Sequential Unipolar Strength-Interval Curves and Conduction Times during Myocardial Ischemia and Reperfusion in the Dog

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SUMMARY Computerized techniques were employed to generate alternating anodal and cathodal or sequential anodal strength-interval curves during and following 15-minute coronary artery ligations in 14 anesthetized dogs. The right atrium was paced at 2.5 Hz, and unipolar ventricular strength-interval curves with simultaneous conduction times were recorded every 45-120 seconds during ischemia and reperfusion. Within 1-2 minutes of ligation, anodal midcurve and late diastolic thresholds fell sharply, and cathodal thresholds fell slightly or changed little. After 5 minutes of ischemia, anodal thresholds remained low, cathodal thresholds rose, and conduction times increased. At 10-15 minutes of ligation, if the ischemic zone was small, anodal thresholds were low, often approaching cathodal values, and conduction returned toward control values. When the ischemic zone was large, unipolar thresholds and conduction times increased late during the ligation period. Throughout the course of ischemia, the falling limb of the strength-interval curve shifted progressively to the left indicating shorter refractory periods. Following abrupt reperfusion, anodal phase 3 dips promptly reappeared; refractory periods returned toward control, and supernormal conduction was noted. By 3-5 minutes of reperfusion, the falling limb of the strength-interval curve had shifted to the right of control and conduction times increased. Thus, vulnerability to arrhythmias during early ischemia (i.e., 5 minutes) is characterized by low anodal midcurves and late diastolic thresholds, short refractory periods, and slow conduction. During the first minute of reperfusion, anodal excitability is increased during the early dip and conduction times are supernormal. Increases in anodal excitability correlate better with the peak incidence of early ligation and reperfusion arrhythmias than do changes in cathodal excitability.

DURING relatively brief periods of coronary artery ligation, the peak incidence of ventricular arrhythmias occurs within the first few minutes of myocardial ischemia. Thereafter, despite continued ischemia, the incidence of spontaneous ventricular arrhythmias falls off markedly only to reappear promptly when the ischemic segment is reperfused. At the height of the early ligation arrhythmias, dispersion of recovery of excitability has been observed and conduction time within the ischemic segment is prolonged, suggesting a reentrant mechanism for these arrhythmias. However, the electrophysiological basis for reperfusion arrhythmias remains unclear. Using computerized techniques for measuring dispersion of the effective refractory period (ERP) among multiple ischemic and nonischemic areas, Naimi et al. found that dispersion of the ERP within the ischemic segment correlated well with the timing of early ligation arrhythmias but not with the arrhythmias of reperfusion. On the other hand, following arterial reflow, the mean ERP within the ischemic segment shortened abruptly to create a large, albeit short-lived, disparity in ERP between the ischemic and nonischemic segments of the ventricle. However, the importance of this phenomenon in the genesis of reperfusion arrhythmias was lessened by the fact that this shortening of the ERP was most marked following the release of short ligations and less conspicuous following the release of longer ligations when the incidence of spontaneous arrhythmias was higher.

In an effort to elucidate better the electrophysiological basis for the arrhythmias of both acute ischemia and reperfusion, Avitall et al. examined excitability to bipolar stimulation during the falling limb of the ventricular strength-interval curve. However, this study of bipolar stimulation, confined to a narrow window of the recovery period, failed to identify important differences in excitability during ischemia and reperfusion. Since anodal strength-interval curves exhibit dips during early repolarization which frequently reach thresholds well below those found during late diastole and which coincide in timing with the vulnerable period, it was considered that the ventricular response to anodal stimulation might play an important role in the genesis of reperfusion arrhythmias. Harumi et al. demonstrated that the type of ventricular arrhythmias which developed in response to a train of low intensity anodal stimuli was related to the depth of the dip following basic driven and premature beats. Moreover, since the depth of early dips of anodal strength-interval curves are increased by sympathetic...

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stimulation and decreased by stellectomy, a relationship between anodal excitability characteristics and ventricular arrhythmias during ischemia or reperfusion is worthy of consideration, particularly since it is known that sympathetic stimulation lowers ventricular fibrillation threshold in normal and ischemic myocardium and that increased cardiac sympathetic nerve traffic occurs during coronary artery ligation.

The present study, therefore, was designed to study the time course of excitability characteristics of the ventricle to unipolar stimulation during and after acute myocardial ischemia in the dog.

