The Ionic Basis of Electrical Activity in Embryonic Cardiac Muscle

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ABSTRACT The intracellular sodium concentration reported for young, embryonic chick hearts is extremely high and decreases progressively throughout the embryonic period, reaching a value of 43 mM immediately before hatching. This observation suggested that the ionic basis for excitation in embryonic chick heart may differ from that responsible for electrical activity of the adult organ. This hypothesis was tested by recording transmembrane resting and action potentials on hearts isolated from 6-day and 19-day chick embryos and varying the extracellular sodium and potassium concentrations. The results show that for both young and old embryonic cardiac cells the resting potential depends primarily on the extracellular potassium concentration and the amplitude and rate of rise of the action potential depend primarily on the extracellular sodium concentration.

Evidence from many electrophysiological studies indicates that the ionic basis for the transmembrane resting and/or action potentials of adult cardiac tissues from a variety of mammalian and nonmammalian species (Overton, 1902; Draper and Weidmann, 1951; Weidmann, 1955; Weidmann, 1956; Délezè, 1959; Brady and Woodbury, 1960; and Seyama and Irisawa, 1967) is qualitatively similar to that described for the giant axon of Loligo (Hodgkin and Katz, 1949; Hodgkin, 1951; Hodgkin and Huxley, 1952 *a*, *b*) and the frog skeletal muscle (Nastuk and Hodgkin, 1950). Nevertheless, in several studies on cardiac fibers the magnitude of the active membrane potential (overshoot) did not vary as predicted from the sodium equilibrium potential. In both guinea pig (Coraboeuf and Otsuka, 1956) and frog ventricle (Van der Kloot and Rubin, 1962) a large positive overshoot persisted after prolonged perfusion with solutions essentially free of Na^+. More recently it was shown that tetrodotoxin, thought to cause selective inactivation of the Na-carrying system (Kao, 1966), does not abolish the overshoot of the action potential of frog ventricle (Hagiwara and Nakajima, 1965). In addition, several investigators have suggested that part of the current causing depolarization of cardiac fibers

during the action potential may be carried by Ca^{++} (Niedergerke and Orkand, 1966; Hagiwara and Nakajima, 1965; and Reuter, 1967). For these reasons, it seemed desirable to undertake further studies of the ionic mechanism responsible for the transmembrane resting and action potentials of cardiac fibers.

A unique opportunity to study the relationship between transmembrane ionic concentration gradients and transmembrane potentials seemed to be provided by embryonic chick hearts since many studies have shown progressive changes in the concentration of sodium and potassium in skeletal and cardiac muscle during embryonic and early postnatal development (see Discussion).

METHODS

Fertilized eggs of white Leghorns containing 2- to 7-day embryos were obtained weekly from Shamrock Poultry Farm, North Brunswick, N.J. and were incubated at 36.5°C with adequate humidity. Incubation was terminated at the ages of 6 and 19 days, providing young and old embryos. The hearts were removed from the embryos and the ventricles were cut free from the atria. For 6-day embryos, because of their small size the whole ventricles were placed in the tissue bath and held in place by a pair of Teflon-coated silver electrodes which were used to deliver stimuli. For 19-day embryos in some experiments the ventricles were cut in half, pinned in the bath with the endocardial side up, and stimulated through two Teflon-coated silver electrodes. For other experiments, records were taken from the epicardial surface. All preparations were allowed to recover from dissection for 1 hr before control records were taken. The basic driving rate was set at 100/min. Both driving and "extrasystolic" pulses were generated by Tektronix Type 161 pulse generators and were delivered to the stimulating electrodes through a stimulus isolation unit. The delay between the driving stimulus and the extrasystolic pulse could be adjusted so that the premature stimulus would fall at any point in the cardiac cycle.

Modified Tyrode solution was used to perfuse the preparation. This solution had the following composition, in mM/liter: NaCl 140, KCl 5.6, $CaCl_2$ 1.35, $MgCl_2$ 0.5, $NaHCO_3$ 12, NaH_2PO_4 1.8, and dextrose 5.5. For experiments on effects of reduced extracellular sodium concentration, the solution contained 70, 50, or 20% of the normal amount of Na. Appropriate amounts of sucrose were used to maintain normal tonicity of low sodium solutions. High potassium solutions were made by adding 3 M KCl to the control solution. Both the tissue bath and the perfusing solutions were constantly gassed with a mixture of 95% O_2 and 5% CO_2 to maintain the pH of the perfusate at 7.4 and to provide adequate oxygenation of the preparation, even though embryonic tissue is said to be relatively resistant to hypoxia (Paul, 1965). The temperature of the perfusing fluid was maintained at 34.0°C ± 0.5°C and the flow rate was 4.0 ml/min, except during changes in the perfusion fluid. After changes in solution, an equilibration period of 5 min elapsed before records were taken, except during perfusion with Tyrode solution containing 20% of the normal sodium concentration. Since hearts from both young and old embryos quickly became inexcitable in this solution, we began taking records only 1–2 min after changing the perfusate.

