Embryonic Staging: The Carnegie System

3

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Historical Background

The science of embryology in the United States was born out of a unique combination of personalities and events involving philanthropy, the importation of a new system, medical education, friendships made abroad, and artistic talent.

Johns Hopkins (1795–1873) was born to Quaker parents of strong convictions. His father, a tobacco planter, in response to the early abolitionist movement, freed his slaves in 1807. Johns went to work to help his family at age 12, beginning the grocery business and quickly demonstrated an acumen for business. Unable to marry his cousin for religious reasons, he devoted his life to his work and became highly successful, investing in the Baltimore & Ohio railroad, of which he eventually became director in 1847. He was active in banking and real estate and was able to retire at age 52 (Fig. 3.1).

Social involvement and charity were core values for Johns Hopkins. A lifelong abolitionist, during the Civil War he worked with the Rev. Henry Ward Beecher and supported President Lincoln's vision for post-war emancipation. Later in life, he founded Johns Hopkins Orphanage for Colored Children. When the city of Baltimore hit upon financial straits, he gave the city financial support.

Cholera and yellow fever epidemics in Baltimore made a deep impression upon Hopkins who saw the city's need for a medical facility, particularly in view of the advances in medicine that took place during the Civil War. He was also impressed by the German university system, with its emphasis on research. Accordingly, in 1870, he made a will of $7 million in B%O stock to establish a research university, a free hospital with university-based schools of medical and nursing. Half of the endowment went toward the creation of Johns Hopkins University as the first formally research-based institution in the United States. Johns Hopkins Hospital was founded in 1876 and the School of Medicine opened its doors in 1893 (Fig. 3.2).

Johns Hopkins occupies a unique place in the history of American medical education. Based upon the Heidelberg model, Hopkins imposed strict entrance requirements and

Fig. 3.1 Johns Hopkins. (1) Whitehall tobacco plantation: (a) Hannah Hopkins $10,000. (2) B&O Railroad. (3) 1870 Johns Hopkins University. (4) 1889 Johns Hopkins Hospital. (5) 1893 Johns Hopkins Medical School. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Hopkinsp.jpg)

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Fig. 3.2 Johns Hopkins Hospital. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Johns_Hopkins_Hospital_in_Baltimore_1900s.jpg)

academic structures, thus becoming the birthplace of the modern residency system. The original core faculty members, the "big four", were not only renowned contributors in their fields, but colorful characters as well. William Henry Welch (pathology) was a confirmed bachelor and gourmand who could consume up to five desserts in a single sitting. Howard Kelly (obstetrics and gynecology), an evangelist with an interest in saving souls, kept snakes for pets. William Osler (medicine) was a practical joker. William Stewart Halsted (surgery) was pathologically shy, but an iron taskmaster with his students. His later experiments with local anesthesia led to a debilitation addiction to cocaine. In addition to setting up the first Ph.D. programs in medical science, Hopkins established brand new departments for the history of medicine and for art as applied to medicine under the direction of world famous medical illustrator Max Brödel (Fig. 3.3).

Andrew Carnegie (1835–) was a Sottish-born industrialist and philanthropist who made an indelible contribution to the study of human development. His biography is compelling story and the subject of a PBS documentary. Born in Dunfermline, Scotland, he was influenced by his father, William, who engaged in radical politics and was involved in the creation of a Trademen's Subscription Library. At age 13, the family moved to Allegeney, Pennsylvania. He was a voracious reader using a library. When he was denied access to it, he fought for his own education, writing to the owners to the effect that no one should be denied access to learning based on their station in life (Figs. 3.4 and 3.5).

He got his start as a telegraph operator with the Pennsylvania Railroad and transitioned into the railroad car business. In 1885, he started a new venture with the Keystone Bridge Company. This morphed into the Carnegie Steel Company. By understanding the new technology of the Bessamer process, he revolutionized the industry. In 1891, Carnegie Steel was sold to J.P. Morgan of US Steel for the unheard sum of $480 million. Carnegie vowed to devote the remainder of his life to philanthropy.

In keeping with its founder's belief in education, the Carnegie Foundation created 2509 public libraries worldwide. The Foundation sponsored and International Endowment for Peace. Most relevant for us, in 1902, in cooperation with President Theodore Roosevelt, the Carnegie Institution for Science was founded with a $22 million endowment in Washington, DC., as a think tank for the advancement of knowledge. A Department of Embryology was created in 1913 in affiliation with the Department of Anatomy at nearby Johns Hopkins Medical School, the first purely university-based medical institution in the United States. This effort was captained by Prof. Franklin Mall (Figs. 3.6 and 3.7).

Franklin P. Mall was born in 1862 in Belle Plaine, Iowa. He studied medicine at the University of Michigan (one of his classmates was William P. Mayo). In 1884, he followed

Fig. 3.3 The Four Doctors by John Singer Sargent, portrait in oil, 1906. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Four_doctors_1907.jpg. With permission from Free Art License 1.3 (FAL 1.3): http://artlibre.org/licence/lal/en/)

his research interests in Leipzig, Germany, where he studied under embryologist Wihelm His. There he met pathologist William Welch, the future head of the Johns Hopkins Medical School In 1886, Mall returned to study pathology at the Johns Hopkins Hospital. 1893 saw the opening of the medical school at Johns Hopkins and Mall was immediately appointed by Welch as Chairman of Anatomy (Fig. 3.8 Mall).

From 1910 to 1912, Mall edited with Franz Keibel the Handbook of Human Embryology and published several monographs in conjunction with the Carnegie Institution. With this impetus, he successfully lobbied for the creation of the Embryology Department at Carnegie in 1913, which he chaired until his death in 1917. Mall was succeeded by George Streeter who studied Mall's embryo collection and began work on a staging system consisting of 23 Horizons. Streeter retired in 1940 and work was continued by the third Chairman, Dr. George Corner, whose beautiful three-dimensional drawings can be accessed through the Carnegie Institution and through the UNSW website.

In 1973, the entire embryo collection was transferred to the University of California Davis Medical School along with Drs. Ronan O'Rahilly and Fabiola Müller. They further refined the Carnegie system and their work, *Developmental Stages in Human Embryos*, was published as Carnegie Institution Publication No. 637 in 1987. Following O'Rahilly and Müllers retirement to Europe, the collection was transferred in 1990 to the National Museum of Health and Medicine in Washington, DC, where it remains as a resource for research. In 2005, the Carnegie Department of

Fig. 3.4 Andrew Carnegie (1835–1919) was born in Dunfermline, Scotland to William Carnegie, a craft weaver who espoused radical politics and created a Tradesmaen's Subscription Library. He emigrated to the United in 1848 and settled in Allentown, Pennsymvania. He subsequently built Carnegie Steel which he sold to J.P. Morgan in 1901 for the sum in today's US dollars of $10 billion. As a champion of philanthropy used his immense wealth to build 2,508 public libraries world-wide, create an International Endowment for Peace, and establish the Carnegie Institute for Science which included a Department of Embryology. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Andrew_Carnegie_by_Francis_Luis_Mora.jpg)

Fig. 3.5 Carnegie Homestead Steel Works, Johnston, PA. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Homestead_Steel_Works,_Homestead,_Pa._(det.4a10138).tif)

Fig. 3.6 Carnegie Institution, cartoon. The Institution was founded with an astounding $22 million (almost half a billion today). (Reprinted from Washington Evening Star; 1911)

Embryology moved to the Maxine Singer building on the Johns Hopkins Homewood campus.

Our foray into the history of embryology at the Carnegie Institution would not be complete without recognizing the unique contributions made by medical artist Dorcas Padget, a self-taught neuroembryologist and researcher whose work is the foundation of our understanding for the vascular development of the head and neck. Her story is inextricably woven with that of pioneer neurosurgeon Walter Dandy at Johns Hopkins and of Max Brödel, world famous medical illustrator and the founder of the Department for Art as Anatomy at Hopkins (Fig. 3.9 left).

Dandy was himself a talented artist. As a student at Hopkins, his work was noted by Franklin Mall, who stimulated Dandy to reconstruct an early human embryo from his collection. Brödel was Dandy's instructor as he worked drawings of the neurovascular supply of the pituitary (Fig. 3.9 right) As chairman of Neurosurgery at Hopkins, Dandy's neurosurgical research progressed to the point where a full-time illustrator was needed for his department. Dorcas Padget was born in Albany, NY, in 1906 and was interested in science throughout her life. She received a full scholarship to Vassar College, where she studied art under Professor Treadwell. Recognizing her talent, in 1926, he referred Padget to Max Brödel to specialize in medical art.

Fig. 3.7 Carnegie Institution for Science. (Reprinted from Wikimedia. Retrieved from: https://commons.wikimedia.org/wiki/File:Administration_Building_-_Carnegie_Institution_of_Washington.JPG. With permission from Creative Commons License 3.0: https://creativecommons.org/licenses/by-sa/3.0/deed.en)

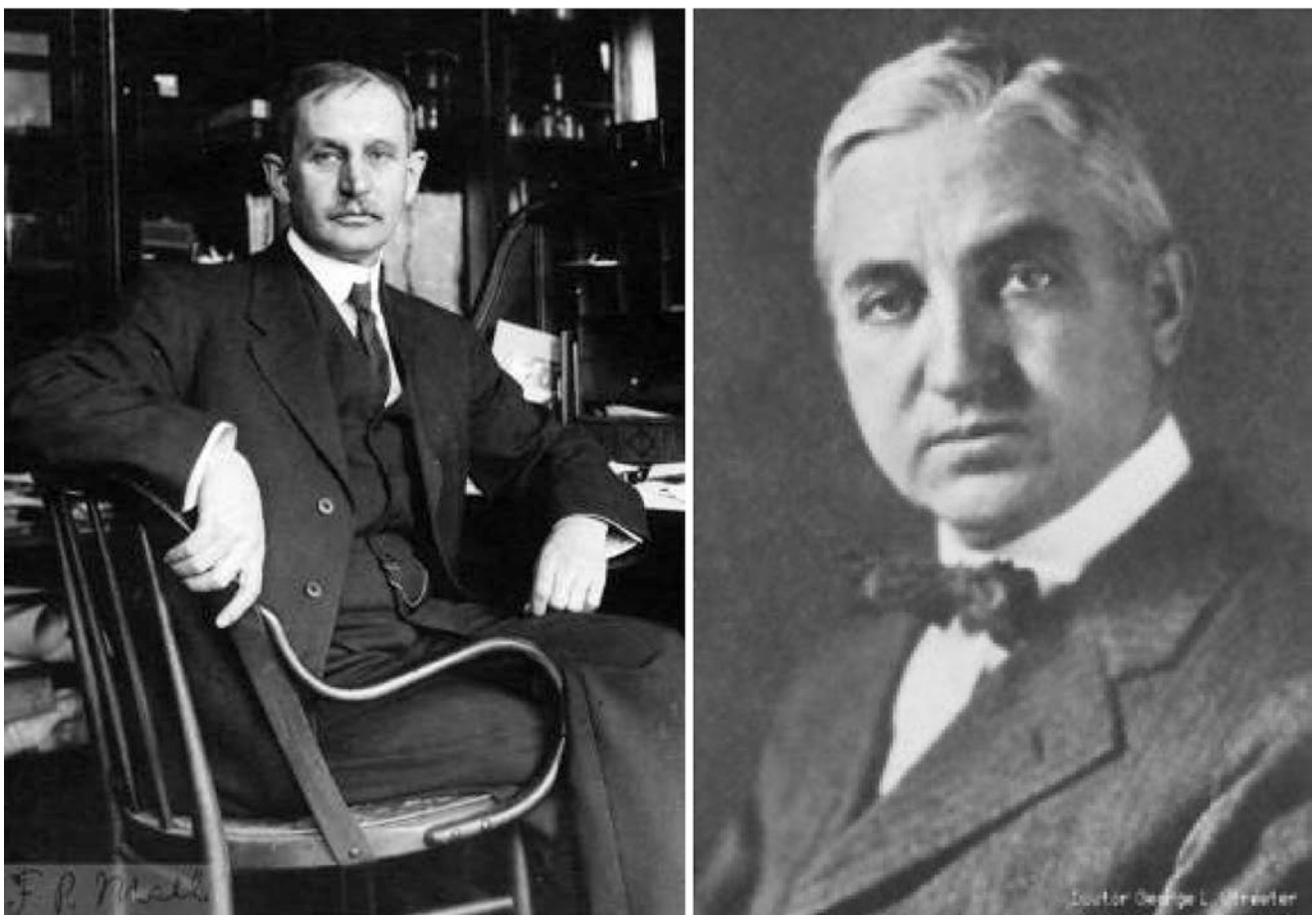

Fig. 3.8 (Left) Franklin P. Mall and (right) George Streeter. The first and second chairmen of the Carnegie Institution Department of Embryology. (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

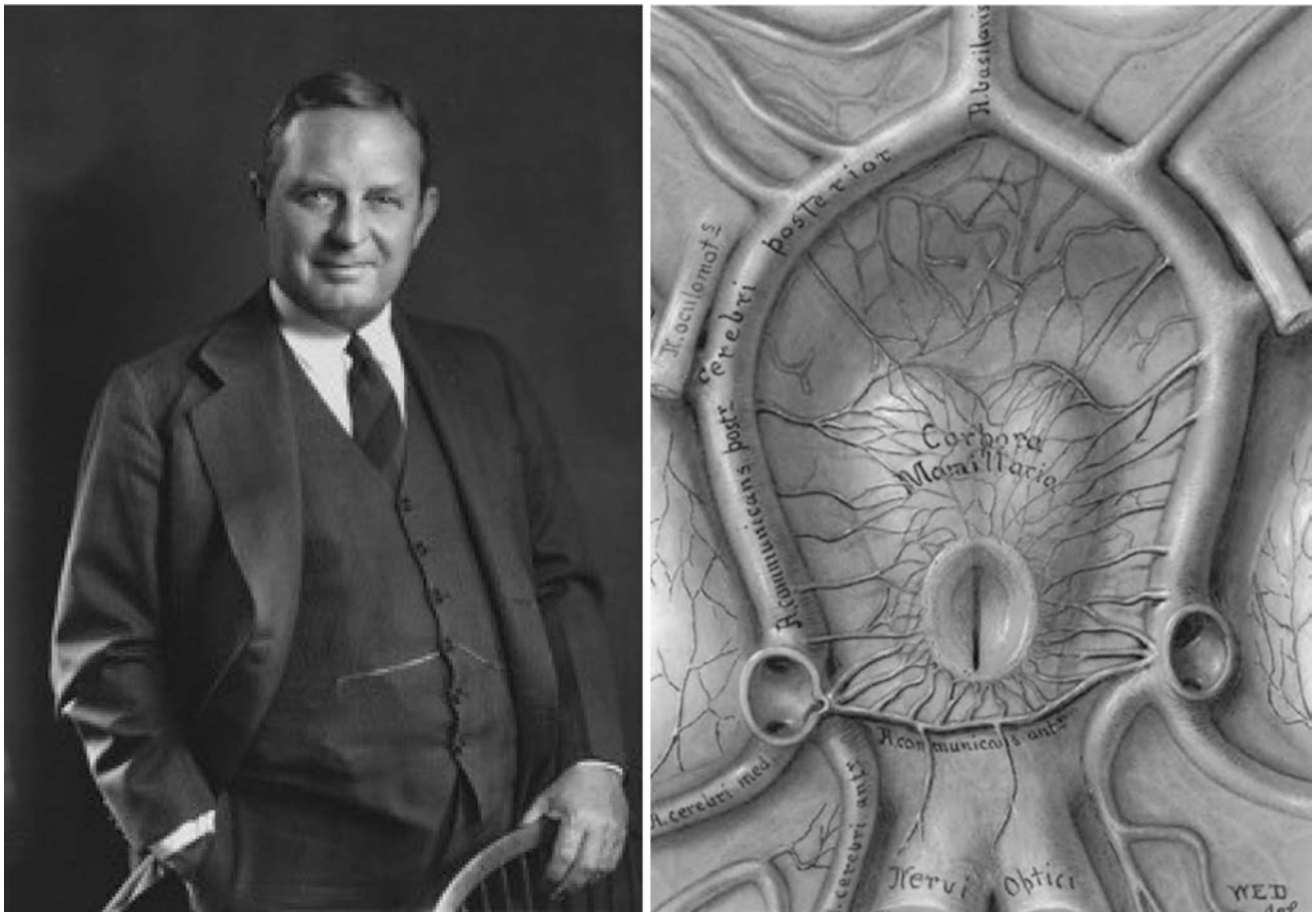

Fig. 3.9 (Left) Walter Dandy, Johns Hopkins neurosurgeon and medical artist. He was influenced by Mall and by Brödel. (Right) Dandy's illustration of the vascular supply to the pituitary. (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

Brödel offered her a 1-year position with the proviso of permanent employment upon graduation. Padget was exceptional from the start and won a full-time position. But the need to help her sisters get through college combined with a steady income resulted Padget forgoing her final year at Vassar. Though she would carry out world class research, she never got her degree and never achieved her goal to become a physician (Fig. 3.10).

For the next 22 years, Padget's work was the backbone of Hopkins Neurosurgery publication efforts, providing the illustrations for many articles and for Dandy's book, *Surgery of the Brain*. Her work is characterized by careful attention to didactic points, elegant design, and exquisite detail. Her depiction of the extirpation of a large hypophyseal cyst is a masterpiece of three-dimensional design and see-through techniques to show multiple planes (Fig. 3.11 left).