Methods

Experiments were performed in 14 mongrel dogs anesthetized with sodium pentobarbital, 30–35 mg/kg, iv. The chest was opened through a median sternotomy and the heart suspended in a pericardial cradle. Ventilation was controlled by a Harvard respirator via a cuffed endotracheal tube. The sinus node was crushed and the right atrium was paced using a bipolar electrode. The basic driving stimulus (S1) was 3 msec in duration, delivered at a rate of 150 beats/min. A loose ligature was placed around the left anterior descending coronary artery, generally just proximal to the last large diagonal branch, and a test occlusion was made to define the ischemic segment. An Ag-AgCl disc electrode, 3 mm in diameter, was placed on the epicardium in the center of the ischemic segment. Secondary anodal and cathodal stimuli (S2), 3.5 msec in duration, were delivered to the disc electrode at an average rate of 60–80 S2 pulses per minute. The indifferent electrode was an Ag-AgCl plate implanted subcutaneously in the groin. A bipolar recording electrode consisting of two curved silver wires, 4 mm apart and embedded in flexible plastic, was sutured to the outflow tract of the right ventricle 4–6 cm from the stimulating disc electrode. Femoral arterial pressure and lead 2 of the electrocardiogram were monitored during an experiment and arterial Po2 was maintained above 100 mm Hg with supplemental inspired oxygen. The sternotomy was covered with thick pads or clamped in a closed position to reduce mediastinal cooling.

Computerized Strength-Interval Curve Program

Each strength-interval curve was generated under complete computer control. Hardware consisted of a Digital Equipment Corporation PDP 8/e computer with 16 K memory core, a real-time programmable clock, dual DECtapes, two-channel D/A converter, 10-bit A/D converter with eight-channel multiplexer, 12 channels of digital input-output, and an LA 36 terminal. This system interacted through a custom-built digital interface with a series of Tektronix pulse and waveform generators and a Grass S-48 stimulator and delivered primary and secondary stimuli via Grass isolation units and a Grass CCU-1 constant current unit. Since the real-time control and premature beat (PVC) detection tasks were complex, computer processing resources were conserved by allowing standard stimulators and waveform generators to control the shape and duration of stimuli. Visual monitoring of stimuli was accomplished by using two Tektronix oscilloscopes (502A and high persistence 5103N).

The strength-interval curve was begun in late diastole and "tracked" leftward into the refractory period. After the late diastolic threshold was measured, the range of current steps to be used was established, generally reaching 3–4 times late diastolic anodal threshold. Before the experiment, the number of current steps to be used, the detail of the time axis, and the curve-following logic were specified. Generally, increasing current steps of approximately 10% of late diastolic threshold (LDT) were used to a level of about 125% of LDT. Current steps of approximately 20% of LDT were continued to a level of twice LDT, and 100-μA steps were employed above this level to a maximum of 1.5–1.8 mA. The response to S1 (R1) was signaled to the computer by a digital pulse coincident with the arrival of R1 at the stimulating electrode. During the flat, late diastolic portion of the strength-interval curve, the interval between R2 and the computer-generated S2 was decremented by 5-msec steps, whereas the rising portion of the curve and the early dip portion of the curve generally were examined at 2-msec intervals. The curve-following logic identified the latter two portions of the strength-interval curve by a specified number of consecutive rising or falling thresholds (generally three). Once the current levels were specified, a calibration curve was generated by varying the input voltage to the constant current unit and monitoring the voltage across the series resistor producing an internal table of input-output voltage pairs. This table was used to generate approximate currents at the specific levels; the actual current delivered at each S2 was measured across the series resistor and used to construct the strength-interval curve.

During an experiment, the computer monitored three inputs; a digital pulse specifying the occurrence of R1, an analog voltage across the series resistor, and an analog signal from the recording electrode for the detection of a propagated premature beat (R2). Two outputs were used to generate S2, a voltage fed to the constant current unit specifying the magnitude of S2 and a digital pulse to the logic interface specifying the timing of S2.

