Ventricular function and morphology in chick embryo from stages 18 to 29

EDWARD B. CLARK, NORMAN HU, JAMES L. DUMMETT, GREGG K. VANDEKIEFT, CHERYL OLSON, AND ROBERT TOMANEK

Division of Pediatric Cardiology, Department of Pediatrics, and Department of Anatomy and The Cardiovascular Center, University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242

Clark, Edward B., Norman Hu, James L. Dummett, Gregg K. Vandekieft, Cheryl Olson, and Robert Tomanek. Ventricular function and morphology in chick embryo from stages 18 to 29. Am. J. Physiol. 250 (Heart Circ. Physiol. 19): H407-H413, 1986.—We evaluated wet and dry ventricular and embryo weights, hemodynamic parameters of ventricular function, and myocardial myocyte organelle composition in the developing chick embryo. Phasic and dP/dt ventricular pressure were measured with a servo null pressure system, and phasic, mean, and dV/dt dorsal aortic velocities were measured with a 20-MHz pulsed-Doppler meter. Ventricular and embryo weight increased geometrically with development, but at different rates, so that the ventricle-to-embryo weight ratio decreased from 0.02 to 0.001 just prior to hatching. Ventricular systolic and end-diastolic pressure increased from 1.31 ± 0.05/0.33 ± 0.03 mmHg at stage 18 to 3.45 ± 0.10/0.82 ± 0.03 mmHg at stage 29, while dP/dt increased from 23.04 ± 1.32 to 79.55 ± 3.69 mmHg/s over the same period. Dorsal aortic dV/dt increased from 878 ± 17 to 2,076 ± 65 mm/s² from stage 18 to 29. Myocyte percent volume of myofibrils increased from 16.7 ± 0.9% at stage 18 to 23.6 ± 1.1% at stage 27 and diminished to 18.4 ± 0.8% at stage 29. Mitochondrial percent myocyte volume remained constant at about 11%. These data define the parameters of normal ventricular function and morphology during embryonic development in the chick.

ventricular weight; ventricular blood pressure; myocardial myocyte; dP/dt; dV/dt

THE HEART IS THE FIRST functioning organ in the embryo. Myoblasts derived from the embryonic splanchnic mesoderm are organized in a homogenous layer of cells surrounding the cardiac tube. In the chick embryo, myofilaments begin to form within the myoblasts at stage 9* (29-33 h of incubation), and the heart tube starts to pulsate at stage 10 (33-38 h). Blood flow to the embryonic and extraembryonic circulation starts at stage 12 (45-49 h of incubation) (11). During the 21-day incubation period, the heart provides circulatory support for the embryo while undergoing morphogenesis from the cardiac tube to the complex, four-chambered heart.

Embryologists have long hypothesized that there is an interrelationship between form and function in the developing cardiovascular system. The difficulty in defining the relationship has rested on the lack of techniques to quantify the hemodynamics of the early circulation. Recently, we applied pulsed-Doppler technology and servo null pressure system to measure cardiovascular function in the chick embryo (5). We found a marked increase in hemodynamic function with development. We hypothesized that developmental changes in morphological characteristics of the embryonic heart parallel the increase in hemodynamic function. In this paper, we describe the changes in ventricular and embryo weight during development (from stages 18 to 43). We also measured the proportional volume of mitochrondria and myofibrils in myocardial myocytes, ventricular pressure, and dorsal aortic blood velocity from stages 18 to 29, the period of rapid cardiac morphogenesis. These results define the changes in ventricular function that occur with development in the chick embryo.

METHODS

Fertile white Leghorn eggs were incubated in a forced-draft, constant-humidity incubator and staged by the system of Hamburger and Hamilton (9).

