Pathogenesis of persistent truncus arteriosus and dextroposed aorta in the chick embryo after neural crest ablation

Makoto Nishibatake, M.D., Margaret L. Kirby, Ph.D., and L. H. S. van Mierop, M.D.

ABSTRACT To investigate the contribution of cranial neural crest cells to the developing cardiovascular system in the chick embryo, cauterization of various regions of cranial neural crest was performed. Five regions may be distinguished, each of which contributes mesenchyme to pharyngeal (branchial) arches 1 through 4 and 6. Ablation of arch 3, 4, and 6 regions resulted in a high incidence of persistent truncus arteriosus (PTA) associated with anomalies of the aortic arch. Dextroposed aorta (DPA) or anomalies of the inflow tract were found in all ablation groups. Although anomalies of the aortic arch arteries were induced in all ablation groups and were usually associated with intracardiac anomalies, those of the third and right fourth aortic arch were most frequent in the arch 4 and arch 4+6 groups. Anomalies of the sixth aortic arch were most frequent after extensive ablations that included the arch 6 region. We speculate that PTA is a direct result of the decreased population of mesenchymal cells derived from the arch 3 through 6 neural crest regions. DPA or anomalies of the inflow tract may be related to altered hemodynamics due to anomalies induced by neural crest ablation. Anomalies of the aortic arch arteries may be caused by either the direct or indirect process.


IT HAS BEEN SHOWN in quail-chick chimeras that the cranial neural crest supplies ectomesenchymal cells to the pharyngeal arches, which contain the aortic arch arteries. Moreover, cranial neural crest cells extending from the auditory placode to somite 3 migrate to the outflow tract of the heart to participate in aorto-pulmonary and truncal septation in the chick embryo. Surgical removal of these premigratory cells results in a high incidence of conotruncal anomalies, which include persistent truncus arteriosus (PTA) and double-outlet right ventricle (DORV) in combination with hypoplasia or absence of pharyngeal pouch derivatives, i.e., thymus and parathyroid glands. These anomalies induced by ablation of cranial neural crest have morphologic features that resemble those of the DiGeorge syndrome in man.

DiGeorge syndrome in man is associated with interrupted aortic arch type B, PTA, and tetralogy of Fallot. In addition, many clinically recognized syndromes, which include malformations of the face, ears, and cardiovascular system, appear to be due to disorders of neural crest cells or their derivatives. Studies of normal development using chimeras and of abnormal development induced by surgical ablation of the neural crest would potentially bridge the gap between embryology and teratology. We have hypothesized that cranial neural crest cells play an important role in the pathogenesis of certain forms of congenital heart defects, especially conotruncal anomalies. However, the role of the cranial neural crest, as a whole, in cardiovascular embryogenesis is still unclear. This prompted us to extend our ablation study to the entire cranial neural crest region, and to examine derivatives of the aortic arch arteries in addition to our morphologic analysis of intracardiac anomalies.

Materials and methods
Fertilized Arbor Acre chicken eggs obtained from a local hatchery (Seaboard Hatchery, Athens, GA) were incubated in forced-draft incubators at 38°C with a relative humidity of 97%. After 30 to 40 hr of incubation, they were “windowed” and prepared for microsurgery as reported by Narayanan. The stage of development of the embryos was determined according to Hamburger and Hamilton, and the overlying vitelline membrane was torn. Surgical ablation by microcautery was performed on the embryonic neural fold at stages 8 through 11. To maintain consistency, all microsurgery was performed by the
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The cranial neural crest is located in the neural fold extending from the diencephalon through the level of the fifth somite. Based on quail-chick neural crest transplantation studies, we divided the cranial neural crest into five regions (figure 1). These regions are considered to contain presumptive mesenchymal cells for the first, second, third, fourth, and sixth pharyngeal arches. Neural crest-derived mesenchymal cells from the regions of somites 4 and 5 have not been found in any of the thoracic viscera.

In the first set of experiments, bilateral lesions of single arch levels of neural crest were produced at stages 8 through 11. In the second set of experiments, bilateral lesions were produced at combined arch levels, i.e., combined arch 1 and 2 regions (arch 1-2), combined arch 4 and 6 regions (arch 4-6), the somite 2 through 3 region (S 2-3), and the somite 3 through 5 region (S 3-5).

