Cells from the cardiac neural crest are essential for the normal development of both the heart and the great vessels. If cardiac neural crest is ablated surgically from Hamburger-Hamilton stage 9 chicken embryos, they will develop anomalies of both the heart and great vessels that are similar to anomalies that occur in humans. In the absence of cardiac neural crest, another area of neural ectoderm (nodose placode) provides replacement cells that are less competent than those of the neural crest. In this study, both the cardiac neural crest and the nodose placodes have been surgically ablated. A syndrome of unusual prevalence (47%) and severity was found among the survivors of this surgery, which was characterized by a large undivided aorta that arched dorsally without right or left deviation to become the dorsal aorta. There was no other tributary to the formation of the dorsal aorta. There were no ducti arteriosi, and the pulmonary arteries were both ectopic and hypoplastic. The brachiocephalic arteries were asymmetric and hypoplastic. The association of the aorta with the anlagen of the thyroid and thymus glands, as well as with the inferior ganglion of the vagus nerve, indicated that the solitary surviving aortic arch arch is that of arch III in this syndrome. These results establish a biological limit of the plasticity of the neural ectoderm and give a probable cellular basis for a lethal congenital septal defect. (Circulation 1989;80:1469–1475)

Cells from the cardiac neural crest migrate into the developing heart and great vessels, where they differentiate into ectomesenchymal cells. These ectomesenchymal cells of neural crest origin are critical to the normal development of the heart and great vessels. If the cardiac neural crest is ablated surgically from early chicken embryos, the hearts of 85% or more embryos will develop conotruncal anomalies that are similar to defects observed commonly in the clinic, particularly variations of persistent truncus arteriosus. Furthermore, the great vessels in the embryos with conotruncal defects have an associated elastin disorder similar in many respects to that of humans born with cyanotic congenital heart disease. Therefore, this experimental model has obvious relevance to human congenital heart malformation. The relation between this model and human malformation has been discussed in detail previously.

In the absence of cardiac neural crest, ectomesenchyme is provided to the heart and great vessels by the nodose placode. The nodose placode serves normally to provide sensory innervation to the heart via the distal or inferior vagal ganglion but normally provides no mesenchymal phenotypes. The ability of the nodose placode to provide surrogate ectomesenchyme in the absence of cardiac neural crest has been described as an example of the “remarkable plasticity of the neural ectoderm.” It is also an example of the close and interdependent relations among the placodes of the neural ectoderm and the neural crest. It may be inferred that a given phenotype may be expressed by contiguous regions in the neural ectoderm but may be expressed perfectly only by its normal progenitor. A hierarchy of competence to support great vessel development, therefore, may be hypothesized, in the order of cardiac neural crest > nodose placode > other neural ectoderm.

The results of the present study supported this hypothesis. A lethal syndrome of high prevalence (47%) was found among embryos that underwent ablation of both cardiac neural crest and bilateral nodose placodes. The principal feature of this syn-

From The Heart Development Group, Department of Anatomy, Medical College of Georgia, Augusta (T.H.R., M.L.K.), and the Department of Pediatrics, J. Hillis Miller Health Center, University of Florida College of Medicine, Gainesville, Florida (L.H.S.v.M.).

Supported by National Institutes of Health grants HL-36059 (T.H.R., M.L.K., L.H.S.v.M.) and HL-42164 (T.H.R.) and by a research grant from the American Heart Association, Georgia Affiliate (T.H.R.). M.L.K. is an Established Investigator of the American Heart Association.

Address for correspondence: Thomas H. Rosenquist, PhD, Professor of Anatomy, Medical College of Georgia, Augusta, GA 30912-2000.

Received March 30, 1989; revision accepted June 20, 1989.
drome is survival of only one great vessel that appears to be derived from a solitary aortic arch artery.

