9. PHARYNGEAL ARCHES

Letty Moss-Salentijn DDS, PhD
Dr. Edwin S. Robinson Professor of Dentistry (in Anatomy and Cell Biology)
E-mail: lm23@columbia.edu

READING ASSIGNMENT: Larsen 3rd Edition. **Chapter 12**: pp. 352; 358-365; 405-412

SUMMARY:

The phylogenetic significance of pharyngeal arches is discussed briefly, followed by a description of the ontogenetic development of these structures. Five pair of pharyngeal arches are transiently present in the period of 20-35 days of embryonic development. The significant role of the cephalic neural crest for the development of the facial region and the visceral neck is stressed. There is a spatial and developmental relationship between the rhombomeres of the hindbrain and the pharyngeal arches, which is controlled by a Hox code. The components of each pharyngeal arch include an aortic arch, a specific cranial nerve and associated muscle, and a cartilage skeleton. The adult derivatives of each of these components are reviewed.

LEARNING OBJECTIVES:

You should be able to:

a. List the developmental stages and the rostrocaudal sequence in which the pharyngeal arches are visible externally in the human embryo.
b. List the evolutionary changes (including the acquisition of the pharyngeal arches) from simple chordates to early vertebrates.
c. Describe pharyngeal arches, pharyngeal pouches, pharyngeal grooves, pharyngeal membranes, and pharyngeal clefts.
d. Describe somitomeres. List their number in the cephalic region, and describe their contribution to the pharyngeal arch derivatives.
e. Describe the migration, and final distribution of midbrain and hindbrain neural crest in the head, neck and heart regions.
f. Describe the boundary between cephalic neural crest and trunk neural crest, and explain the significance of this distinction for the neural crest derivatives.
g. Describe the developmental relationship between rhombomeres and pharyngeal arches, with reference to the expression of homeotic genes (without memorizing expressions of specific gene combinations for each segment).
h. For each pharyngeal arch list the derivatives of its basic structural elements (artery, nerve, muscle, cartilage skeleton).

GLOSSARY:

Branchial arches. See: pharyngeal arches.
Pharyngeal arches. A series of five paired swellings that surround the embryonic foregut from day 20 to day 35 of development.
Pharyngeal grooves.The ectodermally lined depressions between the pharyngeal arches.
Pharyngeal membrane. A thin membrane, composed of ectoderm and endoderm, formed as the result of a contact between a pharyngeal groove and a corresponding pharyngeal pouch.
Pharyngeal pouches. The endodermally lined depressions between the pharyngeal arches.
Visceral arches. See: pharyngeal arches.

INTRODUCTION

Pharyngeal arches are structures that are critical to the understanding of the development and the anatomy of head and neck. Sometimes they are referred to in the literature as ***visceral arches***, or ***branchial arches***, but the term ***pharyngeal arches*** is currently preferred.

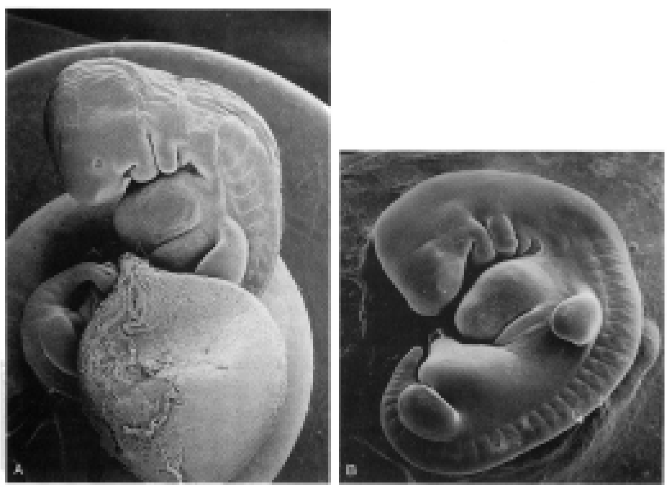

Fig. 9-1. *A,* The form of this embryo is characteristic of a 4-week human embryo just subsequent to the folding process. Note the relatively large definitive yolk sac. *B,* A three-dimensional incipient human form is apparent in this 5-week embryo. The yolk sac has been removed. *(A,* Scanning electron micrograph courtesy of Dr. Arnold Tamarin.)

What are pharyngeal arches?

Pharyngeal arches are a series of five paired swellings that may be seen to surround the embryonic foregut from day 20 to day 35 of development (Fig. 9-1). These arches are numbered 1,2,3,4, and 6. This seems strange, but the reason for this numbering is that some vascular component of a fifth pharyngeal arch occasionally develops transiently and then is lost. Although pharyngeal arches are externally visible for only a brief period of time, their derivatives continue to be present. The adult anatomy of the head and neck is easier understood if the original organization of the pharyngeal arches is kept in mind.

