Relating Extracellular Potentials and Their Derivatives to Anisotropic Propagation at a Microscopic Level in Human Cardiac Muscle

Evidence for Electrical Uncoupling of Side-to-Side Fiber Connections with Increasing Age

Madison S. Spach and Paul C. Dolber

SUMMARY. Elucidation of the mechanisms of cardiac conduction disturbances leading to reentry will require resolution of the details of multidimensional propagation at a microscopic size scale (<200 μm). In practice, this will necessitate the combined analysis of extracellular and transmembrane action potentials. The purpose of this paper is to demonstrate the relationships between the time derivatives of the extracellular waveforms and the underlying action potentials in the experimental analysis of anisotropic propagation at this small size scale, and apply these relationships to human atrial muscle at different ages. The extracellular waveforms and their derivatives changed from a smooth contour during transverse propagation in young preparations to complex polyphasic waveforms in the older preparations. The major problem was to estimate the size and location of small groups of fibers that generated the complex waveforms in the older preparations. We found dissimilarities in the derivatives that distinguished source (bundle) size from the distance of the source to the measurement site. The differences in the extracellular waveforms and their derivatives indicated that there was electrical uncoupling of the side-to-side connections between small groups of fibers with aging. These changes produced a prominent zigzag course of transverse propagation at a microscopic level which, in turn, accounted for the increased complexity of the waveforms. The waveform differences also correlated with the development of extensive collagenous septa that separated small groups of fibers. The electrophysiological consequence was an age-related decrease in the "effective* transverse conduction velocities to the range of the very slow conduction (<0.08 m/sec) which makes it possible for reentry to occur in small regions of cardiac muscle with normal cellular electrophysiological properties. (Circ Res 58: 356-371, 1986)
nisms found in animal preparations are applied to humans, since anisotropic propagation may be different in human cardiac muscle. Because we have been unable to find information on this point, we thought it was important not only to study human cardiac muscle but to investigate anisotropic excitation spread at a microscopic level.

Based on these considerations, we have studied two-dimensional propagation at a microscopic size scale in human atrial muscle bundles. Extracellular (rather than intracellular) potential measurements with a microelectrode were required for the analysis at a microscopic level. The major problem in evaluating nonuniformities of excitation spread was to resolve the location of the source of each deflection of the polyphasic extracellular potential waveforms. Masuda and Paes de Carvalho (1975) encountered a similar difficulty in their analysis of complex waveforms in the atrial region of the dog sinus node. They concluded that "it would seem impossible to ascertain a priori whether a given biphasic complex is generated by a small bundle near the electrode or a larger bundle further away." However, we found that it is theoretically possible to determine the difference between the size of the source (bundle size) and the distance to the source by examining the first and second time derivatives of the extracellular waveforms, which reveal the fine details of the original waveforms.

In this paper, we apply the relationships between the derivatives of the extracellular potentials and the underlying action potentials to the study of anisotropic propagation at a microscopic size scale. The goals of the study were to: (1) confirm experimentally theoretical predictions (Spach and Kootsey, 1985) that it is possible to determine the time of $V_{\text{max}}$ of the underlying transmembrane action potential from extracellular potential waveforms of any shape; (2) determine the fall-off of the amplitude of extracellular potential waveforms vs. their associated time derivatives at different distances from bundles of different sizes; and (3) relate the electrophysiological results to the distribution and geometry of collagenous connective tissue septa of the preparations. The stepwise approach toward these goals was important in order to evaluate the possibility of interpreting extracellular waveforms in terms of the underlying structure. The differences found in the extracellular waveforms and their derivatives at a microscopic level in different preparations provided evidence that with advancing age there is uncoupling of the side-to-side electrical connections between parallel-oriented cardiac fibers.

Methods

Experiment

For the analysis to be made in the most consistent and repeatable way possible, we found atrial pectinate muscle bundles to be the most suitable, because all such bundles demonstrated anisotropic properties (i.e., properties with different values when measured along axes in different directions). Further, these muscle bundles were readily available, since they are located in the apex of the right atrial appendage, which is removed routinely at cardiac surgery prior to artificial pumping of blood for circulatory assist. After approval of investigational protocols by an institutional committee for guidelines for human subject research, atrial specimens were obtained at cardiac surgery in 37 subjects who had no clinical evidence of hemodynamic or electrophysiological dysfunction of the right atrium (e.g., the P-waves of the electrocardiogram were normal and the right atrial pressure was normal at cardiac catheterization). Surgery was performed for the following conditions: coronary artery disease (15, ages 48–65 years), Wolff-Parkinson-White syndrome (17, ages 10–50 years), congenital heart disease without right atrial dilatation: aortic stenosis and ventricular septal defects (5, ages 1–10 years). The Wolff-Parkinson-White patients had arrhythmias due to the accessory pathway at the atrioventricular (AV) sulcus, and six subjects with coronary heart disease had ventricular tachyarrhythmias but none had atrial arrhythmias; i.e., there was no clinical evidence of an underlying pathologic state of the right atrium. Drug therapy was stopped 18–24 hours before surgery in all subjects. No attempt was made to standardize the size of the specimens, since the surgeons removed different amounts of the apical portion of the atrial appendage in different patients.

Each specimen was immediately placed in cooled perfusate solution at 2–5°C, brought to the laboratory, and pinned to the floor of a rectangular tissue bath (4 cm × 5 cm) and maintained at a temperature of 35°C. The composition of the perfusate, in mM, was as follows: NaCl, 128; KCl, 4.69; MgSO$_4$, 1.18; NaH$_2$PO$_4$, 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 a cannula to produce a high flow rate along a 1-cm-wide area on the surface of the preparation. The flow rate was 60 ml/min and the volume of fluid in the bath was 6 ml.

