A Combined Morphological and Electrophysiological Study of the Atrioventricular Node of the Rabbit Heart


ABSTRACT

Thirteen rabbit atrioventricular nodes were studied both morphologically and electrophysiologically. Two of these nodes were subsequently reconstructed in three-dimensional fashion. From the morphological standpoint, it was shown that the atrioventricular node was divided into a smaller enclosed portion and a larger open portion by a fibrous collar derived from the central fibrous body. Three distinct nodal cell types were identified within these nodal segments. The open node was mostly occupied by transitional cells which merged proximally with the atrial myocardium. As they entered the enclosed node, some of these transitional cells merged into a knot of midnodal cells. Others passed circumferentially round this knot and together with the midnodal cells, joined with a tract of lower nodal cells. The latter cells were continuous with the His bundle anteriorly, but they also extended into the open node posteriorly. It was possible to correlate the activation sequence of the node accurately with the disposition of these cells. Using both reconstructions and techniques to mark cells from which action potentials had been recorded, it was shown that the transitional cells correlated with the AN zone of the node. Cells with N potentials were located in the environs of the midnodal cell knot. The anterior portion of the lower nodal cells correlated with the NH nodal zone. The posterior extension of the lower nodal cells and the overlay fibers of the anterior transitional cells both functioned as dead-end pathways. Histologically distinct tracts were not identified within the internodal atrial myocardium.

KEY WORDS
- AN cells
- N cells
- NH cells
- transitional cells
- lower nodal cells
- midnodal cells
- internodal atrial myocardium
- dead-end pathways

Early electrophysiological investigations of the atrioventricular node indicated that the area producing atrioventricular delay was larger than the area delineated as nodal by morphological techniques (1, 2). Using action potential morphology, this electrophysiological zone of delay was divided into three areas which were designated the AN, the N, and the NH zone, respectively. At the time of these early studies, Paes de Carvalho and de Almeida (2) stated that only accurate marking techniques would enable the morphological counterparts of these zones to be elucidated. Some investigators have subsequently demonstrated morphologically distinct zones which appear to them to be comparable with those zones described electrophysiologically, but no direct correlation has been made (3, 4). Since the results of one of these morphological studies fit so closely with the findings of recent electrophysiological investigations in which multiple microelectrodes and surface electrodes were used (5, 6), it seemed desirable to attempt a direct comparison by performing electrophysiological and morphological studies on the same preparation. This investigation was therefore carried out to compare the sequence of activation of the rabbit atrioventricular node directly with its architecture and to correlate cells from morphologically known zones with their action potentials.

Methods

Thirteen adult rabbits were anesthetized with sodium pentobarbital (20 mg/kg, iv). The chest was opened, and the heart was rapidly removed. The
preparation, containing most of the right atrium, the interatrial septum, the right atrioventricular ring, and a small piece of the interventricular septum, was placed in a tissue bath perfused with modified Tyrode's solution as described previously (5). Temperature was maintained at 37.0 ± 0.5°C, and pH was 7.35 ± 0.05.

Surface electrodes were placed on the atrium and the His bundle to record surface electrograms and to stimulate both areas. Transmembrane potentials from cells in the atrioventricular nodal area were recorded with either a brush electrode consisting of ten microelectrodes or single microelectrodes (5). The recorded signals were stored on tape, using an Ampex 1300 tape recorder at a tape speed of 15 inches/sec. For time measurements, the tapes were played back at a speed of 1.875 inches/sec, and the complexes were recorded on a 16-channel Elema inkwriter at 120 mm/sec, resulting in a time resolution of 1 msec. A detailed description of the recording and stimulating procedures has been published (5).

