly in contact with the haemolymph. Hence both the cell types are surrounded by connective tissues which are quite identical though few points of similarities exist, as because the process of diffusion is always in action in between this two fluids which are separated by the cardiac tissue; and the haemolymph made particles migrate from intraventricular space to the extraventricular regions. Andrews and Little (1971) suggest for a definite role of ventricular wall in ultrafiltration. The connective tissues around both the cell types are fibrillar in nature because both the cell types contract during their life time. It seems that the dispersed connective tissue cells either participate in holding the epicardial cells and the neighbouring myocardial cells or help in building structural framework of the heart. The dense and evenly dispersed chromatin material of the connective
tissue cells indicate their inactive nature regarding intranuclear synthesis; and these cells are rich in mitochondria. This may be helpful for the connective tissue elements to be at par with the epi- and myocardial cells as far as the cellular contraction is concerned; the glycogen store serves as the raw material for the anaerobic phosphorylation. This is quite common in molluscan smooth muscles. The membrane delimited structures represent both secretory and excretory products.

In the connective tissue part of the granular amorphous material and the fibriller elements are common around both the epicardial and the myocardial muscles. It is assumed that the extracellular granular substance may represent a part of the constituents of the pericardial fluid in case of the epicardial layer, whereas, in case of myocardial cells this represents the plasma components of haemolymph fluid. The lack of periodicity in the fibriller elements raises doubts as to their collagen nature as commonly seen in the cardiac tissues, but their fibriller nature justifies limited amount of contraction.

It should be mentioned here that thick population of cross-banded collagen fibrils has been reported in case of *V. mercenaria* (Hayes and Kelly, 1969). It is noteworthy that the connective tissue cells are distributed around the epicardial cells only. The structure of the nucleus of these cells indicates their inactive nature but on the contrary, the association of ribosomal particles with the nuclear envelope and the RER system suggests the capability of these cells regarding protein
synthesis. The dense matrix of the mitochondria and the remarkable number of the cristae to increase the internal surface of these organelles remind one of the highly active cardiac tissues of both vertebrates and invertebrates.

It seems logical to assume that both the defined and undefined structures around the myocardial cells are part and parcel of the haemolymph fluid. The reason behind the absence of typical non-cardiac connective tissue elements around the myocardial cells may be that they have attained the status of forming syncitium hence less support is required to hold the cells together or allow them to remain in direct contact with the haemolymph. It is doubtful if collagen net hypothesis (Mullins and Cunderoth, 1965) for the transmission of force holds good in _V. marginalis_, and in this respect it differs from _V. mercenaria_ (Hayes and Kelly, 1969). There is no indication of neuromuscular junction in the microphotographs observed. The spherical microstructures with concentric rings probably represent calcium spherules commonly seen in the connective tissue of freshwater bivalves and pulmonates (A.S.M. Saleuddin, York University, 1979, personal communication) and these are composed mainly of amorphous CaCO₃. The number of spherules decreases during the early part of shell regeneration but they are also connected with acid-base balance of the organisms. Concentric rings of these spherules are developed during their growth and saturation. It may be mentioned here that the muscle fibres of main arteries of prosobranch gastropods remain embedded in connective tissues
containing prominent deposits of fats and calcium salts (Fretter and Graham, 1962).

In *Anodonta cygnea*, Esser (1934) described sarcoplasmic axis, while in muscle fibre the nucleus is surrounded by a contractile cortex of parallel fibrils that pass into branches. Kirpichnegaova (1955) also claimed that sarcoplasmic core is surrounded by a layer of fibrils parallel to the long axis of the fibre. But this is not true for both the epicardial and myocardial cells in case of *A. marginalis*. In this animal the epicardial cells are devoid of contractile elements, whereas a thick population of contractile filaments has been found in the myocardial cells at the cortical region parallel to the long axis of the muscle cell under the sarcolemma. Besides this fact, in both the cases the spindle shaped contractile units resemble the auricular cells of *A. cygnea* (Esser, 1934) though the cytoplasm to nuclear ratios are different.

The reason why a few micrographs of myocardial cells exhibited features like those of intermediate muscles is not clear. It may represent either one of the developmental stages or the physiological state of the individual muscle cell.

The epicardial cells are strikingly dissimilar to the myocardial muscles in their possession of a number of quite prominent microvilli bathed only in the pericardial fluid. The reason for the absence of extraneous fuzzy coat on the epicardial surface is
uncertain but the microvilli together with some exocytotic vesicles indicate both their absorptive and secretory nature.

