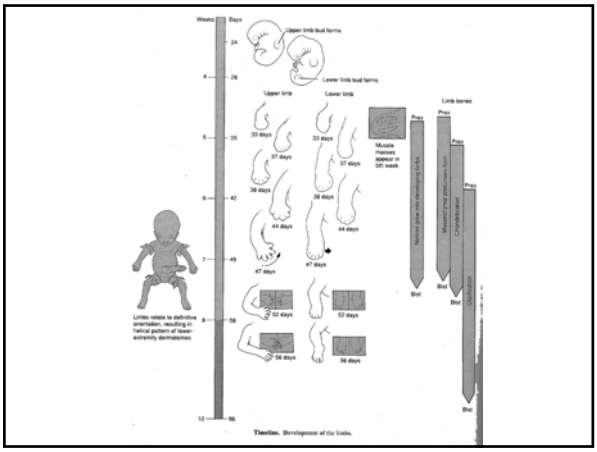
Limb Development

Overview of Limb Formation

Initiation of Limb Development
- Limb Field
- Limb Bud

Outgrowth of the Limb Bud
- Apical Ectodermal Ridge (AER)
- Limb Bud Mesoderm
- Morphogenetic Signaling

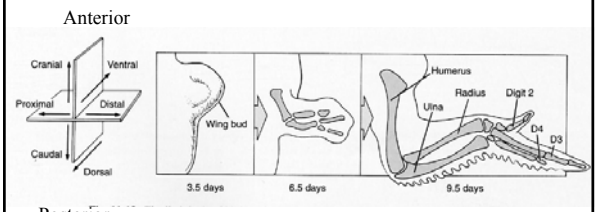
Development of Limb Tissues
- Skeleton
- Musculature
- Innervation
- Vasculature



Limb Development

Limb Bud
- Lateral Plate Mesoderm
- Growth Reduction in the Flank

Limb Axes



Axis	Signaling center	Molecular signal
Proximodistal	Apical ectodermal ridge	FGF-2, FGF-4, FGF-8
Anteroposterior	ZPA	Sonic hedgehog
Dorsoventral	Dorsal ectoderm	Wnt-7a (dorsal)
	Ventral ectoderm	En-1 (ventral)

Clinical Terms

- Meromelia - Absence of part of the limb
- Amelia, Ectromelia - Absence of 1 or more limbs
- Phocomelia - Short, ill-formed limb (flipper limb)
- Hemimelia - Stunted distal limb
- Acrodolichomelia - Enlarged autopod (hand,foot)
- Adactyly - Absence of all digits
- Ectrodactyly - Absence of digits (one or more)
- Polydactyly - Extra digits
- Syndactyly - Fusion of digits

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Initiation of Limb Development

Limb Morphogenetic field = population of cells committed to give rise to a particular organ when transplanted to a neutral site.

Fate maps – Cell marking to identify the cells that participate in limb formation

Regulation - Cells within a field can modify their fates to make up for deficiencies.

Specification – Cells fix their fate - Determination

FGFs Initiate Limb Formation

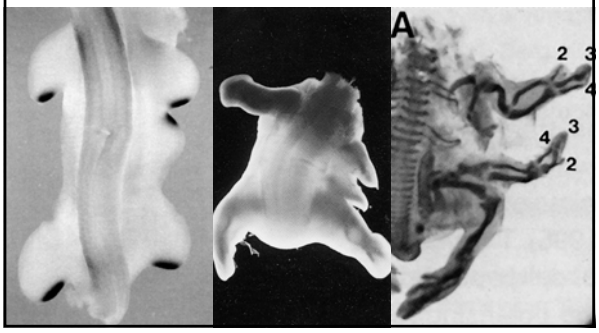
Establishment of the limb field involves of growth factors particularly FGFs

Assay – Microcarrier bead implantation to flank (limb competent tissue)

Endogenous gene expression
FGF10 (mesenchyme) → FGF8 (ectoderm)

FGF10 Knockout causes a limbless phenotype

FGF Induces Supernumerary Limbs



RA is Involved in Limb Initiation

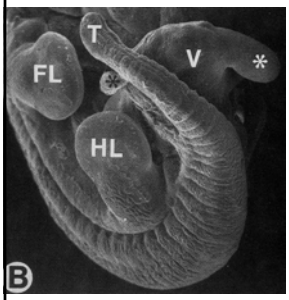
RA bead implantation mimics ZPA grafts – induces ZPA