Embryonic and ventricular weights. Wet and dry embryo and ventricular weights were measured from stage 18 (3 days) to stage 43 (19 days). An embryo was removed from the shell after the extraembryonic membranes were stripped away. It was then gently blotted to remove excess water and weighed on a Mettler balance accurate to ±10 µg. A ventricle including the conotruncus was weighed after the vessels distal to the aortic sac and atria were trimmed off. For dry weight, the embryo or heart was heated in an oven at 80°C for 3 h and transferred to a desiccator containing silica gel for 12-24 h. At stages 18-29, 9 or 10 hearts were pooled for each measure of wet and dry weight, while from stage 31 to 43 hearts were weighed individually.

Myocyte proportion measurements. Fractional myocardial cell volume was determined for mitochondria, myofibrils, glycogen, and sarcoplasm at stages 18, 21, 24, 27, and 29. We harvested a minimum of six ventricles at each stage, which were immediately fixed in isotonic 2.5% glutaraldehyde with buffered 0.1 M sodium cacodylate and subsequently embedded in Spurrs plastic. Random thin sections cut from the ventricle were stained with uranyl acetate and lead citrate and photographed on a Hitachi H600 electron microscope. The micrographs were printed at a final magnification of ×24,000.

We used a point counting technique to analyze the
micrographs after cell vacuoles and nuclei were excluded. Fifty micrographs from five embryos were counted for each stage with an overlay grid of 1,092 intercepts per micrograph. The number of intercepts (points) falling on a given structure ($P_i$) and the total number of points ($P_{cell}$) are related to the relative volume fraction ($V_i$) of the structure per total cell volume ($V_{cell}$) as follows: $V_i / V_{cell} = P_i / P_{cell}$.

In this study we determined the relative fractions (volume densities) of mitochondria, myofibrils, and glycogen. All other cytoplasmic organelles (e.g., ground substance, sarcoplasmic reticulum, lipid) were categorized as sarcoplasm.

The individuals counting the micrographs (Dummett and Vandekieft) were unaware of the embryo stage. Intra- and interobserver errors were determined by $t$ test on repeat counts of 10 micrographs. The intraobserver correlation was high, with the lowest $P$ value of 0.25 for an individual and 0.17 for the two observers. Interobserver correlation was also high as the lowest $P$ value was 0.62.

Hemodynamic measurements. For in ovo physiological measurements, an egg was removed from the incubator and positioned on a dissecting microscope stage. We opened the shell and removed the outer and inner shell membranes to expose the embryo.

We measured ventricular pressure with a servo null pressure system that is accurate over the range of 0–30
mmHg (y = 0.995x - 0.23, r² = 0.99; SEE = 0.11 mmHg) and has a rapid response from 10 to 90% in 20 ms (5).

dP/dt was electronically derived from the pressure curve by a differentiating channel that was calibrated with a ramp voltage generator. The 5- to 7-μm-diameter tip of the glass pressure cannula was inserted through the ventricular wall to measure the ventricular pressure (Fig. 1). The phasic and differentiated ventricular pressures were recorded (Fig. 2A). The zero pressure was measured by positioning the micropipette tip in the extraembryonic fluid at the level of the ventricle. An individual value for dP/dt was determined by averaging the peak value of 40 consecutive pressure curves.

We measured dorsal aortic blood velocity with a 20-mHz pulsed-Doppler velocimeter that is accurate over a range of 0-16 mm/s (y = 0.85x - 1.15, r² = 0.99, SEE = 0.49 mm/s) (5). dV/dt was electronically derived from the velocity curve by a differentiating channel that was calibrated with a ramp voltage generator. The 1-mm piezoelectric crystal was positioned at a 45° angle over the dorsal aorta at the level of the sinus venosus. Phasic and differentiated dorsal aortic blood velocities were recorded (Fig. 2B). An individual value for dV/dt was determined by averaging the peak value of 40 consecutive velocity curves.

The data are shown as means ± SE of the mean for embryo and ventricular weight, ventricular pressure, dorsal aortic blood velocity, and proportional measurements of cell volume. The relationship of ventricular to embryo weight was determined by ratio analysis as well as by log-log plot.