The algorithm used for PVC detection used a first-difference, threshold duration technique. The signal from the recording electrode was examined during a specific window after S0, typically ranging from 30 to 180 msec after S2 to allow for variations in conduction time. Before each curve, a threshold was "learned" by generating a late diastolic PVC with a high intensity S2 and examining the response (R2) at the bipolar recording electrode located at the right ventricular outflow tract. The signal was digitized at a 1-kHz sample rate and the first differences formed between points separated by 8 msec. A threshold was set to a fraction of 40% of the maximum first-difference, ignoring sign. A PVC was considered to have occurred when, during the specified window after S0, the first difference exceeded the threshold continually for at least 4 points. Having recognized a PVC, the computer recorded the intensity of the S2 which generated it and the R1-S2 interval. The time from the delivery
of S2 at the stimulating electrode to the detection of R2 at the recording electrode was defined as the conduction time. Secondary stimuli were delivered after each S2 until a PVC was elicited; following four recovery beats, secondary stimulation was resumed at the next shorter R1-S2 interval. The strength-interval curve was displayed on a storage oscilloscope and continuously traced on a strip chart recorder for visual monitoring. Data also were stored on magnetic tape for later retrieval and analysis. On some occasions, curve-following logic and both time and current steps were altered at the moment of coronary arterial reflow to examine more rapidly specific portions of the strength-interval curve (i.e., phase 3 dips). Under most circumstances, the time required to generate a complete anodal strength-interval curve was 45–120 seconds. Simpler curves without dips could be generated in 30–45 seconds.

Experimental Design
In the control state, a minimum of three strength-interval curves were recorded to confirm that a steady state existed. Coronary artery ligations were maintained for a period of 15 minutes. Strength-interval curves were recorded continuously during the ligation period and for 10 minutes following reperfusion of the ischemic segment. One to three ligation-release experiments were performed in each dog and a minimum period of 20 minutes was allowed between consecutive ligations. In some experiments, anodal strength-interval curves were recorded continuously during and following coronary artery ligations, whereas, in others, cathodal and anodal curves were recorded alternately through the course of the experiment. Spontaneous arrhythmias, other than occasional PVCs, were uncommon in these experiments. On those few occasions when ventricular tachyarrhythmias developed, the computer program indicated the possibility of invalid data and those curves were excluded from this analysis.

To compare different anodal strength-interval curves quantitatively, a four-point analysis of the curve was employed. Each point consisted of a coordinate of current intensity and timing (R1-S2 interval). Point 1 identified the longest R1-S2 interval at which the ventricle was refractory to the maximum current employed (generally 1.5–1.8 mA). Point 2 identified the current and time coordinates of the nadir of the phase 3 dip. Point 3 provided the coordinates of the highest point in the strength-interval curve between the dip and late diastolic threshold, and point 4 was chosen at the shortest R1–S2 interval in late diastole where thresholds were constant. During ischemia, the early dip frequently disappeared and flattening of midcurve thresholds was observed. In these instances, the threshold current for point 2 and/or point 3 was chosen at a time interval following point 1 which corresponded to that observed in the control curve. Numerical values are expressed as mean ± standard error.

Results

Control State
The average LDT using anodal stimulation was 620 ± 75 µA. Phase 3 dips were present in all dogs and, in every instance, reached thresholds below those found in late diastole. The average dip threshold was 393 ± 62 µA. A shallow dip during the supernormal period sometimes was observed. Thresholds well above LDT consistently were found between the dip and late diastole with a peak value that averaged 205% of LDT. With cathodal stimulation, LDT averaged 101 ± 9.4 µA. Although a slight notch occasionally was observed in the falling limb of cathodal strength interval curves, phase 3 dips or supernormal periods were not seen. The shortest R1-S2 interval at which a response was elicited by maximum current intensities (1.5–1.8 mA) averaged 8.5 msec longer with cathodal stimulation than with anodal stimulation.

Conduction times of late diastolic PVCs were similar with cathodal and anodal stimulation and remained unchanged during the flat, late diastolic portion of the strength-interval curve. However, with either form of unipolar stimulation, higher conduction times were encountered during early repolarization. Although very low thresholds were encountered at the nadir of the dip of anodal strength-interval curves, the conduction times of these corresponding PVCs were not shortened (vide infra).

