THE TECHNIQUE of single fiber recording of transmembrane potentials by means of an intracellular microelectrode lead has been employed to study the effect of variations in heart rate and of induced extrasystoles on the electrical activity of the papillary muscle of the dog heart. The object of this investigation was to determine the nature of the alterations in membrane activity that are produced by variations in cycle length and also to demonstrate their relationship to the concomitant changes noted in the cardiac electrogram. Earlier studies of a similar nature (1, 2), in which the monophasic action potential was employed, have clearly demonstrated the value of this type of approach to the study of the genesis of the various deflections of the electrogram and electrocardiogram. However, the monophasic action potential only approximates the time course of depolarization and repolarization of the muscle membrane at any one point and admittedly introduces a variable degree of uncertainty in all interpretations derived from its configuration (3). Therefore, direct recording from single cells is advantageous.

In man and in the intact animal the best available index to the duration of electrical activity of the ventricle is the Q-T interval of the standard electrocardiogram. Obviously the Q-T interval does not indicate the actual duration of electrical activity in any particular area of ventricular muscle but depends for its duration partly upon the temporal dispersal of depolarization and repolarization in the heart. Thus this interval originates in the first depolarization manifest on the body surface and terminates in the latest repolarization which gives rise to changes in potential of the limbs or precordium. The disadvantages inherent in this technique are minimized by recording directly from the heart with contiguous punctate electrodes (differential recording); little information of this latter type is available. In spite of the inadequacies of the method, many attempts have been made to use the Q-T as an indication of changes in the duration of the refractory period of heart muscle (4, 5). For example, the duration of the Q-T interval has been employed in several studies to determine the effect of changes in heart rate upon the duration of electrical systole and various formulations expressing this relationship are available (6-8). In many cases results have been influenced by the operation of factors other than cycle length at extreme rates, such as variations in sympathetic activity and blood levels of adrenaline and nor-adrenaline. These obviously alter both conduction velocity and the rate of repolarization. A more recent study employing the direct measurement of excitability in the exposed dog heart (9) demonstrated that a linear relationship exists between the duration of the total refractory period and heart rate. The observation that the duration of the relative refractory period remained constant at all rates studied indicated that the time course of repolarization was not influenced by changes in heart rate. The observed changes in total refractoriness had resulted from an increase or decrease in the duration of the absolute refractory period alone.

This observation, suggesting that there are various processes involved in the recovery of excitability which have differential sensitivity to various conditions, warrants further examination. Therefore, the effect of a single variable, heart rate, on the repolarization
process has been studied. This was done by directly recording the membrane potentials of the isolated driven papillary muscle of the dog ventricle by means of an intracellular microelectrode. In addition, because of the similarity of the variable—cycle length—in both cases, the changes in the course of repolarization associated with induced extrasystoles have also been studied. The unipolar electrogram has been recorded simultaneously with the membrane action potentials to demonstrate graphically the relationship between these two records both under control conditions and during the induced alterations in the course of membrane activity.

METHODS

The papillary muscles employed were obtained from mongrel dogs anesthetized with pentobarbital sodium, 30 mg/kg. All tissue was removed from the heart within 1 minute following cessation of circulation and transferred immediately to a modified Tyrode’s solution maintained at 38°C and aerated with a mixture of 95% O₂ and 5% CO₂. The techniques employed have recently been described in detail (10). It suffices to say here that each preparation was driven to beat rhythmically at the desired rates by means of a pair of pin electrodes inserted into the cut end of the muscle; these same electrodes were employed to deliver the test shocks producing extrasystoles. An interval of 30-60 minutes was allowed for equilibration in the Tyrode’s solution and the disappearance of any injury potentials before recording was begun.

Transmembrane potentials were recorded in the conventional manner by means of a glass capillary microelectrode with a tip diameter of less than 1 µm filled with 3 M KCl and connected to the cathode follower by means of a chlorided silver spiral. Unipolar electrograms were recorded with direct coupled amplification from a fine silver electrode mounted in a micromanipulator. This electrode was carefully positioned under direct microscopic visualization so that it just failed to make contact with the epicardial surface of the papillary muscle and thus any tissue injury resulting from pressure was avoided. The level of the fluid was not changed during recording, the preparation remaining completely immersed, since it has been demonstrated (11) that lowering the fluid below the surface of the preparation distorts the recorded electrogram not only by varying the shunting effect of the bath but also by altering the rate of repolarization of surface fibers because of local cooling due to exposure and evaporation. A common indifferent electrode immersed in the bath served both the cathode follower and the d.c. amplifier. In all electrograms positive deflections are recorded above the baseline.

Mechanical activity was recorded with a miniature strain gauge attached to the tendinous end of the papillary muscle and contractions were virtually isometric.

