TRANSMEMBRANE recording from single fibers of the atrioventricular (A-V) node of the rabbit heart has shown action potentials exhibiting rising phases with a much slower time course than those of plain heart muscle.1,2 It has also been shown that the area in which such nodal potentials are recorded is not sharply delimited, regions being found at each side of the nodal area in which action potential shapes change gradually to those of the atrium or His bundle.2 An important fraction of the A-V delay was ascribed to a narrow region of the node where slowing of the action potential upstroke and of the propagation process are most conspicuous.2 Determinations of apparent propagation velocity in this area showed values as low as 0.02 M. sec., a figure in good agreement with that found subsequently by Scher and his colleagues,3 using a different technic. On the basis of these experimental facts, the conclusion has been reached that delay in the excitation of the His bundle is most probably explained by a low propagation velocity within the A-V node2 which has tentatively been attributed to decremental conduction2,4 (see page 806). However, more precise data on the sequence of activation of the A-V node were needed before a further understanding of the mechanism of delay could be reached.

**Methods**

Rabbits weighing around 1.5 Kg. were either killed with a single blow or anesthetized with intravenous injection of sodium pentobarbital. The heart was excised and dissected in a constant temperature (35 C.) bath under continuously flowing modified Tyrode solution (NaCl 137 mM; KCl 2.7 mM; CaCl2 2 mM; NaHCO3 12 mM; NaH2PO4 7.2 mM; MgCl2 0.5 mM; glucose 5.5 mM) saturated with a mixture 5 per cent carbon dioxide in oxygen. The endocardial surfaces of the right atrium and the right ventricle were exposed and the whole preparation pinned on a paraffin bed in the tissue bath. Such a preparation contains both the physiologic pacemaker and the tissues involved in atrioventricular excitation. All these parts are easily accessible to microneedle recording.

Figure 1 shows a sketch of part of the preparation. Well-known landmarks are inferior vena cavea (IVC), coronary sinus (CS), tricuspid valve (AVV), interatrial (IAS) and interventricular (IVS) septa. Right atrial roof (RAR) and right ventricular wall (RVW) are also noted, as well as the previously described sinoatrial ring bundle (SARB).5 Mapping of the normal activation of A-V node was performed in preparations regularly driven from the atrial roof. Intracellular recording from as many as 40 points, located mostly in the coronary sinus region, provided data on both time of arrival of activity (time of activation) and action potential shapes at the various parts of the preparation. The exact location of each point was read from Vernier scales attached to the micromanipulator. The time of activation of a given cell was measured as the latency between the time of stimulation and beginning of the action potential. The results of the latency measurements and action potential observations could then be plotted accurately on a map of the preparation.

Transmembrane potentials were recorded by means of 3 molar KCl-filled micropipettes (tip diameter less than 0.5 μ), connected to a negative capacity cathode-follower (grid current less than 10^-12A) and DC preamplifier. A Grass camera (Model C4-F) was employed to photograph the screen of a Tektronix oscilloscope (Model 532). A low resistance 100 mv. source inserted in the circuit between tissue bath and ground provided an accurate calibration signal. A 1,000 cycle square pulse signal was used to test the input capacity neutralization for each microelectrode. The preparation was driven at regular frequency throughout the experiment (1.5 to 2 cycles/sec.). Stimuli (short rectangular pulses) were applied either to the atrial roof or to the interventricular septum, in order to initiate both normal and retrograde A-V propagation. This was accomplished by means of 2 coupled stimulators and stimulus isolation units.

**Histologic Technic**

Preparations, such as those described above, for the mapping experiments were used also for histologic studies. Immediately after dissection, fixa-
tion was performed, either with Baker’s formalin-calcium, or with Bouin’s fluid. In one case, Bouin’s fixative was perfused through the coronary arteries before dissecting. After fixation for 24 hours, the preparations were dehydrated through graded ethanol, cleared in toluene and embedded in tissuemat. Serial sections were cut at 8 or 10 μ. After hydration, slides were stained by Mallory’s trichromatic technic. A slight modification of this method was needed to suit Bouin-fixed specimens.

