The Morphological Basis for Impulse Conduction in the Fowl Heart

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Department of Anatomy, January 1972.

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SUMMARY

The structures involved in initiating and conducting the cardiac impulse to the contractile myofilaments are the plasma membranes of the cells in normal and specialised myocardium, their intercellular junctions and the sarcoplasmic reticulum. These have been studied by a variety of light and electronmicroscopic methods. The stability of intercellular junctions has been investigated by divalent cation chelation and the size of dissociated cells has been measured. Extracellular labelling techniques have been used to confirm the absence of both a transverse tubular system and a subunit pattern at the nexus of intercellular junctions.

The impulse conducting system of the fowl consists of:

1) a sinoatrial node, lying between the right venous valve and the right superior vena cava;
2) an atrioventricular node, lying in the caudal end of the interatrial septum;
3) an atrioventricular bundle continuing from the atrioventricular node;
4) an interatrial node lying between the atrioventricular node and the left interatrial septum;
5) a right Purkinje ring, which issues from the caudal end of the atrioventricular node in the fibrous ring;
6) an extensive subendocardial and periartrial Purkinje network in the atria and ventricles.

In addition to this well described system in birds, peculiar muscle fibres resembling those of nodal tissue are present at the
bases of the valve cusps of the aorta and pulmonary artery in the fowl.

The ultrastructure of normal atrial and ventricular fowl myocardium differs from normal mammalian myocardium in the arrangement of cells in a fibre. The cells of fowl myocardium are much narrower than those in mammalian hearts but are similar in diameter to those of amphibians and reptiles. No transverse tubular system is present in the normal myocardium of the fowl but an extensive sarcoplasmic reticulum makes many contacts with the plasma membrane. The membrane specialisations present at intercellular junctions are similar to those in amphibians and reptiles in that only a few small nexuses are present compared with the large number and size of nexuses in the myocardium of mammals.

The cells of the SA node of the fowl are intermediate in structure between normal and Purkinje cells. The AV node is composed of cells which show a range of ultrastructure varying from true Purkinje cells to normal cells.

The Purkinje cells of the fowl are similar to those of mammals. The structure of the impulse conducting system of the fowl is compared with its structure in other birds, mammals and cold blooded vertebrates.

The factors influencing intercellular and intracellular conduction in mammals birds and amphibians are compared and discussed.
INTRODUCTION

The classical experiments of Stannius (1852) comprising the separation of the various chambers of the frog's heart by ligatures applied at the sinu-atrial (SA) and atrio-ventricular (AV) junctions, provided the basis for the subsequent more detailed studies concerning the site of origin and the spread of depolarisation through the heart. The morphology of impulse conduction may be studied at three levels; the point of origin of the depolarisation and the spread of depolarisation through impulse conducting pathways to the contractile tissue of the heart; intercellular conduction within the impulse conducting system (ICS), from the ICS to unspecialised cells and between unspecialised cells; conduction along and into individual cells.

Impulse Initiation and Conducting Pathways

Mammals

In the mammal the depolarising impulse originates in the specialised tissue of the SA node (Keith & Flack 1907, Lewis 1910), at the junction of the right precaval vein with the right atrium and it then spreads through the musculature of both atria to the AV node (Tawara 1906), a collection of specialised tissue situated in the dorso-caudal part of the atrial septum. The only muscular connection between the atria and ventricles in the mammal along which the impulse may pass, is generally held to be that of the AV bundle (His 1893), the atrial muscle being elsewhere separated from that of the ventricles by the fibrous ring at the bases of the AV valves.
The AV bundle is continuous above with the AV node while distally it divides into two limbs, right and left, each of which passes on the corresponding side of the ventricular septum beneath the endocardium to become continuous with the subendocardial network of large pale-staining fibres, first described by Purkinje (1845). The latter ramify through the greater part of each ventricle and ultimately become directly continuous with ordinary muscle fibres.

During the latter half of the nineteenth century, following on the description of Purkinje fibres, numerous workers studied these structures and their investigations have been reviewed by Davies and Francis (1946). They maintained variously that they were transitional types of muscle fibres continuous throughout with the ordinary myocardium, persistent embryonic cardiac muscle, embryonic muscle able throughout life to replace damaged heart muscle, or even not muscle at all but a special motor-end apparatus necessary for the heart's action. A few investigators, however, notably Gegenbaur (1877) and Minervini (1899) pointed out that at no stage in its development does ordinary cardiac muscle have the structure of fully formed Purkinje cells. Nevertheless, the functional significance of these cells for the propagation of the impulse was not appreciated until a later date, after their association with the muscular connection had been established (Tawara 1906).

Until Paladino (1876), Kent (1893) and His (1893) claimed to have found muscular connections between the atria and ventricles, it had generally been believed that the musculature of the atria in mammals was completely separated from that of the ventricles by
fibrous tissue and the prevailing neutrogenic theory held that the cardiac impulse was conveyed from the atria to the ventricles by nerves. His (1893) described a small muscular bundle connecting the atrial and ventricular septa in the adult mouse, neonatal man and neonatal dog. The bundle was described as arising in the posterior wall of the right atrium in the AV groove near the atrial septum and passed forwards on the upper border of the ventricular septum to a point near the aorta where it divided into right and left limbs, the latter ending in the base of the ventral cusp of the mitral valve.

The bundle described by His (1893) was accepted as the pathway for the conduction of the impulse from atria to ventricles but experimental evidence was not forthcoming for a decade. Dissociation between atrial and ventricular contraction after cutting compressing or ligaturing the bundle was observed by Humblet (1904), Hering (1905), Erlanger and Hirschfelder (1905) and Cohn and Trendelenberg (1910).

A noteworthy advance was made by Tawara (1906) who showed for the first time that the Purkinje fibres beneath the endocardium of the ventricles are the terminal ramifications of the two limbs of the AV bundle, and further that this system, comprising the bundle, its two limbs and the ventricular Purkinje network, is completely isolated by connective tissue from the neighbouring ventricular musculature right up to the point where the terminal Purkinje fibres become continuous either with the immediately subjacent ventricular muscle, or with the deeper musculature, after penetrating for some
distance into the myocardium. He also observed that the bundle commenced in the atrial septum as a complicated muscular network, the AV node, which was continuous with the general atrial muscle fibres near to the opening of the coronary sinus. Unlike the bundle and its limbs, which consisted of Purkinje fibres in all the mammals he examined, the AV node comprised a network of much finer muscle fibres. The bundle was described as penetrating the AV fibrocartilagenous septum and passing to the upper border of the ventricular septum where it divided into two limbs. The right limb passed downwards for a short distance, separated from the endocardium on the right side of the septum by a thin layer of ventricular muscle; it then passed into a muscular ridge, the moderator band, (trabecula septomarginalis) which conveyed it across the ventricle into the anterior papillary muscle where it split into branches which spread beneath the endocardium of the right ventricle. The left limb passed down the left side of the ventricular septum immediately beneath the endocardium and divided into a number of branches which passed across the cavity as tendon like strands, the main ones reaching the anterior papillary muscle where they split into branches which ramified beneath the endocardium of the whole left ventricle.

The fine structure of the component cells of the ICS has been the subject of many recent papers (Johnson & Sommer 1967, Rhodin et al 1961, Trautwein 1963) and will be discussed under a separate heading.
Birds

In contrast with the numerous investigations on the mammalian cardiac conducting system, few studies of this system have been made in birds. As in the early days of investigation into the mammalian ICS, there were many conflicting reports as to the presence or absence of an ICS in birds (Davies & Francis 1946), until Mangold & Kato (1914) noted the effects on the electrocardiogram of cuts and ligatures in various parts of the heart of the bird. They concluded that the following structures were present in the heart of the bird; SA node near the termination of the right precaval vein, and AV bundle with two terminal branches in the ventricular septum. They could, however, find no histological differentiation of any specialised muscle tissue in these sites.

That the spread of the impulse in the bird's heart must be through a specialised distributing system as in mammals was deduced by Lewis (1915) from his electrical studies. The Purkinje fibres of the bird's heart were studied later by Tang (1922), who concluded that they were a part of a special conducting system. The distribution of the Purkinje fibres in the bird's heart was investigated by Holmes (1923) who found numerous Purkinje fibres in both right and left atria.

Drennan (1927), by naked eye dissection of the ostrich heart, demonstrated an AV bundle which passed from the dorsal part of the base of the atrial septum into the depth of the ventricular septum. Here it inclined towards the endocardium on the left side of the septum and gave off a branch to the muscular right AV valve.
The AV node in the bird's heart was described by Ohmori (1928) in the lower part of the atrial septum. From this node he traced the AV bundle into the depth of the ventricular septum where it divided into right and left limbs which passed to the corresponding sides of the septum to become continuous with the ventricular Purkinje fibres.

The entire ICS in the black swan was described by Davies (1930). The SA node, of similar structure to that of the mammal, was shown to be present at the junction of the right precaval vein with the right atrium. It extended through the entire thickness of the atrial wall, and the nodal fibres were continuous with large Purkinje fibres which ramified both beneath the endocardium and periarterially within the myocardium throughout most parts of both atria to join the ordinary myocardial fibres. There were no Purkinje fibres in the lower part of the atrial septum so that, as in the mammal, the specialised fibres of the AV node established continuity with the ordinary myocardial muscle in the atrial septum.

The AV node, similar in structure to that of the mammal, continued into the AV bundle. About one quarter of the way down the ventricular septum, the AV bundle divided into right and left limbs. These approached the corresponding sides of the septum where they divided into a plexus of Purkinje fibres, which ramified beneath the endocardium throughout the right and left ventricles and also penetrated the thickness of the ventricular walls mostly in relation to branches of the coronary vessels, to become continuous with the ordinary ventricular muscle at varying depths from the
surface. The AV bundle and its two limbs consisted of large typical Purkinje fibres.

Davies described a special early branch from the right limb of the AV bundle in the black swan, which passed directly into the right AV valve and became continuous with the muscle fibres.

There was, in addition, a ring of Purkinje fibres lying in the connective tissue between the muscular laminae of the right AV valve, which established continuity with the musculature.

The conducting system of the heart of the house sparrow has been described by Yousuf (1965). The specialised conducting tissue, SA node, AV node, and AV bundle, was found to develop earlier than the nervous elements.

Gossrau (1969) has described the conducting system in the heart of the pigeon. He described the ICS as consisting of SA node, AV node, AV bundle, Purkinje ring and a diffuse Purkinje system.

The fine structure of the ICS in birds has received little attention (Hirakow 1966, Gossrau 1968) and this is discussed subsequently.
Fish, amphibians and reptiles

Many investigators claim that in the hearts of lower vertebrates the muscle connecting the cardiac chambers has special histological characteristics which differentiate it from the general myocardium, although they do not agree as to the nature and extent of these specialised connections. Other authors maintain that there is muscular continuity between the several chambers but deny its specialised nature, while a few observers find only a nervous connection and deny that there is any muscular continuity between the atria and ventricles.

Breunig (1904) observed that the AV canal is simple in fish and by invagination becomes more complicated in amphibia and reptiles although still without histological specialisation.

Haberlandt (1913, 1917) found no specialised muscle in the AV region of the frog heart and determined experimentally that all parts of the circumference of the funnel have the same capacity to initiate automatic contractions of the ventricle. Prakash (1954) observed that of the two sinus venosi of the heart of the frog tadpoles, the sinus venosus sinister performs the same function as the SA node of mammals.

Carlson (1905) found no histological difference in the muscle joining the ventricle to the bulbus in the salamander, although he claimed that the pause in the wave of contraction was here greater than at the AV junction. Gyevai (1958) pointed out the absence of a specialised conducting system in the heart of the crested newt. SA node, AV node and AV bundle have been shown to be present in the
heart of the salamander (Prakash 1960).

Swett (1923) failed to find any specialised tissue either at the SA or AV junction of the heart of the alligator.
Intercellular Conduction

With improved techniques in electron microscopy it has become evident that cardiac cells are separate units (Van Breeman 1953, Sjostrand & Andersson 1954, Muir 1957a, Fawcett & Selby 1958). Since myocardium is cellular and not a syncytium, the problem arises of transmission of the cardiac impulse from one cell to the next. That impulse transmission between cardiac cells is electrotonic and not chemical is suggested by the fact that transmission will occur in either direction across a junction. No vesicles have been detected adjacent to the membranes at intercellular junctions, and transmission cannot be blocked by inhibitors of known chemical transmitters. The factors influencing electrotonic intercellular conduction are, the structure and specialisations of the apposed membranes between cardiac muscle cells, and whether these membranes are in contact or separated by an extracellular gap.

Junctional complexes

Unspecialised membranes. The membranes of myocardial cells are to a large extent unspecialised. The unspecialised membrane has the typical unit membrane structure which appears as two dark lines with an intermediate light zone when stained in block with uranyl acetate (Farquhar and Palade 1963). The inner leaflets of the unit membranes of apposing cells are, at unspecialised areas, separated by 15nm, leaving an extracellular gap of about 10nm. The membrane specialisations occurring in mammalian myocardium correspond in general appearance to the zonula adherens, macula adherens and zonula occludens of epithelial junctional complexes (Farquhar & Palade 1963).
Zonula adherens (Farquhar & Palade 1963), fascia adherens (Fawcett & McNutt 1969), myofibrillar insertion plaque.

The term zonula adherens is employed by Farquhar & Palade (1963) to describe a type of intercellular relationship occurring as a ring around adjacent epithelial cells which is characterised by the presence of an intercellular space of approximately 20nm occupied by apparently homogeneous amorphous material of low density and by conspicuous bands of dense material located in the subjacent cytoplasmic matrix, frequently with myofibrillar insertion. In mammalian myocardium this type of specialisation extends over areas of variable size and shape and it is for this reason that Fawcett & McNutt (1969) have applied the term fascia adherens. The sarcolemmal surface of the membranes in this type of specialisation is, in myocardium, reinforced by a thick layer of densely staining interwoven fine filaments. It is this dense mass of filaments that the thin filaments of the terminal sarcomeres insert into, at the end of the cell. For this reason the term myofibrillar insertion plaque is used to describe this type of specialisation in the present study.

The fascia adherens is present on lateral membranes of amphibian myocardial cells. This has been termed cardiac adhesion plaque (Baldwin 1970). Although cardiac adhesion plaques and myofibrillar insertion plaques can be considered to be the same, the term cardiac adhesion plaque will be used in this study to describe insertion plaques on lateral membranes of myocardial cells. Macula adherens, desmosome.

This type of specialisation in myocardium is characterised by the presence of an intercellular gap between the outer leaflets of the opposite unit membranes of about 24nm. This intercellular gap contains a central disc of dense material. There are also dense cytoplasmic plaques disposed parallel to the inner leaflets of each cell membrane and the plaques receive the attachment of non-
myofibrillar fibrils whose diameters (10nm) are intermediate between those of the thick (12nm) and thin (6nm) filaments.

Zonula occludens, (Farquhar & Palade 1963), quintuple layered junction (Muir 1965), tight junction, nexus.

This type of specialisation occurs in many tissues including epithelial cells where it is characterised by fusion of adjacent cell membranes, resulting in obliteration of the intercellular space. Within the obliterated zone the dense outer leaflets of the adjoining cell membranes converge to form a single intermediate line. The equivalent specialisation in mammalian myocardium was, until 1967, described as being similar in structure to that found in epithelial cells and was consequently referred to as a pentalaminar, (Karrer 1960, Palade 1959, Sjostrand et al 1958), or tight junction.

Work on the tight junction, employing block staining with uranyl acetate (Farquhar & Palade 1963) and extracellular labelling techniques (Revel & Karnovsky 1967), has demonstrated that in myocardium the pentalaminar or tight junction is in fact only a close apposition with a gap of 1.8nm between the outer leaflets of the membranes of the apposing cells. In tangential sections of such areas the membranes exhibit closely-packed subunits with a centre to centre spacing of 9nm separated by channels 3-4nm wide which fill with lanthanum to form a regular hexagonal pattern.

Since the current view that this type of specialisation is not occluded, quintuple layered, or tight, it is referred to in this study as the nexus (Dewey & Barr 1962), as this is the least confusing term. The term nexus was first used by Dewey & Barr (1962)
to describe a junction between cells which appeared to be a fusion of the outer leaflets of the unit membranes of apposing cells. This imprecise term is derived from the Latin nectere = to bind and consequently describes the close attachment of the membranes without implying fusion.

Woodbury & Crill (1961) have approached the problem of action potential propagation in the rat atrium by assuming that the gap between cardiac cell membranes at an intercalated disc is everywhere 8 nm. The ratio of the area of apposition to the gap distance is very large. Their analysis shows that under these conditions propagation of action potentials requires the disc membrane to have an extremely low resistance.

Tarr & Sperelakis (1960) found a weak degree of electrical coupling between neighbouring cells, suggestive of a high cell to cell resistance. Woodbury (1962) from measurements of electrotonus in cardiac muscle, concluded that the cell to cell resistance is low. Weidmann (1966) from experiments on the longitudinal diffusion of $^{42}$K along a bundle of parallel fibres from sheep ventricle, concluded that the cell to cell resistance is low as compared to that of the membrane separating the myoplasm from the extracellular space. Weidmann's calculations, based on fibre thickness in sheep myocardium (15 nm) and on a value for intracellular $K^+$ (150 mM), suggest that each $cm^2$ of disc membrane has an electrical resistance of 3 ohms or less and that the permeability of the disc to labelled $K^+$ is at least 5000 times greater than the permeability of the surface membrane to the outward movement of radiopotassium.
There is conflicting evidence in the literature, concerning the effect on intercellular resistance when cells are immersed in hypertonic solutions. Some authors (Barr, Berger & Dewey 1968) have concluded that electronic coupling is disrupted when cells are immersed in hypertonic solutions. Other authors concluded that electronic coupling between cells is unaffected by similar treatment (Cobb & Bennett 1969). Barr, Dewey & Berger (1965) have shown from experiments on frog atrium that longitudinal resistance increases when atrial muscle is immersed in hyperosmotic solutions of sucrose, and that the nexuses are disrupted.

In view of the above evidence on nexal disruption and the evidence of a high degree of electrical coupling between cells, it has been suggested that nexuses are sites of electrotonic coupling between adjacent cardiac cells and between smooth muscle cells (Dewey & Barr 1962, Dewey & Barr 1964). Similar regions have been demonstrated at certain electrical synapses (Robertson 1961, 1963, Bennett et al 1963, Dewey & Barr 1964), between glial cells (Gray 1961, Peters 1962), and in various epithelia, (Dewey & Barr 1964, Farquhar & Palade 1963, Robertson 1960, Sjostrand 1960).
**Intracellular Conduction**

Conduction of depolarisation along and through a cell depends on the shape and size of the cell, the physiological characteristics of the sarcolemma (Katz 1966) and the presence or absence of a transverse tubular system.

**Cell shape and size**

The ventricular cells of mammalian myocardium have an average diameter of 8-10μm. Individual cells vary in shape, with many showing deep clefts and branches, and may have diameters as large as 20μm. The cells are joined by intercalated discs which traverse the fibre in a stepwise fashion.

Recent studies of the contractile myocardium of birds have shown (Gossrau 1969, Jewett, Sommer & Johnson 1971) that the fibres of the bird are narrower than those of mammals.

The cardiac muscle fibres of amphibia and reptiles are of a diameter similar to that indicated for birds, from 5-8μm.

**Transverse tubular system**

The ventricular fibres of mammalian myocardium possess a transverse (T) tubular system. The continuity of these tubules with the surface in muscle has been well documented, (Page & Solomon 1960, Simpson & Oertelis 1962, Franzani-Armstrong 1963, Nelson & Benson 1963, Endo 1964, Huxley 1964, Page 1964, Peachey 1965, Simpson 1965).

The diameter of the tubules of mammalian cardiac muscle varies somewhat with the method of preparation but it is of the order of 150-200nm as compared to about 40nm for the T tubules of skeletal muscle.
muscle. The tubular system in myocardium opens at the level of the Z line. At the mouths of the T tubules the basement membrane coating can be traced without interruption into the tubules. No T tubular system has been demonstrated in cat atrial muscle, (McNutt & Fawcett 1969) or in the specialised tissues of some mammalian hearts, (Sommer & Johnson 1968).

No T tubular system has been described in avian, reptilian or amphibian myocardium.

