Electrophysiology of an Insect Heart

FRANCES V. McCANN

From the Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire

ABSTRACT Bioelectric activity in single cells of the moth myocardium has been measured *in situ* with intracellular microelectrodes with particular reference to the bizarre ionic medium which bathes the tissues. Resting potentials averaged 47 mv, inside negative with respect to outside, despite a value of 11 mv calculated on the basis of transmembrane potassium concentration gradients. Action potentials overshoot as much as 12 mv in the virtual absence of extracellular sodium. Two "types" of action potentials have been recorded; one that resembles vertebrate atrial action potentials is found in the cephalic region of the tubular heart, and the other, similar in contour to vertebrate ventricular action potentials, is found in the areas posterior to the first abdominal segment. Histological sections indicate no structural differences between the two areas. Typical cardiac pacemaker type potentials occur but are not topographically localized. The effects of the omission from the perfusion fluid of the four major cationic constituents, Na^+, K^+, Ca^{++}, and Mg^{++}, on resting and action potentials may be summarized as: no effect, hyperpolarization, prolonged repolarization, and depolarization, respectively.

INTRODUCTION

The myocardium of the adult moth is uniquely suited for studies of a fundamental nature regarding cardiac excitability because of its primitive nature, its bizarre ionic medium, and its periodic reversal of direction of heart beat. The heart, a thin-walled, tubular structure composed of striated muscle, extends from the thorax along the middorsal line of the abdomen and ends in a bulb-like expansion in the anterior portion of the ninth abdominal segment. The heart is without chambers, valves, or histologically differentiated tissue; *e.g.*, nodal or conducting tissue. Eight pairs of ostia open along the tubular structure serving as channels through which the blood flows into and out of the heart. It is generally agreed that the blood plays no major role in respiration. The pulsations of the heart distribute the blood so as to bathe the tissues directly as it courses through an "open" circulatory system. In view of the apparent histological homogeneity, it is suggested that this heart may serve as a biological simplification of the more complex vertebrate myocardium and a system in which fundamental problems of cardiac automatism might be studied.

The hemolymph has been shown to contain negligible amounts of Na^+, and quantities of K^+, Mg^{++}, and Ca^{++} sufficiently large so as to be considered toxic and even lethal to vertebrate tissue (4, 5, 26). Since this presents a naturally occurring system in which Na^+ is virtually absent, an investigation of the electrical activity takes on special significance.

A third distinguishing pecularity of the moth heart is the phenomenon of "beat-reversal." Periodically the direction of beat, which is usually from caudal to cephalic, reverses its direction, and the beat is initiated in the cephalic region and propagates toward the caudal region. Reversal may occur in many different time sequences (26), but when the beat has been reversed, the frequency of beat is slower (11) and the conduction velocity is also slowed (26).

This report describes the normal electrical activity in single cells of the moth myocardium and the influence of cation removal on bioelectric activity.

METHODS

1. *Electrical Recordings*

Electrical activity in individual cells was recorded by means of conventional microelectrodes, less than 0.5 μ outside diameter, filled with 3 M KCl. Electrode resistance ranged from 20 to 30 MΩ. Microelectrodes were coupled to a dual-beam Tektronix 502 oscilloscope through transistorized negative capacitance amplifiers. Stimulation was carried out by means of a Grass S-4A stimulator with isolation unit through chlorided silver wires. Permanent records were obtained by photographing the screen of the oscilloscope with a Grass kymograph camera.

2. *Moths*

Adult moths of the species *Telea polyphemus* were used exclusively in the preliminary studies since the ionic composition of the hemolymph had been previously analyzed (4). Due to difficulties in obtaining large numbers of viable adults, it was necessary to use two other species; *viz.*, *Samia cynthia* and *Samia cecropia*. Analyses were made of the Na^+ and K^+ content of the hemolymph of these two species and these were found to approximate those of *Telea polyphemus*. In this instance species differences were not reflected in the magnitude and time course of normal electrical potentials.

3. *Physiological Supporting Medium*

A solution composed of ionic species according to analytically determined amounts does not support the tissue in a viable state for prolonged periods of time. A solution modified from that used by Bélar was found to maintain the *status quo* of the electrical activity of the heart (26). The final solution was composed of 1.9 mM Na^+, 45 mM K^+, 5 mM Ca^{++}, and 25 mM Mg^{++}, pH adjusted to 6.8. No effect was produced by the addition of organic constituents, *viz.*, glutamine, glycine, or glutamic acid.

4. *Preparation*

All preparations were *in situ* and maintained at room temperature, about 22°C. The moth was decapitated, and the major nerve center, the mesothoracic ganglion, was destroyed. It was then pinned down, ventral side up, and a ventral midline incision was made extending from the caudal tip of the abdomen to the midthoracic region. The visceral material was carefully removed and the heart thus exposed. The dense tracheolar network was carefully pulled aside from the surface of the heart but attachments were left intact as far as possible. Areas where the alary muscles were attached to the myocardium were generally avoided so that the intracellular action potentials recorded would not be from muscle cells which are not primarily myocardial in structure and function.