Sham-operated embryos were prepared in parallel with experimental embryos. Sham operations included windowing of the eggs, staining of the embryos, and tearing the vitelline membrane. All embryos that survived until incubation day 11 were removed from the egg. The thoracoabdominal wall was opened to expose the cardiovascular system. Saline was injected into the right ventricle, followed with Carnoy's fixative for rapid fixation. Injection was performed from the left ventricle in cases in which the right ventricular cavity looked unusually small. The embryos were stored in 10% neutral-buffered formalin for at least 1 day before examination.

The cardiovascular system was observed from the frontal aspect after the surrounding tissues and the anterior wall of the right ventricle were removed. The heart was elevated cephalad to expose the dorsal side of the aortic arch system. When the atrioventricular connection was unclear from the right ventricular view, the posterior atrial wall and/or posterior left ventricular wall were removed to enable examination of both atrioventricular valves. The chi-square test was used when statistical analysis was required. A p value less than .05 was considered to indicate a significant difference.

Although the normally developing cardiovascular system of the chick embryo resembles that of the human fetus, there are major differences. The right-sided atrioventricular valve of the chick embryo is composed of one muscular flap without chordae tendineae or papillary muscles (the name "tricuspid valve" will be used in this article for convenience). The ventricular septum of the chicken embryo has no membranous part. The aortic arch system of the chick differs from that of man in the following ways: bilateral brachiocephalic arteries derived from the third arch arteries (3AA) arise from the aortic root, the definitive aortic arch is right-sided and is derived from the right fourth arch artery (R4AA), the ductus arteriosus is present bilaterally (both vessels persist until hatching), and primary subclavian arteries developed from the sixteenth segmental arteries are replaced by secondary subclavian arteries arising from the 3AA in the chick embryo.

The terminology of the anomalies of the aortic arch derivatives are based on the descriptions by Rychter and Hodach et al. Embryologic terms will be applied for the names of aortic arch artery derivatives to avoid confusion. As Rychter pointed out, in cases in which absence or anomalous origin of the right subclavian artery is associated with an interrupted right aortic arch, it is difficult to determine whether R3AA or R4AA is absent. These cases will be considered absent R4AA in this report.

FIGURE 1. The levels of cranial neural crest that provide ectomesenchyme to the pharyngeal arches. Ablations of single or combined levels of neural crest are indicated. D = diencephalon; Mes = mesencephalon; Met = metencephalon; Mye = myelencephalon; Oto = otocyst.
Results

Viability of the various groups of embryos after neural crest ablation to 11 days of incubation ranged from 31% to 42%; that of the sham-operated group was 38%. In total, 150 experimental and 31 sham-operated embryos were included in the analysis.

Table 1 summarizes the major cardiovascular malformations after neural crest ablation. The rate of cardiovascular anomalies indicates the percent of the embryos with cardiovascular malformations after surgery. Simple punched-out type ventricular septal defects (VSDs), persistence or remnants of the left fourth aortic arch (L4AA), and absence or anomalous origin of subclavian arteries were found frequently.

PTA was found only after ablation of neural crest contributing to arches 3, 4, and 6, singly or combined (figure 2). A remarkably higher incidence was observed in the arch 4 group. PTA was found frequently in the arch 4-6 and S 2-3 groups, both of which had large ablations of neural crest from arch 4 and 6 areas.

On the other hand, dextroposed aorta (DPA) was found in all ablation groups (figure 2); it was most frequent after lesions of arches 3, 6, and 1-2, and S 3-5 were induced. The distribution and the incidence of inflow tract anomalies were similar to those of DPA and such anomalies were found in all operated embryos.

Among anomalies of aortic arch derivatives, hypoplasia or absence of 3AA or R4AA were distributed among all the ablation groups; however, these two anomalies were of significantly higher incidence in the arch 4 group compared with the other experimental or the sham-operated groups. These anomalies were relatively frequent after ablation of the neural crest contributing to arches 3 and 6 and in the arch 4-6 group.

Absence or hypoplasia of the sixth aortic arches (6AA) was not found in the arch 6 group, but it was relatively frequent in the arch 4, arch 4-6, S 2-3, and S 3-5 groups.

There was no significant correlation between site of ablation and ventricular origin of the PTA or the number of truncal valve cusps (table 2). Dysplastic truncal cusps were observed frequently. Usually the PTA was of type I or II (Collet and Edward)19; one embryo was not classified because there was no recognizable lung tissue. Associated aortic arch anomalies in embryos with PTA were variable; however, PTA with absent R4AA was noted in 14 embryos. Seven of eight embryos with PTA in the arch 4-6 group had unilateral or bilateral 6AA anomalies, whereas five of 13 embryos with PTA in the arch 4 group had 6AA anomalies.

