Susceptible Periods during Embryogenesis of the Heart and Endocrine Glands

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One of the original principles of teratology states that, "Susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence" [Wilson JG. Environment and Birth Defects. New York:Academic Press, 1973]. The time of greatest sensitivity encompasses the period of organ formation during weeks 3–8 following fertilization in human gestation. At this time, stem cell populations for each organ's morphogenesis are established and inductive events for the initiation of differentiation occur. Structural defects of the heart and endocrine system are no exception to this axiom and have their origins during this time frame. Although the function and maturation of these organs may be affected at later stages, structural defects and loss of cell types usually occur during these early phases of development. Thus, to determine critical windows for studying mechanisms of teratogenesis, it is essential to understand the developmental processes that establish these organs. *Key words*: adrenal glands, embryology, heart, pancreas, parathyroid, pituitary, thymus, thyroid. — *Environ Health Perspect* 108(suppl 3):555–561 (2000).
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Heart

The period of greatest risk for inducing abnormal cardiac development occurs during weeks 3–5 of gestation. Primary targets include genes that regulate establishment of the cardiogenic field and cardiac differentiation, neural crest cells that contribute to partitioning of the aorticopulmonary channel, and endocardial cushions. Heart cells originate near the primitive streak at approximately day 15 of gestation. Prospective outflow tract cells lie most cranially in the streak, followed by ventricular, then atrial cells (*1*). These cells migrate through the streak to the cardiogenic area cranial to the cephalic neural folds. Here the cells lie in the splanchnic mesoderm of the lateral plate, adjacent to the underlying endoderm that guides their migration along a fibronectin rich path (Figure 1) (*2*). This endoderm is also responsible for inducing cardiac progenitor cells to form cardiac myocytes (*3*). Cells in the cardiogenic region form a horseshoe-shape tube with the open ends pointing caudally (Figure 1). As lateral folding of the embryo occurs, the two sides of the horseshoe are brought together and fuse in the midline to establish the heart tube (Figures 2, 3). By day 23, the heart tube begins to bend to form the cardiac loop (Figure 4). The outflow region bends caudally and to the right, while the atrial region moves dorsocranially and to the left. Looping causes the heart to fold in such a manner that the atria, ventricles, and outflow tract assume their adult position but remain unseptated (Figure 5).

Septation occurs from days 27–37 of development with formation of the atrial, ventricular, and conotruncal septa. In the atria, this process depends upon downward growth of overlapping septa from the roof of the chamber to meet endocardial cushion tissue surrounding the atrioventricular canal. Prior to contact with cushion tissue, cell death by apoptosis creates a hole in the first septum to grow downward. The second septum grows over this opening but never reaches the cushions to fuse. In this manner it acts as a valve, such that blood flow from the right atrium (receiving oxygenated blood from the placenta) into the left is maintained. At birth, when the lungs become functional and oxygenated blood returns from these organs into the left atrium, the valve will be pressed closed to create an anatomically complete interatrial septum.

Cushion tissue is also important for closing the interventricular septum. Muscle tissue from the expanding ventricles forms a column of tissue comprising the ventral portion of the interventricular septum. Endocardial cushion tissue, derived from the region of the atrioventricular canal, grows downward to join the muscular column and completes the interventricular septum, which then has muscular and membranous portions. Most defects of this septum occur in the membranous portion, derived from cushion tissue (Figure 6).

The outflow tract or conotruncus is also septated into the aortic and pulmonary channels by endocardial cushion tissue. Two cushions growing from the sides of the conotruncus assume a spiral course and fuse in the midline. Their spiral course is essential to establish the appropriate connections for the aortic and pulmonary trunks. As these cushions grow downward, they meet and fuse with cushion tissue surrounding the atrioventricular canal, thereby completing the septation of this region (Figure 6).

Finally, the superior and inferior endocardial cushions surrounding the atrioventricular canal grow together to separate this region into two channels. Then, together with the right and left cushions, they form the mitral and tricuspid valves (Figure 6).

It should be appreciated from the previous discussion that endocardial cushions are a key factor for normal heart development. Not surprisingly, they are also involved in the origin of many cardiac defects, including atrial and ventricular septal defects and abnormalities of the conotruncal region, such as tetralogy of Fallot, transposition of the great vessels, pulmonary stenosis, and others. Thus, the cushions themselves are a potential target for teratogens and merit further investigation. They are bounded by the myocardium, which secretes a hyaluronate-rich matrix (cardiac jelly) that expands the space between the myocardial and endothelial cells. At the time of cushion differentiation, the myocardium secretes EDTA-soluble proteins that, in combination with fibronectin, move through the matrix and transform some of the endothelial cells into mesenchyme (*4*). This epithelial-to-mesenchymal transformation and delamination of these endothelial cells require expression of *MSX-1*; influx of Ca; decreased expression of transforming growth factor β (*TGF-β*); increased expression of substrate adhesion molecules, including integrins; and an increase in urokinase plasminogen activator (*4–8*). In short, a complex orchestra of molecular and cellular events that present targets for disruption is essential for cushion differentiation and, ultimately, heart morphogenesis.

