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ELECTROPHYSIOLOGY OF SINGLE CARDIAC CELLS*

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THE subject of cardiac electrophysiology is old, but much if not all of the experimental material on which this lecture is based has been obtained within the past nine years. The technical advance which made possible the study of electrical activity of single cardiac fibers was the development of the microelectrode by Ling and Gerard[1]. A microelectrode is a glass capillary drawn to a tip diameter of less than one micron at one end and filled with a concentrated solution of potassium chloride. The tip of such an electrode can be inserted through the membrane of the cardiac fiber without causing appreciable injury[2]. The electrolyte solution makes electrical contact with the interior of the fiber and the glass capillary acts as an electrical insulator. When such an electrode is paired with another extracellular electrode, it is possible to record the transmembrane potential from single fibers in all parts of the heart.

The potential difference across the membrane of a resting fiber is

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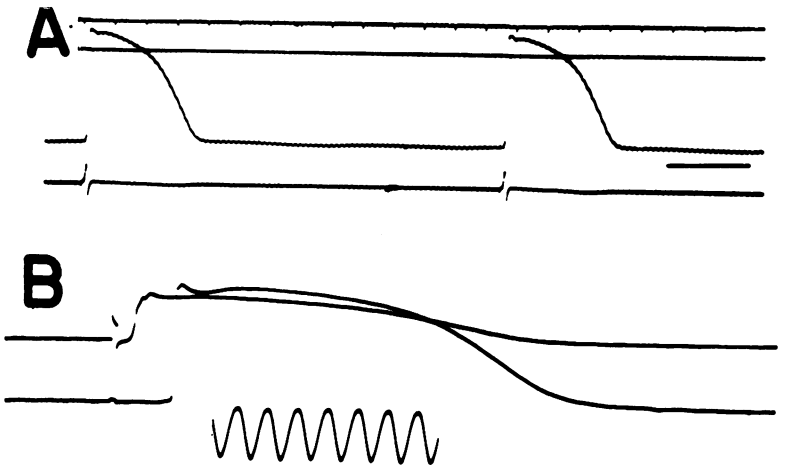

Figure 1—A. Isolated dog papillary muscle. Top trace shows time marks at intervals of 100 and 500 msec. Next trace is line of zero potential. Third trace from top shows transmembrane action potential recorded from a single fiber through an intracellular microelectrode paired with an indifferent electrode. Bottom trace shows electrogram recorded with a unipolar surface electrode adjacent to the microelectrode. B. Isolated cat papillary muscle. Simultaneous records obtained through a small suction electrode (upper trace) and an intracellular microelectrode (lower trace). Equal amplification for both traces. Stimulus artefact precedes the action potential. Sine wave represents 100 mv. and 60 c.p.s.

called the resting potential; that seen during normal activity is called the transmembrane action potential. The type of record obtained when a microelectrode is paired with an external electrode is shown in Figure 1A. When the microelectrode is just outside the membrane of a ventricular fiber, propagated electrical activity is recorded as a typical cardiac electrogram showing R and T deflections. After the electrode has penetrated the membrane, the record shows a steady potential difference, with the inside of the fiber negative to the outside. This potential difference amounts to about 90 mv. in most cardiac fibers. With the onset of electrical activity, the record of the transmembrane potential shows a rapid depolarization, which continues until the inside of the fiber is positive by about 20 to 30 mv. with respect to the outside. This phase of reversed membrane polarity is often referred to as the overshoot. The transmembrane potential then returns to zero and remains steady for 50 to 100 msec. This phase of the ventricular action potential is usually called the plateau, and is followed by a phase of fairly rapid repolarization which restores the normal resting potential. If the local electrogram is recorded at the same time and from the same

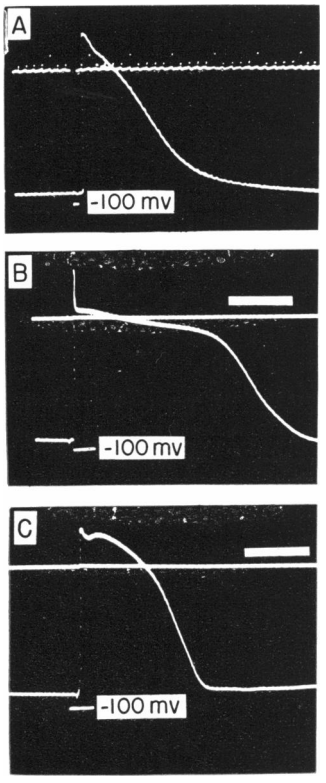

Figure 2—Transmembrane action potentials recorded from single fibers of dog heart. A—Atrium; B—Purkinje fiber; C—Ventricle. Time calibration in A on line of zero potential at intervals of 10 and 50 msec. Horizontal bar in B and C represents an interval of 100 msec.

location as the transmembrane action potential, it is seen that depolarization is simultaneous with the intrinsic deflection of the R complex and repolarization with the T deflection[3]. It is also apparent that the transmembrane action potential is strikingly similar in its voltage-time course to the more familiar monophasic action potential recorded between normal and injured muscle[4]. A comparison of records obtained through an intracellular microelectrode and a small suction electrode[5] is shown in Figure 1B.

