Persisting Zones of Slow Impulse Conduction in Developing Chicken Hearts


We performed a correlative electrophysiological and immunohistochemical study of embryonic chicken hearts during the septation period (Hamburger and Hamilton stages 13–31 [2–7 days of incubation]). The analyses yield conclusive evidence for slow conduction, up to 7 days of development, in the outflow tract, in the atrioventricular canal, and in the sinoatrial junction. The conduction velocity remains approximately 1 cm/sec in the outflow tract and increases in the ventricle 20-fold to approximately 20 cm/sec between 2 and 7 days of development. Transmembrane potentials of myocytes in the outflow tract and atroioventricular canal slowly rise (<5 V/sec), whereas in the atrium and ventricle, the upstroke velocity is eightfold to 13-fold higher. In the outflow tract, repolarization is completed only after the start of the next cycle. Because of the persistence of slow conduction, the myocardium flanking the developing atria and ventricle is thought to represent segments of persisting “primary” myocardium, whereas the more rapidly conducting “working” myocardium of the ventricle and atria is thought to represent more advanced stages of myocardial differentiation. The persisting primary myocardium was characterized by a continued coexpression of both the atrial and ventricular isoforms of myosin heavy chain. The developing atria and ventricle could be demarcated morphologically from the primary myocardium because the free walls of these segments only express their respective isoforms of myosin heavy chain. The slowly conducting myocardial zones appear to be essential for the function of the embryonic heart because 1) they provide the septating heart with alternating segments of slow and relatively fast conduction necessary for consecutive contraction of the atrial and ventricular segments and 2) their sphincterlike prolonged peristaltic contraction pattern can substitute for the adult type of one-way valves that start to develop at the end of septation. (Circulation Research 1992;71:240–250)

KEY WORDS • embryonic chicken hearts • outflow tract • conduction system • conduction velocity • transmembrane potentials • myosin heavy chain isoforms

In the “primary” myocardium of early tubular hearts, the conduction of the depolarizing impulse, which initiates contraction of the myocytes, is slow and spreads uniformly,1 resulting in a peristaltic contraction pattern.2 Shortly after contractile activity can first be observed in the embryonic heart, however, the developing “working” myocardium of the emerging atrium and ventricle begins to contract synchronously. In addition, the atrium and ventricle can be seen to contract sequentially.2,3 These observations indicate the development of cardiac “segments” with different conduction velocities. The existence of such segments is further suggested by the presence of an adult type of electrogram,4–6 including an atrioventricular delay, in these embryonic hearts, in spite of the absence of morphologically identifiable components of the definitive conduction system.7,8 The regulation of the conduction of the impulse in the developing myocardium most likely also has mechanical implications, because the embryonic heart does not yet possess one-way valves. Hence, its dynamic competence must depend on a strict temporospatial coordination of the onset and duration of the contraction of the myocardium. Therefore, the following questions arise: Along what routes does the electric impulse spread through the embryonic heart in the absence of a conduction system? Are there regional differences in the contractile properties of the myocardium?

Possible answers were suggested by the observed developmental changes in the distribution pattern of myosin heavy chain (MHC) isoforms in septating chicken hearts.5–12 These studies showed that the slowly conducting primary myocardium of the tubular heart is characterized by a coexpression of atrium- and ventricle-specific MHC isoforms and that the development of the more rapidly conducting working myocardium of the atrial and ventricular segments can be monitored by the regional loss of this coexpression. These data suggested to us that the cellular expression pattern of MHC isoforms might be used as a parameter for myocardial differentiation, with single expression and coexpression representing more and less advanced stages of myocardial differentiation, respectively. Therefore, it was hypothesized that the coexpression zones that remain in the sinoatrial junction, the atrioventricular canal, and
the outflow tract during the septational period identify slowly conducting myocardium. This hypothesis was corroborated by the observation that during the septational period the impulse is indeed slowly conducted in the myocardium of the atrioventricular canal.\textsuperscript{13,14} Therefore, we decided to study the electrophysiological properties of the myocardium that retains its coexpression of MH isoforms during septation, i.e., between Hamburger and Hamilton\textsuperscript{15} (H/H) stages 13 and 31 (embryonic days [EDs] 2–7) in the chick embryo. The study yields conclusive evidence for slow conduction of the depolarizing impulse in the myocardium of the outflow tract, the atrioventricular canal, and the sinoatrial junction.

