Sequence of Atrial Depolarization at Different Stages of Development of the Chick Embryo

By Gershon Hait, M.D., and Richard H. Licata, Ph.D.

ABSTRACT
Simultaneous left and right atrial surface electrograms were obtained from the right and left atria of 30 chick embryos. These were divided into 3 equal groups that were studied at 15 to 16, 10 to 11, and 5 to 7 days of incubation. The embryos were removed from the shell with their circulation intact and temperature maintained constant. In an early stage of development (5 to 6 days), the left atrium and its appendage were larger than the right, and left preceded right atrial depolarization. At and after 7 days of incubation, the right atrium and its appendage were larger than the left, and right preceded left atrial depolarization. The intervals for interatrial conduction and for atrioventricular conduction were about the same at all stages of development. Since the length and width of the heart at 15 to 16 days of incubation were about 3 times those of a heart at 5 to 7 days of incubation, it is proposed that the higher the stage of development of the chick embryo the greater must be the conduction velocity between the two atria and from atrium to ventricle. This was assumed to be related to the degree of development of the specialized conduction pathways that are probably absent or underdeveloped in early stages of development.

ADDITIONAL KEY WORDS
intact circulation and constant temperature electrogram left atrial pacemaker sinoatrial pacemaker left S-A node interatrial and atrioventricular conduction velocity

Little information is available about possible pacemaking areas in the left atrium (1-6). Mindful of the ontogenic development of the embryonic heart, we concerned ourselves in the present study with the possibility that the left atrium at some early stage of development could initiate its rhythm independently and that its depolarization might precede right atrial depolarization. At the conclusion of our study we became aware of the interesting relationship between the interatrial and the atrioventricular conduction velocity and the various stages of development of the chick embryo's heart and measured the intervals for interatrial conduction and atrioventricular conduction.

Materials and Method
Simultaneous left and right atrial surface electrograms were recorded from 30 chick embryos. Eggs were supplied from the same source weekly and were uniformly incubated in our laboratory. The allocation of 10 eggs for each of the 3 groups was done at random; one group of eggs was incubated 5 to 7 days, another for 10 to 11 days and the third for 15 to 16 days. The embryo was removed from the ovum so as to preserve as much of the circulation as possible and avoid hemorrhage. After cracking the shell, pieces of shell were peeled carefully away from the shell membrane. This procedure was best accomplished by immersing the entire egg in water that had been heated to 98°F. After removal of an adequate area of shell, the exposed shell membrane was further carefully separated from...
the remaining shell so that the entire embryo was removed within its membrane. The membrane was then incised along a line free of blood vessels, and the embryo and its choriovallantoic membranes and attached yolk sac were released. The yolk sac was cut beyond the terminal venous sinus and its contents were allowed to dissipate in the water; the albumen sac or its remnant was removed at the same time and the embryo and its membranes were spread widely in the water and carefully floated on a grid or a glass Maximow slide. In this manner the embryo with its entire circulation was transported to the micromanipulator grid where it was spread out. The amniotic membrane was then cut and the embryo was placed on its back. The chest was opened by incising the thoracic body wall laterally and by removing the central part of the thoracic cage. The embryo with its intact circulation was then ready for placement of the exploring electrode on the heart surface.

A mechanical micromanipulator consisting of two unipolar electrodes and one indifferent electrode was constructed. This permitted coarse and fine vertical, horizontal, and circular movements. A dissecting microscope was placed just above the micromanipulator.

A heating chamber 4½ x 3½ inches was constructed from ¼-inch lucite. The roof of this chamber was also the bottom of a bath. The lucite was cut off the bottom of this bath (roof of the heating chamber) and was replaced by glass, which is a better heat conductor. The bath was filled with Ringer’s solution. A heating unit and thermoregulator (Chatham Control Corp.) were used to maintain the temperature at 98.9°F ± 1°F. A pump provided constant circulation of water. The entire chamber was attached to the base of the micromanipulator.

A 3/32-inch copper wire frame 1 x 2 inches with copper screen attached over it was used as the indifferent electrode and was connected to the indifferent electrode socket. This grid was attached to the bottom of the bath by screws; it was removable and easy to clean. The bath and copper grid were cleaned before each new experiment.

