The Electrophysiological Organization
of the Embryonic Chick Heart

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ABSTRACT   Both intracellular and surface electrodes were employed to record electrical activity from embryonic chick hearts between the ages of 3 and 20 days. Cells from the sinus venosus, sinoatrial (SA) valves, atrium, atrioventricular (AV) ring, and ventricle were localized and characterized on the basis of shape, amplitude, rise time, and duration of transmembrane potentials. The differences in transmembrane potentials from these various regions provided the basis for a hypothesis concerned with the distribution of pacemaker potentiality and one related to the origin of the His-Purkinje system. Action potentials recorded along the entire embryonic AV ring were comparable to those of the adult rabbit AV nodal cells in both configuration and sequence of activation and were thus categorized into three functional regions (AN, N, NH). Histological sections of 7 and 14 day hearts demonstrated muscular continuity between the right atrium and ventricle across the muscular AV valve.

Hoff et al. (9) have shown that the development of the embryonic electrocardiogram reflects the sequence in which the various chambers of the heart make their appearance. Furthermore, it has been observed that the embryonic electrocardiogram is adult in character (presence of P-QRS-T waves), even though the specialized conducting tissues of the heart are not apparent in anatomical studies (1, 9). Intracellular recordings from cells in the embryonic chick sinus venosus, atrium, and ventricle have revealed differences in action potential configurations (7, 15, 20). Several investigators also have recorded both pacemaker and non-pacemaker activity from embryonic chick heart cell cultures (2, 6, 16, 30). However, records of transmembrane potentials which relate to the functional organization of the embryonic heart at several stages of development have not been obtained. In the present study, transmembrane recordings are used to demonstrate the existence of well defined electrophysiological patterns which are prominent before the completion of cardiac

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development and remain throughout adult life. In many instances, the intra-
cellular recordings from the chick embryo heart must be compared with and
related to adult mammalian cardiac action potentials either because of the
scarcity of electrophysiological data concerning embryonic and adult avian
hearts or because homologous cardiac structures are known to exist in birds
and mammals.

METHODS

Fertilized eggs of White Leghorns were placed in a temperature (37.5°C) and hu-
midity (55 per cent) controlled incubator. Incubation was terminated at a desired
developmental stage between the ages of 72 hours and 20 days (just before hatching).
The age of the embryos was determined by corroboration with the Hamburger-
Hamilton classification (8). For embryos older than 7 days, the procedure, following
removal from the egg shell, involved decapitation and rapid excision of the heart.
The heart was immediately placed in a constant temperature, transilluminated tissue
chamber into which flowed an oxygenated mixture of modified Tyrode solution at
33°C. The composition of this solution in mM was: NaCl 140; KCl 5.6; $CaCl_2$ 1.35;
$MgCl_2$ 0.5; $NaHCO_3$ 10.0; NaH_2PO_4 1.8; dextrose 5.5. The solution was gassed with a
mixture of 95 per cent O_2 and 5 per cent CO_2, both at room conditions and in the
tissue chamber. The heart was then dissected by a three-stage procedure similar
to that reported for the adult rabbit heart (24). Next, the preparation was fixed to a
paraffin block in the tissue bath to fully expose the endocardial surface of both the
right atrium and right ventricle (Fig. 1). This procedure was modified for embryos
less than 1 week of age because of limitations imposed by the dimensions of the younger
hearts. Under these circumstances, the decapitated embryo was placed in the tissue
chamber on its right side and pinned to the paraffin bed. The arterial end of the
heart was cut free from the body wall and positioned so that the chambers of the
heart were readily accessible for penetration by microelectrodes.

The microelectrodes were 3 M KCl-filled glass micropipettes (DC resistance 10 to
40 MΩ) rigidly mounted on a micromanipulator equipped with calibrated vernier
scales (0.1 mm/division). Both the microelectrode and the extracellular reference
electrode were connected to the recording system by means of identical Ag/AgCl/
KCl half-cells. Recording was affected by a high input impedance preamplifier (DC
resistance greater than 10^{10} Ω) provided with an input capacity neutralization system.
The signals from both amplifiers were channeled into a dual trace oscilloscope and
photographed by an oscillographic camera. Stimuli applied through a pair of fine
teflon-coated silver electrodes positioned in the atrial roof enabled the heart rate to be
controlled in a frequency range between 2.5 to 3.5 cycles/second. When the prepara-
tion was driven during an experimental trial, the rate was constant.