Intracellular potentials were recorded with conventional glass microelectrodes filled with 3 M KCl and having resistances between 10 and 20 MΩ. To analyze propagating depolarization at a size scale of 200 μm or greater, we used extracellular electrodes made of flexible tungsten wire, 50 μm in diameter and insulated except at the tip. However, during transverse propagation in some preparations the 50 μm in diameter extracellular electrode was too large to permit adequate resolution of the site of origin of each of the multiple deflections; i.e., there were electrically separate groups of fibers within 50 μm, each group firing asynchronously. We first used glass microelectrodes for intracellular measurements to identify the separate groups of cells, but this was not practical, if for no other reason than multiple cell penetrations within a small region caused membrane damage (prominent local injury potentials) and altered the microscopic spread of excitation. Glass microelectrodes with a tip diameter of 1–2 μm yielded high quality extracellular waveforms, but the tip was not visible, making it impossible to verify the exact sites of the measurements. The problem was resolved by using extracellular metal microelectrodes (Diamond Electro-Tech, Inc.) which were insulated except for an exposed tip of approximately 1 μm (Fig. 1A) and which had a resistance of 1–2 MΩ. 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 and intracellular electrode were positioned 7 cm away from the recording site. The rise time (10–90%) for both intracellular and extracellular recording systems was less than 30 μsec.
A pacemaker stimulus 1.0 msec in duration and 1.5 times threshold was applied to the endocardial surfaces of atrial muscle bundles at a rate of one per second. A PDP-11/44 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 20,000 and 50,000/sec (12-bit samples). With the fastest upstrokes (400 V/sec) and with the most rapid deflections of complex extracellular waveforms, we noted no change when the sampling rate was increased above 40,000/sec. The computer stored the data and displayed the waveforms concurrently on a Tektronix 4014 unit with a persistent screen so that each new waveform could be superimposed on the waveforms of the previous beats. This provided a way to (1) detect immediately small changes in the shape of the waveforms and check their reproducibility, and (2) document continually that the sequence of excitation did not change.

To analyze the spread of excitation along the longitudinal and transverse axes of the fibers, we moved the extracellular metal microelectrode in steps of 10–50 μm, as determined by the micrometer of the micromanipulator. The position of the tip was documented from photographs made with a Nikon F250 35-mm camera through a dissecting microscope (effective resolution better than 5 μm). Photographs were necessary since movement of the microelectrode tip transiently penetrated the cell membrane, during which time we recorded action potential upstrokes with an amplitude of 30–60 mV. The timing (but not amplitude) of V_max from these impalements gave the same results as the glass microelectrode impalements. When available, we used the time of V_max measured from these transient metal microelectrode impalements for some of the time correlations with derivatives of complex extracellular waveforms.

After the conclusion of each experiment, the measured waveforms stored digitally were redisplayed and photographed for initial analysis. After selection of appropriate waveforms, the original digitized values were transferred to a HP-9000 computer for detailed analysis and automatic plotting. The transmembrane potential V_m was obtained by subtracting the extracellular potential from the intracellular potential (V_m = φ_i − φ_e), where φ_i is the intracellular potential and φ_e is the extracellular potential. The first time derivative of V_m, and the first and second time derivatives of the extracellular waveforms were obtained numerically, and the values were plotted in time steps of 20, 25, or 50 μsec. In the original extracellular waveforms, the peak-to-peak noise level was typically 15–25 μV, which corresponded to a noise level in the first time derivative of 0.1–0.2 V/sec and in the second time derivative of 0.5–1.0 V/sec. To minimize the sensitivity of the derivatives to the slight noise in the original waveforms, we used established techniques for finding the derivatives of slightly smooth data (Ralston, 1965). When possible, isochrone maps were constructed from the extracellular recordings, taking the time of the peak negative deflection of the derivative as the instant of excitation. Conduction velocity was calculated as the distance traveled normal to the isochrone per unit time. When isochrones were not constructed, the effective velocity was estimated by dividing the distance between two electrodes by the difference in time at which the maximum negative slope of the extracellular potential occurred at each electrode. The relationship between the velocities of different groups of subjects was analyzed statistically using Student’s t-test for unequal means. The level of significance was taken as 0.01.

Morphology

All preparations were placed in Bouin’s fixative and routinely processed and embedded in paraffin; sections were cut at 7 μm. Because the preparations were usually small, it often happened that the orientation of the specimens in the blocks was poor; furthermore, we were often forced to choose between orienting bundles of interest for cross-sections or longitudinal sections (i.e., we could not do both for most single small bundles).
To delineate the distribution of collagenous connective tissue, we used a modification of the picrosirius red technique of Junqueira et al. (1979). The modifications were: use of a 10-minute wash in running water after initial hydration of the sections and the addition of excess picric acid to the picrosirius red staining solution as recommended by Sweat et al. (1964), and 1-minute treatment of the sections in 0.2% aqueous phosphomolybdic acid (PMA) between the running water wash and the picrosirius red staining step. PMA treatment, which eliminated all background staining (including that due to picric acid), increased contrast for photomicrography. PMA also increased the refractive index of the muscle cytoplasm; accordingly, photomicrographs were taken of sections that were temporarily mounted in either trans-cinnamaldehyde (refractive index 1.6195) or a mixture of trans-cinnamaldehyde and diethylene glycol monobutyl ether (formulated to give a refractive index of 1.60).

Theory

Although the physical laws governing the extracellular potentials generated by ionic currents are well known (Lorente de Nó, 1947; Rosenfalck, 1969), we have found no information about the relation between the extracellular depolarization waveforms and their time derivatives near the current sources. Also, at the membrane surface, it is important to know how the measurable quantities of the transmembrane and extracellular potential waveforms and their derivatives are related to each other and, in turn, to the underlying nonmeasurable quantities of the depolarizing current and conductance. The theoretical relationships between the potentials at the membrane surface and the internal membrane variables, the sodium conductance and current, were first examined by computer simulation; those results, along with the details of the calculations, are reported in a separate paper (Spach and Kootsey, 1985). The purpose here was to determine how the extracellular potentials are related to their time derivatives as the measurement site is shifted away from the membrane surface of bundles of different sizes.

We simplified the problem as much as possible by using a single transient sodium current mechanism with the equivalent electrical circuit of a continuous cable. This conduction model would be representative either of propagation in a long thin cell or of plane-wave propagation in a two- or three-dimensional group of tightly coupled cells. A macroscopic rather than single-channel current description was desired because propagating transmembrane and extracellular potentials are determined by the macroscopic membrane currents as a function of time and space. Thus, we considered that models in the form of the Hodgkin-Huxley equations (Hodgkin and Huxley, 1952) for the macroscopic Na* current were most appropriate for this study because of the small time scale they describe and fit. We chose the Ebihara-Johnson description (Ebihara and Johnson, 1980) of the fast Na* current because it is based on voltage clamp data in cardiac muscle under normal conditions.