Platinum wire, 0.0001 inch, was used for studying embryos 15 to 16 and 10 to 11 days old and 0.00005-inch wire for embryos 5 to 7 days old. One end of the platinum wire was soldered to a shielded wire and, except for the tip, was housed in a glass tube 3/16 inch in diameter. To prevent injury to the epicardial surface, a club-shaped tip with a smooth end was prepared. After each experiment, the platinum electrode tip was cleaned by heating to bright red in a flame.

Each of the two recording unipolar electrodes was connected to a preamplifier (Tektronix type 122), and both preamplifiers were connected to a Dual Beam Oscilloscope (Tektronix type 502). Low frequency response of the preamplifiers was set at 0.2 cycle/sec and high frequency response at 1 kc/sec. A gain of 1000 was used in the preamplifiers. For obtaining permanent records, a polaroid camera was used with type 47 3000 speed film. Each record was taken at speeds of 0.2 sec/cm and at 50 msec/cm.

Each of the two type 122 preamplifiers was powered by five 45-volt B batteries (Burgess type M30) as well as a 6-volt storage battery.

Serial electrograms were recorded from one to three points on each surface of the two atria. The first recordings were obtained 1 to 12 min after the removal of the chick embryo from the egg. The work area was enclosed by copper mesh.

**Results**

Left and right atrial surface electrograms were always recorded simultaneously. In the figures, the upper tracing was always recorded from the right atrium and the lower tracing from the left atrium.

The chick embryo’s electrogram inscribed a record similar to that of an adult mammalian heart. Accordingly, the P wave was associated with atrial depolarization, the QRS-T complex with ventricular depolarization and repolarization, and the P-R interval with the period commonly referred to as A-V nodal delay. In man, repolarization of the atrium occurs during depolarization of the ventricles and is therefore usually concealed. In the recording from the chick embryo, one can usually see the unconcealed atrial repolarization (Ta) between the P wave and the QRS complex. When the electrode was placed over the ventricular surface, the electrogram consisted of small P and Ta waves followed by a QRS complex of high amplitude and a T wave (Fig. 1a). Characteristically, there was a reverse relationship of the amplitudes of the P wave and QRS complex when the electrogram was obtained from the atrial surface. Since the electrode placed over the atrial surfaces was relatively far from the ventricular mass, the amplitude of depolarization of the atrium (P wave) was greater than that of depolarization of the ventricle (QRS) (Fig. 1c). The electrode that was placed on the surface of
Simultaneous electrograms obtained from various places over the heart of a 10-day-old chick embryo: (a) 26 min after removal of the embryo from the egg. Upper tracing was obtained from an electrode placed over the upper right side of the ventricular mass near the junction with right atrium. Lower tracing was obtained from electrode placed over the upper left side of the ventricle near the junction with left atrium. In both tracings, note that the P wave was followed by Ta, QRS, and T waves. In the lower tracing note the dome-and-dart shape of the P waves. (b) 28 min after removal from the egg. Note that the P_R in the upper tracing preceded P_L in the lower, but the simultaneous electrical counterpart of P_R is clearly visible and seems to explain adequately the dome-and-dart configuration in P in lower tracing of a. (c) 30 min after removal from the egg. In the upper tracing, the electrode was placed over the right atrium and in the lower, the second electrode was placed over the left atrium. Note that when electrodes were placed over atrial surfaces and were therefore far from the ventricular mass they recorded a much greater amplitude of atrial than of ventricular depolarization. Also note that P_R preceded P_L. (d) 35 min after removal from the egg. In the upper tracing, the electrode was placed over right side of the ventricle and in the lower, was placed over the right atrium. In both upper and lower tracings atrial depolarization appeared simultaneously.