The transmembrane action potentials recorded from single fibers of atrium, ventricle and Purkinje system are similar in magnitude but are quite distinctive with respect to shape and duration (Figure 2). In most mammalian hearts, the record of electrical activity obtained from a single atrial muscle fiber does not show a prominent plateau, but in

other respects it differs little from the ventricular transmembrane action potential[6] of the same heart. The transmembrane potentials recorded from single Purkinje fibers, on the other hand, have several distinguishing characteristics[2]. The resting potential is greater than that recorded from fibers of atrium or ventricle, the rate of depolarization is more rapid and the overshoot is larger. Moreover, at slow to moderate heart rates, the Purkinje fiber action potential is considerably longer than that of ventricular muscle; this is due largely to the duration of the plateau, but in part also to the final phase of repolarization. The difference in duration of ventricular and Purkinje fiber action potentials persists even at rapid heart rates or in the presence of ventricular extrasystoles, and thus limits the transmission of activity from ventricular muscle into the specialized conducting system[7].

A variety of studies has shown that, in single fibers of nerve and skeletal muscle, maintenance of the normal resting potential depends primarily on the concentration gradient of potassium across the fiber membrane and the selective permeability of the resting membrane to this ion[8]. If the resting potential of cardiac muscle is similarly created by a potassium concentration gradient across a membrane predominantly permeable to this ion, the approximate magnitude of the potential at 37° should be given by the relationship:

$$E = 61.5 \log_{10} \frac{[K_i]}{[K_o]}$$

where K_i and K_o are the concentrations of potassium inside and outside the fiber. Since the ratio of potassium concentrations in mammals is 30:1,

$$E = 61.5 \log_{10} \frac{30}{1} = 92 \text{ mv.}$$

Thus, the predicted and measured resting potentials are reasonably similar. Moreover, if the extracellular potassium concentration is increased, the resting potential falls as a linear function of the log of the extracellular concentration (Figure 3). When the extracellular potassium concentration is decreased below normal, the resting potential usually falls or remains unchanged (Figure 3). If permeability of the membrane to K^+ is increased, however, by addition of acetylcholine[9] or by calcium depletion[10], the resting potential in low potassium solutions comes considerably closer to the predicted value.

The upstroke of the action potential of nerve and vertebrate skeletal muscle has been shown to result from an increase in permeability of the

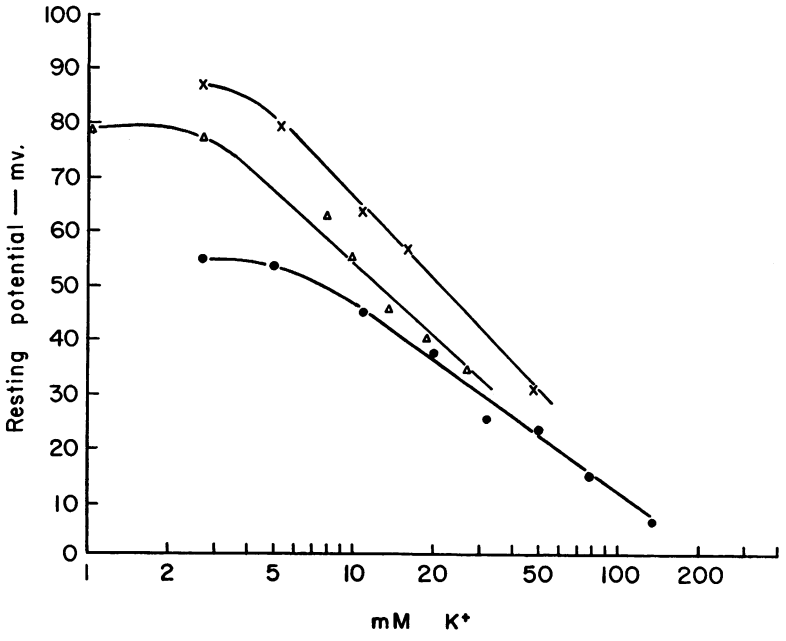

Figure 3—Effect of the concentration of extracellular potassium on the resting transmembrane potential of single fibers. Ordinate = resting potential in mv; abscissa = extracellular K+ concentration in mM/liter. o-o = cat atrium (redrawn from Burgen and Terroux, 1953). x-x = dog papillary muscle. Δ-Δ = rabbit atrium. Each point represents the average of three or more determinations. The extracellular Ca++ concentration in mM/1. for these experiments was as follows: o-o = 1.9; x-x = 2.7: Δ-Δ = 1.9.