The process of cushion formation is further complicated by the fact that neural crest cells are essential for formation of the truncal cushions that separate the distal portion of the outflow tract into the pulmonary artery and aorta (*9*). These crest cells, originating in hindbrain rhombomeres 6,7, and 8, migrate into pharyngeal arches 3, 4, and 6 and some, the cardiac crest, continue into the outflow tract where they augment the endothelial cells in populating the truncal cushions. From the hindbrain, these crest cells carry a specific homeobox (*HOX*) code into the arches (*10*) where they support development of structures

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derived from this region, including the aortic arches, thymus, parathyroids, and thyroid. Crest cells are particularly sensitive to toxic insult from a variety of factors, including alcohol and retinoids. They often respond by undergoing apoptosis, in part because they apparently lack antioxidative stress proteins (*11*). Thus, they are a primary target to be investigated and insults early in their formation, during the latter part of week 3, and their migration, during the beginning of week 4, may result in combinations of seemingly unrelated birth defects. For example, DiGeorge and velo–cardio–facial syndromes may result from a deletion of the q11 region of chromosome 22 but can also be produced by retinoids, alcohol, maternal diabetes, and bis(dichloroacetyl)diamine, an inhibitor of sperm production (*12*). Interestingly, abnormalities characteristic of these syndromes include facial, heart, and glandular defects involving the thymus, thyroid, and parathyroids. The target for these teratogenic insults is crest cells from the hindbrain. Other characteristic defects produced by insults to cardiac crest include, tetralogy of Fallot, transposition of the great vessels, and persistent truncus arteriosus.

In addition to crest and endothelial cells that populate the endocardial cushions, cells in the septum primum that undergo programmed cell death during week 5 of development are potentially susceptible to toxicants. For example, if too many of these cells undergo apoptosis, then atrial septal defects will occur. Interestingly, in contrast to these septum primum cells, endothelial and myocardial cells are resistant to cell-killing teratogens. Recent evidence suggests that although these cells express genes responsible for apoptosis, they do not upregulate these genes following a toxic insult, even when other tissues undergo extensive cell death (*13*).

Genetic regulation of heart development is rapidly being elucidated, thereby providing

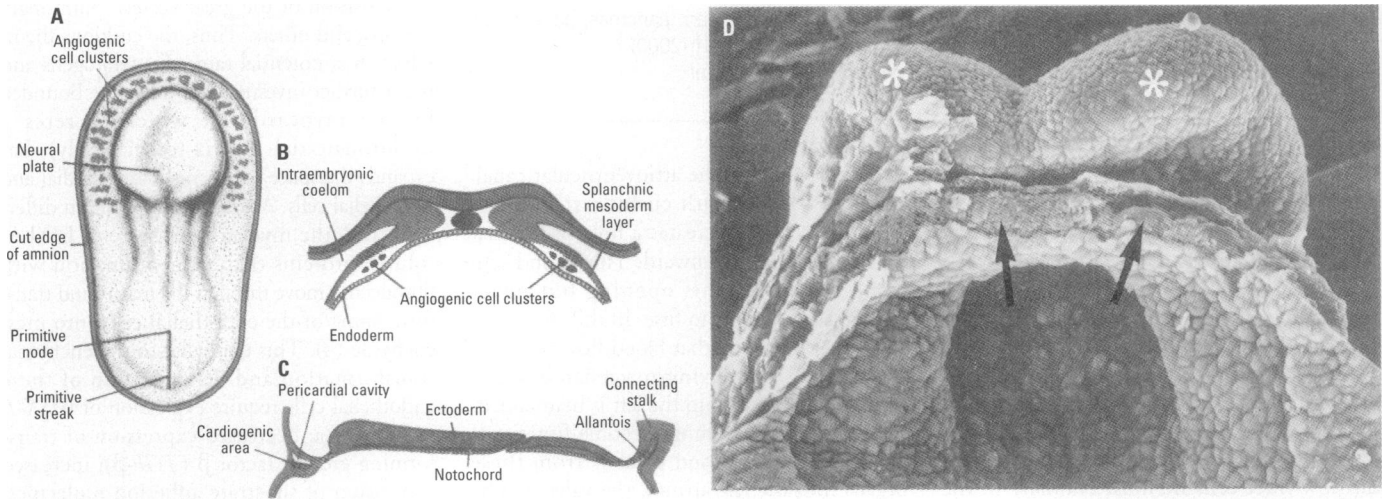

Figure 1. (*A*) Drawing of the dorsal surface of a human embryo on day 18 postfertilization, illustrating the presence of angiogenic cell clusters in the cardiogenic region cranial to the neural plate. These primordial cells have migrated through the primitive streak at the caudal end of the germ disc and now reside in the splanchnic mesoderm shown in cross section in (*B*) and longitudinal section (*C*). (*D*) Scanning electron micrograph of a mouse embryo at a slightly later stage (approximately day 19, human; day 7, mouse) showing coalescence of the cardiac cells into a horseshoe shaped tube (arrows) lying in the pericardial cavity, beneath the head folds (asterisk) of the neural plate.

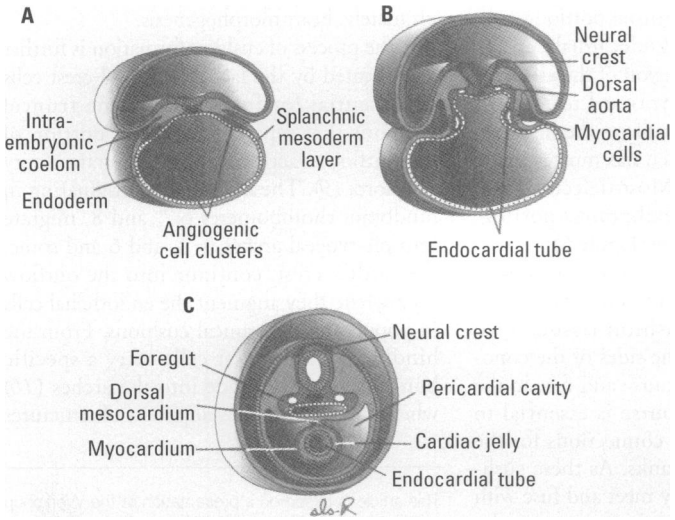

Figure 2. (*A–C*) Drawings of cross sections through the cardiogenic region of 18-, 19-, and 22-day (postfertilization) embryos. During this time, the embryo folds laterally in a ventral direction, causing the two sides of the cardiac tube to come together and fuse in the midline (*C*). By the time of fusion, myocytes form and the first cardiac contractions are initiated. Simultaneously, the neural plate folds dorsally to form the neural tube and neural crest cells from the hindbrain region (*C*) begin migrating to the cardiac outflow tract to participate in partitioning this region into aortic and pulmonary channels.