Subtyping of DPA in accordance with Lev et al.20 is shown in table 2. In two embryos with DORV and subpulmonary VSD, the pulmonary valve overrode the ventricular septum, resembling Taussig-Bing anomaly in humans. Two embryos with DORV and a noncommitted type VSD showed peculiar elongation of the right ventricular outflow tract, which appeared similar to “bulboventricular heart,” as reported by Goor and Lilliehi.21 Subaortic muscular stenosis was found in five embryos with DORV; all of these had absence or hypoplasia of R4AA. After arch 1 ablation one embryo showed DORV with L-transposition in which the aortic valve was located to the left of the pulmonary valve.

In 26 of 27 embryos with inflow tract anomalies the anomaly was associated with conotruncal anomalies;
in the only exception an infundibular VSD and severe hypoplasia of R4AA was observed (table 2). Tricuspid atresia was usually the muscular type with no dimple on the floor of the right atrium. This is the type most common in man (figure 3, B). In all cases of double-inlet left ventricle or straddling tricuspid valve, the tricuspid valve morphologically resembled the mitral valve, in mirror image. Left (nine cases) or right (one case) juxtaposition of atrial appendages was found in embryos with inflow tract anomalies (figure 3, C and D).

The 3AA was absent or hypoplastic in 31 embryos (unilaterally in 26, bilaterally in five). Instead of the affected 3AA, the ipsilateral common carotid artery

![FIGURE 2. Conotruncal malformations (frontal views). A, Normal heart of 11 day chick embryo. B, PTA with tricuspid atresia and absence of the R3AA. A large VSD is located in the infundibular septum. C, DORV with subaortic VSD and double-inlet left ventricle. Arrow indicates the subaortic infundibulum. There is left juxtaposition of the atrial appendages. D, DORV with subpulmonary VSD and absence of the right fourth aortic arch. The large VSD is located in the subpulmonary infundibular septum, and the pulmonary valve overrides the ventricular septum. The right aortic arch is absent. Ao = aorta; P = pulmonary trunk; RV = right ventricle.](image)
TABLE 2
Types of anomalies

<table>
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<tr>
<td>(A) PTA</td>
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<tr>
<td>(1) Origin of PTA</td>
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<tr>
<td>Right ventricle</td>
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<td>Left ventricle</td>
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<tr>
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<tr>
<td>(B) DPA</td>
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</tr>
<tr>
<td>(1) Type of VSD</td>
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<td>DORV</td>
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<td>Subpulmonary VSD</td>
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<tr>
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<tr>
<td>VSD with overriding of the aortic valve</td>
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<tr>
<td>Subaortic VSD</td>
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<td>Doubly committed VSD</td>
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<td>(C) Inflow tract anomalies</td>
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<td>(1) Subtypes</td>
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<td>(with or without TS)</td>
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<tr>
<td>Double-inlet left ventricle</td>
<td>7</td>
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<td>(1 with common atrioventricular valve)</td>
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<td>(2) Associated anomalies in the outflow tract</td>
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<td>PTA</td>
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<td>20</td>
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<td>Infundibular VSD with R4AA hypoplasia</td>
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Arose from the ipsilateral subclavian artery in three and from the common carotid artery in one. In embryos with a hypoplastic 6AA, the ductus arteriosus derived from the distal part of the 6AA was absent (figure 4, D). Isolated right 6AA hypoplasia was found in one embryo.

VSD was a common intracardiac anomaly in this series and was usually located in the proximal infundibular septum as an isolated anomaly or in association with minor aortic arch anomalies. VSD was found in all ablation groups.

Persistent L4AA in which the vessel was considered to be large enough to allow effective blood flow was noted in 14 embryos. We could not find any correlation between the occurrence of persistent L4AA and specific neural crest ablation sites; however, in most of these embryos (12/14) there was associated absence or hypoplasia of other major aortic arches, and persistent L4AA played the role of an alternative or a supplemental pathway for these affected arch arteries.

A remnant of the L4AA, anomalous origin, or absence of the subclavian artery was found in 24, 48, and 34 embryos, respectively. They were often associated with other major anomalies in all the groups, but as isolated anomalies they were uncommon and were found less frequently in the sham-operated group.

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Major extracardiac anomalies were ventral wall defects with ectopia cordis (49 experimental embryos, 0 in sham-operated group), hyoid or mandibular defects (10 embryos), exencephaly (nine embryos), hemimicrocephaly (three embryos), microphthalmia (eight embryos), and short neck or torticollis (25 embryos). Glands were not examined in the present study to allow focus on the anatomy of aortic arch derivatives.