The presence of a T tubular system may be expected to add to the total membrane capacitance in myocardium and so reduce the speed of conduction as it has been shown to do in skeletal muscle, (Falk & Fatt 1964, Freygang 1965, Peachey 1965, Gage & Eisenberg 1967).
The Present Investigation

From a review of the literature on the morphology of the ICS in myocardium, it is evident that although the ICS has been fully described for most mammals there has been no full description in one of the most common and perhaps most economically important birds, the fowl, Gallus domesticus. In order to fill this gap in the knowledge of the ICS in birds, a study of this system in the fowl has been carried out and forms the first part of this thesis.

The problem of intercellular conduction in myocardial tissue has not been resolved. As the preceding review of the literature shows, there are many conflicting reports as to the form and function of the nexus and other specialisations of the sarcolemma and their relationship to intercellular conduction. As the cell diameter decreases, the requirement for an area of low resistance increases, if electrotonic intercellular conduction is to take place (Katz 1966). During a preliminary investigation of the myocardium of the fowl no nexuses were found although the cells were narrower than mammalian myocardial cells.

No T tubular system was seen in the myocardium of the fowl which indicated that the fowl was closer in morphology to cold blooded animals which do not have a myocardial T tubular system. Although the fowl appears to differ from mammals in that no T tubular system is present, it is similar in that it is a warm blooded animal with a rapid heart rate.

On this evidence it was thought that a fine structural study of the myocardium of the fowl might add to the present knowledge of
intercellular and intracellular conduction and such a study forms the second part of this thesis.

A short account of the ultrastructure of fowl myocardium has appeared in the literature (Sommer 1968).

Processing

Decalcification
7% ethanoic acid 2 hr.

(Except for mass fixed blocks)

95% ethanol (all blocks) 1 hr.

Absolute ethanol 1 hr.

Absolute ethanol 1 hr.

Clearing
1st chloroform 1 hr.

2nd chloroform 1 hr.

Impregnation
Soft wax (37°C) 1 hr.

Hard wax (50°C) 1 hr.

The tissues were placed in molten hard wax in a vacuum oven for 2 hr., or until vapour had stopped coming from the tissue, then blocked in hard wax.
MATERIALS AND METHODS

Light Microscopy

The hearts of 20 fowls of both sexes whose ages ranged from 1-18 months were examined. After dislocation of the neck, the thorax was opened and the heart removed, cut into blocks less than 5 mm thick and immersed in phosphate buffered formaldehyde or Bouin's fluid for 24 hr. or Heidenhain's susa for up to 12 hr. (Culling 1963).

After fixation each block was trimmed then processed automatically. Automatic processing ensures uniformity between specimens and by agitation reduces the processing time.

Processing

Dehydration

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time</th>
</tr>
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<tbody>
<tr>
<td>75% ethanol</td>
<td>2 hr.</td>
</tr>
<tr>
<td>(Except for susa fixed blocks)</td>
<td></td>
</tr>
<tr>
<td>95% ethanol (all blocks)</td>
<td>1 hr.</td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td>1 hr.</td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td>1 hr.</td>
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</tbody>
</table>

Clearing

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st chloroform</td>
<td>1 hr.</td>
</tr>
<tr>
<td>2nd chloroform</td>
<td>1 hr.</td>
</tr>
</tbody>
</table>

Impregnation

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft wax (37°C)</td>
<td>1 hr.</td>
</tr>
<tr>
<td>Hard wax (58°C)</td>
<td>1 hr.</td>
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</tbody>
</table>

The tissues were placed in molten hard wax in a vacuum oven for 2 hr., or until vapour had stopped coming from the tissue, then blocked in hard wax.
Serial sections of each block were cut on a Spencer microtome at 6-10μm.

**Staining**

Sections were dewaxed in xylene and passed through a graded series of ethanols to water before staining. The mercury deposit formed in tissue fixed in susa was removed with iodine - sodium thiosulphate treatment. The sections were placed in 0.5% iodine in 80% ethanol for 3 min., rinsed in tap water then immersed in 3% sodium thiosulphate for 3 min. following which they were again rinsed in tap water.

Sections were stained in Ehrlich's haematoxylin and eosin, Heidenhain's iron haematoxylin, periodic acid-Schiff or lead tetra acetate Schiff, according to Culling (1963). The most effective stain for demonstration of the ICS of the fowl was found to be the following modification of Masson's trichrome technique.

**Solutions**

**Weigert's iron haematoxylin**

1(a) Haematoxylin 1g.

(b) Absolute ethanol 100ml.

2(a) 30% Solution of aqueous ferric chloride 4ml.

(b) Concentrated hydrochloric acid 1ml.

(c) Distilled water to 100ml.

Equal parts of 1 and 2 are mixed immediately before use.

**Ponceau fuchsin**

(a) Acid fuchsin 0.3g.

(b) Ponceau de xylidene 0.7g.

(c) 1% Acetic acid 100ml.
Procedure

1. Formaldehyde fixed tissue is post chromed in Zenker's fluid overnight (Culling 1963).

2. Stain in Weigert's haematoxylin for 20 min.

3. Differentiate in 1% hydrochloric acid in absolute ethanol until only nuclei are stained.

4. Blue sections in tap water.

5. Stain in ponceau fuchsin for 5 min.

6. Rinse rapidly in distilled water.

7. Mordant in 1% aqueous phosphomolybdic acid for 5 min.

8. Stain in 2% light green in 2% acetic acid for 30 sec.

9. Differentiate in 1% acetic acid for 30 sec.

10. Dehydrate, clear and mount in Clearmount.

The arrangement of the ICS was studied in projections of every tenth section from serial sections of blocks cut in various planes. These enlargements were mounted on cards. Each section was then examined and the position of the Purkinje cells, nerves and connective tissue marked on the cards. Although this method did not produce an exact scale model, it allowed a greater understanding of the path of the ICS and its relationship to atrial or ventricular muscle.

Electron Microscopy

A study of normal myocardium was carried out using the hearts of 100 brown leghorns, of both sexes, whose ages ranged from 1-18 months. After the thorax was opened and the brachiocephalic arteries were ligatured, the pericardium was opened and the heart excised by cutting the great veins, the brachiocephalic arteries cranial to the ligatures and the descending aorta.
After excision, blocks of myocardium 1mm$^3$ were cut from the atria and ventricles. The blocks were then immersed in one of the following fixatives for 1 hr. at 4°C.

1. Millonig’s buffered osmium tetroxide (Glauert 1965).
2. Osmium tetroxide in cacodylate (Glauert 1965).
3. Osmium tetroxide in veronal acetate (Glauert 1965).
4. 1% Osmium tetroxide in avian ringer.
5. Luft’s buffered permanganate (Glauert 1965).
6. Phosphate buffered formaldehyde (Glauert 1965).
7. Phosphate buffered glutaraldehyde (Glauert 1965).
8. Karnovsky’s fixative (Karnovsky 1965).

Fixation in glutaraldehyde was followed by fixation in buffered 1% osmium tetroxide, after a wash for 24 hr. in 0.1M cacodylate at 4°C.

**Fixation by Perfusion**

After excision of the still-beating heart, the aorta was cannulated with a 3mm diameter cannula, with a terminal flange of 5mm. The cannula was connected to the perfusion apparatus (Muir 1967), and perfused with oxygenated avian ringer (Cleugh et al 1961) for up to 5 min. at 37°C. This brief period of perfusion washed the nucleated erythrocytes from the coronary circulation and so ensured uniform perfusion with fixative. During the period of perfusion the rate of depolarisation was recorded by means of an oscilloscope and if the rate of contraction was between 180-250 beats per min. the hearts were removed from the apparatus, still beating, and fixed by perfusion through the aorta with 20ml. of fixative. Blocks of 1mm$^3$
were then cut from the atria and ventricles and immersed in fixative. Blocks were fixed in osmium, glutaraldehyde and permanganate fixatives for 1 hr. at 4°C. Specimens fixed in formaldehyde were fixed for 5 min. then transferred directly to 1% buffered osmium tetroxide for 1 hr. Fixation in glutaraldehyde was followed by a wash for 24 hr. at 4°C in 0.1M cacodylate before fixation in 1% buffered osmium tetroxide for 1 hr.

The tissue blocks were then processed according to the following scheme.

**Dehydration**

10% ethanol 1 hr.
Abs. ethanol 30 min.
" " 
" "

**Clearing**

Propylene oxide 30 min.

Blocks were embedded in Araldite in plastic boats 3cm x 2cm x 0.5cm for 24 hr. at room temperature. The Araldite was then renewed and the boats placed in a 60°C oven for 48 hr.

**Preparation of Araldite.**

1. Thoroughly mix half a lb. of Araldite resin with half a lb. of hardener (HY964).

2. Mix 5ml. of accelerator (DY064) with 20ml. Dibutyl phthalate BDH.

3. The final Araldite medium is prepared by adding 1ml. of 2 to 20ml. of 1 and mixing thoroughly.
All blocks were mounted on 7mm diameter dowel rods with sealing wax, and cut on a Porter Blum MT1 microtome, using glass knives. Sections 1um thick were cut for light microscopy.

Thick sections, on glass slides, were stained on a hotplate with 0·5% toluidine blue and 0·5% thionin in 1% borax. Excess stain was removed with tap water, before examination by light microscopy. The blocks were trimmed so that selected areas could be cut for examination by electron microscopy.

Thin sections were cut at a microtome setting of 40-100nm on glass and diamond knives. Sections were collected on uncoated Athene 483 grids. Thin sections were stained in either uranyl acetate, lead citrate or both. The staining procedure was as follows:-

Uranyl acetate.

1. Grids were immersed, sections up, in a centrifuged, saturated solution of uranyl acetate in 50% ethanol for 5 min.

2. Grids were immersed in 50% ethanol for 15 sec. then placed on filter paper, sections up.

Lead citrate.

1. Grids were placed, sections down, on drops of 0·5% lead citrate in 0·5% sodium hydroxide in freshly boiled distilled water for 2 min.

2. Grids were agitated in 0·5% sodium hydroxide in freshly boiled distilled water for 15 sec.
3. Grids were agitated in freshly boiled distilled water for 15 sec. then placed on filter paper, sections up.

When double staining was used the sections were stained in uranyl acetate then lead citrate.

The SA and AV nodes demonstrated by the light methods previously described could not be examined by electron microscopy using small blocks because of the size of the nodes and the importance of orientation. It was thought that small blocks would not yield sufficient information on the passage of the main bundle through the interventricular septum and its final connection with the AV node. For this part of the study, blocks 5mm x 7mm were prepared from the inter atrial and interventricular septa of 12 hens, 14 weeks old. All blocks were processed as previously described. Serial sections of prepared blocks were cut on glass and diamond knives at 1um thickness using the planimeter advance of the L.K.B. ultramicrotome (this gave a measure of the distance cut) with intermittent thin sections cut at 700 A.

Cell Separation Methods

Attempts were made to separate myocardial cells in order to appraise the general adhesion between cells and the adhesion at sites of membrane specialisation. The hearts of 20 birds, of both sexes, whose ages ranged from 1-18 months, were used.

A. Removal of extracellular cement.

The hearts were briefly perfused with avian ringer before perfusion with experimental solution after which small blocks were cut from the right ventricle and shaken in the experimental solution.
The resulting suspension was filtered through a silk stocking and dried on glass slides. Small blocks were also taken from the right ventricle and processed for electron microscopy.

The perfusing solutions used were:-

1. Calcium, magnesium-free avian ringer (CMF)

2. CMF + 0.01% ethylene diamine tetra acetic acid (EDTA) 15 or 30 min.

3. Avian ringer with 0.5% trypsin added.

4. Avian ringer + 0.5% trypsin for 15 min. followed by CMF + EDTA for 15 min.

5. Small blocks from the right ventricle were also immersed in $3 \times 10^{-3}$ M sodium tetraphenyl boron (NaTPB) for 40 min.

B. Mechanical disruption.

Hearts were briefly perfused with avian ringer as before, followed by perfusion with avian ringer with 300mM sucrose or 300mM glycerol added, until they stopped beating. Small blocks were taken from the right ventricle of hearts that had been perfused with avian ringer with sucrose or glycerol added, and also from hearts perfused only with avian ringer. The blocks were disrupted by shaking in the perfusing solutions and dried onto glass slides. Small blocks were also taken for electron microscopy.

**Estimation of Cell Length**

The preceding methods of cell separation produced suspensions of cells which were smeared on glass slides, fixed in 4% formaldehyde in phosphate buffer for 5 min. and stained with haematoxylin and eosin. This allowed single cells to be measured using the light microscope.
Extracellular Labelling

The absence of T tubules in fowl myocardium was confirmed, using lanthanum nitrate as an extracellular label. Using this label, the tissue was also examined for the presence of any surface specialisation of the membranes into hexagonal subunits such as is seen at the nexus in mammalian myocardial cells (Revel & Karnovsky 1967). The hearts of 30 animals, whose ages ranged from 1-18 months, were used.

Perfusion.

To perfuse hearts with lanthanum nitrate it was necessary to find a suitable physiological carrier for the label. Avian ringer caused precipitation of the lanthanum due to the presence of carbonate and phosphate ions. A modified Locke's solution was used.

Control solution.

\[
\begin{align*}
\text{NaCl} & : 9g. \\
\text{KCl} & : 0.42g. \\
\text{CaCl}_2 & : 0.24g. \\
\text{Glucose} & : 1g. \\
\text{'Tris' buffer} & : 0.02M
\end{align*}
\]

Make up to 1 litre with distilled water.

Labelling solution.

Control solution + 0.05, 0.1 or 1% lanthanum nitrate added.

Control tissue was obtained by removing the hearts as previously described, and perfusing them with control solution for 15 min. The hearts were then fixed by perfusing them with 1% osmium tetroxide added to the perfusing solution, and processed for electron
microscopy. Labelled tissue was obtained by perfusing hearts with control solution for 2 min. then with labelling solution for 13 min. and fixing the hearts by perfusion with 1% osmium tetroxide in control solution.

**Immersion**

Hearts were perfused with avian ringer for 2 min. then fixed by one of the following methods:–

1. Perfusion with Karnovsky's fixative with 1% lanthanum nitrate added. Blocks were removed from the right ventricle then immersed in the fixative for 1 hr. followed by a wash in 0·1M cacodylate with 1% lanthanum nitrate added, for 1 hr., then post-fixed in 1% osmium tetroxide in 0·1M cacodylate with 1% lanthanum nitrate added, for 1 hr.

2. Perfusion with 1% osmium tetroxide in 0·1M cacodylate with 1% lanthanum nitrate added. Blocks were then removed from the right ventricle and immersed in the fixative for 1 hr.
RESULTS

Light Microscopy

For ease of description, the terms apex, base, left, right, dorsal and ventral, will refer to the heart after removal from the bird, so that the left surface of the heart is occupied by the left atrium and left ventricle and the interventricular septum passes from the dorsal to the ventral aspect of the heart. Plate 1.

The heart of the fowl has four chambers, as in mammals, but the apex is formed only by the conical left ventricle. The right ventricle is wrapped round the upper part of the left ventricle. The left AV valve is composed of two membranous cusps with typical chordae tendinae attached to papillary muscle. The right AV valve is completely different from the AV valves of mammals and from the left AV valve of birds in that it is composed of a muscular reflection from the right wall of the right ventricle and also of a reflection of atrial muscle from the right wall of the right atrium. The atrial and ventricular layers of the valve are separated by the fibrous ring at the base of the valve, and by a connective tissue sheet throughout its substance.

The normal myocardium of the fowl is made up of bundles of fibres which stain densely. With Masson's trichrome stain the bundles stain a cherry red colour, frequently showing myofibrillar banding. The banding pattern on fibres is more clearly demonstrated when the tissue is stained with iron haematoxylin. In these respects the fibres are similar to mammalian myocardial fibres. Unlike mammalian myocardial fibres, when stained with periodic acid Schiff or
lead tetra-acetate Schiff, no glycogen deposits can be demonstrated in either normal or specialised fibres, but this is probably due to the methods employed since abundant glycogen can be demonstrated by electron microscopy.

The SA node of the fowl is a crescent shaped area lying to the right of and caudal to the right superior vena cava, between the right venous valve and the orifices of the right superior vena cava and the inferior vena cava. The tissue occupies the whole thickness of the right atrial wall at this point although it may be separated from the right atrial lumen by a sheet of normal fibres. It is composed of narrow fibres loosely packed with connective tissue. A few of the fibres are pale staining although many differ from normal atrial fibres in diameter only. Plates 2, 4, 5a.

The endocardial edge of the SA node contains a few typical Purkinje cells. Although no direct continuity between these Purkinje cells and the atrial subendocardial Purkinje network was observed it is possible that connections exist.

Many nerve bundles are seen in the area of the node and passing into the node itself.

A nodal artery is present as well as many small capillaries.

As in mammalian hearts, a fibrous ring is present between the atrial and ventricular myocardium in the fowl. This insulating ring is penetrated by AV connections, specialised and unspecialised.

**AV node**

An AV node is present in the attachment of the interatrial septum to the fibrous ring close to the point where the septum joins the dorsal wall of the heart. The node extends above the fibrous
ring for a distance of 1mm in the adult heart, and is composed of loosely packed, narrow, pale staining fibres embedded in connective tissue. The nodal cells are continuous with the normal atrial myocardial cells of the interatrial septum. Plates 3, 4, 5b, 6a.

**Interatrial bundle**

A bundle composed of true Purkinje cells issues from the AV node above the entry of the right Purkinje ring into the AV node. This bundle passes along the edge of the fibrous ring to end in normal atrial muscle of the left interatrial septum.

**AV bundle**

An AV bundle continues from the lower part of the AV node at the level of the fibrous ring. The cells are typical large pale Purkinje cells. The bundle, which is up to 50 cells wide, passes ventrally and downwards towards the apex of the heart, in the interventricular septum. The individual fibres have connective tissue sheaths although there is not a connective tissue sheath around the bundle as a whole. No nerves were seen close to or following the course of the AV bundle. Plates 3, 4, 6b.

**Right Purkinje ring**

Before passing through the fibrous ring the AV bundle gives off a large branch of Purkinje cells which passes into the dorsal part of the muscular right AV valve lying between the atrial and ventricular muscle layers and in close contact with both. The bundle passes ventrally and towards the free edge of the valve until it has reached a point about midway across the valve where it turns towards the base of the heart, still moving ventrally. It then passes round the
dorsal side of the base of the aorta, to reach the interventricular septum. In the interventricular septum the bundle is composed of up to 8 cells, each within a connective tissue sheath. The bundle moves down the septum, joining the main AV bundle one-third of the way towards the apex. Plates 3, 4, 7a, 7b.

**Left and right branches**

Just below the junction of the main AV bundle with the right Purkinje ring the bundle divides into two smaller right and left branches. These pass towards the right and left sides of the interventricular septum and split up to become continuous with normal myocardium and subendocardial Purkinje cells. Plates 3, 4, 8a, 8b, 9a.

**Peri-arterial and subendocardial Purkinje cells.**

An extensive network of true Purkinje cells is present in both atrial and ventricular myocardium as bundles of large pale staining cells following the course of coronary vessels and capillaries, and ramifying throughout the endocardial layer of the heart. These cells appear similar to the Purkinje cells of the AV bundle and its branches. Plates 4, 9b, 10a, 10b.

Serial lum sections did not reveal Purkinje cells in situations other than those described above although it was difficult to say that in atrial muscle Purkinje cells did not exist interposed between normal cells. This difficulty was due to the thinness of the atrial wall in many places.

**Unspecialised AV connections**

To the right of the insertion of the AV node into the fibrous ring, a narrow strip of atrial fibres appears to pass round the edge
of the fibrous ring. The fibrous ring at this point is thin and discontinuous. A few atrial fibres appear to pass into the interventricular septum. No atrial fibres, however, were seen to continue into ventricular fibres although it is possible that lateral contacts were made between atrial and ventricular cells.

Other nodal tissue

Morphologically 'nodal' tissue is present in the ventricular myocardium close to the attachments of the cusps of the aortic and pulmonary artery valves. This tissue is composed of slender pale staining fibres, loosely packed with connective tissue. It forms two rings, which are not in contact with each other, around the bases of the aorta and pulmonary artery. Although this tissue is composed of morphologically nodal fibres, there is no involvement with the ventricular Purkinje network, and no nerve bundles are present.

Plate 11a.

Electron Microscopy

Normal myocardium

Atrial and ventricular myocardium will be described as one, since they are almost identical. Any dissimilarities will be described under the appropriate headings.

