ANNUAL PROGRESS REPORT

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GRANT TITLE: The Effect of Cementation and Autogenous Bone Grafting on Allograft Union and Incorporation (grant no.: N00014-93-1-0745)

REPORTING PERIOD: 1 Jan 1996 - 31 Dec 1996 (12 months)

AWARD PERIOD: 1 May 1993 - 30 April 1996

OBJECTIVE: To investigate six methods of allograft bone implantation stabilized by an interlocking nail; to determine the optimal method for implanting allograft bone with an interlocking nail following significant segmental bone loss.

APPROACH: Initial biomechanical data are obtained from studies with cadaveric bone and idealized allogafts (2.5-cm in length) stabilized with an interlocking nail (ILN) in femora obtained from mature beagle dogs. The bones are divided into 5 groups of 6 each: intact controls, 5.0-mm diameter ILN fixation, 5.0-mm ILN fixation plus intramedullary PMMA cement surrounding the nail in the allograft segment, 6.0-mm ILN fixation, and 6.0-mm ILN fixation plus intramedullary cement.

The incorporation of segmental allograft bone is studied in a mature beagle dog model. The 25 dogs (donor plus 24 treated) are immunologically mismatched for sequential transfer of allograft segments. Pre-operatively, the dogs are evaluated by radiography, dual energy X-ray absorptiometry (DXA), and force plate gait analysis. Allograft segments are harvested bilaterally, double frozen in liquid nitrogen, and stored at -80°C until transplantation to the next dog. Allografts are stabilized with an ILN. The six treatments are variously paired to yield the following groups overall: 1-ILN fixation (n=11), 2-ILN plus intramedullary cement (n=11), 3-ILN plus cement and autogenous cancellous bone graft periosteally at the host-allograft junction (n=6), 4-ILN plus cancellous graft periosteally at the host-allograft junction (n=7), 5-ILN plus cancellous graft within the medullary canal of the allograft (n=7), and 6-ILN plus cancellous graft periosteally at the host-allograft junction and within the medullary canal of the allograft (n=6). Post-operative radiographs, DXA scans, and gait analyses are serially performed over 6 months. The dogs are euthanatized at 6 months, the femora harvested, the nails removed, and biomechanically tested. The bones are then fixed in ethanol for histologic processing. Porosity, new bone formation and allograft bone incorporation are evaluated.

ACCOMPLISHMENTS (last 12 months): All data analysis for this study has now been completed. Biomechanical results from cadaveric specimens were previously reported. Biomechanical testing of bones from the *in vivo* study indicated a strength advantage globally for groups 1, 3, and 6 listed above ($P<0.05$). Locally, at the osteotomies, group 3 had greater stiffness. Overall, there was greater stiffness at distal osteotomies than proximal. At 4 weeks after surgery, the 3 groups with autogenous cancellous bone added to the periosteal surface (groups 3, 5, and 6) had greater callus area than the 3 groups without added cancellous bone (groups 1, 2, and 4)($P<0.05$). There was no difference between groups at 12 weeks. There was no difference in bone mineral density of femora between groups at 12 or 24 weeks after surgery. All groups lost density at the femoral head, perhaps resulting from reduced activity. There was

no difference in peak vertical ground reaction forces between groups. However, all groups had a significant reduction in peak vertical force at 4 weeks after surgery and had returned to pre-operative levels by 12 weeks after surgery. Bone union was greater at lateral and medial quadrants than cranial and caudal quadrants ($P<0.05$). Comparing groups, there was greater complete union with group 6 than 2 at the proximal osteotomy and with group 5 than 2, 3, or 4 at the distal osteotomy. Overall there was greater partial union at the distal site than proximal (85.7% compared to 69.4%; $P<0.05$). Group 6 resulted in greater bone porosity in periosteal and endosteal regions than other groups. New bone formation in mid-cortical and endosteal regions of the allograft was reduced compared to adjacent host bone. Within the allograft, there was no difference between groups for new bone formation. In adjacent host bone, groups 5 and 6 had more new periosteal bone than groups 2 or 4. Tissue at the osteotomies had greater bone content with groups 3 and 6 and more fibrous tissue with group 2. There was more bony tissue at the distal osteotomy than proximal, and more in lateral and medial quadrants than cranial and caudal. The mean remaining gap at the osteotomies was greatest with group 2 and least with group 6.

SIGNIFICANCE: The *in vivo* results indicate the addition of intramedullary cement by itself (group 2) is not advantageous, despite cadaveric data suggesting superior biomechanic properties. By itself, the addition of cement results in decreased bony tissue and increased fibrous tissue at the osteotomies. There is also greater gap size remaining at 24 weeks. However, adding autogenous cancellous bone to the periosteal surface of bone with intramedullary cement (group 3) results in superior mechanical properties, no decrease in union rate, and improved histologic scores. The combination of autogenous cancellous bone added to endosteal and periosteal surfaces (group 6) has the greatest amount of remodeling activity, demonstrated by new bone formation, increased porosity, and the smallest remaining gap size. Additionally, biomechanical properties with this group are not significantly different from those with group 3. These results have potential significance for the long term incorporation of allograft bone.