Discussion

Quail-chick transplantation studies have shown that the cranial neural crest provides all of the mesenchymal cells in the facial skeleton and pharyngeal arch derivatives. The pharyngeal arches contain the aortic arch arteries. Neural crest cells form the walls of the aortic arch arteries (excluding endothelium), as well as the connective tissue of the thymus and the parathyroids and the calcitonin-producing cells of the thyroid gland. Kirby et al.\textsuperscript{22} have shown that cranial neural crest cells located over somites 1 through 3, i.e., arch 4 and 6 regions, migrate to the aorticopulmonary septum of quail-chick chimeric embryos. A more recent study using chimeras has demonstrated that only neural crest cells from the arch 3, 4, and 6 regions migrate to the heart. The population of the cells derived from the arch 4 region is fourfold that from the arch 3 or 6 regions.\textsuperscript{2}

In the conotruncal region of the early chick heart, two separate groups of mesenchymal cells can be recognized: one group, derived from the endocardium, populates the conus and proximal truncus at stages 12 through 19, and the other group migrates from the adjacent aortic arch region and appears in the distal truncal cushion tissue at stages 17 through 18 and rapidly spreads into the proximal truncus.\textsuperscript{23, 24} When aorticopulmonary partitioning begins the latter group of mesenchymal cells condenses to form a reversed Y shape in the truncal ridges. This complex advances upstream to the heart with fusion between two truncal ridges.\textsuperscript{25} Thus, unlike other cushion tissues, only truncal cushion mesenchyme performs aorticopulmonary and truncal septation against the direction of blood flow.\textsuperscript{26} A similar pattern of mesenchymal cell kinetics

![Image of cardiovascular anatomy](http://circ.ahajournals.org/)

**FIGURE 3.** Inflow tract anomalies (transverse sections). A, Atrial view of a normal avian embryonic heart. B, Atrial view of a heart with tricuspid atresia and PTA. Only a shallow dimple is found on the floor of the right atrium. Only one vessel (PTA) comes off from the heart. C, Atrial view of a heart with double-inlet left ventricle and DORV. The tricuspid valve is composed of two leaflets like the mitral valve. The tricuspid valve deviates to the left, and the mitral valve is relatively small. The aorta is dextroposed. D, Ventricular view of the same specimen as shown in C. Dotted line shows the ventricular septum. Both the tricuspid valve and the mitral valve drain into the left ventricle, and the two great arteries arise from the right ventricle. TV = tricuspid valve; MV = mitral valve; Ao = aorta; P = pulmonary trunk; RV = right ventricle; LV = left ventricle.
in the early embryonic conotruncal region has been found in rat and human embryos.\textsuperscript{27, 28} The location and the appearance of mesenchymal cells of extracardiac origin are consistent with those of mesenchymal cells derived from neural crest.

Pathogenesis of PTA. According to Lev and Sapher,\textsuperscript{29} PTA is defined as the presence of only one large trunk, emanating from the heart, which gives off the coronary, pulmonary, and systemic arteries.

PTA is uncommon among congenital heart diseases;

\begin{figure}[h]
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\includegraphics[width=\textwidth]{aortic_arches.png}
\caption{Anomalies of the aortic arches. A, Normal aortic arch system of incubation day 11 embryo, frontal view. Bilateral brachiocephalic arteries derived from 3AA branch into ipsilateral common carotid arteries and subclavian arteries. R4AA develops into the definitive (right-sided) aortic arch. The R6AA derivatives are behind the aortic root. B, Absence of the R3AA. Note right common carotid arises from R4AA (arrow) and bilateral subclavian arteries arise from the aortic root. These share their proximal parts, forming a peculiar T shape. C, Normal dorsal aortic arch system. The heart was turned to the cephalad. Bilateral 6AAs arise from the pulmonary trunk and are confluent with the dorsal aorta through a thick bilateral ductus arteriosus. They also give rise to thin pulmonary artery branches. Only the R4AA connects the ascending aorta to the descending aorta. D, Bilateral hypoplasia of the 6AAs with PTA. Compared with C, the bilateral 6AAs are hypoplastic and supply only the lungs. Both 6AAs are absent. Bilateral 4AAs are persistent to form double aortic arches. PA = pulmonary artery branch; DA = ductus arteriosus.}
\end{figure}
however, it has drawn special attention of many investigators because it seemed to be explained by a failure of the septation in the embryonic truncus arteriosus. Although there are several minor differences, this concept has been generally accepted.

