Effect of Changes in Circulating Blood Volume on Cardiac Output and Arterial and Ventricular Blood Pressure in the Stage 18, 24, and 29 Chick Embryo

Armin J. Wagman, Norman Hu, and Edward B. Clark

We studied the hemodynamic effects of changing volume loading in the chick embryo, before autonomic innervation, to test the hypothesis that the Frank-Starling mechanism functions in the embryonic myocardium. Dorsal aortic blood velocity was measured by pulsed Doppler. Heart rate and aortic diameter were also measured to calculate cardiac output and stroke volume index. Vitelline arterial and ventricular pressures were measured with a servo-null micropressure system in stage 24 embryos. Infusing isotonic solution intravenously resulted in linear increases in stroke volume index for stages 18 (y=388x+6.89), 24 (y=466x+7.86), and 29 (y=549x+4.96). The slopes and intercepts were statistically the same for all three stages. Similar volume loading in stage 24 embryos initially increased mean arterial pressure linearly, but at higher loading conditions, the rate of rise lessens. Thus, volume loading resulted in a decrease in vascular resistance. Withdrawing blood from stage 24 embryos resulted in a decrease in ventricular peak systolic and end-diastolic pressures. With reinfusion of the blood, systolic and end-diastolic pressures initially rose above baseline levels and later returned to normal. We conclude that a length-tension relation is present in the preinnervated embryonic heart and that vascular resistance changes inversely with loading conditions. We speculate that these mechanisms are the primary hemodynamic control mechanism in the early chick embryo. (Circulation Research 1990;67:187–192)

In the mature animal, control of the cardiovascular system occurs through extrinsic and intrinsic mechanisms. The extrinsic mechanisms operate through the neurohumoral and autonomic nervous systems. Both mechanisms play an important role in hemodynamic regulation, but the embryonic cardiovascular system functions prior to autonomic innervation of the heart and before the adrenal gland forms. Therefore, extrinsic mechanisms are probably not present.

We hypothesized that intrinsic mechanisms, in particular the Frank-Starling mechanism, control cardiac function at early stages of development. We sought to define the responses to changes in circulating blood volume to test this hypothesis. We studied the embryo at a time when the heart undergoes rapid growth and morphogenesis from a muscle-wrapped tube to a nearly septated four-chambered mature heart. These stages precede the development of autonomic innervation of the heart and vascular system.1,2 Our data demonstrated a length-tension relation in the embryonic heart and embryonic regulation of hemodynamics by altering peripheral vascular resistance.

Materials and Methods

Fertile white leghorn chicken eggs were incubated blunt end up in a forced-draft constant-humidity incubator to Hamburger-Hamilton stage 18 (3 days), stage 24 (4 days), or stage 29 (6 days).

The shell was carefully opened at the blunt end where the air sac is located. With microdissecting forceps, the outer shell membrane was gently removed.

Cardiac output was calculated from mean dorsal aortic blood velocity and dorsal aortic diameter. Velocity was measured with a 20-MHz pulsed Doppler velocity meter (model 545C-4, University of Iowa

From the Department of Pediatrics and Cardiovascular Center at the University of Iowa Hospitals and Clinics, Iowa City, Iowa. Supported by National Institutes of Health grants HD-14723 and HL-14388. A.J.W. was supported by National Institutes of Health training grant HL-07413. E.B.C. is a recipient of National Institutes of Health grant RCDA HD-00376.

Address for reprints: Armin J. Wagman, MD, Division of Pediatric Cardiology, Department of Pediatrics, University of Florida School of Medicine, J. Hills Miller Health Center, Box J-296, Gainesville, FL 32610.

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FIGURE 1. Recordings showing ventricular pressure measured in a stage 24 embryo heart. Top tracing: Phasic ventricular pressure. Bottom tracing: The first derivative of pressure with respect to time (dP/dt). End-diastolic pressure was measured just before the rapid rise in pressure during systole. This corresponded to dP/dt=0 (Figure 1). Vascular resistance was calculated by dividing mean vitelline arterial pressure by the mean dorsal aortic blood flow.

In the first group of experiments, circulating blood volume was increased by injection of isotonic chick Ringer’s solution through a 10-μm-diameter glass cannula inserted into a vitelline vein. After insertion of the cannula, a baseline recording was made of the heart rate and dorsal aortic blood velocity. Then a specified volume of isotonic solution was injected, at a constant rate, using a graduated syringe (Hamilton Co., Reno, Nev.). Throughout the injection period, there was a continuous recording of heart rate and dorsal aortic blood velocity. At the end of the experiment, dorsal aortic diameter was measured. Dorsal aortic diameter remained constant throughout the cardiac cycle and with the injection of isotonic solution. From these measurements, we determined baseline stroke volume index and maximum stroke volume index after volume infusion. For control studies there was insertion of an intravenous cannula but without injection of fluid. Measurements were made for 1 minute.

