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Role of extracardiac factors in heart development

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Summary. Many factors extrinsic to the developing heart play important roles in determining its final form. The neural crest has been shown to provide ectomesenchyme to the pharyngeal apparatus and outflow tract, as well as the postganglionic innervation of the heart. Ablation of the neural crest providing ectomesenchyme to the outflow tract results in various cardiac malformations. These malformations have in common either outflow and/or inflow tract malalignment. Although the reason for this malalignment is not understood, it is thought that hemodynamic parameters during early cardiac morphogenesis may be disrupted causing cardiac dysmorphogenesis. The most likely area for this alteration to occur is in the pharyngeal apparatus which houses the aortic arch arteries. Various possibilities are discussed. The innervation of the heart by neural crest-derived autonomic neurons and nodose placode-derived sensory neurons is outlined, and the interactions between the two progenitive sites is discussed.
Key words. Cardiac morphogenesis; neural crest; nodose placodes; chick embryos; heart malformations.

Much of the information and most of the cellular precursors needed for cardiac morphogenesis are contained in the primitive endothelial tube from which the structure of the fully formed heart is derived. However, many factors extrinsic to the developing heart play important roles in determining its final form. These factors include vascular resistance, seeding of the outflow tract with extracardially derived ectomesenchyme, and innervation. Circulating factors such as polypeptides and hormones which are derived from endothelium and a variety of other sources probably play some role in differentiation and growth of the heart although almost nothing is known about the influences of circulating factors on the early period of cardiac morphogenesis.

The neural crest has been shown to be of great importance in cardiac morphogenesis because of direct involvement of neural crest-derived ectomesenchyme in outflow tract septation, and also because the neural crest is important for maintenance of the aortic arch arteries which provide the major conduits for blood leaving the developing heart[4, 16]. The neural crest[24, 25] and nodose placodes[8, 48] provide the innervation of the heart and there is increasing evidence that innervation plays a role in maturation of signalling mechanisms in the myocardial membrane[9] and myocardial growth[7, 41]. Hence, the neural crest plays a multifaceted role in development of the heart (fig. 1).

Neural crest

The neural crest arises from the neural folds which develop from the lateralmost part of the neural plate[15, 30, 49]. As the neural plate closes to form the neural tube, neural crest cells are released from the neural folds[46]. The neural crest cells extend processes and actively migrate away from the vicinity of the neural folds. The neural crest is divided into two regions based on the potential for formation of ectomesenchyme[15, 30, 49]. Cranial neural crest (fig. 2) extends from the mid-diencephalon to the caudal limit of somite 5[34]. Neural crest cells derived from this cranial region have the prospective potency to differentiate into mesenchyme which has been called ectomesenchyme because of its unique origin from the ectoderm. Ectomesenchyme derived from cranial neural crest provides a variety of mesenchymal derivatives that are important in development of the face, pharyngeal appara-