Van Praagh and Van Praagh proposed a different hypothesis: that “truncus arteriosus communis with VSD” is tetralogy of Fallot with pulmonary atresia and partial or complete absence of the aorticopulmonary septum. They based their hypothesis on two main facts: the similarity of morphology of the right ventricular outflow tract in PTA and tetralogy of Fallot with pulmonary atresia, and the high incidence of tricuspid truncal valve in specimens with PTA.

A great deal of controversy has arisen between these two opposing hypotheses concerning the pathogenesis of PTA. Thiene et al. and Crupi et al. pointed out the absence of the infundibular septum in specimens with PTA as compared with those with tetralogy of Fallot with pulmonary atresia, in which a remnant of the infundibular septum was found. Crupi et al. described variability in the number of truncal valve cusps, frequently associated unevently divided cusps or raphes of those cusps, and a wider variety of the locations of coronary artery ostia in PTA compared with tetralogy of Fallot with pulmonary atresia. From the embryologic point of view, morphologic findings in a human embryo with PTA, experience in the pig embryo, and findings concerning PTA in the Keeshund dog strain support the hypothesis that PTA is due to the failure of fusion of the truncoconal ridges.

We have shown in previous studies, as well as this study, that relatively large-sized neural crest ablations from the arch 3-6 regions induce a remarkably high incidence of PTA. Our findings suggest that the most likely pathogenetic mechanism of PTA in the chick embryo is a deficiency of mesenchymal cells derived from neural crest over the arch 3-6 regions, which leaves the embryonic truncus arteriosus undivided. We have not found any evidence to support the hypothesis that PTA is a part of the spectrum of tetralogy of Fallot with pulmonary atresia.

Frequent association of aortic arch anomalies with PTA has been described in the reports of PTA in humans. Van Praagh and Van Praagh described interruption or coarctation of the aortic arch (L4AA in humans) and absent ductus arteriosus (distal part of 6AA) with PTA. This classification of PTA is mainly derived from these findings, i.e., Van Praagh’s type 1 and 2 may be considered PTA with bilateral hypoplasia of the 6AA, his type 3 may be PTA with unilateral absence of the 6AA, and his type 4 could be PTA with hypoplasia or absence of the L4AA.

Rothko et al. also found either absent ductus arteriosus or interruption of the aortic arch in 15 of 19 cases with PTA. They proposed that these aortic arch anomalies were a secondary effect of fusion of the two ventricular outflow blood streams at the semilunar valve level, which they stressed as the pathogenesis of PTA. They criticized the two previously proposed hypotheses on the grounds that both require two separate primary events to explain the PTA and associated aortic arch anomalies. However, they did not clarify the primary cause of altered hemodynamics. We agree with this assessment of the importance of hemodynamics in cardiovascular morphogenesis in the embryo and propose that removal of neural crest causes altered hemodynamic variables in the aortic arches.

Van Praagh’s classification helps to clarify our results. We believe that the aortic arch anomalies associated with PTA observed in this study are a part of systemic anomalies due to the absence of mesenchymal cells derived from cranial neural crest. When neural crest cells that should populate pharyngeal arches 3 and 4 are mainly affected, the resulting PTA is associated with 3AA or R4AA anomalies, whereas when neural crest cells that should migrate from the arch 6 region and the caudal half of the arch 4 region are affected, PTA with 6AA and/or R4AA anomalies occur. If neural crest cells over the arch 3-6 regions are evenly affected, associated aortic arch anomalies are variable, or embryos with PTA may have relatively normal aortic arches.

Pathogenesis of DPA. Because of the difficulty in identification of short conal muscle tissue by gross examination in the day 11 chick embryonic heart, we included both DORV with bilateral conus and VSD with overriding aorta in the entity of DPA.

Many studies of cardiovascular teratology in the chick embryo have produced a high incidence of DPA by mechanical manipulation of the outflow tract. These direct manipulations result in a spectrum of DPA similar to that caused by removal of the neural crest. Although our previous study showed that small ablations of the neural crest from the arch 4-6 regions resulted in a high incidence of DORV, we cannot attribute the pathogenesis of DPA in our experimental series to decreased population of neural crest derivatives in the conotruncal region. DPA was found in all ablation groups. Because the quail-chick chimera study has shown that neural crest cells from the arch 1-2 regions do not migrate into the heart, DPA produced by ablation of these regions could only be related to altered hemodynamics induced by abnormal formation
of the corresponding aortic arch arteries rather than a direct effect on the heart.