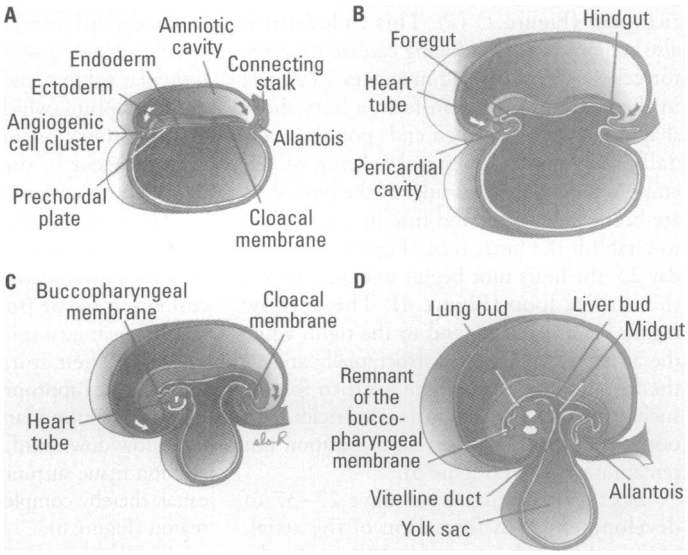

Figure 3. In addition to lateral folding from days 18–22 of gestation (Figure 2), the embryo folds cranially and caudally. In the cranial region, this folding causes the heart tube to move into the thoracic region.

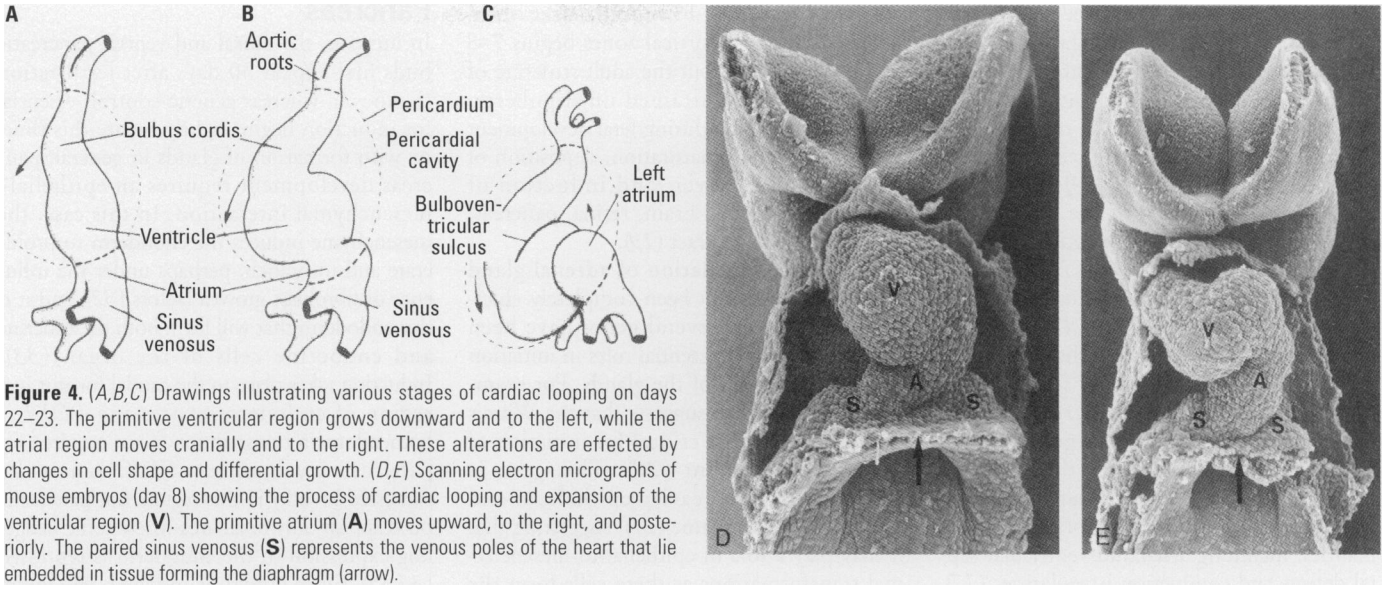

Figure 4. (*A,B,C*) Drawings illustrating various stages of cardiac looping on days 22–23. The primitive ventricular region grows downward and to the left, while the atrial region moves cranially and to the right. These alterations are effected by changes in cell shape and differential growth. (*D,E*) Scanning electron micrographs of mouse embryos (day 8) showing the process of cardiac looping and expansion of the ventricular region (**V**). The primitive atrium (**A**) moves upward, to the right, and posteriorly. The paired sinus venosus (**S**) represents the venous poles of the heart that lie embedded in tissue forming the diaphragm (arrow).

