Structural and Electrophysiological Changes in the Epicardial Border Zone of Canine Myocardial Infarcts during Infarct Healing

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SUMMARY. Structural and electrophysiological properties of the epicardial muscle which survives on the surface of transmural infarcts of the canine heart (epicardial border zone) were studied at different times after occlusion of the left anterior coronary artery (LAD). Isolated preparations were superfused in vitro, transmembrane potentials recorded, and impulse propagation mapped. In preparations from subacute infarcts (1 and 5 days), resting potential, action potential amplitude, upstroke velocity, and duration were all significantly reduced. Well-defined directional differences in propagation occurred. Propagation was more rapid in the direction perpendicular to the left anterior coronary artery than in the direction perpendicular to the base of the heart, because of the uniform anisotropic structure of the surviving muscle fibers which were arranged in tightly packed bundles oriented perpendicular to the left anterior coronary artery. The only ultrastructural abnormalities found in these muscle fibers was an accumulation of large amounts of lipid droplets. As the infarcts healed, resting potential, action potential amplitude, and upstroke velocity returned to normal by 2 weeks, although action potential duration decreased further. Lipid droplets had disappeared, and connective tissue had invaded the epicardial border zone, separating the muscle bundles. By 2 months, action potentials were normal, but the muscle fibers were widely separated and disoriented by the connective tissue (parallel bundles no longer were found). In these regions with a nonuniform anisotropic structure, the well-defined directional differences in impulse propagation were lost. However, activation was very slow, perhaps because of diminished connections between cells. The persistence of slow conduction in healed infarcts may contribute to the occurrence of chronic arrhythmias. (Circ Res 56: 436-451, 1985)

A BORDER zone of muscle cells survives on the epicardial surface of transmural canine infarcts caused by complete occlusion of the left anterior descending coronary artery (LAD). The importance of this region stems from the observation that reentrant excitation occurs here, causing ventricular tachycardia during the first week, and possibly later, following the coronary occlusion (El-Sherif et al., 1977a, 1977b, 1981; Wit et al., 1982; Mehra et al., 1983). The goal of our study was to characterize some of the electrophysiological, histological, and ultrastructural properties of muscle cells in this epicardial border zone and to determine whether these properties change with time as the infarct heals (Ursell et al., 1982a, 1982b). Definition of the evolution of electrophysiological and structural properties may help to elucidate underlying mechanisms causing reentry in this region.

Methods

Surgical Production of Myocardial Infarction

Myocardial infarction was produced in 47 mongrel dogs weighing 10-15 kg by a two-stage ligation of the LAD, by the technique described by Harris (1950). A detailed description of our surgical procedure has been given in previously published reports (Friedman et al., 1973a, 1973b). The chest then was closed in layers, and an airtight seal was established. Thirty-one dogs survived the occlusion. These dogs developed ventricular arrhythmias consisting of multiple premature depolarizations or ventricular tachycardia by 20-24 hours after the surgical procedure. The spontaneously occurring arrhythmias subsided by the 3rd day.

At various times, ranging from 1 day to 18 months after coronary occlusion, the surviving dogs were reanesthetized with sodium pentobarbital (15-30 mg/kg, iv) for the electrophysiological study. Five animals were studied at 1 day, eight at 5 days, six at 2 weeks, seven at 2 months, and four at 8-18 months. We chose these times because they represent periods during the subacute (1-5 days), healing (2 weeks), and healed (2-18 months) phases of myocardial infarction. We characterized the anatomical and electrophysiological properties of the muscle on the epicardial surface of the infarct at each of these different stages. In addition, three sham-operated dogs that had undergone the identical surgical procedure, including coronary artery dissection, but without occlusion, were included in the study. Five normal dogs that had not undergone any previous surgery served as controls.

In Vitro Electrophysiological Studies

After induction of anesthesia on the day of the study, electrocardiograms were taken from all the dogs. The heart
blade from the epicardial aspect of the infarcted left ventricle while it was still in situ. The infarcted region was visually identified by its mottled appearance. The excised myocardium was immediately pinned to the waxed base of a 25-mm superfusion chamber, epicardial surface upward, and superfused with oxygenated Tyrode's solution at a rate of 50-75 ml/min. The composition of the Tyrode's solution was (in mM/liter), NaCl, 137; NaHCO3, 24; dextrose, 5.5; NaH2PO4, 1.8; MgCl2, 0.5; CaCl2, 2.5; and KCl, 4.0. The solution was briskly gassed with a 95% O2-5% CO2 mixture, both in the reservoir and in the bath. The pH of the Tyrode's solution in the tissue bath under these conditions was 7.30-7.35. The temperature was maintained constant at 36.5 ± 0.5°C. The isolated preparations were stimulated through Teflon-coated bipolar silver wire electrodes with isolated square pulses 1-2 msec in duration, at twice diastolic threshold. The stimuli were generated by a Devices pulse generator (type 2521). The basic stimulus cycle length was 1000 msec for all experiments.