The embryonic hearts were dissected for the histological sections in the same
manner previously described for the microelectrode studies. The hearts were im-
mediately placed in Bouin's fixative for at least 24 hours. A sagittal cut was then made
so that each strip of tissue included areas of atrium, AV ring, AV valve, and ventricle.
The preparations were dehydrated in dioxane and placed in a 1:1 mixture of dioxane
and paraffin before being embedded in paraffin (paraplast). Sections were cut at 7

microns. The slides were stained by a modified Masson's trichrome using Harris hematoxylin.

RESULTS

A. *Morphology of the Embryonic Heart*

The present study is concerned with embryos of 72 hours to 20 days. A comprehensive review of the morphological development of the avian heart during
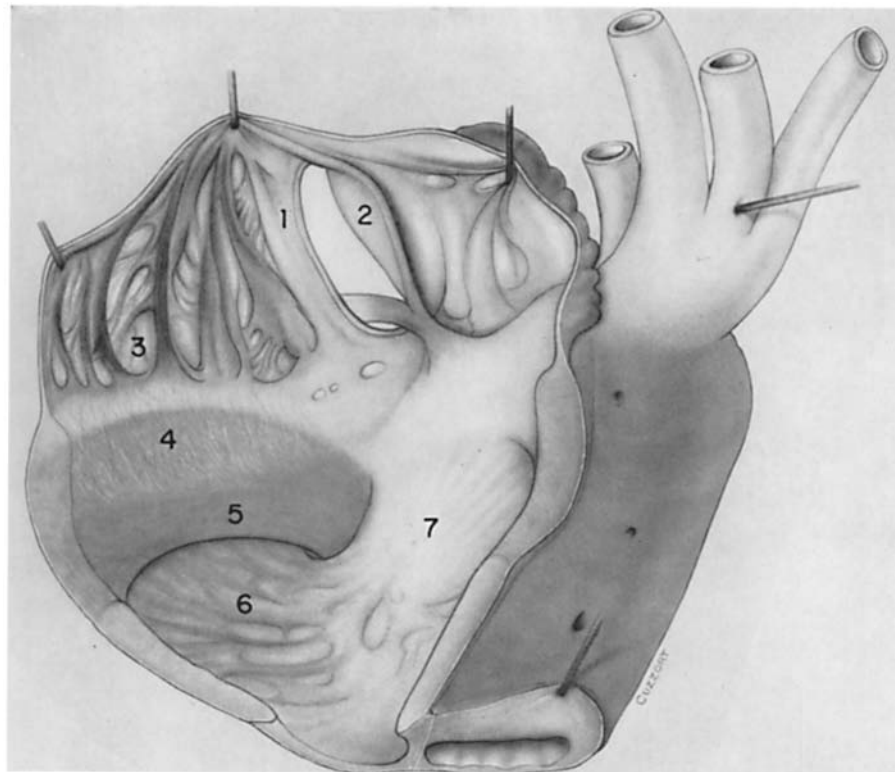

FIGURE 1.   Drawing of a transilluminated 17 day embryonic chick heart preparation. (1) Right sinoatrial valve; (2) left sinoatrial valve; (3) right atrial roof; (4) atrioventricular ring; (5) atrioventricular valve; (6) right ventricle; (7) interventricular septum.

this period may be found elsewhere (29). It is sufficient to briefly describe two representative hearts under investigation. Fig. 3 contains a ventral and dextral sketch of a 72 hour heart (26). The apex of the ventricle is the most caudal portion of the heart and the ventricle is separated from the atrium by the atrioventricular canal. The sinus venosus is a sac-like dilation separated from the atrium by a groove. The atrial chamber is expanded laterally and an atrial sulcus is apparent as the external manifestation of the interatrial septum. At this stage, the interventricular septum is absent. The structures of the