Upon Dandy's death in 1946, Padget began work at the Carnegie Institution. She performed painstaking dissection and tracking for arterial vessel in 22 separate embryos and her monograph, published in 1948, is the foundation of neurovascular developmental anatomy. As the Carnegie Horizons had not yet been worked out and were not available to her, she divided the embryos into seven stages which fit remarkably well into the subsequent system. Over the remainder of her career, Padget authored numerous papers on congenital anomalies of the nervous system. Despite a long struggle with cancer beginning in the 1950s, she remained productive

Fig. 3.10 (Left) Max Brödel and (right) Dorcas Padget. Brödel was brought to Johns Hopkins to found the first program in medical illustration in the U.S. Brodel secured an appointment for Dorcas Hagar with the JHU Neurosurgery Department. Portrait of Dorcas Hagar Padget by Audrey Juliet Artnott (also a student of Brodel). (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

until her death at Johns Hopkins Hospital in 1973. Her work set the standard for medical art and her contributions to neu-

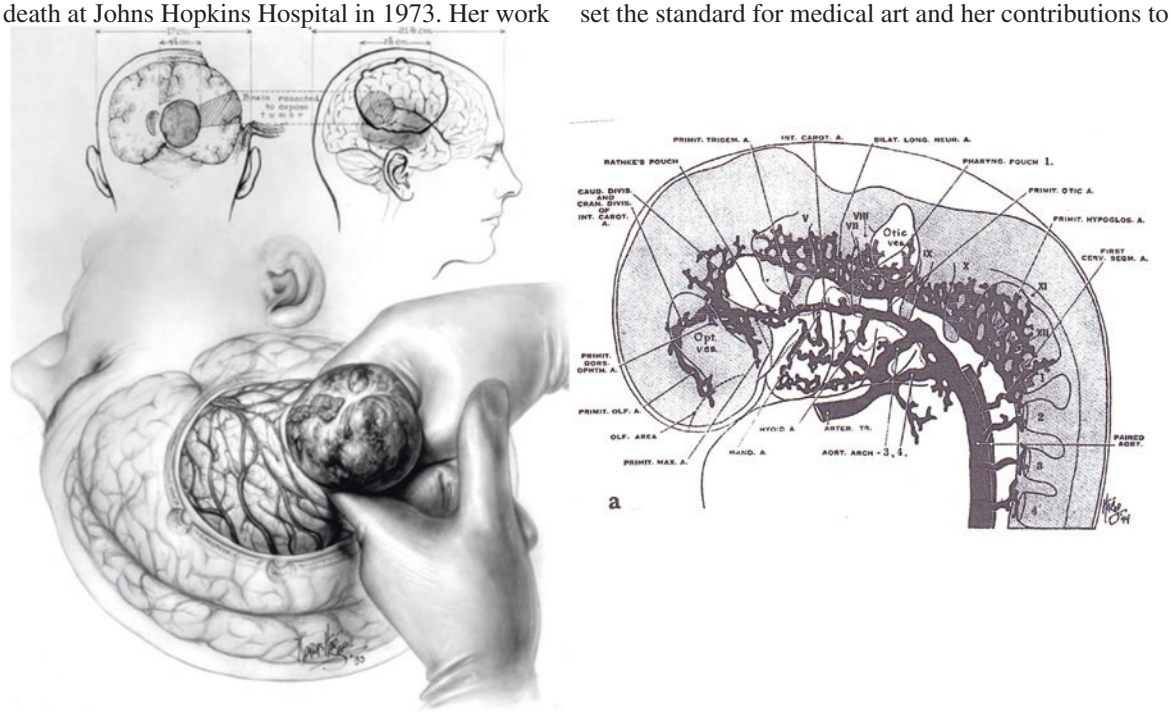

Fig. 3.11 Artwork by Dorcas Padget. (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

roembryology are indisputable (Fig. 3.11 right).

How to Use This Chapter

Human development is a four-dimentional process in which multiple anatomic structures change over time. To understand it, we must create in our minds a videotape of the embryo on multiple levels, from external features, to assembly of individual parts such as the tongue, to the relationships among structures and system such as the evolution of blood supply to the neck. To accomplish this, a reference system to keep track of these changes is required.

Original descriptions of the human embryo suffered from a lack of standardized parameters. Embryos of the same age could vary in size. Gestational age can be inexact. These problems were addressed by Streeter, Corner, and others at the Carnegie Institution by the creation of a staging for embryos based on reproducible landmarks. The system was first described in a series of monographs published by the Carnegie Institution. It was codified by O'Rahilly R and Muller F, *Developmental stages in human embryos*, Publication 637, The Carnegie Institution of Washington, DC, 1987. The Carnegie system was fully implemented in O'Rahilly R, Müller F: *Human Embryology and Teratology*, 3rd ed. New York, Wiley-Liss, 2001.

How can one go about learning the Carnegie system? Modern embryology references such as Carlson refer to the staging system, but no systematic description is available. O'Rahilly and Müller provide reference to the system throughout the text in a very useful way, but leave a description of the Carnegie system to a separate reference work by the same authors (Fig. 3.12).

A comprehensive outline of the Carnegie system can easily expand into a synopsis of embryonic development in general…one can easily get lost with this approach. Our purpose here is to simply outline some of the major events, characterized by major external features with emphasis on the face. Certain key features of the brain, vascular, digestive, and respiratory systems are also listed, inasmuch as these have bearing on craniofacial development.

Devoting time to study this system will give you a means to think about development in a dynamic and esthetically satisfying way. You are encouraged to plow through the descriptions, referring to accompanying figures. Legends contain additional information on visible structures such as the extremities.

A full visual account has been assembled at University of New South Wales by Dr. Mark Hill: https://embryology.med.unsw.edu.au/embryology/index.php/Main_Page.

Monographs on the Carnegie system and by Dr. Mark Hill and on the Kyoto University Embryo Collection by Dr. Mark

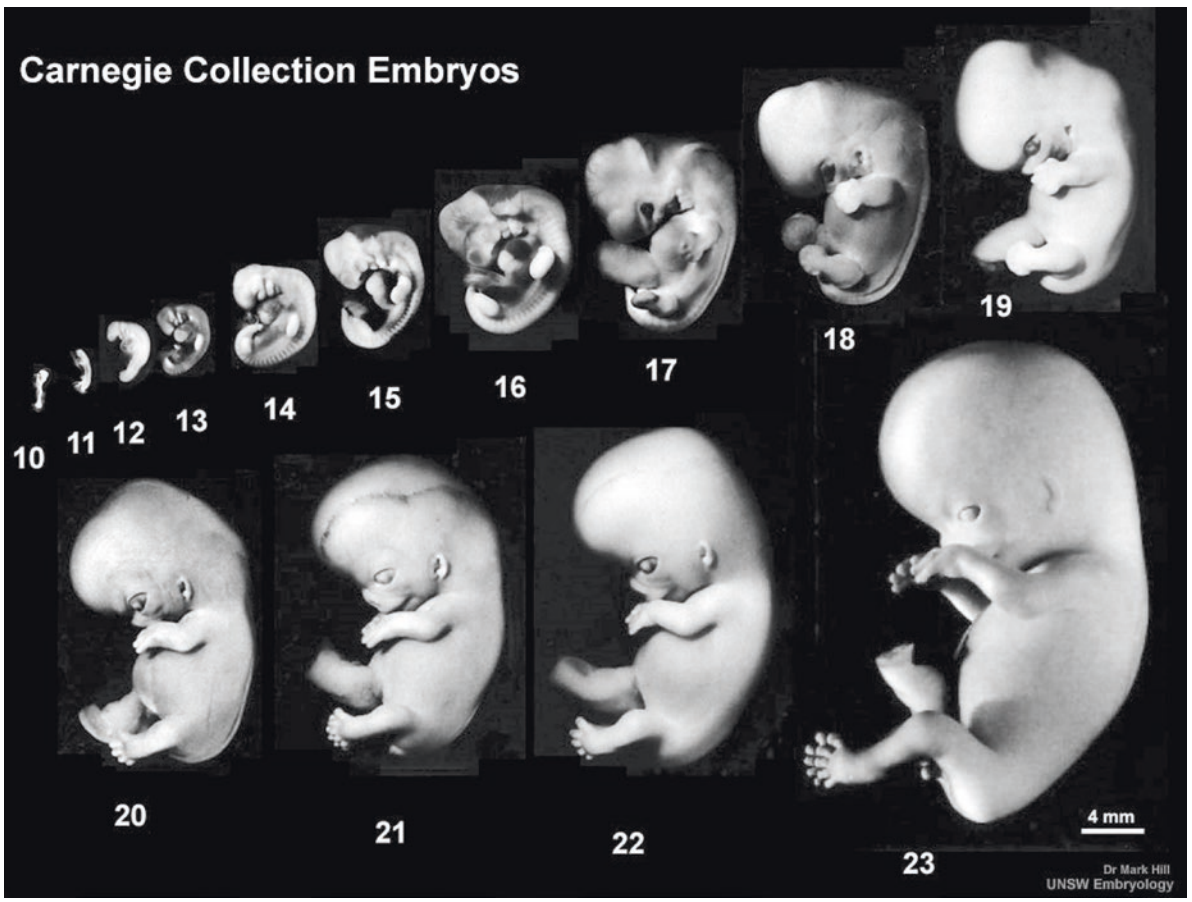

Fig. 3.12 Carnegie stages summary. (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

Hill, Dr. Shigehito Yamada, and Dr. Cecilia Lo are available online through the iBooks store and are compatible with both iPad and laptop.

Stage-By-Stage Description

Major characteristics of the 23 stages are listed. These emphasize key developmental events and external features. In subsequent chapters, this form of stage-by-stage summary will be useful to chart the events of individual structures. Our purpose here is to gain a general visual orientation to key events in development. Although our focus is on the head and neck, many aspects of staging have to do with anatomic features that are easy to identify. We constantly stress developmental events in the CNS, so these are included in the capsule summaries. Hopefully, with repetition, these structures will become second-nature for the reader. One should range back and forth from the capsule illustrations to the figures. This material is closely correlated with the embryology texts of Carlson and O'Rahilly. Note that there is not a consensus on the exact number of days allotted to some of the stages. The numbering used in this text is as per Carlson. For an alternative, consult the UNSW website.

Stage 1 (24 h, 0.1–0.15 mm): Fertilization (Fig. 3.13)

Fertilization takes place in the ampulla of uterine tube

Stage 1a: The *penetrated oocyte* contains all genetic material plus redundant chromosomes enclosed within a single cell membrane.

Stage 1b: The *pronuclear embryo* characterized by resuming arrested meiosis II, followed by anaphase and telophase, in which redundant chromosomes are packaged as the second polar body and expelled from the cell. Pronuclear embryos have two separate haploid components, male pronucleus and female pronucleus. These move to the midline of the cell and press together.

Stage 1c: The *syngamic embryo* has fused pronuclear cell membranes—parental chromosomes come together and assume a position on the spindle. The genome is now formed.

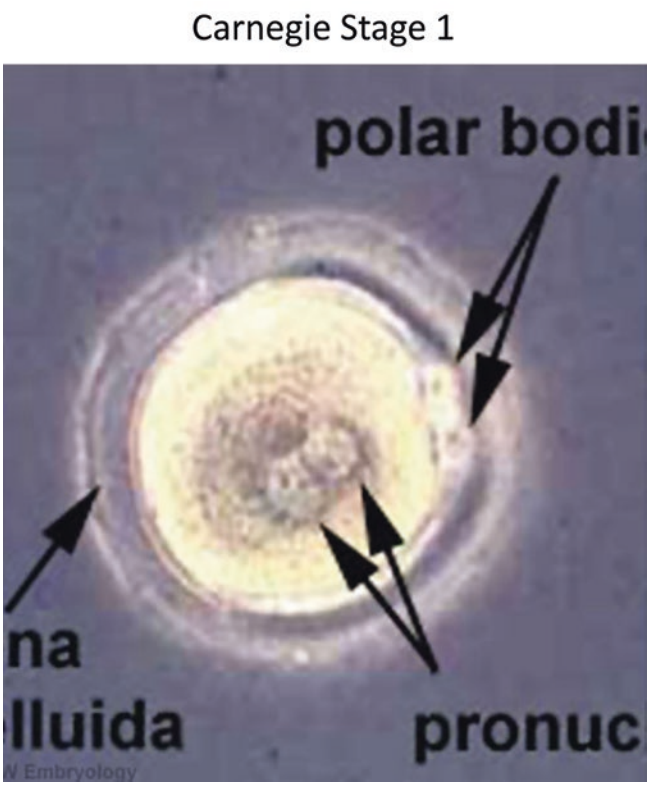

Fig. 3.13 Stage 1: 1 day, 0.1–0.15 mm. (1) Haploid pronuclei have not joined. (2) Polar bodies. (3) Zona pellucida. (Reprinted from Carlson BM. Human Embryology and Developmental Biology, 6th edition. St. Louis, MO: Elsevier; 2019. With permission from Elsevier)

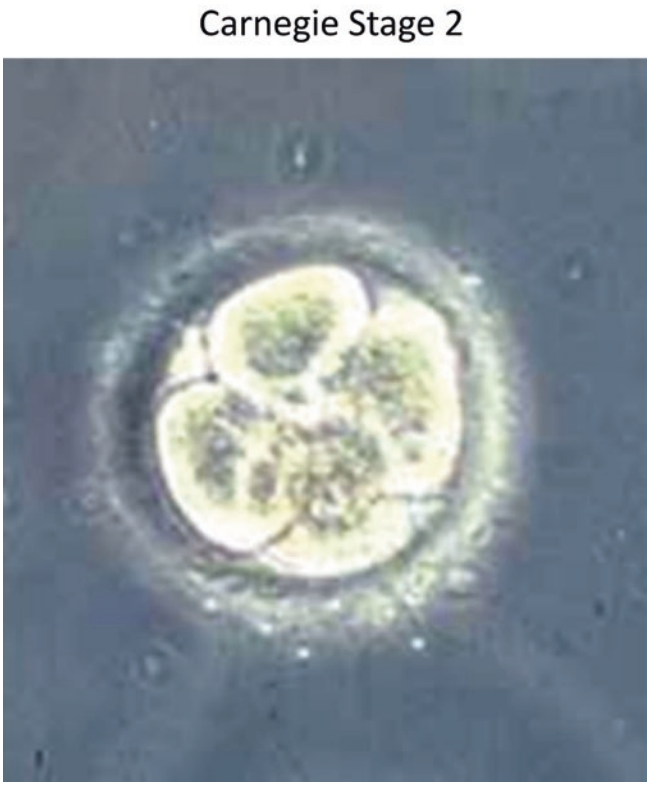

Fig. 3.14 Stage 2: 2–3 days, 0.1–0.2 mm. Early mitosis: (1) No G1 or G2 phase. (2) Reduction in volume. (Reprinted from Carlson BM. Human Embryology and Developmental Biology, 6th edition. St. Louis, MO: Elsevier; 2019. With permission from Elsevier)

Stage 2 (2–3 Days, 0.1–0.2 mm): Cleavage (Fig. 3.14)

Zygote undergoes cleavage with up to 16blastomeres, no change in size but DNA content rapidly expanding. At morula stage, fate of individual blastomeres not yet determined.

Stage 3 (4–5 Days, 0.1–0.2 mm): Free-Floating Blastocyst (Fig. 3.15)

Embryo passes through four phases.

Cavitation produces a blastocyst. No change in volume, so the fluid is produced by the metabolism of the cells and not be importation from the exterior. *Collapse and expansion* take place prior to escape from zona pellucida. Collapse is a sudden event, happening in less than 5 min. *Hatching* refers to escape at days 7–8 post-insemination via the zona pellucida. Embryo now gets bigger. External trophoblast cells tightly bound together.

Stage 4 (6 Days): Attachment of Blastocyst (Figs. 3.16, 3.17, and 3.18)

Adplantation presages the onset of implantation. Outer trophoblast fimbriae create multiple adhesion sites. Trophoblast differentiates into inner cytotrophoblast and outer syncytiotrophoblast which dissolves the epithelium of the endometrium. Embryo is now bilaminar with appearance of hypoblast just below the inner cell mass (epiblast).

Stage 5 (7–12 Days, 0.1–0.2 mm): Implantation, Bilaminar Disc, Trophoblast Development, No Villous Development (Figs. 3.18 and 3.19)

Thick outer syncytiotrophoblast does not have cell boundaries. Thin inner cytotrophoblast has cell boundaries. Extraembryonic mesoblast, the chorion, is present but lacks villous structures.