The literature describing the intercellular junctions specially in molluscs are confusing. Structures, like, nexus, attachment plaque, or dense bodies, desmosomes, homodesmosomes and intermediary junctions are having more or less similar descriptions. The epicardial cells show extremely intimate cell to cell associations in the form of desmosomes, septate desmosomes, and tight junctions. In each case, cellular apposition starts with desmosomes with a wide intercellular space between the two dense plaques. This intercellular space is the region for cellular diffusion and ionic exchange, but the desmosome is followed by septate desmosome and tight junctions, both of which act to maintain cell to cell alignment. The horse-shoe shaped cellular junction commonly seen in epicardial cells is like other smooth muscles where out-pushing of one cell penetrates in the form of an invagination into an adjacent cell in a ball and socket manner. This sort of cell surface apposition may be responsible for cellular shape but cell interfaces also produce balloon-like intercellular spaces of varying diameters to interrupt tight junctions. These intercellular spaces may also allow cellular diffusion. Cellular integrity is maintained in a completely different way in myocardial cells with the help of varied surface modifications, distinct from their epicardial counterparts. It is quite evident that the lateral microstructures generally are engaged to establish cellular connections.
laterally whereas the terminal microstructures are to form the end to end cellular attachments. Hayes and Kelly (1969) proposed completely different ideas regarding the occurrence of the 'scalloped surface' of myocardial cells. They are of opinion that the surface evaginations of muscle cells are the result of two distinct factors: (a) relative positions of the attachment plaques during contraction, and (b) development of intracellular force (Kelly and Hayes, 1969). But in the present case, instances where both lateral and terminal processes take part in establishing cellular fusion amongst more than one cell indicate that the main tendency of the myocardial cells in _L. marginalis_ is to establish cellular connections and fusion as elaborately as possible with a definite scheme of producing syncitium and in this process they are supplemented by both terminal and lateral processes as and when the situation permits. Hence, unlike the higher groups of animals, in this freshwater bivalve the myocardial units elaborate cell to cell junctional complexes to suit the functional needs. Amongst the intercellular communicating devices: (a) nexuses commonly found in cell 'intrusions' (Gabella, 1975), are quite fair in number and generally are at to the terminal processes, but they are not that much elaborate as has been described elsewhere. It is claimed that they may occupy 6% of the surface area of the smooth muscles. The reason for this may be that the cell surfaces are sensitive to the fixation for TEM studies, through the tolerance varies from tissue to tissue. In nexuses, the outer layer of the adjacent membrane may even fuse, but in _L. marginalis_
the pentalamellar system is with a light gap separating them and that is the usual case for the smooth muscles. Nexuses are normally 14 nm wide but in L. marginalis it is 210 Å to 300 Å. Nexuses for the smooth muscles are 0.1 to 0.2 μm across and generally with no unusual modification at the cytoplasmic sides. In Mytilus edulis ABRM is connected by nexuses (Twarog, et al., 1973). The nexuses only operate to join the cells here and after glutaraldehyde and osmium tetroxide fixation they appear to be septilaminar structures (Dewey and Barr, 1964; Revel and Kannovsky, 1967; Brink, Kensler and Dewey, 1979), and the total width of the junction was 17.2 nm, and the electron-lucent region between extracellular lamellae was measured 2.6 nm. Septate and gap junctions have been described in other tissues of bivalves (Flower, 1971). The nexuses (gap junctions) have been shown to be involved in electrical coupling between cells (Barr, et al., 1965; Sheridan, 1971; Barr, et al., 1968; Pappas, et al., 1971) but further electrophysiological data have been accumulated on cellular uncoupling (Barr, et al., 1965; Pappas, et al., 1971; Olivera Castro and Loewenstein, 1971). On the other hand, the unique experiment on the effect of lanthanum observed with freeze-fracture technique (Brink, et al., 1979) indicates that the array of pits and protein particles in a nexus can be changed. It is assumed that nexuses are responsible for both the cellular coupling and uncoupling depending on the physiological situation and environmental state of the muscle. (b) Membrane apposition and tight junction like
structures have been described (Zs.- Nagy and Salanki, 1970) in the adductor muscle of Anodonta cygnea. This structure is assumed to be the site of cellular coupling and takes part in the conduction of cellular massages. In L. marginalia, the tight junctions seem to carry out the same function. (c) Lateral processes taking part in a very complicated and elaborate manner to establish cell fusion laterally are extremely striking features in this Indian freshwater bivalve. These lateral processes were clear also from the ultramicrographs presented by Rutherford (1972) in his study on 'The structure of the ventricle of Elliptio complanatus, a freshwater lamellibranch', but he avoided the description of these processes. In L. marzinalis, the old world pelecypod, it is quite evident that the lateral processes establish intercellular connections in the form of cytoplasmic bridges, which may be complete or incomplete but always carry swarms of mitochondria associated with numerous glycogen particles. This observation supports the case in L. mercuraria (Kelly and Hayes, 1969), where the lateral evaginations are loaded with chondriosomes and glycogen particles.

The survey of different micrographs presenting different developmental stages of these lateral intercellular bridges reveals that mitochondria seem to migrate from one cell to the neighbouring one accompanied by glycogen particles. In course of their development the initially slender lateral processes fuse with the neighbouring ones to increase in bulk and width resulting in the formation of many large vesicular structures which may contain (i) membrane-bound particles, (ii) extraneous fuzzy coat or
(iii) dense plaque-like structures. These vesicular structures are seen at the cortical region, and hence their exact role, if they migrate from the periphery to be placed finally in the contractile elements is not clear. At the same time the part played by the mitochondria (lodged inside the 'bridges') both with electron-dense and electron-lucent matrices and stored particles is not understood. It is too early to suggest any role for both mitochondria and glycogen here. It may be argued that the mitochondria may have some role in conveying molecular or ionic messages to the neighbouring cells aided by glycogen as convoys of ready energy.

