Several characteristic phenomena of excitability were found in the cardiac muscle during the relative refractory period when the excitability was studied by electrical stimulations with surface electrodes. According to Brooks and his associates, subsequent to Hoffman et al., Cranefield et al., and Van Dum et al., the characteristic aspects of the excitability of cardiac muscles during the relative refractory period may be stated as follows: (1) formation of a distinct ‘dip’ in the strength-interval curve, (2) presence of a definite supernormality in anodal excitability, and preferential occurrence of excitation at the anode, and (3) existence of ‘no-response phenomenon’ and ‘vulnerable period to fibrillation’. It was also reported that the cardiac muscle could be stimulated with a comparatively weak anodal current during the diastolic phase. The anodal threshold was reported to be only two to three times as high as the cathodal, the value distinctly low as compared with that in other excitable tissues. At the present time, however, the underlying mechanisms for these characteristics of the cardiac muscle are not yet clear electrophysiologically.

For the purpose of elucidating such characteristic aspects of the cardiac muscle excitability, it may be of utmost importance to study the excitability cycle of the single muscle fiber by means of intracellular techniques in stimulation as well as in recording action potential. When these techniques are employed, confusing effects of asynchronous fiber activation and a possibility of irregular current flow through muscle fibers adjacent to a stimulating electrode can be eliminated. Not many of such single fiber studies have been reported thus far on the cardiac muscle. Weidmann has described briefly the changes in membrane threshold potential and relative strength of threshold current throughout one cardiac cycle in a sheep Purkinje fiber displaying a pacemaker potential. Hoffman et al. also carried out an experiment of this sort in the Purkinje fibers of the dog and demonstrated that the recovery of the excitability following the activity was closely related to the membrane repolarization.
However, the excitability to the anodal current, and quantitative aspects of strength interval relations both to cathodal and anodal stimulation were not fully studied by the intracellular techniques in their experiments.

In the present experiment, the responses of the single Purkinje fibers of the dog to cathodal and anodal currents were studied with the currents being applied through an intracellular electrode. Strength-interval curves so obtained came out to be largely different in many aspects from the results obtained thus far by the conventional surface electrodes. However, some of these apparent discrepancies between the intracellular and extracellular strength-interval curves seemed to be interpretable, as is discussed later.

METHODS

Samples. All experiments were carried out in the superficial fibers of the sub-endocardium of the free wall of the right ventricle of the dog. This type of fibers, which are considered to be the terminal Purkinje fibers, have certain characteristics in electrical activity different from the ordinary ventricular muscle fibers. Insertion of two microelectrodes in these fibers was relatively easy since the course of the fibers was roughly traceable with a microscope and the resting potential was quite stable for a long time during the experiment. Similar experiments which were attempted in ordinary ventricular muscle fibers were not successful because of the difficulty in double impalement with microelectrodes. The method for preparing the samples was the same as previously reported.

Stimulation. The sample was pinned to a paraffin block which was fixed on the bottom of a small Tyrode bath made of Lucite. The sample was driven electrically at a constant rate (85 per min.) with a pair of silver silver-chloride electrodes which were insulated with vinyl tubes except at their tips and were placed on an edge of the sample.

Test stimulation was applied intracellularly to fibers through a microelectrode, and the change in transmembrane potential of the fiber was picked up with another microelectrode. Inter-electrode distance was set at about 100 μ in most cases. Drive stimulus was triggered by the sweep start impulse of an oscilloscope, and the test stimulus (rectangular pulse) was delivered by an electronic stimulator with a variable delay from the drive stimulus. A 50 MΩ resistor was put in series with the stimulator output and the test stimulus electrode. Current strength was measured on the oscilloscope screen by the potential drop across a resistor of 10 KΩ which was inserted between an electrode (silver silver-chloride electrode) in Tyrode solution and the ground.

Microelectrodes. Ling Gerard type microelectrodes with the tip diameter of about 0.5 μ were used both for stimulating and recording. They were filled with 3M KCl solution by means of a direct filling method. Their resistance ranged from 5 to 10 MΩ.

The method for recording membrane potential and the composition of the Tyrode solution used in this experiment were the same as those in the previous report.

