The U wave is the only waveform in the electrocardiogram whose genesis still remains controversial. Since the original description of this wave by Einthoven, several theories have been proposed to explain the physiological mechanisms responsible for its formation. In a recent article, Lepeschkin has extensively reviewed the relative strengths and shortcomings of these theories. Of the two major hypotheses advanced to date: (1) the development of negative afterpotentials in the ventricular myocardium, and (2) repolarization of the Purkinje system, Lepeschkin apparently preferred the first, the negative afterpotential theory. This hypothesis was also favored by several members of an earlier panel discussion held in 1957.

Repolarization of the intraventricular conducting system as the mechanism for the U wave was first proposed by Hoffman and Cranefield. Their theory was based mainly on the electrophysiological characteristics of Purkinje fibers. Previous clinical observations of ventricular parasystole have led us to a similar conclusion. Subsequent studies in our laboratory on the electrocardiographic correlation of bundle branch block and the appearance of the U wave lent further support to this hypothesis. In order to test this hypothesis, experiments were carried out on isolated canine Purkinje fiber-ventricular muscle preparation perfused in a tissue bath, where transmembrane potentials were simultaneously recorded from the Purkinje and ventricular fibers and the effects of several factors clinically known to cause prominent U waves were studied.

Materials and Methods

Comparison of the Action Potential Characteristics of Purkinje and Ventricular Muscle Fibers under Conditions Accentuating the U Wave

Experiments were carried out on canine Purkinje fiber-ventricular muscle preparations perfused in a tissue bath. Adult mongrel dogs of either sex were anesthetized and heparinized by intravenous injection of pentobarbital sodium (30-35 mg/kg body weight) and heparin. The thorax was opened and the heart was quickly removed and transferred into a cool oxygenated perfusate. Several small pieces of right ventricular muscle with attached false tendons were then dissected and pinned to the paraffin block in a lucite perfusion chamber having a capacity of approximately 70 ml. The perfusate was a modified...
Chenoweth's solution with the following composition in mM/L: NaCl, 119.8; KCl, 4.5; CaCl₂, 2.4; MgCl₂, 2.1; NaHCO₃, 25.0; and dextrose, 10.0. The solution was saturated with 95% O₂ + 5% CO₂ both in the reservoir and the chamber, and the rate of exchange was approximately once every two minutes. The bath temperature was maintained at 37 ± 1°C.

The preparation was electrically driven through a small bipolar electrode attached to the ventricular muscle. The stimuli generated by a laboratory stimulator (American Electronics Laboratory) were in the form of square pulses of 2 msec duration, twice the threshold intensity, delivered through a stimulus isolation unit.

Glass microelectrodes filled with 3M KCl and having tip resistances of 10-30 megohms were used to simultaneously record transmembrane potentials from the Purkinje and ventricular muscle fibers. Transmembrane voltages were amplified with a neutralized input capacity amplifier and Tektronix amplifiers, and displayed on two Tektronix oscilloscopes (type 532). Tracings were photographed from one of the oscilloscopes using a Grass camera, usually at a paper speed of 50 mm per second.

The effects of varying frequencies of stimulation were studied by changing the rate of driving by increments of 30 beats per minute, usually ranging from 30 to 180 per minute. Whenever the stimulating frequency was altered, a minimum of 90 seconds was allowed before transmembrane records were obtained at a new rate, as it has been shown that action potential characteristics stabilize within 60 seconds after changes in stimulating frequency.

After series of control records were obtained and the action potential characteristics were considered stable, one or more of the factors clinically known to accentuate the U wave were tested. These conditions include (1) hypothermia, (2) low extracellular potassium concentration, and (3) quinidine. Hypothermia was produced by resetting the thermostat for the bath at 32°C. For lowering the extracellular potassium, the reservoir was switched to a new one containing a perfusate with 2.1 mM potassium instead of 4.5 mM. The effects of quinidine were tested by adding quinidine gluconate into the reservoir to make a concentration of 5 mg per liter. In all these studies, a period of 30 minutes was usually allowed before recordings were resumed. Each of these test periods was followed by a return to the control perfusing conditions in order to assure reversibility of the observed changes.

