Journal of Rehabilitation Research and Development

Rehabilitation R & D Progress Reports 1990

XVII. Wound and Fracture Healing

A. Pressure Sores

B. Fracture Healing

C. Other

XVII. Wound and Fracture Healing

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A. Pressure Sores

[606] Electrical Muscle Stimulation for the Prevention of Pressure Sores

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Sponsor: *VA Rehabilitation Research and Development Service (Project #B351-RA)*

Purpose—Pressure sores represent a severe and costly problem for many disabled individuals, especially those who are wheelchair-dependent and have sensory loss. It was originally hypothesized by our group that electrical muscle stimulation (EMS) can help prevent pressure sore formation based on the rationale that: 1) tissue undulation produced from EMS (a "short-term" effect) will dynamically allow blood flow to ischemic areas; and, 2) the changes in vascular and muscle tissue produced from "chronic" EMS will lead to a reduction of pressure sores. This research program focuses on evaluation of short-term dynamic effects of EMS for pressure sore prevention.

Progress/Methodology—Work over the past 4 years on short-term dynamic effects of EMS for pressure sore prevention has demonstrated that: 1) EMS can reduce pressure under the ischial tuberosity in a seated individual with redistribution to other parts of the seating interface at very low stimulation intensities; 2) similar low-intensity stimulation of the gluteus maximus produces tissue undulation and shape reconfiguration of the buttocks under load; 3) EMS can produce increased muscle blood flow in seated individuals; and, 4) increased blood flow in skin and subcutaneous tissue can occur using EMS. Continuing work on EMS for pressure sore prevention has focused over the past year on small-scale, short-term clinical trials with newly spinal cord injured subjects.

For the most recent studies involving clinical trials with newly spinal cord injured subjects, a time series experimental design (A-B-A-B) was utilized. Phase A involved sitting with no EMS for a predetermined time, which produced redness under the ischial tuberosities that persisted for at least an hour following the sitting period. Phase B involved sitting for the same amount of time while EMS was provided via surface electrodes and a commercial stimulator. Parameters used to compare the EMS phase (A) versus the no-EMS phase (B) included skin temperature, size of erythematous area, and degree of erythema.

Results—The effects of extended sitting on skin erythema and temperature without any EMS were analyzed to serve as a baseline for comparison with EMS trials. Results showed a consistent skin temperature pattern after sitting with experimentally induced erythematous areas remaining elevated even after one hour of pressure relief. Results from seven subjects comparing EMS sitting trials with trials having no intervention showed a reduction in the area, intensity, and temperature changes of the erythematous area induced through extended sitting.

Future Plans—Results from this research program have provided a considerable amount of support for the use of EMS in pressure sore prevention. Multi-center outpatient trials of EMS for pressure sore prevention have been

proposed to and provisionally approved by the VA Rehabilitation Research and Development Service Evaluation Unit.

Recent Publications Resulting from This Research

Electrical Muscle Stimulation for Pressure Variation at the Seating Interface. Levine SP et al., J Rehabil Res Dev 26(4):1-8, 1989.

Electrical Muscle Stimulation for the Prevention of Pressure Sores: Tissue Shape Variation. Levine SP et al., Arch Phys Med Rehabil 71(3):210-215, 1990.

Blood Flow in the Gluteus Maximus of Seated Individuals During Electrical Muscle Stimulation. Levine SP et al., Arch Phys Med Rehabil 71(9):682-686, 1990.

[607] Enhancement of Wound Healing Using Synthetic Skin, Electric Stimulation and Hyperbaric Oxygen Therapy

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Purpose—Decubitus ulcers, commonly referred to as bed sores, affect millions of Americans each year. They arise from prolonged bed rest and ischemia that occurs when areas of skin are compressed in weightbearing areas of the body.

Our group has been involved in evaluating several techniques to promote dermal and epidermal healing of bed sores. Previously, we have demonstrated that type I collagen in the form of flakes promoted healing of stage II and III bed sores in 85% of patients treated as compared to controls. The purpose of our current studies is to evaluate the use of collagen flakes containing hyaluronic acid and an aerosol form of collagen type I for treatment of bed sores.

Methodology—Five patients with chronic bed sores were treated with collagen flakes containing 1% or 5% hyaluronic acid while an additional five patients were treated with an aerosol form of collagen produced by Micro-Collagen Pharmaceutics (Bangor, PA). All patients signed informed consent forms and were treated daily for 3 weeks prior to treatment with collagen using a standard protocol consisting of a daily saline wash, followed by application of a wet-to-dry gauze dressing. In cases where necrotic tissue was present, wounds were debrided prior to collagen treatment. The surface area was measured weekly by placing a clear plastic sheet over the wound and tracing the wound perimeter. After 3 weeks of treatment, the wounds were treated once a day using collagen flakes containing hyaluronic acid or a collagen aerosol spray after the saline washing step. Treatment was followed by the application of saline-wetted gauze and the dressing was secured to the surrounding normal skin using adhesive tape. Wounds were treated with collagen for a total of 12 weeks.