RESULTS

**Ventricular and embryonic weights from stages 18 to 43** (Table 1). Both wet and dry ventricular weights increased during development. However, the ratio of heart to embryo weight decreased from 0.02 to 0.001 just prior to hatching. When expressed as log ventricular wet weight versus log embryo wet weight, there was a linear correlation (Fig. 3). The dry weight log-log relationship was similar to the wet weight relationship (log<sub>dry</sub> y = 0.8295 log<sub>wet</sub> x - 1.634, r = 0.995).

**Myocyte proportion measurements from stages 18 to 29** (Table 2, Fig. 4). The percent volume of mitochondria in the myocytes remained constant from stages 18 to 29 at about 11%. However, the percent volume of myofibrils increased gradually from 16.7% at stage 18 to 23.6% at stage 27, resulting in a gradual decrease in mitochondria-to-myofibril ratio. Between stages 27 and 29, the percent myofibril volume decreased from 23.6 to 18.4%. Glycogen volume percent was variable. Sarcoplasm comprised approximately 60% of the myocyte volume during this

**TABLE 1. Ventricular and embryonic weights of chick embryo**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Wet ventricle</th>
<th>Solid ventricle</th>
<th>Wet embryo</th>
<th>Solid embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.35±0.01</td>
<td>0.03±0.002</td>
<td>17.7±0.9</td>
<td>1.02±0.05</td>
</tr>
<tr>
<td>21</td>
<td>0.42±0.03</td>
<td>0.04±0.002</td>
<td>34.1±1.9</td>
<td>1.66±0.11</td>
</tr>
<tr>
<td>24</td>
<td>0.97±0.08</td>
<td>0.05±0.003</td>
<td>79.5±3.6</td>
<td>3.06±0.09</td>
</tr>
<tr>
<td>27</td>
<td>1.54±0.11</td>
<td>0.10±0.004</td>
<td>149.2±4.8</td>
<td>6.61±0.19</td>
</tr>
<tr>
<td>29</td>
<td>3.18±0.18</td>
<td>0.14±0.006</td>
<td>267.5±7.5</td>
<td>15.36±0.30</td>
</tr>
<tr>
<td>31</td>
<td>4.56±0.15</td>
<td>0.34±0.001</td>
<td>516.5±29.7</td>
<td>46.03±0.94</td>
</tr>
<tr>
<td>34</td>
<td>6.78±0.14</td>
<td>0.88±0.02</td>
<td>1016.5±15.1</td>
<td>71.38±1.68</td>
</tr>
<tr>
<td>36</td>
<td>10.31±0.54</td>
<td>1.62±0.10</td>
<td>2820.0±44.0</td>
<td>162.43±2.69</td>
</tr>
<tr>
<td>38</td>
<td>39.44±1.06</td>
<td>4.27±0.08</td>
<td>4230.0±187.0</td>
<td>369.69±4.65</td>
</tr>
<tr>
<td>40</td>
<td>65.77±1.65</td>
<td>8.50±0.13</td>
<td>9690.0±110.0</td>
<td>1230.70±34.46</td>
</tr>
<tr>
<td>43</td>
<td>102.65±3.06</td>
<td>14.66±0.45</td>
<td>19123.0±275.0</td>
<td>3198.26±106.56</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n, no. of preparations.
Hemodynamic measurements from stages 18 to 29 (Table 3). At stage 18, the ventricular early diastolic pressure was near zero, and the end-diastolic pressure was accentuated presumably by the contributed volume of atrial systole (Fig. 2A). With development, systolic, end-diastolic, and ventricular dP/dt pressures increased. Among embryos of the same stage, ventricular dP/dt was unrelated to heart rate.

The dorsal aortic blood velocity tracing showed only antegrade blood movement, suggesting that the conotruncal cushions act as valves in the outflow tract of the embryonic heart. (Fig. 2B) Aortic blood velocity increased with development as did dV/dt. However, among embryos of the same stage, we observed no relationship between dV/dt and heart rate.