Materials and Methods

Preparations

Fertilized white leghorn eggs were obtained from a commercial supplier (Fa. Drost, Loosdrecht, The Netherlands) and incubated at 37.5°C and 80% humidity for appropriate periods (2–7 days). Embryos were staged according to the classification of Hamburger and Hamilton.\textsuperscript{15} Hearts were dissected from the embryos and transferred to a tissue bath. The bottom of the tissue bath was covered with a dark-colored soft Teflon cushion to enhance the visibility of the hearts and to allow the affixation of the hearts with fine glass needles (microelectrode tips) placed through small parts of noncardiac tissues at the inflow and outflow sides of the hearts. The hearts were superfused with warm (35°C) oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) Tyrode’s solution of the following composition (mM): Na\textsuperscript{+} 156.5, K\textsuperscript{+} 4.6, Ca\textsuperscript{2+} 1.5, Mg\textsuperscript{2+} 0.7, H\textsubscript{2}PO\textsubscript{4} 0.5, Cl\textsuperscript{−} 137.0, HCO\textsubscript{3} 28.0, and glucose 10.0. The pH was 7.4. The preparations were allowed to equilibrate for 30 minutes before the experiments were started. Maximum experimental time was 2 hours.

Electrophysiology

Hearts at H/H stages 19–29 (EDs 3–6) were driven from the atrium or sinus venosus with a stimulus strength of twice diastolic threshold. Cycle length was chosen slightly shorter than the spontaneous cycle length. The hearts at H/H stages 13 and 14 (ED 2) and at H/H stage 31 (ED 7) were allowed to beat spontaneously. Hearts at 7 days of development had grown to such a size and their ventricular walls were so thick that, if kept under the same conditions as younger hearts, they developed tachycardia, followed by a partial atrioventricular block and finally by cardiac arrest. Since these phenomena were not observed in younger hearts, even after 5 hours in vitro, we assume that the superfusion condition was adequate for the metabolic demands of these hearts. In the hearts at H/H stage 31 (ED 7), lowering of the temperature of the superfusion solution (from 35° to 30°C), opening the right and left ventricles near the apex, and allowing the hearts to beat spontaneously (i.e., at a rate similar to that used to drive hearts at H/H stage 29 [ED 6]) did yield an electrophysiologically stable preparation.

Extracellular recordings and marking experiments. Extracellular recordings were performed with small platinum electrodes (diameter, 25 μm). A reference electrode was placed on a fixed position on the ventricle during all experiments. Some electrodes were glued together in such a way that one electrode contained two or three terminals at distances varying from 40 to 170 μm. After recording of local activations, the same electrode could be used to mark its location on the myocardium by applying a current (6–8 mA at 4 Hz for 30 seconds). The (single or compound) exploring electrode was placed at various locations on the heart, starting from the most downstream part of the outflow tract and subsequently on more upstream parts of the heart. This procedure minimized the possibility that the marking procedure would affect the conduction path-

<table>
<thead>
<tr>
<th>ED</th>
<th>OTF</th>
<th>OFT</th>
<th>Ventricle</th>
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<tbody>
<tr>
<td>2</td>
<td></td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.7±0.3 (n=2)</td>
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<tr>
<td>4</td>
<td>0.8±0.2 (n=7)</td>
<td>11.3±0.2 (n=3)</td>
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<tr>
<td>5</td>
<td>0.5</td>
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<tr>
<td>6 and 7</td>
<td>1.3</td>
<td>20.0</td>
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ED, embryonic day; OTF, outflow tract; n, number of experiments, if more than one. When three or more calculations were available, the SEM was calculated; when only two calculations were available, the range is given.