A single experiment was conducted on each of the rabbit hearts. In two experiments, a detailed map of the spread of excitation during antegrade and retrograde conduction was developed, and the preparation was then used to make a three-dimensional reconstruction demonstrating the nodal architecture. In four other experiments, a Wenckebach type of atrioventricular nodal block was produced by stimulating the atrium at a rapid rate; the general sequence of activation was then recorded in the atrioventricular nodal area. These preparations were sectioned and correlated by careful microscopic examination of the sections. These studies gave us an impression of the nodal zones in terms of their electrophysiological behavior; we could approximately locate areas showing typical responses and correlate them with the reconstructed nodal architecture. In the last seven experiments, cells producing known action potentials during the Wenckebach phenomenon were identified by injecting cobalt through the microelectrode. The brush microelectrode was used in the first of these experiments, and in the subsequent six experiments cobalt was injected through a single microelectrode. In each case, the electrodes were filled with a 2.6M KCl solution to which a 0.5% CoCl₂ solution had been added (7, 8). After an action potential had been recorded, the microelectrode was connected to the positive pole of a direct-current source to produce electrophoretic migration of cobalt ions into the cell. The total amount of charge passed varied in our experiments from 3 to 90 μCoul. With a few exceptions, the microelectrodes were discarded after cobalt migration had been induced. In all experiments, photographs of the preparation were made through a dissecting microscope at a magnification of 25 or 40x with the microelectrode (or the microelectrode brush) in different positions. Scale drawings were made of each preparation from these photographs. Since in most experiments the number of recording locations was limited, varying from three to eight, most of the recording positions of either the microelectrode brush or the single microelectrode were marked by placing a small bipolar surface electrode on the interventricular septum just below the microelectrode position. A strong current was then passed through the surface electrode so that a superficial, localized burn was produced on the interventricular septum. Similar burns were made at the end of the experiment on the interatrial septum. These burns served not only as landmarks during histological examination of the sections but also as indicators of the plane of sectioning. In experiments in which extensive activation maps were made, using as many recording sites as possible, burns were made at only a few positions. Immediately after recordings were concluded, a block of tissue containing the atrioventricular nodal area was excised and prepared for histological examination. This block always consisted of the adjacent segments of the interatrial and interventricular septa and included the ostium of the coronary sinus and the posterior portion of the aortic root. The block was frozen in isopentane previously cooled in liquid nitrogen. It was then oriented on a cryostat tissue holder and placed on the rapid-freeze stage of an AO Cryo-Cut cryostat. Three blocks were oriented to allow sectioning in a plane parallel to the ventricular septum, considering the septum as being placed on its apex; however, this plane did not allow adequate differentiation of the atrioventricular ring. The remaining hearts were all oriented to allow sectioning at right angles to the ventricular septum in the vertical plane. The blocks were then sectioned in the AO Cryo-Cut to give slices 20 μm thick.

All sections were collected on glass slides and after drying were stained. The first six blocks were all stained with either Gomori's trichrome stain or hematoxylin and eosin. The subsequent blocks contained cobalt solution that had been injected through the microelectrodes. Following sectioning, therefore, these slices were treated for 2 hours with a solution of alpha-nitrosobeta-naphthol (7, 8). This produced orange deposits of cobalt within the tissue, and, to prevent masking of this deposit the sections were counterstained only with hematoxylin. This procedure allowed adequate delineation of cell types within the node.

Subsequent to the examination of the tissue sections, we decided that accurate reconstruction of the nodal architecture was necessary. Consequently, two of the hearts were reconstructed using Perspex sheets to represent the sections. In one heart, sectioned parallel to the septum, each section was drawn on a 2-mm thick Perspex sheet using a magnification factor of 100x. In each section the area occupied by either transitional, midnodal, or lower nodal cell types was indicated by a different color. The musculature of the atria and the ventricles was also displayed in outline, and the fibrous ring was indicated. The sheets were subsequently stacked in sequence, and the nodal architecture was displayed by transillumination of the entire block.

The other heart from which accurate action

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potential sequences had been recorded was sectioned at right angles to the septum. In this preparation the node extended through three hundred sections. One section out of every five was reconstructed on a 1-mm thick Perspex sheet using a magnification factor of 100x. As in the previous heart, specialized cell zones, musculature, and fibrous tissue were drawn in different colors. The surface marking of each section was then cut round with a mechanical saw and the surplus Perspex removed so that only that Perspex representing the tissue remained. Constant reference points were marked on each sheet and drilled through. The sheets were then assembled on a base plate containing five rods in the positions of the reference points. Each sheet was separated from its neighbor by 9-mm thick washers so that each drawing was 1 cm from its neighbors. The whole assembly was then transilluminated from behind. An artist’s impression of this reconstruction is shown in Figure 2.

