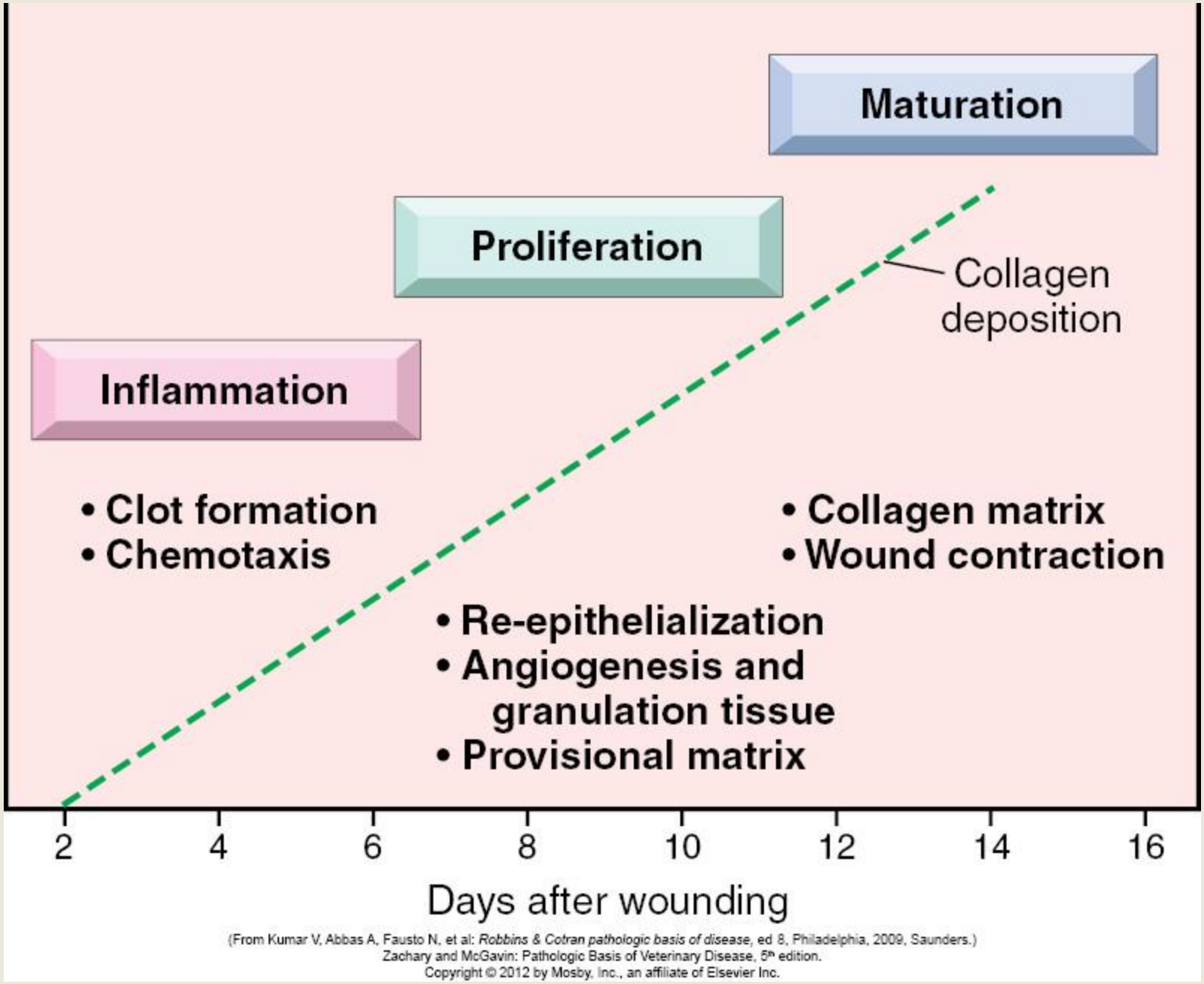
MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

DEPARTMENT OF VETERINARY PATHOLOGY

OVERVIEW OF WOUND HEALING (TISSUE REPAIR)



(From Kumar V, Abbas A, Fausto N, et al: *Robbins & Cotran pathologic basis of disease*, ed 8, Philadelphia, 2009, Saunders.)
Zachary and McGavin: Pathologic Basis of Veterinary Disease, 5th edition.

Tissue repair (healing):
Restoration of tissue architecture and function after an injury

Occurs by two types of reactions

1. **Regeneration**
2. **Scar formation**

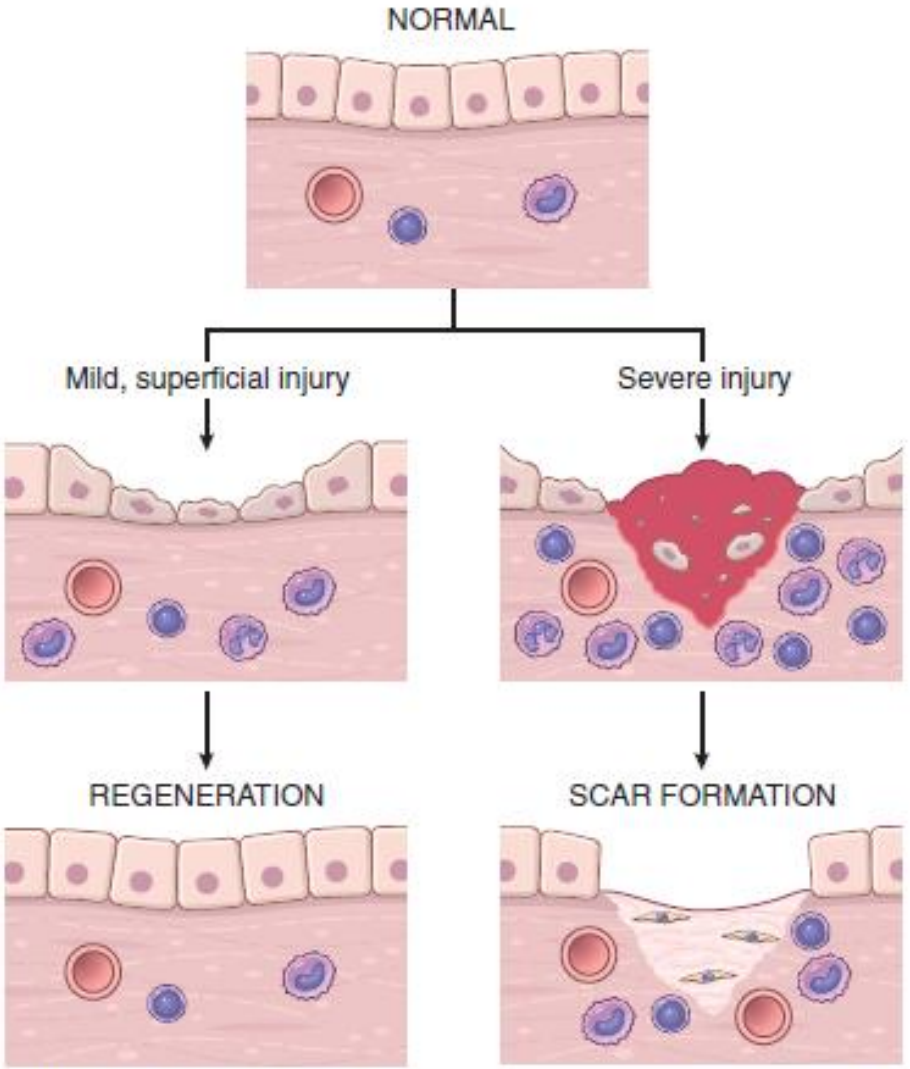


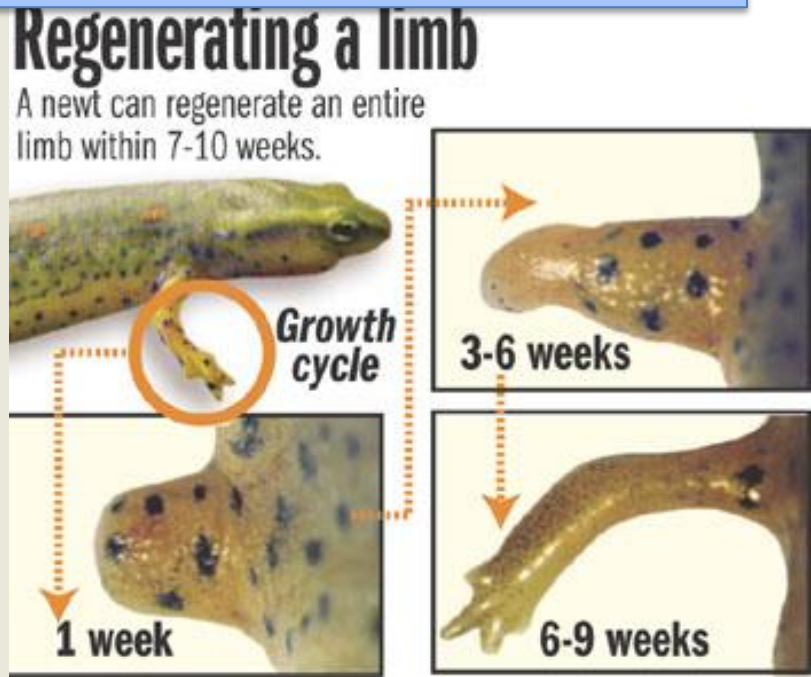
Figure 2–24 Mechanisms of tissue repair: regeneration and scar formation. After mild injury, which damages the epithelium but not the underlying tissue, resolution occurs by regeneration, but after more severe injury with damage to the connective tissue, repair is by scar formation.

1 REPAIR BY REGENERATION

⌂ Replacement of cells by those of an identical type

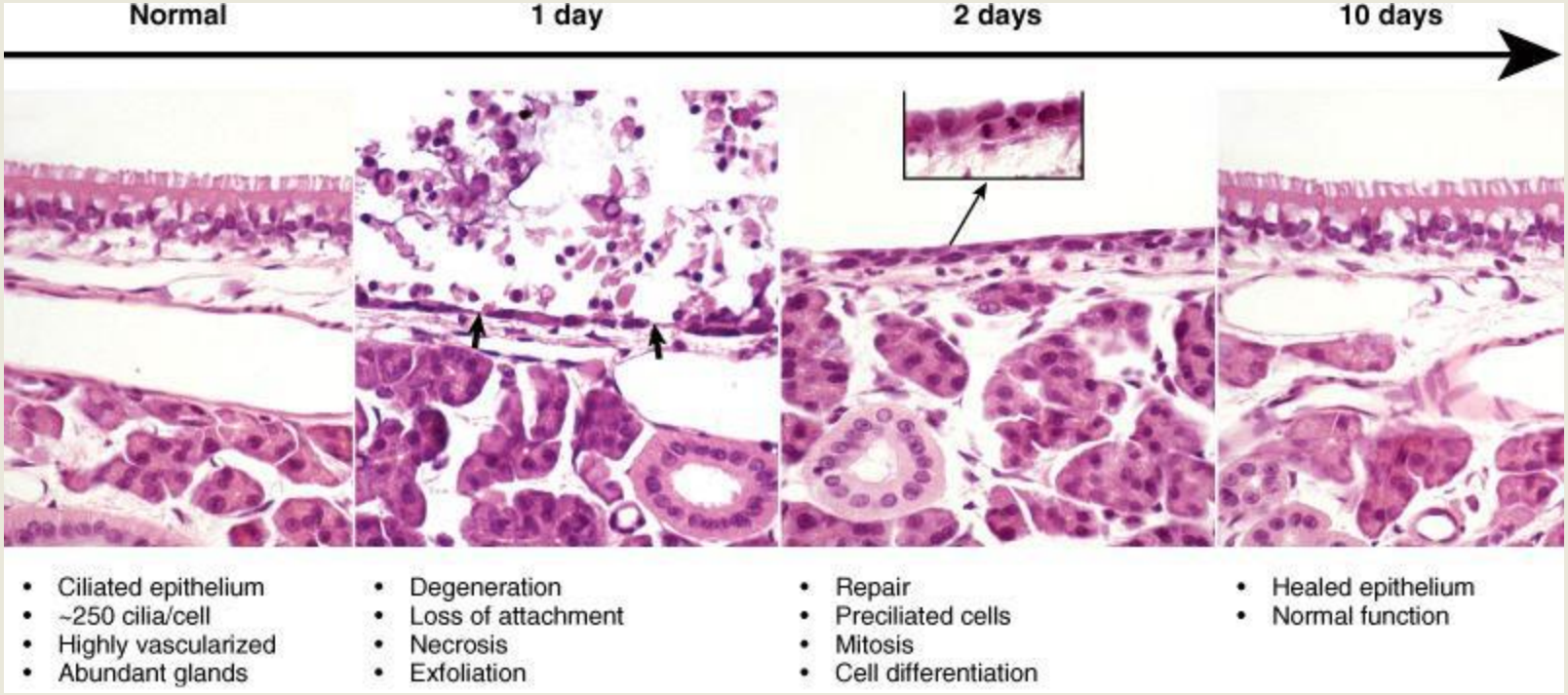
Requires:

- Tissue capable of parenchymal regeneration
- Maintenance of the architectural (CT) framework/ basement membranes



1 REPAIR BY REGENERATION

- Repair starts early in the inflammatory process
- Mediators often both pro-inflammation and pro-repair



2 REPAIR BY SCAR FORMATION

- Injured tissues incapable of regeneration
- If the supporting structures of the tissue are severely damaged
- Repair occurs through connective (fibrous) tissue
- Process that results in scar formation.
- Provides enough structural stability
- Loss of function

1 REPAIR BY REGENERATION
Cell and tissue regeneration

General principles of cell proliferation and the functions of the ECM in this process

- The Control of Cell Proliferation
- Proliferative Capacities of Tissues
- Stem Cells
- Growth Factors
- Extracellular Matrix