In all the above experiments, the action potential duration was measured at 100% repolarization.

Correlation of Repolarization Process in Purkinje and Ventricular Muscle Fibers with the U Wave in the Electrocardiogram Recorded in Intact Dogs

In two experiments, hypothermia was produced in anesthetized dogs by the use of extracorporeal circulation. After control electrocardiograms were recorded, the femoral artery and vein were exposed through an incision, canulated, and connected to a heat exchanger. The rectal temperature was lowered to approximately 32°C, usually within 90-120 minutes. Conventional 12-lead electrocardiograms were repeatedly recorded with a Sanborn electrocardiograph machine, at a paper speed of 50 mm per second. When the development of prominent U waves was noted, the body temperature was recorded and hypothermia discontinued. Then the animal was sacrificed and Purkinje fiber-ventricular muscle preparations were obtained for tissue bath studies as in the first series of experiments.

Two additional dogs were used for the study of low extracellular potassium concentrations. In these experiments, animals were dialyzed with the use of a two-layer Kill hemodialysis unit and a potassium-free dialysate bath, according to the methods previously reported by Seller et al. Before and after the start of dialysis, repeated electrocardiographic recordings were made to detect changes in the T-U waves. Dialysis was continued until prominent U waves developed, during which several blood samples were drawn for the determination of the potassium level. The animal was then sacrificed and Purkinje-ventricular muscle preparations dissected for later tissue bath studies.

In the tissue bath, transmembrane potentials were recorded simultaneously from the Purkinje and ventricular fibers as in the previous series. A range of stimulating frequencies was chosen to include the heart rates as observed in individual hypothermia or hypopotassemia experiments in intact animals. After satisfactory control records were obtained, either the bath temperature or the potassium concentration in the perfusate was lowered in a manner similar to that described earlier. In these cases, a particular temperature or potassium concentration as observed in individual experiments in the intact animal was chosen to reproduce the conditions under which the U waves were accentuated.

In these experiments, the ends of the T and the U waves were difficult to identify, and only the apices of these waves were used to measure the Q-T and Q-U intervals. Hence, the action potential duration in Purkinje and ventricular fibers was measured at 80% of repolarization, in contrast to 100% repolarization as used in the first part of the present study.

Results

The Effects of Stimulating Frequency

Figure 1 shows the comparison between the action potential duration of Purkinje and ventricular muscle fibers at various frequencies of stimulation. It is readily noted that the action potential duration is inversely related to the stimulating frequency in both fiber types, but the change is much greater in Purkinje fibers than in ventricular muscle. Thus, the difference between their action potential durations is increased at slower rates, attaining a value of 100 msec at the frequency of 30 beats/min compared with 40 msec at 180 beats/min.

In addition to the changes in duration, the action potential configuration also undergoes serial alterations with changing frequencies. Figure 2 illustrates such changes in Purkinje fibers. From these records, the duration and the slope of repolarization in both phase 2 and 3 have been determined in a manner shown in figure 3. The slope or the rate of repolarization was expressed in mV/sec. The results are shown in figures 4 and 5. Figure 4 reveals that the duration of both phase 2 and 3 is increased with the lowering of stimulating frequency. In addition, the rate of repolarization is decreased at lower frequencies, and the changes appear similar for both phase 2 and 3 (fig. 5).
The Effects of Low Potassium, Low Temperature, and Quinidine

The effects of these three factors are summarized in figures 6, 7, and 8. When potassium concentration in the perfusate was lowered, the difference between the action potential duration of the Purkinje and ventricular muscle fibers was markedly increased (fig. 6, open circle). This increase was much more pronounced at the stimulating frequency of 60 beats/min than at 120 beats/min. With lowering of the bath temperature to 32°C, similar marked increase in the difference between the action potential duration of Purkinje and ventricular muscle fibers was noted (fig. 6, x's). Again, such increase was more marked at lower frequencies of stimulation. In contrast, the addition of quinidine to the perfusing solution at the concentration of 5 mg per liter little affected the difference in action potential duration (fig. 6, open triangle).