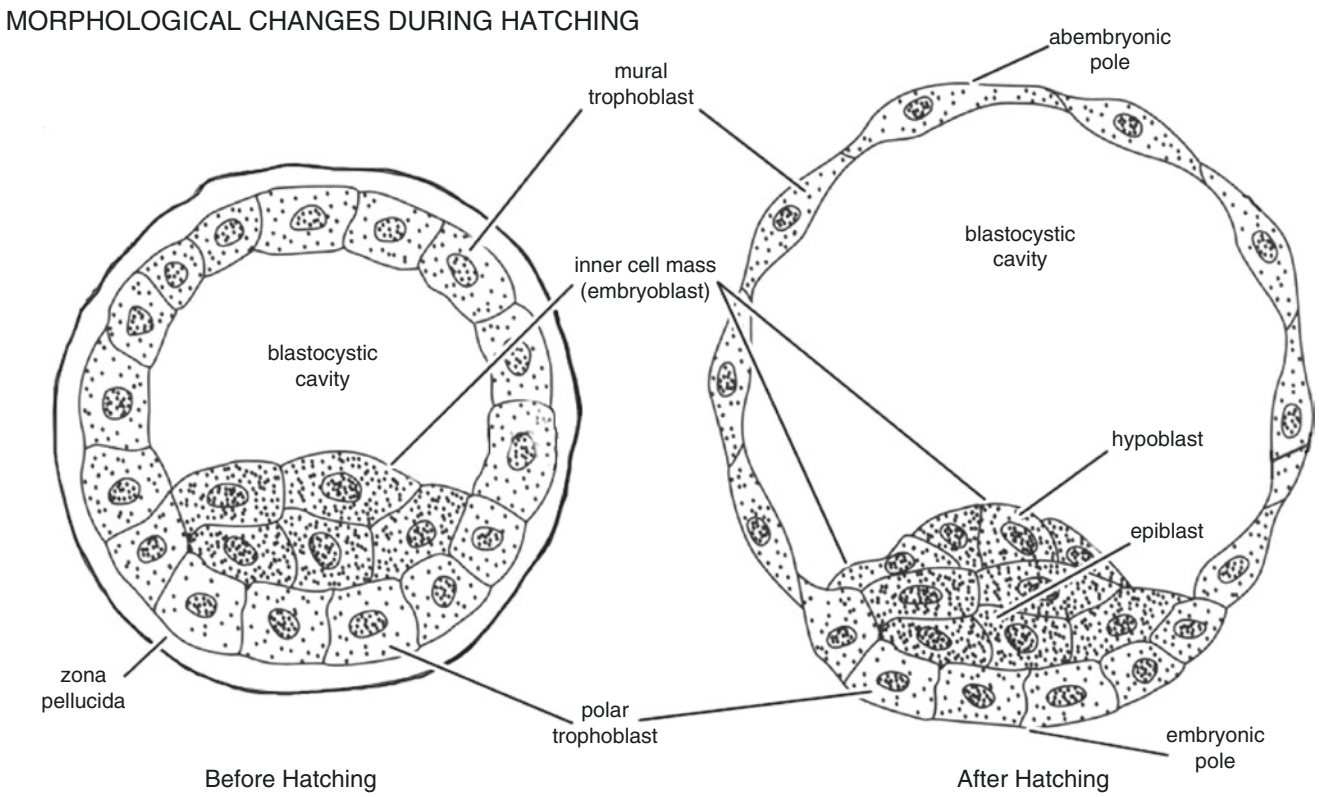

Fig. 3.15 Stage 3: 5 days, 0.1–02 mm. (1) Free floating blastocyst. (2) Zona pellucida > "hatch". (3) Inner cell mass. (4) 8 cells = TOTIPOTENT. (5) Hypoblast not present—hypoblast defines the next stage 4. (6) Squamous trophectoderm. (7) Must "hatch" in order to implant. (Reprinted from Veeck LL, Zaninović N. An atlas of human blastocysts. New York, NY: Parthenon Publishing Group; 2003. With permission from Taylor & Francis)

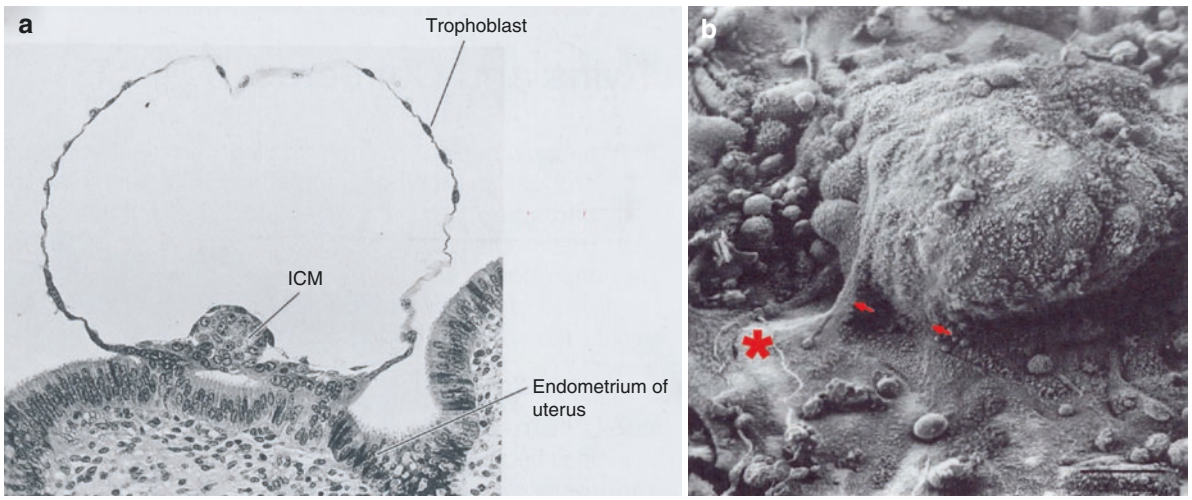

Fig. 3.16 Stage 4 is reserved for the attaching blastocyst that is adhering to the endometrial lining. Attachment is complete at 6 days. Implantation is completed during stage 5. (**a**) Photomicrograph showing beginnings of hypoblast immediately under the epiblast. Cells are now PLURIPOTENT—they can produce all cell types *except* umbilical cord and placenta. (**b**) Attachments of the blastocyst. (**a**: Reprinted from Gilbert SF, Barresi M. Developmental Biology, 11th ed. Sinaurer: Sunderland, MA, 2016. Reproduced with permission of the Licensor through PLSclear. **b**: Reprinted from Bentin-Ley U, Sjogren A, Nilsson L, et al. Presence of uterine pinopodes at the embryo-endometrial interface during human implantation *in vitro*. *Human Reprod.* 1999; 14:515–520. With permission from Oxford University Press)

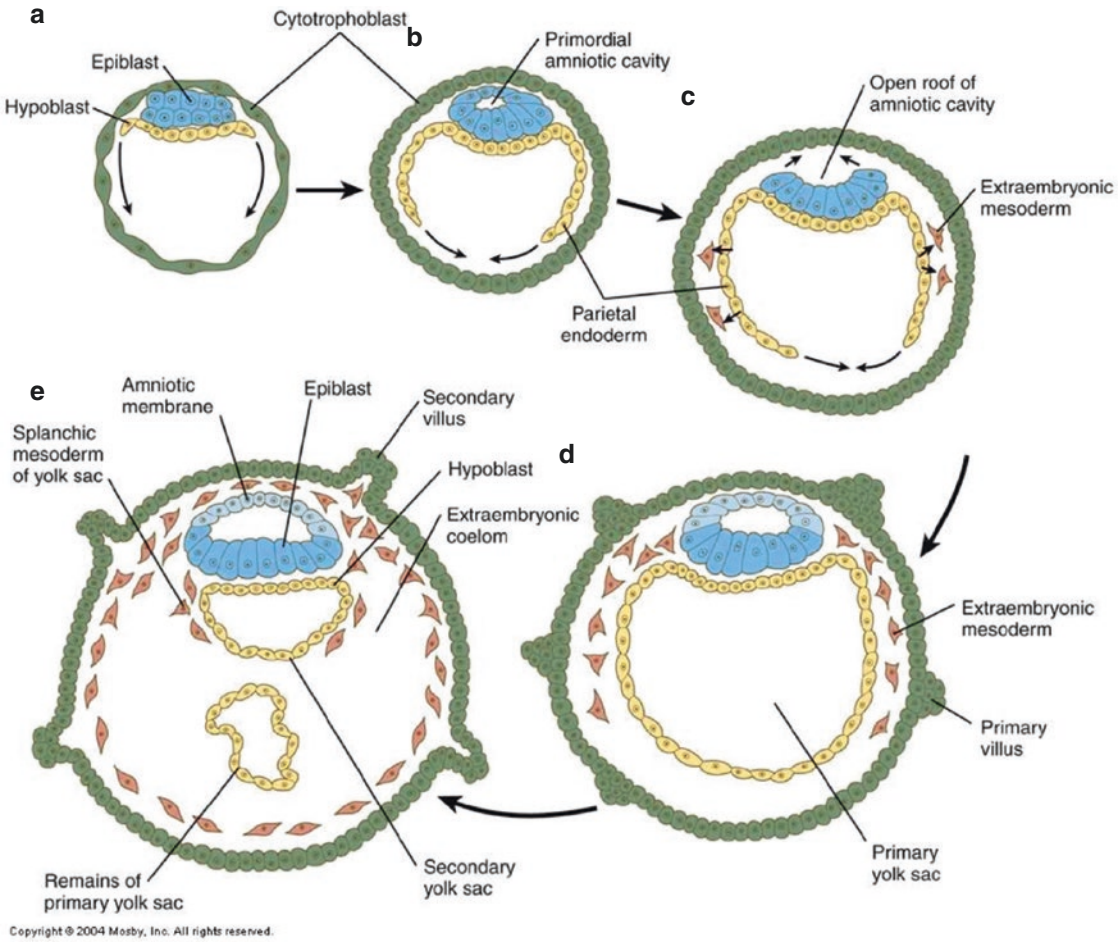

Fig. 3.17 Carnegies stages 4–5 extraembryonic gastrulation. Cytrophoblast (green)—syncytiotrophoblast not shown; epiblast (blue), hypoblast/extraembryonic endoderm (yellow); extraembryonic mesoderm (red). **A**, 6d = implantation starts; **B**, 7.5d = implantation complete, **C**, 8d = extraembryonic mesoderm; **D**, 9d = extrembryonic ectoderm = amnion; **E**, 2 weeks = EEM invades villi; surrounds primitive endoderm remnant (secondary yolk sac) > vitelline vessels. (Reprinted from Carlson BM. Human Embryology and Developmental Biology, 6th edition. St. Louis, MO: Elsevier; 2019. With permission from Elsevier)

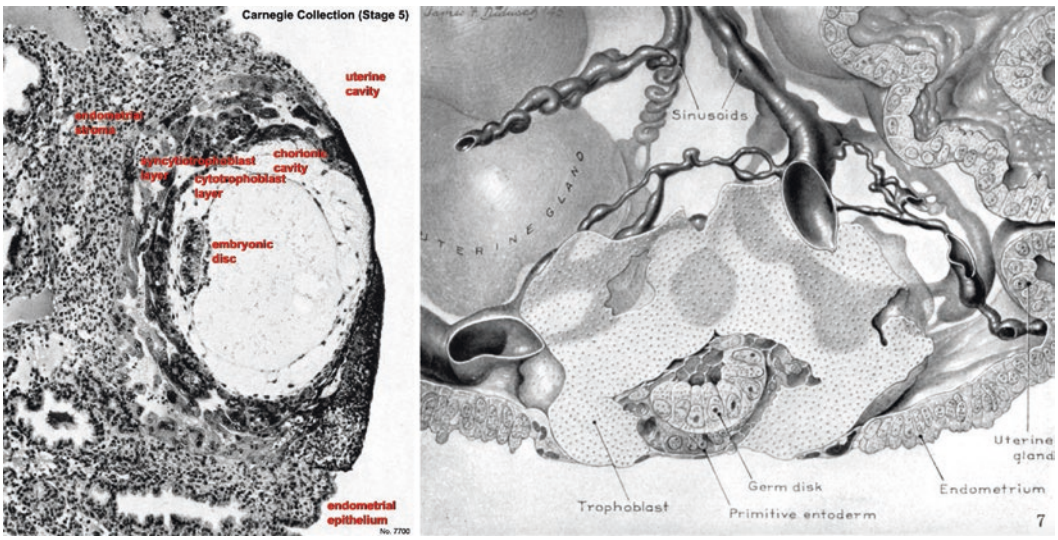

Fig. 3.18 Stage 5 0.1–02 mm, 7–12 days. (1) Implantation complete: trophoblast differentiates, amniotic cavity. (2) Decidual reaction. (3) Bilaminar embryo. (4) Hypoblast > extraembryonic mesoderm (pink) > blood vessels. (5) Primary umbilical vesicle, yolk sac. Note new layer of extraembryonic mesoderm (EEM) produced by the hypoblast. The EEM surrounds the entire embryo and invades into projections of the cytotrophoblast into the syncytiotrophoblast called *primary villi*. When these are filled with EEM, they become *secondary villi*. Left: conceptus is fully implanted with a bilaminar disc and primary yolk sac. Right: Schematic drawing by James F. Dudisch. (Left: Reprinted from Hertig AT, Rock J, Adams EC, Mulligan WJ. On the preimplantation stages of the human ovum: A description of four normal and four abnormal specimens ranging from the second to the fifth day of development. Contrib Embryol. Carnegie Institution of Washington 1954;35:199–220. Right: Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

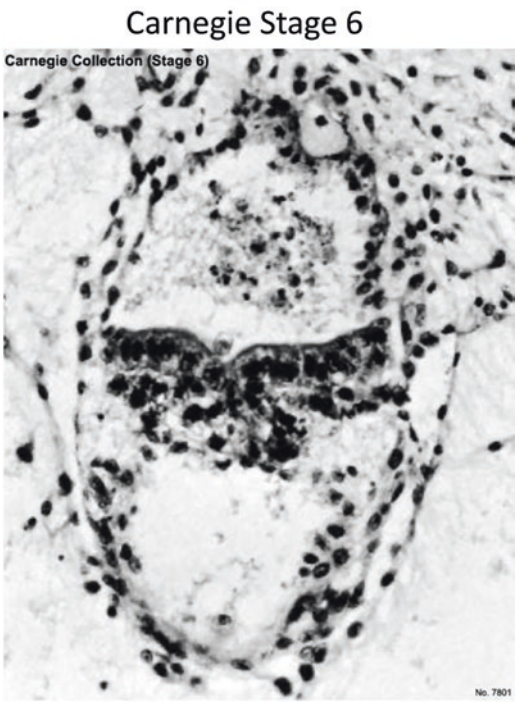

Fig. 3.19 Stage 6a: Bilaminar embryo just before gastrulation. 0.2 mm and 14–15 days stage *VERY FAST.* SEM showing the primitive streak. (1) Chorionic villi (functional placenta). (2) Trophoblasts invade decidua > maternal lakes. (3) Spiral arteries *held open* by trophoblast. (4) Connecting stalk (extraembryonic mesoderm) contains: (a) diverticulum of the allantois, (b) omphaloenteric duct, (c) amniotic somatopleure. (5) Blood islands. (6) Primitive streak: caudal > cranial: (a) interaction with endoderm, (b) tissue movements begin at stage 7. (Reprinted with permission from Johns Hopkins University Department of Medical Art—Gary Lees. Carnegie Institution—Sonya Bajwa)

Stage 5a: 7–8 days with solid trophoblast. Endometrial stroma is very edematous. On the inner surface of the trophoblast, a two-layer embryonic disc has distinct epiblast and hypoblast.

Stage 5b: lacunae form within the syncytiotrophoblast. These communicate with each other and with the maternal sinusoids, but maternal blood is scant. Within the endometrium, "fingers" of syncytiotrophoblast become filled with cytotrophoblast. These are the future villi. Yolk sac appears and is bounded by an external exoceloemic membrane. The embryonic disc now has two forms of cells: the epiblast epithelium being pseudostratified columnar and the hypoblast is simple cuboidal.

Stage 5c: large lacunar spaces interconnect and are blood-filled. At the caudal end 5 of the embryonic disc is a clump of extraembryonic mesoblasts. Hypoblast lines the coelomic cavity. Extraembryonic mesoderm appears between hypoblast and trophoblast.

Stage 6 (13–18 Days, 0.2–0.3 mm): Gastrulation Begins (Figs. 3.20 and 3.21)

This stage is marked by the initiation of gastrulation by formation of the primitive streak and cell movements, as discussed previously. It results in a tri-laminar embryo.

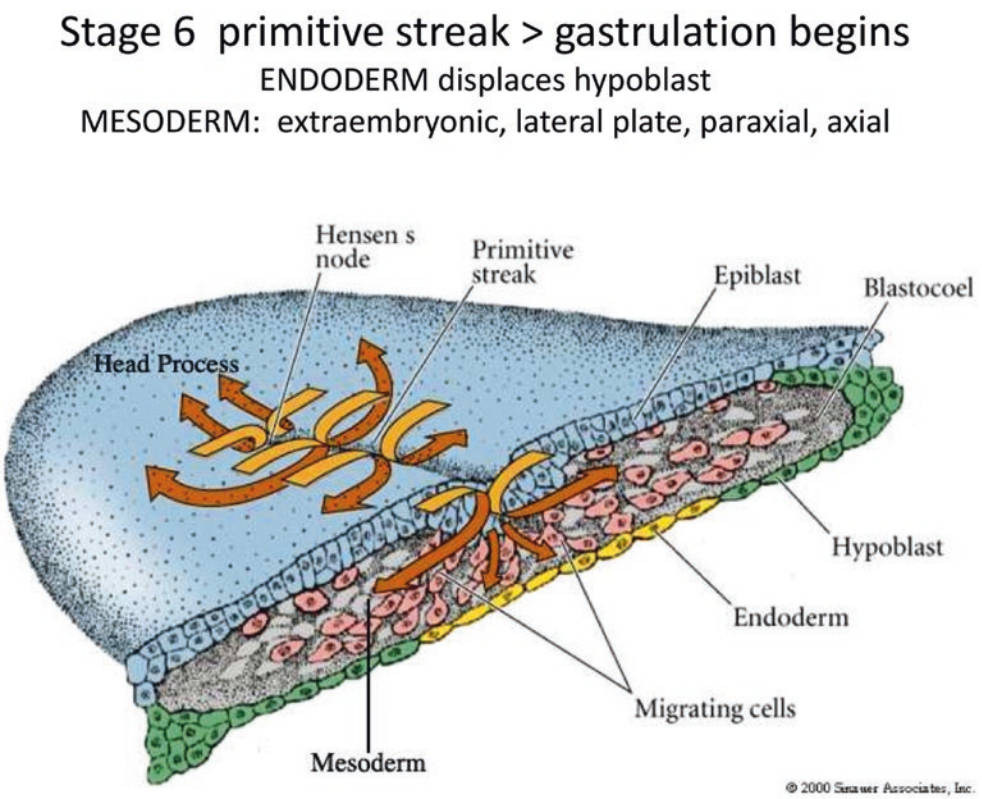

Fig. 3.20 Stage 6b gastrulation. (Reprinted from Gilbert SF, Barresi M. Developmental Biology, 11th ed. Sinaurer: Sunderland, MA, 2016. Reproduced with permission of the Licensor through PLSclear)

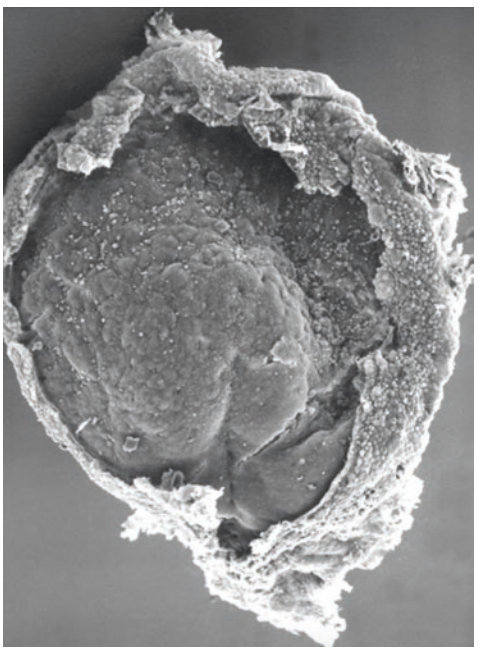

Fig. 3.21 Stage 7: 0.4 mm, 15–17 days. (1) Gastrulation. (2) Blood vessels: (a) chorionic villi, umbilical vesicle. (3) Rostral to PS, notochordal process: (a) arises from the primitive node, (b) extends to the prechordal plate, (c) floor breaks down, (d) True notochord present. (4) Order of ingresssion: (a) intraembryonic endoderm, (b) extraembryonic mesoderm, (c) intraembryonic mesoderm: PAM then LPM. (5) Epiblast and endoderm fuse at two sites: (a) bucco-pharyngeal membrane, (b) cloacal membrane. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

Stage 7 (19–22 Days, 0.4 mm): Notochord (Fig. 3.22)

Gastrulation continues with the formation of mesoderm and the caudal-cranial development of a notochordal process which develops in the opposite direction of gastrulations. The establishment of notochord sets the stage for induction of the neural plate and eventual embryonic folding.

