HEALING AND REPAIR

9.1 OVERVIEW

The body attempts to heal damage induced by local injury very early in the process of inflammation. However, for convenience only, repair is discussed as a separate entity. The student should be reminded that inflammation and repair are closely intertwined.

9.2 TERMINAL OBJECTIVES

Once this section is completed, each student should be able to perform the ***following tasks?***

- **1. Discuss the differences and similarities between "healing by regeneration" and "healing by connective tissue replacement."**
- **2. Outline the sequence of events in the healing of labile cells following a mild skin injury.**
- **3. Discuss the regenerative and/or reparative capabilities of the following tissue components:**
- **-- Brain -- Bone**
- **-- Kidneys -- Surface epithelium**
- **-- Nerves -- Fibrous connective tissue**
- **-- Skeletal muscle -- Pancreas**
- **-- Cardiac muscle -- Endothelium**
- **4. Predict the type of healing that will occur in labile, stable and permanent tissues under the following circumstances:**
    - **-- Destruction of the parenchyma and stroma by the injurious agent.**
    - **-- Destruction of the parenchyma only by the injurious agent (stroma still intact).**
- **5. Outline the sequence of events that occur in healing by "first intention."**
- **6. Differentiate healing by "first intention" from healing by "secondary intention" on the basis of:**
    - **(1) tissue destruction,**
    - **(2) inflammatory exudate,**
    - **(3) granulation tissue produced,**
    - **(4) wound contraction,**
    - **(5) scar formation and**
    - **(6) time required for completion of the reparative process.**
- **7. Discuss granulation tissue, "proud flesh," and keloid formation as they relate to healing by connective tissue replacement.**

- **8. Describe the formation of granulation tissue and explain the mechanisms involved.**
- **9. Discuss the possible role of chalones in wound healing.**
- **10. Outline the sequence of events identified in the healing of bones.**
- **11. Provide appropriate answers for the post-instructional self-examination questions outlined at the end of this section.**

9.3 KEY WORDS

9.4 GENERAL CONSIDERATIONS

Repair of injuries, though discussed separately, is intimately associated with the inflammatory response. The healing process begins early in the inflammatory process and results in repair of the injury by replacement of dead or damaged cells with healthy cells. The body uses two distinct processes to effect repairs:

- (1) regeneration, which is the replacement of injured tissue with cells of the same type; and
- (2) replacement by connective tissue.

Most injuries are repaired by a combination of these processes. Obviously, it is most advantageous for repairs to occur by regeneration because this will restore the organ to normal functioning capabilities. The extent to which a repair occurs by regeneration is governed largely by several factors including the regenerative capacity of the cells involved and the severity of the injury.

9.5 REGENERATION

Based on their regenerative capabilities, the cells of the body are divided into three groups: labile cells, stable cells, and permanent cells. Labile cells proliferate normally throughout life replacing cells that are continually being destroyed. In this group are such cells as surface epithelia and the hematopoietic cells of the spleen, lymphoid organs and bone marrow. Stable cells replicate at very low levels under normal circumstances, but can be stimulated to divide rapidly in response to various stimuli. Cells in this group include the parenchymal cells of most glandular organs, vascular endothelial cells, and mesenchymal cells such as fibroblasts, smooth muscle cells, osteoblasts, and chondroblasts. Permanent cells are cells that either do not replicate in postnatal life or do so to an insignificant extent. This group includes nerve cells, skeletal muscle cells, and cardiac muscle cells.

Injuries in organs or tissues composed largely of permanent cells will undoubtedly be repaired by connective tissue replacement. Injuries in organs composed largely of labile or stable cells are repaired either by regeneration or by a combination of regeneration and connective tissue replacement. The extent of the injury is a major factor in determining which of these occurs. Because the scaffolding provided by stroma and basement membranes is so critical, if the injury is such that these structures are preserved, it is more likely that injury by regeneration will occur. However, if these structures are also damaged, then repair by connective tissue replacement becomes more likely.

9.6 REPAIR BY CONNECTIVE TISSUE REPLACEMENT

This type of repair predominates when injuries occur in tissues formed largely of permanent cells or when the injury results in extensive damage to stromal framework and supporting connective tissues. In these situations, the injured tissue is replaced by fibroblastic cells, usually in the form of granulation tissue, which eventually results in the formation of a scar.

