The Discontinuous Nature of Propagation in Normal Canine Cardiac Muscle

Evidence for Recurrent Discontinuities of Intracellular Resistance that Affect the Membrane Currents

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SUMMARY When the propagation velocity of action potentials is modified by changing the internal resistance of a cell, cable theory predicts that the shape of the action potential upstroke should not change; changes in velocity associated with changes in the upstroke usually are attributed to changes in membrane properties. However, we observed, in normal cardiac muscle, that changes in the upstroke with velocity occur under conditions in which the membrane properties could not have changed. Propagation in atrial and ventricular muscle was studied, in which the velocity of propagation was different at different angles with respect to the cell orientation. Fast upstrokes were associated with low propagation velocities (in a direction transverse to the long cell axis) and slower upstrokes were associated with high propagation velocities (in the direction of the long cell axis). Such changes in the shape of depolarization can be accounted for by the discrete cellular nature of cardiac muscle. The recurrent discontinuities in intracellular resistance cause propagation to be discontinuous on a microscopic scale. The presence of discontinuities in intracellular resistance reverses the usual association of high velocity and high safety factor for propagation: propagation at a low velocity is actually more resistant to disturbances in membrane properties than is propagation at a higher velocity. This inverted relationship suggested that propagation could continue in a direction transverse to the long axis of the cells when block occurs in the longitudinal direction, with resultant reentrant propagation. Such prediction was confirmed in the study of the propagation of premature action potentials in atrial muscle. Circ Res 48: 39-54, 1981

IN a given cell, the propagation velocity of action potentials can be altered in only two ways: by changing the membrane properties or by changing the axial resistivity in the direction of propagation. As an example of the first mechanism, changes in propagation velocity in cardiac muscle have been produced through modulation of membrane properties (presumably the fast sodium current mechanism) by changing the outside potassium concentration (Dominguez and Fozzard, 1970). A correlation between propagation velocity and the fast sodium mechanism had been suggested previously by the theoretical analysis of Pickard (1966) who derived an approximate expression for the dependence of propagation velocity in nerve on the maximum rate-of-rise of the sodium conductance, resting and threshold potentials, the membrane capacitance, and the axial resistance. The general nature of the relationship is a positive correlation between propagation velocity and maximum sodium current, large sodium currents producing high velocities. It is generally concluded that, when the propagation velocity is varied by changing the time course of the membrane permeabilities, the shape of the action potential also is changed. For example, it has been shown (Singer et al., 1967) that, when the propagation velocity is decreased by stimulating at a more depolarized transmembrane potential, the maximum rate-of-rise of the action potential also decreases. The time constant of the foot of the action potential also has been reported to be larger when the propagation velocity is low (Dominguez and Fozzard, 1970). The exact functional dependence of these shape changes on propagation velocity has not been determined experimentally in cardiac muscle, but it has been assumed that the relationships are the same as in nerve and skeletal muscle (Fozzard, 1979).

The second mechanism of variation of the propagation velocity, with axial resistance in the direc-
tion of propagation, is predicted on theoretical grounds (Hodgkin and Huxley, 1952). This mechanism for the variation of propagation velocity has received little experimental study in cardiac muscle. Lieberman et al. (1973) attributed propagation velocity changes in cultured strands of chick embryonic heart muscle to decreased electrical coupling between cells. Others have suggested that the coupling might be changed through variations in intracellular resistivity (Sperelakis et al., 1970) or in the number and distribution of cell junctions (Woodbury and Crill, 1961). Variations in propagation velocity with direction have been observed in cardiac muscle (Sano et al., 1959). Clerc (1976) also observed similar variations in propagation velocity with direction and attributed the variations to directional differences in cell-to-cell connections. He also concluded from his measurements that the shape of the action potential did not change with direction or velocity and considered this result to be in agreement with theoretical predictions of the relationship between velocity and axial resistance.

We have varied the propagation velocity in both of the ways discussed above, looking carefully for shape changes in the upstroke of the action potential as the velocity was varied. We found changes of shape in both circumstances—whether the velocity was varied through premature action potentials or through changes in the direction of propagation. However, the variations were in exactly the opposite direction in the two cases. When the velocity was decreased in a Purkinje strand by premature stimulation, the maximum rate-of-rise of the action potential decreased and the time constant of the foot increased, as expected from continuous cable theory. On the other hand, when the velocity was decreased by changing the direction of propagation with respect to fiber orientation, the maximum rate-of-rise increased and the time constant of the foot decreased.

We have studied both of these cases carefully, looking for an explanation of this apparently anomalous propagation consistent with the known morphology of cardiac muscle. We were unable to find a satisfactory explanation on the basis of the commonly used continuous cable theory. However, if propagation in cardiac muscle under many circumstances is recognized as a kind of discontinuous propagation, the observed changes in action potential shape with direction and velocity are accounted for. We explore the implications of such a mechanism for the propagation of action potentials in the normal heart. We also use the concept to predict how premature action potentials should propagate in a multidimensional structure of cells with uniform membrane properties; e.g., uniform "recovery of excitability." This prediction was confirmed experimentally, showing that a reentrant arrhythmia can be generated in a muscle with uniform membrane properties and anisotropic cell coupling.

Methods

Electrical Measurements

We studied in vitro preparations from the hearts of 42 dogs (weight 14-24 kg). Each dog was anesthetized with pentobarbital sodium (30 mg/kg, iv). The hearts were excised rapidly and the preparations were pinned to the floor of a circular tissue bath, 15 cm in diameter, and maintained at a constant temperature of 35°C. Preparations included the crista terminalis and surrounding tissue from the atrium, papillary muscle from the right ventricle, and Purkinje strands on the surface of the left ventricular septum. The composition of the perfusate, in m\(\text{M}\), was as follows: NaCl, 128; KCl, 4.69; MgSO\(_4\), 1.18; Na\(_2\)HPO\(_4\), 0.41; NaHCO\(_3\), 20.1; CaCl\(_2\), 2.23; and dextrose, 11.1. The solutions were gassed in a reservoir with a mixture of 95% O\(_2\)-5% CO\(_2\) and perfused through the tissue bath at a rate of 100 ml/min.

The details of the extracellular and intracellular electrodes and the recording techniques have been described previously for in vitro measurements of the atrial septum, functionally single Purkinje strands, and anisotropic ventricular muscle (Spach et al., 1972, 1979). Intracellular potentials were recorded by glass microelectrodes filled with 3 M KCl and having resistances between 8 and 20 M\(\Omega\). The extracellular electrodes were made of flexible tungsten wire, 50 \(\mu\)m in diameter, and insulated except at the tip. Each extracellular electrode was connected to one input of an AC-coupled differential amplifier, having a frequency response flat between 0.1 and 30,000 Hz. The (separate) reference electrodes for each extracellular or intracellular electrode were positioned 7 cm away from the recording site. To minimize the capacitance between the glass microelectrode and the bathing solution, the fluid level was allowed to rise no higher than 4 mm above the surface of the preparation. The overall rise time (10-90%) for both extracellular and intracellular recording systems was less than 30 \(\mu\)sec (Spach et al., 1971).