Pharyngeal arches are **unique to vertebrates** (a subphylum of the phylum Chordates), and are an adaptation for the way we exchange respiratory gasses and feed ourselves. Vertebrates share the basic body plan of the Chordates, with a central notochord, a dorsal hollow neural tube, a ventral digestive tube, pharyngeal gill slits and segmented mesoderm laterally.

The simplest Chordate is a sedentary, passive filter-feeding creature. It has a limited brain size and uses gill slits to breathe. Vertebrates have evolved to become predators that hunt for food.

What adaptations were needed in order for this evolutionary change to take place?

- mobility (not our main concern today)
- development of organs of special sense to detect the prey
- development of a large neural circuitry (brain) to integrate input and responses
- an effective jaw apparatus (pharyngeal arch derivative)
- an improved gill apparatus (another pharyngeal arch derivative)

The evolutionary development of the pharyngeal arches depended on the recruitment of a group of cells: the neural crest cells, which would constitute a significant part of the tissue mass in the pharyngeal arches. Neural crest cells probably were not a novel vertebrate cell population. They may have originated from protochordate dorsal midline epidermis. What was new was their acquisition of migratory abilities and their multipotency in differentiation.

What do pharyngeal arches look like?

The pharyngeal arches are bilateral/paired swellings that surround the foregut of the embryo. They are wedged between the developing heart and brain. Note that the pharyngeal arches develop in **a rostral to caudal sequence**. On approximately day 20 of development, the first arch develops, followed by the second and the third. By the time that arches 4 and 6 develop, the first two arches are no longer distinctly visible externally. Textbooks often show diagrams with five distinct arches, but because they are developing and disappearing sequentially, this is not a correct representation.

CELLULAR SOURCES

Pharyngeal arches are formed by cells that are derived from **ectoderm, endoderm, mesoderm** and **neural crest**.

Ectoderm

Ectoderm lines the external surfaces of the pharyngeal arches. The ectodermally lined depressions between the pharyngeal arches are called **pharyngeal grooves**.

Endoderm

Endoderm lines the internal (foregut) surfaces of the pharyngeal arches. The endodermally lined depressions between the pharyngeal arches are called **pharyngeal pouches**.

The derivatives of the pharyngeal grooves and pouches will be discussed in a future lecture (10). Occasionally, pharyngeal pouches and corresponding pharyngeal grooves come so close together that a single membrane, the **pharyngeal membrane** is formed. This membrane is very thin, composed of ectoderm and endoderm. In some instances this membrane rips and forms what is essentially a gill slit (**pharyngeal cleft**) between two pharyngeal arches. The slit is extremely transient (usually it does not last more than a few hours). If the slit does not close, it may form a tract (fistula) from the pharynx to the outside of the neck.

Mesoderm.

Recall in embryonic development the absence of mesoderm at the buccopharyngeal and cloacal membranes. The axial mesoderm immediately caudal (posterior) to the buccopharyngeal membrane is called the **prechordal plate**. The prechordal plate lies anterior (rostral) to the notochordal process, and is intimately fused with the area of thickened endoderm immediately anterior to it, which is part of the future buccopharyngeal membrane. Evidence suggests that the prechordal plate may be the source of mesodermal cells of the anterior head region.

The more "traditional" paraxial mesoderm initially develops into whorl-like structures called **somitomeres** (Fig.9-2, Fig.9-3). Most somitomeres proceed to become somites. However, in the head region the **first seven pairs of somitomeres** remain as incompletely segmented structures and never form somites. These somitomeres are located next to the caudal end of the forebrain, the midbrain and the cranial half of the hindbrain. Because of the presence of these 7 somitomere pairs, the first somite derived **postcephalically** is really the eighth segment of the body.