membrane to Na^+ and a sudden influx of this ion[11]. The evidence for the cause of the action potential upstroke in cardiac muscle is less conclusive[12]. However, in Purkinje fibers Weidmann[13] has demonstrated a marked drop in the electrical resistance of the membrane during depolarization. Also, in Purkinje fibers and ventricular muscle, both the maximum rate of rise of the action potential and amplitude of the reversal are decreased when the extracellular sodium concentration is lowered[2, 14, 15]. Although there is some disagreement between theory and the effect of sodium depletion on tissues from various parts of different hearts[12], nevertheless the effect of a marked lowering of the extracellular sodium concentration on the transmembrane action potential of single cardiac fibers (Figure 4) strongly supports the concept that the change in transmembrane potential on stimulation is due to positive charge carried inward across the membrane by Na^+.

The mechanisms responsible for the subsequent voltage-time course

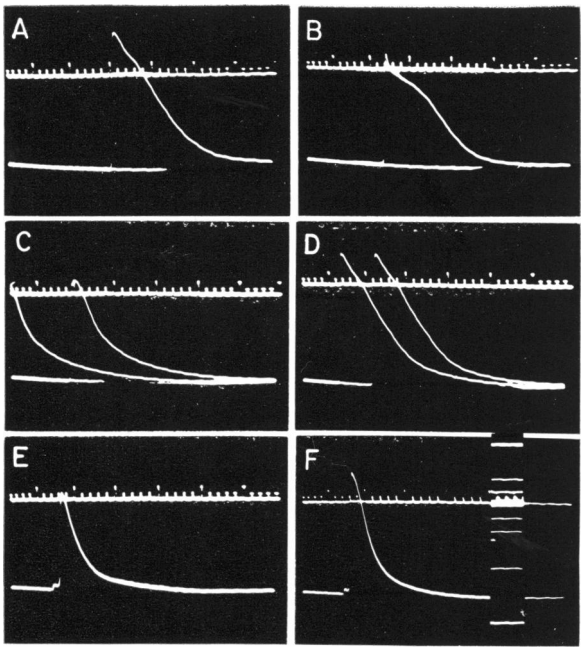

Figure 4—Transmembrane potentials recorded from single fibers of dog atrium showing the effects of decreasing the extracellular sodium concentration. Top trace represents line of zero potential and shows time calibrations in intervals of 10 and 50 msec. Voltage calibration in F = +50, +20, +10, —10, —20, —50 and —100 mv. A—control; B—50% NaCl replaced by sucrose; C—75% NaCl replaced by sucrose; D—return to 100% NaCl; E—75% NaCl replaced by choline chloride; F—return to 100% NaCl. All records obtained 25-30 minutes after changing solutions. See text for discussion.

of the cardiac action potential are less certain. Weidmann[13] has shown that the electrical resistance of the Purkinje fiber membrane increases during the plateau to three times the resting value and then returns to normal during repolarization. Wilde[16] has demonstrated a periodic loss of fiber potassium from the perfused turtle heart more or less synchronous with the S-T segment and T wave. Thus, it is tempting to assume that the current responsible for repolarization of heart muscle is an outward current of K^+ similar to that shown for the squid giant axon[17, 18], and that the relatively steady membrane potential during the plateau results from low membrane permeability to both Na^+ and K^+. However, there is no clear evidence for an increase in permeability of the cardiac fiber membrane to potassium during the phase of rapid repolarization[12, 13]. The postulated potassium efflux may thus result solely from the change in transmembrane potential[13]. Moreover, there are many

aspects of repolarization of cardiac fibers which remain unexplained. Among these perhaps the most suggestive are the marked differences in the voltage-time course of recovery in different types of fibers (see Figure 2), the effect of heart rate and temperature on the duration of the plateau[3, 19] and the shortening of the cardiac action potential caused by a sudden increase in the extracellular potassium concentration[20]. Also of considerable interest is the demonstration that repolarization can be initiated by an anodal stimulus of appropriate strength applied at any time during the plateau of Purkinje fibers[21] or ventricular muscle fibers[22], and that anodal currents weaker than this seem to cause a regenerative change in membrane potential, both during the plateau and during the terminal phase of repolarization[22]. These observations require some revaluation of the apparent decrease in membrane permeability during the latter part of the plateau suggested by impedance measurements.

