ALTERATIONS IN ENDOCARDIAL ACTIVATION OF THE CANINE PAPILLARY MUSCLE EARLY AND LATE AFTER MYOCARDIAL INFARCTION

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ABSTRACT  Permanent coronary occlusion produces time-dependent changes in surviving subendocardial cellular properties. We compared the functional alterations in Purkinje (P) and ventricular muscle (VM) activation early (24 hr) and late (4 weeks or greater) after permanent coronary occlusion in an in vitro preparation of canine papillary muscle. High-density extracellular (1 to 2 mm resolution) and selected intracellular recordings were made in five animals early and seven animals late during stimulation of a free-running P strand. Activation patterns of P and VM layers from ischemic and unaffected papillary muscles were compared in the same animal. Average P layer conduction velocity was determined in normal and ischemic regions with the use of a linear array of recording and stimulating electrodes. Purkinje activation was altered little in the early phase of infarction, while healing was associated with a generalized 25% reduction in P layer conduction velocity and localized block and fragmentation of P waveforms. Intracellular recordings at sites of nonsynchronous P activation revealed electrotonic interaction between cell groups. At 24 hr, small groups of VM were present but with abnormal activation patterns in regions of necrosis with fragmented and delayed extracellular waveforms produced by partially uncoupled groups of cells. Local delay and block could be modulated by rate and site of stimulation. After healing, VM activation abruptly stopped at the visual infarct border, marked by a characteristic "end potential." These studies demonstrate important differences in the functional attributes of the P and VM layers studied early and late after coronary occlusion. Alterations in cell-to-cell relationships are likely very important in determining abnormalities of activation in both settings.


THE IS A GREAT DEAL of experimental interest in the alterations in cardiac activation resulting from ischemia since some of these changes appear to be important in the genesis of arrhythmias that complicate myocardial infarction.1–3 Since a number of different ischemic preparations have been used to study activation properties, it is difficult to make comparison due to variations in location and type of ischemic damage among preparations.9–11 Alterations in endocardial activation have been of particular interest because of evidence implicating these abnormalities as important in human ventricular arrhythmias.12–16

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The cellular electrophysiologic, histologic, and ultrastructural alterations that occur in the canine subendocardium in the days and weeks after coronary occlusion have been extensively studied.17–19 These data suggest a gradual disappearance of subendocardial ventricular muscle (VM) and gradual partial reversal of cellular electrophysiologic abnormalities observed acutely in Purkinje (P) cells. Extracellular alterations such as fibrosis also occur over time and have been suggested to slow conduction and distort local activation in infarcted myocardium by reducing cell-to-cell connections.20–23 Therefore, the overall influence of ischemia may not be totally reflected in the electrophysiologic properties of surviving cells; detailed extracellular activation mapping is necessary to determine such effects. Repetitive responses after a single premature stimulus have been initiated in ischemic canine preparations in vitro.24, 25 While these have been ascribed to reentrant excitation, studies to determine the pathway to reentry and the alterations in
activation that might support reentry in these preparations have been lacking.

The activation pattern of P and VM on the endocardial surface of the canine papillary muscle is well established. Likewise, the anatomy of the fiber orientation and the pattern and distribution of acute and chronic ischemia are predictable and reproducible. These features permit comparison of well-defined regions of both normal and ischemic papillary muscles, since selective coronary occlusion affects one, and spares the remaining muscle. The present study was designed to compare alterations in P and VM activation resulting from acute and chronic ischemic injury.

Methods

Surgical preparation. Healthy mongrel dogs weighing 15 to 25 kg were anesthetized with sodium pentobarbital (Nembutal, 30 mg/kg iv) and ventilated. A left lateral thoracotomy was performed, the pericardium was opened, and the coronary arteries were exposed. A two-stage permanent coronary occlusion was performed on the proximal left anterior descending or the circumflex coronary artery. A second thoracotomy was performed at 24 hr or a minimum of 4 weeks after permanent coronary artery occlusion.