17 day heart are shown in Fig. 1. However, there are a few anatomical features worthy of mention. The right atrioventricular valve is a muscular structure composed of auricular and ventricular muscle (3). A sheet of fibrous-like
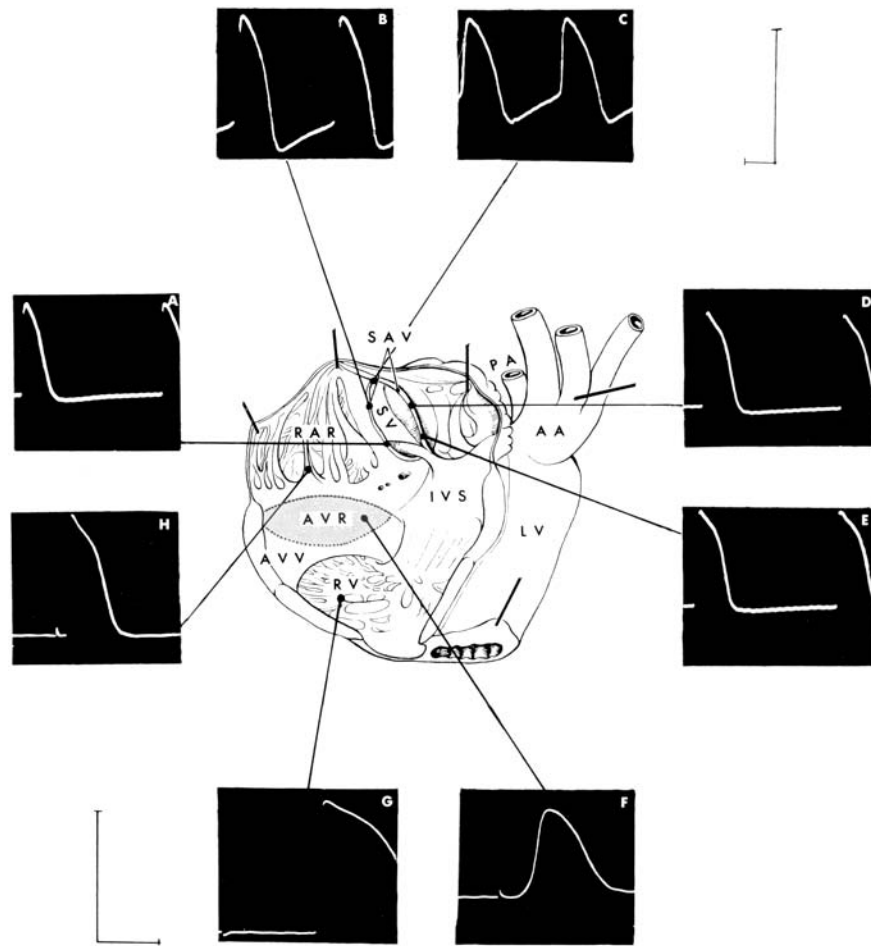

FIGURE 2.   Transmembrane potentials from the 17 day embryonic chick heart. Vertical bars represent 100 mv. Horizontal bars represent 50 msec. Calibration for F, G, H in lower left corner. *SAV*, sinoatrial valves; *SV*, opening into sinus venosus; *RAR*, right atrial roof; *IVS*, interventricular septum; *AVV*, atrioventricular valve; *AVR*, atrioventricular ring.

tissue, the atrioventricular ring, extends from the crest of muscle around the AV opening over a major portion of the valve's ventral surface. The band of muscular tissue which stretches across the sinus venous from the left to the right valve is the sinus septum. This structure separates the entrances of the left precava and coronary veins from the sinus venosus (28).

