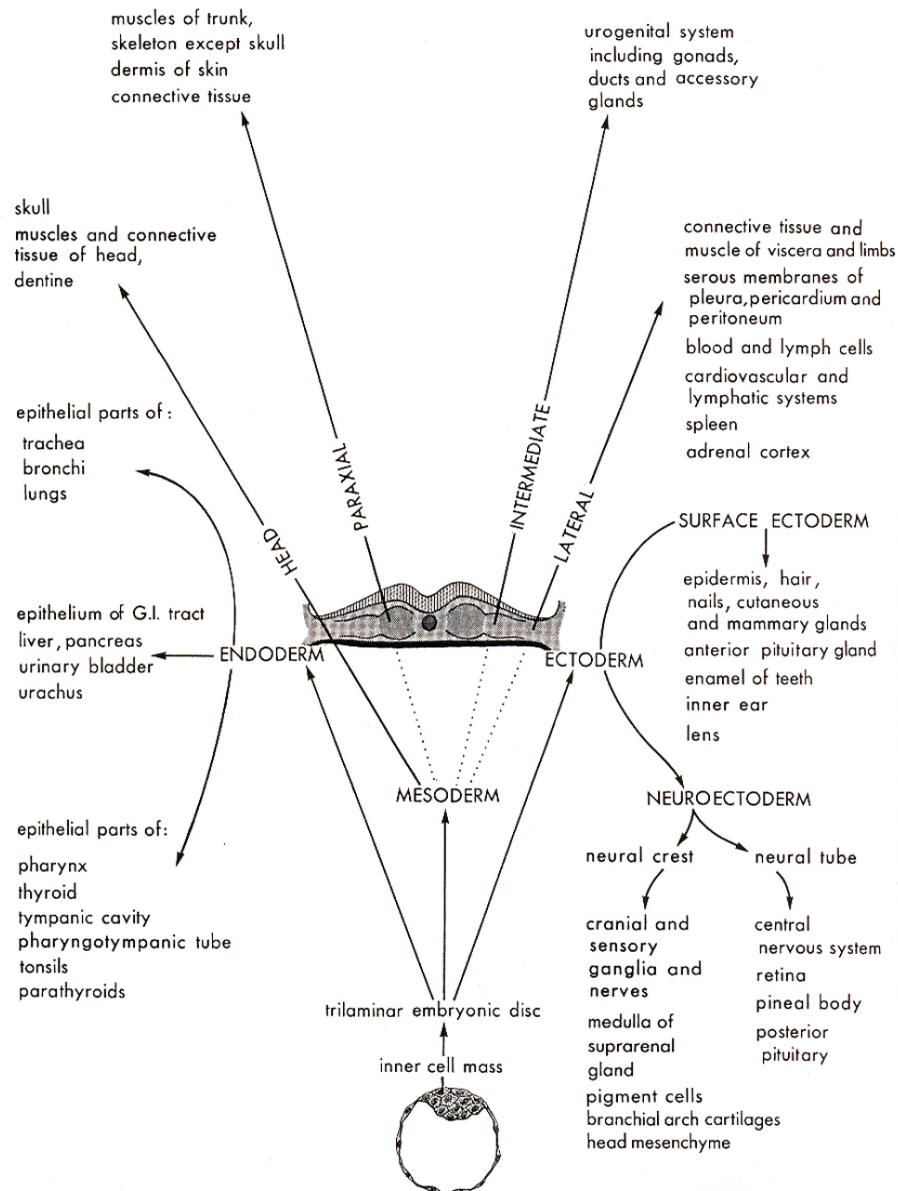


Lecture 2 - Making babies: Organ formation in the Ectoderm, Mesoderm, Endoderm and Neural Crest

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Lecture Objectives

- To examine how the main organ systems are formed between the 4th and 8th week of embryonic development. This general overview will set the stage to understanding how the human body is constructed.