The Control of Cell Proliferation

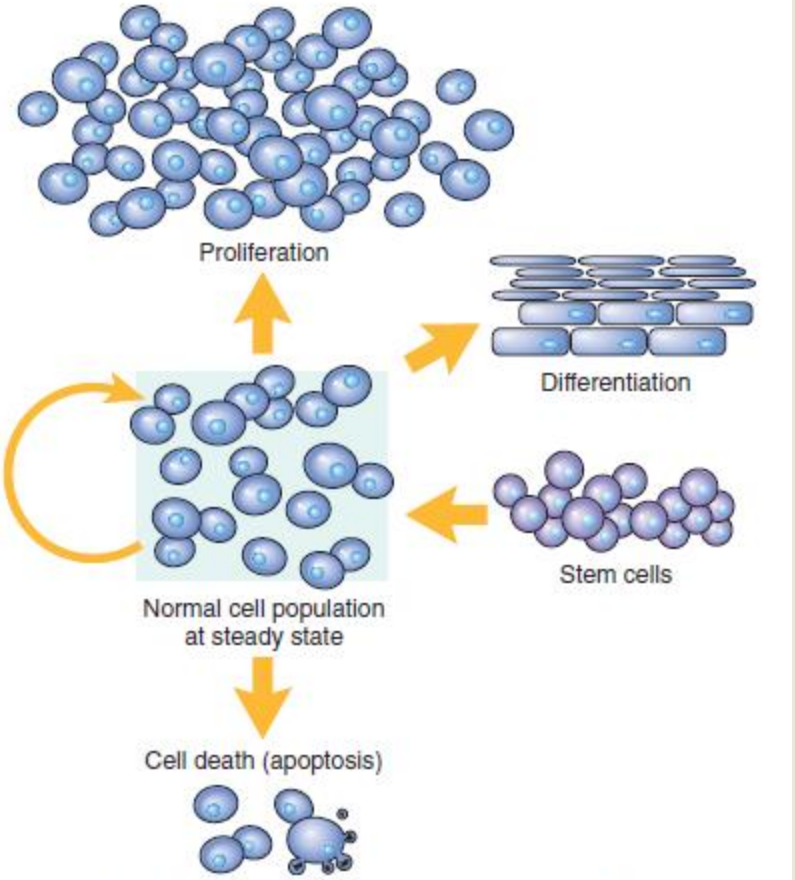
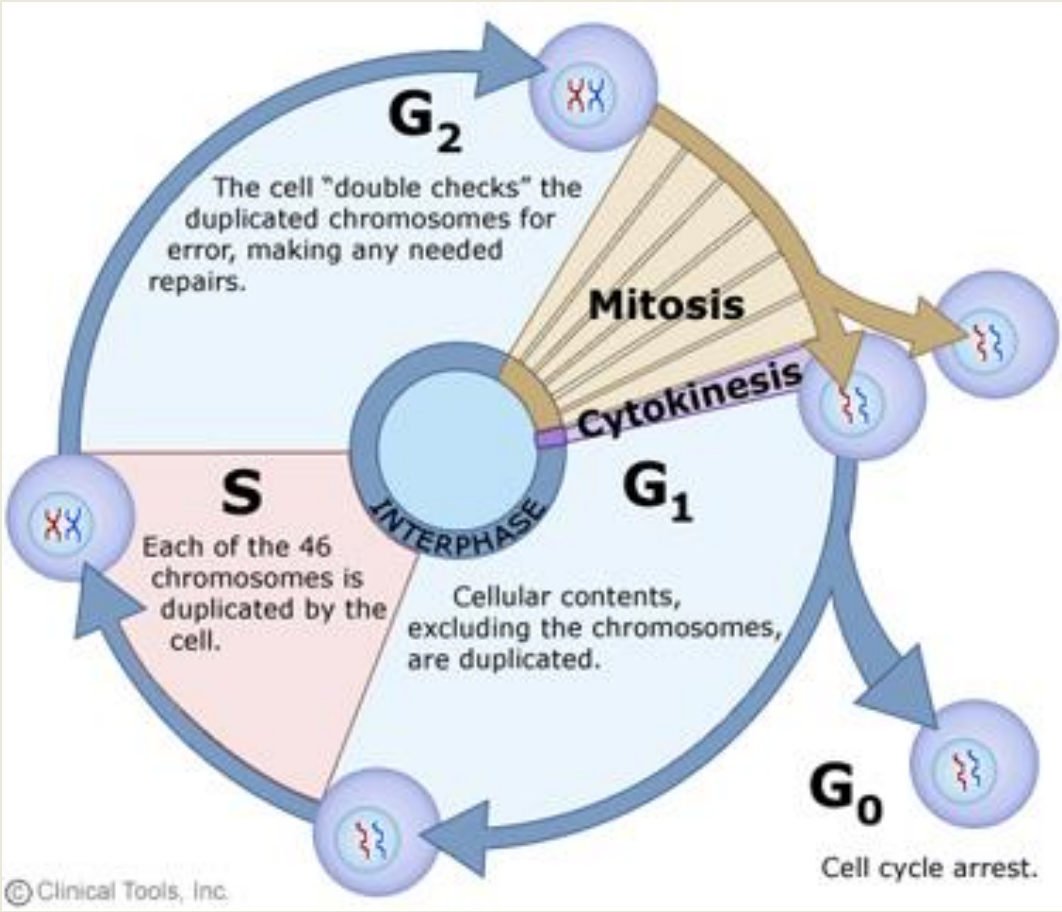


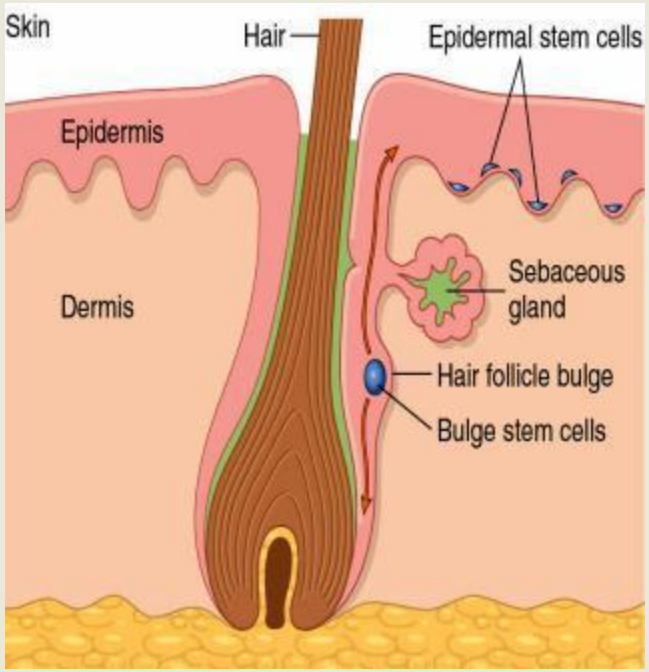
Figure 2–25 Mechanisms regulating cell populations. Cell numbers can be altered by increased or decreased rates of stem cell input, cell death by apoptosis, or changes in the rates of proliferation or differentiation.
(Modified from McCarthy NJ, et al: Apoptosis in the development of the immune system: growth factors, clonal selection and bcl-2. Cancer Metastasis Rev 11:157, 1992.)

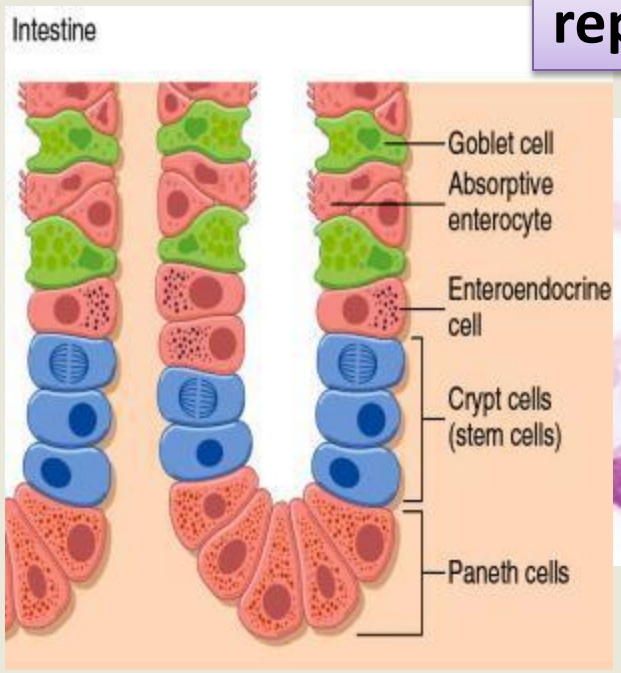


Proliferative Capacities of Tissues

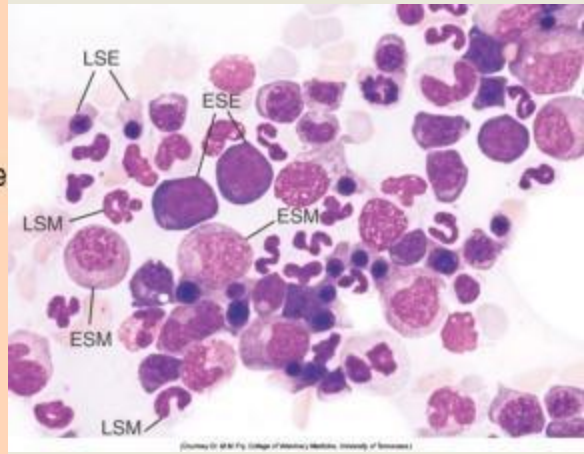
① Labile (continuously dividing) tissues
② Stable tissues
③ Permanent tissues

①Labile (continuously dividing) tissues





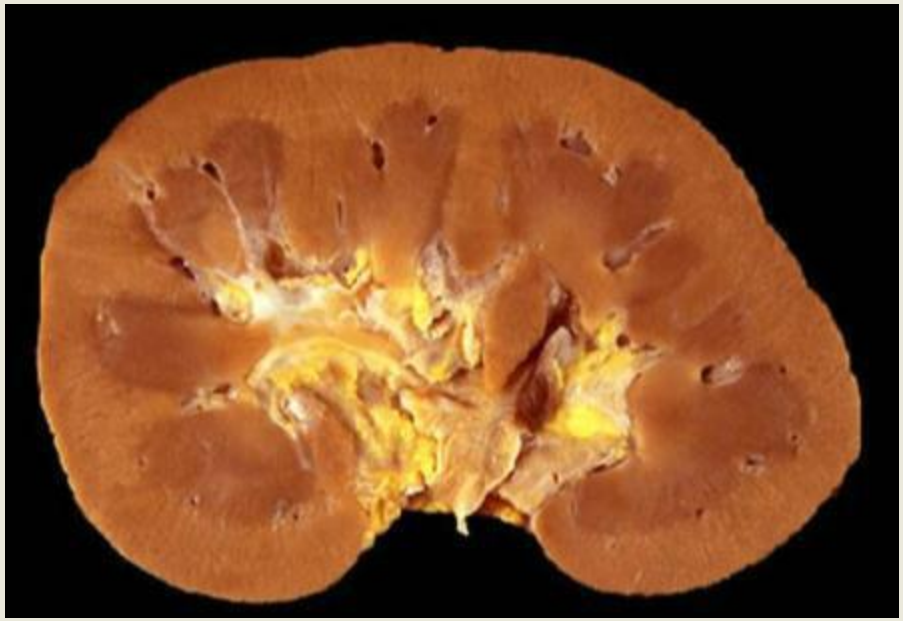
repair by regeneration

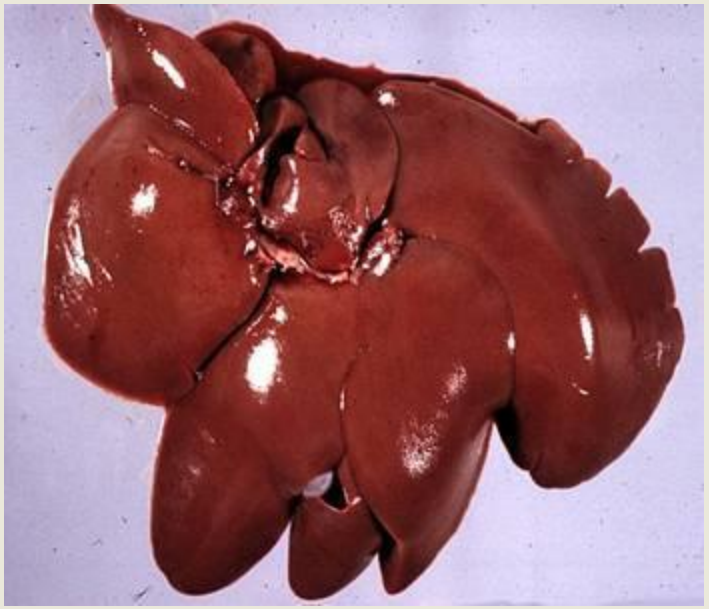


- >1.5% cells in mitoses – More stems cells
- **Examples:**
  - Skin / mucosal epithelium
  - Lymphoid Cells
  - Hematopoietic Cells