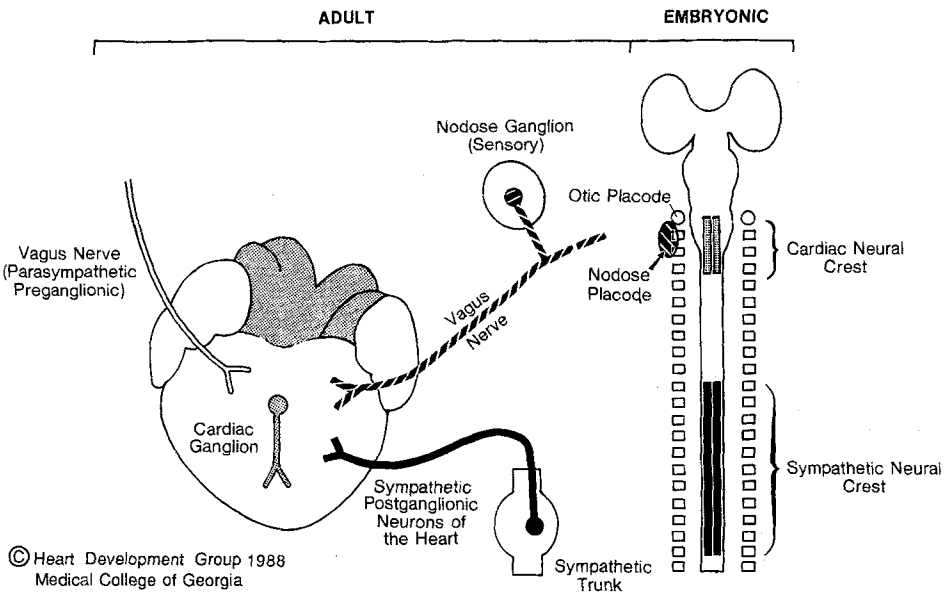

Figure 1. Diagram illustrating the embryonic origin of the various components of cardiac innervation as well as the outflow tract. The truncal septum and parasympathetic postganglionic neurons arise from cardiac neural crest located between the otic placode and the caudal limit of somite 3. Sympathetic postganglionic neurons are located in the first thoracic sympathetic ganglion. These neurons arise from the neural crest located between somites 10 and 20. The sensory innervation of the heart arises from the nodose placode which gives rise to the inferior ganglion of the vagus nerve.

tus, glands of the neck and the outflow region of the heart[16,30] (fig. 3). In addition to its ectomesenchymal potential the cranial neural crest differentiates into neurons and supporting cells of the peripheral nervous system and melanocytes[30].

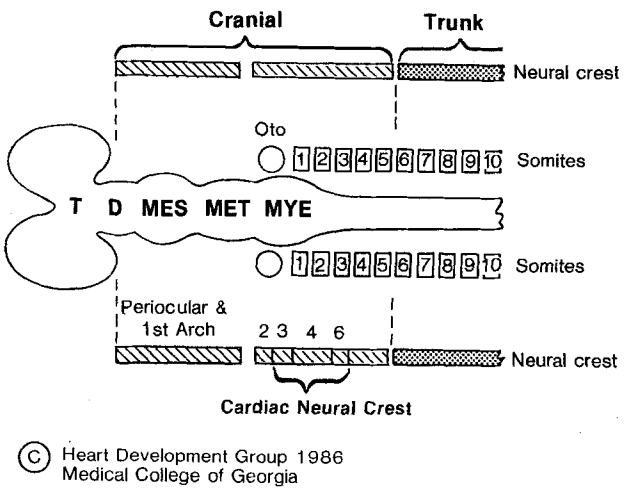

Figure 2. Diagram illustrating the location of various parts of the neural crest and the areas of neural crest that seed the pharyngeal arches. Cranial neural crest extends from the mid-diencephalon to the caudal part of somite 5. Trunk neural crest begins at somite 6 and extends to the caudal limit of the neural tube. The neural crest from the mid-diencephalon to the mid-metencephalon seeds ectomesenchyme to the periocular and 1st arch regions. A small gap in the neural crest is present in the mid- to caudal metencephalon[34]. The neural crest extending from the caudal metencephalon to the mid-otic placode seeds arch 2. Arch 3 is seeded by crest extending from the mid-otic placode to the rostral limit of somite 1. The neural crest adjacent to somites 1 and 2 seeds arch 4 and the area adjacent to somite 3 seeds arch 6. The region which seeds arches 3–6 is referred to as cardiac neural crest. T, telencephalon; D, diencephalon; MES, mesencephalon; MET, metencephalon; MYE, myelencephalon; Oto, otic placode.

Trunk neural crest (fig. 2) extends caudally from somite 5 and is distinguished by its inability to differentiate into mesenchyme[32]. The prospective potency of trunk neural crest includes neurons and supporting cells, adrenal medulla and melanocytes[15].

The cranial neural crest that participates directly in heart development[36] has been mapped using quail-chick chimeras[31]. This region of neural crest has been called cardiac neural crest and it extends from the level of the mid-otic placode to the caudal limit of somite 3[16,26]. The cardiac neural crest migrates from the neural fold into pharyngeal arches 3, 4 and 6 (fig. 3). In the pharyngeal arches the crest cells provide the support for the endothelium of the aortic arch arteries[29]. Some cells migrate from the pharyngeal arches into the outflow tract where they form the aorticopulmonary septum and populate the truncal folds[19]. Cells from this area also seed the cardiac ganglia (discussed below). The ectomesenchymal cells form very distinctive whorls in the truncal folds and these whorls seem to be active in closure of the truncal septum[19,36].

