Developmental Hemodynamic Changes in the Chick Embryo from Stage 18 to 27

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SUMMARY. We report the hemodynamic parameters of stage 18, 21, 24, and 27 chick embryos (from 3 to 5 days of incubation). Dorsal aortic blood velocity and mean vitelline artery blood pressure are measured with a 20 MHz pulsed-Doppler meter and servo-null pressure system respectively. We also measure heart rate, dorsal aortic diameter and embryo weight of each developmental stage. From these data, we calculate mean dorsal aortic blood flow, mean dorsal aortic blood flow per cardiac cycle, mean dorsal aortic blood flow per milligram embryo weight, vascular resistance and cardiac work. Blood flow increases geometrically with each embryo stage but remains constant when normalized for embryo weight. Mean arterial pressure increases linearly and vascular resistance decreases geometrically. Cardiac work increases in proportion to embryo weight. These results define the parameters of normal hemodynamic function during early embryonic development.


BLOOD FLOW in the embryo begins soon after the tubular heart starts to pulsate and continues during subsequent morphogenesis of the cardiovascular system. Since form and function are interrelated, it is important to understand the contribution of each in cardiac development. Structural changes during formation of the heart have been studied extensively (DeHaan 1965). Analysis of experimental studies in cardiac development leads many investigators to hypothesize that functional alteration is a mechanism for morphological abnormality (Rychter, 1962; Jaffee, 1970; Rosenquist and Bergsma, 1979; Pexieder, 1981). Little information is available on the hemodynamic changes during organogenesis. VanMierop and Bertuch (1971) and Girard (1973) measured heart rate and blood pressure in the chick embryo. Faber et al. (1974) reported cine-photographic estimated stroke volume and heart rate in the chick embryo. However, there is no integrated description of the hemodynamic characteristics during embryonic development in bird or mammal.

The chick embryo is a useful model for the study of hemodynamic changes during early development because avian and mammalian hearts are similar at the early stages and access to the embryo is relatively easy. It is only with the completion of organ development that the morphological differences between the avian and the mammalian heart are apparent. Birds have a muscular tricuspid valve, right aortic arch, and bilateral ductus arteriosus. In this paper, we describe a method for measuring blood pressure and dorsal aortic velocity and report the normal hemodynamic parameters in the chick embryo from the third to the fifth day of incubation.

Methods

Fertile White Leghorn chicken eggs were incubated blunt end up in a forced-draft constant humidity incubator to Hamburger-Hamilton (1951) stage 18 (3 days), 21 (3.5 days), 24 (4 days), and 27 (5 days). We chose those stages because there is an approximate 2-fold increase in embryo mass between stages. Each egg was removed from the incubator, positioned on a dissecting microscope stage, and surrounded by a warm air curtain at 38°C; the embryo was exposed by opening a window in the shell and removing the overlying membranes.

The velocity of dorsal aortic flow was measured with a 20 MHz directional pulsed Doppler velocity meter, described by Hartley and Cole (1974), which was adapted and built at the University of Iowa (Marcus et al., 1982). The Doppler probe is a 1-mm piezoelectric crystal mounted at the tip of a 18-gauge needle. The chick embryo floats with its right side up and the dorsal aorta horizontal. Using a micromanipulator and protractor jig, we positioned the crystal at a 45° angle to the dorsal aorta at the plane of the sinus venous (Fig. 1). At this position, the anterior and posterior cardinal veins are separate from the dorsal aorta, thus causing no interference between the venous and arterial streams. We arbitrarily chose a 45° angle for consistency in the velocity calculations. We tested the accuracy of the pulsed Doppler velocity meter against a graduate-measured velocity over a range of 0 to 16 mm/sec (Fig. 2A). The Doppler crystal was placed at a 45° angle to the long axis of a 3-mm internal diameter polyethylene tubing. A suspension of human red blood cells in chick Ringer's lactate was pumped at varying flows with a roller pump. Regression analysis revealed the correlation to be linear (y = 0.85 X + 1.15 $r^2 = 0.99$ S.E.E. = 0.49 mm/sec).