Results
1) Map of Activation of the A-V Nodal Region

Figure 2 shows a synthesis of 12 mapping experiments. The location of the stimulating electrodes in the right atrial roof is not within the limits of the drawing. Arrows indicate the approximate direction of propagation of activity, as evaluated from the timing data. Numbers correspond to the time of activation of the point (in milliseconds after the time of application of the driving stimulus).

On the basis of the shape of the observed action potentials, 3 functional layers could be described in the region below the coronary sinus. These layers appear as hatched areas in figure 1 and are outlined by dashed lines in figure 2. The N layer corresponds to the part of the A-V node, where slowing of both propagation velocity and action potential rising phase is maximal. Despite being a narrow region, it accounts for a 25 to 30 msec. delay in A-V transmission. Rising phases may be as long as 30 msec. and propagation veloc-
head (see histologic results). It is worth mentioning that the sinus-atrial ring bundle (SARB), a structure which can undertake pacemaker functions, is always found to end over the atrial border of the AN layer of the A-V node.

In mapping experiments in which the retrograde excitation of the A-V node was studied, 3 regions could be detected which nearly coincided with the 3 layers described above. Propagation was slowed in the NH layer, attained maximum slowing in the N layer and was again accelerated in the AN layer before reaching atrial muscle. These observations show clearly that the AN and NH layers are not only able to slow down impulses but also to speed them up, depending on the direction of propagation. They are by no means regions of decremental conduction. The same does not hold true for the N layer, as will be apparent from the following results.

2) Normal and Retrograde Activation of A-V Nodal Cells

Figure 3 shows transmembrane action potentials recorded from 5 different cells in the nodal region during normal (atrial stimulation) and retrograde (ventricular stimulation) activation. The location of each cell (A, B, C, D, and E) is noted in figure 2 with corresponding letters. These records were obtained during regular driving of each chamber, extrasystolic beats being carefully avoided.

Cell A is located in atrial muscle. Its normal and retrograde action potentials look alike. Cell B lies in the AN region, close to its atrial border. Slowing of the rising phase is already detectable. It is worth mentioning that the retrograde action potential shows a slow foot at the beginning of the rising phase, though the maximal rising velocities are similar in normal and retrograde activation. Cell C is located in the N layer, close to its boundary with the NH layer. A slow foot is seen in both normal and retrograde activation, but it is more clearly shown in the normally propagated action potential. A higher maximal rising is seen in the retrograde beat. In cell D, located in the NH layer close to the N layer, maximal rising velocities are again equal in normal and retrograde activation. The slow foot in the rising phase is much more evident in the normal beat. Cell E is a typical NH cell. The transition to the long Purkinje-like action potential of His bundle is clear. A marked slow foot is seen only in the normal beat and maximal rising velocities are similar in normal and retrograde activation. This situation is the opposite of that observed in the AN layer.

On the basis of the above results, one may
say that the action potential of a cell in any of the transitional layers (AN or NH) will show a slow foot whenever the impulse, before reaching that cell, crosses a zone which presents a conduction velocity lower than the conduction velocity of the region where the cell is located. In other words, a slow foot appears when the propagation velocity of impulses is increasing. This is the case in the AN layer during retrograde propagation, and in the NH layer during normal propagation.

The N layer is the only region where maximal rising velocities in the same cell are different during normal and retrograde activation. Rising velocity decays in proportion to the distance crossed by the excitation wave in the N layer. This observation points to the existence of decremental conduction within this layer.

A statistical evaluation of resting and action potential parameters in the various nodal layers has been omitted, since one is dealing with nonuniform groups exhibiting gradually changing characteristics. An idea of what typical values are can be obtained from figure 3, where the dashes seen above the stimulus signals represent the zero potential level. No emphasis has been put either on the pacemaker-like diastolic depolarization of nodal fibers or on the lower values for resting and action potentials observed in the N layer. These facts have been reported elsewhere.2

3) Observations on Partially Blocked Preparation

In one experiment, A-V block appeared spontaneously 2 hours after dissection. Before complete block ensued, the phenomenon known as Wenckebach cycles could be observed. A cell of the NH layer has been impaled during the occurrence of one such cycle (preparation driven from the atrial roof). Superimposed tracings of a whole cycle are seen in figure 4. The first action potential of the group shows a slow foot, as expected from a NH cell during atrial stimulation. The rising phase is rather smooth. The second beat exhibits a still slower foot. The fast rising upstroke takes off later than in the first beat, forming with the slow foot an actual step. The same step is exaggerated in the third beat, and in the fourth beat the NH cell fails to develop an active response. Only an aborted local potential is seen, caused by conduction decrement in the N layer. Obviously, occurrence of decremental conduction in a partially blocked preparation does not mean that the same occurs in a normal preparation. The significance of these findings will be discussed later in this paper.

