Optical Recordings in the Rabbit Heart Show That Defibrillation Strength Shocks Prolong the Duration of Depolarization and the Refractory Period

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The present data were obtained using the technique of optical recording with the voltage-sensitive dye WW781. This technique, unlike electrical methods, was able to provide uninterrupted recordings free of artifacts during defibrillation shocks. Optical recordings were made from sites on the ventricular epicardium of perfused rabbit hearts during electrical pacing. Continuous recordings of the electrophysiological responses of an intact heart to defibrillation threshold–strength shocks were made. It was shown that these shocks were able to stimulate normal-appearing action potentials in nonrefractory myocardium. A new and unexpected finding was that defibrillation threshold–strength shocks were also able to evoke a sustained depolarizing response from myocardium already undergoing an action potential. This prolonged the time that the myocardium remained in the depolarized state. Prolongation of the depolarized state was accompanied by an equal prolongation of the refractory period. There was no indication that this depolarizing shock response was due to damage of the myocardium by the shock, to heterogeneous electrical responses in the optical recording area, or to the methods used in this study. It is hypothesized that these shocks were able to elicit a new action potential in already depolarized myocardium by hyperpolarizing portions of the myocardium’s cellular membranes and, in so doing, to reactivate the fast sodium current. This effect, if prevalent in a fibrillating ventricle, could play a role in the defibrillation process by effectively resynchronizing electrical activity. (Circulation Research 1991;69:842–856)

The application of a strong electrical shock across the heart has long been known to terminate ventricular fibrillation, although the mechanism for termination is still uncertain. Investigation into the electrophysiological effects of defibrillating shocks has been hampered by the electrical artifacts attending them. These artifacts, arising chiefly from the voltage gradients produced by the shock current, are large enough to overload electrophysiological recording instruments during application of the shock. Often, in addition to being unable to record electrical activity from the heart during the shock, restoration of normal recording is delayed by lingering electrode artifacts and electronic recovery of the amplification system. Although special instrumentation and recording electrodes have been developed that permit rapid recovery of normal recording, it is not yet possible to record cardiac electrical activity during a defibrillation shock using electrical instrumentation. Optical recording, using a voltage-sensitive dye, is not affected by these shock artifacts, because it directly and exclusively senses cardiac membrane voltage. This technique was used in this study to investigate the electrophysiological effects of defibrillation threshold (DFT)—strength shocks applied during a paced rhythm. The aim was to determine the electrophysiological responses of the myocardium to shocks delivered during various phases of the cardiac cycle to obtain information that might enable prediction of the effects of these shocks when applied during fibrillation.

It is generally believed that defibrillation shocks are excitatory agents that depolarize (stimulate) the excitable portions of the myocardium to cause de-

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fibrillation. This report confirms that DFT-strength shocks are able to stimulate action potentials in excitable myocardium. The focus of these studies, however, was on the effects of shocks applied during the cardiac action potential at times when the myocardium has been assumed to be refractory to stimulation by the shock. It was found that these shocks evoked a sustained, depolarizing response that caused the total depolarization time to outlast the normal action potential duration.

**Materials and Methods**

*Experimental Preparation*

All experiments were carried out on isolated, intact hearts obtained from 4–5-kg New Zealand White rabbits. A total of 21 hearts were studied. Rabbits were intravenously injected with 40–50 mg/kg body wt pentobarbital and 5,000 units heparin. The hearts were removed through a midsternal incision and immersed in cold Tyrode's solution. Hearts were mounted on a Langendorff apparatus, and the coronary system was continuously perfused via a cannula in the aortic root with Tyrode’s solution under a pressure head of 53 mm Hg. This perfusion pressure was slightly greater than the optimal perfusion pressure of 60 cm water previously found for the Langendorff-perfused rabbit heart. The Tyrode's solution consisted of the following (mM): NaCl 130, NaHCO3 24.2, KC1 4, CaCl2 1.8, MgCl2 0.6, Na2HPO4 1.2, and dextrose 11.1. Solutions were continuously gassed with a 95% O2–5% CO2 mixture giving a pH of 7.35–7.40. Solution temperature was controlled with a thermostatic water bath, and the left ventricular endocardial temperature was monitored by a thermistor that was maintained in the range of 36–38°C. The coronary and cavitary blood was first flushed out after the heart was connected to the perfusion system. During perfusion of the first 16 hearts, the coronary effluent was collected, filtered, and returned to the perfusion reservoir. A total volume of ~1.6–2 liters Tyrode’s solution was continuously recirculated throughout these experiments. In the last five hearts, the coronary effluent was discarded while fresh Tyrode's solution was added to the perfusion system. This latter perfusion method did not produce any noticeable differences in the behavior of the hearts but simplified the experimental procedure. The left ventricle was drained with a piece of tubing inserted through the mitral valve. All hearts were weighed at the end of the experiment. Hearts used in this study weighed an average of 15.9±1.8 g with a range of 12.1–18.6 g.