RESULTS

1. Critical membrane potential and current threshold to cathodal stimulus throughout a cardiac cycle.
As previously reported by MATSUEDA et al., the majority of the superficial fibers of the subendocardium of the canine ventricle exhibit an action potential intermediate in shape between that from the Purkinje fiber (in the false tendon) and that from ordinary ventricular muscle fiber. Their action potential is composed of a rapid depolarization phase, high overshoot, sharp spike, and a plateau somewhat longer than that of the ordinary ventricular muscle. Their resting potential is about 90 mV under the normal conditions. These fibers are most probably the terminal arborizations of the impulse conducting system and their transitions to the ventricular fibers.

In this type of fibers the critical membrane potential to cathodal* stimulation was measured at various phases of one activity cycle of repetitive activation. The duration of the test pulse was fixed at 20 mSec., a duration sufficiently long in view of the principal utilization time for the fiber (5.5 mSec. on the average). The criterion for the critical membrane potential was the probability of 50% for firing action potential when the membrane was depolarized to that level by a cathodal pulse. A series of typical records is illustrated in Fig. 1.

* In the intracellular stimulation cathodal means outgoing current and anodal means ingoing current with respect to the cell.

The critical membrane potential was found to be quite constant throughout the diastolic phase including the terminal phase of repolarization of action potential. However, at the point slightly earlier, namely in the later part of the rapid repolarization phase (phase 3), the critical level was raised sharply. Such an abrupt change in critical membrane potential occurred when the repolariza-
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...tion proceeded to $-60\text{--}80\text{mV}$ in membrane potential. And the fiber failed to respond even to the maximum current ($2.4\times10^{-6}\text{A}$) when the test pulse was given before the membrane was repolarized to approximately $-60\text{mV}$.

The current threshold during the diastolic phase was $3.5\times10^{-7}\text{A}$ on the average, the value well in accord with that reported previously by one of the authors\(^{13}\). It remained constant throughout the diastolic phase and rose sharply but quite smoothly at the later part of phase 3. As shown in Fig. 2 the overall shape of the strength-interval curve was smooth and hyperbolic, showing neither

![Fig. 2. Strength-interval curve of the terminal Purkinje fiber. Intracellular stimulation. M.P. shows the time course of membrane repolarization of the fiber.](image)

![Fig. 3. Critical membrane potential (C.M.P), current threshold (I) and the membrane potential (M.P.) at the later phase of the repolarization. Ordinate: membrane potential in mV and current strength in 10^{-7}A. Abscissa: "interval" of excitation cycle in mSec.](image)
irregularities nor dip.

The relations of current threshold and critical membrane potential to the time course of repolarization of transmembrane potential are shown in Fig. 3. At the terminal portion of the action potential a slight decrease in critical depolarization seemed probable, as the membrane potential was changing from 90 to about 75 mV, while the critical membrane potential did not change so much. However, no obvious supernormal phase could be observed in the intracellular strength-interval curve, which was nearly parallel to the curve for the critical membrane potential.

2. Responses to anodal stimulation

During the diastolic phase the fiber did not respond to anodal current as long as its resting potential was maintained within normal range (80-95 mV). The current applied gave rise to only an electrotonic displacement of membrane potential and no apparent sign of active response was seen both during the passage of the current and after breaking the current. The maximum current used in this experiment was $2.8 \times 10^{-6} \text{A}$, that is, about eight times the diastolic threshold to cathodal stimulation. Somewhat stronger currents, which were tested in other experiments, also failed to elicit any response.

On the other hand, on the break of anodal current there arose a characteristic spike response during phase 2 and the first half of phase 3 as shown in

![Fig. 4. Post-anodal responses of the terminal Purkinje fiber. Intracellular stimulation. The pulse of a constant strength (2.4×10^{-6} \text{A}) was applied at various intervals of the cardiac cycle. Time signals and voltage calibration are the same as in Fig. 1.](image)
Fig. 4. While this spike was not accompanied by a pleateau as is usually the case in cardiac muscles, it was followed by a slow potential or an incomplete pleateau only when the break of stimulus current was set in a very limited phase in the middle portion of phase 3, where the membrane potential had repolarized to a range of 45 to 55 mV. A propagated action potential could be recorded from a distance, only in the cases where such a slow potential followed the spike. The current strength required to elicit the propagated action potential at this limited phase was greater than $1.2 \times 10^{-6}$ A. Out of this phase, the propagated action potential could never be obtained even though currents stronger than $2.8 \times 10^{-6}$ A were given. Consequently, the overall shape of the anodal strength-interval curve was a simple downward directed spike located at about 15 to 20 mSec. earlier than the upward inflection point of the cathodal curve (Fig. 2).