A number of investigators have related the excitability of a single cardiac fiber to the transmembrane potential, and several aspects of this work are of particular interest. Jenerick and Gerard[23], in studies of single muscle fibers, showed that excitation depends upon the lowering of the resting potential to a certain critical value which they called the threshold potential. This observation contrasts with the idea that an effective stimulus is one which displaces the membrane potential by a fixed amount, regardless of the value of the resting potential. It has been shown with reasonable certainty that excitation of cardiac muscle similarly depends upon lowering the transmembrane potential to a certain critical value which can be called a threshold potential[24]. This demonstration aids considerably in understanding the mechanisms responsible for changes in excitability as well as the spontaneous firing of cardiac pacemakers. If we consider excitability in terms of the current requirement for an electrical stimulus, a change in excitability may result from a change in the value of the threshold potential or the resting potential or both, as well as from a change in the current voltage relationship of the membrane. For example, an increase in the extracellular concentration of calcium has been shown to lower excitability by reducing the value of the threshold potential[24], while calcium depletion enhances excitability by raising the threshold potential and thus bringing it closer to the resting potential. A moderate increase in the extracellular concentration of potassium increases excitability primarily by decreasing the resting potential at a time when the threshold potential is unchanged.

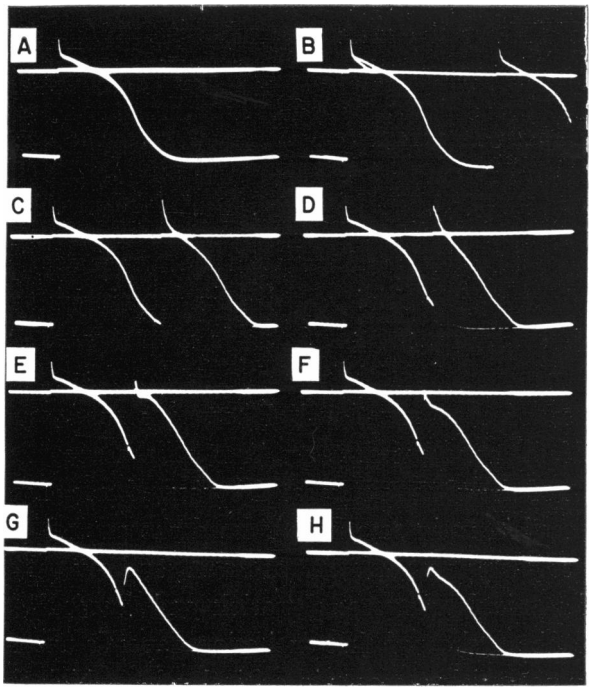

Figure 5—Transmembrane action potentials recorded from a single dog Purkinje fiber showing a normal response (A) and graded responses to stimuli applied at various times during repolarization (B-H).

On the other hand, the changes in excitability which follow excitation depend largely on other mechanisms. In cardiac muscle, as in nerve, the maximum density of the inward sodium current with results from a given depolarization depends primarily on the level of the resting potential prior to stimulation. This relationship depends upon inactivation of the sodium transport system at low levels of membrane potential[11]. Under steady-state conditions, the relationship between the maximum rate of rise of the action potential and the magnitude of the resting potential is described by an S-shaped curve with maximum rates of rise at resting potentials above 90 mv., one-half maximum at -70 mv. and complete "inactivation" at resting potentials lower than 40-50 mv.[14]. For this reason, the recovery of excitability during repolarization depends largely on the level of the transmembrane potential. The absolute refractory period lasts from the upstroke of the action potential until repolarization has restored the membrane potential to approximately -45 to -55 mv. Stimuli applied later during repolarization elicit responses

which are graded both with respect to rising velocity, amplitude and duration (Figure 5). The end of the effective refractory period, when the response to a stimulus becomes propagated, appears at a transmembrane potential of -65 -70 mv.[7], and full recovery of excitability is coincident with the full restoration of the resting potential.

This sort of a description of the recovery of excitability, which employs the level of transmembrane potential rather than the stimulus strength required at various times after the upstroke of the action potential, presents several advantages[7]. For example, it is possible to make direct comparison between excitability in tissues which have action potentials of different shapes and durations and to understand the effects of many agents, such as local anesthetics or antiarrhythmic agents, which have profound effects of excitability but cause little change either in the duration of refractoriness or in the stimulus current required for a response[24].