Figure 1 shows the electrical circuit used to deliver shocks to the heart. There were two defibrillation electrodes: a stainless-steel cup electrode under the cardiac apex (apical cup) and a narrow band of stainless-steel mesh (5 mm wide) that encircled the base of the heart (basal band). The apical cup had an area of 177 mm2, and the basal band, depending on the size of the ventricle, ranged from ~350 to 500 mm2 in area. In most experiments a single defibrillator was used. It was a battery-powered high-energy stimulator (model 2326, Medtronic Inc., Minneapolis, Minn.) manufactured for experimental use. The positive pole of this unit (defibrillator 1) was connected to the basal band electrode. The negative pole was connected to the apical cup electrode through a resistor. The shock current from the defibrillator flowed through the heart between the two shock electrodes. The defibrillation current was measured with a differential amplifier that recorded the voltage drop across a sensing resistor in the circuit. The defibrillator generated standard monophasic, truncated exponential shock-current waveforms having a 63% tilt and 4–6-msec duration. The truncated exponential waveform is generated by the discharge of a capacitor, within the defibrillator, through the external electrical circuit formed by the heart, shock electrodes, and sensing resistor. The total impedance of the external circuit was ~75 Ω. Shock strength could be set to any of 12 possible energy settings over a range of 0.0075–3.0 J. The shock energies were not directly computed from the current and voltage waveforms but were taken from the dial settings of the defibrillator. In eight hearts, a second battery-powered defibrillator (defibrillator 2) was connected in parallel to defibrillator 1. This second unit (model 2394, Medtronic) was used to deliver a shock either before or after the defibrillation shock delivered by the first unit (Figures 7 and 8).

The cardiac rhythm was monitored through bipolar electrodes in contact with the ventricle or through an
"electrocardiogram" taken between an electrode on the aortic root and an electrode within the right ventricle. The heart was paced through a platinum wire bipolar electrode placed on the right ventricular outflow tract or posterior left ventricle. In some experiments pacing stimuli were delivered directly to the optical recording site through platinum bipolar pacing leads. These leads flanked the optical pickup (described below) so that stimulation current passed through the cells within the optical recording area. All pacing stimuli were constant-current pulses 2 msec in duration. Synchronization of pacing stimuli, shock delivery, and data acquisition system operation was controlled by a program running on a personal computer (8 MHz, Fountain Turbo XT). A personal computer–based (12 MHz, model 286-12, Dell Computers) data acquisition system digitized three channels of analog information, an electrogram, shock-current waveform, and optical signal, at 4,000 samples/sec/channel for storage on disk.

Experimental Protocol

At the start of each experiment, the DFT of the heart was determined. Fibrillation was initiated by rapid pacing (25 Hz, 1–4-second train duration), and a defibrillation shock was applied within 10–30 seconds after fibrillation induction. If the shock failed to defibrillate, the same-strength shock was reapplied, or the next highest strength was tested. Successful episodes of fibrillation and defibrillation were repeated until the lowest shock strength capable of reliably defibrillating on the first attempt was found. The criterion for reliable defibrillation was three consecutive defibrillations on the first attempt. Three minutes was allowed to elapse between these attempts. Coronary perfusion was maintained throughout fibrillation. The average DFT for the 21 hearts used in this study was found to be 0.060±0.036 J/g heart. This is 18% higher than the DFT of 0.051±0.026 J/g found for the in situ canine heart in a study using combinations of atrial and ventricular apex defibrillation electrodes. This argues against the belief, based on the classic observations of a minimum (critical) mass of ventricle required to support fibrillation, that the rabbit heart is too small to fibrillate. In fact, a number of reports have been published in which fibrillation was studied using a perfused rabbit heart preparation. The rabbit heart though, unlike the canine or human heart, is capable of spontaneous defibrillation. But the fact that the rabbit heart exhibits a DFT at all, as shown in this and another study, shows that it is not always on the verge of spontaneous defibrillation. Had the rabbit ventricle been on the verge of spontaneous defibrillation, then it should have shown a DFT much lower than that for the canine heart.

The experimental protocol involved recording optical action potentials from the epicardium of the left or right ventricle (see next section for recording method) during ventricular pacing at a constant 300-msec cycle length (200 beats/min) and during application of test shocks at various coupling intervals to the paced beat. Each optical recording typically encompassed a control action potential during the last paced beat followed by a shocked action potential. In some experiments an additional shock, rather than a pacing stimulus, was used to elicit the second action potential (see Figures 7 and 8 of "Results"). The primary independent experimental variables in this study were the shock strength and shock coupling interval (CI).

Refractory period determinations were also performed to measure the influence of defibrillation shocks on the time course of return of cardiac excitability after the shock. In one series of hearts, the refractory period at the optical recording site was measured using pacing stimuli applied through the bipolar electrodes flanking the optical pickup. These stimuli were set to four times the diastolic pacing threshold, and the CIs of the extrastimuli were changed in 5-msec increments. In other experiments, a remote bipolar electrode, at least 1 cm from the pacing site, was used to determine whether extrastimuli, applied at various CIs after the shock, were able to excite a propagated impulse. This electrode had closely spaced contacts to differentially cancel a greater portion of the shock-voltage artifact than the widely separated electrocardiographic leads. The effective refractory period was taken as the time between the basic drive stimulus and the earliest extrastimulus that elicited a propagated impulse.

Optical Recording Technique

A voltage-sensitive dye was used to record cardiac membrane action potentials by nonelectrical means. This technique has been previously used in a variety of cardiac preparations. The heart was stained with the fluorescent oxonol, voltage-sensitive dye WW781 (dye XXV of Gupta et al and available as W-435 from Molecular Probes, Inc., Eugene, Ore.). Dye concentration in the perfusate was 1–2 mg/l (1.3–2.6 μM). Voltage-sensitive dyes bind to cellular membranes and transduce transmembrane potential into an optical signal. They give rapid, linear responses to changes in membrane potential that are due to, in the case of WW781, membrane potential driven shifts of dye molecule populations between the membrane and bathing solution phases. Optical recording was used in this study because the dye transduces only the membrane potential and is insensitive to extracellular or intracellular voltage gradients developed by the shock. Such artificial voltages overload sensitive electrical recording instrumentation. Optical recording is well suited for directly studying the effects of defibrillation shocks, since recordings that are free of these artifacts can be obtained during the shock. Axon voltage-clamp experiments have shown that WW781 is able to linearly sense membrane potential changes within microseconds and is unaffected by the gating of ionic channels or large membrane current fluxes. Another oxonol dye, an analogue of WW781, was found to linearly
sense membrane potential over a 500-mV range in experiments on lipid vesicles.27