RESULTS

Normal Records From the Papillary Muscle.

Even though the papillary muscle preparation has been employed for many years to study mechanical and electrical events in cardiac muscle, a statement concerning normal records seems indicated in view of certain discrepancies between our records and those of others (12, 13). The unipolar electrogram was employed because of the simplicity of interpretation of such records and because of their relative freedom from technical artifacts. Bipolar electrograms were thought unsuitable for these experiments because of the greater complexity of their derivation and because simple variations such as interelectrode distance can greatly modify the records obtained.

Such a unipolar electrogram recorded from the papillary muscle of the dog ventricle demonstrates quite nicely all the expected changes in the magnitude and direction of its
deflections as the position of the exploring electrode is varied with respect to the origin of the wave of excitation (13-15). These changes are indicated in the typical tracings reproduced in figure 1. When the exploring electrode is near the origin of activity, the major depolarization deflection is negative and the T wave is positive. When the electrode is at the center of the muscle, depolarization gives rise to a biphasic plus-minus deflection and repolarization a biphasic minus-plus T wave. Finally, records obtained from the end of the muscle most distant from the origin of the impulse demonstrate a predominantly positive 'R' and a negative 'T'. The expected slight displacement of the R-T segment is also noted in each case.

The membrane potentials of the papillary muscle are generally similar to those recorded from the intact, in situ dog ventricle (16). The usual configuration of the membrane action potential is seen in figure 1D. While there are slight variations between individual cells, the surface fibers of the muscle are homogeneous from end to end with respect to the over-all magnitude and shape of the membrane potentials. The relationship between the unipolar electrogram recorded from a given point and the membrane potential changes at the same location are shown in figure 1. The expected simultaneous occurrence of the intrinsic deflection of the electrogram and the upstroke of the action potential, and also the temporal relationship between the T wave of the electrogram and the repolarization limb of the action potential has been demonstrated previously (16, 17).

The temporal relationship between electrical and mechanical activity is seen in figure 1E. It is obvious that contraction of the papillary muscle is unlikely to produce any artifacts during the upstroke of the action potential or during the early part of the plateau; actually, movement artifacts are most common during the stage of rapid repolarization.

Conduction velocity in these preparations, calculated from the onset of depolarization at points near to and distant from the driving electrodes, averaged 1.2 m/sec. Interelectrode distance was measured with a calibrated ocular micrometer and a magnification of 20X; the onset of depolarization at each location was determined from the microelectrode recordings.

Effect of Changes in Heart Rate. Initiation of activity following quiescence. The term resting potential has been employed to describe the static membrane potential of cardiac muscle during diastole, even though this tissue normally demonstrates automaticity and thus has no true 'resting' potential. Furthermore, it has been stated on the basis of both excitability studies and monophasic recording that cardiac muscle does not attain a constant level of excitability or polarization for quite some time after each depolarization (frogs, 0.2-10 sec. (11); cats (18); turtles, 20-30 sec. (19). This suggests that the true resting potential might differ from the diastolic membrane potential usually recorded. On the other hand, excitability studies of the in situ dog ventricle (9, 20) have shown that a constant level of excitability is attained early in diastole and remains unchanged until the next depolarization occurs.
The results obtained by direct measurement of membrane potential of the dog papillary muscle both during quiescence and on resumption of rhythmic activity are seen in figure 2A–D. In the majority of fibers studied the resting membrane potential of any single fiber was identical before, during and after periods of activity preceded and followed by pauses of one to two minutes duration. The occasional record that demonstrated a drop in membrane potential on the initiation of regular activity showed other evidences that the intracellular electrode had been dislodged by movement. In these instances the membrane potential continued to decline and the action potential showed progressive deformation with each contraction.

As previously noted in many preparations (21, 22) the configuration of the electrogram T wave and the duration of the R-T interval change progressively with resumption of activity following a period of quiescence and require several to many seconds to stabilize. This progressive change in the course of repolarization is clearly demonstrated by the recorded membrane action potentials of the papillary muscle (fig. 2) and consists of a change in both the time and rate of repolarization. The typical course consists of first a long and then a short action potential followed by one slightly longer than the second and then another short one. This alternation continues with a progressive decrease in duration until stabilization finally occurs. The records in figure 2 clearly demonstrate the relationship between the time of repolarization of the membrane and the time of the electrogram T wave and also between the rate of repolarization and the amplitude of the T wave. The first action potential, showing a long plateau and rapid repolarization during phase three elicits a delayed, large T wave. The next action potential, demonstrating a shorter plateau and more gradual repolarization, gives rise to an earlier and more shallow T. This same relationship between the rate of change of the membrane potential during repolarization and the magnitude of the T wave is also seen in the studies of extrasystoles (fig. 6).

