Comparative Ultrastructure of the Sinus Node in Man and Dog

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MYOCARDIUM was one of the first tissues to be studied with the electron microscope, but the ultrastructure of the cardiac conduction system has heretofore been described only in subhuman species. Electron microscopy has been employed to examine the conduction system of the dog,¹⁻⁴ rabbit,⁵,⁶ frog,⁷ sheep,⁸,⁹ rat,¹⁰,¹¹ and steer.¹²,¹³ Interest in the specialized tissue of the heart which comprises the conduction system has been intensified in recent years by experiments which record membrane action potentials of single cells of the sinus node, A-V (atrioventricular) node and bundle, and Purkinje fibers.

There are several reasons for the relative paucity of published observations on the fine structure of the conduction system. The regions to be studied are small, and with current methods of preparing tissue for electron microscopy one must utilize samples of a size (usually about 1-mm cubes) that it is difficult to be certain the sample obtained is actually from the area intended. A second problem concerns the nature and extent of postmortem changes in conduction system tissue, but this can be circumvented in the experimental animal by obtaining samples in vivo. Because of the likelihood of significant destruction of the critical centers involved by such sampling in vivo in man, there is no reasonable alternative to postmortem study of these particular regions in the human heart.

Methods

Our attempts to solve these problems were based on the following reasoning. From several years of experience with obtaining tissue for light microscopic study of the sinus node in man and the dog,¹⁵ we were confident of being able to select the proper tissue. Once such tissue was obtained, alternate 1-mm slices were utilized for light microscopic examination. Slices for electron microscopy had the central portion of sinus node (fig. 1) excised as a 1-mm cube which was then diced with a razor blade into further fragments, random samples of which were alternately selected with half for light microscopic and the other half for electron microscopic study. The remainder of the slice from which the electron microscopic specimen was obtained was then processed for light microscopic study. When those samples selected for electron microscopy were embedded, an initial shaving of the specimen was obtained and examined with phase illumination for additional confirmation that the tissue was sinus node. Finally, the appearance of this tissue could be compared with that already described by others, and in particular with the findings of Trautwein and Uchizono,⁶ who sampled tissue which had been selected on the basis of intracellular recording of membrane action potentials exhibiting typical pacemaker activity in the rabbit.

The nature and extent of postmortem change in human and canine sinus nodes were evaluated as follows. Hibbs and Black¹⁶ reported that "autolytic" changes in myocardium are relatively few within the first 10 hours after death. The principal changes that they found were rapid loss of cytoplasmic glycogen (within minutes) and less rapid clumping of nuclear chromatin. The fine structure of cellular membranes and organelles were changed little on comparison with tissue obtained in vivo. To examine this question further, we obtained sinus node and right atrial myocardium in vivo from the dog, and compared this with identical tissue obtained 3 hours postmortem.
Human right atrial myocardium was obtained in vivo during open cardiac surgery and compared with human right atrial myocardium obtained 3 hours postmortem, and with in vivo and postmortem canine right atrial myocardium. Finally, human sinus node obtained 3 hours postmortem was compared with the canine sinus node obtained both in vivo and postmortem, and with human right atrial myocardium obtained in vivo and postmortem. We are satisfied that true sinus node was studied and that the postmortem changes are relatively few and easily detected.

Human right atrium obtained in vivo and all postmortem tissue (both human and canine) were cut into cubes no larger than 1 mm and immediately immersed in cold phosphate-buffered 6.5% glutaraldehyde. After 2 hours in glutaraldehyde the tissue was transferred to 0.2 M sucrose for several hours and then post-fixed in osmium for 2 additional hours. Dehydration in a graded alcohol series was followed by placement in propylene oxide for embedding in Araldite. Sections were made with a Porter-Blum ultratome and stained with uranyl acetate and lead hydroxide. Canine sinus node obtained in vivo was fixed by direct perfusion of the sinus node with glutaraldehyde through its nutrient artery, employing a method developed in this laboratory for physiological and pharmacological experiments. After initial fixation in vivo, sinus node and neighboring right atrial myocardium were further processed in the same manner as tissue obtained postmortem. The tissue on grids was examined with an RCA EMU-2 electron microscope. Original magnifications of 1,600 to 6,400 diameters were enlarged photographically as desired. A few photographs were made with a Hitachi HU-11A electron microscope.

The sinus nodes from human hearts were all obtained 3 hours postmortem from patients dying of noncardiac disease. Three different nodes were studied, one from a 2-year-old child and two from adults 38 and 45 years of age. Sinus nodes of three dogs were fixed in vivo and of two additional dogs, 3 hours postmortem.

Results

Ultrastructure of the sinus node was so similar in human and canine hearts that the descriptions will be presented together and any significant exceptions noted in the appropriate discussion.

General Organization of the Sinus Node

Overall structure of any tissue is not best appreciated by electron microscopy alone, but with an orientation based on gross and light microscopic studies it is possible to examine certain organizational morphology with much greater detail with the electron microscope. The sinus node is composed of individual cells distributed in a mass of supporting collagen, and the external (basement) membrane of these cells is attached to the collagen. In addition to being occupied by collagen fibers, the intercellular spaces contain capillaries, fibrocytes, and nerves. No direct contact between nerve endings and cell surface was found.