A pacemaker stimulus 0.5-1.0 msec in duration and of amplitude 1.5-2.0 times threshold was applied to the surface of the preparation by a unipolar electrode at the rate of one per second. A PDP-11/20 computer system (Barr et al., 1976) controlled the rate and synchronized the pacing stimuli with the data recording. The outputs of the recording amplifiers were sampled at a rate between 6,600 and 20,000 per second (12-bit samples). The computer stored the data and displayed the waveforms concurrently on a Tektronix 4002 display unit. The output of each recording amplifier also was displayed on one channel of a Tektronix 565 analog oscilloscope with 3A3 dual-trace amplifiers. Photographs of both displays were taken to ensure that the digitizing rates were sufficient for accurate digital reproduction of the waveforms. A dissecting...
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microscope equipped with a Nikon F250 35-mm camera was used to document the positions of the stimulus and recording electrodes.

The transmembrane potential $V_m$ was calculated by subtracting the extracellular potential (recorded as close as possible to the impalement site) from the intracellular potential. It was important to position the extracellular electrode very close to the impalement site because slight changes in the distance between the electrode and the site caused noticeable changes in the waveforms. Initially, we used glass microelectrodes (1-μm tip diameter) to record the extracellular potential. We found it difficult to position the extracellular microelectrode very close to the impaled cell without creating additional injury which, in turn, affected the local propagation velocity. These difficulties in positioning the extracellular microelectrode were due in part to the near invisibility of the electrode tip. The problems in the extracellular recording were solved by replacing the glass microelectrode with a tungsten wire electrode, similar to those used for other extracellular recording. One merit of the wire electrode was that it helped to identify sites with minimal local movement for intracellular measurements. We compared the extracellular recordings made by glass microelectrodes and by tungsten wire electrodes for the anisotropic muscle as we had made previously for Purkinje strands (Spach et al., 1972). We advanced the glass microelectrode into the muscle gradually until the first signs of injury potential were evident, waited for the small injury to disappear, and then compared the extracellular recording from this electrode with that from a wire electrode resting on the surface at the same site. The waveforms were nearly identical in shape, and the amplitudes were identical or slightly smaller (<10%) from the glass microelectrode. We were able to achieve the most reliable results by first positioning the tungsten extracellular electrode on the surface, then inserting the intracellular microelectrode as close to it as possible.

Care was taken to impale cells only at the surface of the preparation. The microelectrode was advanced slowly and, once the intracellular potential appeared, the electrode was not advanced further. We studied changes in propagation velocity at one site by analyzing the records (intra- and extracellular) associated with the impalement of a single cell, thus keeping constant many parameters that could not be measured. We varied the propagation velocity at the impalement site either by stimulating prematurely (to modulate the fast sodium-current mechanism) or by changing the direction of propagation. We were able to achieve constant recording conditions (as defined by the shape, amplitude, and timing of the intracellular and extracellular waveforms) from a single impalement for 3 to 5 minutes in each of nine atrial and six ventricular preparations. Detailed editing of the recordings from these experiments showed reproducible intracellular and extracellular waveforms for each of the numerous excitation sequences.

Since our results depended critically on the shapes of the recorded waveforms, we took meticulous care to be sure that the stimulus current did not interfere with the recorded waveform. Three specific precautions were taken: (1) Each stimulus site was located several millimeters from the impaled cell [several resting space constants (Woodbury and Crill, 1961)]. (2) We checked to make sure that there was an interval of constant resting potential between the stimulus artifact and the recorded waveform. (3) We also checked to make sure that propagation was initiated within 200 μm of each stimulus electrode, as determined by extracellular activation mapping. Any irregularities in propagation encountered within the immediate vicinity (radius of 1.5 mm) of the impalement site, such as may be caused by connective tissue divisions, were grounds for moving the observation site. Such irregularities show up in the extracellular waveforms as rapid notches (Spach et al., 1979).

At the conclusion of each experiment, the preparation was examined histologically by light microscopy to verify the fiber orientation in the area of measurement.

Membrane Effects

To study the situation in which velocity changed through modulation of the fast sodium-current mechanism, we chose small, functionally single Purkinje strands on the left ventricular septal surface (diameter 125–250 μm). In these strands, the activation wavefront remains perpendicular to the long axis of the cells, even for premature action potentials. Three extracellular electrodes were spaced 2 to 3 mm apart along the strand for velocity measurements (Fig. 1A). The stimulus electrode was positioned 10 mm away from one of the outside recording electrodes, a distance of many resting space constants. After control (extracellular) waveforms had been recorded, a cell was impaled at the site of the middle extracellular electrode. The impalement was rejected if the nearby (middle) extracellular recording was changed visibly. Given that the intracellular and extracellular waveforms were satisfactory, action potentials that propagated at different velocities were initiated by premature stimuli introduced before the fibers were repolarized completely. A premature stimulus was injected after every 15th regular stimulus that occurred at the constant rate of 1/sec.

Directional Effects

Two to six stimulating electrodes were located around a single intracellular recording site. The propagation velocity was greatest when the wavefront proceeded down the long axis of the cells,
The potentials were measured during a single cell impalement with the electrode arrangement shown in panel A. In B, the measured $V_{\text{max}}$ is plotted as a function of propagation velocity. The relationship between the maximum axial current (calculated from our measurements) and the propagation velocity is shown in panel C. Each data point was computed from measured $V_{\text{max}}$ and $\theta$ values by Equation 3. The solid line was calculated from the fitted curve in panel B. The units on the vertical axis are arbitrary.

The intracellular ($\phi_i$) and extracellular ($\phi_e$) potentials were measured during a single cell impalement, smallest along the perpendicular axis, and varied continuously and monotonically along intermediate axes. The interval between stimuli was kept constant at 1000 msec, but the site of stimulation was changed randomly between the stimulus electrodes every 10-15 beats to change the direction of propagation.

Five extracellular recording electrodes also were positioned around the impalement site in a cross-shaped pattern, so that there were three along the axis of the fibers and three along an axis perpendicular to the fiber direction. The center electrode, common to both axes, was the site for the intracellular impalement. The intracellular microelectrode first was positioned just above the surface of the preparation and control recordings made of the extracellular waveforms. The microelectrode then was advanced into the tissue within 50 µm of the extracellular electrode tip; care was taken not to advance the microelectrode beyond the superficial cell layer. If there was any change in the extracellular waveform, the microelectrode was withdrawn until the waveforms returned to control shapes. If they did not recover, the entire array was moved to a different area of the preparation. The impalement also was rejected if the resting potential was less negative than -80 mV. If the resting potential changed by more than 2 mV during the course of an experiment, the procedure was terminated.

To get a value for the propagation velocity for each stimulus site, a detailed activation sequence was recorded for each stimulus site after the conclusion of the intracellular measurements. Extracellular waveforms were recorded at 70-90 additional positions within a region (6 mm × 12 mm) around the atrial impalement site or 14-21 additional positions in a region (3 mm × 3 mm) around the ventricular impalement site.

All measurements were made after 2 hours of superfusion. In the ventricular preparations, mechanical motion occurred only on the surface after this time and depolarization did not extend further than 1 mm beneath the surface (Spach et al., 1979). In the atrial preparations, mechanical motion did not change with time; tension was applied to stabilize the preparation for cell impalements.