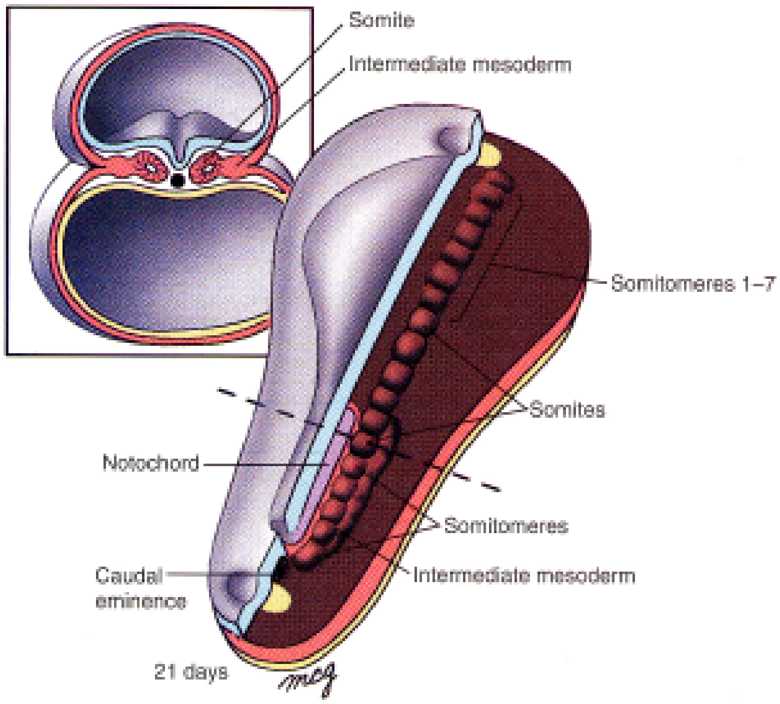

Fig. 9-2. Sections through a 21-day embryo. The cranial and caudal portions of the paraxial mesoderm have become organized into somitomeres, and the four occipital and first two cervical somitomeres have differentiated into somites. The seven most cranial somitomeres never become somites. The dotted line indicates the level of the transverse section. At this level, the lateral plate mesoderm contains the rudiment of the intraembryonic coelom.

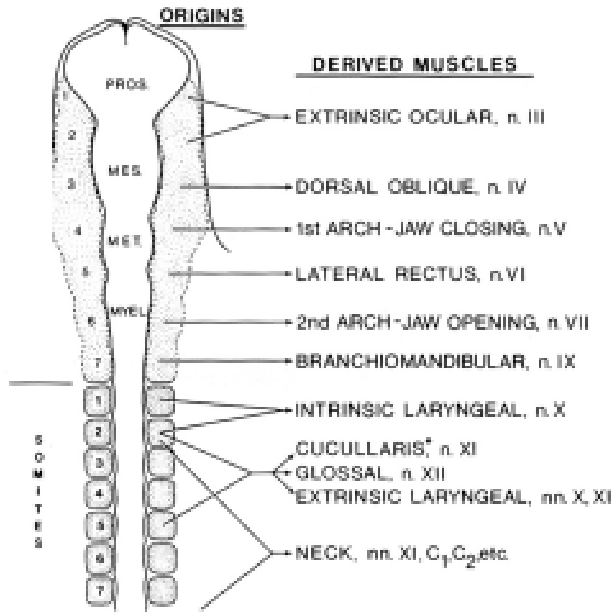

Fig. 9-3.

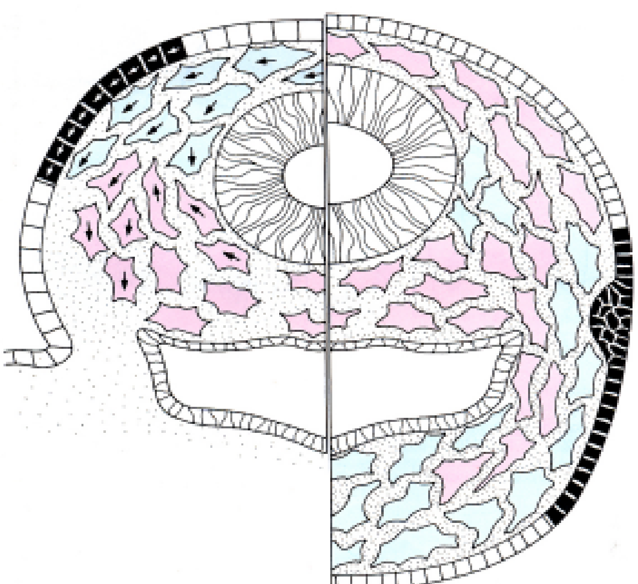

Fig. 9-4.

Neural crest cells.

Migration of the neural crest cells in the **trunk** occurs *after closure* of the neural tube and the principal pathway of migration is through the anterior portion of the somites. In the **head** region neural crest cells start migrating well *before* the tube closes and these cells migrate predominantly between ectoderm and mesoderm. In the general pattern of motion it is important to realize that neural crest cells are not the only ones that move. Think of this motion **as neural crest migration AND relocation of associated ectoderm** (Fig.9-4)!

The neural crest cells that contribute to the development of head and neck are associated primarily with **mid- and hindbrain**. The first cells to move from mid- and hindbrain regions have the most ventral destination and make the largest contribution to the pharyngeal arches. The **midbrain neural crest** moves around the developing eye into the region around the forebrain and behind the eye into the first pharyngeal arch. The **hindbrain neural crest** contributes primarily to arches # 2,3,4, and 6. Neural crest cells that migrate later from the midand hindbrain regions do not move as far away from the neural tube and give rise to meninges and ganglia (Fig.9-5).

