Third to Eighth Week:
The Embryonic Period

The **embryonic period** or period of **organogenesis,** occurs from the **third to the eighth weeks** of development and is the time when each of the three germ layers, **ectoderm, mesoderm,** and **endoderm,** gives rise to a number of specific tissues and organs. By the end of the embryonic period, the main organ systems have been established, rendering the major features of the external body form recognizable by the end of the second month.

Derivatives of the Ectodermal Germ Layer

At the beginning of the third week of development, the ectodermal germ layer has the shape of a disc that is broader in the cephalic than the caudal region. Appearance of the notochord and prechordal mesoderm induces the overlying ectoderm to thicken and form the **neural plate**. Cells of the plate make up the **neuroectoderm** and their induction represents the initial event in the process of **neurulation.**

NEURULATION

Once induction has occurred, the elongated, slipper-shaped neural plate gradually expands toward the primitive streak.

By the end of the third week, the lateral edges of the neural plate become more elevated to form **neural folds,** and the depressed midregion forms the **neural groove**. Gradually, the neural folds approach each other in the midline, where they fuse. Fusion begins in the cervical region and proceeds cranially and caudally. As a result, the **neural tube** is formed. Until fusion is complete, the cephalic and caudal ends of the neural tube communicate with the amniotic cavity by way of the **cranial** and **caudal neuropores,** respectively. Closure of the cranial neuropore occurs at approximately day 25 (18- 20 somite stage), whereas the posterior neuropore closes at day 27 (25 somite stage). Neurulation is then complete, and the central nervous system is represented by a closed tubular structure with a narrow caudal portion, the **spinal cord,** and a much broader cephalic portion characterized by a number of dilations, the **brain vesicles**. As the neural folds elevate and fuse, cells at the lateral border or crest of the neuroectoderm begin to dissociate from their neighbors. This cell population, the **neural crest**, will undergo an epithelial to mesenchymal transition as it leaves the neuroectoderm by active migration and displacement to enter the underlying mesoderm.(**Mesoderm** refers to cells derived from the epiblast and extraembryonic tissues. **Mesenchyme** refers to loosely organized embryonic connective tissue regardless of origin). Crest cells from the trunk region leave the neural folds after closure of the neural tube and migrate along one of two pathways:

1)a dorsal pathway through the dermis, where they will enter the ectoderm through holes in the basal lamina to form **melanocytes** in the skin and hair follicles; and

2)a ventral pathway through the anterior half of each somite to become **sensory ganglia, sympathetic and enteric neurons, Schwann cells,** and **cells of the adrenal medulla**. Neural crest cells also form and migrate from cranial neural folds, leaving the neural tube be- fore closure in this region. These cells contribute to the **craniofacial skeleton** as well as **neurons for cranial ganglia, glial cells, melanocytes,** and other cell types .Induction of neural crest cells requires an interaction between adjacent neural and overlying ectoderm. **Bone morphogenetic proteins (BMPs) ,**secreted by non-neural ectoderm, appear to initiate the induction process.

By the time the neural tube is closed, two bilateral **ectodermal thickenings,** the **otic placodes** and the **lens placodes,** become visible in the cephalic region of the embryo. During further development, the otic placodes invaginate and form the **otic vesicles,** which will develop into structures needed for hearing and maintenance of equilibrium.At approximately the same time, the **lens placodes** appear. These placodes also invaginate and, during the fifth week, form the **lenses** of the eyes.

In general terms, the ectodermal germ layer gives rise to organs and structures that maintain contact with the outside world:

(*a*)the central nervous system;
(*b*)the peripheral nervous system;
(*c*)the sensory epithelium of the ear,nose,and eye;and
(*d*)the epidermis, including the hair and nails.

In addition, it gives rise to subcutaneous glands, the mammary glands, the pituitary gland, and enamel of the teeth.

Neural Crest Derivatives

- Connective tissue and bones of the face and skull
- Cranial nerve ganglia
- C cells of the thyroid gland
- Conotruncal septum in the heart
- Odontoblasts
- Dermis in face and neck
- Spinal (dorsal root)ganglia
- Sympathetic chain and preaortic ganglia
- Parasympathetic ganglia of the gastrointestinal tract
- Adrenal medulla
- Schwann cells
- Glial cells
- Arachnoid and pia mater (leptomeninges)
- Melanocytes

Derivatives of the Mesodermal Germ Layer

Initially, cells of the mesodermal germ layer form a thin sheet of loosely woven tissue on each side of the midline. By approximately the 17th day, however, cells close to the midline proliferate and form a thickened plate of tissue known as **paraxial mesoderm**. More laterally, the mesoderm layer remains thin and is known as the **lateral plate.** With the appearance and coalescence of intercellular cavities in the lateral plate, this tissue is divided into two layers:
(*a*) a layer continuous with mesoderm covering the amnion, known as the **somatic** or **parietal mesoderm layer;** and
(*b*) a layer continuous with mesoderm covering the yolk sac, known as the **splanchnic** or **visceral mesoderm layer**. Together, these layers line a newly formed cavity, the **intraembryonic cavity,** which is continuous with the extraembryonic cavity on each side of the embryo. **Intermediate mesoderm** connects paraxial and lateral plate mesoderm.