The presence of microtubules in lateral processes indicates their role in the cytoplasmic movement through the lateral processes. These processes seem to be important regarding cellular excretion also as because single and confluent excretory vesicles are found here, together with exocytotic vesicles. (d) Desmosome-like structures present in myocardial cells have already been described in epicardial units. Zs-Nagy and Salanki (1970) have described desmosomes in *Anodonta cygnea* in the adductor muscles where the muscle cells are shorter than the total length of the adductors. The 'attachment plaques' (Hayes and Kelly, 1969) which may be equivalent to desmosomes found in other tissues (Gabella, 1973) are quite evident in the present preparations. These are like the 'fusiform bodies' described in invertebrate smooth muscles (Hanson and Lowy, 1957, 1961), and are similarly composed of thin myofilaments covered by dense amorphous substance.
Extraneous fuzzy coats are reported near these attachment plaques. These are distinguished from the nexuses having dense formation of fibrous material on the cytoplasmic faces of the two adjacent membranes. The thin filaments converge towards and merge with them. The asymmetrical attachment plaques or semidesmosomes with bundles of myofilaments are frequently found to be drawn to the cytoplasm. This helps to develop an infolding of the cell membrane in apparently contracted cells in this specimen as has been described elsewhere in other smooth muscles. Twarog (1967) noted this 'attachment plaque' in the ABRM of Mytilus. Rutherford (1972) described this structure in Elliptio, the freshwater bivalve, as rectangular structures underlying the sarcolemma. The 'half desmosome' (Twarog, 1967) or the attachment plaque is regarded as the attachment sites of the thin filaments to the sarcolemma (Hayes and Kelly, 1969). The free (Hayes and Kelly, 1969) fusiform bodies are generally known as dense bodies. Early workers (Prosser et al., 1960) described the dense bodies as non contractile protein structures. But Lane (1965), on the other hand, opined that the number and the shape of these structures depend on the physiological state of the muscle cell, while Harman et al. (1962) called them as 'grapple plaque' in human smooth muscle and suggested that they act as traction sites and transmit tension both mechanically and electrochemically. It is assumed that the attachment plaques are responsible to maintain the structural integrity and anchor the thin contractile filaments - the actins. Regarding the function of free dense bodies Panner and Honig (1967) compared
those structures with the Z-lines in striated muscles. This view has been supported by recent evidence (Szente-Györgyi, Cohen, Kendrick-Jones, 1971), which reports that the thin filaments on each half of dense body have opposite polarity.

The terminal processes are unique features in freshwater Old World lamellibranch, *Lemellidens*. No earlier description of such structures has been followed either in new world freshwater bivalve, *Elliptic* (Rutherford, 1972) or in marine *Venus* (Kelly and Hayes, 1969). The piller-like discrete cellular entities between the terminal ends of adjacent cells very clearly indicate that these terminal processes can fold themselves like collapsible gates to pull the cells together and the mitochondria and the swarms of glycogen particles.

In non-cardiac smooth muscles of molluscs especially in gut muscles, the finger-like invaginations that bent at right angle below the cell membrane to run parallel to the longitudinal axis of the cell have been described. This gives rise to profuse branching in the form of vesicular structures in the cortical region of the cells. This type of complicated cell invaginations has not been found either in epicardial or in myocardial cells in the lamellibranch under consideration. In fact, cell membrane invaginations or pinocytotic canals are less marked in epicardial cells than in myocardial cells. The latter cell type shows wider pinocytotic tubules with fuzzy coat and in some rare
cases with extracellular fibrillar elements or sometimes with half desmosomes or dense plaque-like structure. This is because the myocardial cells exhibit regional fuzzy coat, the surface modifications are more varied than it is in epicardial cells. The exact role of these invaginating processes are not known; may be, they carry some messages from the extracellular sites to the deeper contractile elements through their counterparts in the peripheral region of the cell, or, they are only the resultant features of a tendency to increase the surface areas of the cells. Kelly and Hayes (1969) described vesicles to be the part of sarcoplasmic reticulum in Venus (Mercenaria) mercenaria. Discrete particles bound to these membranous structures in case of myocardial cells resemble those described elsewhere in molluscan smooth muscles.

The smooth muscles are usually activated by rise in myoplasmic free calcium, and it seems probable that the great bulk of this activating calcium has to be translocated from the surface sites, either from the extracellular fluid by diffusion via plasma membrane or from known or unknown storage sites lying immediately below the plasmalemma. Therefore, it is expected that the activation process in nonstriated muscle would be slow, in comparison to the skeletal muscles (Syson, 1974). It is evident that in most visceral muscles there is little dissemination of the transient surface depolarization on the myofilaments. The small vesicles or tube-like internal membrane bound features certainly increase the membrane surface area over which current
flows. Hence, in _L. marginalis_ the short tube-like extensions of the plasma membrane into the peripheral part of the sarcoplasm may have the same vital role as in other molluscan smooth muscles.

