18-2.1. Development of Appendages or Limbs

The paired fore-limbs and hind-limbs of frog make their appearance in tadpole larval stage and derived from the epidermal ectoderm and the mesoderm of somites and lateral plate. The development of a limb is started by thickening of lateral plate mesoderm just beneath the epidermis of a presumptive limb area which may lie either behind the branchial region (for fore-limb) or just in front of anus (for hind-limb). Soon, the epidermis lying over the mesenchyme mass becomes slightly thickened and bulges outward to form a **limb-bud.** Due to active multiplication of mesenchymal and epidermal cells, each limb-bud grows and gets flattened. After a limb-bud has grown so far that its length exceeds its breadth, the differentiation of its subordinate parts sets in. Its distal portion flattens and broadens to become a circular **hand** or **foot plate,** which soon becomes pentagonal, the projecting points indicating the rudiments of the digits. While the tips of the digit rudiments keep growing out further, extensive necrosis (cell death) occurs in the intervening sections. Mesodermal and ectodermal cells of these sections die and are consumed by macrophages (**Saunders** and **Fallen,** 1967). As a result the digits become separated by distinct incisions. Now, the limb rudiment rotates so that its tip bents downward and it further differentiates.

In recent years, the morphogenesis of the limb of some higher vertebrates such as *Calotes* and chick has been well understood. So a brief discussion of limb morphogenesis in amniota at this juncture will greatly enlighten us.

Limb morphogenesis in amniota. The first visible sign of limb development is the accumulation of proliferating cells of the parietal layer of the lateral plate mesoderm. The mesoderm continues to proliferate, and causes the limb forming area to be elevated above the level of the body. Thus, a **limb ridge** is formed, consisting of the mesoderm covered by the ectoderm.

In addition to the lateral plate mesoderm the somitic mesoderm also contributes cells from the ventral end of the somites to the proximal and central limb mesoderm. Thus, the forelimbs tend to receive more mesoderm from the somites than the hindlimbs. The somitic mesodermal cells are found to contribute the musculature (myotubes), while the lateral plate mesodermal cells form cartilage, bone, soft tissue and muscle connective tissue of limb.