B. *Localization and Description of Action Potentials*

Transmembrane action potentials recorded from SA valves, atrium, AV ring, and ventricle of a 17 day heart are shown in Fig. 2. These records are representative of action potentials obtained from embryonic hearts between 7 and 20 days of age. A pacemaker type action potential recorded near the junction of the SA valves is shown in Fig. 2C. Among the features to be noted are a marked diastolic depolarization (20 mv) and a rather slow upstroke. In addition, the amplitude is low and the peak is rounded. Fig. 2 shows records obtained from the right (A, B) and left (D, E) SA valves. There is an apparent diminution in the diastolic depolarization and an increase of upstroke velocity as the distance from the pacemaker cell type (C) increases. This is particularly demonstrable in the left SA valve, where very fast upstrokes and prominent plateaus can be observed. A typical membrane potential recorded from a pectinate muscle in the right atrial roof is shown in Fig. 2H. The distinguishing features include a constant level of membrane potential during phase 4 (diastolic period), a rapidly rising phase 0 (upstroke), a noticeable phase 1 (spike), and phase 2 (plateau). A record from one of the cell types located in the AV ring is characterized by a slowly rising, rounded action potential of reduced magnitude (Fig. 2F). At the AV ring region overlying the AV valve, penetration beyond the surface layers yields recordings from the ventricular portion of the valve (not shown in Fig. 2). A representative action potential recorded from a ventricular fiber consists of a rapid depolarization phase, a prominent plateau, and a steady membrane potential during the diastolic period (Fig. 2G). Action potential configurations of cells from the ventricular portion of the right AV valve resemble those of right ventricular myocardial cells except for the duration of the plateau phase (see below for a quantitative analysis and comparison). Occasionally, action potentials resembling those of Purkinje fibers in configuration, *i.e.* showing a prominent phase 1 and phase 2, were recorded from embryonic atrium and ventricle. This may be correlated with the Purkinje-like action potentials recorded from adult avian atria and ventricles (21).

Characteristic action potential configurations recorded from 3 day embryonic hearts are shown in Fig. 3. Records obtained from the sinus venosus (Fig. 3a), AV constriction (Fig. 3c), and ventricle (Fig. 3d) are markedly similar to those of the previously described older heart cells. However, action potentials from atrial fibers (Fig. 3b) bear similarities to the ventricular potentials at this early stage.

The amplitude and duration of transmembrane potentials recorded from the right atrium, right AV valve (ventricular portion), and right ventricle were compared. The results obtained for hearts between 14 and 20 days are summarized in Table I. The duration of action potentials of cells from the

ventricular portion of the AV valve is significantly greater than for cells from the right ventricle ($P < 0.01$). The action potential duration of right atrial cells is one-fourth to one-half the values obtained from right AV valve and right ventricular cells, respectively. Although membrane potentials recorded
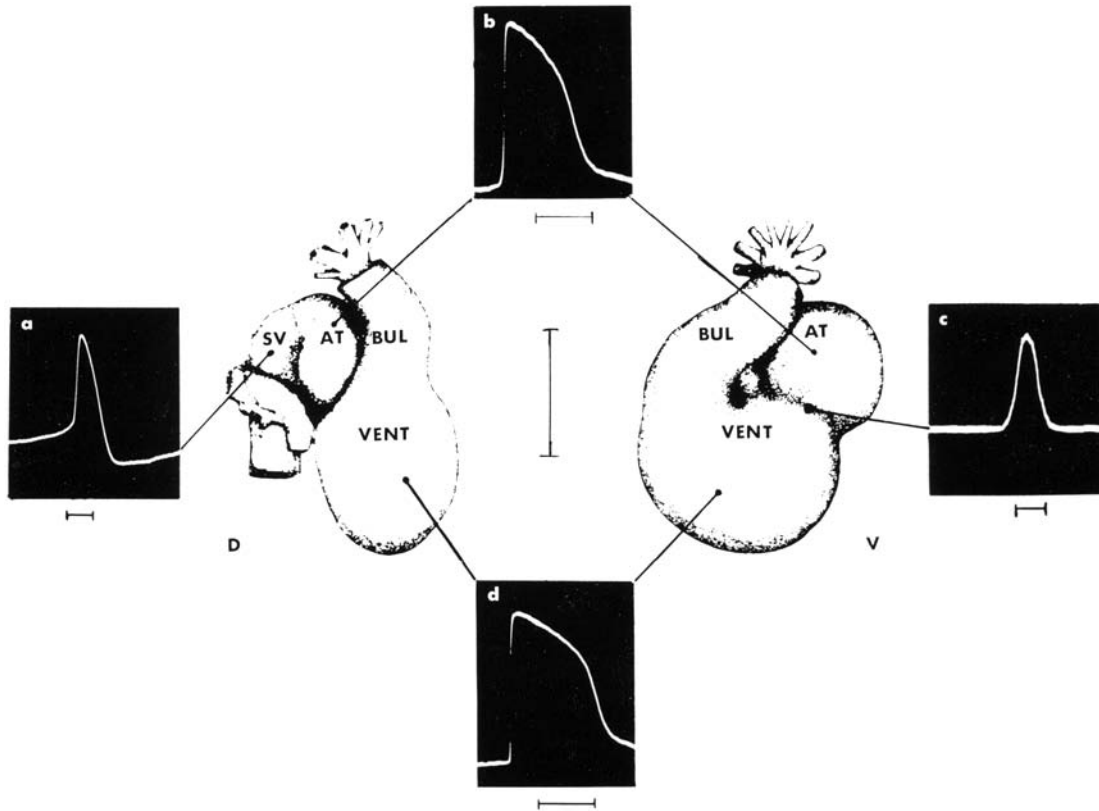