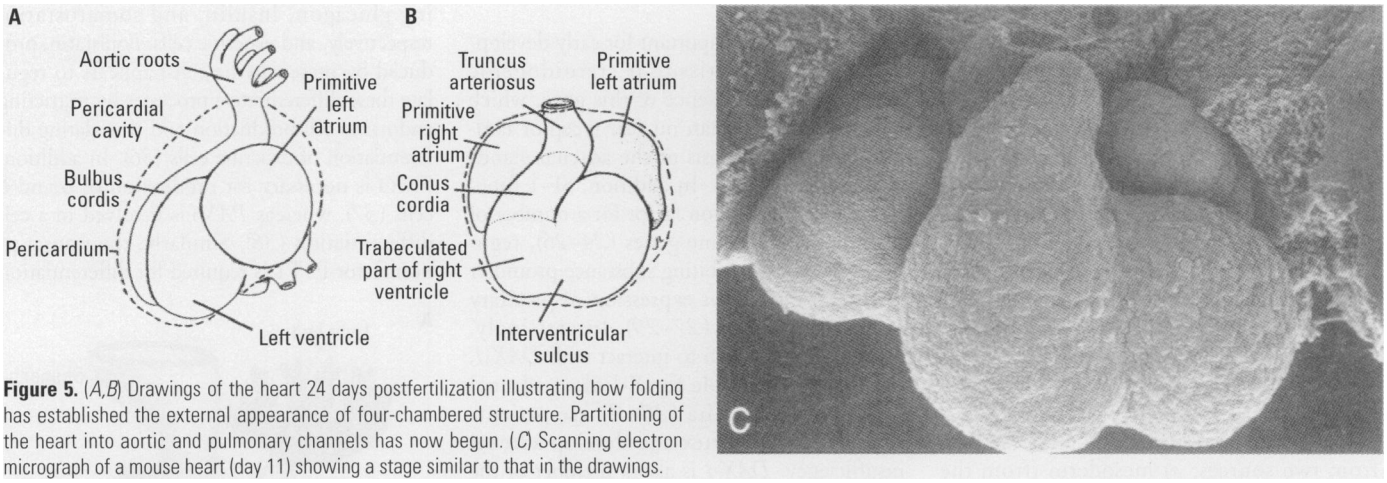

Figure 5. (*A,B*) Drawings of the heart 24 days postfertilization illustrating how folding has established the external appearance of four-chambered structure. Partitioning of the heart into aortic and pulmonary channels has now begun. (*C*) Scanning electron micrograph of a mouse heart (day 11) showing a stage similar to that in the drawings.

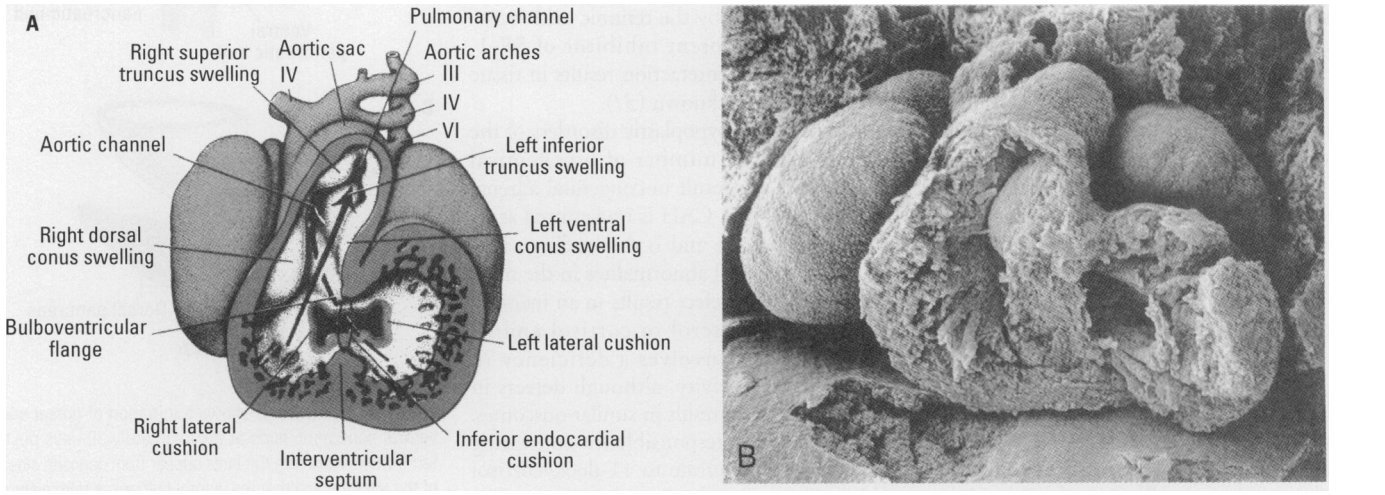

Figure 6. (*A*) Drawing of a 35-day heart showing advanced stages of septation of the atrioventricular canal, ventricles, and outflow tract. Endocardial cushion tissue (swellings in the outflow tract) is essential for partitioning of each of these regions and the atrial septum. Thus, many cardiac defects involve these cushions and affect these areas. In addition, neural crest cells contribute to septation of the outflow tract in the truncus arteriosus, such that abnormalities in the migration, proliferation, or viability of these cells result in defects, including transposition of the great vessels, tetralogy of Fallot, and others. Since crest cells also contribute to formation of the face, many children with facial clefts also have cardiac defects. (*B*) Scanning electron micrograph of a mouse heart (day 12.5) showing a stage similar to the drawing in *A*. Note the cushion (swellings) tissue in the outflow tract and the ventricular septum.

candidate genes to be investigated for potential interactions with environmental influences. As mentioned previously, neural crest cells carry a *HOX* code into the pharyngeal arches and heart. These genes can be respecified by retinoids and may also have their expressions disrupted by other factors (*14*). Genes that regulate establishment of the cardiogenic field also contain a homeodomain and belong to the *NK* family (*15*). *NKX2-5* (also known as *CSX*) is the homolog of the cardiac-specifying gene *tinman* in *Drosophila*. In vertebrates, *NKX2-5* specifies the cardiogenic field and is also important for septation and development of the conduction system. Targeted disruption of this gene in mice arrests cardiac development at the tube stage prior to looping (*16*). In humans mutations in this gene result in a variety of heart abnormalities, including artial and ventricular septal defects and conduction irregularities (*17*). *TBX-5* contains a DNA-binding motif known as a T-box and is also important for septation. Mutations in this gene are responsible for some cases of Holt-Oram syndrome, characterized by limb and heart abnormalities, such as atrial and ventricular septal malformations and conduction defects (*18*). *BMP-2* is expressed by the endoderm underlying the cardiogenic field and maintains *NKX2-5* expression, whereas *Wnt* genes expressed in the cardiac region define the limits of the field by restricting *NKX2-5* expression (*15*). All these genes are expressed early in week 3 of development.

