Electrotonic Interaction between Muscle Fibers in the Rabbit Ventricle

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ABSTRACT Transmembrane potentials were recorded simultaneously from pairs of ventricular fibers in an isolated, regularly beating preparation. A double-barrelled microelectrode was used to record the potentials from, and to polarize, one fiber. A single microelectrode was used to record from a distant fiber. The existence of two systems of fibers, termed P and V, was confirmed. Histological evidence for the existence of two types of fibers is also presented. Electrotonic current spread was observed within both systems, electrotonic interaction between the two systems was rare and always weak. In the case of those pairs of fibers showing electrotonic interaction, the distance for an e-fold decrease in magnitude of the electrotonic potentials was found to be from 300 to 600 μ in P fibers and from 100 to 300 μ in V fibers. However, no electrotonic interaction could be observed in the majority of V fiber pairs. Moreover, the magnitude of the electrotonic potential did not decay monotonically with distance in any one direction. It is concluded that the rabbit ventricle cannot be regarded as a single freely interconnected syncytium.

INTRODUCTION

Using fibers in the rabbit right ventricle, Johnson and Tille (1960, 1961 *b*) found that voltage-current relationships, obtained by passing hyperpolarizing current during the repolarization phase of the action potential, were linear and that no regenerative repolarization could be elicited. They concluded that the membrane conductance of ventricular fibers is independent of the membrane potential during the repolarization phase of the action potential. However, Noble (1962) has calculated that the syncytial nature of ventricular muscle could make impossible any clear interpretation of the results obtained by Jonhson and Tille. This would be the case if each fiber possessed low resistance connections with some of its neighbors. The reason is that in an interconnected syncytium spreading in more than one dimension changes in membrane resistivity caused by the application of current at a single point would not result in detectable changes in polarization resistance. Thus before any progress can be made in the investigation of the membrane properties of

ventricular fibers, some information must be obtained about the number and geometry of functional interconnections between these fibers. The results contained in the present paper have been obtained in an effort to determine the relative position of rabbit ventricular muscle fibers between the extremes of a pure cable and a freely interconnected syncytium. Some of the results are in agreement with those of Tarr and Sperelakis (1964). Thus, it will be shown that many pairs of ventricular cells do not show strong electrotonic interactions. However, some pairs of cells do show such interactions and electrotonic spread may then be detected over longer distances than reported by Tarr and Sperelakis.

MATERIALS AND METHODS

Rabbits were stunned by a blow on the neck and the hearts excised. The right ventricular wall was removed and pinned out, endocardial surface uppermost, on a polythene slab in a silver organ bath. The bath was heated electrically and was maintained at 37 ± 0.5°C by an electronic feedback circuit. The preparation was bathed in the following solution (concentrations are given in mM) NaCl, 118; KCl, 5.9; $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. The solution was run through the bath at about one drop per second and was aerated in the bath by a 95% O_2, 5% CO_2 mixture. The muscle was stimulated regularly at 1.25 or 1.6 impulses per second by a bipolar silver electrode situated on the edge of the preparation.

Apparatus A double-barrelled microelectrode (DBME) and a single-barrelled microelectrode (SBME), both filled with 3 M KCl solution, were mounted in two independent micromanipulators situated on opposite sides of the preparation. Two independent systems were used to record simultaneously the transmembrane potentials from two different cells. The distance between the tips of the DBME and the SBME was measured with the aid of a wire cross-piece in one ocular of a stereo microscope. The accuracy of this measurement was estimated to be ±25 μ. The distance between the tips was rarely less than 100 μ; it can therefore be assumed that in almost all cases the electrodes were located in different cells. The magnitude of the polarizing current which was passed through one barrel of the DBME could be monitored on the oscilloscope screen; however, because only two beams were available the current could not be displayed simultaneously with the two microelectrode records.

The stimulating and recording arrangement was essentially the same as that described by Johnson and Tille (1961 *a*). Some of the apparatus was altered as follows:— a Grass camera was substituted for a Shackman camera; Tektronix 160 series modules were used instead of the Grass stimulator; the main oscilloscope was a Tektronix 502; the monitor CRO was omitted; a second recording channel with an identical electrometer input stage was added.