There are two distinctly different cells within the sinus node, and a third group of cells which may be considered as transitional forms (figs. 2 to 5). One of these cells resembles ordinary working myocardium in all respects and is found principally (but not exclusively) at the margins of the sinus node. The other cell is clearly the main cell of the sinus node itself and can conveniently be designated a P cell for the following reasons: It is pale in contrast to working myocardium, many of its features resemble those of embryonal primitive myocardial cells, it also has many similarities to Purkinje cells, and there is good reason to consider that these cells may be the actual site of pacemaking. Most of the central portion of the sinus node is composed of clusters of P cells, but the homogeneity of the node varies both in different areas of one node and even more on comparison of nodes from different hearts. The clusters of P cells are not spherical but elongated and correspond to the interweaving bundles of sinus node "fibers" seen with the light micro-

Figure 1

Photomicrographs (light microscope) of the central portions of human and canine sinus nodes demonstrating the general organization of the node about a central artery and indicating the region from which specimens were obtained for electron microscopic examination. Both prepared with Goldner trichrome stain.

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scope. The shape of the individual polyhedral cells is ovoid or round, with the dimensions of a single P cell averaging 5 to 10 \( \mu \) in greatest diameter, making them much smaller than ordinary myocardial cells. P cells contain few myofibrils and sarcosomes, and the cytoplasm is strikingly empty in contrast to that of working myocardium.

Transitional cells have increasing numbers of myofibrils and sarcosomes which gradually orient in a fashion similar to working myocardium, and the cytoplasm additionally becomes increasingly electron-dense (fig. 6). All stages of transition can be found, with some cells being quite similar to P cells and others virtually identical to working myocardium. Some transitional cells appear inhomogeneous internally, with one half resembling a P cell (scattered sarcosomes, sparse myofibrils) and the other half resembling working myocardium (numerous myofibrils, linearly arrayed sarcosomes). Such cells were in areas between P cells and working myocardium. Considering the appearance of intercellular contacts within the sinus node, it is not surprising that some cells would have such widely differing internal content and external margins. That side adjacent to P cells resem-

![Figure 2](#)

*Figure 2*

A low-power electron photomicrograph of human (child) sinus node, demonstrating clusters of polyhedral P cells. For the sake of simplicity not all the P cells were labeled P. Adjacent strands of collagen (C) are seen at the top.
This low-power electron photomicrograph of canine sinus node was obtained from tissue fixed by direct perfusion of glutaraldehyde into the beating sinus node through its nutrient artery. The better preservation of most structures compared to the postmortem human sinus node in figure 2 is apparent, but the similarity of general organization is clear. Two entire P cells (P) are seen as well as the tip of a third one protruding into a transitional cell (Tr). Collagen (C) is seen on the left; it is directly bound to the basement membrane of the P cell. W indicates working myocardial cells, the different intracellular organization from P cells being obvious. Solid short black arrows and white ones indicate typical sarcosomes, gr indicates granules in one P cell which appear to have discharged from two adjacent faintly outlined structures (see also fig. 6), ce indicates a centriole (see also fig. 5). Two capillaries are present in this section and one is labeled. d indicates two sets of desmosomes. The upper are a contact between the tip of P cell and the transitional cell; the lower are a similar contact between the left lower margin of one P cell and a tip inserted into it by what is probably (from the appearance of the sarcosomes) another P cell, although the contents are not well enough defined to be certain of its identity.
bled the P cell and made contact with it through a few scattered desmosomes, while that side adjacent to working myocardium resembled it and made contact through numerous desmosomes or even fully developed intercalated discs.

**Membranes**

P cells have a sarcolemma composed of an

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**Figure 4**

Contrast in P cell (P), transitional cell (T) and working myocardial cell (W) from another human (adult) heart. f indicates a point of junction of two cells and bridging by basement membrane as the plasma membranes continue apposite. A longer segment of intercellular contact is seen between the P and T cells, with two of several desmosomes indicated by d. A lysosome is seen adjacent to the letter P. Note the small diameter of the myofibrils in the P cell, and the paucity of myofilaments per fibril, in contrast to the myofibrils of the T and W cells. The random distribution of sarcosomes in the P cell is well shown, but details of the internal structure of the sarcosomes can be accurately interpreted only from the specimens prepared in vivo. The white lines outline an area which is enlarged in figure 12.
external basement membrane and an internal plasma membrane (figs. 7 to 10). Our microscopic resolution was inadequate to determine separate components of the plasma membrane. The basement membrane covers clusters of P cells while most of the direct intercellular contact is between plasma membranes only. The plasma membrane always appeared the more sharply defined, with the basement membrane less electron-dense but slightly thicker. Thickness of the plasma membrane was relatively constant in any single preparation and varied only slightly between preparations, measuring from 40 to 70 Ångström units from internal to external surface. The basement membrane varied more in thickness, even in the same preparation, measuring from 40 to 120 Ångström units from internal to external surface.

