Fine structure of the bundle-branches¹

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Fine structure of the normal left and right bundle-branches was studied with the electron microscope in one human and two canine hearts and correlated with light microscopical observations from 10 human and 10 canine hearts. There was no significant species difference. Though the left bundle-branch was comprised of typical Purkinje cells, there were numerous interspersed cells with the appearance of ordinary working myocardium. Geometric arrangement of the cells in the proximal portion of the left bundle-branch was in one thin sheet divided by numerous longitudinally oriented collagen partitions, resembling a direct continuation of the histological geometry of the His bundle. Intercellular junctions within the left bundle-branch were through intercalated discs which contained many long profiles of gap junction, in contrast with working myocardium, where gap junctions are much smaller. The right bundle-branch was a thin cylinder rather than a sheet and did not contain any apparent partitioning pattern. Typical Purkinje cells are comparatively few in the right bundle-branch and cells with numerous myofibrils (appearing as working myocardium in light micrographs) predominated. Junctions between these cells contained fewer gap junctions than those in the left bundle and very long profiles were less numerous. Widened Z bands were conspicuous in the right bundle-branch cells of one dog. Some of the physiological implications of these fine structural features are discussed relative to electrical properties of the two bundle-branches.

For optimal understanding of any biological activity it is essential to appreciate and correlate structural appearance with functional behaviour. Electrically specialized cells of the heart are a special challenge for such correlation. It would be ideal to know the complete fine structure of one cell for which all electrical properties had been accurately defined, but there are formidable problems which have so far prevented anyone satisfactorily attaining that goal. As a currently suitable compromise, electron microscopical studies have been made of cells taken from specific regions of the heart with general knowledge of the electrophysiological behaviour of those regions. Examples include the sinus node (Kawamura, 1961b; James *et al.*, 1966; Kawamura and James, 1971), AV (atrioventricular) node (Kawamura, 1961b; James and Sherf, 1968; Kawamura and James, 1971), His bundle (James and Sherf, 1971), and Purkinje cells of false tendon in the ventricle (Kawamura, 1961a; Kawamura and James, 1971).

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This study was done to examine the fine structure of normal cells in the left and right bundle-branches and to compare their appearance in the human and canine heart. Goals included an assessment of the relative structural homogeneity or heterogeneity of individual cells in the two bundle-branches, the nature of their intercellular junctions, and their histological geometry. Since electron microscopy does not readily permit suitable assessment of large samples of tissue, for studies of fine structure we concentrated our attention on two regions where one could with confidence obtain cells representative of the left and right bundle-branches: the proximal few millimetres of the left bundle-branch just beneath the membranous septum, and the moderator band of the right ventricle. These observations were supplemented with certain light microscopical examinations.

Subjects and methods

Tissue for electron microscopy was obtained from two normal dog hearts and one normal human heart. That from the dogs was excised and immediately placed in cold phosphate-buffered 6.5 per cent glutaraldehyde within less than two minutes after the hearts were

removed during general anaesthesia with sodium pentobarbitone (30 mg/kg intravenously). That from the normal human heart was excised at necropsy about 3 hours after death from a gunshot wound of the abdomen. Changes caused by this time interval after death are recognized and have been the subject of specific previous electron microscopical studies (Hibbs and Black, 1963; James *et al.*, 1966).

The general region of the left bundle-branch is readily identifiable with gross dissection, and removal of small tissue samples from the mid-portion just beneath the membranous septum in the left ventricle is feasible, though in our experience the precise definition of the anterior and posterior margins of the left bundle-branch is not possible with gross dissection. To assure that the sample for electron microscopy actually represented the left bundle-branch, the area from which the sample was removed was embedded *en bloc* and histological sections were prepared for light microscopical examination. In both canine and human hearts the right bundle-branch often lies deep within the ventricular septum for much of its course after it leaves the His bundle, emerging only as it courses into the moderator band (Truex and Copenhagen, 1947). For this reason, samples of right bundle-branch were obtained from that portion of the moderator band facing the direction of the His bundle, since much of the remainder of the moderator band is comprised of ordinary working myocardium.

