Microcinephotography of the Developing Heart in Neural Crest-Ablated Chick Embryos

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Microcinephotography was used to study heart development in a neural crest model of heart defects, that is, persistent truncus arteriosus, interrupted aortic arch, double outlet right ventricle, or single ventricle and tricuspid valve anomalies. These defects were created in chick embryos by ablation of premigratory neural crest destined for the aortico-pulmonary and truncal septa, as well as the third and fourth aortic arch arteries. When embryogenesis reached the looped cardiac tube stage of development (Hamburger-Hamilton stage 18), 19 experimental and 15 control embryos were filmed at 100 frames per second under controlled environmental conditions. Analysis of the microcinephotography films showed the following significant distinguishing characteristics of the developing heart in the experimental embryos: altered conotruncal shape in 100%, depressed contractility and dilation of the primitive ventricle in 84%, decreased emptying of the bulbus cordis in 79%, incompetent truncal cushions in 68%, incomplete looping of the cardiac tube in 58%, and fourth right aortic arch artery without blood flow and third right aortic arch artery with increased flow in 53%. These abnormal characteristics suggested that there were functional and morphological changes in the developing heart of experimental embryos before the time when the predicted structural heart defects would be apparent. It is proposed that the primitive ventricle might attempt to compensate for depressed contractility by ventricular dilation. The incompetent truncal cushions could be secondary to the depressed contractility or secondary to the neural crest ablation that is known to cause persistent truncus arteriosus, an interrupted aortic arch, or both. The absence of blood flow in the right fourth aortic arch artery that will become the definitive aorta correlates with the expected incidence of interrupted aortic arches in this neural crest-ablation model of heart defects. It is speculated that the incomplete looping of the cardiac tube might hinder normal developmental alignment of the outflow and inflow tracts, producing a spectrum of lesions of maldevelopment of the tricuspid valve and dextroposition of the aorta. (Circulation 1990;81:1047–1057)

The neural crest is located in the neural folds along either side of the neural plate. As the neural plate closes, the neural crest cells are released from the neural folds and migrate throughout the embryo. The neural crest provides both neurons and ectomesenchyme to the developing heart. The ectomesenchyme, derived from the region of premigratory neural crest located between the midotic placode and the caudal border of somite 3, has been referred to as the cardiac neural crest. The cardiac neural crest cells migrate through pharyngeal arches 3, 4, and 6 with some of the cells continuing their migration to the heart where they populate the aortico-pulmonary and truncal septa with ectomesenchyme, as well as form parasympathetic postganglionic neurons. A large number of the migratory neural crest cells do not get as far as the heart and stay in the pharyngeal arches where they form a mesenchymal sheath around the aortic arch arteries and later become the tunica media of the persisting aortic arch artery derivatives. In the developing cardiovascular system, there are five pairs of aortic arch arteries with the right fourth aortic arch artery becoming the definitive aorta in the chick. The third aortic arch arteries persist as the brachiocephalic arteries. The sixth aortic arch arteries are precursors of the bilateral ductus arteriosus.

Ablation of premigratory neural crest destined for the aortico-pulmonary and conotruncal septa and for the third and fourth aortic arch arteries leads to a 95% cardiovascular anomaly rate affecting the out-
flow tracts, inflow tracts, and aortic arch artery derivatives.\textsuperscript{8} Nishibatake et al\textsuperscript{10} showed that 65\% of the experimental embryos developed persistent truncus arteriosus. An additional 25\% had outflow tract anomalies with the common finding of a dextroposed aorta.\textsuperscript{8} These anomalies included double-outlet right ventricle or single ventricle with dextroposition of the aorta. Independent of the outflow tract anomalies, there seemed to be a 20\% incidence of inflow tract anomalies associated with maldevelopment of the tricuspid valve. These anomalies were a spectrum of lesions including tricuspid atresia, straddling and overriding tricuspid valve, and double-inlet left ventricle.\textsuperscript{8} Fifty percent of the experimental embryos developed aortic arch artery anomalies; for example, interruption of the definitive aorta and abnormalities of the subclavian arteries. The aortic arch anomalies were highly associated with persistent truncus arteriosus, which contrasts with the independence of inflow and outflow tract anomalies. This strong association of persistent truncus arteriosus with an interruption of the aorta is also found in humans and is proposed to be because of neural crest abnormalities.\textsuperscript{9}

