OUT ON A LIMB

THE REGENERATION LABORATORY

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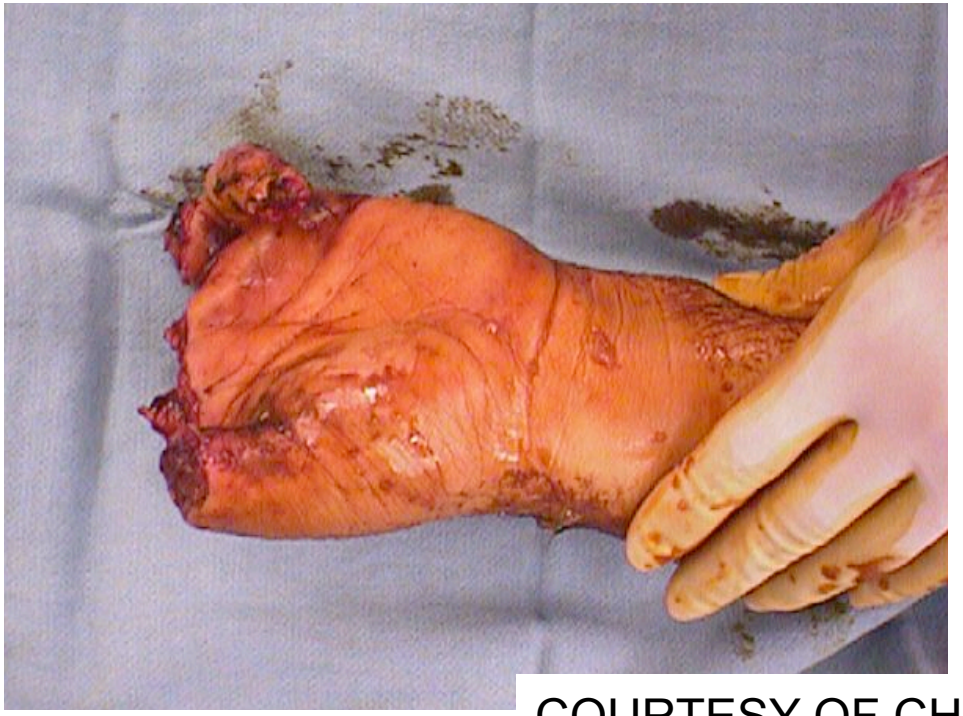
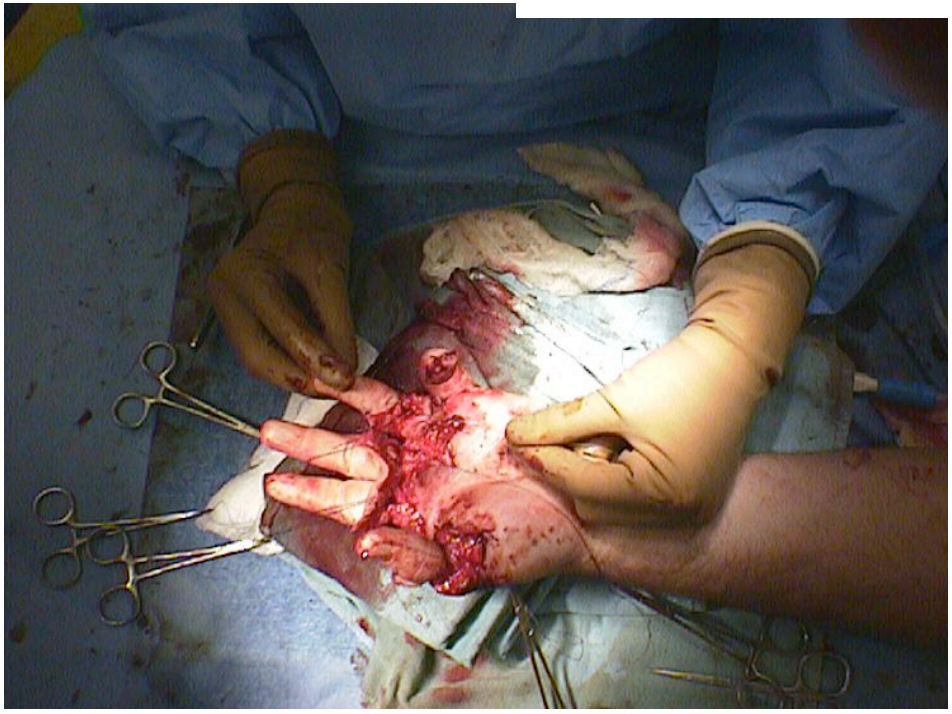