Arrangement of cells

The fibres are composed of up to 5 or 6 individual cells, enclosed within a sheath of basement membrane which follows the contours of the peripheral cells, invaginating for a short distance between cells in places. The basement membrane sheath is about 46nm thick. The fibres are up to 30um wide while the individual cells,
sectioned near their nuclei, are about Sum in diameter with a range of 2-12um. Typical collagen fibrils are found inserting into the basement membrane. Plates 12-16.

The individual myocardial cells composing the fibres interdigitate in a fashion similar to those of mammalian myocardium, with intercalated discs passing in a stepwise fashion across the ends of the cells or across the ends of branches from the cells. The intercalated disc is considered to be the entire membrane forming the step-like boundary of the cell and not only the transverse portions of the steps.

The sarcolemma or cell membrane

The cell membrane of the normal myocardial cell can be divided into lateral membrane and disc membrane, for the purpose of description. The lateral membranes can be further subdivided into those on the surface of the fibre, which are in contact with the basement membrane, and those within the fibre separated from each other by a gap of 20nm.

The lateral membranes of the cell are for the most part typical unspecialised membranes. The inner leaflet of the unit membrane is 2.5nm thick. When fixed in aldehydes with postfixation in osmium it is unusual to see more than the dense inner leaflet of the unit membrane. When the whole unit membrane is shown, the less dense outer leaflet is separated from the dense inner leaflet by a centre to centre spacing of 7nm.

Membrane specialisations

Examination of the membrane specialisations present in fowl myocardium indicates that although examples of all the classical types of membrane specialisation may be seen, there exist examples of specialisations which although possessing the major characteristics of one type also show features which are common to others. This suggests that a continuous spectrum of membrane specialisations exists although most of the specialisations present can be distinguished as myofibrillar insertion plaques (CAPs on lateral membranes), desmosomes or nexuses.

Typical desmosomes are present as the main type of specialisation on apposed lateral membranes. They are composed of a cytoplasmic
disc 10nm thick in each cell, separated from the inner leaflet of the unit membrane by a gap of 10nm. Filaments frequently insert into the dense cytoplasmic disc. A thin dense disc 5nm thick is also present in the extracellular space between the apposed unit membranes, whose inner leaflets are about 40nm apart. The desmosomes, although apparently randomly distributed along the lateral membranes, are often found at the level of the Z disc. Plates 17-22.

Cardiac adhesion plaques are also found on lateral membranes of apposed cells. They appear as filamentous networks on the cytoplasmic side of the membrane extending for a distance of up to 4µm, and they are 50nm thick. They are commonly found as small discs associated with Z disc attachment. The apposed membranes at this type of specialisation are 30nm apart with no central disc as is found in desmosomes. The filamentous mass is on occasions organised so that it appears as a row of dense spots, 16nm in diameter, parallel to the cell membranes of both apposed cells, when the cell is cut longitudinally. The spots have a periodicity of 4nm. Plates 21-25.

The disc membrane is much more specialised than the lateral membrane. Typical desmosomes such as are found on lateral membranes are also present as specialisations on transverse and longitudinal areas of the disc membrane.

Myofibrillar insertion plaques are present as the main specialisation on the disc membrane, extending along most transverse membranes. This type of specialisation consists of a dense fibrillar network, similar to the cardiac adhesion plaque, extending
up to 66nm into the cytoplasm from the inner leaflet of the unit membrane and receiving insertions from the thin filaments of terminal sarcomeres. The inner leaflets of the unit membranes are, at this type of specialisation, 30nm apart.

Since the unit membrane appeared to be 7nm in thickness, apposed membranes, with a total diameter from the centre of the inner leaflet of one unit membrane to the centre of the inner leaflet of the apposed unit membrane of less than 14nm, were considered to be nexal membranes. Few nexuses were seen. These nexal regions were up to 100nm long and present on transverse and longitudinal areas of the disc membrane. Plates 26-36.

The figures for gap width at the various membrane specialisations were obtained in the following way. Negatives showing membrane specialisations were placed in an enlarger and the image, at 100,000 times, was focused onto Imm\(^2\) graph paper. The specialisation profile was then traced out. The figures are given below. This method of obtaining gap widths is biased since the negatives were not taken randomly and the sample may not have included many transitional types of specialisation.

<table>
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<tr>
<th>Specialisation</th>
<th>Mean</th>
<th>Standard Dev.</th>
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</thead>
<tbody>
<tr>
<td>Desmosome</td>
<td>37.5nm</td>
<td>3.75</td>
<td>26</td>
</tr>
<tr>
<td>MIP</td>
<td>28.95nm</td>
<td>3.9</td>
<td>21</td>
</tr>
<tr>
<td>CAP</td>
<td>29.18nm</td>
<td>3.9</td>
<td>16</td>
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The surface area of the normal myocardial cell cannot be obtained by considering the cell to be a cylinder since the disc membrane undulates to a large extent and the cell branches widely. A figure for the ratio of true disc length to apparent disc length was obtained in the following way. Negatives containing complete transverse parts of the disc were projected onto Imm\(^2\) graph paper.
The disc profile was then traced out and the true length measured with a map measurer. The apparent length was measured from where the longitudinal membrane first turned in a transverse direction to where it finally turned longitudinally. From these tracings (Fig.36b) the ratio of myofibrillar insertion plaque to disc membrane was also calculated using a map measurer.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Mean</th>
<th>Standard Dev.</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real Disc</td>
<td>1.79</td>
<td>0.43</td>
<td>23</td>
</tr>
<tr>
<td>Apparent Disc</td>
<td>0.82</td>
<td>0.083</td>
<td>23</td>
</tr>
<tr>
<td>MIP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Disc</td>
<td></td>
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The ratio of surface to volume for normal cardiac cells was obtained by projecting negatives of cells cut in transverse section onto Imm² graph paper. The cell outlines were traced at a final magnification of 4,500 X. (Fig.36c). The method described by Mobley and Page (1972) of line integration and point counting was used to calculate the surface to volume ratio. The formula used was

\[
\frac{S}{V} = \frac{C}{2AP}
\]

where, C is the number of intersections of lines marking cell membrane with the lines of a 5mm grid in an area of 14cm²; P is the total number of intersections of a 5mm grid falling on cell cytoplasm and A is the distance between the grid lines divided by the magnification of the reproduction.

<table>
<thead>
<tr>
<th>Ratio Surface</th>
<th>Mean</th>
<th>Standard Dev.</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.49</td>
<td>0.37</td>
<td>22</td>
</tr>
</tbody>
</table>

**Transverse tubular system**

The sarcolemma showed no invaginations typical of a transverse tubular system although it was frequently irregular and indeed at the Z disc level.

**Sarcoplasmic reticulum**

The SR exists as a tubular network below the sarcolemma and passing between the myofibrils. The walls of the SR tubules show a
unit membrane pattern. The tubules anastomose and divide to produce a network with tubules varying in diameter from 36nm to 80nm. The tubules contain no stainable material, except where there is specialisation of the tubule wall. The network is not specialised over any particular myofibrillar band, and there is no clear association of the SR with any organelle except the sarcolemma.

The SR has a special morphology when associated with the sarcolemma, forming dyads. The tubule approaches the sarcolemma and then runs parallel to it with an intervening cytoplasmic gap of 16nm. The lumen of the tubule at this point is enlarged and occupied by a dense material. The tubule membrane is specialised on its cytoplasmic side in the form of a periodic density with a spacing of 25nm. This density appears to extend from the SR membrane to the sarcolemma and round the whole circumference of the tubule at this point. The SR is also present as stacks of terminal cisternae at the sarcolemma. Plates 37-43.

Myofilaments

The contractile material of fowl myocardium is similar to that found in skeletal and cardiac muscle in other vertebrates. The myofilaments are bundled together into myofibrils. The myofibrils branch and anastomose showing a typical 'Felderstructure' (Kruger, Duspiva and Furlinger 1933). They are transected by Z discs, in relaxed muscle, at regular intervals of 2·16um. The Z discs are made up of dense filamentous material and are 105nm thick.

The I band, composed of filaments 6nm in diameter, extends for a distance of 0·2um on either side of the Z disc. The A band is composed of typical thick filaments 12nm in diameter. The H zone, 130nm wide, is present in relaxed muscle. The M line is indistinct.

The Z discs of peripheral myofibrils appear to be attached to the sarcolemma since an indentation of the sarcolemme is produced at that point, when the myofibrils contract. There is frequently an extension of the Z disc material along the membrane, for up to 12um, to form cardiac adhesion plaques as described above.

Z discs and other myofibrillar bands are in register in all myofibrils in the same cell and with myofibrils in the constituent cells of the same fibre, due to intercellular junctions occurring at the level of the Z disc. Plates 44,45.
Leptomeres

A subsidiary myofibrillar structure is frequently present in apparently normal myocardial cells. When cut in longitudinal section this takes the form of dense lines 0.25 nm thick, 1.00 nm long and 1.50 nm apart, with elongated fibrils, 0.6 nm thick, running between each dense line. These organelles are found most frequently at the cell surface and tend to follow the direction of the adjacent myofibrils although they are also oriented in other planes.

Nucleus

A single centrally situated nucleus, 1.4 nm long and 3 nm in diameter, is present in each cell. It is filled with a typical granular nucleoplasm, with aggregations of densely staining chromatin. The nuclear envelope is frequently indented and when cut in tangential section, nuclear pores may be seen. Plates 50–54.

Golgi and endoplasmic reticulum

At the poles of the nucleus a vacuolated Golgi apparatus is often present, closely associated with rough endoplasmic reticulum containing ribosomes. Polyribosomes are also present between the myofibrils. Plates 52–55.

Dense granules

Dense granules, similar to those found in the atria of many mammals (Jamieson and Palade 1964), are present in both atria and ventricles of the fowl. The granules are usually present close to the Golgi complex and show what may be interpreted as several stages of development. They have an inner dense core up to 1.30 nm in diameter with an outer clear area bounded by a membrane. More
granules of this type are found in atrial than in ventricular myocardium. Plates 52-55.

Mitochondria

Mitochondria of various shapes and sizes are found in abundance between the myofibrils. They are usually packed with cristae, 20nm in diameter, but contain no granules such as have been demonstrated in the mitochondria of many mammals. The mitochondria are elongated and frequently touch at their ends. They show no particular association with any banding pattern.

Glycogen

Single granules, 25nm in diameter, are present throughout the cytoplasm of the cell varying in amount between cells in the same block. Plate 56.

Vesicles

The sarcolemma on its lateral aspect shows pinocytosis. Many vesicles can be seen just within the membrane and also several forming. A few vesicles are often present on the disc membrane also. These vesicles are typically uncoated, although a few coated vesicles have been seen. Plate 57.

Lipid

Lipid droplets, 1.5μm in diameter, are present just below the sarcolemma and also deep in the cell, frequently associated with mitochondria. They are rounded bodies enclosed within a fuzzy membrane.

Lipofuscin droplets

Dense bodies of irregular shape and size are present in the
cytoplasm. They are composed of dense granules of various sizes enclosed within a membrane.

Periarterial and Subendocardial Purkinje Cells

The Purkinje cells following the course of blood vessels are morphologically the same as those underlying the endocardium. The Purkinje cells found in these two situations are typical in that their diameter is about twice that of normal myocardial cells, and they contain little organised contractile material.

Arrangement of the cells

Fibres, up to five or six cells across, are found along the course of blood vessels, whereas the subendocardial fibres are usually formed from fewer cells. Each fibre has a coat of basement membrane 46nm thick, following the contours of the cells and invaginating between them. External to this coat there is a layer of collagen fibrils attached to the outer surface of the basement membrane. Enclosing this network of collagen fibrils there is a single cell layer of fibrocytes. Plates 60, 61.

The Purkinje cells are elongated and have flattened ends. They frequently branch and anastomose but do not show step-like intercalated discs, typical of normal myocardium. The cells are up to 30um wide but average 24um. They show a greater variety of shape than normal myocardium, with narrow branches and deep clefts. Plate 62.

Purkinje cell membrane

The Purkinje cell has a larger surface area than normal myocardial cells, but shows far less specialisation of its membranes,
The membrane area can be divided into lateral membrane, along the length of the cell, and terminal membrane, at the flattened ends of the cell. Plate 63.

**Lateral membrane**

The sarcolemma is a typical unit membrane showing the same structure as in normal myocardium. Myofibrillar insertion plaques are present as the main type of specialisation. They are similar to the myofibrillar insertion plaques found at the ends of normal myocardial cells, showing a fibrillar network extending from the cell membrane into the cytoplasm for up to 60nm. The length of the specialisation varies up to 4µm. The apposed membranes at this specialisation are 30nm apart measured from the inner dense line of each unit membrane. Myofibrillar insertion plaques are also present on the outer lateral membranes of cells on the periphery of bundles without there being an apposing membrane, although this is less frequent than on apposed lateral membranes.

The typical desmosomes present on lateral and disc membranes of normal myocardium are less numerous on the lateral membranes of Purkinje cells. They show the typical cytoplasmic plaques with a central extracellular disc.

Small nexuses of the size demonstrated in normal myocardium are present in the lateral membranes of the Purkinje cell. They frequently show the pentalaminar structure and are 12nm thick and up to 0.2um in length, each covering an area of up to 0.22µm². These nexuses occur at irregular intervals and are much more numerous than the nexuses found in normal myocardial cells. Plates 64-69.
Terminal membranes

The myofibrillar insertion plaque is the main type of specialisation present on terminal membranes. Extending along the membranes for up to 4µm, the myofibrillar insertion plaques are similar to those in normal myocardium and on lateral membranes, in that they receive the insertion of thin filaments from terminal sarcomeres. The membranes of the apposed cells at this type of specialisation are 30nm apart. The extracellular space is more dense than between unspecialised membranes.

Typical desmosomes are even rarer than on apposed lateral membranes.

Nexuses much larger than those found between lateral membranes are present as components of the terminal membranes. They extend for up to 4µm and each may cover an area of about 12.5µm². This type of nexus occurs frequently on the terminal membrane and shows the typical pentalaminar structure. Plates 70,71.

T Tubules

No T tubular system is present in the Purkinje system of the fowl.

SR

A sparse SR system is present, below the sarcolemma extending throughout the sarcoplasm as a tubular network.
found in association with masses of filaments, 10nm thick and also joined to the ends of myofibrils. Plates 72-81.

Nucleus

One or two rounded nuclei are present in the Purkinje cell. They are about 8um in diameter. Their shape does not appear to be influenced by adjacent myofibrils as in normal myocardium, and they are frequently deeply invaginated with irregular outlines. The nucleus is filled with dense granular nucleoplasm and contains dense chromatin material. Plates 82, 83.

Golgi and endoplasmic reticulum

An extensive Golgi complex is found throughout the cytoplasm of the cell and not, as in normal myocardium, only at the poles of the nucleus. The Golgi is otherwise typical in that it appears as aggregations of tubules and vesicles.

Associated with areas of Golgi are elements of rough endoplasmic reticulum which appear as tubules with ribosomes on their cytoplasmic sides. Plates 81, 82, 84.

Mitochondria

The mitochondria present in the Purkinje cell show varied shapes, and are usually slightly smaller than those present in normal myocardium. They are found scattered throughout the sarcoplasm, while those in normal myocardium are compressed in columns between the myofibrils. No differences in structure of mitochondria from specialised or unspecialised myocardium are apparent.
Myofilaments.

The myofilament content of Purkinje cells is in complete contrast to the orderly and in-register arrangement of the contractile apparatus of normal myocardium. Apparently normal myofibrils are found in the periphery of the cell, frequently attaching to the lateral membranes at myofibrillar insertion plaques. The myofibrils extend for up to 10 sarcomeres, attached at one end but no complete sarcomere is visible at the other end. Small pieces of sarcomeres and isolated sarcomeres are also found throughout the sarcoplasm. The complete sarcomeres are apparently normal and consist of Z discs 100nm thick, I bands 0.2μm wide and A bands 1.7μm wide. The filaments composing the I band are 6nm in diameter while the thick filaments of the A band are 12nm in diameter.

Where the Z disc meets with the membrane there is frequently an extension of the Z disc material along the membrane.

Together with fragments of myofibrils there is a mass of filaments 10nm thick throughout the sarcoplasm. These filaments, intermediate in diameter between the thick and thin filaments of normal sarcomeres, are randomly orientated and do not appear to be organised into a contractile system.

As in normal myocardium there is a subsidiary myofibrillar structure in Purkinje cells. The leptomeres are often found just below the sarcolemma. They lie along the membranes, frequently in ridges raised from the surface of the cell. Large aggregations of leptomeres, up to 4μm in diameter, are also found throughout the cytoplasm of the cell. The spherical aggregations of leptomeres are
Glycogen

A few glycogen granules are present but in appreciably lower concentration than in normal myocardium.

Vesicles

As in normal myocardium, pinocytotic vesicles are present along the lateral membranes of the cell. These vesicles are usually uncoated although a few coated vesicles have been seen.

Lipid

No lipid droplets are present in the Purkinje cell sarcoplasm.

Lipofuscin

Dense bodies similar to those present in normal myocardium are present throughout the sarcoplasm.

Transitional Purkinje Cells

Branching off from the true Purkinje cells of periarterial and subendocardial bundles are cells which, although similar to true Purkinje cells, do not entirely satisfy the criteria of a true Purkinje cell.

The cells are elongated and less than 10um in width. They are bundled together in a similar fashion to normal myocardium. Apart from the difference in width, the main distinction is in the quantity and arrangement of their myofilament content. They contain a larger proportion of organised myofibrils than true Purkinje cells, and fewer sarcomere fragments are present. They contain a larger number of filaments, intermediate in diameter between thick and thin filaments. These filaments are arranged in the sarcoplasm parallel to the long axis of the cell.

The sarcoplasmic reticulum is better developed than in true Purkinje cells and no T tubular system is present. Plates 85-89
Normal cell - Purkinje cell junctions.

A junction between a normal cell and a Purkinje cell was seen on only one occasion. No nexus was seen at this junction. Plate 89b.

The SA Node

The SA node is composed of narrow fibres most of which are intermediate in structure between Purkinje fibres and normal atrium. The nodal fibres are formed from usually four cells enclosed within a basement membrane sheath. There are large quantities of collagen between the fibres. Nerve axons are also seen in the extracellular space between the fibres and often outside the basement membrane sheath.

The nodal cells are typically 3um in diameter. They tend to be rounded in shape when cut in cross section. Intercalated discs are present but they are more irregular than in normal myocardium and show many features in common with terminal membrane apposition in Purkinje cells. The membrane carries cardiac adhesion plaques, myofibrillar insertion plaques, a very few desmosomes and nexuses typical of those found in Purkinje cells. The cardiac adhesion plaques, myofibrillar insertion plaques and desmosomes are similar to those found in normal cells although there are greater numbers of cardiac adhesion plaques. The nexuses observed were found throughout the nodal tissue on lateral and terminal membranes of nodal cells. They were found to measure 14nm wide and 500nm long. Plates 90-96.

No T tubular system is present in nodal cells. The sarcoplasmic reticulum is present to a greater extent than in Purkinje cells. The distribution of fibrils within the nodal cells is intermediate between that of Purkinje cells and normal cells.
Fibrils are found both at the periphery and in the centre of the cell. There is more interfibrillar space than in normal cells and this is loosely packed with fine filaments and mitochondria. The mitochondria are typical of these in Purkinje cells.

The distribution of ribosomes, rough endoplasmic reticulum and Golgi is similar to that in Purkinje cells. The nodal cells appeared to have a single nucleus.

No lipofuscin bodies were observed; neither was there any intracellular lipid.

Other cells found in the nodal area were a few normal cells, Purkinje cells and a very few cells which showed many characteristics of smooth muscle cells, but which also resembled Purkinje cells. The Purkinje-like cells contained filaments which were oriented mainly longitudinally. On only one occasion was evidence of Z discs or a banding pattern observed (Plate 96C). These cells appear to be isolated from the main mass of the node.

Serial sections cut at lum showed that the fibres of the node towards the endocardial surface often contained Purkinje cells and it is possible that they were part of the atrial Purkinje network. No branches were seen to pass out from the node. The nodal fibres on the fringe of the node were infiltrated by normal atrial fibres.

The AV Node

Unlike the SA node, the AV node is not composed of cells of one type. The cells composing the node are Purkinje cells, nodal cells, and normal cells. The distribution of these cells varied from one area of the node to another. Close to the periphery of the node many normal cells were found between fibres composed of nodal and Purkinje cells. Throughout the node Purkinje and nodal cells
appeared in almost even amounts except at the upper and lower ends of the node. It was often difficult to decide whether cells were nodal or Purkinje since many of those present appeared to be intermediate in structure between Purkinje cells and nodal cells of the SA node.