After an initial equilibration period of 60 minutes, transmembrane action potentials were recorded from subepicardial fibers at 25-35 sites over the entire epicardial surface of the preparations with glass capillary microelectrodes filled with 3 M KCl. The tip resistances of the microelectrodes were 10-30 megohms, and tip potentials were less negative than 5 mV. At each recording site, action potentials were recorded from the most superficial subepicardial fiber, i.e., the first transmembrane potential recorded as the microelectrode was advanced downward by the micrometer drive of the micromanipulator. Action potentials were also recorded from underlying fibers. Membrane potentials were recorded between the microelectrode—which was coupled through a silver/silver chloride junction to a high input impedance amplifier with capacitance neutralization—and a reference half-cell (Ag/AgCl black pellet) which connected the bath to ground. Membrane potentials were displayed on a Tektronix 565 oscilloscope along with a reference bipolar electrogram which was recorded with Teflon-coated silver wire electrodes. Membrane potentials and electrograms were photographed with a Grass C4 oscilloscope camera. Action potentials were displayed simultaneously at oscilloscopic sweep speeds of 50 and 1-0.2 msec/cm. Resting membrane potential, amplitude, and duration of the action potential at 50% and 90% repolarization were calculated from photographs taken at the 50 msec/cm sweep speed, whereas Vmax of phase 0 (the steepest deflection of the upstroke) was calculated from photographs taken at the 1-0.2 msec/cm sweep speeds. The depth of the fluid over the preparation was maintained at a minimal level (<5 mm) to minimize capacitance in the recording system. Capacitance neutralization of the amplifier was adjusted when necessary (Friedman et al., 1973a).

We determined the mean value for each of the measured parameters for each preparation. For the control and each time period, the mean of the mean values for each preparation within the group was calculated. We then compared the mean values of the different groups. A statistical difference was evaluated first by using an analysis of variance F-test on the groups of data and then by either an unpaired Student's t-test or a modified t-test for populations of unequal variance (Snedecor and Cochran, 1967). All results are presented as mean ± sp; P values less than 0.05 were considered significant. An effort was made to collect data equally from all regions of each preparation. However, the increased thickness of the endocardium in the healed infarct preparations sometimes prevented recordings from being obtained at some sites.

To elucidate whether the changes in transmembrane potentials and structure which occur during the healing process influenced conduction characteristics, we mapped impulse propagation while stimulating the preparations at a regular rate from different sites. Extracellular waveforms were recorded through unipolar or bipolar Teflon-coated silver wire electrodes with a 0.3-mm tip diameter. When unipolar recordings were obtained, the indifferent electrode was placed in the bath, approximately 3 cm from the recording electrode. Activation maps were constructed from electrogram recordings obtained at 70-100 sites within regions measuring approximately 200 mm². In our initial experiments, we recorded electrograms sequentially by moving a "roving" recording electrode in 1-mm intervals with the micrometer drive of the micromanipulator. Activation time at each site was measured in relation to a fixed reference electrode near the stimulation site and plotted on a diagrammatic representation of the preparation. Periodically, while activation sequence was being determined, the "roving" electrode was returned to sites at which electrical activity had previously been recorded to verify that activation time and electrogram waveform morphology had not changed; if they had, the experiment was not continued and the data were discarded. With this technique, it took 20-30 minutes to complete a map. Later experiments were done with a multiple electrode-recording system which enabled us to record unipolar electrograms simultaneously from 96 sites. An electrode array resembling a bristle brush was constructed by embedding 96 Teflon-coated silver wires in a sheet of 2-mm-thick plexiglass, 2.5 × 2.5 cm. Each wire extended about 2 mm through the plexiglass sheet, thus forming the bristles of the "brush." Each electrode contact was 1 mm from the others. The "brush" was attached with an aluminum rod to the mechanical stage of a micromanipulator and positioned on the surface of the tissue preparations. Each electrode lead was fed into one of a group of 96 preamplifiers with automatic gain controlled by a microprocessor. The input has a bandwidth of 10-1000 Hz. The signals were multiplexed and sampled at a frequency of 4000 samples/sec (each signal sampled at a 0.25-msec interval) and digitized by an 8-bit analog-to-digital converter. The digitized signals were converted to Miller code by means of an Ampex pulse code modulation system, and were stored on wide-band tape (Ampex). The digitized signals were then put on the disk of a PDP 11/34 computer and the electrograms displayed in groups of seven on a Tektronix 4012 graphics terminal. The moments of activation were marked by hand with the cursor. Activation times in relation to a reference time were then calculated by the computer, and activation maps displayed. Placement of the electrode brush on the tissue did not hinder adequate superfusion which occurred through the bristles. Stable electrograms were recorded throughout the experimental period. With this multiple electrode array, a complete activation map of a single stimulated impulse could be readily constructed. As a consequence, the electrode was in position on the tissue for less than 5 minutes at a time.

We also mapped activation in some of the preparations from healed infarcts with a microelectrode. This was done when electrograms showed a high degree of fractionation (contained multiple components) (Gardner et al., 1982).
making it difficult to assign a moment of activation to them, or when impulse propagation was studied in very small regions. Transmembrane potentials were recorded sequentially from multiple sites, as described above for the "roving" extracellular electrode. Activation at each site was measured in relation to a reference electrode near the stimulation site.

Electron Microscopy and Histology

Tissue for electron microscopy was obtained at two different times during each experiment. First, fresh tissue directly adjacent to the patch of epicardial muscle removed for the electrophysiological study was excised from the in situ beating heart, blocked, and cut into 1-mm³ pieces, and fixed immediately. Tissue for electron microscopy was also obtained from some of the Tyrode's superfused preparations after the microelectrode studies had been completed. Samples were cut from the epicardial surface in either of two ways: (1) a 3 × 3-mm sample of tissue, 1–2 mm thick, was excised and cut into 1-mm³ pieces, or (2) a core of tissue 1.5 × 1.5 mm, approximately 4 mm deep, was cut and divided into superficial (epicardial) and deep portions. All tissue was fixed and embedded as previously described (Friedman et al., 1975; Fenoglio et al., 1976).

Care was then taken to orient tissue in the embedding mold so that sectioning would occur perpendicular to the plane of the epicardial surface. When oriented in this way, the epicardial surface formed one margin of the section.

