Electrophysiologic substrate for ventricular tachycardia: correlation of properties in vivo and in vitro

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ABSTRACT Cardiac electrophysiologic studies were performed in three control dogs and in nine dogs with previous (8 to 22 days) anterior myocardial infarction. During programmed stimulation, no control dog had inducible ventricular fibrillation (VF) or tachycardia (VT); three dogs with infarcts had inducible VF and six had inducible VT. Recordings in vivo were made via a plaque electrode containing 10 bipolar electrodes (3.0 × 1.5 cm) placed on the epicardial surface of the anterolateral left ventricle. Subsequently, epicardial strips (2 mm thick) from beneath the plaque were prepared for studies in vitro. Electrogram durations were significantly greater in dogs with infarcts than in control dogs both in vivo (p < .05) and in vitro (p < .001). Electrogram amplitudes were significantly lower in dogs with VT in vivo (p < .05) and in vitro (p < .001). In control animals activation was continuous and most rapid in the direction of fiber orientation; there were areas of slow and/or discontinuous conduction in all dogs with infarcts. In one case, sustained reentrant beating in vitro was caused by functional unidirectional block and microreentry at a site of continuous electrical activity during VT in vivo. Reentrant beating in vitro persisted in 0.5 cc of isolated tissue. We conclude that broad low-amplitude electrograms in vivo and in vitro depict local areas of slow and/or discontinuous conduction, that the intrinsically asymmetric nature of cardiac activation due to fiber orientation is accentuated by infarction and may predispose to intraventricular reentry, and that intraventricular reentrant circuits that may be present on the epicardial surface may persist in a volume of myocardial tissue as small as 0.5 cc.


ALTHOUGH the electrophysiologic basis for ventricular tachycardia (VT) in the context of chronic myocardial infarction usually is thought to be reentry,1–9 the size and nature of intraventricular reentrant circuits remain to be determined. It has been shown that broad fractionated electrograms that can often be recorded in vivo at the edge of infarcts8 may be associated with reentrant circuits. Electrophysiologic abnormalities observed in vitro and pathologic changes have been implicated as constituents of the substrate for VT.10–20 However, none of these reports has described the spatial relationships of the properties in vivo and in vitro. Consequently, there remains a gap in our knowledge of the exact nature of the electrophysiologic substrate for VT. We have used a canine preparation of experimental myocardial infarction to try to bridge this gap.

The purposes of the present study were to establish the relationship between electrophysiologic properties in vivo and in vitro in dogs with and without inducible VT and to determine the nature of the electrophysiologic substrate for this condition.

Methods

Experiments in vivo. Cardiac electrophysiologic studies were performed in 14 mongrel dogs. Eleven (10 to 16 kg) were studied 8 to 22 days after experimental anterior myocardial infarction by means of an occlusion-reperfusion method previously used in this laboratory.13 Three (8 to 11 kg) were control animals without myocardial infarction. Each dog was anesthetized with pentobarbital sodium (30 mg/kg) and ventilated via a tracheostomy; the heart was exposed via a mid sternotomy. Surface electrograms, a lead II electrocardiogram, and central aortic pressure were monitored continuously. Rectal temperature was maintained at 38° to 38.5°C throughout the experiment with a thermally regulated operating table.

A hand-held bipolar recording electrode (interelectrode distance 3 mm) was used to review the morphology of electo-
grams (40 to 500 Hz) at 28 predetermined ventricular epicardial sites. Whereas in control dogs all epicardial sites exhibited narrow discrete electrograms, in dogs with infarcts there were always broad fractionated electrograms in the infarcted region over the anterior left ventricle. The remainder of the left ventricle and all of the right ventricle exhibited narrow discrete electrograms in each case.

After epicardial mapping to locate the regions of broad fractionated electrograms, a 20-pole plaque electrode (figure 1) was then sewn onto the anteroseptal left ventricular epicardium, with the long axis of the plaque approximately parallel to the major diagonal branches of the left anterior descending coronary artery. In dogs with infarcts the plaque was located over the region with broad fractionated electrograms. Plaque location and orientation were similar in all experiments. Bipolar Teflon-coated, stainless-steel wire plunge electrodes were used for stimulating the subepicardium of the ventricles (see below).

Simultaneous recordings were made of the 10 bipolar plaque electrograms (40 to 500 Hz), body surface electrocardiogram lead II, and stimulation artifacts on paper at 250 mm/sec via a 16-channel electrostatic strip chart recorder (Gould ES 1000). The amplitudes and durations of electrograms were digitized and measured with a Hewlett Packard 9825 calculator system.