**Data Analysis**

After the conclusion of an experiment, the waveforms stored digitally were redisplayed and photographed for detailed inspection. Isochrone maps were constructed from the extracellular record; we took the time of the peak negative derivative (intrinsic deflection) as the instant of excitation. Conduction velocity then was calculated as the distance traveled normal to the isochrone per unit time.

Transmembrane potential waveforms were calculated by taking the differences between the recorded intracellular and extracellular potentials. $V_{\text{max}}$ was obtained from the recorded intracellular or calculated transmembrane potential by numerical differentiation. The values of $V_{\text{max}}$ obtained depend critically on two aspects of the recording system. First, it is essential that the high-impedance intracellular recording system be fast enough to respond to the rapid rates-of-rise that occur. Negative-capacitance compensation frequently is used to increase the speed of response of such a system, but if not properly adjusted, such compensation can itself cause errors in the calculated $V_{\text{max}}$. Second, it is important that the proper extracellular potential be recorded. If the extracellular electrode is not close enough to the impalement site (horizontally as well as vertically), the intra- and extracellular waveforms may be misaligned in time. In the atrium, the large anisotropy of propagation can produce drastic changes in the extracellular potential as the electrode is moved away from the region of fast propagation. In this situation, the potential can drop by as much as 80% in 100 µm.

The time constant of the foot of the action potential ($\tau_{\text{foot}}$) was calculated graphically by plotting the first 8 mV of depolarization on semilogarithmic paper and fitting a straight line by eye. The rela-
sionship between the time constant of the foot and the velocity or $V_{\text{max}}$ was analyzed statistically using multiple linear regression and Student's $t$-test for paired observations. The level of significance was taken as 0.01%.

Results

Membrane Effects

Figure 1A shows typical intra- and extracellular potential waveforms from one impalement in a functionally single Purkinje strand. Note that as the resting potential or take-off potential was made more negative by delaying the premature stimulus, both the velocity of propagation ($\theta$) and the maximum rate-of-rise ($V_{\text{max}}$) increased, as well as the peak-to-peak amplitude of the extracellular potential. In Figure 1B, $V_{\text{max}}$ from these waveforms and additional recordings from the same cell are plotted as a function of the measured propagation velocity. Such an increase of $V_{\text{max}}$ with velocity is consistent with similar observations made in Purkinje fibers (Dominguez and Fozzard, 1970) and in the atrium (Sano et al., 1967) when the resting potential and velocity were changed by altering the extracellular potassium concentration. The data points in Figure 1B seem to fall along a straight line, within the accuracy of these measurements. However, a square-root relationship was predicted on the basis of a continuous cable model and an approximate representation of membrane properties (Hunter et al., 1975). It is not clear which assumption in their calculation (one-dimensional propagation, continuous fibers, constant membrane properties, etc.) might not be appropriate for this situation. Sano et al. (1967) measured both the propagation velocity and $V_{\text{max}}$ in rabbit atrium with elevated extracellular potassium and with different rapid stimulation rates. They did not analyze the relationship between $\theta$ and $V_{\text{max}}$ directly, but when we replotted the data from their paper in the same manner as our Figure 1B, the result was also fitted well by a straight line; i.e., $\theta$ decreased with $V_{\text{max}}$.

Figure 2A shows $\tau_{\text{foot}}$ from this same impalement plotted as a function of $\theta$ and $V_{\text{max}}$. As the premature action potential was delayed more and more, $\tau_{\text{foot}}$ decreased approximately linearly as both $\theta$ and $V_{\text{max}}$ increased ($r = -0.99, P < 0.01$). However, a rectangular hyperbolic relationship between $\tau_{\text{foot}}$ and $\theta$ was predicted by Tasaki and Hagiwara (1957) on the basis of continuous, one-dimensional cable theory. Again, it is not clear whether the difference between our results and their theoretical prediction is due to insufficient accuracy of measurement or to inappropriate assumptions (for our tissue) in the theoretical calculation. At least there is agreement in the direction of the change in $\tau_{\text{foot}}$ as $\theta$ increases.

The same direction of change in $\tau_{\text{foot}}$ with $\theta$ was found by Dominguez and Fozzard (1970) as they changed the extracellular potassium concentration from 4.0 to 7.0 mm, although they did not have enough points to define a curve.

Thus, we found that when we observed the relationship between propagation velocity and maximum rate-of-rise or time constant of the foot of the action potential by modulating the membrane properties, keeping the relationship between the direction of propagation and the cell geometry constant, both variables changed in the direction predicted theoretically and observed previously.

Directional Effects

The changes in shape of the action potential upstroke with velocity as the direction of propagation was varied were similar for all preparations from the atrium or the ventricle. We present the results of one impalement from each chamber.

Two activation sequences from an atrial preparation, the crista terminalis, are shown in Figure 3. The crista terminalis is a large, flat muscle in which the parallel orientation of the fibers underneath the endocardium is very apparent. Both activation sequences were initiated by point stimuli from an extracellular electrode. Note that the two sequences are very similar in appearance, although they were initiated at different sites on the muscle, displaced both longitudinally and transversely. In both cases, there was rapid conduction in the longitudinal direction (along the long axis of the fibers) and slow propagation in the transverse direction (perpendicular to the long axis of the fibers). The transverse propagation velocity varied somewhat from point to point in the mapped region, but it was reasonably uniform in the vicinity of the impalement site. In the nine successful crista terminalis experiments, the maximum velocities fell in the narrow range between 0.9 and 1.2 m/sec and the minimum velocities ranged between 0.05 and 0.12 m/sec. These low values are within the range of velocities observed by Cranefield et al. (1972) for very slow conduction of "slow response" action potentials.

In the righthand part of Figure 3, typical intracellular and extracellular waveforms are shown from a single impalement for the two situations of high velocity (parallel propagation) and low velocity (transverse propagation). Note that the intracellular potential rises faster when the velocity is low; i.e., $V_{\text{max}}$ is higher and $\tau_{\text{foot}}$ is lower, the exact opposite of the effects seen with premature action potentials. We will proceed now to examine possible explanations of this apparently anomalous result and consider its implications.

It has long been assumed in the nerve and muscle literature that a major determinant of the speed of propagation of action potentials is the peak magnitude of the sodium current (Hodgkin and Katz, 1949). Weidmann (1955) pointed out the connection between $V_{\text{max}}$ and the magnitude of the fast sodium current, making it possible to estimate this current in cardiac cells. Thus $\theta$ and $V_{\text{max}}$ were associated closely at an early date, even though the connection was not made in a quantitative theory until rela-
tively recently (Hunter et al., 1975). So close has been this association that some workers have measured only $V_{\text{max}}$ and considered it an indirect measure of propagation velocity. The few instances in which both $\theta$ and $V_{\text{max}}$ were measured supported the relationship between high velocity and high $V_{\text{max}}$. For example, atrial fibers with higher $V_{\text{max}}$ values have been thought to have special membrane properties producing large sodium currents and thus fast conduction (Wagner et al., 1966). Indeed, it has become widely accepted that, within some atrial muscle bundles, such fibers are organized into a specialized, narrow tract that produces preferential rapid conduction within the bundle (Wagner et al., 1966; Hogan and Davis, 1968). Also, decremental conduction and unidirectional block generally are attributed to action potentials propagating into an area of increased refractoriness; this implies a reduction in fast sodium current.