RESULTS

1. *Histology*

Studies of teased fibers by polarized light microscopy reveal that the myocardium is composed of striated muscle fibers, with banding patterns such as have been described for striated muscle (17), arranged in overlapping layers and oriented at right angles to each other. There appear to be two such layers, giving the wall a depth of two cells, and this is also demonstrated by the intracellular electrode studies. The fibers are not arranged in as orderly a fashion as in the vertebrate myocardium, but branching is apparent. The fibers are long, about 30 μ in diameter, multinucleate, with a well defined sarcolemma, and are oriented in spiral fashion. Cell boundaries are indistinct and structures similar in appearance to intercalated discs cannot as yet be identified with certainty.

2. *Resting Potential*

In accordance with the Hodgkin-Huxley model of membrane electrical behavior, it has become conventional to calculate the magnitude of the resting and action potentials of an excitable cell by substituting the numerical values of intracellular and extracellular K^+ and Na^+ concentrations, respectively, into the Nernst equation (12). Table I presents a comparison of the predicted magnitude of such potentials from a mammalian cell and from an herbivorous insect (adult moth). Since the ionic composition of insects often reflects their dietary intake, there is great variation in the values obtained. It is, however, consistent with those insects whose diet consists almost exclusively of one particular plant that the ionic concentrations are of these magnitudes (5, 8, 9, 26).

The resting potential of the adult moth heart, measured with an intracellular microelectrode, averages −47 mv, but values as large as −53 mv

have been recorded. These values were obtained by placing the silver-chlorided ground lead directly on the surface of the heart as close to the recording electrode as possible. At this time, no artificial bathing solution was used, the tissue being immersed in its own hemolymph. The balanced salt solution that was used in later experiments was tested for its suitability by adding it to a preparation and noting that the bioelectric potentials were not altered in magnitude or time course. This empirical value, some 4 times

TABLE I

THE NERNST EQUATION FITTED WITH IONIC CONCENTRATIONS INSIDE (i) AND OUTSIDE (o) MAMMALIAN AND INSECT CELLS

Mammalian values from Hoffman and Cranefield (13), insect data from Carrington and Tenney (4).

	Insect Moth 20°C		Mammal Cat 37.5°C	
	Hemolymph* o	Tissue‡ i	Plasma* o	Tissue‡ i
K^+	54	88	4.8	151
	$\frac{[K_o]}{[K_i]} = 0.61$		$\frac{[K_o]}{[K_i]} = 0.032$	
	E_K§ $= -11.6$ mv		$E_K = -92.6$ mv	
Na^+	2.5	20	159	6.5
	$\frac{[Na_o]}{[Na_i]} = 0.13$		$\frac{[Na_o]}{[Na_i]} = 25$	
	$E_{Na} = -52.5$ mv		$E_{Na} = 84$ mv	
	$\frac{Na^+}{K^+} = 0.046$ $\frac{Na^+}{K^+} = 0.22$		$\frac{Na^+}{K^+} = 33$ $\frac{Na^+}{K^+} = 0.043$	

* meq/ liter.

‡ meq/ kg weight tissue.

§ E_K, for example, is calculated from the Nernst equation, $E_K = (RT/F) \ln ([K_o]/[K_i])$.

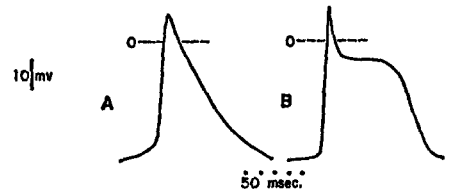
larger than that predicted on the basis of the potassium concentration, corresponds more closely with that predicted on the basis of a Na^+ potential (-52 mv) than with the K^+ value (-11.6 mv).

3. *Action Potentials*

1. MAGNITUDE

The hemolymph normally is virtually devoid of Na^+. The concentration is so low that the values obtained approach the limits of accuracy of the analytical

technique employed (4). No correction was introduced for the extracellular space. However, data obtained from an analysis of the Na^+ content allow a calculation of the predicted potential as shown in Table I. Since the ratio of $\frac{[Na_o]}{[Na_i]}$ is less than one, the predicted sign of the potential due to Na^+ would be negative, that is, the ratios of both $\frac{[Na_o]}{[Na_i]}$ and $\frac{[K_o]}{[K_i]}$ compartments are in the



Resting Potential	(mv)	−47.0	−47.0
Action Potential	(mv)	56.5	59.5
Overshoot	(mv)	9.5	12.5
Rise Time	(msec.)	69.5	48.6
Maximum Rate Rise	(mv/sec.)	601	980
Fall Time	(msec.)	472	665
Maximum Rate Fall	(mv/sec.)	96.0	71.5
Duration	(msec.)	610	750

FIGURE 1. A quantitative description of the two types of action potentials recorded in the moth heart. Only the potential on the left, *A*, is observed in the first anterior abdominal segment, while the one at the right, *B*, is found in the regions posterior to the first segment. This and the following figures are tracings of original photographic records.

same direction, greater than one. Action potentials measured with intracellular electrodes exceed the resting potential by 3 to 12 mv, reaching a maximum amplitude of 65 mv.