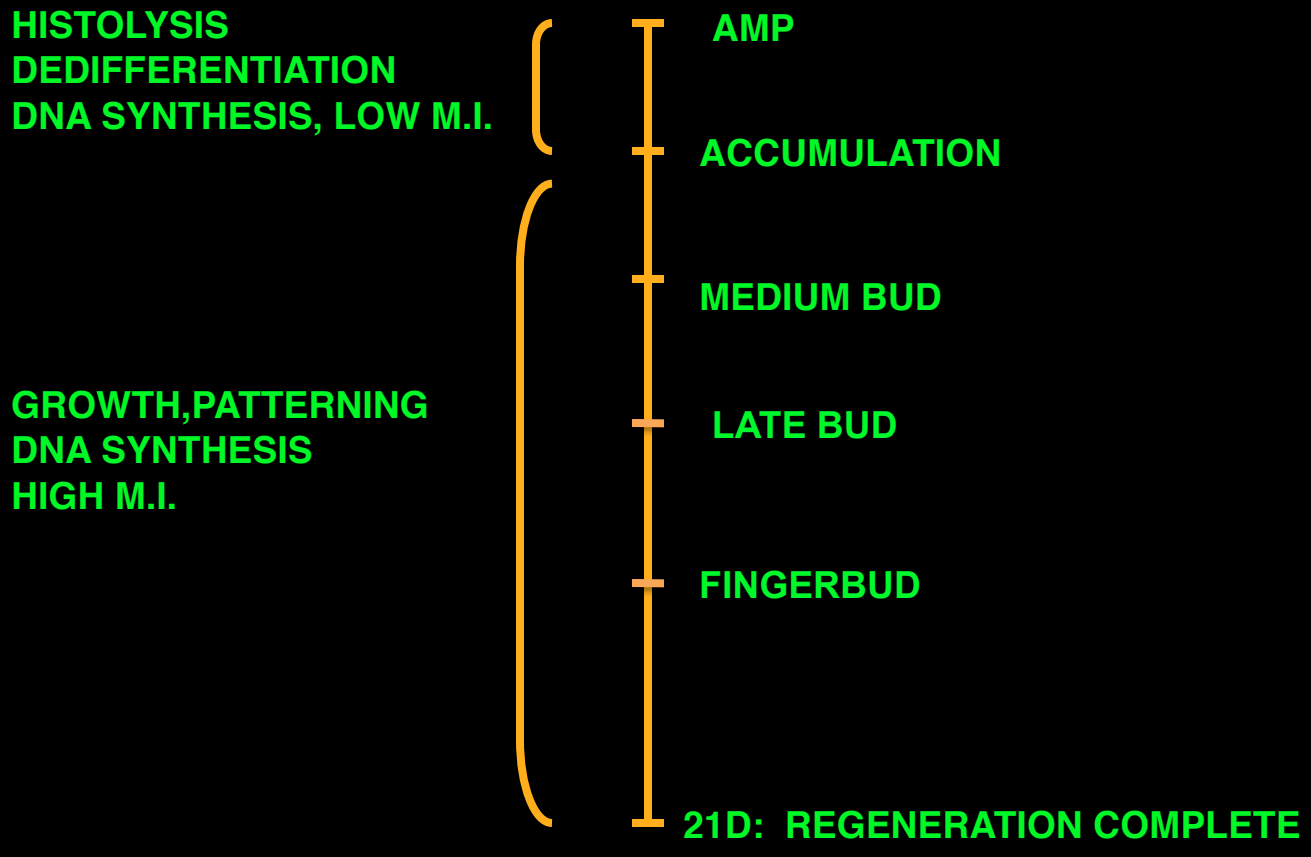
COURTESY OF CHRIS ALLAN, MD

