

Effect of Soft-Tissue Trauma on the Early Periosteal Response of Bone to Injury

Patricia S. Landry, PhD, Andrew A. Marino, PhD, Kalia K. Sadasivan, MD, and James A. Albright, MD

Objective: To determine whether the periosteal response to skeletal trauma is impaired when muscle is also injured, thereby providing a possible explanation for why fractures with extensive soft-tissue damage may take longer to heal.

Methods: A bone defect was made in the tibia of male Fisher rats, and the proliferative response, osteoblast concentration, and callus formation that occurred within 7 days were measured in the presence and absence of simultaneously administered model soft-tissue injury (removal of 10% of the anterior tibialis muscle from a region within 2 to 3 mm of the bone defect). Measurements were made by using autoradiography, quantitative histology, and morphometry.

Results: Addition of the muscle injury increased proliferation in the cambium and in the fibrous periosteum on day 1, but had no effect thereafter; proliferation of fibroblasts in the loose

connective tissue above the periosteum was not affected. Addition of the muscle injury resulted in increased osteoblast levels 2 to 5 days after injury but had no effect on the amount of callus produced.

Conclusion: The inflammatory milieu created by the muscle injury unexpectedly resulted in an increased periosteal response to skeletal trauma, suggesting that inflammatory mediators generated in response to wounding of soft tissues are unlikely to account for delayed fracture healing. These findings may indicate that surgical trauma associated with internal fixation by using plates and screws may not be as deleterious to the fracture-healing response as previously thought.

Key Words: Wound healing, Fracture repair, Delayed healing, Inflammatory response.

Musculoskeletal trauma typically results in injury to both bone and soft tissue, thereby triggering the processes of wound healing and fracture repair. Clinical and experimental evidence suggests that extensive soft-tissue injuries may delay or even prevent fracture healing,¹⁻⁴ but the basis of the link between the two healing processes is not known. Hormonal levels, changes in blood supply, and mechanical forces are important factors in bone healing that may be affected by soft-tissue injury.⁵⁻⁸ Another possibility is that the soluble agents that mediate wound healing could directly impact on fracture repair. Such an interaction could explain why fractures with extensive soft-tissue damage, caused by the injury itself or by subsequent surgical interventions, may take longer to heal.

The inflammatory phase of wound healing is characterized by movement of cells from nearby tissue and from the circulation into a fibrin network at the injury site and by the synthesis of cytokines that activate cells and induce expression of regulatory molecules and extracellular matrix.⁹⁻¹¹ Similar complex coordinated events also occur in fracture repair, resulting in formation of osteoblasts from cells in the periosteum and in the marrow and the expression of bone matrix proteins.^{8,12,13} It is possible that one or more of the agents that mediate wound healing could interact directly

with the regulatory system governing osteogenesis, thereby antagonizing fracture repair.

We previously developed an animal model for studying periosteally mediated bone healing. The purpose of this study was to determine whether the normal bone-healing response manifested in the model was impaired when soft tissue was also injured. This determination was accomplished by comparing the extent of bone healing in the presence and absence of a model soft-tissue injury.

MATERIALS AND METHODS

Animals

Male Fischer rats (Harlan, Indianapolis, Ind) were used. They were caged individually with a light/dark cycle of 12:12 and fed and watered ad libitum. A minimum of 1 week was allowed for the animals to adjust to their housing conditions.

To restrict the focus of the study to possible impacts on the contribution of periosteal cells to bone repair, a bone defect was created that did not communicate with the medullary canal. The defect was 1.1 mm in diameter and 0.5 mm deep, and was made unilaterally in the anteromedial tibia inferior to the saphenous artery bifurcation.¹⁴ In some animals, a model soft-tissue injury was created during the same operation by removing a portion of the anterior tibialis muscle located within 2 to 3 mm of the bone defect. Preliminary studies showed that removal of approximately 10% of the muscle (which was approximately 600 mg) resulted in significant swelling, bleeding, and clot formation, compared with the effect caused by the bone injury alone. As an additional operative control, a third group of rats received only the muscle injury. The average amount of muscle recovered from all rats that underwent the procedure was 67 ± 3 mg.