Preparation in vitro. Papillary muscles were studied 24 hr after left circumflex occlusion in five surviving animals. Additional studies were performed on papillary muscles removed from seven animals surviving 4 to 12 weeks after permanent occlusion of the left circumflex artery (n = 4) or the left anterior descending artery (n = 3). For all of the animals, the second thoracotomy was done under the same anesthesia as the first procedure and the heart was rapidly excised. The left ventricle was opened along the interventricular septum to expose the anterior and posterior papillary muscles. Part of the endocardial surface and 2 to 5 mm of underlying muscle of one or both papillary muscles was removed by sharp dissection and pinned in a 25 ml chamber with the endocardial surface facing upward for study. The preparation was superfused at a flow rate of 20 ml/min with warmed (37°C) Tyrode’s solution composed as follows (mM): NaCl 125, NaH₂PO₄ 0.4, NaHCO₃ 24, KCl 4, MgCl₂ 1, CaCl₂ 2.7, and dextrose 5.5. The control solution was externally aerated with 95% O₂ and 5% CO₂ with a resulting pH of 7.35.

Specimens were stimulated by square-wave pulses 0.5 to 1.0 msec with an amplitude 1.5 to 2.0 times diastolic threshold. Bipolar stimulation was accomplished through a pair of 125 μm diameter silver wires insulated except at the tips via a stimulus isolation unit (Grass Medical Instruments). The preparation was repetitively stimulated on a free-running P strand or directly on the endocardial surface. In the latter case the lowest necessary stimulating current was used to avoid direct stimulation of the underlying VM while activating the subendocardial P layer.

Extracellular recording technique. We used modified bipolar electrodes made from a twisted pair of Teflon-coated 75 μm diameter silver wires (A-M System, Inc.) with one lead cut 0.5 to 1.0 mm shorter than the other. Both leads were kept in the bath solution, with the positive input touching the endocardial surface and the negative input directly above the same surface location. The signal from each modified bipolar electrode was initially amplified with an AC amplifier (ADS521 instrumentation amplifier, Analog Devices, Inc.) with gain of 100, high-frequency cutoff of 10 kHz, and low-frequency cutoff of 5 Hz. The resulting signal was further amplified to a signal amplitude of about ±5 V for analog-to-digital conversion. For each recording, 100 msec of data (sampled with 12-bit precision at 10,000 samples per channel per second) was stored on magnetic disk with use of an interactive data acquisition system operating on a VAX 11/780 computer (Digital Equipment Corp.). For the initial activation sequence mapping of each preparation extracellular recordings were made serially at 1 to 2 mm vertical and horizontal spacing on the endocardial surface of the papillary muscle with use of a calibrated grid map meter and electrode micromanipulators. One or more additional surface recordings were made from fixed reference points during the mapping procedure (elapsed time about 30 min) to ensure stability of the endocardial activation pattern during the period of study. All preparations equilibrated for at least 1 hr in the chamber before any data acquisition.

Data analysis. Digitally recorded waveforms were subsequently redisplayed on a Tektronix 4014 high-resolution graphics terminal. An interactive computer program determined local P and VM activation times relative to the stimulus artifact. The peak negative point of the waveforms produced by P and VM layer activation at each surface site were used to approximate local activation time. This procedure has been used extensively by us in previous studies in which we documented the local activation times by microelectrode recordings. On the basis of these recordings, P and VM isochronal activation maps were constructed and sites of local P-VM junctions were determined, as previously described, as sites with a short P-VM delay and an all-negative waveform corresponding to VM activation. We estimated average linear conduction velocity using a four-electrode array of recording electrodes arranged along the long axis of the papillary muscle. Most commonly, this array straddled the visual infarct border to measure point-to-point activation times in visually normal and infarcted regions. To ensure linear activation relative to the electrode array, the stimulating electrode was placed directly on the endocardial surface at the top or in the middle of the line of electrodes (see figure 11).

Microelectrode recordings. We made intracellular recordings to study components of complex surface potential waveforms using conventional glass microelectrodes filled with 2.5M KCl with tip resistance of 15 to 25 Mohm. Intracellular recordings were made at sites selected by surface potential recordings and were recorded simultaneously with the local surface potential to allow correlation of the cellular activation time with the components of the surface potential.

Anatomic analysis. After the completion of each study, detailed scale drawings were made of each specimen, noting the locations of recording and stimulating electrodes and with particular attention to the location of the infarct border determined visually. Selected muscles were fixed and sectioned every 0.5 to 2 mm and the sections were stained with hematoxylin and eosin or Masson’s trichrome. The extent and distribution of myocardial infarction on stained sections were compared with the scale drawings to confirm the validity of the visually chosen infarct and noninfarct zones.