A fiberoptic pickup, shown in Figure 2A, was used to detect optical signals. The pickup consisted of a bundle of seven plastic optical fibers (Mitsubishi ESKA acrylic), each 250 μm in diameter and 36 in. long. As shown in Figure 2B, six of the optical fibers were “fluorescence return fibers” surrounding a central laser output fiber in the pickup. The red light (wavelength, 633 nm; power, 20 mW) from a helium-neon laser (model HN-20, Jodon Inc., Ann Arbor, Mich.) was focused onto the face of the central fiber. Approximately half of the light emerged from the other end of the fiber to impinge on the dye-stained myocardial tissue (Figure 2A). The dye in the heart fluoresced in response to the incident laser light, and a portion of this fluorescence plus scattered laser light was picked up by the six outer (fluorescence return) fibers and returned to the fluorescence detector. The light returning from the heart first encountered a long-pass filter (3 mm thick, model RG645, Schott Corp., Duryea, Pa.). The laser light was blocked by this filter, and the longer wavelength fluorescence components passed through to the photodiode (model FIL-100C, United Detector Technology) for transduction into an electrical current. This current was amplified and converted into a voltage signal by a two-stage amplifier (106 V/amp, 50-μsec time constant). The output of this amplifier was an optical action potential representing the transmembrane action potential of the cells under the optical pickup. The fiberoptic pickup was placed in contact with the surface of the ventricle and moved from site to site by a manipulator. A shutter was placed between the laser and the lens and was opened only during acquisition of an optical recording.

Optical recordings of ventricular electrical activity show the same action potential time course as that simultaneously recorded by a microelectrode.19 However, while the microelectrode records the transmembrane voltage of a single cell, the fiberoptic pickup senses the aggregate transmembrane activity of a population of cells within the optical recording volume. The optical recording volume is a cylinder that is estimated to be 700 μm in diameter and several hundred microns deep (author’s unpublished data). Because of the time required for propagation of an impulse through this recording volume, the upstroke phases of these optical recordings are longer than those recorded by a microelectrode (author’s unpublished observations). Upstrokes of optical action potentials have been shown to be as fast or faster than microelectrode recordings when the optical recording area is restricted to one or a few cells.21,28 In this present study the optical action potential shows a rapid upstroke when all cells within the recording volume are simultaneously excited as they are by a defibrillation shock (see Figure 4).

In most experiments, D600 (methoxyverapamil, Sigma Chemical Co., St. Louis, Mo.) was added to the perfusate to give a concentration of 2 μM to suppress contraction. This permitted optical action potentials to be recorded without the distortion caused by cardiac contraction. This dose was found to block 90% of the inward calcium current in cat ventricular muscle by McDonald et al.29 After administration of D600, there was an increased coronary flow of Tyrode’s solution. Use of D600 also eliminated changes in heart geometry with contraction, and so the distribution of shock current through the heart remained constant during all phases of the cardiac cycle. Optical recordings obtained in four hearts not treated by D600 were compared with those taken in the presence of D600, and as it will be later described, no qualitative differences were found. There was no noticeable effect of D600 on DFT.

Data Analysis

The optical calibration bars in the figures indicate a 1% change in the optical signal with respect to the background fluorescence level. The optical signal cannot be interpreted to give either a direct or relative indication of transmembrane voltage. The height of the upstroke as a percentage of the background fluorescence varies from recording site to recording site and with time at the same site. This is because of nonuniform staining by the dye, dye washout, and bleaching of the dye by the laser light.

There is high-frequency noise on the optical tracings that is due to electronic noise within the fluorescence detector and to variations in the laser output power. A smoothing algorithm was used to filter these tracings through use of a moving average. This algorithm averaged all of the data points within a window and, while sliding this window through all of the data points in the tracing, plotted these averaged values. A 5-μsec-wide smoothing window was used on all tracings except for those tracings in Figure 4 showing action potential upstrokes that were unfiltered. A baseline correction procedure was also applied to the optical tracings to compensate for

![Diagram of the fiberoptic recording system. Panel A: Arrangement of the optical components used to record an optical action potential (OAP) from ventricular myocardium stained with voltage-sensitive dye. Panel B: Face-on view of the fiberoptic pickup showing the laser output fiber and the fluorescence return fibers. See “Materials and Methods” for an explanation of these components and their operation.](http://circres.ahajournals.org/content/84/2/845)
Figure 3 shows an example of a set of recordings acquired during an experiment. The electrocardiogram (tracing a) shows a ventricular stimulus artifact followed by a QRS complex caused by ventricular activation during the last two stimulated beats of the basic paced rhythm. Atrial activation preceded the second stimulus artifact. The second ventricular stimulus was followed by a QRS complex, which was then interrupted by the application of a DFT-strength shock 80 msec after the pacing stimulus. The shock promptly overloaded the amplification system, and electrode polarization artifacts caused the amplifier to swing between its positive and negative limits long after the shock was complete. (It must be noted that these shock artifacts were exacerbated by the placement of the recording electrodes. Closely spaced bipolar electrodes would show recovery within tens of milliseconds after the shock.) The current passed by the truncated exponential shock-current waveform is shown with a 10 times expansion in the time base in the inset below tracing a.