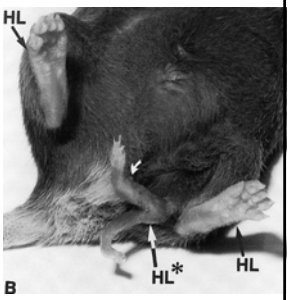
Inhibiting RA synthesis → Limbless phenotype

Retinaldehyde Dehydrogenase Knockout → Limbless phenotype

RA → Hoxb8 → Shh → Bmp2 → limb formation

RA Induces Extra Limbs





Arms or Legs??

Question: what makes hind and fore limbs different?

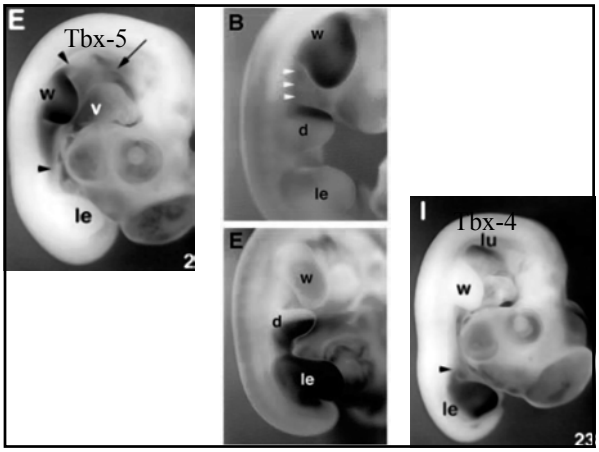
In general the cells are similar and they behave identically

Tbx genes are expressed early and distinguish fore from hind limb.

Tbx–4 is hind limb-specific; Tbx-5 is forelimb-specific

Ptx1 gene controls Tbx4 - hindlimb – Changing Ptx1 expression can change a wing into a leg.

Ptx1 and Tbx genes encode for transcription factors



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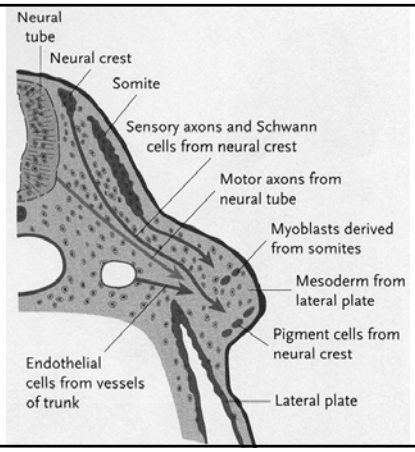
Limb Bud

Early limb bud is composed of lateral plate mesoderm

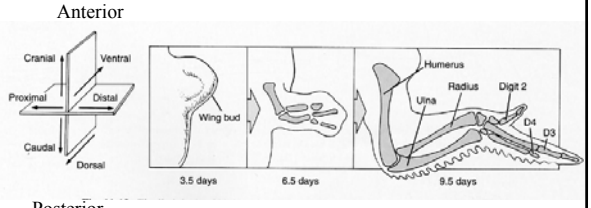
Migratory cells invade the limb bud:
- Myoblasts from somites
- Pigment cells and Schwann cells from neural crest
- Axons innervate the limb bud
- Angioblasts

Migrating cells do not invade the growing limb apex



Limb Axes

Anterior



Posterior

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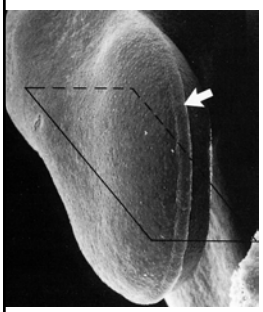
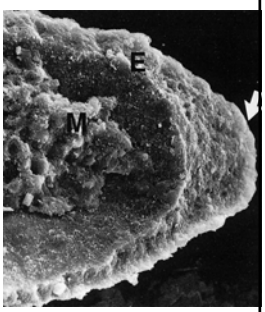
Apical Ectodermal Ridge (AER)