Stage 8 (23–24 Days, 0.5–3 mm): Neurulation (Fig. 3.23)

The embryo is shaped like a pear. Primitive node and notochord visible.

Characterized by the primitive pit, notochordal canal, and neurenteric canal. Its mesoderm is presomitic, meaning that somitomeres have formed (up to 18), the first 7 of which represent the so-called mesoderm supplying the orbit and pharyngeal arches 1–3. The initial seven somitomeres do not undergo transformation to somites.

At this time, uteroplacental circulation was established. Decidua capsularis covers over the embryo.

Fig. 3.22 Stage 8: 17–19 days. 1.0–1.5 mm. (1) Gastrulation complete. (2) Notochordal process extends *opposite* to primitive streak. (3) Segmentation: cranial > caudal. (4) Homeotic code for each segment. (5) Hindbrain Otx-2 induces midbrain and forebrain. (6) Headfold. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

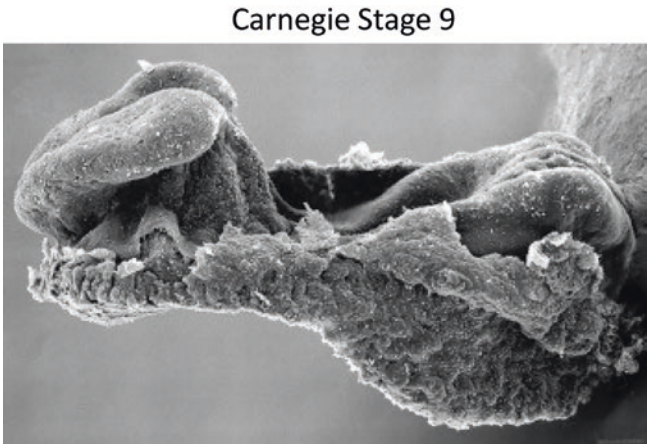

Fig. 3.23 Stage 9: 19–21 days, 1.5–2.5 mm, 1–3 somites. (1) Neural groove, neural folds, neural crest migrates. (2) Lateral plate mesoderm vacuolates. (3) Endoderm opens into yolk sac. (4) Folding of dorsal aortae. (5) Connection of DA with PHC. (6) First pharyngeal arch (first aortic arch—transient). (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

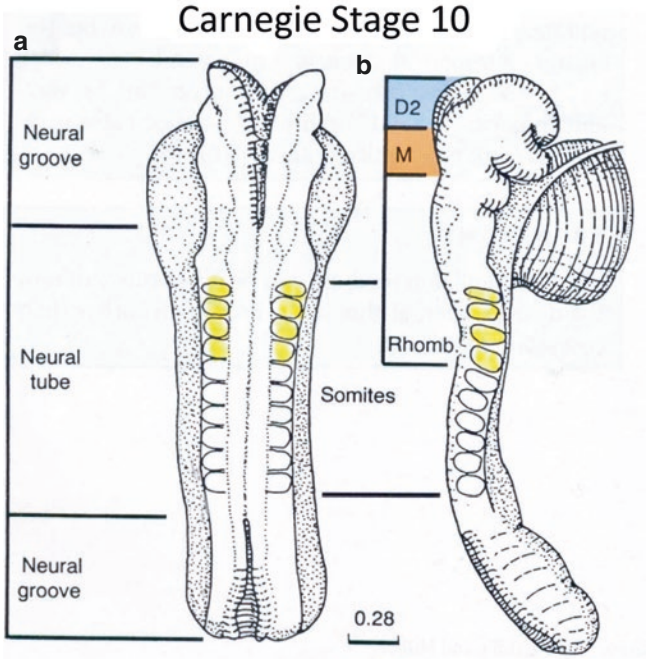

Fig. 3.24 Stage 10: 22–23 days, 2–3.5 mm, 4–12 somites. (1) Neurulation. (2) Heart tubes. (3) Second pharyngeal arch (second aortic arch—transient). (Reprinted from O'Rahilly R, Müller F. The Embryonic Human Brain: An Atlas of Developmental Stages, 3rd ed. Hoboken, NJ: Wiley-Liss; 2006. With permission from John Wiley & Sons)

Stage 9 (25–27 Days, 1.5–2.5 mm, 1–3 Somites, First Aortic Arch) (Fig. 3.24)

Histologic Features

The "embryo proper" is defined by the neural groove.

Embryonic disc sufficiently developed to body systems.
Primitive streak involves 1/4 to 1/3 of length of embryo.
Mesoderm organized longitudinally. Intermediate mesoderm is present, but no kidney structures present, so not identifiable.
Coelom splits lateral plate mesoderm.
Pericardial cavity (intraembryonic coelom) is a horseshoe that starts with occipital and develops via fusion of adjacent cavities.
Notochordal cavity
Notochordal plate

Vascular System

Blood vessels arise in diverse sites: chorion, connecting stalk, umbilical vesicle, and the embryo proper. In embryo, the primitive head plexus and primitive hindbrain channels are flanked by dorsal aortae that connect to the heart via the first aortic arch arteries. Few or no blood cells at this stage. Circulation is ebb-and-flow.

Heart: Endocardial plexus has three parts: atrial (connected to vitelline circulation), ventricular, and conal (bulbar outflow tract).

Digestive System

Foregut assembled from folding. Caudal portal not defined. Foregut related to the neural groove. Although a pit is present in the ventral foregut wall, the thyroid does not appear until stage 10.

Nervous System: Major Divisions of the Brain

Neural folds make their debut, but become dominant later in stage 10. Note that neural folds are separated from each other by a rostral notch which leads directly to the oropharyngeal membrane—at r0. Neural folds are closest together at the junction of the hindbrain and spinal cord. It is here, at stage 10, that neural tube closure will commence.

Neural crest: Head ectoderm differentiating—midbrain, trigeminal, facial, otic, and occipital zones have mitotic figures.
Eye: Optic primorida not yet present.
Ear: Otic disc appears.

Stage 10 (28 Days, 2.5–3.5 mm, 4–12 Somites, Second Aortic Arch) (Figs. 3.25, 3.26, and 3.27)

Characteristics of this stage are as follows: fusion of neural folds in progress, optic sulcus often present, pharyngeal arch 1 visible, cardiac loop seen, laryngeal sulcus, intermediate mesoderm identifiable (future genitourinary system).

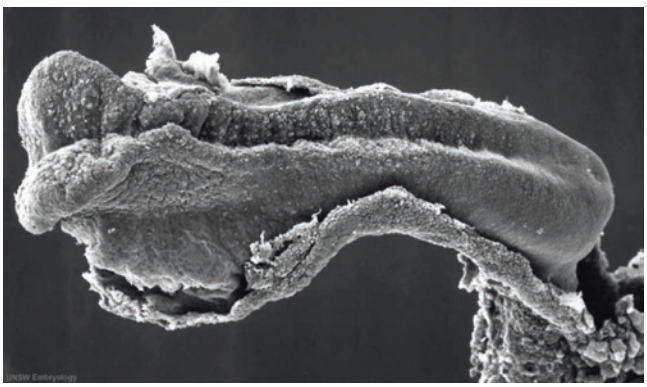

Fig. 3.25 Stage 10 SEM showing 12 somites, posterior PAM still in somitomeric form. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

Histologic Features

Primitive streak: Limited to the posterior (caudal) end of the embryo (where the sperm entered). With the rapid growth of the embryonic plate, the length of the primitive streak (PS) in relation to the embryo gets progressively smaller. Caudal to the neurenteric canal are dense axial cells representing PS. Caudal embryo consists of thickened endoderm of the hindgut, the PS, and, alongside of the neurenteric canal, undifferentiated mesenchyme that will later become differentiated mesoderm. Above, is the neural plate.
Somites: 4–12 pairs are present, the first four being the occipital somites. Recall that Sm11 becomes S4 and that, in tetrapods, this is no longer part of the trunk, but is added on to the cranial base. Fishes have a three occipital somite cranial base.
Notochordal plate: This is present senso stricto only where the notochordal cells have become completely separated from the underlying endoderm. Rostral to the neurenteric canal, the notochordal plate remains fused with the endoderm and forms the roof of the foregut. This zone is marked in the future by Rathke's pouch and demarcates the location of the future basisphenoid.
Neurenteric canal: Although this was prominent at stage 9, it is now disappearing.
Prechordal plate: This most-anterior mesoderm lies just beneath the prosencephalon. A basement membrane separates it from the brain. Prochordal cells migrate during stage 9–10. They are interposed between the eye fields and push out transversely at 180 degrees. It was thought that PCM was the source of the extraocular muscles, but Noden has shown them to originate from somitomeres.
Umbilical vesicle: This structure is trilaminar on SEM—mesothelium, mesenchyme, and endoderm.

Cardiovascular System

Second aortic arch artery develops from the conus. Pericardial cavity is present and communicates with intraembryonic and extraembryonic coeloms. These spaces serve through stage 10 as passive conduits for nutritional fluids, while the blood vessels are getting organized to handle cardiac-based circulation.

Heart: Cardiac contractions begin at this stage. The wall of the heart consists of endothelium, cardiac jelly, and a thin covering of myocardium. Three steps in heart development are recognized during stage 10. (1) The endocardial primordium consists of a plexus on either side of the foregut. (2) These endocardial tubes fuse to form a single tube. At the time of 7–10 somites, an S-shaped flexion forms. It has, from anterior to posterior, a conotruncus, that will give off aortic

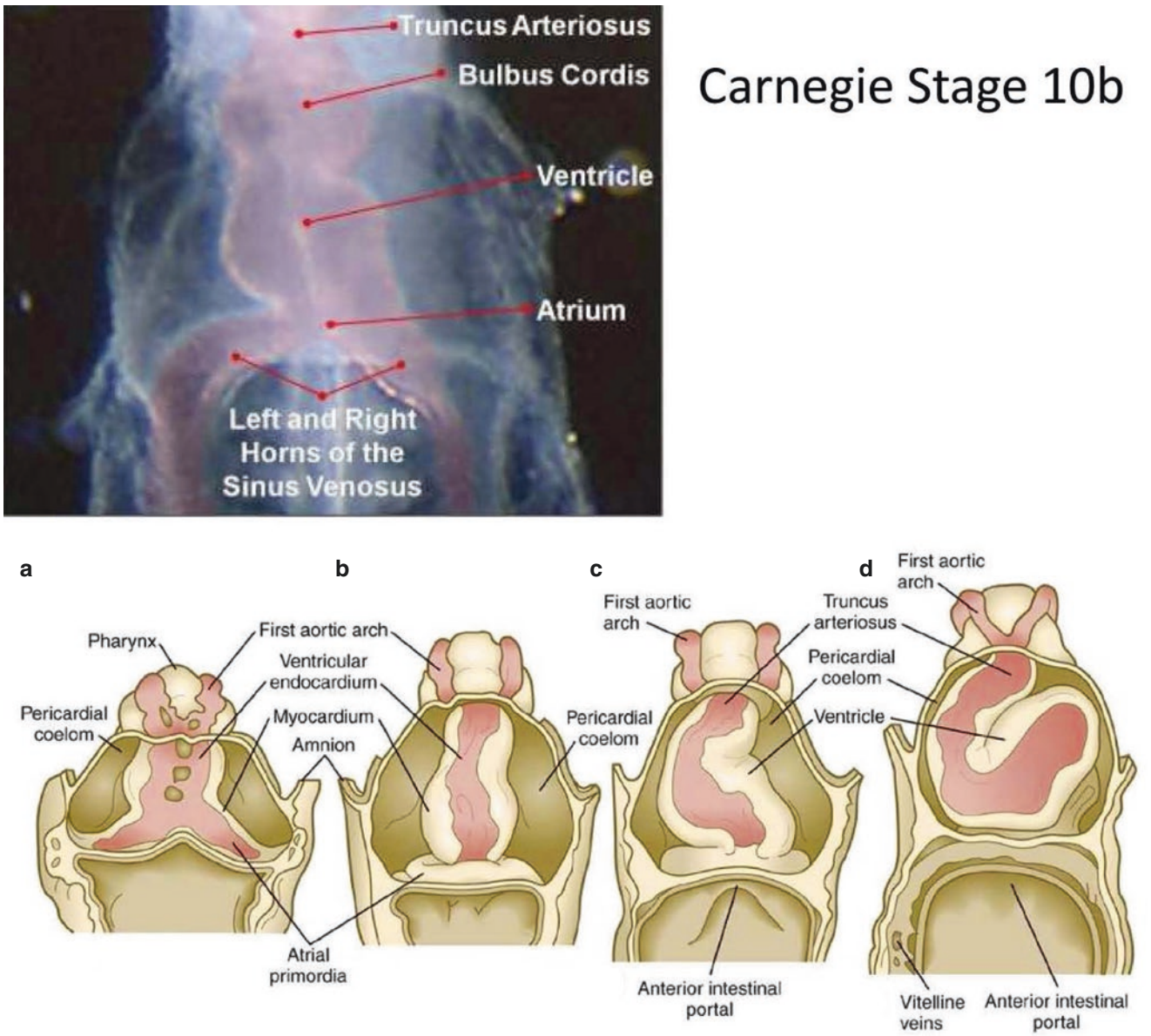

Fig. 3.26 Stage 10 showing folding of the heart and first aortic arches. (Left: Courtesy of Prof. Kathleen K. Sulik, University of North Carolina. Right: Reprinted from Carlson BM. Human Embryology and Developmental Biology, 6th edition. St. Louis, MO: Elsevier; 2019. With permission from Elsevier)

arch arteries, ventricles, and, caudally, the future sinus venosus (not seen at stage 10). (3) S-curve is formed by the ventricular loop, causing asymmetry.

Digestive/Respiratory Systems

The first pharyngeal arch is visible, clumped around the first aortic arch artery. At the apex of the foregut, the oropharyngeal membrane is present. It will eventually demarcate the zone between oral ectoderm infolded from the pharyngeal arches and the true endermal lining of the gut. Nonneural ectoderm of the most anterior neural folds produces paired adenohypophyseal placodes that will be tucking inside the future mouth to form the nonneural pituitary gland; thyroid primordium in the ventral wall of the foregut. Ventral pharynx has a laryngotracheal sulcus at the caudal end of which is the pulmonary primordium.

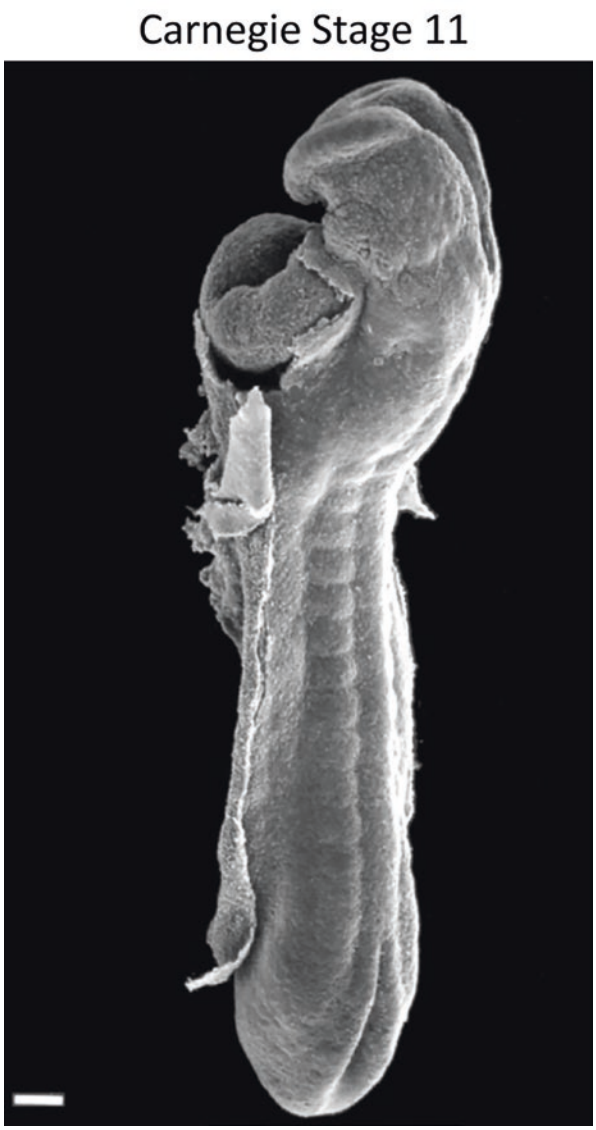

Fig. 3.27 Stage 11: 23–26 days, 2.5–4.5 mm, 13–20 somites. (1) Ectoderm: rostral neuropore closes. (2) Third pharyngeal arch (third aortic arch—persists). (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

Nervous System: The Neural Tube and Optic Primordium

Neural folds elevate. The hindbrain subdivides, first into the rhombomeres as described by O'Rahilly with four segments, A–D. A terminal notch seen at stage 10 in the forebrain marks the future telencephalon. The neural folds of diencephalon are divided into an optic part (D1) and a post-optic part (D2). A midline thickening of D1 will become the chiasm. Rhombomere D (r8–r11) and the cervical spine are grown fastest and closure of the neural folds starts here. CNS gets longer proportional to addition of new somites. Closure of the neural groove gets started at time of somite 5 (neuromere c1). It proceeds cranially and caudally with the caudal neuropore always located opposite the latest pair of somites.

Neural crest: NC cells form the cranial ganglia and migrate around the brain and into the future face.

Eye: At the 7–8 somite stage, the neural folds of D1 form the optic primordium which extends medially as the chiasmatic plate. An indented zone of D1 will be the optic sulcus. The ventricular surface of D1 is smooth but that of D2 has a rounded prominence that projects into the ventrical as the future thalamic nuclei.

Ear: Otic plate/placode contributes cells to the vestibule-acoustic (facial) neural crest.