Results

MORPHOLOGICAL FINDINGS

To provide a sound basis for electrophysiological correlations, we must first describe nodal architecture as we have defined it. When we considered the atrioventricular junctional area with the atrioventricular annulus aligned in the horizontal plane, the atrial septum was superior to the ventricular septum. We chose to view the septum from its right side (Fig. 1). The main landmarks of this junctional region were the large coronary sinus posteriorly and the central fibrous body anteriorly. The latter was a clearly visible structure lying posterior to the root of the aorta. The tricuspid valve ring formed the inferior extent of the atrial segment of this junction, but, owing to the low origin of this ring relative to the mitral ring, the lower part of the atrial septum overlaid the interventricular septum (see Fig. 4). Another constant feature of the area was a blood vessel that bisected the area of the atrial septum between the coronary sinus and the fibrous body. The vessel originated in the interatrial septum and passed downward and forward into the tricuspid valve septal cusp. The superior area of the junctional region was the atrial septum itself. The atrial septal myocardium forming this area was split into muscular bands by the orifices within the septum. Thus, three main bands approached the fibrous annulus. The anterior band approached from in front of the fossa ovalis. The middle band approached between the fossa ovalis and the coronary sinus. This band itself was composed of two segments; one descended between the superior vena cava and the inferior vena cava and the other passed between the inferior vena cava and the coronary sinus from the posterior atrial wall. The two segments merged in the region of the sinus septum. The third main band was present beneath the orifice of the coronary sinus and formed a muscular bundle in the atrial margin of the tricuspid valve septal cusp. This bundle was in direct communication with the inferior extension of the crista terminalis as it descended behind the coronary sinus.

When studied histologically, the distal extent of the atrial septum between the coronary sinus and the central fibrous body was almost exclusively occupied by cells with histological characteristics of specialized conducting tissue (Figs. 1, 3, 4). The cells were not, however, of uniform morphology throughout the node but could be delineated into distinct cellular zones. The dimensions and the interrelationships of these different zones were clearest when we considered the specimen sectioned in the frontal plane and subsequently spatially reconstructed (Fig. 2). It was found that the node was divided into two parts by a fibrous collar which encircled the anterior part of the node. This anterior or enclosed node was much smaller than the posterior or open node (Fig. 2, bottom). The
enclosed node was that part which was directly continuous with the His bundle. The latter structure passed superficially on the right side of the fibrous body before turning deeply into the annulus and perforating it to reach the ventricular septum. Since the enclosed node was surrounded on its superficial, deep, superior, and inferior sides by the fibrous collar, the atrial musculature was able to contact this part of the node only via its junction with the open nodal component. The fibrous collar itself was derived from the central fibrous body. When traced posteriorly, it became attenuated and was continued into the substance of the atrial septum as the Tendon of Todaro. The posterior or open node was a much larger area, and its deep boundary was formed by the fibrous annulus, which separated it from the ventricular septum (Figs. 3, 4). Superiorly the open node was continuous with the myocardium.

Diagrams showing the structure of the atrioventricular node. Top: Artist's impression of a reconstruction of the septum from which detailed activation sequences were recorded. Bottom: Diagrammatic representation of the area occupied by the different nodal cells. The lower nodal cells (LNC) extended throughout the node and were joined in the posterior node by the lower fibers of the posterior group of transitional cells (TC). The upper fibers of the posterior group, together with the middle group and the anterior group of transitional cells, passed into the enclosed node to form the knot of midnodal cells (MNC). In addition, some circumferential transitional cells passed around the upper nodal knot to impinge directly on the lower nodal cells. Note that the fibers of the anterior group of transitional cells passed in an anterior direction from the junction of the open node and the enclosed node and overlaid the fibrous collar. CS = coronary sinus, and AM = atrial myocardium.

Drawings of the photomicrographs illustrated in Figure 4 demonstrating the different areas of the node occupied by the histologically differentiated nodal cell types. Note that the interatrial septum (IAS) and the interventricular septum (IVS) were obliquely orientated so that the distal segment of atrial myocardium occupied by the nodal cells overlaid the ventricular myocardium and was separated from it by the fibrous annulus. a–c: Sections through the open node, and running from posterior to anterior. The posterior group (PG) and the middle group (MG) of transitional cells (TC) occupied the entire thickness of the distal atrial tissue and abutted inferiorly against the posterior extension of the lower nodal cells (LNC). The overlay cells of the anterior group (AG) of transitional cells are already visible in section c; the backward portion of the fibrous collar is indicated by the arrow. Section c also shows the connection with the deep portion of the interatrial septum. d: Section taken through the junction of the open and enclosed nodes. The fibrous collar is better formed (arrows) and the anterior group of transitional cells is clearly visible. Note that the node had a trilaminar arrangement, being composed of circumferential transitional cells within the collar, midnodal cells (MNC), and lower nodal cells. e and f: Sections taken through the enclosed node. The trilaminar arrangement can be seen. Note the fibrous collar separating the node from the overlay extension of the anterior group of transitional cells, again indicated by the arrows. The enclosed node overlaid against the central fibrous body (CFB); the origin of the mitral valve (MV) is indicated.