Abnormal cardiogenesis induced by neural crest ablation has been investigated almost exclusively in fixed specimens.\textsuperscript{4,5,8,10} One previous study\textsuperscript{11} indicated that hemodynamic changes can precede heart defects after cardiac neural crest ablation. Microcinematography has been used previously to evaluate cardiovascular malformations and cardiac functions in studies of chick embryos.\textsuperscript{12–14} Cinephotography films of the developing chick embryo at days 3–4 of incubation were first used in the early 1970s by Faber\textsuperscript{12} who determined the area of the primitive right ventricle, bulbus cordis, or both in end diastole and end systole. From these areas, he derived embryonic stroke volumes and cardiac output. He also injected different types of volume expanders and showed that the increase in stroke volume was the mechanism for increase in cardiac output in early development.\textsuperscript{12} Subsequently, Gilbert and colleagues\textsuperscript{13} have used microcinematography extensively to assess morphological and hemodynamic changes in chick embryos to determine the teratogenic effect of agents such as isoproterenol,\textsuperscript{13} epinephrine,\textsuperscript{15} theophylline,\textsuperscript{16} and caffeine.\textsuperscript{17} A modification of this microcinematography technique has also been used by Ruckman and colleagues\textsuperscript{14,18} to assess the effects of hypoxia and ethanol on cardiac function in the embryonic chick. Therefore, it was anticipated that both structural and functional abnormalities in experimental chick embryos with cardiac neural crest ablation could be further analyzed using an in vivo microcinematography technique.

**Methods**

**Neural Crest Ablation**

Fertilized Arbor Acre chicken eggs (Seaboard Hatchery, Athens, Georgia) were incubated in a forced-draft incubator at 38° C and 97\% relative humidity. After 25–30 hours of incubation, they were "windowed" and prepared for microsurgery as reported by Narayanan.\textsuperscript{19} The stage of development of the embryos was determined according to Hamburger and Hamilton.\textsuperscript{20} Each embryo was stained with neutral red, and the overlying vitelline membrane was torn. Surgical ablation by microcautery was performed on the embryonic neural fold at stages 8–10. In these experiments, neural fold was ablated between the midotic placode and somite 2, bilaterally. This region corresponds to presumptive cardiac neural crest destined for pharyngeal arches 3 and 4. After microsurgery, the eggs were sealed with cellophane tape and reincubated in the same high-humidity incubator for an additional 24 hours, after which they were transferred to a second incubator at 37° C and 70\% relative humidity. Control eggs were transferred to incubators in parallel with experimental eggs. The control embryos were not "windowed" until microcinematographic filming.

**Microcinematography**

Experimental and control eggs were transferred to a heated sand bath. Using a thermistor probe placed on the surface of the yolk adjacent to the embryo, the temperature of the sand bath was adjusted so that the embryo was maintained at a constant temperature of 37.5° C. The embryos were viewed at day 3 and were filmed when they had reached stage 18.\textsuperscript{20} At this stage of development, the heart is a looped cardiac tube with the truncus arteriosus and bilateral aortic arch arteries 2, 3, and 4 being normally present.\textsuperscript{20} At stage 18, the embryo lies on the yolk sac such that right-sided cardiovascular structures can be seen. These structures are delineated by red blood cell streams that are seen through the transparent embryonic tissue. Left-sided structures are visible if the embryo is turned over on the yoke sac surface. Limb buds do not obscure the cardiac tube or aortic arch arteries until later in development.