Rough surfaced ER system with dense granular canaliculi observed in epicardial cells, lacks patterned orientation. But, on the other hand, in myocardial cells varied forms of cytoplasmic vesicular structures of different origin have been found. Besides, (i) the excretory vesicles in the lateral processes described earlier, (ii) the pinocytotic vesicles and (iii) sacs formed by the fusion of the lateral processes are distinct, and they structurally vary from each other. Extremely striking are similar membrane bound structures which are found in (a) close association with the mitochondrial surfaces deep in the tissue and (b) amongst the contractile filaments. Kelly and Hayes (1969) reported close association of mitochondria with the flattened sacs they described at the central region of the contractile units.

The extracellular calcium mediating electro-chemical (EC) coupling in smooth muscles has been proved (Washizu, 1967; Bulbring and Tomita, 1970). And in that case the activity of the sarcoplasmic reticulum is very important (Somlyo, 1972). Evidence suggests that the microsomes isolated from smooth muscle, bind calcium. And in case of smooth muscles where there is lesser number of ER system some sort of calcium storage site is likely to be present within the smooth muscle fibres. The micropinocytotic vesicles
or the inpushings of the plasma membrane gradually separate from the surface and enter the outer sarcoplasm just below the plasma membrane. These maintain a distance of 4-5 nm between them and the mitochondria. This observation leads to the possibility that either the surface vesicles or the mitochondria or both are involved in the process of calcium release and uptake, associated with contraction and relaxation. In the smooth muscles of guinea-pig microsomes actively separate calcium in the presence of magnesium and A.T.P. (Hurwitz, et al., 1973). But it is not clear whether they originate from preexisting cell structures in the form of mitochondria or are derived from any reticular or vesicular systems. Syson (1974) prepared vesicular entities from separate mitochondrial and plasma membrane fractions, in order to follow calcium uptake and release. Syson's observations on smooth muscle indicate that the Ca\(^{++}\) uptake by mitochondria may be due to simple passive diffusion of Ca\(^{++}\) into mitochondrial fraction, followed by electrostatic bindings; but the level is too small to account for the Ca\(^{++}\) requirement of the cell in electrical coupling. Mitochondria play some role in Ca\(^{++}\) translocation but this role may be only a general 'mopping up' during relaxation. The greater binding tenacity of the plasma membrane vesicular fraction indicates that it is this fraction which may be responsible for the ultimate pumping of sarcoplasmic calcium down to levels low enough to permit relaxation. Different visceral muscles differ in relative balance of mitochondria and surface vesicle and in some, mitochondria may play a more significant role. These hypotheses suggest that the terminal
stages of calcium control in EC coupling are far from being solved as yet. It may be that the Ca\textsuperscript{2+} storage within visceral muscle cells is low, and that the binding activity of reticulum or plasma membrane vesicle or mitochondria, or all of these agencies may be adequate to reduce myoplasmic calcium to explain relaxation satisfactorily. Calcium pump in nonskeletal muscles such as heart contains relatively little reticulum, and it has been suggested that the free Ca\textsuperscript{2+} concentration within these is controlled not by a reticulum, but by the plasmalemma itself. In other words, Ca\textsuperscript{2+} is pumped inward and outwards of the whole muscle cell during activity (Hasselbach, 1964). Such a mechanism could, of course, operate only in exceedingly slow muscles, because it would clearly upset the delicate Na\textsuperscript{+}/K\textsuperscript{+} balance in the plasmalemma, on which the depolarization and repolarization of fast skeletal muscles depend, it would also present a very long diffusion pathway.

Ultrastructural examination (Watts and Pierce, 1976) showed cholinesterase reaction products localized only on the extensive regions of cell membrane in Modiolus demissus, and intracellular staining was never observed. In I. macrinalis, myocardial cells produce a very 'non-regular' type of vacuolar system, exhibiting peroxisome-like structures, etc. The ER system is not well marked in these types of cells, and if they play any protective role via metabolism of any toxic substance is not known. These microbodies have not been reported either in Elliptio,
(Rutherford, 1972) the freshwater bivalve, or in marine Venus (Kelly and Hayes, 1969). However, Owen (1972) described lysosomes and peroxisomes in bivalves.

Little is known about the nucleus of the bivalve, Elliptio complanatus (Rutherford, 1972). They are centrally located and irregular in outline the reason of which may be contraction prior to fixation. Like Elliptio both epi- and myocardial cells in Lamellidens are mononucleated with nearly centrally located nucleus. But the nuclei in case of epicardial cells are considerably prominent in comparison to those of myocardial cells. In epicardial cells the spherical nuclei with 186 Ǻ perinuclear space is quite distinct from those of myocardial cells where the nuclei are oval to elongated in shape and may exhibit irregular foldings and the perinuclear space is 250 Ǻ. But in some cases the outer membrane may throw extensive swellings 2000 Ǻ in diameter on average indicating their supposed role in the formation of cytoplasmic vesicular structures or it may also be the result of inadequate fixation.