Submitted for publication January 1, 1999.

Accepted for publication December 8, 1999.

Department of Orthopaedic Surgery, LSU Medical Center in Shreveport, Shreveport, Louisiana.

This research was supported, in part, by grants from the Hamilton Foundation and the Orthopaedic Trauma Association.

Address for reprints: Andrew A. Marino, PhD, Department of Orthopaedic Surgery, LSU Medical Center in Shreveport, P.O. Box 33932, Shreveport, LA 71130-3932; email: amarino@lsuic.edu.

The rats were killed 1, 2, 3, 5, and 7 days after operation (five rats in each group at each time interval). Additionally, three rats that received an incision of the skin only, with wound closure, and 10 noninjured animals (20 tibias) that served as nonoperative controls were killed. All rats were given tritiated thymidine 1 hour before killing (intraperitoneal, 1 μ Ci/g of body weight, diluted with sterile water to a final volume of 0.5 mL, specific activity 2 Ci/mmol, ICN Biomedicals, Irvine, Calif).

All operations, radioisotope injections, and killings (carbon-dioxide suffocation) were carried out between 10:00 AM and 2:00 PM to minimize potential effects caused by circadian rhythms. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Tissue Preparation

The region of the tibia that contained the defect was recovered, processed, and sectioned completely at 4 μ m in the longitudinal plane. A trio of sections was selected from the middle of the defect, from halfway between the middle of the defect and its medial edge, and from halfway between the middle and lateral edge. In the rats that did not receive the bone defect, sections were selected and analyzed as if the defect were present.

One section in each trio was processed for autoradiography and counterstained with van Gieson's stain, the second was stained with methyl-green and thionin, and the third with toluidine blue and basic fuchsin.^{15,16} The criteria for identification of pertinent cell types based on morphologic and histochemical properties were described previously.¹⁴

Measurements

Quantitative determinations of proliferation, osteoblast concentration, and callus formation were made within ± 3 mm from the center of the defect. Proliferation was assessed by counting the number of labeled cells (≥ 5 grains/nucleus), and osteoblasts were counted in the methyl-green/thionin sections. The osteoblast was identified as a cambial cell (previously shown to be alkaline-phosphatase positive¹⁴) that contained a nucleus with nucleoli, extensive basophilic cytoplasm, and a prominent Golgi apparatus. Cell concentrations were determined separately in the cambium, fibrous periosteum, loose connective tissue, and within the defect by dividing each count by the length of the cortical bone surface along which the cells were located. The mean of the three representative sections (expressed as cell-count per millimeter of bone) was used in all subsequent calculations. Each reported mean is the grand mean (\pm SE) of all the rats that received a particular treatment.

Periosteal callus thickness was measured (toluidine-blue sections) from the original bone surface to the superficial edge of the callus; the measurements were made at 200- μ m intervals along the cortical surface by using a computer-based system (Bioquant System IV, R&M Biometrics, Nashville, Tenn). Callus present on day 7 was estimated by using DeLesse's principle.¹⁷



FIG 1. Callus formation in the cambium after bone injury at 3 (A), 5 (B), 7 (C) days after operation. The bone defect is on the left (arrow). Toluidine blue/basic fuchsin stain. Bars are 100 μ m.

Statistical Analysis

The data were analyzed by using analysis of variance and the *t* test at $p < 0.05$. Because we hypothesized that the muscle injury would retard fracture repair, the *t* tests were one-sided.