Standard recordings were made during programmed stimulation. Rectangular pulses of 2 msec were delivered at twice diastolic threshold in turn at each end of the plaque electrode. Basic drive \((S_1,S_1)\) at a cycle length of 300 msec was delivered for 8 beats followed by an extrastimulus \((S_2)\) introduced at the shortest possible coupling interval. These recordings were made before and separately from induction of VT (see below). A standardized protocol was then used to test for inducibility of ventricular arrhythmias. Single \((S_2)\), double \((S_2)\), and triple \((S_2)\) ventricular extrastimuli were introduced during fixed-rate ventricular drive at twice diastolic current threshold. The protocol was performed first with right and subsequently with left ventricular stimulation at a basic cycle length \((S_1,S_1)\) of 350 msec. In the absence of ventricular fibrillation (VF) or sustained (10 sec) VT, the protocol was repeated with a basic cycle length \((S_1,S_1)\) of 300 msec. Pacing or countershock was required to restore sinus rhythm in every case of VT and VF. In the continued absence of VF or sustained VT, the entire protocol was repeated at four times diastolic current threshold.

During sustained VT, simultaneous recordings were made of 10 plaque electrograms, the electrocardiographic lead II, and an electrogram from the hand-held mapping probe. The mapping electrode was used to measure the timing of local ventricular activation (major sharp deflection) at 36 predetermined epicardial sites. Isochronic maps during VT were generated in vivo with these data.

**Experiments in vitro.** After completion of the electrophysiologic study in vivo the heart was excised and an epicardial strip was prepared as follows: Incisions were made normal to the epicardial surface around the perimeter of the electrode plaque. An epicardial strip 2 mm thick from beneath the plaque was then removed, mounted in the tissue bath, and superfused with oxygenated Tyrode’s solution at 37°C. A period of 60 min was allowed for equilibration before proceeding with the electrophysiologic examination in vitro for the reasons described previously.  

Bipolar stimulating electrodes were placed at each end of the epicardial strip, and a fixed bipolar reference recording electrode was placed at a convenient location on the strip. A roving bipolar electrode (0.6 mm interelectrode distance) was used to record local electrograms over the surface of the strip. Extracelluar recordings (0.1 to 500 Hz) were made from 18 to 45 predetermined sites onto paper at 100 mm/sec via an eight-channel Mingograph ink-jet recorder.

Standard recordings were made during programmed stimulation. Rectangular pulses of 2 msec were delivered at twice diastolic current threshold in turn at each end of the epicardial strip. Basic drive \((S_1,S_1)\) for 1000 msec was delivered for 5 beats, followed by an extrastimulus at the shortest possible coupling interval.

A standardized protocol was used to test for inducibility of sustained nonstimulated beating in seven isolated strips. Single, double, and triple extrastimuli were introduced during fixed-rate (60/min) drive at twice diastolic current threshold in turn at each end of the epicardial strip.

Recordings were made during sustained and multiple inductions of reentrant beating in one preparation. The timings of local electrograms (major sharp deflections), with respect to a fixed reference, and amplitudes were manually digitized with a Hewlett Packard 9825 calculator system.

**Pathology.** Each heart was examined by inspection and palpation before application of the plaque electrode. The direction of superficial fiber orientation was noted. After excision of the epicardial strip, the area of infarction deep to the strip was noted. At the conclusion of the experiments in vitro, three standard blocks for histologic analysis were removed from each epicardial strip to confirm superficial fiber orientation as follows: Cuts were made (1) normal to the epicardial surface and normal to the direction of maximal velocity of propagation of wave fronts (measured from isochrone maps in vitro), (2) near the center, and (3) near each end of the epicardial strip. Each of the blocks was then set in paraffin and sections were cut, mounted, and stained with hematoxylin-eosin. Two additional sections were cut in one case, parallel with the epicardial surface —
one from an area with very slow conduction and the other from an area at which microreentry occurred.

**Effective recording depth of electrodes.** Estimates of the effective recording depth of one of the plaque bipoles in vivo and the roving electrode in vitro were made with a calibrated micromanipulator in the tissue bath during fixed-rate pacing (S1, 1000 msec). Amplitudes of electrograms were recorded at the tissue surface and at 50 μm intervals (up to 1500 μm for the electrode in vivo and up to 1100 μm for the electrode in vitro) above the tissue surface. Log-linear regressions of electrogram amplitude vs distance were then calculated.