Tracing b of Figure 3 shows two consecutive optical action potentials: the first was recorded during pacing in the absence of a shock (the control action potential) and the second was interrupted by a defibrillation shock applied during the plateau phase (the shocked action potential). The time and duration of the shock were marked by the thick black bar above the shocked action potential. The control and shocked action potentials were extracted from tracing b and superimposed so that their upstroke phases were aligned (tracing c). The shock CI was measured as the time from the midpoint of the upstroke to the beginning of the shock. Tracing c shows that the shock rapidly depolarized the optical recording from its plateau level and that the depolarization remained for an extended period of time. The optical signal after the shock eventually recovered its resting potential after phases of slow and then rapid repolarization, like those seen in the control optical action potential. The total duration of the depolarized state indicated by the optical recording, from upstroke to postshock repolarization, outlasted that of the control action potential. The additional depolarization time (ADT) was quantified as the time difference between the repolarization phases (phase 3) of the shocked and the control action potential. This terminology is used to describe the effects of the shock on the optical recording even though, as hypothesized in "Discussion," the shock may actually restimulate a new action potential.

The figures for this paper were prepared by converting the acquired data into a format acceptable to a graphics software package (CHART by Zenographics). This software was used to create and label figures portraying optical tracings and was also used to compose plots of the numeric data.

**Results**

**Shocks Applied During Diastole Stimulate Action Potentials**

The effects of shocks delivered during electrical diastole (i.e., while the myocardium was at resting
As the shock strength was increased to 0.15 times the DFT (tracings c and d), the latency between the shock and the action potential upstroke decreased (tracing d). This was interpreted to indicate that a larger volume of myocardium was directly activated by the shock and so decreased the transit time of the propagated activation wave to the recording site. The next highest shock strength, 0.3 times DFT (tracings e and f), caused the upstroke to be generated during the shock and shows direct stimulation of the recording site by the shock. The upstroke of this optical action potential was also faster than those of the propagated upstrokes (compare tracing f with tracings b and d). This is probably due to nearly simultaneous activation of all cells within the recording volume. Increasing the shock strength (tracings g and h, 0.4 times DFT) caused a further decrease in the delay from the start of the shock to the beginning of the upstroke. The last two highest shock strengths (tracings i and j, 0.7 times DFT; tracings k and l, 1.0 times DFT) caused the upstroke to be completed before the end of the shock and increased the amplitude of the optically recorded upstroke to a higher level than that attained during activation by a propagated impulse; this level was possibly due to the large electric field of the shock. These tracings show that action potentials stimulated by DFT-strength shocks retained a normal appearance, and they did not show any indication of either shock artifact or distortion during delivery of the shock except for the changes in upstroke velocity and amplitude. This same process of action potential excitation was found at 35 other sites on this heart and at 17 sites on three other hearts where the strength of shocks applied in diastole was similarly varied. Under these circumstances, DFT-strength shocks appear to behave as widely presumed, namely, as agents for large-scale stimulation of the ventricle.

**Shocks Applied During the Action Potential Prolong the Duration of Depolarization**

Figure 3 (tracing b) shows that a shock applied during the course of a normal optical action potential prolonged the total duration of the depolarized state compared with control. This was always observed when shocks of DFT strength or higher were applied during the action potential plateau. This effect occurred throughout almost all of the plateau phase as is illustrated in Figure 5A, which shows the outcome of DFT-strength shocks applied at various times during repolarization. The black bars under the shocked action potential indicate the time and duration of the shock. In tracings a–e, the normal repolarization time course (dashed line and arrow head) has been superimposed on the recording of the shocked action potential. The CI of the shock was increased from 13 to 134 msec in ~20-msec increments in tracings a–g. In tracing a, the shock was applied while the myocardium was at its highest level of depolarization, and in tracing g, the shock came shortly before complete repolarization of the action.
potential. The shocks in tracings a–e were applied before the action potential was 50% repolarized, at times when the myocardium might be expected to be refractory to stimulation by depolarizing current. At the earliest CI (tracing a), the shock caused a depolarizing deflection but did not increase the total duration of the depolarized state over that of the control action potential. Successively later shocks were able to extend the duration of the depolarized state and delay full repolarization of the membrane beyond its normal time. Tracings f and g show that shocks applied when the action potential was nearly repolarized directly stimulated premature action potentials. The upstrokes of these shock-stimulated action potentials, like those shown in Figure 4, were larger and more rapid than those of the normal action potentials that were elicited by the pacing stimuli.

The most significant effects of the shocks in tracings a–e in Figure 5A were on the time that the optical action potentials remained in the depolarized state. These tracings show a growing separation between the repolarization phases of the control (arrowhead) and shocked action potentials with increasing shock CI. This indicates that, as the shock came later in the action potential, the myocardium remained in the depolarized state longer. This dependence was quantified using the procedure illustrated in Figure 3 (see “Materials and Methods”). Figure 5B shows a plot of ADT versus CI obtained from the experiment shown in Figure 5A. Seven of the 13 data points in this plot are labeled by the letters corresponding to the tracings in Figure 5A. The plot shows a positive, monotonic dependence on the shock CI, without discontinuities or inflections, as
The shocks were defibrillation threshold strength (0.75 J). Panel B: Plot showing the shock CI dependence of both the additional depolarization time (ADT, solid line) and the local RPP (dashed line). Defibrillation threshold shocks (0.75 J) were applied at 13 different CIs. Panel C: Scattergram plotting RPP against ADT for all of the measurements that were taken from 14 sites on five hearts. These shocks were applied over the entire time course of the action potential at each measurement site. The dashed line has a slope of 1 and illustrates the ideal result of an RPP accompanied by an equal amount of ADT. A least-squares linear regression analysis shows that the 171 data points in this plot are best fit by a line having a slope of 1.02, a value that is very close to the ideal. Defibrillation threshold shocks were used in all cases except in one series where 13 data points were obtained using 1.2 times defibrillation threshold shocks. Panel D: Plot showing effective refractory period prolongation (ERPP) by defibrillation threshold–strength shocks applied at various CIs in a heart untreated by WW781 dye and D600. The effective refractory period of the unshocked action potential was 119 msec. The defibrillation threshold was 0.75 J.

the shock was applied throughout all phases of the cardiac excitability cycle.