Methods

Fertilized Arbor Acre chicken eggs (Seaboard Hatchery, Athens, Georgia) were incubated in a forced-draft incubator at 99% humidity and 38° C. Cardiac neural crest and nodose placode were ablated surgically from 132 chicken embryos at Hamburger-Hamilton stages 8–10 with a microcautery unit designed and built by the Department of Biomedical Engineering at the Medical College of Georgia. For the neural crest ablation, the two neural folds were thermally coagulated for the entire length of the cardiac neural crest, from midotic placode to the caudal limit of somite 3. For the nodose placode ablation in the same embryo, the ectoderm was thermally coagulated on both sides, dorsal and lateral, to somites 1 and 2. Sham-operated embryos were stained with neutral red, and the vitelline membrane was torn as if in preparation for ablative surgery. Sham and experimental embryos were returned to an incubator maintained at 99% humidity and 38° C for 24 hours and then were transferred to an incubator with 70% humidity at 37° C. Embryos were collected after 3.5, 4, 5, 6, 7, 8, 9, 10, and 11 days of incubation and placed in “methacarn.” Embryos that have undergone simultaneous ablation of the cardiac neural crest and the nodose placodes do not survive beyond approximately Hamburger-Hamilton stage 38 or incubation day 12. After they were embedded in paraffin, the embryos were sectioned at 10 μm, mounted serially on glass slides, and stained with Spicer’s13 method.

The outflow vessels of selected sham and experimental embryos were reconstructed from serial sections with the aid of the computer program PC3D (Multidimensional Computing, Durham, North Carolina).

Results

Twenty-two (16.6%) of the experimental embryos survived to harvest. Three were collected on each of days 3.5, 4, 5, 6, and 7; two were collected on each of days 8, 9, and 10; and one was collected on day 11. Thirty sham-operated embryos (68%) survived. Three were collected on each of days 3.5, 4,

![Figure 1](http://circ.ahajournals.org) Left: Schematic serial reconstruction of the lumina of the cardiac outflow region of a control 8-day embryo. Panel A: View from ventral to dorsal. Panel B: View from dorsal to ventral. Note the bilateral symmetry of the embryo. Right: Serial reconstruction of the lumina of the cardiac outflow region of an 8-day embryo that had undergone ablation of the cardiac neural crest and the nodose placodes. There is no development of the aortic arches on the left side. A single aortic arch that has been retained on the right as an undivided truncus arteriosus (TA) is the source of all of the arteries. Vessels are hypoplastic compared with the control vessels on the left. R, right; L, left; V, ventricle; PA, pulmonary artery; BR, brachiocephalic artery; SC, subclavian artery; CC, common carotid artery; Ao, aorta.

![Figure 2](http://circ.ahajournals.org) Left: Schematic serial reconstruction of the lumina of the cardiac outflow region from a control 4-day embryo. Panel A: View from dorsal to ventral. Panel B: View from ventral to dorsal. Note the excellent bilateral symmetry of the vessels. Right: Serial reconstruction of the lumina of the cardiac outflow region from a 4-day embryo that had undergone ablation of the cardiac neural crest and the nodose placodes. Only aortic arch 3 has developed bilaterally; a right arch 4 is atretic and apparently regressing. Right arch 3 is in a direct line with the truncus arteriosus (see B) and is larger than the left arch 3 so that there is essentially no bilateral symmetry. R, right; L, left; V, ventricle; T, truncus; S, aortic sac; 2, 3, and 4, aortic arches; DAo, dorsal aorta; PA, pulmonary artery.
FIGURE 3.  Panel A: Photomicrograph of a section from a control 8-day embryo. Derivatives of the third and sixth aortic arches are bilaterally symmetric. They are approximately equal in luminal diameter and medial thickness with aortic arch artery 4. Panel B: Photomicrograph of a section from an experimental 8-day embryo. The large persistent truncus arteriosus (T) is the only retained arch artery. The pulmonary arteries (arrows) arise independently from the truncus. There is no contralateral development of any vessels except a small pulmonary artery. The large, flaccid, and thin-walled esophagus and bronchi are typical of the experimental embryos. B, bronchi; E, esophagus; L, lung; DA, dorsal aortae. Original magnification, ×120.

5, 6, 7, and 8; four were collected on each of days 9, 10, and 11.

Ten of the experimental embryos showed the same cardiovascular anomaly. In these embryos, a large undivided outflow vessel arched dorsocaudad without right or left deviation and became the dorsal aorta with no other tributaries. There was no ductus arteriosus or typical symmetric brachiocephalic arteries. All of the pulmonary arteries arose ectopically. The pulmonary arteries and the brachiocephalic arteries were severely hypoplastic. This configuration gave the embryos a peculiar asymmetry. All of the outflow vessels arose on one side of the embryo, rose dorsally on one side of the esophagus, and then branched right or left (Figures 1–4). Seven of the embryos showed exclusively left development and three exclusively right.

