CHAPTER II

Histological studies on the heart of Lamellidens marginalis.
A survey of the earlier works on the histological features of the heart of the bivalve molluscs indicates a poor information content on this subject. It is believed that the muscular arrangement of the heart is rather irregular and chaotic in Ostrea, Anodonta, Venus and Pecten. According to Brück (1914), Jullien (1935), Brunet and Jullien (1936a, b), there exists a reticular arrangement in the contractile system of some bivalves and this pattern is denser in ventricle of the heart. According to these workers, the muscle fibres seem to anastomose with one another in Pecten and Ostrea. The heart of fresh-water bivalves (Unionidae) has smooth fibres (Cate, J. Ten, 1929; Motley, 1933) although Takatsuki (1934) described cross-striated muscle fibres in oyster heart.

Suzuki (1934) described the existence of nerve cells in the heart wall of Ostrea, at the base of the auricles, at the auriculo-ventricular junction, and in the ventricle of the aortic emergence. But Motley (1933) could not establish the existence of nerve cells in the hearts of Unionidae. Similar report has also been made in Anodonta by Esser (1934).
The present study is based on the light microscopic investigations on the cardiac tissues of *Lamellidens marginalis*, a fresh-water unionid bivalve of India. In fact, there are more than one species of the genus *Lamellidens*, viz., *L. conocephalus*, *L. corrianus*, etc. Among these only *L. corrianus* has been examined by Narain (1972) from the point of the structure of the heart and the circulating amoeboocytes.

The present report gives a comprehensive histological picture on the different morphological components of the heart of *L. marginalis*. Here the heart is comprised of a large median ventricle, two prominent auricles and the valves guarding the two auriculo-ventricular apertures. Additionally, histological information on the epicardial cells, myocardial muscle fibres or cells has been detailed out considerably. An attempt has also been made to correlate these observations with those known in other bivalves, both fresh-water and marine forms. In particular the structure of the heart of a related species of *L. marginalis*, viz., that of *L. corrianus* has been referred to whenever necessary. It has also been suggested that a thorough study on the histology of the heart of this genus would throw newer information/data with regard to the arrangement of different parts of the hearts, the orientation of the myocardial fibres towards the formation of 'nodal complexes', the existence of pericardial glands, etc.
It is unfortunate that the hearts of several invertebrate types have been histologically examined (Sanger, 1979). This picture is simply inadequate and at times highly wasteful to exclude the morphological and histological studies of hearts of many invertebrates. A wider coverage on this subject would ensure the convergence, divergence and even existence of unusual histological patterns or organization in the invertebrate hearts. Such attempts are exceedingly meaningful histo-physiologically on a phyletic scale. The various questions of cardiac rhythmicity and control mechanisms may be settled either by a universal physiological plan or by alternative ways, provided adequate knowledge has been garnered from a wide variety of bivalves, both related and unrelated forms. The empirical demonstration of connective tissue elements in the cardiac tissues of bivalves, like, the present form, requires special attention in view of cell growth and division and particularly during relaxation and contraction. Some of these questions have been touched in this thesis.
MATERIAL AND METHODS

For histological observations two distinctly different techniques were adopted for specimen preparations: (A) serial sections of the heart were stained in haematoxylin (Iron alum), Masson's trichrome and Bromphenol Blue for histological observations; (B) teased muscle fibers were stained in Aniline Blue. For both techniques specimens were allowed to settle in for two days and specimens of mean length, breadth and height of 9.7 cm., 4.7 cm., and 2.3 cm. respectively were selected for histological observations and were subjected to techniques (A) and (B).

(A) The heart of Lamellidens marginalis is very delicate as far as the structure and the texture of the organ is concerned. This pumping organ collapses when it is taken out of the body and is quite difficult to manipulate during the usual processing schedules for making paraffin blocks, and rather difficult to cut serial sections in its collapsed and twisted form. The sections of the collapsed heart is never ideal for histological observations because the contour of the heart is lost and tissues lose respective topographical positions. To avoid such eventualities a technique has been
developed to fix hearts in non-collapsed condition prior to dropping into the alcoholic Bouin's fluid. Alcoholic Bouin's fluid and air introduced inside the cavity of the pumping auricles just with the help of a 10 ml. hypodermic syringe by penetrating the non-cardiac tissues and the primary ctenidia and reaching the auricular lumen. This procedure avoids the rupture of the pumping muscular chambers of the heart. The ventricle was then dissected out with right and left auricles intact for immediate fixation in the alcoholic Bouin's fluid. Routine processings were followed by paraffin blocks. Serial sections were cut and stained in Iron alum haematoxylin, Masson's trichrome and Bromphenol Blue. Light microscopic observations were made with both high and low power objectives and microphotographs were taken.