Recordings of transmembrane potential were obtained by glass microelectrodes

(Ling and Gerard, 1949), and only superficial cells were impaled. The perfused embryonic hearts beat vigorously, particularly when the sodium concentration in the perfusing solution was reduced. This made intracellular recording a difficult task and repeated impalement usually was necessary. The microelectrodes were filled with

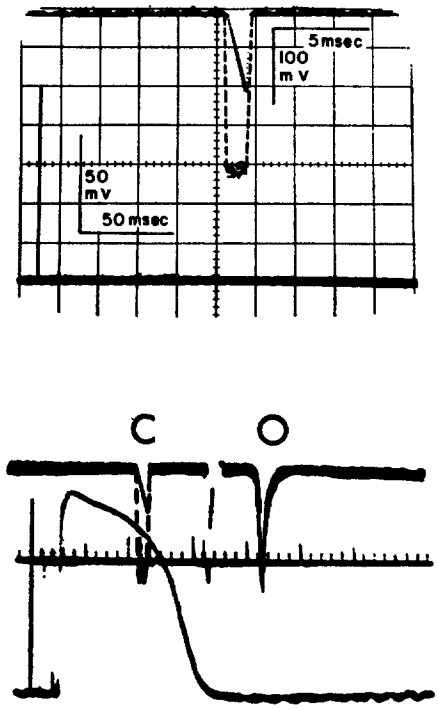

FIGURE 1. The upper panel is the control record obtained without an action potential. The calibrating sawtooth of 100 v/sec is shown as downward slope on the upper beam The first derivative of this pulse, recorded through the microelectrode, appears as a rectangular downward deflection. The base lines for the sawtooth and its dV/dt are superimposed. On the lower beam, which sweeps at a slow speed, the same sawtooth appears as a spike of 100 mv. Calibrations of time and voltage for the upper and the lower traces are shown on the right upper and left lower corners of this figure. Record retouched. The lower panel shows a record taken during an experiment. On the lower beam, the calibrating sawtooth pulse is followed by an action potential. The sawtooth and the differentiation of both the sawtooth and phase 0 of the action potential are displayed on the upper beam which sweeps at a fast rate. The deflections for the control dV/dt of 100 v/sec and dV/dt of the action potential upstroke are labeled *c* and *o*, respectively. (Retouched record)

filtered 3 M KCl (Nastuk and Hodgkin, 1950) by repeated boiling and cooling. Electrodes having a DC resistance of between 20 and 30 megohms were chosen for this study. Both the microelectrode and indifferent reference electrode were connected to the recording system by means of identical Ag/AgCl/3 M KCl half-cells.

An amplifier with provision for neutralization of input capacity (Amatniek, 1958)

with a DC input resistance of greater than 10^{12} ohms and a grid current of less than 10^{-12} amp was used to record through the intracellular microelectrodes. Transmembrane potentials were displayed on a Tektronix RM 565 dual beam oscilloscope and differentiated by an operational amplifier (Tektronix, type 0 or type 3A8) to provide a measurement of the maximum rate of change of potential during phase 0 of the action potential. A sawtooth wave of 100 mv was injected between the bath and ground to permit calibration of the magnitude of recorded potentials, the rate of change of potential, and the adequacy of capacity neutralization. The recorded amplitude of the differentiated signal was linearly related to the maximum rate of change of membrane potential from 0-500 v/sec. Fig. 1 shows the calibrations used in this study. A sawtooth wave of 100 mv, with a slope of 100 v/sec, and the resulting output of the differentiating amplifier are displayed on the upper trace. The same sawtooth waveform is displayed at a lower sweep speed on the bottom trace to provide a calibration step of 100 mv.

The action potential and time marks were displayed on one beam of the oscilloscope at a sweep speed of 50 msec/cm, using a dual trace amplifier (Tektronix RM 565) while dv/dt of the action potential and the control 100 mv sawtooth were displayed on the other at a sweep speed of 5 msec/cm. The latter sweep was started after an appropriate delay. The trace showing the time marks was used as the zero reference line for the action potentials. Traces were photographed with a Grass kymograph camera on Kodak Linagraph orthofilm. The system used in the present study was similar to that which has been reported previously by Bigger et al. (1968) from this laboratory.