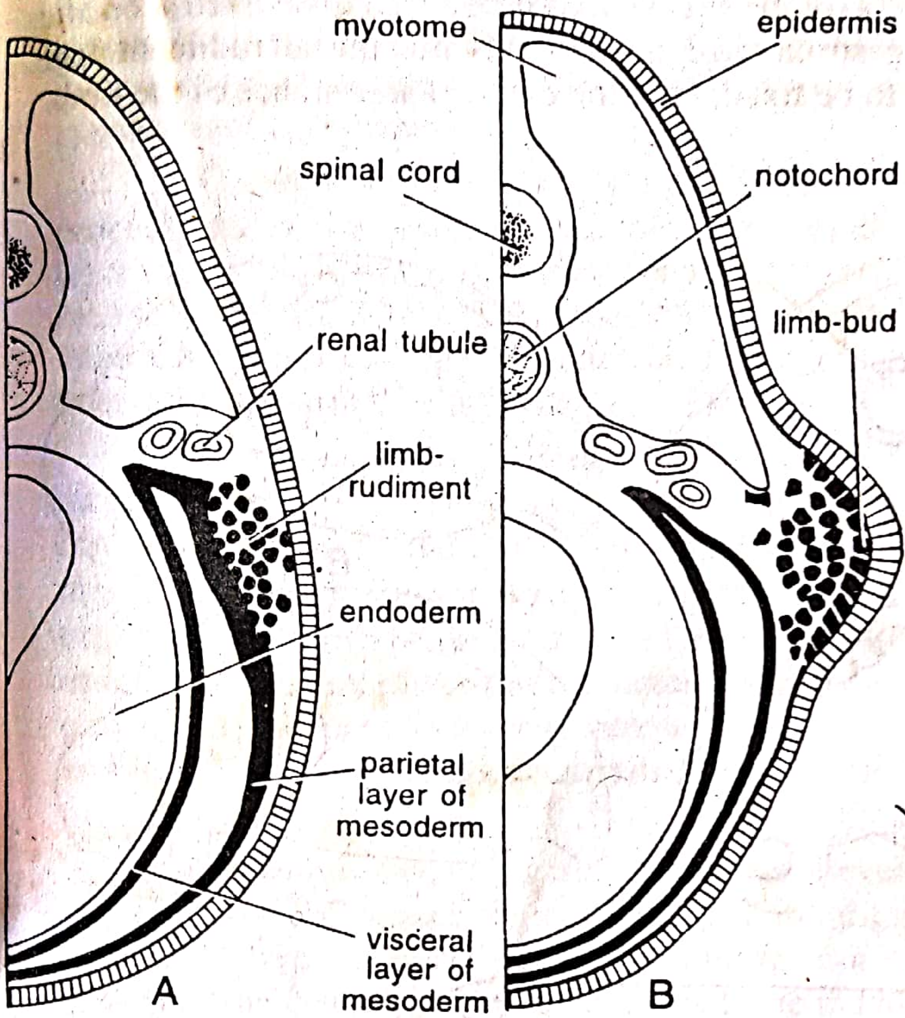

Fig. 18.13. Origin of limb-mesoderm and formation of a limb-bud of an amphibian embryo (after Balinsky, 1970).

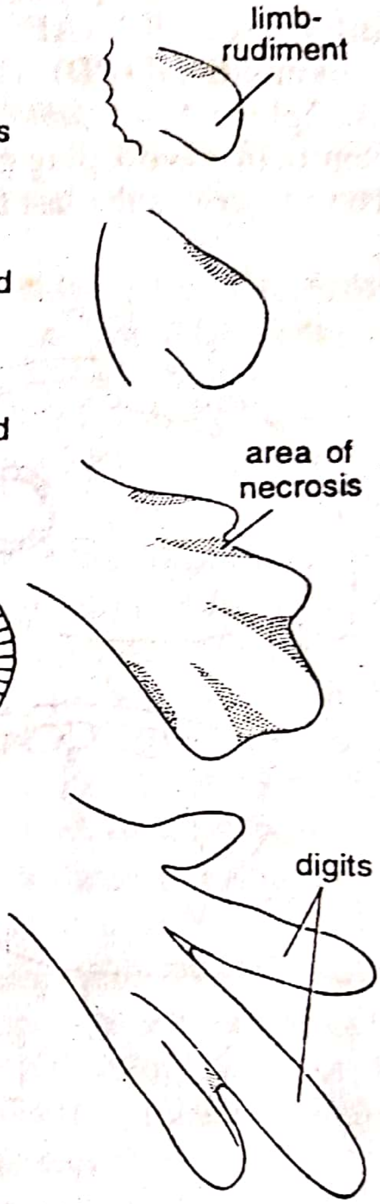

Fig. 18.14. Differentiation of a limb-bud into hind-limb of a vertebrate embryo (after Balinsky, 1970).

Further, in the embryos of some tetrapods (*Calotes* and chick) limbs develop from the anterior and posterior ends of the **Wolffian crests** or **ridges** in the flank region. However, in amphibians the potency to develop limbs is present in the entire flank region.

1. Limb field. During the development of certain organs of the embryo such as a limb or an eye, an area of embryo becomes committed to follow a certain path of development. It is called a **morphogenetic field.** If this area is transplanted, it develops along a specific predetermined path. Such "determined areas" or "fields" have certain interesting properties : (1) They include more than 'presumptive areas' that ultimately develops in a particular organ (*i.e.*, limb). (2) These areas have considerable regulative ability. Thus, it is possible to combine two such areas or remove half or more of a given area and still get a well formed organ. (3) Moreover, these areas, if necessary, can incorporate competent cells from neighbouring areas to form a particular organ. A limb field is clearly demonstrated in the amphibian *Ambystoma* (Fig. 18.15).