Comparison of Anodal and Cathodal Strength-Interval Curves during Coronary Ligation and Reperfusion

Alternating anodal and cathodal strength-interval curves were recorded during 10 ligation-release experiments in six dogs. Representative curves from two such experiments are shown in Figures 1 and 2. In an additional eight dogs, anodal strength-interval curves were recorded continuously during 16-ligation-release experiments. The most consistent change in the anodal strength-interval curve during the first 2–3 minutes of ischemia was a fall in threshold, particularly in the segment of the curve between the dip and late diastole. Characteristically, LDTs fell, dip thresholds changed little or rose somewhat, and the high initial thresholds between these two points fell dramatically. Not infrequently, PVC thresholds fell to the level of the dip at all R1-S2 intervals from the dip to late diastole (Fig. 3). These changes generally were maximal at 2–5 minutes of ligation, but on some occasions were well developed within the first 1–2 minutes of ischemia. The falling limb of the anodal strength-interval curve shifted progressively to the left during ischemia, indicating shortening of the refractory period.

During early ischemia, the cathodal strength-interval curve was altered substantially less than the corresponding anodal curve. As with the latter, the falling limb of the cathodal curve shifted to the left and, in three of six dogs, LDTs fell transiently during the first 2–3 minutes of ligation. Thereafter, cathodal LDT generally rose during ischemia while anodal thresholds were falling, and the difference between the two lessened as ischemia was maintained (Fig. 1). The behavior of unipolar strength-interval curves during the late ligation period (i.e., 10–15 minutes of ischemia) was more variable than during early ischemia and appeared to depend, at least in part, on the size of the ischemic segment. In most experiments, the ischemic segment was kept relatively small both to avoid
spontaneous ventricular arrhythmias and to permit multiple ligations in the same dog. In these instances, LDTs for both anodal and cathodal stimulation remained low during the 10- to 15-minute period of ligation (Fig. 1). In one instance, LDTs of both anodal and cathodal stimulation were the same during the late ligation period. When the ischemic segment was larger, shifts in unipolar strength-interval curves early during ligation were similar to those described above, but by 5-10 minutes of ischemia, both anodal and cathodal thresholds rose rapidly throughout all portions of curve (Figs. 2 and 4). Rarely, the ischemic segment at this time was inexcitable at all R1-S2 intervals to current intensities of 1.5-1.8 mA.

During abrupt reperfusion of the ischemic segment, marked changes in the unipolar strength-interval curves were noted. The falling limb of both anodal and cathodal...
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Results

Strength-interval curves moved to the right following arterial reflow overshooting the control curve at 3–5 minutes. Since curves were “tracked” leftward into the refractory period, dip portions of anodal strength-interval curves were inscribed towards the end of the time periods reported. Within seconds of reperfusion, LDTs for anodal stimuli rose substantially and thresholds in the middle of the curve rose dramatically (Figs. 2 and 3). Frequently, phase 3 dips promptly reappeared and were broader and deeper than during the control state (Figs. 1–3). Thus, while the falling limb of both anodal and cathodal strength-interval curves rapidly shifted to the right following arterial reflow, the prompt appearance of broad, deep dips in anodal strength-interval curves greatly increased ventricular excitability to polarizing stimuli delivered early during repolarization. After 5–10 minutes of reperfusion, thresholds to anodal stimuli at all R1-S2 intervals to the right of the dip generally rose to levels higher than the preligation values. Cathodal strength-interval curves, in a much less dramatic fashion, approached control levels following reperfusion and also maintained LDTs above preligation values during most of the 10-minute recovery period. However, in two dogs with large ischemic segments and very high thresholds during the late ligation period, LDTs to both anodal and cathodal stimuli fell to levels below control following reperfusion.

Analysis of Serial Anodal Strength-Interval Curves during Ischemia and Reperfusion

To depict trends in anodal strength-interval curve shifts during ligation-release experiments, the 4-point analysis shown in Figure 5 was employed. These four coordinates of threshold current and timing represent the average values derived from 16 experiments, and the values shown represent those obtained in the control period.

**Figure 3** Anodal strength-interval curves. Left panel: Coronary artery ligation. The 0 to 2-minute curve is shifted slightly to the right of control, and both midcurve and late diastolic thresholds have fallen markedly. At 12–13 minutes of ischemia, midcurve thresholds have risen slightly and the falling limb has shifted to the left of control. Right panel: Ligature release. Within seconds of arterial reflow, diastolic thresholds rise above control, the dip reappears, and the falling limb remains to the left of control. After 10 minutes of reperfusion, the curve is shifted to the right and upward, and dip threshold is the same as control.