Ultrathin sections were cut with diamond knives on a Porter-Blum MT-2 ultramicrotome, stained with lead citrate and uranyl acetate, and examined in a Siemens Elmiskop electron microscope. The epicardium could be identified in each section, and thus the location of ventricular muscle cells relative to the epicardial surface was known. In sections from deeper blocks not containing the epicardial surface, identification of the infarct by its ultrastructural characteristics enabled us to determine the relationship of surviving muscle to necrotic muscle.

After excision of tissue for electron microscopy, the entire preparation of epi-myocardium which had been studied in the superfusion chamber was fixed in 10% neutral buffered formaldehyde. The side that had been closest to the LAD was marked with India ink. After fixation, the entire tissue was sliced into five pieces cut perpendicular to the LAD border and perpendicular to the plane of the epicardium. The slices then were processed for histology according to routine methods. Five-micron-thick sections were cut from the face of each paraffin-embedded block. Each histological section, stained with hematoxylin-phloxine-saffron, showed the full thickness of the tissue preparation from the epicardial surface to where the muscle was cut from the ventricular wall. From these sections, we determined the thickness of the epicardial sheet of ventricular muscle that survived over the infarcts in these hearts, as well as the orientation of the muscle fibers. In five experiments on subacute infarcts, the number of surviving (intact) muscle cell layers that constituted this sheet was determined at 40–50 equidistant points along the length of each section. This analysis was done on sections cut at three different levels in each block, i.e., 15 sections per experiment. We then plotted the number of surviving layers on a graphic representation of the ventricular epicardial surface to provide a topographic map of the surviving epicardial muscle.

Results

In this paper, we report only data from those infarcts which were transmural, because reentrant excitation has been mapped on the epicardial surface of this type of infarct (El-Sherif et al., 1981; Wit et al., 1982; Mehra et al., 1983). The infarcts were present in similar locations on the anterior left ventricle in all dogs. The medial border was adjacent to the LAD and extended from near the apex, approximately 5 cm toward the base. The infarcts often extended to the lateral free wall of the left ventricle.

**Figure 1.** Changes in maximum diastolic potential (MDP), total action potential amplitude (APA), and rate of phase 0 depolarization (V max) of surviving epicardial muscle fibers with increasing time after coronary occlusion. Column heights represent mean values for the first "layer" of muscle fibers beneath the epicardial surface in preparations from noninfarcted hearts (stippled columns) and in each group of infarcted preparations (ruled columns) studied at the time indicated on the abscissa. Brackets indicate ± standard deviation. The number of impalements from which mean values were calculated in this and Figure 2 are noninfarcted—353 impalements in eight preparations; 1-day infarcts—283 impalements in five preparations; 5-day infarcts—283 impalements in eight preparations; 2-week infarcts—169 impalements in six preparations; 2-month infarcts—180 impalements in seven preparations. Asterisks denote values significantly different from controls (P < 0.01).
Characteristics of Epicardial Muscle, 1 and 5 Days after Coronary Artery Occlusion (the Subacute Phase)

Transmembrane Potentials

At any one site in the noninfarcted preparations, transmembrane potentials could be recorded from at least five to eight cells as the microelectrode was advanced downward. The mean values for maximum diastolic potential, action potential amplitude, phase 0 V_{max}, and action potential duration of the control, normal ventricular muscle in the first epicardial muscle cell layer are shown by the control bars in the graphs in Figures 1 and 2. The transmembrane potentials recorded from subepicardial ventricular muscle fibers of the three sham-operated dogs were no different from the normals, which had had no prior surgery. The data from the sham-operated animals, therefore, were combined with the nonoperated normals for comparisons with the infarct preparations.

At many of the recording sites in the infarcted preparations, transmembrane potentials could only be recorded from one to three cells, as the microelectrode was advanced downward. Only the transmembrane potential characteristics of the first cell layer were quantified in the graphs shown in Figures 1-4, but there did not seem to be major differences in action potential characteristics that were dependent on how far into the subepicardium the microelectrode was advanced. The transmembrane potentials of the surviving epimyocardial fibers 1 and 5 days after coronary occlusion were different from those muscle fibers in normal preparations. Figure 1 shows that the mean values for maximum diastolic potential, action potential amplitude, and V_{max} from epicardial muscle fibers in normal, noninfarcted preparations (solid columns) and in preparations from hearts with 5-day-old infants (ruled columns) studied at the times indicated on the abscissa. Note that most fibers in normal preparations had maximum diastolic potentials of 90 mV or more and amplitudes of 100 mV or more, whereas a large percentage of fibers in the infarcted preparations had much lower values.
significantly less than at 5 days. A change that was particularly striking was that the action potentials had little plateau phase during repolarization, and the action potential duration was decreased, more so in 5-day-old than in 1-day-old infarcts (Fig. 2). Although this characteristic can result from acute anoxia caused by diminished Po2 in the superfusion solution (Carmeliet, 1978), acute anoxia was not the cause here, since, under these experimental conditions, a well-defined plateau was recorded from muscle in normal preparations. These characteristics remained relatively stable over the 2- to 3-hour time period of the experiment.

There was variability in the transmembrane potential characteristics from one recording site to another. This variability for the 5-day infarcts is illustrated in Figures 3 and 4; similar variability also was found for 1-day infarcts. Severely depressed diastolic potentials (<-70 mV) and action potential upstrokes (V max, <60 V/sec) were sometimes seen under our experimental conditions. We did not encounter action potentials that had the characteristics of slow responses, as defined by Cranefield (1975).