From each sample site approximately 20 small cubes no more than 1 mm in maximal dimension were fixed with glutaraldehyde and osmium, embedded in Araldite, sectioned on a Porter-Blum ultratome, and examined with a Philips 300 electron microscope. Details of these methods have been published (James *et al.*, 1966; James and Sherf, 1968; James and Sherf, 1971; Kawamura and James, 1971).

For comparison with the electron microscopical findings, for orientation into intercellular geometry, and for the question of comparative cellular homogeneity (or its lack), light microscopical examinations of human and canine left and right bundle-branches were made in 10 canine and 10 human normal hearts. The tissue was fixed with 10 per cent neutral formalin, embedded in paraffin, and sectioned at 6 to 8 μ intervals. Goldner trichrome stain was used for cell detail, and a simple Van Gieson stain was employed to demonstrate the collagen framework of the region, as an indicator of cell arrangement (James and Sherf, 1971).

Results

Comparison of human and canine bundle-branch cells

Except for changes attributable to the fact that the human tissue samples for electron microscopy were obtained three hours after death while those from

FIG. 1 This light photomicrograph is from the midportion of human left bundle-branch and illustrates direct continuity of a working myocardial cell (dark arrow) with a Purkinje cell (white arrow); (Goldner trichrome stain. $\times 585$). See Fig. 8 and 12 for comparative features demonstrated in electron micrographs.

the dogs were obtained within a few minutes, there were no apparent differences between cells of the human and canine bundle-branches. Changes previously identified as beginning within several minutes after death include loss of intracellular glycogen, clumping of nuclear chromatin, variable dilatation of components of the sarcoplasmic reticulum, and variable swelling and distortion of the mitochondria (Hibbs and Black, 1963; James *et al.*, 1966). Some mitochondria appeared to be well preserved in the

human tissue in the present study; however, this structural integrity varied not only from cell to cell but even within individual cells. Features that did not seem to be significantly altered in the human tissue included the appearance of external cell membranes (both at intercellular contacts and toward the extracellular space), the intracellular distribution of virtually all organelles (including mitochondria), and the distribution and appearance of myofibrils.

FIG. 2 In this electron micrograph a cross-section of intercellular junction within the human left bundle-branch is shown. The three dark arrows indicate gap junctions (formerly called nexus formations or close junctions). Most of the other areas of darkened membranes in the junction represent desmosomes. ($\times 24508$.) The area enclosed is magnified in Fig. 3.

Left bundle-branch

Cell types Although the principal cell seen was one usually considered a Purkinje cell, there were numerous cells indistinguishable from ordinary ventricular working myocardium. This was apparent both at the light microscopical and ultrastructural level. Two areas where cells with the appearance of working myocardium become increasingly prevalent, as would be expected, are at any point where the left bundle-branch penetrates the septum and

where the left bundle-branch is a relatively greater distance from the His bundle. Though there is a gradual increase in intracellular content of myofibrils with increasing distance from the His bundle, in many locations a Purkinje cell joined directly to a working myocardial cell (Fig. 1). In addition to Purkinje cells and working myocardial cells, there was a third group of cells intermediate between these two types and exhibiting various features characteristic of either; these may be considered

FIG. 3 This excerpt from Fig. 2 illustrates the prevalence of gap junctions (white arrows) present within a cross-section of intercalated disc between two human left bundle-branch cells. The gap junctions vary in length. The narrow black arrows indicate desmosomes. ($\times 67430$.)

transitional cells (James *et al.*, 1966; James and Sherf, 1968, 1971).

In this study the following definition of a Purkinje cell is applied. It is both shorter and broader than a working cell, measuring 20 to 40 μ in width and 20 to 60 μ in its long axis. Larger Purkinje cells are found in artiodactyla than in man or in the dog (Kawamura and James, 1971). Two other distinguishing features of Purkinje cells are the paucity of their myofibrils and the comparative simplicity of their intercalated discs. The scanty myofibrils, which contain only sparse myofilaments, are generally disposed about the periphery of the cell, leaving the perinuclear region rather empty in

appearance (obvious even with light microscopy). As there were only a few wispy myofibrils, there are still other distinguishing features of Purkinje cells: their intercalated discs contain few fasciae adherentes, and the external membranes do not have the scalloped appearance typical of working myocardium. Infrequency of fasciae adherentes in the intercalated discs of Purkinje cells fits with the small number and size of myofibrils, since the myofilaments insert into the disc through the fasciae adherentes. Scalloping of the sarcolemma normally occurs in registry with the Z band of sarcomeres, the point at which transverse tubules enter the cell; consequently, with a sparsity of myofibrils in

FIG. 4 The gently weaving appearance of a typical intercalated disc between Purkinje cells is seen in this electron micrograph of canine left bundle-branch. Note the exceptionally long gap junction indicated by the three small black arrows. Gap junctions of this length were seen only in the intercalated discs of Purkinje cells. ($\times 30344$.)