Use of intracellular microelectrodes to record the transmembrane potentials of single fibers have clarified many other aspects of the excitability of the cardiac muscle. Weidmann[21] has shown that the supernormal period appears before the end of repolarization and that a supernormality results from the restoration of a normal threshold potential at a time when the transmembrane potential is less than the resting value. Recent studies have shown that the refractoriness of cardiac muscle to applied cathodal stimuli and to propagated action potentials is the same (Figure 5)[7], and have given a clearer idea of the safety factor of propagation in cardiac muscle. Several of the interesting effects of anodal stimulation of heart muscle have also been studied using intracellular microelectrodes. The anodal supernormality observed during the dip of strength-interval curve[6, 22] has also been demonstrated by stimulating a single cardiac fiber through one intracellular electrode and recording the transmembrane potential through another (Figure 6). Also, it has been shown that when premature repolarization is initiated locally by application of a pulse of anodal current, this early repolarization propagates throughout the extent of the preparation both in isolated Purkinje fibers[21] and in ventricular muscle [22]. The possibility of propagated repolarization in the intact heart, although not yet demonstrated, is of considerable interest.

In addition to studies such as those just described, the intracellular microelectrode has been indispensable in recording the electrical activity

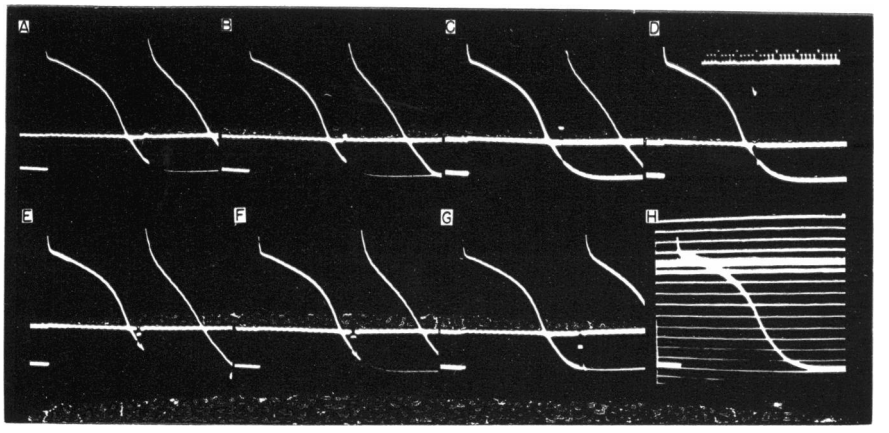

Figure 6—Transmembrane action potentials recorded from a single isolated dog Purkinje fiber through one intracellular microelectrode and effects of stimuli applied through another intracellular microelectrode. Current strength of stimuli is shown by the amplitude of the rectangular pulse on the upper trace. A-C: Response to threshold cathodal stimuli applied progressively earlier during repolarization. D: Ineffective strong cathodal stimulus. E: Weak anodal stimulus (downwards deflection) applied at same interval as cathodal stimulus of D evokes response. F-G: Increasing threshold to anodal stimuli applied progressively later during repolarization. H: Voltage calibration in steps of 10 mv. Time calibration shown in D in intervals of 10 and 100 msec.

of single fibers of the sino-atrial node[25] and the atrioventricular node[26-28], and thereby demonstrating the mechanisms responsible for pacemaker activity in the former tissue and the site and nature of conduction delay in the latter. In Figure 7 the transmembrane potential recorded from a single atrial muscle fiber is compared to the record obtained from a single pacemaker fiber in the sino-atrial node of the rabbit heart. Several marked differences are apparent. Most important, the record from the pacemaker fiber does not show a steady level of resting potential during diastole. Instead, after reaching a maximum value at the end of the phase of repolarization, the transmembrane potential of the pacemaker fiber decreases slowly until, at the level of the threshold potential, the slow depolarization merges smoothly with the upstroke of the locally arising action potential. This type of slow depolarization was first recorded from single pacemaker fibers in isolated bundles of Purkinje tissue[2] and from single fibers in the sinus venosus of the frog heart[29]. The same change in transmembrane potential is seen in records from all cardiac pacemakers, both normal and ectopic, as well as in spontaneously active excitable cells of other types. Slow diastolic depolarization is thus the mechanism underlying the intrinsic rhythmicity of cardiac muscle.

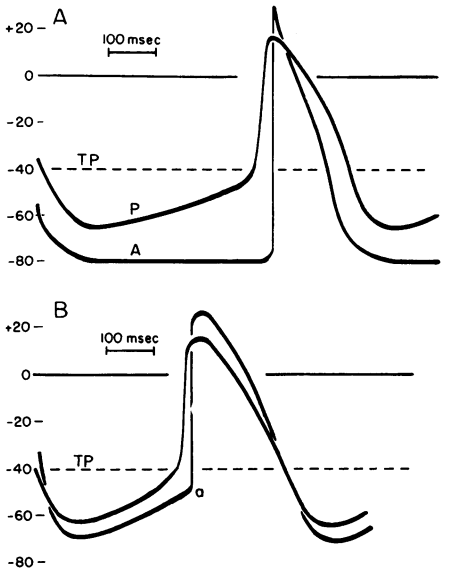

Figure 7—Tracings of transmembrane action potentials recorded from single fibers of rabbit heart. A: Pacemaker fiber of the sino-atrial node (P) and atrial muscle fiber (A). B: True pacemaker fiber and latent pacemaker. Note sharp inflection at (a). Time calibration as indicated. Voltage calibration in millivolts. TP represents level of threshold potential.