Stage 11 (29 Days, 2.5–4.5 mm 13–20 Somites, Third Aortic Arch) (Figs. 3.28, 3.29, and 3.30)

External: Rostral neuropore closing. second pharyngeal arch. Otic invagination.

Internal: Optic vesicle. Sinus venosus, oropharyngeal membrane breaks down—relates to future division between pharyngeal arches 2 and 3.

Histologic Features

Somites: 13–20 pairs. Note that somite 1 is quite small and lies adjacent to r8 vagal/accessory neural crest. S1 has almost no contact with overlying ectoderm. Somitomeres/somites form every 6 h. Process of forming 44 units involves 11 days (day 20–31).

Coelom: This refers to a walled-off space present in the intraembryonic or extraembryonic mesoblast (primitive undifferentiated mesoderm). Mesoblastic cells pass through the primitive streak and migrate outward, coalescing to form vesicles, the tubes, then cavities. A horseshoe-shaped cavity forms in the rostral end of the embryo and extends backwards on either side to become confluent with the extraembryonic coelom. These passages along extraembryonic fluids with nutrients gain access to the interior of the embryo during stages 9–10 until the cardiovascular system takes over. From the internal surface of the coelom mesoblastic cells the visceral lining layers of the internal organs are produced. Coelomic epithelium can readily transition to mesenchyme or vice versa. At the level somite 1, the coelom is involved with the sinus venosus and liver. It contributes the mesenchymal elements to the liver, whereas the epithelial elements come from the gut.

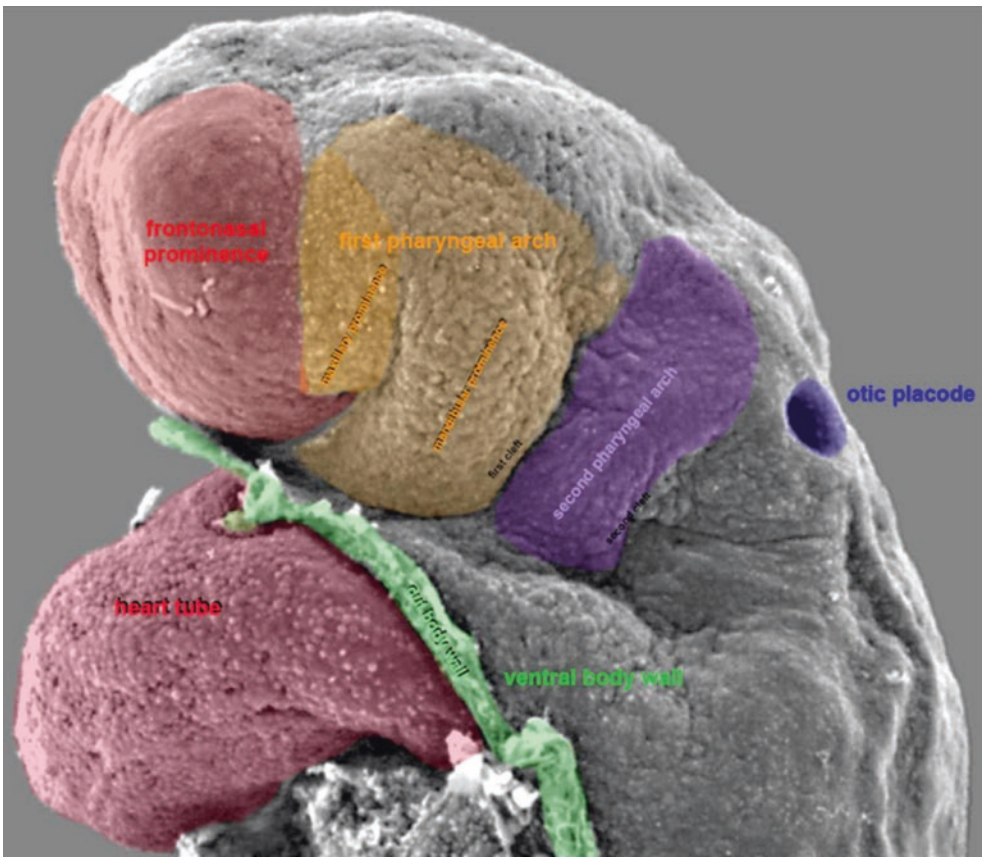

Fig. 3.28 Stage 11 showing the pharyngeal arches: the second pharyngeal arch is well shown, third arch is in formation. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

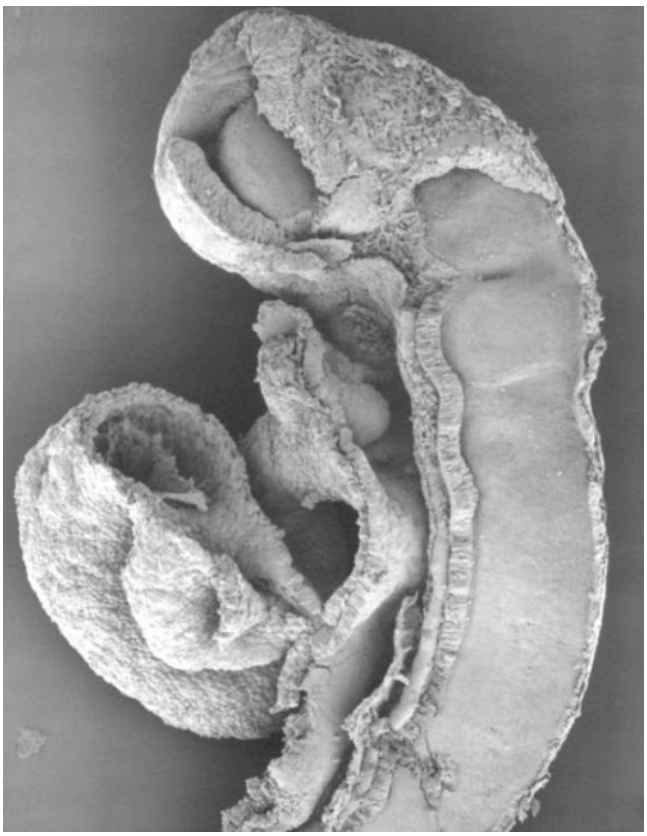

Fig. 3.29 Stage 11: midline section showing rhombomeres, bucco-pharygeal membrane, yolk sac. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

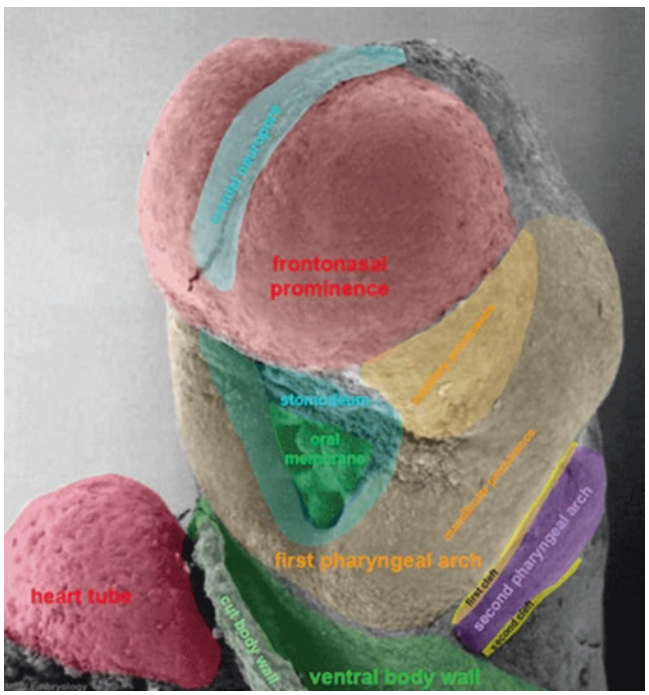

Fig. 3.30 Bucco-pharyngeal membrane constructed from non-neural ectoderm externally and endoderm internally. Lining of oral cavity is first, second, and third arch but second arch is overtaken. BPM marks the boundary in the pharynx between third arch and fourth arch. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

Cardiovascular System

The third aortic arch artery develops from conus. Most embryonic vessels are simple endothelial tubes except in the heart. Cardiac endothelium is surrounded by jelly and enclosed by contractile myocardial tissue, itself derived from coelomic epithelium and bathed in coelomic fluid. Vascular system maturation is cranial-caudal. Capillaries from the rostral umbilical vesicle communicate with the heart. By 16 somites, the sinus venosus is present. Common cardinal veins have not yet reached the atria, so the true circulation is not yet established. Embryonic blood cells are very few, but blood islands are active in the umbilical vesicle and will produce hematopoietic cells in the next stage. CNS angiogenesis is proceeding. In sum, the embryo at stage 11 lives via a system of endothelial channels that transport a virtually cell-free fluid via ebb-and-flow and the primitive pulsations of the myocardium. By the end of stage 11, the sequence of cardiac structures is: right atrium, left atrium, atrioventricular canal, left ventricle, right ventricle, conotruncus, and aortic arch arteries.

Digestive and Respiratory Systems

The second pharyngeal arch is present. Oropharyngeal membrane breaks down in anticipation of third arch at next stage. Esophago-pulmonary groove is present. Liver diverticulum grows into septum transversum.

Nervous System: Closure of the Rostral Neuropore

Closure of rostral neuropore is bidirectional; from hindbrain forward to midbrain and from optic chiasm backward toward roof of diencephalon to form the lamina terminalis with closure complete to this level at somite 14. By somite 20, the entire forebrain is closed. Neural ectoderm becomes isolated from contact with amniotic fluid; compensatory proliferation of capillaries all over the tube takes place. As the walls of the tube thicken, capillaries send in penetrating branches to supply deep-lying cells at ventricular cavities. Forebrain is still quite simple with D1 being the optic primordium and midline chiasmatic plate, the caudal limit of which is the optic recess. The floor of D2 contains the neurohypophysis, opposite of which lies, in the future mouth, the adenohyphysis in the apex of the oropharyngeal membrane. Rostral to D1 is telencephalon created by the fusion of the rostral neuropore and marked by lamina terminalis and the commissural plate. Mesencephalon at this point is large and poorly-defined consisting, at this point, of a single segment (it will eventually have two mesomeres).

Neuromeres: As defined by O'Rahilly, neuromeres are visually identifiable. Recall that this is from morphology, not genetic mapping (as per Puelles and Rubinstein), so it is more simplistic (see Chap. 5 on the neuromeric system). Rhombomeres are identified by ganglia, with trigeminal defining r2, faciovestibulocochlear defining r4, glossopharyngeal defining r6, and vagal determining r7. Midbrain has in stage 11 a single mesomere.

Neural crest: Cells are produced at all levels, but appear to be "given off" at this stage. MNC spreads toward the optic region.

Eye: Right and left optic primordia are a U-shaped continuum at the optic chiasma. At 14 somites, optic evagination occurs with continuity between the optic ventricle and forebrain. At 14–16 somites, it acquires surrounding midbrain neural crest. At 17–19 somites, the optic vesicle is formed. It is kept separate from overlying ectoderm by the ensheathing neural crest.

Ear: Otic disc is sharply demarcated from surrounding ectoderm. Recall the neural ectoderm arises from stem

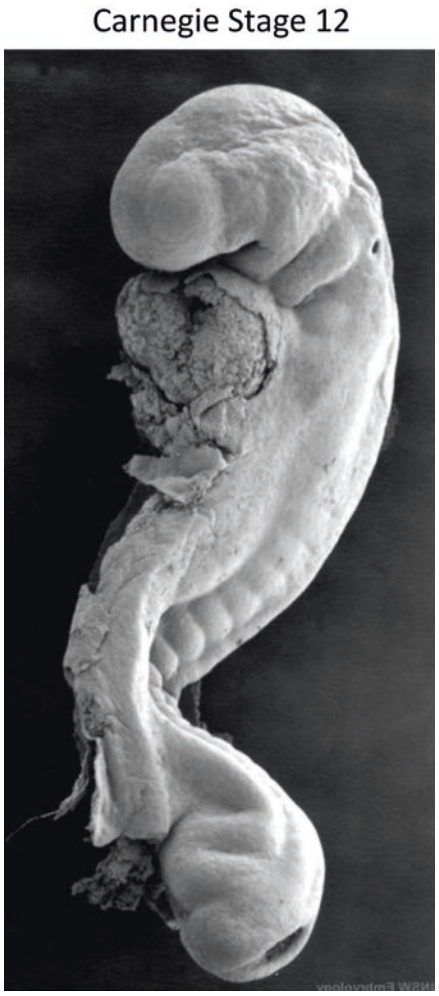

Fig. 3.31 Stage 12: 26–30 days, 3–5 mm, 21–29 somites. (1) Ectoderm: caudal neuropore closes, forebrain developing. (2) Placodes: optic, otic (open to the skin). (3) Mesoderm: stomodeum, heart large, upper limb bud. (4) Fourth pharyngeal arch (fourth aortic arch—persists). (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

cells in the epiblast and is segregated from the epibast at stage 8.

Stage 12 (30–31 Days, 3–5 mm, 21–29 Somites, Fourth Aortic Arch) (Fig. 3.31)

External: 21–29 somite pairs present. third pharyngeal arch visible. The first and second arches are fusing and their respective aortic arteries are disintegrating. Otic vesicle almost closed. Caudal neuropore is closing. Upper limb buds appear.

Internal: present are the interventricular septum, lung buds, and the dorsal pancreas.

External Form

Four occipital somites are lined up with the primordia of the roots of hypoglossal nerve. With the three pharyngeal arches, three points of contact (bars) exist between ectoderm and endoderm. Furthermore, each arch is subdivided into dorsal and ventral sectors; these correspond to neuromeres (first arch r2–r3, second arch r4–r5, and third arch r6–r7). Just caudal to third arch is condensed mesoblast of the future larynx. This will be enclosed at stage 13 by the fourth pharyngeal arch. Note the third, fourth, and fifth pharyngeal arches become internalized. Cervical sinus, the boundary between the externalized first/second arch complex and the cervical somatic derivatives, is located here. Otocyst maintains a pore.

Rostral neuropore closed at stage 11 and caudal neuropore closes at stage 12. Shape of embryo changes to a C-shaped curve with the increasing mass of the spinal cord, somites, and surrounding mesoderm.

The extensive aperture between gut and umbilical vesicle (yolk sac) undergoes a pursestring-like contraction with definition of the umbilical stalk. This will include the paired umbilical arteries and veins. Upper limb bud appears opposite somite 8–10 (neuromeres c4–c7).

Cardiovascular System

Fourth aortic arch artery develops from conus.

During stage 11, embryonic nutrition depends upon exocoelomic fluid brought into direct contact with the interior of the embryo by the coelomic cavities. This was an ebb-and-flow circuit, abetted by early contractions of the primitive heart. By 30 somites, the requirements of the embryonic mesoblast exceed this system. The brain and skin ectoderm remain independent of this system, being nourished by amniotic fluid. When the neural plate rolls into the neural tube and sinks beneath the skin, a new system is required. As early as stage 10, a primitive head plexus and primitive hindbrain channels develop alongside the CNS. These are supplied by dorsal segmental arteries from the dorsal aorta, primitive trigeminal, facial, otic, and hypoglossal arteries. Simultaneously, a separate plexus differentiates in situ in the mesoblast lateral to the neural tube. These channels will connect with the external carotid system to form the vasculature of the future scalp. At 14 somites, umbilical arteries and veins extend to the allantoic diverticulum and thence to the connecting stalk.

Stage 12 presents us with the first connected circulatory circuit. This requires (1) overhaul of the venous end of the heart, (2) creation of dedicated CNS vasculature, (3) cardinal veins, (4) the liver plexus, and (5) modifications in the vitelline plexus into large trunks leading to the atria of the heart. Enlarged parts of vitelline plexus become right and left atria, each side of which enlarges to make common cardinal vein. Note that left atrium becomes cut off from the sinus venosus which now leads exclusively to right atrium. Sinus venosus receives all venous blood flow from the embryo. Placenta does not yet exist.

Digestive/Respiratory Systems

Stage 12 is marked by a dramatic expansion in epithelium of the gut. In the pharynx, the roof proliferates compared to the floor which remains thin. Respiratory tissue expands.

Nervous System: Closure of Caudal Neuroport, Secondary Neurulation Begins

The roof of rhombencephalon becomes thin. The basal plate contains the motor nuclei. Twelve rhombomeres are present. The eighth rhombomere was originally considered to cover the entire medulla, but now it was recognized by Puelles that r8 is broken up into distinct genetic units (r8–r11). Telencephalon enlarges. Area adjacent to the former rostral neuropore is termed the commissural plate. Diencephalon has future thalamic structures. Midbrain now has two segments, m1 and m2. Nasal discs become thickened.

Eye: Optic neural crest at its maximum. Beginning of the *sclera.*

Ear: Otocyst, originally open to the external surface via a pore. Represents a detached island of neurectoderm having simple skin epithelium; has nothing in common with surrounding skin.

Stage 13 (32 Days, 4–6 mm, 30–31 Somites, Fifth Aortic Arch—Abortive) (Fig. 3.32)

Summary External: 30 + somite pairs (somite count no longer useful after this stage). fourth pharyngeal arch forms; otic vesicle now closed; optic disc not indented.

Internal: retinal and lens discs present; trachea begins; right and left lung buds are visible; heart has septum primum.