Neural crest ablation

Removal of the cardiac neural crest before it migrates, by extirpation or ablation of the neural folds, results in persistent truncus arteriosus, a condition in which only a single arterial vessel arises from the heart[33]. This single vessel usually emanates from the right ventricle or straddles the ventricular septum[33]. A very few cases have been noted where the vessel arises from the left ventricle[26]. The occurrence of persistent truncus arteriosus after cardiac neural crest ablation is not surprising, since it could

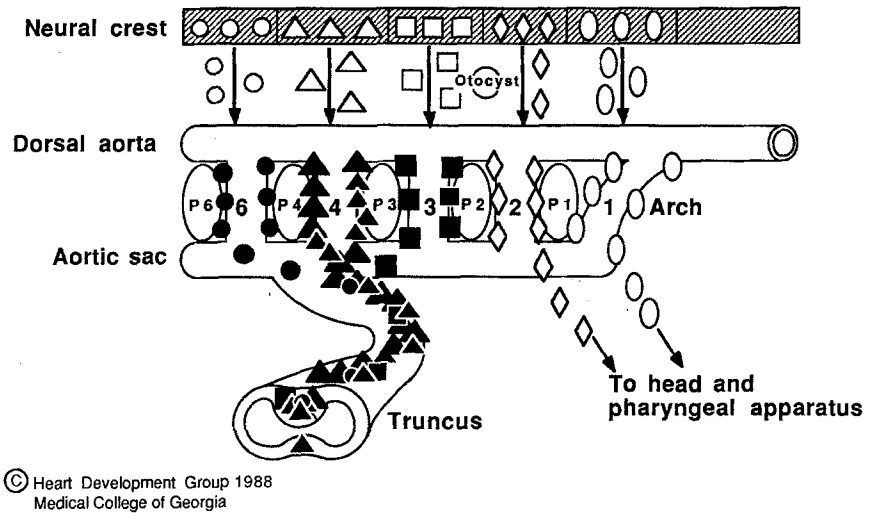

Figure 3. Diagram illustrating the neural crest seeding pharyngeal arches 1–6. Ectomesenchyme provides the support for the aortic arch arteries that traverse the pharyngeal arches. Neural crest from arches 3–6 migrates into the outflow region of the heart. This ectomesenchyme reacts with the neural crest antibody EC-8 while pharyngeal arches 3–6 are present. Ectomesenchyme in arches 1 and 2 does not react with EC-8 antibody. Aortic arch arteries 1 and 2 disappear early in development while arch arteries 3–6 persist (except for the left 4th) as permanent arteries of the thorax. Arch 4 provides the greatest quantity of cells to the outflow septa [36]. In the chick heart, most of the neural crest cells are found in the aorticopulmonary and truncal septa. The dorsal truncal cushion is the most active in septation and the greatest number of neural crest derived cells is found there. Pharyngeal pouches 1–6 are designated P1–P6.

be predicted that the neural crest-derived whorls in the truncal folds would be active in the process of truncal septation. Absence of these cells results in the inability of the truncal septum to close. The more puzzling result is the unpredictable origin of the single vessel.

Removal of a very small area of the cardiac region or cranial neural crest outside of the cardiac region does not result in PTA, but rather in a spectrum of heart defects which have all been classified together as dextroposed aorta (DPA) [33]. Subtypes of this malformation include double outlet right ventricle, tetralogy of Fallot and Eisenmenger's complex [33]. The variations of this malformation have some common features with the variations in PTA even though the DPA type malformations have two completely divided vessels originating from the heart.

Neural crest ablation has also been shown to cause changes in atrioventricular alignment. Several inflow tract anomalies have been noted including tricuspid atresia, tricuspid stenosis, straddling of the tricuspid valve with or without tricuspid atresia and double-inlet left ventricle [33]. A few atrioventricular canals have been found following neural crest ablation [33]. All of the inflow tract anomalies occur with either PTA or DPA although a single case of anomalous inflow tract was found in a heart with infundibular VSD with right fourth aortic arch hypoplasia [33].

A detailed study of the systemic veins has not been done; however, a recent study of pulmonary vein development using plastic casts of the cardiovascular system showed that the pulmonary and systemic veins were normal in hearts with PTA following cardiac neural crest ablation [38]. Evidently the absence of cranial neural crest does not influence development of the great veins.