Recording of Transmembrane Potentials The DBME was used to record the transmembrane potential from, and to introduce polarizing current into the first cell. Because of the low polarization resistance of cardiac muscle fibers (Weidmann, 1952;

Johnson and Tille, 1961 *a*) currents of up to 10^{-6} amp were used. This resulted in large potentials being applied by the constant current generator to the polarizing barrel of the DBME (the resistances of these electrodes were of the order of 20 MΩ) and in large transients being recorded by the recording barrel. The duration of these transient spikes is of the same order as the time constant of cardiac fibers; for this reason the method of balancing out the dc artefact during current flow with the help of the membrane time constant cannot be used. During current flow, therefore, the absolute value of the membrane potential cannot be recorded by the DBME; only intrinsic changes in membrane potential (e.g. action potentials) can be determined by this method. An estimate of the voltage displacement caused by the polarizing currents was obtained when required by superimposing the crests of action potentials recorded before and during current flow. When the absolute value of the membrane potential in the first fiber was not important, the recording by the DBME was used mainly to ensure that the conditions of impalement by the DBME remained constant.

An independent recording circuit headed by a SBME was used to record the transmembrane potential from a second cell. Apart from fast transients at the beginning and end of a current pulse (caused by the proximity of the SBME to the polarizing barrel of the DBME) no potential changes attributable to the flow of the polarizing current were ever recorded with the SBME in the extracellular fluid. It was therefore concluded that the resistance of the extracellular fluid was sufficiently low for the fluid to be considered at zero potential everywhere and that the potential recorded by the SBME when intracellular was the true transmembrane potential at all times.

Histological Histological specimens were obtained from freshly excised hearts and also from preparations which had been used for electrophysiological experiments. They were fixed in a solution of 5% formaldehyde in normal saline, embedded in paraffin, sectioned at 8 or 10 μ, and stained either with the periodic acid-Schiff reaction (PAS) or with Mallory triple stain (Mallory, 1938). Similarly prepared slides of sheep papillary muscles were used for comparison.

RESULTS

P and V Fibers Johnson and Tille (1961 *a*) described the existence of two types of fibers, termed P and V fibers, under the endocardial surface of the rabbit right ventricle. The criteria used for distinguishing the two types of fibers were as follows: (*a*) The two types of fibers had different action potential shapes (see Fig. 2). (*b*) P fibers had a higher polarization resistance, longer membrane time constant, and lower threshold current than V fibers. (*c*) There was no change in the amplitude of the V fiber action potential when hyperpolarizing currents of moderate intensity (less than about 5×10^{-7} amp) were passed throughout the rising phase. When the current intensity was increased further, a fast spike developed at the crest of the action potential and the action potential amplitude began to increase linearly with increasing cur-

rent intensities. In P fibers all values of hyperpolarizing current increased the amplitude of the action potential.

When an electrode is advanced into the muscle, P fiber action potentials can be recorded only from the first, and sometimes from the second fibers under the endocardium. The probability of impaling a P fiber in the surface of the wall of the rabbit right ventricle is quite small and appears to be different in different rabbits. However, P fibers can be usually found without difficulty on, and near near the ends of, small ventricular trabeculae. Thus not only the bulk, but also most of the surface of the rabbit ventricle consists of V fibers. Occasionally fibers with intermediate properties are also impaled.

Displacement of the Crest of the Action Potential An intracellular DBME was used to introduce rectangular pulses of hyperpolarizing current and to record the changes in transmembrane potential in the same fiber. The current pulses were begun about 80 msec before, and were maintained throughout the whole duration of each fourth action potential. A separate SBME was used to record the transmembrane potential in a distant fiber. Records from both electrodes were displayed and photographed simultaneously. An example of such a record is shown in Fig. 1.

Three examples of the time course of the electrotonic potential throughout three different action potentials are shown in Fig. 2. The first is a typical example of a result from a P fiber; the crest of the action potential in the distant fiber is displaced by up to 20% of the resting state displacement. The second example is typical of most of the V fibers impaled; at the distant electrode the displacement of the crest of the action potential is almost equal to the resting state displacement.

The third example shows the behavior observed in a small proportion of V fibers; there is no displacement at the crest or during the resting state. The slow increase of the electrotonic potential during the repolarization phase and its disappearance on repolarization have been observed by Cranefield and Hoffman (1958) in dog and cat papillary muscle using large extracellular polarizing electrodes.