WORK PLAN (next 12 months): Present abstracts from the *in vivo* study at national meetings (International Society of Limb Salvage, Orthopaedic Research Society, and American College of Veterinary Surgeons) and submit manuscripts based on this project. Also, the project is a major portion of a doctoral dissertation that will be defended spring, 1997.

PUBLICATIONS, REPORTS AND ABSTRACTS (last 12 months):

1. Hanson PD, Warner CG, Frassica FJ, Markel MD. Effects of cancellous bone and bone cement on segmental allograft strength. *Vet Surg* 1996;25:425-426. *(abstract)*

   Presented at (a) the 31st Annual Scientific Meeting, American College of Veterinary Surgeons, November, 1996; (b) as a seminar to Genetics Institute (Cambridge, MA), May, 1996; (c) as a seminar to Merck Research Laboratories (Rahway, NJ), June, 1996.

2. Hanson PD, Frassica FJ, Warner C, Vanderby R, Markel MD. An *in vivo* study on the effect of intramedullary bone cement and autogenous cancellous bone on segmental allograft/host bone strength. Accepted by the Orthopaedic Research Society for the 43rd Annual Meeting, February, 1997.

artery during dissection. Viability of bone in the myo-osseous flap was questionable on postoperative scintigraphy, but successful incorporation of the graft occurred within 16 weeks.

Resolution of the primary problem was achieved in 14 dogs. Two dogs with osteomyelitis developed progressive disease necessitating amputation. Donor site morbidity was limited to seroma formation in four dogs, and superficial wound infection in one dog.

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CESARIAN SECTION IN THE MARE: A COMPARISON WITH OTHER METHODS FOR CORRECTING DYSTOCIA AND A COMPARISON OF METHODS FOR HYSTEROTOMY CLOSURE. DE Freeman,* JK Johnston,* GJ Baker,* L Hungerford, T Lock, P Sertich. University of Illinois, Urbana, IL; and New Bolton Center, Kennett Square, PA.

This study compares cesarian section with other methods for correcting difficult dystocias in mares and to assess the need for a hemostatic suture for hysterotomy closure. Data from 116 mares treated for dystocia or that had a cesarian section at two university hospitals were analyzed in five groups, as follows: dystocia corrected by cesarian section was group DCS (n = 48); elective cesarian section was group ECS (n = 10); cesarian section at the same time as colic surgery was group CCS (n = 8); assisted vaginal delivey was group AVD (n = 22); and controlled vaginal delivery under general anaesthesia was group CVD (n = 28). Data was examined by analysis of variance and chi square statistics with significance set at $P < .05$. All mares in group ECS survived and survival rate in group CCS was 38%. Survival rate in all mares that had cesarian section, excluding group CCS, was 88% (51/58). In mares with dystocia, groups DCS (85%) and AVD (86%) had the highest survival rates and group CVD had the lowest (71%). Of the 66 mares that had a cesarian section, the hemostatic suture was used in 31 mares (47%). Three mares that had a hemostatic suture (10%) and two mares that did not (6%) had severe uterine hemorrhage within 12 hours after surgery. Two mares that did not have a hemostatic suture died within 18 hours after surgery from uterine hemorrhage, and these represented 6% of mares that did not have this suture. Cesarian section is a safe method for correcting difficult dystocias in mares and should not be regarded as a last resort. The hemostatic suture could be omitted under certain circumstances; however, if it is not used, the hysterotomy should be closed with full thickness sutures that are sufficiently tight to compress vessels in the uterine wall.

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SYNOVITIS AND CORTICOSTEROIDS ALTER TRANSCRIPTION OF ARTICULAR CARTILAGE MATRIX PROTEINS. SL Fubini,* RJ Todhunter,* DN Gu, JN MacLeod. Cornell University, Ithaca, NY.

Objective. Determine how the transcription of type II procollagen, aggrecan core protein, and fibronectin by chondrocytes in articular cartilage is affected during acute synovitis and after a single intra-articular injection of the corticosteroid methylprednisolone acetate.

Methods. Acute synovitis was induced in 10 ponies by repetitive intra-articular injection of 0.5 ng lipopolysaccharide. Comparisons were made between four experimental joint groups: control, LPS-induced acute synovitis, corticosteroid treated, and LPS-induced acute synovitis with corticosteroid treatment. Total RNA was isolated directly from the cartilage tissue and steady state levels of type II procollagen, aggrecan core protein, and fibronectin mRNA were compared by Northern blot analysis using equine cDNA probes.

