cate as in (8). The medium contained 0 or 2 mg of glucose per milliliter and various insulin concentrations (0 to 2500 μU/ml), but no isoprenaline. Insulin responsiveness was calculated as the percentage inhibition of basal glycerol release at the maximum effective concentration of the hormone. The results of 15 experiments (mean ± standard error) showed that insulin responsiveness was 40.3 ± 3.2 percent without and 56.1 ± 5.7 percent with glucose present ($P < .01$, paired t-test).
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Neural Crest Cells Contribute to Normal Aorticopulmonary Septation

Abstract. *By analyzing the hearts of quail-chick chimeras, it was found that neural crest cells at the level of occipital somites 1 to 3 migrate to the region of the aorticopulmonary septum. Bilateral removal of this neural crest population prior to migration causes malformation of the aorticopulmonary septum resulting in common arterial outflow channels or transposition of the great vessels.*

Transposition of the great vessels and other defects in the formation of the aorticopulmonary septum represent profound disturbances of the basic architecture of the heart. Aorticopulmonary septal defects are clinically referred to as conotruncal abnormalities and include transposition of the great vessels, overriding aorta, double-outlet right ventricle, and persistent truncus arteriosus (*1*). Transposition of the great vessels is the most common of these defects in humans and is found in 12 percent of neonates with congenital heart defects (*1*). The pathogenesis of transposition of the great vessels and related aorticopulmonary septal defects is unknown (*1*). The present study shows that depleting the heart of cells derived from occipital neural crest cells can result in aorticopulmonary septal defects.

We recently reported the removal of the parasympathetic postganglionic innervation to the heart (*2*). Cardiac parasympathetic postganglionic neurons arise from the occipital region of neural crest and migrate toward the heart while it is a simple tube. In that study, the neural crest population that seeds the cardiac ganglia was removed prior to its migration. In the present study, we demonstrate that defects in the aorticopulmonary septum result when the neural crest region containing presumptive cardiac ganglion cells is removed.

Fertile White Leghorn or Arbor Acre chicken eggs and Japanese quail eggs were incubated for 24 to 30 hours in a humidified atmosphere at 37.5°C. Both quail (donor) and chick (host) eggs were opened and prepared for microsurgery (*3*) at stage 9 of development (*4*). The procedure described by Narayanan and Narayanan (*5*) was followed for the interspecific transplantation of cranial neural crest between quail and chick embryos. The chick neural fold over somites 1 to 3 was excised bilaterally with a modified Wenger vibrating needle (*5*). The neural fold consists of the presumptive dorsal part of the neural tube, the neural crest, and some adjacent surface ectoderm (*6*). Two different experiments were performed using the chick embryos with bilaterally excised neural folds. In the first series, chick neural fold was replaced with homotypic quail neural fold. In the second series, the chick neural fold was removed with no futher manipulation of the embryo. After surgery, the eggs were sealed, returned to the incubator, and allowed to develop for an additional 4 to 14 days (total development of 5 to 15 days). Embryos that received sham operations were processed in parallel with each group of microsurgically manipulated embryos. Embryos were fixed by perfusion through the left ventricle with 10 percent neutral buffered Formalin. Appropriate portions of the embryos were embedded in paraffin and processed for serial light microscopy. Feulgen Rossenbeck staining was used for chimeras and hematoxylin and eosin staining for control embryos or embryos with extirpations. All of the embryos described in the results were grossly normal at the time of perfusion.

Thirteen quail-chick chimeras ranging in total incubation age from 6 to 9 days were examined. Clusters of quail cells could be found near the branchial arch arteries and truncus arteriosus of 6-day chick embryos. The developing truncal septum was composed of chick mesenchymal cells interspersed with quail mesenchymal cells (Fig. 1). By day 7 the truncal septation was complete in the chick embryo. Quail and chick mesenchymal cells could be recognized from days 7 to 9 in the aorticopulmonary septum and in the tunica media of the aorta and pulmonary trunk. Other clusters of quail cells, which were identified as developing cardiac ganglia, were segregated from the cells associated with the developing tunica media as reported previously (*2*).