Results

Activation 24 hr after coronary occlusion. We were able to compare P and VM activation in normal anterior versus abnormal posterior papillary muscles after circumflex occlusion in five dogs. Previous work has shown that infarction after circumflex occlusion generally involves the posterior, but routinely spares the anterior, papillary muscle.
P layer. Twenty-four hour infarction did not exert a marked effect on activation in the P layer. Isochronal activation maps of P activation from the anterior and posterior papillary muscles from the same animal are compared in figure 1. In this preparation the visual infarct region (confirmed by later histological study) included nearly the entire posterior papillary muscle, with sparing of the basal region. In both anterior (control) and posterior preparations during stimulation of the free-running P strand, endocardial P activation commenced at the apex of the muscle and proceeded rapidly toward the base in roughly parallel isochrones. Conduction velocity, estimated from parallel isochrones, did not differ between anterior and posterior muscles. Simultaneous recordings made at sites A to E from the anterior and posterior muscles are shown in the lower half of figure 1.

VM activation. VM activation was uniformly unaltered in the anterior papillary muscle. As has been shown previously in papillary muscles studied without coronary occlusion,26–28 we noted VM activation to first take place at focal sites near the base of the muscle. VM activation proceeded normally from these junctional sites toward the apex of the muscle, as illustrated by the simultaneously obtained surface recordings labeled A to E and placed below the anterior muscle P activation map. This pattern of P-VM activation was stable despite decreasing cycle lengths of pacing as short as 200 msec.

The VM activation pattern was grossly abnormal in the posterior (24 hr infarction) papillary muscle, as illustrated by the simultaneously obtained surface recordings labeled A to E placed below the posterior muscle P activation map. In contrast to the large biphasic VM activation signals recorded from the anterior muscle, waveforms from the posterior muscle were reduced in amplitude and frequently had multiple components of VM activation. While activation of VM in the posterior preparation appeared to proceed in general from basal junction sites toward the apex, as seen for the anterior muscle, the fractionated nature of the VM activation waveforms made constriction of isochronal activation maps impossible.

Local alterations in activation due to 24 hr ischemia. More detailed recordings were made by microelectrode at sites of fractionation of surface potential VM waveforms. An example of such studies is shown in figure 2, which illustrates recordings made near site D on the posterior papillary muscle of figure 1. Intracellular recordings within a 0.5 mm radius of the surface electrode showed action potentials with upstrokes simultaneous with either the first VM activation component (V1) or with the second VM activation component (V2).

The local VM activation abnormalities were not fixed. We observed that some abnormalities in VM activation were dependent on the direction of propagation, as illustrated in figure 2. In this example, all four microelectrode recordings are from the same cell

**FIGURE 1.** Isochronal P layer activation maps for an anterior papillary muscle (normal) and a posterior papillary muscle (24 hr infarction) from the same dog. For both muscles stimulation was at a BCL of 1000 msec on the attached apical P strand. The surface recordings shown below each activation map were done simultaneously at the points labeled A, B, C, D, and E on each muscle diagram.

**FIGURE 2.** Simultaneous recording of a surface potential from site D of the posterior papillary muscle diagrammed in figure 1 and a microelectrode recording from the site. The microelectrode is in a cell in the group of cells that activates synchronously with the second VM activation complex of the surface potential recording. A, The results of stimulation on an apical P strand. B, Results of stimulation applied to the muscle surface 3 mm from the recording site.
penetration, although some steady depolarization had occurred between the recording of panels A and B. In A, the surface potential recording and the intracellular recording are shown in response to stimulation of a free-running P strand at a basic cycle length (BCL) of 1000 msec (above) and 400 msec (below). In both, the activation of the first VM component is associated with a small step depolarization in the cell recorded by the microelectrode, indicating electrical coupling between these two groups of VM cells, but of insufficient degree to permit synchronized firing. In B, the preparation was stimulated by a second electrode positioned 3 mm toward the base from the recording site with the use of a stimulus sufficient to provide direct VM layer activation at the stimulus site. The surface potential and the intracellular recordings were substantially altered by the change in stimulus location. At a BCL of 1000 msec (above) a single VM activation signal was present, but with pacing at a BCL of 400 msec two VM activation components again appeared with a much greater delay than that seen during P strand stimulation (panel A). Higher pacing rates gave rise to more complex patterns of activation, as shown in figure 3. For figure 3 the same surface potential recording electrode site was used as for figure 2. The microelectrode was in another cell of the same group that produced the second VM activation signal. A continuous 18 sec recording of simultaneous intracellular and surface potential signals in response to repetitive stimulation on the attached P strand at a BCL of 250 msec is shown in panel A, along with selected time-expanded segments (B, C, and D). During the 18 sec period 1:1 conduction occurred from the P layer to the V1 cell group, but periodic failure of activation occurred for the V2 cell group, as evidenced by the microelectrode recording. The 1:1 activation of the V1 cell group produced some current flow into the V2 cell group during each cycle, as shown by the electronic deflections recorded by the microelectrode. However, the V2 activation followed a complex repeating cycle lasting approximately 14 sec during which there was about 6 sec of roughly 6:1 conduction (panels B and D) followed by roughly 8 sec of 1:1 activation of the V2 group (panel C).