It was always found that the total duration of depolarization increased as the CI of shocks having DFT strength or higher increased. This was demonstrated at a total of 40 sites on 16 hearts where this protocol was executed. Figure 5C summarizes the results obtained from 10 of these hearts. As in Figure 5B, the ADT was plotted against the shock CI, and all of the tracings show a smooth, monotonic increase with shock CI. Differences among these curves could be, in part, due to the naturally arising variations in the normal action potential duration. The most important factor, however, was probably the inherent nonuniformity in the distribution of effective shock strength throughout the ventricle.33

Prolongation of Depolarization by Shocks Was Associated With Prolongation of the Refractory Period

A functional significance of a prolonged depolarized state induced by DFT-strength shocks would be
a prolongation of the refractory period. The local refractory period was therefore measured at the site of the optical recording by applying premature stimuli through the electrodes built onto the fibroptic pickup (see "Materials and Methods"). Tracing a of Figure 6A shows a pair of normal action potentials evoked by direct stimulation of the optical recording site during the pacing rhythm. Tracing b again shows a pair of normal action potentials but also a premature action potential stimulated after the second impulse. This extrastimulus (ES1) was the earliest that could excite an optically recorded premature action potential. Tracing c shows the effect of a DFT shock applied 84 msec after the upstroke of the second action potential (black bar). Comparison of tracings a and c shows that this shock prolonged the depolarization of the myocardium by 49 msec. In tracing d, both a DFT shock, 84 msec after the upstroke (black bar), and an extrastimulus (ES2) was applied to the second action potential. The premature action potential was the earliest that could be stimulated (ES2) after the shocked action potential, and it came later than that shown in tracing b. The time difference between the earliest extrastimuli that were able to excite an action potential in the presence and absence of a shock is the amount of refractory period prolongation caused by the shock and, in this example, was 52 msec, approximately the same as the prolongation of depolarization.

The prolongation of both the depolarized state and the refractory period by a DFT shock was measured at 12 different CIs in the experiment illustrated in Figure 6A, and the data from this experiment are plotted in Figure 6B. The ADT is plotted along the left vertical axis, the refractory period prolongation (RPP in panel B) is plotted along the right vertical axis, and shock CI is on the horizontal axis. The solid line shows the dependence of ADT on shock CI; the dashed line shows the corresponding dependence of RPP on shock CI. Both of these lines show a parallel and nearly equal rise across a range of shock CIs that spanned all phases of the cardiac excitability cycle.

The scattergram plot in Figure 6C summarizes the results of ADT and RPP measurements performed at 14 sites on five hearts using 11–13 different shock CIs. DFT shocks were used throughout except in one series, which used shocks of 1.2 times the DFT. The dashed line has a slope of 1 and is used to show an ideal relation in which the RPP exactly corresponds to the additional depolarization induced by the shock. The 171 experimental data points cluster around this ideal relation, and a least-squares linear regression analysis performed on the data yields a slope of 1.02 and a coefficient of determination of 0.98.

Possible Mechanisms for the Optical Manifestation of Prolonged Depolarization in Response to Shocks

Experiments were performed to determine whether the additional depolarization induced by DFT-strength shocks was related to the optical methods used in this study. The optical signal registers the aggregate electrical activity of the cells within the recording volume (see "Materials and Methods"), so the electrical activity in separate populations of cells would be indistinguishable on the optical recording. When this is taken into consideration, it is possible that some cells within the recording volume might have been excited during pacing while other cells might have remained quiescent during normal pacing because of conduction block. Then, the delivery of a shock during the plateau of the paced action potential would stimulate these quiescent cells. Their response would be superimposed on the action potential from the paced cells and thus show a long-lasting depolarization that would be interpreted as additional depolarization of all the cells. A second possibility is that there might be a population of cells that, because of depressed excitability, produced action potentials only when stimulated by shocks of DFT strength and not during pacing. If such cells existed, then the shock might excite these depressed cells, and their optical action potential would be added to the optical signal from the population of cells that responded during pacing and thus give the appearance of additional depolarization of all of the cells in the recording volume.

The first of the above possibilities was tested and discounted by a protocol that applied a 2 msec rectangular waveform shock through the defibrillation electrodes instead of the normal pacing stimulus to stimulate the action potential. Figure 7 shows recordings obtained from one of these experiments. The strength of the stimulation shock (SI in Figure 7) was set to the lowest setting needed to directly stimulate the optical recording site. This stimulating shock caused the upstroke to be slightly faster and larger than the upstroke of the control action potential. Under these conditions, the SI shock excited all of the cells in the recording volume (see Figure 4). A DFT-strength test shock (S2 in Figure 7) was applied with various coupling intervals to the stimulus shock starting early in the plateau (tracing a) and ending at near complete repolarization (tracing g). Tracings a–e have the repolarization phase of a control, shock-stimulated action potential (dashed line indicated by arrow head in Figure 7) superimposed on the shocked action potentials. Whereas tracing a shows no additional depolarization, tracings b–e show an increase in the duration of the depolarized state with a shock CI similar to that seen in Figure 6. This protocol was repeated at 13 sites on five hearts, and in all cases the DFT-strength shock (S2) produced an additional period of depolarization on the optically recorded action potential.