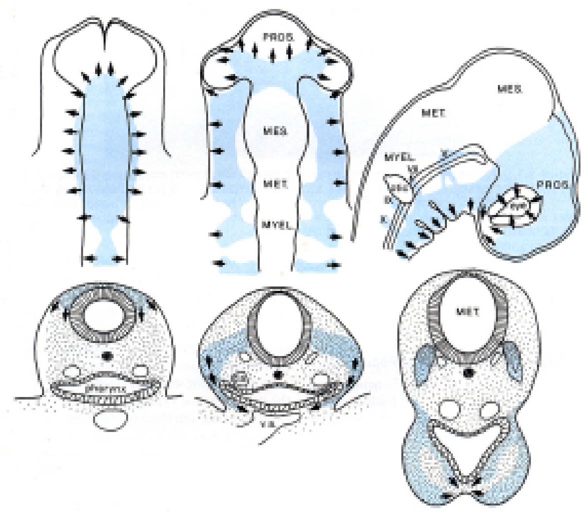

Fig. 9-5.

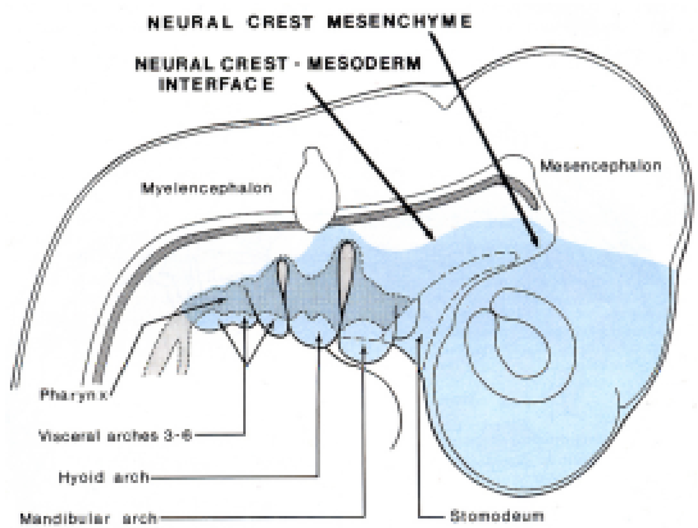

Fig. 9-6.

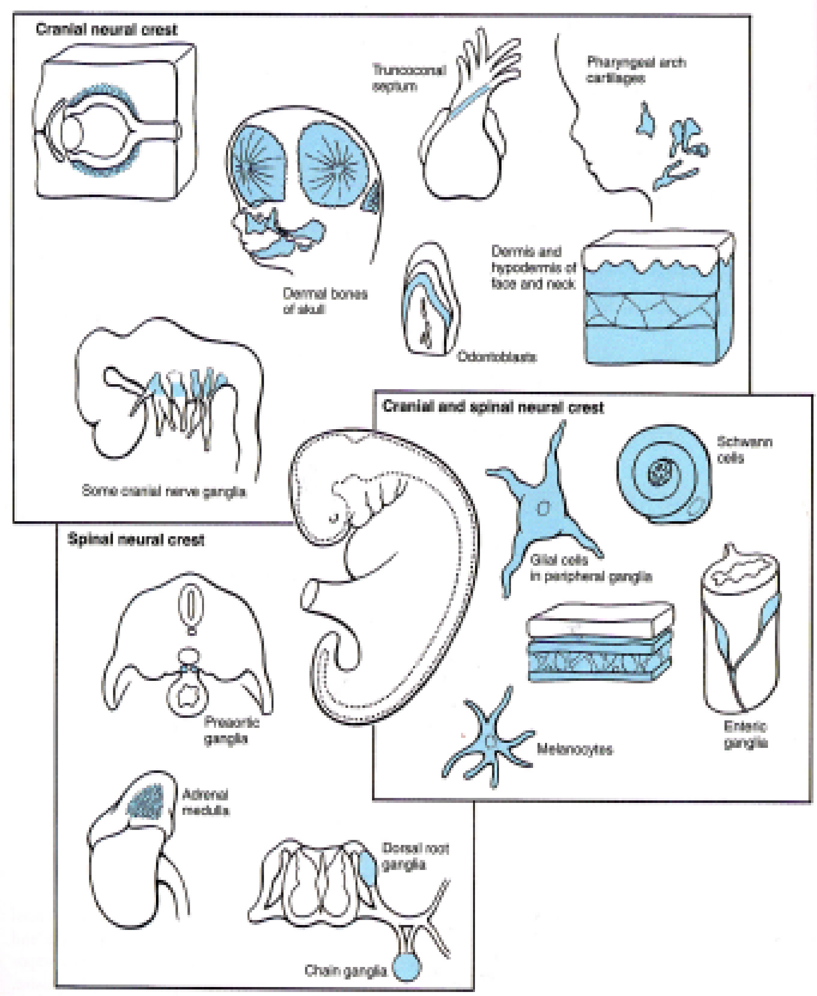

Fig 9-7. Neural crest cells migrating from both cranial and trunk regions of the neural tube give rise to a variety of tissues in the embryo.