In the typical mammalian heart, many fibers show more or less slow depolarization during diastole. Surrounding the sino-atrial node there is a large group of fibers which show marked diastolic depolarization and are best classified as latent pacemakers (Figure 7). They differ from the true nodal pacemakers in that slow depolarization is interrupted, at a level of membrane potential greater than the threshold potential, by the rapid upstroke of the propagated action potential. When pacemaker activity in sino-atrial fibers is arrested, cells in this group assume the role of pacemaker. In addition to these fibers, which have action potentials intermediate in shape and amplitude between sino-atrial node and atrial muscle, there are specialized fibers distributed around the margin of the sino-atrial ring which also show some slow diastolic depolarization and may become true pacemakers under appropriate conditions[30]. Fibers in the bundle of His as well as in the peripheral Purkinje system reveal little or no slow diastolic depolarization under normal conditions. However, either of these fiber types can become a pacemaker. This event is always associated with the appearance of slow diastolic depolarization

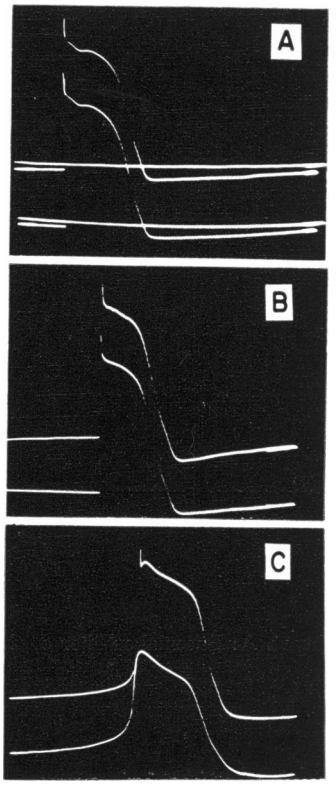

Figure 8—Transmembrane action potentials recorded from two different locations in a single isolated dog Purkinje fiber. A: Record in Tyrode solution showing slow diastolic depolarization terminating in a steady level of membrane potential. B & C: Effects of l-epinephrine, 1:100,000. Note increase in slope of slow depolarization and appearance of pacemaker-type action potential on lower trace in C.

and a concomitant decrease in the velocity and amplitude of the action potential upstroke (Figure 8). The appearance of pacemaker activity has not been observed in the ordinary muscle fibers of either atrium or ventricle. The mechanism which causes slow diastolic depolarization and permits spontaneous initiation of impulses thus appears to be restricted to specialized cardiac tissues.

Transmembrane potentials recorded from fibers of the sino-atrial node characteristically reveal a low resting potential, slow rising phase of the action potential and the reduced or absent overshoot. The second and third of these characteristics may be directly caused by the low resting potential, since Weidmann[14] has shown that rising velocity and amplitude of the cardiac action potential are decreased if the resting

potential is lowered. Also, there are many similarities between action potentials of pacemakers and premature action potentials elicited in other cardiac fibers by stimulation during the terminal phase of repolarization (See Figure 5). However, the cause of the low resting potential of these pacemaker fibers is uncertain. From what has been said about the dependence of the resting potential on potassium, it might be assumed that the low value recorded from fibers in the sino-atrial node is caused by a reduced permeability of the membrane to potassium or a low fiber content of this ion. An alternative possibility is that the resting membrane of fibers in the sino-atrial node is more permeable to sodium than is the case in atrium or ventricle, and that the augmented inward Na^+ current subtracts from the potential difference caused by a normal potassium concentration gradient and potassium permeability. Unfortunately, there have been no studies of the membrane resistance of single sino-atrial fibers; also, accurate information on the content of Na^+ and K^+ of nodal fibers is not available. It has been observed, however, that the resting potential of nodal fibers is changed only slightly by an increase in the extracellular potassium concentration from 2.7 to 13 mM., and that pacemaker activity persists, although under this condition adjacent atrial muscle is depolarized and inexcitable[31].