The microelectrode recordings from the regions of

**FIGURE 3.** A continuous simultaneous recording of the surface potential and a microelectrode penetration as in figure 2. The microelectrode was in another cell of the same group of cells with the delayed activation. Stimulation was done on the attached apical P strand at a BCL of 250 msec. B, C, and D, Intervals of A labeled B, C, and D at an expanded time scale.
this papillary muscle and those from the other four preparations in which some part of the posterior papillary muscle had multiple discrete VM activation signals at locations in the ischemic region shared several features. First, the activation times of cells penetrated near the surface electrode were always coincident with one of the VM activation signals. We found no evidence of continuous conduction between the two or more groups of cells. Second, the cell groups were in all cases separated in the plane of the endocardial surface. In the example shown in figures 1 and 2, the cells with later activation times were to the right of the surface electrode and those with the earlier activation times were to the left of the surface electrode. As the microelectrode was advanced into the endocardial surface all VM cells penetrated had the same activation times. Third, the electrotonic interactions observed between VM cell groups were not observed between P cells and VM cells. An example of this (figure 4) shows recordings of fractionated VM signals from an ischemic region in a different preparation from that in figure 1. Signals in this preparation were recorded after stimulation on an attached free-running P strand.

The P cell and the VM cell recordings in figure 4 were not made simultaneously. We advanced a microelectrode to the endocardial surface within 200 μm of the surface electrode and penetrated a P cell. We recorded simultaneously the P cell activity and the surface poten-

**FIGURE 4.** Microelectrode recordings from a P cell and an underlying VM cell at a region in an ischemic papillary muscle that showed fractionated VM activation at the site of recording. The recordings were made separately at each cycle length for the P cell and the VM cell and then time aligned by the surface potential recording at the same surface location. The arrows indicate deflections due to electrotonic influence. See text for discussion.
tial at a BCL of 1000 msec (panels A and B) and at a BCL of 400 msec (panels C and D). We then advanced the microelectrode slightly and the next cell penetrated was a VM cell from which we made recordings (again with simultaneous surface potential recordings) at BCLs 1000 and 400 msec. The surface potential recordings allowed us to align in time the P and VM cell recordings with the surface potential to illustrate the alterations produced by the shorter BCL. At a BCL of 1000 msec (panels A and B) there was 1:1 activation of both the P cell and the VM cell, but the VM cell had a noticeable prepotential that did not begin at the time of P cell activation. Also, when VM activation occurred there was no visible electrotonic alteration in the waveform of the P cell action potential. At a BCL of 400 (panels C and D) the P cell continued to have 1:1 activation, but the VM cell had 2:1 activation. There were then two clear electrotonic interactions with the VM cell — one before activation and one after activation — but there was again no apparent interaction between the VM cell and the P cell at this site.

**Anatomic correlates.** Histologic sections of selected posterior papillary muscle regions shown to be abnormal electrophysiologically revealed scattered small groups of surviving cells surrounded by frankly necrotic and eosinophilic staining cells extending nearly up to the superficial cell layer (figure 5). We did not attempt to quantitatively relate the histologic findings to the detailed activation mapping. The section shown here is a transverse section of the posterior papillary muscle used for figures 1 to 4, approximately at the level of site D.

**Delayed effects of ischemia on papillary muscle activation**

_Gross alterations in papillary muscle activation._ In anterior and posterior papillary muscles chronic infarction resulted in similar electrophysiologic and histologic abnormalities and will be described together. Chronic ischemia had its greatest effect on the VM layer. Char-
characteristically, we could find no electrophysiologic evidence for persistent propagating VM waveforms in the ischemic zone. We detected normal VM signals in the visually normal zone, but a characteristic alteration in VM waveform occurred at the visible infarct border. As shown in figure 6, persistent P signals were detectable throughout the specimen, even deep within the ischemic zone. In contrast, as recordings were made nearer the infarct border the VM waveform began to reverse in polarity and became completely positive at or near the infarct border. This pattern of voltage reversal and subsequent loss of identifiable VM signal was seen in all infarcted specimens studied and signaled cessation of muscle propagation at the boundary of normal and ischemic zones. The positive or "end" potentials were seen only at the infarct border and generally were recorded just inside the normal zone.