Figure 5

The striking difference in organization and content of P cells and working myocardial cells is illustrated in this section of canine sinus node, with particular contrast between their respective sarcosomes (sa). Two profiles of sarcoplasmic reticulum (sr) are indicated, but a number of other profiles cut both transversely and longitudinally may be seen in the same cell. ce indicates a centriole. The micron bar is in a capillary.
In the sarcolemma the distance between the two membranes was relatively constant, although the inhomogeneity of the basement membrane sometimes made the space less sharply defined and difficult to measure accurately; the intermembrane distance in the sarcolemma ranged from 70 to 180 Angström units. The plasma membrane was essentially the same thickness in the canine and human (both adult and child).

**Figure 6**

Three transitional cells (T) are shown here for comparison to two P cells (P) and working myocardium (W) from canine sinus node. The progressive increase in complexity of sarcosomes (Sa) from P to T to W is apparent, as is the increasing electron density of the cytoplasm, the increasing number of myofibrils and the orientation of sarcosomes between the fibrils. Desmosomes (De) are seen between a P and T cell, while an intercalated disc (D) is seen between two T cells in the left upper corner. Two lysosomes (Ly) are seen in one T cell. Membrane-bound granule-containing inclusions (Gr) are seen in a P cell. The ubiquitous collagen (COL) is present. Ce is the centriole.
sinus nodes. The basement membrane was comparable in thickness in the canine and child's sinus nodes but was consistently slightly thicker in the adult hearts.

In contrast to working myocardium in which the cells are elongated and intercellular contact is principally at intercalated discs at each end, P cells are in contact with each other

![Image of P cells](image)

**Figure 7**

Membranes of typical P cells (P) of human (child) sinus node are shown here. Scalloping of the membrane in one area of the P cell on the right is unusual, the margins nearly always being smooth. The short black arrow in the left upper part indicates an intercellular cleft lined by both basement and plasma membranes, while the intercellular junction indicated by the adjacent short open arrow has only apposite plasma membranes. A desmosome (d) along this junction is indicated, and similar desmosomes may be seen at the opposite side of this cell, the latter joining both P cells with the tip of a third protruding between them. The diffusely distributed black specks are artifacts.
in all directions and there are no intercalated discs. Transitional cells, however, have increasingly well-developed discs, and the occasional observation of such discs within the sinus node is due to the node's inhomogeneity, containing principally P cells (no discs) but at various points variable numbers of transitional cells (some with discs). Most intercellular contact between P cells is without any apparent specialized junctions, but scattered desmosomes can be identified. Desmosomes usually occurred as a single pair of thickenings on apposing plasma membranes of P cells, increasing in number and frequency in the transitional cells. The overall dimension of a single desmosome (both components) was

Figure 8

The intercellular cleft (IC) between these two P cells from a child's sinus node is lined by a double membrane (short black arrow). Along the area of direct intercellular apposition of plasma membranes only (open short arrow) the intermembrane distance remains constant and there are only sparse specialized junctions such as the desmosomes, one of which is indicated (d).
about 1,000 Angström units in length along the axis of plasma membrane apposition, and 700 to 900 Angström units in thickness across both components perpendicular to the membranes. The thickening of the plasma membrane which represented the desmosome always protruded more into the cytoplasm than into the intercellular space, but there was always a distinct protrusion also in the latter direction, making the intercellular distance less at the desmosome than at other points between apposing plasma membrane. The exact

![Figure 9](image_url)

**Figure 9**

*P* cells of a canine sinus node obtained postmortem (compare to the human sinus node illustrations) show considerable distortion of sarcosomes but good preservation of myofibrils and membranes. Intercellular clefts (ic) are lined by a double membrane, with the basement membrane (b) bridging over adjacent cells while direct intercellular contact is between plasma membranes only. N is a nucleus and n is a nerve. D indicates some of the desmosomes, but note that long segments of intercellular contact contain no such specializations. Intensely active pinocytosis is particularly noted in the area indicated by the two short black arrows, details of which are shown in figure 11. The area outlined in white here is enlarged in figure 10.
Figure 10

Distance between apposite plasma membranes, indicated by three short black arrows, seemed to remain constant both in the canine (here) and human (fig. 8) sinus nodes, with no apparent...
distance between cells at the desmosomes was difficult to measure accurately because of the consistent presence of electron-dense material at this point, but it appeared to be about 60 to 100 Angström units.

Although the basement membrane generally covers adjacent P cells, there are also numerous infoldings which form intercellular clefts so that the clusters of P cells have a sponge-like arrangement relative to the extracellular space. The distance between two plasma membranes of contiguous P cells was relatively constant in the range from 70 to 150 Angström units. No fusion of apposing plasma membranes in the form of a nexus could be identified, although the limits of our resolution could not exclude this possibility. In the regions of intercellular clefts where two adjacent plasma membranes parted, a basement membrane appeared; no clefts lined by plasma membrane alone were observed. Many pinocytic vesicles invaginated from the plasma membranes in all P cells and appeared more numerous than in neighboring transitional or working myocardial cells (figs. 11 and 12). Pinocytosis was not confined to the external surface of P cells, since similar infoldings of plasma membrane were present even along lines of intercellular contact where basement membrane was absent; the process was always much more prevalent on the external cellular surface, however.