Adrenal Glands

The adrenal or suprarenal glands develop from two sources: *a*) mesoderm (from the urogenital ridge) that forms the cortex and *b*) neurectoderm (neural crest cells) that forms the medulla. Development is initiated in week 5 postfertilization when epithelial cells covering the cranial end of the urogenital ridge near the root of the mesentery proliferate and penetrate the underlying mesenchyme. These cells form the fetal cortex, which becomes very large. Shortly thereafter a second wave of cells from the epithelium penetrates the mesenchyme, surrounds the first cells, and forms the definitive cortex. These cells will eventually differentiate into the zona glomerulosa, zona fasciculata, and zona reticularis, while the fetal cortex rapidly regresses after birth. The significance of its large size and its function during the fetal period is unknown.

As the fetal cortex is being formed during week 5, neural crest cells from the 6th–12th thoracic segments accompany the growth of nerves from these same regions into the gland. These cells form the medulla of the gland and differentiate into chromaffin tissue. Differentiation begins by week 12 and continues until 12–18 months after birth. Differentiation of cortical zones begins 7–8 months after birth, but the adult structure of this region is not attained until puberty. Cortisol production during fetal development is critical for lung maturation, deposition of glycogen in the liver, and induction of enzymes in the fetal brain, retina, pancreas, and gastrointestinal tract (*19*).

Molecular regulation of adrenal gland development has not been completely elucidated. However, several genes have been identified that play essential roles in initiation and differentiation of the glands. For example, recent evidence suggests that the Wilm's tumor gene *WT1* is critical for initiation of cortex development (*20*). This gene is expressed in body cavity epithelium and underlying mesenchyme (*21*), suggesting that it may play a role in epithelial-to-mesenchymal transformations as these cells form the adrenal cortex during the initiation of gland development (*22*).

Another gene important for early development of adrenal tissue is steroidogenic factor 1 (SF-1). Absence of this gene, which belongs to the orphan nuclear receptor family, results in agenesis of the adrenal glands and gonads (*22,23*). In addition, SF-1 functions as a transcription factor for a number of steroidogenic enzyme genes (*24–26*), regulates Müllerian-inhibiting substance promoter (*26*), and modulates expression of pituitary gonadotrope cells (*27–29*). Interestingly, SF-1 has been shown to interact with *DAX-1*, the gene responsible for X-linked adrenal hypoplasia congenita (*30*). These patients lack an adrenal cortex and develop adrenal insufficiency. *DAX-1* is also a member of the nuclear hormone receptor superfamily and acts as a dominant negative regulator of transcription mediated by the retinoic acid receptor (*30*). It is a potent inhibitor of SF-1, although how this interaction results in tissue differentiation is unknown (*31*).

In addition to hypoplastic disorders of the adrenal glands, a number of biochemical abnormalities can result in congenital adrenal hyperplasia (CAH). CAH is transmitted as an autosomal recessive and is the leading cause of ambiguous sexual abnormalites in the newborn. Usually the defect results in an inability to convert cholesterol to cortisol and/or aldosterone and involves a deficiency in 21-hydroxylase activity, although defects in other enzymes may result in similar outcomes. 21-Hydroxylase is responsible for converting 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone. As a result, adrenocortical-stimulating hormone (ACTH) levels increase, which causes hyperplasia of the adrenal cortex and an increase in steroid metabolites derived prior to the block in the biochemical pathway.

Pancreas

In humans, the dorsal and ventral pancreatic buds first appear 30 days after fertilization (Figure 7), whereas genetic controls essential for induction begin slightly before this time. As with formation of glands in general, pancreas development requires an epithelial–mesenchymal interaction. In this case, the mesenchyme induces the endoderm to proliferate and to branch, perhaps under the influence of fibroblast growth factors (*32*), and it is this endoderm that will form both the exocrine and endocrine cells of the organ (*33*). Induction takes place in the caudal foregut in a region of endoderm expressing *PDX1*, a homeodomain transcription factor required for pancreas development. *PDX1* is actually expressed broadly in this region, but pancreatic buds appear only in an area where sonic hedgehog expression in the endoderm is repressed (*34,35*). Once growth is initiated, endoderm cells differentiate into α, β, or δ cells, producing glucagon, insulin, and somatostatin, respectively, and exocrine cells. Follistatin, produced by mesenchyme cells, appears to regulate these differentiative processes by restricting endocrine cell production and stimulating differentiation of exocrine cells (*36*). In addition, *PAX4* is necessary for production of β and δ cells (*37*), whereas *PAX6* is involved in a cell differentiation (*38*). Similarly, the transcription factor PTF1 is required for differentiation