Conduction

We determined activation patterns by mapping impulse propagation 1–3 hours after the preparations were isolated. The preparations were first driven from electrodes on the LAD margin (margin located adjacent to the left anterior descending coronary artery prior to excision from the heart) and then from electrodes on the basal margin (margin toward the base of the heart prior to excision). The activation patterns from the two different stimulation sites were compared. Identical results were obtained with the different techniques for mapping, described in Methods. The characteristics of conduction in the 1- and 5-day-old infarcts were similar. Figure 5 shows data from representative experiments on 5-day infarct preparations. The map at the top left shows activation of the epicardial muscle when the stimulating electrode was at the LAD margin, and the map at the bottom left shows activation of the same region when the stimulating electrode was at the basal margin. As is evident in the figure, the isochrones are widely spaced when activation occurred in the direction perpendicular to the LAD margin (top left), and are close together when activation occurred in the direction perpendicular to the basal margin (bottom left). Thus, the speed of activation is dependent on the direction of activation. The panel at the right in the figure shows the apparent conduction velocities (calculated in directions perpendicular to the isochrones) for another similar preparation. The most rapid apparent conduction velocity of 0.25 m/sec was in the direction perpendicular to the LAD margin; the slowest apparent conduction velocity of 0.07 m/sec was in the direction perpendicular to the basal margin. The conduction velocity also varied monotonically between these two axes. Although apparent conduction velocities varied in different preparations, this pattern was always found with a ratio of most rapid to slowest apparent conduction velocity of between 2.5 and 4:1. These conduction characteristics are those of a uniformly anisotropic structure, as described by Spach et al. (1981). In fact, as shown by the results of the histological studies described in the next section, the structure of the surviving epicardial muscle in the subacute infarcts is uniformly anisotropic. The direction of most rapid conduction in these experiments was always parallel to the long axis of myocardial fiber orientation; the direction of slowest conduction was perpendicular to the long axis.

Histology and Ultrastructure

The infarcts on which the electrophysiological studies were done were transmural; they extended from the subendocardium to the subepicardium. Beneath the epicardium was a rim of normal-appearing ventricular muscle cells overlying the infarct zone (Fig. 6). Within the infarct zone at 1 day (and beneath the normal-appearing cells), the myocardial fibers were separated by interstitial edema and, in a few cases, hemorrhage. These fibers were somewhat hyperchromatic and lacked nuclei, histological char-
characteristics of necrotic myocardial cells. A few scattered polymorphonuclear leukocytes were present. Tongues of necrotic cells extended from the bulk of the infarct deep within the ventricular wall up toward the epicardium, reaching to within 100–200 myocardial cell layers, and, occasionally, to within 10 cell layers, of the epicardial surface. At 5 days, there was a marked interstitial inflammatory infiltrate composed of polymorphonuclear leukocytes and mononuclear cells, including lymphocytes, plasma cells, macrophages, and scattered fibroblasts. The epicardium contained a mixed mononuclear and polymorphonuclear cell inflammatory infiltrate, as well as fibrinous exudate, indicative of postinfarction pericarditis. The infarcted myocardial cells were shrunken and hyperchromatic, containing an intensely acidophilic homogeneous cytoplasm without cross-striations (Fig. 6). Myocardial cell nuclei were absent. Many myocardial cells were undergoing phagocytosis by adjacent macrophages. There were no histologically normal bundles of myocardial fibers within the infarct. By 5 days, virtually every infarct extended to within 1–30 muscle cell layers of the epicardium, and in six preparations, extended directly to the epicardial surface in focal areas. The intact myocardial fibers comprising this epicardial zone of surviving muscle at 1 and 5 days were always arranged in parallel in closely packed fascicles (Fig. 6). Many of the individual myocardial cells were separated by interstitial edema. The long axis of the fibers was perpendicular to the left anterior descending coronary artery and extended from the coronary artery toward the lateral left ventricle and apex, the same orientation as epicardial muscle fibers in the noninfarcted anterior left ventricle (Greenbaum et al., 1981). Therefore, the epicardial border zone has a uniformly anisotropic structure (Spach et al., 1981). As mentioned previously, the most rapid conduction velocities which were found during mapping studies, such as the one shown in Figure 5, were in a direction parallel to the long axis of the myocardial fibers; the slowest conduction velocities were in a direction perpendicular to the long axis.

Ultrastructurally, these surviving ventricular mus-
FIGURE 6. Surviving subepicardial muscle fibers in 1- and 5-day infarcts. Panels A and B show a section of epicardium 1 day after coronary occlusion. At low magnification (panel A), there are a few scattered inflammatory cells in the epicardium (E). Directly subjacent to the epicardium is a thin rim of parallel-oriented intact myocardial fibers ( delimited by arrows). Deep to the epicardial surface are many layers of muscle cells largely devoid of nuclei. There are foci of wavy fibers, as well as mild interstitial edema, scattered inflammatory cells, and hemorrhage, histological features of recent infarction. At high power magnification (panel B), the subepicardial rim of myocardium consists of two or three layers of cells that have intact cell membranes, cross-striations, and central ovoid nuclei (arrows). Beneath these surviving fibers are dying muscle cells without nuclei or with pyknotic nuclei. At 5 days (panels C and D), the histological appearance is similar but better defined. In panel C, a fibrous pericarditis is well-developed. The thin rim of surviving myocardium (arrow) is sharply demarcated from the subjacent myocardial cells separated by interstitial edema and a marked inflammatory infiltrate. At high-power magnification (panel D), the intact myocardial cells of the thin rim appear normal. The bars represent 50 μm. Hematoxylin-phloxine-saffron.