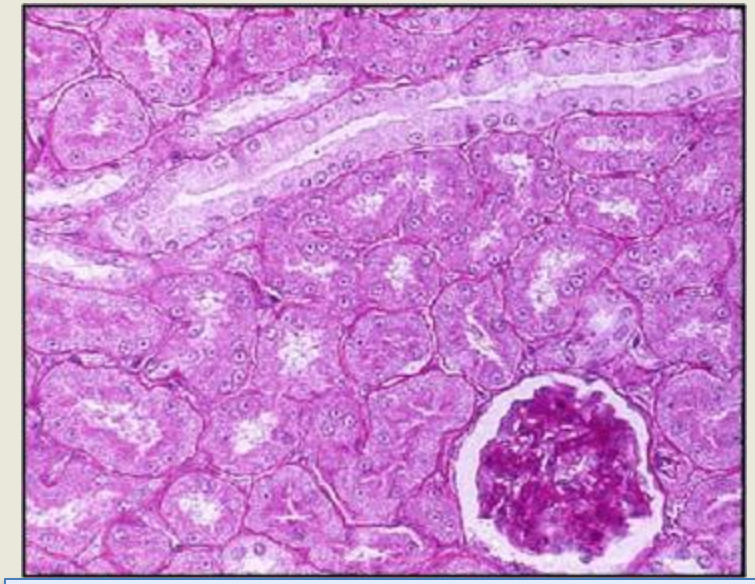
②Stable tissues

repair by regeneration &/or fibrosis

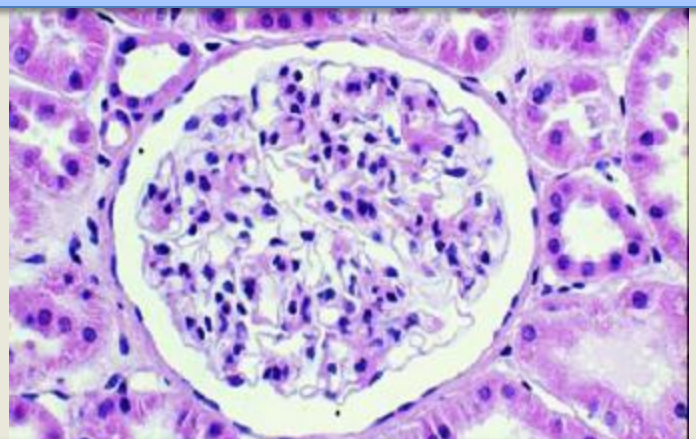




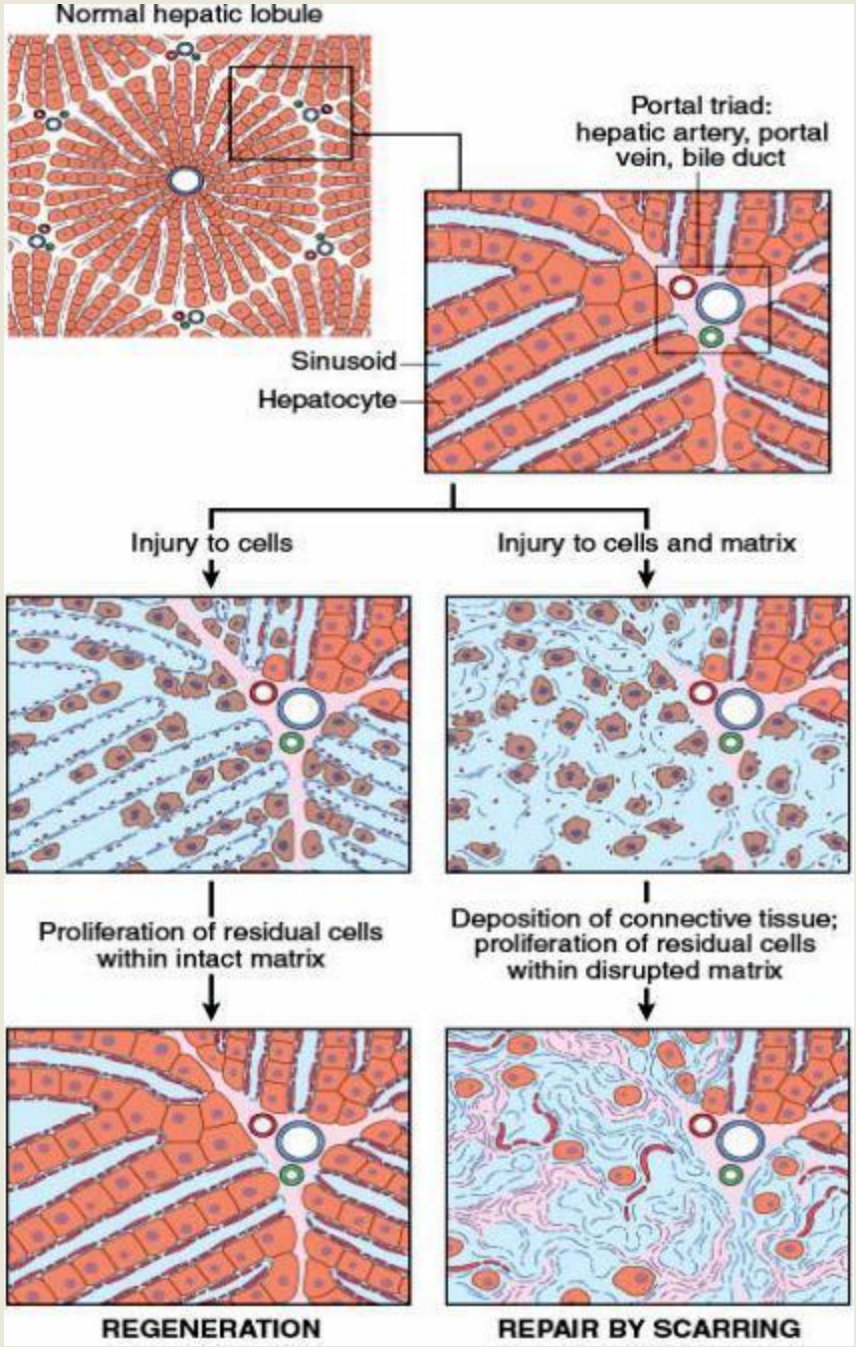
- <1.5% of normal adult cells in mitosis
- Examples:
  - Hepatocytes
  - Renal tubular epithelium
  - Endothelium
  - Mesenchymal cells (fibroblasts) Smooth muscle cells



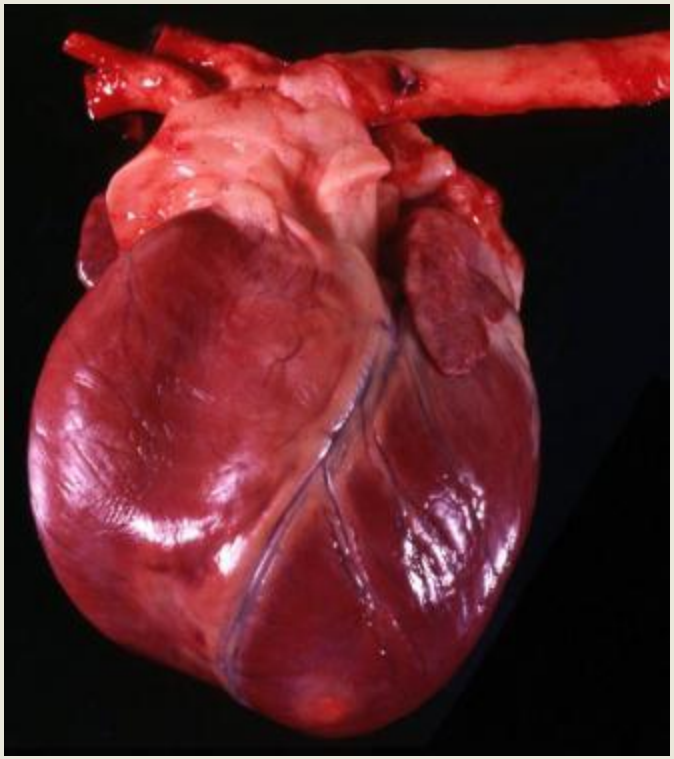
Need intact basement membranes for renal tubules or hepatocytes to regenerate

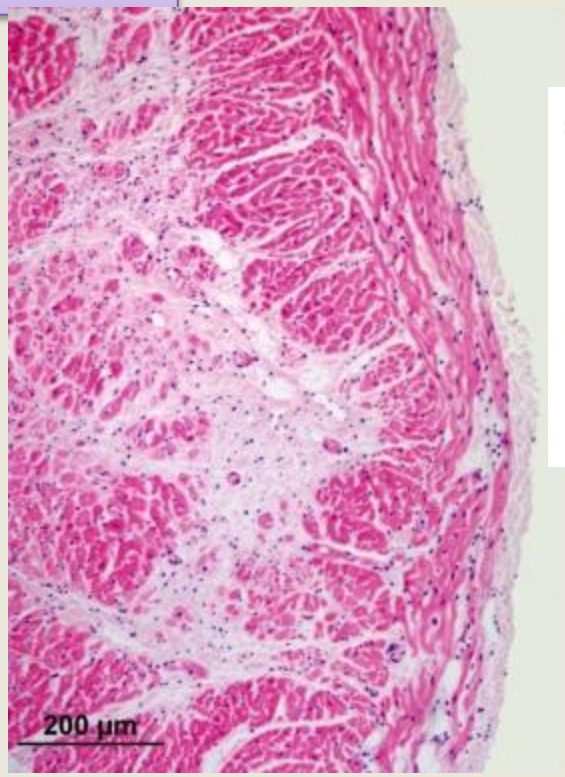


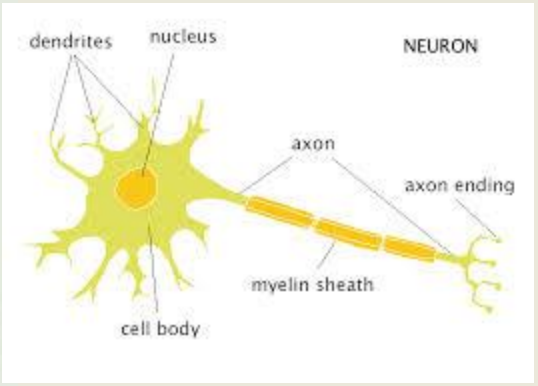
Glomeruli do not regenerate



③Permanent tissues





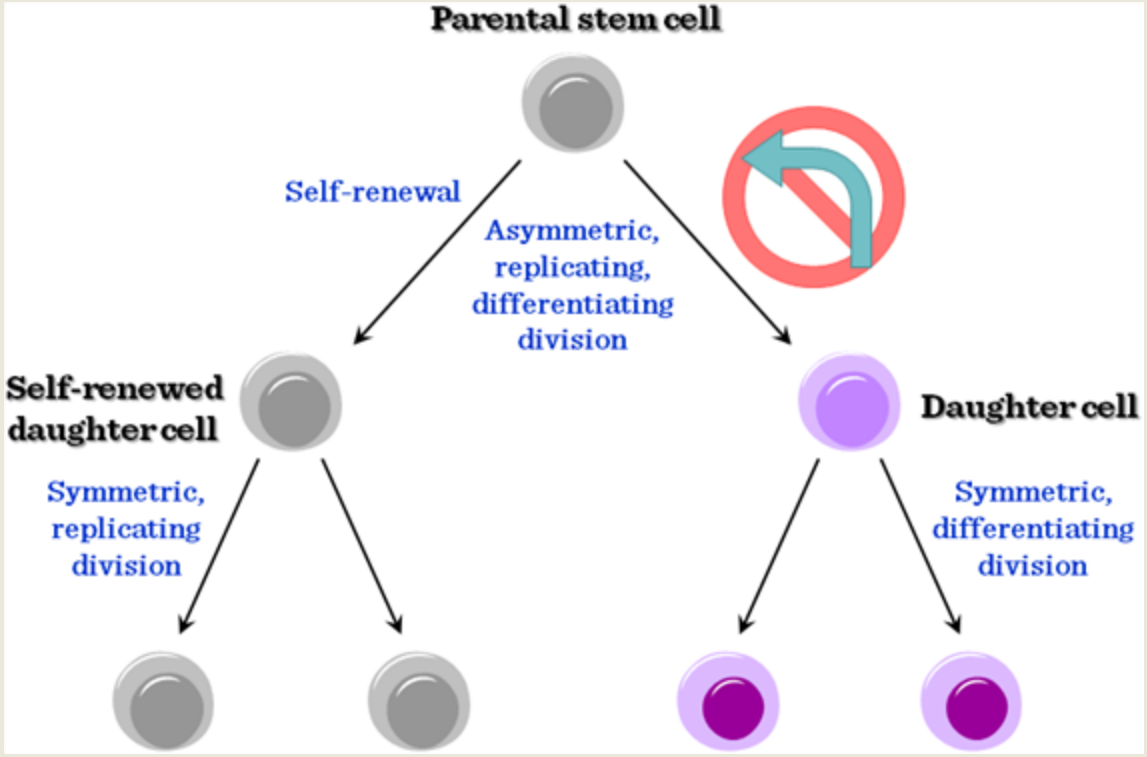


- Cells don’t divide, No regenerative ability
- Examples:
  - Neurons
  - Cardiac myocytes
  - Lens epithelium

Stem Cells

two important properties
①Self renewal capacity
②Asymmetric replication

mature cells die, the tissue is replenished by the differentiation of cells generated from stem cells



Two type of stem cells :

① *Embryonic stem cells (ES)*

- **Totipotent cells:**
  - Fertilized egg
  - Give rise to body
- **Pluripotent:**
  - After 4 days of fertilization
  - Give rise to any tissue

② *Adult stem cells*

- **Multipotent**
- Give rise to limited tissue
- Blood cells, skin cells

induced pluripotent stem cells – ES gene in to fibroblasts or skin epithelial cells

Growth Factors

- Polypeptide growth factors act in
  - Autocrine
  - Paracrine
  - Endocrine
- Stimulate the survival and proliferation of particular cells
- Promote migration, differentiation, and other cellular responses

Table 2–9 Growth Factors Involved in Regeneration and Repair

Growth Factor	Sources	Functions
Epidermal growth factor (EGF)	Activated macrophages, salivary glands, keratinocytes, and many other cells	Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration; stimulates formation of granulation tissue
Transforming growth factor-α (TGF-α)	Activated macrophages, keratinocytes, many other cell types	Stimulates proliferation of hepatocytes and many other epithelial cells
Hepatocyte growth factor (HGF) (scatter factor)	Fibroblasts, stromal cells in the liver, endothelial cells	Enhances proliferation of hepatocytes and other epithelial cells; increases cell motility
Vascular endothelial growth factor (VEGF)	Mesenchymal cells	Stimulates proliferation of endothelial cells; increases vascular permeability
Platelet-derived growth factor (PDGF)	Platelets, macrophages, endothelial cells, smooth muscle cells, keratinocytes	Chemotactic for neutrophils, macrophages, fibroblasts, and smooth muscle cells; activates and stimulates proliferation of fibroblasts, endothelial, and other cells; stimulates ECM protein synthesis
Fibroblast growth factors (FGFs), including acidic (FGF-1) and basic (FGF-2)	Macrophages, mast cells, endothelial cells, many other cell types	Chemotactic and mitogenic for fibroblasts; stimulates angiogenesis and ECM protein synthesis
Transforming growth factor-β (TGF-β)	Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes, smooth muscle cells, fibroblasts	Chemotactic for leukocytes and fibroblasts; stimulates ECM protein synthesis; suppresses acute inflammation
Keratinocyte growth factor (KGF) (i.e., FGF-7)	Fibroblasts	Stimulates keratinocyte migration, proliferation, and differentiation

ECM, extracellular membrane.