After each experiment tip potentials of the microelectrodes (Adrian, 1956) were measured and corrected for by the following method: A base line was set with the input lead of the amplifier connected to a second indifferent electrode, identical to that used during recording of transmembrane potentials. The input lead then was connected to the microelectrode; the magnitude of the shift in base line was due to the tip potential of the microelectrode. Breaking the microelectrode tip caused the tip potential to disappear and the base line to return to the original control level. Microelectrodes having tip potentials smaller than 10 mv were used for this study and the base line for the recorded action potentials was corrected for tip potential.

RESULTS

Electrophysiological studies were carried out to investigate the ionic basis for excitation of young and old embryonic chick hearts and to determine whether or not the transmembrane potentials recorded from the ventricles of young and old embryos would permit an evaluation of the functional significances of the intracellular sodium and potassium concentrations reported by Klein. To do this it was necessary to determine the extent to which the transmembrane potentials of embryonic chick ventricle could be described in terms of the ionic hypothesis (Hodgkin, 1951; Draper and Weidmann, 1951; and Weidmann, 1955) by studying the extent to which changes in electrical activity induced by alterations in extracellular ionic environment might be compatible with predictions based on this hypothesis.

A. *The Resting Membrane Potential*

Because of the small size of the embryonic heart and the lack of dense connective tissue, immobilization of the preparation was difficult. For this reason many records of transmembrane potential were distorted by movement

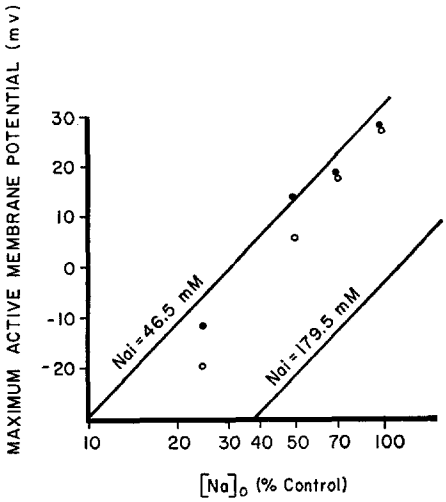

FIGURE 2. Maximum active membrane potential as a function of log $[Na^+]_o$. Top line is V_{Na} calculated for $[Na^+]_i$ = 46.5 mM, and the bottom line is V_{Na} calculated for $[Na^+]_i$ = 179.5 mM. Both lines have a slope of 60.92 mv/decade. Overshoot values obtained during perfusion with 20% sodium Tyrode solution before the hearts became inexcitable are arbitrarily plotted at $[Na^+]_o$ of 25% of the normal. Stimulation rate 100/min, temperature 34°C. Open circles are mean values obtained from 6-day embryonic hearts; closed circles are mean values obtained from 19-day embryonic hearts. The sources of these data are as follows:—

Age of embryos		Normal Tyrode	Tyrode solution containing 70% Na	50% Na	20% Na
6 day	No. of hearts studied	28	20	20	20
	No. of impalements	168	124	156	94
19 day	No. of hearts studied	26	24	24	24
	No. of impalements	152	113	186	107

artifact and many impaled cells were injured by movement of the heart relative to the microelectrode. Only records from stable impalements were measured. Such impalements were defined in terms of the abrupt appearance of the resting potential on advancing the microelectrode and a constant value for resting potential and action potential throughout numerous cardiac cycles.

The average resting potential recorded from the ventricle of 6-day embryos

in control Tyrode solution was 59.3 mv ± 4.8 mv (168 impalements, 28 hearts). The value for the 19-day hearts was 62.1 mv ± 6.0 mv (152 impalements, 26 hearts). These values were somewhat lower than the potassium equilibrium potentials of 67.7 and 70.7 mv calculated from Klein's data on intracellular potassium concentration for embryos of the same ages. Measurements made on preparations of 20 young hearts (374 impalements) and 24 old hearts (406 impalements) showed that when these hearts were perfused with Tyrode solution containing 70, 50, or 20% of normal Na there were small