9.7 GRANULATION TISSUE

Early in the inflammatory process, fibroblasts and vascular endothelial cells start to proliferate. Sometimes this begins as early as 24 hours after injury. By three to five days, a specialized type of tissue appears that is known as **granulation tissue**. This specialized tissue is composed largely of these proliferating fibroblasts and newly formed blood vessels.

The newly formed blood vessels form by budding of pre-existing blood vessels. The process resulting in the development of these newly formed blood vessels is called **angiogenesis** or **neovascularization**. This process is not only important in healing but is also involved in the progressive growth of parenchymatous tumors. It occurs in four basic steps:

- (1) enzymatic degradation of the basement membrane of the parent vessel,
- (2) migration of endothelial cells toward the angiogenic stimulus,
- (3) proliferation of endothelial cells,
- (4) maturation of endothelial cells and organization into capillary tubes.

These newly formed vessels have leaky inter-endothelial junctions, thus granulation tissue tends to be edematous.

The proliferating fibroblasts in granulation tissue have increased amounts of rough endoplasmic reticulum and tend to appear as plump cells in histologic sections. These granulation tissue fibroblasts also develop similarities with smooth muscle cells: i.e. indented nuclei, prominent bundles of cytoplasmic fibrils, and increased amounts of contractile proteins, and thus they are referred to as **myofibroblasts**.

Granulation tissue will generally have considerable numbers of macrophages. Initially, their main purpose is to eliminate injuring agents that survive the neutrophilic attack. Even after the injuring agent has been destroyed, macrophages remove extracellular debris and ultimately they participate in "blanching" of the wound, a process by which the excess granulation tissue is removed. In addition, granulation tissue may have varying numbers of neutrophils, lymphocytes, and eosinophils.

9.8 FIRST INTENTION HEALING (Primary Union)

This type of healing occurs when there is essentially no contamination of the wound and the edges of the wound are approximated thus closing the wound. The best

example of this situation is the surgical incision where contamination of the wound is minimized and the wound is closed by suturing.

Once the wound is sutured, the incision space fills with blood, which contains fibrin and blood cells and which subsequently clots. The surface of this clot becomes dehydrated and forms a scab. Within 24 hours, neutrophils appear at the edges of the incision and the epithelium at the edges of the incision begins to proliferate. It migrates under the scab and forms a thin continuous epithelial layer. By 72 hours, macrophages are usually the most numerous inflammatory cells and granulation tissue starts to develop. Collagen fiber are present but do not bridge the incision site. The epithelial cells continue to proliferate under the scab and the epidermal covering over the incision becomes thicker. By day 5, the incision space is filled with granulation tissue and collagen fibers begin to bridge the incision. The epidermis returns to its normal thickness and keratinized architecture.

During the second week, there is continued accumulation of collagen fibers and proliferation of fibroblasts. Inflammatory cells and edema disappear and the process of blanching begins. Blanching refers to the process whereby collagen fibers accumulate and excessive vascular channels regress causing the area to become lighter in color.

By the end of one month, there is a connective tissue scar that is devoid of inflammatory cells and is covered by an intact epidermis. The damaged adnexal structures are permanently lost and the tensile strength is still well below its maximum.

9.9 SECOND INTENTION HEALING (Secondary Union)

This type of healing occurs when injuries result in more extensive loss of tissues such as with infarction, inflammatory ulceration, and large surface wounds. In these situations, due to the large tissue defect, repair by regeneration is minimal and the defect is filled by granulation tissue.

Second intention healing differs from first intention healing in several ways. First of all, the greater injury invokes a more intense inflammatory response. Secondly, much more granulation tissue is formed. And thirdly, wounds that are repaired by second intention healing undergo a phenomenon known as "wound contraction" whereby specialized granulation tissue fibroblasts called myofibroblasts contract and dramatically reduce the size of the wound.

9.10 COMMON ABERRATIONS OF THE HEALING PROCESS

Exuberant Granulation

Exuberant granulation is characterized by excessive formation of granulation tissue such that a mass of granulation tissue protrudes from the wound and prevents re-epithelialization. Such excesses are commonly referred to as **"proud flesh".** This is a commonly encountered problem in the management of wounds in horses.