DISCUSSION

Understanding the relationships between ventricular morphology and hemodynamic function in the chick is important in the eventual understanding of mechanisms of cardiac development.

Ventricular weight changes rapidly during development in the chick. We found that the relationship between ventricular and embryo weight was not linear as the ventricular-to-embryo weight ratio declined during development. Our measurements of embryo weight agree with those reported by Romanoff (16) and Van Mierop and Bertuch (18).

The linear log-log, ventricular-to-embryo weight ratio is similar to the heart-to-body weight ratio noted among animals. Relative heart weight is greatest in newborn animals and declines as the animal matures (15). Among mature animals of different sizes, the larger the animal the relatively smaller its heart weight (8). The greater relative heart weight early in development may relate to the circulatory requirements of the embryo. In the chick, vascular resistance is initially high and decreases as new resistance units are added to the circulation (5).

Intracellular organization of the myocytes is also changing rapidly with development. Myofibrils first appear in the chick cardiac myocytes at stage 9 but account for only a small proportion of the cell volume (11). Between stages 18 and 29 we noted an increase and subsequent fall in the proportion of myofibrils. The myofibrils became more organized with development, and assumed a more orderly arrangement, presumably parallel to lines of stress. However, we could not quantitate the alignment process, since there is no reference point for comparison.

In the lamb and rat, mitochondrial and myofibril volume increase during development. Brook et al. (2) noted a progressive increase in the number and organization of intracellular organelles beginning in the 29-day-old fetal lamb. Hirakow and Gotch (10) noted that in the rat
cardiac myocyte, myofibrils increased from 22% at day 16 to 35% at day 25 (birth) while mitochondrial percent volume increased from 17 to 35%. On the basis of these data, we hypothesize that there is a further increase in the percent volume of mitochondria and myofibrils during later development in the chick embryo. The relationship of myofibril organization to heart function is unclear. At the electron microscopic level, the

### TABLE 3. Hemodynamic measurements

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Systole, mmHg</th>
<th>End-diastole, mmHg</th>
<th>dP/dt, mmHg/s</th>
<th>Ventricular Pressure</th>
<th>Mean velocity, mm/s</th>
<th>dV/dt, mm/s²</th>
<th>Heart rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>20</td>
<td>1.31±0.05</td>
<td>0.33±0.03</td>
<td>23.04±1.32</td>
<td>30</td>
<td>2.99±0.12</td>
<td>878±17</td>
<td>146±2</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>1.61±0.04</td>
<td>0.34±0.02</td>
<td>31.98±1.28</td>
<td>30</td>
<td>4.22±0.08</td>
<td>931±14</td>
<td>155±2</td>
</tr>
<tr>
<td>24</td>
<td>20</td>
<td>1.96±0.05</td>
<td>0.40±0.03</td>
<td>44.64±1.81</td>
<td>30</td>
<td>6.40±0.15</td>
<td>1,790±23</td>
<td>172±2</td>
</tr>
<tr>
<td>27</td>
<td>20</td>
<td>2.35±0.08</td>
<td>0.56±0.03</td>
<td>52.91±2.31</td>
<td>30</td>
<td>11.45±0.22</td>
<td>1,740±35</td>
<td>183±3</td>
</tr>
<tr>
<td>29</td>
<td>21</td>
<td>3.45±0.10</td>
<td>0.82±0.03</td>
<td>79.55±3.69</td>
<td>30</td>
<td>15.33±0.62</td>
<td>2,076±63</td>
<td>208±5</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n, no. of preparations.