Results—Results obtained in this study suggest that wounds treated with collagen flakes containing hyaluronic acid or a collagen aerosol showed healing that was similar to wounds treated with control collagen flakes. Some patients treated with collagen flakes containing 5% hyaluronic acid showed evidence of local hemorrhage within the ulcer. In comparison, wounds treated with collagen flakes containing 1% hyaluronic acid showed no evidence of hemorrhage and healed with similar kinetics to that reported previously for collagen alone. A 50% wound area reduction was observed in patients treated with collagen flakes containing 1% hyaluronic acid or the collagen aerosol over a time course of about 6 weeks.

Although collagen flakes have been shown to improve healing of bed sores, they are difficult to pack under skin flaps and often result in discomfort to the patient. Results of studies conducted using a collagen aerosol spray indicate that it offers advantages such as ease of application and reduced discomfort with respect to use of collagen flakes. In addition, reduction of the wound area observed with the spray is similar to that observed with the flake form of the material.

Implications—Our results indicate that initiation of healing of bed sores is promoted by type I collagen and is not further promoted in the presence of hyaluronic acid. Use of an aerosol form of collagen offers distinct advantages over flakes or other physical forms and results in comparable wound area reduction.

[608] Identification and Evaluation of a Comprehensive Skin Care Program to Prevent Skin Breakdown in Spinal Cord Injured Patients

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Purpose—The purpose of the study was to identify and evaluate a comprehensive skin care education program to prevent recurrent skin breakdown in patients with spinal cord injuries (SCI). A further purpose was to evaluate two methods of gaining the subjects' cooperation in practicing skin protection behaviors. Subjects in Group I received a behavioral intervention while those in Group II received a psychological intervention.

Results/Implications—A thorough review of existing skin care education programs revealed none adequate for the purposes of the study. A major problem was that the reading and comprehension levels required were too high. Thus, a skin care education program was designed and produced as a low-literacy work book titled, *Your Skin: An Owner's Manual.* Copies are available from our Center.

Data are still being analyzed, but the following findings may be useful. Blacks and paraplegics with complete injuries are greatly over-represented in this population. Approximately two-thirds of the subjects showed evidence of cognitive impairment. Whether this existed before the SCI or was due to closed head injury at the time of the SCI could not be determined.

Although not statistically significant (the sample was too small), it appears that the behavioral intervention was more effective with this population than the psychological intervention.

The study was terminated in September, 1990.

[609] Treatment of Pressure Ulcers

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Purpose—Development of improved clinical protocols may help reduce the staggering morbidity statistics resulting from pressure ulcers in people with spinal cord injury (SCI). In order to develop optimal active treatment protocols for pressure ulcers, basic wound-healing research is essential. The objectives of this study are to: 1) determine, in cell culture, the optimal concentration of oxygen and optimal pressure for fibroblast activation and macrophage deactivation; 2) determine, in cell culture, the optimal dosage of platelet derived growth factor (PDGF) to stimulate fibroblast activation; 3) determine, in rabbits, optimal use of oxygen for healing of both subcutaneous porous implants and full-thickness skin defects; 4) determine, in rabbits, the optimal dosage, timing, and type of growth factor drug delivery for healing of both subcutaneous porous implants and full-thickness skin defects; 5) test, in rabbits, the optimal oxygen therapy in combination with the optimal growth factor delivery systems for full-thickness skin grafts, and full-thickness skin defects; and, 6) evaluate the efficacy of various therapeutic approaches on pressure ulcers in patients with SCI.

Methodology—The optimal concentration of oxygen and optimal pressure for fibroblast activation and macrophage deactivation will be determined by growing cells in a controlled environment at various predetermined combinations. The activation of cells will be determined by analysis of cellular metabolism, production of collagen (fibroblast), and cell growth. The optimal concentration of platelet-derived growth factor for cellular activation and deactivation will be determined by *in vitro* tests of cellular metabolism and cell growth. The PDGF exhibiting the greatest potential will be attached to polyactic and collagen matrices. The matrices will be tested in cell culture to determine the load of growth factor necessary to elicit increased cellular metabolism, collagen production, and cell growth.

Progress/Preliminary Results—During the first 24 months of the grant period, the following progress has been made: 1) primary cultures of fibroblasts and macrophages have been obtained from rabbits; 2) test results of the oxygen concentration and duration for fibroblast activation are in progress. Oxygen concentrations of 32% for up to 10 hours a day cause increased proliferation. Higher concentrations and/or durations of oxygen slowed fibroblast growth to below that of the control; 3) tests on the concentration of oxygen and duration for macrophage deactivation are still being analyzed. Initially, it seems to indicate decreased cellular proliferation with increasing oxygen concentration and duration of exposure; 4) tests using PDGF to increase the activity of fibroblasts are on-going. Results at this time indicate that PDGF increases proliferation significantly in the 3-9 units/ml range—indicating that small amounts of PDGF produce significant increases in proliferation. No adverse effects of PDGF on fibroblasts proliferation have been determined; 5) test substances of collagen and collagen with PDGF have been tested *in vitro*. Collagen with PDGF has produced increased proliferation. Tests are on-going to determine the loading and attachment methods that are optimal; and, 6) *in vivo* testing of optimal oxygen levels on a rabbit model have been initiated.

Future Plans—*In vitro* testing will continue and *in vivo* testing will be initiated during this 5-year project.