The external surface of the P cells is smooth, with noticeable absence of the marginal scalloping typical of working myocardium. This is most likely related to the paucity of myofibrils, since the transitional cells develop indentations of the membranes in register with the Z bands of their increasing numbers of myofibrils. As more myofibrils appear in the transitional cells, they tend to orient parallel to each other and insert at the plasma membrane, where desmosomes appear in concomitantly increasing number to form intercalated discs. The transitional cells most resembling ordinary working myocardium have fully scalloped membranes and fully developed intercalated discs. Transitional cells made contact with both P cells and working myocardium as well as with each other. P cells made contact only with transitional cells and each other, no direct contact between P cell and working myocardium being seen.

Sarcosomes

P cells contain relatively few sarcosomes (mitochondria) in comparison to working myocardium, but their distribution differs even more (figs. 1 to 9). In working myocardium the sarcosomes are packed between myofibrils with some lying directly beneath the plasma membrane, whereas those in P cells are distributed totally at random within the cell. In transitional cells as more myofibrils appear, the sarcosomes assume an orientation in relation to them, until in those transitional cells with many myofibrils the sarcosomes line up between them just as in working myocardium. The size and shape of sarcosomes in P cells is impressively variable, even when allowance is made for tangential sectioning. Sarcosomes in both P cells and working myocardium were comparable in average size and were rarely more than 1 μ in largest dimension, averaging about 0.5 μ, but both the shape and size were more variable in P cells.

Our postmortem specimens demonstrated more distortion of the sarcosomes than has been reported in myocardium examined after death by others, making interpretation of internal mitochondrial configuration of postmortem specimens less reliable than in the specimens fixed in vivo. In one human 3-hour postmortem specimen (that of a 2-year-old

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fusion or nexus formation. The long black arrow with a D indicates one desmosome. The intermembrane gap at the desmosome is not only narrowed by intrusion of the desmosome, but there is also an accumulation of electron-dense material. The basement membrane (bm) does not enter into the intercellular junction with the plasma membranes (pm). Two layers of the nuclear membrane (nm) are indicated, with the inner layer appearing to contain more than one component. Nuclear chromatin is clumped within the nucleus (N), characteristic of postmortem change. Note the variable size and directions of myofibrils.
Figure 11

Pinocytotic vesicles (PV) formed by the plasma membrane (pm) are shown; the ones marked by asterisks appear to be dissolving or discharging their content into the cytoplasm. The less
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child without chronic disease and no heart disease), the sarcosomes were less altered and comparable to the canine specimens obtained in vivo, whereas those of the two adult humans with chronic disease (cancer) resemble those of dog sinus node obtained 3 hours postmortem. The descriptions of sarcosomes here are based on their appearance in P cells of canine sinus node fixed while beating in vivo and are compared with those of adjacent working myocardial cells in the same heart fixed in the same manner at the same time.

Sarcosomes of P cells are surrounded by a double-layered membrane, each component of which is sharply defined and of approximately the same thickness. Each layer of the membrane is about 30 Angström units thick, and the distance between layers is also about 30 Angström units. These measurements of sarcosomal membrane thickness and intermembrane distance are similar to those of sarcosomes in neighboring working myocardial cells. But the internal appearance of the P cell sarcosomes was strikingly different from that of working myocardium, exhibiting a much simpler internal structure with very few cristae mitochondrialis and little intercristal matrix (fig. 13). Instead of the orderly transverse spiral arrangement of cristae seen in working myocardial cells, those of the P cells exhibited a variety of distributions, including a rosette type of arrangement with peripheral cristae and a central clear space which was observed only in P cells. The individual cristae of sarcosomes of P cells appeared thicker than those in working myocardium, but any real difference was exaggerated by their consistently fewer numbers per mitochondrion. The cristae of P cell sarcosomes were coarsely homogeneous, with a suggestion of granularity, and each fold varied in thickness from 200 to 400 Angström units.

The difference in electron density of the intercristal matrix was most apparent on comparing adjacent P cells and working myocardium. Because such comparison in the same sinus node demonstrated a consistent difference in sarcosomes of P cells, we feel the simpler organization and pleomorphism of these sarcosomes is real and not an artifact. This interpretation is supported by the identical appearance of our working myocardial sarcosomes to those published by Sonnenblick and associates, who also used the dog and fixed their tissues in vivo with intracoronary perfusion of glutaraldehyde, as we did.

In transitional cells the increasing numbers, more organized distribution, and greater internal complexity of the sarcosomes paralleled the increasing number of myofibrils.

Myofibrils

Although there was a general parallelism of numbers of sarcosomes and myofibrils, particularly in transitional cells, in the P cells with almost no visible myofibrils, there was still an appreciable number of randomly distributed sarcosomes. Even though allowance was made for the plane of sectioning missing some myofibrils in such cells, there was nevertheless a disproportion of sarcosomes to myofibrils in the P cells. In P cells the few myofibrils present contained only small numbers of myofilaments. The orientation of myofibrils was random in all directions, rather than in a single axis, as they appear in the elongated working myocardial cells (figs. 1 to 6). Most myofibrils in P cells did not attach to the plasma membrane but some of this appearance was of course due to the plane of sectioning. Similarly, desmosomes often appeared on apposite plasma membranes without attached or even neighboring myofibrils; although it is not possible to say that single or small numbers of obliquely sectioned myofilaments were not present without being visible in our preparation. Myofibrils commonly contained only a few myofilaments in P cells, and in some areas there appeared to be single myofilaments; however, we were un-

well-defined basement membrane (bm) does not participate in this process. Two (or more) layers of the membrane (nm) of the nucleus (Nu) are shown above. An intercellular cleft (IC) is seen.