2. CONTOUR

A slow depolarization and a period of prolonged repolarization resulting in a "plateau" are evident in myocardial cells of the moth heart. Despite the absence of morphological specialization at least two types of action potentials occur (Fig. 1). These types differ mainly with regard to the slope of the slow repolarization phase. Action potential type "A" is the only type that occurs consistently within the limits of the first abdominal segment. Occasionally it can also be demonstrated at scattered sites throughout the myocardium. At the present time it can be localized to the first abdominal segment but cannot be excluded from other areas. This potential appears similar in contour to that described for atrial muscle in vertebrate hearts (22). Type B is found

throughout the myocardium except in the first segment. It is observed in many modified forms; *i.e.*, at times there is a marked slowing of the initial phase of the upstroke indicating a pacemaker component (Fig. 2, upper trace). Spontaneous alterations in the time course of these potentials occur frequently and give the impression that contours resembling those described for various delineated areas of the vertebrate heart are present. An added bit of caution is warranted in evaluating and assessing such influences here. It can be said that all the action potential types described for the vertebrate myocardium have been observed in this heart, but at the present time they can be assigned neither to specific locations nor to specialized functions. In this regard it is of

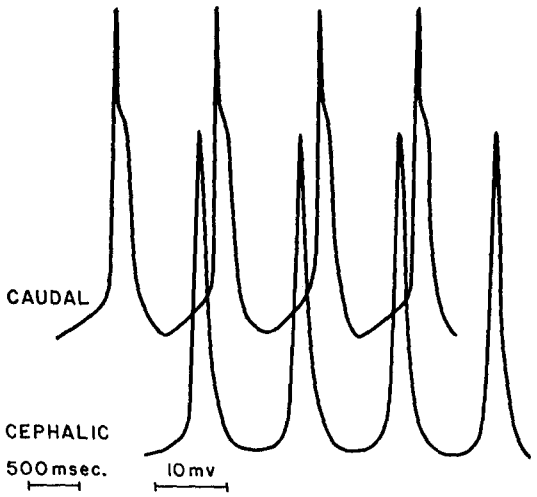


FIGURE 2. The two types of action potentials recorded simultaneously from the caudal and cephalic regions of the moth heart. The action potentials in the caudal region display a pronounced pacemaker component during this particular recording; however, this is not a localized event, and either of the two types may exhibit the slow initial rise indicative of pacemaker activity.

special note that a pacemaker component can be observed in all the types, suggesting that any area or, more specifically, any fiber can initiate activity anywhere in the myocardium. This would support the thesis that cells need not be morphologically modified in order to exhibit the property of automatic activity.

Fig. 1 includes a description of the two types of action potentials and a quantitative evaluation of the voltage and time course. The values are representative of the potentials measured. It will be noted that the time course is not markedly different from that given for vertebrate tissues, except that the rate of rise is extremely slow. The average maximum rates of rise of 0.8 v/sec. and 1.2 v/sec. for the two types of potentials (Fig. 1) are exceedingly small as

compared with magnitudes such as 350 v/sec. for mammalian myocardial fibers, or even 50 v/sec. for frog and turtle ventricular fibers (13).

The relationship between action potentials recorded from the cephalic and caudal regions simultaneously is shown in Fig. 2. In this instance the beat originated in the caudal region (upper trace); a pacemaker component is evidenced by the initial slow rise. The caudal action potential thus precedes the cephalic. This precedence does not always occur, as the conduction velocity is so low that even though a cell is acting as pacemaker, a cell distant

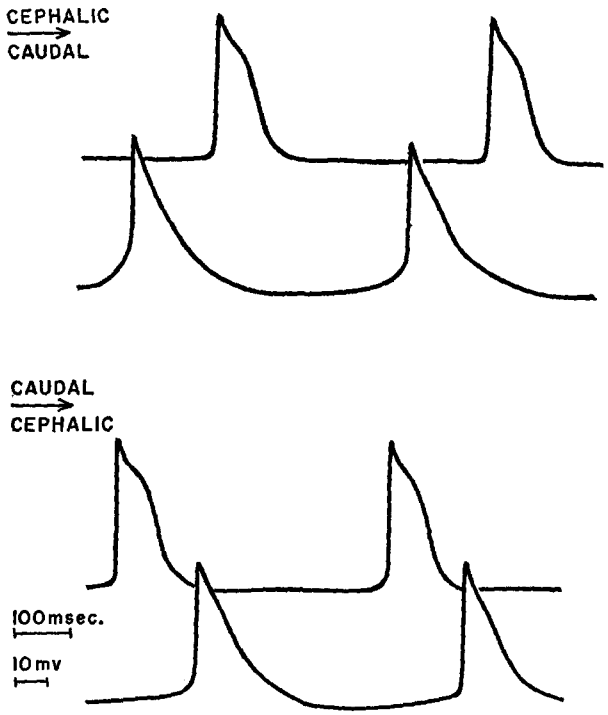


FIGURE 3. Beat reversal. The top pair of traces shows the beat originating in the cephalic region and passing on to the caudal region; the reverse occurs in the two lower traces. The contour of the action potential does not change with beat reversal.

to it may be invaded by an impulse originating at an ectopic locus causing it to reach threshold before the pacemaker reaches its maximum depolarization. Such a phenomenon has been observed in the mammalian A-V node and is interpreted as a consequence of delayed conduction (13). The linear conduction velocity in the moth heart is exceedingly low, ranging from 20 to 10 mM/sec.