Fifteen control and 20 experimental embryos, stage 18 of development, were selected for microcinematography filming. A high-speed Redlake Locam II camera was mounted on an Olympus stereomicroscope. The embryo could be viewed through the camera for appropriate positioning and lighting, as well as through binocular lenses on the microscope for accurate staging. Lighting was provided by two fiber-optic light sources. The secondary light source was continuous. The primary light source was focused through a lens system attached to a strobe (Strobex Model 236) that was externally triggered by the camera’s shutter opening. Each embryo was filmed for 5 seconds at a film rate of 100 frames per second. External timing marks on the film ensured correct timing of the shutter opening. In a typical embryo, there were 40 frames per cardiac cycle. The best resolution of cinemographic detail was seen using Kodak Ektachrome High Speed Daylight Film #7251, producing 37.5 million pixels per frame. Each egg was positioned in the sand bath the same distance from the microscope lens yielding an
approximate ×30 magnification factor onto the film. Each embryo was filmed with a micrometer to allow exact calculation of the magnification factor.

The embryos were analyzed using a specially designed motion picture projector for high-speed cinephotographic film. The films were viewed at 25 frames per second (one sixth of the normal heart rate) and at slower speeds, as well, for stop-frame analysis. Black-and-white still-frame photographs for publication were reproduced from the color cinephotographic film with some loss of resolution.

Microcinephotography Film Analysis

Two investigators independently analyzed microcinephotography films. Interrater reliability was judged as high with Pearson correlation coefficients in the range 0.94–0.98 on the dependent variables. The films from 15 control and 19 of 20 experimental embryos had a high degree of interrater reliability and were used for subsequent evaluation. Microcinephotography descriptions of each embryo were obtained. From these descriptions of the cardiovascular system in each embryo, eight characteristics were selected as distinguishing features between the control and experimental groups. These distinguishing characteristics were distinctly abnormal if present in the experimental embryos. All the films were reviewed and rated on each of these eight characteristics (Table 1). On review of the films, some control and experimental embryos exhibited a less severe form of the abnormal characteristics and were, therefore, rated as mild. Using a digitizing pad, the dimensions and areas of the primitive ventricle, conotruncus, and bulbus cordis were obtained (Table 2). The control and experimental groups were compared using χ² tests with the level of statistical significance chosen as p values less than 0.05.

Because the microsurgery performed was expected to cause several types of heart defects, the experimental group was reevaluated separately to see if there was a clustering of characteristics that might be correlated with the predicted outflow tract, inflow tract, or aortic arch anomalies in each group (Figure 4). Within the experimental group, eight χ² tests were performed on each of the characteristics in Figure 4.

Results

Composite Description of the Cardiovascular System

Figures 1–3 show still-frame photographs and corresponding drawings of typical control and experimental embryos. Right-sided structures are shown in diastole (Figures 1A and 2A) and systole (Figures 1B and 2B), whereas left-sided structures are shown in diastole alone (Figures 1C and 2C). A particular subgroup of experimental embryos that showed pronounced incomplete looping of the cardiac tube and dilation of the primitive right ventricle are depicted in the diastolic right-sided projection of Figure 3.