In all of our preparations (both 24 hr and chronic infarction), stimulation of the P layer resulted in activation of the VM layer. Therefore there were at least some regions of successful P-to-VM conduction in all preparations. An example of such a site is shown in figure 7, which illustrates the persistent antegrade P activation as well as the normal basal-to-apical activation of the surviving VM layer from a junctional site near the infarct border. As in our previous studies of P-to-VM junctional sites in normal papillary muscles, the VM signal was all negative and occurred with a short (7 msec) delay after the local P activation.

The resulting isochronal ventricular muscle activation in the chronically infarcted papillary muscle is illustrated in figure 8.

**Alterations in P activation.** Chronic infarction had several types of effects on the P activation pattern. The first effect was a generalized apparent slowing of activation,
as indicated by a uniform distortion of the usually broad and parallel isochronic activation of the P layer. This general effect is illustrated in figure 8. During P strand stimulation the normal zone activation did not differ from activation of noninfarcted papillary muscles studied early (n = 5) or late (n = 2) after coronary occlusion. Depending on the severity of the generalized slowing, an actual change in the apparent direction of propagation was observed (figure 8).

More localized areas of conduction abnormality were noted, especially at higher resolution of mapping. These commonly occurred at the visual infarct border. An example of such a localized area of delay is shown in figure 9. In this example P activation is occurring from stimulation of a distant P strand. Broad parallel isochronic activation lines are distorted by an apparent localized region of block surrounded by relatively unaltered activation. This resulted in a rotation of the wavefronts from both sides of the area of block and ultimate collision in the ischemic zone below the infarct border.

Very localized abnormalities of activation were also seen deeper within the infarct zone. These consisted of low-amplitude multicomponent waveforms whose activation was temporally related to the passing P wavefront. In addition, individual components of the waveforms closely resembled small P signals. To more precisely measure the local activation times associated with these extracellular recordings, we made limited intracellular recordings (figure 10). We were able to demonstrate action potentials that temporally corresponded to individual components of the extracellular recording by probing a very small region with the microelectrode. We also recorded action potentials with multiple ridges and notches that corresponded temporally to the extracellular deflections. These findings suggest electronic influence between the recorded cell and the nearby electrically partly uncoupled fibers.

Despite the obvious alterations in P layer activation, the activation pattern was extremely stable; no changes in activation were noted despite more rapid pacing of the preparations, even during pacing at a BCL of 250 msec or less.

**Average P conduction velocity.** To determine more precisely the amount of slowing of P propagation in the infarct zone, we arranged stimulating and recording electrodes as previously described (figure 11). We performed additional activation mapping around the electrode array to ensure the absence of gross localized nonlinear abnormalities. We only accepted data when the P waveform could be easily recognized at all sites measured. Eight measurements were obtained in five
Preparations and the estimated average conduction velocity in normal and ischemic zones is shown in Figure 12. The average normal zone conduction velocity was 2 m/sec and declined to approximately 1.5 m/sec in the ischemic zone. This roughly 25% decrease in conduction velocity was statistically significant by a paired analysis.

**Anatomic correlates.** Areas of chronic infarction could readily be visually distinguished from noninfarced regions. The presence, extent, and distribution of infarction, as manifested by ingrowth of collagen demonstrated on histologic sections, corresponded closely to scale drawings made at the time of study based on visual inspection. The extent of infarction was variable when present; it most commonly involved the base of the papillary muscle and spared variable areas of the tip or apex of the muscle in both infarced anterior (left anterior descending occlusion) and posterior (circumflex occlusion) muscles. There were no visual or histologic differences between specimens studied at 4 weeks and those studied at up to 12 weeks. Representative anatomic alterations are illustrated in Figure 13. A dense region of subendocardial fibrosis was generally seen at the base of the muscle and extended commonly to the midpapillary level. This dense fibrosis resulted in a well-demarcated infarct border. This border corresponded closely with the site of distortion of isochronal P activation as well as VM end-potential recordings. Mature subendocardial fibrosis was uniformly covered by a layer of surviving cells three to 10 cells thick. These cells were themselves physically partly separated by a network of collagen. The fibrosis was most prominent in the deep regions of the infarct and were least prominent at the infarct border, and there was no discernible collagen in the normal zone. Fractionation of the P waveform was noted predominantly in regions deep within the infarct, while abnormalities of the P waveforms were not characteristic of other regions.