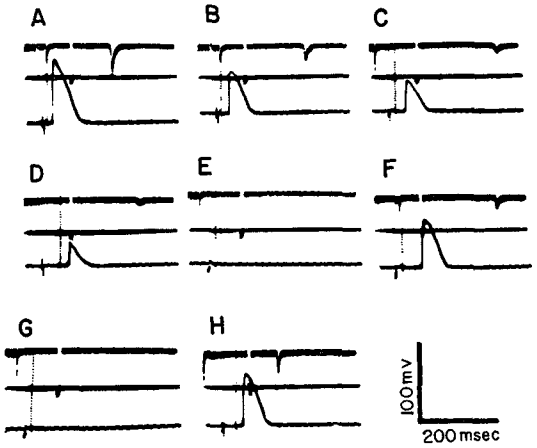

FIGURE 3. Effects of increased extracellular potassium concentration on electrical activity of ventricular myocardium from a 19-day chick embryo. The amplitude of the downward spikes on top trace indicates the dV/dt of the corresponding action potentials. These spikes are preceded by a calibrated dV/dt of 100 v/sec which appears as a downward square wave on the top trace. Second trace is the zero reference line. Stimulus artifacts are seen preceding action potentials. A is the control record. The potassium concentration in the perfusate was increased from 5.6 mM to 20 mM for B, and B, C, D, E are sequential recordings obtained during depolarization. The preparation became inexcitable in E. F, G, and H are records taken during recovery. For F a strong stimulus was used to elicit a response. For G the stimulus strength was reduced to show that excitability still was decreased and for H a stimulus of control value elicited a response. Stimulation rate 100/min; temperature 34°C. See text for discussion.

variable changes in the value of resting membrane potential; however, the average value did not change.

When the potassium concentration in the Tyrode solution was increased from 5.6 to 20 mM, the resting membrane potential decreased from approximately 60 mv to 40 mv (mean of 20 cells from 6 hearts). The depolarized cells became inexcitable at this level of membrane potential. Results were the same both for young and old hearts. These findings show that the resting membrane potential is dependent primarily on the extracellular potassium concentration, as it is in adult mammalian cardiac tissues.

B. *The Overshoot of the Transmembrane Action Potential*

During perfusion by control Tyrode solution, the overshoot of the transmembrane action potential (or the maximum active membrane potential) was 28.3 ± 2.0 mv for ventricles of 6-day embryos and 28.4 ± 2.2 mv for ventricles of 19-day embryos. If one assumes, as will be shown later, that excitation of the embryonic ventricle was dependent upon an increase in permeability to sodium and an influx of sodium during phase 0 of the transmembrane action potential (Hoffman and Cranefield, 1960), it is possible to calculate the approximate magnitude of overshoot expected on the basis of intracellular and extracellular sodium concentrations. If one uses Klein's data of 179.5 and 46.5 mM sodium for young and old embryos, the calculated values for overshoot are −3.8 and +31.6 mv for 6- and 19-day embryonic ventricles,

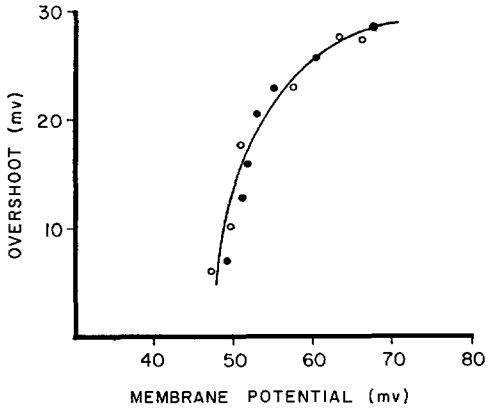

FIGURE 4. Relationship between the overshoot and the level of membrane potential in 19-day embryonic chick hearts. Membrane potential was lowered by raising the concentration of potassium in the perfusate. Closed circles represent mean values obtained from 20 cells of 6 hearts during depolarization; open circles during recovery after restoration of the normal perfusate. Stimulation rate 100/min, temperature 34°C.

respectively. These calculations are made for an extracellular sodium concentration of 153.8 mM at 34°C.

If we assume that the overshoot of the transmembrane action potential results largely from the permeability of the membrane to sodium and the transmembrane concentration gradient for this ion, the finding of an overshoot of +28.3 mv for the 6-day embryos implies that intracellular sodium activity must be lower than the extracellular activity. An overshoot of 28.3 mv would be expected if the maximum value for intracellular sodium activity was 52.7 mM.

The magnitude of the overshoot decreased when the sodium concentration of the Tyrode solution was reduced. Fig. 2 shows the relationship between the maximum active membrane potential, in millivolts, and the logarithm of

extracellular sodium concentration. When the concentration of sodium in the Tyrode solution was between 100 and 50% of the control value, the decrease in magnitude of the reversal was roughly proportional to the decrease predicted by the Nernst equation and had a slope of approximately 60.92 mv per 10-fold change in sodium concentration. The findings were the same for both the 6- and 19-day hearts. However, when the hearts were perfused with Tyrode solution containing only 20% of the normal sodium concentration, the decrease in overshoot was greater than that expected for a sodium electrode. Since both old and young preparations soon became inexcitable during perfusion with Tyrode solution containing 20% of the normal sodium concentration, it does not seem proper to plot data obtained prior to inexcitability