Changes in cycle length. The effect of variations in heart rate on the duration and magnitude of the membrane action potential was studied in the following manner. In one series, the microelectrode was inserted into a single fiber and then the papillary muscle was driven at a wide range of rates. Each increment or decrement in rate was small and after sufficient time for equilibration had elapsed, the action potential of the same single fiber was photographed. In another series a number of fibers was studied at each new rate in an attempt to evaluate the effect of individual variations on the results obtained. The duration of the action potential was measured from a projection of the record on an enlarged time scale. Because of the very gradual slope of the termination of the repolarization phase and because of minor variations in the magnitude of the action potential, the duration was measured from the onset of rapid depolariza-
In all fibers, as rate was progressively increased, sooner or later a regular alternation in the shape of the membrane action potential appeared (fig. 3A, D, E). This consisted of a more gradual repolarization on alternate beats with shortening of the plateau and a decrease in the slope of the recovery limb or phase 3 of the action potential. This phenomenon is discussed below in conjunction with studies of extrasystoles.

The magnitude of the resting potential and overshoot, or active membrane reversal, are relatively insensitive to changes in cycle length, as seen in figure 5C and D. It is only at very rapid rates that a small decrease in either measurement is encountered. It should be noted that this decrease in resting potential does not result from a cycle length so short that depolarization occurred before the repolarization from a previous action potential had been completed. Similar results have been mentioned by others (23).

As expected, the only significant change in the configuration of the electrogram produced by marked variations in heart rate was in the duration of the R-T interval. Rapid rates did not alter the polarity of the T wave in any of the papillary muscles studied. A representative series of records is reproduced in figure 3C and D.

**Extrasystoles.** Extrasystoles were produced at various intervals following the completion of repolarization of the previous driven beat by means of the same electrodes and employing the same intensity of stimulus as for driven beats. Usually the extrasystole was introduced at intervals of 7-10 normal cycles. The course of repolarization and the duration of the extrasystole occurring during electrical diastole was only slightly different from that of the driven beat—the plateau was usually slightly prolonged and the slope of the final phase of recovery increased. The conduction velocity

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**Fig. 4.** A, superimposed tracings of records from fiber shown in fig. 3A. Time scale on zero potential line (0) in 50-msec. intervals. Minus 100 mv calibration shown. B, superimposed tracings of records from single fiber shown in fig. 3B. Drive stimulus artifact (a) labeled.

In figure 3A and B the action potentials of two single fibers at rates ranging from 70-285 beats/min. are reproduced. Similar single fiber records have been traced and superimposed in figure 4A and B to facilitate comparison of the action potentials for each cycle length. As seen, alterations in heart rate produce significant changes only in the duration of the plateau, or phase 2 of repolarization. As cycle length decreases the plateau becomes progressively shorter and while the recovery of resting potential starts earlier, it proceeds to completion at essentially the same velocity in each case. Only at very rapid rates is a minor increase in the slope of the repolarization limb of the action potential noted.

The relationship between heart rate and the duration of the action potential was linear between 60 and 300 beats/min. in all fibers and at still faster rates in some. This relationship was the same for both single fiber records (fig. 5A) and for tracings obtained from many fibers (5B). These results are nearly identical to those obtained from an earlier investigation (9) wherein the effect of cycle length on the duration of the total refractory period of the in situ dog ventricle revealed a linear relationship for a similar range of rates. In any fiber, at some rate in excess of 300, each action potential commences before recovery from the preceding depolarization has been completed and thus the effect of heart rate on action potential duration is complicated by the influence of prematurity of each beat and a linear relationship no longer maintains. As noted previously (11) at extremely slow rates the expected prolongation of the action potential fails to occur and a constant duration persists in spite of further increases in cycle length (fig. 5B).
of the extrasystole was the same as that of the driven beats. However, the action potential of the subsequent driven beat was altered more markedly, repolarization commencing earlier than normal and progressing more gradually to completion (fig. 6). Again, conduction velocity was unchanged. These changes in the course of repolarization of cell membrane demonstrate quite clearly the nature of the alterations produced in the R-T interval and T wave of the simultaneously recorded unipolar electrogram (fig. 6). The possibility that the stimulating current employed to produce the extrasystoles might have directly influenced the course of subsequent repolarizations was ruled out by two observations: First, the same stimulus introduced during the end of the absolute refractory period of a driven beat (when no extrasystole resulted) brought about no change in the magnitude or shape of the subsequent action potential or electrogram. Secondly, in preparations demonstrating spontaneous rhythmicity and driven at a rate only slightly faster than the intrinsic rate, extrasystoles occasionally resulted from the pacemaker activity of the muscle and demonstrated changes identical to those seen in the electrically induced extrasystoles.