**Discussion**

These studies were designed to compare activation under normal conditions with that occurring early and late after acute coronary occlusion in the same region of interest. Alterations in activation due to ischemia alone may be directly contrasted to normal activation by this approach. The papillary muscle preparation reported here is especially well suited for this purpose since ischemic damage is limited to one of two papillary muscles and the undamaged muscle may serve as a control. The distribution of ischemic damage in the affected muscle is also relatively predictable and usually divides the ischemic muscle into a normal and abnormal region, further enhancing direct comparison. Alterations in activation at the border of normal and ischemic regions are easily studied with this preparation. We emphasize that our velocity measurements necessarily represent an average velocity in each region since irregularities in conduction could have occurred below the resolution of our recording technique.

![FIGURE 9. Localized activation abnormality at the infarct border zone. Detailed (1 mm resolution) P mapping was performed at the visible border between the ischemic zone (IZ, stippled) and normal zone (NZ). Recordings were not made in the region of deep surface depressions on the lower left, as indicated. Isochronal activation lines are drawn based on 2 to 5 points falling within 0.1 msec of the activation times shown. A focal area of delay surrounded by more rapidly activated regions causes rotation of wavefronts, with collision at 22 msec. This focal area did not distort the isochronal activation pattern when determined at 2 mm resolution.](image)

![FIGURE 10. Prolonged local P activation. Intracellular (IC) recordings were made at a site of fractionated extracellular P waveforms deep in the ischemic zone of a healed infarction. In the panels on the left, a qualitatively normal appearing action potential corresponds roughly to one of several extracellular deflections (arrows). On the right, at a slightly deeper level an action potential is recorded, with several notches on the upstroke (triangles) corresponding temporally with the extracellular deflections.](image)
FIGURE 11. Extracellular measurement of average P conduction velocity. The arrangement of stimulating and recording electrodes is shown on the left. Recordings are made from both normal zone (NZ) and ischemic zone (IZ) pairs, as well as a pair straddling the visible border between NZ and IZ (stippling). On the right, recordings from each of these sites are shown, from top to bottom as on the left. Note the normal P and VM signals in the upper panel, an end potential in the second panel (at border), and no VM signal in the bottom two panels. The P activation time is marked by the cursor position chosen visually. The point-to-point conduction velocities calculated from activation times over the grid-determined distance is shown for each pair on the left (m/sec).

The present study has shown that important alterations in activation occur early and late after permanent coronary occlusion. However, the changes that occur in P and VM activation are quite different at early and late study. The P layer appears to be affected little in the early stages of infarction and its activation characteristics resemble those of the normal anterior papillary muscle, despite histologic evidence of extensive necrosis in the VM layer of the posterior muscle studied. The P and VM layers continues to be linked by discrete and well coupled junction sites, as in the normal situation.

The activation of the surviving VM was extensively distorted in the early phase of ischemic injury. The VM waveform was diminished in amplitude and no longer consisted of a single smooth inscription. Rather, split and fragmented signals were recorded from regions of necrosis. The components of the extracellular signal were produced by closely spaced groups of cells whose activation times were very asynchronous and coincident with extracellular deflections. The delay noted between such groups of VM cells could be markedly influenced by the direction of activation, resulting in

FIGURE 12. Normal (NZ) and ischemic zone (IZ) P conduction velocity. The calculated P conduction velocities (see figure 11) in NZ and adjacent IZ are shown for five preparations. The mean velocities are indicated by the circular symbols.
FIGURE 13. Anatomic alterations late after coronary occlusion. Longitudinal sections of affected papillary muscles are shown. Top, The borders of ischemic (IZ) and normal (NZ) zones. The specimen is oriented with the IZ at the upper left and the NZ to the lower right. There is an abrupt zone of subendocardial fibrosis with continuation of a surviving tissue layer. Bottom, This surviving cell layer (here from a different specimen) was consistently found throughout the ischemic region. Note the dense underlying mature scar and separation of surviving fibers by connective tissue.
some cases in a severalfold increase in local VM activation time. Higher rates of pacing resulted, in some cases, in complex patterns of conduction and block between individual components of fragmented VM extracellular potentials. Intracellular recordings revealed distortions of the action potential and local non-propagating potentials during block, demonstrating electrotonic interactions between groups of VM cells.