That the action potentials from the cephalic and caudal regions exhibit distinctive shapes is evident from Fig. 2, and these do not change when reversal of the direction of conduction has occurred. Fig. 3 illustrates the

action potentials recorded from single cells in the caudal and cephalic regions simultaneously during a period of beat reversal. The top pair of traces shows the sequence of activity as the beat originates in the cephalic region and then arrives at the electrode in the caudal region. The lower two traces show the initiation and progression of the impulse in the reverse direction. There is no marked alteration in the time course of the potentials as reversal occurs. This suggests that the mechanism which initiates and controls the process of reversal is not ionic in nature; if it were, one might expect some rather dramatic shifts in rates of rise or fall. The complex geometrical arrangement of the cardiac muscle fibers undoubtedly allows for the reinvasion of partially depolarized cells, which effectively disturbs the normal course of the peri-

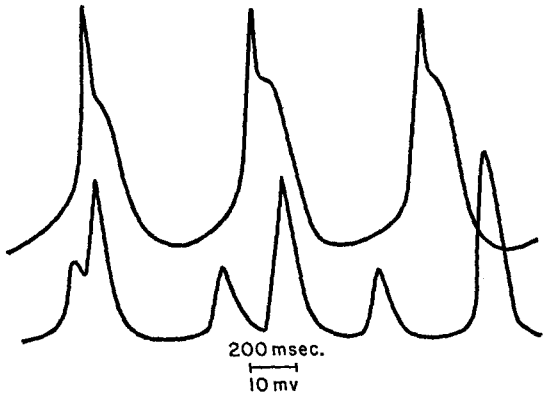


FIGURE 4. Two microelectrodes recording the electrical activity in the caudal region (top trace), and the cephalic region (bottom trace). In this recording the pacemaker component in the top trace is not as pronounced as in Fig. 2, nor is the action potential conducted to the anterior segment, as can be seen in the disturbance of the normal rhythmic arrival of the impulse from one end to the other, and the appearance of small, slow transient potentials which occur during different times of the beat cycle.

staltic wave. Fig. 4 illustrates the complex type of potential recorded from the cephalic region (lower trace) during a dual recording in which the action potentials of the caudal region do not arrive at the cephalic region in a 1:1 sequence.

The unusual ionic composition of the hemolymph makes the moth heart a singularly attractive tissue on which to test the contribution of various ion species to the generation and maintenance of bioelectric potentials. In the following experiments the heart was bathed in a fluid (*cf.* Methods) in which, alternately, a single cation species was omitted. The chloride compartment was maintained at normal levels by the addition of choline chloride. Four solutions were tested; *viz.*, sodium-free, calcium-free, potassium-free, and magnesium-free. Photographic records were taken at 5 minute intervals over

a period of 2 hours. The activity recorded was all spontaneous. In order to eliminate differences in electrical activity which might occur normally from fiber to fiber, it is imperative that *each fiber must serve as its own control.* Each experiment was, therefore, conducted on one fiber, *i.e.* the electrode impaled a cell, and was maintained in that same cell throughout the experimental period, which included full recovery to the original level of activity. Only those experiments in which the electrode could be successfully retained over this period are reported.

A. *Omission of Na^+*

A 2 hour perfusion of the heart with a solution containing choline chloride substituted for NaCl, altered neither the magnitude of the resting potential nor the magnitude or time course of the action potential in a total of twelve experiments. Replacement of NaCl by an osmolar equivalent of sucrose did not influence these quantities. There was no disturbance of the electrical activity by the addition of ouabain observed over a period of 30 minutes, a treatment believed to attack specifically the sodium pump mechanism of cardiac tissue (23, 24).

B. *Omission of K^+*

The omission of K^+ from the bathing solution produced an alteration of the electrical activity as shown in Fig. 5 *B-D*. The series shown in *A* depicts normal activity, where the interval between beats is 350 msec. Hyperpolarization of the membrane accompanied these changes so that in the normal condition (*A*) the membrane potential was 35 mv, 1 hour after perfusion with K^+-free solution (*B*) it was 36 mv, and 2 hours after perfusion (*C*) it was 40 mv. The increase in threshold, *i.e.* identified here as that point during the depolarization event that marks the abrupt upstroke of the action potential, and the slowed rate of pacemaker depolarization effect a slowing of the heart rate, increasing the interval between beats from a normal of 350 msec. to 700 msec. after a 2 hour period.

The addition of normal amounts of K^+ to the bathing medium restores the heart to normal activity within a period of 15 minutes. If, now, the potassium compartment is again withdrawn, the response of the heart to this condition is much faster than previously, the same pattern of slowing becoming evident after only 10 minutes. Repeating this procedure a third time produces an almost immediate alteration in the electrical activity as seen in *D*, where the first two action potentials represent the normal response, and as K^+-free solution is readded, the generation of the action potentials is abruptly abolished and only a subthreshold oscillation is seen. The repeated withdrawal and replacement of potassium appears to "sensitize" the membrane to the

loss of this cation in such a way that while the changes in *B* and *C* occur over a period of 2 hours, those in *D* occur immediately.

