Ventricular Pressure–Area Loop Characteristics in the Stage 16 to 24 Chick Embryo

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The accurate description of embryonic cardiovascular function requires the adoption of standard measurement techniques to the small scale of the developing heart. In the mature heart, the analysis of ventricular pressure and volume accurately defines function. Because in vivo measures of volume are not feasible in the embryonic heart, we tested the hypothesis that ventricular pressure–area loops accurately define ventricular function in the stage 16 to stage 24 white Leghorn chick embryo. We simultaneously measured ventricular pressure with a servo–null pressure system and recorded video images at 60 Hz. The pressure waveform was superimposed onto the video image in real time. Video fields were planimetered for epicardial ventricular cross-sectional area and ventricular pressure. Pressure and area data were smoothed using a fast Fourier transform filter and plotted. Data are reported as mean±SEM, n≥4, and were tested by regression analysis and analysis of variance (p<0.05). Heart rate increased from 90±7 beats/min at stage 16 to 130±13 beats/min at stage 24. All pressure–area loops displayed diastolic filling, isometric contraction, ejection, and isometric relaxation, similar to pressure–volume loops of the mature heart. Isometric contraction time increased from 42±5 to 62±4 msec (p<0.05), while isometric relaxation time was 124±12 and 120±10 msec (p>0.05) between stages 16 and 24, respectively. The maximum ratio of instantaneous ventricular pressure to area identified end systole better than peak ventricular pressure or minimum ventricular area. Thus, pressure–area relations define ventricular function in the embryonic chick heart. (Circulation Research 1991;68:226–231)

The accurate measurement of ventricular function in the mature heart has been a primary goal of clinicians and scientists for over a century.1 In the chick embryo, ventricular pressure, ventricular cross-sectional area, and cardiac output can be accurately measured shortly after the onset of cardiac contraction.2,3 Although the early embryonic heart lacks formed valves or autonomic innervation, ventricular blood pressure, stroke volume, and heart rate are tightly regulated.4 Cardiac output increases in proportion to embryo weight so that blood flow indexed for embryo weight remains constant.5 In addition, the structurally simple embryonic heart accelerates ventricular growth in response to experimentally increased developed pressure.6

In the mature heart, pressure–volume (PV) analysis allows the precise definition of ventricular filling and ejection characteristics and the time of end systole.7,8 Because the cardiovascular system operates as a closed system, the PV diagram also provides a unique method to define the influence of the vascular system on ventricular function.9 We therefore adapted the method of PV analysis to the embryonic ventricle to more precisely measure ventricular function and to define ventricular/vascular interactions during cardiovascular development.

We tested the hypothesis that ventricular pressure–area (PA) loops define cardiac function in the stage 16 to 24 chick embryo. Each PA loop displayed diastolic filling, isometric contraction, ejection, and isometric relaxation.10,11 Isometric contraction time increased from 42±5 to 62±4 msec (p<0.05), while isometric relaxation time was 124±12 and 120±10 msec (p>0.05) between stages 16 and 24, respectively.

PA loop analysis of the embryonic ventricle integrates dynamic morphological and functional measures of cardiovascular function and complements our long-term investigation of the relation of form and function in the developing heart.

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**Materials and Methods**

We studied white Leghorn chick embryos at stage 16 (2.3 days, \(n=4\)), stage 18 (2.8 days, \(n=8\)), stage 21 (3.5 days, \(n=9\)), and stage 24 (4 days, \(n=8\)) of a 46-stage (21-day) incubation period.\(^{12}\) Developmental landmarks including somite number, limb size, and cardiac morphology identify each stage. Embryo wet weight doubles between each selected stage.\(^5\) Fertile eggs were incubated blunt end up in a forced draft incubator at 38.5°C. When incubated blunt end up, the blastodisk floats to the top of the egg and the embryo develops beneath the air cell. Access to the embryo was gained by opening the shell and removing a small region of the inner and outer shell membranes. The embryo was then positioned for imaging on a photomicroscope stage.

Experiments were performed and analyzed at an integrated physiology and morphometry workstation (Figure 1). Video images were acquired using a photomicroscope (model M400, Wild Leitz USA, Inc., Rockleigh, N.J.), a video camera (model 70, Dage-MTI, Michigan City, Ind.) with a grade 1 Newvicon tube, a fiber-optic light source (Dolan-Jenner Industries, Woburn, Mass.), a VHS video recorder (model VR9670, Magnavox North American Phillips, Jefferson City, Tenn.), and a time/date generator (model VTG-33, FOR.A, West Newton, Mass.). The Dage camera generated 60 sequential video fields per second in the interlaced mode. The image field was 1,000–2,000 \(\mu\)m in diameter, and the effective raster spacing was 4–8 \(\mu\)m. One pixel in the image equals 0.25% of the epicardial diameter. Real time \(\pm 0.005\) second was recorded on each field. A 10-\(\mu\)m scale scribed-glass standard was recorded in the plane of each embryo after imaging.\(^3\)

We simultaneously measured intraventricular blood pressure with a servo-null pressure system (model 900, WPI, New Haven, Conn.). A fluid-filled 7-\(\mu\)m-tip glass capillary tube was positioned with a micromanipulator (Leitz, Wetzlar, FRG) to puncture the embryonic ventricle in the region of the primitive right ventricle. Zero transtip pressure was measured by immersing the electrode in the extraembryonic fluid adjacent to the ventricle. The analog signal gain was increased 10-fold by a direct-current amplifier (Nelson Technical Services, South Old, N.Y.), sampled at 500 Hz by an analog-to-digital board (RC Electronics, Santa Barbara, Calif.), and qualitatively reviewed on the computer in digital oscilloscope mode.

