Spectrum of Congenital Cardiac Anomalies Produced in Chick Embryos by Mechanical Interference with Cardiogenesis

By Ira H. Gessner, M.D.

Direct surgical manipulation of the developing embryo heart is one of several physical and chemical methods that are known to produce congenital cardiac anomalies in experimental animals. This procedure involves the concept that the pattern of blood flow through the embryonic heart is of prime importance in cardiogenesis. It is thought that the primitive cardiac tube is molded by the streaming of blood through it, thus determining subsequent development and location of the ventricular, bulbar, and aortico-pulmonary septa. Abnormalities in blood streaming might then be expected to produce abnormal development of the heart. Experimental methods designed to interfere mechanically with cardiogenesis should provide information on the validity of this hypothesis, and should increase general knowledge concerning the origin of congenital cardiac defects.

This report is concerned with a procedure involving temporary mechanical interference with normal cardiac development in the chick embryo. This manipulation distorted the bulboventricular outflow tract, and produced a spectrum of congenital cardiac anomalies, including abnormal positioning of the aorta and also ventricular septal defects.

Methods

White leghorn eggs, obtained from a commercial hatchery, were incubated at 38.5 to 39.5°C for a time calculated to place the embryos at stage 19 to 20 of Hamburger and Hamilton. This stage represents approximately 72 hours of incubation. After candling the egg to mark the
A series of drawings illustrating the normal and three components of the spectrum of anomalies described in the text. The large drawings represent a three dimensional approach to the predominant features found in each group, while the smaller drawings represent a direct superior view of the ventricular skeleton, emphasizing valve location. The coronary ostia are indicated in all drawings. a: Normal relations. b: Group 2. Ventricular septal defect is present. c: Group 3. Ventricular septal defect is larger, and the aorta appears to "override" the septum. Rightward displacement of the aorta is indicated in the smaller drawing by the dotted outline of normal aortic valve position. d: Group 4. Both great vessels arise entirely from the right ventricle. Muscular tissue intervenes between the aortic and mitral valves. Location of the ventricular septal defect is such that flow from the left ventricle is directed predominantly toward the aortic orifice. RV: Right ventricle. LV: Left ventricle. M: Mitral valve. A: Aortic valve. P: Pulmonary valve. T: Tricuspid orifice.

site of the embryo, a hole was punched in the air sac and a window cut in the shell over the embryo. The device used for manipulating the heart was a fine wire ramp, figure 1, similar to that of Stephan, and constructed in the following manner from 5-0 stainless steel surgical wire. The J-shaped base of the device was designed to rest on the embryonic membranes so that no movement of the wire could occur. The wire was then bent upwards at a right angle and then downwards so as to form a ramp. Each end of the wire was bent upwards slightly to reduce the possibility of trauma from the wire points. Using sterile technique, the membranes were cut over the heart. The tip of the wire ramp was inserted under the truncosomal portion of the heart tube, and then set down so that the heart tube rested on the wire. Since the chick embryo lies almost always on its left side, the effect of this procedure was to lift the truncosomal end of the cardiac tube anteriorly and to the right. Although all of the wire ramps were made in the same manner and positioned similarly in the embryo, it was not possible to control precisely or to measure the amount of displacement of the heart, nor the tension applied to it.

Once the wire was in place, the window was covered with cellulose tape, and the hole in the shell over the air sac closed with paraffin. The egg was returned to the incubator and ob-
served every 12 hours to insure that the wire remained in place. The wire ramp was removed with sterile forceps after 24, 48, or 60 hours, and the embryos were then left undisturbed and allowed to complete development. Sham operated controls were exposed to the entire operation as described, except that the wire was removed immediately after the heart loop had been suspended on it.

Twenty out of a total of 74 experimental embryos and 8 of 10 sham controls survived to at least 18 days of development and these 28 specimens form the basis of this report. The embryos were fixed in 10% buffered formalin after external examination of the heart and great vessels in the fresh state. After fixation, a complete examination of all the vessels derived from the aortic arches, the great systemic and pulmonary veins, and a detailed examination of the heart itself was done by means of a dissecting microscope.

**Results**

All eight sham operated control embryos developed normal hearts and normally placed great vessels. The 20 experimental embryos, however, presented a spectrum of abnormalities, illustrated in a series of drawings, figure 2b, c, and d, and figure 3. This spectrum correlated with the length of time the wire remained in place, table 1, to the extent that 24 hours of wire placement produced one phase of the spectrum, while 48 hours or longer produced a second phase.