C. *Omission of* Ca^{++}

Omission of Ca^{++} from the bathing fluid produced a marked slowing of the heart by prolonging the period of repolarization. Fig. 6 *A-E* illustrates the action potential of a single cell over a period of 2 hours' exposure to a Ca^{++}-free bathing solution. There is a small loss of resting potential, but the effect on the time course of the action potential is pronounced.

In addition to the marked slowing of the heart beat frequency the beat itself shows an alteration in the mechanical response. The beat appears double, the first portion being more forceful while the second portion is weaker and of shortened duration. The electrical expression of this response is illustrated in

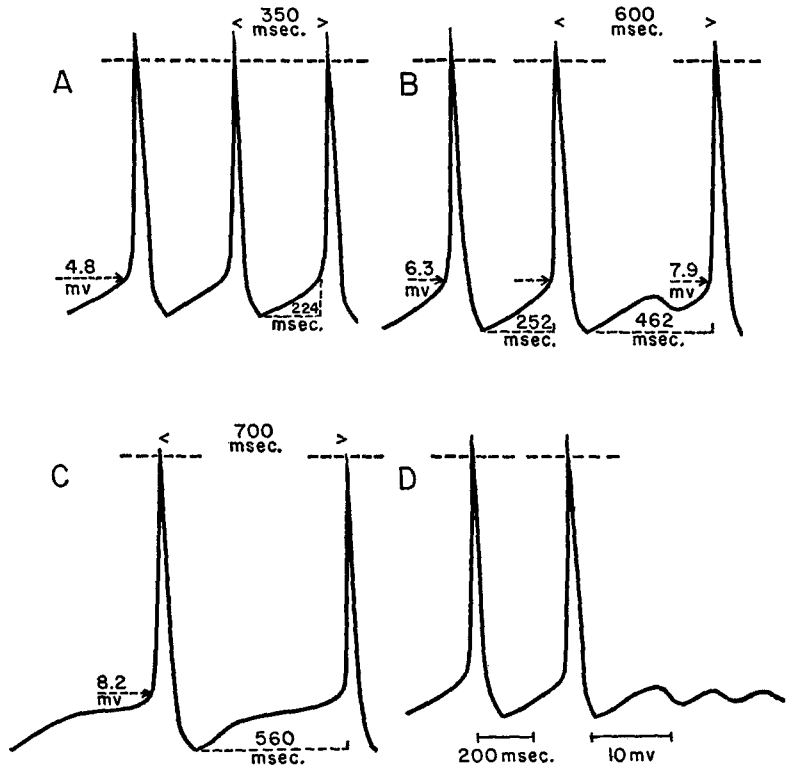


FIGURE 5. The effect of potassium omission on the electrical activity in a single cell of the moth heart. *A*, normal control, *B* and *C*, 1 and 2 hours, respectively, after perfusion with a potassium-free solution. The replacement and subsequent withdrawal of potassium effects the changes seen in *D*, where the spike-like portion of the action potential is obliterated and only subthreshold activity remains.