In the late phase of ischemic injury both the P and VM layer activation were significantly affected. Electrophysiologic evidence of a persistent VM layer was absent in the chronic preparation. Detection of VM waveforms with a reversed polarity (end potential) at the visual border of infarction with subsequent loss of any apparent VM signal was consistent with the loss of a propagating VM wavefront.\(^{31}\)

The P layer survived in the chronic phase of infarction, but was altered in ways not observed in the normal preparations or those of early ischemia. P activation started normally at the apex of the muscle but was slowed in the region of visual infarction. In addition to a generalized slowing, local activation abnormalities were observed both at the infarct border and deep in the ischemic P layer. These local abnormalities were associated with marked changes in the direction of the activation wavefront or localized regions of fragmentation of the extracellular P signal. Intracellular recordings at sites of fragmented and prolonged P activation demonstrated disparate cellular activation and distortion of individual action potentials consistent with electrotonic cellular interactions. Despite these alterations, P activation propagation through the ischemic regions remained intact, even with more rapid pacing. The overall effect of chronic ischemic damage on conduction velocity was an approximately 25% reduction in the infarct zone, from a mean of 2.0 to 1.5 m/sec. The layer of cells in the ischemic zone was three to 10 cells thick and corresponded closely to the distribution determined visually.

Many earlier studies have documented the electrophysiologic sequelae of coronary occlusion in surviving subendocardial cells.\(^{17}{}^{19},\,3^{2}\) These studies have invariably demonstrated a number of abnormalities, including partial depolarization and a decline in action potential amplitude and maximum upstroke velocity (\(V_{\text{max}}\)). The abnormalities noted in surviving P cell action potentials are the most severe early in the infarction, but tend to reverse or totally normalize in the days and weeks after coronary occlusion. In addition, electrophysiologic and histologic evidence for a viable subendocardial VM layer disappears very early, and does not appear to recover subsequently. Fenoglio et al.\(^{33}\) showed that after 14 hr of coronary occlusion some ischemic regions had no surviving VM layer. However, limited numbers of VM action potentials were noted at 5% of the recording sites; the spatial distribution of these sites was not described. Our approach was somewhat different in that microelectrode recordings were made at sites of surviving VM identified by extracellular mapping. Such an approach does not permit assessment of relative numbers of surviving P and VM cells. Earlier attempts to correlate the cellular electrophysiologic findings with functional properties of the tissues have been limited.

The temporal pattern of cellular injury in the P layer, as previously reported, suggests that the expected functional effects of ischemia are greatest in the early phases of ischemia, and relatively less later on. However, our data suggest that overall P activation is little affected in the early phase (24 hr), but may be significantly affected in the chronic situation. Early and late effects of ischemia on VM activation cannot be compared since we also have found VM activity to be absent at late study.

Why is there an apparent discrepancy between the type and severity of cellular abnormalities previously documented and those observed in our studies of tissue function? Several factors are likely relevant. First, our study and previous ones may not be directly comparable because of differences in the regions sampled. Studies of early cellular abnormalities have generally been performed over a broad region of interest, including the center of the infarction. These data would not be relevant to function in the border region we have chosen to study. It is important to examine a well-defined region, as we have, because considerable inherent regional differences may exist, particularly in P layer activation.\(^{34}\) Our systematic study of this less severely damaged border region may explain our finding persistent VM activity at 24 hr, while earlier studies focused on more severely ischemic regions. Preparations studied at 24 hr after infarction show considerable improvement in the first hour or more of superfusion, which may account for the variability that makes comparisons difficult. We allowed a minimum of 1 hr for stabilization of the preparation and used reference electrodes to ensure stability of activation. An additional potential source of difficulty in translating acute cellular alterations into the functional attributes of tissue activation involves the suggested relationship between \(V_{\text{max}}\) and conduction velocity, which for a continuously propagating medium linearly relates \(V_{\text{max}}\) to the square of conduction velocity.\(^{35}\) In the absence of other modifiers of conduction velocity,
a large change in $V_{\text{max}}$ results in a modest change in conduction velocity.

In the late phase after coronary occlusion we noted alterations in P activation, as outlined above, at a time when previous studies had suggested near or complete reversal of P action potential abnormalities. The discordance between cellular recovery and impaired tissue function may be accounted for by extracellular modulation of conduction velocity by ingrowth of dense fibrous tissue over time. The loss of cells and cell-to-cell connection that occurs in chronic infarction has been associated with both generalized and local abnormalities of activation in other preparations. The changes in P layer activation noted in the present study, specifically generalized slowing, local conduction block, and fragmentation as well as electrotonic distortion of action potentials, are strikingly similar qualitatively to abnormalities noted in canine epicardial muscle overlying a chronic infarction and in isolated human tissue.