Electrotonic Current Spread The same experimental procedure was used as before. The method used for calculating the decay of the electrotonic potential with distance involves the estimation of the potential displacement at the site of application of current. The estimation employed here is based on the assumption that the crest of the P fiber action potential and the brief spike which develops at the crest of the V fiber action potential at the site of the DBME (see Fig. 1) are both periods of low membrane resistance. In the calculation of the values of V_o employed in Fig. 3 an allowance was made for the displacement of the action potential crests of up to 20% of the resting state displacement. This correction was estimated from the displacement of the crest of the

action potential recorded with the SBME. However, it is possible that the drop in membrane resistance during the rising phase is not very pronounced; the displacement of the crest would then be comparable to the resting displacement and the values of V_d/V_o given in Fig. 3 would all be overestimated.

FIGURE 1. An example of the effect of hyperpolarizing current on V fiber action potentials. The figure is a superposition of two oscilloscope sweeps; no current was passed during the first sweep, constant hyperpolarizing current (5.3×10^{-7} amp) was passed through one barrel of the DBME throughout the duration of the second sweep. The lower two traces were recorded with a DBME; the top two traces were recorded with a SBME 200 μ away. The displacement between the two top traces gives the magnitude of the electrotonic potential recorded 200 μ away from the site of application of current. Calibration, 50 mv, 50 msec.

The decline of the electrotonic potential with distance is shown in Fig. 3. In these experiments the membrane potential displacement was measured before the upstroke of an action potential. Each point in Fig. 3 represents a different pair of cells; the preferable experiment of maintaining the DBME in the same cell and moving the SBME to different positions proved too difficult in a contracting preparation. Nevertheless, Fig. 3 should be a reasonable representation of the decline of electrotonic potential with distance from the

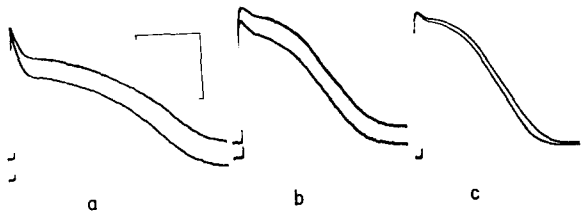

FIGURE 2. Three examples of the effects of hyperpolarizing current recorded at a distance from the site of application of current. Each record consists of two superimposed oscilloscope sweeps; no current was passed during the first sweep, constant hyperpolarizing current was passed throughout the whole of the second sweep. Record *a* is from a P fiber, records *b* and *c* are from V fibers. These records were obtained with the SBME; for simplicity the concurrent DBME records are not shown. The separations between the electrodes were 400 μ, 200 μ, and 800 μ in *a*, *b*, and *c* respectively and the polarizing currents were 2.1×10^{-7}, 5.3×10^{-7}, and 5.0×10^{-7} amp in *a*, *b*, and *c* respectively. Calibration, 50 mv, 50 msec.