The nuclear pores are much more evident in case of epicardial cells, though the pore complex in detail is not evident in any of the cell types. The reasons may be amenable to the techniques followed for TEM preparations, as because the nuclear pores are very delicate and sensitive to the fixation procedures. But nuclear pore is usually seen in other molluscan smooth muscles, and on average they are 13 nm across. In epicardial cells, the conspicuous nucleoli with dense granular core and the filamentous cortex that in turn establish
associations with extensive heterochromatin at the nuclear periphery together with the rough nuclear membrane indicate active RNA synthesis. On the contrary, in the myocardial nucleus the strikingly scanty amount of peripheral chromatin, the highly dispersed chromatin material with different electron opacity and presence of single or double prominent nucleoli with filamentous pars amorph a also indicate that ribosomes are profusely synthesised in these cells.

In Venus (Mercenaria) round or ovoid mitochondria have been described (Kelly and Hayes, 1969), but Rutherford (1972) observed these chondriosomes as elongated structures in Elliptio. The shape of this cellular organelle varies from elongated to ovoid in Lamellidens depending on the plane of sectioning. In both the cell types mitochondrial cristae appear in sharp negative contrast and a fair number of them are haphazardly oriented. This is expected usually in any smooth muscle whose mitochondria take a very active role. Frederic and Chevremont (1953) observed that mitochondria make periodic contacts with the nucleus in fibroblast cells. This behavioural pattern has also been noted in both epicardial and myocardial cells of the specimen in question. In myocardial cells the level of a highly synthetic machinery in the chondriosomes can be measured by the presence of discrete and diffuse particles along with the ribbon-like structures in the matrix. Fine granular inclusions in the mitochondrial matrix have also been found in Venus, the marine bivalve (Kelly and
Hayes, 1969). The topographic positions of organelles point towards their multiple functions: (1) They supply energy to the contractile filaments materialising contraction and relaxation. (2) They receive messages from the subsarcolemmal cisternae which remain in intimate association with them. (3) They carry information from one cell to another via the lateral processes and (4) They supply power for the contraction or folding of the terminal microstructures. The mitochondrial division in the cardiac cells of Lamellidesma establishes the fact that they increase their number depending on the requirements of the cells, and the vacuoles in the inner chamber of a few indicate that lysis (?) of this structure may take place after a very active life span.

Rutherford (1972) did not describe any cytosome-like structures in Elliptia, but in L. marginalia varied forms of cytosome-like bodies have been found dispersed in the cytoplasm of the epicardial cells, but they are meagre in form and number in myocardial cells where they are only peripherally oriented. The reason of this type of distribution is not clear. The yellow pigment granules (Notte, et al., 1965) occur in the neural cells of many species of bivalves and snails. Cytosomes (De Duve and Wattiaux, 1966) show some similarities with the vertebrate lysosomes as the name indicates but at the same time they show some different functions even in the nerve cells of Amoeba ovumae (Zs. - Nagy, 1967; Zs. - Nagy and Kerpel Fronius, 1970a). Cytosomes of all the non-neural tissues including heart muscles
are identical with the neural cytosomes (Zs. - Nagy, 1967; 1969; Zs. - Nagy and Borovyagin, 1972), and they produce oxidative energy even under anoxic conditions. Generally two types of limiting membranes are found (Zs. Nagy, 1973) associated with the cytosomes: (1) outer limiting membrane with internal one displaying regular unit membrane character, or, (2) five layered complexes of membranes. In spite of only these two varieties, several types of cytosome-like structures have been observed specially in the epicardial cells of _A. marginalis_. These are: (a) membrane delimited structure with highly electron-dense granular materials with lipid-like bodies (as has been described by Nagy, 1973), (b) dense microstructures, (c) larger varieties with electron-dense granular materials and clear vesicles or dense microstructures. In further support to Nagy's observations (1973) here these cellular components are not found in association with the contractile filaments. But their abundance in epicardial cells and accumulation in the lateral processes of myocardial units and further their intimate association with the mitochondria need careful explanation.

Workers are still in dispute regarding the function of cytosomes. Certain cytosomes display acid phosphatase activity (Meek and Lane, 1964; Lane, 1966; Zs. - Nagy and Borovyagin, 1972), but the detailed morphological study and the lipid contents of cytosomes differ markedly from lysosomes (De Duve and Wattiaux, 1966), and on the other hand, any of the respiratory enzymes is not reported in lysosomes (Gahan, 1967). But the total function of the cytosomes is more than lytic as has been
indicated by their activation during anoxia (Zs. Nagy and Borovyagin, 1972). 'Anoxic endogenous oxidation' attributed to nerves (Zs. Nagy and Ermini, 1972b; Zs. Nagy, 1971a, 1973) may be suggested for the total animal tissue (except adductors) cytosomes also. Hence, respiratory enzyme activity and cytosomal anoxic energy dependent Sr** accumulation (Zs. Nagy, 1967, 1971a, Zs. Nagy and Kerpel Fronius, 1970a, 1970b; Kerpel Fronius and Zs. Nagy, 1973) may take part in the anoxic metabolic processes, and it may even be true for the cardiac tissues of bivalves.