**Figure 4** Effect of a large ischemic segment on anodal strength-interval curve. The curve recorded early during ischemia is shifted to the left with reduced midcurve and late diastolic thresholds. By 5 minutes of ischemia, however, thresholds have increased markedly at all R1-S2 intervals. Numbers in parenthesis indicate the conduction time in milliseconds corresponding to a given PVC threshold.
were actually higher than point 3. At 15 minutes of ligation, LDTs had drifted upward toward preligation levels, midcurve thresholds rose further, and a shallow early dip (point 2) reappeared.

During the first minute following arterial reflow (Fig. 6), point 1 changed little, dip thresholds fell, midcurve thresholds rose substantially, and LDTs (point 4) rose toward preligation levels. Although the average dip threshold remained above control levels during the first minute of reperfusion, great variability was noted from one experiment to another. Thus, in some experiments, deep wide dips were demonstrated clearly during early reperfusion (Figs. 1-3); in others, thresholds remained elevated at the moment this portion of the curve was explored. After 10 minutes of reperfusion, the falling limb of the anodal strength-interval curve had shifted to the right of the control curve with little further change in dip threshold. Midcurve thresholds rose to levels well above control with peak values occurring later during diastole. LDTs after 10 minutes of reperfusion averaged 24% higher than preligation levels.

Conduction Times during Coronary Artery Ligation and Reperfusion

In the bottom panel of Figure 5, average conduction times are given for all PVCs generated by the threshold state and at 2–3 minutes, 5 minutes, and 15 minutes of ligation. Points 1 and 2 are shifted progressively to the left during the 15-minute period of ischemia, indicating shortening of the refractory period. Occasionally, however, the first strength-interval curve recorded after ligation revealed a transient rightward shift of the falling limb of the curve, as shown in Figure 3. This brief rightward shift was thought to be due to myocardial cooling coincident with coronary ligation and was not observed if measures were taken to prevent a fall in mediastinal temperature (vide infra). At 2–3 minutes of ischemia, midcurve thresholds (point 3) fell markedly from an average of 1260 μA to 650 μA while dip thresholds (point 2) had risen to the same level. After 5 minutes of ischemia, LDTs had fallen slightly more than at 2–3 minutes, whereas thresholds at points 2 and 3 were somewhat higher than at 2–3 minutes of ischemia. Since the early dip was generally no longer in evidence after 5 minutes of ischemia, average thresholds for point 2, measured at an interval following point 1 which corresponded to the relative position of the dip in the control curve,
stimuli shown in the upper panel of this figure. At 2-3 minutes and at 5 minutes of ligation, conduction times were greater than control at all corresponding R1-S2 intervals. However, after 15 minutes of ischemia, conduction times had returned to control levels during the early recovery period but remained prolonged during late diastole (point 4). A more detailed analysis of PVC conduction times is shown in Figure 7. For this experiment, conduction times of the serial anodal and cathodal strength-interval curves shown in Figure 1 are plotted. Control state, early ligation, and late ligation curves are shown for both anodal and cathodal stimulation. During the control state, conduction times remained constant throughout the flat, late diastolic portion of both anodal and cathodal curves and then progressively increased as the R1-S2 interval shortened. Supernormal conduction during the dip of anodal curves was not observed. Marked prolongation of conduction times was observed during the early ligation period (2-4.5 minutes) while, later during ligation (12-15 minutes), conduction times had returned toward repolarization levels, particularly at the shorter R1-S2 intervals. A similar trend based on average values is shown in Figure 5.

During the first minute of arterial reflow (Fig. 6), average conduction times fell to levels below those observed either during the control state or at 15 minutes of ischemia at all R1-S2 intervals except during late diastole (point 4). Since conduction times during late diastole were the first to be recorded following ligation release, their measurement preceded the full effect of reperfusion. The observation that late diastolic conduction times within seconds of reperfusion were the same as those found just prior to ligation release (Fig. 6) supports this view. On the other hand, as reperfusion evolved, shortened conduction times measured earlier in diastole were observed. At an R1-S2 interval of 145 msec, for example, conduction times recorded later during the first minute of reperfusion averaged 11 msec less than control, despite threshold levels which were 600 μA greater than control. After 10 minutes of reperfusion, conduction times were greater than control at all comparable R1-S2 intervals except during late diastole.