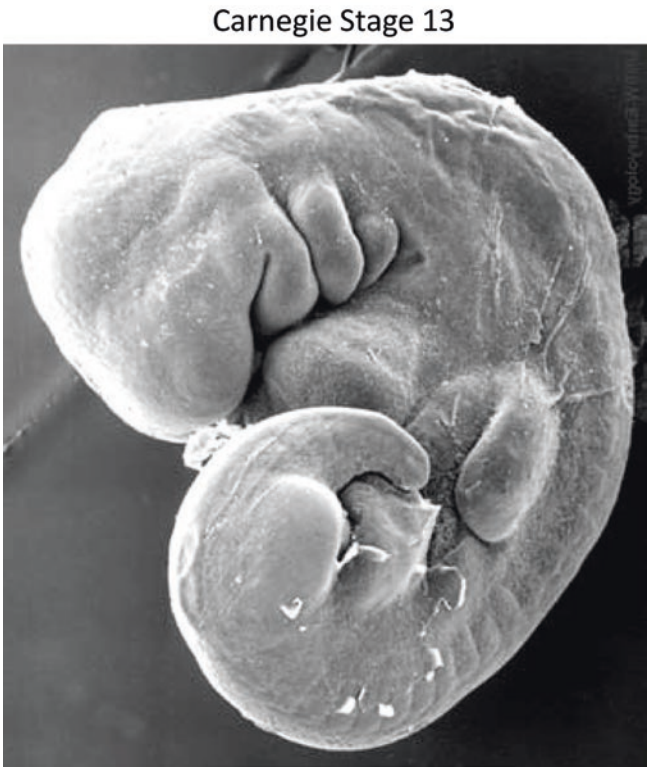

Fig. 3.32 Stage 13: 26–30 days, 26–30 mm, 30–31 somites. (1) Nasal placodes. (2) Lower limb bud. (3) Fifth pharyngeal arch (fifth aortic arch—fails or disintegrates). (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

External Form

Three-dimensional form of the embryo determined by the shape of the rapidly growing CNS. Through the glossopharyngeal arch runs the third aortic arch artery and just behind it is a depressed zone where the surface ectoderm is in contact with the pharyngeal endoderm. The floor ceases to be visible in stage 14 due to growth of tissues in front of and behind this depression such that the floor of this triangular area becomes covered over, thus forming the cervical sinus. A new fourth pharyngeal arch is forming in this region. Both upper and lower limb buds are seen.

Cardiovascular System

This stage is marked by further development of embryonic-material circulation. fifth aortic arch artery from conus and dorsal aorta appears and then fails to develop. The enormous CNS is well perfused through the surface capillary network, even though distinct arterial structures are in formation. The lateral plate mesoderm that will form lungs and gut has numerous small arteries in situ. The caudal end of the embryo is rapidly developing and contains a vascular plexus from which arise the umbilical arteries. These progress backward into the connecting stalk via which they unite with the chorionic circulation. Interchange now occurs between the embryonic blood in the chorionic villi and maternal blood circulating in the intervillous spaces. Venous return via a plexus in the connecting stalk dumps into the right and left umbilical veins and thence into the sinus venosus of the heart.

An additional circulation develops between the body and the vitelline plexus consisting of a single vitelline artery from the splanchnic aorta and right and left vitelline veins that connect with the liver sinusoids. This hepatic plexus empties into the sinus venosus. Thus, sinus venosus received venous return from three sources: (1) from the embryo via the cardinal veins, (2) from the placenta via the umbilical veins, and (3) from the yolk sac via the vitelline veins to the liver. The venous system was initially symmetrical but, with the advent of the sinus venosus and the hepatic plexus, a one-sided portal system becomes established. We shall not spend more detail on this here.

The heart begins to undergo functional partitioning in stage 12.

The liver has the following blood vessels. (1) The intrinsic hepatic plexus arises from coelomic mesoderm. (2) This intrinsic system receives flow from the yolk sac via the vitelline veins. (3) The left umbilical vein makes an anastomosis with the hepatic plexus to form a single ductus venosus that empties into the inferior vena cava. Hepatocardiac veins coalesce to drain the hepatic plexus into the sinus venosus.

Digestive/Respiratory Systems

In stage 12, certain zones of endoderm become determined to form lung, dorsal pancreas, and liver. In the floor of the pharynx, the two lobes of the thyroid primordium appear. Pharyngeal pouch 3, interposed between the second and third pharyngeal arch masses, produces thymus.

Trachea appears. The right primary bronchus descends vertically, whereas the left bronchus is directed laterally.

Nervous System: Neural Tube Is Closed, Cerebellus Appears from r1

In form, the CNS is still tubular, with basal and alar plates. Early neurons are emerging as ventral roots; dorsal roots are

lagging behind in development. Rhombomeres are clearly distinguished. Motor columns are present in the brain stem and those of the spinal cord are in continuity with hypoglossal. Midbrain has two distinct segments. Diencephalon also has two main components: D1 gives rise to the optic evagination and D2 to the thalamus.

Eye: Optic retina, pigmented retina, and optic stalk are seen. On the outer surface of the retinal disc, a marginal zone of nuclei forms a meshwork which will eventually send fibers backwards into the optic nerve. Pigmented retina is much thinner. The optic vesicle is covered by a basement membrane external to which, in the mesenchyme that originated from MNC, angiogenesis is taking place. Retinal disc moves outward to come into contact with overlying p5 ectoderm, the optic placode. Induction of the lens results.

Ear: Otic vesicle interacts with surrounding mesoderm likely from PAM of somitomeres 5–7 plus facioacoustic neural crest from r4-r5 causing angiogenesis. The vestibular part of vestibulocochlear ganglion appears.

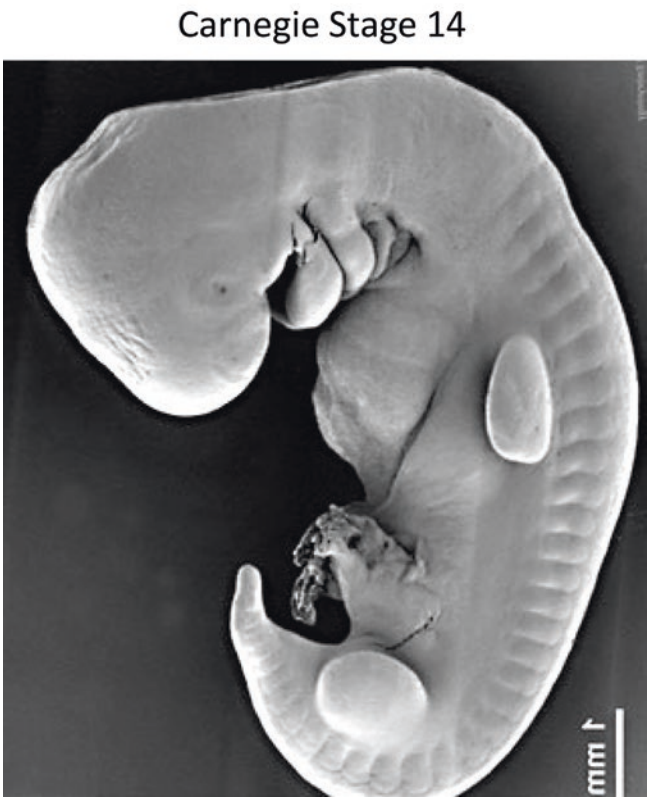

Fig. 3.33 Stage 14: 31–35 days, 5–7 mm, 32+ somites. (1) Endoderm: gut development. (2) End of pharyngeal arch period. (3) No sixth pharyngeal arch (sixth aortic arch is a plexus for pulmonary circulation). (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

Stage 14 (33–35 Days, 5–7 mm, Sixth Aortic Arch for Pulmonary Circulation) (Figs. 3.33 and 3.34)

External: Lens disc invaginated and open to the exterior, lengthened upper limb buds.

Internal: Fifth pharyngeal arch, ventral pancreas, dorsal growth of the lung sacs, cerebellar hemispheres.

External Form

Cervical bend at the level of sclerotomes 5–6 (*Nackengrube of His*) anticipates the later flexure sequence of the vertebral column. first and second arches have not yet coalesced; third pharyngeal arch is beginning to become internalized and concealed with respect to them. second arch has notable dorsal (r4) and ventral (r5) zones. Because the head is largely translucent at stage 14, the following can be observed: the optic cup, trigeminal ganglion with V2 and V3 cells flowing into the dorsal/maxillary (r2) and ventral/mandibular (r3) zones of first arch, faciovestibular mass of neural crest enters dorsal (r4) zone of second arch, otic vesicle with well-defined endolymphatic appendage, and both glossopharyngeal and vagus nerves. Nasal plate is flat; it does not have the distinct elevated rim that defines it at later stages.

Vascular System

Sixth aortic arch artery present. Its formation and destiny are unique. Recall that first aortic arch is the passive deformation of the dorsal aortae leading to the heart as it is retropositioned beneath the pharynx. Aortic arches 2–5 were formed from the union of a dorsally directed sprout from the aortic sac and a ventrally directed sprout from the dorsal aortae. The two components of aortic arch 5 never unite and it aborts. Aortic arch 6 is formed but loses contact with the dorsal aorta to become dedicated to the lung. No remnant corresponding to AA6 exists from the dorsal aortae. The four divisions of the cardiac tube are still visible. Atrioventricular cushions are not united. The outflow tract is still a single lumen but beginnings of septation are taking place.

Digestive System

Stage 14 marks the close of the aortic arch period. There is no outcropping of endoderm distal to AA6. Thus, there can be no sixth pharyngeal arch. Furthermore, the fate of AA6 is strictly pulmonary. The fifth pharyngeal arch survives by perfusion from the external carotid supply dedicated to the fourth pharyngeal arch (superior thyroid). Fifth arch neural crest from r10 to r11 produces the arytenoids.

Prior to stage 14, the pharyngeal pouches were simply lateral extension of pharyngeal endoderm interposed between the aortic arch arteries and making contact with the external ectoderm such that barriers to neural crest migration were established. Thus, neural crest flowing down around the aor-

Fig. 3.34 Stage 14, 32 days 35 somites Adjacent to upper limb bud is liver and above that, the heart. Four pharyngeal arches seen, with the fifth arch internalized. Optic placode has a pit to the surface ectoderm. (Courtesy of Prof. Kathleen K. Sulik, University of North Carolina)

tic arch arteries created the mesenchymal expansions known as the pharyngeal arches, a process much like filling up a balloon with water.

The pharyngeal pouches are now pocket-like, each with future derivatives. Thymus and parathyroid tissues are identified. Thyroid is elongating downward, but remains connected to the epithelium of the oropharynx. It has two defined lobes and an intervening isthmus.

Merger of the first and second arches affects the oropharyngeal distribution of second arch. Sensory representation of second arch is eliminated from the epithelium (although it persists in the gustatory tissues of the tongue). Thus, the pharyngeal lining has V2 and V3 apposed to third arch IX. This reduction in representation of second arch to the tubotympanic recess means that the Eustachian tube represents the interface between first and third arches with the lining of the tube being somatic sensory to V3 and conducts r6-r7 fibers of vagus into the ear canal as the Arnold's nerve.

Intestinal growth proceeds with individual zones defined by homeotic genes. Ventral pancreas arises as an evagination from the bile duct.

Respiratory System

Arytenoid swellings corresponding to the fifth pharyngeal arch appear in the pharyngeal floor. Trachea and esophagus are separate structures. The epithelium structure of the lungs, previously dominant, is now surrounded by mesenchyme from the adjacent coelomic walls—these being ultimately derived from visceral lateral plate mesoderm. The proliferation of the coelomic cells took place at stage 12. As they migrate to surround the pulmonary and alimentary epithelium, they leave behind an external "slime trail" of cells which constitute the lining mesothelium.

Within the coelomic mesenchyme ensheathing the lungs, an extensive capillary network develops by angiogenesis. These channels connect with the sixth arch pulmonary arteries.

Nervous System: Future Cerebral Hemispheres Are Defined

The neural tube corresponding to spinal cord develops from cranial to caudal, from medial (ventral) to lateral (dorsal), and from internal to external. It has three zones: (1) the ventricular zone contains the stem cells from which ependymal cells, glial cells, and neurons will develop; (2) the mantle zone at stage 14 contains the earliest neurons which give rise to ventral roots; and (3) the marginal zone constitutes free space for the neurons to grow into. Hindbrain cranial nerves have formed and individual hypoglossal roots are uniting. In the midbrain, oculomotor is now present. Cerebellum arises from the alar plate of r1. In the telencephalon, neural crest migration is now in contact with the brain at the olfactory area.

Eye: Optic cup is present. In the outer uveal layer, a distinct capillary network appears. Lens placode communicates with the surface with a definite pit.

Ear: The optic vesicle does not change much in size, but elongates due to the growth of the cochlear duct. Endolymphatic duct appears. Within the walls of the vesicle, a thickening develops for the future labyrinth.

Stage 15 (36–37 Days, 7–9 mm) (Fig. 3.35)

External: lens vesicle now closed; nasal placodes develop pits, hand plates.

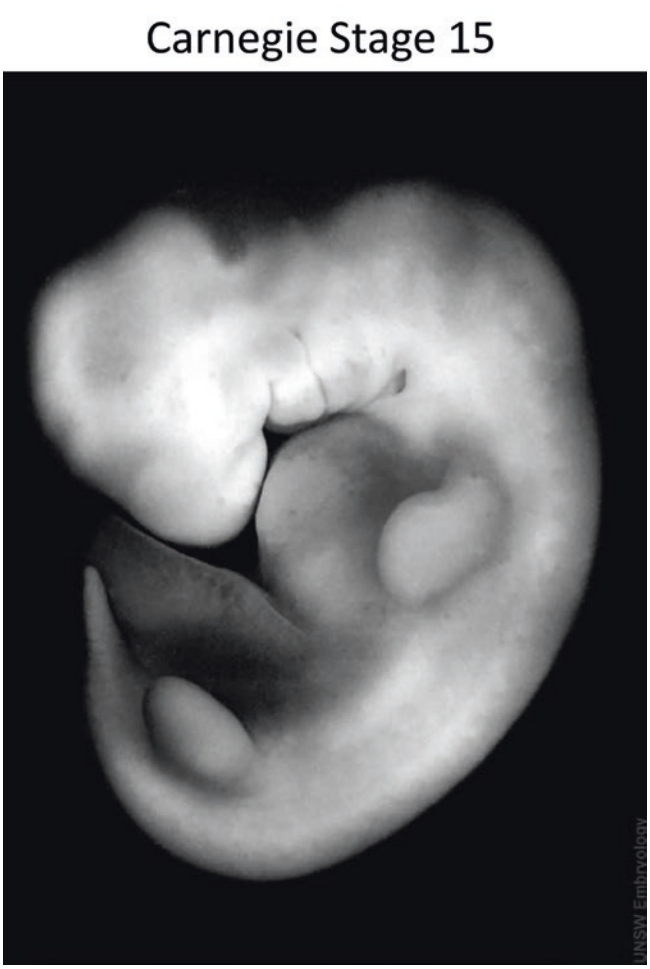

Fig. 3.35 Stage 15: 35–38 days, 7–9 mm. (1) Ectoderm: lens pit, otocyst, nasal pit. (2) Hand plate. (3) Umbilical cord. (4) Head: maxillary/mandibular fields, cervical groove. (5) This is the last stage at which somites can be seen on the surface. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

Internal: heart has foramen secundum, intestine for loop with distinct cecum, lobar buds, ureteral pelvis, defined cerebral hemispheres, retinal pigment.

External Form

Lens vesicles are closed and the external pit with which they communicated disappears. Nasal discs appear to sink into the MNC mesenchyme. Proliferation of mesenchyme around the rim of the discs created elevated prominences, the future nostrils. Ventral first arch forms auricular hillocks. In stage 15, the superficial tissues are thin, permitting visualization of somites, muscles, and ganglia along the length of the embryo. In subsequent stages, interposition of proliferating mesenchyme makes them no longer visible.

Cardiovascular System

Arterial part of the heart is distinct from the venous part of the heart which was derived from the vitelline plexus. Mesenchyme is distributed asymmetrically between ventricles and atria. Blood flow through the atrioventricular canal is now divided into left and right streams. Semilunar valves appear. Recall the cardiac neural crest has descended into the heart to contribute to valves and conduction system.

Thyroid primordium now detaches from the pharynx and descends. Primary bronchi are located in front of esophagus which has lengthened.

Respiratory System

Through stage 15, caudal trachea remains in close proximity to anterior wall of esophagus. This presents opportunities for congenital anomalies to occur. Esophagus begins to separate from trachea.

Nervous System: Diencephalon Develops Longitudinal Zones

Brain surrounded by identifiable primary meninx. Mesenchyme of secondary meninx is present but not defined. Rhombomeres remain identifiable. Geniculate ganglio of VII separates from the vestibulocochlear ganglion of VIII. Cochlar part of ganglion VIII now distinct from vestibular part. Abducent nerve seen. Decussation of trochlear nerve constant.

Eye: Lens now has its own capsule and contains lens fibers. Neural crest cells surrounding lens but nothing formed as yet. Lens vesicle and optic cup just below the surface. Surface ectoderm constitutes epithelium of future cornea.

Ear: Mesenchyme condenses to form otic capsule (not yet chondrified). Groove on the membranous labyrinth designates future semicircular ducts. Fibers from vestibular reach the otocyst. From the ventral segment of second arch, a subsegment forms the tragus.

Stage 16 (38–40 Days, 8–11 mm) (Figs. 3.36 and 3.37)

External: *Nasal pits* coming to midline, retinal pigment, arch 3 is disappearing from the surface, auricular hillocks, lower extremity (thigh, leg, and foot).

Internal: Foramen secundum; intestinal mesentery is present and rotation of the gut is starting; bipartite pelvis, no longitudinal cerebral fissure; neurohypophyseal evagination; lens pit D-shaped; semicircular ducts appear as thickenings.

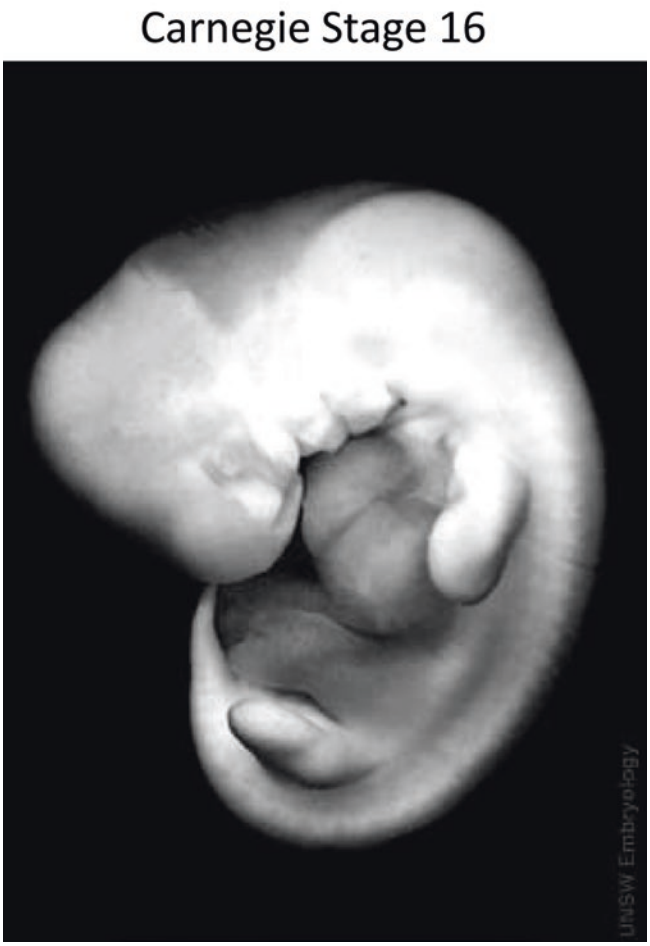

Fig. 3.36 Stage 16: 37–42 days, 8–11 mm. (1) Nasal pits now positioned ventral. (2) Nasolacrimal groove. (3) Eye: retinal pigment. (4) Auricular hillocks. (5) Arm. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

External Form

Midbrain and thalamic regions larger than stage 15. Details of special regions emerge.