FIGURE 3.   Transmembrane potentials from the 72 hour chick embryo heart. D, dextral view. V, ventral view. Vertical bar represents 50 mv. Horizontal bars represent 100 msec. (Drawings from (26). *Early Embryology of the Chick, by B. M. Patten, Copyright 1951, McGraw-Hill Book Co. Used by permission.*)

from 7 and 8 day hearts are qualitatively similar to those reported for the older hearts, the difference in action potential duration between cells of the AV valve and ventricle does not appear to be significant.

C.   *A Comparison of Membrane Potentials Recorded from Embryonic Chick AV Ring and Adult Rabbit AV Node*

On the basis of physiological evidence, the AV node of the rabbit heart has been divided into three functional regions; upper (AN), middle (N), and lower (NH) (22, 23). Membrane potentials recorded from cells in each of

these zones are presented in Fig. 4B (22). Action potentials recorded from corresponding areas of the embryonic AV ring are shown in Fig. 4A. There is a striking resemblance between the configurations of action potentials recorded from similar regions of the two structures: the potentials showing a rounded peak in cells of the upper zone, the decrease in upstroke rising velocity in cells of the middle zone, and the gradual, smooth transition from phase 4 to phase 0 in cells of both the middle and lower zones. Action potentials resembling those obtained from cells in the upper AV ring were recorded from the fibers of the adult rabbit heart close to the tricuspid valve (24). A progressive increase in activation time (atrial stimulus artefact to action

TABLE I

AMPLITUDE AND DURATION OF ACTION
POTENTIALS RECORDED FROM EMBRYONIC CHICK
ATRIUM, AV VALVE, AND VENTRICLE*

	$n\ddagger$	Amplitude Mean ± SE	Duration§ Mean ± SE
		mv	*msec.*
Right atrium	57	83.5 ± 1.3 (67–105)	25.8 ± 0.7 (16–39)
Right AV valve (ventricular portion)	31	81.3 ± 2.2 (55–106)	102.6 ± 11.3 (71–150) ‖
Right ventricle	30	86.1 ± 2.8 (55–128)	50.8 ± 2.5 (27–85) ‖

Numbers in parentheses indicate range of observations.
* Embryos ranged in age from 14 to 20 days.
‡ Number of impaled cells.
§ Measured between points on upstroke and downstroke at 50 per cent of total amplitude.
‖ Group comparison of AV valve and ventricular action potential durations resulted in $P < 0.01$.

potential upstroke) is evident in the transmembrane potentials (Fig. 4) as the microelectrode is moved across the AV ring and AV node.