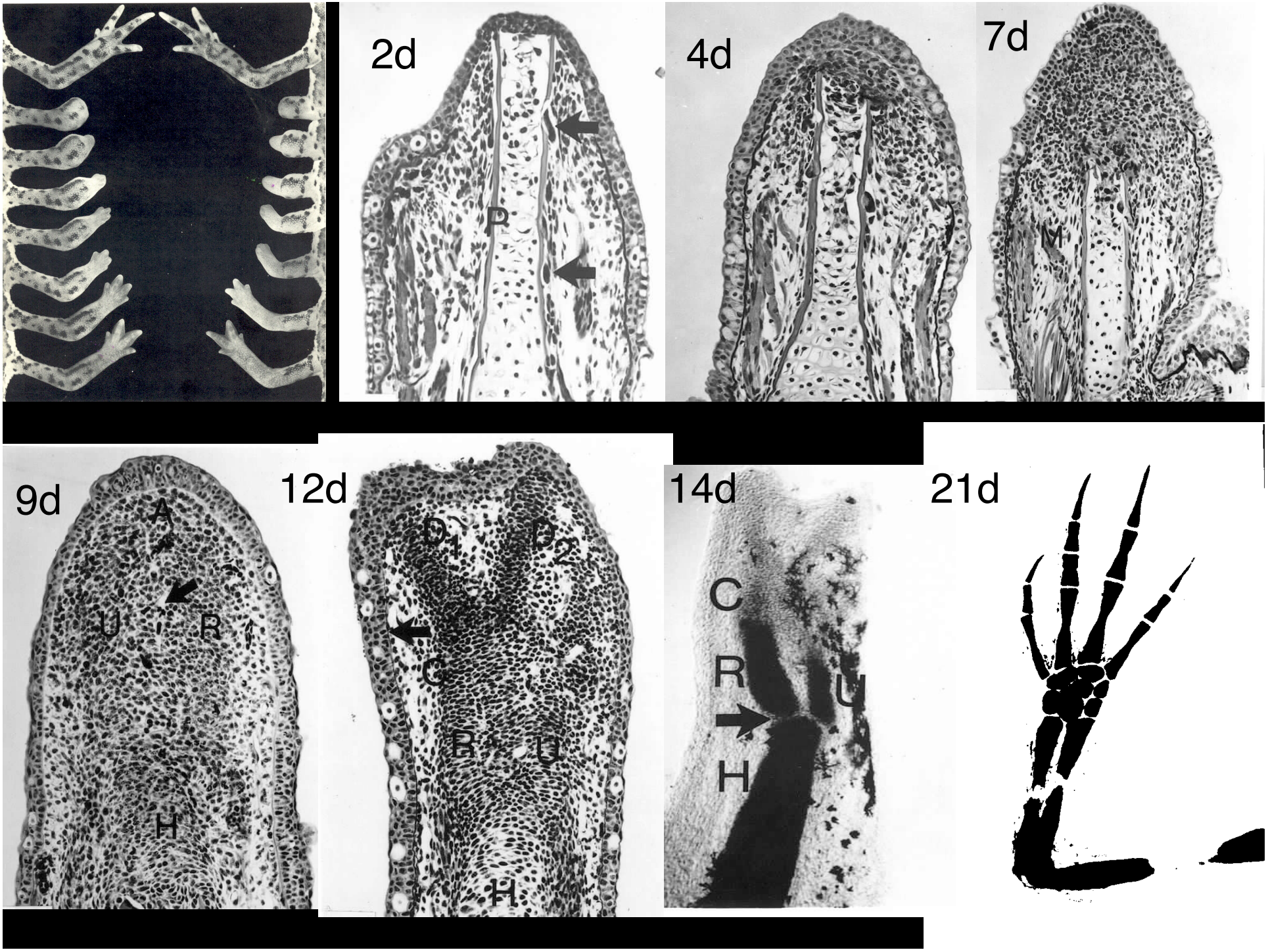


CAN WE REGENERATE DIGITS?