The Purkinje cells of the node were typical of Purkinje cells in other parts of the heart in all respects except diameter. They had few myofibrils which were usually distributed around the periphery. The membrane specialisations present were myofibrillar insertion plaques, cardiac adhesion plaques, nexuses and a very few desmosomes. Plates 97-102.

Many of the nodal cells were similar to those in the SA node and were about 4μm in diameter. They were enclosed by basement membrane into fibres of three or four cells. The fibres were loosely packed together with connective tissue and fibrocytes. Nerve bundles were also seen to pass between the fibres although few nerves were seen within the basement membrane sheath.

The nodal cells had irregular intercalated discs. Myofibrillar insertion plaques and cardiac adhesion plaques were common but desmosomes and nexuses were rare. The myofibrils were distributed round the periphery and in the centre of the cell. The interfibrillar space contained rounded mitochondria and fine filaments.

The SR was rarely seen although subsarcolemmal cisternae were present showing the same structure as in normal tissue. No T tubular
system was seen. Ribosomes and Golgi were distributed throughout as in Purkinje cells.

The nodal cells of both the SA and AV nodes resembled transitional Purkinje cells found in other parts of the heart.

The AV node was seen to give off three branches. The main branch, composed of large Purkinje cells, left the node and passed through the fibrous ring ventral to the node. This formed the AV bundle. The cells composing the AV bundle were typical Purkinje cells in all respects. The bundle was composed of about 25 cells bound into fibres of two or three cells. Fibrous tissue was found between the fibres but not surrounding the bundle as a whole.

Plate 103.

Another branch left the node from its tapering dorsal end and progressed, close to the fibrous ring, from the node to normal tissue at the left side of the interatrial septum. This branch was composed of about ten typical Purkinje cells.

A third branch left the AV node at its ventral end to form the right Purkinje ring. This branch was also composed of typical Purkinje cells.

To the right of the main septal artery and one-third of the way down the interventricular septum the main bundle split into two smaller branches which were also composed of true Purkinje cells. These bundle branches made their way to the left and right surfaces of the interventricular septum where they became continuous with Purkinje cells of the subendocardial Purkinje network.
A Purkinje bundle also branches off from the bifurcation of the main bundle into left and right links. This bundle which formed the ventricular part of the right Purkinje ring was seen in a few cases to be a branch of the right bundle branch rather than of the main bundle.

'Nodal' Cells of the Aortic and Pulmonary Artery Valve Cusps

The muscle cells at the bases of the cusps of the aortic and pulmonary artery valves are peculiar in that they appear, morphologically, to be nodal cells.

The tissue is composed of a loose network of narrow fibres, separated by connective tissue. The fibres are three to four cells across and are surrounded by an envelope of basement membrane with attached collagen fibrils. There is frequently a layer of fibrocytes such as surrounds true Purkinje cells.

The cells show a very variable outline, with many long branches. They are up to 4μm in diameter. The cell membrane shows specialisations typical of normal myocardial cells. Myofibrillar insertion plaques and desmosomes are present, although intercalated discs are absent and cells abut onto each other in a fashion similar to Purkinje cells.

Although narrower than true Purkinje cells and similar in diameter to transitional Purkinje cells, they resemble true Purkinje cells in their cytoplasmic content.

Typical myofibrils are often present throughout the cell sarcoplasm. Fibrils 10nm thick, similar to those in true Purkinje cells, are present to about the same extent as in true Purkinje cells.
The cells contain a centrally situated nucleus with associated Golgi complex. Many mitochondria are present larger than those found in true Purkinje cells. Many pinocytic vesicles are found similar to those in normal myocardium and true Purkinje cells.

Plates 104-108.

Nerve Bundles

Many large compound nerve bundles are present in fowl atrial and ventricular myocardium, following the course of coronary vessels. The nerve bundles are composed of myelinated and unmyelinated axons.

Nerve axons with associated Schwann cells are found throughout normal myocardium and Purkinje tissue, and naked nerve endings have been seen associated with atrial and ventricular myocardial cells.

Plates 109-115.

Cell Separation

Smear preparations were examined using the light microscope. Fragments were considered to be separate cells if they contained a single nucleus. The results of the cell separation methods employed are given in Table 2. where a + sign indicates cell separation.

The methods used however give no quantitative information on the adhesive properties of membrane specialisations.
The tissue taken for examination by EM was not shaken as was the tissue for light microscopy, so the amount of separation was less. Cells were considered to be separated if the extracellular gap at intercalated discs was greater than 40nm with no trace of any connection across the gap at myofibrillar insertion plaques.

As is shown in Table 2 suspensions of single cells were only obtained by perfusing hearts with CMF-EDTA for 2 hr. or Trypsin + CMF-EDTA for 1 hr. In both cases many fragments of more than one cell were found. Where single cells were found they were elongated with step-like margins. Striations could be clearly seen, and also

<table>
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<tr>
<th>Perfusion Fluid</th>
<th>Time</th>
<th>Electron Microscopy</th>
<th>Light Microscopy</th>
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<td>CMF</td>
<td>15 min.</td>
<td>-</td>
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<tr>
<td>CMF</td>
<td>30 min.</td>
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<td>CMF-EDTA</td>
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<tr>
<td>CMF-EDTA</td>
<td>30 min.</td>
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<tr>
<td>CMF-EDTA</td>
<td>1 hr.</td>
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</tr>
<tr>
<td>CMF-EDTA</td>
<td>2 hr.</td>
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<tr>
<td>Ringer + Trypsin</td>
<td>15 min.</td>
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<td>Ringer + Trypsin</td>
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<tr>
<td>Trypsin + CMF-EDTA</td>
<td>15 min.</td>
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<td>Trypsin + CMF-EDTA</td>
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<tr>
<td>Trypsin + CMF-EDTA</td>
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<td>High Sucrose</td>
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a dense nucleus, about 14\textmu m long in the centre of the cell. The cells appeared to be wider than when examined in the EM but this may have been due to the method of preparation.

Separation of cells in pieces taken for electron microscopy occurred after perfusion for 30 min., 1 hr. and 2 hr. with CMF-EDTA, or for 1 hr. with Trypsin + CMF-EDTA.

The discs of these cells, prepared as above, were widely separated with the undulating disc surface of one cell being an exact replica of the undulations on the disc membrane of the cell from which it had separated. Separation to this extent occurred only at the transverse portions of the disc membrane and not at all at apposed lateral membranes. Although the junctional complexes present in the desmosomes may have been released there was no obvious separation at desmosomes with any of the methods used. Plates 116-119.

**Cell length**

Perfusion of hearts with CMF-EDTA for 2 hr. or Trypsin + CMF-EDTA for 1 hr. provided suspensions of single cells, each containing a single central nucleus. The length of single cells was found to vary from 118\textmu m to 160\textmu m with the average length being 140\textmu m. Plate 120.

**Extracellular labelling**

Perfusing hearts with control solution containing 'Tris' buffer instead of bicarbonate and phosphate buffers did not produce any alterations in the heart rate or ECG over a period of 1 hr. Blocks removed from hearts perfused with the control solution were examined
and compared with tissue fixed after perfusion with avian ringer. There was no detectable morphological difference between the two methods of preparation.

The labelling solutions, prepared by adding 0.05, 0.1 or 1% lanthanum nitrate to the control solution, produced a slowing effect on the heart, which increased with increased concentration of lanthanum. With 0.05% lanthanum, hearts could be perfused for up to 30 min. without showing any deleterious effects. With 0.1% lanthanum, slowing of the heart occurred after 15 min. perfusion, and after 10 min. perfusion with 1% lanthanum, the heart stopped beating altogether.

After perfusion with 0.05% lanthanum, very little deposit was present in the extracellular space. Lanthanum could be seen lining the capillaries and passing across endothelial cells in vacuoles. Lanthanum also penetrated the intercellular space between the endothelial cells. Basement membrane sheaths of fibres close to capillaries had deposits of lanthanum attached as large granules.

After perfusion with 0.1% lanthanum, precipitate was present as for 0.05% lanthanum but was also found on the basement membranes of fibres away from capillaries. Smaller granules of lanthanum were also present closely packed together with fine precipitate in the extracellular space between apposed lateral membranes and throughout the extracellular space of intercalated discs. Lanthanum was present only where it was bound to the basement membrane or where the intercellular space was less than 15nm wide. Fine lanthanum
deposit was also present filling pinocytotic vesicles beneath the sarcolemmal surface.

Perfusion with 1% lanthanum a precipitate similar to that found after perfusion with 0.1% lanthanum with no increase in the amount of precipitate present. Lanthanum was present on all basement membranes and filling narrow intercellular gaps.

The precipitate found at intercalated discs with perfusion with 1% or 0.1% lanthanum was large and irregularly spaced and appeared to have been partly washed out.

Labelling by immersion produced a similar picture to perfusion with 1% lanthanum. The precipitate of lanthanum was found on basement membranes and filling pinocytotic vesicles.

Both methods of labelling failed to penetrate the thick coat of the Purkinje cell. Precipitate was found on collagen fibrils surrounding Purkinje bundles and on the basement membrane coat but little precipitate was present in the intercellular space.

The labelling of extracellular space was not consistent although in many blocks showing heavy labelling vesicles at the surface of the cells were filled with lanthanum. In these blocks no structures suggestive of a T tubular system were demonstrated, which indicated that a T tubular system was not present.

In the labelling experiment no nexuses were seen and therefore no information on the structure of the nexus was obtained. Electron microscopy of normal tissue however does suggest that there is a hexagonal substructure at the nexus.

Plates 121-128.
was not heavy enough to demonstrate any specialisation of the abundant nexal membranes of Purkinje cells. Plates 121-130.

This study, agrees with the observations of Gosseau (1962), which were published subsequent to these investigations.

The SA node of the foal is similar in position to that of the mode in other birds. Foster (1970) described the SA node of the black swan and pigeon as being beneath the epithelium at the base of the right venous valve. In these studies, the node is situated in the middle and upper part above the valve. It extends towards the just above the orifice of the inferior vena cava, in a position near the opened opening of the right superior vena cava. According to Gosseau (1962), however, the SA node of the pigeon lies on the anterior wall of the right atrium directly below the opening of the right superior vena cava. Curving round, the node extends to the neighbourhood of the left venous valve, the venous postcavital valvulae (Kern 1925). Rarely does the SA node extend to the right venous valve. In the cock, as in the pigeon, the node is found below the right superior vena cava (Gosseau 1962).

In a similar position to the SA node of birds, reports an area of the sheep (Copenhaver and Truce 1952), cat (Bliss and Burke 1953), monkey (Copenhaver and Truce 1952), rat (Burt 1955), and man (Segro 1926, Copenhaver and Truce 1952). Copenhaver and Truce (1952) described the SA node of the sheep as being transverse between the cardiac muscle which surrounds the base of the superior vena cava.
DISCUSSION

The SA Node - Light Microscopy

The position of the SA node in the fowl, as described in this study, agrees with the observations of Gossrau (1969), which were published subsequent to these investigations.

The SA node of the fowl is similar in position to that of the node in other birds. Davies (1930) described the SA node of the black swan and pigeon as being beneath the epicardium at the base of the right venous valve. In these birds, the node is widest in the middle and tapers off above and below. It extends upwards from just above the orifice of the inferior vena cava, to a point some distance below the opening of the right superior vena cava. According to Gossrau (1969), however, the SA node of the pigeon lies in the dorsal wall of the right atrium directly below the opening of the right superior vena cava. Curving round, the node extends to the neighbourhood of the left venous valve, the musculus pectinatus valvularis (Kern 1926). Rarely does the SA node extend to the right venous valve. In the duck, as in the pigeon, the node is found below the right superior vena cava (Gossrau 1969).

In a similar position to the SA nodes of birds are the SA nodes of the sheep (Copenhaver and Truex 1952), ox (Blair and Davies 1935), monkey (Copenhaver and Truex 1952), rat (Muir 1955), and man (Segre 1926, Copenhaver and Truex 1952). Copenhaver and Truex (1952) described the SA node of the sheep as being interposed between the cardiac muscle which surrounds the base of the superior vena cava,
and that which forms the anterior wall of the right atrium. Muir (1955) described the SA node of the adult rat as being an inverted horseshoe in the medial wall of the right superior vena cava.

The relative positions of the SA node in the mammals and birds in which it has been described are shown in Plate 2A.

There is no general agreement as to whether a histologically modified SA node is present in amphibians or reptiles and so a comparison with birds, including the fowl, is not possible. Prakash (1960) described the SA node in the invaginated portion of the wall of the sinus venosus of the lizard, lying in close proximity to the SA orifice at the base of the left SA valve. On the basis of this study, if an SA node is present in reptiles it lies in a similar position to that of mammals and birds.

The SA node of the fowl is composed of fibres, narrower than normal myocardium, which stain less densely than adjacent normal atrial fibres, in agreement with the description of Gossrau (1969) of the SA nodal fibres in the duck, goose, and other birds. However this description does not agree with the description of the SA node of the black swan and pigeon by Davies (1930) who found that the nodal cells were of a larger diameter than normal myocardium.

The SA nodal fibres of birds are similar to those of mammals. The fibres of the rat SA node vary in size (Muir 1955); some are smaller than the surrounding myocardial cells, others are larger, having the appearance of typical Purkinje cells. The nodal fibres of the sheep are narrower than normal fibres, having fewer myofibrils
and are more irregular than normal atrial cells (Pace 1924, Copenhaver and Truex 1952).

The SA node of the fowl contains nerve bundles but no ganglia. Gossrau (1968) has demonstrated high concentrations of acetylcholinesterase in the fowl SA node consistent with the presence of nerves. The present description of the SA node agrees with that of Davies (1930) in that the main part of the node was seen to be in contact with Purkinje cells which could have been part of the atrial Purkinje network. The connection of the SA node with the Purkinje network was not proved in this study. This is mentioned again in the section entitled 'Further Work'.

The association of the fibres of the SA node with other than normal atrial fibres is disputed in birds and mammals. In the sheep, rat and monkey, the SA nodal fibres establish contact with only the adjacent normal atrial fibres. In man, due to the great variation in the size of cells (Truex 1960), it is not possible to state that the fibres of the SA node do not make contact with Purkinje cells. In the ox, the fibres of the SA node make contact with short lengths of Purkinje fibres which are in contact with normal atrial fibres (Blair & Davies 1935).

The SA Node - Ultrastructure

The ultrastructure of the SA nodal fibres of the fowl described in this study varies in several aspects from the ultrastructure of the SA node in mammals.

In general the nodal fibres of the fowl, cow (Rhodin, del Missier & Reid 1961, Hayashi 1962), rabbit (Torii 1962) and
dog (Kawamura 1961) lie parallel to each other with very few fibres at right angles to the main direction of the node. The reports of intercellular junctions between nodal cells, however, vary from one author to another. Rhodin et al (1961) describe many desmosomes in the node. Kawamura (1961) describes intercalated discs with myofibrillar insertion. Challice (1966) and Torii (1962) describe desmosomes and myofibrillar insertion plaques in the node of the rabbit. These reports appear to differ from the structure of the SA node of the fowl as described in this study in that many nexuses were seen in the fowl SA node.

The nodal cells in all species in which they have been described contain myofibrils distributed throughout the cytoplasm.

The differences which exist between the description of the fowl SA node and the descriptions by other authors of SA nodal tissue may be explained by poor fixation. An accurate comparison between the structures observed by other authors in the SA node and the ultrastructure of the node in the fowl cannot be made since many earlier studies were made on poorly fixed material (Kawamura 1961, Hayashi 1962, Torii 1962, Challice 1966).

The atrial Purkinje network

An extensive network of typical Purkinje cells is present throughout the endocardium of the right and left atrium of the fowl and also following the course of coronary vessels. This is similar to the network described by Davies (1930) in the black swan, and pigeon, and by Gossrau (1969) in the pigeon and other birds. Prakash (1955) failed to find a Purkinje network in the heart of the fowl.
In contrast, few mammals possess true Purkinje fibres in the atria. In man and the ox a few large cells have been seen which resemble Purkinje cells (Blair & Davies 1935, Copenhagen & Truex 1935).

No direct connection between the SA node and the AV node, via the Purkinje system, could be demonstrated with the methods employed. It is possible however that such a connection exists. Davies (1930) described connections between the SA node and the Purkinje network in the bird, he concluded (Davies 1942) that the impulse originating in the SA node region reaches the AV node by spreading in a wave-like manner over atrial muscle in general and not by special pathways. This was also the conclusion of Lewis (1925) from his electrocardiographic studies.

The AV Node – Light Microscopy

The position of the AV node in the fowl agrees with the descriptions of the AV nodes in the black swan and pigeon (Davies 1930) and with the description by Gossrau (1969) of the node of the pigeon and other birds. The position of the AV node in birds is similar to that occupied by Tawara’s node (Tawara 1906) in the mammal and similarly is in contact with normal atrial muscle at its cranial end and with large Purkinje fibres of the AV bundle caudally.

Compared with the very obvious AV connections in birds and mammals, the AV node is apparently absent in reptiles or at least lacking in many species of the class. Davies & Francis (1946) and Davies, Francis & King (1952) stated that there is no histological
specialisation of the muscle fibres in any part of the heart in cold
blooded vertebrates. Buchanan (1956) and Gyevai (1958) also failed
to find any specialised system in the hearts of poikilothermic
vertebrates. Only Prakash (1960) described an AV node in the lizard.
According to Prakash (1960) the AV node of the lizard is a prominent
well-defined and conspicuous body situated ventral to the SA node
and very near the AV orifice.

The structure of the node in birds and mammals is similar in
that it is composed of narrow pale staining cells, held together in
a loose mesh by connective tissue, as in the SA node.

Anderson & Latham (1971) have recently described the AV node in
the human. Their description differs from previous descriptions
both in the cell content of the node and the shape of the node.
They did not observe any Purkinje cells, as described by James (1961).
Although Kistin (1949), Copenhaver & Truex (1952), James (1961) and
Titus, Daugherty & Edwards (1963) found that the AV node was composed
of cells of uniform morphology, Anderson & Latham (1971) found that
the node was composed of nodal cells and transitional cells.
DeFelice & Challice (1969) also found transitional cells in the AV
node. The observations of Anderson & Latham (1971) confirmed those
of James (1961) that the upper end of the node connects with normal
atrial muscle through several branches. Although no branches were
seen to pass out from the upper part of the AV node in the fowl,
three dimensional reconstructions were not made and it is possible
that such nodal connections exist.
The AV Node - Ultrastructure

It would appear from the literature that the structure of the AV node in the fowl (as described in this study) is unlike the AV node in other animals. While the node in the fowl is composed mainly of parallel fibres, the AV nodes in the cow, dog and rabbit are formed from networks of branching and anastamosing fibres. The cells of other nodes appear to be uniform in structure. This difference may be due to the fact that the AV node of the fowl is smaller than the AV node of the cow, dog and rabbit in which case there is less true nodal tissue with the fibres of the three main branches playing a larger part in its structure.

The differences found between the intercellular junctions present in the fowl and those described by other authors may be due in part to the better preservation of structure provided by the fixatives used in this study.

Interatrial bundle

The bundle from the AV node to the left interatrial septum has been described previously by other authors. Blair & Davies (1935) found in the human a prolongation from the AV node to the left atrial component of the interatrial septum piercing the upper part of the trigonum fibrosum dextrum. Shaner (1929) described an interatrial node in the early foetal calf heart which disappeared in later stages of foetal life gradually fading into ordinary atrial muscle. It is possible that the interatrial bundle found in the fowl in this study is present in young animals only, since it was observed in animals three and a half months old and not in eighteen month old hens.
The AV Bundle

The position of the AV bundle of the fowl, continuing caudally from the AV node and moving in a ventral and caudal direction in the ventricular septum, is similar to the AV bundle in other birds (Davies 1930, Prakash 1955, Yousuf 1965 and Gossrau 1969), and to the position of the AV bundle in mammals (Holmes 1921, Davies 1942, Davies, Francis, Wood & Johnson 1955, Truex 1960).

The composition of the AV bundle is also alike in birds and mammals, consisting of true Purkinje fibres with longitudinal fibrillae at the periphery. The cells vary in size along the length of the bundle. In the fowl, as in other birds (Davies 1930), the bundle possesses no fibrous sheath. On the other hand, the bundle in mammals is separated from the normal ventricular myocardium by a connective tissue sheath (Tawara 1906, Curran 1909).