Nasal discs have elevated borders and appear concave; moving ventrally. Nasofrontal groove marks the beginning of the nose.

Hyoid arch appears massive because of precursors of hillocks. Both first and second arches have grooves marking dorsal and ventral segments.

Cervical sinus was visible at stage 14, its floor is formed by third and fourth arches. At stage 16, these are receding and the sinus is closing.

Hand has a central carpus and digital flanges. Lower extremity has 3 growth centers. Rostrolateral (femoral, obturator nerve of lumbar plexus), caudomedial (peroneal and tibial nerve of sacral plexus), and foot (extension of tibial nerve).

Spinal ganglia present throughout the trunk.

Face

Roof of the mouth slopes ventrally between the nasal pits (Hinrichsen)—no sign of the upper jaw. Skin ectoderm is thickened over areas of activity (lower jaw, hyoid).

Between head ectoderm and brain is thin primary meninx carrying blood vessels to brain wall and cranial nerves. Nasal and otic proliferation.

Maxillary fields are bulging inferolateral to eye and nose, fields are widely separated. Premaxillary condensations.

Cardiovascular separation of aortic and pulmonic circulations into fourth and sixth AAs. Pulmonary develops bronchial tree.

Nervous System: Neurohypophysis Evaginates

Ascending fibers of posterior columns (dorsal funiculus) reach the medulla. Rhombomeres still present. Cells of somatic and visceral efferent nuclei of 5, 7, 9, 11 migrating laterally. Isthmus; Groove between cerebellum and midbrain. Midbrain still has two parts. SANS forms a solid trunk.

Eye forms D-shaped lens cavity. Perilentil vessels present from posterior and anterior chorodoidal. Shallow grooves appear above and below the eye; these mark the future eyelids.

Ear: Auricular hillocks all present long endolymphatic sac Wall thickening in otic vesicle preseages semicircular canals.

Stage 17 (41–43 Days, 11–14 mm) (Fig. 3.38)

External: Head enlarged due to brain. Trunk straighter with slight lumbar curve. Nasal pit now ventral, so nostril not seen on profile; full complement of ear hillocks both first and second arches; hand plate has digital rays; foot now a rounded plate. Surface manifestations of somites now

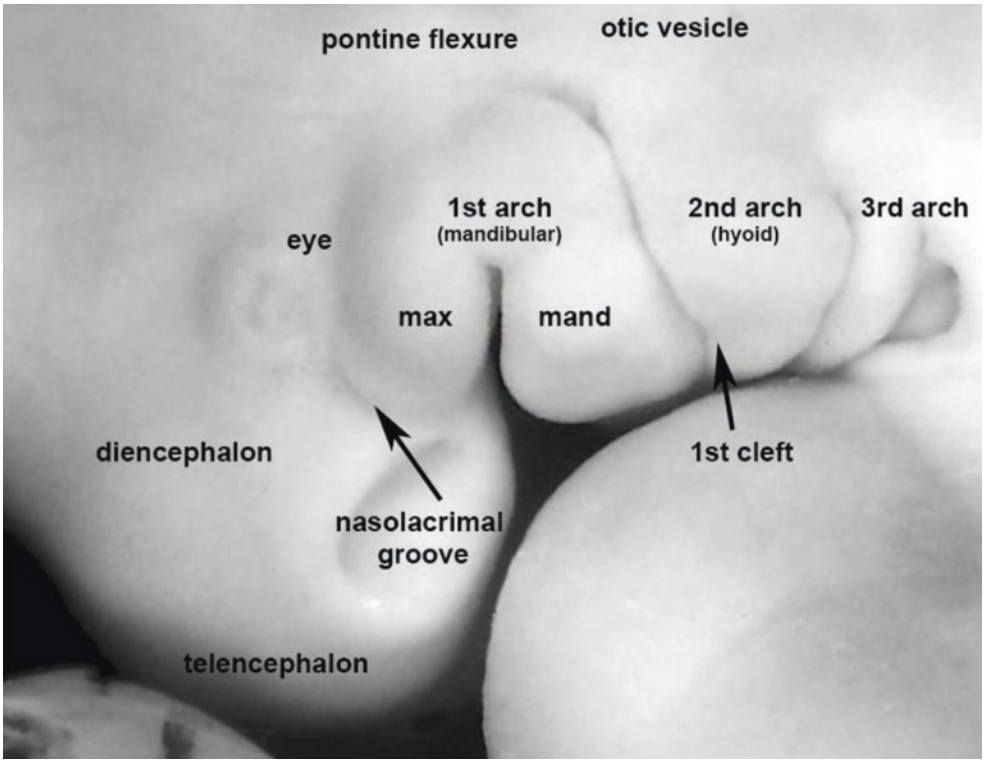

Fig. 3.37 Stage 16 showing nasolacrimal groove. At the top of the first cleft, adjacent to the otic vesicle, is future external auditory canal, flanked by auricular cartilage hillocks from first and second arches. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

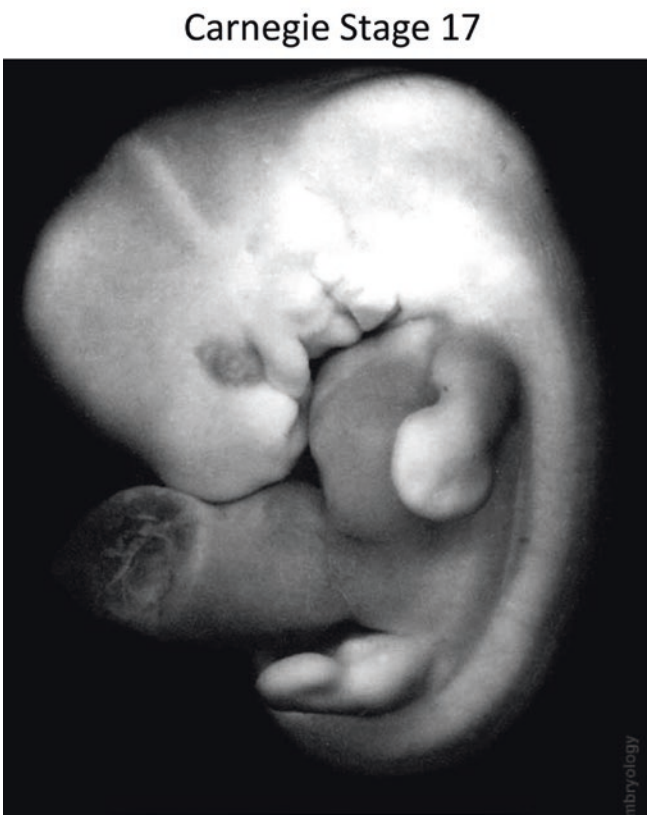

Fig. 3.38 Stage 17: 42–44 days, 11–14 mm. (1) Lens Pit, eye pigment. (2) External acoustic meatus. (3) Nasal pit moved ventrally (well seen here). (4) Mesonephric ridge. (5) Hand digital rays. (6) Thigh, ankle, footplate. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

seen only in lumbar region—mesenchymal development of the neck and thorax.

Internal: Foramen primum being eliminated, foramen secundum and semilunar cusps appear, fusion of A-V cushions (stages 16–18); palate begins; fusion pancreatic buds; bronchial tree has segmentations; functioning mesonephros with calices; olfactory bulb; retinal fissure almost closed and lens is crescent-shaped, semicircular canals imminent but not present; ear bones present.

External Form

Head and neck proportions increase—now have same profile area as entire body; fronto-mesencephalic length increased, just in front of precerebellar notch. Nostrils can only be seen on decapitated specimens; auricular hillocks fully developed—those of second arch are larger. First arch ventral hillock is tragus dorsal 2 hillocks crus helicis—hillocks are coalescing.

Upper limb bud has finger rays. Crenation characteristic of fingertips starts, but is prominent in next stage. Lower limb has rounded digital plate (paddle). Evidence of growth of muscles and the pelvic girdle. Surface marking from somites only lumbar and sacral.

Face

Furrows between processes (fields) are smoothing out—coalescence elevates the furrows. Intermaxillary field. Nasal passages end in cul-de-sac, the *hinteren Blindsack* of Peter, terminate in bucconasal membrane.

Pits become respiratory passages—formation of MNC fields. Regional specialization of epithelium. Outlines of nose seen, median rim of nostrils.

Six zones of odontogenic epithelium.

Nervous System: Future Olfactory Bulges, Future Amygdaloid Nuclei

Rhombomeres are beginning to recede; migration from medioventral cell column to pharyngeal arch (visceral) motor nuclei still in progress; Trigeminal is first to form. Geniculate and vestibulocochlear ganglia now separate. Olfactory tubercle and future olfactory bulb are prominent; olfactory fibers can be separated into medial (vomeronasal and terminal) vs. lateral (olfactory).

Eye: Retinal fissure almost closed. Retina differentiates. Lens vesicle is crescentic. Lower eyelid fold.

Ear has large endolymphatic sac, cochlear duct elongating. Walls of vestibular labyrinth thinning. Semicircular ducts not present. Six hillocks present and develop anterior to posterior and ventral to dorsal: first arch tragus (1), crus helicis (2, 3); second arch antitragus (6) helix (5, 4). Concha and external meatus formed from the first pharyngeal cleft. Eustachian tube forms from first pharyngeal pouch. Note anterior wall is first arch and posterior wall is second arch. Mucosal representation of second arch between the first and second pouches is eliminated such that first arch mucosa abuts third arch mucosa.

Stage 18 (44–45 Days, 13–17 mm) (Figs. 3.39 and 3.40)

External form alone is not sufficient to stage, internal features have to be included. Distinct finger rays present and, in older stage 18 embryos, elbow is present.

Nasal Passages/Respiratory

Nasal tip and frontonasal angle appear. Columella and septum identifiable; the latter has the vomernasal organ. Nasal passages end blindly in early embryos, but break down later to enter the pharynx. Vomeronasal organ present. Choanae result from breakdown of buccopharyngeal membrane.

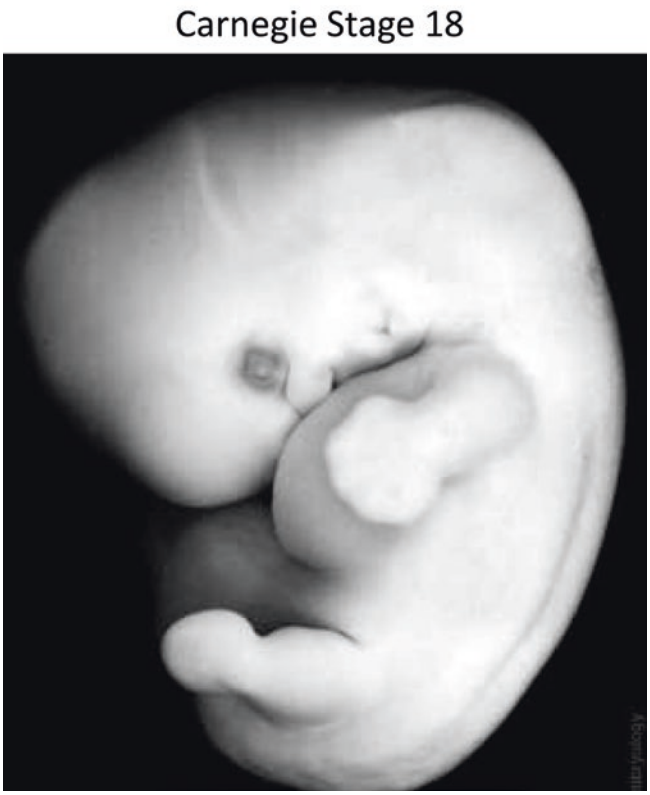

Fig. 3.39 Stage 18: 44–48 days, 13–17 mm. (1) Eyelid. (2) Semicircular canals × 3. (3) Footplate. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

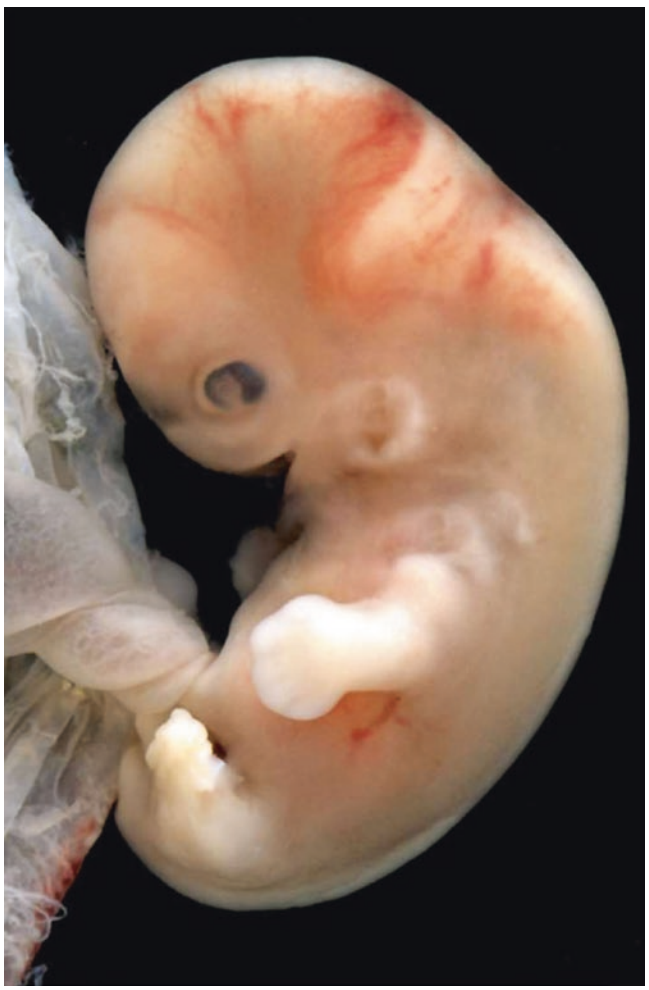

Fig. 3.40 Stage 18 bright field. (Courtesy of Stephen O'Connor, MD)

Trachea structure and epithelium radically differ from the esophagus. Larynx becomes specialized.

Mouth

Upper lip not differentiated. Auricular hillocks are starting to fuse with those rostral to the external auditory canal (from the first arch) and more advanced than those caudal to it (from the second arch). Premaxillary and maxillary fusion for primary hard palate. Secondary hard palate appears as lateral elevations. Submandibular gland first appears thicken epithelium between tongue and jaw.

Nervous System: Future Corpus Striatum, Inferior Cerebellar Peducles, Dentate Nucleus

Hindbrain is most developed with motor nuclei more mature than sensory nuclei. Choroid plexus is present in advanced stage 18. Outer swelling of the cerebellus marks the flocculus. Adenohypophysis now isolated from the mouth. Olfactory bulb developed.

Eyes: Upper eyelid fold present. Grooves mark future conjunctival sacs. Lots of eyelid pigment. The retinas are polygonal. Hyaloid system developed. Scleral thickenings appear with attachment sites for muscles. MNC invades between the lens epithelium and the surface ectoderm. Lens cavity filled in with primary lens fibers.

Ear: Hillocks are fusing. Hillocks rostral to auditory cleft (first arch) are more mature than those caudal to it (second arch). and ventral dorsal. 1–3 semicircular canals emerging from the epithelium of the membranous labyrinth. Cochlear duct is L-shaped. Malleus and incus are chondrifying and stapes now identified with stapedium muscle.

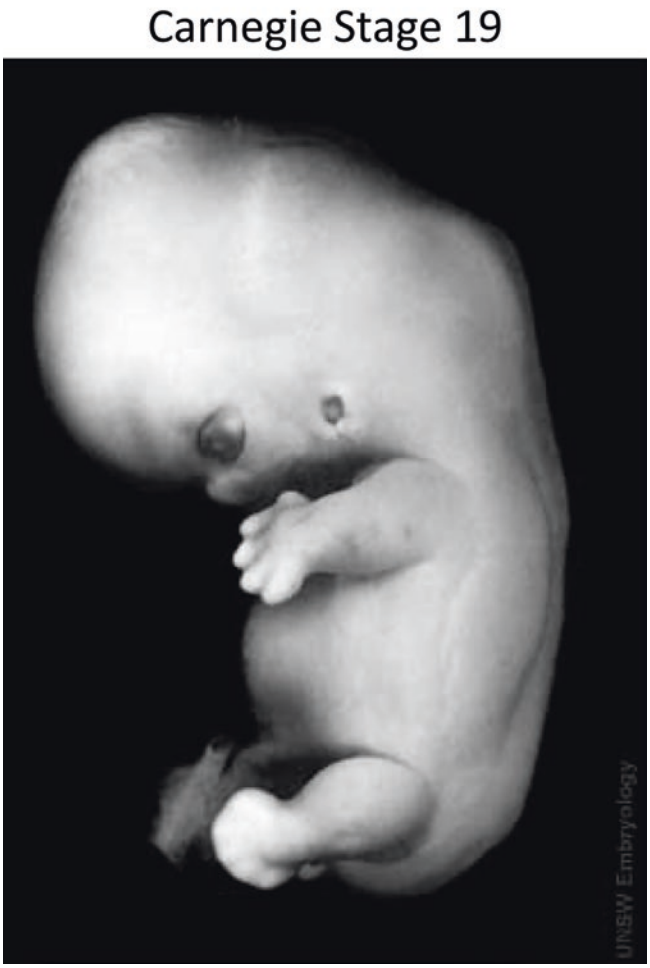

Fig. 3.41 Stage 19: 48–51 days, 16–18 mm. (1) Eye. (2) Auricle. (3) Ossification continues. (4) Foot rays. (5) Straightening of the trunk. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

Stage 19 (46–48 Days, 16–18 mm) (Figs. 3.41 and 3.42)

External form.