The AER is an inducer of limb outgrowth

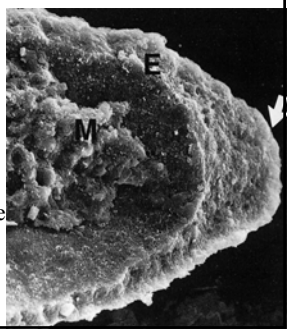
Apical Ectodermal Ridge (AER)

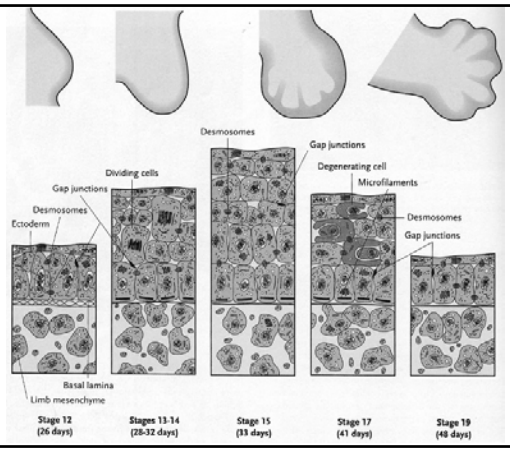
Transient embryonic structure

Multilayered Ectoderm

Connected by Gap Junctions

Basal lamina separates AER from underlying mesenchyme



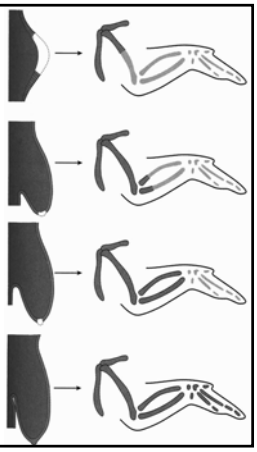


AER Removal

AER removal results in limb truncation

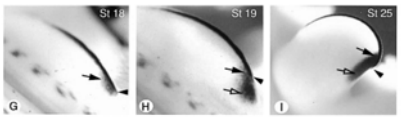
Limbless mutant – the AER fails to form

AER signal is Fibroblast Growth Factors – FGF2, FGF4, FGF8

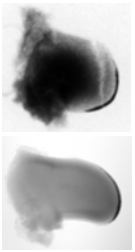


FGFs Expressed in the Limb

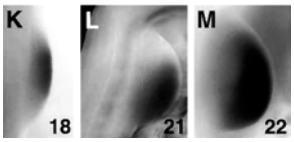
FGF 8 - AER



FGF 4 - AER



FGF 10 - Mesenchyme



Fibroblast Growth Factors

FGF-2, FGF-4, FGF-8 and FGF-9 are produced by the AER

FGF family is large
- Heparan sulfate binding
- Heparan sulfate is required

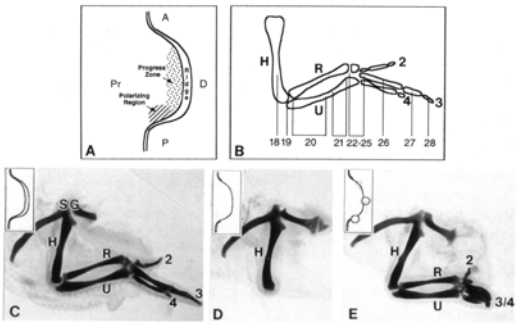
FGFR – FGF receptor
- Transmembrane receptor tyrosine kinase

FGFs will stimulate limb outgrowth after AER removal

FGFs will induce regeneration of amputated limb buds

FGFs will induce flank supernumerary limbs

AER removal and FGF beads



FGFs can replace the activity of the AER

Proximal Distal Axis

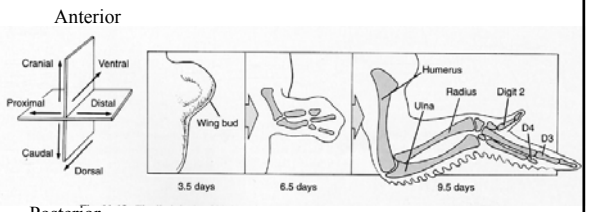
FGF2, FGF4, FGF8 produced by the AER can replace AER function

FGF8 is considered to be the endogenous signal

The AER and FGFs function to maintain gene expression in the Progress Zone (Shh, Fgf10, Msx, Hoxd13, many more)