For propagation in one dimension along a uniform structure, the relationship between the transmembrane potential $V_m$ and the net transmembrane current per unit area $I_m$ is a function of time and space governed by the cable equation:

$$I_m = a \frac{d^2V_m}{2R_i \, dx^2} = C_m \frac{dV_m}{dt} + I_m,$$  \hspace{1cm} (1)

where $a$ is the radius of the cylinder, $R_i$ is the internal resistivity, and $C_m$ is the membrane capacity. We approximated the ionic current $I_n$ (per unit area) during the depolarization phase of the cardiac action potential by the fast, transient sodium current in parallel with a constant leakage (repolarization) conductance:

$$I_{ion} = \frac{G_{Na} m^3 h (V_m - V_{Na}) + g_l (V_m - V_L)}{2}.$$  \hspace{1cm} (2)

where $G_{Na}$ is the maximum Na* conductance, $g_l$ is a constant, and $V_{Na}$ and $V_L$ are the sodium and leak equilibrium potentials, respectively.

Values of 1.0 $\mu F/cm^2$ and 450 Ohm-cm were used for $C_m$ and $R_i$ respectively. In an attempt to approximate the effective size of a small bundle of fibers, we assigned a value of 25 $\mu m$ to the radius. Equation 1 was solved over a length of 6 resting space constants, and the transmembrane and extracellular potentials were computed at the midpoint of the cable where there was uniform propagation (i.e., no end effects). The ordinary differential equations for $m$ and $h$ were solved by the predictor-corrector or modified Euler method (e.g., Gerald, 1970) with a time increment of 2 $\mu sec$. The cable equation (Eq. 1) was solved by the Crank-Nicolson implicit method (1947) using a length increment ($\Delta x$) of 0.014 resting space constant.

The extracellular potential $\phi_x$ at a point on the membrane surface and at varying distances from the surface was derived by solving the following equation for a one-dimensional cable, as done previously (Spach et al., 1973):*$^*$

$$\Phi_x (P, \tau) = \frac{a R_e}{2} \int \frac{I_m(x, \tau)}{[a + d^2] + [x' - x]^2} \, dx,$$  \hspace{1cm} (3)

where $a$ is the radius of the cylinder, $R_e$ is the resistivity of the extracellular fluid, $I_m(x, \tau)$ is the net transmembrane current (Eq. 1) at point $x$ at time $\tau$, $d$ is the perpendicular distance between site $x'$ at the membrane surface and the observation point $P$, and $(x' - x)$ is the distance along the cylinder between point $x$ and point $x'$. We used a value of 150 Ohm-cm for the extracellular resistivity of the fluid comprising the homogeneous volume conductor (Geselowitz et al., 1982).

In bundles of different sizes, the actual effective radius of the active tissue producing the extracellular potentials is not known. To approximate the effective size of different bundles (cables), we assigned values of 25, 75, 150, and 250 $\mu m$ to the radius in Equation 3 (the assumption was that each large cable represented a compact group of synchronously firing small bundles). Previous evaluation of Equation 3 at the membrane surface (Spach and Kootsey, 1985) demonstrated that in going from a radius of 25 $\mu m$ to 250 $\mu m$, any distortion of the final $\Phi_x$ waveforms due to timing errors produced by the small contributions of currents from distant membrane was negligible (<16 $\mu sec$). Therefore, we considered that the approximation of multiple simultaneously firing small bundles by a single cable to be valid at the microscopic size scale of this study. We maintained the same underlying shape and velocity of a uniformly propagating transmembrane action potential (shown in Fig. 2) for all calculations of $\Phi_x$ (Eq. 3). That is, once the values of $I_m$ had been computed using Equa-

* For the idealized geometry of a one-dimensional cable, we assumed that the surface integral term (Spach et al., 1972, see Equation 1) can be neglected, as shown by Plomsey (1969, pp. 240-248).
tion 1 for the small cable (radius = 25 μm), these values of \( I_{m} \) were substituted into Equation 3 to allow calculation of \( \phi_e \) for the small cable and for the three larger cables. The extracellular potential waveforms were calculated at the surface of each cable and at nine additional radial distances to a maximum of 1000 μm.

The values were computed on the HP 9000 computer for detailed analysis and plotting of the theoretical waveforms. The first time derivatives were obtained numerically from the computed values of \( \phi_e \), \( I_{m} \), and the transmembrane potential. The extracellular waveforms, along with their first and second time derivatives, were plotted for each time instant at 20-μsec intervals.

### Results

To enhance comparison of the extracellular potential waveforms and their time derivatives, we present representative results from subjects of widely different ages. We use the term "derivative" to mean the first time derivative of the original potential waveform; the second time derivative is specified as such in each case.

### Status of the Preparations

Of the total 37 specimens, 23 provided successful experiments from subjects whose ages varied between 1 and 65 years. These 23 specimens were from subjects in the following age categories: 1-14 years (nine subjects), 15-39 years (four subjects), and 40-65 years (10 subjects). None of the 14 unsuccessful preparations responded to electrical stimulation; we made no attempt to restore them by the addition of acetylcholine or epinephrine (Trautwein et al., 1962; Gelband et al., 1977). Intracellular measurements in four of the nonviable preparations revealed a constant transmembrane potential of approximately −30 mV. The viability of the preparations appeared to be related to the amount of apical appendage removed at surgery; none of the specimens smaller in area than 1 cm² responded to stimulation. Since there was no clinical evidence of disease of the atrial appendage, we attributed their low resting potentials and lack of excitation response to the lack of healing over, or to injury from, the surgical removal of such small specimens, rather than to any underlying pathological state. All specimens larger in area than 1 cm² responded well for 4-12 hours and had membrane resting potentials more negative than −74 mV, thus allowing us to interpret the mechanism of depolarization to be the fast sodium current. In no case was there evidence of diastolic depolarization (e.g., none of the preparations beat spontaneously) and in none did we find slow upstroke action potentials (\( V_{max} < 20 \text{ V/sec} \)).