We calculated a percent increase in stroke volume index as a function of the injected volumes. The injected volumes used for each embryo stage are listed in Table 1. A different group of embryos was studied at each volume, so that each embryo received one injection. For stage 18 embryos, these infusions corresponded to 0.9-7.7% of circulating blood volume. For stage 24 embryos, these infusions corresponded to 1.0-14.9% of circulating blood volume. For stage 29, these corresponded to 1.9-7.6% of circulating blood volume. To compare the response of the different developmental stages, the injection volume was normalized for average embryo wet weight. Percent change in stroke volume index was plotted against normalized injection volumes.

Volume infusion experiments were also done in stage 24 embryos while measuring arterial blood pressure. The same injection volumes were used as listed in Table 1 for the stage 24 embryos. Control studies were done in the same fashion as described above. Five embryos were studied at each injection volume and in the control experiments.

In other experiments, we examined the effect of altering circulating blood volume on ventricular pres-

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**Table 1. Injection Volumes Used in Stroke Volume Index Experiments in Stages 18, 24, and 29 Chick Embryos**

<table>
<thead>
<tr>
<th>Stage 18</th>
<th>Stage 24</th>
<th>Stage 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume (μl)</td>
<td>n</td>
<td>Injection volume (μl)</td>
</tr>
<tr>
<td>0.12</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.25</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>0.50</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>7.5</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>10.0</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>12.5</td>
</tr>
</tbody>
</table>

n, Number of embryos studied at each injection volume.
sure in stage 24 embryos first by removing blood and then by reinfusing it. After insertion of a glass cannula into a vitelline vein, the pressure cannula was inserted into the ventricular cavity. First, to decrease intravascular volume, 10 μl blood was withdrawn intravenously from the embryo at 0.5 μl/sec. After 15 seconds, the same 10 μl blood was reinfused at the same rate. Five embryos were studied. Ventricular pressure and heart rate were recorded continuously. In some experiments, dorsal aortic blood velocity was simultaneously recorded.

Data are expressed as mean±SEM and analyzed by analysis of variance and analysis of covariance. Vascular resistance calculations are expressed as mean±SEE and analyzed by linear regression and Kendall's τ. Statistical significance was defined by a value of p<0.05.

Results

Dorsal aortic blood velocity increased in response to volume infusion (Figure 2). Stroke volume index increased since it is proportional to dorsal aortic blood velocity. For all experiments and control studies, there was no significant change in heart rate (Table 2). Therefore, at stages 18, 24, and 29, stroke volume index increased linearly as a function of injection volumes (Figure 3). Injection volume was normalized for wet embryo weight in Figure 3D. There were no statistical differences in the slope or intercept of the response among the different stages. Stroke volume index did not change during control studies (Table 2).

Phasic and mean arterial pressure increased in response to volume infusions in stage 24 embryos (Figure 4). At the lower injection volumes, the percent change in mean arterial pressure increased linearly. But with volumes greater than 5 μl, the rate of rise of mean arterial pressure begins to level off (Figure 5). The rise in stroke volume index was linear at all injection volumes. Thus, vascular resistance decreased with increased volume loading: y = -0.027x + 1.11, r = -0.84, p = 0.001 (Table 3). Mean arterial pressure did not change during control studies (Table 2).

During the withdrawal of blood, ventricular peak systolic pressure, ventricular end-diastolic pressure, and mean dorsal aortic blood flow (i.e., stroke volume index) all decreased. With reinfusion of the blood volume, these measurements increased (Figure 6). An example of the effect of withdrawal and reinfusion of 10 μl blood on peak ventricular systolic and end-diastolic pressures is shown in Figure 7. During blood withdrawal, systolic and end-diastolic pressures decreased. When the volume was reinfused, peak systolic and end-diastolic pressures increased above the baseline pressures, but after a short time, these returned to the baseline measurements.

Discussion

The Frank-Starling mechanism has been studied extensively in fetal and neonatal animals. Numerous studies8-10 have shown that it functions in fetal and neonatal lamb hearts, but its importance as a control mechanism has been debated.