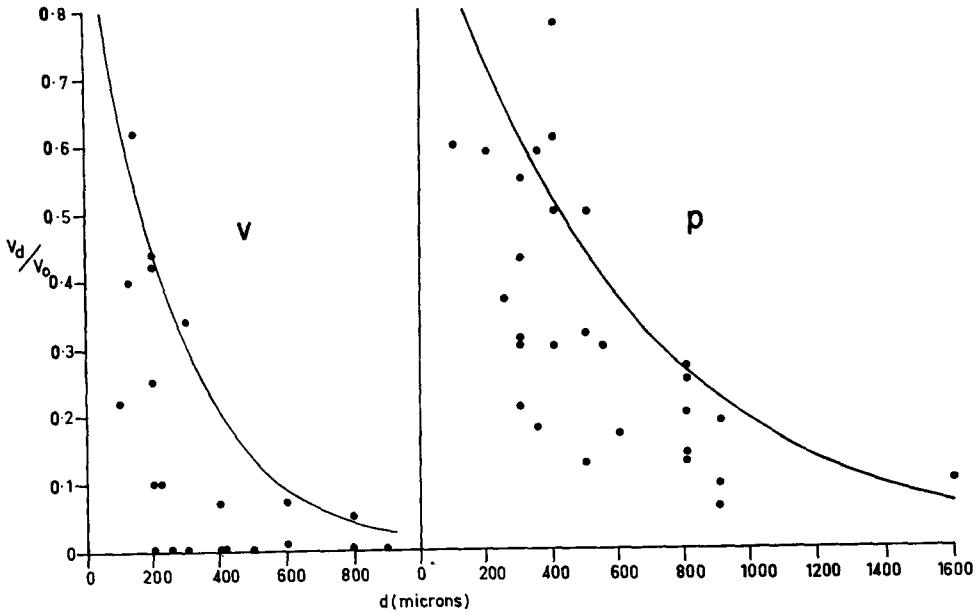

FIGURE 3. Relationship between the magnitude of the electrotonic potential (plotted as a fraction of the potential displacement at the point of application of current) and distance from the point of application of current in V and P fibers. Each point represents a different pair of fibers. The two solid lines are exponential curves with length constants of 300 and 600 μ respectively. These are intended to give an indication of the spatial spread occurring along the pathways of strongest interaction. It should be noted, however, that these pathways are relatively rare in the case of V fibers.

point of application of current. Two distinctions between P and V fibers are apparent. The distance for an e-fold decay of the electrotonic potential of P fibers is about twice that of V fibers. Furthermore, electrotonic interaction was observed in all pairs of P fibers with a separation smaller than about 1 mm, whereas many pairs of V fibers showed no observable interaction, even at small distances.

The ratio of interacting to noninteracting pairs of V fibers shown in Fig. 3 is biased in favor of the interacting ones. During these experiments, many

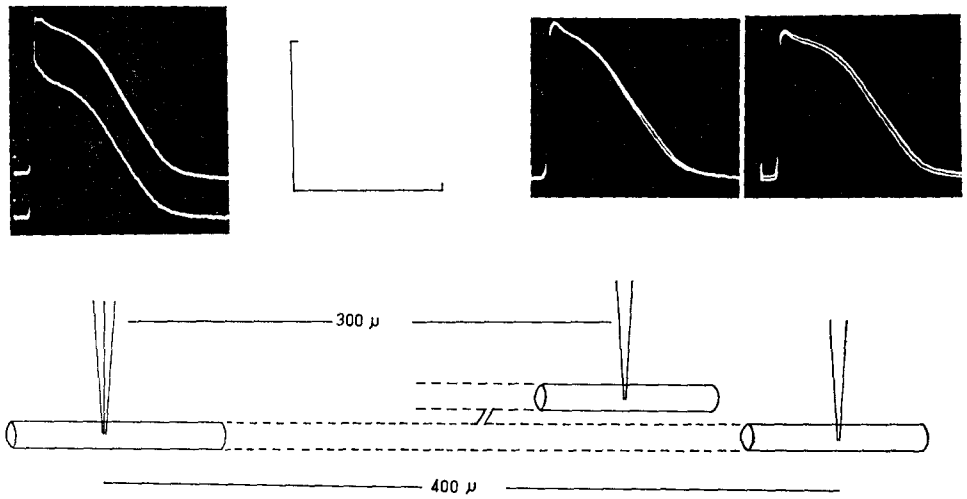

FIGURE 4. The decay of the electrotonic potential with distance is not monotonic. Each oscilloscope record is a superposition of two sweeps; no current pulse was passed during the first sweep, a hyperpolarizing current pulse was passed throughout the duration of the second sweep. The DBME and SBME were first inserted into two V fibers at a distance of 400 μ and the two outermost records were obtained. The SBME was then inserted into another V fiber at 300 μ from the DBME and the middle record was obtained with the DBME now passing twice as much current. All three impalements were made in the same straight line. Calibration, 100 mv, 100 msec.

noninteracting pairs were impaled but no records were taken. In fact, interacting pairs of V fibers were very difficult to find. Moreover, the electrotonic potential in V fibers did not decay monotonically with distance in any one direction. Fig. 4 shows the potential displacement in a V fiber at a distance of 400 μ from the polarizing electrode and a much smaller potential displacement in another V fiber at a distance of 300 μ in the same direction.

Interaction between P and V Fibers It soon became apparent that electrotonic interaction between P and V fibers was either absent or was extremely weak. Fig. 5 illustrates this point. No electrotonic potential was recorded in a

P fiber when the DBME was used to polarize a nearby V fiber; however, a large electrotonic potential was observed when the SBME was advanced into a V fiber, immediately underneath the P fiber.

On no occasion was current observed to spread from P to V fibers. However, in two cases out of at least forty, a small electrotonic potential was observed in a P fiber, when a nearby V fiber was polarized. Nevertheless, intracellularly initiated action potentials were observed to propagate both from P to V fibers (see Fig. 7) and from V to P fibers.