The changes produced by these factors in the duration of individual phases of repolarization in Purkinje fibers are shown in figure 7. It is noted that the duration of phase 2 was greatly prolonged by hypothermia, particularly at the stimulating frequency of 60 beats/min, whereas low potassium had little effect and quinidine even shortened this phase. Thus, the effects of these factors on phase 2 are inconsistent. On the other hand, the duration of phase 3 repolarization was markedly increased by lowering of the potassium concentration, and tended to be prolonged also by low temperature and quinidine (fig. 7).

Figure 8 summarizes the effects of these three factors on the slope of phase 2 and 3 in Purkinje fibers. The slope of phase 2 repolarization was decreased with low temperature, unchanged with low potas-

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PURKINJE REPOLARIZATION AND THE U WAVE

Effects of stimulating frequency on the duration of phase 2 and phase 3 repolarization in Purkinje fibers in a representative experiment.

sium, and slightly increased with quinidine. In sharp contrast, the rate of repolarization during phase 3 was markedly decreased by all three factors. The degree of this decrease was similar at both slower (60/min) and faster (120/min) rates of stimulation.

In ventricular muscle fibers, detailed measurements of these variables were not made, since they were supposed to be related only to the T wave and not the U wave.

Time Relationships Between the Electrocardiographic U Wave and the Transmembrane Potentials

Figure 9 illustrates the correlation between the electrocardiograms and the membrane action potentials before and after lowering of potassium concentra-

tion in one of the two dogs. The top records are the lead II electrocardiograms obtained from the anesthetized, closed-chest animal during the control period (left) and after the serum potassium level was lowered to 2.1 mEq per liter with hemodialysis (right). The sinus rate remained stable at 200 beats per minute throughout the procedure. In the control record, the T wave was of low amplitude. What appears to be the second peak of a bifid T may well be a U wave. After hypokalemia was produced, the QRS amplitude was increased. The ST segment was depressed and a large diphasic T-U complex developed. A small notch seen on the upstroke of this T-U complex probably represents the summit of the T, whereas the following larger peak is most likely the U wave.

The bottom two panels show the transmembrane potentials recorded from the Purkinje-ventricular muscle preparation dissected from this same animal. Under the control perfusing condition and at a stimulating frequency of 200 beats per minute (left), the action potential duration (at 80% of repolarization) of the ventricular fiber measured 152 msec while that of the Purkinje fiber was 162 msec. When the potassium concentration of the perfusate was lowered from 4.5 to 2.1 mM, the action potential duration of the ventricular muscle was slightly shortened to 140 msec (right). In contrast, the repolarization process was markedly retarded in the Purkinje fiber, due particularly to a prolongation of its phase 3. Thus, the action potential duration of the Purkinje fiber measured 180 msec. It should be pointed out that if the Q-T interval of the electrocardiogram in the presence of hypokalemia is measured between the onset of the Q wave and the small notch on the upstroke of the T-U complex, it measures approximately 136 msec, a value

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*Figure 4*

Effects of stimulating frequency on the duration of phase 2 and phase 3 repolarization in Purkinje fibers in a representative experiment.

*Figure 5*

Rate of repolarization in phase 2 and phase 3 of the Purkinje action potential as plotted against the stimulating frequency in a representative experiment.

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Changes in the duration of phase 2 and phase 3 repolarisation in Purkinje fibers produced by low K, low temperature, and quinidine, at stimulating frequencies of 60 and 120 beats/min. Mean values from three hearts.

Figure 7

Changes in the rate of repolarisation in phase 2 and phase 3 of Purkinje action potential caused by low K, low temperature, and quinidine, at stimulating frequencies of 60 and 120 beats/min. Mean values from three hearts.