RESULTS

Cortical Surface

After injury to bone, callus first formed on the cortical surface adjacent to the defect, and then progressed into the defect beginning around day 5 (Fig. 1). The histologic changes were described previously.¹⁴

The bone injury produced a large sustained increase in proliferation of cells in the cambial layer of the periosteum (Fig. 2). The cells were previously identified as osteoprogeni-

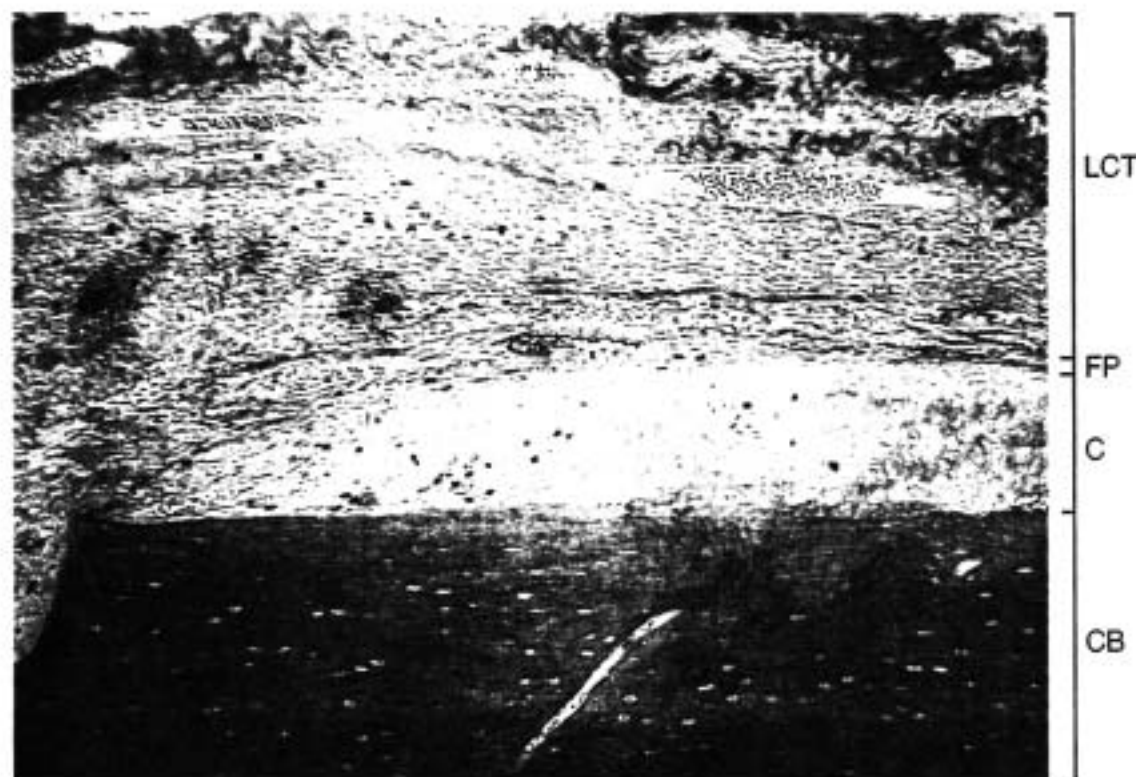


FIG 2. Autoradiograph showing proliferative response 3 days after bone injury. Elevated proliferation occurred in all tissue compartments (seen as black dots at 10 \times). Autoradiograph stained with van Gieson's. Cortical bone (CB), cambium (C), fibrous periosteum (FP), loose connective tissue (LCT), defect on left.

tors and osteoblasts on the basis of morphologic and histochemical evidence.¹⁴ Addition of the soft-tissue injury increased cambial proliferation on day 1 but not thereafter (Fig. 3). Proliferation of fibroblasts in the fibrous periosteum also occurred in response to the bone injury (Fig. 2); its time dependency and the effect of the soft-tissue injury were similar to the corresponding results in the cambial periosteum (Fig. 4). Proliferation of fibroblasts in the loose connective tissue above the periosteum was not affected by the injury to muscle (Fig. 5).