Discussion

Studies of the time course of changes in excitability during acute myocardial ischemia have yielded variable results which are best explained by differences in the mode of stimulation used and the duration and severity of ischemia at the time measurements were made. Using cathodal stimulation, Elharrar et al.19 recently demonstrated decreased diastolic thresholds during the first 1-3 minutes of myocardial ischemia which quickly gave way to a rapid rise in excitation thresholds. Brooks et al.9 studied excitation thresholds to both bipolar and unipolar stimulation during experimental myocardial ischemia and generally found them to be increased. Both cathodal and anodal strength-interval curves recorded 30-35 minutes after coronary ligation showed a shift in the falling limb to the left and an increase in diastolic thresholds. Tsuchida,20 on the other hand, found cathodal diastolic thresholds to fall slightly after 30 minutes of ischemia and, in the majority of his experiments, thresholds remained less than control 2 hours after coronary ligation. In our own studies using unipolar stimulation, a decrease in excitation thresholds during acute ischemia was primarily a feature of anodal stimulation. Although diastolic thresholds to cathodal stimulation fell slightly during the first 2-3 minutes of ischemia in some dogs, they invariably were greater than control at the time of peak vulnerability to arrhythmia (i.e., 3-8 minutes of ischemia). Thus, during this peak incidence of spontaneous ligation arrhythmias,1,3 excitability to anodal stimuli was enhanced while cathodal excitability was decreased. Directional changes in excitation thresholds following coronary ligation are also a function of the extent of the ischemia. When myocardial ischemia is severe enough, thresholds rise rapidly and the tissue may become virtually inexcitable (Fig. 4). Nonetheless, the tendency for anodal thresholds to fall while cathodal thresholds rose in the same dog during ischemia was a rather consistent finding in the present study.

Despite the variable behavior of excitation thresholds during acute myocardial ischemia, there appears to be a more uniform response of refractory period duration. In 1935, Wiggers and Wiggers21 demonstrated that the myocardial action potential shortened within 1 minute of coronary artery ligation, and there is general agreement...
among investigators that the ERP shortens during acute myocardial ischemia.4,5,8,20,21 In the present study, as long as the size of the ischemic segment was relatively small, the falling limb of both anodal and cathodal strength-interval curves shifted leftward, indicating shortening of the refractory period. The transient rightward shift in the curve observed during the first 1-2 minutes of ischemia was not observed in closed-chested dogs. In other studies in our laboratory (unpublished observations), subepicardial temperature fell by 1-3°C within the first 1-2 minutes of coronary ligation only in open-chested dogs and was associated with a short-lived 15- to 20-msec prolongation of epicardial ERPs within the ischemic segment. These findings suggest that the initial rightward shift in strength-interval curve following coronary ligation in open-chested dogs is due to cooling of the ventricle. Indeed, in the absence of severe ischemia, the falling limb of the strength-interval curve shifted progressively to the left throughout the course of 15-minute ligations. Recent studies relating transmembrane potentials in the intact pig heart to changes in local refractoriness indicate that, during early ischemia, decreases in action potential duration, amplitude, and resting potential were associated with shortening of local refractory periods, while later during ischemia postrepolarization refractoriness developed.22 A possible expression of this latter phenomenon in the present study might be expected only in those instances in which the ischemic segment was large and the strength-interval curve was shifted to the right and upward (Fig. 4).

Despite the impressive fall in anodal thresholds during acute ischemia, there is reason to believe that changes in PVC conduction times correlate better with vulnerability to arrhythmias during this phase of ischemia than do excitation thresholds. Notwithstanding progressive shortening of the refractory period (leftward shift of the strength-interval curve) and often persistently low thresholds to unipolar stimulation during the entire 15 minutes of ischemia (Fig. 1), the time course of conduction time prolongation closely parallels that of heightened vulnerability at 3-8 minutes of ischemia and relative electrical stability at 10-15 minutes of ischemia. Indeed, the early onset of conduction time prolongation may be most abrupt (Fig. 7) and unrelated to changes in excitation thresholds, yet conduction may inexplicably return toward control levels at 10-15 minutes, despite continued ischemia. Scherlag and associates have emphasized the dominant role of delayed activation in the genesis of early ligation arrhythmias24,25 and the present study tends to support this view. Tsuchida50 reported that conduction velocity was remarkably depressed in ischemic myocardium during the early phase of the relatively refractory period. While the present study demonstrated prolonged conduction times at all R1-S2 intervals during early ischemia (i.e., 5 minutes), later during ligation conduction times had returned to normal throughout early repolarization but remained prolonged during late diastole (Figs. 5 and 7). This finding suggests a mechanism by which the ventricle may be especially protected from early (R on T) PVCs during the late ligation period. However, this prolongation of late diastolic conduction times may help explain the clinical observation that in the wake of an acute myocardial infarction, life-threatening ventricular tachyarrhythmias frequently may be initiated by late-coupled PVCs.26,27