Scheme illustrating the origin and derivatives of the three germ layers: ectoderm, endoderm, and mesoderm. Cells from these layers make contributions to the formation of the different tissues and organs; e.g., the endoderm forms the epithelial lining of the gastrointestinal tract, and the mesoderm gives rise to its connective tissues and muscles. From Persaud's book.

- The period of organogenesis constitutes the period of highest sensitivity to **teratogens** that cause congenital malformations (Figure from Langman's inner cover).

- New findings have extended the critical stages for human congenital malformations. First, the **Zika virus** infects brain cortex stem cells called radial glia which divide during all three trimesters. The emerging Zika epidemic causes microcephaly, and epilepsy in milder cases. Second, recent genome sequencing of patients with cardiac and other malformations reveals the presence of heterozygous loss-of-function **mutations** that are absent from both parents. Thus, *de novo* mutations in the gametes (for example in histone-methylation genes) result in genetic **haploinsufficiency** to a higher degree than was previously suspected in humans.

4) THE FOURTH TO EIGHTH WEEKS - Organogenesis.

Overview of organ systems:

- a) Ectodermal organs. The **neural plate** and closure of the **neural tube**. Surface ectoderm and its derivatives. The three **brain vesicles**. **Cerebrospinal fluid** (CSF) and the **four brain ventricles**. (6 slides)
- b) Mesodermal derivatives. **Notochord**, **somite**, **intermediate mesoderm** (kidney) and **lateral plate mesoderm**. **Visceral (splanchnic) mesoderm** and **parietal** or body wall **mesoderm** (also called somatic mesoderm). **Mesentery** formation. Disaggregation of somite cells into **sclerotome**, **myotome**, and **dermatome**. (6 slides)
- c) Endodermal organs. Derivatives of **foregut**, **midgut** and **hindgut**. (2 slides)
- d) Neural crest derivatives. **Pharyngeal arches**, **cranial bones** and **melanocytes**. The **peripheral nervous system**. Diseases of the neural crest. (6 slides)

4a. DERIVATIVES OF THE ECTODERMAL LAYER - NEURULATION

- **Neural plate** is induced by proteins, such as Chordin and Noggin, secreted by the underlying mesoderm (primitive node, the human equivalent of Spemann's organizer). Neural induction.
- **Neural folds** elevate and fuse in the midline, forming the **neural tube** (Langman's Fig. 6.2, 6.3).
- **Neural closure** proceeds anteriorly and posteriorly, like a zipper. The **anterior neuropore** closes at day 25 and the **posterior neuropore** closes at day 27 (Langman's Fig. 6.3, 6.4). At these stages the **somites** and **pharyngeal arches** can also be seen very well (Langman's Fig. 6.3 and 6.4).
- The neural tube or **central nervous system (CNS)** consists of the **spinal cord**

and three **brain vesicles: forebrain** (telencephalon including cerebral cortex, and the diencephalon), **midbrain**, and **hindbrain** (giving rise to cerebellum and medulla oblongata) (1 slide). At early stages, CNS anatomy is simpler.

- The brain has four **ventricles** (two **lateral ventricles** in forebrain, the **third ventricle** in the midbrain, and the **fourth ventricle** in the hindbrain) filled with **cerebrospinal fluid (CSF)**.
- The hindbrain is divided into eight segments (called **rhombomeres**) from which **cranial nerves** arise (Langman's Fig. 18.40). Nerve I (olfactory) originates from telencephalon. Nerve II (optic) from diencephalon. Nerve III (oculomotor) from mesencephalon. The rest (IV to XII) arise from hindbrain segments. In the spinal cord, one pair of spinal nerves arises per vertebral segment.
- An organizing center is found in the **midbrain-hindbrain isthmus**. The inductive activity of this **isthmic organizer** (mediated by the secretion of FGF8 and Wnt-1) is required for **cerebellum** development.

Clinical correlations: Failure of neuropore closure causes **spina bifida** and **anencephaly** (Langman's Fig. 6.7).