Two embryos, in which the wire was in place for 24 hours, were entirely normal, figure 2a. In two more embryos with 24 hours of injury, group 1, there was definite widening of the left ventricular outflow tract with no other abnormality present. Widening of the left ventricular outflow tract, or aortic vestibule, can be quantitated by two measurements as in figure 3. The first is the distance from

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<th>Length of time wire in place</th>
<th>Number of embryos</th>
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<th>Group 1*</th>
<th>Group 2</th>
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*Group 1: Widening of the left ventricular outflow tract unassociated with other defects.

Group 2: Widening of the left ventricular outflow tract with a subaortic ventricular septal defect ranging in size from 0.2 mm × 0.4 mm to 0.5 mm × 0.8 mm.

Group 3: A ventricular septal defect ranging in size from 1.0 mm × 1.5 mm to 1.0 mm × 2.0 mm, combined with abnormal position of the aorta (overriding), but with maintenance of continuity between the aortic and mitral valve annuli.

Group 4: The double outlet syndrome, including origin of both great vessels from the anatomic right ventricle, muscular separation between the aortic and mitral valve annuli, and a ventricular septal defect ranging in size from 0.8 mm × 1.2 mm to 1.0 mm × 1.8 mm.

†Two of the 24-hour embryos, and one of the 60-hour embryos had other types of defects as explained in the text.
the right leaflet of the aortic valve to the lateral extent of the left ventricular lumen immediately below the mitral valve, i.e., the maximum width of the left ventricular orifice. The second is the length of the left ventricular lumen measured from the aortic annulus to the apex. In the eight normal control hearts, these values averaged 1.6 and 6.2 mm, respectively, a width:length ratio of 0.26.* In the two hearts in group 1, these measurements were 2.0 mm:5.0 mm, and 2.5 mm:6.0 mm, an average width:length ratio of 0.41. Thus, in the group 1 embryos, there is clearly a much greater distance across the left ventricular orifice in relation to left ventricular length, than is found in the normal chick heart.

In five chicks, group 2, the left ventricular outflow tract was widened and a ventricular septal defect was found just beneath the right leaflet of the aortic valve in the membranous portion of the septum, figure 2b. The size of these oval shaped defects ranged from 0.2 mm × 0.4 mm to 0.5 mm × 0.8 mm. From the right ventricle, the ventricular septal defect was located just behind the medial edge of the tricuspid orifice, the valve of which, in the chick, consists of only a single lateral, muscular leaflet.7 A photograph of one of these specimens is shown in figure 4. Of these five embryos, the wire was in place for 24 hours in two, for 48 hours in one, and for 60 hours in two.

In two embryos, group 3, in which the wire was in place for 48 and 60 hours, the aorta was displaced further to the right and anteriorly. A larger ventricular septal defect measuring 1.0 mm × 1.5 mm and 1.0 mm × 2.0 mm was present, and the aorta gave the appearance of overriding the septum, figure 2c. In both of these hearts, however, the aortic annulus retained its fibrous relation to the mitral annulus, with no muscle tissue separating them. The pulmonary valve was normal, and without evidence of right ventricular outflow tract obstruction.

In six embryos, group 4, the aorta was located directly to the right of the pulmonary artery, both vessels arising directly from the right ventricle, with both semilunar valve annuli at the same coronal and cross-sectional level. Of these six hearts, the wire had been in place for 48 hours in three, and for 60 hours in three. In all, the left ventricular outflow tract ended in a membranous defect ranging in size from 0.8 mm × 1.2 mm to 1.0 mm × 1.8 mm, and the aortic annulus was no longer in fibrous continuity with the mitral annulus, a band of muscle tissue now separating the annuli by 0.8 mm to 1.2 mm, figure 2d. A photograph showing the ventricular skeleton of such a heart, figure 5b, illustrates the marked rightward and anterior location of the aorta in comparison with the normal, figure 5a. A photograph of the left ventricular cavity of the same heart, figure 6, shows the outflow tract ending in a ventricular septal defect.

Three experimental embryos illustrated car-

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*The actual measurements, in millimeters, for the eight control hearts are 1.2:5.5, 1.5:6.0, 1.5:5.8, 1.6:6.0, 1.8:6.2, 1.8:7.0, and 2.0:7.0.
Experimental defects in chick hearts

Two cases in which the duration of injury was 24 and 60 hours, ectopia cordis was present. In one, only the apex of the heart protruded through a midline defect just below the sternum. In the other, the entire anterior body wall was open below the midsternal area. Both of these hearts had a severely hypoplastic right ventricle which gave off a single arterial trunk. This persistent truncus arteriosus gave rise to an aortic arch and the pulmonary arteries. The left ventricle was much larger than normal in both, communicating with the truncus arteriosus through a ventricular septal defect of 1.0 mm × 1.5 mm. Both hearts had essentially a common atrium, and one had tricuspid atresia.