Figure 8

Discussion

Although many other possibilities have also been suggested to date, we feel that the three major theories explaining the U wave formation are: (1) negative afterpotential in the ventricular myocardium, (2) delayed repolarization of certain portions of the ventricles, and (3) repolarization of the Purkinje system. Ideally, the genesis of an electrocardiographic waveform could be, and should be established only when a close correlation between the given deflection and certain electrical events occurring in the heart is directly demonstrated in man or intact animals. In order to prove the negative afterpotential theory in the genesis of the U wave, for instance, one must show that production and abolition, at will, of such afterpotential in the ventricular myocardium always result in the appearance and disappearance, respectively, of the U wave in the electrocardiogram. Unfortunately, such direct evidence has never been presented for any of the several hypotheses, mainly because of the great technical difficulties involved. Hence, one must still rely upon indirect, supporting evidence, either clinical or experimental, to defend any proposed theory.

As one of those indirect approaches, correlation of certain electrophysiological events with the behavior of the U wave in various clinical settings has frequently been attempted. It is generally agreed that the conditions accentuating the U wave include, among others, hypokalemia, hypothermia, administration of quinidine, and bradycardia. Hence, in the present study, the effects of these factors on the action potential characteristics of Purkinje fibers and the ventricular muscle were investigated, in order to find evidence either for or against the Purkinje repolarization hypothesis.
The first series of the present study indicates that these four conditions tend to produce common electrophysiological alterations in canine Purkinje fiber-ventricular muscle preparations. For instance, the difference between the action potential duration of Purkinje and ventricular fibers was markedly increased by lower frequencies of stimulation, lowered extracellular potassium level, and hypothermia. Such increase resulted from a greater prolongation of the action potential duration in Purkinje compared with ventricular muscle fibers (figs. 1 and 6). Quinidine produced little change in this difference at the concentration studied. Regarding the mechanism of delay in Purkinje repolarization, alterations in phase 2, or plateau, apparently did not play a significant role, since low potassium, hypothermia, and quinidine showed contrasting effects on both the duration and the slope of this repolarization process (figs. 7 and 8). On the other hand, the duration of phase 3 was greatly prolonged by low potassium concentration and longer
cycle length, and slightly prolonged by hypothermia and quinidine (fig. 7).

However, the most consistent change in the action potential characteristics of the Purkinje fibers caused by these factors was seen in the rate of repolarization during phase 3. Indeed, this slope was markedly decreased by low potassium, low temperature, as well as quinidine (fig. 8). Reduction of the rate of phase 3 repolarization associated with slower heart rate is also evident in figure 5. Thus, longer and slower phase 3 in Purkinje action potentials appears always related to the development of prominent U waves under these several conditions. An increased disparity of repolarization process between Purkinje and ventricular muscle fibers may also play some role, except in the presence of quinidine.

Furthermore, in the second series of experiments, it was demonstrated that the timing of the electrocardiographic U wave recorded in intact animals rendered either hypokalemic or hypothermic closely corresponded to that of the prolonged phase 3 repolarization in Purkinje action potential studied in the tissue bath under similar perfusing conditions (figs. 9 and 10). On the other hand, the inscription of the T wave in the electrocardiogram seemed to coincide with the phase 3 repolarization of the ventricular muscle fibers under the control and the test conditions, as would easily be expected. These results appear to suggest Purkinje repolarization as the likely mechanism for the U wave formation, at least under the conditions studied.

In contrast, none of these several factors did produce significant negative afterpotentials in transmembrane records from the ventricular muscle fibers. Hence, the present study failed to support the afterpotential hypothesis.

Past experimental studies on the genesis of the U wave have often attempted to correlate the timing of this waveform with certain electrical events occurring in the ventricles. For instance, one of the major arguments for the negative afterpotential theory arises from the finding that the afterpotential, as recorded with suction electrodes, corresponds in time to the U wave in epicardial electrogram. Furthermore, it has been argued that the amplitude of such afterpotential is 5 to 25% of that of the ventricular action potential proper, and this value is similar to the usual U/T ratio. Lepeschkin also pointed out that the slope of the afterpotential closely resembles that of the terminal portion of the U wave. These observations were considered to lend strong support to this theory of U wave genesis.