During the first 1-2 minutes of arterial reflow, the most striking increase in excitability to unipolar stimulation was noted during the early dip of anodal strength-interval curves. At the same moment, anodal midcurve and LDTs rose briskly and, while cathodal thresholds gradually fell, they generally remained higher than control levels throughout most of the recovery period. During reperfusion, the falling limb of both anodal and cathodal strength-interval curves shifted progressively to the right and by 3-5 minutes had exceeded control levels. On the other hand, reported measurements of the ERP following reperfusion (bipolar stimulation) have indicated abrupt shortening within the first 1-2 minutes of ligation release6,28 and prolongation after 5 minutes.29 In the face of our observations, it would seem likely that the shortened ERPs observed immediately following arterial reflow are due to the appearance of early dip thresholds in bipolar strength-interval curves rather than a further leftward shift in the falling limb of these curves. Since ERPs generally are measured using thresholds 2-3 times LDT, relatively shallow early dips would be encountered by this method. Support for the importance of shortened refractory periods in the genesis of reperfusion arrhythmias is provided by an examination of the late reperfusion period. At this time, when the danger of reperfusion arrhythmia has long passed, conduction time prolongation is substantial (Fig. 6) and entirely comparable to that which existed during the peak incidence of early ligation arrhythmias. However, the strength-interval curve at this time is shifted to the right of control, and thus refractory periods are prolonged and no arrhythmias are observed.

Since reperfusion arrhythmias are more often observed after longer ligations3-5 when the ischemic injury is presumably more intense, the two dogs with very high excitation thresholds during the late ligation period are of particular interest. In only these two dogs did both cathodal and anodal LDTs fall below control levels following reperfusion. These findings suggest an oscillatory behavior of the membrane at the time of reperfusion, the lowest excitation thresholds during reperfusion following the highest thresholds just prior to release. This phenomenon is also apparent when one compares the anodal dip thresholds before and after ligation release in Figures 1 and 2. In the instance when late ligation dip thresholds were high (Fig. 2), dip thresholds following reperfusion fell dramatically; when thresholds remained low throughout the ligation period, the fall in dip thresholds following ligation release was less marked (Fig. 1).

A similar phenomenon may be operative with respect to conduction following ligation release. The shortened conduction times observed during the first minute of reperfusion may be the consequence of hyperpolarization. Trautwein and Dudel59 studied the myocardial action potential of the isolated papillary muscle during hypoxia and re-oxygenation and found an overshoot in action potential amplitude and duration during re-oxygenation, the former occurring more promptly than the latter. A
similar sequence in the reperfused ischemic segment should be associated with shortened conduction times initially and an overshoot rightward shift in the strength-interval curve subsequently, just as was observed in the present study. Since it is reasonable to assume that reperfusion of the ischemic segment is inhomogeneous, particularly during the early reflow period, closely juxtaposed areas of increased and decreased excitability and conduction are to be expected. In this regard, some of our experiments revealed prolonged conduction times well into the first 1-2 minutes of reperfusion while most others demonstrated prompt superconductivity.

It should be emphasized that at no time during reperfusion were conduction times found to be shorter than those observed during late diastole in the control state. It is, of course, possible that an experiment designed to examine late diastolic excitability and conduction toward the middle or end of the first minute of reperfusion might have identified conduction times shorter than control. Nonetheless, the claim that supernormal conduction was observed during early reperfusion refers specifically to conduction measured at comparable R1-S2 intervals. Furthermore, as a consequence of the design of the experiment, the effect of early reperfusion upon the conduction of late diastolic PVCs remains undefined.