FIGURE 1. Conduction in the ventricle at Hamburger and Hamilton\textsuperscript{15} stage 14 (embryonic day 2). OTF, outflow tract; SA, sinoatrial junction; AV, atrioventricular canal; V, ventricle; ↓, local activation of V; EN, endocardium; MY, myocardium. Panel A: Photograph of left aspect of a heart at Hamburger and Hamilton stage 14 identifying the myocardial position of recordings 1–3 shown in panel B. Bar, 0.2 mm. Panel B: Recordings at a calculated conduction velocity of 0.9 cm/sec. This heart was allowed to beat spontaneously (cycle length, ∼285 msec). Horizontal bar, 50 msec; vertical bar, 0.5 mV. Panel C: Transverse hematoxylin-stained section through an embryonic day 2 heart illustrating the thin myocardial wall and the endocardial jelly (asterisks) lining the entire cardiac tube except part of the SA. Bar, 0.2 mm.
way of the impulse. Approximately 60% of the marks could be traced in the immunohistochemically processed serial sections of the hearts. The fixed distance between the recording electrode terminals allowed a calculation of conduction velocity. It was found (see "Results") that the ventricle was activated from the atioventricular canal toward the greater curvature (≤H/H stage 21 [ED 3.5]) or in reverse order (≥H/H stage 23 [ED 4]) and that the outflow tract was activated from its junction with the ventricle toward its junction with the branchial arteries. Conduction velocities were therefore calculated on the assumption that the compound recording electrode was placed in such a way (Figures 1A, 2A, 3A, 6A, and 7A) that the activation front ran perpendicular to the axis through the electrode terminals. If this assumption is not accurate, our calculations overestimate the real values, and the calculated conduction velocities (summarized in Table 1) could even be lower. However, our calculated conduction velocities in the developing ventricle are comparable to those reported previously.\textsuperscript{15}

Transmembrane potentials. Transmembrane potentials were recorded from isolated H/H stage 23 (ED 4) hearts by conventional floating glass microelectrodes filled with 2.5 M KCl (tip diameter, =1 μm; resistance, 20–40 MΩ). The signals were fed into a high-input impedance amplifier, photographed from an oscilloscope (model 5111A, Tektronix, Beaverton, Ore.), and written on a recorder (model MT 8500, Astro-Med, Inc., West Warwick, R.I.). The upstroke of the action potentials was electronically differentiated to determine the maximum upstroke velocity.
**Immunohistochemistry**

After completion of the electrophysiological experiments, the hearts were fixed for 2 hours by immersion in a mixture of methanol, acetone, acetic acid, and water (9:9:2:4 by volume) at room temperature and photographed. Thereafter, the hearts were dehydrated in acidified 2,2-dimethoxypropane, embedded in Paraplast (Paraplast Plus, Sherwood Medical, Athy, Ireland), and serially sectioned (5 μm). Sections were alternately incubated with antibodies specific for MHC isoforms of adult chicken atria or adult chicken ventricles. The antibodies have been characterized previously. The antibody-antigen reaction was made visible with the peroxidase anti-peroxidase technique. After determination of the plane of sectioning, the MHC isoform expression patterns were projected onto the photographs of the hearts that were made before embedding and sectioning. The areas where coexpression of atrial and ventricular MHCs was found are depicted as a dotted pattern in the photographs shown in Figures 3, 4, 6, and 7.

**Validation of Methods**

**Signal analysis and interpretation.** The interpretation of deflections in the electrograms as local myocardial activations underneath the electrode terminal is supported by several lines of evidence: 1) Simultaneously recorded electrograms on the outflow tract and transmembrane potentials of neighboring myocytes unequivocally showed that both the extracellular and intracellular signals occurred at the same moment in the heart cycle (Figure 5A). 2) Deflections in the electrograms of the outflow tract are not remotely sensed ventricular repolarization signals, because ventricular repolarization signals appeared at a constant moment in the heart cycle, whereas the outflow tract deflections appeared at variable moments depending on the position of the terminal on the outflow tract (Figure 2B). 3) Deflections in the electrograms disappeared selectively, when the myocardium underneath an electrode terminal was damaged, as shown by using a stationary terminal first as a recording electrode, then as a stimulator, and finally again as a recording electrode (compare tracing 3 [before marking] and tracing 3' [after marking] in Figure 2B).

**Functional damage to the myocardium and recognition of the marks in the serial sections.** Functional damage to the myocardium by marking was restricted to a very small area. The deflection recorded by a terminal located on the outflow tract only 140 μm upstream from a marking terminal remained unaltered with respect to the timing in the heart cycle and the amplitude, showing that the pathway taken by the activation front was not affected by the myocardial damage 140 μm downstream (Figure 2B, tracings 2 and 2').

Marks could be identified in the sections as little holes in the myocardium or as exploded myocardial cells from which the myosin-containing fragments were easily recognizable (Figures 4E and 4F).