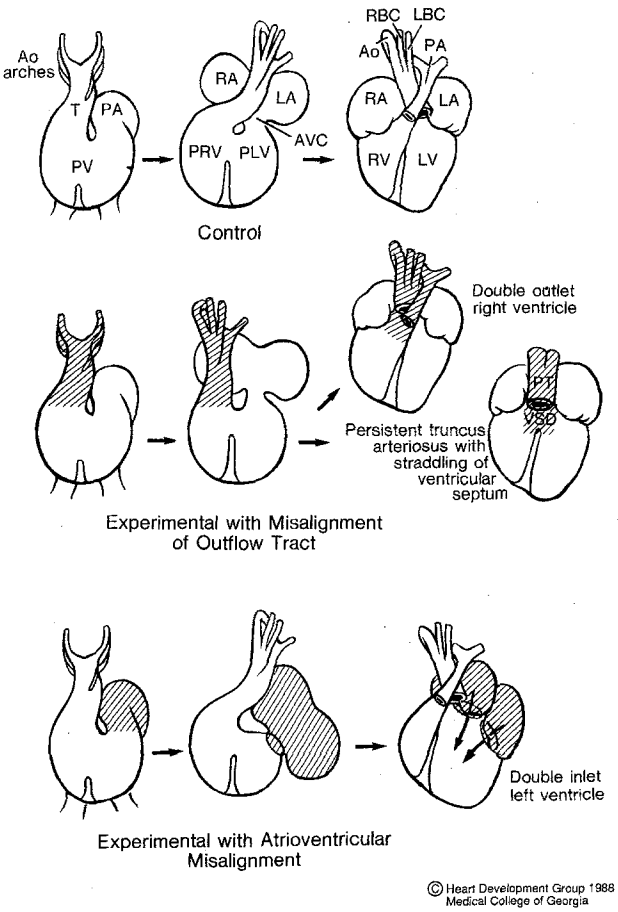

Figure 4. Malalignment of the inflow and outflow portions of the heart are common in neural crest related heart defects. Unlooping of the cardiac tube in the looped tube stage is thought to result in the outflow vessel (PTA) or vessels (DPA including double outlet right ventricle) originating from the right ventricle while it could also account for atrioventricular malalignments such as double inlet left ventricle.

Although the position of the outflow vessel or vessels in PTA and DPA is unpredictable, the most notable common feature is the malalignment of the outflow tract (fig. 4). This is also the common feature in the spectrum of inflow tract anomalies that have been described (fig. 4). As the malalignment can be produced by removal of cardiac or noncardiac cranial neural crest, it seems reasonable to hypothesize that the cardiac neural crest found in the aorticopulmonary and truncal septa plays little role in the alignment of inflow and outflow vessels in the heart.

Aortic arch arteries

Although only a limited extent of the cranial neural crest participates directly in heart development [16], cells from all levels of the cranial neural crest migrate through the pharyngeal arches [29]. The mesenchyme in the pharyngeal arches is mostly ectomesenchyme [29]. Except for endothelium, the walls of blood vessels which are formed from arch arteries are derived from ectomesenchyme [29]. Previous studies have shown that the neural crest in the pharyngeal arches is important in the maintenance of the aortic arch arteries traversing the pharyngeal arches [4]. For instance, neural crest ablation is associated with a high incidence of aortic arch anomalies [33]. These anomalies occur individually and in association with malformations of the outflow tract including PTA and DPA [33].

A recent study using intravascular injection of chick embryos between 48 and 168 h of incubation showed that cardiac neural crest ablation produced a delay in the disappearance of aortic arch arteries 1 and 2 [4]. In addition, arch arteries three, four, and six failed to develop to the proper sizes in some animals. Closure of the arch arteries in some animals and maintenance in others produced greater variability in experimental animals than in controls. The lack of a predictable pattern of maintenance and closure of these arteries was the most notable finding of these experiments. It was also determined in the same study that the morphology of the heart significantly deviated from normal several days prior to the development of the outflow septa [4].

Preliminary data have shown that there is a loss of bilateral symmetry in the aortic arch arteries [5]. In addition, it was common for individual arch vessels to have increased diameters while others in the same embryo were decreased [5]. Thus blood flow is altered at a very early stage of vascular development by neural crest ablation and the alterations are maintained during maturation.