We measured the internal diameter of the dorsal aorta at the level of the sinus venous with a filar micrometer eyepiece calibrated against a 10-μm scribed glass standard. The sharp margin of blood in the dorsal aorta could be seen through the translucent embryo body. The dorsal aorta did not pulsate visibly to confound measurement.

We recorded phasic and electronically integrated mean
dorsal aortic blood velocity (Fig. 2B), and computed heart rate by counting phasic pulsations between time lines. Blood flow was calculated from the equation, $Q = \bar{V} \frac{d^2}{4}$, where $\bar{V}$ is mean dorsal aortic blood velocity and $d$ is aortic diameter. Dorsal aortic blood flow per cardiac cycle was calculated as the quotient of mean dorsal aortic blood flow divided by heart rate.

Calculation of mean dorsal aortic blood flow from measured mean aortic velocity underestimates cardiac output, because blood flow to the head and myocardium originates before the point of measurement. However, accurate measurement of blood velocity proximal to the dorsal aorta cannot be made for two reasons. In embryos at these stages, the conotruncus contracts with each cardiac cycle. The changing cardiac lumen diameter makes it impossible to calculate flow. In addition, two laminar blood streams flow through the conotruncus in a spiral course (Bremer 1932). The constantly changing angle of flow relative to horizontal precludes calculation of the incident angle of the ultrasound beam.

Systolic, diastolic, and electrically integrated mean blood pressure were measured with a servo-null pressure system described by Falchuk and Berliner (1971) and manufactured by WP Instruments (Fig. 3A). We inserted a 5-μm diameter tip, drawn glass micropipette electrode in a first-order vitelline artery for pressure measurement (Fig. 1). We assumed that vitelline artery pressure and dorsal aortic pressure were similar. In vitro validation against a standing
milligram of embryo weight is similar at each stage (Fig. 4B). Calculated dorsal aortic flow per cardiac cycle doubles with each successive stage (Fig. 4C).

We observe a gradual increase in heart rate, systolic, diastolic, and mean blood pressure (Table 1) (Fig. 4D). The ratio of blood pressure to blood flow (vascular resistance) decreases with development (Fig. 4E). This decrease is even more pronounced when the ratio is corrected for embryo weight (Table 1). The index of cardiac work approximately doubles with each stage studied (Fig. 4F).

**Discussion**

Delineation of the functional characteristics of the embryonic cardiovascular system is important in the eventual understanding of the development of the heart and vascular bed. This paper describes the normal parameters of cardiovascular function and provides a perspective on changes during development. In the chick embryo, the heart tube begins to pulsate at 33–38 hours of incubation (stage 10) and blood flow begins at 45–49 hours (stage 12). The heart forms from a tubular structure which connotes to a cardiac loop and then septates to a four-chambered adult form (DeHaan 1965).

Previous investigators have described individually heart rate, blood pressure, and estimates of cardiac output. Embryonic heart rate in the chick is the most extensively studied cardiac function. Other investigators have reported a gradual increase in heart rate during development similar to that observed in our study (Romanoff 1960). Measurement of heart rate at

**Results**

The mean wet weight of embryos approximately doubles between the successive stages chosen for this study (Table 1). We find that the diameter of the dorsal aorta increases with development, but the rate of increase declines as the embryo matures. Dorsal aortic blood velocity increases geometrically between the developmental stages studied. With the increase in dorsal aortic diameter and blood velocity, calculated dorsal aortic blood flow doubles between successive stages (Fig. 4A). Dorsal aortic blood flow per
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TABLE 1
Selected Hemodynamic Characteristics of the Chick Embryo at Stages 18, 21, 24, and 27