### Relation between Extracellular and Transmembrane Potential Derivatives

#### Simulation Predictions

Figure 2 summarizes the computer simulations (Spach and Kootsey, 1985) that relate the extracellular and transmembrane potentials, and their derivatives, to each other and to the nonmeasurable internal membrane variables \( g_{Na} \) and \( I_{Na} \) for a uniformly propagating action potential. In panel A (top), the asymmetric shape of the biphasic extra-

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**Figure 2.** Theoretical predictions: simulations relating extracellular and transmembrane potentials, and their derivatives, to each other and to the sodium current and conductance. Panel A: computed extracellular and transmembrane potentials (top) and their first time derivatives (bottom) for a uniformly propagating action potential. The extracellular potential \( \phi_e \) was computed for a location at the surface of a cylinder with a radius of 25 μm. The conduction velocity was 0.9 m/sec. Panel B: computed temporal relationships between the first derivative of \( V_m \) and the nonmeasurable internal membrane variables \( I_{Na} \) and \( g_{Na} \). The first time derivatives of \( I_{Na} \) and \( g_{Na} \) are shown below. The transmembrane potential \( V_m \) in panel A was used in all calculations of \( \phi_e \) in subsequent simulations.
cellular waveform with a more prominent minimum than maximum is accounted for by the asymmetry in the shape of depolarization of \( V_m \). The lower row of panel A shows the derivatives of the extracellular (above) and transmembrane (below) potentials. The maximum negative slope during the course of the biphasic extracellular waveform occurred at the same time as \( V_{\text{max}} \) of the transmembrane potential. Panel B (top) shows the computed temporal relation between \( V_m \) and the internal membrane variables. \( V_{\text{max}} \) occurred between the time of turn-on and the peak values of \( g_{\text{Na}} \) and \( I_{\text{Na}} \). The computed derivatives of \( g_{\text{Na}} \) and \( I_{\text{Na}} \) (lower row), however, show that \( V_{\text{max}} \) coincided closely in time (difference <20 \( \mu \text{sec} \)) with the maximum rate of increase in \( g_{\text{Na}} \) and \( I_{\text{Na}} \). Thereby, both the negative peak of the first derivative of the extracellular potential and \( V_{\text{max}} \) of the transmembrane potential provide a marker for the instant of the maximum rate of increase of the depolarizing current and its conductance. Further simulations (Spach and Kootsey, 1985) showed that the coincidence in time between \( V_{\text{max}} \) and the maximum negative slope of the extracellular potential waveform was unchanged for variations in the shapes of the waveforms with (1) marked changes in the membrane properties (e.g., changes in \( E_{\text{Na}} \) and \( G_{\text{Na}} \)) and resting potential, and (2) propagation at sites where the shapes of the transmembrane and extracellular waveforms were quite different and changed from site to site; e.g., at sites where \( \phi_e \) was entirely negative (where impulse conduction begins) and where \( \phi_e \) was largely positive (at collisions or where the cable ends). A major practical significance is that the negative peak of the derivative of the extracellular waveform gives the same fiducial point as \( V_{\text{max}} \) of \( V_m \) and, thereby, it can be used to measure small differences in the timing of local excitation between points at a very small size scale—which we have found to be very difficult from inspection of the original \( \phi_e \) and \( V_m \) waveforms.

**Experimental Confirmation**

To determine if the above relationships could be confirmed experimentally for different shapes of the extracellular and transmembrane potential waveforms, we examined the measurable quantities \( V_m \) and \( \phi_e \) while the velocity was altered by changing the direction of propagation and by initiating premature stimuli. A typical result is shown in Figure 3. In panel A (1) the transmembrane potential rises
faster and the amplitude is greater during slow transverse ($\theta_T$) propagation than during fast longitudinal ($\theta_L$) propagation. Thus, the changes in the shape of the upstroke of $V_m$ with changes in the direction of propagation in anisotropic human cardiac muscle are the same as those found in dog atrial and ventricular muscle (Spach et al., 1981). The directionally dependent differences in the shape of depolarization were equally marked, or accentuated, when the action potential was initiated at a less negative take-off potential by early premature stimuli [panel A (2)]. However, the derivatives in each case (panel B) confirmed the theoretical prediction that the maximum negative slope of the extracellular waveform occurs at the same time as $V_{max}$ of the transmembrane potential (time difference <50 $\mu$sec), whether the shape differences were due to changing the direction of propagation or to initiating progressively earlier premature action potentials.

Age-Related Differences in the Anisotropic Propagation Properties

Preparations from Subjects 1-14 Years Old

Figure 4 shows the pattern of excitation spread and the waveforms of a preparation from a 2-year-old male, a result that was representative of the youngest age group. The major feature was characteristic uniform anisotropic excitation spread. There was a gradual transition in velocity from the longitudinal (fast) axis to the transverse (slow) direction, resulting in a relatively broad area of fast longitudinal conduction with an elliptic shape of the isochrones. The velocities parallel to and perpendicular to the long fiber axis were 0.59 and 0.12 m/sec, respectively ($\theta_L/\theta_T$ approximately 5). Intermediate velocities occurred dependent upon the direction of propagation at different angles to the long fiber axis, similar to results in dog papillary muscle (Spach et al., 1981). The associated extracellular waveforms maintained a smooth contour as the magnitude of the deflection decreased in association with the transition from fast longitudinal to slow transverse propagation. The derivatives of the waveforms also maintained a smooth contour in association with a decrease in the magnitude of the negative peak, although, at site 3, the derivative was slightly uneven prior to the major deflection. Intracellular measurements demonstrated the expected directionally different shapes of the upstroke, as well as the coincidence in time of $-d\phi_e/dt_{max}$ and $V_{max}$ (arrows at bottom of Fig. 4). In bundles from subjects 1-14 years old, the longitudinal (maximum) velocity $\theta_L$ in each preparation occurred within the range of 0.43 to 0.62 m/sec (mean 0.50 m/sec) and the transverse (minimum) velocity $\theta_T$ varied between 0.09 and 0.14 m/sec (mean 0.11 m/sec), a $\theta_L/\theta_T$ ratio of the means of 4.5.

We looked for evidence of local conduction delays at a microscopic level by measuring the instant of local excitation, taken as $-d\phi_e/dt_{max}$ at sites 20-100 $\mu$m apart (less than a cell length), along the long axis of the fibers in the zone of fast conduction and at

**FIGURE 4.** Youngest age group (1-14 years): representative pattern of excitation spread (top) and extracellular waveforms and the associated first time derivatives (bottom). The isochrones are separated by 1 m/sec. Propagation was initiated at a point (asterisk) using a 50-$\mu$m stimulus electrode. The points on the outline of the preparation mark the locations of the extracellular recordings from which the activation map was constructed. The thin dashed lines denote the orientation of the fibers. The circled points indicate the three sites where the depicted waveforms were measured; the accompanying arrows on the drawing of the preparation indicate the direction of propagation normal to the isochrones at each waveform site. The arrows at the bottom of each panel mark the times of $V_{max}$ of the underlying action potentials. * = stimulus site. (The preparation was from a 2-year-old male).
sites 10–20 μm apart (about a cell diameter) along an axis in the transverse direction where there was slow conduction. The waveforms and their derivatives maintained a smooth contour, and there was a monotonic increase in the local excitation times without evidence of local "jumps" or delays in time in either direction.