See figure 9 for general orientation.
Several features of intercellular junction are shown in detail in this enlargement from the section illustrated in figure 4 (adult human sinus node). Contrast in size between small myofibrils in P cells and larger ones characteristic of transitional cells is indicated by F. The small white F in the left lower corner indicates junction between the T cell and a working myocardial cell, most of which is not shown here. Two of several desmosomes are indicated by D; note the markedly increased electron density of the intermembrane gap at these points. Along the long line of plasma membrane apposition (area outlined with white lines) the intermembrane distance remains relatively constant except at the point indicated by ic, where an intercellular cleft.
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able to exclude the possibility that the latter represented longitudinal profiles of sarcoplasmic reticulum. Cross striations were present in nearly all fibrils and were similar to those of working myocardium, although their definition was poor in those fibrils with only a few filaments. Sarcomere length varied in different myofibrils of P cells, and this plus their random (nonlinear) orientation suggests that their contraction or relaxation may be asynchronous.

Sarcoplasmic Reticulum

P cells exhibited little sarcoplasmic reticulum and its distribution was not recognizable organized within the cell. Transitional cells exhibited an increasing content of sarcoplasmic reticulum commensurate with their increasing content of myofibrils and the organization of sarcosomes between them. The few profiles of sarcoplasmic reticulum visualized in P cells had no features distinguishing them from those in working myocardium, except for lack of relation to myofibrils. In neither site were significant numbers of ribosomes seen attached to the reticulum. In a few sites an infolding of plasma membrane forming sarcoplasmic reticulum could be recognized, but this was in transitional rather than in P cells. In the P cells the infolding was almost exclusively due to pinocytosis, although a concentration of some sarcoplasmic reticulum adjacent to the plasma membrane is a plausible alternative interpretation. Golgi complexes were not seen in any P cells but were present (though rare) in transitional cells.

Nuclei

All postmortem specimens exhibited clumping of nuclear chromatin. Canine sinus node fixed in vivo showed no unusual features in the nuclei of P cells. Their size was relatively large, occupying one third or more of the interior of some P cells. The nuclear chromatin was finely granular and evenly dispersed. Nucleoli were present in some P cell nuclei, being centrally located, small and densely reticulated. A double membrane was regularly present about the nuclear surface of P cells from both postmortem and in vivo specimens.

Because of the extensive invaginations of basement membranes within clusters of P cells forming intercellular clefts, the possibility of each cluster being a single multilobulated cell was considered. The demonstration of two plasma membranes crossing the entire diameter could conceivably be an infolding of a single cell, for example. We do not believe this is the case because the size of such adjacent cells was comparable to single cells visualized in other regions, because infolding without complete crossing between cells was rare in P cells, and because there were nuclei for each adjacent cell in most sections. Single P cells with more than one nucleus were not found.

Other Organelles

Centrioles were found in several P cells of the canine sinus node fixed in vivo (figs. 3 and 5) and in one postmortem human P cell. In each instance the centriole was centrally located within the cell, and in the two which included the nucleus in the section, the centriole was adjacent to the nucleus.

Lysosomes were present in both canine and human P cells, exhibiting highly variable shapes and containing both coarsely granular and solid inclusions (fig. 6). They occupied no consistent location within the cell, being present both in central and peripheral regions.

Membrane-bound inclusions with dark granules were present in a number of P cells of the canine sinus nodes fixed both postmortem and in vivo (figs. 3 and 6). The membranes in most instances contained two layers and sometimes more. Some of these structures had partially opened and appeared to be dis-

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charging their granular contents into the cytoplasm, while others appeared to be intact and packed with granules. The diameter of these rounded structures was about 0.5 μ and the granules, which were round and uniform in size, were about 60 Angström units in diameter. Similar but less well-defined structures were seen in some human P cells.

Cytoplasmic granules which we presume to be glycogen were abundantly present in work-

**Figure 13**

Contrast between the simple sarcosomes (M) or mitochondria of P cells, shown in A, and those of working myocardium, shown in B and C, is illustrated here from canine sinus node fixed in vivo. Even with lower magnification for the working myocardium (same in B and C), the much larger number of cristae mitochondriales per sarcosome and the greater electron density of intercristal matrix is obvious.

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ing myocardial cells of the canine heart fixed in vivo (fig. 6), but absent in the postmortem specimens. Some of the granules were clustered in rosettes. P cells contained relatively few cytoplasmic granules, and this accounted largely for their clear appearance; this difference in cytoplasmic granules was consistent in adjacent P cells and working myocardial cells of the sinus node fixed in vivo and seems unlikely to be artifactual.

**Discussion**

Both human and canine sinus nodes are composed principally of a characteristic cell, the P cell. Since the same type of cell has been demonstrated to be the predominant one in the pacemaking region of the rabbit heart selected because of its characteristic membrane action potential,\(^6\) it seems likely that these cells are also the site of pacemaking in both human and canine hearts. The sinus node is not homogeneously composed of P cells, but their frequency is highest in the central portion of the node. There are not only transitional cells scattered throughout the sinus node, but also some cells which have all the features of ordinary working myocardium. The proportion of P cells and transitional cells varies from one heart to another, and the functional efficiency of the sinus node may possibly bear some relationship to the content and focal concentration of P cells. Although the fine structure of P cells and transitional cells is best appreciated with the electron microscope, once their appearance becomes familiar their general distribution can be easily detected with the light microscope.