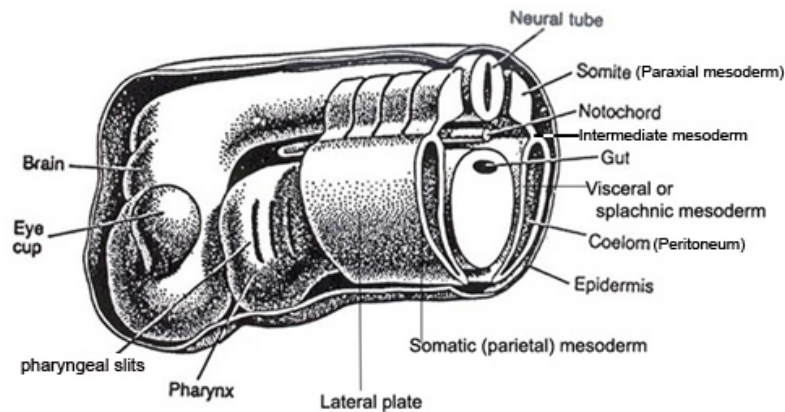
If the neuropore does not close, **Alpha-Feto Protein (AFP)** secreted by fetal liver passes from cerebrospinal fluid to the amniotic fluid and from there into the mother's plasma. This provides the basis for a diagnostic test for neural tube closure defects.

Having a blood test for fetal pathologies from the mother's plasma is very practical. Recently, it has been found that 10% of DNA sequences found in plasma come from fetal tissues (which undergo apoptosis and release nucleosomes into the maternal circulation). Using high-throughput sequencing one can now diagnose chromosomal trisomies without need of amniocentesis.

Incidence of neural tube closure defects is about 1:1000 births. However, it was as high as 1:100 in regions of China in which green foliage was not included in the diet. Diet supplementation with the vitamin folic acid (so-called because it is present in foliage) decreases the incidence of neural closure defects by as much as 70%. (Folic acid works by facilitating methylation of DNA at cytosine residues; DNA methylation keeps genes repressed).

4b. DERIVATIVES OF THE MESODERMAL LAYER

The Chordate general body plan:



There are four characteristics common to the body plan of all **chordates**: 1) a dorsal CNS, 2) a notochord, 3) pharyngeal gill slits (pharyngeal arches) and 4) a postanal tail.

- In all chordates the mesoderm is subdivided (Langman's 6.8) into:

- 1) **Axial mesoderm** or **Notochord**. The notochord is a flexible rod used by aquatic larvae for swimming. In the mammal the notochord functions as a signaling center and eventually disappears, except for forming the pulp of the intervertebral discs (nucleus pulposus). During embryonic development we see many transitory structures that act as signaling centers and then disappear in the adult (other good examples are the primitive streak and the somites).
- 2) **Paraxial mesoderm**. Somites represent the segmented body plan of the vertebrate (Langman's Figs. 6.9, 6.8). **Somites** are blocks of epithelial cells that are formed by segmentation in a rostro-caudal sequence from paraxial mesoderm in a mesenchymal to epithelial transition. These cells then disaggregate and form a multitude of tissues.
- 3) **Intermediate mesoderm** is also segmented and gives rise to a **nephric tubule** (kidney) in each segment (Figs. 6.9, 6.13) and the **urogenital ducts**.
- 4) **Lateral plate** mesoderm is not segmented and becomes subdivided (split down the middle) by the formation of the **intraembryonic** or **coelomic cavity** (future **peritoneum** and **pleural cavities**) (Langman's 6.13). The coelom is covered by **mesothelium** (mesothelium means epithelium originated from mesenchyme, an example of mesenchymal to epithelial transition). The mesothelial

membranes (also called **serous membranes**) in the human body are the **peritoneum**, **pericardium** and lung **pleura**. Formation of the coelom generates two subdivisions in the lateral plate mesoderm:

- a) **Parietal** (or **Somatic**) **mesoderm** that forms the **body wall**, and
- b) **Visceral** (or **Splachnic**) **mesoderm** (Gr. Splachno, viscera) which surrounds the endodermal organs. Later, in this layer **blood islands** form, indicating the beginning of **vasculogenesis**.

