Mechanical function of the septating embryonic heart

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FABER, J. JOB. Mechanical function of the septating embryonic heart. Am. J. Physiol. 214(3): 475-481. 1968.—Intracardiac pressures were measured by direct puncture of the cardiac cavities in chick embryos of incubation ages between 3 and 7 days. The frequency response of the manometer was flat within ±3% to 40 Hz and its compliance was less than 40 μl/100 mm Hg pressure. The cardiac cycle of the embryonic heart was found to be comparable to that of the adult heart. No reflux could be detected in pressure recordings from the duct of Cuvier and atria, or ventricles during ventricular systole and diastole, respectively, in spite of the absence of true cardiac valves. Peak systolic ventricular pressures rose from approximately 20 to 60 mm of water between the 3rd and the 7th incubation day. End-diastolic pressures were variable but mean end-diastolic pressure remained under 10 mm of water. Isovolumically beating ventricles developed peak systolic pressures which depended strongly on end-diastolic pressures. Ventricles beating isovolumically at constant end-diastolic pressures and driven electrically exhibited a positive and a negative Bowditch staircase after acceleration and deceleration of heart rate, respectively.

Intracardiac pressures in embryonic hearts; inotropic effect of heart rate on embryonic hearts; Frank-Starling mechanism in embryonic hearts; Bowditch staircase in embryonic hearts

The structure of the early embryonic heart is different from the structure of the fetal heart on gross anatomical as well as on electron microscopic levels of resolution. It seemed, therefore, interesting to investigate whether the embryonic heart already possesses the typical physiological properties of cardiac muscle. This paper describes some simple experiments to demonstrate that the Frank-Starling mechanism and Bowditch’s staircase phenomenon can be revealed in the septating embryonic heart of the chick.

The early development of the chick heart (14, 17) is similar to that of the human heart (2), except that the time scale of development is different. Two cardiac primordia fuse to form a single tube. Fusion proceeds from the cranial (outflow) end towards the caudal (inflow) end. During the 2nd day of incubation, the cranial end of the cardiac tube begins to beat, although the pacemaker activity is located in the caudal end (19). The cardiac tube bulges and bends toward the right side of the embryo and the caudal (atrial) end moves cranially into a position behind and slightly above the ventricular portion. This happens between the 2nd and the 3rd day. (Our earliest measurements were taken at the end of this development.) In the meantime, endocardial masses separate the ventricular from the atrial cavities, and the truncus arteriosus from the ventricles.

The ingrowing cardiac septa separate the atrium, ventricle, and atrioventricular canal into left and right portions during the following 3 days. This process is completed on the 6th day of incubation, except for small openings that remain in the ventricular and atrial septa. The truncus arteriosus becomes divided into an aorta and a pulmonary artery during the 7th and 8th days. Although the embryonic heart now resembles the adult heart in external form, there is a profound difference in internal structure since nothing resembling cardiac valves has yet been formed.

The embryo is transparent during the first 8 days of development. One can see the blood enter the heart and follow its ejection into the truncus arteriosus with each beat. Endocardial cushions close off the atrioventricular canal before each ventricular contraction and similar masses obstruct the passage between ventricle and truncus arteriosus during ventricular diastole. Visual inspection (15) shows that these valve mechanisms are effective. Cardiac contraction in the 2-day-old embryo appears to be no more than a peristaltic wave running over the cardiac tube. But from the 3rd day onward, it is an orderly sequence of atrial and ventricular (and truncus) systoles.

We have made observations on embryos between just under 3 days to just over 7 days incubation. Younger embryos were too fragile for study and older embryos were so enveloped in their allantoic membranes that they could not be taken out of the shell with intact circulations. An embryo 4.5 days old is shown in Fig. 1.

Received for publication 25 September 1967.