Several aspects of the light microscopic appearance of the sinus node require revision in view of the present findings. In previous reports describing the human\(^1^4\) and canine\(^1^5\) sinus nodes studied with the light microscope, two components of the sinus node were described as present within its collagen framework. These were sinus node fibers and syncytial cells. It is clear now that the sinus node fibers of light microscopy are multicellular, and since most of them contained many myofibrils, it seems most likely that they correspond to transitional cells seen with the electron microscope. The term “syncytial” cells was given to a distinctly separate structure usually attached to several sinus node fibers but containing a relatively clear cytoplasm, comparatively few myofibrils which coursed in all directions, and a single rounded nucleus. Because these cells resembled a sheet of cytoplasm with poorly defined margins, they appeared (through the light microscope) as a syncytium. Due to the functional connotations relative to the myocardium as a syncytium, the term “syncytial” cell was an unfortunate choice. Now with the demonstration of a distinct surrounding membrane in the P cells, which seem to be the same cells, the previous term should be abandoned.

From their light microscopic appearance it was previously postulated that these cells may be the actual site of pacemaking within the sinus node,\(^1^4\) based on their central location, primitive appearance, regular attachment to sinus node “fibers,” and proximity to numerous nerve endings. This hypothesis was strengthened by the demonstration of cells with similar appearance which behaved as pacemakers in cultures of chick heart\(^2^2\) and further by the recording of typical pacemaker potential with microelectrodes impaling such cells.\(^2^3\) It was further supported by the demonstration of similar cells in the rabbit heart which were located by their electrophysiological characteristics and examined with the electron microscope by Trautwein and Uchizono.\(^6\) Current evidence thus indicates that the P cells are most likely the site of pacemaking within the sinus node.

With this in mind the special ultrastructural features of the P cells may be summarized as an introduction to a consideration of each of their possible functional significances. Transitional cells represent all stages of intermediacy between the typical P cell and the typical working myocardial cells, so that the clearest comparison and contrast is between the latter two. P cells are smaller than working myocardial cells and rounded in shape rather than elongated. Their cytoplasm appears exceptionally clear, containing relatively little granu-
lar material (presumably glycogen), and there are few myofibrils, each of which contains few myofilaments. The myofibrils present in P cells course in random directions but except for their small size and random course have the same fine structure as those in working myocardial cells. Sarcosomes in P cells are also randomly distributed within the cytoplasm, their internal structure is simpler than in working myocardium, and their size and shape is more variable. Sarcosomes of P cells contain fewer cristae mitochondriales and the intercristal matrix is either sparse or certainly less electron-dense. Intercellular contact between P cells is in all directions, with their plasma membranes becoming apposite and their basement membranes covering a number of adjacent cells. There are relatively few points of specialized contacts such as desmosomes, and these are usually independent of the few myofibrils. P cells are grouped in elongated clusters and transitional cells connect them with ordinary working myocardium. The intimate relationship between cells of the sinus node and its supporting collagen framework which is so striking in light microscopy has been reported to be even closer on electron microscopic examination, with direct contact between basement membranes of P cells and collagen fibers; our findings confirm this observation. Intercellular clefts between P cells communicate with the free surface of clusters of these cells, forming overall a spongy structure surrounded by collagen.

Conduction in the sinus node has been demonstrated to be slow. If one accepts the functional interpretation of intercalated discs as representing sites of boosted or accelerated intercellular conduction rather than as sites of increased intercellular resistance, then the paucity of specialized intercellular contact points in the P cells is one possible explanation for retarded intranodal conduction. The distance between apposite plasma membranes of adjacent P cells appears too great (70 to 150 Angström units) for any conduction without special contacts. Even the shape of the P cells, which are polyhedral, implies conduction is not one of their primary functions, since both conduction and contraction can occur more efficiently in elongated cells. Finally, the poorly developed sarcoplasmic reticulum in P cells, which is thought to facilitate intracellular conduction in working myocardium, is still another structural explanation for slow conduction in the sinus node.

No direct contact being observed between P cells and working myocardium, it seems likely that intranodal conduction occurs from P cells only through contact with each other and with transitional cells, the latter acting as the bridge to working myocardium. Contacts between transitional cells and working myocardium occurred through numerous adjacent desmosomes and also through typical intercalated discs. Contacts between transitional cells and P cells occurred through few scattered desmosomes, while contacts between P cells themselves occurred principally through apposed plasma membranes with even fewer desmosomes. Recent evidence indicates that conduction in myocardium at the region of the intercalated discs may not occur through the specialized thickenings themselves but at the intervening membrane to membrane contacts visualized as “steps” in the disc, and shown to be the site of special structure, including fusion of adjacent membranes into a “nexus.” The observation of such intimate contact between myocardial cells is particularly important in view of the ionic composition on either side of such junctions (high potassium and low calcium concentrations on both sides) which suggests the absence of significant diffusion potential and therefore presumably low resistance.