Sixteen embryos with extirpation of

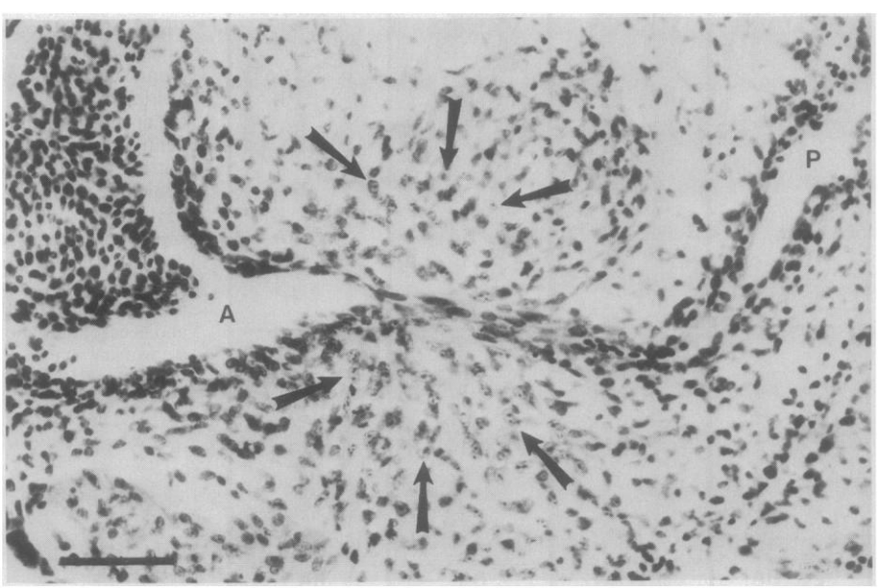

Fig. 1. Cross section of the truncal region during septation in a 6-day chick embryo with a bilateral quail neural fold transplant at the level of somites 1 and 2 at stage 10. Typical quail cells (arrows) can be seen in the region of the developing septum. *A*, aorta; *P*, pulmonary trunk. Scale bar, 50 μm.

neural folds over somites 1 to 3 were examined on incubation days 8 to 15. Six of these embryos were fixed by immersion rather than perfusion because they died before day 8. Only gross structural defects were examined histologically in these embryos. The hearts from the ten perfused embryos showed varying reductions in the number of cardiac ganglia. Fifteen of the 16 hearts had aorticopulmonary septal malformations. The most common septal defect (which was present in 14 of the hearts) was classified as persistent truncus arteriosus or common ventricular outflow channel (Fig. 2, panels 1 to 4). Transposition of the great vessels was present in one heart (Fig. 2, panels 5 to 8), and one heart was entirely normal by histological examination (Fig. 2, panels 9 to 12). The latter heart also showed a full complement of cardiac ganglia. It is thought that too small an amount of neural crest was removed or that the lesion was placed incorrectly in this embryo. The hearts with common outflow channels had other cardiac defects including ventricular septal defects in eight and a single-chambered ventricle in three (Fig. 2, panels 1 to 4). The heart with transposed vessels had both high and low ventricular septal defects (Fig. 2, panel 5) with a slight defect in the aorticopulmonary system (Fig. 2, panel 7).

Observation of whole hearts, before they were processed for histological examination of internal architecture, revealed grossly detectable differences between the control and experimentally manipulated groups. Hearts from control embryos demonstrated typical overall shape as well as the normal relative proportions contributed by the four heart chambers. The hearts, which subsequent histological examination revealed to possess common ventricular outflow channels, exhibited a blunting of the apex of the heart and some distortion of the size, shape, and position of the left atrium. The single heart with transposed great vessels exhibited an abnormally shaped ventricular region, that is, it was markedly elongated and bent so that the right margin was distinctly concave. None of the 12 control hearts examined had either external or internal malformations.