The above results in young preparations, therefore, are consistent with an anisotropic propagation medium consisting of tightly coupled cells in both the longitudinal and transverse directions; i.e., the distribution of the sites of electrical coupling between fibers provides pathways for intracellular current flow in all directions. We hasten to add that even though the extracellular waveforms could be accounted for by a model of tightly coupled cells in a uniformly anisotropic medium (Spach et al., 1979), there was some heterogeneity. We often found waveforms characteristic of nonuniform anisotropy near the junctions of major bundles (branching sites). Transverse (but not longitudinal) propagation in these areas usually produced extracellular waveforms that had 2 or 3 small (50 μV to 150 μV) deflections superimposed on the main deflection of 1–2 mV.

Preparations from Subjects 40–65 Years Old

A representative result of the older age group is shown for a preparation from a 42-year-old male in Figure 5. The major characteristic of excitation spread was the abrupt transition from fast longitudinal to slow transverse conduction. This is illustrated by the abrupt change in amplitude and shape of the extracellular waveforms and their derivatives at sites less than 60 μm apart in going from fast longitudinal propagation (site 1) to slow transverse propagation (site 2). Within the pectinate muscle, there was a narrow zone of fast longitudinal propagation (open arrow in drawing) with most of the bundle being excited by slow transverse spread that was quite irregular in nature, as reflected by the complexity of the low amplitude extracellular waveforms and their derivatives. There was no intervening zone of intermediate velocities at different angles to the long fiber axis between fast longitudinal and slow transverse conduction, as was present in the uniform anisotropic bundles of the subjects under 15 years.

The abrupt, rather than gradual, spread of excitation from the longitudinal to the transverse direc-

![Figure 5. Oldest age group (40-65 years): representative pattern of excitation spread (top) and the extracellular waveforms and the associated first time derivatives (bottom). The prominent open arrow on the outline of the preparation indicates the narrow region of fast propagation along the longitudinal axis of the fibers. The "sawtooth" curve denotes the irregular zigzag course of excitation spread in a direction transverse to the long axis of the fibers. The arrows at the bottom mark the times of V_max. * = stimulus site. (The preparation was from a 42-year-old male.)](http://circres.ahajournals.org/doi/fig/10.1161/01.RES.83.2.363/fig-5)
tion was manifest even in the large extracellular waveforms recorded in the narrow zone of fast conduction. The derivatives revealed irregularities in the terminal negative component (Fig. 5, site 1), a feature not encountered in bundles of the young subjects. In panel 1 (bottom) there are three small deflections during the 2 msec following the major deflection of the derivative. These small but definite irregularities in the terminal phase of the large biphasic waveform of fast longitudinal propagation were due to the irregular spread of excitation in the transverse direction in adjacent fibers. This is evidenced by the following points regarding the waveforms in the three panels, which represent the three recording sites in the drawing at the top (Fig. 5). (1) With increasing distance from site 1, there was a marked decrease in amplitude of the large biphasic waveform and its derivative, with virtually no change in the time of the deflection or in that of the negative peak of the derivative. (2) There were multiple small deflections in the waveforms at sites 2 and 3, so that the associated derivatives had multiple negative peaks at approximately the same time as those at site 1, i.e., during the 2 msec following the major deflection. (3) Intracellular action potentials were measured transiently at several sites, and the time of $V_{\text{max}}$ coincided with one of the negative peaks in the derivative waveform (arrows at bottom of Fig. 5), similar to the time alignment of the derivative peaks during transverse propagation shown in Figure 3.

In bundles from the older subjects, the longitudinal velocity varied between 0.58 and 0.78 m/sec (mean 0.69 m/sec), and the effective transverse velocity varied between 0.04 and 0.11 m/sec (mean 0.07 m/sec), a $\theta_1/\theta_1$ ratio of the means of 9.8. Thus, the average longitudinal velocities were greater ($P < 0.01$) and the average effective transverse velocities were lower ($P < 0.01$) than the respective velocities of the preparations from the young subjects. The quite low average effective velocity (0.07 m/sec) in the transverse direction occurred in ordinary (non-specialized) atrial muscle that had fast upstrokes arising from resting potentials more negative than $-74 \text{ mV}$; i.e., in the absence of slow upstroke action potentials that have been considered necessary to produce "very slow conduction" (<0.1 m/sec) (Cranefield, 1975). Therefore, we next examined the complexity of the extracellular waveforms, looking for a way to explain the development of the quite low effective transverse conduction velocities in the older subjects in the presence of normal action potentials.

We looked for conduction delays by measuring the instant of local depolarization, measured as $-\Delta \phi/\Delta t_{\text{max}}$ at sites 10 to 100 $\mu$m apart, as done in the young preparations. All of the older bundles yielded the same type of result. In the zone of fast conduction along the long axis of the cells, there was a monotonic progressive increase in the excitation times without evidence of local "jumps" or delays (or changes in waveform shape). In the transverse direction in the zone of slow conduction, however, the pattern was quite complex with both delays and "jumps" of the local excitation times.

The above results in the older age group suggest an anisotropic propagation medium comprised of cells that are tightly coupled along the longitudinal axis of the fibers, but along the transverse axis there are recurrent areas in which side-to-side electrical coupling of adjacent groups of parallel fibers is absent. Thus, when compared to the tightly coupled medium with uniform anisotropic propagation of the young preparations, there is a progressive loss with age of side-to-side electrical coupling between groups of parallel-oriented atrial fibers. Such a medium would account for the uniform fast propagation without delays in the direction of low axial resistivity (the long axis of the fibers) in the presence of nonuniform slow propagation with delays along the axis of high axial resistivity (the transverse direction). The periodic absence of side-to-side electrical coupling between groups of fibers should also produce a zigzag course of transverse propagation. A significant zigzag component of excitation spread, in turn, should generate numerous deflections (and their varied shapes) in the extracellular waveforms. Although not shown in Figure 5, additional measurements during transverse propagation often demonstrated local excitation times consistent with microscopic spread along the longitudinal axis of small group of fibers (in either direction), a finding that did not occur in the young preparations. Thus, we conclude that in changing from longitudinal to transverse propagation, the conduction velocity decreases not only because the effective axial resistance is greater, but also because the conduction pathway, being quite irregular (zigzag), is longer. In such complex preparations, the question arises of whether the preceding relationship of the peak negative derivative of $\phi_1$ to $V_{\text{max}}$ also could be used to relate each deflection of the polyphasic waveforms to its site of origin.