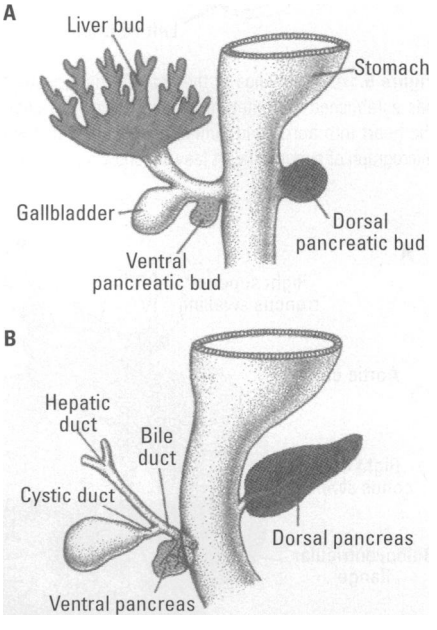

Figure 7. (*A*) Drawings showing initiation of dorsal and ventral pancreatic buds at approximately 30 days postfertilization. Note that the buds appear from opposite sides of the gut tube and that the ventral bud arises from a common outgrowth that will form the liver and gall bladder. (*B*) A slightly later stage in pancreas development showing the ventral bud moving posteriorly to join the dorsal bud. Endoderm from the gut tube forms the parenchyma of the pancreas, gall bladder, and liver; whereas surrounding mesoderm forms the connective tissue.

and/or proliferation of the exocrine cells (*39*). Definitive β cells appear in the pancreas by the 3rd month, although an initial wave may be formed earlier during weeks 4–6 (*36*). These earliest cells express insulin and glucagon but do not associate to form islets. Insulin production begins by the 5th month.

As the two buds increase in size, the ventral bud moves into closer proximity to its dorsal counterpart. By week 6, the two buds fuse and the duct connecting the dorsal bud to the foregut usually degenerates (Figure 8).

Malformations of the pancreas may be most commonly related to inductive events. For example, excessive growth of pancreatic tissue, including annular pancreas, nesidioblastosis, and heterotopic pancreas, may all be related to abnormal signaling during initial phases of development (*34,35*). Whether or not pancreatic function can be affected by epigenetic factors acting on cell differentiation is not clear. In any case, however, the pancreas may have more than one window of susceptibility: an early one during weeks 4–6 and a later one at weeks 10–12 when cells are differentiating.

Thymus and Parathyroids

The thymus and parathyroids are also derivatives of pharyngeal endoderm arising from outpocketings of endoderm known as the pharyngeal pouches (Figures 9, 10). Thus, during week 5 of gestation, the thymus and superior parathyroid arise from proliferations of the 3rd pharyngeal pouch and the superior parathyroid from the 4th (Figure 10). While the parathyroids assume a final position posterior to the lobes of the thyroid, the thymic anlagen migrate to the anterior mediastinum where they fuse in the midline (Figure 11).

Induction and development of these tissues again involve epithelial mesenchymal interactions with much of the mesenchyme derived from neural crest cells. As mentioned previously, these cells are quite sensitive to toxicants. Adverse effects on crest cells by alcohol, retinoids, maternal diabetes, and bis(dischloroacetyl)diamine results in DiGeorge syndrome, which includes partial or complete loss of the thymus, parathyroids, and thyroid; craniofacial defects; and heart abnormalities (*12*). The origin of the glandular defects is thought to be due to decreased numbers of crest cells, leading to incomplete formation of the pharyngeal arches, and disruption of epithelial–mesenchymal signaling essential for gland differentiation.

Thyroid

The thyroid gland develops from endoderm of the floor of the pharynx at a point that later demarcates the foramen cecum. The evagination appears during week 5 and this evaginated tissue migrates caudally in the midline to the level of the trachea as a bi-lobed structure (Figure 11). Differentiation occurs by weeks 8–9 and thyroid hormone synthesis begins by weeks 10–12. Thyroid hormones are essential for brain development where they *a*) promote neuronal and glial proliferation and determine the time for terminating proliferation and beginning differentiation; *b*) play a role in neuronal migration by regulating microtubule synthesis and assembly and controlling the scaffolding for this cell movement; *c*) enhance neuronal outgrowth by regulating growth factors such as nerve growth factor; *d*) stimulate development of dopaminergic and cholinergic systems; and *e*) promote myelination (*40*). These effects of thyroid hormone on brain development occur in three phases: phase I is regulated by maternal sources of the hormone, extends from weeks 3–12, and encompasses the beginning stages of cerebral neurogenesis and migration; phase II is regulated by fetal production of the hormone from 12 weeks to birth and overlaps many periods of neuronal proliferation, migration, and differentiation throughout the brain; phase III occurs during the postnatal period as the central nervous system continues to differentiate (*40*).

This important action of thyroid hormone on brain development and its effects on growth make it a potential target for environmental factors, but few studies documenting such effects exist. Examples include endemic iodine deficiency that causes a decrease in thyroid hormone resulting in many neurological deficits (*41*). Also there is some evidence suggesting that dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) can reduce thyroid hormone concentrations and affect learning and memory capabilities (*42*).

Anterior Pituitary

The pituitary gland is derived from two sources: the posterior lobe arises from neuroepithelium of the diencephalon, the infundibulum; the anterior and intermediate lobes from an outpocketing of epithelium in the oral cavity (itself a derivative of neural ectoderm from the anterior neural ridge), called Rathke's pouch (*43*) (Figure 12). Development begins early in week 4 with inductive signals from the diencephalon initiating formation of Rathke's pouch. The homeobox gene *TTF-1* (*NKX2-1*) is expressed in the diencephalon and is essential for this inductive influence (*44*). By week 8 of development, the pouch pinches off from the oral cavity and the cells undergo rapid proliferation (*43*). As differentiation continues, five cell types form in the anterior pituitary: *a*) corticotropes, which produce ACTH; *b*) thyrotropes, which synthesize thyroid-stimulating hormone; *c*) somatotropes, which produce growth hormone; *d*) gonadotropes, which make lutenizing (LH) and follicle-stimulating hormones (FSH); and *e*) lactotropes, which secrete prolactin. Corticotropes appear at approximately week 8 of development; thyrotropes on week 11; somatotropes and lactotropes by week 11; and gonadotropes for LH on week 12 and those for FSH on week 13.