An analog device sampled the pressure waveform at 15.75 kHz and placed a marker proportional to instantaneous pressure onto each horizontal video line in real time (model PONV, Ogden Scientific, Spencerport, N.Y.). The device also placed zero, full scale, and pressure baseline markers onto video fields for calibration of the pressure scale. The composite video fields were recorded on \(\frac{1}{2}\)-in. VHS tape.

Individual video fields were analyzed at a workstation that included a minicomputer (Premium/286, AST Research, Irvine, Calif.), a monitor (Multisync II, NEC Information Systems, Boxborough, Mass.), a frame grabbing board (model Targa M8, Jandel Scientific, Corte Madera, Calif.), JAVA video analysis software and a mouse (Microsoft, Redmond, Wash.), and a multipurpose video monitor (model PVM1271Q, Sony Corp.).

The video measurement protocol for each embryo included 1) calibration of measurement software for length (millimeters) and area (square millimeters) with the scribed standard; 2) planimetry of sequential video fields for ventricular epicardial area (square millimeters) and scaled ventricular pressure (millimeters); 3) planimetry of the zero, baseline, and full scale pressure markers; and 4) selection of maximum (end-diastolic) and minimum ventricular area images. The data analysis protocol included 1) calculation of heart rate from the cycle length of consecutive end-diastolic images; 2) transformation of the scalar pressure data into hemodynamic data (mm Hg);

![Figure 1: Workstation diagram for pressure and area data acquisition and analysis.](http://circres.ahajournals.org/DownloadedFrom)
3) fast Fourier transform low-pass filter (three point) of pressure and area data; 4) pressure and area data plotted as PA loops; 5) calculation of diastolic filling time as the time from minimum area to the onset of ventricular contraction; 6) identification of end systole as the point of maximum instantaneous pressure to area ratio for each cardiac cycle; 7) calculation of isometric contraction time as the number of video fields from the onset of ventricular contraction to 95% of end-diastolic area, and isometric relaxation as the number of video fields from end systole to the first rise in ventricular pressure; and 8) determination of the point of maximum systolic pressure (t_max, P_max), end-systolic pressure and area (t_est, P_est, A_est), and minimum ventricular area (t_min, A_min) for each cardiac cycle.

The mean values for each variable were compared by analysis of variance and regression analysis with significance assigned to p<0.05. We previously documented that intraobserver and interobserver error of area measurement by planimetry was not significant. Intraobserver and interobserver error of pressure measurement by planimetry was not significant (p>0.05).

**Results**

Heart rate increased from 90±7 beats/min at stage 16 to 130±13 beats/min at stage 24. Ventricular pressure and area increased across the stage range (Figure 2). A negative ventricular pressure occurred at the onset of ventricular filling in all embryos. Ventricular PA loops contained diastolic filling, isometric contraction, ejection, and isometric relaxation (Figure 3).

From stage 16 to 24, isometric contraction time calculated from the PA plots increased from 42±5 to 62±4 msec (p<0.05), while isometric relaxation time remained similar at 124±12 to 120±10 msec (p>0.05, Figure 4).

Peak ventricular pressure, maximum pressure to area ratio, and minimum area were identified in all embryos (Figure 5). At the time of the maximum pressure to area ratio (end systole), ventricular pressure was less than peak pressure (Figure 6), and ventricular area was larger than minimum area (Fig-
The time of peak ventricular pressure occurred 5±4 to 29±6 msec sooner than end systole (p<0.05), and the time of minimum area occurred 52±13 to 124±12 msec after end systole (p<0.05) from stage 16 to 24, respectively (Figure 8).

**Discussion**

The precise definition of terms is crucial in quantifying cardiac function. At the simplest level, systole is the period of active ventricular contraction and ejection, and diastole is the period of ventricular relaxation and filling. Preload is the force exerted on the ventricle while in a passive state, and afterload is the force against which the ventricle contracts. However, the measurement of any one of these parameters of cardiac function and the study of their regulation during cardiovascular development are complicated by their complex interdependence. We therefore adapted the PV method to the embryonic heart to define ventricular function and ventricular/vascular interactions.