2. Limb polarity. A developed limb has three polarities : **anteroposterior (AP**; also called craniocaudal), **dorsoventral (DV)** and **proximodistal (PD)**. These polarities are not determined simultaneously. For example in *Ambystoma* (axolotl), the AP axis is believed to be fixed soon after neurulation (yolk plug gastrula stage) and DV axis after the tail rudiment stage. The PD axis is the last to be fixed, after the external appearance of the limb.

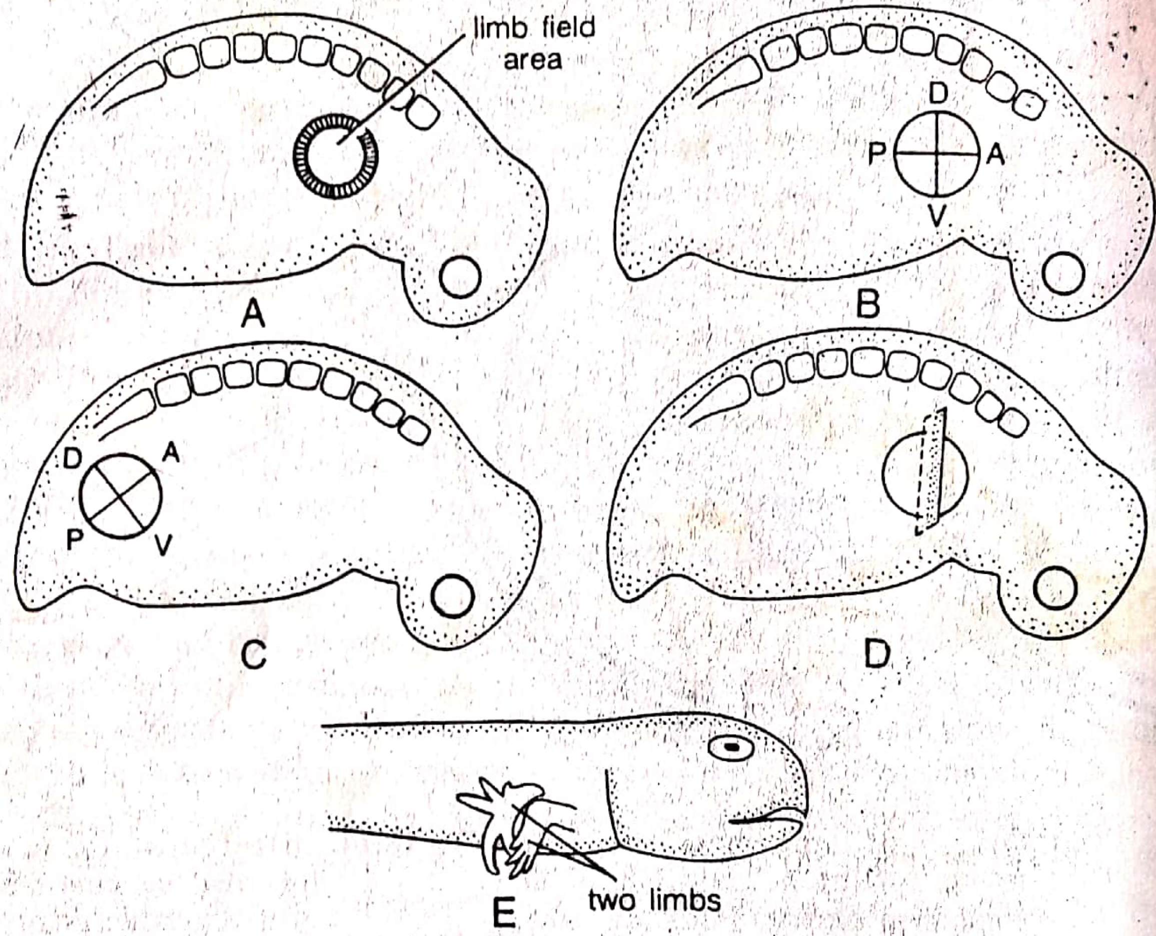

Fig. 18.15. Limb field in *Ambystoma*. A—Limb field area (hatched + stippled) is larger than presumptive limb area (LA : stippled); B–E—Experiments with limb field at tail bud stage; B—Limb disc transplanted at a normal limb site (orthotopic) in another embryo or C—at a different site (heterotopic) such as flank, produces a normal limb. At the time of transplantation dorsoventral (DV) or anteroposterior (AP) or both axial polarities of the limb disc can be reversed to test if these were determined; D—Limb field is split into two by a membrane or extraneous tissue. E—It (*i.e.*, D) produces two limbs.

3. Growth of limb. The growth of the limb bud is asymmetrical from the very beginning. The limbs grow more rapidly along the PD axis than along the AP or DV axis. The dorsal surface of the early limb bud appears convex, while the ventral surface is rather flat. Slightly later the limbs undergo DV flattening at the distal end, and come to acquire a free margin. A distinct ectodermal ridge called **apical ectodermal ridge** or **AER** develops around the margin of limb bud. The AER is located somewhat ventrally and becomes nipple-shaped at

the distal end. As development proceeds, the distal end of the limb becomes paddle shaped. In the blade of the paddle the rudiments of the digits are formed, each digit pointing to a corner in a paddle.

4. Growth of mesoderm. The growth of different parts of the limb mesoderm is reflected in their characteristic cell proliferation rates. There is no migration of the mesoderm from one area to another, but only a relative expansion of each area.

5. Growth of AER. The apical ectodermal ridge (AER) is formed probably due to the action of the underlying mesoderm. The AER is two cell layer thick, but is pseudostratified. The cells of AER of amniotes (*e.g.*, rat) are metabolically active. This is reflected in the fact that these cells contain more RNA, more glycogen and high contents of enzyme alkaline phosphatase than other epidermal cells (**Milarire, 1956**).