(B) Considerable amount of haemolymph was syringed out from the actively pumping heart. A 10 ml. hypodermic syringe was introduced inside the ventricular cavity via non-cardiac tissues with an intention not to rupture the heart while collecting the haemolymph fluid. Slow speed centrifugation (4000 rpm) proved to be useful to get haemolymph free of corpuscles. This cell-free haemolymph was brought to room temperature and cardiac tissue from the (i) epicardial layer was collected from (a) ventricular 'Moše' region, (b) other parts of the ventricle and (c) auricular wall together with
the (ii) myocardial tissue from the (a) 'Node' region, (b) other parts of the ventricle and (c) auricular wall. These parts of the heart were teased in the serum as the medium. These teased muscles were stained in Iron alum haematoxylin and Aniline Blue and were observed under the light microscope both at low and high power objectives and microphotographs were taken.
OBSERVATIONS

(A) The following organs are described from the stained serial sections of the heart:

(i) **Auricles**: In most of the cases, the auricles cannot withstand the rigours of the processings to allow paraffin block (Fig. 4). However, some of the sections permitted to record several features of the auricles (Figs. 1, 2 and 3). Histological observations revealed that auricles are composed of delicate and highly convoluted wall that is composed of epicardium and comparatively thick myocardial layer (Fig. 5). The epicardial layer is formed with flat rectangular cells with nearly centrally placed prominent single nucleus (Fig. 5). The myocardial cells are elongated with central oval nuclei and the muscle fibers look vacuolated with lightly stained cytoplasm or sarcoplasm (Fig. 5). The auricles communicate with the ventricle via a couple of wide auriculo-ventricular apertures guarded by valves (Fig. 4). Auricular epicardial walls extends over the outer surface of the in-folded auriculo-ventricular valves (Fig. 4) and very prominent pillar shaped or finger-like distinct cells,
known as pericardial gland cells (Figs. 4, 8 and 12) are observed at this region forming distinct patches of glandular cell aggregates.

(ii) Pericardial glands: Pericardial glandular cells are columnar or finger-like in shape (Figs. 8, 9, 11 and 12). These distinct cell type is recognizable as mosaic with the epicardial cells (Figs. 9 and 11). These cells are provided with very prominent but almost round nuclei. Each nucleus has densely packed chromatin materials and a nucleolus (Figs. 12, 13 and 14). A thick population of pericardial glands is found at the junction of the auricles and the ventricle, i.e., near the two auriculo-ventricular apertures around the auriculo-ventricular valves (Fig. 4). Besides, these two sites, pericardial glands have irregular distribution at the two lateral sides of the ventricle (Fig. 20) and in particular the ventro-lateral surfaces of the ventricle are marked by a remarkable accumulation of these glandular cells (Figs. 4, 13 and 14).

(iii) Auriculo-ventricular valves: These valves are composed of characteristic cardiac tissues and are observed on the 'middle chamber' (?) of the ventricle only when the auriculo-ventricular apertures appear in sections (Figs. 2 and 4). These valves are found on the floor of the 'middle chamber' at the two ventro-lateral sides of the
ventricle (Figs. 1 and 4). The sections of the valves exhibit strikingly prominent accumulation of tissues in different contours or outlines in different sections (Figs. 4, 15 and 16). This is suggestive of the fact that the heart was arrested in different physiological states of cardiac output. The infolded nature of the valves is clear from different preparations (Figs. 1 and 2). In some cases, the tissue volume or mass is not constant along the entire length of the valve (Figs. 2 and 15). In some regions especially at the free ends of the valves some wider parts follow the narrow portions indicating thereby that the valvular muscles are vigorously active and have been fixed at the different modes of action (Fig. 15). Each and every section of the valvular tissue comes in cross-section when transverse section of the heart was attempted (Figs. 15, 16 and 18). Histological studies reveal that the very prominent muscle fibres are held together by connective tissue matrix (Figs. 16 and 18). The muscle cells here are with dense cellular outlines with oval to circular prominent nucleus with densely packed chromatin granules and a nucleolus (Figs. 16 and 18).