Neural crest from the **forebrain** region does not contribute to the pharyngeal arches, but is involved in the development of the nasal placode and the nose.

In a lateral view of the head and neck region of the developing embryo, you can draw a line between the **somatic** region (containing brain, spinal cord) and the **visceral** region (containing foregut). Neural crest migrates into the visceral region and therefore mesenchymal derivatives of neural crest are only found in the visceral region. The only anatomic structures that cross the line between visceral and somatic neck are nerves of the pharyngeal arches (Fig.9-6).

Head neural crest vs.trunk neural crest

Head (cephalic cranial) neural crest is very different from trunk neural crest because of its ability to form connective tissue (connective tissue proper, bone, cartilage, etc).

Because cephalic neural crest can form ***mesenchyme*** (embryonic connective tissue), it has a much broader set of derivatives than trunk neural crest: including fibroblasts, chondrocytes, osteocytes and cells that form dental tissues (Fig. 9-7).

The boundary between head neural crest and trunk neural crest is at the level between 5th and 6th somites. Recall the position of the pharyngeal arches wedged between the developing heart and brain. Neural crest cells that contribute to arches 3,4, and 6 may also migrate beyond the arch into the area of the developing heart (Fig.9-8). These cells contribute to the initial development of the truncoconal septum, responsible for the separation of the arterial outflow. The development of the truncoconal septum that separates pulmonary trunk and aorta is made possible by the fact that cephalic neural crest can form mesenchyme. Clinically this is an important point, because developmental

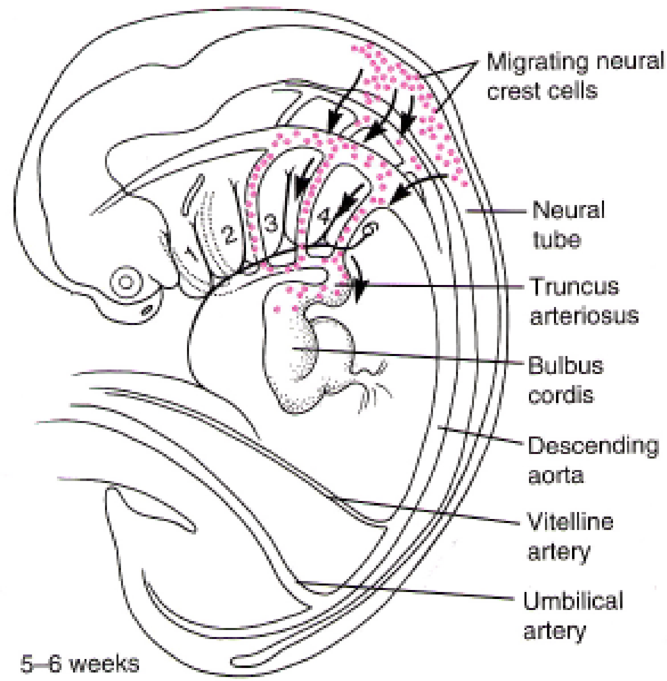

Fig. 9-8. Formation of the truncoconal septa from neural crest cells. Neural crest cells migrate from the hindbrain through pharyngeal arches 3, 4, and 6 and then invade the truncus arteriosus to form the truncoconal septa. (Modified from Kirby ML. 1988. Role of extracardiac factors in heart development. Experientia 44:944.)

problems in the cephalic neural crest may result in problems affecting both pharyngeal arches and heart. **Many syndromes have combinations of craniofacial and heart malformations, because of the commonality of their neural crest cells**.

PATTERNING OF ARCHES - SEGMENTATION

Segmentation gives us repeating structures; a basic plan. The intercostal areas in the adult body illustrate this point: in dissection it becomes apparent that, despite minor variations, “if you’ve seen one, you’ve seen them all.”

Many types of segmentation are apparent in the embryologic period, and occur at approximately the same time in development. While **somites** appear from day 19 to day 35 as externally visible structures, **pharyngeal arches** are visibly present from day 20 to day 35. Other examples of segmentation include **rhombomeres** (eight segments of the neural tube, located in the hindbrain, or rhombencephalon), and segmentation of the **intermediate mesoderm** which occurs during the development of the urogenital system.