Extracellular Matrix (ECM)

- Provides mechanical support to
- Acts as a substrate for cell growth and the formation of tissue microenvironments.
- Regulates cell proliferation and differentiation
- Scaffolding for tissue renewal *:* If the ECM is damaged, repair can be accomplished only by scar formation.
- Two form : ❶ **Interstitial matrix:** ❷ **Basement membrane:**

❶ Interstitial matrix:

- Present in the spaces between cells
- Synthesized by mesenchymal cells (e.g., fibroblasts)

⌂ **Collagens:**

- Tensile strength
- Fibrillar type in healing
- Vitamin C required for synthesis

⌂ **Elastin:** tissues to recoil

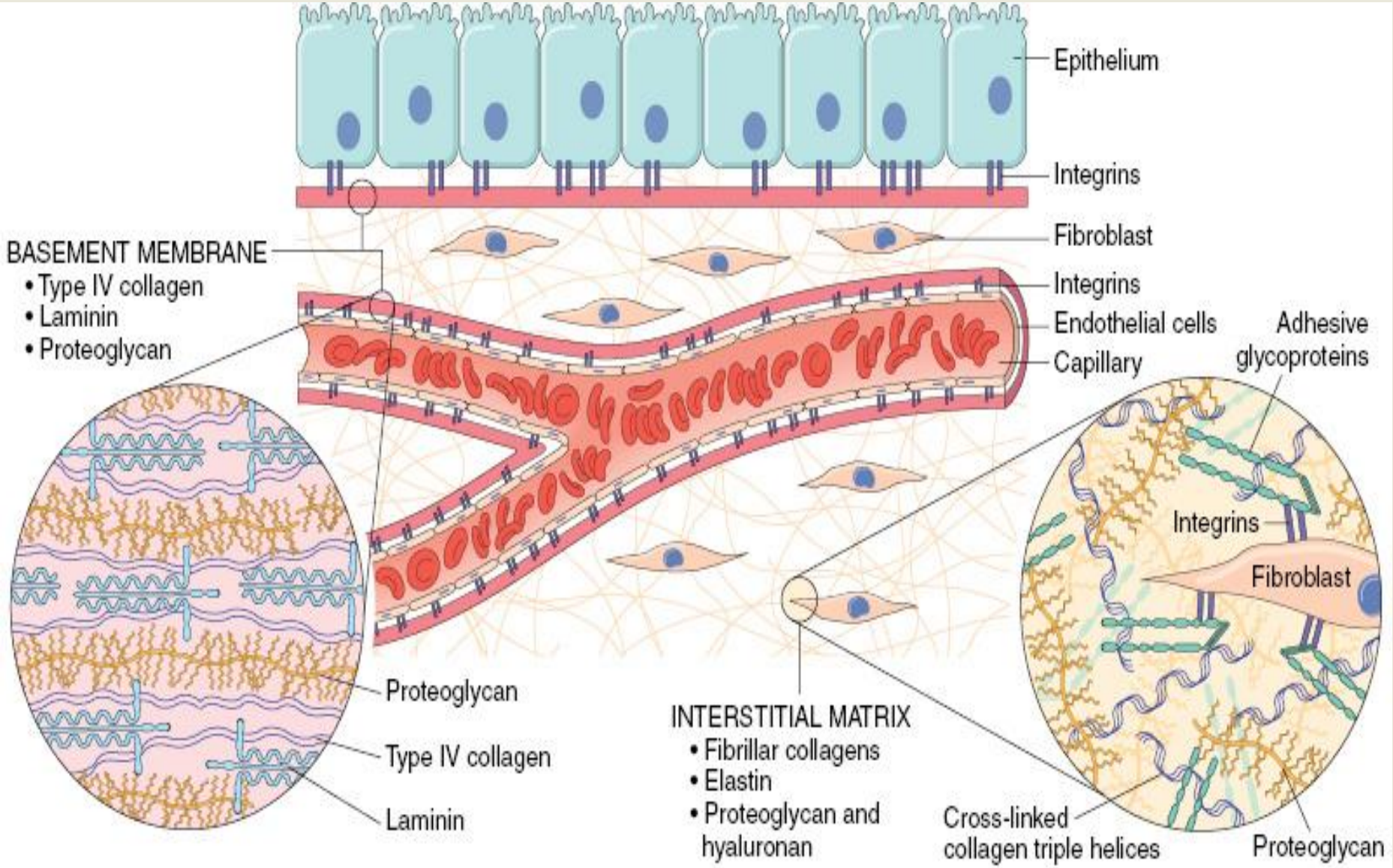
⌂ **Fibronectin & Integrins :** Adhesive Glycoproteins - cell-to-cell adhesion, cells to the ECM

⌂ **Proteoglycans & Hyaluronate :** hydrated compressible gels conferring resilience and lubrication

⌂ other elements

❷ Basement membrane:

- Highly organized around epithelial cells
- synthesized by overlying epithelium and underlying mesenchymal cells
- Nonfibrillar type IV collagen
- Laminin : Adhesive Glycoproteins - cell-to-cell adhesion, cells to the ECM



(From Kumar V, Abbas A, Fausto N, et al: *Robbins & Cotran pathologic basis of disease*, ed 8, Philadelphia, 2009, Saunders.)

Zachary and McGavin: Pathologic Basis of Veterinary Disease, 5th edition.

❷ REPAIR BY SCAR FORMATION

- Injured tissues incapable of regeneration
- If the supporting structures of the tissue are severely damaged
- Repair occurs through connective (fibrous) tissue
- Process that results in scar formation.

Four components

① Angiogenesis (neovascularization)
② Migration & proliferation of fibroblasts
③ Deposition of extracellular matrix
④ Maturation and reorganization of fibrous tissue

Timeline:

- Begins within 24 hours of injury!
- Fibroblasts & endothelial cells migrate to the site of injury & proliferate
- 3-5 days see early granulation tissue
- Wks to months see ↑ collagen and ↓decreased vessels (scarring)

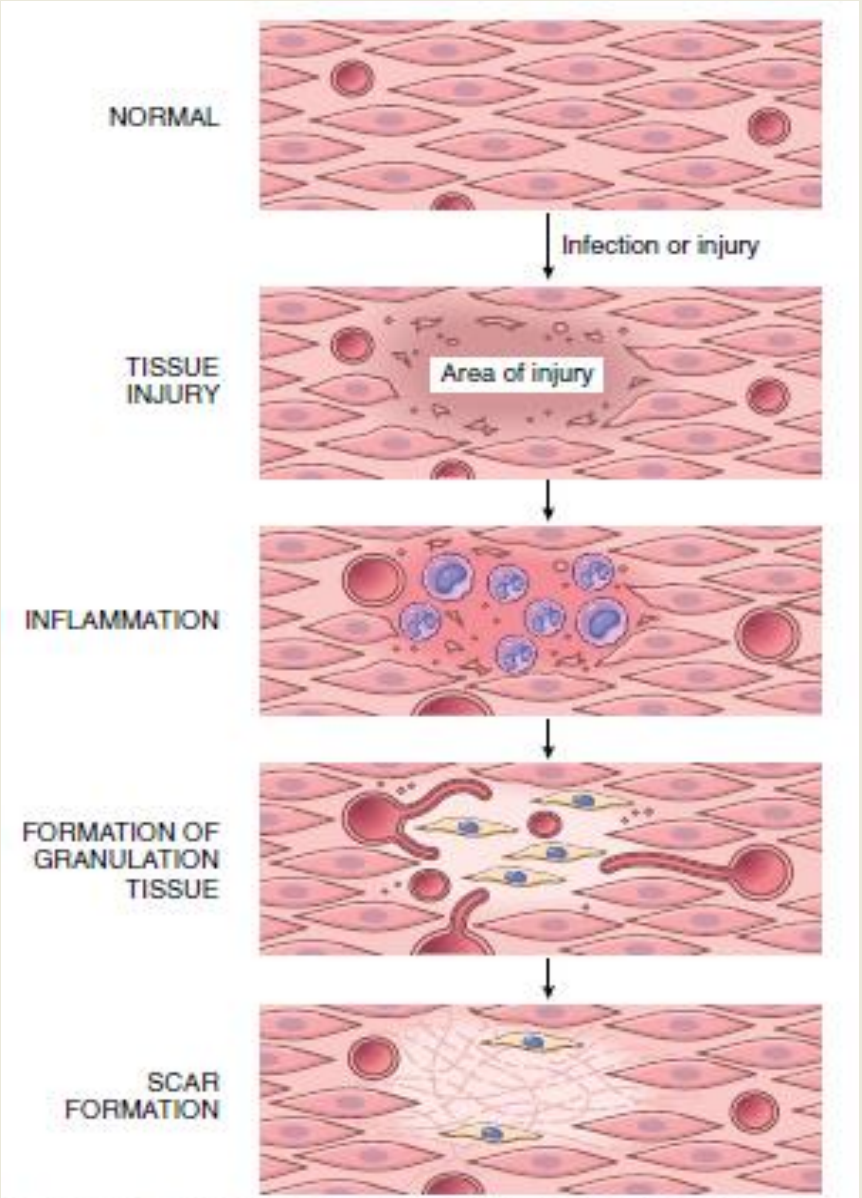


Figure 2–29 Steps in repair by scar formation. Injury to a tissue that has limited regenerative capacity first induces inflammation, which clears dead cells and microbes, if any. This is followed by formation of vascularized granulation tissue and then deposition of ECM to form the scar. ECM, extracellular matrix.

① Angiogenesis

the process of new blood vessel development from existing vessels, primarily venules.

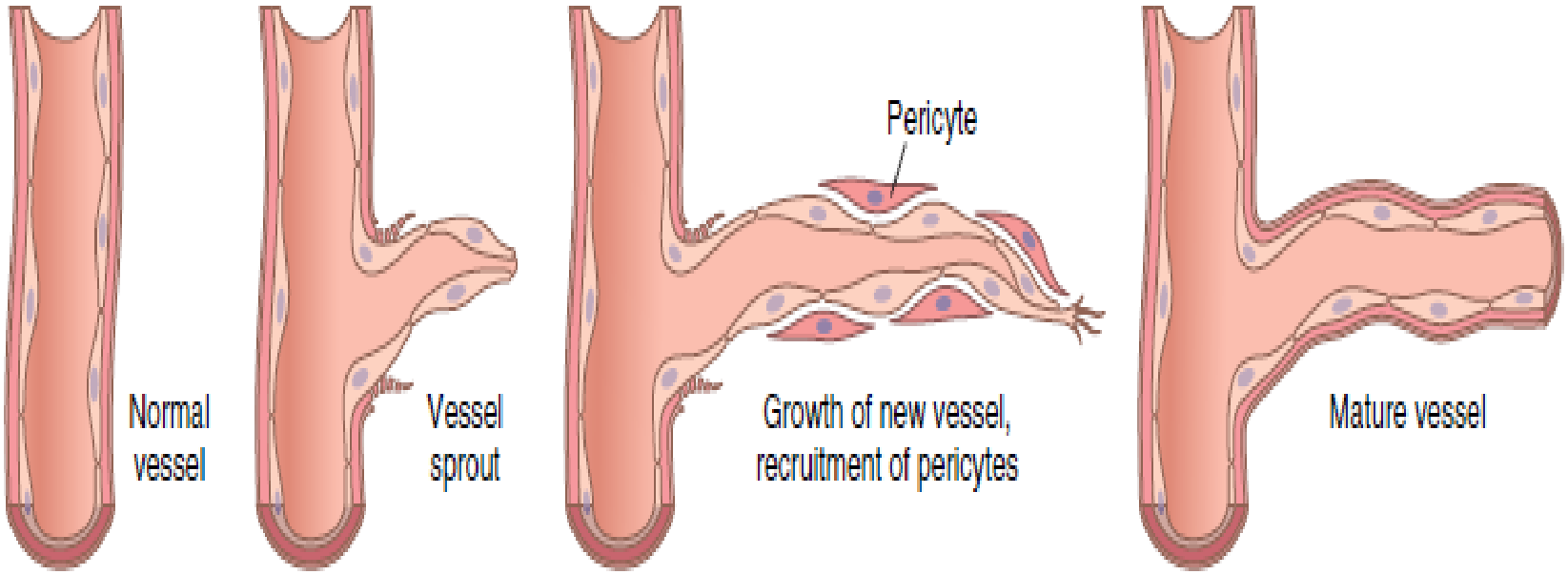
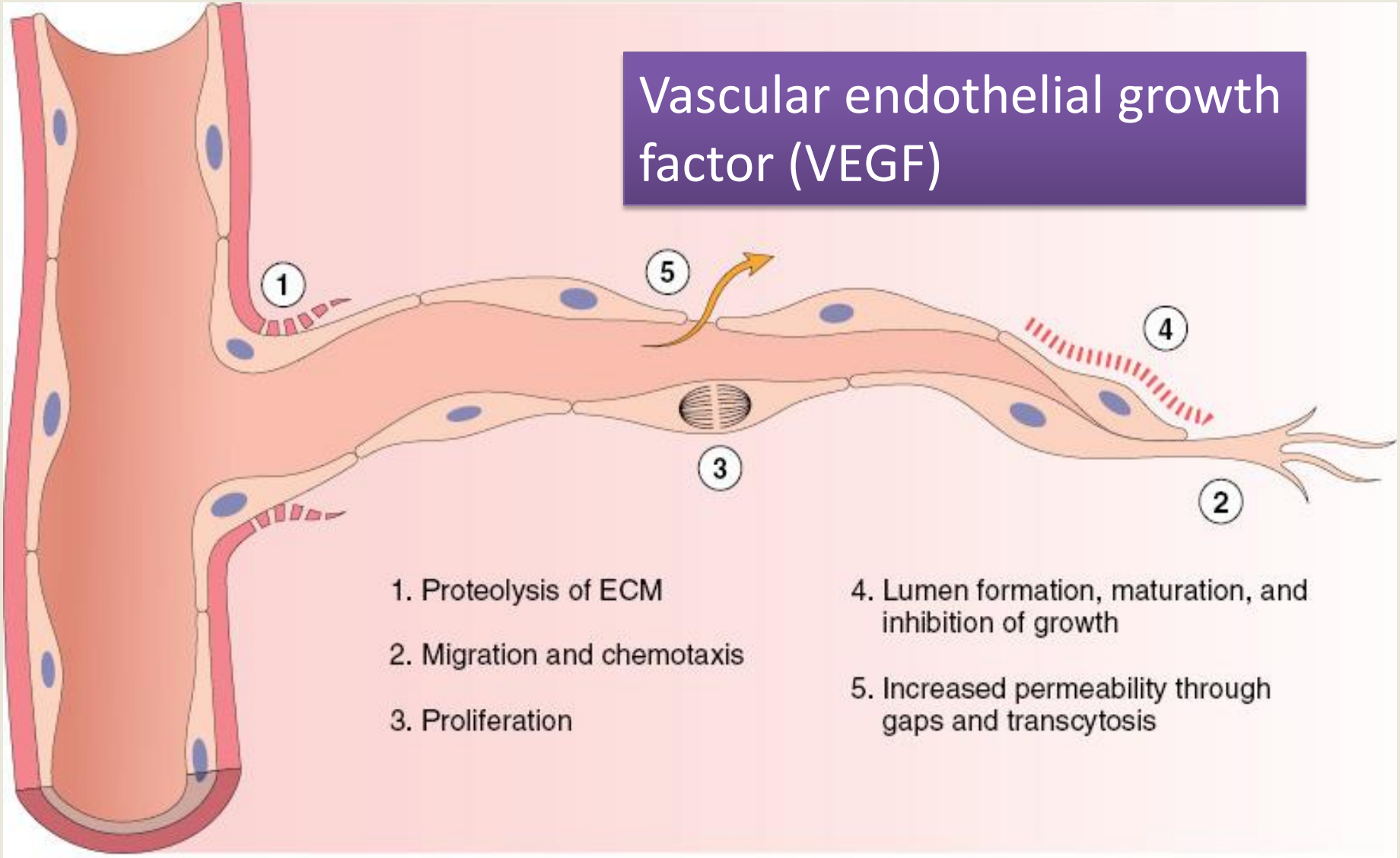