**Results**

Experimental results of the whole septal period will be divided into three periods: H/H stages 13 and 14 (ED 2), H/H stages 19–27 (EDs 3–5), and H/H stages 29–31 (EDs 6 and 7).

**H/H Stages 13 and 14**

Figure 1 shows hearts at 2 days of development. It proved difficult to obtain interpretable electrograms from hearts at H/H stages 13 and 14. Their very thin myocardial wall (which is only a few cells thick; see panel C) underneath the small electrode terminals
causes activation signals of low amplitude that barely rise above the noise in the amplified recordings. Furthermore, these isolated tiny hearts were prone to mechanical damage during subsequent immunohistochemical processing. Out of five hearts, only one triple ventricular electrogram could be recorded (panel B), from which we calculated a ventricular conduction velocity of approximately 0.9 cm/sec. This slow conduction velocity is comparable with that found in the outflow tract of slightly older embryonic hearts. At this stage the ventricular myocardium is still characterized by a prominent coexpression of isomyosins.10

H/H Stages 19–27

Figures 2–5 show hearts at 3–5 days of development. Hearts between H/H stages 19 and 27 (EDs 3–5) possess a relatively long myocardial outflow tract that is characterized by a prominent coexpression of isomyosins (Figures 3A, 4A, and 4C). Hearts in this period of development are easily handled experimentally; thus, a
sufficient number of embryos could be studied to obtain statistically significant parameter values.

Activation of the ventricle. The electrograms recorded by compound extracellular electrodes placed at various locations on the ventricular myocardium (Figures 2A, 3A, and 4A) reveal a rapid spread of the impulse (Figures 2C, 2D, 3C, and 4B). A comparison of tracings R, 2, and 3 in Figures 2C and 2D shows that terminals C2 and D2 are activated slightly before terminals C3, D3, and R, indicating that the activation front in this heart at H/H stage 21 (ED 3.5) is conducted from the atrioventricular canal toward the greater curvature. This pattern of conduction represents the original caudocranial direction of the conduction of the impulse in the myocardial wall. Similar analyses of the activation of the ventricular myocardium at and after H/H stage 23 (ED 4) (tracings 1–4 in Figure 3 and tracings E and T in Figure 7C) show that from 4 days of development onward the apex is activated earlier than ventricular myocardium, which is closer to the atrioventricular canal. This change in activation pattern is thought to be due to preferential conduction of the impulse through the developing trabeculae. Day 3 of incubation is characterized by a rapid development of the ventricular trabeculae (see below). A conduction velocity of approximately 11 cm/sec was calculated (Table 1).

Zones of slow conduction. Three zones with a low conduction velocity, relative to the conduction velocity of the ventricular myocardium, could be identified at these stages (H/H stages 19–27); these zones are the atrioventricular canal, the sinoatrial junction, and the outflow tract. The stimulus–ventricle delay when stimulated from the atrium was 80 ± 12 (mean ± SEM) msec (n = 10) (see Figure 4D). The assumption that the slowly conducting zone of the atrioventricular canal measures approximately 100–200 μm implies a conduction velocity of less than 0.5 cm/sec across the atrioventricular canal. When the stimulating electrode was moved from the atrium to the sinus venosus in H/H stage 23 (ED 4) or H/H stage 27 (ED 5) hearts, a considerable increase of the stimulus–ventricle delay was detected (compare tracings 2 and 3 in Figure 4D). Thus, an additional slowly conducting zone exists between the stimulation site on the sinus venosus and the relatively fast conducting myocardium of the ventricle, i.e., in the sinoatrial junction. Electromograms of the outflow tract (Figures 3B, 4B, and 4D) show deflections that appear after ventricular activation, reflecting a delay between the activation of the ventricle and the outflow tract. Moving the exploring electrode in a downstream direction away from the groove between the ventricle and the outflow tract reveals increasing delays between the activation of the ventricle and the outflow tract (Figures 3B and 4B). Conduction velocities in the outflow tract between H/H stages 19 and 27 (EDs 3–5) did not change and were approximately 0.7 cm/sec (Table 1).