Two categories of cell types are observed in histological preparations. One group having faintly stained cytoplasm and the others with densely stained cytoplasm; some empty looking muscle fibres are also observed (Figs. 15, 16 and 18). Valvular tissue is supported by longitudinal and transverse muscle elements derived from the ventricular wall (Figs. 2, 4 and 17).
Ventricle: The ventricle appears to be a very spacious and spongy sac-like structure (Figs. 1, 4, 19 a, b and 20) exhibiting different contours or outlines (Figs. 1 and 19 a, b) depending upon the different physiological states of activities. Ventricle envelops the rectum. This structure is outlined by a very thin epicardial layer (Figs. 6 and 7) and the myocardial layer is composed of bundles of muscle fibres that form a network which traverses the lumen in some places to obliterate the ventricular cavity to a great extent (Figs. 1, 19 a, b and 20). The endocardial layer is absent here (Figs. 1 and 19 a, b) and hence loosely oriented myocardial fibres are in direct contact with the haemolymph fluid.

(A) Epicardial layer: Epicardial cells are characterised by very prominent nuclei and little of cytoplasm (Figs. 6 and 7). These epicardial units are prominent rectangular cells with densely stained cytoplasm and central dense nucleus and sometimes found in close association with the pericardial gland cells at the two ventrolateral sides of the ventricle (Figs. 11, 12, 13 and 14). In case of epicardial layer the cells are found with round prominent nuclei having densely stained chromatin lumps and scanty amount of cytoplasm (Figs. 6 and 7). Sometimes the amebocytes of the pericardial fluid penetrate the epicardial layer (Fig. 21).

(B) Myocardial layer: Different shapes and sizes of the ventricle are observed in histological preparations (Figs. 1, 2, 4 and 19 a, b). In some sections considerable accumulations of the
muscle bundles are prominently deflected at any one particular site or sites of the ventricular chamber (Fig. 19 a, b). Histologically the muscle bundles are encapsulated and supported by connective tissue (Figs. 7, 11, 22 and Plate 1). At the two lateral sides of the ventricle muscle bundles of the nodal regions come in transverse sections (Figs. 19 b and 20). Transverse sections of the muscles are specially observed at the two lateral sides of the ventricle (Figs. 4, 19 a, b and 20). Muscle bundles of the myocardial layer are extended from the sub-epicardial region towards the lumen of the ventricle (Figs. 1, 2, 6, 10, 11, 19 a, b and 20). Myocardial muscle fibres are composed of elongated cells tapering at the two ends hence spindle shaped with nearly centrally placed densely stained single nucleus (Fig. 22 and Plate 1). Some of the cells exhibit densely stained cytoplasm; the cytoplasm of some are lightly stained (Plate 1). There are intercommunicating muscle bundles and cells are apparently joined end to end but no syncitial formation is observed (Fig. 22 and Plate 1).

At the two lateral sides of the ventricle, near the periphery accumulation of strikingly different cell types are observed (Figs. 23 and 24). These cells are distinct from the longitudinal and transverse muscle fibres (Fig. 23) around them. These unidentified cells come in cross-section when transverse sections of the heart is cut (Figs. 23 and 24). But these cells are not noticeable in each and every transverse
section of the heart (Figs. 1, 4, 19 a, b and 20). These cells are found in nature, with big round densely stained nucleus packed with chromatin granules and the cytoplasm to nuclear ratio is nearly 2:1 (Figs. 23 and 24).

Rectum: In each and every serial cross-sections of the ventricle as well as of the heart, sectional (cross-sectional) view of the rectum is found with a ventral broad typhlosole (Figs. 1, 4, 19 a, b and 20). Internally the rectum is lined by ciliated columnar epithelium, each cell of which is provided with densely stained prominent nucleus. The middle layer of the rectum is composed of a very specialized tissue composed of cells with densely stained nuclei and of spongy appearance with intercellular spaces. The outermost layer of the rectum inside the ventricle is quite distinct from the rest of the gut tissue as it is provided with two or three layers deep peripheral region composed of gradually tapering spindle shaped cells with nearly centrally placed prominent nuclei resembling that of the cardiac tissue (Fig. 25).

(B) Teased and stained muscle fibres of the heart:

Both epicardial and myocardial cells collected from the different parts of the ventricle and auricles do not show any regional differences.
(i) **Epicardium of the 'node' region**: Teased muscle bundles from node region reveal that the muscle fibres are composed of myofibres joined end to end; cytoplasm is moderately stained and there is a single but centrally placed round to oval nucleus with densely stained chromatin materials (Figs. 26 and 27).