**Statistical methods.** Dogs were separated into three groups: group 1 (no inducible VT or VF), group 2 (inducible VF), and group 3 (inducible sustained VT). Electrograms were excluded as technically unsatisfactory if the largest component did not exceed 0.1 mV or if the signal-to-noise ratio did not exceed 2. Amplitudes (mV) were measured as maximum peak-to-peak and durations (msec) were measured as the interval between earliest deflection exceeding 0.1 mV to latest deflection exceeding 0.1 mV. Differences between durations and amplitudes of electrograms in vivo and in vitro for each group were examined by analysis of variance.

**Results**

Electrophysiologic properties were studied in 14 dogs. None of the control dogs had inducible VF or sustained VT (group 1), five dogs with infarcts had VF (group 2), and six dogs with infarcts had VT (group 3). Complete maps of activation sequence were obtained during VT in dogs 4, 5, and 6 of group 3. There was continuous electrical activity in dog 4 (group 3). Sustained reentrant beating in vitro was induced in the epicardial strip from dog 4 (group 3) and complete maps of activation sequence were obtained. Two group 2 dogs were excluded from the analysis, one because refractory VF prevented completion of the study in vivo and one because no satisfactory electrograms could be recorded in vitro due to very dense epicardial scar tissues. The electrophysiologic properties of three control and nine dogs with infarcts are presented below.

**Experiments in vivo.** Technically satisfactory electrograms could be recorded at all electrode sites in vivo during fixed-rate pacing at each end of the electrode plaque. Although there was some variation in the amplitudes and durations of individual electrograms when recorded with pacing at the apical vs the basal end of the plaque, and with fixed-rate vs fixed-rate plus extrastimuli drive, there still were no significant differences between the means, standard deviations, or ranges of values within individual experiments. For this reason, and to simplify presentation of the results, only values associated with fixed-rate stimulation at the apical end of the plaque are given.

Table 1 summarizes characteristics of electrograms

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<td><strong>Electrogram characteristics in vivo</strong></td>
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<tr>
<td>R/S</td>
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<td>Group 1 (control)</td>
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<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>30/30</td>
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<td>Group 2 (VF)</td>
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<td>1</td>
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<td>2</td>
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<td>30/30</td>
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<td>Group 3 (VT)</td>
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<td>5</td>
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<tr>
<td>6</td>
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<tr>
<td>60/60</td>
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<tr>
<td>p&lt;.05&lt;sup&gt;a&lt;/sup&gt;</td>
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R/S = number of electrograms recorded/number of sites at which recordings were attempted.

<sup>a</sup>p values with respect to group 1 (controls), by analysis of variance.

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in vivo. There were considerable variations of amplitudes and durations among the 10 sites recorded in each animal. However, on the average, amplitudes were lower and durations were longer among infarcted hearts compared with control hearts. These differences were considerably greater among animals with VT than among those with VF.

Whereas only six of 30 group 1 electrograms were less than 1 SD (12.0 mV) below the mean amplitude (19.6 mV) for controls, 17 of 30 and 58 of 60 group 2 and group 3 observations, respectively, fell below this value. Similarly, whereas only four of 30 group 1 electrograms were more than 1 SD (17 msec) above the mean duration (41 msec) for controls, nine of 30 and 46 of 60 group 2 and group 3 measurements, respectively, fell above this value.

Figure 2 depicts characteristic electrograms from a control dog with no inducible arrhythmia (group 1, No. 1) and from a dog with infarct in which VT was induced (group 3, dog 4). In each case the recordings were made while the heart was paced at a basic cycle length of 300 msec from a bipolar stimulating plunge electrode located in the subepicardium at the apical end of the plaque electrode. Although there was considerable overlap in the dimensions of individual electrograms in the examples shown, the average amplitudes were less and durations were greater (3.7 mV, 56 msec; 10.8 mV, 48 msec) for the dogs with infarcts than for the controls.

Figure 3 illustrates VT (cycle length 175 msec) in one of the dogs with infarct (group 3, dog 4). The macroscopic infarct was centered beneath the electrode plaque, and the epicardial surface of the infarcted region did not extend beyond the edges of the plaque. It can be noted in the isochronic map that ventricular activation commenced on the anterior left ventricle, spread initially toward the septum anteriorly, then swept cranially and caudally around the infarct. The advancing wavefronts met at the left ventricular base along with another wavefront emerging from the lateral aspect of the infarct. Ventricular activation beyond the edge of the infarct was complete within 100 msec. However, electrical activity was present throughout the cardiac cycle in the epicardium subjacent to the plaque electrode (figure 4).