Limb Axes

Anterior



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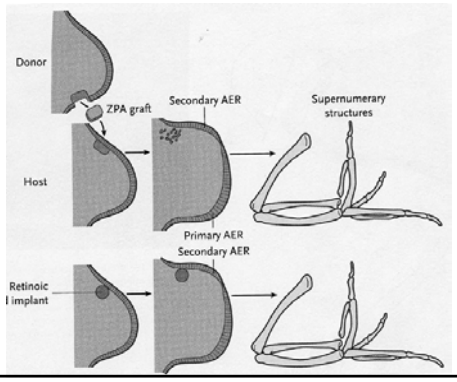
Zone of Polarizing Activity - ZPA



ZPA

ZPA grafts to the anterior induces supernumerary limbs

Supernumerary limbs are mirror symmetrical with normal limb

Stimulates mesenchymal cell proliferation

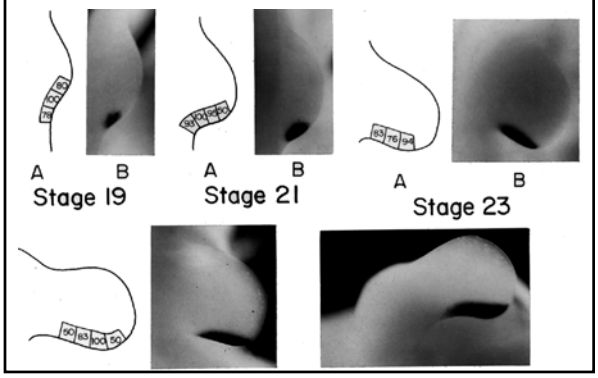
Induces changes in the AER

ZPA signal is conserved – e.g. human ZPA induces chick supernumerary limbs

RA induces the ZPA

The ZPA signal is Sonic Hedgehog

Sonic Hedgehog



Sonic Hedgehog (SHH)

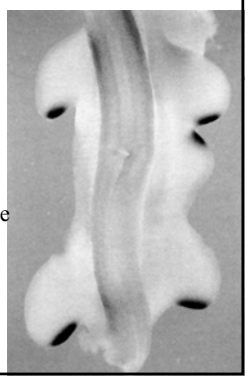
SHH is the ZPA signal

SHH is a secreted cholesterol linked-protein – a Morphogen

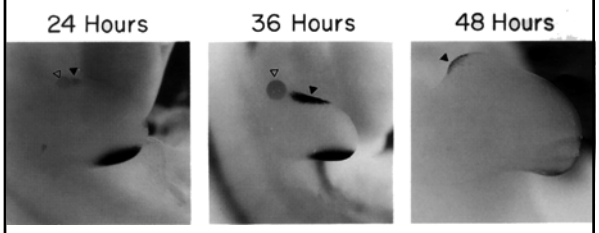
SHH receptor is PATCHED – a transmembrane protein

SHH signaling pathway is responsible for some types of cancers

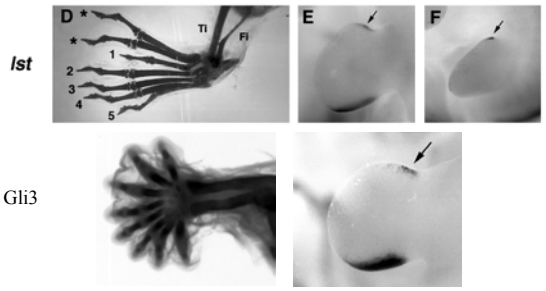
Other Hedgehog related genes
Indian Hedgehog
Summer Hedgehog



RA Induces Shh



Polydactylous Mutants and Shh Repressor Activity



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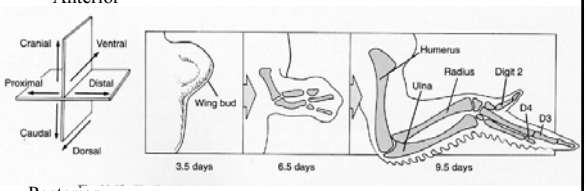

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Ectoderm Controls D/V Pattern

The AER separates Dorsal Ectoderm from Ventral Ectoderm

AER position is controlled by the expression of Radical Fringe (RFng)

Dorsal Mesenchyme Pattern is controlled by the dorsal ectoderm - production of Wnt7a – a secreted factor. Induces Lmx1 (a homeobox containing gene) and ventral to dorsal transformation. Wnt-7a knockout displays dorsal to ventral transformation.