Purkinje cells and minimal external scalloping, there are only rare profiles of transverse tubules to be found.

Other important features of the intercalated disc of Purkinje cells include a larger number and size of gap junctions than are seen in working myocardium, and a simpler or less interdigitated profile on cross-section than occurs in working myocardium (Fig. 2-4) (Kawamura and James, 1971). Since the gap junctions are generally considered to be the sites of lowest intercellular electrical resistance, their multiplicity and large size fit with the general concept of more rapid conduction in Purkinje cells. When the intercalated disc is viewed across the entire Purkinje

cell cut in its long axis, it is seen to weave gently from one external margin of the cell to the other, often in an oblique direction (Fig. 5). By contrast, the intercalated disc of a working myocardial cell crosses almost perpendicular to the long axis of the cell, though often making steps from the level of one Z band to that of more proximal or distal sarcomeres. This difference in appearance of intercalated discs is attributable at least in part to the pronounced difference in myofibril content of the two types of cells, and the appearance of the discs of working cells is more in keeping with the maximal intercellular adhesive strength necessary for their primary contractile function.

FIG. 5 Here the entire intercalated disc is shown between two Purkinje cells within human left bundle-branch. Lateral margins of one Purkinje cell are indicated with the two white arrows, and three points on the intercalated disc are indicated with the black arrows. ($\times 6536$.)

Working myocardial cells are characteristically packed with longitudinally arrayed myofibrils which have numerous mitochondria sandwiched between them (Fig. 6). Myofibrils in Purkinje cells are not only sparse and contain few myofilaments, but they sometimes course in various directions within the

cell. While some of this appearance, as well as some other features such as the scalloping of sarcolemma, is attributable to the contractile state of the cell at the time of fixation, the random orientation of Purkinje myofibrils is too prevalent to be exclusively an artefact of preservation or preparation.

FIG. 6 The characteristic orderly array of myofibrils with mitochondria sandwiched between them is illustrated in this electron micrograph of septal working myocardium adjacent to the canine left bundle-branch. The white letter E is on one erythrocyte within a capillary. ($\times 14450$)

Intercellular junctions Intercalated discs of working myocardial cells and Purkinje cells differ as discussed above. The only cells in which relatively long profiles of gap junction were observed were Purkinje cells, but not all sections of their intercalated discs contained these; in some sections of Purkinje cells the discs contained no larger a number or longer profile of gap junctions than are seen in working myocardium. Quantifying the portion of intercellular interface which is occupied by gap junction requires three-dimensional reconstruction which was not attempted in this study. In general terms, a much larger portion of the discs of Purkinje cells is comprised of gap junctions than is true for working myocardium, and the only *long* profiles of such junctions were seen in Purkinje cells. This was equally true whether the cells were observed in cross-section or longitudinal section.

FIG. 7 Longitudinal partitioning of the proximal human left bundle-branch is demonstrated with a van Gieson stain, in which the red collagen photographs as black. The arrow indicates the general direction of the intracardiac course of this left bundle-branch. ($\times 76$.)

Both working myocardium and Purkinje cells have junctions in a variety of directions, not only end-to-end. However, the number and extent of lateral junctions and of multicellular junctions were greater for Purkinje cells than for working myocardium. In the left bundle-branch most Purkinje cells have multiple lateral as well as end-to-end connexions, sometimes making the long axis of the cell an arbitrary assessment, particularly since these cells normally tend to be short and broad.

Histological geometry In the region of the left bundle-branch sampled near the His bundle, the cellular arrangement resembles that of the His bundle itself in being longitudinally partitioned by collagen (Fig. 7). This pattern persists for at least the first centimetre of the left bundle-branch. Just as in the His bundle, however, the partitioning is not one of complete separation, periodic crossovers being seen between strands. Furthermore, for each strand there are rows of two or more Purkinje cells which interconnect freely within the strand.