1 This study was supported by grant HE 6336 of the National Heart Institute. An abstract of this work appeared in the Federation Proc. 26: 663, 1967.
METHODS

Chicken eggs of a variety of unspecified subspecies were incubated at temperatures between 37 and 38 °C (3). To prepare the embryo for the experiment, the egg was taken out of the shell and put into a cup. The vitelline circulation was carefully cut away from the rest of the yolk and the intact embryo with its vitelline circulation were transferred to a petri dish containing a disc of filter paper moistened with small amounts of normal saline. Very often the vitelline circulation was torn during this maneuver; such preparations were discarded. The petri dish was placed on a warming plate under a dissecting microscope (Fig. 2). The embryo was kept at 37 °C (verified by thermocouple measurement) if normal intracardiac pressures were recorded. Other experiments were usually done at room temperature.

Pressures were recorded by means of a Statham P23Gb pressure transducer. The fluid domes of these gauges have two connections. Figure 2 shows how one was mated to a hypodermic needle on which a glass pipette was glued and that the other connection was closed off with a three-way stopcock. The stopcock could also connect the gauge to a syringe filled with heparinized normal saline, with which the system could be flushed, or to a manometer filled with saline and mounted on a rack and pinion, with which calibration pressures were obtained after each measurement. The entire gauge assembly, was mounted on a micropositioner. It was moved forward in order to puncture the cardiac chamber with the tip of the micropipette.

Pipettes with outside tip diameters of 100–150 μ were found to be quite satisfactory. Tips smaller than this tended to become obstructed by the sponge-like interior surface of the myocardium. Once a cavity was punctured, pressures remained stable for periods ranging from 5 min to approximately 0.5 hr.

The pressure transducer was used with a polygraph (a Beckman-Offner RB, or a Grass P7). The recordings were curvilinear, with the exception of a few that were made with a rectilinear pen or photographed from the face of a monitoring cathode-ray oscilloscope.

The hydraulic system was calibrated by means of a sinusoidal pressure generator which generated pressures at frequencies between 10 and 55 Hz (cycles/sec). Comparison of the output of the pressure assembly as used in the experiments with the output of a reference pressure gauge (Statham P23Gb) which was not “loaded” with a narrow needle showed that the amplitude response of the hydraulic portion of the recording system was constant within ± 3% between 0 and 40 Hz, for all pipettes with outside tip diameters equal to, or greater than 85 μ (Fig. 3). The reference gauge itself was calibrated by means of the transient method (5) and was found to have a natural frequency in excess of 400 Hz and to be less than critically damped. Therefore, we may safely assume that the reference gauge faithfully recorded pressure in the frequency range used. The frequency response of the recording system was limited by the frequency response of the polygraphs. According to our calibrations, the polygraphs (as used in the experiments) recorded amplitudes within 10% of true values up to 10 Hz and within 50% of true values 2. The device is a barrel of a 10-ml syringe covered with a tough rubber membrane. A ball bearing mounted slightly eccentrically on the shaft of a variable-speed motor presses against the membrane and subjects it to sinusoidal displacements. The nipple of the syringe connects to a hose with a T tube at the end, and the arms of the T tube are connected to the test and reference gauges. The system is filled with fluid; the T tube must be entirely free of bubbles. It is essential to verify that the pressures oscillations recorded by the reference gauge are truly sinusoidal, for instance by comparing them against the signal from an electrical sine-wave generator on a double-beam cathode-ray oscilloscope.
values up to 20 Hz (Beckman) and 25 Hz (Grass). This frequency response was considered adequate for our purpose, since no detectable change in wave form occurred if the polygraph frequency cut-off switches were set at higher values. Noise levels at these higher values were, however, objectionable.

The volume distensibility of the assembly was found to be of the order of 40 μl/100 mm Hg pressure. This means that in the smallest preparations, in which I visually estimated the intraventricular volumes to be of the order of 50 μl, approximately 3% of the intraventricular volume moved into the recording system during ventricular contraction. In older embryos this fraction was considerably less.