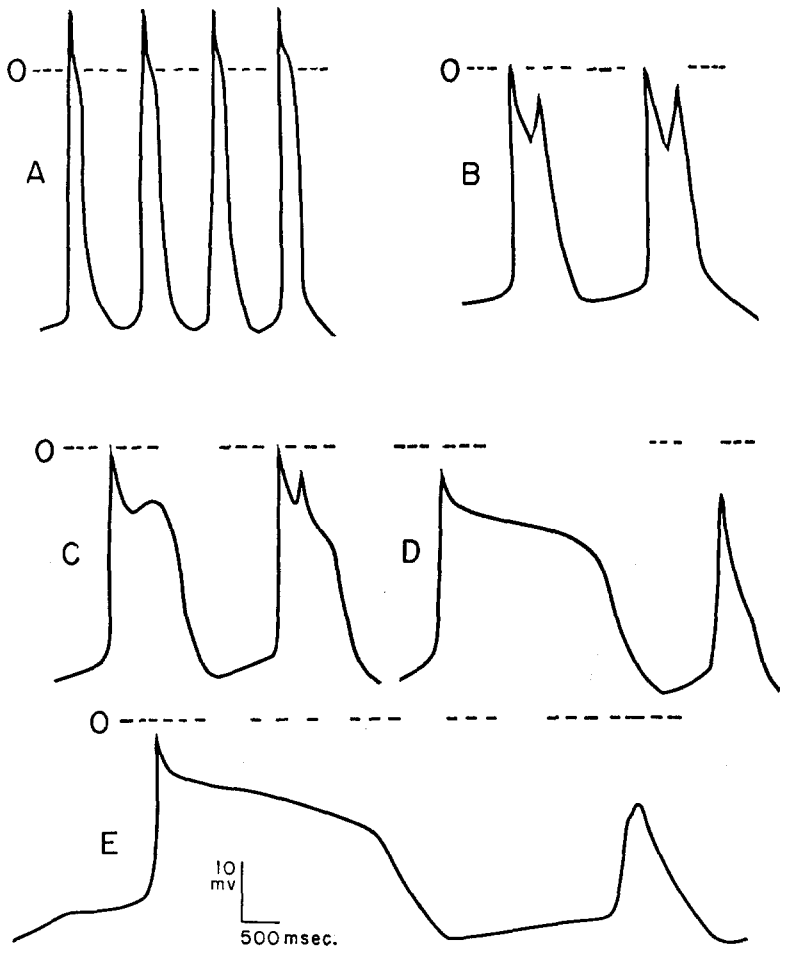


FIGURE 6. The omission of calcium from the bathing solution produces a marked slowing of the heart rate by slowing the repolarization phase. *A*, normal control, *B*, after 30 minutes' perfusion with a calcium-free solution, *C*, after 1 hour, *D* and *E*, after 1½ and 2 hours, respectively. Note the appearance of two potentials of different contours, *D* and *E*.

Fig. 6 *D* and *E*. As normal solution is readded, recovery proceeds stepwise through the observed intermediate action potential types.

D. *Omission of* Mg^{++}

Omission of Mg^{++} from the bathing solution produced an increase in the frequency of the heart rate and a loss of resting polarization which is nearly complete after a period of 2 hours, in a total of eleven experiments. Fig. 7 *A-D* illustrates the effects of Mg^{++} removal on the resting and action potentials over a period of 2 hours. The normal control potentials shown in *A* exhibit a

slow pacemaker type component, the slope of which gradually increases (*B*, *C*) as magnesium ion is withdrawn. The initial increase in frequency is accompanied by a decrease in the time for repolarization; *i.e.*, the plateau becomes markedly diminished (*B*). The rate continues to increase (*C*) as the diastolic phase is acutely reduced, and the membrane resting potential has become reduced to almost half of its original value. The heart rate continues to increase, contractions are uncoordinated and feeble, and depolarization becomes almost complete (*D*). After 3 hours of perfusion, the resting potential

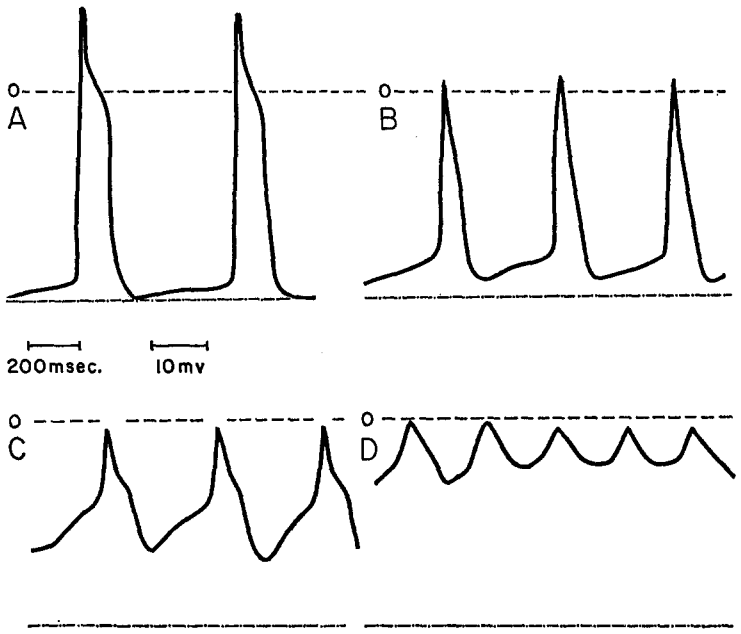


FIGURE 7. The effect of Mg^{++} omission on bioelectric activity. *A*, normal control, *B*, after 1 hour's perfusion with magnesium-free solution, *C*, after 90 minutes, and *D*, after 2 hours. Dashed line represents zero potential, lower line indicates original level of resting polarization.

is diminished to about 5 mv, and action potential activity ceases. Further perfusion results in an irreversible loss of all electrical activity.

The restoration of Mg^{++} to the bathing solution is accompanied by a restitution of resting and action potential activity. While replacement of the other ion species resulted in the reestablishment of the normal electrical potentials through a stepwise reversion of the intermediate effects, the replacement of Mg^{++} produced intermediate effects not seen as Mg^{++} was progressively removed. Fig. 8 *C*, *D* illustrates the recovery of activity observed when Mg^{++} was readded to the bathing medium. The potentials in *A* represent normal activity before Mg^{++}-free solution was applied. The potentials in *B*

are the remnants of activity after 2 hours' perfusion with a Mg^{++}-free solution. Recovery is recorded in *C* as, after one-half hour, the membrane potential has become partially restored. As the original level of polarization is restored, the action potential displays a burst of spike-like responses (*D*), which interrupt its repolarization phase. As the falling phase of the spike-like action potential approaches closer to the original resting level of polarization, the individual repetitive responses increase in amplitude. Magnesium, therefore, appears to play a major role in the maintenance of the level of polarization and excitability of the cardiac cell with consequent influences on the heart rate.

