EMBRYOLOGY:
Development of the Limbs

Recommended Reading: Larsen, pp. 315-347
Langman, pp. 154-160, 168-169
Moore, pp. 355-362

OBJECTIVES: Following the lecture the student should be able to:

1.) Define the time course of the development of the limbs.
2.) Define the role of the apical ectodermal ridge in limb development.
3.) Explain how the mesodermal core goes about specifying which segment of the limb will form.
4.) Define the components of the limb that are derived from the somites, lateral plate mesoderm and neural crest.
5.) Define the muscles that are derived from the ventral and dorsal muscle masses of the limb bud.
6.) Explain how limb bones and joints develop.
7.) Define common terms and definitions of limb malformations,
Four stages of limb development exist for both the upper and lower limbs:
1.) Initiation: represented by limb bud formation
2.) Specification of limb pattern: when growth of the 3 axis occurs
3.) Tissue formation and limb morphogenesis: when limb elements form
4.) Growth: occurring into early adulthood

I. The upper and lower limb form from a simple structure called the limb bud that consists of-
   1.) outer ectodermal cap
   2.) an inner mesodermal core from which develops the musculature, skeleton, tendons, dermis and other connective tissue.

II. Limb development takes place over 4-week period from 5th to 8th weeks.
   1.) The upper limb bud appears at 24 days.
      a.) Hand plate, forearm, arm and shoulder can be distinguished at day 33.
      b.) Digital rays (precursor to fingers) are present in digital plate (precursor to hand) by day 38 (fig. 1).
      c.) The entire upper limb undergoes horizontal flexion so as to lie in the parasagittal plane at day 47.
      d.) Fingers are clearly delineated and begin developing distal swellings called tactile pads by day 52.
      e.) All regions of the arms are developed by Day 56.

   2.) The lower limb bud appears at 28 days.
      a.) The thigh, leg and foot become distinct at day 37.
      b.) Foot plate becomes apparent on the caudal side of the distal end of the bud at 38 days.
      c.) Toe rays become visible in the digital plate of the foot at 44 days.
      d.) Lower limbs begin to flex toward a parasagittal plane at 47 days.
      e.) Legs and toes are well defined at 56 days.

Note: It is convenient to describe the limb by reference to three axes: the proximodistal axis, from base to tip; the antero-posterior axis, from "thumb" to "little finger"; and the dorso-ventral axis, at right angles to these.

III. The limbs develop through a series of inductive interactions.
   1.) Just after limb bud formation, the mesodermal core induces a thickening of the ectoderm, the apical ectodermal ridge, which forms a rim around the tip of the bud (fig. 2).
2.) The apical ectodermal ridge induces the mesodermal core to differentiate into the proximo-to-distal components of the limb.
   a.) Chick experiments have shown that if the ridge is removed during development the distal structures yet to be laid down are not formed. e.g. excising the ridge from a wing bud at an early stage of produces a wing consisting of an upper arm only; the same operation at slightly later stages produce a with upper arm and forearm but not hand.

Figure 1: The development of the upper and lower limb buds occurs between the 5th and 8th weeks. Nearly every stage in the development of the lower limb bud takes place several days later than in upper limb bud (taken from Larsen’s “Human Embryology”, 1995, Churchhill Livingstone Publ.).
3.) The ridge defines a site of outgrowth; it maintains a progress zone, an undifferentiated population of mesenchyme cells beneath it from which successive parts of the limb are progressively laid down. Chick experiments have shown that the age of the ectoderm makes no difference to the character of the structures laid down in the mesoderm,, rather it merely marks out the site of the progress zone and tells the mesoderm to proceed with its own developmental program (fig. 3).

4.) Defects in signaling in the proximodistal axis likely lead to truncated limb formation (fig. 4).
Figure 3: Experiment on chick embryos demonstrating the roles of the apical ectodermal cap and the mesodermal core in limb bud development (taken from Larsen’s “Human Embryology”, 1995, Churchill Livingstone Publ.).

Fig. 4: Examples of reduction defects; (Left) Amelia: entire limb missing and, (right) meromelia: part of the limb is missing.
IV. Patterning of the AnteroPosterior Axis

Through transplantation studies it has been determined that the caudal tissue region responsible for digit-determining activity is called the zone of polarizing activity (ZPA) (fig. 5). This area of tissue appears to contain a morphogen which is expressed in a craniocaudal gradient across the limb bud. One candidate morphogen that establishes this axis is Sonic Hedgehog.