In the head and neck region there is a matching up of rhombomeres with pharyngeal arches, most probably because the rhombomeric segmentation directs a segmented migration of neural crest cells. Neural crest cells emerge from all rhombomeres (r) and sort into distinct exiting streams **adjacent to even-numbered**

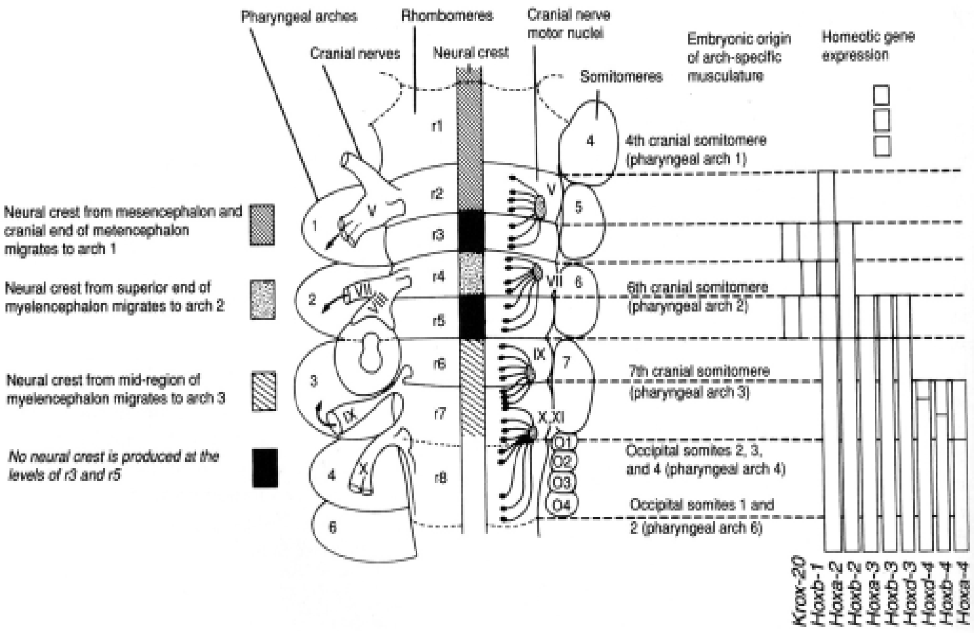

Fig. 9-9. Stylized depletion of the brain stem showing the spatial relationships of the pharyngeal arches, neuromeres, cranial nerves, cranial nerve motor nuclei, neural crest domains, somitomeres and somites, and Hox, Eux-1, Pax-2, and Kmx-20 gene expression. (Krox-20, Eux-1, and Pax-2, like the Hox genes, have been implicated in transcription regulation.) (Data from Lumsden A, Keynes R. 1989. Segmental patterns of neuronal development in the chick hindbrain. Nature [London] 337:424; Wilkinson DC, Bhatt S, Cook M, et al. 1989. Segmental expression of Hox-2 homeobox-containing genes in the developing mouse hindbrain. Nature [London] 341:405; Jacobson AC. 1992. Somitomeres: mesodermal segments of the head and trunk. In Hanken, Hall BH [eds]: The Vertebrate Skull. University of Chicago Press, Chicago; and Krumlauf R. 1993. Hox genes and pattern formation in the branchial region of the vertebrateTIG 9:106.)

rhombomeres. The first pharyngeal arch receives neural crest cells from r2, the second arch from r4, and the third arch from r6. There is a **unique expression of homeodomain genes** that control regional specificity of development - in this case, segmentation pattern. If the expression of this unique combination of homeobox genes is blocked, the normal derivatives of the pharyngeal arches do not form (for an example: see below under 3. Cartilages). This tells us that through the expression of homeotic genes, the rhombomeric segmentation of the hindbrain and the developmental pattern of the pharyngeal arches are coordinated (Fig.9-9).

However, it is increasingly becoming clear that the pharyngeal endoderm plays a major role in directing the segmentation process in this region.

COMPONENTS OF A PHARYNGEAL ARCH

Structure

If you look at a coronal section of an embryo, you can see that a pharyngeal arch has **ectoderm** on the outside, **endoderm** on the inside, and **mesenchyme** between the two epithelia. Mesenchyme derived from neural crest cells lies at the periphery, and the central core is filled with mesodermal mesenchyme. **Each arch has its own artery, nerve, cartilage rod "skeleton", and a group of muscle cells**.

1. Arteries. The first structure to develop in each arch is the ***aortic (pharyngeal) arch artery***. This is a communicating blood vessel between dorsal aorta and ventral aorta (aortic sac in humans). The arteries, like the arches, develop in a rostro-caudal sequence (Fig. 9-10). While the fourth aortic arch artery is forming, the arteries of the first and second arches are breaking up into capillary plexuses, and pretty much disappear. The third arch artery becomes part of the **common carotid**, the fourth arch artery becomes the **arch of the aorta**, and the sixth becomes the **pulmonary artery**. The endothelial cells of the arteries are mesodermally derived. The surrounding tissue layers (pericytes, smooth muscle cells) develop from neural crest derived cells. (Fig.9-11).