In the ultrastructure of the ventricle of Elliptio, the presence of golgi apparatus has not been described (Rutherford, 1972). This is also true in Venus (Kelly and Hayes, 1969; Hayes and Kelly, 1969). But the present study reports a prominent golgi complex with its elaborately dispersed cisternae revealing the glandular nature of the epicardial cells. In the myocardial cells this structure has not been adequately identified but near the nuclear region some of the components of the golgi apparatus await further elucidation.

According to Revel (1964) glycogen particles are of α-type in both Venus (Kelly and Hayes, 1969) and Elliptio (Rutherford, 1972) ventricular fibres. In the latter form the average diameter of glycogen rosette is 532 Å, with a range from 478 to 586 Å (Rutherford, 1972), but in Venus, glycogen rosettes are 500 to 550 Å in diameter whereas, in case of Lamellidens they are 700 Å. Two varieties of glycogen particles have been found here
α and β particles. In epicardial cells, it is assumed that
the glycogen particles serve as the substrate for both the
oxidative and anaerobic oxidation, and the function of glycogen
is presumably manyfold in myocardial cells, where they remain
in close association with the contractile filaments nearly
intermingled with them in the form of rosettes and discrete
particles, and also in the lateral and terminal processes. These
particles are also found swarming around the vesicular structures
resembling 'diads' deep in the contractile elements.

In Lamellidene the presence of ribosomes and polysomes, which
are not described in Elliptio (Rutherford, 1972), indicates
synthetic nature of the cell in case of epicardial units, as
these granules are found in association with the ER system.
But in this cell type their association with the sarcolemma is
not clear. In myocardial cells the huge population of ribosomes
and polysomes in association especially with contractile elements
reminds the suggestion given by Davies (1965), that polyribosomes
take part to prepare myosin, but the reason of their population
in the lateral processes is not defined.

In pelecypods an unique approach has been made for understanding
the structural and functional relationship of the ABM
(Apouinary Sobieszek, 1973) and adductor muscles (Hanlon and
Lowy, 1961, 1962; Morrison and Odense, 1974) either by
adopting better technique for cell preservation or by the use of
X-ray diffraction studies (Lowy and Vibert, 1967) or optical
diffraction analysis of electron micrographs (Apolinary Sobieszek, 1973). Although all of these studies provided a guideline to analyse the structure and types of the contractile elements involved in muscle physiology of bivalves, little such approach has been made the better understanding of cardiac muscle movements. And it is to be clear if the rhythmic activity of most of the cardiac units should be explained in the light of catch mechanism. 'Catch' mechanism (Nieuwenhoven, 1947; Twarog, 1954; Johnson, 1954, 1958) or a modified form of catch mechanism (Jewell, 1959) have received support from Philpott, et al. (1960). They were of the opinion that the two separate systems: (a) Actomyosin (phasic) and paramyosin (tonic) systems may be present in the same muscle. Rüegg (1964) holds the same view. But Lowy, et al. (1964) have in opposition argued that the characteristic protein of the thick filament of catch muscles (tropomyosin A) is present in the thick filaments of the obliquely striated part of the adductor in Crassostrea sp. and this muscle cannot maintain tonic contractions. It is likely that the different responses characteristic of tonic and phasic muscles may have their origin in the totality of organization; hence may not solely depend on the structural characteristics of their myofilaments. An organization that provides rapid provision and removal of calcium ions, at the active sites (Weber, 1966) and excitation - contraction coupling (Huxley and Straub, 1958; Huxley and Taylor, 1958) is one
'Long filaments' (Rutherford, 1972) have been found arranged peripherally both in Venus (Kelly and Hayes, 1969) and Elliptio (Rutherford, 1972). But close examination of the plates (plate 2 through 4, page 427 to 431) presented by Rutherford (1972) on the ultrastructure of Elliptio reveals that in Lamellidens the population of myofilaments is more dense.

In ABRII of Mytilus edulis (Apolinary Sobieszek, 1973) very regular arrangement of thick and thin filaments has been reported. In Placopecten magallanica, Crassostrea virginica, Arctica undata (Morrison and Odense, 1974) series of types of muscles were observed, while the striated and smooth types exhibit 'vague' (Morrison and Odense, 1974) differences. But in both the types either fixed in 'in situ' condition, or, in stretched mode, no pattern is evident in the fibres. Absence of a regular pattern of organization of the myofilaments, dense bodies, and sarcotubular system with associated mitochondria is correlated with slow and sustained (tonic) contraction in the smooth muscles. Haphazard orientation of myofilaments in case of Lamellidens indicates that this type of cardiac units may be grouped with tonic muscles. But their elaborate vesicular system and orientation of chondriosomes impart a bit of speciality to this type of contractile cells. But phasic contraction is related to the specialized organization of muscle fibres into sarcomeres, consisting of myofilaments, dense bodies, transverse and longitudinal sarcotubules and parallel column of mitochondria. The latter arrangement together with a
'diad' association between sarcolemmal invaginations, and the sarcotubules appears to be significant in relation to the rapid movement of calcium ions to and from the active sites on the myofilaments, and to EC coupling.