Transmembrane potentials. Figure 5 and Table 2 show transmembrane potentials at H/H stage 23. The electrophysiological properties of the myocytes in the relatively fast and in the slowly conducting myocardial areas were investigated in hearts at H/H stage 23 (ED 4) by impaling myocytes in the atrium, in the atrioventricular canal, in the ventricle, and in the outflow tract (Figures 5A–5D, tracing 3). The transmembrane potentials were simultaneously recorded with the signals from extracellular electrodes placed on the ventricle and the outflow tract (Figures 5A–5D, tracings 1 and 2). The parameters of the recorded transmembrane potentials are summarized in Table 2. The transmembrane potentials of myocytes in the outflow tract are characterized by a slow upstroke velocity of approximately 5 V/sec, by an amplitude of approximately 50 mV, and by a long duration (140 msec at the 80% level) (Figure 5A, tracing 3). The “plateau” phase of the transmembrane potentials in the outflow tract continues during the initial phases of the next cardiac cycle (Figure 5A, large arrowhead). In the atrioventricular canal the transmembrane potential parameters also have a low depolarization rate and amplitude but are of shorter duration than those in the outflow tract (Figure 5C, tracing 3). In intact hearts, it proved difficult to impale myocytes of the atrioventricular canal. The zone is probably very small, because most action potentials were either of the atrial or of the ventricular type and coincided with
either the atrial or the ventricular activation moments, respectively (compare with Arguello and colleagues\textsuperscript{13,14}). The depolarization rate in atrial and ventricular myocytes was eightfold to 13-fold higher than that of the atroventricular canal and of the outflow tract (Table 2).

Correlations between electrophysiological and immunohistochemical parameters. After completion of the electrophysiological experiments, the hearts were processed immunohistochemically to reveal their isomyosin heavy chain expression patterns. Marks in slowly conducting myocardium were only found in coexpress-

### Table 2. Parameters of Transmembrane Potential in Cardiomyocytes at Hamburger and Hamilton Stage 23 (Embryonic Day 4)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>APA (mV)</th>
<th>MDP (mV)</th>
<th>APD (msec)</th>
<th>V\textsubscript{max} (V/sec)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>30%</td>
<td>80%</td>
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<tr>
<td>Atrium</td>
<td>2</td>
<td>62</td>
<td>-58</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>AV</td>
<td>1</td>
<td>46</td>
<td>-65</td>
<td>38</td>
<td>56</td>
</tr>
<tr>
<td>Ventricle</td>
<td>9</td>
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<td>-62±3.1</td>
<td>71±4.4</td>
<td>95±5.6</td>
</tr>
<tr>
<td>OFT</td>
<td>4</td>
<td>48±2.1</td>
<td>-42±1.2</td>
<td>86±10.7</td>
<td>140±10.2</td>
</tr>
</tbody>
</table>

\(n\), Number of experiments; APA, action potential amplitude; MDP, maximum diastolic potential; APD, action potential duration; \(V\text{\textsubscript{max}}\), maximum upstroke velocity; AV, atroventricular canal; OFT, outflow tract. Values are given as mean±SEM for three or more experiments.

Data for the AV action potential are from an impalement other than that in Figure 5C, which was written on the paper recorder only.
ing myocardium, i.e., in the area that is depicted in the figures as a dotted pattern (Figures 3A, 4A, and 4C), whereas marks in relatively fast conducting myocardium were found in myocardium that expresses the ventricle-specific isomyosin only. The boundaries between fast ventricular myocardium and slow outflow tract myocardium at the groove between the ventricle and the outflow tract proved to coincide with the transition of monoexpression of ventricle isomyosin to coexpression of atrial and ventricular myosins (Figures 3A and 4A). Therefore, coexpression of atrial and ventricular myosins is a convenient histological guide to delineate slowly conducting myocardium in the cardiac wall.

**H/H Stages 29–31**

Figures 6 and 7 show hearts at 6 and 7 days of development. Between 6 and 7 days of development,
septation becomes complete. The single tubelike outflow tract of the hearts at H/H stage 23 (ED 4) becomes transformed into two channels. The septal and parietal endocardial ridges within the outflow tract myocardium fuse and give origin to the cusps of the aortic and pulmonary valves, the primordia of which can be recognized in the heart at H/H stage 29 (ED 6).17-19

A zone of slow conduction continues to exist in the outflow tract in these stages (Figures 6A, 6B, 7A, and 7B). However, whereas the ventricle–outflow tract delay at the distal rim of myocardium of the outflow tract was still 120 msec at H/H stage 27 (ED 5) (Figure 4B), it had diminished to 70 msec at H/H stage 29 (ED 6) (Figure 6B) and to only 45 msec at H/H stage 31 (ED 7) (Figure 7B). A conduction velocity of approximately 1.3 cm/sec could be calculated in the outflow tract of the heart at H/H stage 29 (ED 6) (Figure 6B). Conduction velocity in the ventricular free wall at H/H stage 31 (ED 7) was approximately 20 cm/sec (Table 1).