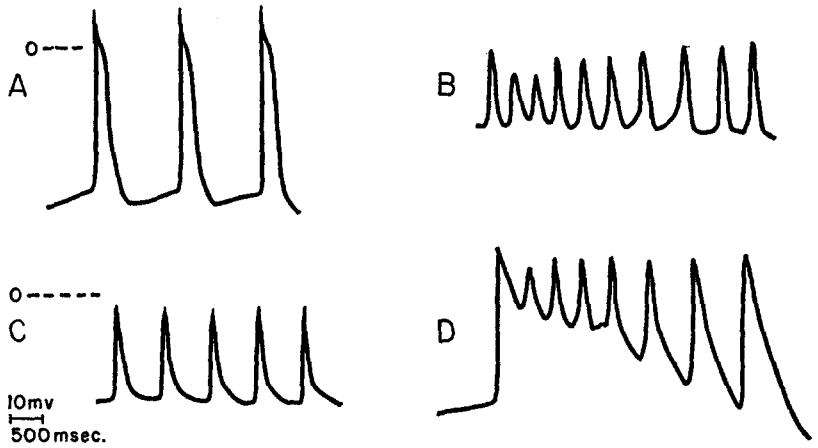


FIGURE 8. The sequence of recovery of electrical activity following perfusion with magnesium-free solution. *A*, normal activity, *B*, potentials remaining after 2 hours' perfusion with magnesium-free solution. *C*, partial recovery of a stable resting potential and regular beat, *D*, the abrupt reappearance of spike-like responses.

DISCUSSION

Several questions fundamental to our understanding of the phenomena of automatism and bioelectricity may be examined in the light of this study.

The hearts of higher animals attain a degree of organization such that bioelectric potentials of different contours can be topographically localized to morphologically and functionally distinct tissues. Thus, action potential types are associated with atrial, nodal, conducting, and ventricular tissues (7, 13, 22). Pacemaker activity is localized to nodal or specialized conducting tissue, and is identified as an initial slow depolarization occurring just prior to the rapid upstroke of the action potential. When disturbances of rhythm occur, the site of origin of ectopic foci is generally regarded as being in some area of the "specialized" system of the myocardium. That atrial or ventricular muscle itself can initiate spontaneous activity remains to be conclusively demonstrated (13).

The morphologic complexities encountered in the hearts of higher forms are not present in the adult moth heart. Although the muscle tissue is arranged in a complex interlacing network, the tubular structure consists of striated muscle from one end to the other, and there is nothing, at least at the present time, to suggest that one end is morphologically distinct from the other. It is surprising, therefore, that a heart which is structurally so primitive does, nevertheless, generate action potentials whose contours resemble those generally associated with the myocardia of higher animals. Not only is the long plateaued action potential so characteristic of ventricular tissue present, but also there are potentials similar in contour to those associated with specific areas of the vertebrate myocardium.

Potentials similar to pacemaker potentials can occur during the initial phase of any of the action potential types in the moth heart. Thus, pacemaker activity is not localized to specific areas or tissues. The evidence presented in this study indicates that the moth heart generates bioelectric potentials similar in contour to those identified with specific areas of the myocardia of higher animals in the absence of visible tissue differences. A question to be re-examined, therefore, is how is individual fiber specialization expressed? It appears from these studies, at least at primitive levels, that it transcends the morphological level and may be in the still dark areas of electromechanical coupling or the contractile event itself. Such a localization has been suggested in studies of the automatic activity encountered in insect flight muscle (20).

Since, at the present time, there is no cellular morphological basis on which to assign action potential types to specific locations, the question arises whether the shapes of the action potentials are determined not by location but by the role the cell plays at a particular time. It seems that in this heart a single muscle fiber functions in a number of capacities, *i.e.* pacemaker, conduction, and/or contraction and that the performance characteristics of the cell, expressed in its electrical activity, might be altered during the process of reversal.

Recording from the two ends of the heart simultaneously dispelled this notion. The contour of the action potential does not change during beat reversal (*cf.* Fig. 3). The factors initiating and controlling beat reversal are not understood, but it has been inferred by recording conduction velocities during cephalic to caudal transmission (26) that a "preferred direction" of conduction also is incorporated into this seemingly homogeneous muscle mass.

Another area of fundamental importance involves the generation and maintenance of bioelectrical activity in the presence of ionic concentrations quite bizarre by vertebrate standards. The ionic requirements for the generation of bioelectric activity have been set forth in the basic equations of the Hodgkin-Huxley hypothesis (12), and have been extended to include cardiac muscle fibers (2, 21). An extension of this approach to the insect heart poses

several interesting considerations. Sodium, and ion recognized as playing a major role in the production of the upstroke and overshoot of the action potential, appears to contribute little if anything to these events in this tissue. Evidence for the existence of non-sodium requiring systems has been presented for crayfish muscle (10) and even for guinea pig auricle (6), but further interpretation as to the significance of these data has not been forthcoming. The moth heart presents a naturally occurring system in which the sodium gradient is opposite to that found in the vertebrates (*cf.* Table I), and indeed the concentration of extracellular sodium is so small as to be barely detectable.