One heart, in which the wire was in place for 24 hours, demonstrated left heart and great vessel hypoplasia involving the left atrium, left ventricle, and the aorta. No atrial or ventricular septal defects were present.

In all cases, the coronary arteries were identified as arising from the two aortic sinuses located on either side of the aortico-pulmonary septum, figure 2a, b, c, and d. The course of the coronaries was not followed. No anomalies of the great systemic or pulmonary veins were seen.

Numerous variations in the development of the great arteries were present. Although the
TABLE 2

Summary of Aortic Arch Anomalies

1. Absent left 6th arch with origin of the left pulmonary artery from the right 6th arch.
2. Atresia of the right 4th arch. Definitive arch of the aorta formed by the right 3rd arch.
3. Atresia of the right 4th arch. Definitive arch of the aorta formed by the left 3rd arch.
4. Persistence of the left dorsal aorta.
5. Absent right pulmonary artery. Hypoplastic aorta.
6. Hypoplastic left 6th arch.
7. Persistence of the left dorsal aorta connecting the left 6th and 3rd arches.
8. Absent left ductus arteriosus.
9. Absent left ductus arteriosus, with left 6th arch giving off the left internal carotid artery as well as the left pulmonary artery.
11. Bilateral 4th aortic arches, left smaller than right.

majority of these anomalies occurred in those hearts with origin of both great vessels from the right ventricle, aortic arch abnormalities were also seen in hearts with lesser degrees of intracardiac anomalies. One of the hearts with so-called overriding aorta and a large ventricular septal defect (VSD), had a missing left sixth arch, and another with only a VSD had a persistence of a portion of the left dorsal aorta. One heart, which was internally normal except for a widening of the left ventricular outflow tract, demonstrated bilateral fourth arches which joined at the descending aorta. All of the arch anomalies found are listed in table 2.

Discussion

The chick heart is a four-chambered organ in which embryological development is similar to that of mammals, although the chick does retain the right fourth aortic arch as the definitive arch of the aorta, and both sixth arches persist, so that a ductus arteriosus is present bilaterally. At stage 20 the heart is predominantly a coiled tube in which ventricular development is just beginning. The atrial septum has begun to form (50 to 55 hours) but the ventricular septum does not appear until the fourth day. The endocardial cushions also form during the third and fourth days. Growth of the ventricles during the fourth and fifth days together with leftward rotation of the bulbus maintains the central location of the truncus arteriosus. Development of the aortico-pulmonary septum occurs on the fifth and sixth days, and this unites with the bulbar ridges, so that by the seventh day, division of the aortic and pulmonary trunks is complete down to the ventricles.

Two distinct streams of blood can be identified in the chick embryo heart, while it is in a simple tubular stage. It is thought that the direction of flow of these spiral streams is particularly important in the development of the aortico-pulmonary septum and the bulbar ridges, and the subsequent fusion of these components with endocardial cushion tissue to complete the ventricular septum. Bremer stated that these two streams pursue definite, different courses, and that between the streams are developed the endocardial cushions, and the ventricular and bulbar septa. Barry, also using the chick embryo, arrived at similar conclusions.

Several investigators have reported the effects of procedures that involve surgical interference with the developing heart. Stephan described a technique for the experimental occlusion of aortic arches in the chick by the use of a fine wire. He was able to produce variations in aortic arch development, and he also obtained some intracardiac anomalies. Rychter applied fine metal clips to the third, fourth, and sixth pairs of aortic arches in all possible combinations. He also applied the clips to portions of the heart loop itself. Among his results, was the consistent production of specific types of ventricular septal defects when particular combinations of aortic arches were occluded. Suppression of both the right and left sixth (pulmonary) arches resulted in a triangular shaped ventricular septal defect located just beneath the aortic valve. Occlusion of all left ventricular arches, i.e., both third and both fourth, produced teardrop shaped defects located anteriorly beneath the pulmonary valve. He concluded that these defects arose because the blood stream from the ventricle whose outlet had been so markedly narrowed, was forced to pass across the unclosed ventricular...
EXPERIMENTAL DEFECTS IN CHICK HEARTS

septum into the opposite ventricle. This prevented the normal fusion of the right and left bulbar ridges, and the atrioventricular cushions which ordinarily complete the ventricular septum.

The manipulation described in this report probably interfered with normal bulbar rotation, forcing the truncus arteriosus to remain in a more anterior and rightward location than normal. The degree of this abnormality of position was related to the length of time the wire ramp was left in place. If the wire was left in place for 48 hours, or longer, the aorta usually originated from the right ventricle with muscular separation between the mitral and aortic valves. A similar origin of both great vessels from the right ventricle in the chick was reported by Rychter, and also by Le Douarin after local X-irradiation of the chick heart at stage 16 to 18. Neither of these authors commented specifically on the separation of the aortic and mitral valve annuli by muscular tissue in these hearts, but from the description they give, this seems likely to have been present.