Figure 4 presents the same 10 simultaneously recorded plaque electrograms inscribed during the initiation of sustained VT (A) and during the termination of the same tachycardia (B). Programmed electrical stimulation was used both to initiate (S2, S3, S4) and to terminate (S5, S6) the VT. During the initiation of VT, diastolic bridging of epicardial activation was seen at site 2 (figure 4, A), and during termination of the tachycardia, epicardial activation persisted at sites 1 and 2 after the other sites became electrically quiescent (figure 4, B). Note that taken together, there was continuous electrical activity at sites 1 and 2 during the sustained arrhythmia. The locations of electrode sites within the plaque were described in figure 1. As shown in figure 3, epicardial activation at the edge of the plaque adjacent to sites 1 and 2 spread counterclockwise around the apex of the heart.

These findings are very suggestive of sustained reentry in the epicardium but are still not conclusive. Continuous electrical activity at a site or sites during VT may simply be caused by activation at “dead ends” that are not part of the reentrant circuit controlling rhythm of the heart at the time. In the absence of more detailed mapping in vivo, this possibility cannot be ruled out. However, when the epicardium subjacent

![FIGURE 2. Simultaneous recordings of surface electrocardiogram, 10 bipolar plaque electrograms, and time (100 msec between marks) during fixed-rate pacing at the apical end of the plaque at a basic cycle length of 300 msec. Electrogram numbers correspond with the sites depicted in figure 1. Narrow vertical lines to the left of each electrogram indicate stimulation time. Broad vertical bars to the right of each electrogram are amplitude calibration marks (1 mV). A. Control (group 1, dog 1); B, infarct (group 3, dog 4).]
FIGURE 3. Isochronic map of epicardial activation during sustained VT (group 3, dog 4). This planar projection depicts the ventricles as if divided at the septum posteriorly and then laid flat, epicardial surface uppermost. The location of the plaque electrode is indicated as the shaded rectangular area (without isochronic curves) on the anterior left ventricle (ALV). The orientation of the plaque is indicated in the lower left. The macroscopic infarct was centered on the myocardium subjacent to the electrode plaque. Activation times (msec) are given with respect to the local electrogram at plaque electrode site 5 (top left of the plaque in this projection). The individual bipolar electrograms from the plaque are shown in figure 4. See text for description of the pattern of spread of activation over the epicardium. PRV = posterior right ventricle; ARV = anterior right ventricle; PLV = posterior left ventricle.

to the plaque was examined in vitro (figure 6) counter-clockwise reentrant beating occurred near site 1 in vivo. Site 1 in vivo appears to the lower left of the plaque in figure 3 and to the upper left of the isolated strip in figures 5, 6, and 6.

Experiments in vitro. Ventricular electrograms were recorded in vitro both during fixed-rate pacing and after extrastimuli were introduced at each end of the isolated strip. As in the experiments in vivo (table 1), the amplitudes and durations of individual electrograms varied somewhat at pacing at the left compared with the right end of the strip, and with fixed-rate drive stimuli compared with extrastimuli. Nevertheless, there were no significant differences between the means, standard deviations, or ranges of values within individual experiments. For this reason, and to simplify presentation of the results, only values associated with fixed rate stimulation at the left end of the strip (corresponding with the apical end of the electrode plaque in vivo) are given.

Table 2 summarizes the characteristics of electrograms recorded in vitro. The number of sites at which recordings were attempted varied between 18 and 45. Whereas technically satisfactory recordings (clear electrograms greater than 0.1 mV) were obtained at all group 1 sites (108 recordings from 108 sites), technically satisfactory recordings were obtained at only 91 of 108 and 129 of 229 group 2 and group 3 sites, respectively. Other sites in the experiments in vitro were electrically inactive. In one experiment (group 3, dog 5) no electrically viable cells were identified. Since detailed three-dimensional anatomic reconstruction was not attempted in this study, it is not possible to say which mechanism was responsible for failure to obtain recordings in every case. Several explanations may account for failure to obtain satisfactory electrograms in some cases. There may have been no viable cells within the recording range of the electrode; electrically viable cells within the recording range of the electrode may have been disconnected from neighboring cells as the epicardial strip was isolated from the rest of the heart, or previously viable cells may have been injured in the isolation process. Since all group 1 recording sites yielded satisfactory recordings, the latter explanation seems unlikely to be correct.