The visceral and parietal lateral plate mesoderm remain connected to each other by the **dorsal mesentery**, bringing blood vessels and nerves along the entire length of the gut. (Langman's Fig. 6.13). Development of the coelom and mesenteries helps one understand abdominal anatomy.

I will show you all these mesodermal subdivisions in embryo histological sections through the discussion microscope at Embryology Labs.

SOMITE DIFFERENTIATION: Somites generate our segmented body plan by subdividing the paraxial mesoderm. Three pairs of somites are formed per day (i.e., one every eight hours, starting at day 20). The somite forms an epithelial vesicle that later disaggregates and disperses as mesenchyme (epithelial-mesenchymal transition). Somites give rise to many of the tissues in our body through three groups of cells (Langman's Fig. 6.11):

- 1) **Sclerotome** – gives rise to hard tissues such as bone, cartilage and tendons of the future **vertebral column** and skeleton. Formation of the vertebral bodies is induced by the notochord. There are 7 cervical, 12 thoracic (connected to ribs), 5 lumbar, 5 sacral and 8 to 10 coccygeal vertebrae in humans.
 - 2) **Myotome** – gives rise to the **skeletal muscles** (i.e., voluntary) of the vertebral column, body wall and limbs.
 - 3) **Dermatome** – gives rise to cells of the **dermis** (subcutaneous connective tissue).
- Importantly, each dermatome and myotome is innervated by nerves originating in the CNS next to the original somite, even after extensive cell migrations during embryonic development. This segmental body organization is important for neurological diagnosis in the adult (Langman's Fig. 12.8). The human body plan is segmented because as embryos we have somites.

4c. DERIVATIVES OF THE ENDODERMAL GERM LAYER

- **Endoderm** is divided into **foregut**, **hindgut** and **midgut** by the appearance of

head and **tail folds** that deform the flat embryo. The midgut connects to the yolk sac (Langman's Fig. 6.17). A good animation of this process can be seen at: <https://www.youtube.com/watch?v=yXUv4MPuNTA>

- In the anterior an **oropharyngeal membrane** and in the posterior a **cloacal membrane** are formed. They result from the direct apposition of endoderm and ectoderm (with no mesoderm in between). Later in development they are perforated at the level of the velum (between the mouth and pharynx) and the anus, respectively. Oropharyngeal perforation takes place at 4 weeks, cloacal membrane perforation at 7 weeks.
- The **foregut** gives rise to the epithelium of the **pharyngeal endoderm** (including **pharyngeal pouches** derivatives such as **tonsils, thymus, thyroid** and **parathyroid glands**), **trachea, lung buds, esophagus, stomach, liver, gall bladder, pancreas** and part of the **duodenum** (Langman's Fig. 6.19).
- The **midgut** gives rise to the epithelium of **posterior duodenum, small intestine, ascending colon** and part of **transverse colon**.
- The **hindgut** gives rise to the epithelium of the rest of the **transverse colon, descending colon, sigmoid, rectum, urinary bladder, prostate and urethra**.
- Each of the main subdivisions of the gut - foregut, midgut and hindgut - has its own separate artery. This is important for gastrointestinal surgery.

4d. THE NEURAL CREST

- **Neural crest** cells migrate away from the neural folds by an epithelial-mesenchymal transition and undergo extensive cell migrations to colonize a multitude of organs. (Langman's Fig. 6.5, Table 6.1).
- The neural crest cells proliferate extensively after leaving the neuroectoderm. Given its important contributions to embryonic development, particularly in the head region, the neural crest is sometimes called the fourth germ layer.
- The neural crest forms all **melanocytes** of the skin, sensory neurons of the **dorsal root spinal ganglia**, the **suprarenal (adrenal) medulla**, and the **Schwann cells** that cover peripheral nerves. In addition, the **autonomous (visceral) nervous system**, both **sympathetic** (adrenalin) and **parasympathetic** (enteric ganglia producing acetylcholine) is of neural crest origin (Langman's Fig. 6.5).
- Neural crest cells of the head region give rise to most of the bones of the

cranium and the **pharyngeal arches** (Langman's Fig. 17.1, 17.12). The neural crest of **pharyngeal arch I**, both in its **maxillary** and **mandibular** subdivisions, is particularly sensitive to teratogenesis by retinoic acid, which can cause ectopic (more anterior) expression of Hox genes. This can result in **cleft palate**, **micrognathia** (small jaw) and other malformations.