4) Histologic Results

Baker's formalin-calcium has been found the more suitable fixative to be used for the demonstration of the specialized system of the rabbit heart by Mallory's trichromic staining method. When dealing with Bouin-fixed material, it has been observed that fuchsin staining was readily removed by the aniline-blue-orange G mixture. A modification of the classical technic was used to overcome this difficulty, consisting of a very quick rinse in water after staining with fuchsin and staining in the aniline-blue-orange G mixture directly from the phosphotungstic acid solution. The optimum plane of incidence for cutting serial sections has been found to be one as closely parallel to the floor of the coronary sinus ostium as possible. In this way, the A-V node is sectioned before the ventricular muscle, due to its location immediately under the endocardial surface and to the angle formed between that floor and the muscle. Conne
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tions of the A-V node with either the His bundle or the atrial muscle can be found in different planes by following a series of sections, since they are not at the same anatomic level. Under these conditions, the A-V node can be traced just beneath the endocardial surface, extending in depth from 0.7 to 0.8 mm. from the endocardial surface. It forms a kind of head in the trajectory from the atrial muscle to the His bundle and is recognizable just to the left of the fibrous septum, when looking at the preparation as depicted in figure 5. Clear connections with atrial muscle and His bundle could be shown, and at these points there are no sharp limits between the 2 interconnected tissues; this makes it difficult to follow the spread of A-V node into atrial muscle. The His bundle is also very clearly shown, just under the endocardium (fig. 6). It follows a somewhat curved trajectory from the A-V node into the ventricular side of the preparation. As seen from figure 6, it is entirely surrounded by a connective tissue sheath, shortly after leaving the A-V node. Further following the His bundle into the depth of the preparation, one can observe its division in 2 branches.

The cells at the atrial and ventricular borders of the A-V node are oriented in the direction of its long axis and disposed approximately parallel to the A-V fibrous ring (figs. 7A and C). In the middle of the node, however, such an arrangement does not appear to exist (fig. 7B). The cell diameter is greater in the middle region (about 11 μ) than at the borders (about 7 μ) and the nucleus is characteristically surrounded by a broader zone of clear "empty" cytoplasm. This aspect is better observed in formalin-calcium-fixed, Mallory-stained sections. These features suggest a histologic distinction between the middle and the borders of the A-V node; however this finding remains to be reinvestigated.

Discussion

1) Mapping of Excitation

One major question about the results is whether the electrophysiologic A-V node, identified as the only site where continuity of the A-V excitation process can be traced, corresponds to the structure described by Tawara. The only direct answer would be obtained through the labeling and subsequent histologic identification of a cell from which a typical nodal action potential had been recorded. Sano and his colleagues, recently have attempted to do this and succeeded in roughly labeling the region where the microelectrode had been inserted. They concluded that "the potential had originated from the cells in the atrioventricular node, or at least in the special junctional tissue adjacent to it." The histologic examination of our preparations showed that the A-V node lies just beneath the endocardial layer, in the region from which nodal action potentials have been recorded. Furthermore, the connective tissue-insulated His bundle (fig. 6) can be identified unmistakably in the electrophysiologic mapping (fig. 2), both by its Purkinje-like action potential and by the drastic change in the time of activation observed when the microelectrode is moved towards the adjacent atrial muscle. As the interrelations of the regions functionally identified as A-V node and His bundle agree well with the histologic picture (compare figs. 2 and 5), we may assume that

Figure 5
Section through the "head" of the A-V node. Note the parallel arrangement of the fibers at the borders, different from the middle region. Dashed lines show the position of the His bundle, which lies in a more superficial plane. IAS: interatrial septum; IVS, interventricular septum. Formalin-calcium-fixed, Mallory-stained, 10 μ section. (X 82.)
the functional A-V node coincides, at least in part, with the histologic A-V node. However, definite conclusions as to the identification of the 3-layered figure obtained from the mapping experiments with the 3 histologic regions of A-V node cannot be drawn before a successful labeling of A-V nodal cells is achieved.