Also unexplained is the mechanism responsible for slow diastolic depolarization. Weidmann[32] has demonstrated a progressive increase in membrane resistance of Purkinje fibers during the phase of slow depolarization, suggesting a decrease in ionic permeability. An increasing sodium permeability as a cause of depolarization is thus unlikely, even though a lowered extracellular concentration of Na^+ abolishes slow depolarization in Purkinje fiber pacemakers[2]. This result would be expected regardless of the membrane characteristics directly causing slow depolarization, and merely shows that there is some inward Na^+ current during the period of slow depolarization. Moreover, in Purkinje fibers, potassium excess decreases and potassium depletion augments slow diastolic depolarization, while in the sino-atrial node a comparable change in the concentration of either Na^+ or K^+ has little if any effect on pacemaker activity. In summary, although most of the evidence suggests that pacemaker activity is directly related to altered ionic permeability and most likely a decreasing permeability to potassium, a clear experimental demonstration of this mechanism is lacking.

Records of the transmembrane potentials of pacemakers suggest at

least three distinct mechanisms which will influence heart rate. The cycle length will be increased if the slope of diastolic depolarization is reduced, if the threshold potential is decreased or if the resting potential is increased. Obviously, changes in the cycle length may also result from a simultaneous change in more than one of these factors. Each of these mechanisms has been shown to operate under certain conditions. The effects of autonomic mediators are primarily on the slope of slow depolarization; vagal stimulation decreases and sympathetic stimulation increases the rate at which the membrane potential approaches the threshold level[33-35]. Acetylcholine acts in much the same manner as vagal stimulation, although a difference between amphibian and mammalian hearts has been noted. In the former[34, 35], vagal stimulation causes an actual hyperpolarization of the pacemaker membrane. In the rabbit atrium, on the other hand, arrest of a sino-atrial pacemaker by acetylcholine is associated with a steady level of membrane potential between the maximum resting potential and the threshold level. Concurrently, the pacemaker site shifts to some other area in either the atrium or upper His bundle. This effect of acetylcholine on pacemaker tissue is apparently due to an increase in the permeability of the membrane to potassium [35, 36]. The resulting augmented K^+ efflux would be expected to maintain a higher resting potential regardless of the ionic currents responsible for slow depolarization, and, as in the case of sodium depletion, supplies only suggestive evidence for the cause of pacemaker activity. The increase in rate caused by sympathomimetic agents resembles that resulting from sympathetic nerve stimulation. In both instances, acceleration is due primarily to an increase in the rate of diastolic depolarization, although the level of the threshold potential has been reported to increase, remain constant or decrease[37-39]. This latter observation is difficult to evaluate because of concomitant shifts in the pacemaker site. Both epinephrine and nor-epinephrine are equally potent in accelerating a sino-atrial pacemaker or in producing pacemaker activity in previously quiescent Purkinje fibers (Figure 8), and in high concentrations both agents cause multifocal pacemaker activity in the specialized conducting system[6].

The slope of diastolic depolarization of Purkinje fibers is influenced markedly by changes in temperature, while the threshold potential remains the same[19]. Moderate changes in the extracellular concentration of calcium alter the frequency of Purkinje fiber pacemakers mainly by

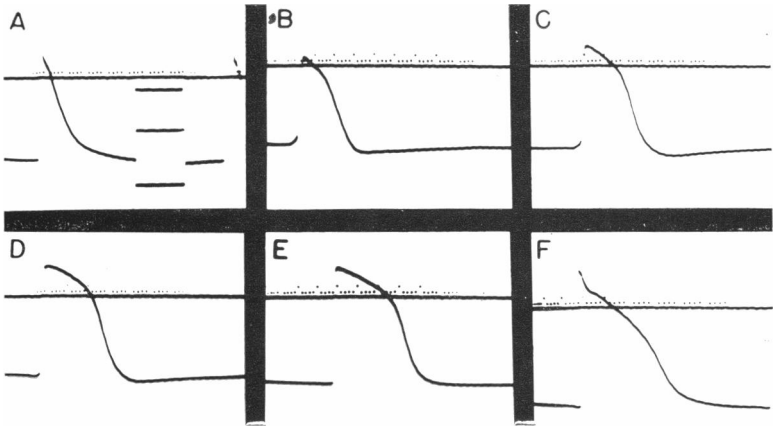

Figure 9—Transmembrane action potentials recorded from single fibers of atrium (a), upper node (b), mid and lower node (c, d, e) and upper His bundle (f). Upper trace represents line of zero potential and shows time calibration in intervals of 10 and 50 msec. Voltage calibration in (a) from above down, shows in mv: —10, —50 and —100. Overshoot in (a) is larger than that commonly recorded. See text for discussion.