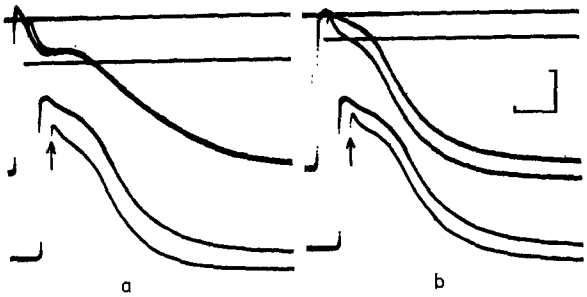

FIGURE 5. An example of the absence of a resistive connection between adjacent V and P fibers. Each half of the figure is a superposition of two oscilloscope sweeps; no current was passed during the first sweep, during the second sweep a hyperpolarizing current pulse was begun shortly after the upstroke of the action potential and was continued throughout the rest of the sweep. The displacement of the two top (straight) traces indicates the magnitude of the applied current.

In *a* the SMBE (top action potential trace) was situated in a P fiber and the DBME (bottom action potential trace) was in a V fiber. No potential displacement can be observed in the P fiber. The SBME was then advanced into a V fiber, immediately underneath the P fiber, and record *b* was obtained. A considerable potential displacement is observed in the new fiber even though the applied current has been reduced. The current traces and the SBME records are synchronized in time, the DBME record is displaced by about 15 msec (the beginning of the current pulse on the DBME records is indicated by arrows). These records were photographed on a Tektronix 565 oscilloscope. Calibration, current, 3×10^{-7} amp; voltage, 25 mv; time, 25 msec.

During these experiments, i.e. with the stimulating electrode placed near the periphery of the preparation, it was observed that the action potential upstroke in a P fiber always preceded the upstrokes in nearby (>200 μ distant) V fibers by 1 to 2 msec. Similar delays have been observed by Matsuda (1960) and Alanis et al. (1961). This delay showed no significant increase with distance from the stimulating electrode. This observation also suggests the presence of isolated points of contact between the two systems of fibers.

Electrotonus at Long Distances The maximum distance at which electrotonic interaction was observed in V fibers was 800 μ. In P fibers electrotonic potentials could often be observed at distances of more than 1 mm. Fig. 6

shows the waveform of such a potential in response to a rectangular current pulse applied 3 mm away. The electrotonic potential shows the characteristic delay which is to be expected form cable theory (Hodgkin and Rushton, 1946) when the recording is made in a cablelike structure several length constants away from the site of application of current.

Although these results agree with those of Tarr and Sperelakis (1964) in showing that electronic interaction between pairs of V fibers is relatively rare, they also disagree with their finding since these authors did not obtain any "substantial" interaction between ventricular fibers of frogs and cats at dis-

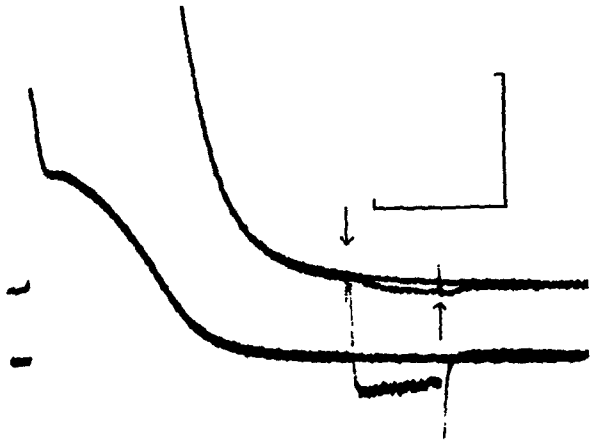

FIGURE 6. Demonstration of an electrotonic potential recorded in a P fiber, 3 mm away from the point of application of current. The figure is a superposition of two oscilloscope sweeps. Top rcording with SBME, bottom recording with DBME, both electrodes were located in P fibers. Arrows denote the make and break of the current pulse. The *dc* artefact on the DBME during the current pulse is not properly balanced out. Calibration, voltage, top trace 10 mv, bottom trace 50 mv; time, 100 msec.

tances greater than 45 μ. This difference can probably be explained by the different magnitudes of the polarizing current employed in the two cases.