These results suggest several explanations for the pathogenesis of the truncal septum of embryos with neural crest extirpations. The first is that the cardiac ganglia induce septal formation. No direct evidence is available that would support such an induction. It is possible that surgical manipulation of the embryo might disturb other cell populations or cause abnormal circulation in the early embryo which could result secondarily in cardiac malformations. However, conotruncal malformations do not occur after removal of other areas of neural crest (*7*), nor do they occur in chimeric embryos. Thus, the most likely possibility is that the neural crest over somites 1 to 3 which provides cardiac ganglia to the heart also seeds the truncus arteriosus with mesectodermal cells which in some way are necessary for proper aorticopulmonary septum formation. These neural crest cells could play any of several roles in septal formation including the formation of an essential cell type or matrix component or the organization of mesodermal components. LeLievre and Le Douarin (*8*) have shown previously that the transplanted neural crest cells that migrate into the tunica media of the visceral arch arteries undergo smooth

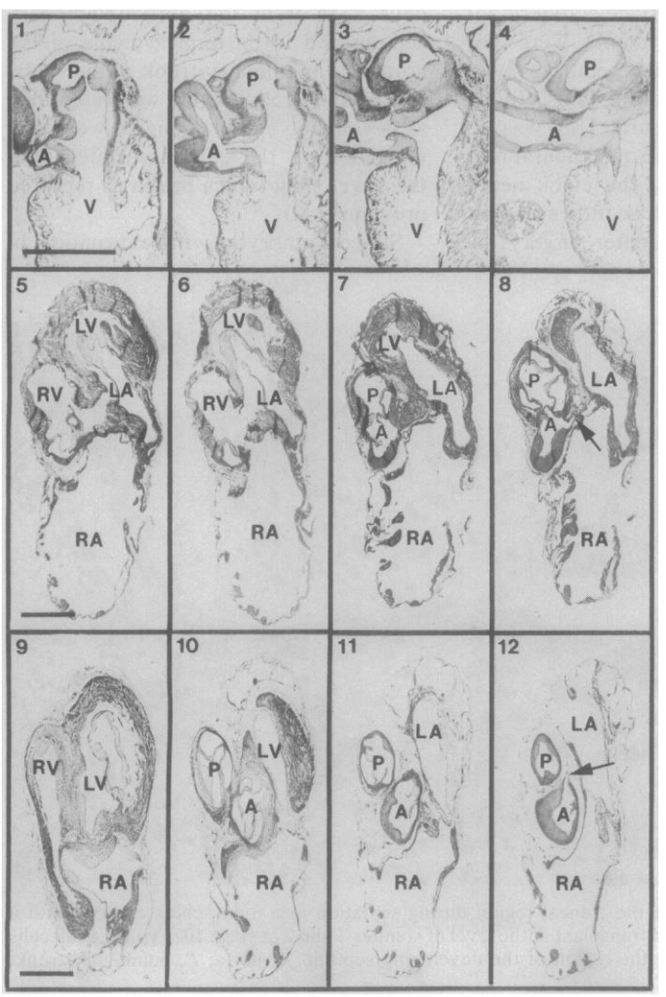

Fig. 2. Hearts from representative embryos with neural crest extirpations over occipital somites 1 to 3 at stage 9 or 10. Scale bar, 1 mm. Sequence 1 through 4 is from an 8-day (total incubation) embryo sectioned sagittally. The common outflow from the ventricle to both the aorta and pulmonary trunk can be seen best in panels 1 and 2. No ventricular septum was present in this particular heart, but this was not the case for most of the hearts with common outflow channels. Sequence 5 through 8 shows transverse sections of a heart from a 15-day embryo with transposition of the great vessels. A ventricular septal defect can be seen in panel 5 and a small aorticopulmonary defect is seen in panel 7. The arrow in panel 8 shows the origin of a coronary artery from the aorta. This heart is matched with the one below (sequence 9 through 12) which is from an embryo the same age and is essentially normal. The arrow in panel 12 indicates the origin of a coronary artery. *P*, pulmonary trunk; *A*, aorta; *V*, ventricle; *LV*, left ventricle; *RV*, right ventricle; *LA*, left atrium; *RA*, right atrium.

muscle differentiation and elaborate elastin fibrils. However, no active role in differentiation has previously been proposed for these cells. Our chick chimeras have a significant population of neural crest–derived quail cells located in the aorticopulmonary septum (Fig. 1).

Thompson and Fitzharris (*9*) have described in detail the normal development of the truncal septum. The mesenchyme in the truncus is derived from two distinct sources: endocardium and a population of cells which migrate caudally into the cardiac jelly from the aortic arch. Rychter (*10*) has also described the participation of the 6th aortic arch mesenchyme in the formation of the aorticopulmonary septum. We believe that this population of caudally migrating mesenchymal cells corresponds to the neural crest from the occipital region and provides the impetus for aorticopulmonary septum formation. With this view of truncal septation any teratogenic agent that affects occipital neural crest formation or migration could cause septal malformations. Agents already known to produce transposition of the great vessels are trypan blue, dextroamphetamine, x-rays, anoxia, and avitaminoses (*11*).