Is it possible that the marked differences in propagation velocity we observed in the crista terminalis were caused by differences in membrane properties, possibly in cells organized into a narrow tract?

Two aspects of the results in Figure 3 demonstrate that the differences in velocity cannot be accounted for by differences in membrane properties or a specialized tract. First, by comparing the upper and lower excitation sequences in Figure 3, note that the pattern of propagation is determined by the site of the stimulus electrode and the fiber orientation. We also stimulated at several other sites across the muscle and always found the same result: there was rapid conduction from the stimulus site along the long axis of the fibers and slow conduction in a transverse direction. Such propagation patterns can be generated only by a muscle that is anisotropic, but that has uniform properties (including membrane properties) along any one axis. If the rapid propagation was produced by a single tract of fixed location (Joyner et al., 1975), the pattern of propagation would have changed.

**Figure 2** Membrane and directional effects on the foot of the action potential. The points in panel A are from action potentials measured in a single cell when the propagation velocity was changed with premature beats in a Purkinje strand. The records are from the same impalement shown in Figure 1A. Panel B is from the ventricle, where the velocity was changed by altering the direction of propagation.

**Figure 3** Directional effects on the action potential in the crista terminalis. The activation sequences for two stimulus sites are shown on the left. The intracellular ($\phi_i$) and extracellular ($\phi_e$) potential waveforms shown on the right were recorded at the indicated site when excitation proceeded in the longitudinal and transverse directions. The points on the outline of the preparation indicate the locations of extracellular recordings from which the activation maps were constructed.
markedly with the stimulus site—whether the tract differed in membrane properties or in resistive connections. Thus, our recorded sequences provide definitive proof that the preferential conduction (fast or slow) was not caused by a specialized tract of fixed location.

Second, we observed a higher speed of depolarization (higher $V_{\text{max}}$ and lower $T_{\text{foot}}$) when the velocity was low, the opposite of what would be predicted if the velocity difference was due to a tract with specialized membrane properties. Furthermore, in going from the upper to the lower excitation pattern in Figure 3, the velocity at the site of cell impalement changed without a change in membrane resting potential, which remained at a high negative value (~85 mV).

It is interesting that propagation velocities as low as 0.05 m/sec can be observed in normal atrial muscle during ordinary excitation. Similar low velocities are routine in the atrioventricular node and in depressed tissue (Cranefield et al., 1971). The very low propagation velocities we observed were achieved without the low resting potential, small amplitude, or slow rate of rise associated with equally slow propagation due to “slow response” action potentials (Paes de Carvalho et al., 1969; Cranefield et al., 1972). The more slowly propagating action potentials that we recorded originated from resting potentials around ~85 mV and had fast upstrokes (even faster than those of faster propagating action potentials).

Two questions naturally arise about the changes in action potential upstroke with velocity described above. Is a similar relationship observed in ventricular muscle and how do $V_{\text{max}}$ and $T_{\text{foot}}$ vary between the upper and lower velocity limits? In a muscle with different propagation velocities along the two major axes, if the velocity were measured in a direction at an angle to both perpendicular axes, the resulting velocity value would lie in between the velocity values along the two axes. In other words, as the direction of measurement is varied continuously from one major axis to the other, the apparent velocity will vary monotonically and continuously. Thus, we can study intermediate propagation velocities by choosing the direction of measurement at various angles with respect to the fiber orientation. There is a problem with this approach, however, if the ratio between the velocities along the major axes is large. In such cases, for example the crista terminalis in which we found the ratio to be about 10, the variation with angle is so steep that the study of intermediate velocities is not practical. In a previous paper (Spach et al., 1979), we found a lower ratio of velocities in anisotropic propagation in ventricular muscle. Thus, we chose to continue the study of velocity and depolarization shape in a papillary muscle from the right ventricle.

Figure 4 shows the arrangement of the stimulus electrodes around the impalement site and four propagation patterns measured in one ventricular preparation typical of the six studied. The isochrones shown in Figure 4B represent propagation in a localized region around the impalement site and were derived by interpolating between excitation times at 14 extracellular recording sites. The velocities parallel to (stimulus site 4) and perpendicular to (site 1) the long fiber axis were 0.50 and 0.19 m/sec, respectively (ratio approximately 2.5), with intermediate values from stimulus sites 2 and 3. In the six successful experiments, the maximum velocity in each preparation occurred within the narrow range 0.47 to 0.52 m/sec and the minimum velocity varied between 0.14 and 0.20 m/sec.

Figure 5 shows the intracellular and extracellular potentials at the impalment site of Figure 4 for each of the four velocities and for a collision. The collision was included with the other measurements because it represents an approximation to zero velocity; i.e., there is no propagation away from the recording site. The presence of a collision was verified by the extracellular waveform; positive uniphasic waveforms have been shown to be characteristic of terminating propagation (Spach et al., 1971). It can be seen in Figure 5 that there was a change in the shape of the upstroke with velocity. As the velocity decreased, $T_{\text{foot}}$ changed and the peak amplitude increased steadily, with the largest amplitude associated with the collision. When propagation occurred in a direction other than the long axis of the fibers, the action potentials frequently had irregular notches after the peak of the upstroke. The possible significance of these notches will be discussed later.

In Figure 6A, the transmembrane potentials ($V_{\text{m}}$), calculated by subtracting the measured intracellular and extracellular potentials, are shown for the recordings of Figure 5. The beginnings of the depolarization are shown on expanded scales in

![Figure 4](https://example.com/figure4.png)

**Figure 4** Changes in propagation velocity with direction in the papillary muscle. Panel A shows, on a schematic drawing of the preparation, the locations of the stimulus electrodes (numbered) with respect to the recording site. Panel B shows the direction of propagation (illustrated by isochrones) and the associated velocities from excitation initiated at each of the stimulus sites. The distance scale applies to panel B only, where the magnification is greater than in panel A.
From continuous one-dimensional cable theory, Hodgkin (1954) concluded that the shape of the transmembrane potential as a function of time (including the upstroke) should not change "if the velocity is altered by factors which change the current distribution in the fiber without affecting the membrane." Clerc (1976) applied this conclusion to anisotropic propagation in cardiac muscle. He assumed that the change in velocity with direction in such a preparation could be represented by a change in longitudinal resistance in the direction of propagation and concluded, from this argument, that the shape of the action potential should not change as the velocity was varied (by varying the direction of propagation). He interpreted his measurements in calf ventricular muscle to agree with his theoretical conclusion.

We examined the transmembrane potential carefully during depolarization to see whether shape...
changes (e.g., changes in $V_{\text{max}}$ or in $\tau_{\text{foot}}$) could be found as the velocity was varied by changing direction. The prediction of no change in the shape of the upstroke was based on an application of cable theory (Hodgkin, 1954) written in terms of the transmembrane potential. Therefore, it was not sufficient to examine the intracellular potential for shape changes; it was critical to know whether $V_{\text{m}}$ changed as we varied the direction of propagation.