Cells in the expanded osteoprogenitor population differentiated into osteoblasts, leading to a sustained increase in

osteoblast concentration. Addition of the muscle injury resulted in increased osteoblast levels during days 2 to 5 ($p < 0.05$, analysis of variance); the comparison on day 3 was statistically significant (Fig. 6). However, the muscle injury had no apparent effect on the amount of callus produced ($p = 0.12$) (Fig. 7).

Defect

Healing occurred by growth of callus¹⁴ from the defect periphery (Fig. 1). Too little ingrowth occurred by day 7 to adequately permit assessment of the impact of the muscle injury.

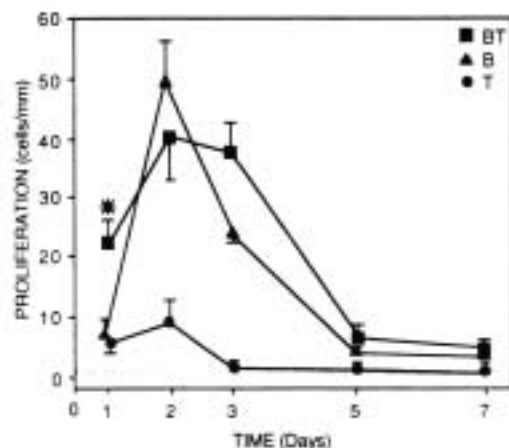


FIG 3. Proliferative response in the cambial periosteum of the region of interest after operation (on day 0). B, bone injury; T, soft-tissue injury; BT, combined injuries. The baseline (shading) was 0.3 ± 0.1 cells/mm as determined from 10 control rats (20 tibias). Asterisk indicates $p < 0.05$ for BT versus B.

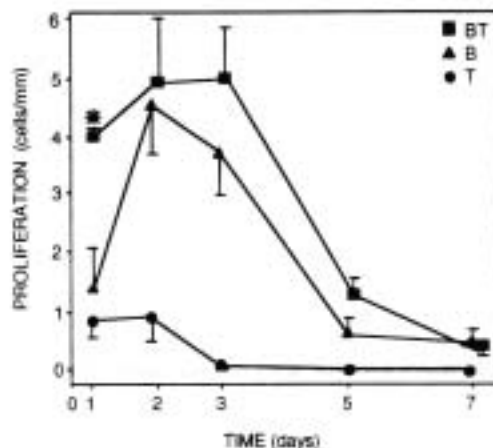


FIG 4. Proliferative response in the fibrous periosteum of the region of interest after operation (on day 0). B, bone injury; T, soft-tissue injury; BT, combined injuries. The baseline (shading) was 0.1 ± 0.1 cells/mm as determined from 10 control rats (20 tibias). Asterisk indicates $p < 0.05$ for BT versus B.

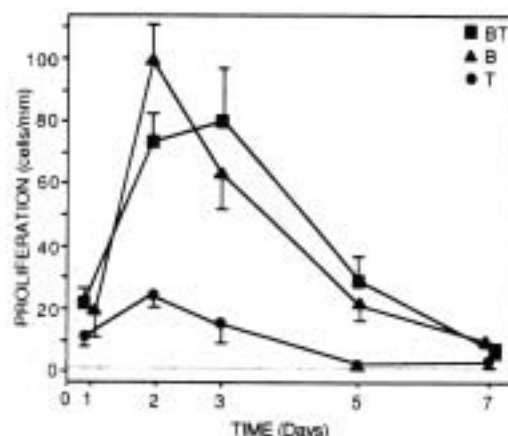


FIG 5. Proliferative response in the loose connective tissue of the region of interest after operation (on day 0). B, bone injury; T, soft-tissue injury; BT, combined injuries. The baseline (shading) was 0.4 ± 0.1 cells/mm as determined from 10 control rats (20 tibiae).