RESEARCH SYSTEM: REGENERATING AXOLOTL LIMB

LIMB REGENERATION: SEQUENCE OF EVENTS




2d
P
4d
7d
M
9d
A
U
R
H
12d
D1
D2
C
R
U
H
14d
C
R
U
H
21d

KEY SCIENTIFIC QUESTIONS

- How is the initial (accumulation) blastema formed?
- How are the boundaries of the proximal-distal axis established?
- What are the patterns of cell proliferation within these boundaries?
- How is tissue patterning within the blastema achieved and how is it linked to proliferation?
- How is the continually changing morphogenesis of the blastema achieved?
- How are all these things related?

CURRENT METHODOLOGIES

- Grafting and ablation
- Histology
- Immunohistochemistry
- Gene expression (FISH, PCR)
- Proteomic analysis

SYSTEM COMPONENTS

- **Cell types:**

  --immune cells

  --wound epidermis

  --blastema cells (derived from muscle, skeletal, dermal and Schwann cells)

  --limb nerves
- **ECM:**

  --fibronectin

  --collagen I

  --hyaluronic acid

  --proteoglycans

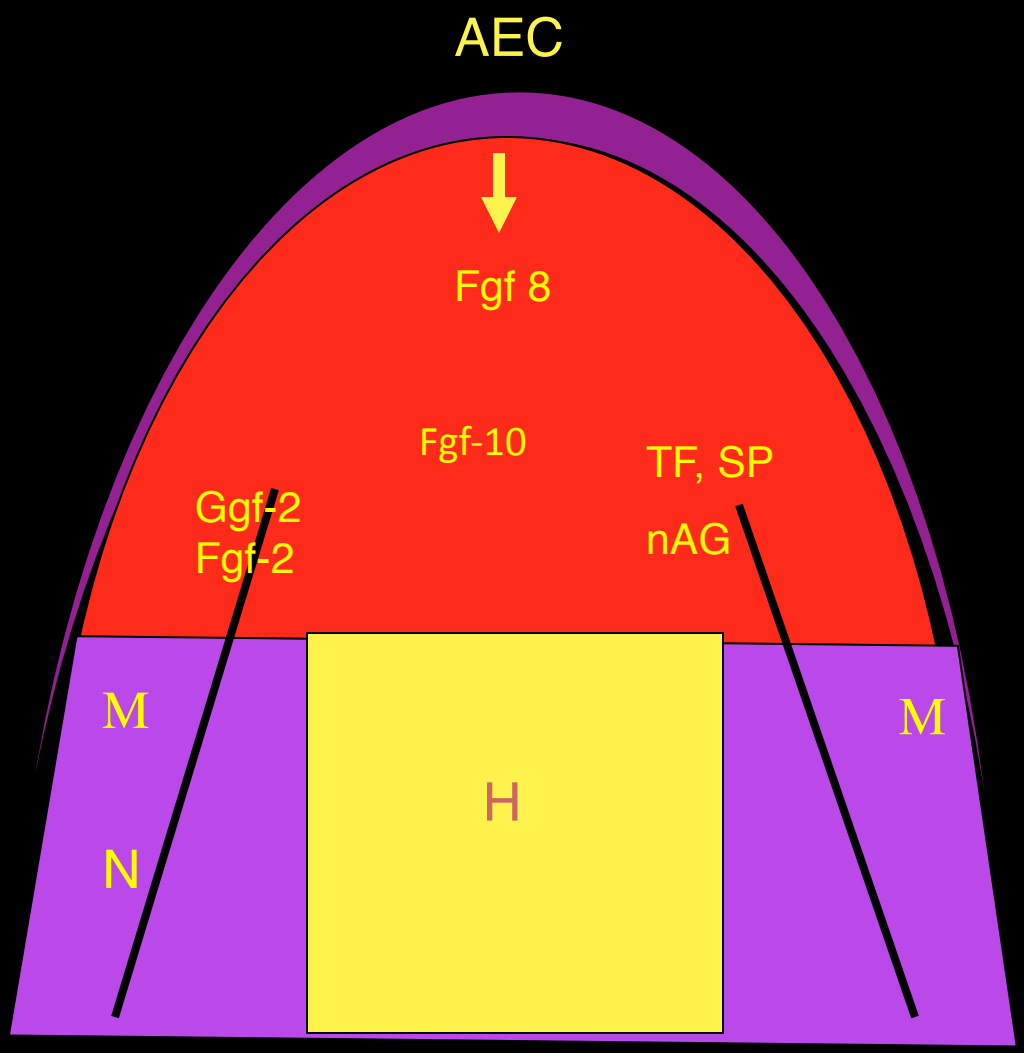
KEY COMPONENT BEHAVIORS

- Cell proliferation
- Cell adhesion
- Cell motility
- Signaling (cell interaction)
- Self-organization
- Differentiation
- Morphogenesis