The same qualitative differences in electrogram amplitudes and durations between groups seen in vivo were also seen in vitro. Electrograms recorded from
patterns in vitro in a control dog (group 1, dog 1) and a dog with infarct (group 3, dog 4). Isochronic maps during fixed-rate pacing are shown at the top, with the original analog data from which the isochronic maps were drawn presented at the bottom. In each control preparation, activation across the strip was grossly uniform, with most rapid conduction in the direction of superficial fiber orientation. In contrast, each of the strips from dogs with infarcts exhibited at least one region of slow conduction (<0.3 m/sec) in the direction of fiber orientation. Sustained reentrant beating was induced by programmed stimulation in the epicardial strips from one group 2 (attempted in 3/3), one group 3 (attempted in 3/6), but in neither of two group 1 preparations (attempted in 2/3).

Figure 6 illustrates the mechanism of induction and maintenance of sustained reentrant beating in one of the epicardial strips (group 3, dog 4). Figure 6, A (similar to figure 5, B), illustrates the pattern of activation during fixed-rate pacing. Note that there was an area of constant conduction block to the lower right of the strip. This area was electrically inert at all times and did not contribute to the initiation or maintenance of reentrant beating.

In figure 6, B, an extrastimulus at 215 msec produced functional bidirectional block toward the upper left of the strip (dark bars). The advancing wavefront was distorted and swept counterclockwise around the area of block. Reentry was not possible because the distorted (retrograde) wavefront reached the area of block less than 107 msec after anterograde activation. This meant that the wavefront arrived during the refractory period. After the second extrastimulus was introduced at 410 msec (figure 6, C), anterograde block occurred in the same region, but the distorted retrograde wavefront arrived at the region of anterograde block about 60 msec later than after the first extrastimulus and was than able to reenter.

The first nonstimulated reentrant beat is depicted in figure 6, D. Figure 6, E, is a more detailed map of the established arrhythmia. After the recordings in figure 6, E, were obtained, an area to the right of the strip was resected without interruption of the arrhythmia (at the left). The reentrant beating persisted even after the lower portion of the strip also was resected. Figure 6, F, shows the size of the final piece of epicardium in which the reentrant beating persisted ($2.2 \times 1.1 \times 0.2$ cm).

Note that the site of the vortex of the reentrant beating described above coincided with the site of continuous electrical activity recorded in vivo during VT (figure 4). Also note that the direction of reentrant beating...
TABLE 2
Electrogram characteristics in vitro

<table>
<thead>
<tr>
<th>Group</th>
<th>Amplitude (mV)</th>
<th>Duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R/S</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Group 1 (control)</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>45/45</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>18/18</td>
<td>6.6 ± 2.9</td>
</tr>
<tr>
<td>3</td>
<td>4/45</td>
<td>5.0 ± 3.1</td>
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<tr>
<td>Group 2 (VF)</td>
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<tr>
<td>1</td>
<td>36/36</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>36/36</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>19/36</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>Group 3 (VT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25/36</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>40/45</td>
<td>1.9 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>13/36</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>4</td>
<td>37/40</td>
<td>1.8 ± 1.4</td>
</tr>
<tr>
<td>5</td>
<td>0/36</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>14/36</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>129/229</td>
<td>1.6 ± 1.3</td>
<td>0.1-5.4</td>
</tr>
</tbody>
</table>

R/S = number of electrograms recorded/number of sites at which recordings were attempted.
*p values with respect to group 1 (controls), by analysis of variance.

(clockwise) was the same in vivo and in vitro. These observations suggest that the reentrant circuit identified in vitro was the one responsible for VT in vivo.

Pathology. Whereas the beating hearts of control dogs were normal to inspection and palpation, those of dogs with infarcts all exhibited hypokinesia or akinesia of the anteroapical left ventricle and were "woody" to palpation in the same area. In the dogs with infarcts, myocardium deep to the excised epicardial strip comprised mostly pale scar. In three dogs with infarcts, additional myocardial strips (3 × 1.5 × 0.2 cm) beneath the epicardial strip were studied in the tissue bath. No intracellular or extracellular responses could be elicited at any site on these cut surfaces during bipolar stimulation of the tissue.