| Table 1. Distinguishing Characteristics of the Developing Heart in Experimental Embryos |
|-----------------------------------------------|------------------------------|------------------------------|--------------|
| Characteristic and severity                  | Control                      | Experimental                 | p            |
| Incomplete looping of cardiac tube (n)       |                              |                              |              |
| Normal                                       | 15 (100)                     | 8 (42)                      | 0.0003       |
| Severe                                       | 0 (0)                        | 11 (58)                     |              |
| Total abnormal                               | 0 (0)                        | 11 (58)                     |              |
| Dilated primitive right ventricle (n)         |                              |                              |              |
| Normal                                       | 11 (73)                      | 3 (16)                      | 0.0002       |
| Mild                                         | 3 (20)                       | 1 (5)                       |              |
| Severe                                       | 1 (7)                        | 15 (79)                     |              |
| Total abnormal                               | 4 (27)                       | 16 (84)                     |              |
| Altered conotruncal shape (wide and short) (n) |                              |                              |              |
| Normal                                       | 12 (80)                      | 0 (0)                       | 0.0001       |
| Mild                                         | 3 (20)                       | 1 (5)                       |              |
| Severe                                       | 0 (0)                        | 18 (95)                     |              |
| Total abnormal                               | 3 (20)                       | 19 (100)                    |              |
| Fourth right aortic arch artery without flow (n) |                              |                              |              |
| Normal                                       | 14 (93)                      | 9 (47)                      | 0.0044       |
| Severe                                       | 1 (7)                        | 10 (53)                     |              |
| Total abnormal                               | 1 (7)                        | 10 (53)                     |              |
| Third right aortic arch artery, increased flow (n) |                              |                              |              |
| Normal                                       | 14 (93)                      | 9 (47)                      | 0.0044       |
| Severe                                       | 1 (7)                        | 10 (53)                     |              |
| Total abnormal                               | 1 (7)                        | 10 (53)                     |              |
| Depressed contractility of primitive ventricle (n) |                              |                              | 0.0001       |
| Normal                                       | 14 (93)                      | 3 (16)                      |              |
| Severe                                       | 1 (7)                        | 16 (84)                     |              |
| Total abnormal                               | 1 (7)                        | 16 (84)                     |              |
| Decreased emptying of bulbus cordis (n)       |                              |                              | 0.0004       |
| Normal                                       | 12 (80)                      | 4 (21)                      |              |
| Mild                                         | 3 (20)                       | 3 (16)                      |              |
| Severe                                       | 0 (0)                        | 12 (63)                     |              |
| Total abnormal                               | 3 (20)                       | 15 (79)                     |              |
| Incompetent truncal cushions (n)              |                              |                              | 0.0012       |
| Normal                                       | 14 (93)                      | 6 (32)                      |              |
| Mild                                         | 0 (0)                        | 6 (32)                      |              |
| Severe                                       | 1 (7)                        | 7 (36)                      |              |
| Total abnormal                               | 1 (7)                        | 13 (68)                     |              |

Major criteria for distinguishing characteristics: Incomplete looping, inflow and outflow portions of cardiac tube separated by width of dorsal aorta; dilated ventricle and altered conotruncal shape, severe rating corresponded to digitized measurements of 1.5-fold the control mean; fourth aortic arch without flow, no red blood cells visualized through arch; third aortic arch with increased flow, greater density and column width of red blood cells; depressed contractility, decreased wall motion; decreased emptying, more red blood cells remaining in bulbus cordis at end systole; and incompetent truncal cushions, red blood cell column moving retrograde filling proximal conotruncus.

Numbers in parentheses indicate percentages.

Composite microcinephotography descriptions of typical control and experimental embryos are presented. Using a qualitative scale, eight microcinephotography characteristics were rated and found to show significant differences between control and experimental embryos (Table 1). The first five of
Incomplete Looping of the Cardiac Tube

The first distinguishing characteristic of the experimental embryos was the incomplete looping of the cardiac tube that was severe in 58% of the embryos (Table 1). In all embryos, the smallest venous structures showed a column of red blood cells lined up, one behind another. As the veins progressively joined together, the individual columns of red blood cells looked like small wires forming a bundle or larger stream. The veins emptied into the sinus venosus, which was seen from the right side (Figures 1A and 2A). In the experimental embryos, the sinus venosus appeared moderately dilated. The blood streams entered into the spherical primitive atrium, which was best seen from the left side (Figures 1C and 2C). In the control embryos, the bulbus cordis overlaid the primitive atrium, such that it could not be visualized in the right-sided projections (Figures 1A and 1B). In the experimental embryos, incomplete looping of the cardiac tube was a distinguishing characteristic. In 42%, there was a slight incomplete looping of the cardiac tube so that, in the right-sided projections (Figures 2A and 2B), the primitive atrium could be visualized. This slight degree of incomplete looping was judged as a normal variant because it was not significantly different from that in control embryos. The left side (Figures 1C and 2C) of the looped tube showed the primitive atrium that propelled the red blood cells through a thick-walled, funnel-shaped atrioventricular canal into a smooth-walled primitive left ventricular cavity with an internal size smaller than that of the primitive right ventricular chamber.