However, as has been stressed earlier by Hoffman and Suckling, the polarity, contour, and amplitude of the T wave should depend upon various factors including spatial relationship between the recording electrode and the wave of recovery, rate of change of membrane potential, and local variations in the duration of active state. Similar considerations must apply also to the contour of the U wave. Thus, Lepeschkin has stated that the U wave results from potential difference between the ventricular muscle with larger negative afterpotential and that with smaller afterpotential, with the latter positive relative to the former. More specifically, Schaefer has pointed out in an earlier panel discussion that the usual distribution of the U waves could be explained by afterpotentials only when the base of the heart and the inner layers of the ventricles develop greater negative afterpotentials as compared with the apex and outer layers, respectively. The third theory of delayed repolarization in certain portions of the ventricles is vulnerable to the same criticism. In this regard, it may simply be stated that the theory advocated by Furbetta et al. invoking delayed repolarization of the papillary muscles appears to have little electrophysiological support from either recordings of monophasic potential or more recent transmembrane records. The possibility of delayed repolarization of ventricular myocardium other than the papillary muscles would also need further experimental studies.

Although it has been theorized that afterpotentials occur in stretched cardiac fibers and the subendocardial tissue is subjected to a greater stretch than the subepicardial muscle, actual distribution of afterpotentials has not been demonstrated under any experimental conditions. Nevertheless, from the above discussion, it becomes apparent that the correlation between the contour of the action potential and that of electrocardiographic deflections is only indirect. Hence, neither should the similarity between the slope of the afterpotential and the terminal portion of the U wave be considered as evidence to support the afterpotential theory, nor should the dissimilarity of the slope of phase 3 in Purkinje action potential and the U wave be taken as evidence against the Purkinje repolarization theory.

On the other hand, it has been shown that the action potential duration is the longest in Purkinje fibers several millimeters proximal to the Purkinje-ventricular junction, while it shortens progressively from the terminal Purkinje system to the transitional fibers and then to the ventricular muscle. Furthermore, assuming the presence of a similar action potential duration in both apical and basal Purkinje fibers, the latter, being the last to be depolarized, might well repolarize later than the former. Then, under normal conditions, repolarization process in the peripheral intraventricular conducting system would produce a vector force directed from the subendocardium to the

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cavity and from the apex to the base. From these considerations, the Purkinje repolarization theory appears to more easily explain the usual polarity of the U wave than the other two theories, as we have previously pointed out.6 Prolongation of the Q-U interval with hypokalemia and hypothermia is also readily explained by the prolongation of the action potential duration in Purkinje fibers (figs. 9 and 10), although the present study does not provide information as to why slower phase 3 repolarization in Purkinje fibers could increase the amplitude of the U wave under these conditions.

A discussion seems appropriate on the frequently asked question of whether the mass of Purkinje tissue is sufficient to account for the U wave amplitude in the surface electrocardiogram.4 In this regard, one must first point out that, under usual circumstances, the U wave is indeed inconspicuous except in the midprecordial leads where the recording electrodes are closest to the ventricles.

Secondly, we have previously suggested that,6 if one takes into consideration those transitional fibers between the peripheral Purkinje and the working myocardial fibers which show intermediate action potential duration,11,12 there may well be enough mass of tissues. However, no data is available to calculate the precise percentage of this tissue mass in the ventricles. It may also be pointed out that the negative afterpotential theory may also be subject to the same criticism of inadequate mass, unless all the ventricular muscle fibers produce such afterpotential.

Furthermore, although mass might be a major determinant of the amplitude of electrographic deflections, there are other important factors which modify the latter, as mentioned earlier in this discussion. For instance, Hoffman and Cranefield have stated that the spread of activation in the Purkinje fiber network is highly directional, and hence, repolarization of this system may produce potential differences which are less subject to cancellation than are those of ventricular muscle.4 That the amplitudes of the repolarization wave could be markedly modified without any change in ventricular mass, as in the presence of hyper- and hypopotassiumemia, further illustrates the role of other variables.

In summary, the Purkinje repolarization hypothesis appears to most satisfactorily explain various characteristics of the U wave, although much still remains to be clarified on this electrocardiographic waveform.

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