[610] Use of Direct Current Stimulation in Pressure Sore Healing

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Purpose—A newly developed animal model for stage 3 or 4 pressure ulcers was used to study the influence of direct current (DC) stimulation on denervated wound healing. In two groups of animals, one group was treated daily with DC stimulation, while the other was used as a control.

Methodology—Changes in wound area, volume, perfusion, histology, and collagen were recorded as dependent variables. The exponential wound-healing model of Vodovnik and Stefanovska was used to calculate healing time constants. Results indicated reduced area and volume time constants in DC-stimulated animals, exceeding control values.

Results—Denervated tissue perfusion was estimated at the wound edge by measuring transcutaneous oxygen partial pressure. Healing tissues with or without stimulation showed greater than normal perfusion at the same anatomical sites. Stimulation initially reduced perfusion, then increased above and finally equalized with the unstimulated control site.

Histology showed a reduced inflammatory period followed by enhanced cellular proliferation with DC stimulation. Stimulation also induced earlier wound maturation. Histomorphometry, quantifying the vascular density and cross-sectional area in the granulation tissue, showed advanced neovascularization with stimulation, significantly exceeding control values in the healed wounds.

There was no change in the collagen concentration of granulation tissue with applied DC stimulation. The concentration of hydroxyproline was the same in both the control and the stimulated tissues. Protein solubility tests also showed no significant increase in the soluble fractions of the DC-stimulated healed wounds.

Overall, the effect of DC stimulation was to enhance healing time constants and neovascularization without significantly affecting collagen synthesis of healed wounds.

Recent Publications Resulting from This Research

An Experimental Pressure Sore Model for Functional Electrical Stimulation: Continuous Pressure Application on Monoplegic Pigs. Negami S et al., in Advances in External Control of Human Extremities, D.B. Popovic (Ed.). Nauka, Yugoslavia, 535-541, 1990.

Orientation and Biomechanical Properties of DC Electrically Stimulated and Healed Pigskin Pressure Sores. Kambic HE et al., in Advances in External Control of Human Extremities, D.B. Popovic (Ed.). Nauka, Yugoslavia, 519-524, 1990.

Wound Healing and Perfusion of Pressure Ulcers in Direct Current Stimulated Denervated Tissues. Reger SI et al., in Advances in External Control of Human Extremities, D.B. Popovic (Ed.). Nauka, Yugoslavia, 525-534, 1990.

[611] Therapeutic Intervention for Healing Pressure Sores with Electrical Stimulation on Persons with Spinal Discontinuities

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Sponsor: *National Institute on Disability and Rehabilitation Research; Commission of the European Communities, Directorate-General for Science, Research and Development, International Scientific Cooperation, Brussels; Research Communities of Slovenia, Ljubljana, Yugoslavia*

Purpose—The purpose of this project is to conduct a carefully controlled and quantified study on the effects of subthreshold tetanizing currents and a double blind study of the effects of DC currents on wound healing. Pressure sores in patients with spinal discontinuities and wounds due to peripheral vascular diseases are included in the study. Our purpose is also to find out the mechanisms by which electrical currents influence healing.

Progress—Subthreshold tetanizing and DC currents applied through skin electrodes are used. The first phase will address the problem of quantification and control. After the phenomenon is demonstrated with statistical significance, efforts will be concentrated on the mechanisms by which electrical currents influence healing.

Major efforts are devoted to the continuation of the clinical study which includes 60 patients with 90 decubitus ulcers, and 40 patients with wounds due to vascular diseases. All data are in a dBASE IV database, thus enabling simple access to relevant information and improved data processing.

Methodology/Results—In order to elucidate mechanisms which are involved in accelerated wound healing when electrical stimulation is applied, several basic studies have been started. Preliminary results seem to indicate that wounds to which AC pulses are applied heal twice as fast as control wounds, and somewhat faster than wounds treated with DC.

Endogenous skin potentials have been measured between wounded and normal skin on healthy subjects. It was found that the wounded area is positive relative to normal skin. During the healing process this potential difference slowly disappears.

Bacteriological analysis of samples taken from decubitus ulcers were performed. While the results for other bacteria are not quite consistent, it seems that electrical stimulation has a bacteriostatic effect on *pseudomonas aeruginosa*.

An *in vitro* study of effects of electrical stimulation on fibroblast proliferation and migration has been initiated. Since fibroblasts are responsible for most collagen formation, their role in wound healing is important.

Some additional techniques such as thermography, oxymetry, and magnetic resonance imaging (MRI) have been introduced. Preliminary data from thermography produced an interesting time course of temperature increase after electrical stimulation.

Recent Publications Resulting from This Research

Restorative and Regenerative Functional Electrical Stimulation. Vodovnik L, Stefanovska A, in Proceedings of the Osaka International Workshop on Functional Neuromuscular Stimulation, J. Kawamura, T. Tamaki, K. Akazawa (Eds.), Osaka, Japan, 11-27, 1989.

Accelerated Wound Healing of Lower Extremities by Means of Electrical Stimulation. Karba R et al., in Advances in External Control of Human Extremities, D.B. Popovic (Ed.), Nauka, Yugoslavia, 509-517, 1990.