Correlations between electrophysiological and immunohistochemical parameters. The areas where coexpression of atrial and ventricular MHCs were found were depicted as a dotted pattern on the photographs of the hearts (Figures 6A and 7A). In all hearts at H/H stages 29–31 (EDs 6 and 7), markings made in slowly conducting areas were only found in coexpressing myocardium.

Conduction Velocity in the Ventricular Trabeculae

A close examination of the ventricular electrograms revealed fractionated ventricular activation signals most prominently when the exploring electrode was placed above the (developing) ventricular septum, from H/H stage 23 (ED 4) onward (Figure 3C). Moreover, these electrograms showed that the apical part of the ventricle is activated slightly before the ventricular myocardium that is closer to the atrioventricular canal (Figures 2C and 2D, tracing R, 2, and 3; Figure 3C, tracings 1–4). These findings suggest that the activating impulse is preferentially conducted through the ventricular trabeculae. To further test this hypothesis, we used hearts at H/H stage 31 (ED 7), because their compact ventricular myocardium is sufficiently thick to allow a distinction between the moment of activation of the trabeculae and the compact working myocardium of the free ventricular wall. We placed one extracellular electrode on the ventricular trabeculae through the opened left ventricle (arrow T in Figure 7A) and a second extracellular electrode on the corresponding location at the epicardial side (arrow E in Figure 7A). The simultaneously recorded electrograms (Figure 7C) reveal that the electrogram recorded on the endocardial side is a single deflection and that the electrogram recorded on the epicardial side is fractionated; the first part of the fractionated deflection coincides with the single deflection recorded on the trabecular side. The first part of the fractionated deflection therefore probably represents the remotely sensed endocardial signal, suggesting that the trabecular portion of the ventricular wall is activated earlier in the heart cycle than the compact outer wall. Fractionated deflections were never seen in the outflow tract.

Correlations between electrophysiological and immunohistochemical parameters. It is noteworthy that the rapidly conducting ventricular trabeculae coexpress atrial and ventricular isomyosins during the entire septational period (the present study and References 8, 10, and 12). Despite the unexpected dissociation of conduction velocity and isomyosin expression pattern in the ventricular trabeculae, coexpression of both isomyosins remains a reliable histological guide to delineate the slowly conducting primary myocardium that flanks the developing atrial and ventricular segments.

Discussion

Conduction Velocity and Development

The measurement of conduction velocities in avian embryos (Table 1, Figure 8, and References 13 and 14) has shown that conduction is slow (≈1 cm/sec) and nearly uniform in the primary myocardium of tubular hearts at H/H stages 13 and 14 (ED 2), resulting in a peristaltic contraction pattern. Conduction velocity markedly increases in the developing working myocardium of the ventricles and atria, but it remains low in the flanking myocardium, namely, the sinoatrial junction, the atrioventricular canal, and the entire outflow tract (Figure 8). Although slow conduction in the atrioventricular canal of developing chicken hearts was recognized previously,4,13,14,21,22 the presence of a zone with a low conduction velocity in the sinoatrial junction was not reported earlier to our knowledge. The relatively late activation of the outflow tract, compared with the ventricle, was visualized previously as a C wave in the electrocardiogram of ED 3 chicken embryos,23,24 but data beyond ED 3 were lacking, and conduction velocities were not measured. Conduction velocities in all three slowly conducting areas are of similar magnitude, i.e., less than 1.5 cm/sec (Table 1, Figure 8; compare with References 13 and 22), indicating that the myocardium flanking the working myocardium of the developing atrial and ventricular segments represents the remaining primary myocardium.