(ii) **Myocardium**: Myocardial muscle fibres are composed of elongated cells with characteristically spongy cytoplasm with centrally-placed nuclei having prominent nucleoli. These muscle cells are joined end to end (Figs. 28, 29, 30 and 31).
Fig. 1. Photomicrograph of transverse section of the heart (H) of Lamellidens marginalis, showing the ventricle (V), a part of an auricle (AU) and auriculoventricular valve (AV). X 90.

Fig. 2. Enlarged view of the auricular (AU) region particularly displaying the auriculoventricular valve (AV). X 200.

Fig. 3. Figure demonstrates the sectional view of auricular pouch (AP). X 200.

Fig. 4. Diagrammatic representation of the transverse section through the ventricular region of the heart of Lamellidens. Sectional view of the typhlosole (TY), auriculoventricular valve (AV), transverse and longitudinal sections of muscle bundles (MUS), and glandular epicardial cells (arrow) are displayed.

Fig. 5. Histological preparation of the auricular wall specially showing the epicardial (EC) cells. X 2,300.

Fig. 6. Figure represents the epicardial cells (EC) of the ventricular wall. X 2,300.
Fig. 7. Association of the cardiac muscle cells and blood corpuscles (arrow). X 2,300.

Figs. 8 & 9. Microphotographs representing the distinct glandular cells (arrows) amongst the epicardial cells (EC) of the heart. X 2,300.

Fig. 10. Sectional view of a part of the ventricular wall showing the epicardial (EC) and myocardial (MC) cell layers. X 2,300.

Fig. 11. A part of the sectioned heart showing the lateral position of the epicardial glandular cells (arrows). Rows of myocardial muscle bundles (MY) extends towards the lumen of the heart from the periphery. X 900.

Figs. 12-14. Figures represent magnified views of epicardial glandular cells (arrows). X 2,300.
Figs. 15 - 18. Histological preparation exhibits auriculo-ventricular valve of Lamellidens. Fig. 16 shows the attachment sites of the valve to the ventricular wall (arrow) while Fig. 18 shows the nature of cytoplasm (CY) of the cells of the valvular tissue.

Fig. 15. X 2,300; Fig. 16. X 2,300;
Fig. 17. X 2,300; Fig. 18. X 3,000;

Fig. 19a & b. Photographs demonstrate the different muscular assemblages (arrows) during the contractile movement of the ventricle.

Fig. 19a. X 90; Fig. 19b. X 90.
Fig. 20. Hand drawing represents schematically the sectional view of the ventricle showing striking orientations of the cardiac tissues.

Fig. 21. A blood cell penetrating through the ventricular wall of the heart. X 3,000.

Fig. 22. Microphotograph represents typical spindle shaped myocardial cells with nearly centrally placed nucleus (N). X 2,300.
Plate - 1. Longitudinal and transverse section of myocardial muscle bundles. X 900.
Figs. 23 & 24. Photomicrographs of unidentified cells (arrows) intermingled with the myocardial muscle bundles (MY) at the two lateral sides of the ventricle. Fig. 23. X 900; Fig. 24. X 4,500.
Fig. 25. A part of the rectum showing the outer layer of spindle shaped cells (arrow). X 2,300.

Figs. 26 & 27. Teased and stained muscle cell/fibres of the epicardial layer at the 'node' region of the ventricular wall. X 2,300.

Figs. 28 - 31. Same preparation as above of the myocardial cells from the other region of the ventricular wall. X 2,300.
In the fresh-water mussel the heart is covered by the epicardium. In *Anodonta cygnea* (Esser, 1934), branching fibres are composed of sarcoplasmic axis. Auricle is composed of typically molluscan type of heart cells. Cells are spindle-shaped with central nucleus which is surrounded by a contractile cortex of parallel fibrils that pass into branches. According to Esser (1934) the cardiac muscle in *Anodonta* is like the smooth muscle of intestinal aorta. In *Elliptio complanatus* (Rutherford, 1972), the ventricular tissue is composed of smooth muscles. In *Venus mercenaria* or *Mercenaria mercenaria*, the marine bivalve (Kelly and Hayes, 1969), the ventricle is composed of smooth muscle fibres.