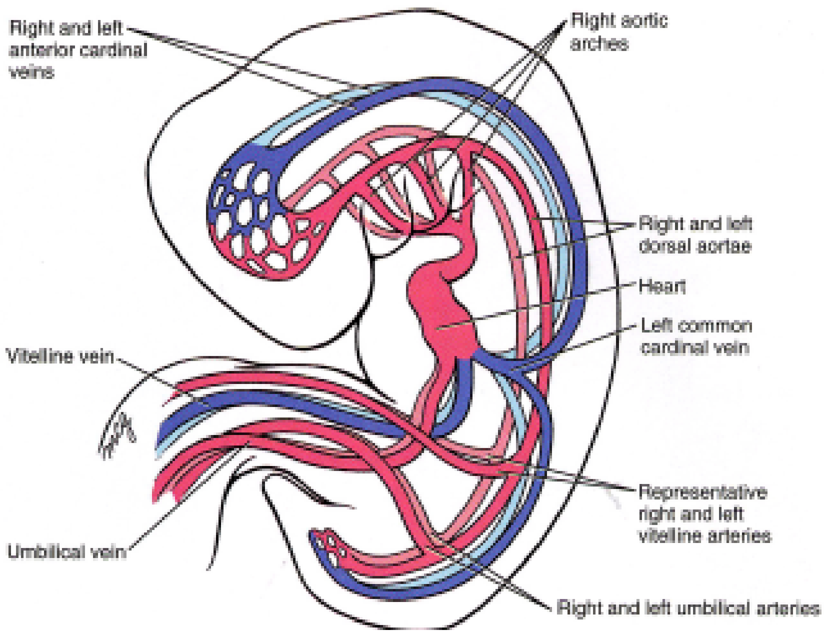

Fig. 9-10. Schematic depiction of the embryonic vascular system in the middle of the fourth week. The heart has begun to beat and to circulate blood. The outflow tract of the heart now includes four pairs of aortic arches and the paired dorsal aortae that circulate blood to the head and trunk. Three pairs of veins– umbilical, vitelline, and cardinal– deliver blood to the inflow end of the heart.

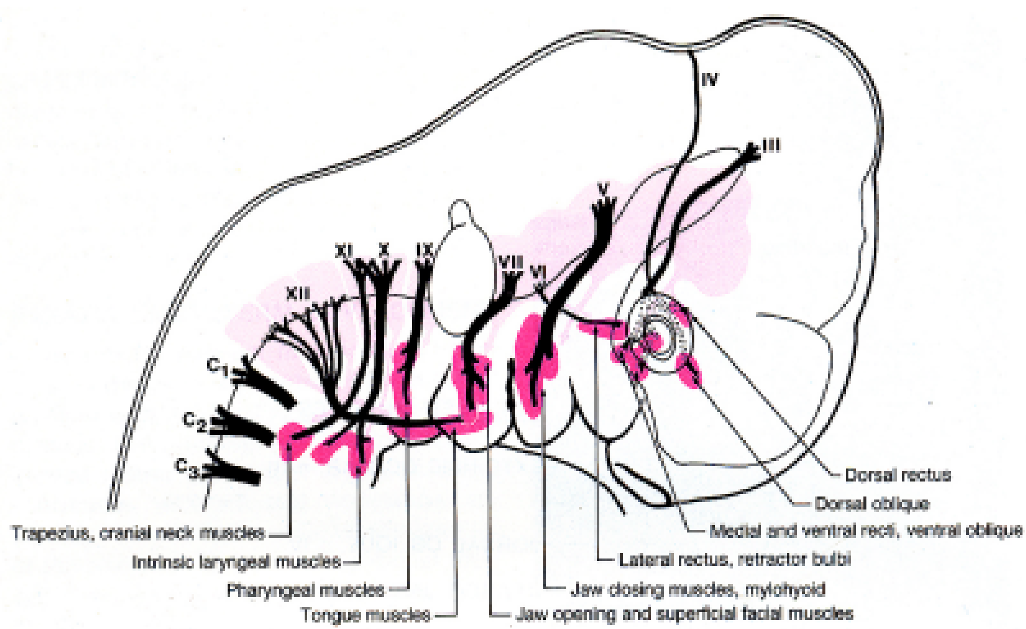

Fig. 9-11.

2. Nerves and muscles. Each arch has its own cranial nerve with both afferent and efferent branches innervating the arch itself (an additional afferent "pretrematic" branch is found in the adjacent, rostral arch). The cranial nerve grows into the arch sometime after the development of the arteries. As it enters the arch, it brings along with it mesodermally derived muscle cells that it will innervate.