Muscle Injury Alone

A low-level transient proliferative response occurred that lasted longer in the connective tissue than in the periosteum (Fig. 4 compared with Figs. 1 and 3). Osteoblast levels increased slightly (Fig. 6), and the cells were synthetically active (Fig. 7). The rats that received only the skin incision exhibited a negligible periosteal response (data not shown).

DISCUSSION

Endogenous cytokines occur at bone injury sites,¹⁸ and exogenous cytokines can alter the bone injury response.¹⁹ Bone cells are sensitive to a range of signaling agents, including interleukin-1 β , transforming growth factor β , platelet-derived growth factor, and insulin-promoting growth factor.²⁰⁻²³ Thus, evidence suggests that the skeletal response to trauma is mediated by a key group of regulatory molecules.

It would be reasonable to anticipate that, under some circumstances, mediators of wound healing⁹⁻¹¹ might adversely affect the regulatory system governing fracture repair. The model muscle injury was chosen because it produced histo-

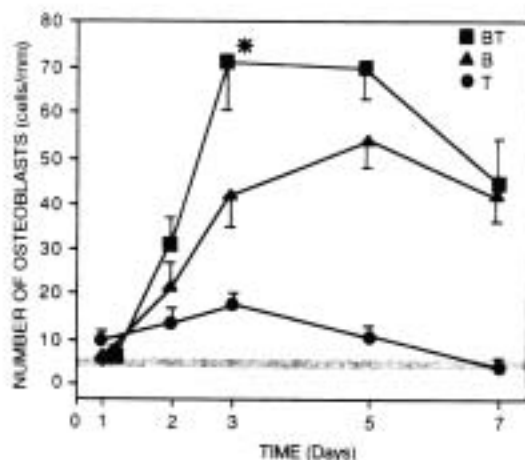


FIG 6. Osteoblast concentration in the cambial periosteum of the region of interest after operation (on day 0). B, bone injury; T, soft-tissue injury; BT, combined injuries. The baseline (shading) was 4.7 ± 0.6 cells/mm as determined from 10 rats (20 tibiae). Asterisk indicates $p < 0.05$ for BT versus B.