We have listed in Table 1 the values of peak amplitude, $V_{\text{max}}$, and $\tau_{\text{foot}}$ from the ventricular impalement (Figs. 5 and 6) and the atrial impalement (Fig. 3). For both preparations, there is a clear and monotonic decrease in $V_{\text{max}}$ and increase in $\tau_{\text{foot}}$ as the velocity was increased. The results for the foot of the ventricular action potential are plotted in Figure 2B for comparison with the results from modulating the membrane properties (Fig. 2A). Note that $\tau_{\text{foot}}$ varied with velocity in the opposite direction with the two methods of velocity change. The direction of variation of $\tau_{\text{foot}}$ with $V_{\text{max}}$ was the same in both cases, however, although the shapes of the variations were not the same.

The changes in the upstroke in Table 1 are not in agreement with the theoretical prediction or the experimental conclusion of Clerc (1976) that the shape should not change with the direction of propagation. Previously, we had agreed with his conclusion based on our own visual comparison of photographs of intracellular waveforms in canine ventricular muscle (Spach et al., 1979), a comparison we now consider inadequate. We see in the two published action potentials of Clerc (see Fig. 5 of Clerc's paper) the same direction of change in $V_{\text{max}}$ with velocity as in our own results in Table 1, in spite of the fact that Clerc's experimental method differed from ours in three ways: (1) he used calf trabeculae; (2) he used bulk electrodes both for transverse and longitudinal stimulation; (3) he flattened the trabeculae, covered the surface with oil, and recorded action potentials from a region in which transverse propagation velocity could be assumed constant as a function of distance in the transverse direction.

It has been assumed that the variation of propagation velocity with direction in cardiac muscle is due to different effective axial resistances in different directions (Joyner et al., 1975; Clerc, 1976). Although this assumption may account for the dependence of velocity on direction, it does not explain our observations of shape changes in the action potential. Is it possible that the reduced velocity transverse to the long fiber axis is due instead to a tortuous path of propagation? In a structure consisting of packed, elongated cells interconnected at their ends, the macroscopic velocity would be reduced because of the zig-zag path of propagation on the microscopic scale. Although this picture of microscopic propagation might account for directional differences in propagation velocity, it is not consistent with our observation of shape changes with velocity. The zig-zag path is analogous to propagation down a folded, one-dimensional cable: the apparent velocity would be reduced, but the transmembrane potential of each membrane element would have the same time course.

There is evidence in our data that the reduction in velocity transverse to the long fiber axis is due to increased axial resistance in that direction. In a previous paper (Spach et al., 1979), we showed that the amplitude of the extracellular potential waveform is quantitatively related to the axial current in the direction of propagation in anisotropic muscle. In particular, the extracellular potential increases as the axial current increases. In the case of propagation velocity reduction with premature action potentials, the extracellular potential and the rate of depolarization decreased together (Fig. 1A). This is in agreement with the previous theoretical prediction on the basis of propagation in a uniform cable structure and modulated membrane properties (Spach et al., 1972). When the velocity was changed through the direction of propagation, however, the amplitude of the extracellular potential and the rate of depolarization changed in opposite directions (Figs. 3 and 5A). When the velocity was lower, the extracellular potential also decreased.

### Table 1  Action Potential Characteristics for Different Propagation Velocities, Corresponding to Different Directions

<table>
<thead>
<tr>
<th>Velocity (m/sec)</th>
<th>Peak-to-peak amplitude of extracellular potential (mv)</th>
<th>Resting transmembrane potential at onset of excitation (mv)</th>
<th>Amplitude of upstroke of action potential (v/sec)</th>
<th>$V_{\text{max}}$</th>
<th>$V_{m}$</th>
<th>$\tau_{\text{foot}}$ (sec)</th>
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<tbody>
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<td>A. Ventricle</td>
<td>0.19</td>
<td>6.2 (−90)</td>
<td>110</td>
<td>106</td>
<td>171</td>
<td>696</td>
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<td></td>
<td>0.22</td>
<td>6.6 (−90)</td>
<td>110</td>
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<td>111</td>
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</tr>
</tbody>
</table>

Sample values from one ventricular impalement (A; Fig. 5) and one atrial impalement (B; Fig. 3).
while the rate of depolarization increased. Thus, we conclude that the axial current also decreased when propagation was slowed in the transverse direction. The correlation of reduced velocity and decreased axial current is consistent with the suggestion that the cause of the slowing is increased axial resistance in the direction of propagation (Clerc, 1976).

A Hypothesis

What is the origin of the shape changes with propagation velocity described above? There is clearly a problem in accounting for these changes because past applications of cable theory have predicted that they should not occur. Since the physical laws on which the cable analysis is based (Ohm’s Law and the conservation of charge) are not in question, one of the assumptions made in applying the analysis to cardiac muscle must be in error. We next looked for a clue as to which assumption would have to be modified or eliminated so that, as the propagation velocity changes with direction, the time course of membrane currents and permeabilities is changed.

We will continue to treat the propagation along any axis in the anisotropic muscle as one-dimensional propagation. Although this is not true in general, it is a good assumption when the excitation wavefronts are parallel over several millimeters and when the direction of propagation is taken perpendicular to the wavefronts.

The assumption also will be made, for the moment, that the action potential propagates along a continuous structure, uniform in any given direction. This is clearly an oversimplification; the granular effects of cell shape and interconnections will be introduced only if necessary to account for our results. Any descriptive parameters for this continuous structure thus represent complex microscopic effects as being distributed on a macroscopic level. Intracellular current, for example, thus will involve many cells and cell-to-cell connections. As the direction of propagation is changed in anisotropic muscle, the intracellular pathway for current flow will be different: a different direction with respect to the cell orientation and different cell-to-cell couplings. Our velocity θ is also a macroscopic variable which represents the speed along a given axis averaged over many cells. It might be expected that, if an attempt is made to measure the velocity over a distance approaching the cell size, there would be considerable variation in the values obtained, as indeed has been observed by Chapman and Fry (1978) for distances less than 500 μm. We found a similar increase in variability for short distances, but velocity was reasonably uniform on a more macroscopic scale (see Figs. 3 and 4).

The resistance to current flow in the direction of propagation depends on the intracellular and extracellular resistivities, the size and shape of the cells, the relative volumes of intra- and extracellular space (cell packing), and the resistance and distribution of cell-to-cell couplings. We will use the word effective axial resistivity, Ra, to include the effects of all of these (Spach et al., 1979).

Hodgkin pointed out (1954) that there is, in a continuous one-dimensional structure, a fixed relationship between the transmembrane current and the shape of the action potential which, for a propagating action potential, involves the velocity and the axial resistance. In particular, if the action potential shape remains constant, the velocity is related inversely to the square root of the axial resistance. The action potential can be made to propagate as rapidly or as slowly as is desired by choosing the appropriate axial resistance; the shape of the action potential remains constant while the spatial distribution of transmembrane potential and the axial current change. As we will see, when these conditions are met, the axial current increases steadily as the axial resistance is decreased (and the velocity is increased). We therefore looked at the relationship between axial current and axial resistance implied by our data (on the basis of the continuous cable approximation discussed above).