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Stage 18</th>
<th>Stage 21</th>
<th>Stage 24</th>
<th>Stage 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo n</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>18 ± 1</td>
<td>34 ± 3</td>
<td>80 ± 4</td>
<td>149 ± 5</td>
</tr>
<tr>
<td>Blood flow n</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Aortic diameter (mm)</td>
<td>0.29 ± 0.003</td>
<td>0.36 ± 0.003</td>
<td>0.40 ± 0.003</td>
<td>0.41 ± 0.003</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>165 ± 3</td>
<td>177 ± 3</td>
<td>183 ± 3</td>
<td>188 ± 3</td>
</tr>
<tr>
<td>Mean dorsal aortic blood velocity (mm/sec)</td>
<td>2.63 ± 0.10</td>
<td>3.89 ± 0.13</td>
<td>6.11 ± 0.23</td>
<td>11.32 ± 0.39</td>
</tr>
<tr>
<td>Blood pressure n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.82 ± 0.03</td>
<td>1.07 ± 0.05</td>
<td>1.29 ± 0.04</td>
<td>1.46 ± 0.07</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.52 ± 0.02</td>
<td>0.63 ± 0.03</td>
<td>0.81 ± 0.04</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular/embryo weight (mm Hg/mm³ per sec per mg)</td>
<td>0.21</td>
<td>0.06</td>
<td>0.02</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

early stages requires direct observation of the embryo. Thus, heart rates reported in this as well as other studies may be different from those present during undisturbed incubation because of temperature fluctuations and the trauma of shell opening.

Measurement of embryonic arterial blood pressure using small volume mechanical transducers has been reported by several investigators. VanMierop and Bertuch (1971) measured arterial pressure from a distal vitelline artery. They used analog signal processing to compensate for pressure distortion from a small diameter pipette. Our measurements are similar to theirs. Girard (1973) avoided small pipette signal damping by inserting occlusive probes in large vitelline arteries. The pressures in his report are different from those we found in our experiments. In part, this may be explained by Girard's use of incubation days rather than developmental staging. Embryos of the same incubation day may vary considerably in the degree of development. The servo-null technique has advantages over those previously used. First, the small tip diameter minimizes hemodynamic disturbance while allowing a pressure recording from a large proximal artery. Second, the rapid response time of the system overcomes signal damping and the need for extensive signal processing.

Cine-photo analysis is the only other technique used to estimate cardiac output in the early chick embryo (Faber et al., 1974). Comparison of the cine-photo cardiac output from their paper and dorsal aortic pulsed Doppler measurements from our study at given embryo weight revealed differences ranging from 10 to 100%. We accept the pulsed Doppler measurements because of the validation study and the small variability in individual measurements, yet we recognize that an independent third technique is not available for comparison.

The increase in cardiac output with development is accounted for primarily by an increase in stroke volume. Rychter et al. (1955) first proposed that cardiac output increases as a result of increasing circulatory blood volume, and Faber et al. (1974), with the intravascular injection of colloid solutions, reached a similar conclusion. The increase in stroke volume may, however, be secondary to metabolic factors. We base this speculation on the observation that cardiac output is proportional to oxygen consumption in adult animals subjected to environmental hypothermia (Thauer 1965). It has also been observed that heat production increases progressively during incubation (Romijn and Lokhorst 1960). The mechanism(s) responsible for the control of cardiac output in the embryo may begin to be operational very early.

Cardiac work increases with developmental stage. This increase may be a result of the development and alignment of contractile units in existing myocardial cells as well as the addition of new cardiac cells. Nakamura et al. (1980) observed that the myofibrils already are highly ordered by stage 12. Therefore, the increase in work between stages is secondary to an increase of contractile elements within existing cells and the addition of new cardiac cells. Evaluation of the relative importance of each mechanism requires a measurement of contractile mass.

The combined measurement of an index of cardiac output and mean arterial pressure permits the calcu-
lution of vascular resistance. Our calculations of resistance include somatic vessels (excluding the head and heart) and the resistance vessels in the extra embryonic circulation. Observation suggests that there are more resistance vessels in the vitelline bed, particularly in the early stages of development. The progressive ontogenic decrease in resistance may be accounted for by a gradual increase in dorsal aortic diameter, the addition of new resistance units in parallel to the circulation, and increased reactivity of the resistance vessels already present. It is possible that blood flow and mechanisms for vascular regulation may not develop concurrently. Definition of the sequence of development of vascular reactivity may provide important insight into the control of the cardiovascular system.

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INDEX TERMS: Dorsal aortic blood flow • Vitelline artery blood pressure • Pulsed doppler • Stroke volume • Vascular resistance
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