Clinical correlations: Diseases of neural crest development are very important:

- **Hirschsprung disease** or megacolon (caused by mutations in the neural crest cell growth factor receptor **c-ret**) results from failure of neural crest migration and absence of parasympathetic neurons in the rectum (Langman's Fig. 18.41), leading to excessive contraction of its musculature (and megacolon upstream from it).
- **Piebald** pigmentation caused by mutation in the neural crest growth factor receptor **c-kit** (slide). This receptor is needed not only for melanocyte migration, but also for blood stem cell (causing anemia) and germ cell (causing sterility) development.
- **Waardenburg syndrome** (caused by mutations in Pax3 transcription factor), presents with frontal blaze of white hair, sometimes microtia and deafness.

Conclusion: Medical Embryology helps you to understand the plan by which the human body and its organs are constructed. It will be useful throughout your medical studies. Hopefully you will return to your Medical Embryology textbook when confronted with individual patients to understand the pathogenesis of diseases of the various organ systems.

This was a very general tour of human embryogenesis from fertilization to the eighth week, at the end of which all organ systems have been formed. We will review the body plan in embryology labs. A period of rapid fetal growth then takes place during the rest of the pregnancy, as will be explained by Prof. Robert Trelease tomorrow.

EMBRYONIC DEVELOPMENT IN DAYS

DEVELOPMENT WEEK 1		DEVELOPMENT WEEK 2		DEVELOPMENT WEEK 3		DEVELOPMENT WEEK 4		DEVELOPMENT WEEK 5		DEVELOPMENT WEEK 6		DEVELOPMENT WEEK 7																																	
Day 1 FERTILIZATION	Day 2 TWO CELL STAGE	Day 3 MORULA	Day 4 EARLY BLASTOCYST	Day 5 LATE BLASTOCYST	Days 6-7 EVENTS DURING FIRST WEEK	Day 8	Day 9 TROPHOBLAST WITH LACUNAE	Day 10-11 EMBRYO IN UTERUS 10-11 DAYS AFTER OVULATION	Day 12	Day 13 UTEROPLACENTAL CIRCULATION BEGINS	Day 14 EMBRYONIC DISC SEEN FROM DORSAL	Day 15 DORSAL ASPECT OF EMBRYO	Day 16 TRILAMINAR GERM DISC	Day 17 MESODERM MIGRATION	Day 18 CUT EDGE OF AMNION	Day 19 FORMATION OF SOMITES	Day 20 APPEARANCE OF SOMITES	Day 21 TRANSVERSE SECTION THROUGH SOMITE REGION	Day 22	Day 23	Day 24-25 VILLUS FORMATION	Day 26 BRANCHIAL ARCHES	Day 27	Day 28	Day 29 ARM AND LEG BUDS	Day 30 DEVELOPING FACE	Day 31	Day 32 EMBRYO IN CHORIONIC CAVITY	Day 33	Day 34 DEVELOPING LIMB BUDS	Day 35 BRANCHIAL ARCHES AND CLEFTS	Day 36 PHYSIOLOGICAL UMBILICAL HERNIA	Day 37 DEVELOPING FACE	Day 38	Day 39 ENDODERMAL DERIVATIVES	Day 40 EMBRYO IN UTERO	Day 41	Day 42	Day 43	Day 44 DEVELOPING FACE	Day 45	Day 46	Day 47	Day 48	Day 49 FETAL MEMBRANES IN THIRD MONTH