**Time Derivatives of Potential Waveforms Near the Sources**

**Theoretical Considerations**

The above results at a microscopic level suggest that for different bundles the rapid spreading out of the ionic currents in extracellular space produces a complex relationship between the amplitude of a potential deflection and its time rate of change at varying distances in the region of the membrane surface. For example, the amplitude of a potential deflection was not proportional to the amplitude of the negative peak of its derivative and, in some cases (not shown), the larger deflections had smaller derivative peaks. Since we were unable to find prior theoretical treatment of the relationships expected
between the two, we computed the relationships for a uniformly propagating transmembrane action potential in bundles of different sizes using Equation 3 (see theoretical section of the Methods). The results are summarized in Figure 6 for 10 radial distances from the surfaces of three bundles (cylinders), the radii of which were 25, 75, and 250 μm.

At the membrane surface, increases in the size of the cylinder had less effect on the magnitude of the derivatives than on the amplitude of the original potential waveforms. For example, the peak-to-peak amplitude of the potential deflection at the surface (panel A) increased in rough proportion to the increases in the radius from 25 to 250 μm. However, a 10-fold increase in the amplitude of the original deflection was associated with slightly more than a 5-fold increase in the magnitude of the first derivative (panel B), and the amplitude of the second derivative increased only by a factor of 2.3. Also, in panel C, the magnitudes of the second derivatives at the membrane surface were approximately the same for cylinders b and c, although the original potential deflection was three times greater at the surface of the larger cylinder c.

Increasing the distance from the sources produced a fall-off in the values of each variable that was relatively different for cylinders of different sizes. The following example illustrates the different effects of cylinder size and distance. In panel A, the largest cylinder (radius = 250 μm) produced a peak-to-peak deflection at a distance of 1000 μm from its surface that was about the same as the amplitude of the deflection produced at the surface of the smallest cylinder (radius = 25 μm). However, the magnitudes of the first and second derivative peaks were considerably greater for the deflection at the surface of the smaller cylinder. The arrow in each panel of Figure 6 marks the distance that the observation site would have to be moved from the surface of the largest cylinder to make the extracellular potential or one of its derivatives, according to the case, equal to the values at the surface of the smallest cylinder. This shows that for equivalent values to be achieved by two bundles of markedly different sizes the reduction in distance became more pronounced in going from the original potential waveform to the first derivative to the second derivative. Thus, a large potential deflection with a relatively small amplitude of the first and second derivatives indicates that the observation site is relatively far from a large bundle and, conversely, a small potential deflection with a relatively large amplitude of the first and second derivatives indicates proximity to a small bundle.

**Comparison of Experiment with Theory**

The above theoretical results suggest that experimentally it should be possible to distinguish variations in the size of a source (bundle size) from variations in the distance of the source from a measurement site by analysis of the amplitude, velocity, and acceleration of each deflection of a polyphasic waveform in nonuniform anisotropic preparations. Panel A of Figure 7 shows a very complex and prolonged polyphasic waveform measured in a preparation from a 62-year-old male. The drawing in panel B shows an outline (broken lines) of the main bundle and its nearby branches, the stimulus site (asterisk), and the location of the tip of the recording electrode (solid triangle) in an area of transverse propagation. To identify the bundle where each deflection originated, intracellular and extracellular measurements were made at sites along
FIGURE 7. Highly complex polyphasic waveform measured in an older preparation (panel A), geometry of the underlying structures with pattern of excitation spread (panel B), and cable model of the location of the sources (panel C). The numbers in panels A and B show the correspondence between each deflection and the structure of origin. In panel A, the letters (a–i) identify each small deflection produced during transverse propagation in area 2 of the major bundle. In panel B, the outline drawing (broken lines) was made from photographs of the main bundle and its branches, the stimulus site (asterisk), and the location of the tip of the extracellular metal microelectrode (solid triangle). (The preparation was from a 62-year-old male.) Panel C shows the complete geometric configuration of large and small cylinders that produced the best theoretical fit of the experimental waveform in panel A, along with its first and second derivatives (see Fig. 8). Table 1 lists the parameter values of the geometric configuration.

The general pathway of excitation spread (panel B, lines with arrows) was derived from additional extracellular measurements (25 sites). This established that the direction of propagation was transverse in area 2 and longitudinal in the other areas of origin.

Figure 8A shows the same experimental polyphasic waveform (top) along with the unsmoothed curves of the first (middle) and second (bottom) time derivatives. The absolute values of the derivative peaks associated with the smallest potential deflections during interval 2 became progressively larger than the other derivative peaks (arising from larger potential deflections) in going to the first derivative and then to the second derivative. In the middle panel (Fig. 8A), the negative peak of the first derivative of deflection 3 and the negative peaks during interval 2 have a similar amplitude. However, the negative peaks of the derivative during interval 2 represent a marked relative increase compared to the very low amplitude of the original potential deflections and, conversely, the negative peak of the derivative of deflection 3 represents a considerable relative decrease compared to the large amplitude of its original potential deflection.
progression of the relative changes was especially pronounced in going from the first to the second derivative; the largest second derivative peaks occurred for the smallest original potential deflections, which were produced by sources located closest to the observation site. On the other hand, the derivatives of the largest original potential deflections, which were due to more distant sources, decreased almost to the noise level of the second derivative curve. Thus, the derivatives provided information similar to that in the combined results of panels A and B of Figure 7; e.g., although the largest deflection originated from area 3, the smallest deflections originated from sources nearest the measurement site.

This example provides a general experimental confirmation of the theoretical predictions summarized in Figure 6. However, a rigorous test would be to simulate numerically the experimental waveform based on the known locations of the bundles of origin (only approximately known for area 2) and the measured times of each deflection, with approximations of different bundle dimensions used as an index of source strength. We attempted such a forward simulation, beginning with a numerical solution for the fast sodium current of a uniformly propagating action potential and ending with superposition of the individual waveforms (Eq. 3), which were computed at the same 10 radial distances from the surface of each cylinder as done in Figure 6. To make the simulation practical, we simplified as much as possible: (1) We used a value of 25 μm for the radius of all of the smallest bundles, and we represented the largest bundles by two sizes, one with a radius of 150 μm and the other with 250 μm. (2) The distance of the membrane surface of each source from the observation site was determined from photographs of the preparation; however, in area 2 we made the final assignment of distance of each of the smallest bundles after repeated trials, using all available computed waveforms with a small amplitude. (3) The local time of depolarization (V_max) of each bundle was assigned as the time of -dΦ_e/dt_max of each deflection in the original experimental waveform (panel A of Fig. 7); however, we adjusted the time of V_max within the error limits (20 μsec) of the experimental sampling rate for the potential deflections during interval 2 to obtain the best visual fit.