raising or lowering the threshold potential; with extreme calcium depletion, however, the slope of diastolic depolarization is increased and multifocal pacemaker activity ensues. Sino-atrial pacemaker fibers are relatively insensitive to cooling, and regular activity persists after propagation in atrial muscle has failed because of a reduced resting potential[40]. Under these conditions, addition of acetylcholine increases the resting potential in atrial muscle and restores propagation of the activity originating in the sino-atrial node. This mechanism would seem to explain the observations reported a number of years ago by Bülbring and Burn[41]. Records obtained from single Purkinje fibers by means of an intracellular microelectrode have helped explain the antiarrhythmic action of quinidine and procaine. Both these agents, in concentrations which do not cause any important change in the amplitude of the resting potential or the amplitude, rising velocity or duration of the action potential, decrease the slope of diastolic depolarization in Purkinje fiber pacemakers. This effect is the same whether pacemaker activity has appeared spontaneously or in response to sympathomimetic agents. In higher concentrations, toxic effects of these same agents, such as conduction delays and appearance of extrasystoles, can be shown to result from a decrease in the rising velocity of the action potential and an actual enhancement of slow diastolic depolarization.

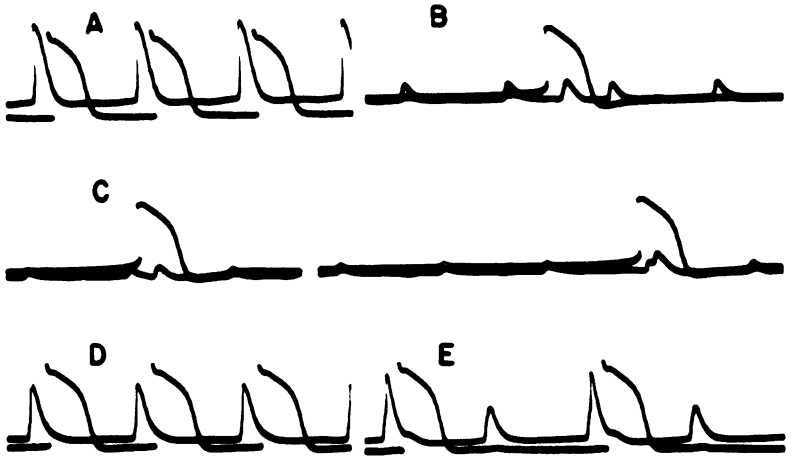

Figure 10—Transmembrane action potentials recorded from a single fiber of the atrioventricular node (top trace) and bundle of His (bottom trace) of the rabbit heart. A: Control. B-E: Segments of a continuous record showing the changes produced by acetylcholine added at B. Note decrease in amplitude of nodal response (B), complete a-v dissociation and appearance of pacemaker activity in the bundle of His (C) and 2:1 relationship between activity in nodal and His bundle fibers (E) during washout of the acetylcholine.

One problem in cardiac electrophysiology which has been studied by a variety of techniques is the nature of normal atrioventricular delay. Use of intracellular microelectrodes to record from single fibers of atrium, atrioventricular node and bundle of His has recently provided new information on the site and cause of this delay. Transmembrane potentials recorded from single fibers at the atrial margin of the A-V node have been found to show several distinctive characteristics. In addition to a low resting potential and some slow diastolic depolarization, these fibers show a slowly rising action potential with a slurred, notched upstroke and small or absent reversal (Figure 9)[26, 27]. Between this site and the upper His bundle, the action potential shows a gradual transition in amplitude and shape; and in the bundle of His the action potential is similar in configuration to that recorded from peripheral Purkinje fibers (Figure 9).

The major part of the normal atrioventricular delay is due to the slow spread of activity through fibers at the atrial margin of the node[27, 28]. Partial and complete block due to acetylocholine, high rate or hypoxia results from partial or complete failure of transmission at this site (Figure 10)[27, 28]. Records obtained from single nodal fibers during normal and altered A-V transmission suggest that, at the atrial

margin of the node, conduction is decremental because of the anatomical arrangement and physiological properties of the fibers at that location[27, 28]. On the other hand, block of retrograde transmission from ventricle to atrium has been shown to take place, at least under some conditions, at the peripheral junction between ventricular muscle and the Purkinje fibers[7, 42]. During complete atrioventricular dissociation, however, if pacemaker activity originates in fibers of the bundle of His, failure of transmission to the atrium is localized to the atrial margin of the node. All of these findings are in remarkably good agreement with the early postulates of Erlanger[43].

In conclusion, use of intracellular microelectrodes to study single cardiac muscle fibers has provided answers to many classical problems in cardiac electrophysiology. Also, as is the case with each technical improvement, the ability to record the transmembrane potential of single cardiac fibers has also given rise to new challenges and new questions which can only be answered by further technical advances.

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