Fine Structure of the His Bundle

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SUMMARY
The fine structure of the His bundle is described on the basis of its light and electron microscopic appearance. Electron microscopy was performed on one human and two canine hearts, and light microscopy on over 400 human and 60 canine hearts. The His bundle was identified by its light microscopic appearance. There were no significant differences in the fine structure of human and canine His bundles. In both, the principal cell was a typical Purkinje cell containing few myofibrils and a large perinuclear clear zone; these cells are shorter and broader than working myocardial cells, and their intercellular junctions (which are obliquely rather than transversely oriented) contain a high proportion of nexus formations. Both the human and canine His bundles are partitioned by fine collagen septa, which are longitudinally oriented with comparatively few crossover connections. The general organization of the His bundle is thus into multiple strands of Purkinje cells, and these strands are largely separated from one another by collagen. This longitudinal separation of Purkinje strands by collagen, plus the specialized nature of intercellular junctions within each strand, form an anatomic basis for suspecting longitudinal separation of conduction within the normal His bundle. Some electrophysiologic implications of these findings are discussed.

Additional Indexing Words:
- Longitudinal dissociation
- Ultrastructure of His bundle
- Histology of His bundle
- A-V node-His bundle junction
- Collagen partitions of His bundle
- Purkinje cells

The normal sequence of electrical activity in the heart is from the sinus node, where the pacemaking impulse originates, to the atrioventricular (A-V) node, where there is a slight delay in transmission, and thence to the His bundle for rapid conduction into the branches distributing to the two ventricles. In a previous study of the ultrastructure of the sinus node, we described the heterogeneous population of cells in the normal pacemaker and discussed how this complex multicellular biologic unit functioned in such a well organized and consistent fashion. A later report similarly described the A-V node and how its ultrastructural appearance could be utilized in understanding its triple roles of triage of atrial signals, conduction to the His bundle (including the consistent slight delay), and alternative or subsidiary pacemaking.

This report concerns a similar examination of the fine structure of the His bundle in man and dog. Both light and electron microscopic observations are included, since optical microscopy is essential for orientation and histologic interpretation (literally defined), while detailed examination of cellular structure and intercellular anatomy is possible only with the electron microscope.

Material and Methods
The electron microscopic studies were conducted on the His bundle of two canine and one human heart. The two canine specimens were
These two light photomicrographs of human His bundle (Goldner trichrome stain) illustrate the region studied with the electron microscope. (A) Section from a tissue block directly adjacent to the one used for electron microscopy. The area excised for study is indicated by the square. (B) A higher magnification of this region, with His bundle tissue comprising the upper two-thirds and interventricular septum, the lower third of the photograph.

For electron microscopy, the His bundle tissue was cut into pieces of no more than 1 mm maximal dimensions. Specimens were immersed in cold phosphate-buffered 6.5% glutaraldehyde for 2 hr, transferred to 0.2 M sucrose for several hours, and then post-fixed in osmium for two additional hours. Embedding was in either Araldite or Epon. Sections were cut on a Porter-Blum ultratome and stained with uranyl acetate and lead hydroxide. Examinations were conducted with a Philips 300 electron microscope.

For light microscopic interpretations, the His bundles of over 400 human hearts (89 of which have been serially sectioned) and over 60 canine hearts have been studied. Previous interpretations of some of this material have been reported, as well as comparative observations on the His bundle of other animals, including the dog.

Results

Both the light and electron microscopic appearances of the canine and human His bundle are similar, and no major species differences were noted.
These two light photomicrographs compare the His bundle (A) and working myocardium (B) as identified from specimens embedded in Araldite and subsequently sectioned for electron microscopy. Note the larger number of parallel myofibrils and the distinct transverse intercalated discs (three black arrows) in working myocardium. Same magnification in (A) and (B) (Grimley stain).

difference could be found. Minor differences will be noted where pertinent. The findings are most clearly considered under four headings: (1) general organization of the His bundle, (2) types of cells in the His bundle, (3) collagen partitioning of the His bundle, and (4) intercellular junctions in the His bundle.