In a continuous, one-dimensional electrical cable, the axial or longitudinal current, iθ, is related to the transmembrane potential by the equation

\[ i_\theta = \frac{1}{r_a} \frac{\partial V_m}{\partial x}, \] (1)

where ra is the axial resistance per unit length. Substituting into Equation 1 the definition of velocity, θ = dx/dt, the axial current is

\[ i_\theta = \frac{k'}{R_a} \frac{\partial V_m}{\partial t}. \] (2)

Here we have substituted the effective axial resistivity, R_a, for the axial resistance, ra, per unit length. The proportionality of ra and R_a is included in the constant k'. Taking the specific instant where the rate of depolarization is maximum, the maximum axial current I_a^max is

\[ I_a^\text{max} = \frac{k'}{R_a} V_m^\text{max}. \] (3)

When the membrane properties (including the membrane capacitance*) are kept constant in a continuous cable, the product of the axial resistance and the square of the velocity is also constant (Hodgkin and Huxley, 1952), so

\[ R_a \theta^2 = k \] (4)

where the constant, k, includes the effects of membrane properties and the cell size. Combining Equations 3 and 4, we have a relationship between I_a^max

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* The membrane capacitance may not be constant in some preparations, but may be a function of frequency; e.g., part of the membrane capacitance may be in series with a resistance (Pozzard, 1979). In sheep Purkinje fibers, the isolation is such that for frequency components associated with the upstroke of the action potential, the effective membrane capacitance is essentially constant and corresponds to the capacitance of the free surface membrane. For other tissues, this may not be the case.
and $R_a$:

$$I_{a}^{\text{max}} = \frac{K}{\sqrt{R_a}} V_{\text{max}}$$

(5)

where $K$ is a new constant (related to $k$ and $k'$). In the calculations that follow, based on Equations 4 and 5, we used measured values of $\theta$ and $V_{\text{max}}$ and arbitrarily selected values of the constants $k$ and $K$ (values were selected to make the resulting numbers comparable to those from previous calculations (Spach et al., 1979)).

According to Equation 5, if the shape of the action potential (including $V_{\text{max}}$) does not change with $R_a$, the maximum axial current, $I_{a}^{\text{max}}$, is inversely proportional to the square root of $R_a$. This relationship is shown as the broken line in Figure 7. Note that $I_{a}^{\text{max}}$ decreases steadily as $R_a$ increases and that $I_{a}^{\text{max}}$ changes slowly with $R_a$ for large values of the resistivity. When $R_a$ is small, both the value and the rate of change of $I_{a}^{\text{max}}$ are large.

Our observation was that $V_{\text{max}}$ was not constant, but that it varied with propagation velocity (as the direction was changed). This in turn implies a different relationship between $I_{a}^{\text{max}}$ and $R_a$. To see what this relationship was, we first plotted the values of $V_{\text{max}}$ from the experiment summarized in Table 1 and Figure 5 as a function of $\theta$ (Fig. 8A) and fitted the points with the empirical function shown in the figure. Next, we calculated (relative) values of $R_a$ for each velocity value, using Equation 4; these are plotted in Figure 8B, showing the relationship between $V_{\text{max}}$ and $R_a$. (Note that both curves are the steepest at high velocities: $V_{\text{max}}$ changes most rapidly with velocity at high velocities and with resistance at low resistances.) $I_{a}^{\text{max}}$ then was plotted (Fig. 7, solid curve with dotted extension) as a function of $R_a$, inserting $R_a$ directly into Equation 5 and getting $V_{\text{max}}$ from Figure 8B.

We wish to call attention to three aspects of the relationship (derived from our measurements) between $I_{a}^{\text{max}}$ and $R_a$. First, the maximum axial current decreased gradually as $R_a$ increased when $R_a$ was large; this is the region of low propagation velocity, i.e. transverse to the long fiber axis. Second, axial current reached a peak at low values of $R_a$ and decreased sharply were $R_a$ to decrease further. Note that the maximum propagation velocity (in the direction of the long fiber axis) occurred at the peak of the $I_{a}^{\text{max}}$ curve. Finally, the curve derived from our measurements follows the inverse square-root relationship ($V_{\text{max}} = \text{constant}$) at high values of $R_a$ and deviates from it more and more as $R_a$ decreases.

What aspect of propagation could be responsible for this relationship? Considering the known morphology of cardiac muscle—individual, elongated cells coupled electrically through junctional complexes—perhaps we are seeing the effects of the nonuniform nature of the effective axial resistivity, $R_a$. It is well known that propagation in a discontinuous structure (such as myelinated nerve) depends on geometric, cytoplasmic, and membrane properties in a way different from that in a continuous structure (such as unmyelinated nerve). Brill et al. (1977), simulating propagation in myelinated fibers, found that as the internodal distance was increased, the propagation velocity increased to a maximum, then decreased gradually for larger internodal distances. It can be seen in their Figure 2A that, in the range where the propagation velocity increased with internode distance, $V_{\text{max}}$ increased and $V_{\text{peak}}$ increased as the velocity increased—the same dependence we observed in cardiac muscle.

The presence of notches on or following the depolarization phase of action potentials when propagation occurs in the transverse direction (Fig. 5) suggests that, indeed, propagation is discontinuous in that direction. Notches might be expected when the intracellular current has to pass through localized regions (cell junctions) of high resistance relative to the cytoplasm. Such junctions would separate the active membrane into patches, loosely coupled with each other. The peak after a notch would be caused by firing of the next patch, after a short delay for charging its capacitance. Heppner and Plonsey (1970) showed such notches in their simulations of propagation across a single junction between cardiac cells, as did Lieberman et al. (1973) during the repolarization phase with longer delays. Indeed, such notches were observed (Figs. 5 and 6) when propagation was in the transverse direction, but never in the longitudinal direction. Given the observed propagation velocity in the transverse direction and the timing between peaks on either side of a notch, the distance between discontinuities can
be estimated to be about 100 µm. Recent studies provide a morphological explanation for such widely spaced discontinuities (J. R. Sommer, personal communication). The intercellular connections in a trabecula of ventricular muscle of about 1 mm in diameter were reconstructed from several thousand serial sections, using a technique of combined light and electron microscopy similar to that used previously for a single (approximately 100 µm in diameter) Purkinje strand (Johnson and Sommer, 1967). Sommer showed that the trabecula was composed of several strands, the frequency and distribution of intercellular connections within each strand being similar to that of the Purkinje strand studied previously. The strands were connected side-to-side, however, at relatively widely spaced intervals—spacing approximately equal to the diameter of the strand. Propagation transversely, then, would be entirely different from propagation in a longitudinal direction. Longitudinally, propagation would be more or less continuous, similar to that in unmyelinated nerve. Transversely, each strand would fire more or less as a unit, with a delay between the firing of such units because of the high resistance of the relatively small and infrequent connections between them.

It should be clear that the features of myelinated nerve fibers that cause saltatory propagation are not analogous to those responsible for such discontinuous propagation in cardiac muscle. In models of myelinated nerves, the node length is kept constant when the internode distance is varied; patches of active membrane of fixed area are coupled by different lengths of passive cable. In cardiac muscle, the total area of active membrane remains constant as the distribution of junctions and membrane is varied. No model or simulation appropriate for such a structure is available in the literature, but work is in progress on such a model and the results will be presented in a subsequent paper.