3. Characters of action potential during the refractory phase

The action potential of the single terminal Purkinje fiber evoked by cathodal stimulation during the relative refractory phase is characterized by its diminished overshoot, slow depolarization velocity and shortening of its duration. These do not seem to differ significantly from those of the ordinary ventricular muscle or of other parts of the heart. It has been pointed out that the graded response can be seen at the junctional region of the Purkinje fiber and papillary muscle in the dog heart during the early phase of relative refractoriness\(^7,8\). However, in this experiment, the occurrence of such distinct graded responses were not confirmed, the changes in action potential being rather slight (Fig. 1).

On the other hand, responses to the anodal stimuli were quite characteristic as stated above, namely, an isolated narrow spike during phase 2 and 3, and the spike followed by a slow potential at the middle portion of phase 3. For the purpose of clarifying nature of these potentials the following observations were made.

(i) The relationship between the height of the post-anodal spike and stimulus strength. As shown in Fig. 5 (A) a progressive increase in strength of anodal current resulted in an increase in spike height. This however, never exceeded the size of the normal spike. This figure illustrates the quantitative relationships between the overshoot of the post-anodal spike and the anodal hyperpolarization (maximum value in membrane potential) at various phases of the action potential. The curves closely resemble the "inactivation curves" of action potential obtained by Weidmann\(^17,18\) in ungulates Purkinje fibers. The figures also illustrates a greater effectiveness of the anodal current in producing the spike at the later phases of repolarization. Fig. 5 (B) shows the effect of the duration of anodal pulse. In this experiment three kinds of duration of stimulus were tested, the time of break being fixed at the same phase of the action potential. The results indicated the longer the pulse the stronger the effect.
FIG. 5. The relationship between the height of the post-anodal spike and the final level of hyperpolarization produced by anodal current. (A) The influence of the phase of action potential: open circles, 315; solid circles, 275; triangle, 230; crosses, 190 mSec. after the onset of the action potential. The duration of the pulse was fixed at 20 mSec. (B) The influence of the duration of pulse: crosses, 5; open circles, 30; and solid circles, 80 mSec. The break of the current was fixed at 275 mSec after the onset of the action potential.

FIG. 6. Decay of the post-anodal spike along the fiber length. Upper: actual records of transmembrane potential from various distances from the site of stimulation. Lower: plots of the height of the post-anodal spike (solid circles) and the amplitude of hyperpolarization during the passage of an anodal pulse (open circles) against the interelectrode distance.
(ii) Effects of lowering in external sodium upon the spike height. The maximum (saturated) height of the post-anodal spike was measured in the medium where the sodium concentration was lowered by substituting sodium with osmotically equivalent sucrose to 60, 40, 30 and 25 per cent of the normal. Along with the decrease in sodium concentration the post-anodal spike height decreased in a similar way to the decrease of the spike in the non-refractory fiber.

(iii) Spread of the post-anodal spike along the fiber. The post-anodal spike did not appear to be conducted along the fiber because it could not be recorded as such from distant parts of the fiber. This was further ascertained by observing the decay of the post-anodal spike along the fiber. As shown in Fig. 6 the spike, which was almost fullsized in the vicinity of the stimulating electrode, decayed exponentially with distance, in the same way as the electrotonic hyperpolarization.

These findings lead to a conclusion that the sodium carrier system at the membrane is the generator of the post-anodal spike in the refractory period as of the normal spike, but the conductivity of the spike in refractoriness is more labile, being controlled by complex factors.

(iv) Conductile action potential elicited by anodal stimulation. As described above, the propagation of excitation could be evoked by anodal stimulation only at a narrow phase of repolarization. In such a case, the fiber in the vicinity of the stimulating electrode exhibited a characteristic action potential, i.e., a spike-and-slow potential. However, the distinction of the spike and the slow potential became less marked as the distance from the stimulated spot increased. Moreover detailed observations disclosed an interesting fact that the rising phase of the slow component was steeper and its summit appeared more advanced at a greater distance than at a close proximity of the stimulated site. Of course, beyond a certain distance away from the stimulus site the evoked action potential appeared successively delayed according to the usual manner of impulse conduction. Although the post-anodal spike underwent a decrement with distance as stated above, the rising phase of the slow potential became prominent with distance, until it took an appearance of a spike so that the overall shape of the evoked action potential turned into the normal shape.