The tracings shown in Figure 8 test the second possibility discussed above, that of a population of cells having depressed excitability. Tracings a–g were obtained from experiments that used normal shocks of DFT strength (SI in Figure 8) to stimulate action potentials during the paced rhythm. A second shock (S2 in Figure 8), having the same duration and tilt as
S1 but 90–95% of the strength of the stimulating shock, was applied with various CIs to S1. The S2 shock was made slightly weaker than the S1 shock to exclude the possibility of that shock stimulating cells that might have a higher threshold than those stimulated by the S1 shock. Control repolarization phases (dashed lines indicated by arrowheads) were superimposed on the shocked action potentials of tracings a–e. It can be seen that S2 was able to prolong the duration of the depolarized state in tracings a–e in the same manner as shown by the tracings in Figure 6. (Tracing d in Figure 8 shows a spontaneous extrasystole arising after repolarization of the shocked action potential. Such shock-induced extrasystoles have been observed by others and they do not necessarily indicate cellular membrane damage, since they are most easily produced by sub-DFT–strength shocks.)

**Figure 7.** Optical recordings showing test action potentials stimulated by low-strength shocks. The optical recordings in tracings a–g show pairs of action potentials that were stimulated by the basic pacing stimulus (left side) and a low-strength shock applied through the defibrillation electrodes (right side). The time and duration of the low-strength shocks are indicated by the black bars labeled S1. The strength of S1 was set to the minimum needed to directly stimulate the optical recording site. A shock of defibrillation threshold–strength (time and duration marked by bar labeled S2) was applied at various coupling intervals with respect to the upstroke of the second action potential. The repolarization phase of a control, shock-stimulated action potential is the dashed line (arrowhead) superimposed on the second action potential of tracings a–e. The coupling intervals of the shocks were 6, 30, 49, 71, 91, 111, and 131 msec. The vertical calibration bars give the size of a 1% change in background fluorescence. The defibrillation threshold was 1.25 J.

**Figure 8.** Optical recordings showing test action potentials stimulated by defibrillation threshold–strength shocks. The optical recordings in tracings a–g show pairs of action potentials that were stimulated by the basic pacing stimulus (left side) and by a defibrillation threshold shock (right side). The time and duration of the defibrillation threshold–strength shocks are indicated by the black bars labeled S1. A shock of 0.95 times defibrillation threshold was applied at various coupling intervals to the upstroke of the second action potential. The time and duration of the second shock is marked by bar labeled S2. The repolarization phase of a control, shock-stimulated action potential is the dashed line (arrowhead) superimposed on the second action potential of tracings a–e. In tracing d, a spontaneous extrasystole (ES) occurs after repolarization of the shocked action potential. The coupling intervals of the shocks were 21, 43, 62, 83, 103, 122, and 143 msec. The vertical calibration bars give the size of a 1% change in background fluorescence. The defibrillation threshold was 1.0 J.
These tracings demonstrate that the shock-induced prolongation of depolarization was not due to a population of cells within the optical recording volume that had depressed excitability. These same results were obtained when this protocol was repeated at 17 sites on three hearts.

The possibility that either the voltage-sensitive dye itself or the use of D600 was responsible for production of additional depolarization by the shock was also examined. Figure 9 shows data from a heart in which undistorted optical action potentials were obtained without the use of D600 during a paced rhythm. These measurements were then repeated at the same site after the administration of 2 μM D600. Figure 9A compares plots of ADT versus shock CI made while perfusing with normal Tyrode’s solution and with solution containing D600. Both plots show an increase in ADT with shock CI, consistent with the data in Figures 5–8, but because of an as yet unknown mechanism, they are not identical. In both sets of tracings shown in Figure 9B, a single, unshocked action potential is superimposed on the responses to shocks delivered during the action potential. It is seen that shocks in the presence of D600 gave rise to larger, longer-lasting depolarizing responses. Treatment with D600 has some direct electrophysiological consequences and, undoubtedly, secondary effects that caused these differences in the shock responses, but these effects alone are not responsible for the additional depolarization elicited by the shock. The results shown in Figure 9 were found at all seven sites on the two hearts studied without D600-containing Tyrode’s.

The data presented in Figure 6D rule out the possibility that the shock-induced prolongation of depolarization and the corresponding prolongation of the local refractory period was caused by the membrane potential–sensitive dye WW781. The effective refractory period (see “Materials and Methods”) in the presence and absence of a shock was determined in three hearts not treated by D600 and WW781. The stimulation site used for these effective refractory period determinations was on the right ventricle, and DFT-strength shocks were applied at 13 different CIs. The increase in the effective refractory period after a shock with respect to a control action potential was called effective refractory period prolongation. Figure 6D plots the effective refractory period prolongation against the shock CI for one of these experiments and shows that effective refractory period prolongation increased with shock CI in the same manner as the prolongation of the refractory period demonstrated in Figure 6B. This result was found in all of the three hearts examined and shows that shock-induced prolongation of depolarization was not due to the use of WW781.