To generate sufficiently rapid threshold measurements for this study, the method used to record strength-interval curves required that secondary stimuli be delivered almost continually. At a heart rate of 150 beats/min, approximately 60-80 S2 pulses were generated per minute, of which 15-20 were threshold. Since it is known that sudden changes in frequency evoke reciprocal changes in refractory period, this testing method itself is capable of producing shifts in the strength-interval curves. During a control state, anodal curves were run continuously in three dogs for a period of 30 minutes. While variations were observed during the first 3-4 curves, particularly in the region of the early dip, thereafter the curves remained quite stable with thresholds rarely differing by more than 1-2 current steps at each R1-S2 interval. Similarly, during a steady state, no significant differences were observed when 12 rather than four recovery beats were allowed after each PVC. Nevertheless, the demonstration that excitability to anodal stimuli is enhanced during ischemia may be exaggerated by the delivery of the anodal stimuli themselves, which in turn may increase electrical dispersion by polarization of nearby cells.

This report reemphasizes the importance of abbreviated refractoriness, prolonged conduction, and nonuniformity of recovery properties in the genesis of ventricular arrhythmias during acute ischemia. In addition, it identifies a possible role for dispersion of conduction during early reperfusion (supernormal conduction in reperfused tissue and prolonged conduction in persistently ischemic tissue) and increased excitability to anodal stimuli both during the early ligation period and immediately after arterial reflow. During the early ligation period, enhanced anodal excitability is confined to the mid and late diastolic portion of the strength-interval curve, while during early reperfusion these thresholds are increased and early dip thresholds are low. Thus, directional changes in anodal excitability correlate better than do cathodal with vulnerability to ventricular arrhythmias. Although it has long been appreciated that the anode is preeminently effective in evoking fibrillation,

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References
Morphological Changes in Isolated Perfused Dog Lungs after Acute Hydrostatic Edema

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SUMMARY Morphometric data from stable (nonedematous) isolated dog lungs, perfused with nearly cell-free perfusates, were compared to similar stereological evaluations of isolated dog lungs after induction of severe acute hydrostatic edema. In the edematous lungs, capillary surface and volume densities were substantially increased. Alveolar surface density was also increased. Thicknesses of the endothelial and type I epithelial cellular compartments of the air-blood barrier were unchanged. Thickness of the interstitial compartment of the air-blood barrier was substantially increased and this, in turn, caused an overall increase in mean thickness of the barrier. Volume densities of the nonparenchymal connective tissue spaces surrounding the extra-alveolar vessels and airways were also increased. In both the endothelial and type I epithelial cells, cytoplasmic volume densities of pinocytotic vesicles were increased. In addition, the number of vesicles opening onto the luminal and albuminal cellular surfaces increased significantly. Transendothelial vesicular passage may contribute to interstitial edema formation, and transepithelial vesicular transport may contribute to alveolar flooding in isolated perfused dog lungs.

IN A PREVIOUS study of isolated dog lungs perfused under stable conditions, morphometric analyses of the air-blood barrier, alveolar and capillary surface densities, and extra-alveolar vessels and airways were presented.1,2 The present report describes the same morphometric parameters in isolated dog lung preparations after induction of severe acute hydrostatic or cardiogenic edema.

Previous microscopic examinations of hemodynamic pulmonary edema demonstrated swelling of connective tissue spaces surrounding the extra-alveolar vessels and airways, fluid accumulation in the alveolar interstitium, and finally alveolar flooding.3-5 It was concluded that these observations represented the sequential pattern of fluid accumulation in acute pulmonary edema.4 Increases in capillary hydrostatic pressure with consequent increases in transendothelial transport have been suggested as a primary means of extravascular fluid accumulation in edematous lungs.6,7 Additional evidence suggesting a contribution of extra-alveolar pulmonary vessels to edema formation also has been presented.7,8

In the present study, established stereological techniques9 were employed to quantitate changes with acute pulmonary edema in air-blood barrier thickness, alveolar and capillary surface densities, and volume densities of the nonparenchymal connective tissue spaces surrounding the extra-alveolar pulmonary vessels and airways. The morphometric results indicated swelling of the nonparenchymal connective tissue spaces and the interstitial compartment of the air-blood barrier. The proportion of the alveolar septa occupied by capillary contents was increased, as were capillary and alveolar surface densities. The interendothelial junctions were unaltered; however, proportions of both the endothelial and type I epithelial

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