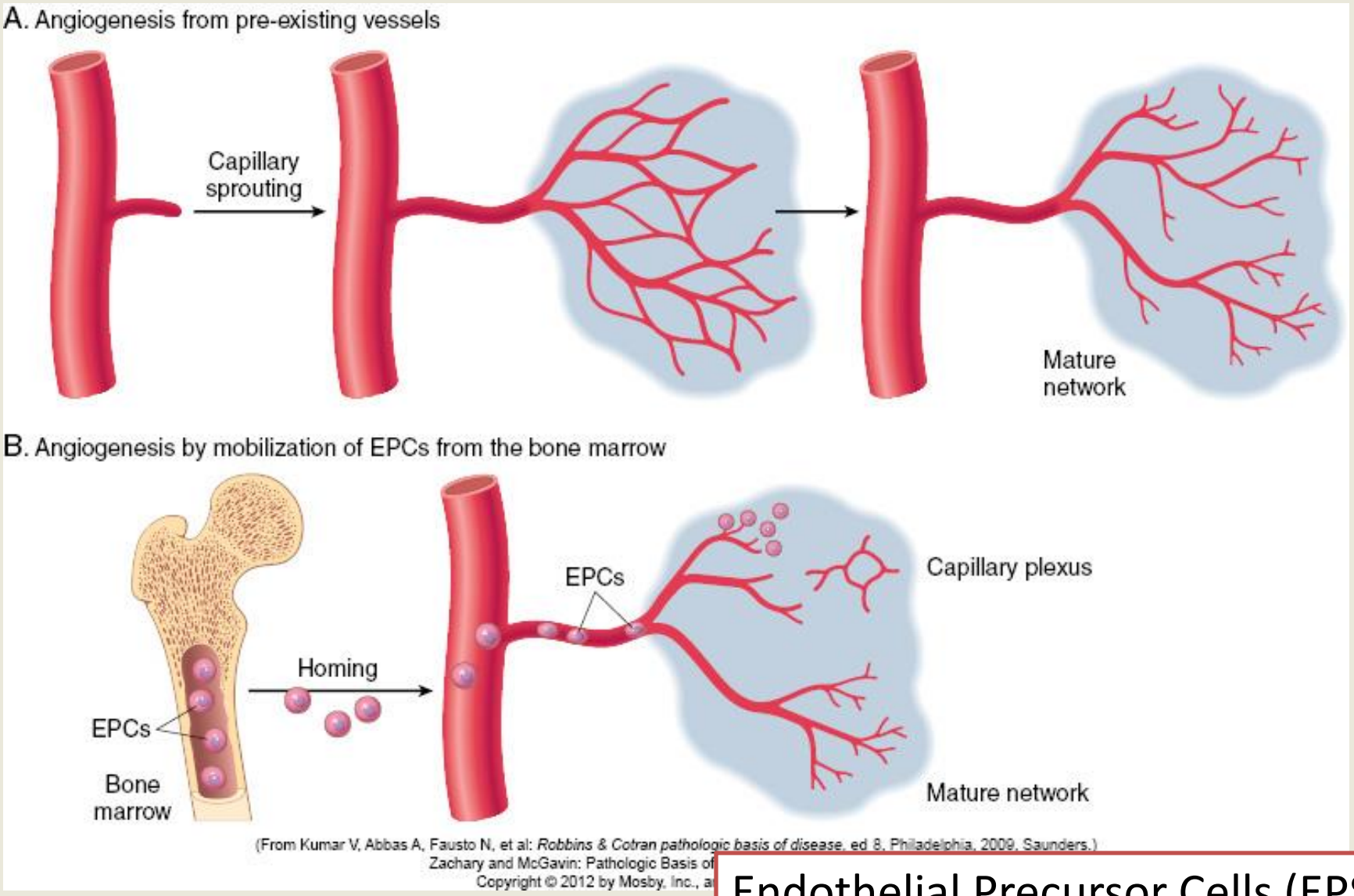
Figure 2–31 Mechanism of angiogenesis. In tissue repair, angiogenesis occurs mainly by growth factor–driven outgrowth of residual endothelium, sprouting of new vessels, and recruitment of pericytes to form new vessels.

① Angiogenesis



(Modified from Motamed K, Sage EH: *Kidney Int* 51:1383, 1997.)
Zachary and McGavin: Pathologic Basis of Veterinary Disease, 5th edition.

① Angiogenesis



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Zachary and McGavin: Pathologic Basis of
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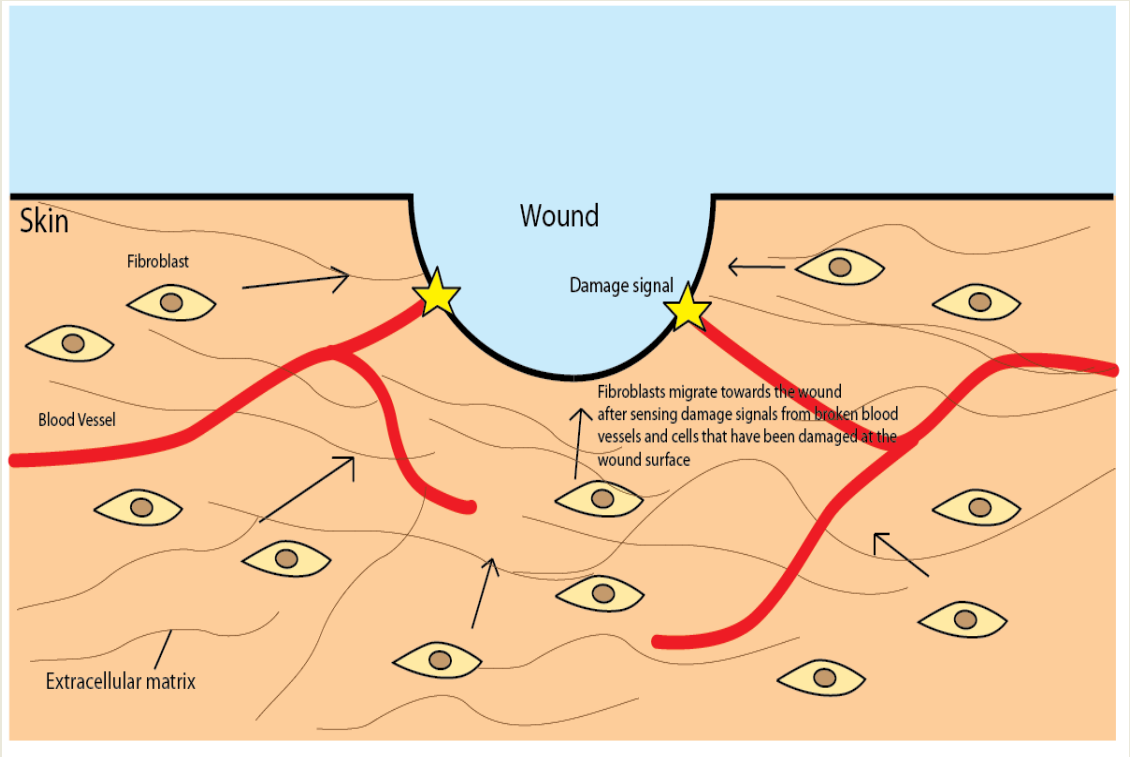
Endothelial Precursor Cells (EPS)

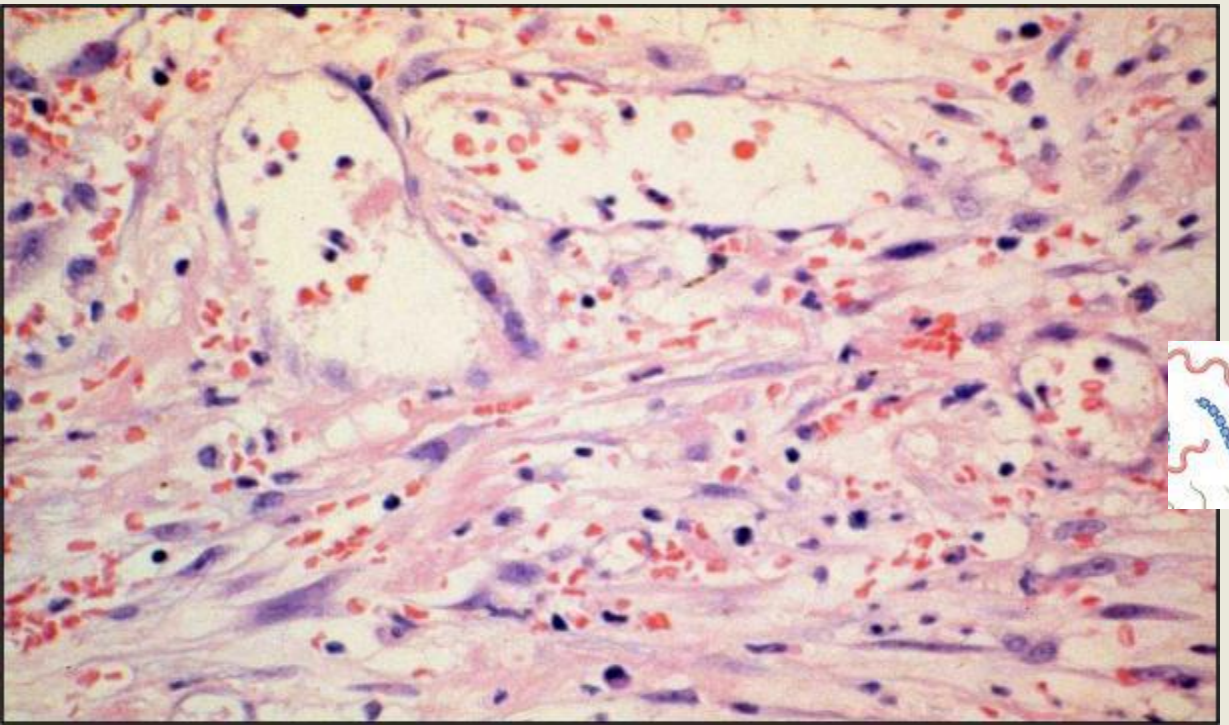
②Migration & proliferation of fibroblasts

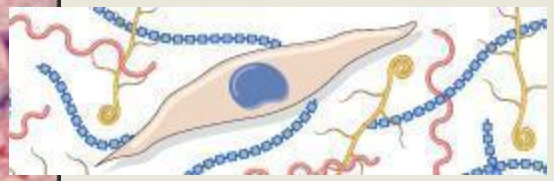
- Inflammatory cells secretes growth factors : PDGF, TGF-β, FGF
- fibroblasts attract toward site of inflammation
- After migration - Proliferation of fibroblasts



③Deposition of extracellular matrix

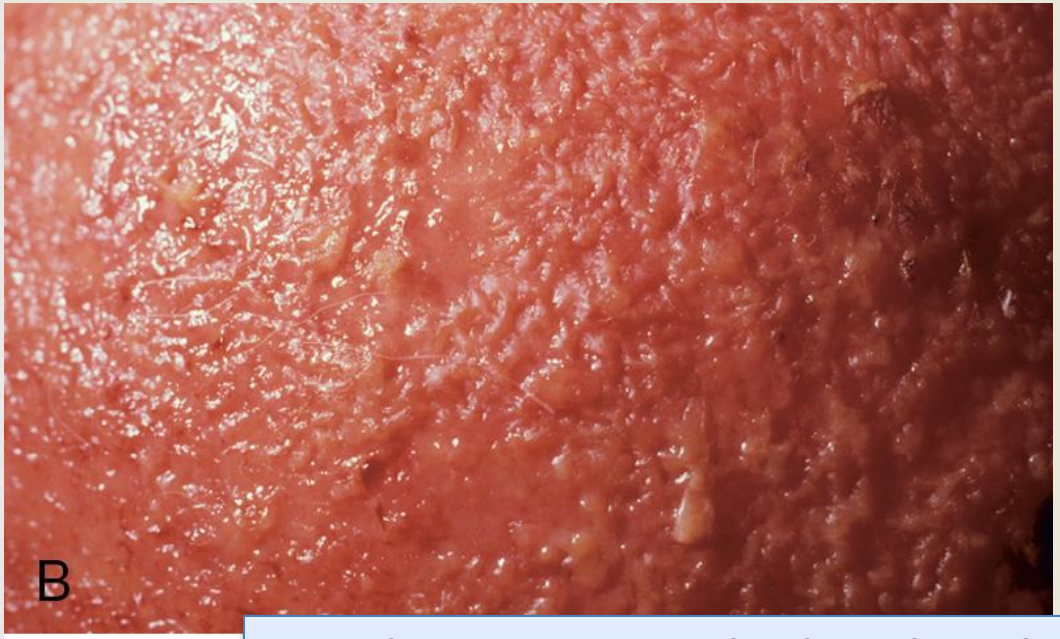
- TGF-β stimulates the production of collagen, fibronectin, and proteoglycans
- PDGF causes migration and proliferation of fibroblasts and smooth muscle cells and may contribute to the migration of macrophages.