Immediately after each experiment, the fluid level in the water manometer was adjusted to the fluid level in the petri dish. "Zero pressure" was recorded by connecting the gauge to the water manometer. This calibration was accurate to ±3 mm water pressure. A scale value was obtained by raising the manometer on the rack by exactly 30 mm; this calibration was accurate within 2%.

No data were available on the magnitude of the surface tension of chick embryo blood. If the surface tension of embryonic blood is markedly different from that of the heparinized saline used in the experiments, the measurements could be in error by a fixed pressure difference depending on the diameter of the tip used. Arguments against this possibility are that the pressures recorded in nonbeating hearts were less than 10 mm of water and that pressures recorded in the same heart with the blood-saline interface near the tip of the pipette were the same as those recorded with the blood-saline interface in a wider part of the pipette. Capillarity of the water manometer was found to be negligible.
The pressure elevation of atrial systole (open atrioventricular canal) is clearly visible.

The truncus arteriosus was cannulated in one 3-day-old embryo (bottom section of Fig. 4). There were two systolic pressure peaks in each cycle because the truncus arteriosus is still contractile in a very young embryo. No intraventricular pressure peak corresponding to truncus contractions were seen and inspection of the embryo showed a tightly closed area between ventricle and truncus immediately after ventricular systole.

I succeeded only once, in a much older embryo, in recording atrial and ventricular pressures simultaneously by means of two pressure gauges (Fig. 5). It is probable that in this experiment the tip of the pipette was not lying free in the ventricular cavity since the downstroke of the ventricular pressure recording appeared to be slurred. However, atrial contraction clearly preceded ventricular contraction and no atrial reflux was apparent during ventricular systole.

On the basis of these and similar experiments, one may conclude that the cardiac cycle of the embryonic heart is similar to that of the adult heart, except that the function of the cardiac valves is taken over by (presumably active) closures of the areas between truncus arteriosus and ventricle and of the atrioventricular canal.

![Graph](image1)

**Fig. 5.** Simultaneously recorded atrial and ventricular pressures for chick embryo of 6 days and 6 hr at 37 C. Ordinates show shape of time line. The large atrial pressure waves are due to atrial contraction. The small atrial pressure waves are interpreted as being due to venous filling during ventricular systole and atrial discharge at commencement of the "rapid filling phase."

![Graph](image2)

**Fig. 6.** Normal intraventricular pressures in embryos of incubation ages between 3 and 7 days.

Normal intraventricular pressures were recorded in 25 chick embryos whose incubation ages were between 3 and 7 days. The results are summarized in Fig. 6. Because of the ventricular septal opening, no systematic pressure differences were expected or found between "right" and "left" ventricles. Damage to the embryonic cardiovascular system always results in a decrease in heart rate. Only preparations whose vitelline circulations were intact after preparation of the embryo (no bleeding, and visible progression of blood in the vessels), which did not bleed from the cardiac puncture, which did not have recognizable malformations, and which had heart rates in excess of 100 beats/min were included in Fig. 6. The rise of peak systolic pressures with incubation age is consistent with a similar rise in arterial blood pressure reported in the literature (7, 8, 20).

The existence of the Frank-Starling mechanism was demonstrated in the isovolumically beating heart. These experiments were done at room temperature to decrease the heart rate. The top section of Fig. 7 shows that peak systolic pressure rose when the outflow tract of the ven-
The heart was then beating isovolumically at a constant filling pressure. The end-diastolic and peak systolic pressures from this experiment plotted in the bottom section of Fig. 7 formed a line, with a slope of approximately 6 mm of water pressure increase in peak systolic pressure for every millimeter increase in end-diastolic pressure, and an intercept at minus 2 mm of water end-diastolic pressure. The accuracy of the intercept is, of course, questionable in view of the possible error of ± 3 mm water in the zero-pressure level; the accuracy of the slope depends only on the accuracy with which increments in pressure were measured (± 2%) and is not significantly limited by instrument errors. The results of experiments on five embryos whose incubation ages were between 3 and 6 days are compiled in Table 1. Even the youngest embryo, which was 3 days and 4 hr old, demonstrated the Frank-Starling mechanism.