The calculated value of 52.2 mv arrived at by using the $\frac{[Na_o]}{[Na_i]}$ in the Nernst equation could, quantitatively, at least, account for the empirically determined resting potential. However, if sodium were responsible for this parameter, then one could predict that manipulation of the sodium concentration would directly alter the resting potential. Perfusion with a sodium-free solution over a period of 2 hours does not influence the electrical activity. Action potentials overshoot as much as 12 mv, enough to be considered significant in the absence of this ion. A further point to be considered is whether ion depletion can be carried out effectively; *i.e.*, 2 hours may be too short a period to alter the transmembrane ratios. Here it would seem that if sodium is to be an effective carrier of current, it must move readily and this would preclude the possibility of its being too tightly bound intracellularly to be effectively depleted.

The presence of a Na^+ pump mechanism may also be questioned. Application of the cardiac glycoside ouabain in concentrations of 10^{-7} and 10^{-6} M did not alter electrical activity when observed for a period of 30 minutes. Since ouabain is believed to function as an uncoupler of oxidative phosphorylation (23, 24), sodium extrusion would be halted, and this event would be expected to be expressed in some alteration in the electrical activity at the membrane. From the foregoing it is concluded that sodium is not the major ion species responsible for carrying current in this system, and there is probably no metabolically active "pump" to extrude intracellular sodium ions.

The maintenance of electrical activity and excitability in these tissues in which extracellular potassium concentrations exceed the limits of tolerance by vertebrate systems has provoked two alternative hypotheses. One invokes the presence of a tissue sheath which acts as a barrier to diffusion (15, 27), the other proposes that ions are not freely ionized but rather are bound probably to the large organic anions which are known to be present in large quantities (5). Therefore, the concentrations of ions measured are not physiologically active.

The presence of a protective sheath seems an untenable hypothesis to explain the events in this system. If the excitable integrity of the tissues were to be dependent on the exclusion of the ions in the extracellular fluid, it would be necessary to interpose such a barrier around every muscle fiber and its end-plate to make the isolation complete. Although there is an adventitia enclosing the moth heart which could conceivably act as such a sheath, it does not pervade the inside of the heart as a lining. Since the fibers inside of the heart are exposed to the same ionic concentrations as those muscle fibers lying under the adventitia at the outer surface of the heart, it is unlikely that a sheath is present or functions in this capacity. The possibility of a selective ion barrier has been examined in the case of the stick insect *Carausius* but here again evidence has not supported its presence (29).

The question of ion binding remains unsettled. Ideally, the composition of a balanced salt solution should mimic that found as the normal bathing medium. However, if such a solution, *i.e.* one containing salts in the same concentrations as analytically determined plus a variety of amino acids, is placed on the preparation, it will not support the tissue in a physiologically unaltered state. The addition of several amino acids, glutamine, glutamic acid, and glycine at separate times, did not offer effective binding sites so as to ameliorate the toxicity of the ionic concentrations. The discrepancy between the magnitude of the resting potential as empirically determined and that predicted by inserting the $\frac{[K_o]}{[K_i]}$ values into the Nernst equation is without explanation at the moment.

The depletion of calcium results in an alteration of excitability very similar to that seen in specialized regions of vertebrate hearts despite the relatively large concentrations present in the insect hemolymph. The prolongation of the plateau is strikingly similar to that described for frog ventricle (28) and dog papillary muscle (14), while a slight exaggeration of the initial slow depolarization may be noted. Here, as has been suggested for dog papillary muscle (14), calcium does not directly control the resting level of polarization but rather regulates, through a control mechanism, the permeability characteristics of the membrane.

The reaction of the heart to magnesium depletion is one of almost total depolarization. Although magnesium depletion is generally regarded as being innocuous to the maintenance of rhythm and electrical integrity of mammalian hearts (25), and even of moth heart (1), the omission of magnesium from the bathing solution produces a marked effect on the polarization and consequent excitability of the myocardial cell. The diminution of magnesium ion in the extracellular compartment results in a depolarization which will proceed to

an irreversible loss of electrical and mechanical activity if treatment is prolonged beyond 2½ hours. The excitability of the cell is thus directly influenced by the presence of magnesium ions. It must be recognized that the regulation of tissue excitability is not mediated through a single ion species, but rather by a complicated interplay as a result of interionic influences whether these be antagonistic or complementary. It is indeed difficult to perceive how a large divalent cation such as magnesium could serve as the major carrier of current. More likely it acts not so much as a primary carrier of current but rather as an agent antagonistic to or regulative of those ions capable of functioning in this role. Such a role for divalent ions has been suggested in crustacean fibers (10).

The participation of anions in the generation and maintenance of bioelectric activity remains to be studied in this system. The influence of anions on electrical activity in the mammalian heart has recently been reviewed (3). Chloride ions contribute increasingly to membrane conductance as depolarization ensues in mammalian hearts, and may thus play a significant role in bioelectric activity (16). Chloride ion, however, is not recognized as the major anion present in insect hemolymph (4). Data from experiments in which sodium chloride was either simply omitted from the bathing solution or was replaced with an equivalent amount of choline chloride in order to maintain the normal chloride concentration indicate that electrical activity is unaltered.

In view of the foregoing we may conclude that the major current-carrying ion remains to be identified, but its effectiveness is greatly influenced by the presence of magnesium ions. This system is unlike most other systems so far studied, in that sodium ion is not the major current carrier as described by the Hodgkin-Huxley hypothesis, nor is the resting potential determined primarily by the intra-extracellular concentrations of potassium ion.

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