Analysis of the P wave in the electrogram taken at a speed of 50 msec/cm demonstrated the asynchronous activation of the two atria (Fig. 2). In the upper tracing (right atrial electrogram, Fig. 2b, c) the initial tallest component corresponded to right atrial depolarization. The second component, which was of a
Electrograms obtained from one embryo in each of the three groups. Upper tracings were obtained from the right atrium and lower tracings from the left atrium, except for the upper tracing in a, which was obtained from the right upper ventricle, between the right and left atrium. (a) From a 16-day-old chick embryo 49 min after removal from the egg. Note that right atrial depolarization $P_R$ preceded left atrial depolarization. Since in the upper tracing the electrode was on the right ventricle at about equal distance from both atria, both right and left atrial depolarizations were of similar amplitudes. (b) From 10-day-old chick embryo 31 min after removal from the egg. Note in the upper tracing that right atrial depolarization $P_R$ was in synchrony with a small wave in the lower tracing. This $P_R$ was followed by a small elevation (upper tracing) synchronous with left atrial depolarization, which was of higher amplitude in the lower tracing $P_L$. (c) From a 7-day-old chick embryo at 47 min after removal from the egg. Note in the upper tracing that right atrial depolarization $P_R$ was followed by a prolonged interatrial interval (interatrial block) and a small wave synchronous with left atrial depolarization $P_L$. In the lower tracing, a small wave synchronous with $P_R$ was followed by a large wave $P_L$. Also note absence of QRS complex (A-V block).

much smaller amplitude, corresponded to left atrial depolarization. In the lower tracing (left atrial electrogram), the small initial component represented the right atrial depolarization and the second tall component that of the left atrial depolarization. Right preceded left atrial depolarization in all 20 embryos 10 to 16 days old.

In 10 embryos 5 to 7 days old it was possible to distinguish microscopically two different stages of development. In five hearts at an earlier stage of development, the left atrial appendage was two to three times larger than the right, and the ventricular apex was rounded rather than pointed in the ventral view. This corresponded approximately to 5 to 6 days of incubation. In histologic section of these hearts we noted that the volume of the left atrium was greater than that of the right, anteriorly. In four of these hearts, left preceded right atrial depolarization (Fig. 3b). In the other five hearts at a more advanced development, the right atrial appendage was larger than the left and the ventricular apex was more conical in shape in the ventral view; this stage corresponded approximately to 6 to 7 days of incubation. In four of these hearts the sequence of atrial depolarization was right preceding left (Fig. 3a). In two embryos, one from the earlier stage (5 to 6 days) and one from the more advanced stage of development (6 to 7 days), the initial depolarization was from the right atrium but reverted to an initial left depolarization 4 and 12 min after the beginning of the experiment (Fig. 4); this latter sequence of depolarization re-
FIGURE 3

(a) Electrograms from a 7-day-old-embryo 33 min after removal from the egg. Note in the upper tracing $P_R$ was followed by a $P_L$ in the lower tracing. Also note presence of $Ta$ and absence of QRS complex (A-V block). (b) From an embryo 5 to 6 days old, 12 min after removal from the egg. Note in the lower tracing that left atrial depolarization $P_L$ preceded right atrial depolarization $P_R$ in the upper tracing.

Intervals between the two atrial components as well as the P-R intervals were measured in all cases at the beginning of each experiment, then 30 min after the removal of the embryo from the egg, and at the end of each experiment (Table 1). The interval that separated the two atrial components was taken to represent an interatrial conduction time during which the electrical impulse was propagated from one atrium to the other. The average interatrial intervals were 8, 6, and 6 msec and the average P-R intervals were 115, 104, and 114 msec in embryos 15 to 16, 10 to 11 and 5 to 7 days old respectively. These findings were particularly significant, since the hearts of embryos 15 to 16 days old were about three times as long and as wide as those of embryos 5 to 7 days old. Both intervals increased progressively throughout the experiment, thus indicating interatrial and atrioventricular blocks. However, although 30 min after removal of the embryo from the egg the average interatrial interval increased only by 1 msec in embryos 15 to 16 days old, it increased by 6 and 10 msec in those 10 to 11 and 5 to 7 days old. The increase in the average P-R interval 30 min after removal of the embryo from the egg was 27, 31, and 65 msec in embryos 15 to 16, 10 to 11 and 5 to 7 days old respectively.