In the routine histologic sections from dogs with infarcts there were areas of disorganization and/or death of superficial fibers. However, since the vast majority of superficial fibers were sectioned transversely, the direction of superficial fiber orientation was considered to be aligned with the direction of maximal conduction velocity in vitro.

Figure 7 comprises two horizontal (additional) sections from one of the infarcted hearts (group 3, dog 4).

Figure 7, A, is a low-power photomicrograph from the upper left part of the tissue, centered on the site of the vortex of sustained reentrant beating in vitro. It shows patchy myocardial scarring. Presumably, during slow fixed rate drive, activation was able to proceed in the direction of fiber orientation (from lower left to upper right). When a premature extrastimulus was introduced, anterograde conduction in the direction of fiber orientation (near the center of this panel) failed. Sustained reentrant beating was then possible around this area of functional block.

Figure 7, B, is a low-power photomicrograph from the lower right part of the tissue where an area of constant block was observed. Activation was always forced to go around this area of block. However, as can be seen, reentry in this area was impossible because there was no continuity of viable myocardial cells across the artery toward the right. The myocardium deep to the artery was dead.

Effective recording depth of electrodes. Electrogram amplitude decreased exponentially as the electrode was lifted away from the epicardial surface (through Tyrode's solution) for both the electrodes recording in vivo (r² = .98) and in vitro (r² = .99). The distances at which electrogram amplitudes fell to 50% of amplitude...
FIGURE 5. Representative examples from experiments in vitro. A, Control animal (group 1, dog 1); B, animal with infarct (group 3, dog 4). The epicardial strips depicted were $3.5 \times 2.0$ cm. The upper panel in each case is an isochronic map during fixed-rate pacing via stimulating electrodes at the left (solid circles). The orientation of the tissue with respect to the electrode plaque in vivo is given in figure 6, F. The direction of superficial fiber orientation is indicated by the arrows at the ends of the strips. Activation times (in msec) with respect to the stimulus (zero) are shown. The electrograms below comprise the analog data from which the isochronic maps were constructed (coordinates for each electrogram correspond to the letters and numbers to the right and below each isochronic map). Broken vertical lines indicate stimulation time (zero). The small arrows above each electrogram indicate the time taken as local activation. The calibration marker in the lower right panel depicts 20 msec (horizontal) and 1 mV (vertical). A, In the control strip, activation spread from left to right, with maximum conduction velocity attained in the direction of fiber orientation. B, In the infarct strip, conduction was slow and nonuniform. Activation at the right spread around an area of anatomic block (see figure 7, B, for the histologic appearance in this area). Note that the area of constant block (lower right of infarct strip) played no part in the initiation or maintenance of reentrant beating in vitro (figure 6).

at the tissue surface were 478 and 267 $\mu$m for electrodes in vivo and in vitro, respectively. In a previous study, Myerburg et al.\textsuperscript{21} demonstrated a similar exponential decrease of electrogram amplitude with increasing distance from the endocardial surface in dogs.

The average electrogram amplitude for recordings in vivo in controls was 19.6 mV (table 1). The distance at which an electrogram of 19.6 mV would be attenuated to 0.1 mV (through Tyrode’s solution) would be 3637 $\mu$m. Since there would be some cancellation of signals through heart muscle (compared with Tyrode’s solution) and since the resistance to current flow would be greater in heart muscle than in Tyrode’s solution, the effective recording depth of the electrode in vivo in controls would probably be less than this. The effective recording depth over infarcted tissue would probably be significantly less than 3 mm. This indicates that the electrograms recorded in vivo primarily represented epicardial activation.

The average electrogram amplitude for recordings in vitro in controls was 4.9 mV (table 2). The distance at which an electrogram of 4.9 mV would be attenuated to 0.1 mV through the Tyrode’s solution would be 1499 $\mu$m. With the same reasoning as above, the effective recording depth of the electrode in vitro in controls would be less than this. Since no direct measurements of amplitude attenuation with depth through the myocardium were made, the results of the above analysis are presumptive. However, since electrically active tissue was not present below the superficial epi-
cardiac strips in vitro, an epicardial origin of the electrograms is likely. Thus the broad low-amplitude electrograms recorded in the present experiments in vivo and in vitro would appear to represent primarily slow and/or discontinuous conduction in the superficial layer of epicardium. In conclusion, sustained reentrant beating can occur in this superficial epicardial layer in areas that exhibit broad low-amplitude electrograms in vivo and in vitro.