Bundle Branches

In the fowl the left and right bundle branches become continuous with the normal myocardium of the interventricular septum and with the Purkinje network of the ventricles. Drennan (1927) and Davies (1930) described a branch from the right bundle branch in birds, which passes up the interventricular septum to end in the muscular right AV valve. The terminations of the right and left branches of the fowl and other birds are different from those in mammals. The left bundle branch of mammals frequently divides into anterior and posterior limbs. These in turn give off numerous fibres which are often visible as pale grey endocardial bands, most clearly seen on the lower septum and surrounding the bases of the papillary muscles.
(Truex, Curry & Smythe 1954). In the ungulate hearts, these Purkinje fibres frequently span the cavity of the left and right ventricles. The right bundle branch divides into two or three terminal branches which are continuous with the Purkinje network of the ventricle. One of the large terminal branches normally traverses the moderator band of the right ventricle when this structure is present.

**Right AV Ring**

The right AV ring, present in the fowl and other birds (Davies 1930, Gossrau 1968, 1969), is absent in mammals. This ring of true Purkinje cells issues from the base of the AV node in the fibrous ring, passing into the fibrous sheet in the muscular right AV valve. Prakash (1955), in his study of the fowl, failed to find the right Purkinje ring.

The relationship between the right AV ring of birds and the AV ring of cold-blooded vertebrates is discussed by Davies (1930).

**Other Nodal Tissue**

In agreement with the findings of Mackenzie & Robertson (1910) in the pigeon, an area of morphologically nodal tissue surrounds the base of the aorta and the pulmonary artery in the fowl. Mackenzie & Robertson (1910) described at the base of the heart, in the region of the attachment of the aorta and pulmonary artery, a mass of 'peculiar tissue' surrounding the openings of these vessels. They noted that the fibres were deficient in striation and that the nuclei were somewhat rounded. The muscle fibres in this area were in contact with the adjacent normal myocardium.
This peculiar muscle tissue was not observed by Davies (1930) in his investigation into the conducting system of birds or by Prakash (1955) or Gossrau (1969) in the fowl. However, in the present study, this tissue was found in all hearts examined, in birds from 1-18 months old. It is suggested, therefore, that this tissue is present as a constant feature of the aortic and pulmonary artery valve cusps of the fowl and other birds.

Similar tissue is present in mammals, only in the SA and AV nodes. The constituent cells of this peculiar tissue have the same electron microscope appearance as the nodal cells of the steer (Rhodin et al 1961), dog (Kawamura 1961), rabbit (Torii 1962) and cow (Hayashi 1962).

The function of this tissue is not known. Since the tissue does not connect the atria with the ventricles, and does not extend between the interventricular septum and the left wall of the right ventricle, it is unlikely that the area is a functional part of the impulse conducting system, although the composition of the area is typical of nodal tissue. While the area does not appear to be a functional part of the conduction system, it may be a secondary nodal area with a rate of self depolarisation less than that of either the SA or the AV nodes.

Another possibility is that the muscle fibres are present in the connective tissue at the base of the membranous valves to give structural support. Although this seems likely in the case of the aorta, due to the high pressure which the valve cusps sustain, the opposite is the case in the pulmonary circulation where the pressure is lower.
Unspecialised AV Connections

Light microscope observation of lum sections has shown that an unspecialised connection could exist. This has previously been suggested although not proved by other authors. Blair & Davies (1935) noticed no direct continuity between the myocardial fibres of the right atrium and those of the ventricular septum although in a few sections the terminal fibres came very close to each other. Kent (1893) noted direct continuity between the myocardial fibres of the right atrium and those of the ventricular septum in the hearts of the rat and the young rabbit and to a lesser extent in the guinea pig and hedgehog. The fibres described by Kent as establishing atrio-ventricular continuity at this site in the monkey appear from his description to have been transitional Purkinje cells. Pace (1924) believed that these fibres were in the nature of insertions.

The work of Lewis & Master (1925) on the electrophysiology of the AV node should have shown up an AV connection other than the AV node but none was observed.

The fibres in the fowl although not observed to be in direct continuity could have formed lateral connections and this is discussed in the section on further work.

Impulse Conduction in the AV Node

Several authors have reported that part of the delay in conduction observed between atria and ventricles in the hearts of many species is due to the AV node (Hoffman & Cranefield 1960, Lewis & Master 1925).

The delay may be due to the architecture of the AV node. The atrial terminations of the AV node in the human and rabbit appear to
be different from those found in this study in the fowl. James (1961), DeFelice & Challice (1969) and Anderson & Latham (1971) report that the atrial part of the AV node in human and rabbit hearts is formed from many branches and that there are many possible pathways from the atria through the AV node to the AV bundle. This lends morphological support to the dual conduction theory (Moe, Preston & Burlington 1956, Hoffman & Cranefield 1960, James 1961) which suggests that two impulses reaching the same point from different directions can cancel each other out. Rhodin, del Missier & Reid (1961) have put forward a mechanical theory for the delay at the AV node. They represent the node as a three dimensional fisherman's net and the AV bundle as a collection of parallel strands, and suggest that a pull at one corner of the net would spread in all directions, thus delaying the pull to be felt at the opposite corner of the net. This idea is however basically unfounded since the pull (if there is any pull in the AV node and AV bundle) must come after the depolarisation of the cells.

The delay at the AV node may also be due to slower inter- and intracellular conduction. There is little morphological evidence to suggest that the passage of the impulse from one nodal cell to another is slower than between normal cells. Conduction along nodal cells, however, may be slowed due to their narrow diameter.

Structure and Function

Although the function of the heart appears to be the same in all species of animals there is a great diversity of morphology.
While the myocardium of birds is similar in ultrastructure to amphibians and reptiles, it forms a heart which is closer in gross anatomy to the hearts of mammals. Is the heart of the bird then an intermediate form between the lower orders and mammals? This may be so in the case of the myocardium but the impulse conducting system does not fit into this scheme. Contrary to the expectation that the ICS should be simple and less well developed than the ICS of mammals, in birds it is more extensive, implying a greater control over and a better distribution of the cardiac impulse.

The well-developed ICS in birds would seem to be related more to the ultrastructure of the heart rather than to the function of the heart as a pump. Although the heart rate of birds is in general greater than that of mammals, many small mammals have heart rates as high as the fowl. Few mammals have an atrial Purkinje system although the volume of atrial tissue can be one hundred times greater than that of the fowl.

It seems possible that the well-developed Purkinje system is present in birds to compensate for a slower rate of impulse conduction in normal fibres or to allow a greater flexibility of heart rate. There is at present little information available on the rate of conduction in either normal or Purkinje fibres of birds.

**Intercellular Conduction**

Although intercellular conduction is a common property of the myocardial cells of mammals, birds and amphibia, the cells in these classes show many morphological differences. The morphological factors influencing intercellular conduction in mammals, birds and
amphibia, will first of all be compared. Their influence on intercellular conduction will then be discussed.

Morphology of Intercellular Conduction in Normal Myocardium Fibres

The myocardial fibre in the bird is composed of 2-6 cells, enclosed within a continuous sheath of basement membrane. The inner membrane of the cells in the fibre are closely apposed with an intervening extracellular gap of about 18nm, whereas the outer membranes are covered with a layer of basement membrane.

The arrangement of cells in the frog ventricular fibre is an extension of the fibre composition in the fowl. The frog ventricular fibre contains 10-15 muscle cells (Staley & Benson 1968), whereas the frog atrial fibres are up to 12 cells wide (Baldwin 1970). The cells are separated, as in the fowl, by intercellular clefts. The basement membrane in general does not become part of the lining of the intercellular clefts, which are 20-40nm wide.

The arrangement of cells in the myocardial fibre of the turtle is similar to that of birds (Fawcett & Selby 1958, Hirakow 1970).

In higher orders the composition of the fibre is different. In the mammal the fibre is usually composed of a single cell, although up to three cells may be present. Thus most of the cell membrane is covered with basement membrane. Cell membranes are only in close apposition at intercalated discs and for short distances on lateral membranes, whereas in birds, amphibia and reptiles, most of the membrane of a cell is in apposition to the cell membranes of other cells.
Cell Shape and Size

The myocardial cells of the mammal have a greater diameter than those of birds, amphibia and reptiles. According to Muir (1965), the cardiac cells of the rat are 19-90um wide. McNutt & Fawcett (1969) conclude that the atrial cells of the cat tend to be smaller in diameter than ventricular cells, when both are stretched to approximately the same sarcomere length. The atrial cells average 5-6um in diameter while the right ventricular papillary muscle cells average 9-10um in diameter.

Intercellular Contacts

The myocardial cells of mammals, birds and reptiles are similar, in that they have step-like ends, with step-like protrusions and invaginations occurring along the length of the cells. In the frog intercalated discs are numerous but simple with no steps (Staley & Benson 1968).

(a) Myofibrillar insertion plaques.

In mammals, myofibrillar insertion plaques, zonulae adherentes, fasciae adherentes are present as the main type of specialisation along the intercalated disc (Muir 1965, Sommer & Johnson 1967, Fawcett & McNutt 1969, McNutt & Fawcett 1969). They exist as a filamentous mass on the cytoplasmic side of transverse elements of the disc membrane at the Z disc level. They receive the insertions of the thin filaments of the terminal sarcomeres.

In lower orders similar structures are present. In birds and reptiles (Fawcett & Selby 1958, Leak 1967), which have step-like intercalated discs, the specialisation is, as in mammals, present on
the transverse parts of the disc. In the frog, where the disc is simple and possesses only a transverse component, typical myofibrillar insertion plaques are present (Barr, Dewey & Berger 1965, Staley & Benson 1968).

(b) Cardiac adhesion plaques.

Structures similar to the cardiac adhesion plaques, described by Baldwin (1970) in frog atrium, have been demonstrated in the fowl, boa constrictor (Leak 1967), and turtle (Fawcett & Selby 1958). These appear as filamentous masses on lateral membranes and on longitudinal parts of disc membranes. They are frequently found at the Z disc level and appear to be connected with the fusion of the Z disc to the membrane.

Although this type of specialisation is present in lower orders, it has not been described in mammals.

(c) The desmosome.

Typical desmosomes, as described by Farquhar & Palade (1963) in various epithelia, are present in disc membranes of rat ventricular muscle (Muir 1965), rabbit papillary muscle (Sommer & Johnson 1967) and cat ventricular and atrial muscle (Fawcett & McNutt 1969, McNutt & Fawcett 1969). These structures have also been demonstrated in the myocardium of birds (Gossrau 1969), reptiles (Fawcett & Selby 1958, Leak 1967), and in the frog (Staley & Benson 1968).

Rayns, Simpson and Ledingham (1969) have investigated the structure of the desmosome, using an extracellular marker, and a tilt stage microscope. The electron opaque line, seen midway between
the opposed membranes (Karrer 1960, Farquhar & Palade 1963), is connected to the adjoining cell membranes on either side by a quadratic array of side arms.

The Nexus

The precise morphology of the nexus, as present in the cardiac muscle cell, has only recently been defined. The nexus (in cardiac muscle) has been termed a tight junction, gap junction and close junction and the distinction between the structures which represent those terms and the structures termed zonula occludens have not always been very clear.

Present evidence indicates that the gap or close junction as found in many tissues tend to take the form of a plaque. The structure of this junction appears to differ according to the method of fixation and staining.

Cobb & Bennett (1969) have investigated the effect of different fixatives on the structure of the nexus. After osmium tetroxide fixation the unit membrane in chick and pigeon gizzard was a triple layered structure measuring 7.3 nm from the centre of the cytoplasmic leaflet of one unit membrane to the centre of the extracellular leaflet of the unit membrane, while the nexus was a quintuple layered structure measuring 16.3 nm on average. With potassium permanganate, the unit membrane measured 6.7 nm and the nexus 12.2 nm. The nexus was found to be better preserved when the tissue was fixed in permanganate than when fixed in osmium.

McNutt (1970) has observed that when membranes are adequately preserved and stained with uranyl acetate but little or no lead
citrate, the nexus has a seven layered appearance which can be converted to a five layered nexus by heavier lead staining. Thus the gap, close and pentalaminar junctions are terms which describe the same structure. This structure is very different from the zonula occludens of Farquhar & Palade (1963). The gap junction and the zonula occludens have been compared by Goodenough & Revel (1970) who show that the macular gap junction is characterised by particles closely packed in a regular polygonal lattice occupying a space 20°A wide between the facing outer leaflets of the junctional membranes. The zonula occludens, on the other hand, consists of long, anastamosing threads or chains of membrane contacts where plasma membrane outer leaflets appear to fuse.

Experiments using extracellular tracers have shown that while the nexus or gap junction can be penetrated by horseradish peroxidase or lanthanum nitrate (Revel 1968), the zonula occludens is impermeable (Goodenough & Revel 1971).

Models of nexal structure have been proposed. McNutt & Weinstein (1970) have produced a model from freeze etch studies of the nexus. They suggest that each of the two apposed or joined membranes at the nexus is composed of two layers. One layer next to the cytoplasm has granules on one surface which fit into indentations in the other layer. The outer layers have hexagonal blocks on their extracellular surfaces. The hexagonal blocks of opposite cells are fused occluding the extracellular space except for that at the sides of the blocks. McNutt & Weinstein (1970) also suggest that the two cells are connected by hydrophylic channels in the centre of the
hexagonal blocks. This model fits present evidence from extracellular labelling and freeze cleaving. McNutt & Weinstein (1970), however, state that their inability to demonstrate the apposed outer layers of the membrane is due to the fact that these layers are fused. These layers are not however fused over their whole surface as they admit. Therefore it cannot be said that the apposed outer layers are fused since the gaps at the sides of the hexagonal blocks cannot be demonstrated either.

Another model has been proposed by Pappas, Asada & Bennett (1971). Their model is similar to that of McNutt & Weinstein (1970) except that they propose a more block-like structure for the nexus, with the extracellular space forming a hexagonal network of tubes through the block. Intercytoplasmic channels pass through the centres of the hexagons.

Nexuses have been described in rat ventricle (Muir 1965), mouse ventricle (Revel & Karnovsky 1963), rabbit papillary muscle (Sommer & Johnson 1967), and cat ventricle and atrium (Fawcett & McNutt 1969, McNutt & Fawcett 1969). The nexuses demonstrated in the myocardium of these mammals are up to 3\(\mu\)m long. Small nexuses have been described in frog atrium (Barr, Dewey & Berger 1964) and a few small nexuses are present in fowl myocardium.

Morphology of Intercellular Conduction in Purkinje Cells

Fibres

Like the Purkinje fibres of birds (Davies 1930, Hirakow 1966, Gossrau 1968), the fibres of mammals are composed of five or six cells bundled together with a basement membrane sheath (Muir 1957b, Rhodin et al. 1961, Sommer & Johnson 1968).
Cell Shape and Size

The Purkinje cells of mammals are similar to those of birds in that they are roughly cylindrical and up to three times as long as they are broad. The Purkinje cells of the fowl are about 22μm in diameter whereas the Purkinje cells of the sheep (Muir 1957b) and cow (Hayashi 1962) are 30-40μm in diameter.

The cells do not show typical step-like intercalated discs but are more irregular at the ends in larger animals.

The lateral apposed membranes of Purkinje cells are separated by larger extracellular gaps than are present in normal myocardium.

Intercellular Contacts

(a) Myofibrillar insertion plaques.

In the Purkinje cells of birds and mammals, myofibrillar insertion plaques are present to a large extent on terminal and lateral membranes. They show a typical filamentous structure with a density in the intervening extracellular space.

(b) Desmosomes.

Few desmosomes are present in fowl Purkinje cells. In contrast, in the steer (Rhodin et al 1961), goat, sheep and dog (Sommer & Johnson 1968), many desmosomes are present giving adjacent cells the appearance of having been spot-welded.

(c) Nexuses.

Although few small nexuses are present in normal fowl myocardium, many large nexuses are present in terminal and lateral membranes of fowl Purkinje cells. Purkinje cells of the sheep, guinea pig, rabbit, cat, goat and dog (Muir 1957b, Sommer & Johnson 1968) are similar to those of the fowl in that they possess large nexuses.
Morphology of Intercellular Conduction in Nodal Tissue

The morphology of nodal fibres in all animals are intermediate between normal fibres and Purkinje fibres and therefore the factors contributing to intercellular conduction are probably the same as have been described for normal and Purkinje fibres.

Fibres

The SA and AV nodal fibres in all animals can be composed of true nodal cells or mixtures of nodal and normal cells. In the fowl Purkinje cells are also added to the fibres.

Cell Shape and Size

The cell shape and size varies due to the mixed composition of the fibres. Cell diameter varies but in most species nodal cells are narrower than normal cells.

Intercellular Contacts

A useful comparison between the present findings and those of other authors cannot be made due to poor fixation in earlier reports.
Stability of Intercellular Contacts

Attempts have been made by varying the osmolarity of incubating solutions (Dewey & Barr 1962, Sedar & Forte 1964, Barr, Dewey & Berger 1965, Barr, Berger & Dewey 1968 and Cobb & Bennett 1969), or by varying the ionic composition of perfusion fluids (Muir 1967) to investigate the stability of intercellular contacts.

According to Dewey & Barr (1962), Sedar & Forte (1964), Barr, Dewey & Berger (1965), Barr, Berger & Dewey (1968), all nexuses of smooth muscle can be disrupted by incubating them in hypertonic solutions, although desmosomes may remain attached. Barr, Dewey & Berger (1965) and Barr, Berger & Dewey (1968) maintain that although nexuses can be disrupted in hypertonic solution, this change can be completely reversed by returning the tissue to an iso-tonic solution. Although the above authors agree that the nexus is labile in hypertonic solutions, this is refuted by Cobb & Bennett (1969) who claim that after treatment with hypertonic solutions nexuses are intact. Although Cobb & Bennett (1969) demonstrate various types of nexuses present in tissue fixed normally, they only show intact nexuses of the bulb and socket type after hypertonic treatment. The stress placed upon a nexus of the bulb and socket type might be less since one cell is being held by the other without the aid of the nexus.

Thus, on the strength of the evidence available, nexuses appear to be labile in hypertonic solution. The small nexuses present in the myocardium of the fowl may or may not be labile in hypertonic solution. Since they were so small and rare in normal tissue it is
not conclusive that no intact or ruptured nexuses were seen in tissue after hypertonic treatment.

According to Muir (1967), although myofibrillar insertion plaques and desmosomes are disrupted by calcium chelation, many normal nexuses remain, in rat myocardium. Similarly nexal membranes remain after calcium chelation and violent agitation whereas other types of membrane specialisation separate. Contrary to the conclusions of other authors (Dewey & Barr 1962; Sedar & Forte 1964; Barr, Dewey & Berger 1965; Barr, Dewey & Berger 1968) this indicates that perhaps the nexus is resistant to mechanical disruption although this does not take into account the effect of calcium chelation on the membrane or the stress and direction of stress imposed upon the nexus by the method of mechanical disruption employed.

More recent studies on the stability of the nexus have confirmed that removal of Ca\[^{++}\] does not appear to effect nexal adhesiveness (Asada & Bennett 1971; Pappas, Asada & Bennett 1971). These authors have also shown, by rather unphysiological methods, that mechanical injury or replacement of Cl\(^-\) with propionate in saline bathing solutions tends to cause nexal separation and increase intercellular resistance.

**Influence of Morphological Factors on Intercellular Conduction**

Whether there is a high or a low intercellular resistance between cardiac muscle fibres will determine the influence of the fibres on intercellular conduction. If there is a low intercellular resistance, as is proposed by several authors (Woodbury 1962, Weidmann 1966), the cells must have a certain amount of ionic
continuity, making them a morphological as well as a physiological syncytium. In this case the fibre of the fowl, which is composed of several cells, may act as a single cell if the amount of ionic continuity is sufficient. Comparing the fibres in mammals and the fowl, if there is sufficient continuity, then on the basis of similar diameter, the fowl and mammalian myocardial fibres should conduct at the same rate.

If there is a high intercellular resistance, as is suggested by Sperelakis (1963), then the cells of the composite fibre of the fowl, reptiles and amphibia may act as many separate cables in parallel, with a slow rate of conduction due to their narrow diameter (Hodgkin 1964).