**Discussion**

**Optical Recordings for the Study of Defibrillation**

To explain the events responsible for defibrillation caused by an electrical shock, it is necessary to record electrical activity from the heart at the time of the shock. However, currents flowing through the myocardium during a defibrillation shock create large voltage artifacts on electrical recordings that prevent observation of electrophysiological events occurring during the shock. These artifacts arise both from voltage gradients directly created within the heart by the shock current and from lingering electrode polarization. Sometimes these problems can be minimized, such as was done in a microelectrode study of the responses of myocardial cell cultures to externally applied electrical fields. In that study a nonpolarizable reference electrode was positioned so that it differentially canceled the voltage gradient imposed by the shock. This cancellation was possible because of the uniform geometry of the external shock field and myocardial preparation, a situation that does not exist in the intact heart. Where it has not been possible to null out the voltage contribution of the shock field, investigators have electronically disconnected the microelectrode amplifier during the flow.
of shock current. Amplifier switching has also been used while recording electrograms from in situ ventricles during cardioversion and defibrillation shocks. The amplifiers were isolated from the heart during the shock, and to minimize the electrode polarization artifact, the high-pass filter was switched to a higher cutoff frequency. Electrogram recordings resumed within 5 msec after the end of a low-energy shock used for cardioversion, but recovery after higher-energy defibrillation shocks required at least 20 msec. Sintered Ag/AgCl electrodes have also been combined with robust amplifier circuitry to eliminate the need to disconnect the amplifiers during the shock or to high-pass filter the electrogram signals. These electrodes dissipated the shock polarization artifact so quickly that there was only about a 20-msec lapse in the electrogram recording during a defibrillating episode. To date, however, there has been no practical way to remove shock voltage artifacts from electrical recordings of the membrane action potential or from electrograms; therefore, the events occurring during the shock have not been described in detail.

Defibrillation shocks exert their electrophysiological effects through their influence on the membrane potential. However, the linkage between externally applied shock current and membrane potential cannot be easily deduced. The shock current passes between electrodes in a path through the intervening tissue of the heart. At the electrode surfaces, this current passes into the fluid film wetting the heart. The current is not constrained to the extracellular conducting fluid spaces but passes into its intercellular conductive pathways as well. Current from extracellular electrodes, such as a defibrillation shock, enters and eventually leaves the intracellular conductive pathway by passing through the myocardial cell membranes. It is this transmembrane current flow that causes the changes in membrane potential that, in turn, evoke electrophysiological responses from the myocardium. Thus, the passage of shock current causes voltage gradients in the extracellular and intercellular conductive domains and changes in myocardial membrane voltage. This latter voltage change is the driving force behind the electrophysiological response of the myocardial cell. An electrical recording method, such as the microelectrode, responds to both the membrane voltage and extracellular and intercellular voltages. Optical recordings, however, using voltage-sensitive dyes respond only to transmembrane voltage (see "Materials and Methods"). This specificity was demonstrated in studies where a voltage-sensitive dye was used to map the changes in membrane potential induced in a single cell exposed to external electric fields whose strength, in one report, exceeded 40 V/cm. It is for this reason that optical recording was used in this present study, since the actual membrane responses of the myocardium to defibrillating shocks could be studied without interference from the artificial voltage gradients produced by the flow of shock current through the extracellular and intracellular spaces.

**Optical Recordings Accurately Portray Prolongation of the Depolarized State Produced by Shocks**

This study has shown that DFT-strength shocks applied during the action potential, at a time when the cell is expected to be refractory to less intense stimuli, evoked a depolarizing response that extended the period of membrane depolarization. The prolongation of the optically recorded depolarization state was accompanied by an equal prolongation of the local refractory period (see Figure 6), demonstrating that the optical recordings were accurately registering the electrical behavior of the myocardial cells and not some shock-related artifact. Results shown in Figures 7 and 8 ruled out the possibility of a heterogeneous population of abnormal cells within the optical recording volume, giving rise to spurious responses. Figures 6D and 9 likewise eliminated the use of D600 or the voltage-sensitive dye as possible sources of experimental artifact. The optical recordings also show that shock-induced depolarization was not due to prolonged depolarization of the myocardial cell such as found by others after very high-energy shocks. In those studies, the normal action potential and excitability of the myocardial cells was disrupted by the large electric field of the shock, resulting in maintained cellular depolarization and refractoriness. This did not occur in the present study, since, as shown in Figures 5 and 7–9, DFT-strength shocks evoked normal action potentials at later coupling intervals. An indication of the normal myocardial behavior seen in our study was given in Figure 6, which showed excitability recovered in concert with the optically registered repolarization of the action potential after shocks of defibrillation strength.

**Possible Mechanism for the Additional Depolarization Induced by the Shock During the Action Potential**

It has been traditionally considered, as pointed out by Plonsey and Barr, that a shock injects a depolarizing current into the cells of the ventricle. Because the optical recordings in the present study show depolarizing responses to the shocks, they would seem to confirm this belief. There are two difficulties with this concept. The first is that injection of brief, depolarizing current pulses during the plateau has been found to have little effect on action potential duration in ventricular muscle. The second point is that, as seen in Figures 5A, 7, and 8, the height of the depolarizing responses varied in amplitude with the shock CI even though the shock strength was constant. Injection of constant depolarizing current pulses should have produced optical responses of nearly constant height such as those shown by Cranefield and Hoffman (see their Figure 3).