The AVR region which is in continuity with and has the same physiologic properties as the AN layer of A-V node behaves like the latter under various experimental conditions. A likely hypothesis for explaining this finding may be drawn from a consideration of the embryologic development of the heart. It has been shown that a P-R interval is clearly recognizable in the electrocardiogram of the 42-hour-old chick embryo (incubation age), a stage at which atrioventricular connections still extend all around the embryonic A-V ring. It also has been shown that A-V propagation is equally effective at any part of the ring. The ultimate development of the fibrous A-V valves in the adult mammal interrupts most of the muscular A-V connections, normally leaving only the A-V node and the His bundle connecting atrium to ventricle. Since the A-V delay remains qualitatively unchanged since early embryonic life, one may assume the A-V node to be the only remnant of the embryonic A-V ring whose ventricular connections have been maintained throughout the development. The AVR region would then correspond to the rest of the embryonic A-V ring.

An implication of this hypothesis, is that any persisting extranodal A-V connection, being a remnant of the embryonic A-V ring, is likely to exhibit definite delaying properties. This possibility should be kept in mind when attempts are made to draw physiologic implications from the histologic finding of an anomalous A-V bundle.

2) Direction of Propagation within the Nodal Tissue

Arrows in figure 2 show the approximate direction of the spread of activation through the A-V node. The fact that propagation within the nodal region is always directed perpendicularly to the 3 physiologic layers (AN, N and NH) is to be expected if excitation propagates freely from atrial muscle into nodal tissue. In such a syncytial system, excitation passes from fast to slow-conducting regions and propagation into the latter will tend to be oriented perpendicularly to the boundary, whatever the direction of propagation in atrial muscle may be. This is due to the fact that the fast-conducting atrial muscle will excite the whole atrial-nodal boundary before excitation has traveled far into nodal tissue from the initially excited point. A similar situation is seen at the superior vena cava-right atrium boundary, where excitation propagates back into the slow conducting pacemaker fibers when atrial muscle is driven electrically.

3) Normal and Retrograde Excitation of the A-V Node

It has been shown that action potential shapes in the nodal area are dependent on the direction of propagation. If the assumption is made that microelectrode records are faithful images of the events occurring exclusively in the immediate cell membrane, the potential shifts thus observed might originate from 3 basic conditions: (1) electrotonic currents; (2) all-or-none conduction of an impulse; and (3) decremental conduction of an impulse.
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Figure 7
Details of the section shown in Figure 5. Left. A-V node near its atrial border. Note the longitudinal arrangement of the fibers. Center. Middle region of the A-V node. Note the greater cell population and the apparent absence of order in arrangement. Right. A-V node near its ventricular border. The distribution of the fibers is similar to that in left section. Horizontal bar in right section represents 10 μ, which applies to all three sections of the figure.

Decremental conduction differs from all-or-none conduction in that both amplitude and rising velocity of the action potential gradually decay with the spread of the excitation; propagation velocity decays along with these parameters. Decremental conduction is observed, for instance, in a semiblocked axon and can then be interpreted as an inability of the cell to maintain actively an all-or-none propagation. Decremental conduction differs from passive physical electrotonus in that it has a propagation velocity of its own.

The records taken from cells in the transitional regions of A-V node (AN and NH) show that the maximal rising velocity is independent of the direction of excitation. This fact suggests that these cells are actively engaged in the conduction of impulses, though their intrinsic conduction velocity becomes progressively lower as the N layer is approached. The fact that the AN and NH layers effectively speed up propagation on its way from the N layer to atrial muscle (retrograde propagation), or to His bundle (normal propagation), constitutes further evidence to support this view.