Except for there being more working cells as one approached the more distal distribution of the left bundle-branch, there was no apparent pattern for the location or distribution of the working myocardial cells seen in the left bundle-branch. They simply were interposed at what seem to be random locations.

Right bundle-branch

Cell types Typical Purkinje cells are much less numerous in the right bundle-branch than in the left, and were in fact infrequently observed either in light or electron microscopical preparations. This was equally true for human and canine hearts. Working myocardial cells were seen more frequently than in the left bundle-branch, though it may be noted that the sampling site for electron microscopical study of the right bundle-branch was a greater distance from the His bundle than was the sampling site for the left bundle-branch.

There was a broader spectrum of cells in the right bundle-branch having mixed features of working myocardium and of Purkinje cells than there was in the left bundle-branch. For example, relatively short cells with more myofibrils than usual for Purkinje cells were abundant (Fig. 8). Furthermore, those cells that contained sparse myofibrils exhibited an even wider variety of orientation of myofibrillar directions than was seen in most Purkinje cells of the left branch (Fig. 9). Broad bands of Z substance were very numerous in the sarcomeres of right bundle-branch cells in one dog (Fig. 10 and 11), less numerous in the right bundle-branch of

FIG. 8 Several cells characteristic of many within the canine right bundle-branch are seen in this low power electron micrograph. Note the appearance of the intercalated disc to left of the cell labelled A, and the one between cells B and C. The latter junction is seen at higher magnification in Fig. 12. From the longitudinal orientation of the myofibrils and the cross-section of nucleus, cell B can be judged as cut near the midline of the cell, which is rather short and broad. ($\times 2500$.)

the other dog, and were occasionally seen in left bundle-branch cells of the human heart; they were not seen in cells sampled from the right bundle-branch of the human heart nor in the left bundle-branch samples of either dog.

Intercellular junctions These were similar to the ones described for cells of the left bundle-branch, except that fewer long profiles of gap junction were seen in the cells of the right bundle-branch (Fig. 12-14). Many cells of the right bundle-branch

FIG. 9 The dizzy array of myofibrils coursing in every direction within one cell of the canine right bundle-branch is shown in this electron micrograph. There are also some widened Z bands, seen better in Fig. 10 and 11. ($\times 9632$.)

contained such a wide array of orientations of their myofibrils that the true long axis of the cell was sometimes impossible to ascertain. Location of their intercalated discs was of little help, since they joined other cells in a variety of directions, just as did the Purkinje cells of the left branch.

Histological geometry Based on light microscopical appearance, neither the proximal nor distal components of the right bundle-branch contain the orderly separation into longitudinal strands which is characteristic of the proximal portion of the left branch. Instead, the right branch as soon as it

FIG. 10 This cell from canine right bundle-branch illustrates many examples of widened Z bands (appearing as darkened smudges between sarcomeres), random orientation of myofibrils, many 'empty' interfibrillar spaces, and random distribution of mitochondria. Compare this cell to the working myocardial cell shown in Fig. 6. N indicates the nucleus. ($\times 7000$.)

leaves the His bundle appears to be a slender cylinder of a thin sheet, and within the cylinder the cells connect with each other in virtually all directions. On cross-section the cylinder is ovoid or round, the shape varying slightly from one heart to another and also in any given heart from proximal

to more distal location in the septum. For most of its course within the septum the right bundle-branch is ensheathed by collagen.

Discussion

While structure is no proof of function, it is never-

FIG. 11 Many of the features shown in Fig. 10 are seen at higher magnification in this electron micrograph of canine right bundle-branch. The widened Z substance, completely random orientation of myofibrils, and the non-relation of mitochondria to myofibrils is apparent. ($\times 14450$.)