A partial answer to the outstanding questions on the cardiac physiology of the bivalve molluscs may be obtained by systematic studies on the histology of the hearts in these organisms. A true myogenic behaviour of the heart of bivalve mollusc may be successfully explained when a comprehensive account of the histology of this organ is established. It is significant to indicate the occurrence of epicardium, myocardium, unusual
histological elements, pericardial glandular tissue, maker', etc. in the heart of Lamellidens marginalis. The dominant role of the myocardium over epicardium or vice-versa depends largely on the extent of the distribution of a particular tissue and the nervous control, if there is any. Apparently there is a growing need to establish a workable picture on the histophysiology of the hearts of bivalve molluscs.

One of the methods of investigation of the molluscan hearts is Histology. The musculature of the heart of L. marginalis is unique; the auricle is extremely thin compared to the wall of the ventricle. Numerous trabeculae and their ramifications are difficult to follow in histological preparations. Same situation has been reported by Zonta (1973), in Parreysia corrugata, regarding the orientation of muscle strands. According to Van Cate (1929) and Motley (1933) the heart muscles of bivalve molluscs are smooth fibres. In L. marginalis, the muscles are of smooth type and the endocardium is absent. Here, the atrio-ventricular valves have been described. These valves maintain a critical balance between the hydrostatic pressure within the heart and the contraction of the muscle fibres. Definite nerve cells or ganglionic supply are absent in the hearts of bivalves so far studied (Motley, 1933; Esser, 1934; Prosser, 1940), and it is believed that myogenic 'pace maker' exists in the organisms. Zacks and Welsh (1953) demonstrated the activity of cholinesterase in the ventricular muscle fibres.
of Venus. Recently, Watts and Pierce (1978) detected
acetylcholinesterase activity on the sarcolemma of Modiolus
demissus demissus by ultracytochemical methods. The demonstration
of such marker chemicals in the heart muscle fibres of bivalve
molluscs can only be extended following a complete histology of
the heart of a particular bivalve form. The present work is
highly significant towards the elucidation of the histological
features of the heart of L. marginalis. This would certainly
facilitate the introduction of cytochemical methods to localize
the various chemical moities required during cardio-physiological
activities. The present thesis highlights the topographical
distribution of auricles, ventricle, valvular tissues and the
rectum in the heart of L. marginalis. The occurrence of
pericardial gland cells though not systematised in the heart of
this bivalve mollusc seems important from the point of protein
synthesis and other metabolic functions. Striking observations
have been forwarded by Zoute (1973) in case of P. corrugata,
where he found glandular epithelium associated with auricular
walls and reno-pericardial canals. It has been claimed that
the 'pericardialdiirise' or the pericardial glands give rise to
the brown cells of the haemolymph (G. Grobben, 1882). However,
Potts (1967) suggested that the association of the pericardial
glands in the heart, either with auricles or with the ventricle
of bivalve molluscs is a primitive feature. A fuller treatment
on the pericardial gland cells particularly their cytochemistry
and ultrastructures is long overdue in bivalves. The participation
of the connective tissue elements and cells particularly at the
auriculoventricular valves and muscle cells/fibres in this bivalve
is unique towards the formation of a very complicated cardiac architecture.

There is one pair of auriculo-ventricular valves situated in the middle (?) chamber or portion of the ventricle of L. marginalis. The valvular tissue is highly characteristic in possessing different cellular elements of which the majority comes from the myocardium. Apart from the occurrence of muscle fibres there are two types of cells with highly differentiating cytoplasm. There is apparent indication that one of these cell types is synthetically active compared to the other.

Although Pierce (1973) indicated the importance of rectum in the taxonomic studies of bivalves, the present chapter, however, does not throw any light on this aspect, i.e. the relation of the rectum with the heart of L. marginalis. It only attempts to show that there are 2-3 layers of spindle-shaped muscle cells (resembling those of the myocardial cells) which cover the rectum. The most important part of this chapter concerns the demonstration of the distinctive features of the epicardial tissue with the myocardium. In F. corrugata also, the epicardium is composed of cuboidal cells, and extends both over the auricles and the ventricle (Domke, 1973). The differences which are outlined here are further corroborated by the transmission electron microscopic results. The occurrence of unidentified cell groups at the two lateral sides of the ventricle of L. marginalis
poses problems. It is necessary to trace these cell clusters in the hearts from several unionid bivalves before a specific or nonspecific role is assigned to them. The compact nature of these cells reminds of the ideas proposed by Irisawa (1978), that pace maker cells are small, while Greenberg (1979) was of opinion that the entire bivalve myocardium is pace maker tissue, and Hill (1979) suggested that 'size criterion does not hold in molluscan heart'. The possibility of one of the haemopoetic centres in the unidentified tissue complex in the heart of L. marginalis however, remains.