The different values of maximal rising velocity of the action potentials during normal and retrograde beats suggest that the cells in the N layer are not actively engaged in the maintenance of an all-or-none response. The very low propagation velocity observed in this region rules out the possibility of pure electrotonic spreading, decremental conduction being the more suitable explanation. This view is favored by observations made during the occurrence of Wenckebach cycles (fig. 4). Though concomitant alteration of the excitation threshold of the NH cell cannot be overruled as a contributing factor, there seems to be an exaggeration of the decrement through the N layer, so that the decremented action potential is gradually less effective in firing the actively responding NH cell.

4) Evaluation of the Data in View of Other Experimental Facts

The A-V node is not a simple structure from the physiologic standpoint. The view of A-V transmission presented above (slow and decremental conduction) can explain most of the data presented in the literature; however some other facts deserve consideration.

The notched upstroke, observed in the nodal action potentials during the action of acetylcholine1,4 and high external potassium concentration,9 has been shown to occur in sinoatrial pacemaker cells under these same conditions, as well as in plain heart muscle cells in a high-potassium medium.9 It is, therefore, possible that such behavior of the A-V nodal cells is not related to peculiar physiologic characteristics. On the other hand, the A-V blocking action of acetylcholine is to be expected if acetylcholine lowers the length constant of the nodal fiber by decreasing mem-
brane resistance. This effect would result in a steeper decrement in the N layer and A-V block.

Other facts that can hardly fit in the proposed picture of the function of the A-V node are the sudden increase in A-V delay observed with early induced extrasystoles and the "echo" phenomenon which may enue. These facts suggest that during the relative refractory period, propagation in the A-V node may follow a more complicated pattern than after complete recovery.

Summary

Electrophysiologic mapping experiments on the excitation of the A-V node of isolated rabbit heart have been performed with intracellular microelectrodes. An area of tissue showing peculiar physiologic characteristics was shown to coincide, at least in part, with the histologic A-V node and His bundle. Three functional regions have been determined in the physiologic mapping of the A-V node. The middle layer (N) shows signs of decremental conduction and is the site of the slowest propagation velocity in the A-V node. The other layers are transitional regions between the N layer and atrium (AN) or the His bundle (NH). The AN layer shows a progressively slower propagation velocity as the N region is approached. The same holds for the NH region. A precise correlation between these findings and the 3 existent histologic layers remains to be determined. The existence of an area of tissue just above the A-V valves and encircling the A-V orifice has been confirmed. This area is continuous with the AN layer of the functional A-V node. Illo eserba designato como lo anulo atrioventricular, in le supposition quo illo es un resto del anulo atrioventricular del embryon. Cyclos de Wenkebach e bloco atrioventricular de origine nodal es explicite tentativamente como resultado de un plus marcate decremento in lo strato N.

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References


Experiments de "cartographia" electrophysiologic relative al excitation del nodo atrioventricular del isolate corde de conilio esseva executate per medio de microelectrodos intracellular. Characteristicas physiologic peculiar esseva constatate in un area coincidente in parto con lo nodo atrioventricular e lo fasce de His. Tres regiones funzional esseva determinate in lo physiologia del nodo atrioventricular. Le strato intermediari (N) exhibi signos de conduction decremental e es lo sito del plus lente propagatio intra lo nodo atrioventricular. Le altere stratos es regiones transitional inter lo strato N e le atrio (AN) o lo fasce de His (NH). Le strato AN monstra un progressive relentation del propagation con le crescente vieinitate del region N. Le mismo vale pro lo region NH. Le precisa correlation de iste constatastiones con le 3 existente stratos histologic remane a determinar. Le existentia de un area de tissu de character peculiar justo supra le valvulas atrioventricular e circundante lo orificio atrioventricular esseva confirmate. Iste area se trova in continuatate con lo strato AN del nodo atrioventricular funzional. Illo eserba designato como lo anulo atrioventricular (AVR), in le supposition quo illo es un resto del anulo atrioventricular del embryon. Cyclos de Wenkebach e bloco atrioventricular de origine nodal es explicite tentativamente como resultato de un plus marcate decremento in lo strato N.


Spread of Activity Through the Atrioventricular Node
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