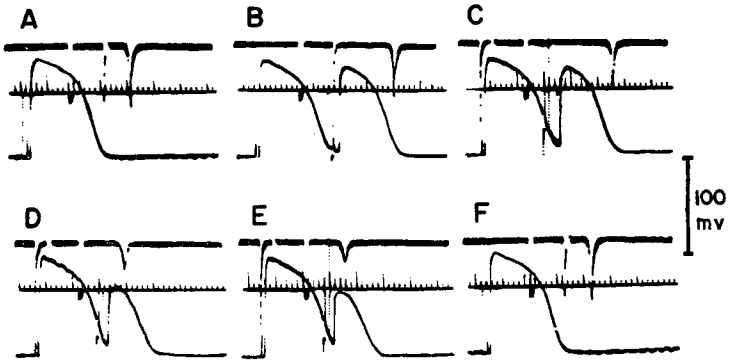

FIGURE 5. Relationship between the level of the membrane potential and both the overshoot and rate of rise of extrasystoles. These representative records were obtained from a 19-day embryonic chick ventricle perfused with modified Tyrode solution at 34°C and driven at 100/min. dV/dt of both the calibrated sawtooth (100 v/sec) and the action potentials of driven (A, F) or extrasystolic beats (B, C, D, E) are shown on the top trace. Zero potential line is indicated by the time marks at 10 and 50 msec. A is the control record of the driven beat. B, C, D, E show that both the size of the overshoot and the rate of depolarization of the extrasystoles decreased progressively as the extrasystoles were elicited from a progressively lower level of membrane potential. F is the control driven record obtained after turning off the extrasystoles.

against a sodium concentration of 20%. Therefore we arbitrarily have selected 25% as being representative of the actual extracellular sodium concentration shortly before the onset of inexcitability.

Although the effect of decreasing extracellular sodium concentration on the magnitude of overshoot provided results which were not in perfect quantitative agreement with predictions based upon the ionic hypothesis, nevertheless the results strongly support the concept that depolarization of both young and old embryonic hearts resulted primarily from a specific, transient increase in permeability of the membrane to sodium and an inward sodium current.

Weidmann (1955) showed that both the size of the overshoot and maximum rate of depolarization during phase 0, for Purkinje fibers, were dependent

upon the level of membrane potential at the time of stimulation. This relationship which, in a sense, describes the voltage-dependent activation and inactivation of the sodium-carrying system, is a crucial aspect of the ionic hypothesis. For this reason, several attempts were made to study the same relationship for the embryonic chick heart. Because of the geometry of the cells and tissue, voltage clamp techniques could not be employed. However, two other means were used to vary membrane potential and record the magnitude of overshoot and maximum rate of change of potential during phase 0. Fig. 3 shows the representative relationship between the level of membrane potential at the time of excitation and both the maximum rate of change of voltage during phase 0 and the magnitude of the overshoot. These data were obtained for a 19-day heart depolarized by increasing the extracellular potas-

TABLE I

RELATIONSHIP BETWEEN THE MAXIMUM RATE OF CHANGE OF POTENTIAL DURING PHASE 0 AND THE CONCENTRATION OF SODIUM IN THE PERFUSATE FOR HEARTS OF 6- AND 19-DAY CHICK EMBRYOS

Age of the embryo	dV/dt max., *v/sec*			
	$[Na^+]_o$ % control			
	100	70	50	25*
days				
6	93.8±4.2	62.5±6.5	35.0±5.4	15.9±2.0
19	100.6±5.9	55.8±7.5	49.1±7.2	18.9±2.0

* Data obtained during perfusion with Tyrode solution containing 20% of the normal sodium concentration before the preparations became inexcitable are arbitrarily plotted as $[Na^+]_o$ = 25%. Driving rate 100/min, temperature 34°C. Values shown in the table are mean ± SD. Material used in this table is the same as that shown in Fig. 2.

sium concentration. It is clear that, as membrane potential was reduced, the overshoot decreased in magnitude. The decrease in membrane potential caused by KCl was completely reversible, and data obtained during recovery were similar to those obtained during progressive depolarization, as is shown in Fig. 4.

In four other experiments premature stimuli were used to elicit action potentials at different times and thus at different levels of membrane potential during phase 3 of repolarization. Fig. 5 shows that the magnitude of these extrasystoles depended similarly on the membrane potential at the time of excitation. These results suggest that for the embryonic chick heart, as in the case of adult cardiac muscle, availability of the sodium-carrying system is a function of transmembrane potential. The findings for the embryonic chick heart were qualitatively similar to those presented by Weidmann for the Purkinje fiber.