Muscle cells, although viable, were affected by the ischemia caused by the coronary artery occlusion. Muscle cells at both 1 and 5 days showed similar changes. The major abnormality identified was the presence of numerous nonmembrane-bound lipid droplets within the sarcoplasm of the epicardial muscle cells (Fig. 8). Although we did not quantify the amount of lipid, more than 90% of the muscle cells contained more than 10 droplets per cell. (Lipid droplets in normal ventricular muscle cells in the subepicardium were scarce.) The number of droplets varied from cell to cell, but, overall, increased progressively in number from muscle cells adjacent to the epicardium to muscle cells adjacent to the frankly necrotic region. Lipid droplets filled major portions of the intact muscle fibers adjacent to the infarcted cells, whereas, at the epicardial surface, the droplets were fewer, and were scattered. Other ultrastructural features of the surviving epicardial muscle were normal. The sarcolemmae of these cells were intact. The intercalated discs, including numerous normal nexi, coursed stepwise transversely between muscle cells. Numerous side-to-side disc connections between the parallel-oriented fibers were also apparent. Cell nuclei contained fine chromatin and large nucleoli. The sarcoplasm contained normal-appearing contractile elements, abundant glycogen, and numerous mitochondria (Fig. 8). T-tubules were present, and Z-bands appeared normal. Scattered dilated segments of sarcoplasmic reticulum and occasional osmiophilic dense bodies were present in some cells. The interstitium contained variable amounts of proteinaceous material separating the surviving myocardial fibers in the thin rim overlaying the infarct. The branching nature of the fibers was accentuated due to the edema.

There was always a distinct sharp but irregular boundary between the intact muscle cells overlaying the infarct and the ventricular muscle cells within the infarct (Fig. 8). The muscle cells within the infarct showed characteristics of irreversible ischemia—discontinuous sarcolemmae and intramitochondrial dense deposits of calcium. Scattered cells were frankly necrotic, with disintegrating cell organelles and masses of coagulated protein. Some of the necrotic cells were being phagocytized by macrophages.

Characteristics of Epicardial Muscle 2 Weeks to 18 Months after Coronary Artery Occlusion (the Healing and Healed Phases)

As the infarcts healed, there were significant changes in the transmembrane potentials, conduc-
FIGURE 7. Graphic depiction of the thickness of the surviving epicardial sheet of muscle over an infarct. The rectangle represents the block of tissue excised from the epicardial surface on the anterior left ventricle. The top margin is toward the base, the bottom toward the apex, the left toward the LAD, and the right toward the lateral left ventricle. Each pattern of shading indicates the number of surviving epicardial muscle cell layers (thickness) according to the key at the bottom of the figure.

Transmembrane Potentials

Transmembrane potentials could be recorded from one to two cell layers at many epicardial sites in the preparations isolated from the hearts with healing or healed infarcts. We did not attempt to advance the microelectrode any further through the subepicardium. It was more difficult to impale the myocardial cells in these preparations, probably because of the increased amounts of connective tissue on the epicardial surface of the infarcts at these times (see below). At sites where membrane potentials were not recorded, we could not be certain that there were no viable muscle fibers; the thick connective tissue may have prevented us from recording action potentials.

The maximum diastolic potential, action potential amplitude, and \( V_{\text{max}} \) of phase 0 of the surviving epicardial muscle at 2 weeks and 2 months were not significantly different from normal \( (P > 0.05) \) (Fig. 1). Fibers with values less than 2 standard deviations from the mean were rarely found. In contrast, the action potential duration at both 50% and 90% repolarization was even shorter at 2 weeks than at 5 days \( (P < 0.01) \), and there was very little evidence of a plateau phase (Fig. 2). The action potential duration and configuration of the surviving muscle fibers in 2-month-old infarcts were not significantly different from those of muscle fibers in noninfarcted preparations \( (P > 0.05) \) (Fig. 2). There was no difference between the characteristics of the transmembrane potentials recorded from the older infarcts, and those from the 2-month infarcts (based on analysis of 105 action potentials in four preparations).

Conduction

The preparations from the 15-day-old infarcts still showed the directional differences in activation that were found in the 1- and 5-day-old infarct preparations (see Fig. 5). There was, however, a marked difference in conduction characteristics in preparations from the 2- to 18-month healed infarcts, compared to the subacute infarcts. The protocol used to study activation in some of the experiments was similar to the one used for the experiments on the subacute infarcts. Activation maps during stimulation at different margins of the preparations were constructed from the timing of electrogram deflections. The maps from a representative experiment on a 16-month-old infarct are shown in Figure 9; similar maps were obtained from healed infarcts of all ages. It is apparent that in part of the preparation there are no longer the marked directional differences in activation time; uniform anisotropy is no longer evident. In the top panel, which shows activation during stimulation at the LAD margin, activation away from this margin occurs relatively rapidly at the left of the preparation, but much more slowly at the right, where the isochrones are closely bunched (compare this with Fig. 5, top panel, where activation across the whole preparation in this direction occurred relatively rapidly). Stimulation from the apical margin (bottom panel) resulted in activation of the left of the preparation that was slower than during stimulation at the LAD margin. This directional difference in activation was not apparent in the right half of the preparation, where activation time was as long as that shown in the top panel.