**FIGURE 6.** Isochronic maps illustrating the initiation of sustained reentrant beating in an isolated strip of epicardium. Panels A to D were constructed from data acquired at 28 to 30 sites during multiple initiations of reentrant beating (one site [---] was electrically silent). Panel E was constructed from a single episode of sustained reentrant beating (64 sites, three electrically silent). See text for further details. A. Last drive train stimulus commenced at stimulating bipolar (solid circles) at time 0 msec. Activation spread across the strip from left to right. Conduction velocity in the direction of fiber orientation toward the upper left part of the strip was 0.29 m/sec (normal 0.50 m/sec approximately). B. An extrastimulus was introduced at 215 msec. Functional bidirectional block occurred in the upper left part of the strip (heavy bars). C. A second extrastimulus was introduced at 410 msec. Anterograde block remained in the upper left part of the strip, but retrograde activation was now possible. D. First nonstimulated beat. At 600 msec the counterclockwise advancing wavefront in the upper left part of the strip reentered toward the left and continued to circulate. The original strip of anterograde block disappeared, and reentry was maintained around a small area at the vortex of the continued beating (panel E). E. Sustained reentrant beating. The pattern of reentrant beating depicted here was maintained for several minutes. F. The broken lines depict the remaining tissue (upper left quadrant) after resection in vitro (see text). The shaded areas are centered on blocks from which photomicrographs were prepared for figure 7. The numbers within this panel indicate the orientation of the tissue with respect to the plaque in vivo. The tissue orientation was the same as in figure 5.
Discussion

Our understanding of the mechanism(s) for reentrant VT has resulted essentially either from studies in vivo in man and animals1-9, 16, 17, 20, 22, or from separate studies in vitro10, 11, 15, 18, 19, 23 in the same species. The relative absence of electrophysiologic studies analyzing and correlating the mechanism of VT in the same heart and using stimulating and recording techniques first in vivo and then in vitro has made difficult the task of resolving the nature of the electrophysiologic substrate for reentrant VT. Endocardial and epicardial

![FIGURE 7. For legend see facing page.](http://circ.ahajournals.org/Downloaded from http://circ.ahajournals.org)
mapping studies in patients with recurrent spontaneous VT have shown that broad fractionated electrograms can commonly be recorded at or near the edge of infarcts during sinus rhythm. During VT these same areas may exhibit continuous activity or local electrograms that coincide with or precede QRS onset. Pace mapping studies have suggested that these areas are close to the reentrant circuit producing VT. Therefore broad fractionated electrograms may be a marker for the electrophysiologic substrate for VT.

Spear et al. described several types of cellular response to electrical stimulation in infarcted tissues excised from patients with recurrent VT and suggested that some of these abnormal responses may be associated with the electrophysiologic substrate for VT. However, there is no evidence to date that any of the described electrophysiologic abnormalities in vitro actually represent the basis for reentrant VT in vivo. Therefore the fundamental mechanism of VT remains to be confirmed.

In the present study we have examined identical epicardial areas within the same heart and correlated results in vivo with those in vitro. Similar stimulating and recording electrophysiologic methods were used both in vivo and in vitro to determine the relationship between observations in the intact heart and observations in isolated strips of epicardium.

We found that in dogs with inducible VT that broad low-amplitude electrograms can be recorded both in vivo and in vitro from the same epicardial regions overlying chronic myocardial infarction. The amplitudes and durations of these electrograms studied both in vivo and in vitro differed significantly from those of control animals and animals with inducible VF. The bipolar epicardial recording electrodes that we used in this investigation recorded activation from only the superficial few millimeters of myocardium.

Conduction over the epicardium in vivo and in vitro was grossly uniform in normal tissues. However, we did observe some asymmetry of activation (as described previously by Spach and Kootsey) due to the relationship of fiber orientation with direction of propagation of excitatory wavefronts.

Although conduction in normal epicardium was grossly uniform, we found conduction in infarcted epicardium to be complex with areas of slow and/or discontinuous conduction in all cases. Predisposition to VF in some cases and VT in others may possibly be a function of the degree of conduction slowing in a particular area. This possibility is suggested by the observation that the amplitude and duration of electrograms in dogs with infarcts and prone to VF are more normal than those recorded in animals with VT. Further support of this observation that more normal ventricular electrograms occur in dogs prone to VF than in dogs prone to VT is the report that the same protocol of programmed stimulation used in this study can sometimes lead to VF in control animals. Wetstein et al. never observed VT after programmed electrical stimulation in any control animal.