The final result is shown in Figure 8B for compar-
TABLE 1
Parameter Values Used in Simulation of Polyphasic Waveform and Derivatives of Figure 8

<table>
<thead>
<tr>
<th>Bundle</th>
<th>Radius of bundle (μm)</th>
<th>Distance to bundle surface (μm)</th>
<th>Time of V max (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A</td>
<td>150</td>
<td>400</td>
<td>1.54</td>
</tr>
<tr>
<td>B</td>
<td>150</td>
<td>400</td>
<td>2.52</td>
</tr>
<tr>
<td>C</td>
<td>150</td>
<td>400</td>
<td>3.36</td>
</tr>
<tr>
<td>2. a</td>
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<td>50</td>
<td>4.32</td>
</tr>
<tr>
<td>b</td>
<td>25</td>
<td>25</td>
<td>5.30</td>
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<tr>
<td>c</td>
<td>25</td>
<td>50</td>
<td>6.10</td>
</tr>
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<td>25</td>
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</tr>
<tr>
<td>e</td>
<td>25</td>
<td>50</td>
<td>6.52</td>
</tr>
<tr>
<td>f</td>
<td>25</td>
<td>150</td>
<td>7.52</td>
</tr>
<tr>
<td>g</td>
<td>25</td>
<td>150</td>
<td>8.52</td>
</tr>
<tr>
<td>h</td>
<td>25</td>
<td>250</td>
<td>11.00</td>
</tr>
<tr>
<td>i</td>
<td>25</td>
<td>400</td>
<td>13.20</td>
</tr>
<tr>
<td>3.</td>
<td>250</td>
<td>600</td>
<td>17.90</td>
</tr>
<tr>
<td>4.</td>
<td>150</td>
<td>1000</td>
<td>23.50</td>
</tr>
<tr>
<td>5.</td>
<td>250</td>
<td>1000</td>
<td>27.70</td>
</tr>
</tbody>
</table>

* The bundles (cylinders) are identified with labels that correspond to those of Figure 7. See text for description of summing each computed waveform for final result of Figure 8B.

ison with the experimental curves in Figure 8A. The complete geometric configuration of the small and large cylinders used for the final computed result is shown in Figure 7C, and Table 1 lists the values of the parameters that were varied for each source (radius of each bundle, distance between cylinder surface and the observation site, and the time of V max). We conclude that the excellent qualitative and quantitative fit of the waveforms strongly supports the concept that in the region close to the membrane currents, the amplitude of the extracellular potential deflection is determined primarily by the magnitude of the membrane ionic currents (source size), and the first and second time derivatives (velocity and acceleration) of the deflection are determined primarily by the distance from the current sources.

Structural Correlations

We considered that the age-related loss of side-to-side electrical coupling between bundles as demonstrated electrically might be associated with the proliferation of connective tissue septa (sheets) in intercellular spaces. Accordingly, we employed our modified picrosirius red technique (see Methods) to investigate the distribution and geometry of collagenous connective tissue septa in pectinate muscle bundles from the subjects of different ages. From this, a correlation between the anisotropic propagation properties and the distribution of the septa at different ages emerged. In the pectinate muscle bundles from the young patients, the septa were short and only incompletely surrounded fiber groups, while the septa in pectinate muscle bundles from older subjects were generally long and often completely surrounded fiber groups. The distribution of septa in different pectinate muscle bundles from young subjects was more homogeneous than in those from older subjects; i.e., some of the pectinate muscle bundles from the older subjects resembled those from younger subjects by having short septa which incompletely surrounded fiber groups. However, most of the pectinate muscle bundles of older subjects showed septa which were quite long (sometimes over 1 mm) and which divided the bundles into smaller fiber groups, themselves composed of yet smaller fiber groups, and so on. The smallest fiber groups delineated by collagenous septa in the older subjects ranged from about 20 to 150 μm in diameter. Representative longitudinal sections of the youngest and oldest subject groups are shown in Figure 9; panel A is from a 12-year-old male, and panel B is from a 62-year-old male. These sections illustrate that the distribution of collagen differed greatly but the absolute amount of collagen appeared to be similar in both preparations. Electrical data from the preparation of panel A is not shown in this paper, but it was qualitatively very similar to that shown in Figure 4; electrical data from the preparation of panel B is presented in Figures 7A and 8A. (The preparation from the 2-year-old subject of Figure 4 was poorly oriented in the block.)

FIGURE 9. Collagenous septa at different ages: longitudinal sections of pectinate muscle bundles from 12-year-old (panel A) and 62-year-old (panel B) subjects stained by the picrosirius red technique; septa appear dark gray to black. Collagen content is similar in the two sections, but septa are generally much longer in panel B than in panel A. Vertical bar in panel A = 200 μm, applicable to panel B as well.
Discussion

The point established by these results is that the anisotropic electrical properties of human atrial muscle change with advancing age to produce increasingly complex pathways of propagation spread at a microscopic level. The age-related differences found in the extracellular waveforms and their derivatives indicate that with advancing age there is progressive electrical uncoupling of the side-to-side connections between groups of parallel-oriented atrial fibers (while there was no evidence of electrical uncoupling along the long axis of the fibers). The consequence was an increase in the number of longitudinally oriented insulated boundaries that correlated with the development of extensive collagenous septa that separated small groups of fibers. Electrophysiologically, this resulted in a pronounced zigzag course of propagation in the transverse direction. This provides a simple but most effective structural mechanism in the presence of normal action potentials for further reducing the effective conduction velocity in the transverse direction to the range of very slow conduction (<0.1 m/sec) that is routine in the AV node and in very depressed fibers (Cranefield et al., 1973). A major implication of the progressive change from uniform to nonuniform anisotropic electrical properties is that it is possible with increasing age for reentry to occur in progressively smaller regions. Our preliminary studies on this point indicate that in nonuniform anisotropic preparations of older subjects (but not from uniform anisotropic preparations from the young group) "micro-reentry" can occur within an area of 1–2 mm² within a single muscle bundle (Spach and Dolber, 1984). This size scale for reentry is much smaller than the 25–30 mm² area shown previously by West and Landa (1962) and Allessie et al. (1977) to be the minimum size needed to accommodate reentry in rabbit atrial muscle (25–30 mm² of the atrial appendage contains multiple separate bundles). This structural mechanism, which occurred in preparations that maintained repeatedly consistent structural anisotropy (parallel orientation of the fibers), also should provide a microscopic basis for understanding numerous mechanisms that involve complex interactions of structure and cellular electrophysiological properties. These include the long-known greater incidence of atrial tachyarrhythmias in older people than in children, the association of increased fibrosis and atrial arrhythmias (Bailey et al., 1968), and the association of complex "fractionated" extracellular waveforms and arrhythmias in infarcted ventricle (Waldo and Kaiser, 1973; Boineau and Cox, 1973; Wit and Josephson, 1985).