The absence of the clear directional differences in activation time which characterized the 1- and 5-day infarcts was particularly apparent in the preparations that had a large number of fractionated electrograms—electrograms with long durations of 10–70 msec consisting of multiple deflections. (Gardner et al., Circulation, submitted). In these preparations, we did not construct activation maps from electrogram recordings because of the difficulty in assigning an activation time to the electrograms. We therefore used recordings of transmembrane action potentials. An activation map of a preparation from a 2-month infarct is shown in Figure 10. The orientation is the same as in the other figures. The isochrones are not uniformly distributed in any direction, closely bunched isochrones occur in all directions, and there is no direction of rapid
FIGURE 8. Ultrastructure of muscle fibers in the surviving epicardial rim of 1 day (panel A) and 5 day (panel B) infarcts. The only ultrastructural abnormality detected at these times was the presence of intrasarcoplasmic lipid droplets (L). The contractile elements, mitochondria, and intercalated discs (arrows) appear normal. The sarcolemmae are intact. In these fields, there is only mild interstitial edema, but in other fields there was more extensive extracellular edema. The myocardial cells, however, were always oriented in parallel array. The bars represent 5 \( \mu \)m. The interface between surviving subepicardial muscle and necrotic muscle was sharp (panel C; 5-day infarct). The cell at the top is a viable cell with abundant lipid, whereas the cells at the bottom are necrotic, demonstrating ultrastructural features of irreversible cell injury—namely, discontinuous sarcolemmae (arrow) and intramitochondrial deposits of calcium. There is abundant interstitial edema and numerous macrophages (M). The bar represents 3 \( \mu \)m.

activation similar to the subacute infarct preparations. It is also apparent from this figure that activation occurs much more slowly than in the preparations from the subacute infarcts. It took the impulse 90 msec to reach the right border, a distance of 3 mm—an apparent conduction velocity of 0.03 meter/sec. However, the parameters of the transmembrane potentials used to construct this map were not significantly different from our normal values. These parameters are shown in the bar graph (experiment 1) in Figure 10. Similar data were obtained in two other experiments in which mapping was done with microelectrodes (Fig. 10, experiments 2 and 3); slow activation with normal action potentials was found.

Histology and Ultrastructure

At 2 weeks, the infarct was composed of granulation tissue with scattered mononuclear cells and small blood vessels. Overlying the infarcts, the epicardium was thickened by dense fibrous tissue, indicative of healed postinfarction pericarditis. There were no intact ventricular muscle cells within the infarcts.

At the epicardial surface, there was still a thin rim of 1–30 layers of histologically intact ventricular muscle cells (Fig. 11). The intact cells were characterized by distinct cell membranes and acidophilic cytoplasm with cross-striations. The cells contained large central ovoid nuclei with evenly dispersed chromatin. The myocardial fiber bundles still had the parallel orientation which was evident at 5 days, probably accounting for the prominent directional differences in activation which were still present at this time. There was increased fibrous tissue adjacent to the healing infarct in this subepicardial region, sometimes separating individual myocardial fibers along their length (Fig. 11). The separation of myocardial fibers was particularly marked at the margin between infarct and intact muscle, but was also apparent all the way up to the epicardial surface.

The surviving epimyocardial cells at 2 weeks displayed a range of ultrastructural appearances rang-
There was a relatively small, well-defined scar on the anterolateral surface of the hearts with 2- to 18-month-old healed infarcts. Cross-sections of the ventricular wall showed variable thinning, with the wall being only one-half to one-quarter the normal thickness. Large amounts of adipose tissue were found in the scar tissue of the 4- to 18-month infarcts, but not in the 2-month infarcts. The thin rim of surviving 1–30 layers of intact muscle cells still persisted, trapped on the epicardial surface of the dense scar (Fig. 11). The organization of the muscle fibers, in regions where they were present in relatively large numbers (20–30 cell layers), was similar to the 15-day infarcts, i.e., parallel-oriented fibers separated by increased connective tissue. In these regions, directional differences in conduction similar to those seen at 1 day to 2 weeks were found. For example, the left side of the preparation from which the maps in Figure 9 were constructed had 20–30 layers of parallel-oriented fibers. In the regions where there were fewer cell layers, the myocardial fibers were markedly separated from each other along their length to such an extent that we could not be certain whether there were side-to-side connections between bundles (Fig. 11). In addition, the individual cells and their orientation were markedly deformed by the ingrowth of fibrous tissue.
from the healed infarct. These myocardial fibers no longer presented a uniformly anisotropic structure—they were no longer parallel to one another—but, rather, were oriented in many different directions (Fig. 11) (Spach et al., 1982). This type of structure was found in the right side of the preparation in Figure 9 and the preparation in Figure 10. Neither region showed the prominent directional differences in conduction characteristic of the subacute infarcts. As in the 5-day-old infarcts, several of the healed infarcts extended to the epicardial surface in focal areas, leaving no intact muscle cells adjacent to the epicardium.

The surviving rim of epicardial muscle in the healed infarcts was comprised of cells showing a range of ultrastructural appearances, from cells with normal shape and intracellular components to cells with a markedly distorted shape (Fig. 13). The distorted shape was particularly evident in those cells immediately adjacent to the infarct, but was also seen in cells just beneath the epicardium. The distortion occurred because the interstitium was expanded by abundant collagen which separated and isolated individual cells and portions of cells. The bizarre configurations of some cells severely limited cell-to-cell interfaces. Attenuated projections of muscle cells were apposed through very short intercalated discs containing numerous nexi. As a result, the total extent of cell-to-cell connections appeared to be decreased. Despite their abnormal shape, the distorted cells contained abundant glycogen, normal mitochondria, and a normal number of contractile elements organized in registry (Fig. 13). Normal appearing T-tubules were distributed throughout the sarcoplasmic reticulum. Intrasarcoplasmic lipid droplets were not present in abnormal amounts. Ventricular muscle cells with reduced numbers of contractile elements were rarely identified. All cells had a completely intact sarcolemma and normal-appearing nuclei. None of the cells had the ultrastructural abnormalities which were present in surviving fibers in 2-week-old infarcts.