Because the morphology of the aortic arch arteries is altered very early in development, control of the blood flow through the pharyngeal region may be a key in normal heart development.

Ventricular function and hemodynamics after neural crest ablation

Ventricular function has been evaluated using cinephotography on day 3 of incubation following ablation of neural crest which would migrate to pharyngeal arch 4 [28]. This lesion results in a high incidence of PTA but day 3 is well before septation of the outflow tract begins [45]. All of the indices of contractility (shortening fraction, wall velocity, and ejection fraction) were markedly depressed in experimental embryos. The experimental embryos appeared to be able to compensate for the decreased contractility by dilation of the primitive ventricle. The average heart rate in control and experimental embryos was not different [28].

Visual evaluation of the cine films suggested that the cardiac tube was less acutely looped in the experimental embryos. The ventricle was dilated in both diastole and systole and the truncus arteriosus was shorter and wider. In embryos with the most depressed contractility indices,

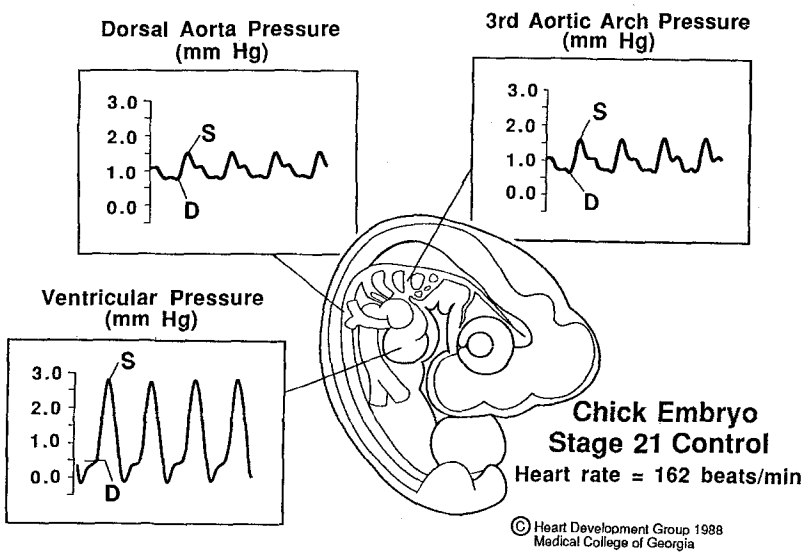

Figure 5. It is possible to obtain blood pressure measurements from various places in embryos from 2–5 days of development. This diagram of a 3-day chick embryo shows actual pressure tracings obtained in a single embryo.

there was evidence of incompetence of the truncal folds such that red blood cell streams regurgitated instead of moving in a continuous pulsatile forward flow[28].

Dorsal aortic blood velocity and vitelline artery blood pressure have also been measured at 3 days of incubation[44]. Embryos with neural crest lesions have significantly greater dorsal aortic blood flow velocity than control embryos. It is also possible to measure pressure in a variety of vessels in the early embryo (fig. 5). Hopefully, information derived from these types of measurements in experimental embryos will provide some insight into the early functional alterations. The flow data suggest that hemodynamic abnormalities occur very early following neural crest ablation and the time at which hemodynamics are altered correlates with changes in aortic arch artery morphology[4,44].

How might ectomesenchyme control blood flow?

Although several studies have shown that ectomesenchyme is essential for development and maintenance of the aortic arch arteries, the contribution of the ectomesenchyme to these processes is unclear. Bockman and Kirby[3] have shown that thymus development from the pharyngeal epithelium is dependent on ectomesenchyme. The nature of the interaction required for thymic induction is not known. However, it is likely that a similar interaction occurs between the aortic arch endothelium and its supporting ectomesenchyme. It is interesting that neural crest-derived mesenchyme populates pharyngeal arches 1 and 2 in which the aortic arch arteries disappear[40], while arches 3, 4 and 6 remain as permanent vessels[40]. Coincidentally, the neural crest antibody EC-8 specifically reacts with ectomesenchyme in pharyngeal arches 3, 4 and 6 while the ectomesenchyme in arches 1 and 2 are nonreactive[6]. This indicates a basic difference in the ectomesenchyme in the two different regions.