With regard to the spread of electrotonus along a cell and across cell junctions with an extracellular gap of 15nm, Katz (1966) has calculated that if the physical properties of the terminal membranes of the cells are similar to those of the rest of the cell, electrical transmission will be completely ineffective, except perhaps in the case of very large giant fibres. For conduction to occur across a 15nm gap, the terminal membrane resistance would have to be reduced from about 2,000 ohms/cm² to less than 1 ohm/cm². If, however, small cytoplasmic bridges, 10nm in diameter existed between the two terminal membranes, then one bridge per square micrometer would be sufficient for a 5μm fibre. Another possibility is that the intercellular connections may be larger, in which case fewer would be required.
Although the desmosome has been suggested by one author as the site of intercellular conduction (Rhodin et al 1961), it seems more likely, on the basis of close apposition of membranes, and the evidence from cell separation experiments (Dewey & Barr 1962; Sedar & Forte 1964; Barr, Dewey & Berger 1965; Barr, Berger & Dewey 1968) that the nexus is the site of intercellular conduction.

If the nexus is the site of electrotonic intercellular conduction, then there is disagreement between the morphology and physiology of impulse conduction in myocardium. From physiological measurements it would seem that narrow cells have a higher input resistance than wide cells (Katz 1966), and also might be expected to have larger areas of contact than wide cells. On the contrary, in the narrow, normal myocardial cells of the fowl the nexuses are few and very small compared with the large nexal areas of normal mammalian myocardium (Revel & Karnovsky 1963; Muir 1965; Johnson & Sommer 1967; Fawcett & McNutt 1969, McNutt & Fawcett 1969) and specialised myocardium of mammals (Muir 1957b, Sommer & Johnson 1968), and birds (Hirakow 1966).

The required area of contact in narrow and wide cells might be less than the size of the nexus in the fowl. This conforms with the physiological analysis of Katz (1966), leaving the extra area of intercellular contact of wide cells to be explained. The variation of nexal size in myocardium may be associated with types of intercellular communication other than impulse conduction.

The explanation of this nexal area variation may lie in the efficiency of communication. Large diameter cells have a greater
volume of ions and nutrients than narrow cells of the same length and therefore for efficient transfer of these intracellular constituents, the communicating corridors must be wider in large diameter cells than in narrow cells. This may account for the large nexal area present in the Purkinje cells of the fowl while the narrow ventricular cells, with a lesser volume of ions, have few, small nexuses.

De Haan & Gottlieb (1968) have found that single cells are more difficult to impale successfully with electrodes than cells in sheets. They attribute this to the fact that myocardial cells in contact are more stable, due to the ability of ions to pass freely between cells. Cells in sheets are more resistant to damage due to their intercellular junctions which make a large reservoir of ions and nutrients available to each cell.

Kanno & Loewenstein (1963) have investigated intercellular communication by observing the passage of colourant between insect salivary gland cells. From their experiments they conclude that molecules as large as bovine serum albumen (M.wt. 69,000) can pass between the cells of the insect salivary gland by diffusion, without any loss to the extracellular space, although this figure has recently been reduced to 10,000 (Loewenstein 1970).

Communication between cells in sheets exists for a short time after injury of one cell, as is illustrated by the injury potential which lasts until the door between the injured and normal cells has been closed by divalent cations (Loewenstein & Kanno 1967).
The main argument against the nexus being the site of electrotonic conduction is the existence of tissues which are electrotonically coupled but which appear to have few or no nexuses. Johnson & Sommer (1969) originally found only one or two nexuses in fowl myocardium. Further observation by Jewett, Sommer & Johnson (1971), however, has confirmed the findings of this study and has shown that nexuses are also present in the heart of the finch and hummingbird.

Leyton & Sonnenblick (1971) have reported that nexuses are lacking in the crab heart, but the fact that such a structural modification is lacking in the Limulus disc and that electrotonic interactions between cells cannot be demonstrated (Abbot, Lang, Parnas, Parmley & Sonnenblick 1969) lends further support to the idea that such specialised junctions are necessary for low impedance pathways in cell-to-cell conduction.

Thus it seems that the nexus is probably involved in electrotonic impulse transmission although it may not be the only structure with this role. Loewenstein (1966) and Bullivant & Loewenstein (1968) have suggested that septate desmosomes are involved in cell-to-cell electrotonic coupling in several invertebrates. Hudspeth & Revel (1971) have shown coexistence of nexuses and septate junctions in an invertebrate epithelium and it would seem that if both structures are involved in impulse conduction then it is unlikely that they should occur together.
Morphology of Intracellular Conduction

It is now widely accepted that T tubules provide for spread of electrical activity from the surface of the muscle cell into its interior (Huxley 1958, Peachey & Porter 1964, Gage & Eisenberg 1967).

A transverse tubular system has been shown to exist in the normal myocardial cells of mammals (Lindner 1957, Simpson & Oertelis 1962, Nelson & Benson 1963, Simpson 1965, Fawcett & McNutt 1969). That these tubules are invaginations of the sarcolemma has been clearly demonstrated (Lindner 1957, Simpson & Oertelis 1962, Nelson & Benson 1962). No T tubular system has been observed either in the atrial myocardium of the cat (McNutt & Fawcett 1969) or rat (Hibbs & Ferrans 1969), in normal or specialised myocardium of the fowl, in ventricular myocardium (Staley & Benson 1969) or atrial myocardium of the frog (Baldwin 1970), or in the myocardium of the boa constrictor (Leak 1967).

According to Girardier (1965) there is a critical size of fibre below which no T tubules are found. This appears to be borne out by the fact that there are no T tubules in the narrow cells of the cat atria (McNutt & Fawcett 1969), whereas there is a T tubular system in the wide cat ventricular cells (Fawcett & McNutt 1969). Although the specialised myocardial cells of mammals possess no T tubular system, they have a greater diameter than normal myocardial cells (Muir 1957b, Sommer & Johnson 1968), contradicting the size theory of Girardier.

An examination of the relationship between the amount of myofibrils present, their distance from the sarcolemma, and the presence of T tubules shows that although specialised myocardial
cells have a much greater diameter than normal cells and contain the same total amount of myofibrils (Muir 1957b), the myofibrils are arranged around the periphery of the cell close to the sarcolemma, and would not require a T tubular system. Similarly, in normal atrial muscle of the cat (McNutt & Fawcett 1969) normal and specialised myocardium of the fowl, and atrial and ventricular myocardium in the frog (Staley & Benson 1968, Baldwin 1970), the myofibrils are close to the sarcolemma and no T tubules are present.

Thus it seems that the presence of a T tubular system is not related to cell diameter but to the amount of myofibrils and their relationship to the sarcolemma.

The presence of a well developed SR in large and small myocardial cells, in mammals and birds, is probably related to the myofibrillar development since only a poorly developed SR is present in the specialised myocardium of mammals and birds where the myofibrils are also poorly developed.

The Effect of a Transverse Tubular System on Conduction Velocity

Estimates of plasma membrane capacitance in muscle cells with T tubules (Katz 1948, Fatt & Katz 1959) are high relative to the values obtained for nerve cells (Curtis & Cole 1938; Hodgkin, Huxley & Katz 1952). Similarly the membrane capacity of the iliofibularis muscle of the frog, which has no T tubules, is much lower than frog muscle with T tubules (Adrian & Peachey 1965). Peachey (1965) concludes from this evidence that the presence of T tubules adds considerably to the membrane capacitance and he has shown that in a 100μm diameter fibre there is 7cm² of T tubule area per cm² of outer surface area.
As in frog skeletal muscle, the T tubular systems in myocardial cells might be expected to have a higher membrane capacitance if T tubules are present.

Although conduction velocity increases with fibre diameter, it is also proportional to membrane capacitance (Hodgkin 1964). As the capacitance increases the conduction velocity decreases for a constant diameter of fibre. Therefore in fibres of similar diameter the conduction velocity will be less in those with T tubules. On this basis, therefore, since the cells of fowl myocardium do not possess T tubules, they should conduct impulses along their length faster than cells of a similar diameter which possess T tubules. The cells of cat atrial myocardium are of a similar diameter to fowl myocardial cells and also do not possess T tubules, suggesting that conduction along cat atrial and fowl myocardial fibres should occur at the same rate.

Conclusions

The light microscope investigations of the impulse conduction system in the fowl have shown that, in birds, this system is not intermediate in structure between mammals and amphibians as might have been expected but is much more specialised. The SA and AV nodes are situated in a similar position in birds and mammals. The interatrial node present in the fowl has not been previously described in birds. The major differences which exist between birds and mammals are the extensive subendocardial and periarterial system and the right Purkinje ring.
It is concluded that the extensive Purkinje system present in
birds exists either to compensate for a slow rate of conduction
through normal fibres or to allow a greater flexibility of heart
rate.

The study of intercellular connections has shown that many
large nexuses are present in Purkinje cells. Few nexuses are present
in normal myocardium. Many nexuses are present in the SA and AV
nodes. A review of the literature has shown that the morphology of
the nexus has now been well described and models of intercellular
communication at the nexus proposed. Although it seems likely that
the nexus is the site of electrotonic conduction there is no concrete
proof. Also the nexus may not be the only site of intercellular
impulse conduction.

No T tubular system is present in the fowl heart. It is
suggested that the presence of a T tubular system in other hearts is
not related to cell diameter but to the concentration and
distribution of myofibrils in the cell.

**Further Work**

From this study it appears that the heart of the fowl is
composed of a whole range of cell types from normal myocardium to
true Purkinje cells. It would be interesting to find out how these
cells are generated. Do they come from a single cell line? Further
investigations into the differentiation of the specialised cells
and the development of the ICS may suggest reasons for the highly
developed ICS in birds.
A study of the development of the ICS may also show whether the interatrial node is restricted to young animals and also provide clues as to its function.

This study has suggested that there may be unspecialised connections between the atria and ventricles. Serial sections for electron microscopy may prove or disprove their existence. Serial sectioning for electron microscopy may also settle the question of connections between the SA node and the atrial Purkinje system.
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Plates 1 - 129.
Plate 1.

A diagramatic representation of the surfaces of the heart removed from the bird. The left side of the heart is occupied by the left atrium and left ventricle. The aorta and pulmonary artery leave the left and right ventricles on the ventral surface of the heart.
Plate 2.

A diagramatic representation of the position of the SA node in the right atrium of the fowl.

RSVC - right superior vena cava.

IVC - inferior vena cava.

LSVC - left superior vena cava.

MPV - musculus pectinatus valvularus.

RVV - right venous valve.

Shaded area - SA node.
Plate 3.

A diagramatic representation of part of the specialised conduction system.

A - AV node.
B - AV bundle.
C - right bundle branch.
D - Left bundle branch.
E - right Purkinje ring.
F - fibrous ring.
G - interatrial bundle.
Plate 4.

A diagrammatic representation of the Impulse Conduction System. AV node and bundle in black. Right AV Purkinje ring in blue. LV - left ventricle. RA - right atrium.
Plate 5a.

SA nodal region. A few of the fibres are pale-staining, many are narrower than normal myocardial fibres. A nodal artery is present. x 50.

Right atrium of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.

Plate 5b.

AV node and AV bundle. The cells of the AV node and bundle, which are pale in comparison with normal myocardial fibres can be seen passing from the atrial tissue, through the fibrous ring, which is stained green, into ventricular muscle. x 50.

Interventricular and interatrial septum of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.
Plate 6a.

The AV node (N) and AV bundle (B) are demonstrated as clumps of pale-staining fibres at the level of the fibrous ring. x 50.

Interventricular and interatrial septum of a 6 months old hen. Fixed by immersion in susa. Stained with Masson's trichrome.

Plate 6b.

The AV bundle is demonstrated as pale-staining fibres, passing through the fibrous ring into ventricular muscle. x 50.

Interventricular septum of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer.
Plate 7a.

The recurrent right AV ring is shown as a bundle of pale-staining fibres in atrial muscle.  x 50.

Interatrial and interventricular septum of a 6 months old cock. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.

Plate 7b.

The pale-staining fibres of the right AV ring are shown at the level of the fibrous ring, passing round the base of the aorta.  x 50.

Interatrial septum of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.
Plate 8a.

AV bundle in the interventricular septum. x 250.

Interventricular septum of a 6 months old hen. Fixed by immersion in susa. Stained with Masson's trichrome.

Plate 8b.

AV bundle in the interventricular septum. x 50.

Material as in 8a, but stained with Heidenhains haematoxylin and Van Giesson.
Plate 9a.

The recurrent branch of the right AV ring in the interventricular septum. x 50.

Interventricular septum of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.

Plate 9b.

Pale subendocardial Purkinje fibres are demonstrated between the densely stained atrial myocardium and the endocardial connective tissue. x 50.

Right atrium of a 6 months old hen. Fixed by immersion in 4% formaldehyde in phosphate buffer. Stained with Masson's trichrome.
Plate 10a.

Peri-arterial Purkinje fibres are demonstrated in the connective tissue surrounding a blood vessel. \(x\) 50.

Interventricular septum of a 12 months old hen. Fixed by immersion in susa. Stained with Masson's trichrome.

Plate 10b.

Large diameter pale-staining Purkinje cells are demonstrated together with narrower dense normal fibres. The nuclei of the Purkinje cells are rounded whereas the nuclei of the normal cells are elongated. \(x\) 250.

Right atrium of a 6 months old hen. Fixed by immersion in 4\% formaldehyde in phosphate buffer. Stained with Masson's trichrome.
Plate 11a.

Peculiar narrow, pale-staining muscle cells from the base of the aortic valve cusps. x 50.

Left ventricle of a 6 months old hen. Fixed by immersion in susa. Stained with Masson's trichrome.

Plate 11b.

A large nerve bundle is shown close to atrial myocardium. x 50.

Right atrium of a 6 months old hen. Fixed by immersion in susa. Stained with Masson's trichrome.
Plate 12.

Transverse and longitudinal sections of normal ventricular muscle are shown. The fibres can be seen from the transverse section to be very irregular in shape. Although the fibres in the longitudinal sections at first sight appear to be single cells, they are actually composed of many narrow cells. x 1800.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Stained with toluidine blue.
Plate 13.

A diagramatic representation of a normal fowl myocardial fibre. The intercalated disc is considered to be the membranes from C-D including longitudinal and transverse portions. Cells A and B run side by side. Their apposed membranes at E are considered to be lateral membranes.
Plate 14.

Typical narrow cells are demonstrated separated by intercellular clefts. (> <). Only one of the intercellular clefts is not lined with basement membrane (A). The sarcoplasm between the myofibrils is occupied by elongated mitochondria. x 8,000.

Right ventricle of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig’s buffer. Postfixed in 1% osmium tetroxide in Millonig’s buffer. Stained with uranyl acetate and lead citrate.
Plate 15.

Two narrow ventricular cells are shown. The characteristic Z (Z), I, and A (A) bands of the myofibrils are clearly shown. Elongated mitochondria are present between the myofibrils. A section of nucleus (Ncl) can be seen between the myofibrils. x 13,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 16.

The relationship of capillaries to muscle fibres is shown. The fibre demonstrated is composed of two narrow cells. The nucleus of one cell is shown, with a Golgi complex at its lower pole. x 7,200.

Right ventricle of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 17.

Two typical desmosomes are demonstrated on lateral apposed membranes at the level of the Z disc.

x 50,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 18.

A typical desmosome is demonstrated on lateral apposed membranes of ventricular cells. Filaments can be seen inserting into the dense cytoplasmic plaques.

x 67,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 19.

A typical desmosome is demonstrated. The dense cytoplasmic plaques can be clearly seen. The unit membrane is shown but the extracellular disc is not visible. 

x 88,000.

Right ventricle of a 6 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 20.

A desmosome is shown as a specialisation of lateral apposed membranes at the level of the Z disc. Two dense cytoplasmic plaques can be seen.

x 156,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 21.

At lateral apposed membranes of two atrial cells, two typical desmosomes are demonstrated, with two cardiac adhesion plaques above them. Between the two desmosomes the membranes are close together forming what might be a nexus.

x 75,000.

Atrial tissue from a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 22.

A typical desmosome (D) is shown on two lateral apposed membranes. A cardiac adhesion plaque (CAP), with dense spots at regular intervals can be seen at the level of the Z disc. A subsarcolemmal cisterna is present below the cardiac adhesion plaque. x 50,000.

Right ventricle of a 12 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 23.

A typical cardiac adhesion plaque is demonstrated as a dense, filamentous mass at the level of the Z disc.

x 100,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 24.

A cardiac adhesion plaque is demonstrated at the level of the Z disc, giving the appearance of an extension of the Z disc material. The extracellular space at this point is more dense than between unspecialised membranes. Subsarcolemmal cisternae are shown above and below the cardiac adhesion plaques.

x 70,000.

Right ventricle of an 18 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 25.

An extensive cardiac adhesion plaque is demonstrated on apposed lateral membranes.

x 23,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 26.

A typical intercalated disc is shown passing in a stepwise fashion across a narrow branching cell (x-x).

x 13,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 27.

A typical step-like intercalated disc is shown (x-x). The myofibrils have disappeared and only the Z bands remain.

x 19,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 1% Potassium permanganate in veronal acetate buffer. Unstained.
Plate 28.

A typical transverse portion of an intercalated disc, consisting of undulating apposed membranes with myofibrillar insertion plaques.

x 60,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer, stained in uranyl acetate and lead citrate.
Plate 29.

A typical convoluted transverse portion of an intercalated disc. Myofibrillar insertion plaques are present as specialisations on the undulating parts of apposed membranes.

x 36,000.

Right ventricle of a 4 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixation in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 30.

A typical step-like intercalated disc is shown passing across a cell. The membrane specialisations present are desmosomes (D), and myofibrillar insertion plaques (MIP).

x 37,500.

Right ventricle of 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 31.

The transverse portion of an intercalated disc is shown with two small nexuses. (N). The dense material on either side of the membranes of this transverse portion is myofibrillar insertion plaque.

x 46,000.

Right ventricle of a 4 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 32.

A typical transverse part of an intercalated disc is shown. A nexal region is demonstrated (N).

x 37,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 1% potassium permanganate in veronal acetate buffer. Unstained.
Plate 33.

Myofibrillar insertion plaques are demonstrated on the apposed undulating membranes at the level of the Z disc.

x 67,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 34.

A typical transverse segment of an intercalated disc with desmosomes (D) and myofibrillar insertion plaques (MIP). Mitochondria are present between the myofibrills.

x 24,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 35.

This transverse portion of an intercalated disc has two areas which by measurement are considered to be nexuses (N). The typical pentalaminar structure is not demonstrated.

x 55,000.

Right ventricle of an 18 months old hen. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in uranyl acetate and lead citrate.
Plate 36.

This intercalated disc is not typical of discs in ventricular or atrial muscle in that a nexus is present (N). A desmosome (D) and myofibrillar insertion plaques (MIP) are also demonstrated. x 55,000.

Right ventricular muscle of a 6 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 36 (b).
A tracing of part of an intercalated disc. The continuous line accompanied by a dotted line represents myofibrillar insertion plaque and continuous line alone represents unspecialised membrane.

Plate 36 (c).
A tracing of part of a transverse section through normal ventricular muscle. Cell membranes are represented by continuous lines. The hatched areas are extracellular space.
Plate 37.

The SR network (SR) is demonstrated.

x 72,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 1% potassium permanganate in veronal acetate buffer. Unstained.
Plate 38.

The SR can be seen in face view overlying the myofibrils and in cross section (△), passing between the myofibrils. x 55,000.

Right ventricle of an 18 months old hen. Fixation by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in lead citrate and uranyl acetate.
Plate 39.

This high magnification plate shows the SR network in face view. The wall of the SR tubule has the typical unit membrane structure. x 208,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 40.

A subsarcolemmal cisterna (▲) is shown. A periodic density is present between the cisterna and the sarcolemma.

x92,000.

Atrium of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 41.

A subsarcolemmal cisterna is shown with a suggestion of openings to the extracellular space (▲).

x 108,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 42.

Subsarcolemmal cisternae are present (A) with specialisations on their sarcoplasmic sides.

x 60,000.

Atrium of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 43.

Like the SR described previously, these SR lamellae demonstrated here, have a periodic specialisation on their cytoplasmic sides. x 120,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 44.

Two narrow ventricular cells are demonstrated, apposed at their lateral membranes. The Z discs in one cell are in register with the Z discs in the other cell. The sarcoplasm between the myofibrils contains elongated mitochondria (Mt) and SR cut transversely (↑). x 21,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 45.

The Z disc in the centre of the plate has split in two, with filaments joining the two sections.

x 36,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 46.

A leptomere can be seen connecting between the Z discs. Filaments run between the dense lines of the leptomere. To the right of the leptomere, tubules of SR can be seen. x 90,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 47.