Anode Break Excitation The termination of a hyperpolarizing current pulse during the latter part of the repolarization phase of a cardiac action potential is often followed by a self-regenerative anode break response. In the rabbit right ventricle this response was of two kinds, both of which are illustrated in Fig. 7. One response took the form of a fast depolarization followed by an approximately exponential decay; such a response declined rapidly with distance and was not associated with a visible contraction. The amplitude of this response was graded; it depended on the magnitude of the current pulse and the time of its cessation. The second type of response consisted of the fast depolarization just described, followed by a more or less de-

layed but all-or-none, plateau; this response was propagated throughout the preparation and was associated with a contraction. This response could only be obtained during the terminal phase of the action potential and both the magnitude of the current pulse and the timing of its termination were critical. If the current was too weak or was broken too early, only the first type of response was observed; if the current was too strong or was terminated too late, the anode break response was abolished entirely. Both types of response could be elicited in P fibers but the second type of response was never observed in a V fiber.

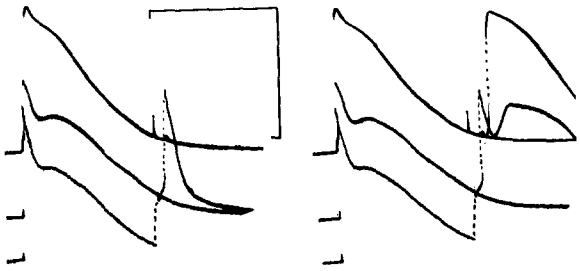

FIGURE 7. Two types of anode break response. Each half of the figure is a superposition of two oscilloscope sweeps. No current was passed during the first sweep, a hyperpolarizing current pulse, terminated in the middle of each record, was passed during the second sweep. The currents were of equal magnitude and were terminated at approximately the same times in the cycle. The top recordings were made with the SBME in a V fiber and the bottom recordings were made with the DBME in a P fiber about 400 μ away. No electrotonic potential displacement can be observed in the V fiber during current flow. On termination of the current pulse on anode break response is developed in the P fiber. When this response has the form of a single spike it has no effect on the V fiber; when the response has the form of a spike followed by a plateau, a full sized action potential is recorded in the V fiber. The upstrokes of the anode break responses have been retouched. Calibration, 100 mv, 100 msec.

An explanation of these phenomena is as follows: When hyperpolarizing current is applied during the action potential, a small region of the muscle is repolarized and its sodium system reactivated. If the applied current pulse is then broken, positive current flows into the repolarized region from its depolarized surroundings activating the sodium system and producing a local response. If, by the time the local response is produced, spontaneous repolarization has reached a stage at which conduction of an impulse is possible, the local response may develop into an ordinary propagated action potential. Sperelakis and Lehmkuhl (1964) have recently described anodal break responses in cultured heart cells.

A further observation was that anode break responses could never be elicited in the resting state except in fibers with obviously low resting potentials.

Histological Observations The PAS stain revealed no significant difference

in glycogen content of the fibers in the rabbit right ventricle (as compared with sheep Purkinje and ventricular fibers). With this stain it was not possible to distinguish two types of fibers.

With the Mallory stain, however, surface fibers differing in several respects from the bulk of the ventricular fibers were observed. Consistent differences between the two types of fibers are summarized in Table I. On the basis of

TABLE I

HISTOLOGICAL DIFFERENCES BETWEEN TWO TYPES OF FIBERS IN THE RABBIT VENTRICLE

Ventricular fibers	Surface fibers
Fibers are always straight	Fibers often form characteristic loops and interconnections, especially near the ends of trabeculae. The circumferences of the loops range from 100 to 400 μ
Fibers form the bulk of the ventricular wall	Fibers are always on the surface almost always running across the direction of the ventricular fibers
Striations always present	Striations were not observed in the looped portions of these fibers; in the straight portions the striations appear to be confined to a thin layer just inside the surface of the fibers leaving a cylinder of clear sarcoplasm inside
Profuse blood supply: close to one blood vessel per muscle fiber	No blood supply was observed
Dense packing of fibers with very little connective tissue in between	Loosely packed with abundant connective tissue between fibers

these findings it seems reasonable to conclude that the surface fibers described in Table I are identical with the electrophysiologically identified P fibers.