General Organization of the His Bundle

There are some semantic problems in defining the His bundle which should be explained. It is simple enough to say that the His bundle begins at the end of the A-V node and that it continues until no more bundle branches remain, but determination of precisely where the A-V node ends and the His bundle begins and where the common bundle ends and its branches begin is a complex matter. Electrophysiologists may functionally define the N-H region as the site of physiologic junction of A-V node and His bundle, but we do not know what this corresponds to histologically, in terms of specific cells. Currently prevalent definitions of A-V node and of His bundle, whether anatomic or physiologic, may require surprising modification when appropriate correlative studies are feasible. However, we must begin somewhere, and this is an effort at definition of the His bundle based on its light and electron microscopic appearance.

One immediate problem is produced by the fact that the histologic organization of the His bundle is not the same in all hearts, even in a single species, and the potential electrophysiologic differences relative to this variation are completely unknown. For example, the A-V node is composed predominantly of slender interweaving transitional cells, although it does contain distinctly different cell types as well, while the His bundle is composed
predominantly of large Purkinje-type cells which are longitudinally oriented. One may then assume that the boundary between A-V node and His bundle should be easy to define, but here are the facts to the contrary. In some His bundles there are some slender transitional cells present all the way to the bundle branches themselves, and the major difference between A-V node and His bundle then becomes the parallel longitudinal orientation of all cells in the His bundle rather than a change in cell type. In other hearts the proximal portion of the His bundle (that nearest the A-V node) is composed of interweaving Purkinje-type cells, but one could with equally sound logic argue that this is actually the terminal portion of the A-V node. With these qualifications in mind, we will define the histologic boundary of A-V node and His bundle as that point at which the cell type becomes predominantly large Purkinje-type and at which most of the cells become oriented parallel in a longitudinal direction and cease interweaving (fig. 3). This transition in intercellular geometry usually corresponds to the point at which the His bundle penetrates the central fibrous body and leaves the A-V node, which is predominantly a right atrial subendocardial structure.

In coursing through the central fibrous body, the His bundle is normally a smoothly outlined structure of ovoid or nearly triangular cross-sectional appearance. An important exception to this description occurs in the human fetal and newborn heart, where the His bundle has numerous outcroppings and
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These two electron micrographs compare cells of working myocardium (A) and of His bundle (B) in the same human heart at the same magnification. Transverse orientation of the dark jagged intercalated disc (solid black arrow) of working myocardium is in contrast to the obliquely oriented and finer line of the intercellular junction between Purkinje cells (open arrows). Note also the comparative density and orientation of myofibrils.

loop connections which may be of considerable significance in the genesis of such electrocardiographic events as preexcitation or reentrant tachycardias. The normal postnatal molding and shaping process which converts the shaggy His bundle of the newborn into the smoothly outlined structure of the adult may play a role in the pathogenesis of lethal arrhythmias responsible for crib deaths. Except in the fetal and postnatal period, the normal His bundle has no important divisions within the central fibrous body, where it passes along the posterior and inferior margin of the membranous interventricular septum to reach the muscular crest. At the septal crest the His bundle promptly begins to provide branches to the left ventricular endocardium, and at a slightly more distant and variably placed point provides a single slender right bundle branch. The right branch usually plunges directly into the septal myocardium, although it sometimes passes along the right endocardium. The left branches virtually always distribute directly beneath the left ventricular endocardium rather than plunge into the myocardium. A third group of divisions are those in which His bundle cells pass directly into the septal myocardium and appear to terminate there. These latter connections are variably located and differ greatly in size, but are rarely more than a few cells in maximal cross-sectional dimension and can seldom be traced for more than a few millimeters into the septum. They are regularly present in the fetus, less often in infants and children, and continue to decrease in frequency (and size) with advancing age. The tissue directly beneath the His bundle is always dense collagen, the result of a normal postnatal developmental process, although in some hearts there is more collagen than in others; the fibrosis present at this site is probably of no physiologic significance, except in contributing to the structural strength of the fibrous skeleton of the heart.
Here we face the second dilemma in defining the extent of the His bundle: where does it end and where do the bundle branches start? There is little problem concerning the undivided portion of the His bundle within the central fibrous body, but once any bundle branch has begun to originate, is it more useful to think of conjoined bundle branches or a dividing portion of common His bundle? We favor the latter definition. As will be discussed later, particularly in the section concerning collagen in the His bundle, this may be a less important matter than initially appears. There is little question that the bulk of the His bundle is comprised of cells eventually coursing into the left bundle branches, and only a comparatively small number of these enter the right branch. In this context one may usefully think of the His bundle as a structure providing A-V connections primarily for the left ventricle, although the smaller number coursing to the right ventricle are nevertheless very important.