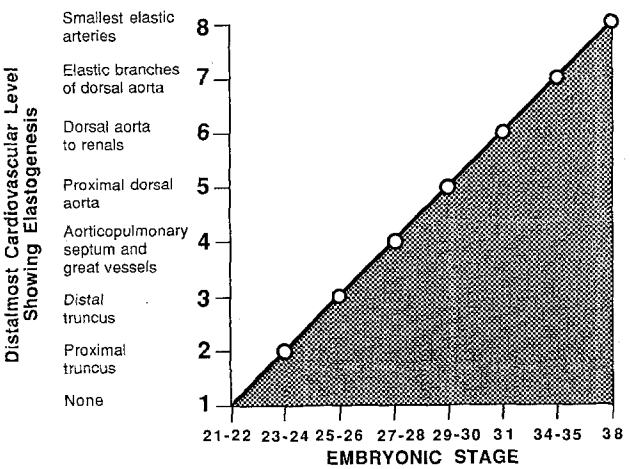

Figure 6. Elastogenesis has been mapped in the developing cardiovascular system of the chick embryo. Elastogenesis begins in the proximal truncus and proceeds downstream between days 3 and 9.

The epitope for the EC-8 antibody is thought to be on the cell surface of the ectomesenchymal cells[6]. The surface glycoproteins and extracellular matrix produced by the ectomesenchyme may have some components which maintain wall properties of the endothelium of the aortic arch arteries. Certainly molecules such as the chondroitin sulfates and hyaluronate that are associated with neural crest could fill this role.

In the outflow tract, the ectomesenchyme produces soluble tropoelastin and aldehyde-rich protein which contribute to the formation of elastic connective tissues[39]. Formation of elastin occurs in a proximal-distal sequence from the truncal whorls (fig. 6). It has been hypothesized that elastogenesis is a critical event in outflow tract septation[39]. Certainly the formation of an elastic connective tissue matrix would impart distinct characteristics to the walls of the aortic arch arteries.

Cardiac innervation

Innervation of both the mammalian and chick heart is from three different sets of nerves. Sympathetic innervation in the chicken is via the first thoracic sympathetic ganglia[1] which are derived entirely from the neural crest[24]. The sympathetic neural precursors can be removed by ablation of the neural fold adjacent to somites 10–20 (fig. 7). Presumably the precursors of the normal ganglia originate from a much smaller length of neural fold[50] but since the trunk neural crest is capable of reconstituting a lesioned area[51], it is necessary to remove at least the length between somites 10 and 20 to achieve a completely sympathetically aneural heart[24]. Although sympathetic nerves are present in the chick heart by days 10 or 11 of incubation[13,22], positive chronotropy in response to sympathetic stimulation does not occur until day 16[14].

Parasympathetic innervation of the heart is from cardiac ganglia located on the surface of the heart and in the outflow tract[11]. The cardiac ganglia are derived from the cardiac neural crest which has been discussed at length above. Removal of the cardiac neural crest does not produce an aneural heart although it does result in PTA as discussed above[20] (fig. 6). The cardiac ganglia are reconstituted from an alternate source that will be discussed below. The cardiac ganglia form from neurons which populate the heart late on the third day of incubation[27]. Fully formed cardiac ganglia can be identified by day 8; however, a negative chronotropic response to parasympathetic stimulation does not appear until day 12[35]. This response can be elicited on day 10 if the embryo is pretreated with cholinesterase inhibitor[35].

Sensory innervation of the heart is from the distal ganglia of the vagus nerves[48] (fig. 1). The neurons of these ganglia arise from the nodose placodes located lateral to somites 1–3[8]. The supporting cells of the ganglia are provided by neural crest located approximately adjacent to the caudal otic placode region and extending to somite