To account for the results shown in this study, it is proposed that the shock excites a new action potential in the same cell during an ongoing action potential. This hypothesis is suggested by the optical
tracings shown in Figures 5 and 7–9, which convey the impression that the shock caused a premature action potential whose upstroke phase started at a depolarized level instead of from resting potential. This impression is strengthened by examination of tracings in Figure 5A, where, as the shock CI was increased, the depolarizing response evoked by the shock in tracing a was smoothly transformed into a shock-stimulated action potential in tracing g. The peaks of the prompt shock responses attained nearly the same height over a range of takeoff potentials during the plateau as they might if the prompt shock response was an action potential upstroke. Another clue is given by the shape of the curves showing the dependence of ADT on shock CI in Figure 5. The curves do not show any discontinuities or inflections even though the CIs spanned all phases of cardiac excitability. This is consistent with a single mechanism underlying the response of the myocardial cell to defibrillation shocks applied both early and late in repolarization. This hypothesis explains the monotonic increase in ADT with shock CI because the duration of the new action potential is added to the time already spent in the depolarized state before the shock.

Figure 10 illustrates how it should be possible to stimulate an action potential from cellular membranes whose inward sodium channels have been inactivated by the depolarization from the ongoing action potential.31 It shows shock current passing from left to right through a cross section of a myocardial cell. A component of this shock current remains in the extracellular spaces and passes around the myocardial cell. The remaining component passes through the cell, entering it on the left side, and leaving it through the right side by passing through conductive ionic channels in its membrane. This inward flow of current (arrows on left side of the cell) causes these portions of the cellular membrane to become hyperpolarized (H in Figure 10) as indicated by the plus and minus symbols. This current, having entered the cell, now leaves it. This outward flow of current (arrows on right side of cell) causes a depolarization of that portion of the cellular membrane (D in Figure 10). This general pattern of bipolar membrane hyperpolarization and depolarization in the same cell has been theoretically predicted and experimentally verified (using optical recording) by others in an isolated, nonmyocardial cell.40,41 The expected electrophysiological response to a shock applied while a myocardial cell is already depolarized is illustrated by two hypothetical action potentials drawn in Figure 10. On the right side (D), the shock (black bar S) causes a rapid depolarization of the membrane from the plateau to higher potentials. On the left side of the cell (H), the shock (black bar S) rapidly hyperpolarizes the membrane downward from the plateau potential. In both hypothetical action potentials (D and H), the normal time course of repolarization is shown by the dashed curves indicated by the arrowheads. The sodium channels in these hyperpolarized portions of the cell membrane are now able to recover excitability in accordance with the kinetics of the voltage-sensitive inactivation gating mechanism of the sodium channel.44 Having regained excitability, these sodium channels become able to undergo activation again. Shortly after the end of the shock, or perhaps during it, depolarizing current spreads from the right side of the cell and initiates a new action potential on the left side of the cell in the diagram. While the shock-induced depolarization produced on the right side of the cell rapidly decays, the new action potential initiated on the left side is able to maintain the entire cell at a depolarized level as it slowly repolarizes. The bipolar changes in membrane voltage of individual cells produced by the shock would be averaged out, and the optical recording would show the upstroke of the new action potential arising from the plateau. The optical recording would remain depolarized throughout the duration of this new action potential and so prolong the time that the optical recording stays depolarized just as the data in “Results” portray.

Significance of Shock-Induced Depolarization for Defibrillation

If the effects of shocks applied during steady pacing shown in the current study are present in a fibrillating ventricle, then the present theories of defibrillation may need to be amended. It has been traditionally
considered that defibrillation shocks do not affect myocardium already depolarized to plateau level potentials but, rather, exert their defibrillatory effects by stimulating excitable portions of the myocardium, thus causing them to be refractory.4,8,45,46 Because the fibrillating ventricle undergoes rapid, unsynchronized activation,11,47 it is likely that a large fraction of it will be in a depolarized state at any moment. Therefore, a strong shock might cause additional depolarization in a large fraction of the ventricle’s myocardial cells. Such a response could be considered beneficial, because, by prolonging the refractoriness of cells already depolarized, it would hinder the continued propagation of impulses surviving after a defibrillation shock. Although it was long recognized that excitable cells could be made transiently refractory by stimulation,4,8,45,46 additional depolarization of already activated myocardium would also make the rest of the ventricle similarly refractory.

If the hypothesized mechanism of action potential reexcitation illustrated in Figure 10 is correct, then a DFT-strength shock could be viewed as having a single effect, that of action potential stimulation in depolarized and repolarized myocardium alike. Occurring during fibrillation, this would lead to a resynchronization of the electrical state, which would in turn act against continued fibrillation even more vigorously than if the shock was only able to stimulate excitable myocardium. This is because, by only stimulating the excitable cells in fibrillation, the shock would leave the rest of the cells to recover excitability in the order of their excitation sequence, which, owing to the disorganization of impulse spread in fibrillation,48–52 would create a large dispersion in refractoriness. On the other hand, if shock-induced depolarization occurs in myocardium in all action potential states, then it would result in near synchronous repolarization after the shock. This could be a potent antiarrhythmic action, because it should decrease the probability that impulses still present in the ventricle after the shock would undergo reentry. Recent preliminary results not only show that DFT shocks produce additional depolarization during fibrillation but that they cause synchronization of repolarization as well.53

Note added in proof. Since this paper was submitted, a report has appeared describing refractory period prolongation in canines by defibrillation threshold–strength shocks.54

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References

38. Weidmann S: Electrical constants of trabecular muscle from mammalian heart. J Physiol (Lond) 1970;210:1041–1054
45. Crampton R: Accepted, controversial, and speculative aspects of ventricular defibrillation. Prog Cardiovasc Dis 1980;23:167–186
47. Wiggers CJ: The mechanism and nature of ventricular fibrillation. Am Heart J 1940;20:399–412

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