DISCUSSION

The configuration of transmembrane potentials recorded from different regions of the embryonic heart was investigated during development. The results show that the magnitude of membrane potentials is greater than those previously recorded by others (7, 20). Pacemaker and non-pacemaker action potentials recorded from 37 hour (20) and 72 hour hearts are similar to those from comparable regions of the fully developed septate heart. Whereas recordings from older hearts do not corroborate an earlier finding of significant differences between the amplitudes of atrial and ventricular action potentials (7), those from younger hearts do confirm the presence of certain similarities between the two cell types. Based on the accumulation of data concerned with the electrical activity of embryonic chick hearts, an attempt will be made
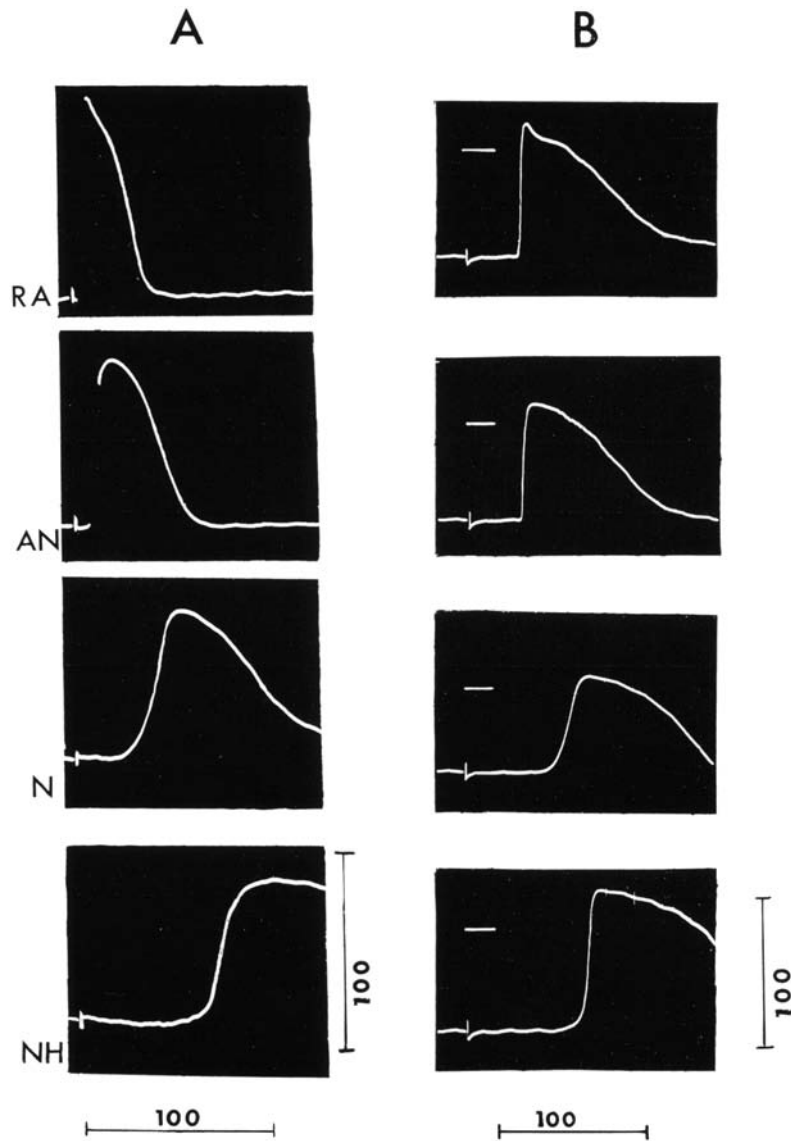
FIGURE 4.   Transmembrane action potentials from embryonic chick AV ring (A) and adult rabbit AV node (B) (22, page 122). *RA*, right atrium. *AN*, atrial portion of AV ring and AV node. *N*, middle portion of AV ring and AV node. *NH*, lower portion of AV ring and AV node. Horizontal bars equal 100 msec. Vertical bars equal 100 mv.

to establish a logical correlation of the distribution of functional properties in the embryonic heart.