At the caudal end of the left ventricular chamber, the cardiac tube made an acute loop with blood flowing through the bulboventricular foramen into the trabeculated primitive right ventricle. Blood was seen to flow through the right-sided structures of the looped cardiac tube and into the aortic arches (Figures 1A, 1B, 2A, and 2B). Fifty-eight percent of the experimental embryos were judged as having a severe degree of incomplete looping of the cardiac tube, such that there was a significantly greater distance between the inflow portions, labeled atrium and atrioventricular canal, and outflow tract portions, labeled conotruncus of the cardiac tube, as seen in Figure 3. Because this tube was less tightly looped, the left-sided structures such as the atrioventricular canal were visible from this right-sided projection.

As seen in Figure 4, the characteristic of incomplete looping of the cardiac tube was found in 58% of the experimental embryos. This was the only characteristic that was not highly interrelated with the other distinguishing characteristics. Incomplete looping of the cardiac tube correlated weakly with depressed contractility or dilation of the ventricle (p=0.02). It operated independently of the other variables, which were subsets of each other.

Dilation of the Primitive Ventricle

The second distinguishing characteristic of the experimental cardiovascular system in the experimental embryos was the pronounced enlargement of the bulbus cordis. The bulbus cordis is composed of the primitive right ventricle, conus cordis, and truncus arteriosus. The conus cordis and truncus arteriosus appeared as a long cylinder that acted as one functional unit. This region is referred to as the conotruncus (Figures 1A, 1B, 2A, 2B, and 3). In the experimental embryos, the primitive right ventricular chamber was markedly dilated in diastole (Figures 1A and 3), with more readily apparent trabeculations in the ventricular wall. The enlargement of the bulbus cordis was because of dilation of the primitive right ventricular chamber with no significant difference in the size of the conotruncal region (compare end-diastolic vs. end-systolic areas in Table 2). In comparing control and experimental embryos, 84% of the ventricles from experimental embryos (Table 1) were markedly dilated, with only 27% of the ventricles in control embryos being dilated to a mild degree. Cinephotographic analysis also showed dilation of the primitive left ventricle, as seen in the comparison of Figures 1C and 2C.

Altered Conotruncal Shape

The conotruncal shape was altered in all experimental embryos (Table 1). Twenty percent of the control embryos were in a category of mildly altered conotruncal shape but none showed the distinctive characteristics of being wider or shorter, as seen in the experimental embryos (Figures 1A and 2A). Data in Table 2 show that the conotruncus in the experimental embryos was shorter in length and wider in