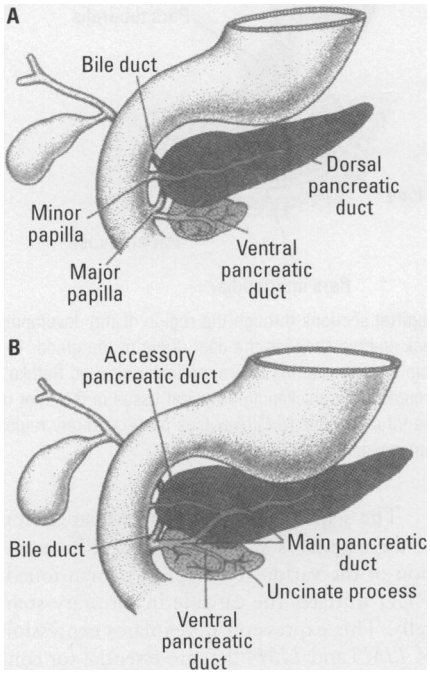

Figure 8. Drawings of pancreas development at week 6 of development showing fusion of the dorsal and ventral buds. The main duct is derived from the distal part of the dorsal bud and the proximal part of the ventral. In 10% of cases, the entire dorsal duct remains to form an accessory pancreatic duct.

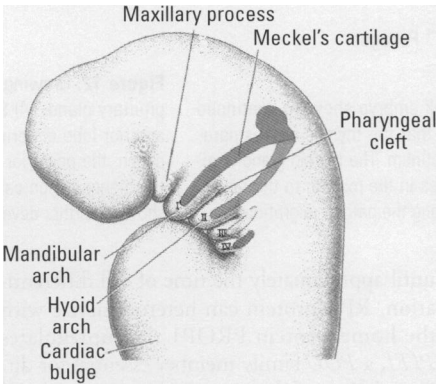

Figure 9. Drawing of the exterior of a human embryo at week 4 of gestation showing the presence of pharyngeal arches. Mesenchyme from these arches contributes to formation of the maxilla, mandible, and external ear. Cartilage from each of the arches contributes to these regions and the larynx. Internally, along the pharynx the arches are lined by endoderm that will contribute to glands in the head and neck. See Figures 10 and 11.

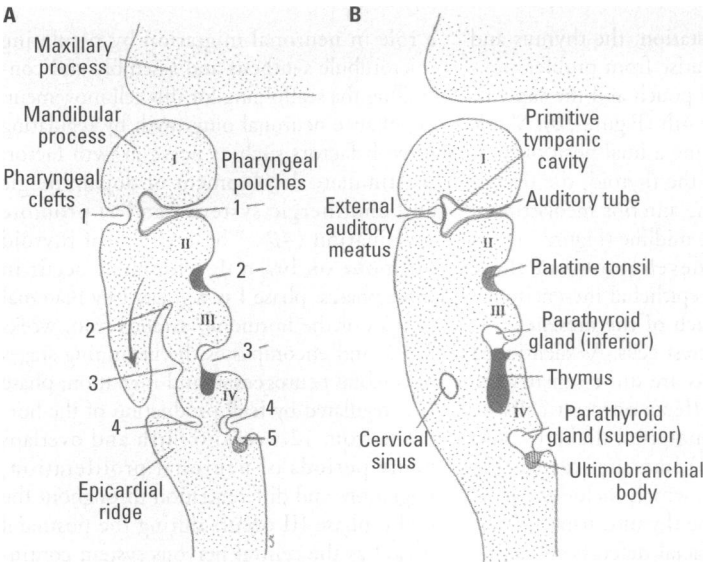

Figure 10. Drawings of a longitudinal section through the pharyngeal region of a 4-week embryo showing endoderm-lined pouches on the interior of the pharyngeal arches. These pouches form the auditory tube (#1), palatine tonsils (#2), inferior parathyroid glands and thymus (#3), and the superior parathyroid glands and ultimobranchial body (#4). Endoderm from these regions forms the parenchyma of the glands and surrounding mesoderm forms the connective tissue. These tissues migrate to their final positions, such that the thymus drags the parathyroid tissue of the #3 pouch into a position inferior to that of similar tissue from pouch #4. See Figure 11.

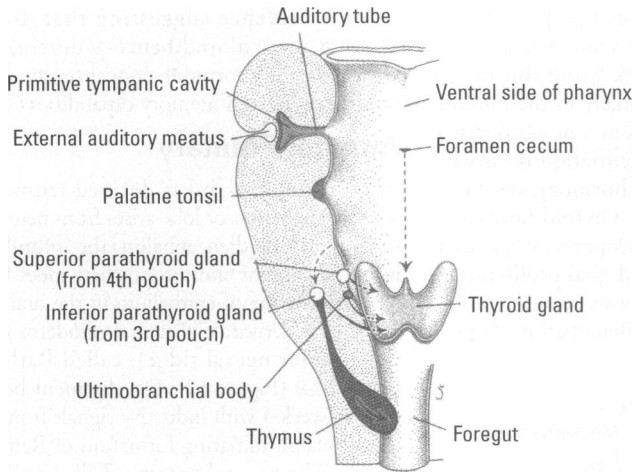

Figure 11. Drawing of the pharyngeal region of a 6-week embryo showing continued migration and development of the endocrine glands. Note that the thymus carries parathyroid tissue with it as it moves toward the superior mediastinum. The thyroid gland originates from endoderm in the floor of the pharynx and migrates in the midline to the larynx. Remnants of tissues from any of these glands may remain along the path of migration.