DISCUSSION

Displacement of the Crest of the Action Potential By analogy with other excitable fibers it is reasonable to suppose that the rising phase of the ventricular action potential is associated with at least a tenfold reduction in membrane resistivity. This implies a minimum threefold reduction in the length constant. Thus, during this period of lowered membrane resistivity a potential displacement measured one resting lenght constant away from the site of application of current, should be significantly reduced or even abolished. If the period of low membrane resistivity includes the crest of the action potential, or if the crest occurs no later than about one resting membrane time constant after the

drop in resistivity, then the measured action potential amplitude should increase when hyperpolarizing currents are used.

Johnson and Tille (1961 *a*) found no increase in the action potential amplitude at the site of application of current when hyperpolarizing current pulses of moderate magnitude were passed throughout the rising phases of V fiber action potentials. They concluded that the membrane resistance decreases briefly during the rising phase but returns to its resting value well before the crest of the action potential is reached. However, it could be argued that their result was due to the polarizing current not being injected into the fibers. This possibility can be discounted on the evidence of results such as that shown in Fig. 1. This illustrates a situation in which a large hyperpolarizing current pulse resulted in the appearance of a fast spike at the crest of the V fiber action potential at the site of the DBME. At the site of the SBME however, the displacement of the crest of the action potential is about equal to the resting displacement. Since in this experiment there is no possibility that the recorded potential is an extracellular artefact, it must be concluded that (*a*) polarizing current does enter V fibers and (*b*) the crest of a V fiber action potential is often displaced by as much as the resting membrane. The appearance of the fast spike at the crest indicates a drop in resistance but both this and the membrane time constant must be extremely brief to allow the membrane to be recharged by the polarizing current by the time the crest is reached. The duration of the depolarization phase of V fiber action potentials is characteristically 2 msec. This places a definite upper limit of 1 msec on the sum of the durations of the resistance drop and of the membrane time constant.

Electrotonic Current Spread Woodbury and Crill (1961) observed in rat atrial trabeculae that an electrotonic potential declines monotonically with distance from the site of application of current. This decline was somewhat faster in a direction at right angles to the fibers than along the direction of the fibers. This indicates that polarizing current applied at a point can spread freely from each fiber to all its neighbors. Because the fibers are densely packed such a system can be approximated in its properties by a thin disc of sarcoplasm bounded by two parallel membranes. This model was used by Noble (1962) as the basis for his explanation of the linear current-voltage relationships obtained by Johnson and Tille (1960) in the rabbit ventricle.

It is clear from the results presented in this paper that the properties of rabbit ventricular muscle are different from those of rat atrium. First, there is a surface network of P fibers with only very sparse resistive interconnections with the V fibers comprising the bulk of the ventricular wall. Both the histological and the physiological evidence indicate that the P fibers form a loose network; they certainly do not form a continuous two dimensional surface over the muscle. The two most commonly observed forms of the P fibers were long unbranching strands of up to ten parallel fibers and extensive two dimen-

sional networks of loops. Thus the relationship between the polarization resistance, R_p, and membrane resistivity R_m, in these fibers could be expected to be of the cable form, i.e. $R_p \alpha R_m^{0.5}$, in the long strands; and to be of the "closed syncytial" form (George, 1961), i.e. $R_p \alpha R_m^{0.25}$, in the looped region.

Second, the results obtained in V fibers show that these fibers are also different from rat atrial fibers. The small probability of obtaining an electrotoinic interaction at small distances and the fact that the electrotonic potential does not decline monotonically with distance favor the conclusion that the V fibers do not form a single freely interconnected syncytium. These fibers may be arranged in small bundles or network systems with many resistive connections within each system, but with few interconnections between the systems.

In order to determine the relation between R_p and R_m, therefore, it will be necessary to determine the spread of current from a single point source. If the fibers are arranged in bundles, the system may approximate closely to the cable form and the current spread should be mainly in one dimension. If, on the other hand, the fibers are arranged in a number of relatively isolated networks, the "disc" model may be more applicable (although it would have to be applied to each network separately and not to the whole ventricle). In this case the spread of current from a single point source would be two or three dimensional. Unfortunately, it is difficult to distinguish between these possibilities experimentally since it is not easy to keep an intracellular current electrode in one cell while a large number of observations are made. However, the present results do show that the disc model may be inapplicable to the rabbit ventricle, although further experiments will be required to settle this question with certainty.

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