Accordingly, the conducted impulse appeared to start at some distance from the site of stimulation. The slow potential recorded from a close proximity might be a result of a conduction of the evoked excitation in the reverse direction, viz, concentrically to the stimulating electrode. This interpretation leads to a conclusion that the response of this type was a mixture of the local post-anodal spike and a cathodal excitation evoked by that post-anodal spike.

4. Anodal excitation in fibers with decreased resting potential or somewhat altered action potential

It is considered to be a general property of excitable cells that the anodal excitability is enhanced in the partially depolarized or slightly deterio-
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rated fibers\textsuperscript{5,6,10,14,19}. This is also true in the terminal Purkinje fiber. As shown above, intact terminal Purkinje fibers do not respond to intracellular anodal stimulation of any strength during the diastolic phase, whereas genuine post-anodal excitation with conduction could easily be evoked in certain samples of depolarized fibers (Fig. 7, A).

![Fig. 7. Post-anodal responses observed in the terminal Purkinje fiber with reduced resting potential (A), and a prolonged negative after-potential (B and C). (B: without extra stimulus, C: post-anodal response).](image)

Such post-anodal excitation was also seen in some fibers exhibiting an action potential with distinctly prolonged negative after-potential (Fig. 7B, C). Such action potential was frequently recorded from the samples which had been preserved in Tyrode solution cooled to about 5°C for 24 or 48 hours. The strength-interval curve was not fully determined in such a case, but an impression was that the sensitive interval in phase 3 to anodal stimulation became significantly broader in such fibers than in the intact fibers.

DISCUSSION

Differences in character between extracellular and intracellular strength-interval curves

Hoffman, Kao and Suckling\textsuperscript{7} have presented a typical example of the strength-interval curves of cardiac muscles obtained by the unipolar extracellular stimulation. In comparison with the intracellular strength-interval curves which were obtained in this study, their curves showed several distinct characters. The most striking difference was in the anodal excitability. Their anodal curve for the extracellular stimulation had a diastolic segment which ran exactly parallel to their cathodal curve, and the anodal threshold there was relatively low, namely only two to three times as high as that for the cathodal stimulation. In the present experiments, however, the fiber could never be fired by intracellular anodal stimulus during the diastolic phase as long as the normal resting and action potentials were maintained, even when currents more than eight times as strong as the mean cathodal threshold were used. The phase where the anodal stimulation produced the
propagating action potential was found to be confined only at a limited period of phase 3, so that the anodal strength-interval curve obtained by intracellular stimulation had a shape of inverted spike located slightly earlier than the upstroke of the cathodal curve (Fig. 2).

The reason for the difference above, may be that the true excitability curve as revealed by the intracellular stimulation is modified by some factors when examined by the extracellular stimulation. One of these factors is that there might be some local injury and/or partial depolarization in the fibers in contact with a surface stimulating electrode, for the cardiac muscle fibers are fairly sensitive against slight compression by an external electrode. Another factor, which may be of greater importance than the above, is that the excitation elicited by an extracellular anodal stimulation is apparently post-anodal but actually cathodal. In the present investigation, it was frequently observed that the start of the evoked action potential did not shift on a sudden increase in duration of an anodal pulse in the “unipolar” extracellular stimulation (Fig. 8, A). Furthermore, it was experimentally confirmed that there were many fibers, surrounding the surface electrode, of which transmembrane potentials were depolarized instead of being hyperpolarized during the passage of the anodal current (Fig. 8, C). These facts suggest a possibility that an extracellular anode was working not solely as an anode but also as a cathode (virtual cathode) especially on the fibers in the vicinity of the electrode (see Fig. 9). This could occur also in the anodal stimulation during the diastolic phase, and the reason for a slightly higher threshold here to the ‘anodal’ stimulation than to the cathodal may be ascribed to a lower density of currents at the site of the virtually cathodal effect.
HOFFMAN and CRANEFIELD cited the argument of H. SCHAEFER in their monograph⁸) that "because of the complexity of current flow in the intact heart, anodal excitation might be apparent only—the result of a 'virtual cathode'". Present results are supporting Schaefer's interpretation.