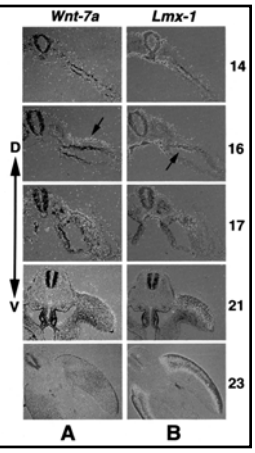
Ventral Ectoderm expresses Engrailed-1 (En-1) – a transcription regulator. En-1 knockout mice display ventral to dorsal transformation

Wnt7a and Lmx1 Expression

Wnt 7a (a secreted factor) is expressed in the dorsal ectoderm

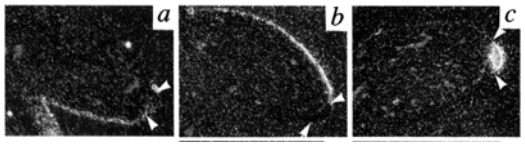
Lmx1 - transcriptional regulator - cysteine-histidine-rich LIM domain and a homeodomain

Lmx1 is expressed in the dorsal mesenchyme



Engrailed-1 (En-1)

- En–1 is expressed in the Ventral Ectoderm
- En-1 is a transcription factor



En-1 Wnt-7a Fgf-8

En-1 Knockout

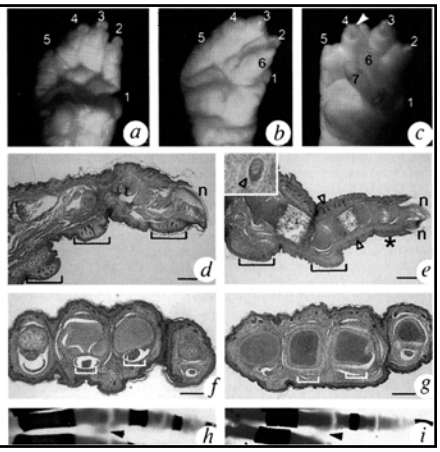
Circumferential nails

Ectopic ventral digits

Absence of ventral tendon

Dorsal flexion

Absent Sesamoid bone



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Cell Death and Digit Formation

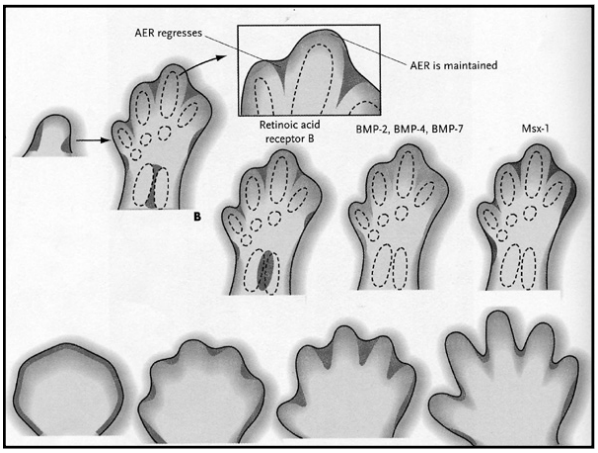
Cell Death – Apoptosis is a normal Developmental Pathway

Interdigital Cell Death – Paddle to Individual Digits

BMP signaling controls Interdigital Cell Death

Msx genes and RA also play a role in cell death

Absence of cell death results in syndactyly



Differentiation – Skeleton

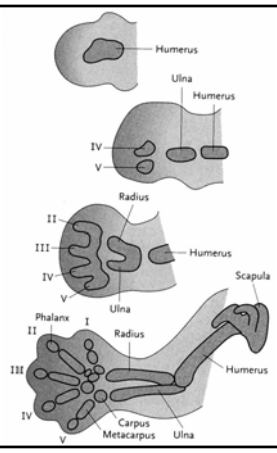
Limb Skeleton – derived from Lateral Plate Mesoderm