Database for Assembling and Analysis of Electrical Wound Healing Data. Kroselj P et al., in Proceedings of ETAN, Belgrade (in Slovene), 1990.

Effects of Electrical Current on Healing and Bacteria Growth in Decubitus Ulcers. Karba R et al., in Proceedings of the 3rd International Symposium on Tissue Repair, Miami, FL, 1990.

Pressure Sore Healing and Endogenous Potential. Jercinovic A, Bobanovic F, in Proceedings of the 10th Anniversary Meeting of Bioelectrical Repair and Growth Society, Philadelphia, PA, 1990.

Self-Organization of Pathological Systems Induced by Electric Currents. Stefanovska A, Vodovnik L, in Proceedings of the 12th Annual International Conference, IEEE Engineering in Medicine and Biology Society, Philadelphia, PA, 1990.

[612] An Analytical Service Demonstration of the Role of Biochemical and Behavioral Indicators in the Prevention of Recurrent Pressure Sores

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Purpose—Our purpose was to confirm the potentiality of a biochemical indicator to predict skin breakdown and the efficacy of specific self-directed behaviors to prevent recurrent pressure sores.

Methodology—This is an observational, prospective, cohort study. Males with spinal cord injury are randomly assigned to two groups. The control group will be interviewed only at the beginning and at the end of the study, and will provide a 24-hour urine sample at each of those times. The experimental group will be interviewed in person initially, and then by telephone every 4 to 6 weeks. They will provide a 24-hour urine sample at the time of each interview. Follow-up will continue for 2 years or until the subject develops a pressure sore, whichever comes first.

The interviews will elicit demographic information, medical history with special emphasis on incidence of pressure sores, and a description of the usual skin care regimen. The urine will be assayed for the content of glucosyl-galactosyl hydroxylysine, a collagen metabolite.

The data analysis will seek to establish the relative risk of developing a pressure sore based on the fluctuations in urinary concentration of the collagen metabolite and/or specific items in the skin care regimen.

Progress—Recruitment of subjects has been completed. There are 40 experimental and 20 control subjects. Compliance continues to be good. Initially, 83% of the subjects believed they were not likely to develop a pressure ulcer within the year. At this time, 22% of the subjects have developed a pressure ulcer. Four subjects have completed the project with skin intact. Preliminary data analysis shows that a larger proportion of controls developed ulcers. Subjects with ulcers tended to be younger and had lower body mass index. The most frequently cited pressure ulcer prevention methods were: weight shifts, not sitting too long, skin inspection, and using the proper cushion.

Implications—Successful completion of this research project will provide a means of identifying patients at imminent risk of developing a pressure sore. More aggressive preventive measures can then be brought into play to forestall an actual skin breakdown. This should translate into a considerable reduction of hospitalization time and costs.

[613] Pressure Sore Prevention: An Effective Stepped Care Approach

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Purpose—The goals of this project include the refinement, validation, and dissemination of a novel system with unique potential to produce durable preventive behavior, and thereby lower the incidence and severity of ischial pressure sores. Its promise lies in its self-correcting, data-based approach; in its stepped, systematic application of a variety of interventions, both established and new; and in its utility in validating any effort at teaching pressure relief behaviors. The key to the system is the Timer-Logger-Communicator (TLC), an electronic device developed by the authors. The TLC continuously and unobtrusively records pressure-relief behavior, and provides data, cues, or immediate feedback to enhance pressure relief behavior.

Progress/Methodology—To date, 45 subjects have been recruited, with five subjects currently enrolled. We are at 90% of our recruitment target and analysis of the data

collected to date is still in progress. All subjects have received standard teaching to prevent pressure sores as a baseline. If needed, experimental interventions were randomly selected and applied. Engineering and software improvements have been made to the TLC, which is used to measure the timing and frequency of pressure relief behaviors.

[614] Bedsore Biomechanics

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Purpose—Bedsores (pressure sores) or decubitus ulcers are localized areas of cellular necrosis resulting from prolonged excessive stresses on soft tissues, and present a major problem in the comprehensive rehabilitation of spinal cord injured patients and others with paralyzing neurological diseases. The type and magnitude of stresses generated in the tissue depend on body build, mechanical properties of the tissue, mechanical properties of the cushion, and posture, etc.

The objectives of this investigation are to study the effect of the following parameters on the internal stress distribution generated in the soft tissue of the buttock during vertical and inclined loading: 1) effect of bone and tissue geometry; 2) effect of mechanical properties of the soft tissue; and, 3) effect of the mechanical properties of the supporting cushion.

Progress—We have developed two types of 2-D physical models of the buttock. In each of these models, PVC gel simulating the soft tissue was cast around a wooden core simulating the bone. The first model had a rounded edge core to simulate blunt bony prominence. The second model consisted of a flat circular (sharp) edge "bone" core to simulate sharp bony prominences.

Each of these models was placed on a representative cushion and loaded. A grid etched on the "soft tissue" model allowed photography for calculating strains and stresses in the tissue. Cushion materials were compared in terms of the tissue-cushion interface pressure and shear stresses generated in the soft tissue.

In addition, we are developing interface pressure transducers using conductive polymers. We are in the process of evaluating various types of polymers for suitability as interface pressure transducers.