Hemodynamic regulation has not been well studied in the embryo. Faber et al11 studied the Frank-Starling mechanism in the embryo in vivo. They showed that an intravenous bolus of either isotonic chick Ringer's solution or a hypertonic dextran solution increased end-diastolic volume as measured by the area on a cinemomicrograph.

Our results support the hypothesis that the Frank-Starling mechanism is functional in the chick embryo heart. The increase in stroke volume index is partly related to increase in preload (resting tension). This is consistent with our previous experiments in which an injection of 5 μl chick Ringer's solution in a stage

![Image](images/figure2.png)

**Figure 2.** Recordings showing an example of the response of dorsal aortic blood velocity in a stage 24 embryo to a 5-μl volume load (6% of circulating blood volume). Stroke volume index is derived from the velocity, aortic diameter, and heart rate (see text). Top tracing: Phasic velocity. Bottom tracing: Mean velocity. Baseline stroke volume index is 0.25 mm/sec cardiac cycle. After volume infusion, there is a 52% increase in stroke volume index.

<p>| Table 2. Changes in Stroke Volume Index and Mean Arterial Pressure During Control Studies and Effect of Volume Loading on Heart Rate in Stages 18, 24, and 29 Chick Embryos |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Stage</th>
<th>Change in control SVI (%)</th>
<th>Change in control MAP (%)</th>
<th>Change in heart rate (%)</th>
<th>Change in heart rate (%)</th>
<th>Change in heart rate (%)</th>
<th>Change in heart rate (%)</th>
<th>Change in heart rate (%)</th>
<th>Change in heart rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1.3±1.3 (5)</td>
<td></td>
<td>-0.6±0.8 (5)</td>
<td>-1.4±1.5 (5)</td>
<td>-5.5±0.6 (5)</td>
<td>-5.5±0.6 (5)</td>
<td>-5.5±0.6 (5)</td>
<td>-5.5±0.6 (5)</td>
</tr>
<tr>
<td>24</td>
<td>-1.8±1.5 (9)</td>
<td>-2.0±2.0 (5)</td>
<td>-2.3±0.4 (9)</td>
<td>-5.5±1.0 (9)</td>
<td>-5.5±1.0 (9)</td>
<td>-5.5±1.0 (9)</td>
<td>-5.5±1.0 (9)</td>
<td>-5.5±1.0 (9)</td>
</tr>
<tr>
<td>29</td>
<td>-2.7±0.8 (5)</td>
<td></td>
<td>-1.7±0.5 (5)</td>
<td>-0.5±0.5 (5)</td>
<td>-0.5±0.5 (5)</td>
<td>-0.5±0.5 (5)</td>
<td>-0.5±0.5 (5)</td>
<td>-0.5±0.5 (5)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers in parentheses represent the number of studies performed for each group of experiments. SVI, stroke volume index; MAP, mean arterial pressure.

*Values indicate maximum change in heart rate during volume loading.
FIGURE 3. Graphs showing response of stroke volume index to volume loading in stage 18 (panel A), stage 24 (panel B), and stage 29 (panel C) embryos. Data points are mean ± SEM. In panel D, the change in stroke volume index is compared among stages by plotting against injection volumes normalized for average wet embryo weight at each stage of development. Data points and standard error bars are omitted for clarity. For panel D, linear regression equations are as follows: y = 388x + 6.89, r = 0.88 for stage 18; y = 466x + 7.86, r = 0.82 for stage 24; and y = 549x + 4.96, r = 0.91 for stage 29. Total number of experimental embryos studied at each stage was 20 for stage 18, 59 for stage 24, and 21 for stage 29.

24 chick embryo resulted in a 22% increase in the atrial pressure.12

The similarity of responses among the different stages was not expected. There are marked differences in development. The stage 18 embryo is a muscle-wrapped bent tube with a single atria and ventricle. By stage 29, the heart has assumed a more mature shape, and there are four distinct chambers with septation nearly complete. There is also a 15-fold increase in wet embryo weight from stage 18 to 29.5 Although the slopes in Figure 3D suggest a slight trend toward an increased response in the

FIGURE 4. Recordings showing an example of the response of vitelline arterial pressure in a stage 24 embryo to a 2.5-μl volume load (3% of circulating blood volume). Top panel: Phasic pressure. Bottom panel: Mean pressure. Baseline mean pressure is 0.9 mm Hg. After volume infusion, there is a 17% increase in mean pressure.