HOW RESULTS ARE EXPRESSED

- Morphology of blastema (mound, cone, flat, indented tip, etc)
- Tissue morphology (usually skeletal)
- Tissue histology
- Cell morphology (mesenchyme, wound epidermis)
- DNA and RNA synthesis
- Mitotic index
- Gene expression
- Protein expression
- Some or all of these after an experimental manipulation (e.g., grafting)

FACTORS PRODUCED BY THE AEC AND NERVES



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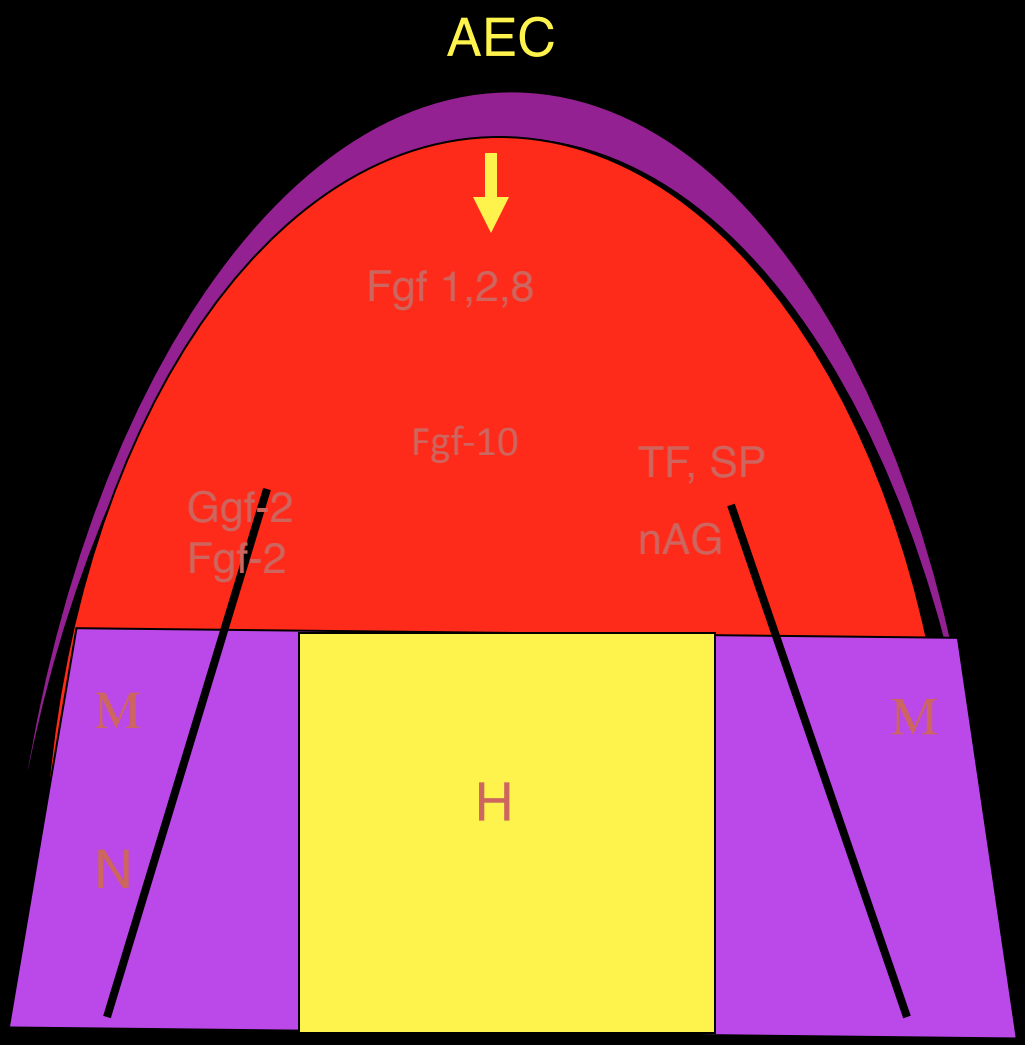
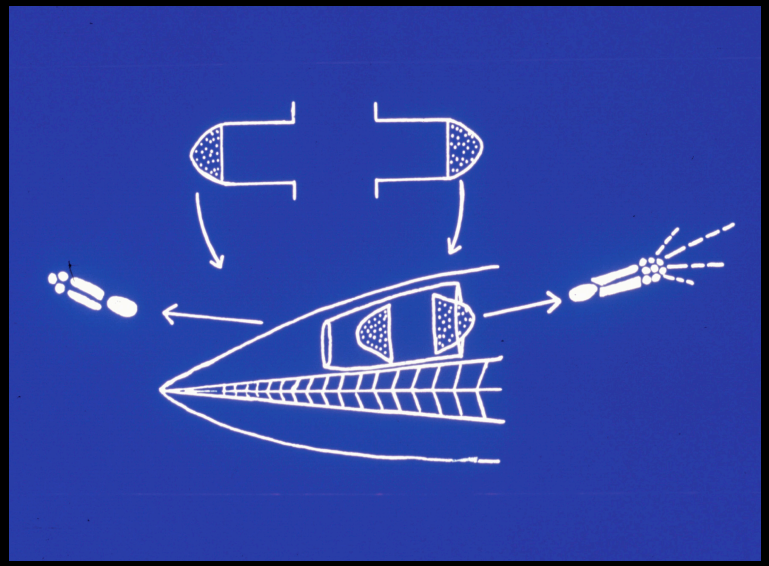
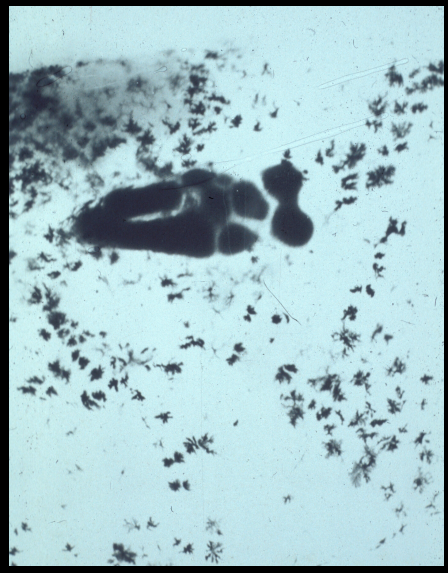
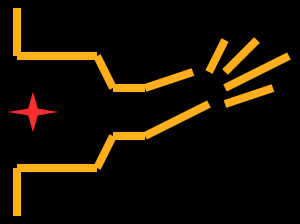