It seems clear from our late studies of P and VM activation that the P layer in ischemic regions remains quite viable electrophysiologically, despite complete loss of the underlying VM layer. Despite rather modest generalized slowing, 1:1 conduction was routinely maintained even to sites deep within the ischemic zone. These data suggest that in some patients developing apparent conduction system dysfunction after myocardial infarction, the delays may reflect loss of underlying muscle rather than distal P layer dysfunction or a specific defect in P-VM conduction. Such a hypothesis would also be consistent with the observed low rate of progression to complete atrioventricular block in stable patients with chronic conduction disturbances complicating coronary artery disease.

**Relevance to arrhythmogenesis.** Activation delay and slow conduction are important conditions in the occurrence of reentrant arrhythmias. Alterations in activation may be fixed, functional, or combine elements of both. Our study was designed to detect fixed alterations in activation rather than functional ones, and therefore may underestimate the amount of slow and delayed propagation present under other circumstances. However, sources of static activation delay that are present early and late in this preparation deserve further comment.

In the preparations studied early (24 hr), the surviving VM layer noted in our study demonstrates delays not noted in the P layer. The local VM activation abnormalities were in some cases dependent on direction of activation, suggesting unique spatial determinants of conduction delay. This spatial component of delay may be related to the spontaneous or induced occurrence of ventricular arrhythmias from specific sites of excitation.

It appears that the P layer in the border region early after infarction does not possess intrinsic slowed conduction under our study conditions. Others have reported induction of presumed P layer reentrant complexes in both early and late phases of infarction in vitro. In these studies delayed and slowed conduction were documented in response to premature stimulation deep in the region of infarction and well away from the border zone that we have studied. Whether these unstimulated complexes are reentrant, where they arise, and what cell populations are involved remain unanswered questions.

In the chronic phase after infarction the VM layer is unlikely to take part in arrhythmias initiated in the preparation in vitro. Ventricular muscle layers deeper than 500 μm into the subendocardium are also unlikely to participate under conditions in vitro since they are not viable in the superfused preparation. The overall slowing of P layer activation is modest. Local regions of conduction block that we have noted at the infarct border could potentially serve as a focus of reentrant excitation if coupled with regions of sufficiently slowed conduction. In the absence of such regions, very local activation abnormalities may be compensated for (“runaround”) and not appreciated unless high-density mapping is performed. Very localized regions of poorly synchronized P layer activation might play a role in the development of conduction delay in response to additional factors known to alter cellular or resistive conditions (premature excitation, local changes in potassium, pH, PO₂, or antiarrhythmic drugs).

The site of reentrant excitation in canine ventricular arrhythmias is controversial. The epicardium overlying an infarction is a site of considerable conduction delay believed to be important in the induction of ventricular arrhythmias after coronary occlusion. More recently, higher density transmural techniques have suggested that the canine subendocardium at the infarct border provides, in some cases, a rapidly conducting “fast pathway” that is part of the reentrant circuit. Our studies of a similar border zone region of the papillary muscle are consistent biophysically with such a role, although our observations apply to a very superficial endocardial layer. Ventricular arrhythmias complicating human myocardial infarction are believed to arise in border regions between normal and infarcted tissue. In addition, these arrhythmias have been localized to the subendocardium at the base of papillary muscles in human ventricular tachycardia.
Microelectrode studies of human subendocardium have also documented the persistence of viable P activity. Participation of surviving P tissue in human ventricular tachycardia is possible but requires further study. Parallel studies of human and canine papillary muscles may provide valuable direct comparison of the effects of ischemia on P activation. Further studies with this preparation are possible to identify modulating factors that influence slow conduction in ischemically damaged tissue.

References

19. Friedman PL, Fenoglio JJ, Wit AL: Time course for reversal of electrophysiologic and ultrastructural abnormalities in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. Circ Res 36: 127, 1975
38. Janse MJ, de Bakker JMT, Wilde AAM, Coronel R, van Capelle FJL, Becker AE: Conduction delay in subendocardial tissue from
Alterations in endocardial activation of the canine papillary muscle early and late after myocardial infarction.
M G Kienzle, R C Tan, B M Ramza, M L Young and R W Joyner

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