Discussion

Epicardial Muscle Survives Transmural Myocardial Infarction

In their studies on ventricular arrhythmias in the canine heart with subacute myocardial infarction caused by LAD occlusion, El-Sherif et al. (1977a, 1977b) recorded continuous electrical activity from the epicardial surface of infarcts during tachycardias. These results not only verified Harris' original observation that subepicardial muscle did not infarct (Harris, 1950) but suggested that reentrant excitation occurred in this muscle to cause tachycardia (El-
FIGURE 12. Ultrastructure of muscle fibers in surviving epicardial rim over a 2-week-old infarct. At this time, although scattered myocardial cells were misshapen, especially adjacent to the healing infarct, the majority were oriented in parallel. The most striking ultrastructural abnormalities of the myocardial cells at this time were changes reminiscent of degeneration, characterized by loss of contractile elements (M) and replacement by large collections of monoparliculate glycogen and mitochondria. In severely affected cells, the few remaining contractile elements were adjacent to the sarcolemmae. Many cells contained osmiophilic dense bodies (thin arrow) and dilated segments of sarcoplasmic reticulum. Although several intercalated discs had unusually serpiginous configurations (open arrows) the majority of the intercalated discs (both end-to-end and side-to-side) appear normal (thick arrows). The sarcolemmae were intact, indicating cell viability. The bar represents 5 μm.

Sherif et al., 1977a, 1977b). Subsequently, impulse propagation in the surviving epicardial muscle was mapped by techniques that enabled electrograms to be recorded simultaneously from a large number of sites. The results of these studies verified that reentrant excitation occurs in the surviving epicardial muscle (El-Sherif et al., 1981, 1982; Wit et al., 1982; Mehra et al., 1983).

The rim of surviving epicardial muscle is what remains after the wavefront of injury—that is, the sequential progression of myocardial damage from endocardium to epicardium (Reimer et al., 1977)—has stopped advancing toward the epicardial surface. Since we found more surviving muscle cell layers at 1 day (up to several hundred) than at 5 days (1–30), it appears that ischemic injury is still progressing between these two times. Not condemned to inevitable death, as has previously been claimed (Hearse and Yellon, 1981), this muscle probably survives and forms a border zone of the infarct because the epicardial region is supplied by collateral branches of both the LAD and left circumflex coronary arteries (Schaper, 1971). After coronary occlusion, blood flow is redistributed from dying intramyocardial regions to the subepicardium (Rivas et al., 1976).

**Structural and Electrophysiological Properties of Surviving Epicardial Muscle: Possible Relationships to Arrhythmogenesis**

We found a reduction in resting membrane potential, action potential amplitude, and upstroke velocity and action potential duration in the surviving ventricular muscle cells of the thin epicardial border zone, 1–5 days after occlusion. Other investigators have reported similar results (Hope et al., 1980; Lazzara and Scherlag, 1980; Spear et al., 1983). Action potential duration was shorter at 5 days than at 1 day, also suggesting more ischemic damage by the later time. The only ultrastructural abnormality of the myocardial cells was the presence of intracellular lipid deposits which appeared similar at 1 and 5 days. Since all of the abnormalities in transmembrane potentials persisted after several hours of superfusion in well-oxygenated Tyrode’s solution, the prolonged period of ischemia in situ prior to the study probably caused alterations in membrane properties which are not totally dependent on an abnormal extracellular environment.

The significance of the lipid within the myocardial cells in relation to the abnormal transmembrane potentials is unclear. Lipid droplets have been identified previously in surviving muscle and Purkinje cells adjacent to experimental myocardial infarcts (Wartman et al., 1956; Friedman et al., 1975; Fengolio et al., 1979; Page and Polimeni, 1977; Bilheimer et al., 1978; Vokonas et al., 1978). Presumably, the lipid is related to ischemia; it has been suggested that, during hypoxia, lipid droplets are derived from circulating plasma lipids which can no longer be metabolized by the mitochondria (Wartman et al., 1956; Page and Polimeni, 1977; Bilheimer et al., 1978). Whether the lipid droplets are the cause of the electrophysiological abnormalities we found at
1 and 5 days, or merely an independent association, cannot be answered by this study. That large amounts of intracellular and extracellular lipid may be arrhythmogenic has been suggested (Opie, 1972).

The abnormalities in the transmembrane potentials at 1–5 days may contribute to the occurrence of reentry. The decrease in resting potential and upstroke velocity may, in part, explain the slowed conduction that occurs in the epicardial muscle during tachycardia (El-Sherif et al., 1977a, 1977b, 1981, 1982; Lazzara and Scherlag, 1980; Wit et al., 1982). The short action potential duration, if accompanied by a decrease in refractory period, might also facilitate the occurrence of reentry. During reentry, reexcitation of the epicardial muscle has occurred within 100–130 msec, suggesting a shortening of the refractory period (Wit et al., 1982). However, studies will still be required to determine the exact relationship of the abnormalities in the transmembrane potentials recorded from in vitro muscle to the in situ arrhythmias.

The structure of the rim of surviving epicardial muscle may also contribute to the occurrence of reentry. The surviving ventricular muscle cells in the epicardial border zone of transmural infarcts constitute a thin sheet overlying the infarct. This viable muscle is often connected to normal myocardium only at its periphery; connections with underlying intramural bundles of surviving muscle are only rarely present. Hence, impulses cannot activate the epicardial muscle from below, and thereby prevent circus movement. Conduction of impulses is confined to an essentially two-dimensional plane on the epicardial surface (Wit et al., 1982; Mehra et al., 1983). At 1–5 days, the muscle fibers in the thin rim were parallel-oriented, making this region a uniformly anisotropic structure. This architecture influences conduction of impulses (Clerc, 1976). Spach et al. (1981) have shown that conduction velocity perpendicular to the long axis of fiber orientation in atria or ventricles is slowed sufficiently to cause reentry. We have also shown in this study that very slow conduction occurs in this epicardial region when activation occurs perpendicular to the long axis of fiber orientation, whereas it is more rapid parallel to the long axis. The slow conduction caused by anisotropy may also contribute to the occurrence of reentrant ventricular tachycardia (Gardner et al., 1981).