Although additional examinations at higher microscopic resolution are indicated, it does not appear at present that “nexus” types of intercellular contact occur between P cells. Apposing plasma membranes of P cells not only did not appear to fuse but were sufficiently separated to suggest that conduction probably does not occur without some specialization such as a desmosome, although it is conceivable that the intercellular space con-
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tains some special material which is not electron-dense. At the desmosomes, however, the space between cells was reduced by the membrane thickening itself, and there was consistently a heavy accumulation of electron-dense material in this space between the P cells. It would seem the most tenable working hypothesis to explain conduction between P cells is that it occurs through the desmosomes.

Metabolism of the sinus node differs from that of ordinary working myocardium in some features, such as resistance to hypoxia and ability to withstand much higher concentrations of extracellular potassium without deterioration of its primary (pacemaking) function.24 The sparse sarcoplasmic reticulum in P cells in comparison to working myocardial cells means P cells have a much smaller surface area (external plus infolded internal) exposed to extracellular fluid, and this observation suggests a possible explanation for their different response to altered extracellular ionic contents (for example, K+). The resistance to hypoxia has been interpreted as indicating a relatively low metabolic rate. The emptiness of the cytoplasm of P cells and the simple structure of the sarcosomes is compatible with such an interpretation, particularly if there is truly less cytoplasmic glycogen and if one can equate the rate of energy production in the mitochondrion directly to the complexity of its internal structure. Slatterback3 has demonstrated that the degree of internal mitochondrial complexity in birds is directly related to heart rate, although it should be noted that these were sarcosomes in ventricular myocardium. Considering the general simplicity of the P cells and the much more highly organized structure of ordinary working myocardial cells, it is possible that we have heretofore been considering the wrong area of the heart as "specialized."

It is tempting to presume that the sarcosomes of the P cells represent a primitive un-specialized state, since the sarcosomes of both human35 and chick36 embryonic hearts are also very simply constructed early in fetal development. This similarity includes the small number of cristae per mitochondrion and a sparse intercrystal matrix. As the embryonic heart matures and myofibrils become organized, the sarcosomes become more complex. The simple appearance of the sarcosomes of P cells is not necessarily an embryonic holdover, however, and at present one may equally logically suspect that they have developed a special hypoxia-resistant function which has to do with constant pacemaking. In the embryonic chick heart the sarcosomes become oriented longitudinally even before the appearance of myofibrils,36 which supports the likelihood that sarcosomes of P cells are originally different from those of working myocardium. Whatever their functional sophistication or primitiveness, however, it appears that the fine structure of the sarcosomes in P cells is distinctly different from that of adjacent working myocardial sarcosomes obtained the same way at the same time in the same heart.

Transitional cells may be presumed to contract, with their strength of movement in direct relationship to their content and longitudinal orientation of myofibrils. If P cells contract, their totally random orientation of the few myofibrils indicates that forces must be multidirectional and weak. Furthermore, if intercalated discs represent a surface specialization facilitating adhesion of two adjacent contracting cells,37 the infrequency of desmosomes and total absence of discs in P cells additionally indicate that contraction is not one of their important functions. It has recently been suggested that the few myofibrils which are present in specialized myocardium represent a means for the adaptation (by some contraction) of this tissue to variation in volume of adjacent working myocardium during systole and diastole.38 Such an explanation seems unlikely for the reasons cited above, particularly the random orientation of the few myofibrils, which would both dissipate and disorganize effective contraction. In view of the relatively undeveloped sarcoplasmic reticulum, however, it is possible that randomly oriented myofibrils may produce a wringing action within the cell which would expedite motion of extracellular fluid in and out of cell.

For many reasons, a functional relationship
tached collagen, that light particularly view of arterial be predominantly the artery and sinus node “fibers” might lead to transmission of arterial motion to the fibers. In view of the ultrastructural appearance, and particularly since these fibers now appear to be predominantly transitional cells, a different functional relationship between expan-
sile arterial motion and pacemaking function of the sinus node may be considered. In view of the spongelike structure of clusters of P cells which are surrounded by closely at-
tached collagen, and the paucity of sarco-
plasmic reticulum with unusually active pin-
cytosis, it is possible that motion of the sinus node artery facilitates the transport of extra-
cellular fluid to the surface of individual P cells by producing a wringing action of the collagen framework. This action may be particularly important in view of the organization of P cells into clusters which are jointly surrounded by a basement membrane, since current concepts hold that the basement membrane in myo-
cardium probably acts as a diffusion barrier just as it does in capillaries.42

Bennett43 has suggested that pinocytotic ac-
tivity at the membrane may be an important index of the ion pumping mechanism or ac-
tive ion transport. Pinocytotic vesicles were conspicuously numerous in P cells, not only along their external surface adjacent to the basement membrane but also in some areas of intercellular contact in apposing plasma membranes. Since the latter sites did not ap-
pear to communicate with the extracellular space, one must consider whether these par-
ticular areas were involved in direct inter-
cellular exchange of ions or other materials (for example, neurotransmitter substances). On the other hand, in view of the poorly devel-
oped sarcoplasmic reticulum in P cells the increased pinocytosis may simply represent some form of cellular metabolism not con-
cerned directly with electrical activity. Even with known slow conduction in the sinus node it seems unlikely that active ion exchange oc-
curs directly or structurally in the pinocytotic vesicle, but the possibility that these vesicles are sites of energy synthesis indirectly responsible for active ion pumping indicates the need for histochemical studies in that region, par-
ticularly for nucleotides or adenyl cyclase, the cyclicizing enzyme.