The present investigations point out that intraventricular reentry may occur in the epicardium. Reentry around a region of functional block may be one of the mechanisms for reentrant VT in the context of chronic myocardial infarction. The fact that sustained reentrant beating was recorded in vitro from the site of sustained reentrant beating in vivo enabled us to demonstrate that sustained reentry may occur in 0.5 cc of epicardium. The study in this animal provides the first direct demonstration that VT recorded in an intact heart may originate from such a small segment of myocardium. Although we chose to examine the epicardium in detail we do not suggest that the endocardium may not be the substrate for intraventricular reentry in some or many cases. Harrison et al. found that sustained VT was commonly associated with stable continuous electrical activity in the epicardium in dogs with 4-day-old myocardial infarction and that nonsustained VT was associated with unstable continuous electrical activity both in the epicardium and in the endocardium. Unquestionably, microreentry may occur in any part of the myocardium, given appropriate conduction velocities and refractory periods within potential reentrant circuits.

Some limitations of our methods must be kept in mind. The plaque electrode comprised relatively large recording bipoles. Nevertheless the electrodes had an effective recording depth of less than 3 mm. This means that the electrograms recorded by each bipoles represented, for the most part, depolarization of the myocardium immediately subjacent to the plaque. Furthermore, in those dogs with broad fractionated electrograms and inducible arrhythmias, myocardium deep to the epicardium was mostly dead and could not have

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**FIGURE 7.** Histologic sections cut parallel with the plane of the surface of the epicardium from an infarct strip (group 3, dog 4). See figure 6 for location of blocks. The scale bars represent 1 mm. See text for details. A, Low-powered photomicrograph centered on the site of the vortex of reentrant beating (see figure 6, F, for location of section). B, Low-powered photomicrograph centered on the site of constant block (see figure 6, F, for location of section).
contributed greatly to the electrograms recorded at the epicardium. In the tissue bath, extracellular electrograms were recorded with a smaller electrode that had a smaller effective recording depth of about 1 mm. Previous theoretical and experimental studies using small unipolar recording methods indicate that surface electrograms represent local activity in the immediately subjacent myocardium.

Although it may be ideal to use identical recording electrodes in vivo and in vitro, this is not feasible for routine work. In fact the present methods have some advantages. On the one hand, our electrodes for recording in vivo were similar in size to those used for intraoperative mapping in man. The ability to reproducibly initiate a sustained VT in a heart that maintained adequate hemodynamic function allowed us to use standard epicardial mapping techniques to record data from the region of interest without the necessity of a multichannel multiplexing system. On the other hand, our methods for recording in vitro permitted considerable resolution of patterns of activation on a minute scale. This allowed us to make direct comparison of data in vivo and in vitro.

**Clinical implications.** It has been thought for many years that a “critical mass” of infarct is required before reentry may occur. However, this mass may be small. Our observations suggest that reentry is possible in a very small volume of myocardium, provided that there are appropriately matched conduction velocities and refractory periods within potential “circuits.” Whether or not such circuits can gain control of the rhythm of the heart will depend on many factors, including entrance and exit block as well as the inherent electrical stability of the circuit itself. In some cases, larger infarcts may predispose to slower, more organized tachycardia, not because the reentrant circuits themselves are physically longer than the reentrant circuits in smaller infarcts, but because larger amounts of scar may provide more “protection” for the circuits.

We have shown that microreentry in vitro in a given piece of myocardium can sustain reentry with similar cycle length (in fact, longer) than the cycle length of VT in the same tissue. Since medical prophylaxis for recurrent VT is sometimes unsuccessful, other treatment modalities are often considered, including pacing, automatic defibrillators, and surgery. We suggest that when surgery is used, all areas with broad fractionated electrograms should be considered potential substrates for VT. Whenever technically feasible, all such areas should be excised or isolated from adjacent normal myocardium.

We conclude that broad low-amplitude electrograms representing local slow and/or discontinuous conduction can be recorded both in vivo and in vitro. The intrinsic asymmetry of cardiac activation (due to fiber orientation) is accentuated by infarction and may predispose to intraventricular reentry, and intraventricular reentrant circuits that may be present on the epicardial surface may occur in a volume of myocardial tissue as small as 0.5 cc.

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