The cardiac muscles show progressive specialization, in which dense bodies become transversely aligned and the contractile units come to lie in parallel with one another, giving rise to the series of sarcomeres seen most clearly in the longitudinal sections of Achatina auricle. It has been suggested by Nisbet and Plummer (1968) that the evolution of phasic contractile system therefore, may be more dependent, upon the development of close associations between myofilaments, vesicular or tubular conducting systems and mitochondria, than upon the variations of biochemical organization in the myofilaments themselves.

The proteins of the contractile apparatus of non-striated muscle are essentially similar to those of skeletal and cardiac muscle, but the thick filaments of the latter are organized in a rather different manner from the myosin filaments of smooth muscle. Like vertebrate striated and smooth muscle and other molluscan smooth muscle, cardiac cells of molluscs are also provided with two kinds of filaments (Hanson and Lowy, 1959; Philpott, Kahlbrock and Szent-Györgyi, 1960). Previously it was thought that in the smooth muscle the demonstration of thick filaments needs adequate technique for the preparation of visceral muscle for electron microscopy. Hence there is an argument that thick
filaments of skeletal and smooth muscle are not basically the same, but instead in the smooth muscles the thick filaments are transient structures. But thick filaments are now demonstrated in tissue sections by routine procedure (Somlyo, et al., 1973). In *Lamellidens* the demonstration of thick filaments is not very difficult as the case in other invertebrate smooth muscles.

In *Lamellidens* like other smooth muscles of molluscs (Hanson and Lowy, 1959; Philpott, Kahlbrock and Szent-Györgyi, 1960) two kinds of filaments have been observed. It is suggested that the thin filaments have the same appearance as those of striated muscle (Hanson and Lowy, 1963) and composed basically of actin (Szent-Györgyi, Cohen, Kendrick-Jones, 1971). Periodicity of about 5 nm has been reported in the thin filaments of one preparation of adductor muscles in a pelecypod (Morrison and Odense, 1974), whereas periodicity of about 5.5 nm has been reported for actin filaments (Lowy and Vibert, 1967). But no such periodicity has been observed in the thin filaments of *Lamellidens* and Rutherford (1972) offered no description of thin filaments whatsoever in the freshwater bivalve, *Elliptio*. Thin filaments may be sinusoidal with a swing towards the attachment plaque or dense body. The thin filaments appear very thin and tenuous in longitudinal section and difficult for detailed substructural description. Kelly and Hayes (1969) described the thin filaments in case of *Venus* as non-rigid structures. In molluscan smooth muscle thin actin filaments may appear to have an electron-lucent core. In ABRM of *Mytilus* the thin filaments
with an average length of 11 μm are arranged very regularly though the degree of order varies from preparation to preparation. Here the thin filaments pack hexagonally in multi-rows, branched in two dimensions and so called 'actin lattice' of vertebrate smooth muscle has been reported (Apolinary Sobieszek, 1973). But no such orientation of thin filaments has been reported in the freshwater bivalve Elliptio, and in case of Lameillidene the arrangement of thin filaments is too haphazard to suggest any pattern; and that may be caused by the fixation procedure applied. In case of marine bivalve Venus, thick and thin filaments show orientation comparable to the lattice observed in the transverse section of a band of cross-striated muscle, but they fail to show any definite pattern (Kelly and Hayes, 1969). Sobieszek (1973) opined that the order of the thin filaments is rather sensitive and this situation is comparable to the contractile apparatus of vertebrate smooth muscle which is also sensitive to the condition used for chemical fixation (Small and Squire, 1972). In Lameillidene the thick filaments are followed by several rows of thin filaments, (the number being variable) oriented in parallel fashion to the thick filaments though their display is not quite ordered. Areas free of thin filaments are also noted here and this character they share with other smooth muscles. It has been suggested that dense bodies of vertebrate smooth muscles are artifacts and not the ordering sites of the thin filaments (Small and Squire, 1972). On the other hand, Twarog (1967) reported the presence of dense bodies in ABRM of Mytilus and he was of the opinion that the dense bodies are formed.
by the aggregation of the thin filaments. Similar hypothesis has been forwarded by Hayes and Kelly (1969). In the report on TEM studies of the ventricular muscle of *Elliptio*, Rutherford (1972) suggested no idea on the probable nature of the dense bodies. In case of *L. marginalis* thin filaments are seen to emerge out of the two sides of the dense body. These dense bodies are observed as attachment plaque on the sarcolemma and two such attachment plaques or half desmosomes are found apposing each other in neighbouring cells. This condition has also been reported in ABMN of *Mytilus* (Sobieszek, 1973). In the latter case dense bodies are on average 1.8 \( \mu \) in length and 0.12 \( \mu \) in diameter, and after a 'careful' study, Sobieszek (1973) opined that these structures in *Mytilus* are 'real' and 'significant' as because their distribution is also uniform throughout the muscle. In the present study, the dense bodies are 0.57 \( \mu \) long and 0.1 \( \mu \) in diameter on average and they may serve as the probable sites for tension propagation along the whole muscle as has been suggested in the case of ABMN of *Mytilus* (Sobieszek, 1973). Here, uniform distribution of this structure has been noted, but it lacks recognizable order. In adductor muscles the arrangement of thin filaments has been reported to be irregular; the dense bodies in *C. virginica* and *A. islandica* were too far apart to show whether any alignment was present (Morrison and Odense, 1974). The presence of contraction bands indicating greater alignment needed for contraction (Lowy and Hanson, 1962) in *Lamellidens marginalis* supports the view of Sobieszek (1973) regarding the function of dense bodies. The
variable diameters (166 Å to 270 Å) of thick filaments in *L. marginalis* indicate that these cylindrical structures gradually taper at the two ends, and the cross-sections of the thick filaments resemble that of smooth muscles. The tapering nature of thick filaments is due to the presence of paramyosin (Lovy and Hanson, 1962). Regulatory proteins are located inside the nodes near the active sites of myosin and the paramyosin that form the core of myosin shows bipolarity. In *Mytilus* ABRM the thick filaments are 25 μ long, and the diameter varies from 10 to 75 nm, whereas in *Elliptio*, Rutherford (1972) reports that paramyosin filaments with average diameter of 315 Å have a range from 319 to 426 Å, and in *Venus* the thick filaments are 350 to 400 Å in diameter, and 1.5 to 2.0 μ in length (Kelly and Hayes, 1969). Apparent overlapping of thick filaments has been observed in between the dense bodies in the adductor muscle of *C. virginica* and *A. islandica*, and also in *L. marginalis*. Although the initial impression given by the thin filaments in molluscan smooth muscles is that of a random distribution, they tend actually to arrange themselves in complete or incomplete circular orbits around the thick filaments. The same situation is observable in *L. marginalis*; several rows of thin filaments run between the thick filaments. In *Mytilus edulis* the ratio of thick to thin filaments is 1:17; in *Lemellidens*, the ratio is 1:12+7 and in case of smooth muscles the ratio is 1:15, which may be up to 1:30.