C. *The Rate of Depolarization*

Table I shows the relationship between the maximal rate of depolarization and the concentration of extracellular sodium ion for 6- and 19-day hearts. It is clear that the maximum rate of rise of the action potential depended on the concentration of sodium ion in the perfusate. The maximal rate of rise of the action potential may be regarded as a rough measure of the maximum inward sodium current. Since both the driving force for the sodium ion and the sodium conductance decrease when the sodium concentration of the perfusate is reduced, one would expect the maximal rate of depolarization to fall. Though the theoretical basis for predicting in a qauntitative way the effect of lowering

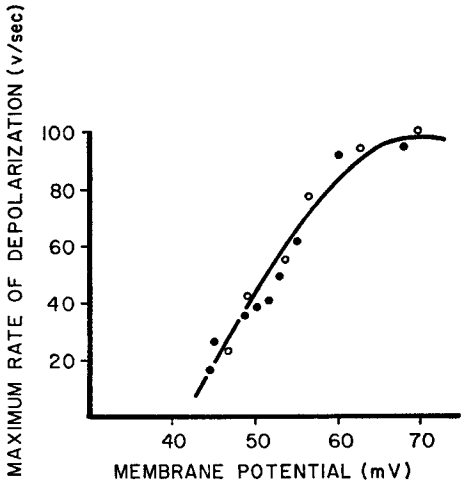

FIGURE 6. Relationship between the maximum rate of depolarization and the level of membrane potential for 19-day embryonic chick hearts. Closed circles represent data obtained from 20 cells of 6 ventricles during potassium depolarization; open circles during recovery. Stimulation rate 100/min, temperature 34°C.

the sodium concentration on the rate of rise is lacking (Weidmann, 1955), these findings certainly can be considered as positive support for the assumption that sodium ions are the main carriers of electric charge during the upstroke of the action potential.

As in the case of the overshoot of the membrane potential, the rate of depolarization also depended upon the level of membrane potential at which excitation occurred. Figs. 3 and 6 demonstrate this relationship for the potassium-depolarized fibers. Furthermore, during recovery from potassium depolarization, an interesting observation was made. The maximum rate of depolarization depended not only on the level of membrane potential, but also on the time during which the level of membrane potential had been maintained at a particular value. Fig. 3 H was taken 15 sec later than Fig. 3 F. Though there was no change of membrane potential between these two re-

cords, there was, however, a marked difference in the rate of rise of the action potentials. Also noticeable was the larger overshoot in Fig. 3 H. The improvement in overshoot was less marked than the change in maximum rate of rise. Associated with the increase in rate of depolarization, a considerable improvement of conduction also was clearly demonstrated.

D. *The Contour and the Duration of the Action Potential*

Electrical recordings obtained from 6- and 19-day embryonic chick ventricles during perfusion with Tyrode solution containing normal and lowered concentrations of sodium are shown in Figs. 7 and 8, respectively.

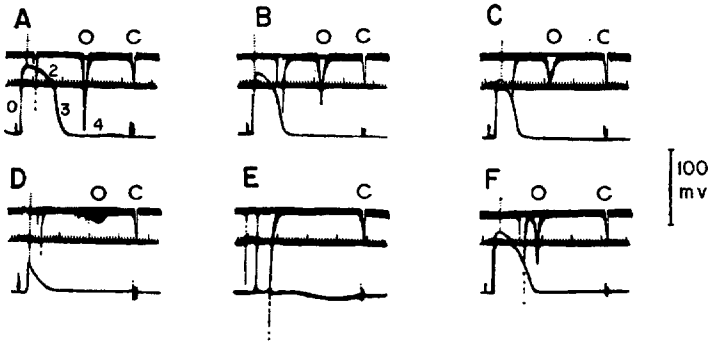

FIGURE 7. Effects of varying the sodium concentration in the perfusate on the electrical activity of 6-day embryonic chick ventricle. Downward spike which is labeled *o* on top trace shows dV/dt of the action potential. This is followed by a 100 mv negative going sawtooth and a calibrated dV/dt of 100 v/sec, which is labeled *c*. Zero reference line is indicated by time marks (10 and 100 msec). Stimulus artifacts are seen preceding action potentials. A is the control. B and C were recorded during perfusion with Tyrode solution containing 70 and 50% of the normal sodium concentration, respectively. The sodium concentration of the perfusate was lowered to 20% of the normal value for D and E. The preparation became inexcitable in E. F was recorded during recovery from low sodium solutions and shows that the changes in electrical activity caused by lowering the sodium concentration in the perfusate were largely reversible. Stimulation rate 100/min, temperature 34°C.

At a driving rate of 100/min the duration of the action potentials was 120–130 msec for both 6- and 19-day ventricles. The action potentials showed a rapid depolarization phase with overshoot which is designated as phase 0. The initial rapid repolarization phase, or phase 1, usually was difficult to demonstrate or was absent. The prominent plateau phase or phase 2 of the action potential, characteristic of ventricular cells of many species, was obvious. This was followed by a phase of rapid repolarization (phase 3). The ensuing electrical diastole is called phase 4. Records from ventricular cells of both 6- and 19-day embryos showed a flat phase 4: this means that the ventricular cells of chick embryos lacked automaticity.