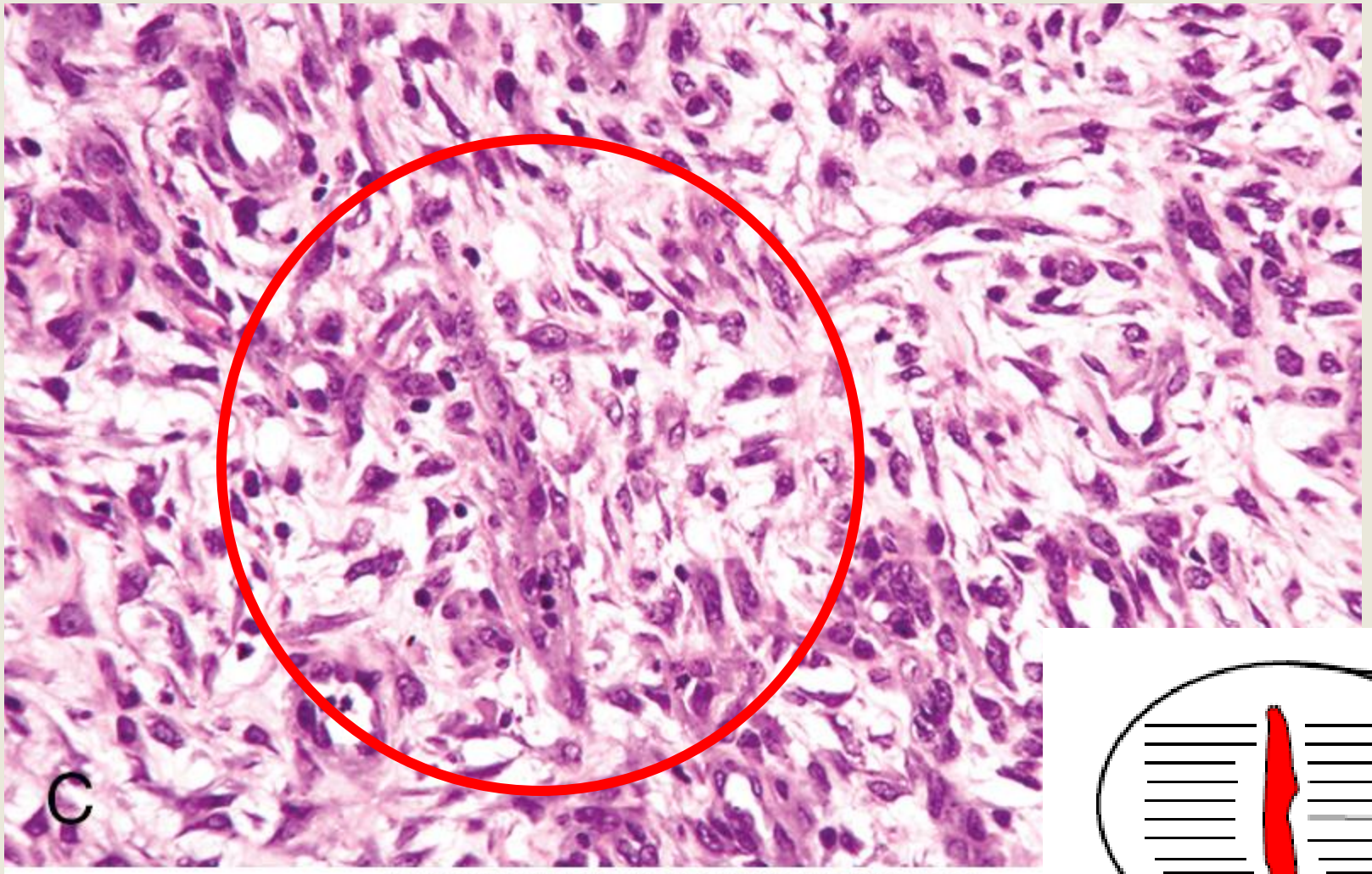


④Maturation and reorganization of fibrous tissue

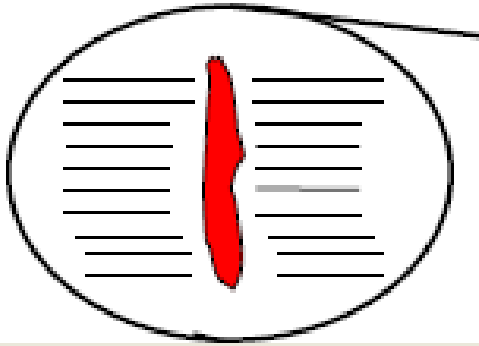
- Development of granulation tissue and subsequent scar tissue formation



Granulation tissue, nonhealing ulcer, skin, distal limb, horse A In the bed of the ulcer, there is extensive fibrosis and granulation tissue. **B,** Gross photograph of the surface of the granulation tissue



(Courtesy Dr. M.D. McGavin, College of Veterinary Medicine, University of Tennessee.)
Zachary and McGavin: Pathologic Basis of Veterinary Disease, 5th edition.



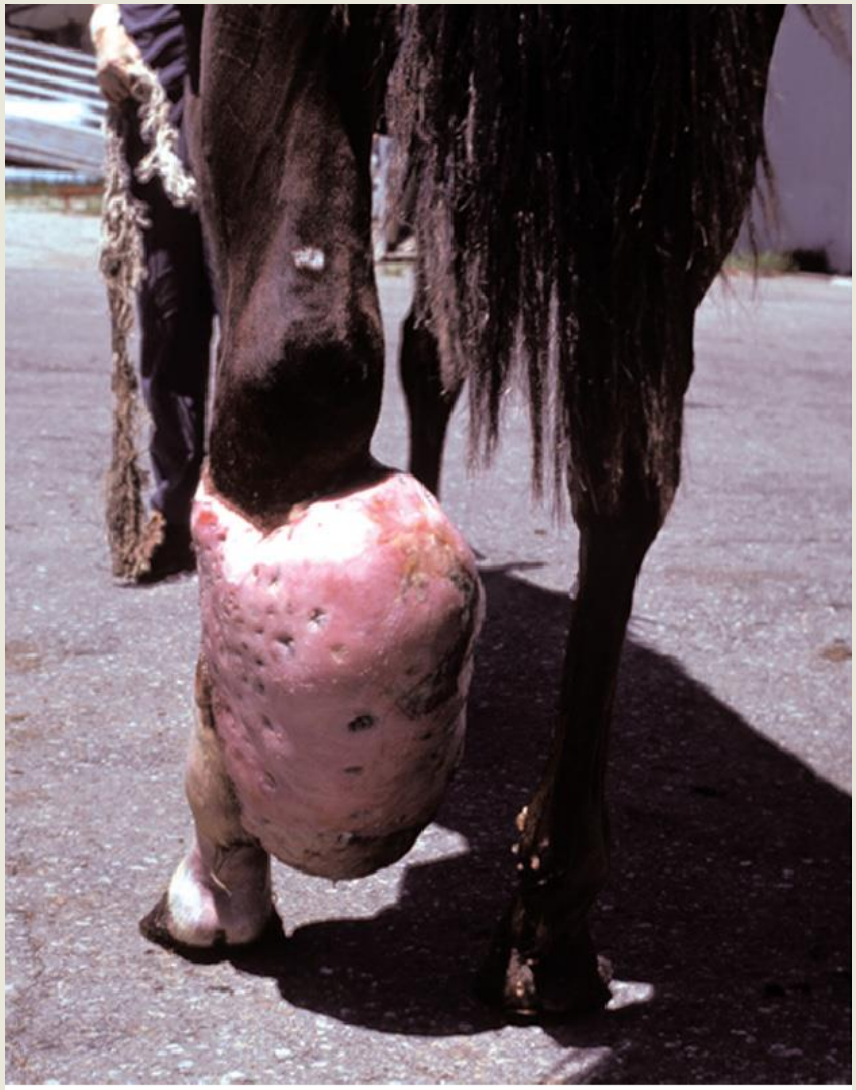
Granulation tissue, nonhealing ulcer, skin, distal limb, horse

C, Photomicrograph of granulation tissue. Note how the new fibroblasts are arranged perpendicularly to the newly formed blood vessels in a rich bed of ECM *(clear spaces)*

What might impair Wound Healing?

- Infection
- Nutrition
- Glucocorticoids
- Mechanical factors
- Poor perfusion
- Foreign bodies
- Type & amount of tissue injured
- Location of injury

Exuberant Granulation Tissue "Proud Flesh"

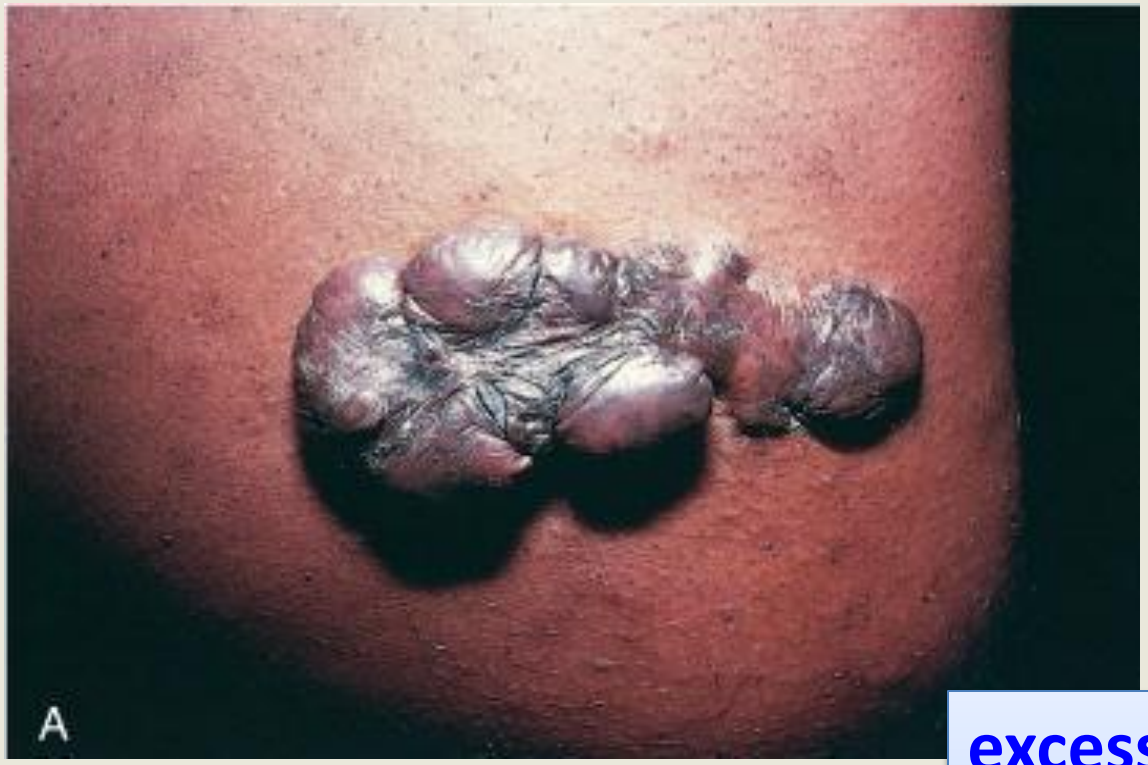


(Courtesy Dr. M.D. McGavin, College of Veterinary Medicine, University of Tennessee.)
Zachary and McGavin: Pathologic Basis of Veterinary Disease, 6th edition.

Healing wounds
May generate exuberant granulation tissue that protrudes
above the level of the surrounding skin and hinders re-epithelialization

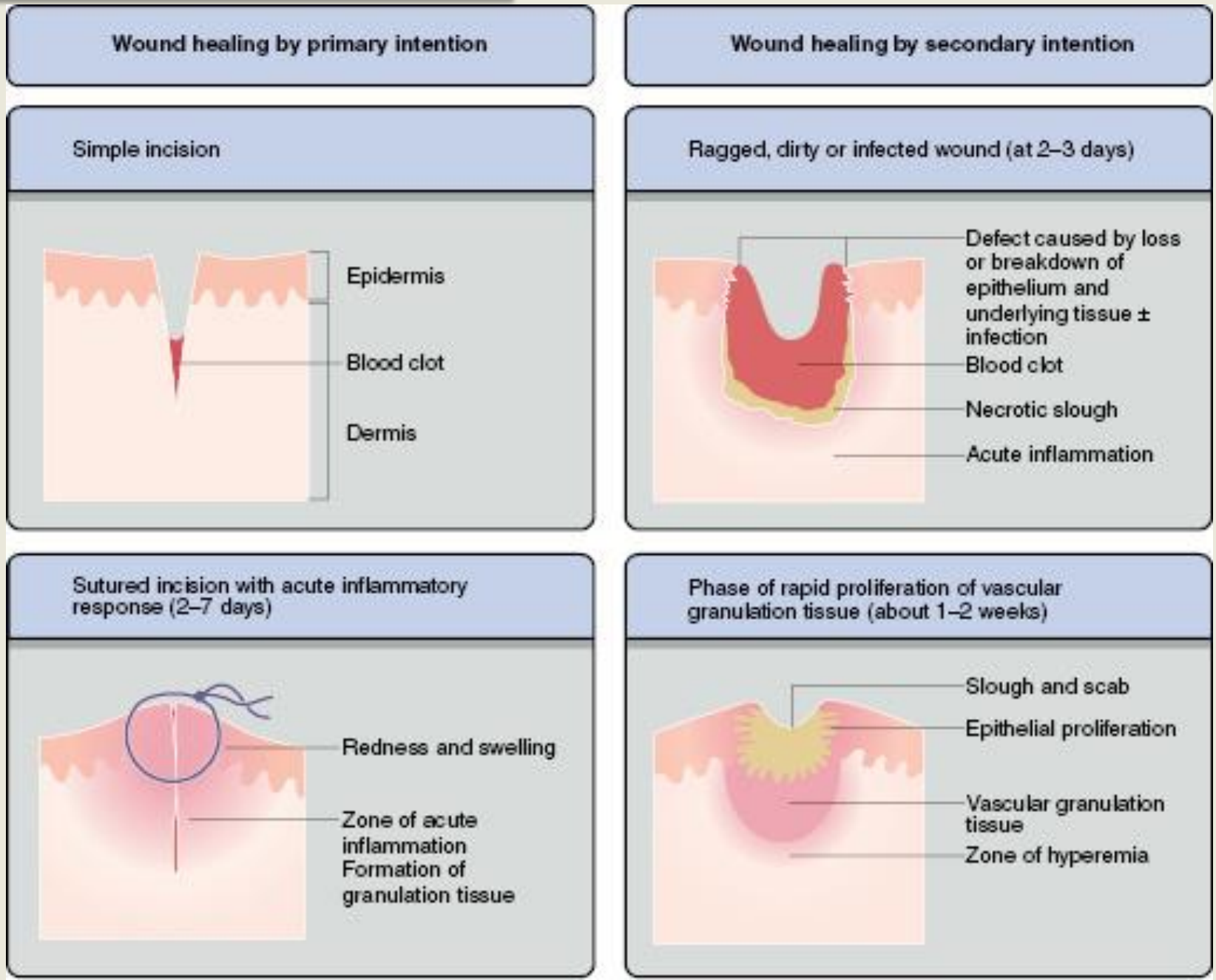
Exuberant granulation tissue (proud flesh), chronic ulcer, skin, distal hindlimb, horse. Note the large proliferating mass of fibrous tissue on the lower portion of the left hindlimb. It often lacks superficial epithelium.