It can be seen in Fig. 7 that after 20 beats, end-diastolic and peak systolic pressures both leveled off to a steady value. The heart was then beating isovolumically at a constant filling pressure. Under these conditions, one can study the effect of inotropic alterations without any superimposed effects of the Frank-Starling mechanism. The probe that obstructed the outflow tract was used as a stimulating electrode and a circular stainless steel second electrode was placed in the saline in the petri dish (Fig 2). The hearts contracted in their normal sequence when stimulated with rectangular pulses from a Grass S5 stimulator even though the electrode was placed on the truncus instead of on the atrium. The results obtained on a heart in spontaneous arrest were particularly clear cut, (top section of Fig 8). The heart showed a typical Bowditch staircase with an increasing peak systolic pressure and an increasing rate of pressure rise during the first few beats of a new series. Spontaneously beating hearts that were electrically driven also showed a positive staircase effect upon acceleration and a negative staircase effect upon deceleration (bottom section of Fig. 8). After a change in heart rate a steady state was usually reached after approximately 10 beats. The steady-state peak systolic pressures were usually somewhat decreased after an increase in heart rate (though not in the example in bottom section of Fig. 8) but if there were any changes in rate of pressure rise or in time to peak systolic pressure, they escaped detection. This was probably due to our inability to increase the heart rate of a spontaneously beating heart to more than approximately two times the initial heart rate. The staircase effect was found to be present in all of the four embryos that were studied. Their incubation ages were 3 days and 21 hr, 4 days and 0 hr, 6 days and 1 hr, and 7 days and 5 hr.

**DISCUSSION**

Paff and co-workers (13) measured intraventricular pressures in chick embryo hearts by a modification of the Landis technique. The hearts were punctured with a micropipette connected to a reservoir. The fluid levels in the reservoir at which flow from the ventricle into the pipette became just continuous and just zero throughout the cardiac cycle were recorded as diastolic and systolic pressure respectively. The pressures recorded by these workers were consistently about 10% higher than the ones shown in Fig. 6. It is possible that the difference merely reflects the pooling of the results of these workers, who assigned the ages of their embryos to classes of 1-day intervals instead of stating them to the nearest hour. Paff and co-workers observed a decline in diastolic pressure (presumably early diastolic pressure) after the 5th incubation day and drew the conclusion that the postsysolic closure of the outflow tract of the ventricles did not become fully effective until after this time. The late decline in diastolic pressure and the conclusion that outflow tract closure is incompetent during the first 5 incubation days were not substantiated by my observations.

Several authors (7, 8, 20) have measured arterial pressures in chick embryos from 2 days incubation age and older. The results are compatible with the intraventricular pressures recorded in the present study (Fig. 6).

**TABLE 1. Relation between end-diastolic pressure and peak systolic pressure in isovolumically beating ventricles**

<table>
<thead>
<tr>
<th>Incubation Age of Embryo in Days and Hours</th>
<th>Temperature, °C</th>
<th>Slope ΔPSP/ΔEDP</th>
<th>Intercept, EDP at PSP = 0 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 and 4</td>
<td>29</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>3 and 21</td>
<td>29</td>
<td>9</td>
<td>0</td>
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<tr>
<td>4 and 22</td>
<td>21</td>
<td>4</td>
<td>-4</td>
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<tr>
<td>4 and 22</td>
<td>21</td>
<td>6</td>
<td>-2</td>
</tr>
<tr>
<td>6 and 6</td>
<td>21</td>
<td>6</td>
<td>+4</td>
</tr>
</tbody>
</table>