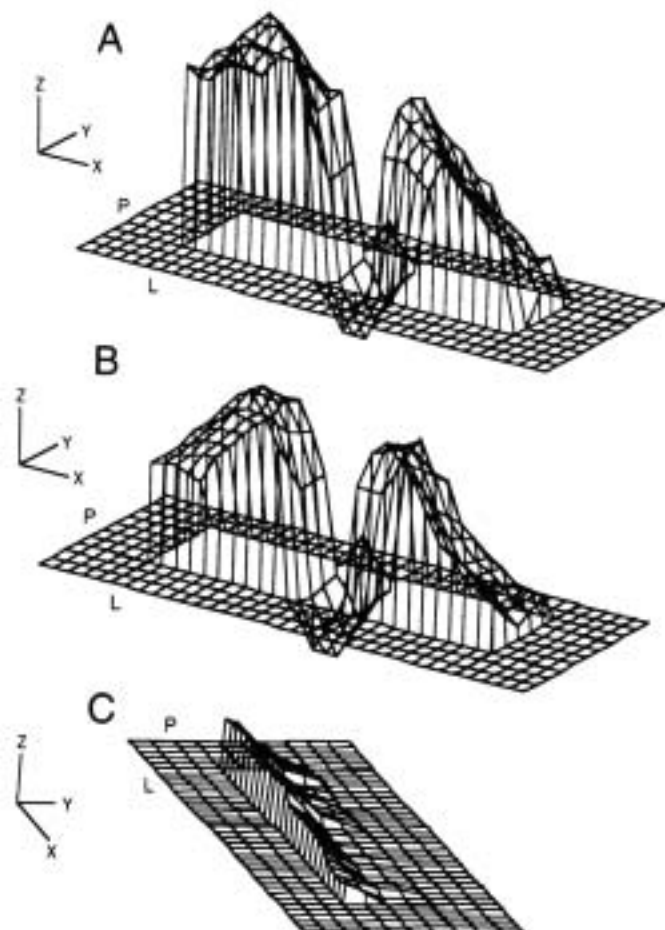


FIG 7. Callus formation in muscle-plus-bone-injured (A), bone-injured (B), and muscle-injured (C) rats 7 days after injury. The rectangular flat regions depict cortical bone surfaces; the bone defect (A and B) is the region below the cortical surface. The results were obtained by measuring the height of the callus at equally distant points (200 μ m) along each of the three representative sections, and averaging over five rats in each group. For illustrative purposes, values between the planes of measurement were obtained by linear interpolation, and the callus outside the region measured was arbitrarily assigned zero height. All heights (Z axis) were scaled to the observed maximal callus thickness (300 μ m). Y scale, 200 μ m/grid; X scale, 100 μ m/grid. P, proximal; L, lateral. Callus volume in A and B was 0.50 ± 0.10 mm³ and 0.37 ± 0.06 mm³, respectively.

logic and clinical signs of inflammation in the region of the bone defect that significantly exceeded the inflammatory response to the bone injury alone. Consequently, if factors derived from soft tissues could interfere with the bone-cell response to injury, then the model muscle injury should have been sufficient to permit observation of the response. The observation that the muscle injury triggered osteogenesis in the absence of bone injury supports this view (Fig. 7C).

However, in contrast to our premise, the inflammatory reaction caused by the muscle injury did not impair the early phases of bone healing. Rather, the opposite result was observed; addition of the muscle injury produced more periosteal proliferation (Figs. 3 and 4), more osteoblasts (Fig. 6), and may even have resulted in more callus formation (Fig. 7). Taken together, these observations do not support the view that a direct effect of the mediators of wound healing on the periosteum could explain delayed fracture healing or formation of nonunions. Other factors such as blood supply and stability of the fracture site may be more important predictors

of the duration and success of bone healing. This study was not designed to address the role of these factors.

Two limitations regarding the clinical implications of our results merit mention. Because the soft-tissue injury was administered surgically, the resulting inflammatory response may not adequately mimic the cytokine milieu triggered by an injury resulting from the application of force such as a crush. Second, the conclusions of this study pertain only to the periosteal repair system. Bone-matrix-producing cells conditioned in the marrow environment may have cytokine receptors that confer sensitivities different from those of periosteal cells.

An unexpected finding was that the soft-tissue injury alone was capable of eliciting proliferation in the cambium and in the fibrous periosteum, differentiation leading to osteoblast formation and callus formation (Figs. 3, 4, 6, and 7). The response to the muscle injury differed from the response to bone injury in both magnitude and duration, but the important point is that a bone-cell response occurred in the absence of injury to bone: this finding suggests that some of the initial triggers in wound healing and fracture response were qualitatively similar. This inference is further supported by the observed pattern of callus formation in relation to the injury site (Fig. 7). The callus that formed on the cortical surface around the bone defect was roughly symmetric around the defect. The muscle injury-induced callus was greater on the lateral edge of the tibia (which was nearer the muscle injury) and was significantly less more medially. Both patterns could be explained by assuming that bone-cell regulatory factors were synthesized or released at the injury sites and diffused through the region of interest, resulting in concentration-dependent signaling.

Why should there be a commonality in the initial signaling patterns of wound healing and fracture repair? From an evolutionary viewpoint, perhaps natural selection favored development of one rather than many systems for triggering healing responses. Vascular disruption accompanies all forms of trauma and consequently could serve as the source of necessary vulnerary factors. In this view, the tissue-specific nature of the healing would be determined by the receptor-linked pathways of the target cells.

In summary, the evidence suggests that the inflammatory milieu created by a surgical injury to soft tissue did not interfere with the early phase of the regulatory system governing bone repair. A clinical implication is that the additional trauma from incisions when internal fixation is performed with plates and screws might not be as deleterious to the fracture-healing response as previously thought.