Preliminary Results—Shear stresses generated in the model soft tissue were significantly larger in magnitude in the case of flat (sharp edge) bone core when compared to the rounded edge model. However, the compressive stresses in the flat (sharp edge case) were lower than the rounded edge model. There were significant stress concentrations in the case of the sharp edge model. Foam cushions led to more uniform stress distribution in the model soft tissue when compared to all others tested during vertical loading. The gel cushion performed better during inclined loading. Inclined loading led to large magnitudes of shear stresses in the buttock model when the model was supported by air and hydro cushions.

Future Plans—Currently, we are investigating the effect of aspect ratio (bone width to tissue thickness ratio) and mechanical properties of the soft tissue on the stresses generated in the soft tissue model during vertical and inclined loading.

Recent Publications Resulting from This Research

Stress Analysis of Cushion Supported Tissues with Respect to the Bedsore Problem. Candadai RS, Masters thesis, University of Akron, 1989.

Cushion Evaluation Based on Stress Distribution in Soft Tissues. Candadai RS, Reddy NP, Canilang EP, in Proceedings of the 13th Annual RESNA Conference, 403-404, Washington, DC, 1990.

Effects of Mechanical Stress on Lymph and Interstitial Fluid Flows. Reddy NP, in Pressure Sores: Clinical Practice and Scientific Approach, 203-220, D.L. Bader (Ed.). London: MacMillan Press, 1990.

B. Fracture Healing

[615] Enhancement of Union of Segmental Defect Fractures II

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Purpose—Application of rigid external fixators is a commonly accepted practice for stabilization of segmental defect fracture injuries. However, rigid immobilization of the fracture site greatly reduces the mechanical stimulus to which the bone is normally exposed. This environment is not optimal for osteogenesis.

The normal mechanical stimulus for osteogenesis in intact bone is intermittent cyclical deformation. Some studies have demonstrated that mechanical stimulation of the developing callous in the form of micromovement (1.0-2.0 mm) and/or loadsharing, results in earlier callous formation, callous with greater cross-sectional area, and an increase in fracture stiffness. However, the optimal temporal, distance, and stiffness parameters over which these mechanical stimuli should be restored to the fracture area have not yet been defined. Our goal is to define these parameters.

Progress—We are currently treating segmental defect fractures created in a canine model with the application of "variable stiffness" external fixators. These fixators allow both micromovement (up to 2.0 mm) and loadsharing (by weightbearing) in the callous area immediately after fixator application, thus ensuring a more normal frequency of axial loading during the healing period.

These devices have replaceable stainless steel springs; this allows us to customize the stiffness of the fixator to the weight of the animal.

Methodology—Forty-five adult mongrel dogs (50-60 lbs) are used. The dogs' radii are approached anteromedially and 2.50 cm osteoperiosteal segments are removed bilaterally, creating transverse diaphyseal defects. The defects in both legs are then supplanted with cancellous autogenous graft (generally accepted as the ideal bone graft material) from the contralateral humeral head. External fixators are applied bilaterally. One fixator has a 150 mm rigid stainless steel tube while the contralateral side has a 130 to 170 mm variable stiffness fixator. Dogs are sacrificed at 4, 8, and 12 weeks. Radiographs are taken immediately postoperatively and at 4, 8, and 12 weeks depending on sacrifice. Following euthanization, both radii are excised and subjected to biomechanical testing by impact torsional testing. Afterwards, the bones are sectioned and stained for computerized video analysis.

Results—A small pilot project has been completed. Radiological results indicate that there are significant differences in cross-sectional areas between legs treated with variable stiffness fixators and control fixators. Additional histological and biomechanical data are necessary for conclusive results.

Future Plans/Implications—Use of these fixators will assist in determining the optimum osteogenic environment for segmental defect fracture healing by helping to define the precise relationships between the degree of fixator stiffness and micromovement, and the rates and patterns of fracture healing. These data will help to determine if the size and strength of callous formation are based on optimization criteria. Our hypothesis is that these results will allow us to *formulate a model of callous healing behavior under certain conditions of stress.* When applied clinically, this model could potentially result in a higher percentage of unions in segmental defect fractures by allowing some control over callous formation and remodeling. This would represent an inexpensive, practical method of treatment that could be utilized immediately with the use of currently available materials (i.e., AO clamps and Kirschner wires). Additionally, we are currently evaluating techniques for the Ilizarov method of bone transfer. Some trials are completed and the data will soon be evaluated. We have also recently completed a biomechanical study on synthetic graft substitutes which indicated that all of the synthetic graft materials were greatly enhanced by the use of aspirated bone marrow. A series of trials using a canine model to find the bone morphogenic fraction of bone marrow aspirate is scheduled.

[616] Periosteum as a Functional Membrane

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Sponsor: *Cappagh Research and Development Trust*

Purpose—The purpose of this study was to set up a periosteal membrane preparation for *in vitro* electrophysiological investigation. Clinically, periosteal membrane acts as a functional limiting membrane to bone formation. Under electron microscopy, the membrane structure shows tight junctions between apposing cells, and the size of the intercellular spaces seems to be influenced by calcitonin and PTH. The importance of the integrity of the periosteal and endosteal membranes has been emphasized by work on limb lengthening and bone transport.