Some of the organelles observed within the P cells are as yet too vaguely defined to attempt any interpretation of their exact function. For example, the membrane-bound inclusions with granules could represent structures for either secretion or ingestion; however, concerning their possible content of neurohormones it may be indicated that the granules are too small to represent catechola-
mines as presently known. The presence of centrioles may be interpreted as indicating that P cells divide, which is also supported by the presence of lysosomes (recently suspected to be important in cell division44) but this pos-
sibility was not confirmed by any observed examples of mitosis among P cells. With the primitive present state of our knowledge con-
cerning the fine structure of the sinus node, it is possible that the most important organelles have not been identified.

The similarity of the appearance of P cells in our study to nodal cells observed in dog2 and rabbit6 by others leaves little doubt of the identity of the principal cell of the sinus node. In the human sinus node the same cell is present. Although this does not make ex-
perimental canine and lapine data transpos-
able to human physiology, it does make qual-
ified comparisons more valid. The exceptional findings of Rhodin and associates,12 who re-
ported that the bovine sinus node is composed of cells indistinguishable from ordinary atrial myocardium, have been challenged by others working with different mammalian hearts6 as well as with bovine sinus node.13 Even with the light microscope the bovine sinus node is clearly different from adjacent ordinary at-
rial myocardium,45 and, we believe, the best present evidence indicates the ultrastructure of bovine sinus node and working myocardium is correspondingly different. The principal cell
of the bovine sinus node seems in all essential features similar to that in human, canine, and lapine hearts. Because the sinus node is not composed exclusively of P cells and because tissue samples employed for electron microscopy are so small, one can readily understand how information obtained from a sample containing mostly transitional cells or working myocardium (both present within and near the node) can be misleading.

With the introduction of high quality intracellular recordings of membrane action potential, the tendency is sometimes to think of the sinus node as a single cell. Interest in examination of single cells of the sinus node with the electron microscope can compound this misconception. The sinus node is not a single cell, nor even a simple aggregation of such cells. It is an intricately organized complex biological unit in which the P cell may act as the actual site of pacemaking, but the function of the entire node as the cardiac pacemaker cannot be considered so simply. Multiple potential sites of pacemaking must be synchronized into a consistent regular signal which can be optimally transmitted out of the node and which can be accelerated or decelerated as required. It has been suggested that clusters of P cells may act together as a functional unit and this may contribute to synchronization of impulse formation. But numerous adrenergic and cholinergic nerve endings, a rich ramification of arterioles and capillaries within the node, and the tantalizing presence of the disproportionately large central artery regularly within the center of the node all contribute to the ultimate function of the sinus node. Although much progress has been made in determining the fine structure of the sinus node and the electrophysiological behavior of single cells within it, we are only beginning to understand its complex and highly integrated function as a biologic unit.

Summary

Ultrastructure of the sinus node was studied in human and canine hearts and found to be similar. The principal cell of the sinus node is a small round pale cell with randomly distributed sarcosomes and sparse myofibrils. These cells have been designated as P cells. They occur in elongated clusters and make contact with each other in all directions but do not make direct contact with ordinary working myocardium. Transitional cells, which have features intermediate between P cells and working myocardium, serve as the connections between P cells and the rest of the heart. Each P cell is surrounded by a plasma membrane and groups of them are bound by a basement membrane. The only specialized junction between P cells is the desmosome, which occurs singly and seldom, with most contact being between apposing plasma membranes. P cells exhibit unusually active pinocytosis but have only a sparsely developed sarcoplasmic reticulum. The sarcosomes of P cells are much simpler in internal structure than are those of adjacent working myocardium obtained at the same time in the same heart in the same way, by direct perfusion with glutaraldehyde into the beating sinus node. Correlation of the electron microscopic appearance of the sinus node with that based on light microscopy is discussed, and some of the possible functional significances of the fine structure are considered.

References

An Historical Sketch of the Stethoscope

In 1851, Dr. Marsh, of Cincinnati, patented a double stethoscope. This had a membrane stretching over its objective end, and two gum-elastic tubes leading from the chest-piece to the ears. In this instrument the ear-pieces were inconvenient; it required two hands for its application, and the sounds conveyed were muffled and confused. These circumstances rendered it of little value. Dr. Cammann was familiar with two of these instruments, Landouzy’s and Marsh’s, and it was chiefly the fact that Marsh’s was patented which induced Dr. Cammann [my father] to devise a better one and give it freely to the profession. His binaural stethoscope, therefore, was not a new invention, but was, and is now, the best instrument of the kind devised. It was only after much labor and considerable expense that a satisfactory result was attained. The instrument was perfected in 1852, and described in the New York “Medical Times” of January, 1855. It is light, durable, easily carried, and a good conductor of sound. The ear-pieces are the best that have been devised, but room for improvement in this respect still remains.—D. M. CAMMANN: New York Med J 43: 465, 1886.
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