This hypothesis of cardiac segmental development, which was suggested by the developmental changes of the expression patterns of atrial and ventricular MHCs,8-10,12,25-29 is supported by the observation that the differentiating working myocardium acquires fast sodium channels (compare with References 30 and 31) and is further underscored by the observation that such ion channels are apparently absent in the remaining primary myocardium; the transmembrane potentials of these cardiomyocytes are characterized by a low resting potential, a slow rising phase, and a low amplitude (Table 2, Figure 5; compare with References 13 and 21). The primary myocardium of the atrioventricular canal and the primary myocardium of the outflow tract further share the property of being more sensitive to the induction of conduction blocks by cardiac glycosides than working myocardium.24,32 In addition to these differences in ion channels, primary myocardium continues to express smooth muscle α-actin33 and to coexpress atrial and ventricular MHCs (the present study and References 8–10 and 12), whereas developing working myocardium no longer expresses smooth muscle α-actin and displays monoeexpression of either MHC. These electrophysiological and biochemical data suggest that the slow conduction velocity of the primary myocardium is one of the manifestations of the persistence of the primordial pattern of gene expression in these cardiomyocytes.
Increases in the conduction velocity in the developing ventricle are commensurate with the increasing size of this cardiac segment. Such a coordinated development ensures that the time necessary to activate the ventricle electrically does not increase and that the cardiac rhythm can be maintained or even increased during the first week of incubation.34 Our observations suggest that before the development of ventricular trabeculae the impulse conduction is in the expected caudocranial direction through the myocardial wall of the ventricle, whereas at and after H/H stage 23 (ED 4), preferential conduction apparently passes through the trabeculae that, in turn, activate the compact myocardium of the ventricular wall (Figures 3 and 7). The latter pattern resembles that observed in later stages, i.e., after the development of the conduction system in H/H stages 31–34 (EDs 7 and 8).7,8 We have recently shown35 that gap junctions are abundant in the ventricular trabeculae but virtually absent in the outer free wall of the ventricle of prenatal rats, suggesting preferential conduction of the activating impulse through the ventricular trabeculae in this species as well.

A Hypothesis of Cardiac Function in the Embryo: Why the Conduction System and Valves Can Be Missed

The local differences in the conduction velocity (Figure 8) and duration of the depolarizing impulse for the myocardium in the embryonic heart suggest hypotheses for the function of these hearts in the absence of a conduction system and in the absence of one-way valves.

The measurement of conduction velocities in embryonic hearts has shown that the sequential contraction pattern of atria and ventricles is determined by the alternation of zones with a fast and a slow conduction velocity (Figure 8): the contraction of the upstream compartment has to be completed before the onset of the contraction of the downstream compartment. This is accomplished by the slow conduction velocity and the relatively great length of the junctional zones of the embryonic heart.

At the same time, these embryonic hearts have to cope with the absence of functional one-way valves. This condition necessitates the contraction of the downstream compartment to occur before the relaxation of the upstream junctional zone. Thus, relaxation of the myocardium in the ventricle and the outflow tract has to await the arrival of the next cardiac cycle if regurgitation during ventricular relaxation is to be prevented. Competence of cardiac function is achieved because of the long-lasting contraction of the ventricular trabeculae, which are mainly responsible for ventricular contraction,36 and because of the unique features of the embryonic outflow tract, namely, its relatively great length, its very low conduction velocity, and the long duration of its action potentials. These features ensure that there is simultaneous electrical activity in the embryonic heart that belongs to cycle n (in the atrium) and to cycle n–1 (in the outflow tract) (Figure 5A). Such a model of outflow tract function in embryos predicts the development of incompetence of the sphincter mechanism if two consecutive cycles no longer overlap. This is exactly what is observed when cardiac rhythm slows down on lowering the ambient temperature.

Slow conduction of the impulse, combined with a long duration of the contraction, as a mechanism to prevent regurgitation has to persist until the cardiac valves have become competent. These valves develop inside myocardial segments that are characterized by slow conduction velocity in the embryo. Therefore, not surprisingly, the development of the semilunar valves,17 for example, coincides with the involution of the outflow tract. This involution can be demonstrated morphologically37,38 but is also evident from the decrease of the maximum ventricle–outflow tract delay from approximately 120 msec at H/H stage 27 (ED 5) to approximately 45 msec at H/H stage 31 (ED 7) (Figures 5–7), although conduction velocity stays more or less the same (Table 1).
Our studies show that the embryonic development of the myocardium is gradual and that regional differences in the degree of differentiation are essential for normal cardiac function in the embryo. The special (electrical) properties of the primary myocardium probably persist into adulthood in part of the sinoatrial and the atrioventricular nodes.

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