A. *The Distribution of Pacemaker Potentiality*

Records from cells which are capable of functioning as pacemakers show a characteristic slow depolarization during phase 4 (10). The steepness of this depolarization is related to the degree of automaticity inherent in the cell. Cells with intrinsically slow rhythmicity are driven by faster pacemaker cells, although the former usually possess some degree of diastolic depolarization. Those cells which are not spontaneously active show a perfectly steady membrane potential during phase 4. In addition, there are cells (dormant pacemaker cells) which may not show characteristic pacemaker potentials but are capable of responding to a steady depolarizing current with a repetitive discharge (30, 31). However, it may generally be stated that the presence of slow depolarization during phase 4 is an indication of rhythmic capability or potentiality.

Embryonic chick hearts consist of tissues capable of spontaneous activity in areas such as the sinus venosus, musculature of the sinoatrial valves (right SA valve corresponds to the rabbit SARB (32)), and AV ring. Varying degrees of pacemaker potentiality are present in the embryonic chick heart as evidenced by the gradation in the slope of diastolic depolarization recorded from the SA valve junction and the SA valves, the latter being less steep than the former. It was never possible to record phase 4 depolarization from myocardial cells of the atrium or ventricle. The findings noted above correlate well with those reported for the adult rabbit heart (10, 24) and turkey heart (21). The possibility still remains that some intrinsically slow pacemaking cells in the atrium and/or ventricle did not appear in the experiments because of the rapidity of the driven heart rate. A definition of the pacemaking areas in the early embryonic heart requires further investigation.

It will be necessary to record intracellularly from embryonic heart cells as they undergo the first contractions (stages 9–10) (8) in order to determine specifically, whether these cells are spontaneously active or are being driven by other cells which possess automaticity. Based on the information presented thus far, the spontaneous beat of the heart tissue must be attributed to the presence of specialized pacemaker cells. Patten (27) was of the opinion that during the early stages of development there exists not one pacemaker, but a "succession of pacemaking zones." The possibility exists that all pacemaker cells originally are situated along the sinoventricular canal and that as the cardiac primordia begin to fuse in the midline, pacemaking areas which possess a higher intrinsic rhythmicity are incorporated into the developing heart. With the development of the heart, the pacemaking cells of the sinoventricular canal region probably become organized into nodes and bundles while keeping their original relationship with the surrounding musculature.

Several investigators have recorded pacemaker activity from ventricular cell clusters, in culture (2, 6, 16, 30). These findings cannot be considered conclusive proof that "non-specialized" embryonic cardiac muscle is capable of spontaneous activity since cells of the specialized conducting tissues may have been present in the inoculum. Furthermore, to what extent these findings result from the procedures employed in producing cell cultures from the
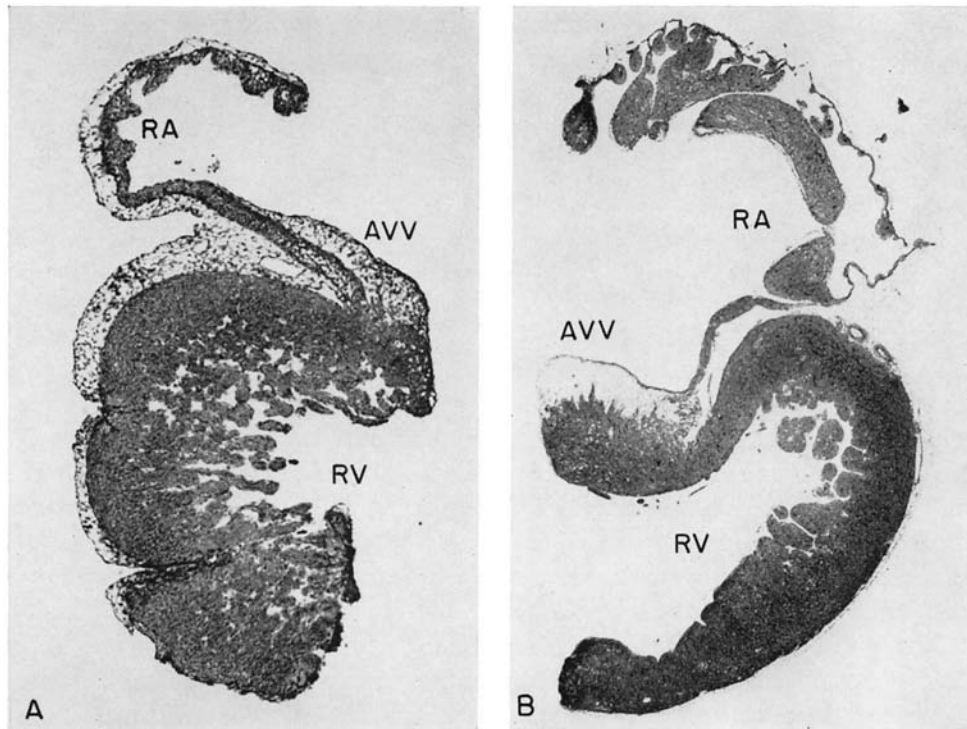