REFERENCES

- Buckwalter JA, Cruess RL. Healing of the musculoskeletal tissues. In: Rockwood CA, Green DB, eds. *Rockwood and Green's Fracture in Adults*. 4th ed. Philadelphia: Lippincott-Raven; 1996:181-222.
- Brand RA, Rubin CT. Fracture healing. In: Albright JA, Brand RA, eds. *The Scientific Basis of Orthopaedics*. 2nd ed. Norwalk, Conn: Appleton and Lange; 1987:325-346.
- Whiteside LA, Lesker PA. The effect of extraperiosteal and subperiosteal dissection. II: On fracture healing. *J Bone Joint Surg Am*. 1978;60:26-30.
- Hulth H. Current concepts of fracture healing. *Clin Orthop*. 1989;249:265-284.
- Wallace AL, Draper ER, Strachan RK, McCarthy ID, Hughes SP. The vascular response to fracture microenvironment. *Clin Orthop*. 1994;301:281-290.
- Henley MB, Chapman JR, Agel J, Harvey EJ, Whorton AM, Swionkowski MF. Treatment of type II, IIIA, and IIIB open fractures of the tibial shaft: a prospective comparison of unreamed interlocking intramedullary nails and half-pin internal fixators. *J Orthop Trauma*. 1998;12:1-7.
- Dorow C, Markgraf E. Therapy of soft tissue injuries: biological strategies. *Zentralbl Chir*. 1997;122:962-969.
- Einborn TA. Enhancement of fracture-healing. *J Bone Joint Surg Am*. 1995;77:940-956.
- Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ. Mechanisms of tissue repair: from wound healing to fibrosis. *Int J Biochem Cell Biol*. 1997;29:5-17.
- Steed DL. The role of growth factors in wound healing. *Surg Clin North Am*. 1997;77:575-586.
- Lawrence WT. Physiology of the acute wound. *Clin Plast Surg*. 1998;25:321-340.
- Andrew JG, Andrew SM, Freemont AJ, Marsh DR. Inflammatory cells in normal human fracture healing. *Acta Orthop Scand*. 1994;65:462-466.
- Lian JB, Stein GS. Development of the osteoblast phenotype: molecular mechanisms mediating osteoblast growth and differentiation. *Iowa Orthop J*. 1995;15:118-140.
- Landry PS, Marino AA, Sadasivan KK, Albright JA. Bone injury response: an animal model for testing theories of regulation. *Clin Orthop*. 1996;332:260-273.
- Dickson GR. Chemical fixation and the preparation of calcified tissues for transmission electron microscopy. In: Dickson GR, ed. *Methods of Calcified Tissue Preparation*. New York: Elsevier; 1984:79-148.
- Anderson C. *Manual for the Examination of Bone*. Boca Raton, Fla: CRC Press; 1982.
- Aberne WA, Dunnill MS. *Morphometry*. London: Edward Arnold Publishers; 1982.
- Krane SM, Goldring MB, Goldring SR. Cytokines. *Ciba Found Symp*. 1988;136:239-256.
- Goldring MB, Goldring SR. Skeletal tissue response to cytokines. *Clin Orthop*. 1990;258:245-278.
- Canalis E. Interleukin-1 has independent effects on deoxyribonucleic acid and collagen synthesis in cultures of rat calvariae. *Endocrinology*. 1986;118:74-81.
- Pfeilschifter J, Mundy GR. Modulation of type β transforming growth factor activity in bone cultures by osteotropic hormones. *Proc Natl Acad Sci USA*. 1987; 84:2024-2028.
- Canalis E, McCarthy TL, Centrella M. Effects of platelet-derived growth factor on bone formation in vitro. *J Cell Physiol*. 1989;149:530-537.
- McCarthy TL, Centrella M, Canalis E. Regulatory effects of insulin-like growth factors I and II on bone collagen synthesis in rat calvarial cultures. *Endocrinology*. 1989; 124:301-309.