FIG. 12 *An intercalated disc between two cells in the canine right bundle-branch is indicated by the three curved arrows, each of which is pointed to a gap junction. The disc shown here is identical to the ones seen in most of the working myocardium. It may be compared to the discs shown in Fig. 13 and 14 (also from the right bundle-branch) which are characteristic of most junctions between Purkinje cells. The cell on the left has more myofibrils than the one on the right, the latter resembling a Purkinje cell. This is a magnified view of one portion of Fig. 8. ($\times 14450$.)*

FIG. 13 A portion of the gently weaving intercalated disc typical of Purkinje cells is shown here from canine right bundle-branch. Short segments of gap junction are indicated with the four black arrows. Longer gap junctions were seen more often in the left bundle-branch than in the right. ($\times 21670$.)

FIG. 14 *Desmosomes of varying length give this intercalated disc a beaded appearance. Random orientation of the sparse myofibrils, non-relation of the mitochondria to myofibrils, and paucity of gap junctions (compared to left bundle-branch) characterize these Purkinje cells from canine right bundle-branch. ($\times 14450$.)*

theless a useful guide. Based on this study of the fine structure of the bundle-branches, there are several functional implications to consider. These thoughts on function will be discussed under four headings: (1) heterogeneity of cell types in the left bundle-branch; (2) several peculiarities of cells in the right bundle-branch; (3) nature of intercellular junctions of both bundle-branches; and (4) histological geometry of the two bundle-branches.

Heterogeneity of cell types in left bundle-branch

Methods in current use to measure conduction velocity in myocardium require certain assumptions such as homogeneity of cell types, uniformity of intercellular junctions, and a consistent histological geometry within the segment of tissue to be studied. For example, if the cells in the left bundle-branch differ significantly in appearance (as shown in the present report) and if their functional properties are as different as their appearance, then measurements of these functional properties may be expected to vary according to the relative prevalence of one or the other cell type. Until it is possible to determine the exact functional properties of individual cells of the myocardium, the pronounced difference in appearance of various cells in the left bundle-branch may best be interpreted as strongly suggesting that these cells also differ in their functional properties (including conduction velocity). Proof or disproof of this interpretation will require physiological measurements different from those in general use today.

Several peculiarities of cells in right bundle-branch

Although cells of the left bundle-branch do differ in their fine structural characteristics, the great majority of them are of the type generally considered as Purkinje cells. By contrast, cells of the right bundle-branch not only differ from each other, but there are comparatively few typical Purkinje cells and a distinctly smaller percentage than in the left bundle. Furthermore, the myofibrils in many cells of the right bundle-branch present a virtual maze of contractile elements coursing in almost every direction. Widened Z bands of the sarcomeres of cells in the right bundle-branch were conspicuous in some samples; even though this feature was also present in some left bundle-branch cells, its prevalence was far greater in the samples of the right bundle-branch. Since it is not possible to make accurate interpretation of sarcomere structure with light microscopy, which would permit a much larger sample size, the prevalence of wide Z bands in our electron micrographs of right bundle-branch

cells may be attributable to a sampling bias of some unrecognized sort.

On the other hand, most features of Purkinje cells are readily identified with light microscopy, and the paucity of typical Purkinje cells in the right bundle-branch was a feature of all 11 human and 12 canine hearts. Whether this 'non-Purkinje' appearance of right bundle-branch cells may be interpreted in the same way as the apparent working myocardial cells of the left bundle-branch is uncertain, but there are two reasons to suspect that the predominant cells in the right bundle-branch have different electrophysiological properties from working myocardium. First, the abundant cells with numerous myofibrils oriented in virtually all directions were a prevalent electron microscopical feature of the right bundle-branch but not of the left; these cells probably appear as working myocardium on light microscopy, though their myofibrillar orientation is indistinct. Second, the conduction velocity of the right bundle-branch *in toto* is more rapid than one would expect from tissue having as many apparent working myocardial cells as appears to be present in the right bundle-branch based on light microscopical study alone. Thus, the numerous cells in the right bundle-branch which differ from the typical appearance of Purkinje cells both in their light and electron microscopical appearance may nevertheless be capable of conducting as rapidly as typical Purkinje cells, or at least more rapidly than working myocardium. This simply further illustrates the complex cytological features which must be considered in attempting to explain even one electrophysiological property, conduction velocity.

What the wide Z bands of many right bundle-branch cells may signify is puzzling. It has been suggested that this appearance of Z bands is indicative of sarcomerogenesis (Legato, 1970). None of the three hearts studied with electron microscopy was hypertrophied, and the right ventricles in particular were not. However, the possibility of focal hypertrophy not discernible on gross examination cannot be excluded, though if this were the case some interesting questions about growth of bundle-branches would arise.