FIGURE 5. Sagittal section of 7 (A) and 14 (B) day embryonic chick hearts (modified Masson's trichrome). *RA*, right atrium. *AVV*, atrioventricular valve. *RV*, right ventricle. A, × 80; B, × 30.

intact heart remains to be determined. There is evidence that cells of the specialized conducting system possess some biochemical, histological, and cytological characteristics of the embryonic heart (4). Questions pertaining to whether all primordial cardiac cells are capable of pacemaker activity or whether pacemaker and non-pacemaker cells are differentiated *de novo* presently are unanswered.

B. *Tissue of the Atrioventricular Ring*

Muscular continuity is demonstrated, histologically, between the atrium and ventricle across the right AV valve in 7 and 14 day embryonic chick hearts (Fig. 5) and adult bird hearts (3). At higher magnifications (17), the

cellular orientation of the AV ring cells is similar to that of the newborn mammalian AV junction (14) and the adult rabbit AV node (23). Studies concerned with the evolution of the vertebrate heart reveal that a fibrous separation of the atria and ventricles occurs simultaneously with the reduction of the AV ring (lower vertebrates) to the AV node (birds and mammals) (19). To date, demonstration of an AV node and bundle of His in the 4 day embryonic heart is lacking. Nevertheless, the electrocardiogram was shown to display a PR interval (1, 9). It is highly probable that, at this stage, the AV ring tissue is responsible for delaying AV transmission. Transmembrane potentials recorded from the embryonic AV ring are similar in both sequence of activation and configuration to the potentials recorded from the adult rabbit AV node (11, 12) and AV ring (24). Also, records from frog AV ring cells (13) are similar to those of the embryonic chick AV ring.

The origin of the AV node has been questioned since the early part of the century (5). Patten (27) was of the opinion that the AV node started its development as the counterpart of the left sinus horn. The migration of the left sinus horn may well account for the presence of specialized fibers in the region of the coronary sinus. However, this hypothesis does not account for the presence of AV delay before the migration of the left sinus horn; *i.e.*, in the tubular heart in which complete muscular continuity is present between atrial and ventricular muscle. The fact that embryonic AV ring cells are shown to possess some of the physiological properties of the adult AV node (18) strengthens an earlier proposal that the adult AV node is a remnant of the embryonic AV ring (22).

C. *Origin of the Bundle of His*

Membrane potentials recorded from the ventricular side of the embryonic chick AV valve are similar to those recorded from the corresponding side of the AV ring muscle in the frog heart (13). Furthermore, several analogies may be drawn between the cells of the ventricular portion of the embryonic AV valve and the adult mammalian His-Purkinje system. Both structures (*a*) are situated in the path of propagation between slowly conducting tissue and the ventricular musculature; (*b*) contain cells whose action potential duration is significantly longer than that of ventricular myocardial cells; (*c*) are comprised of cells which are insensitive to local applications of acetylcholine (17, 25).

It thus might be suggested that cells of the ventricular portion of the AV valve are precursors of the His-Purkinje system. The diffuse cellular structure of the embryonic AV valve (ventricular portion), may coalesce into branching bundles which are separated from one another by the invading connective tissue of the adult AV ring. In birds, such a transformation would be partial, since the AV bundle is known to coexist with the AV valve connections (3). However, in adult mammals, a complete transformation normally would

occur with the presence of a single main bundle that branches into an extensive subendocardial Purkinje network. A definitive statement regarding this hypothesis awaits further electrophysiological and histological studies.

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