PARAXIAL MESODERM

By the beginning of the third week, paraxial mesoderm is organized into segments. These segments, known as **somitomeres,** 1st appear in the cephalic region of the embryo, and their formation proceeds cephalocaudally. Each somitomere consists of mesodermal cells arranged in concentric whorls around the center of the unit. In the head region, somitomeres form in association with segmentation of the neural plate into **neuromeres** and contribute to mesenchyme in the head. From the occipital region caudally, somitomeres further organize into somites. The 1st pair of somites arises in the occipital region of the embryo at approximately the 20th day of development. From here, new somites appear in craniocaudal sequence at a rate of approximately three pairs per day until,at the end of the . 5th week, 42 to 44 pairs are present. There are four occipital, eight cervical,12 thoracic, 5 lumbar, 5 sacral, and eight to 10 coccygeal pairs. The 1st occipital and the last .five to seven coccygeal somites later disappear, while the remaining somites form the axial skeleton. During this period of development, the age of the embryo is expressed in number of somites.

By the beginning of the fourth week, cells forming the ventral and medial walls of the somite lose their compact organization, become polymorphous, and shift their position to surround the notochord. These cells, collectively known as the **sclerotome,** form a loosely woven tissue, the **mesenchyme.** They will surround the spinal cord and notochord to form the vertebral column. Cells at the dorsolateral portion of the somite also migrate as precursors of the limb and body wall musculature. After migration of these muscle cells

and cells of the sclerotome, cells at the dorsomedial portion of the somite proliferate and migrate down the ventral side of the remaining dorsal epithelium of the somite to form a new layer, the myotome. The remaining dorsal epithelium forms the dermatome, and together these layers constitute the dermomyotome. Each segmentally arranged myotome contributes to muscles of the back, while dermatomes disperse to form the dermis and subcutaneous tissue of the skin. Furthermore, each myotome and dermatome retains its innervation from its segment of origin, no matter where the cells migrate. Hence each somite forms its own **sclerotome** (the cartilage and bone component), its own **myotome** (providing the segmental muscle component), and its own **dermatome,** the segmental skin component. Each myotome and dermatome also has its own segmental nerve component.

INTERMEDIATE MESODERM

Intermediate mesoderm,which temporarily connects paraxial mesoderm with the lateral plate, differentiates into urogenital structures. In cervical and upper thoracic regions, it forms segmental cell clusters (future **nephrotomes**),whereas more caudally, it forms an unsegmented mass of tissue, the **nephrogenic cord.** Excretory units of the urinary system and the gonads develop from this partly segmented, partly unsegmented intermediate mesoderm.

LATERAL PLATE MESODERM

Lateral plate mesoderm splits into parietal and visceral layers, which line the intraembryonic cavity and surround the organs, respectively. Mesoderm from the parietal layer, together with overlying ectoderm, will form the lateral and ventral body wall. The visceral layer and embryonic endoderm will form the wall of the gut. Mesoderm cells of the parietal layer surrounding the intraembryonic cavity will form thin membranes,the **mesothelial membranes,** or **serous membranes,** which will line the peritoneal, pleural, and pericardial cavities and secrete serous fluid. Mesoderm cells of the visceral layer will form a thin serous membrane around each organ .

BLOOD AND BLOOD VESSELS

Blood vessels form in two ways: **vasculogenesis,** whereby vessels arise from blood islands, and **angiogenesis,** which entails sprouting from existing vessels. The 1st blood islands appear in mesoderm surrounding the wall of the yolk sac at 3 weeks of development and slightly later in lateral plate mesoderm and other regions. These islands arise from mesoderm cells that are induced by broblast growth factor 2 (FGF-2) to form **hemangioblasts,** a common precursor for vessel and blood cell formation. Hemangioblasts in the center of blood islands form **hematopoietic stem cells,** the precursors of all blood cells, whereas peripheral hemangioblasts differentiate into **angioblasts,** the precursors to blood vessels. These angioblasts proliferate and are eventually induced to form endothelial cells by **vascular endothelial growth factor (VEGF)** secreted by surrounding mesoderm

cells.This same factor then regulates coalescence of these endothelial cells into the . 1st primitive blood vessels.

Once the process of vasculogenesis establishes a primary vascular bed, additional vasculature is added by angiogenesis, the sprouting of new vessels. This process is also mediated by VEGF, which stimulates proliferation of endothelial cells at points where new vessels are to be formed. Maturation and modeling of the vasculature are regulated by other growth factors, including platelet-derived growth factor (PDGF)and transforming growth factor â (TGF-â), until the adult pattern is established. As mentioned,the .1st blood cells arise in the blood islands of the yolk sac, but this population is transitory. The de .nitive hematopoietic stem cells arise from mesoderm surrounding the aorta in a site called theexisting vessels. The 1st blood islands appear in mesoderm surrounding the wall of the yolk sac at 3 weeks of development and slightly later in lateral plate mesoderm and other regions.