Nature of intercellular junctions of both bundle-branches

Since no effort was made to quantify components of the intercellular junctions, results of which would in turn have limited significance because of the unavoidably small size of the samples, it is only possible to make general quantitative observations about the nature of these junctions. Most of the intercalated discs of both left and right bundle-branches were obliquely oriented and gently sweep-

ing across between adjacent cells. From their cross-sectional appearance, the intercalated discs seen in the bundle-branch cells may be estimated to have less total surface area than the complexly interdigitated discs of working myocardium. At the same time, the component comprised by gap junctions in obliquely oriented discs, which are characteristic of Purkinje cells (Kawamura and James, 1971), appears to be much larger than seen in intercalated discs of working myocardium.

In addition to the differences between intercalated discs seen in working myocardium compared to Purkinje tissue, there is a third type of myocardial cell which has still another form of intercellular junction. This is the P cell most prevalent in the sinus node (Kawamura, 1961b; James *et al.*, 1966; Kawamura and James, 1971) but also present in the AV node (Kawamura, 1961b; James and Sherf, 1968; Kawamura and James, 1971). P cells, which are thought to be the site of impulse formation, have the simplest of all myocardial intercellular junctions on electron microscopical examination, containing almost exclusively undifferentiated regions with sparse desmosomes, small and infrequent fasciae adherentes, and only rare small gap junctions. No P cells were found in the present study of the bundle-branches. Since conduction velocity in the sinus node and AV node has been reported to be the slowest in the heart (Hoffman and Cranefield, 1960), one may deduce that the undifferentiated region of intercalated discs is an area of slow electrical spread. In experimental studies causing dehiscence of desmosomes, fasciae adherentes, and undifferentiated regions but having no effect on gap junctions, there is no significant alteration of conduction velocity (Dreifuss, Girardier and Forssmann, 1966; Kawamura and Konishi, 1967; Kawamura and James, 1971). This further supports the interpretation that rapid conduction in the right and left bundle-branches is attributable to the prevalence of gap junctions in those cells.

Histological geometry of the two bundle-branches

In this study the histological geometry of the left-bundle-branch was examined only for the first few millimetres beyond its origin from the His bundle. In that region the distinct longitudinal partitioning of the His bundle simply continued directly into the proximal portion of the left bundle-branch. However, as the left branch coursed away from the His bundle, its partitioned sheet began to fan out, instead of the strands coursing parallel as in the His bundle. This thin and broad fan in the proximal segment of left bundle-branch was separated from the underlying septal myocardium by a sheet of

collagen, and the abundance of this collagen makes sectioning of left bundle-branch samples for ultrastructural studies more difficult than for tissue with less associated collagen. Neither in the His bundle nor in the proximal left bundle-branch do the collagen septa completely isolate adjacent cords of myocardial cells, since crossover connexions are present. Based on this fine structural appearance, one would expect predominantly partitioned conduction (or longitudinal dissociation) within the first portion of the left bundle-branch as well as in the His bundle itself. Several recent clinical and experimental studies support this possibility (Sherf and James, 1969, 1972; Anderson *et al.*, 1970; Myerburg, Nilsson and Gelband, 1972).

Organization of the proximal right bundle-branch is quite different. It also is sheathed from adjacent septal myocardium near the His bundle, but on cross-section the right bundle-branch at that point is round or ovoid instead of being a sheet like the left bundle. Furthermore, the partitioning by collagen so striking in the His bundle and proximal left bundle-branch is not seen in the right bundle-branch. Instead, on light microscopical examination the right bundle-branch cells do not appear to be organized into longitudinally oriented cords but to interweave rather loosely and with no clear pattern except as one multicellular tube. One may thus suspect that the left bundle-branch carries discretely partitioned signals – at least in its proximal portion – whereas the right bundle-branch acts more as a single unpartitioned electrical conduit. However, what the exact functional significance may be relative to the electrophysiological pattern of activation of the left and right ventricles, based on the considerable difference in intrinsic appearance of their respective bundle-branches, remains to be determined.

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