Limbs stick out straight. Toe rays are prominent but no interdigital notches as yet.

Nervous System: Choroid Plexus of the Fourth Ventricle, Medial Accessory Olivary Nucleus

Eye: Lowe eyelid formed. Eyelid folds meet at lateral canthus.

Optic nerve fibers are passing from retina backward, but have not reached the mid-point of the stalk. Submandibular gland mesenchyme is condensing. Will be invaded by epithelium. Half of the diencephalon is covered by hemispheres.

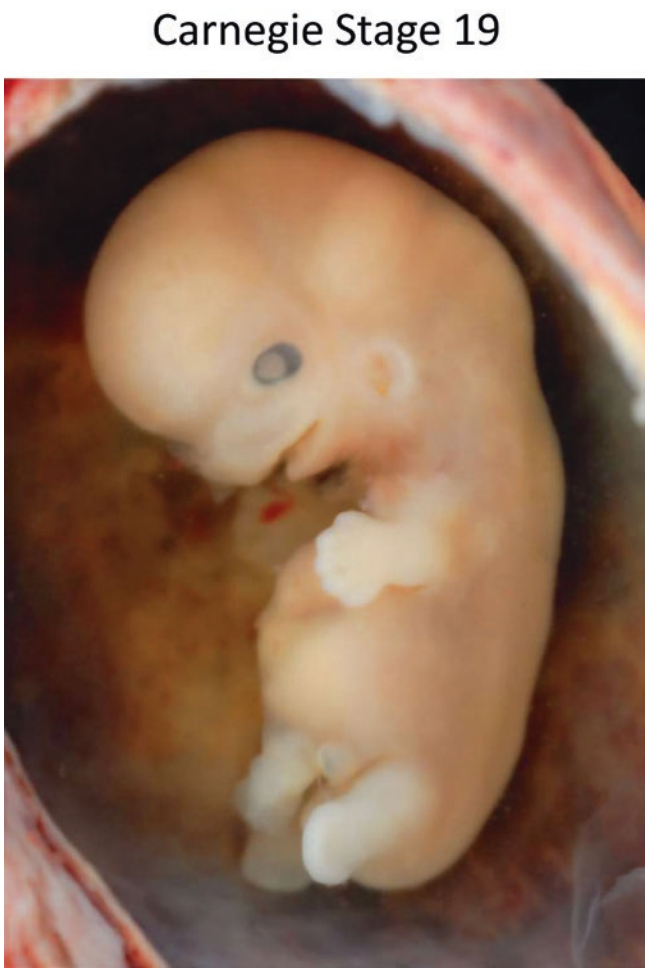

Fig. 3.42 Stage 19 bright field. (Courtesy of Stephen O'Connor, MD)

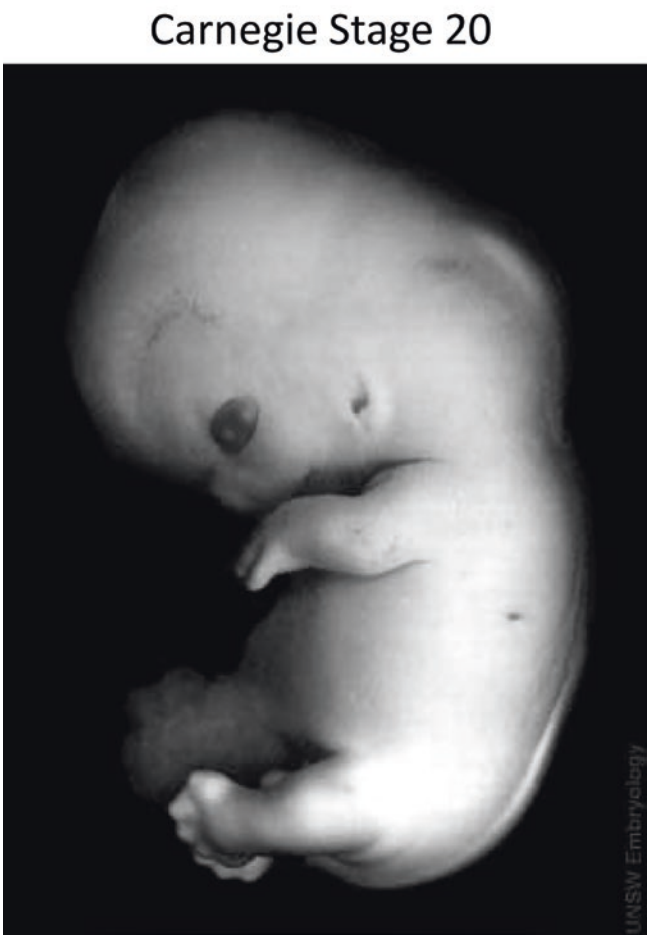

Fig. 3.43 Stage 20: 49–50 days,18–22 mm. (1) Scalp vascular plexus stage 1. (2) Nose. (3) Flexion at elbow and knee. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

Ear: Malleus and incus are present. Otic capsule chondrifies but not unified with skull base. Tip of cochlear begins to curl.

Stage 20 (49–50 Days, 22–24 mm) (Figs. 3.43, 3.44, and 3.45)

Scalp vascular plexus, stage 1.
Elbow flexion present. Fingers distinct but webbed.
Vomeronasal organ well-defined.
Submandibular gland had duct.

Nervous System: Choroid Plexus of Lateral Ventricles, Medial Accessory Olivary Nucleus

Hemispheres cover over 2/3 of the diencephalon. Inferior colliculus present.

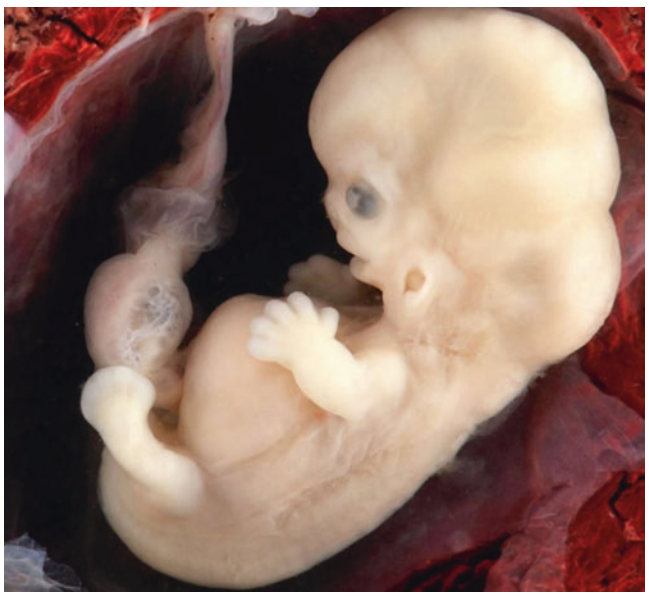

Fig. 3.44 Stage 20 bright field. (Courtesy of Stephen O'Connor, MD)

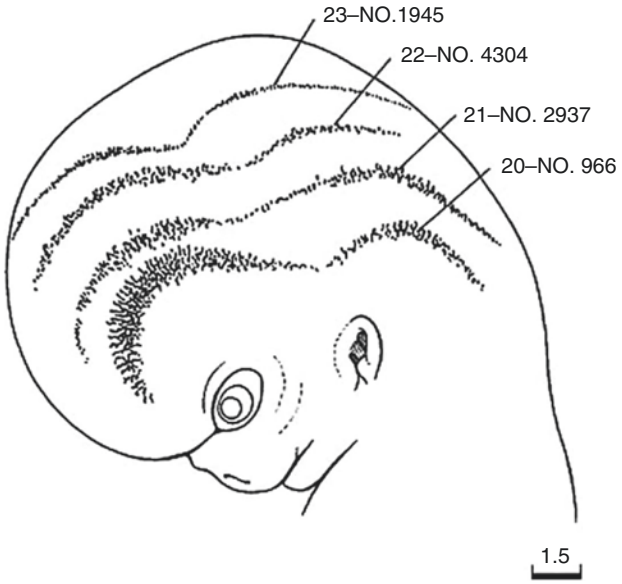

Fig. 3.45 Scalp vascular plexus has four distinct developmental levels that define stages 20–23. Stages determined with respect to the eye-ear line (EEL) and the vertex. Stage 1: less than half the distance between EEL and vertex. Stage 2: half the distance between EEL and vertex. Stage 3: three quarters of the distance between EEL and vertex. Stage 4: plexus greater than three quarters of the distance between EEL and vertex. (Reprinted from Finlay Contributions to Embryology Carnegie Institution 1923; 71: 155–161)

Eye: Upper eyelid formed. Eyelid folds meet at medial canthus. Lens cavity obliterated.

Optic nerve fibers reach the chiasma. Cornea has anterior epithelium, intermediate acellular layer, and posterior epithelium. Trochlea for superior oblique forms stage 20–23.

Ear: Tensor tympani. Otic capsule consolidated with basioccipital and exoccipitals. Cochlea elongates.

Stage 21 (51–52 Days, 2–24 mm) (Figs. 3.46 and 3.47)

Scalp vascular plexus, stage 2.
Fingers are free, toes webbed.
Submandibular gland duct is branching.

Nervous System: Cerebral Hemispheres Have Cortical Plate

Hemispheres cover 3/4 of the diencephalon.
Superior colliculus.

Eye: Levator palpebbrae superioris deliminates from superior rectus. Cells invade intermediate layer of cornea to form substantia propia.

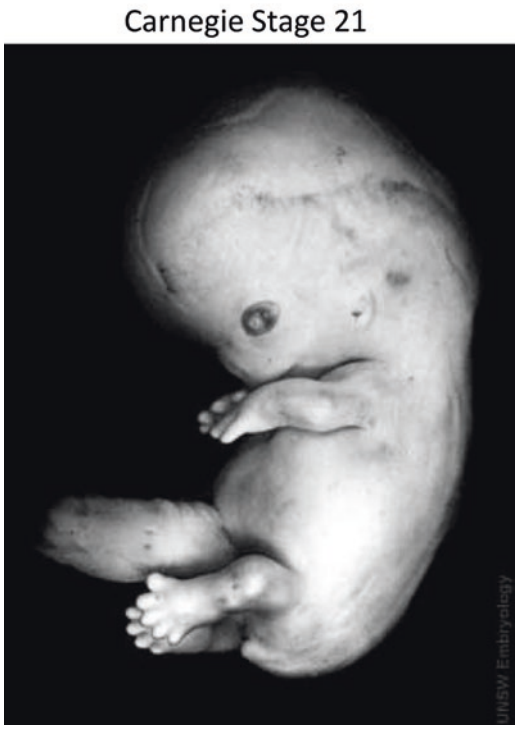

Fig. 3.46 Stage 21: 53–54 days, 22–24 mm. (1) Scalp vascular plexus, stage 2: half way between eye-ear line and vertex. (2) Philtrum. (3) Hand plate with digital webs. (4) Footplate with rays. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

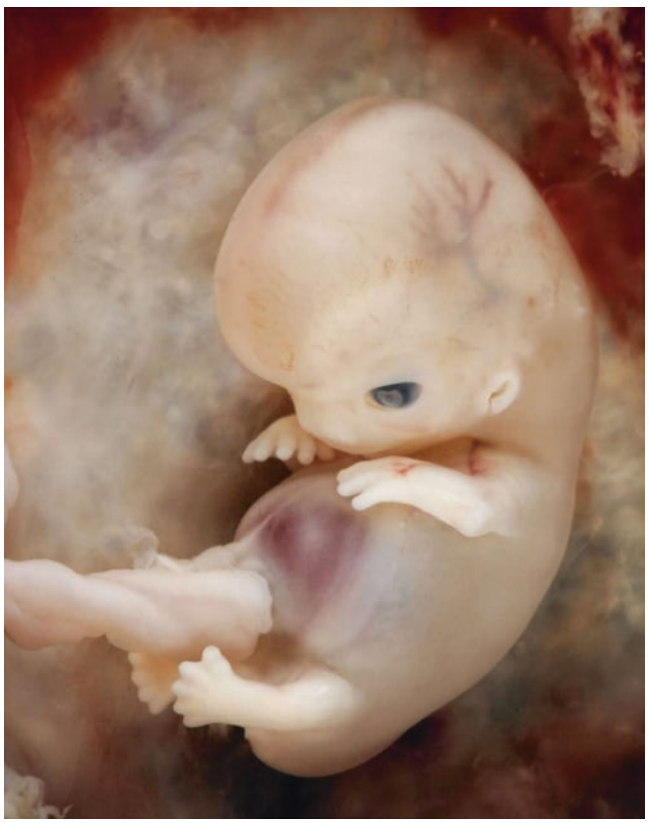

Fig. 3.47 Stage 21 bright field. (Courtesy of Stephen O'Connor, MD)

Stage 22 (53–55 Days, 23–28 mm) (Fig. 3.48)

Scalp vascular plexus, stage 3.

Nervous System: Olfactory Capsule Complete, Internal Capsule Complete

Eye: Eyelids almost closed. Sclera is now definite. Optic nerve now ensheathed.

Ear: Cochlear spiral incomplete.

Stage 23 (56+ Days, 27–31 mm) (Fig. 3.49)

Scalp vascular plexus, stage 4.

Submandibular gland ensheathed in mesoderm.

Eye: Eyelids closed. Retina complete.

Ear: Cochlea has nearly completed 2.5 turns (tip points down). Labyrinth complete.

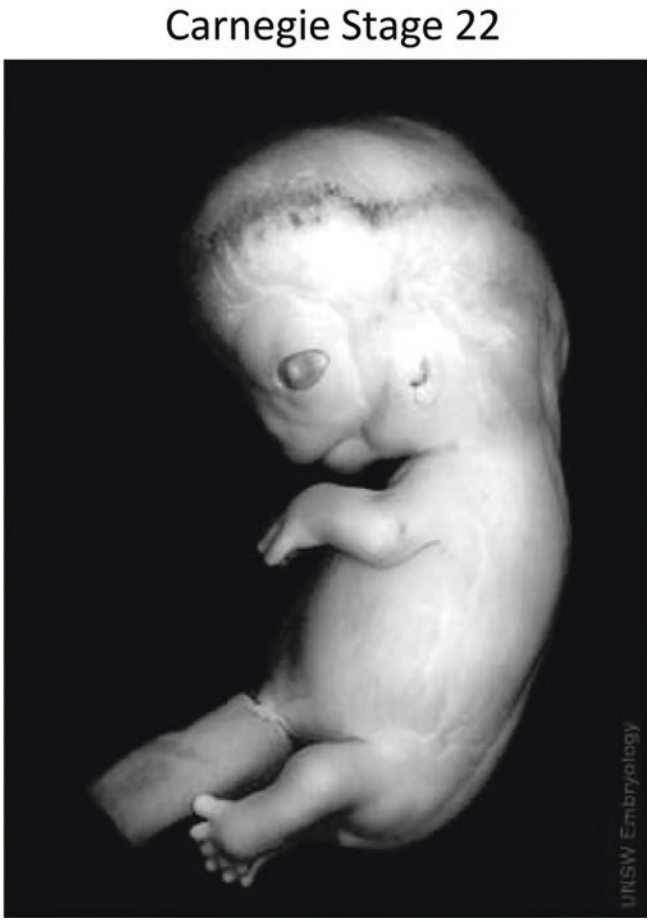

Fig. 3.48 Stage 22: 54–56 days, 23–28 mm. (1) Scalp vascular plexus stage 3: three quarters way between eye-ear line and vertex. (2) Hand plate with separated digits. (3) Foot plate with webbed digits. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)

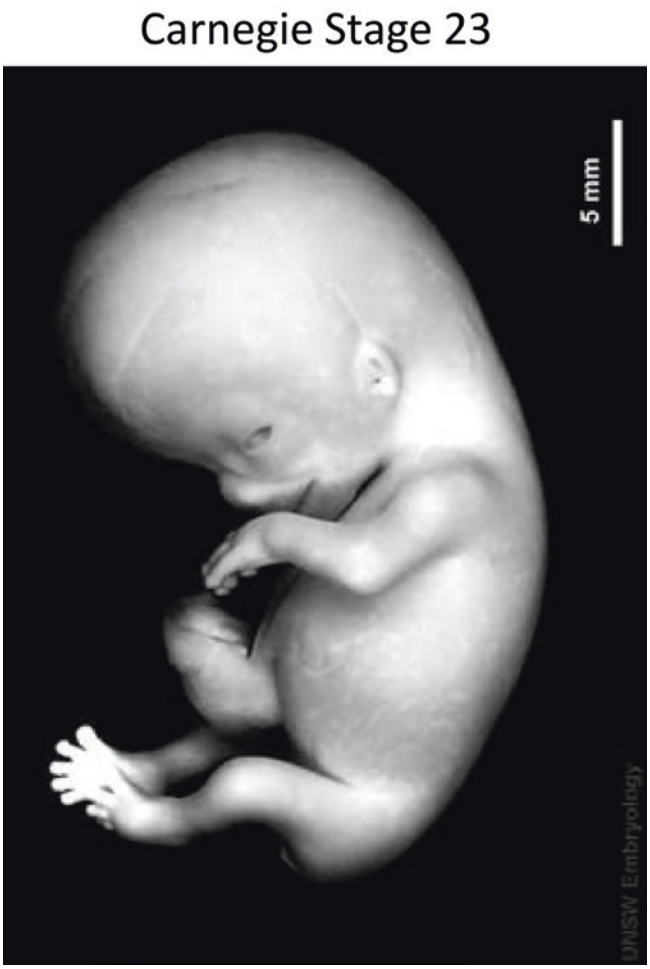

Fig. 3.49 Stage 23: 56–60 days, 27–31 mm. (1) Rounded head. (2) Scalp vascular plexus stage 4: at vertex. (3) Eyelids. (4) Shoulder. (5) Toes separated. (6) Sole of the foot. (Reprinted from The Kyoto Collection, Kyoto University Graduate School of Medicine. Courtesy of Prof. Kohei Shiota and Shigehito Yamada)