The ductus arteriosus that arises normally from aortic arch 6 and the paired symmetrical brachiocephalic arteries that arise normally from aortic arch 3 were absent. Based on the absence of these vessels, we assumed initially that the retained large single vessel was an example of “persistent left fourth aortic arch.” However, several of our data indicated that the arch that is retained in the present study is aortic arch 3. Both individual cross-sections and three-dimensional reconstructions show that the retained arch passes among the anlagen of the parathyroids and the thymus in a relation that is distinctly characteristic of the third arch artery (Figures 4 and 5). Furthermore, the origins of the subclavian arteries in the experimental embryos are not anomalous but are typical if their parent vessel is aortic arch artery 3. This site of origination of the subclavian arteries may be taken as another criterion for confirming the presence of the third arch artery.

That the retained arch was indeed arch 3 was supported further with three-dimensional reconstruction of the vessels of a 4-day experimental embryo.
with the following characteristics. The third arches were retained bilaterally, but the right one was larger and directly in line with the truncus arteriosus. There were no second arches, no left fourth arch, and the right fourth arch was regressing. Finally, the truncus arteriosus was straight compared with age-matched controls (Figure 2). The ultimate vessel pattern that can be extrapolated from the configuration of the vessels of this 4-day embryo is found in the vessel pattern of the 8-day experimental embryo shown reconstructed in Figure 1.

Because of the apparent absence of all of the typical aortic arch arteries in these embryos except arch 3, the syndrome has been designated “solitary aortic arch artery” (SA). Embryos with the SA syndrome also showed changes in the development of the inferior ganglion of the vagus. The “sidedness” of the aortic arch development of a given embryo with SA was related to the presence of the inferior vagal ganglion. Wide variations in the size, shape, location, apparent cell density, and identifiable boundaries were common characteristics of the inferior vagal ganglion in the experimental embryos. The last of these variables, nondiscrete boundaries, was remarkable. For some experimental embryos, there was no apparent cranial boundary of the vagal ganglion, which was continuous with the petrosal ganglion of the glossopharyngeal nerve (nerve IX) (Figures 5 and 6). An alternative description that also suits these data is that the petrosal ganglion of nerve IX was greatly attenuated caudally in the experimental embryos, and no inferior ganglion of the vagus was truly present. With either of these interpretations, the general visceral afferent ganglia of nerves IX and X were essentially continuous.

Embryos collected on day 3.5 of incubation were found to be too immature to yield any reliable information on the effects of the surgical ablation of neural crest-nodose placode as it may relate to the definitive development of the aortic arch derivatives. Therefore, they are not included in the tally of experimental embryos, which consequently becomes

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Panel A: Photomicrograph of a section from an experimental 4-day embryo. Only one large outflow vessel has developed, and it passes dorsad to become the dorsal aorta with no other tributaries (arrow). The greatly diminished pulmonary arteries (PA) arise as described above. Original magnification, ×120. Panel B: Photomicrograph of a section just cranial to the previous section (photographed at higher magnification) shows that the retained arch has a relation with the anlagen of the thymus (TH), the thyroid III (TR), and the inferior ganglion of the vagus (IGV) that is typical of the third aortic arch. S, subclavian artery. Original magnification, ×360.
FIGURE 5. Panel A: In this photomicrograph from an experimental 8-day embryo, the paired cranial branches of the third aortic arch artery (*), the only arch found in this embryo, are parallel to the attenuated ganglion of nerve IX (G) and bear the typical relation to the anlagen of the thyroid and thymus glands (arrows). Original magnification, ×120. Panel B: Higher magnification of the same section, using the same abbreviations. The ganglion can be seen to turn dorsad (arrow), where it was found on subsequent serial sections to join the main body of ganglion IX. Original magnification, ×360. T, trachea; E, esophagus; S, spinal cord.

FIGURE 6. Photomicrograph from experimental 8-day embryo in which the vagus nerve (*) has been found to contain neurons throughout its length. In this section from the neck region the neurons are abundant, giving the appearance of an ectopic ganglion between the jugular vein (upper left) and the carotid artery (right) at the level of the larynx. Subsequent sections taken cranial indicate that this ganglion is a posterior attenuation of the ganglion of nerve IX. Original magnification, ×720.
19. The prevalence of the SA syndrome, therefore, becomes 47% in embryos from which both cardiac neural crest and nodose placode have been ablated bilaterally.