FIGURE 5. Graph showing response of mean arterial pressure in the stage 24 embryo to volume loading. Data points are mean ± SEM. Five embryos were studied at each of six injection volumes for a total of 30.
TABLE 3. Comparison of Total Vascular Resistance Before and After Volume Loading in the Stage 24 Chick Embryo

<table>
<thead>
<tr>
<th>Injection volume (µl)</th>
<th>Baseline (mm Hg/mm³/sec)</th>
<th>After volume loading* (mm Hg/mm³/sec)</th>
<th>Change in vascular resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 (control)</td>
<td>1.11±0.16</td>
<td>1.15±0.16</td>
<td>3.6</td>
</tr>
<tr>
<td>1.0</td>
<td>1.28±0.15</td>
<td>1.20±0.13</td>
<td>-6.3</td>
</tr>
<tr>
<td>2.5</td>
<td>1.04±0.10</td>
<td>0.97±0.09</td>
<td>-6.7</td>
</tr>
<tr>
<td>5.0</td>
<td>0.96±0.11</td>
<td>0.88±0.08</td>
<td>-8.3</td>
</tr>
<tr>
<td>7.5</td>
<td>1.06±0.12</td>
<td>0.87±0.09</td>
<td>-17.9</td>
</tr>
<tr>
<td>10.0</td>
<td>1.01±0.08</td>
<td>0.83±0.07</td>
<td>-17.8</td>
</tr>
<tr>
<td>12.5</td>
<td>1.15±0.11</td>
<td>0.86±0.09</td>
<td>-25.2</td>
</tr>
</tbody>
</table>

Values are mean±SEE. Vascular resistance is calculated from the ratio of mean vitelline arterial pressure and mean dorsal aortic blood flow.

*Values were calculated using peak mean pressure and peak blood flow.

In the mature animal, autoregulation usually results in vasoconstriction in the face of increasing pressure and flow. In the embryo, we theorize that the reverse would occur in the extraembryonic vascular bed. This would result in maintaining a more constant hemodynamic state within the embryo itself. We also theorized that a decrease in circulating blood volume would result in an increase in vascular resistance. Again, this process would maintain a more constant hemodynamic state for the embryo at the expense of the extraembryonic vascular bed, which is less critical to the embryo at least for short periods of time.

The results of the experiment that measured ventricular pressure while decreasing and then increasing circulating blood volume also support both of our hypotheses. The end-diastolic pressure, an index of preload, changed directly with changes in circulating blood volume. This is not a pure response, since the peripheral vascular resistance could not be controlled, but the immediate association between changes in end-diastolic pressure and peak ventricular pressure demonstrate a length-tension relation of the embryonic ventricular myocardium. Stroke vol-

![Graphs](https://via.placeholder.com/150)

**FIGURE 6.** Slow recording of the response of ventricular pressure and dorsal aortic blood velocity to withdrawal and reinfusion of 10 µl blood in a stage 24 embryo. Top tracing: Ventricular pressure. Middle tracing: Mean dorsal aortic blood velocity, which is directly proportional to stroke volume index. Bottom tracing: Phasic aortic blood velocity.
Figure 7. Graph showing ventricular pressure changes during the withdrawal and reinfusion of 10 μl blood in a stage 24 embryo. Baseline peak ventricular systolic and end-diastolic pressures are shown at point A. With withdrawal of blood, the solid line plots the fall in peak systolic and end-diastolic pressures to point B. After a short time to allow parameters to stabilize (dash-dot line between B and C), the blood is reinfused starting at C and ending at D (dashed line). After a short period of time after the end of reinfusion, pressures return to E (dash-dot line) and are nearly equal to the baseline pressures.

The volume index was also altered as a function of end-diastolic pressure.

We favor the following interpretation of the ventricular blood pressure experiments as support for the hypothesis that vascular resistance is related to circulating blood volume. Withdrawal of blood decreases circulating blood volume and elevates vascular resistance. When the blood is reinfused, systolic and end-diastolic pressures rise to levels above baseline because of a higher resistance of the vascular bed. With time, systolic and end-diastolic pressures return to baseline as vascular resistance returns to normal.

We speculate that control of hemodynamics is crucial to the embryo. Circulating blood volume and vascular resistance may be markedly altered by environmental changes. A length-tension relation is important for maintaining normal hemodynamics in the chick embryo. Precise control of hemodynamics may also be critical for normal cardiac development because this may be related to flow. The ability of the myocardial muscle cell to respond with a length-tension relation is a fundamental property of the myocyte and is present even in the early stages of heart morphogenesis.

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References


Key words: cardiac development • hemodynamics • Frank-Starling mechanism • vascular resistance • chick embryo
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