Nonetheless, does the recognition of the discontinuous structure of cardiac muscle help us to understand the relationship we observed between $V_{\text{max}}$ and velocity (Fig. 8A)? Again, a quantitative analysis of this relationship will have to await the results of simulations, but a qualitative argument shows that the introduction of discontinuities can produce results which change in the right direction. A specified set of membrane properties will produce the largest $V_{\text{max}}$ when the membrane is in the form of an isopotential patch. When the membrane is distributed in the form of a cable, each piece of membrane (as it is depolarizing) has to supply charging current for the next piece of membrane downstream, slowing its own depolarization. It is reasonable, then, that $V_{\text{max}}$ might be larger for transverse propagation than for longitudinal propagation; in the transverse direction, the membrane is divided up into relatively isolated patches by relatively high

**Figure 8** The relationship of $V_{\text{max}}$ to propagation velocity (measured) and to effective axial resistivity $R_a$ (calculated from measured velocity). A: Rate of rise of the action potential as a function of velocity, recorded from one impalement in a ventricular preparation, Figure 5. The "crosses" are made up of (1) a vertical line of points representing $V_{\text{max}}$ measurements on several action potentials with the same propagation direction and velocity and (2) a horizontal bar (arbitrary length) to indicate the average $V_{\text{max}}$ value for the group of points. The solid line is an empirical fit to the data points; the equation for the curve is given in the figure. B: Same data as panel A, plotted as a function of $R_a$ calculated from the velocity from Equation 4.
resistance cell junctions, whereas in the longitudinal direction, the membrane distribution more closely approximates a continuous cable. During transverse propagation, then, activity would seem to halt momentarily or to hesitate before moving on to the next unit along the path. The action potential would be expected to be closer to a membrane action potential and, in some ways, similar to that in a collision.

**Predicted Abnormal Propagation**

Assuming that cardiac muscle is best described as a discontinuous structure, we can predict an unexpected kind of propagation that would not be possible in a continuous structure. Uniform propagation occurs if a patch of membrane can, once its permeability mechanism is triggered, supply enough current to depolarize itself and to supply the necessary current to charge the capacitance of the neighboring tissue which has yet to depolarize. The terms “safety ratio” and “safety factor” have been used (Schmitt and Schmitt, 1940; FitzHugh, 1969) to compare the maximum current a cell can supply with the current required to bring it to threshold; if the ratio is greater than one, propagation can occur, and if it is less than one, stable propagation cannot occur.

Uniform propagation was observed along the solid line in Figure 7. The part of the curve near and to the right of the peak (low $R_a$ values) is probably a region of propagation in which the safety factor is low. Two aspects of our results indicate the possibility of a low safety factor in this region: $V_{max}$ is low there, and the effective axial resistance is also low—implying that the cells or regions of membrane are tightly coupled for the flow of current. The amount of axial current in a given direction is determined by the source characteristics of a patch of membrane (described in terms of permeabilities and equilibrium potentials) and by the impedance (or loading) of the surrounding tissue. The combination of low $V_{max}$ and low $R_a$ is, therefore, consistent with a large current load on the membrane and a low safety factor.

If propagation along the axis with low $R_a$ does indeed have a lower safety factor than along the axis with high $R_a$, then propagation in a sheet of muscle should fail first in the direction of low $R_a$ as the ability of the membrane to supply depolarizing current is reduced. We tested this prediction in a crista terminalis preparation, where the normal ratio of longitudinal propagation velocity to transverse propagation velocity is large. If the safety factors differ with $R_a$, as we have proposed, the difference should be most apparent when the velocity ratio is large. We reduced the maximum membrane current during depolarization by stimulating prematurely, activating the fast sodium mechanism before it was reactivated completely late in repolarization. We eliminated other causes of altered propagation (e.g., a change in $R_a$ in a given direction) by comparing recordings from normal and premature beats initiated at a single stimulus site.

The arrangement of the electrodes on the preparation is shown in Figure 9. Eleven to 18 extracellular electrodes were arranged in a cross-shaped pattern around the stimulus electrode, the long axis of the cross parallel to the fiber orientation. We recorded a full sequence of responses in seven preparations; a typical set of recordings is shown in Figure 9. Normal stimuli were applied at intervals of 500 msec and a single premature stimulus was inserted after every 15th normal stimulus. A premature stimulus with a long delay (290 msec) produced a normal propagation pattern of high velocity in the longitudinal direction and low velocity in the transverse direction. High velocities calculated between different pairs of electrodes varied between 0.68 and 0.71 m/sec (cf a range of 0.97 to 1.1 m/sec for a normal stimulus) and the low velocities ranged between 0.11 and 0.12 m/sec.

Starting the premature stimulus in the absolute refractory period and increasing its delay, the earliest distant extracellular potential occurred at a delay of 130 msec (Fig. 9, upper right). The activity being recorded was clearly decremental because it was recorded only by the closest extracellular electrode in the longitudinal direction. As the premature stimulus was delayed to 134 and 145 msec, the decremental propagation in the longitudinal direction grew in strength and was

**Figure 9** Abnormal propagation of early premature action potentials. The drawing (upper left) shows the locations of the stimulating electrode and the recording electrodes on the crista terminalis preparation. The waveforms in the rest of the figure were recorded from extracellular electrodes at the numbered sites. The numbers in the boxes represent the delay time (in msec) between the prior normal stimulus and the premature stimulus.
observed at greater distances. Nothing could be seen in the transverse direction even at 145 msec.

By 155 msec, stable propagation was evident in the transverse direction while longitudinal propagation was still decremental. Note the shape of the potentials from the longitudinal electrodes numbered 1 through 4. At electrode 1, the potential is sharp and biphasic, indicating an action potential approaching and leaving the recording site. Electrode 3, on the other hand, showed a positive uniphasic potential, indicating that the propagation terminated near that site. It might be recalled that the original demonstration of decremental conduction was made in nerves with extracellular electrodes (Schmitt and Schmitt, 1940) on the basis of nearly identical waveform shapes. To see whether the propagation in the longitudinal direction was truly decremental, we calculated the propagation velocity between various pairs of electrodes. The resulting values were 0.38 m/sec (electrodes 0 and 1), 0.24 m/sec (electrodes 1 and 2), and 0.11 m/sec (electrodes 2 and 3). At the same time, the action potential propagated in both transverse directions at about 0.05 m/sec.

Note the later waveforms on electrodes 1 through 4 at a stimulus delay of 155 msec. The waveforms are biphasic, evidence of a propagating wavefront, and their sequence in time indicates propagation toward the stimulus electrode (from electrode 4 toward electrode 1). Such a wavefront could not have originated from the original longitudinal propagation, because it decremented to extinction. Thus the reverse wavefront must have been the result of the original transverse wavefront and is further evidence that the slow transverse propagation was not decremental.