In ABRM of *Mytilus* the surface of thick filaments show regular arrangement of projecting structures, representing the general
symmetry of the Bear-Selby net and are interpreted as myosin projections (Sobieszek, 1972). Projections or cross-bridges were originally observed by Huxley (1957) in vertebrate striated muscles, and are thought to represent the head of myosin molecules projecting from the thick filament surface. There is still some uncertainty regarding the exact arrangement of myosin molecules in the filaments studied in various muscles (Huxley and Brown, 1967; Reddy, 1968; Squire, 1971) and the reason for the limited knowledge (Small and Squire, 1972; Sobieszek, 1972) regarding the electron microscopical evidence that relates the arrangement of the myosin molecules on the thick filaments of muscle may be due to sensitivity of these structures to the preparation method in practice. In the present study cross-bridge arrangement has been observed only in longitudinal sections, and in cross-section a 'Halo region' of semi-dense material has been observed imitating the case in ABRM of Mytilus edulis (Sobieszek, 1972) and similar connections between thick filaments surface and thin filaments were observed. Aggregation of thick filaments is also a common character in these two forms of muscles, and this sort of organization may be a type of artifact (Sobieszek, 1972). Considering all these facts discussed it may be assumed that in the molluscan smooth muscle there exists contractile unit that has the form of a sarcomere (Sobieszek, 1972). Diagonal periodicity of simple appearance has been found in the thick filaments of C. virginica, interwoven type in Arctica islandica and a diamond pattern in Astarta undata have
been reported. In *C. virgo*ino*ce* a transverse periodicity of 5.6 nm and a diagonal periodicity of 53.4 nm (Morrison, Cameron and Odense, 1970) support the observation of Hanson and Lowy (in Csergoly, 1964); the same observation is true also in *C. angulata* in the negatively stained preparation of homogenised opaque muscle (Elliott, Lowy and Squire, 1968) and in *Mercenaria mercenaria* (Cohen, Szent-Györgyi and Kendrick-Jones, 1971).

Morrison and Odense (1974) claimed that in the sectioned material of *Hystilus* ABKM (Twarog, 1967) there are indications of periodicities. But no such characteristics could be noted in the present study.

Myosin aggregates in such a way that the globular region of the molecules projects from the surface of the filaments as lateral projections. These myosin heads act as cross-bridges with actin filaments, mediate the actual sliding of the thick and thin filaments relative to each other and bring about contraction. Although cross-bridges are identified less easily in smooth muscles, but they are similar to those of the striated muscles.

It is an well established fact that speed of muscle action depends not on the structure of the filaments, but more on the number of mitochondria, and the complexity of the sarcotubular system, because the mitochondria are the source of energy for contraction and the calcium ions move to and from the filaments through the sarcotubular system (Hibbet and Plummer, 1968). In *Lamellidens*
no well-developed sarcotubular system has been found but like in the adductor muscles of *C. virginica* and *A. islandica*, the separate vesicles discussed earlier and those maintaining close association with mitochondria and contractile apparatus may have taken up the role of SR system. Both the vesicular structure and the 'diad-like' structures in association with glycogen are unique for *Lamellidens* and are not described in *Elliptio* (Rutherford, 1972) and in *Venus* (Kelly and Hayes, 1969). The glycogen in this complex may help the process of ionic exchange, whereas the glycogen rosettes in the contractile apparatus may serve as the energy source, while the microtubules may help in the relative movement of the contractile filaments. But all these assumptions need further close viewing and test.