Discussion

It is not clear why left atrial depolarization precedes that of the right at the early stage (5 to 6 days) of development. In histologic studies of the hearts we noted that the left atrium was still larger than the right, anteriorly, at 4 to 6 days of incubation (7). It may
Therefore be postulated that the pacemaking cells of the sinus venosus which are incorporated in the atrium are distributed in proportion to the size of their respective atria, resulting in left atrial dominance at this stage of development. Some time between 6 and 7 days of incubation, with the outgrowth of the right atrium, the sinus venosus with its pacemaking cells completes its shift to the right and becomes submerged primarily in the right atrium (8), resulting in right atrial dominance. Such a postulate may perhaps provide an explanation for our observations in hearts of two chick embryos belonging to this stage of development (5 to 7 days). In these cases (Fig. 4), initial right atrial depolarization may have reverted to initial left atrial depolarization during a time when transition from left to right atrial dominance had just taken place, and because the right atrium at that time was more vulnerable when the preparation deteriorated, left sided dominance was reestablished.

Another possible explanation of why left preceded right atrial depolarization during the early stages of development may be related to the left structural homologue of the right sinoatrial node (9, 10). Although Patten (9) suggested that the pacemaking tissue at the left precaval vein was later carried downward and inward to become the A-V node, Licata (10) believed that as the left caval vessels dropped out, regression of the sinoatrial homologue followed. The two primordial nodes had general structural similarity and were both intimately supplied by homolateral blood and innervation.

Histologic sections of our present material showed that at the point of discharge of the right superior vena cava there was a thickening of muscle anteriorly which could be the precursor of the sinoatrial node. A minimal concentration of such a precaval musculature mass was also seen in a similar location on the left superior vena cava. The area comparable to the S-A node as we saw it in the human on the right side was difficult to distinguish from the remainder of the atrial musculature since very little connective tissue separated them, whereas in the human embryo, the separation of the nodal mass from atrial musculature was well defined. Furthermore, in the chick the musculature of this nodelike area spiraled some distance headward within the adventitia of the superior vena cava.
to this area was comparable to that in man.

Throughout, we have assumed that the impulse arose somewhere in the right atrium and after reaching the right atrial electrode propagated through the left atrium, finally reaching the left atrial electrode. In the younger age group (5 to 6 days) we assumed that the impulse traveled in the opposite direction. This assumption is, however, not the only one possible. It is possible that the impulse arose somewhere in the right atrium between the two electrodes and yet reached the left electrode sooner than it reached the right electrode and therefore seemed to have arisen from the left atrium. However, we believe that even if the impulse had originated between the two electrodes, it most probably originated somewhere in the left sinoatrial region since left preceded right atrial depolarization no matter where or how medially over the surface of the left atrium the left electrode was placed.

However one may explain the fact that left preceded right atrial depolarization in the early stages of development, the ultimate answer requires the identification of a diffuse or localized pacemaking area in the sinoatrial region, especially between 2 and 5 days of incubation. To date, localization of tissue with pacemaker potentiality in the left sinoatrial region has been reported to vary (16). No information, however, is yet available about localization of pacemaking areas in the left sinoatrial region by microelectrode methods during early or late stages of development.

Observations of occasional spontaneous contractions have been made in the isolated left atrium of the chick embryo by Cohn (6) and in the adult animal by Erlanger and Blackman (1), Demoor (2, 4), and Hermann et al. (5). Rothberger and Sacks (3) noted that automaticity of left atrium occurred irregularly in the rabbit's heart, but histologic examination of their material by Aschoff did not reveal accumulation of specialized tissue in the left atrium. Serge (17) pointed out that in ruminants the sinus node is shaped like a horseshoe, ending with two nodular formations, one of which is located at the junction between the two atria. In four human hearts, he also found a horseshoe-shaped mass in which the left nodular mass occupied the groove between the musculature of the superior wall of the right atrium and that of the left. Bruni (18) also found an extension of sinus node tissue into the interatrial septum in embryos of the sheep and ox. However, investigations and conclusions about an additional sinus node were not confirmed (19). Clinical evidence to confirm left atrial pacemaker sites is also absent. In 1920 Schrumpf (20) published an electrocardiogram he thought was a "double sinus rhythm;" one group of P waves had a regular rate of 64/min of sinus origin and a second, independent, set of P waves had a rate of 109 beats/min. Marcel and Exchaquet (21) demonstrated a double atrial rhythm in one human fetus of 24 months gestation 11 min after hysterotomy. As in our chick embryos 5-6 days old, they observed left preceding right atrial depolarization. The possibility of a left atrial ectopic focus has been mentioned on a few occasions in connection with an abnormal direction of the P wave (22-27). More recently, Mirowski et al. (28), in an attempt to explain abnormal configuration of the P wave in eight patients with mirror-image dextrocardia and in four normally placed hearts that were not in sinus rhythm, suggested that a dome-and-dart-like P wave might represent left atrial ectopic rhythm.