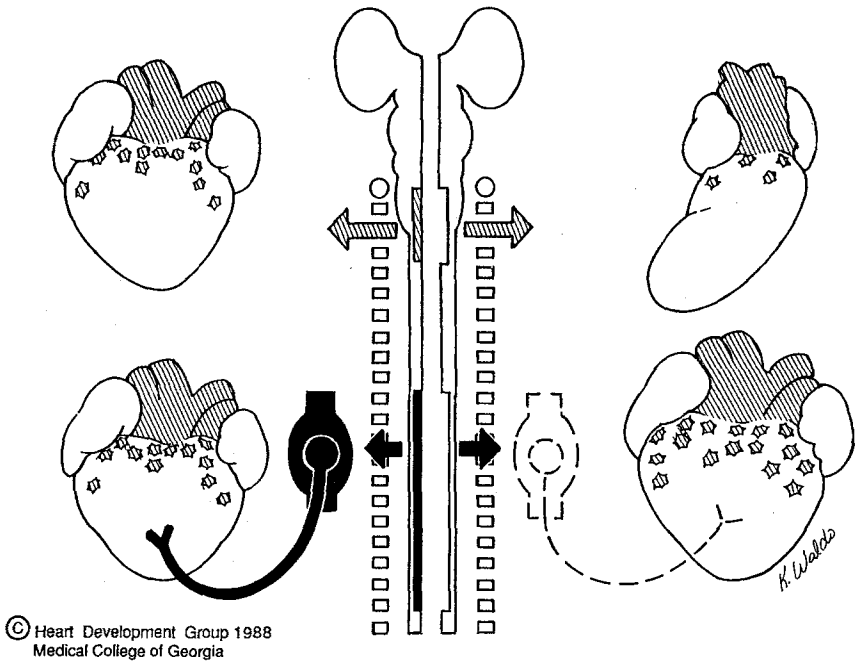

Figure 7. Diagram illustrating the normal source of cardiac innervation from the neural crest and the consequences when that neural crest is removed. Ablation of the cardiac neural crest results in PTA with slightly depleted cardiac parasympathetic ganglia. Removal of trunk neural crest between somites 10 and 20 results in a sympathetically aneural heart; however, the cardiac parasympathetic ganglia proliferate.

3[8]. It is not known at what time during development the sensory innervation to the chick heart becomes functional.

After ablation of the cardiac neural crest, reconstitution of the cardiac ganglia occurs such that the cholinergic innervation of the heart is approximately 70% of normal[20]. However, if the nodose placodes are removed as well as the neural crest, the heart is indeed parasympathetically aneural[17] (fig. 8). Embryos with this 'double' lesion do not survive beyond day 12 of incubation and the hearts from these embryos are extremely abnormal morphologically. Part of this structural aberration results from the fact that the nodose placode is also capable of providing ectomesenchyme to the outflow tract in the absence of cardiac neural crest[18] (fig. 8). After the double lesion, there is a complete absence of parasympathetic innervation and the ectomesenchyme provided by the nodose placode. It should also be mentioned that these hearts probably lack sensory innervation as the nodose placode is the only source of sensory innervation that has been described in the chick heart. However, the possibility exists that sensory innervation might be reconstituted from yet another source. This particular model of aneural heart development should provide many new insights into heart development.

Sympathetically aneural hearts have been shown to have increased cholinergic innervation due to both hypertrophy and hyperplasia of the ganglion cells as well as their terminals[21] (fig. 7). This overgrowth may be dependent on the growth factor-rich environment of the heart[10]. The effects of this increased innervation are not known at the present time. Interestingly β-adrenergic receptor density was founded to be normal in the aneural hearts[43].

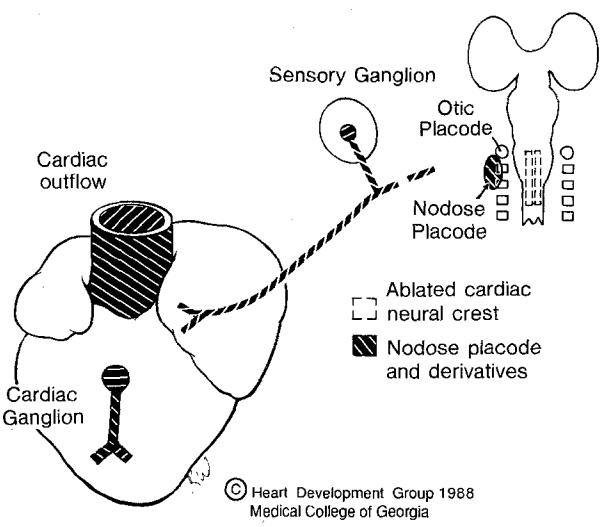

Figure 8. Diagram illustrating why removal of the cardiac neural crest does not result in a parasympathetically aneural heart. When the cardiac neural crest is removed, cells from the nodose placode that normally provide sensory innervation to the heart via the inferior ganglion of the vagus, migrate into the heart and provide cholinergic parasympathetic innervation. These cells are also capable of reinforcing the wall of the outflow vessel with ectomesenchyme; however, the nodose placode-derived ectomesenchyme is not competent to initiate septation of the outflow vessel in the absence of cardiac neural crest.