**Types of Cells in the His Bundle**

The principal type of cell in the human and canine His bundle is that first described by Purkinje in ungulate hearts. This cell (figs. 4-6) is broader and shorter than ordinary working myocardial cells, contains relatively few myofibrils (which in turn contain a small number of myofilaments), has a large perinuclear clear zone (which means that the myofibrils are peripherally located near the sarcolemma), has comparatively less scalloping of sarcolemma (a consequence of fewer myofibrils and, thus, fewer Z lines near the external membrane), and exhibits typical intercalated discs only rarely (figs. 2 and 4). This latter point is discussed in more detail in the section on cell junctions. Other features of these predominant Purkinje-type cells include...
Figure 6

This electron micrograph from canine His bundle illustrates at least one Purkinje cell (with nucleus), several slender transitional cells, and a capillary (Cap). Z bands are seen in some myofibrils oriented in random directions, with scattered mitochondria of various sizes. There are no specialized junctions between these cells, most being separated by collagen partitions of varying thickness.
A typical broad Purkinje cell from canine His bundle is shown in this electron micrograph. Its obliquely oriented line of junction with a second cell is indicated by three small solid black arrows. The lower margin of the cell is indicated with two broader solid black arrows near the bottom of the photograph. C = intercellular clefts; N = nucleus; G = Golgi apparatus; SR = the profusely arborizing sarcoplasmic reticulum.

some random orientation of myofibrils (rather than a strictly longitudinal array as in working myocardium), presence of a Golgi apparatus, an abundance of mitochondria in the perinuclear zone, occasional presence of two separate nuclei, and a variety of randomly
Two slender transitional cells from canine His bundle are indicated with open arrows in this electron micrograph. Erythrocytes in an adjacent capillary are labeled e. Dense strands of collagen separate the cells.

distributed tubular profiles (fig. 7). There is one difference between the predominant cells seen in these His bundles and those described by Purkinje, which are typical of the ungulate heart, and this concerns the shape. Ungulate Purkinje cells are almost spherical or polyhedral and make contact with other cells at virtually their entire periphery, whereas the cells in the canine and human His bundles are elongated and oblong in shape, making contact to some extent along their lateral margins but more often at their terminal ends.

In addition to the predominant cell type there are three others that are found in the myocardium of the His bundle. The slender transitional cell, which is most numerous at the A-V nodal end of the His bundle but may course much further, is identical to its counterpart, which is the predominant cell type within the A-V node itself (figs. 8–10). A second cell type has features intermediate between those of the Purkinje cell and working myocardium, including a larger number of myofibrils and concomitant increased complexity of intracellular organization of mitochondria and sarcoplasmic reticulum. This may also be considered a form of transitional cell, but in contrast to those of the A-V node, this type is at least as broad as a working myocardial cell. As an expedient in nomenclature these may be called broad...
Margins (arrows) of slender transitional cells in human His bundle are shown in this electron micrograph.