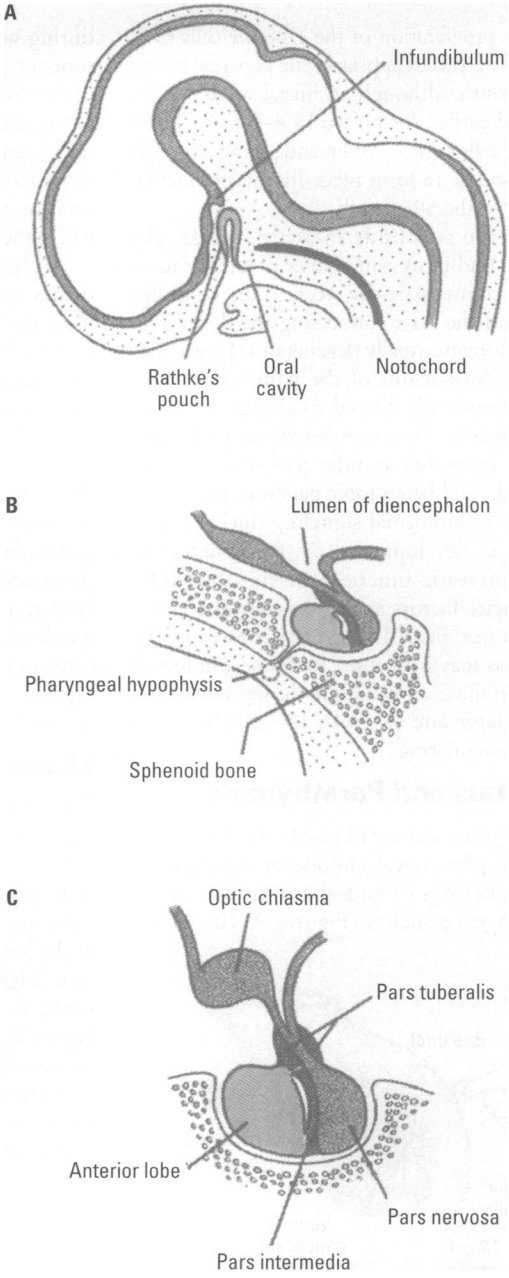

Figure 12. Drawings of sagittal sections through the region of the developing pituitary gland. (*A*) Six-week embryo showing the dual origin of the gland: The anterior lobe is derived from an outpocketing of oral epithelium called Rathke's pouch; the posterior lobe represents a thickening of neural tissue in the floor of the diencephalon called the infundibulum. (*B,C*) Drawings of the pituitary region showing further development in week 11 (*B*) and week 16 (*C*).

Genetic regulation of pituitary development involves a cascade of genes and a nested pattern of homeobox expression to create the different cell types (*45,46*). *PTX1*, a pituitary homeobox gene, is the first to be expressed throughout the epithelium of the stomadeum, including Rathke's pouch, and appears to be the upstream regulator of pituitary differentiation. Its expression is followed closely by that of *RPX* (Rathke's pouch homeobox), whose expression is transiently restricted to the pouch from its initial stages of morphogenesis until approximately the time of cell differentiation. RPX protein can heterodimerize with the homeoprotein PROP1 that upregulates *PIT1*, a *POU* family member essential for differentiation of thyrotrope, somatotrope, and lactotrope cell lineage (*47,48*). Thus, *RPX* appears to be a negative regulator of pituitary differentiation. In addition to these genes, the transcription factors *LIM3*/*LHX3* and *LIM4*/*LHX4* are required for pouch development and subsequently proliferation and differentiation of specific cell lineages (*49*).

The sequential and restricted expression of these genes is also essential for differentiation of the various cell types. As mentioned, *PTX1* initiates the cascade in pituitary stem cells. This expression upregulates expression of *LIM3* and *LIM4* that are essential for continued pituitary development (*50*). Without *LIM3*, pituitary morphogenesis is arrested at the pouch stage. The combined expression of *PTX1*, *RPX*, and *LIM3* leads to the formation of gonadotropes. Expression of *PTX1*, *LIM3*, and *PROP1*, together with downregulation of

Table 1. Periods of susceptibility of the heart and endocrine glands.

Tissue	Sensitive periods	
	Early	Late
Heart	Weeks 3–6	?
Adrenal		
Cortex	Weeks 5–8	7–8 months
Medulla	Weeks 5–8	Week 12
Pancreas	Weeks 4–8	Weeks 10–14
Thyroid	Weeks 5–6	Weeks 8–9
Thymus	Weeks 5–8	?
Parathyroid	Weeks 5–8	?
Pituitary	Weeks 4–9	Weeks 8–13

RPX results in expression of *PIT1* (induced by *PROP1*) and creation of cells destined to form thyrotropes, lactotropes, and somatotropes. To achieve final differentiation of these specific cell types, additional genes have to be expressed. Finally, expression of *PIT1* and *NeuroD1* results in differentiation of coticotropes (*46*).

Abnormalities in the expression pattern of these genes has resulted in agenesis of the pituitary (*TTF1*, *LIM3*) (*44,49*) and growth inhibition (*PIT1*, Snell dwarf mouse; *PROP1*, Ames dwarf mouse) (*50–52*). Whether environmental factors can impact this development is not clear, but retinoids and other factors would seem to be likely candidates to disrupt these processes. One can imagine that the hypothalmic–pituitary–endocrine axis is a very sensitive system with potentially small perturbations capable of having far-reaching consequences.

Summary

In summary, the heart and endocrine glands have a high degree of sensitivity during weeks 3–8 of gestation when the anlage for these organs and tissues is first established. In addition, later periods of sensitivity may occur as the various cell types begin to differentiate as indicated in Table 1.

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