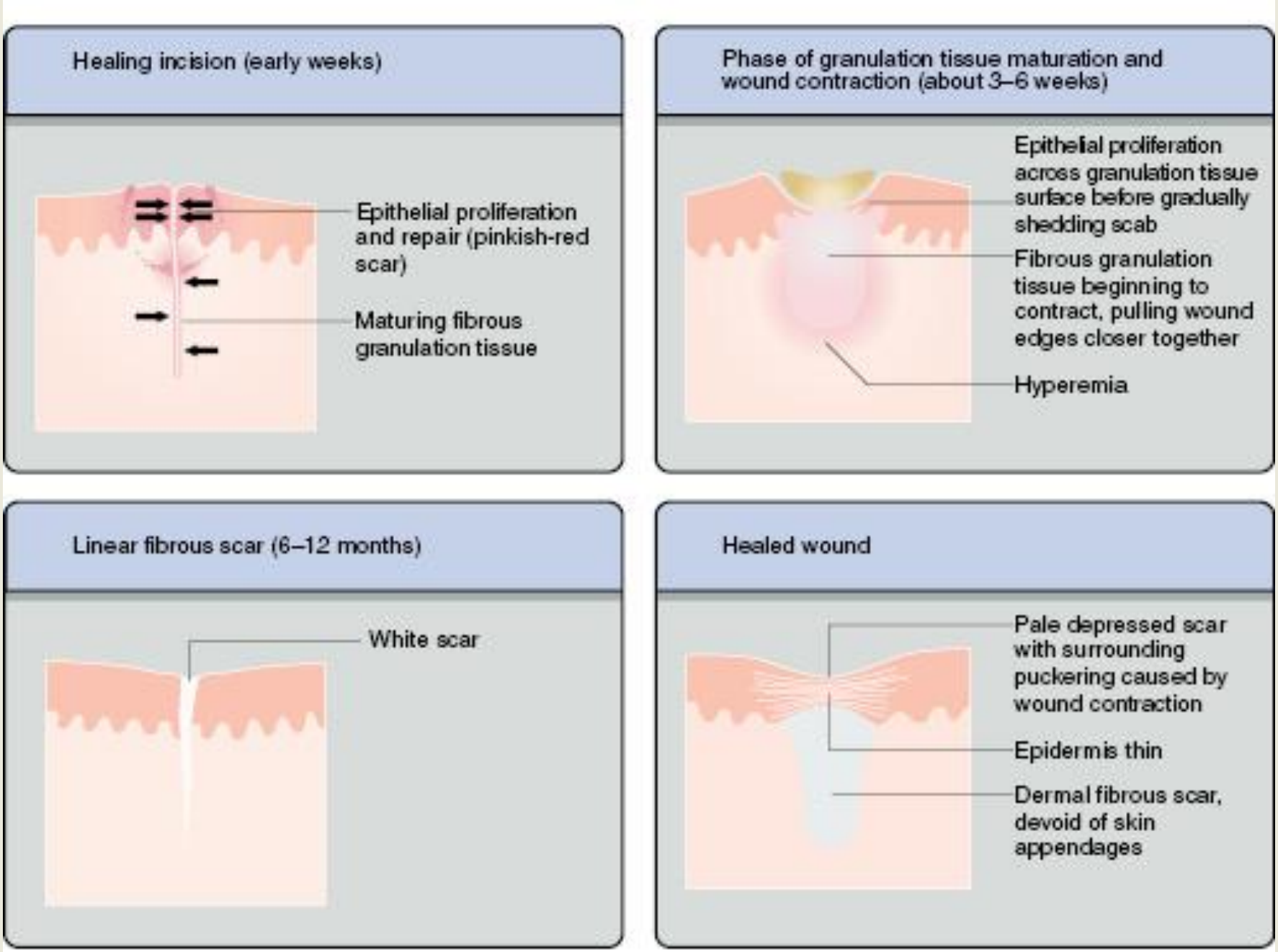
Raised scars “keloids”



excessive amount of scar tissue that grows beyond the boundaries of the original wound and does not regress.

Healing of Skin Wounds

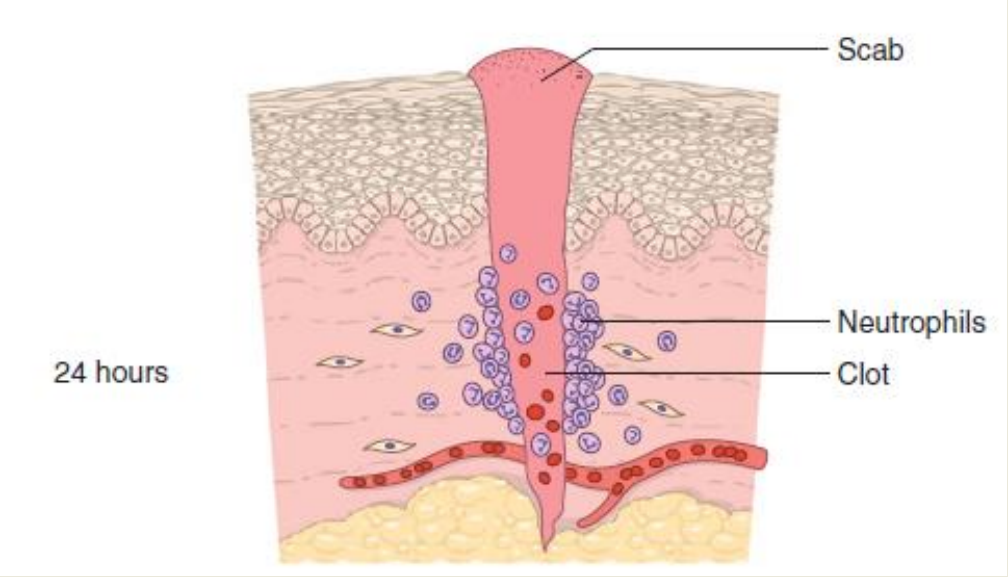



Healing incision (early weeks)
Epithelial proliferation and repair (pinkish-red scar)
Maturing fibrous granulation tissue
Phase of granulation tissue maturation and wound contraction (about 3–6 weeks)
Epithelial proliferation across granulation tissue surface before gradually shedding scab
Fibrous granulation tissue beginning to contract, pulling wound edges closer together
Hyperemia
Linear fibrous scar (6–12 months)
White scar
Healed wound
Pale depressed scar with surrounding puckering caused by wound contraction
Epidermis thin
Dermal fibrous scar, devoid of skin appendages

Healing by First Intention

- Possible when tissue elements in close proximity (eg surgical wound).
- A primary union where regeneration predominates over fibrosis

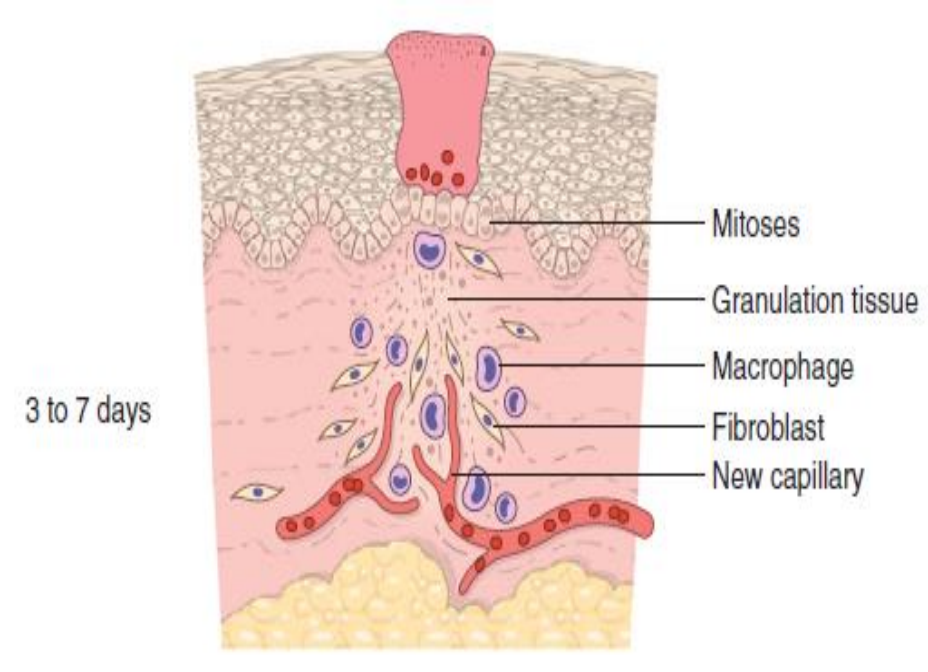
24 hours
- Neutrophils migrate into fibrin clot
- Basal epidermal cells at edges increase mitotic activity



Healing by First Intention

24-48 hours

- Epithelial cells migrate and proliferate
- Deposition of basement membrane



Day 3

- Macrophages replace neutrophils
- Fibroblasts & collagen fibers at margins (vertical)
- Epithelial cells continue to proliferate

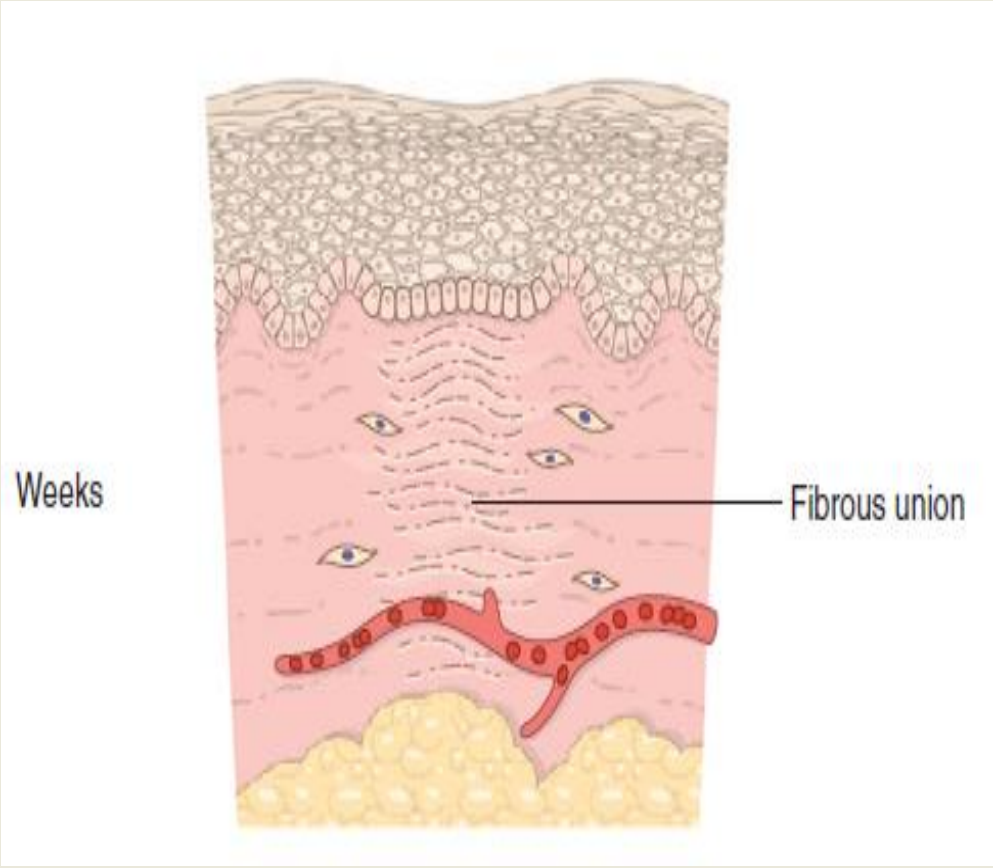
Day 5

- Neovascularization peaks
- Collagen fibers bridge wound (horizontal)

Healing by First Intention

Day 14

- Fibroblasts and collagen accumulation continue
- Decreased leukocytes & edema
- Vascular channels regress



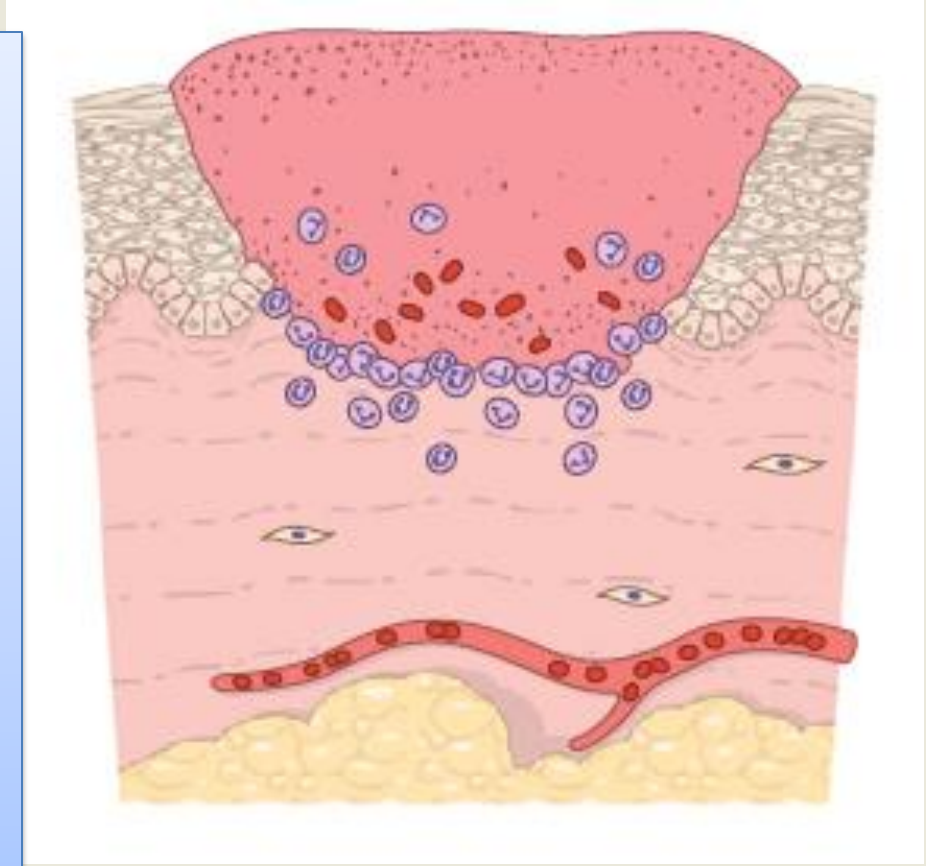
4 weeks

- scar (fibroblasts and collagen)
- few inflammatory cells
- tensile strength increases with time