When the wire was in place for only 24 hours, there was less internal derangement of the heart. Thus, it seems reasonable to conclude that these findings represent a continuum of abnormalities related directly to the length of time during which the outflow tract of the primitive heart tube was distorted. It should be pointed out that this continuum did not include any primary malpositioning of the pulmonary artery. In every case the pulmonary artery maintained its right ventricular origin.

It is difficult to assess the role of altered blood flow in the production of these anomalies. In the cases of double outlet, it is suggested that the left ventricular stream, forced to cross the ventricular septum to reach the aorta, prevented closure of the ventricular septum. In the cases of persistent truncus arteriosus, and those in which the aorta arose directly above the septum, the same phenomenon may have occurred, plus the probability that the aortico-pulmonary septum could not fuse with the bulbar ridges to close the ventricular septum. In those hearts in which only a ventricular septal defect was present, the picture is even less clear. The presence in these hearts of a widened left ventricular outflow tract might suggest that the defect was due simply to an inability of the normal tissues to accommodate to the abnormal degree of their separation. It could be argued also that the direction of the left ventricular stream was changed slightly, crossing the ventricular septum to a minor degree, but still sufficiently to prevent its completion.

Although the initial intent of this work was to interfere primarily with blood streaming, the best interpretation seems to be that the primary effect of the manipulation was a disturbance of growth, preventing migration of the aorta to its normal leftward and posterior position. Altered blood streaming secondary to the disturbance of growth may also have been important in determining subsequent development of the heart and great vessels.

While no direct correlation is possible between the findings in this report and human congenital heart disease, Neufeld et al., in a series of communications, have described, in the human being, the syndrome of double outlet from the anatomical right ventricle with and without pulmonary stenosis. The pathological basis for the diagnosis of double outlet by these authors was the finding of "the ascending aorta to the right of the pulmonary artery and the two semilunar valves in approximately the same coronal and cross-sectional body planes," exactly as was found in the group 4 chick embryos. In several of their autopsied cases, the aortic valve is described as maintaining continuity with the mitral valve. These are included as examples of the double outlet syndrome, however, because, as these authors point out, the aorta did not connect directly with the left ventricle nor straddle the ventricular septal defect, but lay completely to the right of the defect. The aortic valve was considered to be continuous with the mitral valve not because of normal aortic position, but because of an elongated anterior mitral leaflet extending upward and to the right. It is possible that the variation found in these human hearts repre-
sents the differences one may expect in dealing with an anomaly which may include a spectrum of severity. The controversy which persists over what actually constitutes an overriding aorta in the tetralogy of Fallot may well be thought of in the same manner. It is reasonable to consider that causes of congenital heart disease may occur with grades of severity and/or at varying times in cardiac development, resulting in a spectrum of clinical heart disease at birth.

The production of a progression of cardiac anomalies in the chick embryo as described in this report suggests, at least, that injuries may produce a similar range of abnormalities in other species. By contrast, human congenital cardiac malformations are often considered as a collection of independent defects, and frequently some confusion and disagreement arise over variation in anatomic findings. While the spectrum of anomalies discussed here would present quite different clinical and hemodynamic findings in the human being, it may still be useful to keep in mind the concept that certain groups of cardiac anomalies may not be independent, but rather may represent varying degrees of severity of a basic malformation.

Summary

Stage 19 to 20 white leghorn chick embryos were subjected to an operative manipulation in which a wire device was passed under the outflow tract of the cardiac tube, and allowed to remain in place from 24 to 60 hours. The embryos were then permitted to develop until hatching. Twenty-eight embryos, including eight sham operated controls, all of whom were normal, survived to at least the 18th embryonic day, and are included in this report. A spectrum of cardiac anomalies was produced as follows: group 1, widening of the left ventricular outflow tract unassociated with other defects; group 2, widening of the left ventricular outflow tract with a subaortic ventricular septal defect, 0.8 mm or less in its greatest dimension; group 3, a large ventricular septal defect, 1.5 mm or more in its greatest dimension, with varying degrees of abnormal position of the aorta, including "overriding" of the aorta, but with maintenance of continuity between the aortic and mitral valve annuli; group 4, the double outlet syndrome, in which both great vessels arise from the anatomic right ventricle with complete muscular separation between the aortic and mitral valves. In the time period studied, the double outlet syndrome represents the extreme of the continuum. However, it is possible that other forms of this spectrum exist, some perhaps incompatible with embryonic life, and that these may be produced by changing the experimental technique.

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