Although the electrodes were placed approximately in the same places on the surface of both atria in all our experiments, their tips were larger in the studies done on hearts of chick embryos 15 to 16 days old than on those 5 to 7 days old. Nevertheless, the impulse traveled between the two atria at about the
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**TABLE 1**

**Measurements of Interatrial and Atrioventricular Intervals in Serial Electrograms of 30 Chick Embryos at Three Stages of Development**

<table>
<thead>
<tr>
<th>Embryo age</th>
<th>First tracings</th>
<th>Tracings at 30 min*</th>
<th>Last tracings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interval (msec)</td>
<td>P-R, P-R increase</td>
<td>Interval (msec)</td>
</tr>
<tr>
<td>15-16 days</td>
<td>15-16</td>
<td>5-15</td>
<td>85-150</td>
</tr>
<tr>
<td></td>
<td>(7.6)</td>
<td>(8)</td>
<td>(115)</td>
</tr>
<tr>
<td>10-11 days</td>
<td>5-12</td>
<td>5-10</td>
<td>70-130</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(8)</td>
<td>(104)</td>
</tr>
<tr>
<td>5-7 days</td>
<td>5-9</td>
<td>5-7.5</td>
<td>90-150</td>
</tr>
<tr>
<td></td>
<td>(7.6)</td>
<td>(8)</td>
<td>(114)</td>
</tr>
</tbody>
</table>

P-R = Interval between right and left atrial depolarization; numbers in parentheses are average values.
*Minutes after removal of the embryo from the egg.
†Increased time as compared to the first tracing.
‡Number of cases with A-V block.

The P-R interval that we measured (Table 1) was not the "period of A-V nodal delay" commonly referred to. The first part of the ventricle to be excited was rather nearer the apex, and the P-R interval therefore represented the time required for the impulse to traverse through the A-V node, bundle of His, and the Purkinje fibers. The measured P-R interval, therefore, represents the time interval between the ventricle's apex and the Purkinje fibers, rather than the "period of A-V nodal delay." The interval that we measured was almost the same in all three groups (Table 1), even though the three times as long as hearts at 5 to 7 days. The slower conduction velocities in the younger hearts appear to be due to cell-to-cell conduction or to underdeveloped conduction pathways that linked the atrium and the ventricle. The P-R intervals for hearts at 16 to 17 days were about three times as long as hearts at 5 to 7 days. The slower conduction velocity in the younger hearts may be due to a cell-to-cell conduction or to underdeveloped conduction pathways that linked the atrium and the ventricle.
Electrogram from 7-day-old chick embryo 60 min after removal from the egg. Upper tracing was obtained from right atrium and lower tracing simultaneously from left atrium. Note absence of QRS in both tracings (A-V block). Upper tracing shows first and third right atrial depolarization $P_R$, followed by a small wave synchronous with left atrial depolarization $P_L$, which is well seen in the lower tracing. Second and fourth right atrial depolarization $P_R$ was not followed by left atrial depolarization. This 2:1 interatrial block was maintained for a few minutes; many such records were obtained to document its occurrence.

Histologic verification of such a hypothesis is difficult in the chick embryo, since it is not known when the conduction system differentiates. Although the conduction system, S-A, and A-V node have been well described in human and in a few other mammalian species, they have been identified in the adult chicken by some but not by others (31). In histologic sections of our present material, the interventricular septum, which contains the A-V conducting bundle, was not complete before the fifth to sixth incubation day. It was thus reasonable to assume that conduction, at least before this time, was not through a complete conduction system and therefore its velocity was slower. As in interatrial block, the degree of atrioventricular block was in direct relationship to the age of the embryo and to the duration of the experiment (Table 1).

Acknowledgment
We are deeply indebted to Dr. Maurice Lev, Director of the Congenital Heart Disease and Training Center in Chicago, for his continuous interest and encouragement throughout this work.

References
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Circ Res. 1967;20:204-213
doi: 10.1161/01.RES.20.2.204

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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