Methodology—To obtain a sample of intact periosteal membrane free from muscle attachments, the outer table of the skull of mature Sprauge Dawley rats was chosen as a suitable site. Under chloral hydrate anesthesia, two polyethelene washers are inserted subperiosteally and the membrane repaired over them. At 3 weeks, the animals are sacrificed and the skin and galea removed from the skull to expose the implanted washers covered with intact periosteum. Both washer and membrane are removed together and placed in an Ussing chamber for electrophysiological determinations.

Results—The 3-week duration of implantation was chosen because the membrane is fully healed and bone has grown up through the core of the washer to reach the new position of the periosteum. Recorded potentials of the membrane are of the order of 500 microvolts and are difficult to separate from junctional potentials of the agar bridges. Transmembrane resistances of the order of $10^6/cm^2$ have been recorded between the two half-cells of the Ussing chamber.

Future Plans/Implications—It is proposed to use this model to investigate the effect of calcitonin and PTH on the transmembrane resistance of intact periosteal membrane. It may be a function of periosteum to control the ionic milieu and so act as a blood-bone barrier, as has been suggested by tracer studies.

[617] Enhancement of Femoral Head Fracture Healing by Means of AC and DC Electrical Stimulation

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Sponsor: *Republic Ministry for the Research Activity and Technology of Slovenia, Yugoslavia*

Purpose—The purpose of this study is to evaluate and compare the effects of DC and AC electrical stimulation on the healing of fresh femoral head fractures.

Numerous studies dealing with electrical stimulation of bone fractures have reported beneficial effects on healing, irrespective of the type of current (DC or AC) used. In our study, carefully selected and matched femoral head fractures of the same location will be immobilized and randomly assigned to four groups. Two experimental groups of patients will be treated with biphasic electrical stimulation, and with constant direct current of low intensity, respectively. The third group of patients will receive dummy stimulators (delivering no current), and the fourth group of patients will serve as controls.

Finally, the comparison of healing times will provide the estimation of the effects of different treatment modalities, and will simplify making the choice of future clinical routine methods.

Methodology—Totally implantable current sources will be used for electrical stimulation. The stimulators are designed to deliver a defined current for a period of 10 months: after that, they will be removed. 1) *AC electrical stimulation.* Bipolar rectangular electrical current impulses with a mean value of zero, an amplitude of 25 mA, and

a frequency of 0.5 Hz will be delivered through Pt electrodes. These will be inserted in the bone marrow a few centimeters distant on both sides of the fracture. 2) *DC electrical stimulation.* Continuous direct current with the amplitude of 7μA will be used. The negative electrode (cathode) will be inserted directly in the fracture site. The positive electrode (anode) is attached to the stimulator, which will be placed in the muscle tissue, 15 cm distant from the fracture. Actual current density utilizing Pt at the cathode will be 0.7 $\mu A/mm^2$.

Future Plans—The results of all experimental work carried out during 1990 will be gathered and analyzed during 1991.

[618] Biomechanics of External Fixation of Tibial Fractures

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Sponsor: *Scottish Home and Health Department, Chief Scientist's Office; Orthofix Slr., Verona, Italy*

Purpose—External fixation is a method of managing tibial fractures which offers the potential for studying and controlling the biomechanical environment in which the fracture is maintained. The aim of this project is to measure how this biomechanical environment changes as the fracture heals, to determine how this information can be used clinically, and to develop a system which can perform these measurements in a routine clinical environment.

Methodology—A strain-gauged transducer has been built into the fixator body to measure the six components of force and moment which it carries. Externally applied (ground reaction) forces and moments are measured using a Kistler force plate. Use of a VICON kinematic analysis system allows the effect of these forces at the fracture site, and the ratio of shared loading between the fixator and healing bone to be estimated. The data is analyzed for changes occurring through healing, and these are correlated with radiological and clinical observations.

Progress—A transducer-based system has been developed which is capable of giving estimates of the six components of load carried by the fixator, and applied externally during standing, walking, and certain clinical tests. Thirteen patients have been monitored from initial application of fixator through to removal.

Preliminary Results—Several trends are suggested by results to date. Changes in the ratio of shared loading of the axial components of force give an early indication of the stability of the fracture and can be used to help determine the appropriate timing for dynamization.

Changes in the ratio of shared loading of bending moment components are observed later in the healing period and can given an indication of appropriate timing of the removal of such devices. Regular monitoring can give an early indication of pathological healing patterns.

Future Plans—More clinical evidence is required to confirm these results. It is intended to develop a microcomputer-based system to incorporate the findings of this study in a system which can be used routinely in the clinical environment.

Recent Publications Resulting from This Research

A System for the Measurement of Shared Loading Between Fractured Long Bones and External Fixators. Baker RJ et al., in Proceedings of IMechE, 165-170, 1989.

[619] Effect of Acetoazolamide on Fracture Healing

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Sponsor: *Department of General and Orthopaedic Surgery, University College Cork, Ireland*

Purpose—The purpose of this study was to investigate the hypothesis that alteration in the pH at the site of fracture healing might influence the rate of union. Fracture consolidation at the stage of calcification of the callus matrix is associated with a change in the pH of to an alkaline milieu. Patients with head injury and alkalosis often

generate excessive calcified callus at a fracture site. Acetoazolamide as an inhibitor of carbonic anhydrase would be expected to retard alkalinization at the site of callus formation.