Pharyngeal arch	cranial nerve	muscles
1st	CN V	muscles of mastication
2nd	CN VII	muscles of facial expression
3rd	CN IX	stylopharyngeus muscle
4th and 6th	CN X-XI	laryngeal musculature, pharyngeal plexus

3. Cartilages. The cartilage rods are the final structures to develop in each of the pharyngeal arches. The skeletal elements of the pharyngeal arches are derived from the mid- and hindbrain neural crest cells (arches 1-3) or lateral plate mesoderm (arches 4-6).

The first arch will develop into maxillary and mandibular swellings and will give rise to midface and lower face respectively. The first arch cartilage in the maxillary swelling will form the ***incus*** (middle ear ossicle) and the ***alisphenoid*** (a small part of the greater wing of the sphenoid bone). The principal cartilage of the first arch is a rod in the mandibular swelling: Meckel's cartilage. It will form the ***malleus*** (a second ossicle of the middle ear), and two ligaments: the ***sphenomandibular*** and ***anterior malleolar ligaments***. The remainder of Meckel's cartilage disappears without a trace. It does NOT give rise to the mandible!!

The **second arch cartilage**: Reichert's cartilage, forms the ***stapes*** (third ossicle of the middle ear), ***styloid process, stylohyoid ligament,*** and ***lesser horns of the hyoid bone***. The pattern of the cartilage skeleton in the second arch is regulated by the specific expression of Hoxa2, which has been identified as the selector gene specifying second arch fate. Hoxa2 is expressed in the hindbrain with its anterior limit at the r1/r2 boundary. It is the most anteriorly expressed of the vertebrate Hox genes. However, the crest cells that migrate from r2 and are destined for the first pharyngeal arch, switch off Hoxa2 as they migrate. The first pharyngeal arch mesenchyme is thus completely devoid from Hoxa2 expression. Neural crest cells that are destined for the second pharyngeal arch strongly express Hoxa2. Upregulation of Hoxa2 in the first arch results in it assuming characteristics of the second arch.

The **third arch cartilage** contributes to the ***hyoid body***, and the ***greater horns of the hyoid.*** The **fourth and sixth arch cartilages** contribute to the ***laryngeal cartilages***. If you place your index finger along your lower jaw and splay the rest of your fingers out along your neck, this is where the locations of the derivatives of the original pharyngeal arch cartilages still may be found (Fig.9-12).

The anatomical derivatives of the pharyngeal arches should be reviewed in detail after these structures have been dissected in human anatomy.

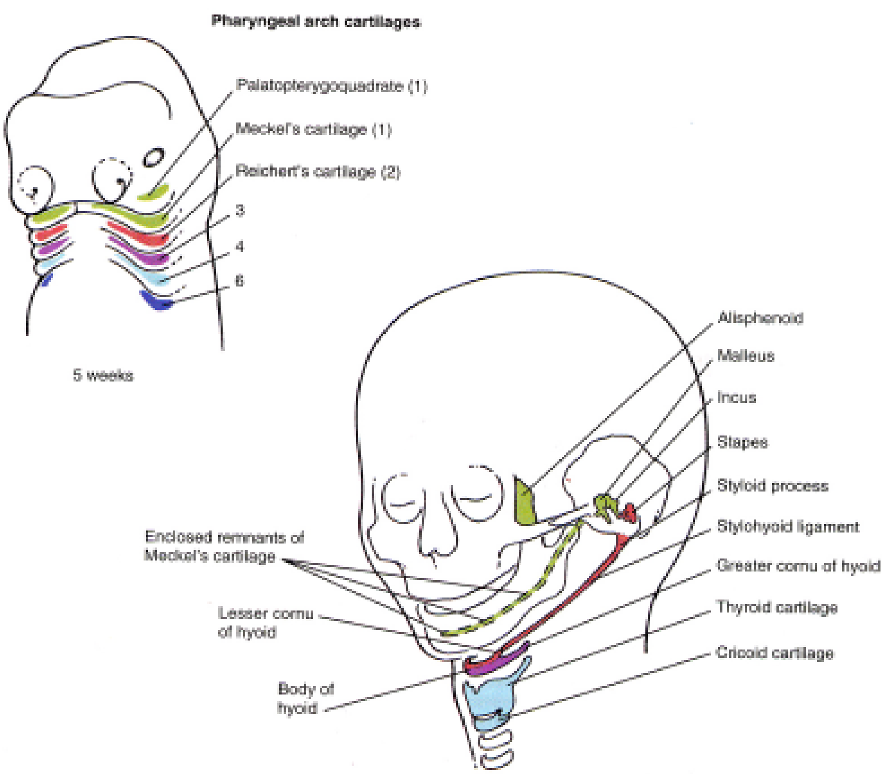

Fig. 9-12. Fate of the pharyngeal arch cartilages. These cartilages give rise to small bone of the orbit, to elements of the jaw skeleton, to the three auditory ossicles, and to the hyoid and laryngeal skeleton.