Derivatives of Extracellular Waveforms Measured at a Microscopic Level

The results emphasize the importance of detailed analysis of extracellular potential waveforms because they provide precise measurements at a very small size scale that reflect multiple underlying cellular electrophysiological events. For example, our experimental results at a microscopic level failed to confirm the theoretical predictions of the Diaz-Rudy-Plonsey model (1983) of the effects of the intercalated discs as a possible cause of the direction-dependent shape differences in $V_m$ and $\phi$, the basis for the initial hypothesis of discontinuous anisotropic propagation (Spach et al., 1981). We found no evidence during propagation along the long axis of the cells that the intercalated discs (approximately 100–140 μm apart in the experimental preparations) caused local delays or that they caused irregularities in the extracellular waveforms as predicted by that model. The delays in excitation spread and the irregularities in the extracellular waveform we found were related to transverse propagation and the absence of side-to-side electrical coupling between small groups of fibers.

Although the results of this paper are encouraging for investigating excitation spread at a microscopic size scale, caution is warranted in extrapolating them to a much larger size scale; e.g., epicardial ventricular and atrial waveforms recorded in vivo. In large preparations, the effective size of the active tissue generating the extracellular waveforms is not known, and the spreading out of the ionic currents in the region of the sources is complex. For example, it is not obvious what changes would be expected for cables larger than those presented in Results. Table 2 lists the values (simulated on the basis of Eq. 3) that would occur in waveforms measured at the membrane surface of a hypothetical bundle (cable) with further increases in size, maintaining the same underlying shape and velocity of a uniformly propagating action potential. The first and second time derivatives achieved a maximum value and then decreased, even though the original potential deflection continued to increase in magnitude with progressive increases in cable (bundle) size. The presence of a theoretical limit in the time rate of change, but not of the potential amplitude, can be attributed to the increase in the small contributions of current that result from the associated increase in distant active membrane.

### Table 2

<table>
<thead>
<tr>
<th>Radius (μm)</th>
<th>$\phi$, Amplitude (mV)</th>
<th>$-\frac{d\phi}{dt}_{max}$ (V/sec)</th>
<th>$-\frac{d^2\phi}{dt^2}_{max}$ (V/sec²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.24</td>
<td>-1.59</td>
<td>-13.98</td>
</tr>
<tr>
<td>75</td>
<td>1.03</td>
<td>-5.18</td>
<td>-33.40*</td>
</tr>
<tr>
<td>250</td>
<td>3.19</td>
<td>-9.06</td>
<td>-32.91</td>
</tr>
<tr>
<td>350</td>
<td>3.94</td>
<td>-9.10*</td>
<td>-27.37</td>
</tr>
<tr>
<td>450</td>
<td>4.46</td>
<td>-8.73</td>
<td>-22.62</td>
</tr>
</tbody>
</table>

The underlying transmembrane action potential in each bundle (cylinder) was that of Figure 2A.

* Maxima of the absolute values of the negative peaks of the first and second derivatives.
Thus, for a given temporal and spatial shape of the underlying transmembrane potential, theoretically there is a unique relationship between \( \phi \), and its first and second time derivatives as a function of the size of the active bundle. That there is a unique relationship likely accounts for the excellent qualitative and quantitative theoretical fit of the experimental complex polyphasic waveform and its first and second derivatives (Fig. 8). In turn, this implies that the relationship between a given experimental extracellular deflection and its first and second derivatives (peak velocity and peak acceleration) should be useful as an inverse method to estimate the size of the underlying active bundles.

**Electrophysiological Significance of Microscopic Collagenous Septa**

Collagenous septa encircling fiber groups mark sites where it is not possible to have electrical coupling between adjacent fiber groups. That is, nexuses cannot be present between those fibers located alongside one another but separated by collagenous septa; nexuses can occur only between the fibers within each of the different groups segregated by collagenous septa. Nonetheless, the presence of fiber-to-fiber apposition does not ensure the presence of side-to-side connections via nexuses; it only permits their presence. Thus, one cannot infer from an increase in the deposition of collagen that the pattern of electrical coupling will have changed; collagenous septa could fill in spaces where no side-to-side connections existed in the first place. Combined electrical and structural studies are necessary to relate changes in collagen deposition to changes in electrical coupling between fibers.

The clear difference in the extracellular waveforms in the young vs. old preparations correlated with a clear difference in the organization of collagenous structures. The greater length of septa and greater degree to which fiber groups were completely encircled by septa in pectinate muscle bundles of old vs. young subjects, taken together with the electrophysiologically demonstrated uncoupling of side-to-side connections between fiber groups with age, strongly suggests that development of extensive collagenous septa is associated with obliteration of existing intergroup couplings. In a related vein, Caulfield (1983) commented that "the encircling of individual myocytes (by collagen) could easily interfere with lateral impulse conduction" in human ischemic heart disease. It is further of interest that collagenous septa subdivided the pectinate muscle bundles into small groups ranging from about 20 \( \mu \)m to 150 \( \mu \)m in diameter, which is approximately the same size (50 \( \mu \)m in diameter) of the cables that produced the computed \( \phi \) results that fit the first and second derivatives of the waveforms measured in these preparations.

The development of extensive collagenous septa need not entail an increase in the collagen content of the heart; that is, there could be a redistribution of collagen (e.g., Montfort and Pérez-Tamayo, 1962). This is illustrated by the young and old preparations shown in Figure 9, where the collagen content was very similar (19% and 23%, respectively, for the two sections shown) although the septa were much longer and they more completely surrounded fiber groups in the older preparation. Similarly, we have observed that collagen in dog ventricular papillary muscle is approximately as plentiful in the form of "rods" as it is in Bachmann's bundle in the form of septa (Spach and Dolber, 1985). Our function-structure correlations in both species strongly indicate that it is the distribution rather than the content of collagen which is of interest when the effect of collagen on electrical properties of cardiac muscle is being considered.

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Address for reprints: Madison S. Spach, M.D., Box 3090, Duke University Medical Center, Durham, North Carolina 27710.

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Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age.

M S Spach and P C Dolber

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