A single leptomere is shown running parallel to the myofibril. x 65,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 48.

Two leptomeres are shown in a typical situation close to the sarcolemma. There is a regular spacing between the dense lines and the same spacing between the last dense line and the Z disc of the myofibril. Fine filaments can be seen running between the dense lines. x 32,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 49.

Two leptomeres are demonstrated lying at right angles to the main direction of the myofilaments. Thin filaments can be seen running between the periodically spaced lines. x 40,000.

Right ventricle of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 50.

A typical nucleus of a normal ventricular cell is demonstrated.

x 13,000.

Right ventricle of a 6 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 51.

An invaginated atrial nucleus is shown, with three nuclear pores demonstrated above the left hand invagination, where the nuclear envelope is cut tangentially.

x 43,500.

Atrium of a 6 months old hen. Fixation by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 52.

This view of one pole of a nucleus demonstrates mitochondria of various shapes and sizes between the myofibrils and a cluster of tubules and vesicles forming the Golgi complex (G) with associated 'atrial' granules (A). Ribosomes are present as dense granules throughout the sarcoplasm and round distended endoplasmic reticulum. A typical desmosome (D) is demonstrated. The nucleus is filled with dense granular nucleoplasm and contains clumps of dense chromatin.

x 24,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 53.

The organelles at a pole of an atrial cell nucleus are shown. Associated with the Golgi complex are what appears to be several stages of development of 'atrial' granules (Jamieson and Palade). (▲). Rough endoplasmic reticulum (RER) is present and ribosomes are scattered throughout the sarcoplasm.

x 40,000.

Atrium of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 54.

The Golgi complex is demonstrated as a collection of smooth vesicles at a pole of an atrial cell nucleus. 'Atrial' granules are also demonstrated. The cristae of some mitochondria are wavy similar to the cristae described in the mitochondria of the ventricle of the cat (Fawcett and McNutt 1969).

x 37,000.

Atrium of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 55.

Granules with dense cores are shown in the centre of the plate, associated with vesicles of Golgi complex.

x 41,000.

Atrium of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 56.

Many single glycogen granules are shown surrounding typical mitochondria.

x 32,000.

Right ventricle of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 57.

Pinocytotic vesicles (PV) are shown close to or opening onto the sarcolemma. A coated vesicle is also shown (CV).

x 90,000.

Right ventricle of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 58.

A centriole (C) is demonstrated close to the Golgi complex of a normal ventricular cell, 55,000.

Right ventricle of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 59.

A diagramatic representation of a Purkinje fibre. A typical branching Purkinje cell is demonstrated (A) with lateral apposed membranes (B) and flattened terminal membrane (C). D - nucleus. (E) - fibrocyte. (F) - basement membrane.
Plate 60.

Three Purkinje cells are shown in a typical position close to a capillary. Whereas one cell (A) is relatively free of Golgi and mitochondria, another (B) contains a diffuse Golgi system, many mitochondria and two leptomeres. x 7,800.

Right ventricle of a 12 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 61.

Three Purkinje fibres are shown. The extracellular space between the fibres contains collagen fibrils. The lower Purkinje cell has a concentration of mitochondria near its centre.

$x 18,000$.

Right ventricle of a 6 months old cock. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in uranyl acetate and lead citrate.
Plate 62.

A typical Purkinje cell is demonstrated with many protrusions, and giving off a narrow branch.

$x \times 18,750$.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 63.

Three typical Purkinje cells are demonstrated. A terminal junction (T-T) is shown where two of the cells are joined end-end. A lateral junction is shown where one cell is apposed to the side of another cell.

x 9,000.

Right ventricle of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 64.

Two Purkinje cells are shown, side by side. The lateral apposed membranes support a nexus (N). A large complex leptomere is present in the right hand cell. x 13,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 65.

Two typical lateral apposed membranes of Purkinje cells are shown. Myofibrils insert into myofibrillar insertion plaques all along the membrane. x 17,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 66.

A cluster of leptomeres is shown close to the lateral apposed membranes of two Purkinje cells. The leptomeres are in close association with normal myofibrils and with the 10 nm fibrils which fill the sarcoplasm.

x 24,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 67.

An apparently normal myofibril is present below the sarcolemma of the left hand cell. At the level of the Z disc a cardiac adhesion plaque (CAP) is demonstrated. A small nexus (N) is also shown. Fibrils can be seen in cross section (↑) passing in a circumferential direction.

x 30,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Post-fixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.

A lateral junction between two Purkinje cells is demonstrated.
Plate 68.

Junctions between four Purkinje cells are demonstrated. (MIP) Myofibrillar insertion plaque. (N) Nexus.

x 14,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 69.

A small nexus is demonstrated between lateral apposed membranes of two Purkinje cells.

x 100,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 70.

A nexus (N) is demonstrated on apposed terminal membranes of two Purkinje cells.

x 90,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 71.

A nexus is demonstrated between the sarcolemma of two ventricular Purkinje cells. The unit membranes of the apposed sarcolemmata can be seen as trilaminar structures which come together as a seven layered, then a pentalaminar structure.

x 290,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 72.

A myofibril is shown inserting into a myofibrillar insertion plaque on an unapposed lateral membrane. The membrane is indented at this point.

x 69,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 73.

The myofibrils of Purkinje cells are unlike the myofibrils of normal myocardium in that they insert into myofibrillar insertion plaques on apposed and unapposed lateral membranes. Myofibrils inserting into myofibrillar insertion plaques at unapposed lateral membranes are demonstrated.

x 58,000.

Interventricular septum of 12 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 74.

A leptomere (L) is shown in association with a mass of filaments. Peculiar cross branches are demonstrated in a normal myofibril (A).

x 50,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 75.

A large Purkinje cell is shown in cross section. In the centre of the cell close to the rounded leptomere there is a concentration of myofilaments, and filaments 10nm thick. Mitochondria are scattered throughout the sarcoplasm which is filled with fine filaments, 10nm thick. Sections of myofibrils are shown around the periphery of the cell.

x 7,200.

Interventricular septum of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 76.

A leptomere is shown on a ridge at the surface of a Purkinje cell.

x 55,000.

Right ventricle of an 18 months old hen. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in uranyl acetate and lead citrate.
Plate 77.

A complex leptomere is demonstrated in a typical ventricular Purkinje cell, close to a mass of thick and thin myofilaments.

x 20,000.

Right ventricle of a 4 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer, Stained in uranyl acetate and lead citrate.
Plate 78.

The coat of the Purkinje fibre is demonstrated. External to the cell membrane is a layer of basement membrane (BM), a layer of collagen fibres, a fibrocyte layer (F), and another collagen layer.

A small leptomere is present within the Purkinje cell. The sarcolemma at this point bulges out, possibly due to the constricting or restricting effect of the leptomere.

x 140,000.

Right ventricle of a 4 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 79.

A typical leptomere is demonstrated continuing in from a normal myofibril.

x 40,500.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 80.

This view of a Purkinje cell is a section cut at right angles to the main direction of the myofibrils. A group of leptomeres, showing the characteristic banding pattern, is present on the right hand side of the plate in association with fragments of myofibrils.

x 20,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide. Stained in uranyl acetate and lead citrate.
A leptomere is demonstrated continuing on from a Z disc in a Purkinje cell. Three rough endoplasmic reticulum lamellae are also shown.

x 40,500

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig’s buffer. Stained in uranyl acetate and lead citrate.
Plate 82.

A highly convoluted Purkinje cell nucleus is shown, containing granular nucleoplasm and aggregations of dense chromatin. The sarcoplasm of the cell contains Golgi complex (G), leptomeres (L), and many mitochondria.

x 10,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 83.

The nucleus and mitochondria of Purkinje cells tend to be more rounded than those of normal myocardium, as is demonstrated in this plate. The sarcoplasm contains irregular fine filaments 10nm thick.

x 24,000.

Interventricular septum of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 84.

Purkinje cell centrioles are demonstrated close to the Golgi complex.

x 48,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 85.

A typical transitional Purkinje cell is shown containing a mass of filaments 10nm wide, oriented in the long axis of the cell. The narrow transitional cell to the right contains a well developed SR which is present as rows of vesicles.

x 18,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 86.

Transitional Purkinje cells are demonstrated. The sarcoplasm contains more organised myofilaments than true Purkinje cell. The cells are of a similar diameter to normal myocardial cells. The 10nm fibrils found in the sarcoplasm are oriented in the long axis of the cell. x 7,800.

Right ventricle of a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 87.

Two transitional Purkinje cells are shown side by side. The apposed membranes support a desmosome (D), and cardiac adhesion plaque (CAP). The cells, which are up to 10um wide, contain masses of filaments 10nm thick.

x 19,200.

Interventricular septum of 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 88.

The transitional Purkinje cell on the right shows a well-developed SR network, which appears as rows of vesicles, unlike the scanty SR of true Purkinje cells.

x 25,000.

Right ventricle of a 6 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 89.

Three transitional Purkinje cells are demonstrated. Myofibrils are present to a greater extent than in true Purkinje cells but are not as well developed as in normal myocardium. The transitional Purkinje cells are of a similar diameter to normal myocardial cells. Many tubules and vesicles of the SR are present in the sarcoplasm, which also contains filaments 10nm thick.

x 12,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 89 (b).
A Purkinje cell - normal cell junction.
X 10,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Plate 90.

A photo-montage of the main part of the SA node showing true nodal cells.
X 3,600.

Atrium of a 3½ month old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 91.

Cells of the SA node with nerves in the extracellular space.

X 12,500.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 92.

A typical SA nodal fibre composed of four cells.

X 17,250.

Atrium of a $3\frac{1}{2}$ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 93.

This figure demonstrates the three types of cells in the SA node. Cell 1- nodal cell; Cell 2- normal cell; Cell 3- Purkinje cell.

X 16,500.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 94.

A purkinje cell forming a fibre with two normal cells at the edge of the SA node.

X 13,500.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 95a & 95b.

Typical membrane specialisations present between nodal cells. MIP - myofibrillar insertion plaque; N - nexus.

(a) X 69,000.

(b) X 80,000.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 96a & 96b.

Typical membrane specialisations present between nodal cells.

N = nexus.

(a) X 32,000.
(b) X 34,000.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
A cell from the SA node possessing characteristics common to normal cells, Purkinje cells and smooth muscle cells. A few cells like this have been seen in the SA node but on only one occasion was evidence of Z discs found. The cytoplasm is filled with longitudinally orientated filaments. The cells bear a greater resemblance to Purkinje cells than to any other type.

X 23,000.

Right atrium of a $3^{1/2}$ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 97.

A photo-montage of the main part of the AV node.

X 2,200.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 98.

Typical nodal fibres containing nodal cells, Purkinje cells and a few intermediate cells.

X 5,500.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 99.

Main part of the AV node.

X 6,900.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate I00a & I00b.

(a) A fibre composed of two cells which possess normal characteristics and one which appears to be a Purkinje cell.
X 10,000.

(b) A fibre composed of four cells which range in structure from 'almost' normal to 'almost' Purkinje.
X 9,500.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate IOIa & IOIb.

Purkinje cells of the AV node. These cells are narrower than periarterial Purkinje cells.

(a) X 6,200.

(b) X 6,200.

Atrium of a 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate IO2 a,b,c, & d.

Nexal regions found between cells composing the AV node.

N - nexus.

(a) X 25,500.

(b) X 20,250.

(c) X 17,550.

(d) X 32,200.

Atrium of a 3.5 months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 103 (a).

A photo-montage of the atrioventricular bundle close to its entry into the interventricular septum. The bundle is composed of typical Purkinje cells.

X 1,900.

Ventricle of a $3^{1/2}$ month old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
Plate 103 (b).

Purkinje cells forming the interatrial bundle.
X 7,500.

Atrium of 3½ months old hen. Formaldehyde fixation. Stained in uranyl acetate and lead citrate.
The irregular shape and arrangement of the muscle cells constituting the peculiar tissue below the aortic valve cusps is demonstrated.

x 15,000.

Peculiar tissue from the base of the aortic valve cusps of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in lead citrate only.
The peculiar cells of this area contain mitochondria which are larger than Purkinje cell mitochondria. As in Purkinje cells, the sarcoplasm of these cells contains filaments 10nm thick. x 15,000.

Tissue from the base of the aortic valve cusps of a 4 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in lead citrate.
Typical cells resembling the nodal cells of mammals are demonstrated. Many pinocytotic vesicles (FV) are present close to the sarcolemma. The sarcoplasm is filled with filaments 10nm thick. A few myofibrils are present (▲).

x 18,750.

Tissue from the base of the aortic valve cusps of a 2 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium in Millonig's buffer. Stained in lead citrate.
The cells composing the peculiar tissue at the bases of the aortic and pulmonary artery valve cusps frequently have narrow processes as is shown in this plate. The mitochondria (Mt) have few cristae.

x 20,000.

Tissue from the base of the pulmonary artery valve cusps of a 2 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in lead citrate.
Fibrocytes from the base of the aortic valve cusps. The cells contain fine filaments and lipid droplets.

x 72,000.

Tissue from the base of the aortic valve cusps of a 4 months old cock. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in lead citrate.
A large nerve bundle is shown passing close by a Purkinje cell. The nerve bundle contains both myelinated (My) and unmyelinated (U) axons.

x 19,000.

Right ventricle of a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Nerve axons within Schwann cells are demonstrated in the extracellular space between two ventricular fibres. An unusually long mitochondrion is demonstrated in the left-hand cell.

x 25,000.

Right ventricle from a 12 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
A Schwann cell enveloping two nerve axons is shown in the extracellular space between two ventricular muscle fibres.

x 45,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium in cacodylate buffer. Stained in uranyl acetate and lead citrate.
Plate 112

A Schwann cell surrounding many vesiculated nerve axons is shown close to a normal ventricular cell.

x 42,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
A nerve ending containing many vesicles is demonstrated at the Z band level of an atrial muscle cell.

x 12,000.

Atrial tissue from a 6 months old hen. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
A naked nerve axon is present (↑) close to a Purkinje cell. x 40,500.

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in uranyl acetate and lead citrate.
A Schwann cell with nerve axons is shown close to a Purkinje cell.

x 54,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer. Stained in uranyl acetate and lead citrate.
The intercalated discs are completely separated at myofibrillar insertion plaques, but no separation has occurred at lateral apposed membranes.

x 9,000.

Right ventricle of a 2 months old hen. Perfused for 5 min. with avian ringer followed by perfusion with CMF-EDTA for 30 min. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
The intercalated disc is completely separated at the myofibrillar insertion plaque.

x 49,000.

Right ventricle of a 2 months old hen. Perfused for 5 min. with CMF, followed by perfusion with CMF-EDTA for 30 min. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Postfixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
The myofibrillar insertion plaques at this transverse portion of an intercalated disc have separated entirely. There is no separation of the longitudinal apposed membranes.

x 30,000.

Right ventricle of a 2 months old cock. Perfused for 5 min. with avian ringer followed by perfusion with CMF-EDTA for 30 min. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Post-fixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Myofibrillar insertion plaques are widely separated. Separation has not occurred at lateral apposed membranes. 

x 25,000.

Right ventricle of a 2 months old cock. Perfused for 5 min. with avian ringer followed by perfusion for 30 min. with CMF-EDTA. Fixed by perfusion with 4% formaldehyde in Millonig's buffer. Post-fixed in 1% osmium tetroxide in Millonig's buffer. Stained in uranyl acetate and lead citrate.
Typical cells from the ventricles of the fowl are demonstrated. The cells are elongated with irregular margins, frequently with large branches.

x 500.

Right ventricle of a 2 months old cock. Perfused for 2 hr. with CMF-EDTA. Fixed in 4% formaldehyde in phosphate buffer. Stained with haematoxylin and eosin.
A heavy deposit of lanthanum is present on basement membranes, surrounding collagen fibrils and filling the extracellular space between the narrow ventricular myocardial cells. Pinocytotic vesicles are also filled with deposit but vesicles in the centre of the cells do not contain deposit.

x 9,600.

Right ventricle of a 6 months old hen. Perfused with avian ringer for 5 min. then fixed by perfusion with 1% osmium tetroxide in cacodylate buffer, with 1% lanthanum nitrate added. Unstained.
Lanthanum precipitate is present on all basement membranes. x 12,500.

Right ventricle of a 2 months old hen. Fixed by perfusion with 0.8% glutaraldehyde and 3% formaldehyde in cacodylate buffer. Post-fixed in 1% osmium tetroxide in cacodylate with 1% lanthanum nitrate added. Unstained.
Lanthanum precipitate is present on basement membranes and in the extracellular space. 

x 22,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 0.8% glutaraldehyde and 3% formaldehyde in cacodylate buffer. Postfixed in 1% osmium tetroxide in cacodylate buffer with 1% lanthanum nitrate added.
Lanthanum precipitate is present on basement membranes (BM) surrounding collagen fibrils (C), endothelial cells, and in the capillary lumen (CAP).

x 12,500.

Right ventricle of a 6 months old hen. Fixed by perfusion with 0.8% glutaraldehyde and 3% formaldehyde in cacodylate buffer. Postfixed in 1% osmium in cacodylate buffer with 1% lanthanum nitrate added. Unstained.
Lanthanum deposit is present filling the extracellular space between the cells, bound to the basement membrane, and surrounding collagen fibrils.

x 30,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 0.8% glutaraldehyde and 3% formaldehyde in cacodylate buffer, with 1% lanthanum nitrate added. Postfixed with 1% osmium tetroxide in cacodylate buffer with 1% lanthanum nitrate added. Unstained.
Lanthanum deposit is present entirely filling the intercellular space.

x 39,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 0.8% glutaraldehyde and 3% formaldehyde in cacodylate buffer. Post-fixed in 1% osmium tetroxide in cacodylate buffer with 1% lanthanum nitrate added. Unstained.
Lanthanum precipitate is present in the extracellular space and filling pinocytotic vesicles. There are no vesicles close to the sarcolemma without lanthanum.

x 30,000.

Right ventricle of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in Millonig's buffer after perfusion with control solution with 1% lanthanum nitrate added. Unstained.
Plate 128

Lanthanum precipitate is present on the basement membranes of the two Purkinje cells and surrounding collagen fibrils. x 12,500.

Interventricular septum of a 6 months old hen. Fixed by perfusion with 1% osmium tetroxide in cacodylate buffer with 1% lanthanum nitrate added. Unstained.
RSVC - Right superior vena cava.

IVC - Inferior vena cava.

LSVC - Left superior vena cava.

MPV - Musculus pectinatus valvularis.

RVV - Right venous valve.

1. The position of the SA node in the black swan (Davies 1930).

2. The position of the SA node in the fowl (this study), the SA node in the rat (Muir 1955), and the SA node in the sheep (Copenhaver and Truex 1952).

3. The position of the SA node in the duck and pigeon (Gossrau 1969).
The ultrastructure of ordinary and Purkinje cells of the fowl heart

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INTRODUCTION

The ultrastructure of ordinary mammalian (Muir, 1965; Fawcett & McNutt, 1969; McNutt & Fawcett, 1969), ordinary amphibian (Staley & Benson, 1968; Baldwin, 1970) and ordinary reptilian myocardium (Fawcett & Selby, 1958; Leak, 1967) has been fully described. Muir (1957), Rhodin, Del Missier & Reid (1961), Hayashi (1962) and Sommer & Johnson (1968) have described the specialized myocardium of mammals. The ordinary and specialized myocardium of birds, however, have received relatively little attention. Didio (1967) has described the ultrastructure of the myocardium of the humming bird. Hirakow (1966) and Gossrau (1968) have published histochemical and ultrastructural observations on the ordinary and specialized myocardium of birds. Certain ultrastructural aspects of avian and amphibian myocardium have been compared by Sommer & Johnson (1969).

The observations of Sommer & Johnson (1969) on the presence of nexuses and T-tubules in fowl myocardium do not agree with the earlier observations of Gossrau (1968) but they do not discuss the reasons for the differences. For this reason it was thought that a further study of the ultrastructure of ordinary and Purkinje cells of the fowl heart was necessary. The purpose of this paper is to describe in general the ultrastructure of ordinary and Purkinje cells of the fowl heart, with particular reference to the structures thought to be involved in intercellular impulse conduction and excitation contraction coupling.