Keloid Formation

Keloid formation also refers to an aberration of wound healing resulting in the formation of large bulging scars but it differs from granulation tissue in that it is caused by excessive collagenization of the wound and not excessive formation of granulation tissue. This phenomenon is a common problem in people of darker complexions but it has not been reported to any significance in animals.

9.11 MECHANISMS INVOLVED IN REPAIR

There are a multitude of complex processes involved in repairs that must be orchestrated. The mechanisms regulating these events are becoming better understood and the more important features involved in this control include:

- (1) the role of growth factors,
- (2) cell to cell and cell to matrix interactions,
- (3) extracellular matrix synthesis and collagenization.

9.11.1 Growth Factors

Current theories contend that cell proliferation is controlled by a delicate counterbalance between growth stimulators and growth inhibitors. Numerous growth factors have been reported, including nutrients, but we will focus on several polypeptides that are normally found in the serum or are produced by cells locally. Some of these growth factors stimulate DNA synthesis directly in competent cells and are referred to as progression factors, while others merely make cells competent to be stimulated for DNA synthesis and are referred to as competence factors.

9.11.1.1 Epidermal growth factor

(EGF) is a polypeptide that was originally purified from submaxillary glands of mice. It was also isolated from human urine where it was referred to as **"urogastrone".** It is mitogenic for a variety of epithelial cells and fibroblasts ***in vitro*. EGF** is a progression factor meaning it stimulates DNA synthesis directly in competent cells.

9.11.1.2 Platelet-derived Growth Factor

(PDGF) is primarily found in the alpha granules of platelets from which it is released subsequent to platelet activation. It is also produced by activated macrophages, endothelium, smooth muscle cells, and a variety of tumor cells. **PDGF** causes proliferation and migration of fibroblasts and smooth muscle cells and it has many other pro-inflammatory properties. It is a competence factor, therefore a progression factor is necessary to stimulate mitogenesis.

9.11.1.3 Fibroblast Growth Factor(s)

(FGFs) are a family of polypeptide growth factors that have numerous activities including stimulation of fibroblast proliferation and angiogenesis.

Remember,

Angiogenesis (neovascularization) involves enzymatic degradation of the basement membrane of the parent vessel, migration of endothelial cells toward the angiogenic stimulus, proliferation of endothelial cells, maturation of endothelial cells and organization into capillary tubes.

9.11.1.4 Transforming Growth Factor Alpha and Transforming Growth Factor Beta

Were originally extracted from sarcoma virus-transformed cells and were thought to be involved in neoplastic transformation. Transforming growth factor alpha is similar to EGF structurally. It binds to EGF receptors and produces similar biologic effects as EGF. Transforming growth factor beta is produced by different cell types including platelets, endothelium, T cells, and macrophages. It inhibits growth in most cell types; however, it stimulates fibroblast chemotaxis and the production of collagen and fibronectin by cells. It also inhibits collagen degradation and it deactivates macrophages.

9.11.1.5 Interleukin-1 (IL-1) and Tumor Necrosis Factor

(TNF) stimulate fibroblastic proliferation and the synthesis of collagen and collagenase. They are believed to play a role in fibroplasia and remodeling of inflammatory connective tissue. In addition**, TNF** has been shown to have angiogenic properties in vivo.

It is important to note that these growth factors are not only mitogenic but also affect contractility, cell locomotion, and differentiation. Also, macrophages, because they tend to exist in high numbers in inflammatory sites and because they are capable of producing so many of the aforementioned growth factors, play a central role in these processes.

9.11.2 Growth Inhibitors

A number of growth inhibitors are known to be produced in inflammation. Transforming growth factor beta has already been described and others include alpha interferon, prostaglandin E2, and heparin.

9.11.3 Cell to Cell and Cell to Matrix Interactions

Normal cells in tissue cultures tend to proliferate until a confluent monolayer is formed at which point proliferation ceases. This density-dependent regulation is controlled by either

- (1) limitation of necessary materials in the environment,
- (2) alterations in the number of receptor sites for growth factors,
- (3) accumulation of growth inhibitors.