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**TABLE 2. Embryonic Heart Dimensions**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conotruncal dimensions (mean±SD) (mm/mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End diastole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>0.306±0.035</td>
<td>0.370±0.035</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Length</td>
<td>0.543±0.60</td>
<td>0.458±0.071</td>
<td>&lt;0.001</td>
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<tr>
<td>Area</td>
<td>0.166±0.028</td>
<td>0.170±0.32</td>
<td>NS</td>
</tr>
<tr>
<td>End systole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>0.170±0.128</td>
<td>0.127±0.147</td>
<td>NS</td>
</tr>
<tr>
<td>Length</td>
<td>0.174±0.139</td>
<td>0.124±0.161</td>
<td>NS</td>
</tr>
<tr>
<td>Area</td>
<td>0.046±0.039</td>
<td>0.037±0.060</td>
<td>NS</td>
</tr>
<tr>
<td>Bulbus cord area (mean±SD) (mm/mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End diastole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.381±0.128</td>
<td>0.463±0.071</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Conotruncus</td>
<td>0.166±0.010</td>
<td>0.170±0.032</td>
<td>NS</td>
</tr>
<tr>
<td>Bulbus cordis</td>
<td>0.548±0.129</td>
<td>0.632±0.083</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>End systole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.038±0.029</td>
<td>0.116±0.050</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Conotruncus</td>
<td>0.046±0.039</td>
<td>0.037±0.060</td>
<td>NS</td>
</tr>
<tr>
<td>Bulbus cordis</td>
<td>0.083±0.052</td>
<td>0.153±0.085</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
diameter but there was no significant difference in area in comparison with the control embryos. In analyzing microcinematographic characteristics (Figure 4), the altered conotruncal shape was a highly sensitive marker for the experimental embryos, with all other characteristics being subsets.

**Aortic Arch Artery Abnormalities**

The next pair of distinguishing characteristics (Table 1) focuses on the right aortic arch arteries. At this stage of development, blood flowed through the conotruncus, which gave rise to a small aortic sac that subsequently divided into three pairs of aortic arch arteries. These second, third, and fourth aortic arch arteries were visible in the control embryo on either the right (Figures 1A and 1B) or left (Figure 1C) sides. Because red blood cell streams outlined the aortic arch arteries and dorsal aorta, there seemed to be minimal movement in the aortic arch artery walls, whereas there was a mild degree of lateral movement in the walls of the dorsal aorta during the cardiac cycle. Fifty-three percent of the experimental embryos (Table 1) showed no evidence of any blood cells passing through the right fourth aortic arch artery (Figures 2A, 2B, and 3), indicating an absence or interruption of this vessel. In each embryo with absent or severely decreased flow through the right fourth aortic arch artery, there was a compensatory increase in the width of the right third aortic arch artery, with an increased density of red blood cells, suggesting more blood flowing through the third aortic arch artery (Figure 4) \( p<0.0001 \). These embryos occasionally had a wider second right aortic arch artery. When there was absence of blood flow in the right fourth aortic arch artery, there seemed to be a compensatory increase of blood flow through the remaining aortic arch arteries.

**Depressed Contractility**

The microcinematography films allowed the evaluation of dynamic characteristics that distinguished experimental from control embryos. The most pronounced difference was the impression of significantly depressed contractility in 84% of the experimental embryos versus 7% of the control embryos (Table 1). The left ventricular wall motion in control and experimental embryos was not as great as the primitive right ventricular wall motion. The left ventricle tended to act more as a conduit for the red blood cells in comparison to the distinct synchronous contractile pattern of the primitive right ventricle. In the experimental embryos, there was a significant decrease in wall motion in the experimental embryos with no specific areas of dyskinesis. In the experimental embryos with the most depressed contractility, there appeared to be a rocking motion of the bulbus cordis that was not present in the control embryos.

**Decreased Emptying of the Bulbus Cordis**

In control embryos during diastolic filling of the primitive ventricle, there was antegrade flow of a bolus of blood into the conotruncal region. The conotruncal cushions were not impeding antegrade flow during diastolic filling. Thus, there was no
The Heart in Neural Crest-Ablated Embryos

Experimental Diastole

isovolumic contraction phase at this point in development. Therefore, the conotruncus and primitive right ventricle, together as the bulbus cordis, represented a unit of diastolic filling. In the control embryos, the primitive right ventricle contracted and ejected nearly all blood at end systole. The conotruncal region propelled the blood through the cushions, such that there were no residual blood cells seen in the conotruncus during end systole and early ventricular filling. Cinephotography showed there was a minimal amount of blood left in 20% of the control embryos but none with a large amount. In comparison, 79% of the experimental embryos had a large residual amount of blood, usually to a severe degree, in the primitive right ventricle and the conotruncus in end systole (Table 1). The end-systolic area again confirmed that the bulbus cordis of experimental embryos was not able to empty as well as that of controls (Table 2). This decreased emptying of blood from the bulbus cordis is another reflection of the decreased ventricular contractility in the experimental group.