transitional cells, but a more suitable designation will undoubtedly be offered when their function is clarified. The third cell type is found in numerous clusters at the proximal margin of the His bundle and is identical to the P cells of the sinus node1 and A-V node.2 These P cells are an important histologic feature of the site of junction of A-V node and His bundle (fig. 3). P cells are round or oval and have an empty-appearing cytoplasm with few organelles, sparse and randomly oriented myofibrils, and a scanty sarcoplasmic reticulum with no apparent organization by current criteria; their membranes are simple, and their junctions with other cells (almost exclusively with P cells and transitional cells) are in all

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Figure 10

A Purkinje cell (external margins indicated by arrows) from human His bundle is shown in this electron micrograph for comparison with figures 8 and 9. For orientation relative to an entire Purkinje cell, see figures 2A and 4B.
Collagen Partitioning of the His Bundle

One of the more striking histologic features of the His bundle is its intricately patterned partitioning by collagen. This is well demonstrated with a simple van Gieson stain without any counterstain (figs. 11 and 12). The partitions are longitudinally oriented and course for the distance of many cells, measuring several millimeters in length. There are occasional crossover connections between partitions but these are sparse, and the prevalent situation is a distinct separation into long slender compartments containing maximal cross-sectional dimensions of only two or three cells. There appear to be two orders of collagen septa, one rather dense and the other quite fine (fig. 12). The density of the collagen septa varies from one heart to another, but in general on cross section appears to be a more prominent feature in the canine than in the human heart, although on longitudinal sections the sheets of collagen are more distinctive in the human heart.

Collagen septa in the His bundle have been noted by others, and they are readily
apparent in cross sections. However, their potential significance as longitudinally oriented dividers cannot be based on the appearance in cross section, since the apparent partitioning may only be an illusion due to the plane of sectioning. To examine this question, we recut 15 human and four canine His bundles with serial sections in a plane perpendicular to those for cross section, so that the paraffin blocks were oriented for sagittal cuts (fig. 13). The purpose was to make sections through the His bundle in a direction as nearly as possible parallel to its long axis. It is those sections which so clearly demonstrate the considerable length of the longitudinally oriented collagen sheaths (figs. 14 and 15), particularly in the human His bundle, and the apparent compartmentation of the bundle into narrow cords of Purkinje cells running in its long axis and with relatively little cross connections between compartments.

Figure 13
This light photomicrograph is of a block of human His bundle (between two open arrows) sectioned in the sagittal plane to demonstrate the considerable length of the longitudinally oriented collagen septa. (See also figures 14 and 15.) Interatrial septum is above and interventricular septum, below; the large open space between them is due to a fold in the membranous interventricular septum which was missed in this cut. (Van Gieson stain.)

Figure 14
These two light photomicrographs illustrate the identical longitudinal partitioning of the His bundle seen in two different human hearts at the same magnification. (Both with van Gieson stain.)
Longitudinal division of the His bundle by collagen is a distinct histologic difference from the A-V node (fig. 15) and from the working myocardium of the interventricular septum (figs. 11 and 13). In fact, the loss of interweaving sheets and septa of collagen and the appearance of longitudinally oriented ones is another useful characteristic for histologically defining the junction of the A-V node and His bundle (figs. 3 and 15). By contrast, at the other end of the His bundle the septation by collagen continues unaltered from the main body of the His bundle directly into the bundle branches. The growth of collagen partitions in various components of the conduction system of the heart is a normal postnatal developmental activity which undoubtedly plays an important role in the normal “maturation” of function in those special structures.