This same phenomenon occurs in vivo and is at least partly responsible for the regulation of cell proliferation in healing. It has been shown that transforming growth factor beta is responsible for limiting proliferation of hepatocytes following partial hepatectomy.

The nature of the matrix appears to influence cell proliferation and differentiation. Such factors include:

- 1) the type of collagen,
- (2) the presence of fibronectin or laminin, and
- (3) the nature of the proteoglycans.

Endothelial cells grown in culture and exposed to growth factors, proliferate faster when grown on type I collagen or laminin than when grown on type IV collagen. On the other hand, when grown on type IV collagen, they tend to form tube-like structures. Fibronectin of fibronectin fragments promote migration of fibroblasts and endothelial cells into an area of injury. This cell to cell interaction seems to be mediated through cell surface receptors which interact with the cytoskeleton to signal locomotion or differentiation. This group of receptors includes **integrins** which are primarily adhesion receptors such as fibronectin receptors, platelet glycoprotein receptors, and leukocyte adhesion molecules.

9.11.4 COLLAGENIZATION OF A WOUND

Collagen ultimately provides the tensile strength of healing wounds. It is produced by the proliferating fibroblasts that are a part of the healing process. The fundamental unit of collagen is the collagen molecule which is called tropocollagen. Based on biochemical composition of the molecules, 11 types of collagen are recognized; however, types I, II, and III are the interstitial or fibrillar collagens.

9.11.4.1 Common Defects Involving Collagen

A critical modification of collagen occurs in the rough endoplasmic reticulum and involves the hydroxylation of proline at the alpha position. This modification is dependent on ascorbic acid **(vitamin C**) and is critical because it is necessary to hold the collagen molecules in the RER. A dietary deficiency of ascorbic acid leads to inadequate collagen production and causes a disease known as scurvy.

There are inherited diseases characterized by defective collagen production as well. One group of such diseases is referred to as Ehlers-Danlos Syndrome **(EDS)**. Based on the underlying biochemical defect, at least 20 variants of EDS are recognized and most are characterized by hyperextensible skin and hypermobile joints. The mode of inheritance varies as well with autosomal

recessive, autosomal dominant, and sex linked patterns all having been reported.

Another inherited disease involves oxidation of lysine of collagen molecules, a critical modification that occurs extracellularly. In humans, this disease is referred to as Marfan's Syndrome. It results in the development of cross linkages between alpha chains which is responsible for most of the structural stability of collagen. Marfan's Syndrome is characterized by malformations in the skeleton, skin, and blood vessels.

9.11.4.2 Wound Strength

Immediately following surgery, properly sutured wounds have approximately 70 of the strength of unwounded skin. After removal of the sutures, usually at 1 week following the surgery, the wound has approximately **10%** of its original strength. Wound strength plateaus at approximately **70 - 80%** of the pre-incision tensile strength in 3 or 4 months and increases minimally from that point on.

9.12 FACTORS THAT MIGHT MODIFY THE REPAIR RESPONSE

Many factors, including nutrition and the presence of other disease states, tend to influence the repair response. Prolonged protein starvation tends to retard the development of tensile strength in healing wounds and conversely, a high protein diet accelerates the rate of tensile strength gain. In addition, a dietary deficiency of ascorbic acid will also reduce collagenization of a healing and result in retardation of the acquisition of wound strength.

Any phenomenon that prolongs infections in a healing wound will result in a decreased rate of wound healing. This includes defects in either numbers or function of neutrophils or macrophages. It also includes defects resulting in increased bleeding because certain blood elements often serve as nutrients for bacteria. Other influences include the presence of systemic diseases such as diabetes, corticosteroid therapy, adequacy of blood supply, the presence of foreign bodies, and the nature of the tissue involved.

9.13 HEALING OF BONE

Bone fractures are categorized as being either **pathological** if they occur in bones with a preexisting abnormality or **traumatic**, if they occur in bones that are otherwise healthy. They are also categorized based on the extent and direction of the fracture. Greenstick fractures are characterized by an incomplete fracture of a bone resulting in a fracture of one side of that cortical bone but not the other. Fractures characterized by splintering of the bone or breakage into multiple pieces are referred to as comminuted fractures. When a fractured end of a bone penetrates the skin it is called a compound fracture. A simple fracture is one in which the bone is fractured into two large pieces and overlying tissues remain intact. Greenstick and simple fractures heal with the best results. Devitalized bone fragments in comminuted fractures cause problems in healing and infection is often a hindrance to healing in compound fractures.