Development of the myocardium

Using neonatal rat hearts, Claycomb[7] has shown that a switchover of myocardial cells from the replication phase

to differentiation can be elicited by exposing the tissue to isoproterenol. This effect is mediated by cyclic AMP. Slotkin[42] has shown that there is a critical period between 2 and 12 days postnatally when exposure to isoproterenol causes a decrease in tritiated thymidine incorporation into DNA. Unfortunately, both of these systems use exogenously applied β-agonist rather than the intrinsic nerve supply.

Using various combinations of lesions to produce aneural and/or malformed hearts in chick embryos, it has been possible to obtain some new insights into myocardial development. Ventricles of sympathetically aneural hearts have greater stores of glycogen as compared to controls[12]. α-Glucan phosphorylase, an enzyme responsible for glycogen hydrolysis is less active in these aneural hearts which may account for the increased glycogen stores. The indicates that myocardial metabolism in sympathetically aneural hearts is altered by decreased capability in the maximal rate of glycogen breakdown and subsequent increased storage of the glycogen substrate. Other metabolic enzymes were shown in the same study to be normal[12], although cyclic AMP is depressed[37].

In a recent study of contractile proteins in aneural and malformed hearts, isomyosin was found to be the same in experimental and control hearts[23]. However, actin isoforms showed some differences. Actin isoform expression was studied using cDNA probes made to the 3′ untranslated region of actin mRNA for α-skeletal and α-cardiac actin isoforms. Cardiac actin mRNA was elevated in sympathetically aneural hearts (approaching adult levels) while it was decreased in parasympathetically aneural hearts. Hearts with PTA showed no differences in actin mRNA isoforms[23].

Conclusions

There are multitudinous extracardiac factors that influence heart development. I have discussed several model systems which are available in chick embryos to study many of these influences. This particular model is very powerful because it allows development to occur in a temporospatially normal environment. However, there are numerous weaknesses inherent in the system. The most outstanding of these is the lack of control of the environment. A relatively new model system involves growth of the heart or various regions of it in rats in oculo. This system has the potential to control the innervation and environment more precisely than in hearts in situ. It has been shown using this system that sympathetically innervated atria grown in oculo have higher intrinsic beat rates and are almost twice the size of noninnervated atria[47]. This is not the case in sympathetically aneural chick hearts in situ where the beat rate is the same as control and the atrial weight is increased over hearts with intact sympathetic innervation[21]. Sympathetically aneural hearts in situ have hypercholinergic innervation which may account for the different results in the two situations[21]. Alternate systems to compare such results should provide new insight into factors important in cardiac morphogenesis.

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0014-4754/88/11-120944-08$1.50 + 0.20/0

Thoughts on concepts of development of the heart in relation to the morphology of congenital malformations

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Summary. In the past, it has often been the case that congenital malformations have been categorized in terms of their presumed embryologic development. The knowledge of development, however, has itself often been derived from studies of the normal heart during its development coupled with inferences drawn from the morphology of the abnormal hearts. This can lead to circular thinking which, often, has little basis in fact. It is our belief that cardiac embryology is an important science which should stand in its own right, but that knowledge of abnormal development should be derived from observation rather than inference. The potential dangers of concepts derived by extrapolation are illustrated with reference to hearts having deficiencies of atrioventricular septation ('endocardial cushion defects') and those with double inlet left ventricle ('single ventricle'). It is shown that description of these hearts is greatly facilitated by eschewing those concepts derived from 'armchair embryology'. Once a clear description is established, the scene is set to understand the real mechanisms underscoring the maldevelopment of these lesions.
Key words. Embryology; atrioventricular septal defect; endocardial cushions; double inlet left ventricle; conduction tissues.