The mesoderm has a dominant role in limb development. In early stages it has the potency to form limb in cooperation with non-limb ectoderm; however, the limb ectoderm does not cooperate with non-limb mesoderm in the similar way. Mesoderm tends to induce, maintain and organize the AER in the competent ectoderm through the action of the **apical-ectodermal-ridge-maintenance-factor (AEMF)**. The AER tends to play a crucial role in limb development as an ''inductor'' of limb outgrowth, and by maintaining the subjacent mesoderm cells in a state of developmental plasticity.

Cell death has an important role in limb development. The interdigital necrotic zone influences the separation of the digits and the extent of the webbing. The cause of cell death is genetic and not a physiological stress. The cells become determined for death in the same manner as for differentiation.

18-2.2. Development of Appendicular Endoskeleton

Side by side of external changes in limb rudiment, internally a skeleton of **procartilage** is formed by the mesenchyme of differentiating limb bud in the form of separate skeletal units. The procartilaginous limb skeleton soon becomes cartilagionus and ultimately becomes bony. In fact, chondrogenesis (cartilage synthesis) proceeds in a proximodistal sequence but most of the mesopodial (wrist) cartilages appear well after the metapodial ones (*i.e.* metatarsus and metacarpus).

The formation of limb skeleton generally proceeds in a proximodistal direction. In frog, the first skeletal part to become differentiate is **stylopodium (humerus or femur)**. Part of the **zeugopodium (radius** and **ulna** of fore- limb; and **tibia** and **fibula** of hind-limb) are laid down next. The **autopodium** (hand or foot) differentiates considerably later. In autopodium, the **metacarpals** or **metatarsals** develop more rapidly than **carpals** or **tarsals.** In the digits, however, the proximal phalanges are laid down earlier than the distal ones.

The **pectoral** and **pelvic girdles** develop in intimate connection with the limbs. The mesodermal material for each kind of girdle is derived from the peipheral parts of that mesenchyme mass, the central part of which develops into the limb-buds.

Ref: Verma & Agarwal.