Figs. 7 B–7 E and 8 B–8 E show the recordings obtained during perfusion of 6- and 19-day embryonic hearts with low sodium solution. Part A of both figures shows the control records. Parts B and C show the changes obtained when the sodium concentration of the perfusate was decreased to 70 and 50% of the normal, respectively. It is clear that, in addition to the changes in overshoot and the rate of rise of the action potential, there was a gradual disappearance of the plateau, and the action potential gradually became triangular. Figs. 7 D, 8 D, 7 E, and 8 E show records obtained during the perfusion with Tyrode solution containing 20% of the normal sodium concentration. The changes in plateau and the duration of the action potential became

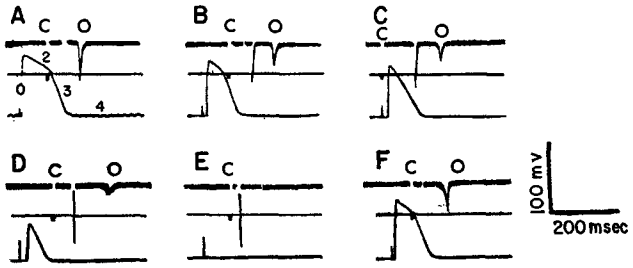

FIGURE 8. Effects of varying the sodium concentration in the perfusate on the electrical activity of 19-day embryonic chick ventricle. Calibrated dV/dt of 100 v/sec and dV/dt of the action potential upstroke are shown on the top trace and are labeled *c* and *o*, respectively. Second trace is the zero potential line. On bottom line, small spikes preceding action potentials are stimulus artifacts. A is the control, B and C show the changes in action potential when the sodium concentration of the perfusate was decreased to 70 and 50% of the normal. D and E were recorded during perfusion with Tyrode solution containing 20% of the normal sodium concentration. Note the progressive deterioration of the action potentials before the preparation became inexcitable in E. F shows the improvement in electrical activity after restoration of a normal sodium concentration. Stimulation rate 100/min, temperature 34°C.

more marked in D; the preparations became inexcitable in E. The progressive deterioration of the action potentials brought about by lowering the sodium concentration of the perfusate was reversible; Figs. 7 F and 8 F show the improvement in electrical activity after restoration of a normal extracellular sodium concentration. The changes in contour of the action potential suggest that sodium ions were responsible for a major part of the sustained depolarization (phase 2) as well as for the depolarization during phase 0.

E. *Excitability and Conduction*

When extracellular Na^+ concentration was reduced there was a progressive decrease in both conduction velocity and excitability until, when $[Na^+]_o$ had been reduced to 30.8 mM, the ventricles became inexcitable. This gives support to the concept that excitation of embryonic hearts was due to a specific

increase in the permeability of the membrane to sodium ions and that the depolarization of the membrane was due to the entry of sodium ions from the extracellular fluid. However, in comparison with other cardiac tissues, it seems that the embryonic hearts became inexcitable at a higher $[Na^+]_o$ than do fibers from some other hearts studied. For example, Draper and Weidmann (1951) found that Purkinje fibers from canine and ungulate hearts became inexcitable when the extracellular Na^+ concentration was reduced to 10–20% of normal. Brady and Woodbury (1960) reported that frog ventricle became inexcitable when sodium was reduced to 10% of normal and Délezè (1959) showed that sheep and calf ventricular myocardium lost excitability at an extracellular Na^+ concentration of 10%. Indeed, it has been reported that frog atria and ventricles retain excitability for as long as 2 hr in isotonic sucrose media (Van der Kloot and Rubin, 1962). We believe that these differences in the dependence of excitability on extracellular Na^+ concentration have been explained, at least in part, by the studies of Brady and Tan (1966) who showed that, with adequate perfusion, frog atrial trabeculae lost excitability in Na-free solution in less than 30 sec and that the results of Van der Kloot and Rubin (1962) could be attributed to inadequate exchange between the solution immediately outside the membrane and the perfusate. Our findings could indicate that the exchange of extracellular fluid with the fluid immediately surrounding the cardiac cells is faster or more complete in the embryonic tissue than in some other preparations. An alternative possibility is that in the embryonic hearts the intracellular Na activity is higher. If excitation failed at a $[Na^+]_o$ of 30.8 mM, one may assume that the activity in intracellular $[Na^+]$ probably was higher than 30.8 mM. This is a reasonable estimate and is compatible with our other calculations of the value of intracellular $[Na^+]$ activity.