The finding that removal of occipital neural crest results in a large percentage (94 percent) of hearts with aorticopulmonary defects should play an important role in determining the pathogenesis of this and related defects in human embryos. One of the major drawbacks to studying this congenital malformation has been its low incidence in laboratory animals. Shaner (*12*) examined 20,000 pig embryos to find 48 abnormal hearts in the early stages of transposition. It is likely that as the microsurgical technique for neural crest extirpation is improved any of the aorticopulmonary septal defects can be produced by more precise manipulation of the area and number of cells removed.

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Nucleotide Sequence Analysis of the T24 Human Bladder Carcinoma Oncogene

Abstract. *The nucleotide sequence of the T24 human bladder carcinoma oncogene was determined, and the coding and noncoding sequences of the genome were identified. The amino acid sequence of p21, the translational product of the T24 oncogene, was predicted from the nucleotide sequence of the oncogene. Comparison of this sequence with that of the normal cellular homolog showed that a single point mutation in the coding sequences of the T24 oncogene resulted in the acquisition of transforming properties. Other differences between the T24 oncogene and its normal cellular homolog were found in the 5′ noncoding and 3′ noncoding sequences, but these differences appear to be due to polymorphism and do not play a significant role in the transformation process.*

The DNA's from certain naturally occurring human tumors can induce malignant transformation of NIH/3T3 cells (*1, 2*), a continuous mouse cell line that is contact-inhibited and is highly susceptible to DNA transfection. Although only about 10 percent of human tumor DNA's have been shown to be capable of transforming NIH/3T3 cells in transfection assays, oncogenes have been detected in tumors representative of each of the major forms of human cancer. An oncogene present in T24 and EJ bladder carcinoma cell lines was the first to be isolated by molecular cloning techniques (*3–5*). This bladder carcinoma oncogene is closely related to the *onc* genes of Harvey and BALB murine sarcoma viruses (MSV), termed v-*has* and v-*bas* (*6*), respectively. Use of cloned DNA's of bladder carcinoma oncogene as sequence probes showed that this oncogene is derived from a sequence of similar structure present in the normal human genome (*3–6*). Unlike the T24 bladder carcinoma oncogene, which exhibited a transforming activity of $> 10^4$ focus-forming units per microgram of DNA, its normal cellular analog did not exhibit transforming activity in transfection assays. It is thus clear that the bladder oncogene arose by the mutation of a normal cellular gene during the process of carcinogenesis. The mechanisms of activation of T24 and EJ oncogenes were studied by constructing several recombinants from parts of the T24 oncogene and its normal homolog (*7*). Analysis of such recombinants for transforming activity and a limited comparison of their sequences showed that the genetic change leading to the activation of this oncogene is a point mutation, with thymine replacing guanine. This substitution results in the incorporation of valine instead of glycine as the 12th amino acid residue of the T24 oncogene–encoded protein p21. These findings provided evidence for a molecular mechanism in carcinogenesis and demonstrated that small phenotypic changes in the structure of certain proteins can lead to dramatic changes in the biological activity of these molecules. Nucleotide sequence analysis of the T24 human bladder carcinoma oncogene was undertaken to elucidate the structural organization of these transforming genes and the primary structure of the proteins encoded by these genes. The sequence of the T24 oncogene (Fig. 1) was compared to the sequence of its normal human analog and those of two related retroviral *onc* genes, v-*bas* (*8*) and v-*has* (*9*). Molecular cloning and characterization of the T24 human bladder carcinoma oncogene has been described (*3*).

Heteroduplex analysis of the T24 oncogene and its normal allele with v-*bas* and v-*has* had indicated that the putative coding sequences of these human genes are distributed among four exons (*6*). The sequences of the entire coding region as well as flanking cellular sequences of the T24 oncogene were determined, and the four exons were identified by comparison of these sequences with those of v-*bas* and v-*has*. Within the nucleotide sequence, the following re-