There is not yet sufficient data to indicate whether...
or not reentry occurs in the surviving epicardial muscle at 2 weeks to 18 months after permanent coronary occlusion, although ventricular tachycardias can sometimes be induced by programmed stimulation at these times (A.L. Wit, unpublished observations). Nevertheless, the further changes in structure and electrophysiology that accompany healing of the infarct may have some relationship to arrhythmogenesis. By 2 weeks after occlusion, the myocardial fibers are separated by an ingrowth of connective tissue. Many of the cells also show ultrastructural features suggestive of degeneration (Maron et al., 1975). These changes are nonspecific, implying no particular etiology. They may reflect ongoing ischemia in the muscle cells or may be a residuum of the initial ischemic event. Alternatively, they may not be related to ischemia, but may be a response to local factors such as lingering proteases from the adjacent myocardial infarct or encroachment by fibrous tissue of the healing infarct. The role of the pericarditis which occurs after infarction is also unknown. Whatever the cause, these degenerative changes in no way predict the fate of the muscle cells. By structural criteria, these cells are viable; they may recover and resynthesize their normal components, or they may further degenerate and die. That many of the cells recover is suggested by the fact that by 2 months the muscle cells remained misshapen, but appeared otherwise structurally normal.

The ultrastructural changes that we found at 2 weeks were associated with repolarization further accelerated from that recorded at 1–5 days. Resting potential and upstroke velocity, however, had returned to normal. Spear et al. (1983) also found similar changes in transmembrane potentials of epicardial muscle cells over infarcts caused by coronary occlusion and reperfusion. Since we did not ultrastructurally mark individual cells from which transmembrane action potentials were recorded, we cannot determine whether some of these potentials were recorded from the muscle cells with abnormal structure or mainly from cells with normal structure.

At 2–18 months, the muscle fibers are markedly distorted and the epicardial border zone no longer has a uniform anisotropic structure. The intercellular contacts are also attenuated. The intracellular ultrastructure and the transmembrane potentials are not significantly different from normal. Recovery may be due to the development of collateral vessels.

Myerburg et al. (1977) have also determined transmembrane potential characteristics of muscle cells surviving in healed infarcts up to 6 months old in feline hearts. The muscle fibers that they studied were in the subendocardium and presented a histological picture similar to the epicardial surface of canine hearts. Although many of the surviving muscle fibers had normal transmembrane potentials, action potentials with abnormal characteristics also were found.

Because of the distortion of the muscle fibers which no longer have a parallel orientation in healed infarcts, the well-defined directional differences in activation time seen in the subacute infarct preparations did not occur in some regions. Activation of the epicardial border zone in the healed infarcts was still very slow, despite the normal transmembrane potentials. We have not yet determined why activation is so slow. Conduction velocity might be normal, but activation may occur over circuitous pathways caused by the distortion of muscle fiber orientation, giving the impression of a slow conduction velocity. On the other hand, conduction velocity might be slowed as a result of decreased coupling between cells caused by their separation by connective tissue.

**Border Zones of Myocardial Infarcts**

There has been considerable controversy as to whether a border zone (a zone of viable but damaged muscle fibers with abnormal physiological properties) exists adjacent to a myocardial infarct (Hearse and Yellon, 1981). A number of studies using histological, biochemical, or histochemical staining techniques have concluded that there is a sharp interface between necrotic and normal tissue on the lateral aspect of infarcts during the first 24 hours after coronary occlusion, and thus no border zone (Hirzel et al., 1977; Factor et al., 1978, 1981; Janse et al., 1979; Yellon et al., 1981). However, others suggest that there is a narrow lateral border zone less than 3 mm wide (Cox et al., 1968; Harken et al., 1978; Gottlieb et al., 1981; Kovanagi et al., 1982). Some studies show biochemical abnormalities and low blood flows in this lateral border zone, but it is difficult to distinguish between immediately damaged cells and interdigitating normal cells and dead cells by some of the techniques that have been used (Hearse and Yellon, 1981). Muscle fibers in this region on the lateral aspect of infarcts do have some structural features that are similar to the epicardial border zone which we described. Their histology appears normal, but numerous lipid droplets are present during the first several days after coronary occlusion (Vokonas et al., 1978; Fishbein et al., 1980; Gottlieb et al., 1981). Besides the lipid, the only other ultrastructural abnormality which has been described is sarcomere relaxation (Gottlieb et al., 1981).

The morphological changes in the lateral border zone in the weeks and months following myocardial infarction (during infarct healing) have not been well documented. There are some data indicating that biochemical abnormalities may persist (Schwartz et al., 1973), although there are the same difficulties in interpreting these data as there are for the studies on more acute infarcts (Hearse and Yellon, 1981). Dusek et al. (1971) have described structural changes in the lateral regions of infarcts which are similar to those that we found on the epicardial surface. They describe the occurrence of a progressive increase in interstitial connective tissue 1–4
weeks after myocardial infarction, and this collagenization led to deformation of the surviving cells. They found, as we did at 2 weeks, that the deformed muscle cells were largely devoid of contractile elements, and that they contained primarily glycogen and scattered mitochondria. Whether cells with normal intracellular ultrastructure were located on the lateral aspect of older infarcts, as we found, is not apparent. It has not been possible to determine whether the electrophysiological properties (action potentials and conduction) of the cells on the lateral aspects of infarcts are abnormal—a finding which would help to establish the existence of a lateral border zone.

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