Anodal excitability of single muscle fibers during phase 3 as observed above probably corresponds to the supernormal phase of the extracellular anodal strength-interval curve reported by HOFFMAN, KAO and SUCKLING⁷). The nature of the excitation during this phase, however, seems to be rather complex, i.e., a mixture of the post-anodal local response and the actual cathodal excitation as analyzed above. It may be reasonable to conclude that the true post-anodal excitation with conduction does not occur in the normal terminal Purkinje fibers.

However, the spike response evoked by an anodal current during phase 2 and 3 is really a post-anodal response. Detailed analysis of the nature of the spike in several aspects revealed that it did not essentially differ from the spike of normal action potential, except its failure of conduction. This failure may be ascribed to the fact that its phase is just the absolute refractory period to the cathodal stimulation.

It has been well known that a hyperpolarizing current of sufficient strength and duration can abolish the action potential of the cardiac muscle at its repolarization phase⁴,⁶). However, the present authors could not produce experimentally such abolition of action potential by intracellular application of anodal currents in the terminal Purkinje fibers, even though currents of sufficient strength and duration were applied at various phases of the action potential. Details of this study will be reported elsewhere (in preparation).

Any of the strength-interval curves for intracellular cathodal stimulation obtained in the present study runs smoothly except at a single, rather sharp inflection, whereas the extracellular curves obtained by HOFFMAN, KAO and SUCKLING⁷) usually showed a gradually rising course with more or less obvious
irregularities or ‘dip’s. According to HOFFMAN and others\textsuperscript{8)} the ‘dip’ was not present in the curve for unipolar cathodal stimulation, while BROOKS and others\textsuperscript{2)} had described that it was present sometimes. The experiments in the authors’ laboratory on the strength-interval curve of the dog ventricle with unipolar cathodal stimulation revealed that about one fifth of 25 samples examined exhibited a distinct dip, but in the remainders, only slight irregularities were found in the refractoriness (unpublished data).

The gradual rise of the upstroke of the extracellular cathodal curve and some irregularities therein may partly be due to the population of fibers which are not uniform in their excitability cycle. However, the more probable cause might be that the cathodal stimulation was not entirely cathodal but could also virtually anodal to some fibers surrounding the stimulating electrode, because of the similar, but reverse in sign, circumstances to the case of the anodal stimulation mentioned above. It was our frequent observation that during the early part of the relative refractory period, including the ‘dip’ area, the excitation occurred not on the make, but on the break of the current. Furthermore, the shape of the action potential recorded from the fibers in the vicinity of the stimulating electrode (the cathode) is quite similar to that evoked by anodal stimulation (Fig. 10), i.e., dissociated spike-and-slow potential. Consequently, the formation of the dip and/or irregularities in the cathodal strength-interval curve are thought to be largely due to the virtually anodal excitations and the abrupt transition of the site of impulse origination from one fiber to another as considered above in the text.

**SUMMARY**

1. Recovery of the excitability of the terminal Purkinje fibers of the dog ventricle was studied by means of intracellular stimulation and recording of membrane potential.
2. The strength-interval curve for the cathodal stimulation was a uniform hyperboloid with a sharp inflection. Their course was closely related to the
membrane repolarization, and the relative refractory period corresponded to the phase of repolarization from \(-60\) to \(-80\) mV.

3. The strength-interval curve for anodal stimulation had a shape of an inverted spike located about 15 to 20 mSec. earlier than the end of the absolute refractory period to the cathodal stimulation. The intact fibers were unresponsive to any anodal stimulation during the diastolic phase.

4. Break of anodal current produced an isolated spike response during phase 2 and in the first half of phase 3, but the response did not conduct. At a somewhat later stage, the anodal stimulation produced a response consisting of a spike and slow potential. In this case the response was conductive.

5. In view of the excitability curve of a single fiber, it is concluded that the excitation elicited by the extracellular anodal stimulation in the diastolic phase may be due to the virtually cathodal effect of the current around the anode, and the 'dip' or some of the irregularities observed in the extracellular cathodal strength-interval curves may largely be ascribable to the virtually anodal effect of the stimulating cathode.

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REFERENCES