Of the experimental embryos that did not manifest the syndrome, two appeared to have normal hearts and great vessels (5 and 6 days old); one had a small ventricular septal defect (8 days old); and seven had persistent truncus arteriosus, with hypoplastic pulmonary arteries and bilateral ductus arteriosus. This distribution of anomalies is consistent with those found among embryos from which neural crest alone has been ablated and may have resulted from inadequate surgical ablation of the nodose placodes.

Discussion

Optimum treatment or prevention of congenital heart defects is contingent upon a thorough understanding of the biological bases of their etiology. Previous experimental studies have indicated that the pathogenetic basis of truncal septal defects is at the cellular level. If ectomesenchymal cells from the cardiac neural crest are absent from the developing truncus, they are replaced by cells originating in the nodose placode. The substitute ectomesenchymal cells cannot produce an aorticopulmonary septum. Ectomesenchymal cells from the same origin produce outflow vessels that are structurally abnormal but adequate to sustain life to the end of gestation. When neither cardiac neural crest cells nor nodose placode cells are available as in the present study, outflow vessel development is severely retarded and is inadequate to carry the embryo beyond the first half of gestation in ovo. Therefore, this study establishes a biological limit of the plasticity of the neural ectoderm and gives a probable cellular basis for a lethal congenital septal defect. The syndrome we have called SA apparently killed most of our experimental embryos before they completed half their gestation. SA does not appear to have a clear equivalent in the clinical literature, as might be expected from the early death of afflicted embryos. Variations in development of the cardiovascular system between avian and mammalian species may, of course, contribute to differences in the expression of a given syndrome without negating the value of a given model. Details of many kinds of interspecific variations in heart development have been given elsewhere.

It is not clear why the third arch artery should survive and predominate rather than the fourth or the sixth after simultaneous ablation of the cardiac neural crest and the nodose placode. When the cardiac neural crest is ablated without simultaneous ablation of the nodose placodes, anomalies of the third and the right fourth arch artery have an identical rate of occurrence (45.5%) and the sixth is anomalous 63.6% of the time. In another study of the effect of neural crest ablation on aortic arch development, Bockman et al found that the diameters of arches 3, 4, and 6 were all reduced significantly. However, the diameter of arch artery 6 was reduced earlier and to a greater degree than that of 3 or 4. Thus, the sixth arch artery appears to depend more on neural crest ectomesenchyme for support of development than does arch 3 or arch 4. But this difference would not help to explain the present results, where both the fourth and the sixth arch artery apparently are undeveloped.

The ductus arteriosus represents not just the dorsal part but all of the sixth arch. Therefore, the complete, bilateral absence of ductus arteriosus indicates the complete absence of the sixth arch arteries. In the present study, bilateral pulmonary arteries arose from the aorta despite the complete absence of the sixth arch. This is consistent with the datum that the pulmonary artery arises as a caudal branch of the aortic sac before the sixth arch is present. Anomalous origin of the pulmonary arteries from the aorta such as that described here is extremely rare in human fetuses surviving to term. Anomalous origin of the left pulmonary artery is thought to be related specifically to aortic arch development including absent arch 6 and has been found to occur twice in 7,329 patients with congenital heart disease. However, none of the three different and unrelated varieties of anomalous origin of a pulmonary artery is believed to be a form of persistent truncus arteriosus.

The common origin of the inferior ganglion of the vagus and the "substitute" cardiac ectomesenchyme from the nodose placode has been established previously. Therefore, the change in the inferior vagal ganglion reported here is a logical correlate of the SA syndrome. If the inferior ganglion of the vagus is absent, an elongated petrosal ganglion of nerve IX may provide compensatory general visceral sensory neurons for innervation of the heart. Indeed, there are many similarities between the inferior vagal and the petrosal ganglia that support the likelihood that such compensation may occur. Despite this putative compensatory innervation, simultaneous ablation of cardiac neural crest and nodose placode causes the hearts to be deficient in both sympathetic and parasympathetic innervations, which could itself be a fatal defect.

References


**KEY WORDS** • aorta • arteries • cardiac neural crest
Solitary aortic arch artery. A result of surgical ablation of cardiac neural crest and nodose placode in the avian embryo.
T H Rosenquist, M L Kirby and L H van Mierop

Circulation. 1989;80:1469-1475
doi: 10.1161/01.CIR.80.5.1469

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/80/5/1469