The records seen in figure 6 demonstrate again the expected relationship between the time of initiation and rate of recovery of the action potential and the time of occurrence and magnitude of the electrogram T wave. The similarity between the changes in subsequent action potentials produced by extrasystoles and the alternation in the shape of the action potential that occurs at rapid rates suggests that in both cases similar factors are operative.

**DISCUSSION**

While there are differences of opinion concerning the exact technique that should be employed to relate the surface electrogram to the potential variations of the heart muscle membrane (11), it is generally accepted that the initial deflection, or R wave of the surface record results from depolarization and the terminal deflection, or T wave, from repolarization. Furthermore, the dependence of the polarity of these deflections upon the spatial relationship between the recording electrode and the wave of excitation and recovery, the rate of change of membrane potential during activity, the velocity of propagation of the wave of activity, and the duration of the active state has been demonstrated in both theoretical and experimental treatments (11, 14, 15). Recently, however, it has been postulated that the configuration of the bipolar electrogram recorded from the papillary muscle (12) and the T wave inversion...
produced by anoxia and other variables (13) indicate that the T wave of cardiac muscle is produced by either afterpotentials or oxidative recovery processes rather than by the potential difference that results from repolarization of the cell membrane. The present work shows that in the papillary muscle preparation under constant conditions the polarity of the T wave of the unipolar electrogram may be positive, biphasic or negative and is primarily dependent upon the spatial relationship that exists between the wave of excitation and the exploring electrode. This contrasts with the conditions which are present in the intact heart, wherein local variations in the duration of the active state contribute to the polarity of the T deflection (1, 11).

Furthermore, the T wave of the electrogram coincides in time with the repolarization of the muscle membrane recorded by the intracellular microelectrode. Moreover, the T wave is present in records obtained from tissues that do not exhibit afterpotentials in the membrane action potential. Finally, the electrogram T wave is seen to vary directly in timing with the time of recovery of the membrane and directly in magnitude with the rate of repolarization of the membrane. These results indicate strongly that the origin of the T wave is indeed the repolarization of the muscle membrane and that both slight and major variations in the time course of this repolarization are represented in appropriate changes in timing and magnitude of the T wave.

The simple relationship obtained between heart rate and the duration of the action potential agrees with the results of previous studies employing different preparations (9) and demonstrates clearly that alterations in the duration of the action potential are primarily the result of either an earlier or later onset of repolarization. The duration of the plateau, therefore, is most directly related to the length of the preceding diastolic interval. When cycle length is progressively shortened the plateau similarly becomes shorter until, at high rates, an inadequate diastolic interval is accompanied by a partial loss of the plateau from alternate beats. Similarly, an interpolated extrasystole produces a partial loss of the plateau and an altered course of repolarization of the subsequent action potential. In like manner, on the sudden initiation of activity the first action potential shows a long plateau; the interval between this and the next depolarization is short with respect to the duration of this plateau and the second action potential of the series shows gradual repolarization and little true plateau. The third action potential of the series again manifests a plateau somewhat shorter than the first. This alternation, resembling that produced by high rates and interpolated extrasystoles, persists until the plateau duration is adjusted to the cycle length. Finally, at very slow rates the action potential duration tends to reach a constant value.

These results suggest the possibility that the length of diastolic interval available for resynthesis of some energy yielding compound
determines the duration of the subsequent plateau. If this were the case, it could be further postulated that repolarization of the muscle membrane is actively delayed and the available energy expended in maintaining the membrane potential near zero by preventing the net outflux of potassium during the major portion of the plateau. The demonstration of low membrane conductance during the plateau (24, 25) is congruent with this hypothesis, as is the observation that the net outflux of potassium from the perfused turtle heart does not commence until the termination of the R-T segment of the electrogram (26).

Postextrasystolic T wave changes resulting from induced extrasystoles have recently been attributed to a persistent and widespread effect of the stimulating current on the repolarization process of the ventricle (27). Our results are in agreement with the interpretation (1) that the effect of cycle length is most important in determining the form and amplitude of the postextrasystolic T wave of the normal heart. The action potential changes produced by both spontaneous and electrically induced extrasystoles obviously result from the prematurity of the beat and the resulting disturbance of cycle length. In addition, these alterations in the time course of the action potential are of such a nature as to adequately explain the T wave changes seen in the simultaneous unipolar electrogram.

SUMMARY

Simultaneous records of the membrane action potential and unipolar electrogram of the papillary muscle demonstrate the direct dependence of the timing, polarity and magnitude of the T deflection of the electrogram on the time course of repolarization of the muscle membrane. A linear relationship between heart rate and the duration of electrical activity of the ventricle is demonstrated by single fiber recordings. The effect of cycle length on the duration of the plateau of ventricular action potential suggests the existence of an active delay of repolarization in cardiac muscle.

REFERENCES