The pattern of propagation shown in Figure 9 (155 msec) represents reentry; the exact pathway of the reentrant beat is not known, but the path length could not be very long because of the time delays observed. We observed simultaneous decremental and nondiminutional propagation along the two axes in all seven preparations studied; reentry of the form shown in Figure 9 was observed in six of these seven studies.

How can we be sure that the patterns of propagation (including decremental conduction and reentry) illustrated in Figure 9 were not due to nonuniform membrane properties? Both unidirectional block and decremental propagation usually have been attributed to nonuniform recovery of excitability (Allessie et al., 1976) when the action potential propagates into an area having a longer refractory period. To ensure that this mechanism was not responsible for our results, we made the following checks: (1) Refractory periods were measured at multiple sites in the entire area of extracellular recording. We used a stimulus of 1.5 to 2.0X threshold and identified the refractory period in the usual way, i.e., the interval at which a 1-msec additional delay just produced propagation to a distant recording site located 2-3 cm from the stimulus electrode. If the refractory periods differed by more than 7 msec throughout the recording area, another region was selected for study. (2) The stimulus electrode and a few of the recording electrodes were moved to other sites within the initial measurement area and a portion of the sequence of premature beats repeated. In all seven preparations and for all sites tested, the pattern of simultaneous decremental and non-decremental propagation was observed for some range of delay of the premature stimulus. The unusual conduction patterns clearly were related to the direction of fiber orientation and to small differences in refractory period. In the experiment shown in Figure 9, for example, the measured refractory periods were 151 msec at the stimulus site and 144 msec at electrode 4, a variation in the opposite direction required to explain the decremental conduction.

It should be emphasized that the predicted pattern of propagation for premature action potentials described above depend on the presence of recurrent discontinuities in intracellular resistance. If the medium of propagation was continuous, propagation would have to fail in both directions simultaneously because, in that case, varying $R_e$ scales only the variation of $V_m$ with distance and not the variation with time.

Discussion

Discontinuous Propagation

Several aspects of the propagation of excitation in cardiac muscle are inconsistent with the representation of the tissue electrically as a continuous structure. In a continuous structure, the propagation velocity can be reduced in two ways: the axial resistance can be increased or the membrane ionic properties altered. In the former case, the theory of propagation in continuous media predicts no change in the shape of the action potential and, in the latter case, the upstroke should be slowed with the velocity. Recording transmembrane action potentials at a single site as excitation arrived from different directions and at different velocities, we found that the shape of the depolarization phase of the action potential does change. Furthermore, $V_{max}$ and $V_{foot}$ varied in the opposite direction with velocity from what would be expected if the velocity change was caused by altered or modulated membrane properties. We also observed notches on or immediately following the upstroke of the action potential when excitation propagated in a direction at an angle to the long axis of the fibers, where cell couplings would be expected (on morphological grounds) to be closely spaced. As we argued, such notches would not be expected in a continuous, uniform structure, but would be produced by patches of membrane coupled by relatively high resistance junctions.

The results from cardiac muscle that demand the
inclusion of discontinuities for their explanation cannot be accommodated by cell branching alone. Strictly speaking, cardiac cells do not branch (Muir, 1965; Sommer and Johnson, 1979), but appear to do so where one cell joins with two or more. Branching in cardiac muscle thus must be thought of in terms of a network of cell junctions, rather than branching cytoplasm. From theoretical studies on branched models with continuous cytoplasm, small delays are produced at branching sites (Lieberman et al., 1973; Goldstein and Rall, 1974), but the amount of slowing observed would require a degree of branching not consistent with the known morphology of cardiac cells. Paes de Carvalho [in a paper which is not readily available, but which he kindly sent to us (Paes de Carvalho et al., 1976)] also found shape changes in the action potential when propagation originated at different sites. He could not account for these changes on the basis of a uniform cable model and implied that branching might be a possible explanation. Our results cannot be explained by any model of propagation in which the cytoplasm is continuous, even if it is assumed to divide, splitting the intracellular current.

Effects on Propagation

We observed directional differences in propagation velocity in both atrial and ventricular preparations, as others have previously (Sano et al., 1959). The propagation velocity was high in the direction of the long axis of the cells and low in the transverse direction. Because we know that cardiac cells are elongated structures coupled electrically through cell junctions, the directional difference in propagation velocity is reasonable because the distribution of cell junctions varies with direction. When this explanation for directional velocity differences is combined with the knowledge of the discontinuous structure, an unexpected corollary results: propagation in each of the two perpendicular directions responds differently to the same change in membrane properties. Propagation can become decremental and stop in one direction while it continues as uniform propagation in the other direction. In fact, uniform propagation ceases and becomes decremental first in the direction having the highest velocity, as the membrane activity is depressed. The low-velocity wavefront continues to propagate as the membrane is depressed still further. Thus, the "safety factor" is lower when the velocity is high and vice versa. The words "optimal" and "preferential" often have been applied to regions of fast conduction, implying that conditions are advantageous, that the propagation is safer there. This is true in a continuous structure if the velocity is changed by modifying the membrane properties. The introduction of discontinuities and modification of the propagation velocity by changing the intracellular resistance reverse the relationship of velocity and safety factor. Under these conditions, slow propagation is safer than fast propagation.

The term "functional dissociation" has been used to refer to situations in which propagation along one pathway behaves in a fashion different from propagation along a parallel pathway. For example, it has been postulated that a difference in membrane properties (reflected in a difference in refractory periods) accounts for the positive and negative waveforms of the dual A-V nodal conduction system (Mendez and Moe, 1966). Allessie et al. (1976) postulated a similar mechanism for the initiation and maintenance of circus movement in atrial muscle. As we have seen in our experiments, "functional dissociation" can be produced in a bundle of muscle with uniform membrane properties because of the differences in safety factors in the longitudinal and transverse directions. Directional block usually has been thought of in terms of one-dimensional propagation along some restricted structure—antegrade or retrograde propagation. Our results show that the angular dependence is more general and can be related to cell orientation.

It has been assumed that a measurement of the refractory period (by finding the time at which a stimulus of specified amplitude fails to produce excitation at a remote site) gives a measure of the cell membrane properties in the vicinity of the stimulus site. On the basis of our results, we now can see that it is much more difficult to perform this experiment than had been imagined. Since propagation of excitation blocks at different stimulus times in different directions (in relation to fiber orientation), it would be necessary to investigate propagation in the entire region around the stimulus site, not at just one point. Furthermore, it would be essential to make sure that any excitation observed was not actually decremental, a determination that would require several recording sites. In other words, the results depend not only on membrane properties, but also on the details of cell connections around the stimulus site.

The requirements for producing reentry in cardiac muscle generally have been thought to include a spatial nonuniformity of some sort, e.g., nonuniform membrane properties to produce nonuniform recovery of excitability (Allessie et al., 1976), possibly with an insulating boundary to provide an anatomical loop around which the impulse could propagate. We have demonstrated that reentry can occur within a small area having uniform membrane properties. Directional block can be produced by directional differences in cell coupling, so the pattern of propagation during reentry is related to the cell orientation, not to regional differences in membrane properties.

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The discontinuous nature of propagation in normal canine cardiac muscle. Evidence for recurrent discontinuities of intracellular resistance that affect the membrane currents.

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