FIG. 4. Electron micrograph of chick ventricular myocytes. A: stage 18; B: stage 27. Major difference between these stages is increased volume density of myofibrils at stage 27. mi, Mitochondria; gl, glycogen; my, myofibril.
randomly arranged myofibrils seem incapable of coordinated myocardial contraction. Only a portion of the myofibrils, those with sarcolemmal attachment, may be functional while others are in various phases of assembly (11). However, when the whole heart is viewed under polarized light, there are hoop-like bands of myofibrils that encircle the heart. (13) Circumferential contraction of the tubular heart would be very efficient. With increasing geometric complexity of the septating heart, the lines of stress and strain would change. We hypothesize that the final organization of the myocardium is in part determined by the hemodynamic forces interacting with the developing ventricular walls.

With the changes in cardiac morphology, there are remarkable changes in hemodynamic function. The ventricular systolic pressure rapidly increased nearly threefold from stages 18 to 29. The systolic pressure noted in our studies were similar to those reported by Paff et al. (14), who used a modified Landis apparatus, and Faber (6), who used an electromechanical transducer. We were not able to make exact comparisons with the other workers' data, because they reported embryo development by incubation day, which is more variable than morphological stage.

We noted a gradual increase in ventricular diastolic pressure, which we hypothesize is due to a decrease in ventricular compliance as the heart walls thicken. The decline in early diastolic pressure to near zero implies that wall stress changes markedly during the cardiac cycle. Changing wall stress is likely an important extrinsic factor in the regulation of myocardial formation (19).

The contour of the ventricular pressure curve in the embryo is markedly similar to that recorded from a mature heart. The distinct early diastolic pressure suggests that the endocardial and bulbar cushions have a valve-like function in the early heart. We speculate that the accentuation in the end-diastolic pressure is due to atrial systolic augmentation of ventricular filling.

Pump performance of the embryonic ventricle increased with development. dP/dt and dV/dt, prejection and ejection phase indexes of cardiac performance, respectively, are directly related to preload, myocardial contractility, and heart rate and are inversely related to afterload. With expansion of the vascular system, we expect that preload increases with development. The progressive increase and alignment of myofibrils would increase myocardial contractility. Afterload, as measured by vascular resistance, decreases markedly from stages 18 to 29 (4).

The relationship between ventricular function and heart rate is less clear. Between stages, dP/dt and heart rate increased. However, among embryos at each stage, we did not find a relationship between dP/dt and heart rate. Investigators who found a relationship between dP/dt and heart rate studied the same fetal animal at different paced heart rates (7, 17). We measured the dP/dt at naturally occurring, rather than at artificially paced heart rates.

At each stage, ventricular systolic pressure was higher than the vitelline arterial pressure (5). At stage 18 the difference was 0.49 mmHg and increased progressively to 1.45 mmHg at stage 29. The aortic arches and dorsal aorta are the vascular channels between these two points in the circulation. We hypothesize that this pressure drop may be important in the mechanism of aortic arch selection. Cardiac embryologists have long suggested that the aortic arches are selected by vectorial distribution of aortic arch blood flow (3). Such a pressure difference could provide the energy necessary for the orientation of vectorial blood flow.

From these studies, an intriguing question arises. Does functional load affect ventricular development? In the earliest stages of heart development, cardiac looping can proceed in the absence of pump function of the heart (12). However, at what point in development does the embryonic ventricle respond to increased work load? There is indirect evidence from human congenital heart defects to suggest that growth can be accelerated in the developing heart (1). The existence of a feedback mechanism by which ventricular function, in part, controls ventricular growth may be the early manifestation of growth processes responsible for ventricular hypertrophy in the mature animal.

The authors thank Sue Kucera and Trudi Fawcett for skillful work with the manuscript and Horst Jordan for the graphs.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-14388 and HL-18629. E. B. Clark is the recipient of Research Career Development Award HD-000376 from the National Institute of Child Health and Human Development. J. L. Dummett and G. K. Vandekieft received Summer Research Fellowship H-107485. Address for reprint requests: E. B. Clark, M.D., Div. of Pediatric Cardiology, Dept. of Pediatrics, Brady 516, Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21205.

Received 12 March 1985; accepted in final form 17 October 1985.

REFERENCES