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dium of the atrial septum (Fig. 2). On the basis of histological characteristics, it was possible to divide the cells occupying the node into three types termed transitional, midnodal, and lower nodal cells (Fig. 2). The transitional cells were found for the most part in the open node, and, as their name suggests, they were intermediate in morphology between the atrial myocardial cells and the other nodal cells (Figs. 3–5). They were distinguished from atrial cells by their pale staining reaction, their smaller size, and by the fact that they were separated from their neighbors by connective tissue septa. Three distinct groups of these cells were recognized within the open node, and they made contact with the three bands of septal myocardium delineated by the orifices within the septum. The posterior group of transitional cells extended for only a short distance proximally before merging with the myocardium beneath and behind the coronary sinus (Fig. 3a). The middle group of transitional cells was much more extensive and connected with both the myocardium of the sinus septum and also the deeper myo-

**FIGURE 4**

Photomicrographs of selected sections of the specimen reconstructed and demonstrated in Figure 2. Drawings of these sections are described in Figure 3, where the areas of specialized tissues are indicated. The magnification factor is indicated by the scale in A and is the same for each section. TC = transitional cells, MG = middle group of transitional cells, AG = anterior group, CTC = circumferential transitional cells of enclosed node, OC = overlay extension of the anterior group of transitional cells, FC = fibrous collar, LNC = lower nodal cells, MNC = midnodal cells, AM = atrial myocardium, and VM = ventricular myocardium.
FIGURE 5

Higher power photomicrographs of histological sections from the specimen reconstructed and demonstrated in Figure 2. These sections demonstrate the histological characteristics of the different specialized cells of the node. A: Section taken from the proximal extent of the middle group of transitional cells (TC), which shows the area of junction with atrial myocardium (AM). The transitional cells were small and pale staining and separated from each other by much more connective tissue than was present among the atrial myocardial cells. B: Shows the distal edge of the posterior group of transitional cells and is a detail of Figure 4A. Note the well-delineated group of lower nodal cells (LNC) and the contact made between these cells and the transitional cells. C: Section through the junction of the open and enclosed nodes. Note that the superficial overlay cells (OC) were separated from the node by the fibrous collar (arrows) but that contact was still made with the deeper portion of the atrial septum. Note also the trilaminar arrangement of the node, formed by the circumferential transitional cells (CTC), the midnodal cells (MNC), and the lower nodal cells (LNC). FA is the fibrous annulus. D: Section through the middle part of the enclosed node. It is a detail of Figure 4F. Note that the trilaminar arrangement was still present but that the lower nodal segment had expanded. The fibrous collar (arrows) was formed at this level, separating the node from the overlay cells. The magnification factor is indicated in D by the scale and is the same for each section.

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The extent of the middle group of transitional cells decreased considerably when the group was traced anteriorly, and in the region of the junction with the enclosed node these cells merged with the anterior group of transitional cells. This group of cells varied considerably in extent from specimen to specimen. In the reconstructed specimen, the group was well formed and could be traced anteriorly and superiorly from the junctional zone into the septum. An extension from this group passed superficially to the fibrous collar and overlaid the anterior node (these overlay cells are well demonstrated in Figs. 3 and 4), but no contact was made with the underlying tissues of the enclosed node. In all specimens studied, these cells were observed to terminate in the tricuspid valve base (Fig. 3d, e). Throughout their extent, the transitional cells were mostly oriented toward the enclosed node, with the exception of the anterior group which first passed posteriorly from the septum, curved round the fibrous collar to join the more posterior transitional cells, and then turned anteriorly into the enclosed node with the other cells. Having entered the enclosed node, some of the cells retained their transitional characteristics and passed circumferentially directly inside the fibrous collar (Figs. 3d, e and 5C). However, the more centrally placed transitional cells merged together and formed a knot of tightly packed spherical cells at the entrance to the enclosed node (Fig. 3d, e). These cells were the midnodal cells; they were situated inside the circumferential transitional cells (Fig. 3d, e). The midnodal cells were small and not separated by much connective tissue (Fig. 5C, D). They formed only a small knot of cells and extended about halfway into the enclosed node (Fig. 2). These cells in turn, together with the circumferential transitional cells, abutted against a well-formed bundle of cells oriented in a direction parallel to the fibrous annulus. These latter cells occupied the lower half of the enclosed node (Fig. 2) and were larger and stained more intensely than the transitional and the midnodal cells (Fig. 5C, D). They were separated by connective tissue septa through which coursed large nerve bundles. They constituted the lower nodal cells. When it was traced anteriorly, the bundle of lower nodal cells expanded rapidly and became continuous with the His bundle. It was not possible to distinguish lower nodal cells from His bundle cells on morphological criteria alone. The demarcation point was made arbitrarily according to the position of the tract of cells. However, the lower nodal cells were not confined to the enclosed node but could also be traced in a posterior direction. Thus, they passed into the open node as a distinct tract of cells occupying the most distal segment of the atrial myocardium (Figs. 3–5). The tract of lower nodal cells was much smaller in the open node than it was in the enclosed node (compare Fig. 3a with Fig. 3e). The transitional cells of the posterior and middle groups were in contact with the superior surface of the lower nodal tract in the open node (Fig. 5B). However, as already indicated, the main orientation of the transitional cells was toward the junction of open and enclosed nodes.