DISCUSSION

It has been repeatedly shown that the content of sodium decreases while that of potassium increases during embryonic and early postnatal development of skeletal and cardiac muscles (Needham, 1931; Barlow and Manery, 1954; McCance and Widdowson, 1956; Dickerson and Widdowson, 1959; Klein, 1960 and 1963; and Vernadakis and Woodbury, 1964). The most striking change of sodium content in cardiac muscle is that reported for the chick heart by Klein (1960). The intracellular sodium was said to be 650 mM, 179.5 mM, and 46.5 mM for 2-day, 6-day, and 19-day embryonic chick hearts (Klein, 1960). During this period of dramatic change in the myocardial sodium content, the concentration of sodium ion in the amniotic, the allantoic, and the extraembryonic fluid remained relatively constant (Smoczkiewiczowa, 1959).

Our electrophysiological data indicate that the ionic basis of resting and action potentials in both young and old embryonic hearts is similar to that

described for most adult vertebrate hearts. Raising potassium concentration in the perfusate caused depolarization; reducing $[Na^+]_o$ caused no consistent change in resting potential. This provided evidence that the resting membrane potential of the embryonic hearts was primarily dependent on the concentration gradient for potassium across the membrane and the predominant permeability of the membrane to this ion. Further, these findings permit some estimate of the intracellular K^+ concentration of the embryonic heart. When the extracellular potassium concentration was raised to 20 mM (Fig. 3), the measured transmembrane potential was −40 mv. At this value for $[K]_o$ one can expect membrane potentials to closely approximate the potassium equilibrium potential, E_K. The value for $[K]_i$ calculated for these conditions is 90 mM. This suggests that the values for intracellular potassium concentration of 67 and 81 mM for 6- and 8-day embryos reported by Klein probably underestimate the true values. Our measurement of resting potential for young and old embryos perfused with normal Tyrode solution ($[K]_o$ = 5.6 mM) gave values of 59.3 and 62.1 mv. These are lower than the value for E_K calculated either on the basis of Klein's data (67.7, 70.7 mv) or the estimated value for $[K]_i$ of 90 mM, given above (72 mv). However, at least two factors contribute to this disparity. First, it is possible that, due to the small size of the embryonic cardiac cells and their vigorous contractions in normal solution, the measured values of resting potential are lower than the actual transmembrane potential. Also, at low and normal levels of extracellular potassium, resting potential of cardiac fibers normally is less than E_K because g_K is lower than at high $[K]_o$.

For both young and old hearts the changes in electrical activity which resulted from decreasing the sodium concentration of the perfusate strongly supported the hypothesis that depolarization during phase 0 of the transmembrane action potential was due to a specific increase of the membrane permeability to sodium ion. Thus, the ionic basis for electrical activity for cardiac cells is already established in embryonic hearts, as early as the 6th day of embryonic chick development.

Many electrophysiologists had recorded transmembrane action potentials with overshoot from young embryonic hearts either in vitro (Fingl, Woodbury, and Hecht, 1952; Meda and Ferroni, 1959), or in tissue culture (Fänge, Persson, and Thesleff, 1956) before Klein reported that these young hearts had a very high sodium content. Lehmkuhl and Sperelakis (1963) also recorded action potentials with overshoot from young embryonic hearts. These studies suggested that much of the muscle sodium in the young embryonic hearts was bound. In this study, action potentials recorded from young and old hearts showed changes which were roughly comparable both quantitatively and qualitatively when the sodium concentration in the perfusate was lowered. All preparations became inexcitable when the sodium concentration of the perfusate was decreased to 20% of the normal values (30.8 mM.) Calculations

based on the values of the action potential overshoot indicated that the maximum activity of sodium ion could be 46.5 mM for 19-day embryonic hearts, while similar calculation indicated that the intracellular sodium activity for 6-day embryonic hearts might be 52.7 mM or lower. This suggests that, if Klein's measurements of total intracellular sodium are correct, no less than 70% of intramyocardial sodium in young embryonic hearts was electrophysiologically inactive. This finding is not inconsistent with Klein's claim (1960) that the exchangeability of intramyocardial sodium was only 7% for chick embryos 7 days old.

CONCLUSIONS

Electrophysiological data obtained from the present study suggest that the ionic basis of resting and action potentials in embryonic chick hearts is similar to that which has been described for most vertebrate hearts. The resting potential results primarily from the concentration gradient for potassium ion across the membrane and the predominant permeability of the membrane to potassium ion. Excitation results from a transient and specific increase in the permeability of the membrane to sodium ion, a consequent inward sodium current, and the resulting depolarization of the cardiac cell membrane.

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