**Intercellular Junctions in the His Bundle**

As was indicated previously, the Purkinje cells, which are predominant in the cytology of the His bundle, do not exhibit typical intercalated discs as seen in working myocardial cells. In working myocardium the discs run almost directly perpendicular to the long axis of the cells, although they may make “steps” from the Z line level of one group of myofibrils to a different level in an adjacent group (figs. 2, 4, and 5B). Discs in working myocardium do contain numerous infoldings and interdigitations between the contiguous cells, but the general appearance in cross section is a jagged perpendicular line. Purkinje cells of the His bundle have much thicker interdigitations between cells and consequently fewer of them in number across the cell; these give the appearance of very large tongue-in-groove joints. The terminal intercellular junctions of Purkinje cells are not in a line perpendicular to the cell’s long axis, as in working myocardium (figs. 2, 4A, and 5B), but are obliquely oriented (figs. 4B, 16, and 17). Furthermore, there are fewer and less dense desmosomes and other sites with...
The typical obliquely oriented intercellular junction (two black arrows) between Purkinje cells of human His bundle is shown in this electron micrograph. Margins of the two adjacent cells are indicated by the open arrows, which lie in an intercellular cleft.

The dark "Z-substance," so that the intercellular junction is more nearly a fine line than a darkly jagged one. Another contrast to working myocardial cells, where lateral junctions between such cells are rare, are the more abundant lateral connections between the Purkinje cells of any given strand in the His bundle. Finally, and perhaps most important of all, the tight junctions or nexuses, which are seen as small or short (in cross section) sites of very close apposition between membranes in intercalated discs of working myocardium,
Figure 17

The obliquely oriented "tongue-in-groove" junction (eight small black arrows) of two Purkinje cells, A and B, from canine His bundle is shown in this electron micrograph. Intercellular clefts filled with collagen partitioning these narrow cords of Purkinje cells are indicated with long slender arrows and C.

Discussion

Because the His bundle is normally deeply encased in the dense collagen of the central fibrous body, it is not surprising that there are so few studies of the transmembrane action potential of its cells. Although the technical difficulty introduced by collagen relative to impalement of single cells may be less in the hearts of young animals (e.g., puppies), the paucity of collagen there may introduce a significant limitation in interpreting findings...
The specialized nature of intercellular junction within the His bundle is shown in this electron micrograph of canine His bundle. Since the myofibrils are almost transversely cut and do not attach at the junction here, this is a rather laterally placed intercellular junction. C indicates a collagen partition; PV, various groups of pinocytic vesicles; and SR, elements of the sarcoplasmic reticulum. Long segments of tight junction (nexus) are indicated by arrows and N; there is probably an additional such junction in a loop just above the upper N. The two open arrows indicate an unusual view of the two apposing plasma membranes and the dark interposed basement membrane forming almost an intermediate line; this interpretation is based on the width of this junction (about 500 Å) and the volume of adjacent pinocytotic activity, suggesting communication with the extracellular space. Method of preparation is given in the text.

Relative to function in the mature or adult heart. Such has already proven to be the case when functional characteristics of A-V conduction in young mammals (dogs, pigs, and
Figure 19

These two light photomicrographs illustrate crossover connections (curved arrows) between adjacent strands of Purkinje cells in human (A) and canine (B) His bundle. (Same magnification, Goldner trichrome stain.) In (A) the broad black arrow indicates a fortuitous section through a focal hemorrhage which interrupts one of the Purkinje strands.

goats) were compared with those of adults of the same species.\textsuperscript{15} This point is emphasized here because one of the histologic differences between the His bundle of newborn babies or puppies and that of their adult counterparts is the development of collagen partitioning in a longitudinal direction.

Another maturational change that may influence function of cells in the His bundle is the specialization of intercellular connections. Thus the simple intercellular junctions seen in embryonal myocardial cells (and in P cells of the adult heart) may not permit the rapidity of conduction observed between cells with numerous nexuses such as are present in the adult His bundle. However, this is a point...
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concerning which it is much more difficult to obtain quantitative data, and it is thus still largely speculative.