There was a high degree of association between several abnormal microcinephotographic characteristics in the experimental embryos as portrayed in Figure 4. The second most frequent characteristic of the experimental embryos was that 84% exhibited depressed contractility of the primitive ventricle as well as ventricular dilation. An important interrelation was the one-to-one correlation between the ratings of depressed contractility and a dilated primitive right ventricular chamber in the experimental embryos (p<0.0001) (Figure 4). This high association suggests the hypothesis that the hearts in experimental embryos might be dilating to compensate for their decreased contractility.

Incompetent Truncal Cushions

Movement of blood through the conotruncal cushions was an abnormal characteristic in 68% of the
Leatherbury et al  The Heart in Neural Crest–Ablated Embryos

Figure 3. Photomicrograph of experimental stage 18 chick embryo with right-sided structures visible in diastole showing incomplete looping of cardiac tube. ACV, anterior cardinal vein; PCV, posterior cardinal vein; OMV, omphalomesenteric vein; SV, sinus venosus; A, atrium; AVC, atrioventricular canal; R2, right second aortic arch artery; R3, right third aortic arch artery; R4, right fourth aortic arch artery; DAo, descending aorta.

Experimental incompleteness in neural crest-ablated embryos in comparison with a mild abnormality in 7% of the control embryos (Table 1). In a typical control embryo, a bolus of red blood cells passed through the conotruncal cushions in what has been labeled a pulsatile nature, with each cardiac cycle.21 The bolus moved between the conal and, then, the truncal cushions, and the cushions closed after the bolus of blood with no flow backwards into the conotruncal region during diastole. In the experimental embryos at the end of systole, the tail end of the bolus of blood could be seen to flow backwards into the most distal portion of the conotruncus, with simultaneous separation of the truncal cushions, which was judged as mild incompetency of the truncal cushions. A third of the experimental embryos showed competent truncal cushions, whereas a second third of the experimental embryos showed mild incomptence of the distal truncal cushions. The last

Figure 4. Diagram showing areas of circles and ellipse that represent percentages of experimental embryos exhibiting abnormal characteristic. Circles within circles represent characteristics that are subsets. Ellipse characteristic is independent of smaller circle characteristics.

Interrelationships of Cinephotographic Characteristics of the Developing Heart in Experimental Embryos

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Altered conotruncal shape</td>
</tr>
<tr>
<td>84%</td>
<td>Depressed contractility &amp;</td>
</tr>
<tr>
<td></td>
<td>Dilated ventricle</td>
</tr>
<tr>
<td>84%</td>
<td>Incompetent truncal cushions</td>
</tr>
<tr>
<td>53%</td>
<td>Absent 4th aortic arch &amp;</td>
</tr>
<tr>
<td></td>
<td>Wide 3rd aortic arch</td>
</tr>
<tr>
<td>58%</td>
<td>Incomplete looping of cardiac tube</td>
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</tbody>
</table>

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third showed a severe degree of incompetency of the truncal cushions with the bolus of blood coming back to the midpoint of the conotruncus (Figure 2C). The red blood cell columns were never seen to regurgitate back into the proximal conotruncus or into the primitive right ventricular chamber. Even in the group judged as severe, the most proximal part of those conotruncal cushions continued to be closed until the next bolus of blood was moving through the conotruncus in late diastole.