In briefly summarizing this somewhat complicated morphological arrangement, it can be stated that the node is divided into open and enclosed segments by a fibrous collar. The larger open node is that part in contact with the atrial myocardium via three distinct groups of transitional cells. These cells merge at the entrance of the enclosed node to form a small knot of midnodal cells, which themselves abut against a larger tract of lower nodal cells in the enclosed node. However, the more superficial of the transitional cells pass directly around the midnodal cells within the fibrous collar to join directly with the lower nodal cells. The enclosed node therefore has a trilaminar appearance (Figs. 2–5). The lower nodal cells are responsible for forming the His bundle; however, they are also continuous throughout the open node and pass posteriorly beneath the coronary sinus.

**SEQUENCE OF ACTIVATION OF THE NODE**

Complete action potential maps of the atrioventricular junctional areas were recorded from the two preparations which were subsequently spatially reconstructed to demonstrate nodal architecture. In Figure 6 the activation sequences of both antegrade and retrograde conduction are shown. The nodal architecture is demonstrated in the insets to the figure. This particular specimen was that from which Figure 2 was made. These activation sequences are in agreement with previous descriptions (5).
There was a dual input to the nodal area in antegrade excitation (Fig. 6A). The posterior input descended beneath the coronary sinus, whereas the anterior input was from the atrial septum. The posterior input corresponded to the posterior group of transitional cells, and the anterior input corresponded to the middle and anterior groups of transitional cells. The fact that the anterior transitional cells were separated from the enclosed node by the fibrous collar was reflected in the activation sequence. Thus, part of the anterior input curved in an S-shaped way to merge posteriorly with the remaining input. The combined input then entered the enclosed node from behind. In the area where transitional cells, midnodal cells, and lower nodal cells overlaid each other, the activation pattern was more complex. Superficial cells were activated earlier than deeper cells, and cells at the superior nodal margin were activated earlier than the more inferiorly located lower nodal cells. The cells which were more or less synchronously excited prior to excitation of the His bundle corresponded to the anterior group of lower nodal cells. In this experiment, no recordings were obtained from the posterior extension of the lower nodal cells. In other experiments, however, they were shown to take no part in normal nodal activation.

For retrograde conduction (Fig. 6B), the activation sequence was approximately reversed except that the earliest "output" to the atrium was via the anterior and middle groups of transitional cells. The crista terminalis, in contrast, was activated relatively late. These findings are again in keeping with previous descriptions (5).

**ELECTROPHYSIOLOGICAL CHARACTERISTICS OF THE NODE**

During the course of the experiments, it was noted that the cells constituting the "mainstream" of the node exhibited differences in electrophysiological behavior. These differences were manifested in activation times, action potential configuration, and response to a Wenckebach phenomenon evoked by rapid atrial stimulation. They are demonstrated in diagrammatic form in Figure 7. Since the terms AN, N, and NH zones are in such widespread usage, we will continue to employ them. However, it is necessary to provide specifications of the terms as we have employed them. The AN cells were activated roughly between 20% and 80% of the atrial-His interval. Their action potential configuration changed gradually from a typical atrial potential with a rapid upstroke to a typical nodal potential with a slow upstroke. These changes were observed as the recording microelectrode was shifted from the atrial margin of the node to positions closer to the His bundle. During the Wenckebach phenomenon, the moment of activation and the action potential configuration remained constant in successive beats even though...
conduction time through the node increased and block occurred. Thus AN cells were proximal to the site where the typical increment in conduction time and conduction block were produced during a Wenckebach phenomenon. Cells with these characteristics were always located in the zones of morphological transitional cells in the open node. N cells were activated late (roughly between 70% and 98% of the atrial-His conduction time), and the upstroke velocity of their action potential was always slow. During the Wenckebach phenomenon, the upstroke velocity and the amplitude gradually diminished in successive activations until a local response was recorded during the dropped beat. These cells were located in a relatively restricted area which could be roughly correlated with the entrance to the enclosed node. However, the area appeared to be larger than that occupied by the knot of midnodal cells. NH cells were activated just prior to the His bundle. Their action potentials had a fast upstroke, and during conduction block they showed either a small local response or no response at all. These cells were clearly distal to the zone where the conduction delay and block occurred during the Wenckebach phenomenon. Their position was coincident with the anterior group of lower nodal cells.