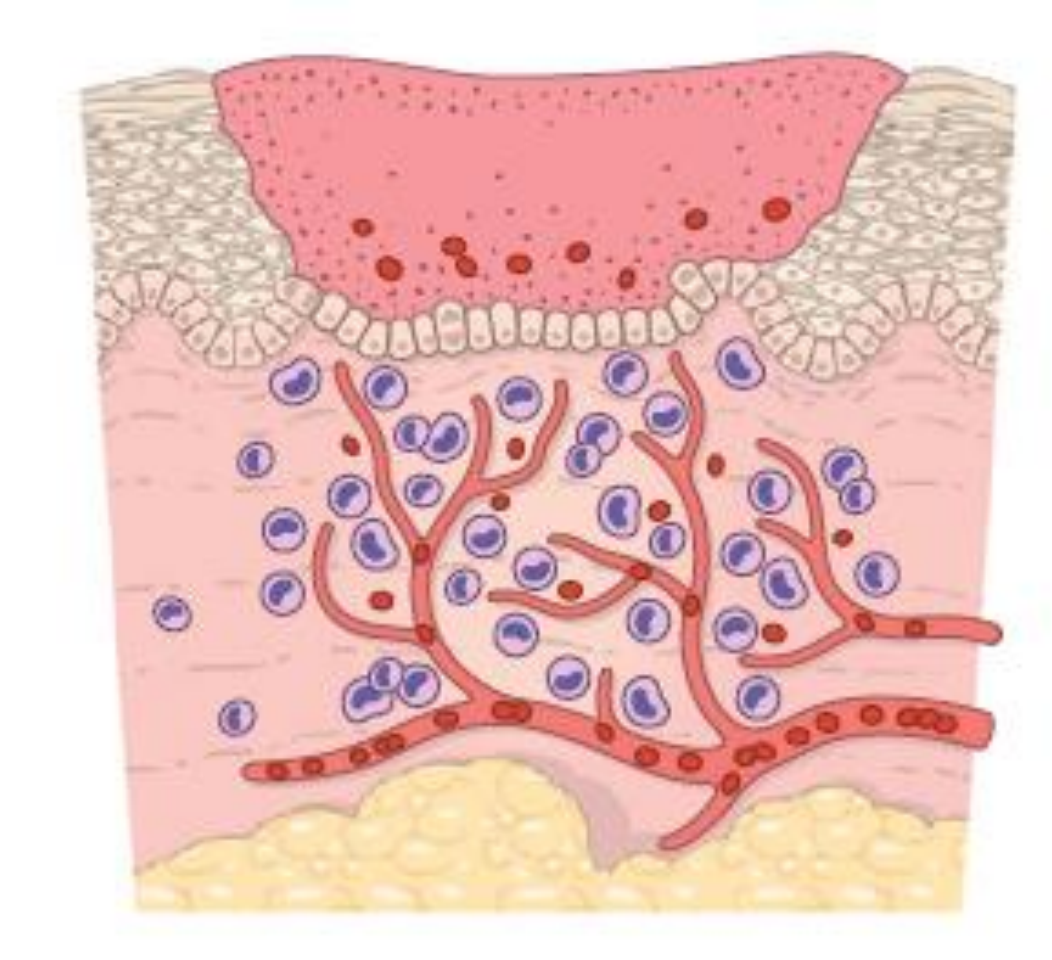
Healing by Second Intention

where there is poor apposition (eg ragged cuts)

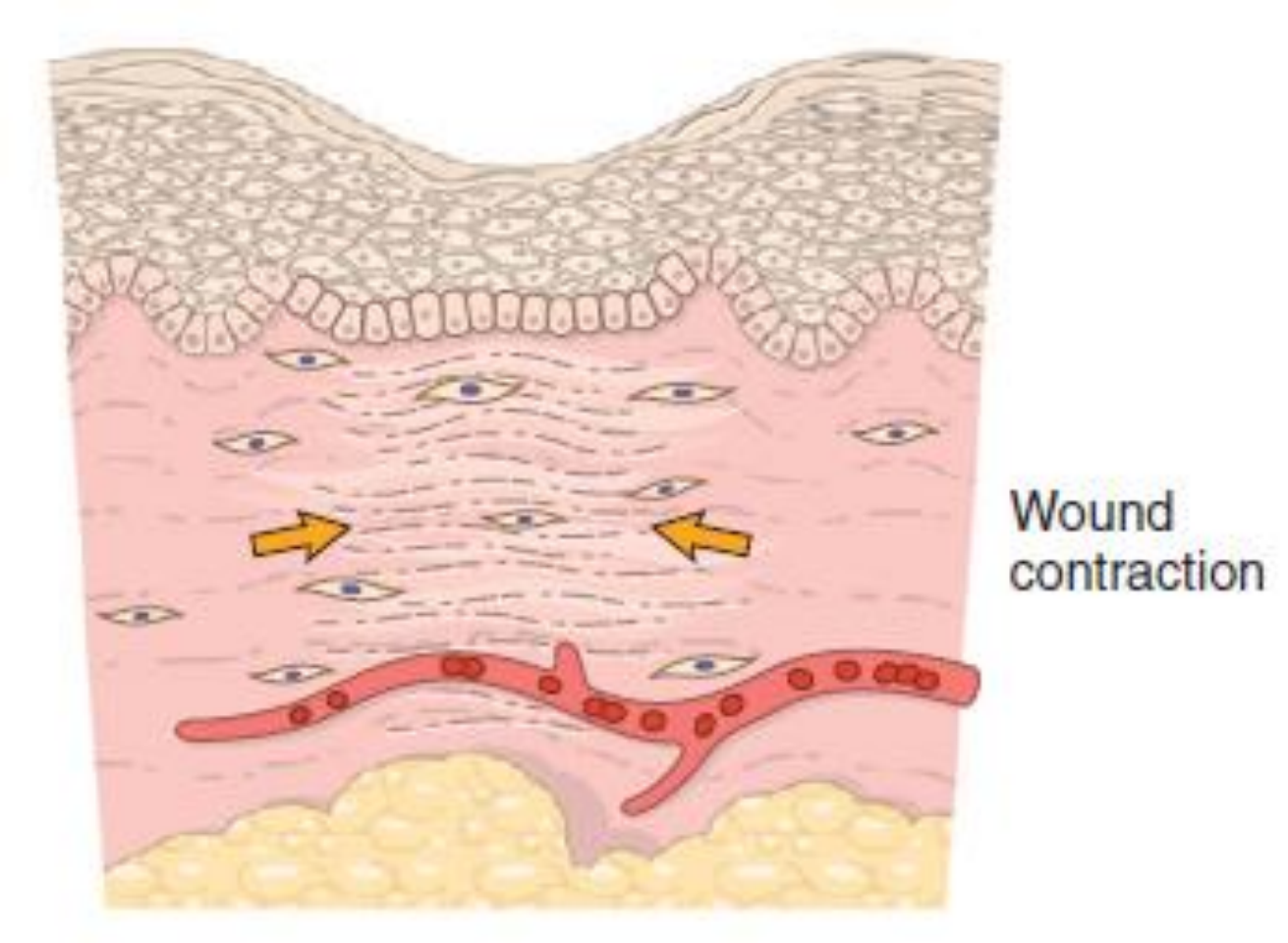
- More complex repair process
- More inflammation (fibrin & leukocytes)
- More granulation tissue
- Contraction due to myofibroblasts
- Often an irregular scar

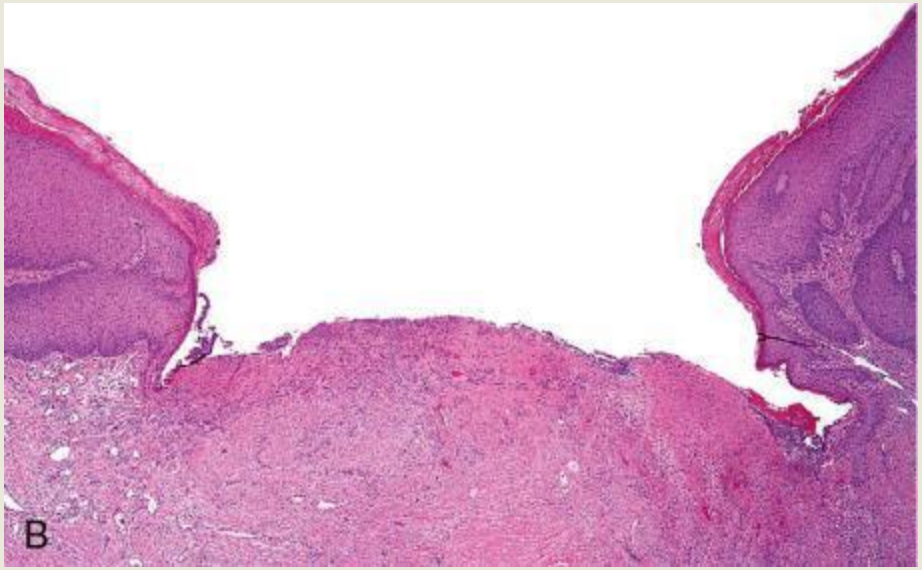


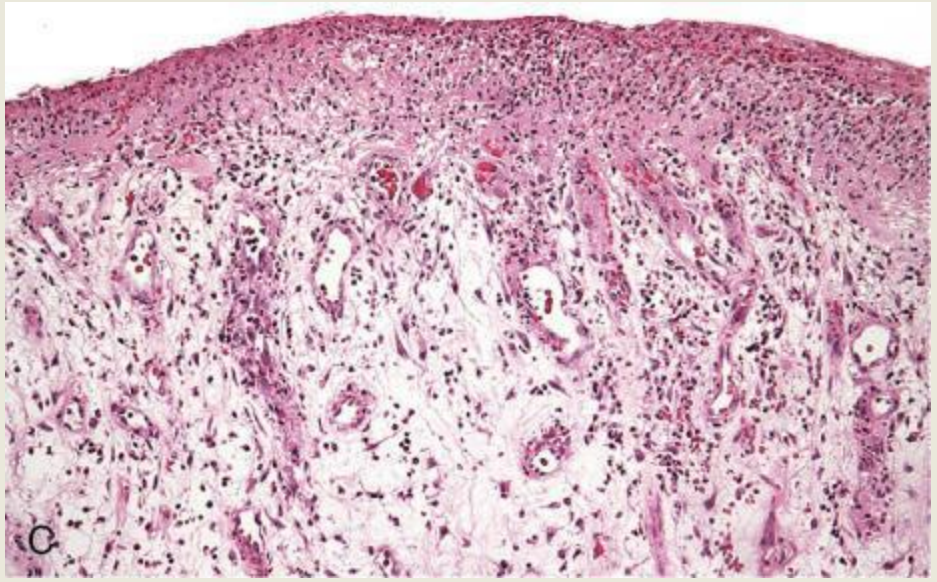
Healing by Second Intention



Healing by Second Intention







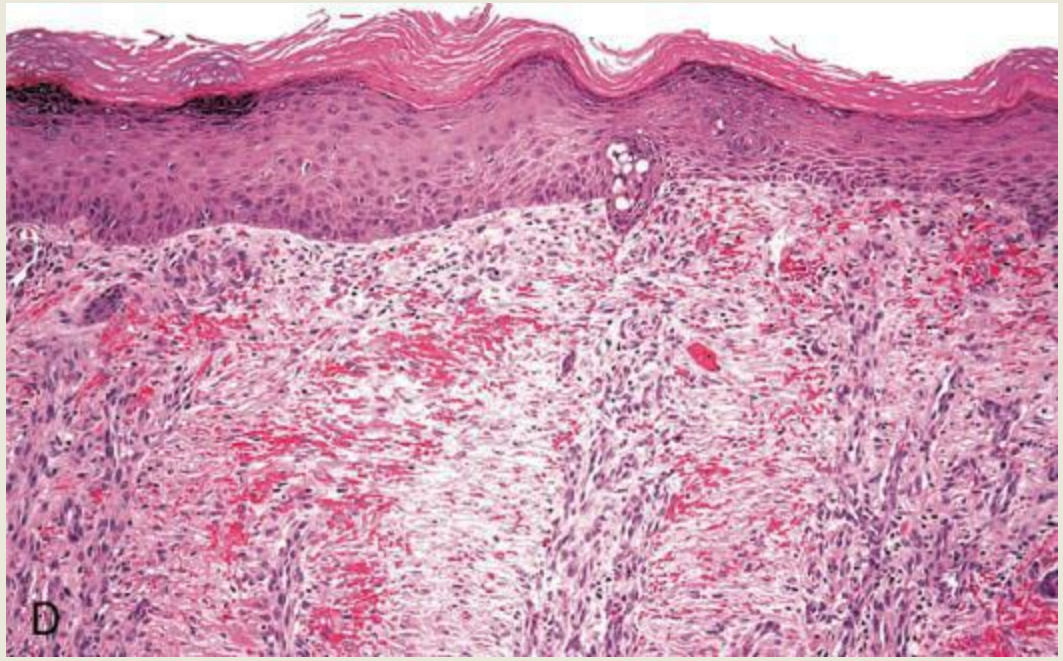


Figure 3-20 (Robbins) Healing of skin ulcers. The histologic slides show: B, a skin ulcer with a large gap between the edges of the lesion; C, a thin layer of epidermal re-epithelialization and extensive granulation tissue formation in the dermis; and D, continuing re-epithelialization of the epidermis and wound contraction

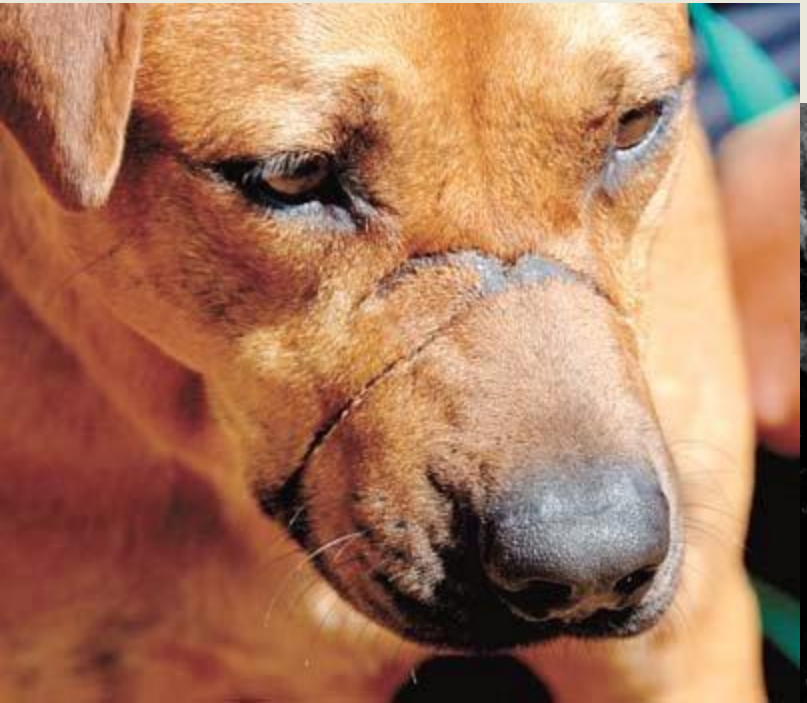
Wound Strength

immediate (if sutured): ~70%

- if not sutured:

1 week : ~10%

3 months : ~ 70-80%



Wound Healing

1. Injury → inflammation (necrosis)

2. Parenchymal cells regenerate (if possible)

3. Migration/proliferation
   - fibroblasts & endothelial cells
   - parenchymal cells

4. Synthesis of ECM (collagen / proteoglycans)

5. Remodeling of parenchymal cells (restore function)

6. Remodeling of connective tissue (wound strength)