Thus, we propose that the pathogenesis of DPA in our neural crest ablation study in the chick embryo is due to altered hemodynamics in the embryonic circulation caused by a disrupted sequence of cytodifferentiation of neural crest cell derivatives, or decreased cell populations in the pharyngeal arches affected.

Pathogenesis of other anomalies. Inflow tract anomalies noted in the present series, which include tricuspid atresia, tricuspid stenosis, straddling of the tricuspid valve, and double-inlet left ventricle, were found in most of the ablation groups. The distribution pattern of inflow tract anomalies was similar to that of DPA. These malformations were always associated with conotruncal anomalies, i.e., PTA or DPA and/or interruption of R4AA.

In teratologic studies using early chick embryos, a spectrum of inflow tract anomalies similar to those reported here have been induced by mechanical constriction of the outflow tract, wires or rings placed at the bottom of the atrioventricular sulcus or flange, and electrical shock to the conotruncal region. In these experiments it is possible that the blood flow patterns downstream from the atriocarotid canal were changed, interfering with the normal rightward shift of the atriocarotid canal.

Because inflow tract anomalies were not produced reliably in this series, we cannot propose a mechanism for the pathogenesis of these anomalies. However, considering the fact that our chimeric study did not reveal any migration of neural crest cells into the atrial wall or atriocarotid cushion tissue, inflow tract anomalies can be assumed to be induced by indirect mechanisms related to the neural crest ablation, most likely through altered hemodynamics in the conotruncal or aortic arch regions in the early stages of development.

Pathogenesis of the major anomalies of the aortic arch arteries, especially interruption or coarctation of the aortic arch, has been explained previously by changes in embryonic hemodynamics, which are mainly induced by abnormal intracardiac structure. The low-flow hypothesis of Van Praagh et al. has been supported by clinicopathologic studies and animal experiments. On the other hand, Van Mierop and Kutsche proposed a different pathogenesis of type B interruption from type A or coarctation of the aorta; type B interruption is considered to be related to disturbance of neural crest–derived mesenchymal cells. Bockman and Kirby have produced Di-George-like morphologic features in chick embryos by removing the neural crest. In the present study, the incidence of the 3AA or R4AA anomalies in the arch 4 group was higher than that in other groups (table 1). Moreover, 6AA anomalies were induced by large ablations that included the arch 6 region. The high incidence of 3AA anomalies in the arch 4 and arch 4-6 groups could be explained by scanning electron microscopic findings in another study, in which both the third and the fourth pharyngeal arches were hypoplastic in many of the incubation day 3 chick embryos after arch 4-6 ablation surgery. These findings support the existence of overlapping populations of neural crest cells in the pharyngeal arch areas and more complicated interaction between the contribution of neural crest cells to the arch regions and hemodynamic effects in the formation of aortic arch arteries.

In summary, we propose different pathogenetic mechanisms for different categories of cardiovascular anomalies. We attribute the pathogenesis of PTA to the decreased population of mesenchymal cells derived from the neural crest over the arch 3-6 regions. DPA is mainly due to the altered hemodynamics in the aortic arch arteries, induced by neural crest ablation in this study. Inflow tract anomalies may be induced by hemodynamic changes that occur with the abnormalities in the outflow tract or the aortic arch arteries. Major anomalies of the aortic arch arteries could be induced by either a decreased population of mesenchymal cells derived from specific neural crest regions or secondary changes in the embryonic circulation induced by structural abnormalities after neural crest ablation.

Many problems remain unsolved. Various cardiovascular malformations have been induced by microsurgery. Such invasive disturbance never occurs naturally in embryos in ovo or in utero. The factors and the processes that affect normal migration and differentiation of the neural crest cells must be clarified.

Although many cardiovascular malformations found in the chick embryo in the present study were similar to those in humans, there are several differences between these two species. For example, tetralogy of Fallot and transposition of the great arteries are common conotruncal anomalies and are frequently found in humans with DiGeorge syndrome, while we observed only a few embryos with those anomalies in neural crest ablation studies. Conversely, DPA or PTA with inflow tract anomalies was frequent in the present study, while such combinations are extremely rare in humans.

Further investigation of these problems surrounding neural crest cells and cardiovascular embryogenesis
may lead us closer to a better understanding of cardiovascular morphogenesis and hopefully to the prevention of congenital cardiovascular malformations.

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