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Derivatives of the Endodermal Germ Layer

The gastrointestinal tract is the main organ system derived from the endodermal germ layer. This germ layer covers the ventral surface of the embryo and forms the roof of the yolk sac. With development and growth of the brain vesicles, however, the embryonic disc begins to bulge into the amniotic cavity and to fold **cephalocaudally.** This folding is most

pronounced in the regions of the head and tail, where the **head fold** and **tail fold** are formed.

As a result of cephalocaudal folding, a continuously larger portion of the endoderm-lined cavity is incorporated into the body of the embryo proper. In the anterior part, the endoderm forms the **foregut;** in the tail region, it forms the **hindgut.** The part between foregut and hindgut is the **midgut.** The midgut temporarily communicates with the yolk sac by way of a broad stalk, the **vitelline duct**. This duct is wide initially, but with further growth of the embryo, it becomes narrow and much longer.

At its cephalic end, the foregut is temporarily bounded by an ectodermal- endodermal membrane called the **buccopharyngeal membrane**. In the fourth week, the buccopharyngeal membrane ruptures, establishing an open connection between the amniotic cavity and the primitive gut. The hindgut also terminates temporarily at an ectodermal- endodermal membrane, the **cloacal membrane**, which breaks down in the seventh week to create the opening for the anus. As a result of rapid growth of the somites,the initial .at embryonic disc also folds laterally, and the embryo obtains a round appearance. Simultaneously,the ventral body wall of the embryo is established except for a small part in the ventral abdominal region where the yolk sac duct and connecting stalk are attached. While the foregut and hindgut are established, the midgut remains in communication with the yolk sac. Initially, this connection is wide, but as a result of body folding,it gradually becomes long and narrow to form the **vitelline duct**. Only much later, when the vitelline duct is obliterated does the midgut lose its connection with the original endoderm-lined cavity and obtain its free position in the abdominal cavity.

Another important result of cephalocaudal and lateral folding is partial incorporation of the allantois into the body of the embryo, where it forms the **cloaca**. The distal portion of the allantois remains in the connecting stalk. By the 5^{th} week, the yolk sac duct, allantois,and umbilical vessels are restricted to the region of the umbilical ring. In humans,the yolk sac is vestigial and in all probability has a nutritive role only in early stages of development. In the second month of development, it lies in the chorionic cavity.

Hence,the endodermal germ layer initially forms the epithelial lining of the primitive gut and the intraembryonic portions of the allantois and vitelline duct.During further development,it gives rise to

(*a*)the epithelial lining of the respiratory tract;
(*b*)the **parenchyma** of the thyroid,parathyroids,liver, and pancreas;
(*c*)the reticular stroma of the tonsils and thymus;
(*d*)the epithelial lining of the urinary bladder and urethra ;and
(*e*)the epithelial lining of the tympanic cavity and auditory tube.

External Appearance During the Second Month

At the end of the fourth week, when the embryo has approximately 28 somites, the main external features are the somites and pharyngeal arches. The age of the embryo is

therefore usually expressed in somites. Because counting somites becomes dificult during the second month of development, the age of the embryo is then indicated as the **crown-rump length (CRL)**. CRL is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks.

During the second month, the external appearance of the embryo is changed by an increase in head size and formation of the limbs, face ,ears, nose, and eyes. By the beginning of the 5th week, forelimbs and hindlimbs appear as paddle-shaped buds.The former are located dorsal to the pericardial swelling at the level of the fourth cervical to the 1st thoracic somites, which explains their innervation by the **brachial plexus.** Hindlimb buds appear slightly later just caudal to attachment of the umbilical stalk at the level of the lumbar and upper sacral somites. With further growth, the terminal portions of the buds .atten and a circular constriction separates them from the proximal, more cylindrical segment. Soon, four radial grooves separating . five slightly thicker areas appear on the distal portion of the buds, foreshadowing formation of the digits. These grooves, known as **rays,** appear in the hand region. first and shortly afterward in the foot, as the upper limb is slightly more advanced in development than the lower limb. While fingers and toes are being formed, a second constriction divides the proximal portion of the buds into two segments, and the three parts characteristic of the adult extremities can be recognized.

Birth Defects

Most major organs and organ systems are formed during the third to eighth week. ***This period, which is critical for normal development, is therefore called the period of organogenesis.*** Stem cell populations are establishing each of the organ primordia, and these interactions are sensitive to insult from genetic and environmental influences. Thus, this period is when most gross structural birth defects are induced. ***Unfortunately, the mother may not realize she is pregnant during this critical time, especially during the third and fourth weeks, which are particularly vulnerable.*** Consequently, she may not avoid harmful influences, such as cigarette smoking and alcohol. Understanding the main events of organogenesis is important for identifying the time that a particular defect was induced and, in turn, determining possible causes for the malformation.