Methodology—A control and study group of Sprauge Dawley rats were used with both groups subjected to a controlled tibial fracture which was internally fixed with an intramedullary fixation device. Both groups were managed postoperatively in similar fashion except that the study group was tube-fed with acetazolamide daily. Animals were sacrificed from both groups at regular intervals and the tibiae removed for analysis. All the tibiae were then tested in tension using an Instron tensile device after the intramedullary Kirschner wire was removed.

Results—The results suggest that fracture healing in both control and study groups proceeded in a similar fashion. Fracture stiffness and ultimate yield point remained at a low level for the first 3 weeks. In the fourth week, the stiffness of the control group was significantly greater than the acetoazolamide-treated group. At 6 weeks, both groups had fully united and showed no statistical differences in mechanical properties.

Future Plans/Implications—The specimens are presently undergoing histological analysis of matrix and mineral morphology to determine if differences exist to define the delay observed in fracture consolidation. The implication of this study is that it may be possible to accelerate the rate of fracture union by systemic alkalinization.

C. Other

[620] Morphologic and Ultrasonic Analysis of Normal and Ischemic Human Wounds

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Sponsor: *VA Rehabilitation Research and Development Service (Project #A210-2RA)*

Purpose—We have engaged in the investigation of deficiencies in the wound healing process in individuals with peripheral vascular disease (PVD) and diabetes mellitus (DM). We hope to identify abnormalities in the repair process which may suggest clinical interventions.

Progress/Methodology—We have utilized standard incised wounds created with a Simplate II bleeding time device to produce uniform wounds on normal elderly subjects, as well as patients with PVD or DM who are awaiting amputation. A variety of time points following wounding have been evaluated and differences in events of repair defined for several variables including PVD, DM, transcutaneous pressure of oxygen ($TcPO_2$), and anatomic locations. We have also studied the timetable for the appearance and disappearance of a number of proteins thought to be important to the repair process, including thrombospondin, filaggrin, laminin, type IV collagen, TGFβ, PDGF, PDGF receptor, SPARC and involucrins. Antibody to stain for the presence of nonenzymatic glycosylation (NEG) of proteins has been successfully used to study human wound tissue. In addition to morphological and immunochemical evaluation of the repair process we have investigated the use of high frequency ultrasound as a method for noninvasive evaluation of the repair process. A scanning laser acoustic microscope (SLAM), and backscatter acoustic techniques have been used for the latter studies.

Results—We have now studied 24 patients with DM, 17 with PVD, and 25 normal elderly subjects. Morphological events of dermal repair are significantly advanced: 1) if $TcPO_2$ is greater than 21; 2) in the superficial wound compartment compared to the deep wound; 3) in controls compared patients with PVD and DM; and, 4) if arm wounds are compared to leg wounds. Epidermal events of repair were not different between controls, patients with PVD or DM. Wounds from diabetic patients stain much more intensely than normals for

NEG. Considerable progress has been made in the use of ultrasound to assess skin and wounds.

Future Plans/Implications—Using biochemical methods, we hope to be able to use our monoclonal antibody to the glucitolysine epitope (NEG) to identify the specific proteins stained in the diabetic wound matrix and to ascertain functional changes in these proteins which may be important in the pathogenesis of diabetic wound failure. We also plan to fully evaluate normal wounds and wounds from patients with PVD and DM for the presence of a variety of growth factor as well as a timetable for appearance and disappearance of those factors. We plan to use the validated human wound model to do comparative trials of growth factors applied directly to these standard wounds in dysvascular extremities.

Recent Publications Resulting from This Research

A Method for Localizing the Early Products of Nonenzymatic Glycosylation in Fixed Tissue. Kelly SB, Olerud JE, Witztum JL, Curtiss LK, Gown AM, Odland GF, J Invest Dermatol 93:327-331, 1989.

The Systematic Study of Partial Thickness Wounds in Normal Elderly Adults and Patients with Peripheral Vascular Disease (PVD) and Diabetes Mellitus (DM). Olerud J, Odland GF, Burgess E, Wyss C, Fisher L, Matsen F, presented at the Society of Investigative Dermatology, 1989.

Correlation of Tissue Constituents with the Acoustic Properties of Skin and Wounds. Olerud JE, O'Brien WD Jr, Riederer-Henderson MA, Steiger DL, Debel J, Odland GF, Ultrasound Med Biol 16:55-64, 1990.

Ultrasonic Assessment of Skin and Surgical Wounds Utilizing Backscatter Acoustic Techniques to Estimate Attenuation. Forster FK, Olerud JE, Riederer-Henderson MA, Holmes AW, Ultrasound Med Biol 16:43-53, 1990.

Ultrasonic Propagation Properties of Articular Cartilage at 100 MHz. Agemura DH, O'Brien WD Jr, Olerud JE, Chun LE, Eyre DR, J Acoust Soc Am 87:1786-1791, 1990.