* PSP is peak systolic pressure and EDP is end-diastolic pressure.
Van Mierop and Bertuch (20) recorded some intracardiac pressures as well. They used pipettes with tip diameters smaller than the ones used in this study (about 50–75 μ) and corrected the relatively poor frequency response of the hydraulic system by electronic means. They presented evidence that this correction was indeed sufficient to compensate for the effects of the hydraulic damping of the gauge. However, the nominal compliance of their pressure gauge was four times higher than the nominal compliance of the gauge used in the present study, and the actual compliance of the hydraulic system must have been considerably higher still because of the flexible connections used between the glass pipette and the gauge. It is possibly because of the "windkessel" properties of their recording system that these authors found that they could record intraventricular pressures in only a few cases and that the youngest embryo in which they successfully recorded intraventricular pressure after slowing the heart rate by cooling was 5 days old. In older, larger embryos the compliance of the recording system is less critical (if the frequency response is adequate) and the published curve (20) for a 6-day-old ventricle beating at a normal rate shows systolic and diastolic pressures well within the range of pressures shown for this age in Fig. 6.

Starling sometimes used different terms to denote the same quantity. Consequently, there is some confusion as to what exactly constitutes a Frank-Starling mechanism. In three pages (p. 18–20) of his Linacre Lecture (18) the words "energy of contraction," "amount of chemical change at each contraction," "total mechanical energy set free as tension at each contraction," "strength of contraction," "free energy," and "contractile stress" are used to refer to one and the same thing. It is, therefore, necessary to stress that these pages show that measuring isovolumic peak systolic pressures as a function of ventricular filling was acceptable to Starling as a demonstration of the mechanism that bears his name. Since Frank discovered the mechanism by the same method, historical reason supports the conclusion that the Frank-Starling mechanism exists in the embryonic heart of the chick. The modern consensus seems to be that the Frank-Starling mechanism is best defined as an increase in the force intercept of the force velocity relation under the condition that the frequency response is adequate and necessary to stress that these pages show that measurements of the mechanism that bears his name.

The Frank-Starling mechanism of the heart has a homologue in the active length-tension relationship of skeletal muscle; both are expressions of the contractile properties of the molecular mechanisms of cross-striated muscle. The existence of the Frank-Starling mechanism in embryonic chick hearts of only 3 days and 4 hr incubation age indicates that the molecular mechanism of contraction in the early embryonic heart is probably the same as that of the adult heart. Electron microscopy of the embryonic chick heart reveals that at an incubation age of about 60 hr almost all myocardial cells contain a few myofibrils, although their density is very much lower than in the adult myocardium (6, 12). But the force per unit wall thickness exerted by the embryonic myocardium can be calculated to be of the order of 30 times less than that exerted by the adult myocardium. The functional and morphological properties of the early embryonic heart are thus mutually consistent.

It has been known for some time that the electrical activity of the early embryonic heart is also very similar to that of the adult heart (4, 9, 10, 19). It is believed, however, that the inotropic effect known as Bowditch's staircase is an adaptation of the mechanism of excitation-contraction coupling to an increase or a decrease in heart rate and is not due to changes in the myocardial action potentials (1, 11). It seems that the activity of the septating embryonic heart is in all known respects similar to that of the adult heart: excitation, excitation-contraction coupling, and contraction follow essentially similar laws in both systems.

Innervation of the embryonic chick heart does not occur until the end of the 3rd day, and autonomic fibers reach the auricular portion of the heart only at the end of the 4th day (17). It is not known whether the heart comes under autonomic control when innervation is established. Medullary cells are not seen in the chick's suprarenal complex until the 5th day of incubation (17). It is unlikely, therefore, that locally produced or circulating catecholamines influence cardiac performance during the first few days of incubation. It is interesting to come across an example where no cardiac regulatory mechanisms seem to be available to the organism with the possible exception of the Frank-Starling mechanism.

I gratefully acknowledge help received from Dr. A. J. Rampone, Dr. J. M. Brookhart, Mr. F. M. Hart, Mr. A. C. Poutala, and Mrs. M. M. Gordon. Mr. F. M. Hart constructed the calibrating device.

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