We emphasize that the transition between these zones was gradual, in particular that between the AN and N zones. Thus, some late AN cells exhibited action potentials influenced by events occurring more distally in the node, since action potential duration was shorter when block was present. Similarly, there was no abrupt transition between cells with slow and fast upstrokes in the N and NH zones.

Certain cells in the atrioventricular nodal area could not be categorized within this simple scheme. Some of the cells can be considered as the "dead-end pathways" that have been previously described (5). They did not participate in transmitting the impulse from the atrium to the His bundle and vice versa. This fact became evident when the moment of activation of such a cell was expressed as a percent of the atrial-His and His-atrial times during antegrade and retrograde conduction, respectively. The sum of these times for a cell in the nodal mainstream added up to approximately 100%. For dead-end pathway cells, the sum was considerably greater than 100%, indicating that they were activated too late in both modes of conduction. These cells were located in two areas; one was consistent with a position in the posterior extension of the tract of lower nodal cells in the open node and the other was consistent with a position in the overlay zone of anterior transitional cells. In some experiments, evidence for longitudinal dissociation was obtained, since some cells showed signs of local block and reentry. Such cells were identified only in the zone of posterior transitional cells.

The correlation of morphological and electrophysiological zones just described was made on the basis of comparison of reconstructions and known microelectrode positions. To provide a more precise correlation, cobalt was injected into known cell types in the final seven experiments, once using a brush electrode and in the other experiments using single microelectrodes. The cobalt was

FIGURE 7

Diagrammatic representation of the different electrophysiological characteristics of cells on the mainstream of nodal activation between the atrial myocardium and the His bundle. The traces show typical action potential configurations recorded during a Wenckebach phenomenon as the microelectrode brush was moved from proximally in the node (top trace) to the nodal-His bundle junction (bottom trace). Note that transitional cells could be correlated with AN cells and that these cells had activation times between approximately 20% and 80% of the atrial-His interval; they were proximal to the zone where the increment in conduction time and the conduction block occurred. The N cells could be correlated with either midnodal or adjacent transitional cells, and they had activation times of 70-98% of the atrial-His interval. These cells produced block and incremental delay. The NH cells correlated with the anterior group of lower nodal cells in the enclosed node, and their activation times were between 93% and 100% of the atrial-His interval. These cells were distal to the zone of block.
subsequently visualized, and the precise cell producing the known action potential was identified. Cobalt was injected 39 times in the seven experiments. We accepted only 16 of the resulting "spots" as indicating electrode position. In each of these 16 cases, a single cobalt spot was found at the expected site in a preparation from which no histological sections were missing. The reasons for rejection of 23 injections were multiple. Sometimes the amount of cobalt was too small to be detected; damage or loss of some sections also prevented identification of some cobalt deposits. Multiple or diffuse cobalt deposits resulted from leakage from broken electrode tips or leakage after the application of current during withdrawal of the electrode. In 1 of the 16 validated positions, insufficient electrophysiological data was present to make an accurate categorization. The locations of the 15 spots related to known action potential types are demonstrated in Figure 8. Four of the spots had potentials of the NH type. All of these spots were located in the anterior group of the lower nodal cells within the enclosed node. Four spots were located in typical N cells. None of these spots was in the middle of the knot of midnodal cells, but 3 were adjacent to this knot. Two were in the junction with transitional cells, and 1 spot was in the junction with the lower nodal cells. The remaining N cell was located some distance behind the enclosed node in a transitional cell of the open node. In another instance, not shown in Figure 8, a diffuse cobalt deposit was identified in the middle of the midnodal cell group following the recording of an N action potential. Four action potentials were recorded that were typical of AN cells, and the corresponding 4 cobalt spots were identified in the transitional cells of the open node. One of these cells is illustrated together with its action potential in Figures 9 and 10. Another of these cells exhibited characteristics of longitudinal dissociation. The three remaining action potentials were all typical of cells in dead-end pathways. One of these cells was localized to the anterior group of transitional cells in an overlay position superficial to the enclosed node. The other two cells were both identified in the open node in the posterior extension of lower nodal cells.