Once a fracture occurs, the fractured ends of the bones must be realigned as closely as possible to their original locations. This realignment is referred to a **reducing** the fracture. Initially, there is hemorrhage into the fracture site which clots and forms a loose fibrin mesh. This clot, referred to as a hematoma, extends for a short distance around the fractured ends of the bones and seals the fracture site. Within 24 - 48 hours fibroblasts proliferate and newly formed capillaries penetrate the hematoma. The hematoma becomes organized and once fibroblastic cells predominate, it becomes known as a soft tissue callus. After several days, newly formed cartilage and bone matrix appear in the soft tissue callus and once these tissues reach significant proportions, the callus becomes a provisional callus or procallus. The provisional callus forms a rather effective temporary splint and it gains strength as more and more bone spicules arise. After 4 or 5 weeks, bone will predominate in the callus and it becomes known as an osseous callus. The osseous callus will eventually be remodeled such that the bone will return to nearly its original contour.

9.13.1 Complications of Bone Healing

Malalignment of a fracture often results in deformity of the bone once healing is complete. Devitalized bone fragments in comminuted fractures often delay bone healing. Inadequate fixation, with fractured ends not being immobilized, often results in nonunion healing or the formation of a fibrous connective tissue callus. The latter situation produces a permanently flexible union between the fractured ends referred to as a false joint **(pseudoarthrosis**). Infection tends to be the most serious complication of bone healing.

9.15 POST-INSTRUCTIONAL SELF-EXAMINATION

Questions:

After completing this section, each student should be in a position to provide appropriate answers for the following questions.

- **1. What is the interrelationship between healing and the inflammatory process?**
- **2. What two processes are involved in the healing or repair of injured tissue.**
- **3. Under what circumstances would you expect perfect regeneration of injured tissue to occur?**
- **4. How would you distinguish healing by regeneration from repair by connective tissue replacement?**
- **5. What cells in the brain and kidneys have little or no capacity to regenerate?**
- **6. What are labile, stable and permanent cells?**
- **7. Under what circumstances would you expect labile and stable cells to restore normal structure following injury?**
- **8. What is the importance of supporting stroma in the repair process?**
- **9. Under what circumstances would you expect labile and stable cells to proliferate in a haphazard manner following injury?**
- **10. At approximately what point in time following injury would you expect**

fibroblast and vascular endothelial cells to start proliferating in an injured area?

- **11. What is considered to be the "hallmark" of the healing process?**
- **12. What are the identifying features of granulation tissue?**
- **13. Explain the manner in which new blood vessels grow into a healing wound.**
- **14. How would you explain the presence of edema in a healing wound?**
- **15. What are "myofibroblasts?"**
- **16. Discuss the differences and similarities between normal fibroblasts and so-called modified granulation tissue fibroblasts?**
- **17. Under what circumstances would you expect wounds to contract during the healing process?**
- **18. What factors influence the quality and adequacy of the repair process?**
- **19. What is so-called "healing by first intention?"**
- **20. Outline the sequence of events that occur during "healing by first intention."**
- **21. What is responsible for "scab" formation over wounds? What is the importance of scab formation?**
- **22. At approximately what point in time would you expect epidermal continuity to be established in primary union?**
- **23. At what point in time would you expect normal tensile strength to be restored in primary union?**
- **24. What events would you expect to occur in secondary union?**
- **25. Distinguish secondary union from healing from first intention.**
- **26. What are keloids? How would you distinguish a keloid from exuberant granulation tissue?**
- **27. Discuss the role of chalones in inflammation and healing.**
- **28. Describe the manner in which a simple fracture heals. What is a simple fracture, a compound fracture and a "greenstick" fracture?**
- **29. What is osteoid? What is a callus?**
- **30. Briefly describe the roles of the following cell types in healing:**
  - **-- Fibroblasts**
  - **-- Osteoblasts**
  - **-- Osteocytes**

- **31. Why is inflammation and repair important for the health and well-being of an animal?**