1. TRUNCATION OF REGENERANTS DEVELOPED FROM EPIDERMIS-FREE CONE STAGE BLASTEMAS GRAFTED TO DORSAL FIN TUNNELS



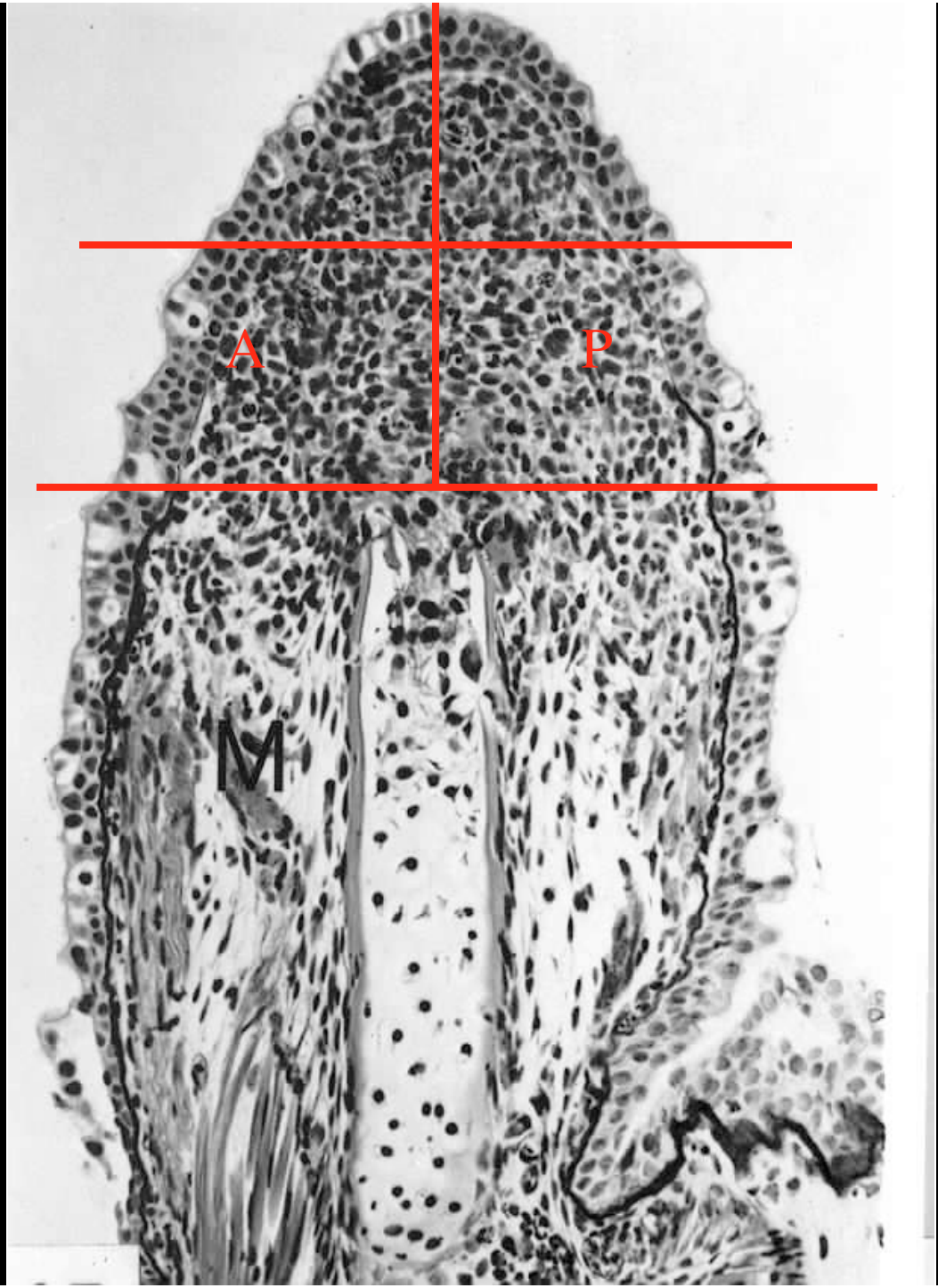


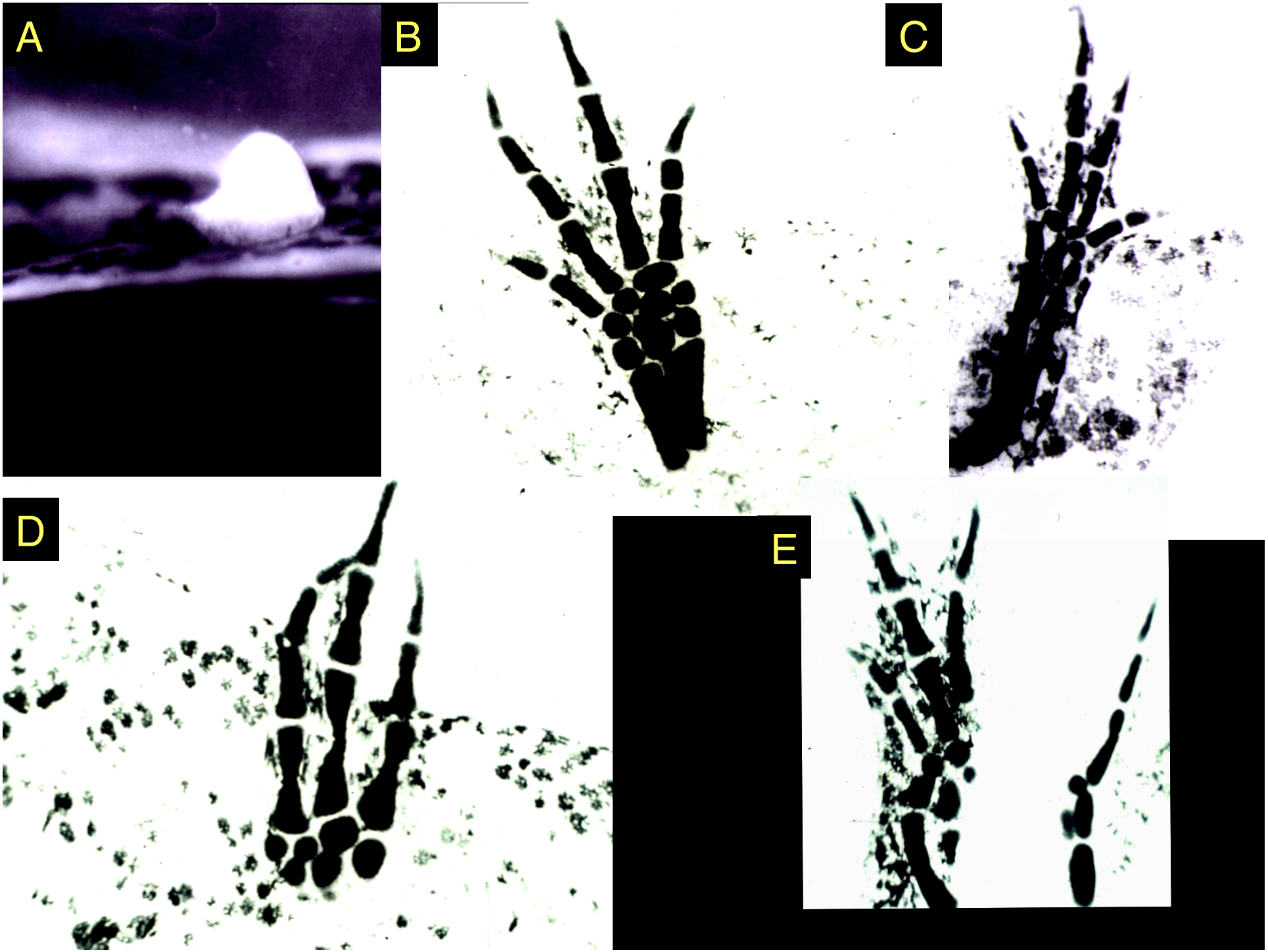
2. DENERVATED CONE STAGE BLASTEMAS DEVELOP A NORMAL PD PATTERN.



3. IN EACH CASE, THE REGENERANTS ARE MINIATURES BECAUSE MITOSIS IS HALTED

SELF-ORGANIZATION




A
B
C
D
E

BIGGEST DEFICIENCY: NEED DATA COLLECTED AT SHORT-INTERVAL TIME POINTS TO RELATE BIOLOGICAL PROCESSES SUCH AS CELL ADHESION, MOTILITY, AND PROLIFERATION TO MORPHOGENESIS AND DIFFERENTIATION OF THE BLASTEMA

A POSSIBLE ONTOLOGY

- Self-organization
  - Patterning
    - Cell interaction
      - Boundary establishment
      - Intercalation
        - paracrine signals
        - juxtacrine signals
  - Morphogenesis
    - Cell proliferation
    - Cell motility
    - Cell adhesion
    - ECM production
    - Differentiation

POSSIBLE APPLICATIONS OF CBO.CBMS/REPOSITORY

- SIMULATION OF A SELF-ORGANIZING SYSTEM THAT HAS BOUNDARIES WITHIN WHICH INTERCALATION OF INTERMEDIATE POSITIONAL IDENTITIES TAKES PLACE
- SIMULATION OF MORPHOGENETIC CHANGES THAT TAKE PLACE DURING BLASTEMA DEVELOPMENT