Discussion
The morphological results of this investigation have established the spatial architecture of the rabbit atrioventricular node. To a certain extent these results confirm a previous description based on histological and histochemical techniques (4), but they extend this investigation in terms of the interrelationships of the distinct nodal zones occupied by different cell types. As Truex and Smythe (9) have rightly pointed out, until a three-dimensional structure has been reconstructed from histological sections it is difficult to be sure of precise architecture. Although we cannot be certain, we believe that the disposition presently demonstrated is in keeping with the original description of the node by Tawara (10). He stated that the "atrial part of the conducting system" consists of two parts: an anterior part forming a tight network which he called "knoten," and a posterior part consisting of small cells running in small bundles separated by connective tissue and gradually merging with atrial

![Diagram](http://circres.ahajournals.org/)
myocardium. The knoten corresponds to our enclosed node, the posterior part to our zone of transitional cells (open node).

Failure to recognize the open node as specialized tissue that is part of the atrioventricular node may account for the apparent discrepancy between the present results and those described in the rabbit node by James (11). It is our opinion that the "node" described by James represents only the enclosed node of our investigation. Indeed, it has already been stated (4) that the extent of the open node and the laminations of both the enclosed and the open node can only be appreciated following study of tissue prepared in such a way that histologically processed sections can be directly compared with sections processed for cholinesterases. With our present knowledge, we believe that we can in fact distinguish the trilaminar arrangement in Figure 4 of James' paper (11). Thus, we conclude that some of the differences concerning nodal architecture are referable to variations in technique and interpretation.

The three nodal zones are more easily distinguished in cryostat-prepared sections than they are in sections prepared after

![Figure 9](image-url)

**Figure 9**

Demonstration of a selected identified cell and its electrophysiological characteristics. Action potentials typical of an AX cell during antegrade conduction (A), retrograde conduction (B) and antegrade 3:2 Wenckebach block (C) are shown. Top trace = atrial electrogram, middle trace = action potential, and bottom trace = electrogram of His bundle showing deflections from overlying atrial muscle (A) and from His bundle (H). Numbers indicate moments of activation (t = 0 corresponds to the stimulus artifact). Note that during the Wenckebach phenomenon the interval between the atrial complex and the action potential upstroke remained constant in successive activations. The 50-mv calibration refers to the transmembrane potential in the middle trace.
embedding in paraffin. These differences are also likely to underlie the variations in interpretation of the anterior overlay fibers. James (11) thought that these cells formed bypass tracts which inserted into the nodal-His bundle junction. Our findings demonstrated that these cells were always separated from the enclosed node by the fibrous collar and that the orientation of the cells was from anterior to posterior, representing part of the S-bend of the anterior input from the atrial septum. Indeed, we subsequently demonstrated that the more inferior of these overlay cells formed a dead-end pathway rather than a bypass tract. Another important difference between our findings and those of James (11) concerns the nature of the interatrial myocardium. James believed that this tissue contained specialized interatrial tracts in the rabbit heart. We were unable to demonstrate such histological specialization. The atrial myocardium was split up into muscle bundles by the septal orifices, but histological differentiation of the musculature was not observed until each of the muscle bands gave rise to the transitional cells of the node. These cells extended only a short distance into the atrial myocardium from the fibrous annulus. The electrophysiological results of this investigation indicated that the atrioventricular node of the rabbit could be interpreted in terms of the AN, N, and NH zones delineated by Paes de Carvalho and de Almeida (2), albeit with the modifications described. There are differences between their representation and that presently reported. Paes de Carvalho and de Almeida (2) indicated that the nodal zones represented three strata throughout the node. In contrast, our findings indicated that the zone of N cells was confined to the junctional area between the enclosed and open nodes. Furthermore, Paes de Carvalho and de Almeida (2) stated that the AN zone was continuous with the atrioventricular ring bundle encircling the tricuspid orifice (Fig. 1, ref. 2). Previous morphological studies have demonstrated that the specialized tissue of the atrioventricular ring is in fact continuous with the lower nodal cells (4, 12), which

FIGURE 10

Low power (A) and high power (B) photomicrographs of the cobalt deposit which was identified in a transitional cell of the posterior group. Note also the burn mark, used to provide approximate location of the electrode, in the lower left corner of A. Similar cobalt deposits were identified for cells in the remaining 14 positions shown in diagrammatic form in Figure 8. TC = transitional cell; VM = ventricular myocardium; and LNC = lower nodal cell.

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anteriorly are correlated with the NH cells. The present results showed that cells of the posterior part of the lower nodal tract in the open node functioned as a dead-end pathway and were not comparable to the NH cells. The results of our study also exclude under normal conditions the possibility that this tract of cells could function as a bypass of the AN and N nodal zones by virtue of its morphological continuity with the atrioventricular bundle. This fact was demonstrated conclusively in the present study, since a cell of the lower nodal tract in the open node showed a full-blown action potential in the presence of conduction block at the level of the NH cells during a Wenckebach phenomenon, indicating that the excitatory wave within the posterior extension was of insufficient strength to excite the much larger anterior part of the tract.