Diastolic filling in the mature heart is determined by the interaction of the passive ventricle and the venoatrial preload system. Similar to data in the mature ventricle,14,15 we found that intraventricular pressure was negative at the termination of isometric relaxation in all embryos. While the exact atrioventricular pressure differential is not known for the embryonic heart, a negative ventricular pressure at the onset of ventricular filling is consistent with the presence of ventricular diastolic suction.16

The mechanism by which negative pressures are generated in the thin-walled embryonic heart is unknown. Radially oriented fibers extend from the endocardium to the myocardium in the extracellular matrix of the early embryonic heart.17 These fibers are stretched as the wall thickens during cardiac contraction and may provide a restoring force during diastole.18 Because the contents of the extracellular matrix can be selectively altered by proteases19 and hyaluronidase,20 measuring ventricular function after these perturbations will further define the role of the matrix in generating diastolic suction in the embryonic heart.

In the mature heart, ventricular systole begins with the rise in pressure that occurs after the completion of

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**Figure 5.** Representative pressure–area loops labeled for peak ventricular pressure, maximum ventricular pressure to area ratio, and minimum area.

**Figure 6.** Peak systolic pressure versus maximum pressure to area ratio for stage 16 to 24 chick embryos. Data plotted are mean±SEM. Second-order regression curves are plotted to raw data.

**Figure 7.** Area at time of maximum pressure to area ratio versus minimum area for stage 16 to 24 chick embryos. Data plotted are mean±SEM. Linear regression curves are plotted to raw data.

**Figure 8.** Time difference between time of end systole defined as the maximum instantaneous pressure to area ratio and time of minimum area or time of maximum ventricular pressure. Data plotted are mean±SEM.
of atrial contraction. This sequence depends on electrical isolation between the chambers and the time delay of atrioventricular node depolarization. These structures are not present in the embryonic heart, and no electrical isolation exists between the atria and ventricles.21

Simultaneous atrial and ventricular myograms of the embryonic heart confirm that ventricular contraction begins before the completion of atrial contraction.22 This overlap is likely responsible for the increase in ventricular area noted after the onset of systole (Figure 3). In addition, the mature ventricle changes shape during isovolumic contraction,23 and a similar mechanism would change the amount of the embryonic ventricle imaged in a single plane during isovolumic contraction. Despite this overlap of atrial and ventricular contraction, we defined the onset of systole as the beginning of isometric contraction displayed in the PA loop.

The presence of isometric contraction and relaxation periods in each PA loop confirms previous observations that the atrioventricular and conotruncal cushions function as valves during the cardiac cycle.24 Retrograde flow through the atrioventricular cushions does not occur during ventricular systole. Forward flow through the conotruncal cushions occurs shortly after the onset of active ventricular contraction.3 The increase in isometric contraction time noted from stage 16 to stage 24 is consistent with the generation of a higher prejection ventricular pressure. The increased isometric contraction time may relate to ventricular-vascular coupling of the ventricle to progressively higher aortic sac pressures.

Systolic function is greatly influenced by the interaction of the ventricle with the arterial system. Arterial impedance is a measure of the property of the arterial system to oppose ventricular ejection.25 Because blood is ejected in a pulsatile manner, resistance must also include components of arterial compliance and blood inertia.26 In the mature ventricle, changes in arterial impedance characteristics predictably alter the shape of the ejection trajectory defined by the upper right corner of the PV loop.27 In the chick embryo, hydraulic load decreases from stage 18 to 29 because of a progressive decline in vascular resistance.28 We found that from stage 16 to 24 the ejection curve of the PA loop became progressively curvilinear and peak ventricular pressure occurred earlier in systole. These changes in ventricular ejection characteristics defined by PA loops are consistent with the increased vascular compliance calculated by Fourier analysis of simultaneous aortic blood pressure and flow.28

Systole ends with the completion of active ventricular contraction. In the mature heart, end systole is best defined as the time of maximum ventricular elastance and can be approximated as the time of maximum pressure to volume ratio.13 The time of end ejection is greatly influenced by vascular impedance and occurs after end systole in the ejecting heart. The right ventricular PV loop has a rounded upper left corner because of the low resistance of the pulmonary vascular bed relative to the inertance and elastance components of pulmonary arterial impedance.29 This low-resistance vascular impedance increases the difference between the time of end systole defined by maximum ventricular elastance and end ejection.

We found that in the embryonic heart, the time of end systole differed significantly from the time of end-ejection area or peak systolic pressure. Following ventricular ejection, the contractile conotruncus provides additional energy for the ejection of blood into the aortic sac. Thus, end ejection is not a valid index for the end of systole in the developing cardiovascular system. The time difference between peak ventricular pressure and maximum pressure to area ratio increased across the stage range. Because of the rapid change in arterial impedance characteristics that occurs during vascular development, instantaneous maximum pressure to area ratio, rather than peak ventricular pressure or minimum ventricular area, best defines the time of end systole.

In the mature heart, pressure and volume data have been used to test mathematical models of mechanics and energetics.7 The analysis and modeling of ventricular mechanics by PA loops in the chick embryo will provide a framework for defining mechanisms of normal and experimentally altered cardiovascular development.

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