MATERIALS AND METHODS

The hearts of 100 Brown Leghorns, of both sexes, aged 1–18 months, were used. After the neck was dislocated the thorax was opened and the brachiocephalic arteries were ligatured. The pericardium was opened and the heart excised by cutting the great veins, the brachiocephalic arteries cranial to the ligatures, and the descending aorta. After excision of the still beating heart, the aorta was cannulated by a 3 mm diameter cannula with a terminal flange of 5 mm. The cannula was connected to a perfusion apparatus (Muir, 1967), and the heart was briefly perfused with oxygenated avian Ringer solution (Cleugh, Gaddum, Holton & Leach, 1961) at 41 °C. This washed the cells from the coronary circulation and so ensured uniform perfusion with fixative. During perfusion the heart rate was recorded with surface electrodes. If it was between 180 and 250 beats/min the heart was removed from the apparatus, still beating, and fixed by perfusion through the aorta with 20 ml of fixative.

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The fixatives used were Millonig’s buffered osmium tetroxide, phosphate buffered formaldehyde (Glauert, 1965), Karnovsky’s fixative (Karnovsky, 1965) and 1% osmium tetroxide in avian Ringer. Blocks of 1 mm³ were cut from the atria and ventricles, and immersed in fixative for 1 h at 4 °C, except for formaldehyde fixed preparations which were transferred after 5 min to 1% buffered osmium tetroxide for 1 h. Observations with the light microscope (Scott, 1971) on the distribution of Purkinje cells in the heart of the fowl made it possible to cut out for electron microscopy blocks containing periarterial and subendocardial Purkinje fibres. The blocks were dehydrated in ethanol, embedded in Araldite, and sectioned on a Porter-Blum MT 1 ultramicrotome, using glass knives. Thick sections were stained for light microscopy in 0-5% toluidine blue and 0-5% thionin in 1% borax. Thin sections for electron microscopy were stained for 5 min in a saturated solution of uranyl acetate in 50% ethanol followed by staining for 2 min in 0-5% lead citrate in 0-5% sodium hydroxide in freshly boiled distilled water. Thin sections were examined in an AEI EM6B electron microscope.

RESULTS

Ordinary myocardium

Atrial and ventricular myocardium will be described as one since they are almost identical. Any dissimilarities will be described under the appropriate headings.

Arrangement of cells

The fibres are composed of up to six individual cells, enclosed within a sheath of basement membrane about 46 nm thick which covers the outer surface of the peripheral cells and invaginates for a short distance between the cells. Collagen fibrils attach to the basement membrane. The muscle fibres are up to 25 µm wide, while the individual cells, sectioned near their nuclei, are about 8 µm in diameter with a range of 5–12 µm.

The individual myocardial cells interdigitate like those of mammalian myocardium with intercalated discs passing in steps across the end of the cells (Fig. 1). Since transverse areas of apposed cells are usually small and separated by relatively large areas of longitudinally apposed membrane, the term intercalated disc will be used to refer to the transversely apposed membranes only.

The cell membrane

The cell membrane of the ordinary myocardial cell can be divided into the lateral and the disc membrane. The lateral membranes can be subdivided into those on the surface of the fibre, which are in contact with the basement membrane, and those within the fibre. The latter are separated from each other by gaps of 20–60 nm.

The lateral membranes of the cell are mainly typical unspecialized unit membranes. The inner leaflet of the unit membrane is 2.5 nm thick. When fixed in aldehydes with postfixation in osmium, only the dense inner leaflet of the unit membrane is

Fig. 1. A longitudinal section through an ordinary myocardial fibre. The arrows indicate intercalated discs passing in a stepwise fashion across the fibre. Mt, mitochondria. Formaldehyde fixation.
usually visible. When the whole membrane is shown, the less dense outer leaflet is separated from the dense inner leaflet by a centre-to-centre spacing of 7.5 nm.

Typical desmosomes are the main type of specialization on apposed lateral membranes. They are composed of a pair of dense cytoplasmic discs, 10 nm thick, separated from the inner leaflets of the unit membranes by gaps of 10 nm. Filaments frequently insert into these discs. A dense disc, 5 nm thick, is also present in the extracellular space midway between the apposed unit membranes, whose inner leaflets are 30 nm apart. Most desmosomes are found at the level of the Z discs (Fig. 2) but some are randomly distributed.

Cardiac adhesion plaques are also found on the lateral membranes of apposed cells. They appear as filamentous networks on the cytoplasmic side of the membrane, extending for a distance of up to 1 μm, and 30 nm thick. They are commonly found as small discs associated with the attachment of Z discs. The apposed membranes at this type of specialization are 20 nm apart, and the extracellular space is more dense than between unspecialized apposed membranes, but no intervening disc is present. The filamentous mass may be organized in circumferential bands running parallel to the cell membranes of both cells. It can appear as a row of dense spots 16 nm in diameter when the cells are cut longitudinally. The bands are spaced at 8 nm intervals (Fig. 3).

Unapposed lateral membranes usually carry no specializations apart from filamentous masses associated with the attachment of the Z disc.

The disc membrane is more specialized than the lateral membrane. Typical desmosomes frequently occur at the margins of tranverse areas of apposed membrane, but myofibrillar insertion plaques are the main specialization on the disc membrane. Each plaque consists of a dense fibrillar network, similar to the cardiac adhesion plaque, extending into the cytoplasm up to 66 nm from the inner leaflet and receiving the insertion of the thin filaments of the terminal sarcomeres. The inner leaflets are 30 nm apart and the extracellular space is relatively dense.

Since the unit membrane appears to be 7.5 nm thick, apposed membranes with a total thickness of less than 15 nm, measured from the centre of the inner leaflet of one unit membrane to the centre of the inner leaflet of the apposed unit membrane, are considered to be nexal membranes. Typical nexuses can only be detected in fairly thin sections. They are usually present between myofibrillar insertion plaques on the undulating transversely apposed disc membranes (Fig. 4). They appear not as bands, but as plaques of irregular shape and size, not exceeding 0.25 μm in width.

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Fig. 2. A longitudinal section through ordinary myocardium showing two apposed cells with a narrow lead of intercellular space between them. Two typical desmosomes (D) are present. Osmium fixation.

Fig. 3. A longitudinal section through an ordinary myocardial fibre showing typical lateral membrane specializations. D, desmosome; CAP, cardiac adhesion plaque; SR, sarcoplasmic reticulum. Osmium fixation.

Fig. 4. Two nexuses (N) are shown on undulating apposed disc membranes of ordinary myocardial cells. Formaldehyde fixation.
Transverse tubular system

The sarcolemma shows no invaginations typical of a transverse (T) tubular system although it is frequently irregular and slightly indented at the Z disc.

Sarcoplasmic reticulum

The sarcoplasmic reticulum is a tubular network which lies below the sarcolemma and passes between the myofibrils (Figs. 5, 6). The tubules, which have walls of unit membrane, vary in diameter from 30 to 80 nm. They contain no stainable material except where the wall is specialized. The network is not specialized over any particular myofibrillar band nor is it clearly associated with any organelle except the sarcolemma.

The sarcoplasmic reticulum has a special morphology when associated with the sarcolemma forming dyads. The tubule approaches the sarcolemma and then runs parallel to it with an intervening cytoplasmic gap of 16 nm (Fig. 7). The lumen of the tubule at this point is enlarged and occupied by a dense material. On its cytoplasmic side the tubule membrane forms a periodic density with a spacing of 25 nm. This density appears to extend from the sarcoplasmic reticulum membrane to the sarcolemma and round the whole circumference of the tubule at this point.

More terminal cisternae of the sarcoplasmic reticulum are applied to laterally apposed membranes with intervening intercellular space than to the unapposed lateral membranes in contact with basement membrane. No morphological difference could be detected between the terminal cisternae at these two sites.

Myofilaments

These are similar to those found in skeletal and cardiac muscle in other vertebrates, and are bundled together into myofibrils. The myofibrils branch and anastomose, showing a typical 'Felderstructur' (Kruger, Duspiva & Furlinger, 1933). Transecting the myofibrils at regular intervals are Z discs, 105 nm thick, composed of dense filamentous material. The I band, containing thin filaments, 6 nm in diameter, extends on either side of the Z disc. The A bands have typical thick filaments 12 nm in diameter. The H zone, about 130 nm wide, is present in relaxed muscle, and the M line is indistinct.

The Z discs of peripheral myofibrils appear to be attached to the sarcolemma, since an indentation of the sarcolemma is produced at that point when the myofibrils contract. As described above, filamentous material is frequently present where the Z disc meets the membrane. Z discs and other myofibrillar bands are in register in all myofibrils in the same cell and with myofibrils in the constituent cells of the same fibre, due to intercellular junctions occurring at the level of the Z disc.

Fig. 5. The sarcoplasmic reticulum (SR) is shown on face view. Z, Z disc. Osmium fixation.
Fig. 6. Tubules of the sarcoplasmic reticulum are shown (arrows) in cross-section passing between the myofibrils in an ordinary myocardial cell. Mt, mitochondria. Formaldehyde fixation.
Fig. 7. A typical terminal cisterna of the sarcoplasmic reticulum (SR) is shown connected to the sarcolemma by a periodic specialization (arrow). Formaldehyde fixation.
Leptomeres

A subsidiary myofibrillar structure is often present in apparently normal myocardial cells. In longitudinal section it comprises a series of dense lines, each 25 nm thick and 200 nm long. They lie 150 nm apart, and there are fine filaments running between adjacent dense lines. These leptomeres are found most frequently at the cell surface and tend to follow the direction of the adjacent myofibrils (Fig. 11) although they are also orientated in other planes. The dense lines of the leptomeres are frequently seen to be confluent with Z discs.

Nucleus and juxtanuclear organelles

A single centrally situated nucleus, about 14 μm long and 4 μm in diameter, is present in each cell. It has a granular nucleoplasm, with aggregations of densely staining chromatin. The nuclear envelope is often indented and nuclear pores are seen. At the poles of the nucleus a typical Golgi apparatus is usually present, as well as rough endoplasmic reticulum containing ribosomes. Dense granules are usually present lying close to the Golgi complex and apparently showing several stages of development. They have an inner dense core, up to 130 nm in diameter, with an outer clear area bounded by a membrane. These granules are more common in atrial than in ventricular myocardium.

Mitochondria

Many mitochondria of various sizes occur between the myofibrils. Most have regular outlines and are about 2 μm in length (Fig. 6); some are long and narrow, extending for up to three or four sarcomeres, while others, usually near the nucleus, are small and rounded.

Glycogen, lipid and lipofuscin

Many single glycogen granules, 25 nm in diameter, are present throughout the sarcoplasm, varying in amount from cell to cell in the same section.

Lipid droplets, 1-5 μm in diameter, are present just below the sarcolemma and also deep in the cell, frequently associated with mitochondria. They are rounded bodies enclosed within an indistinct membrane.

Dense bodies of lipofuscin, of irregular shape and size, are present in the sarcoplasm. They are composed of dense granules of various sizes enclosed within a membrane.
Vesicles

The sarcolemma on the apposed and unapposed lateral aspects shows pinocytosis. Many vesicles can be seen immediately inside the membrane and several are attached to it. A few vesicles are also present on the disc membrane. Most of the vesicles are uncoated.

Periarterial and subendocardial Purkinje cells

The Purkinje cells following the blood vessels are identical to those underlying the endocardium. Both are typical, having a diameter about twice that of ordinary myocardial cells and containing little organized contractile material.

Arrangement of the cells

Each fibre accompanying a blood vessel contains up to six cells, whereas the subendocardial fibres are formed from fewer cells. Each fibre has a coat of basement membrane 46 nm thick following the contours of the cells and invaginating between them. A layer of collagen fibrils attaches to the outer surface of the basement membrane, and outside the collagen is a single layer of fibrocytes.

The Purkinje cells, which are up to 24 \( \mu \text{m} \) wide, are elongated, and have flattened ends. They frequently branch and anastomose. They differ from ordinary myocardial cells in being more variable in shape, with deep clefts and branches, and in having intercalated discs which are not step-like.

Purkinje cell membrane

The Purkinje cell has a greater surface area than ordinary myocardial cells, but its membrane is far less specialized. The membrane can be divided into the lateral membrane along the length of the cell and the terminal membrane at the flattened ends of the cell.

The sarcolemma is a typical unit membrane. Myofibrillar insertion plaques form the main type of specialisation on lateral membranes (Fig. 8), and resemble the myofibrillar insertion plaques of ordinary myocardial cells. They are occasionally present on the outer lateral membranes of cells on the periphery of bundles without there being an apposing membrane. Desmosomes are uncommon on the lateral membranes, but their structure is typical. Relatively many small pentalaminar nexuses, about 14 nm thick and 1 \( \mu \text{m} \) long, occur at irregular intervals on the lateral membranes of Purkinje cells (Fig. 9).

Typical myofibrillar insertion plaques, up to 4 \( \mu \text{m} \) long, are the main specialization on terminal membranes. Typical desmosomes are also present, but are rare. Many relatively large pentalaminar nexuses are present on the terminal membranes (Fig. 10) extending 4 \( \mu \text{m} \) in length.

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Fig. 11. A leptomere is shown running parallel to a myofibril in an ordinary myocardial cell. Osmium fixation.

Fig. 12. A leptomere is shown continuing on from a myofibril in a Purkinje cell. Formaldehyde fixation.

Fig. 13. A Purkinje cell leptomere is shown. The dense line of the terminal leptosarcomere appears to be confluent with the Z disc of a myofibril. Osmium fixation.
Tubular system

No T system could be demonstrated.

Sarcoplasmic reticulum

This seems to be more labile than in ordinary cells. Although a network of tubules, it frequently presents the appearance of rows of vesicles. It is not so extensive in Purkinje cells as in ordinary myocardium, being confined to the periphery of the cell. A few typical terminal cisternae were observed.

Filaments

Unlike the regular array of myofibrils in ordinary myocardium, the contractile material in Purkinje cells appears to be arranged at random. Myofibrils similar in diameter and structure to those in ordinary myocardium are attached over the entire inner surface of the cell membrane. However, these myofibrils are of irregular length, appear to be attached at only one end, and are not orientated in any particular direction. Smaller myofibrils of one or two sarcomeres are present throughout the cytoplasm and do not appear to be organised into a contractile system.

Together with myofibrils composed of normal thick and thin filaments, there is a mass of randomly orientated filaments of intermediate diameter which appears to fill the large sack-like cells.

Leptomeres

Similar to those in ordinary myocardial cells, leptomeres are found lying along the membranes, frequently in ridges raised from the surface of the cells: they appear to be continuous with normal myofibrils (Figs. 12, 13). Leptomeres are also found in the centre of the cell, where many are gathered together in the shape of a ball with mitochondria in the centre. The ball is not formed from any consistent arrangement of leptomeres.

Nucleus and juxtanuclear organelles

Each cell contains one or two rounded nuclei about 8 μm in diameter. Apparently their shape is not influenced by adjacent myofibrils, as in ordinary myocardium, and they are frequently invaginated, with irregular outlines. The nucleus is filled with dense granular nucleoplasm and contains dense chromatin material. The extensive Golgi complex is not confined to the poles of the nucleus, but is otherwise typical: associated with it are elements of rough endoplasmic reticulum. There are no large membrane-bound granules.

Mitochondria

The mitochondria in Purkinje cells are smaller, more rounded and more uniform in shape than those of ordinary myocardium. They also differ in being distributed throughout the cytoplasm.
Glycogen, lipid and lipofuscin

A few glycogen granules are present but in appreciably lower concentrations than in ordinary myocardium. Lipid droplets are absent, but dense lipofuscin bodies are present.

DISCUSSION

The observations of Didio (1967), Hirakow (1966), Gossrau (1968) and Sommer & Johnson (1969) on the general morphology of the ordinary and Purkinje cells of the fowl heart have been confirmed. The ordinary cardiac cells of the fowl are narrower than those of mammals, have a similar myofibrillar arrangement and show step-like intercalated discs. The morphology of the intercalated discs and the T tubular system will be discussed separately since the findings of the present investigation do not agree with those of other authors. The Purkinje cells of the fowl are similar in almost all aspects to those of mammals. They do not have step-like intercalated discs but have irregularly arranged areas of membrane apposition which possess desmosomes, myofibrillar insertion plaques and large pentalaminar nexus. No transverse tubular system is present and the sarcoplasmic reticulum is poorly developed. The few myofibrils are irregularly arranged.

The Purkinje cells have been observed in this study to contain rounded aggregations of leptomeres. Leptomeres have previously been described by Hirakow (1966) who suggested that they represented an aberrant form of muscle fibril arising during development. This is unlikely since they are present in almost all Purkinje cells. It seems reasonable to assume that they are associated with myofibrillar formation or destruction since they are seen in association with large masses of disorganized filaments and also in continuity with apparently normal myofibrils.

The Nexus

Contrary to the findings of Sommer & Johnson (1969) and in agreement with the observations of Gossrau (1968), nexuses were observed in this study as components of the intercalated discs of ordinary cardiac cells of the fowl. Although only a single nexus was seen by Sommer & Johnson (1969), this may have been due partly to the complexity of the apposed membrane at the disc. In most thin sections the intercalated disc membranes are difficult to follow because of the density of myofibrillar insertion plaques. Thus it is possible that the small nexuses which have been observed are usually hidden and can only be seen in the thinner sections and when the plane of section is appropriate for their demonstration.

The nexus has been suggested as the site of electrotonic conduction (Dewey & Barr, 1962, 1964), although there is not complete agreement as to whether a low resistance pathway exists (Sperelakis, 1963). Theoretical considerations of the function of the nexus must take into account whether the outer lamellae of the nexus are actually fused or separated by a small gap. The morphology of the nexus has recently been investigated by McNutt & Weinstein (1970), who conclude that since neither of the outer surfaces of the apposed membranes can be demonstrated by freeze-etching, there is no gap between the two membranes. This is not substantiated by their own evidence of lanthanum penetration. The apposed surfaces at the nexus
cannot have been shown to be separated at regular intervals by a 1-8 nm gap. Since this gap cannot be demonstrated by the freeze etch techniques then the remaining nexal membrane cannot be said to be fused using the same technique.

If the nexus is a gap junction, then conduction will occur more readily between wide cells than between narrow cells (Katz, 1966). It might therefore be expected that larger nexuses would be present between narrow cells than between wide cells. This is not the case, however, since the nexuses between the narrow ordinary cells of the fowl heart, observed on the same section as Purkinje cells, are much smaller and occur less frequently than those of the larger Purkinje cells. Therefore either the nexus is not involved in electrotonic impulse conduction or the nexuses present in ordinary fowl myocardium are sufficient for impulse conduction even in narrow cells, leaving the extra area of nexus in ordinary mammalian myocardium and in the Purkinje cells of mammals and birds to be explained.

If the membranes at the nexus are in fact fused, with hydrophilic channels connecting the apposed cells, as is suggested by McNutt & Weinstein (1970), then a useful comparison of nexal area in birds and mammals cannot be made, since there is no information on the size and distribution of the hydrophilic channels.

T tubular system

Gossrau (1968) described a transverse tubular system in the ordinary cardiac muscle of birds, interrupting the rows of mitochondria pressed between the myofibrils. Sommer & Johnson (1969) failed to find a transverse tubular system, and their observations are confirmed by the present study. Although Gossrau described T tubules as being present, no illustration demonstrating their presence, or the presence of triads, was given. It is possible that the T tubules he observed were transverse elements of the sarcoplasmic reticulum. The basic morphological features of a transverse tubular system are that the tubules are continuous with the extracellular space and that they have a special relationship with the sarcoplasmic reticulum. No invaginations or triads typical of a transverse tubular system were observed in ordinary or Purkinje cells of the fowl heart in the present study or in the earlier study of Sommer & Johnson (1969).

It seems possible from the structure of the ordinary muscle fibre of the fowl heart that the narrow intercellular space is similar to the T tubular system in mammals in that the membranes lining it have specialised relationships with the sarcoplasmic reticulum and that it is a space which is relatively closed off from true extracellular space.

SUMMARY

1. Ordinary and Purkinje cells of the heart of the fowl have been examined.
2. Ordinary myocardial fibres and Purkinje fibres are composed of up to six cells. The ordinary cell is about 8 µm in diameter while the Purkinje cell averages 16 µm in width.
3. Ordinary myocardial cells possess small nexuses while Purkinje cells have many large ones.
4. No T tubular system is present in either ordinary or Purkinje cells. The sarco-
plasmic reticulum is sparse in Purkinje cells and extensive in ordinary cells, in which it makes many specialized contacts with the sarcolemma.
5. Leptomeres are present in both ordinary and Purkinje cells.
6. The factors thought to be involved in intercellular impulse conduction and excitation contraction coupling are discussed.

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REFERENCES