It is evident from our study that reasonable correlations can be made between the electrophysiological and morphological zones of the rabbit atrioventricular node. Previous attempts have been made to correlate nodal architecture and electrophysiology with varying degrees of success. As indicators of electrode position, both the glass left behind in the preparation (3) and the mark produced on the endocardial surface (13) have been used. We have been unable to identify endocardial marks left by microelectrodes, and although we occasionally found pieces of microelectrodes in histological sections we did not think that they accurately identified the recording position, since they could have moved from their original position during handling of the preparation. Sano et al. (14) used a different method to localize the cell. They injected ferricyanide solution via the recording electrode and visualized it with ferrous chloride applied endocardially. A blue ring was identified at the boundary of the two solutions, but the authors mention the "infrequent successful formation of the blue ring."

Two other investigations have been performed using the cobalt marking technique (7, 8); that of Nagata (7) is particularly noteworthy. He was able to divide the node into three zones and to identify the morphological counterparts of these zones. It is again difficult to be sure if these zones correspond precisely with those presently defined, but Nagata (7) noted that the atrial part of the node consisted of specific muscle fibers separated by connective tissue. From this part of the node he recorded action potentials with both slow and fast upstrokes. He observed that in the middle part of the node the specific fibers were more densely packed and in the lower node the fibers were parallel.

Our own correlations are in close agreement with those of Nagata (7). We were able to record AN action potentials from the transitional cells of the open node, including the anterior group of these cells which recurred over the enclosed node toward the anterior septum. N potentials were recorded from the area in the junctional zone between the enclosed and open nodes. Although some of these potentials were recorded from the small knot of midnodal cells identified within the enclosed node, it is also certain that other N potentials were recorded from transitional cells of the open node. We were not able to distinguish between the transitional and midnodal cells of the enclosed node using the electrophysiological techniques. However, close correlation was obtained between the anterior group of lower nodal cells in the enclosed node and the zone of NH potentials. The dead-end pathways were conclusively correlated with the anterior overlay cells and the posterior extension of the lower nodal cells. Because typical N potentials could be recorded from both midnodal and transitional cells and because transitional cells themselves could give rise to action potentials with widely different configurations, it is evident that action potential configuration is determined to only a limited extent by cellular morphology. However, it may be determined to a greater extent by nodal architecture. Thus, in the anterior lower nodal cells where fibers are arranged in a regular bundlelike structure, the upstroke of the action potential is fast and conduction is synchronous and rapid. In contrast, the arrangement of transitional cells in smaller irregular bundles separated by connective tissue may contribute to the gradual decline of upstroke velocity, action potential amplitude, and conduction velocity as the cells become activated later and later in the atrium-His interval. These transitional cells are responsible for a considerable portion of the nodal delay. During a normally conducted
and antegrade impulse, 60% of the total atrial-His interval is involved in traversing these transitional cells; the first 20% is taken up in passage through the atrial myocardium from the stimulating electrode and only the latter 20% is involved with passage through the enclosed node. However, during a Wenckebach phenomenon, the increment in conduction time occurs in the N zone, which we have shown to be composed of the midnodal cells and their surroundings at the junction of the open and enclosed nodes. Since the midnodal cells are tightly packed and do not consist of loose strands separated by fibrous tissue, it is possible that special membrane characteristics of this region are a further contributory factor in the production of incremental nodal delay. This same zone is the site of the block produced in the Wenckebach phenomenon. This finding in itself does not mean, however, that transitional cells more distant from the N zone are incapable of producing block. We have recorded several instances of local block in the posterior group of transitional cells. It is also well known that premature atrial beats with different preceding cycle lengths can be blocked at different levels within the atrioventricular node (15, 16).

Acknowledgment
We are grateful to Mr. D. van der Moot, Mr. R. Verhoeven, Miss S. Teengs, and Miss I. Dijk, Wilhelmina Gasthuis, Amsterdam, and to Mr. P. Howarth, Anatomy Department, University of Manchester, for their valuable technical assistance.

References
14. Sano T, Tashiki M, Shimamoto T: Histological examination of the origin of the action potential characteristically obtained from the region bordering the atrioventricular node. Circ Res 7:700-704, 1959
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ROBERT H. ANDERSON, Dirk Durrer, MICHEL J. JANSE, FRANS J. L. VAN CAPELLE, JACQUES BILLETE, ANTON E. BECKER and DIRK DURRER

Circ Res. 1974;35:909-922
doi: 10.1161/01.RES.35.6.909

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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