Differentiation is Proximal to Distal, Posterior to Anterior

Endochondrial Bone

Ectoderm inhibits Chondrogenesis

Important factors
- BMPs
- Indian Hedgehog (IHH) Growth/differentiation factor-5 (Gdf-5)



Joint Formation

Joints (articulations) - junction between bones

3 Classes of fibrous joints

Dense fibrous tissue - little or no movement, e.g. sutures of skull

Synchondroidal joint – interzone cells differentiate into fibrocartilage, e.g. between pelvic bones

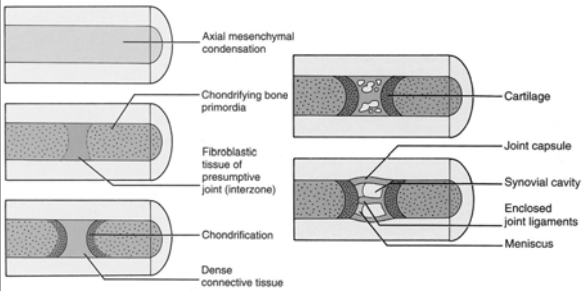
Snynovial joint, complex differentiation of interzonal mesenchyme, e.g. knee and elbow

Synovial Joint

Precartilage rod – transverse splitting

1) Interzone mesenchyme differentiating into fibroblastic tissue
2) Fibroblasts differentiate into 3 layers
   2 cartilage layers with a dense connective tissue in between
3) Central region forms menisci and ligament surrounded by the joint capsule
4) Vacuoles form and coalesce to from the synovial cavity.

Synovial Joint



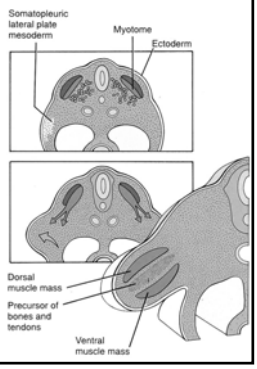
Differentiation – Musculature

Migration of myoblasts from somites

Dorsal and Ventral muscle masses

Tendons form from limb bud mesenchyme interacting with myotubes

Tendons form in the absence of muscle



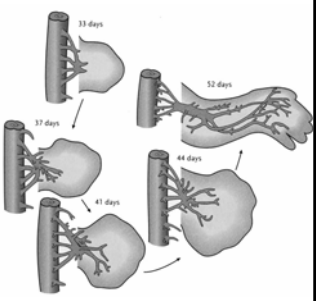
Differentiation – Innervation

Motor Axon from the Spinal cord innervate limb tissues

Local cues guide axons

Axons can regulate for small changes

Sensory axons use motor axons for guidance



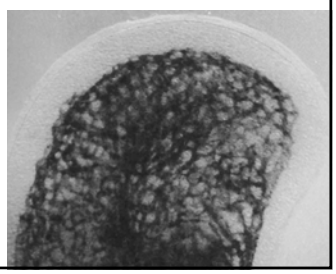
Differentiation – Vasculature

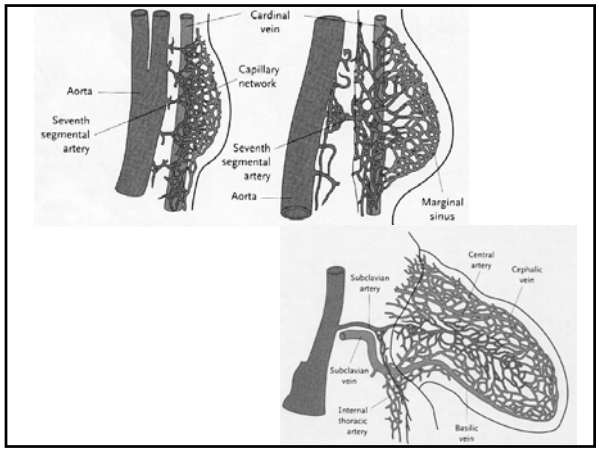
Angioblast - Endothelial cell precursor

Fine capillary network → large central artery

Maginal sinus under the AER – accumulates blood and drains the limb via peripheral veins

Ectoderm inhibits vasculature




Cardinal vein
Capillary network
Aorta
Seventh segmental artery
Seventh segmental artery
Aorta
Marginal sinus
Central artery
Cephalic vein
Subclavian artery
Subclavian vein
Internal thoracic artery
Basilic vein