![Fig. 5](image)

Fig. 5: Transplantation of the zone of polarizing activity of one limb bud to the cranial edge of another will induce mirror polydactyly (taken from Larsen’s “Human Embryology”, 1995).

V. Patterning along the Dorso-Ventral Axis

Through gene inactivation studies it has been established that epithelial-mesenchymal interactions also control patterning across the dorso-ventral axis of the developing limb. Experiments have shown, for example when limb ectoderm is rotated so that, dorsal ectoderm overlies ventral mesoderm, structures subsequently develop that conform with ectoderm polarity. The genes involved in controlling this axis appear to be Wnt-7 and Engrailed 1.
Formation of Limb Elements

I. The limb bones form by ossification of a cartilaginous precursor or, termed endochondral ossification.
   a.) Rod-like condensations of lateral plate mesenchyme form the endochondral bones and their joints.
   b.) Cartilage is deposited around axis of condensation during initial phase of chondrification where chondrocytes differentiate within mesenchyme and begin secreting matrix molecules of cartilage.
   c.) Mesenchyme in the sites of the future joints-the interzones-differentiates into fibrous tissue.
   d.) Ossification commences in a region of the chondrified bone called the primary ossification center. And spreads from this center towards the ends of the bone.
   e.) Osteoblasts (bone-forming cells) differentiate from mesenchymal cells within the chondrified bone and begin to deposit matrix and secrete calcium salt of mineralized bone.

II. The limb musculature develops from ventral and dorsal condensations of somitic mesoderm.
   a.) During the fifth week, somatic mesoderm invades the limb bud and forms a dorsal and ventral condensation.
   b.) Cells within the condensation differentiate into myoblasts (muscle cell precursors).
   c.) The dorsal and ventral muscle masses give rise to muscles of the upper and lower limb.

Congenital anomalies of the limb (Table 2).
   Three categories of defects:
   1.) Reduction defects: part (meromelia) or entire (amelia) limb is missing.
   2.) Duplication defects: supernumerary limb elements are present (polydactyly)
   3.) Malformation of the limb (dysplasia)
### Table 2: Some Common Terms for Limb Malformations

<table>
<thead>
<tr>
<th>TERM</th>
<th>DEFINITION</th>
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<tbody>
<tr>
<td>Meromelia</td>
<td>Absence of part of a limb</td>
</tr>
<tr>
<td>Amelia, Ectromelia</td>
<td>Absence of one or more limbs</td>
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<tr>
<td>Phocomelia</td>
<td>Short, ill-formed upper or lower limbs -named for their resemblance to the flippers of a seal</td>
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<tr>
<td>Hemimelia</td>
<td>Stunting of distal limb segments</td>
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<tr>
<td>Acrodolichomelia</td>
<td>Disproportionately large hands or feet</td>
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<tr>
<td>Ectrodactyly</td>
<td>Absence of any number of fingers or toes</td>
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<tr>
<td>Polydactyly</td>
<td>Presence of extra digits or parts of digits</td>
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<td>Syndactyly</td>
<td>Fusion of digits</td>
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<tr>
<td>Adactyly</td>
<td>Absence of all the digits on a limb</td>
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#### How Long Bones Grow:

Body height increases mainly by growth of the long limb bones-the femur, tibia and fibula in the leg. This occurs by virtue of special growth plates of cartilage called the **epiphyseal plates** (fig. 4), which are situated near both ends of the bones. In each epiphyseal plate, the edge of the cartilage nearer the center of the bone is gradually converted to bone while, at the same time, new cartilage grows outward from the edge further from the center. In this way the bone length

![Figure 4: Bone Development (from “Encyclopedia of the Human Being, Guinness Publ., 1994).](image-url)
progressively increases. Once body growth is complete—at the age of about 25—the epiphyseal plates are converted wholly to bone and no further growth of the long bones is possible.

The activity of the cells of the epiphyses is under the control of the growth hormone **somatotropin**, produced by the pituitary gland. The amount secreted determines the extent of growth and the ultimate height of the individual. If, for any reason the hormone is not produced during the growth period, the individual will suffer from dwarfism; if excess hormone is produced during the childhood, however, the individual becomes a giant. Excess hormone is usually the consequence of a tumor of the pituitary gland.