[621] Diabetic Foot Ulcers: Quantifying the Effects of Nonsurgical Treatments

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Sponsor: *VA Rehabilitation Research and Development Service (Project #A318-2RA)*

Purpose—The purpose of this study was to describe the natural history of healing of diabetic foot ulcers and observe whether specific medical treatments might substantially improve the rate of healing. This required developing an objective method to quantitate the healing progress of cutaneous ulcers. We enrolled subjects and characterized them extensively with regard to aspects of their particular ulcer, status of diabetes, and a variety of physiological measurements, including nerve function and circulation. Patients were randomized to receive, in addition to standard treatment, intensive diabetes management for optimal control of diabetes, nutritional supplementation with zinc and ascorbic acid, and standard medical surgical treatment alone (control).

Methodology—Subjects who presented for treatment of lower extremity ulcers in the presence of diabetes were randomized prospectively to receive the various medical treatments identified above. Subsequently they were followed on a weekly basis in the outpatient setting with measurements which allowed calculation of the rate of ulcer healing. Subjects were followed until either total healing or an alternative definitive medical outcome such as amputation, osteomyelitis, requirement for surgical revascularization, or death occurred. Vascular testing was performed on all subjects, including measurements of transcutaneous oxygen and carbon dioxide tensions, and segmental total Doppler blood pressures and toe blood pressures of the affected extremity. The rate of ulcer healing was quantified over a defined 4-week initial period of treatment, according to a method of tracing the ulcer contours sequentially and photography of the lesions.

Results—Over 100 diabetic individuals with foot ulcers were studied. A subgroup of 46 subjects who had full-thickness skin ulcers completed the protocol and characteristics of wound healing were described. Calculated rates of tissue repair were found to be from 10 to 50% as rapid as published rates for wound healing in people without diabetes. Overall, 83% eventually achieved reepithelialization with the remaining 17% failing to heal for a variety of reasons. Many factors were examined for the possibility that they might predict nonhealing. Particular demographic factors, specific aspects of diabetes such as type or level of glucose control, initial ulcer size, or presence of initial infection were not useful for predicting subsequent healing failure. Significant prediction of failure of tissue repair, however, was obtained by initial

measurements of transcutaneous oxygen and transcutaneous carbon dioxide at the periwound site. These measures of local skin perfusion appear to be much more sensitive and specific for predicting ulcer healing than traditional measurements of limb arterial blood pressure.

Recent Publications Resulting from This Research

Classification of Wounds in Diabetic Amputees. Pecoraro RE, Reiber GE, Wounds 2:65-73, 1990.

Dissociation of Skin Oxygenation from Arterial Blood Pressure in Diabetic Limb Amputation. Pecoraro RE, Reiber GE, in Proceedings of the European Association for the Study of Diabetes, Copenhagen, Denmark, 1990.

Pathways to Diabetic Limb Amputation. A Basis for Prevention. Pecoraro RE, Reiber GE, Burgess EM, Diabetes Care 13:513-521, 1990.

Tissue Repair, Clinical Outcomes and Associations with Healing Failure in Diabetic Foot Ulcers. Pecoraro RE, Ahroni JH, Stensel V, Diabetes 39(Suppl 1):157A, 1990.

[622] Bone-Derived Cells Produce a Chemotactic Factor

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Sponsor: *National Institute of Dental Research, National Institutes of Health*

Purpose—Monocytes arise from stem cells in the bone marrow, enter the circulation, and undergo final maturation to macrophages in peripheral tissue. Monocytes/macrophages are essential to wound healing as demonstrated by delayed or incomplete wound healing in animals depleted of monocytes. The inflammatory, proliferative, and regenerative phases of wound healing require the participation of monocytes/macrophages either through their phagocytic or secretory function. Particularly important is the secretion of growth-promoting factors which are capable of stimulating cellular proliferation and angiogenesis. Monocytes/macrophages may also support the growth of solid tumors through the production of paracrine and angiogenesis factors. Since the majority of monocytes/macrophages which infiltrate a wounded site or a solid tumor are recruited from the peripheral vasculature, factors which regulate monocyte chemotaxis are of considerable importance. The goal of this proposal is to study a monocyte chemoattractant, CF-O, produced by a cell line derived from an osteogenic sarcoma. The proposed studies include deducing the complete amino acid sequence of CF-O through characterization of the CF-O, cDNA, studying transcriptional, translational, posttranslational, and secretory events in the production of CF-O, and describing its binding kinetics and stimulation of monocytes/macrophages. The constitutive synthesis of CF-O by a bone-derived cell line provides an opportunity to study a monocyte chemoattractant that may be important in osseous wound healing and in the growth of osseous tumors. This factor may provide insight into the potential control of monocyte chemotaxis by locally produced chemoattractants.

Recent Publications Resulting from This Research

Identification of Monocyte Chemotactic Activity Produced by Malignant Cells. Graves DT et al., Science 245:1490-1493, 1989.

Mesenchymal Cell Growth Factors. Graves DT, Cochran DL, CRC Rev Oral Biol Med 1:17-36, 1990.

Expression of Transferrin and Vitamin D-Binding Protein Genes in Osteogenic Sarcoma Cell Lines. Adrian GS et al., Exp Cell Res (in press).