To interpret the possible physiologic significance of this study on the fine structure of the His bundle, we wish to stress two particular observations. These are the longitudinal partitioning of strands of His bundle cells for distances of several millimeters and the large size and multiple locations of nexuses between cells within each of these strands. These two points are complementary relative to function by the His bundle for rapid and longitudinally compartmented A-V conduction: the collagen would at least minimize and, for some distances actually prevent, lateral spread of the propagated impulse, while the abundance of specialized intercellular connections within these compartmented strands would be anticipated to do the opposite, viz., preferentially facilitate rapid longitudinal spread of the impulse. There are of course some interconnections between compartmented strands, and although one may assume that these mean intermingling of the components of electrical conduction in the His bundle, there are some interesting other possibilities. For example, considering the extent of longitudinal partitioning and the relative paucity of interconnections, it is conceivable that the entire propagation front is normally compartmented and that the crossovers function only under special circumstances, such as during re-entrant tachycardias or when some components of the longitudinal strands are interrupted by disease (fig. 19).

While the present observations provide an anatomic substrate for the concept of longitudinal dissociation of conduction within the His bundle (under both normal and pathologic circumstances), they of course do not prove the concept. Physiologic experiments to test the hypothesis are extraordinarily difficult to perform, in large measure because of the deep location of the His bundle within collagen. As indicated previously, this limitation applies particularly to the matter of intracellular microelectrode recordings. More distally obtained recordings, such as with close bipolar endocardial electrodes, may or may not represent the entire electrical activity of the His bundle and would, at any rate, tell us little of the postulated individual propagation fronts within the bundle. Similarly, experiments with electrical stimulation through such electrodes are too crude for the purpose since an unpredictable component of the His bundle (and quite likely all of it) would be activated. Cutting of portions of the His bundle or selective focal damage with a microcautery has been attempted,\textsuperscript{17, 18} and some of those experimental observations support the concept of longitudinal dissociation. However, more precise methods for selective localized production of experimental lesions are needed.

Indirect evaluation of function in the His bundle can be made with standard electrocardiography, and on that basis a number of investigators have previously suggested the likelihood of longitudinal dissociation in the His bundle.\textsuperscript{19-21} We have recently reviewed the evidence supporting the concept in electrocardiography.\textsuperscript{22} It should be indicated that, whereas most investigators now readily accept the possibility of longitudinal dissociation in the A-V node, the general thought among physiologists has been that the His bundle functioned more as a solid copper wire. The fine structure of the His bundle does not support that concept but is more in keeping with one of multiple insulated filaments contained in a single common cable.

We may conclude with a consideration of the A-V node and the His bundle functioning together and the mechanisms for their intricate integration. If the A-V node acts as a filter and for triage of atrial signals, providing at its distal end a given propagation front which is under normal circumstances always of the same configuration, then the nature and form of the propagation front in a compartmented His bundle would also remain uniform and consistent for every cardiac cycle. The two obvious ways in which this consistency may be distorted are (a) for the A-V nodal propagation front to be altered (either by a change in the input into the node from the

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atrium or by focal disease or a physiologic influence on the A-V node, e.g., a strong vagal stimulus) or (b) for the compartments in the His bundle to be deranged. Either of these would then be anticipated to alter the sequence of activation of the peripheral ventricular myocardium and, consequently, the form of the QRS complex. Depending on the nature of the alteration, the result may be any form of "bundle-branch block" (although the responsible lesion would be in the A-V node or His bundle and not in a bundle branch), or ventricular aberration, or ventricular preexcitation. Finally, focal interruption of longitudinal conduction within the His bundle may facilitate the passage of a propagated impulse from one compartment to another, particularly if the arrival of the wave front in the second compartment had either been delayed or blocked. The consequence could then be a reentrant tachycardia of the same type now generally considered to develop in the A-V node under special circumstances. Thus the sparsely distributed crossover connections may be thought of as safety structures permitting continued propagation to the ventricles from one compartment to another in the event of structural damage in either, but they may also function as the route by which reentrant tachycardias may be established.

References


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