Within the group of embryos that had dilation and depressed contractility of their right ventricle, 81% of this group or 68% of the total group had incompetent truncal cushions as seen in Figure 4. This significant association (p=0.055) between incompetency of the truncal cushions and depressed contractility suggests that the dilated, poorly contractile primitive right ventricle might secondarily develop incompetency of the truncal cushions. Other causal factors of incompetent truncal cushions are also possible. There was a strong association between aortic arch artery abnormalities and incompetency of the truncal cushions. The absence of blood flow in the right fourth aortic arch artery occurred in 53% of the experimental group, which was a subset of the group with incompetent truncal cushion group (Figure 4). Therefore, there was a definite association between incompetent truncal cushions and aortic arch artery absence (p=0.0015). It is possible that increased resistance to blood flowing through the aortic arch arteries increases afterload, so that incompetency of the truncal cushions develops.

Other evidence suggests that there is not a significant resistance to blood flow across the total sum of aortic arch arteries. Analysis of the aortic arch arteries in Figure 4 shows that there is a one-to-one correlation of absence of blood flow in the right fourth aortic arch artery to the appearance of more blood flow in the right third aortic arch (p<0.0001). This one-to-one association suggests that if the right fourth aortic arch artery does not develop functional patency, the third aortic arch artery compensates to allow the same total amount of blood flow through the aortic arch arteries.

**Discussion**

In the neural crest model of heart defects, microcinematography showed characteristics of functional and morphologic abnormalities. Altered conotruncal shape is a definitive marker for the experimental embryos by the looped cardiac tube stage of development. The next most frequently seen abnormal characteristic was depressed contractility and ventricular dilation in 84% of the experimental group. This pair of characteristics suggests that the primitive ventricular chamber might have been dilating to compensate for its degree of depressed contractility. Normal chick embryos at this point in development follow the Frank-Starling law in response to an increased volume load. It is possible that the experimental embryos compensated for the depressed contractility by ventricular dilation. Sixty-eight percent of the experimental embryos showed incompetent truncal cushions. There are several possible explanations for this abnormal characteristic. The embryos being studied are at a point in development before the truncus arteriosus would be considered structurally defective. At this early stage of development, changes in extracellular matrix or cells that are directly related to the neural crest ablations23 can lead to the incompetent truncal cushions. Another possibility is that the incompetent truncal cushions occur secondarily to depressed contractility of the ventricle. Incompetent truncal cushions have been seen in other teratologic models in the setting of decreased contractility of the ventricle. An alternative hypothesis is that the incompetent truncal cushions, as well as the depressed contractility, are secondary to an increased afterload presented to the primitive ventricle. Increased afterload might be because of increased resistance to blood flow across the total sum of aortic arches, considering that 53% of the experimental embryos had an absent fourth aortic arch artery. The fact that the right third aortic arch artery seemed to have compensated for the absent fourth aortic arch artery does not support this hypothesis.

The characteristics of the developing heart in experimental embryos discussed in Figure 4 were highly interrelated as subsets. Fifty-eight percent of the experimental embryos also had incomplete looping of the cardiac tube. Because this characteristic was not highly related to the remaining characteristics, it seemed to operate as an independent variable. It is speculated that this variable might be secondary to depressed contractility and ventricular dilation. It is proposed that dilation of the primitive right ventricle might impede the process of cardiac tube looping so that the cardiac tube, as it appeared in Figure 3, would have a greater physical distance separating the right-sided outflow tracts and the left-sided inflow tract at this developmental stage. With this reasoning, incomplete looping could subsequently impede further cardiac developmental processes of the outflow and inflow tracts. Particularly, it would impede the aorta from moving over the left ventricle and impede the atroventricular valve division and subsequent migration of the tricuspid valve component over to the right ventricle. It is projected that the defects produced by neural crest ablation, for example, dextroposed aorta in double-outlet right or single ventricles and tricuspid valve inflow abnormalities, might be because of persistence of structural relations seen at this developmental stage caused by incomplete looping.

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References


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L Leatherbury, H E Gauldin, K Waldo and M L Kirby

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