Results. Acute synovitis significantly ($P < .05$) increased steady state levels of type II procollagen mRNA in articular chondrocytes. A single intra-articular corticosteroid injection, however, decreased levels of type II procollagen transcripts approximately six-fold. Levels of mRNA encoding aggrecan core protein were increased two-fold by LPS injections, but were not significantly different in the other three experimental groups. No quantitative changes in total fibronectin expression were observed.

Conclusion. Synovitis and corticosteroid therapy induced gene-specific changes in steady-state mRNA levels of cartilage matrix proteins in articular chondrocytes. The transcription of type II procollagen was found to be more sensitive than aggrecan or fibronectin, consistent with an important role in the pathogenesis of osteoarthritis.

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RENAL ALLOGRAFT REJECTION IN THE CAT: A TEMPORAL AND PATHOLOGICAL DESCRIPTION. CR Gregory,* SM Griffey, LS Yeh, RE Morris. Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA.

Introduction: Although clinical renal allograft transplantation for the cat has been performed for almost 10 years, renal allograft rejection has never been described. In addition, large animal models of organ rejection are essential for the development of new immunosuppressive agents. This study evaluates the temporal, histopathologic, and immunopathologic pattern of renal allograft rejection in the cat.

Methods: Two groups of five, adult, unrelated domestic shorthair cats were obtained from two separate breeding facilities. Heterotopic renal transplantation, with bilateral native nephrectomy, was performed between unrelated pairs. Body weight, packed red cell counts, and serum creatinine levels were determined daily. Euthanasia was performed when the serum creatinine was ≥8 mg/dL, or a cat lost ≥20% of its body weight.

Results: Four cats were excluded from the study: three received kidneys from donors that were approximately half their body weight, and one donor cat had multiple renal arteries bilaterally. Six cats were euthanatized at 18.5 ± 7.8 days when serum creatinines became ≥8 mg/dL; serum creatinine had returned to normal in five of six cats. Body weights remained constant, and graft rejection was accompanied by mild depression and hypothermia. Histopathologically, the allografts revealed a marked to severe diffuse interstitial and perivascular rejection response consisting of CD3+, CD4+, and CD8+ lymphocytes and macrophages. There was tubular necrosis and thickened glomerular membranes.

Discussion: Cats reject renal allografts from unrelated donors in approximately 18 days with histological features identical to that seen in humans and dogs. Cats appears to be an excellent large animal models of renal allograft rejection.

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CENTRAL NERVOUS SYSTEM DISORDERS FOLLOWING RENAL TRANSPLANTATION IN CATS. CR Gregory,* KG Mathews,* LR Aronson, RA Lirtzman, EJ Kochin,* TW Broaddus, IMG Gourley.* Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA.

Introduction: Central nervous system (CNS) disorders have become more frequent during the postoperative period following renal transplantation in cats. This study describes the incidence and severity of postoperative CNS disorders (pCNSD) in feline renal transplant recipients.

Methods: The medical records of feline renal transplant recipients receiving allografts from 1987 to 1996 were reviewed for the frequency, duration, and severity of pCNSD.

Results: Twelve (20%) of 58 patients had pCNSD within 1 hour to 4 days postoperatively. Six cats survived; four had single or multiple seizure episodes, and two had seizure episodes followed by a period of coma. Six cats died; all had episodes of disorientation and/or seizures that progressed to a fatal comatose state, respiratory arrest and/or cardiac arrest. Forty-six patients had no pCNSD after transplantation. There was no significant difference between the two groups in preoperative serum creatinine, blood urea nitrogen, cholesterol, blood pressures, immediate postoperative serum glucose levels, or electrolytes. Preoperative cyclosporine levels were higher in the group with no pCNSD, while a greater percentage (58% vs 28%) of the cats with pCNSD had received human recombinant erythropoietin prior to surgery.

Discussion: pCNSD is a common and often fatal complication of renal transplantation in cats. The cause is probably multifactorial.

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EFFECTS OF CANCELLOUS BONE AND BONE CEMENT ON SEGMENTAL ALLOGRAFT STRENGTH. PD Hanson,* CG Warner, FJ Frassica, MD Markel.* Comparative Orthopaedic Research Laboratory, University of Wisconsin, Madison, WI.

Complications of segmental allograft bone replacement occur in 10% to 15% of cases. This study evaluated the biomechanic effects of adding bone cement to the allograft medullary canal in an in vitro and in vivo segmental replacement model in the dog. Additionally, we evaluated the biomechanical effects of 3 adjunct autogenous cancellous bone graft methods on the construct in an in vivo model.

In Vitro Study. Idealized allografts in the femoral diaphysis from 24 mature beagle dogs were stabilized with an interlocking nail (ILN) technique, divided into groups of 6 each: 5-mm diameter ILN, 5-mm ILN plus intramedullary bone cement, 6-mm ILN, and 6-mm ILN plus bone cement. Six intact bones served as controls. Bones were tested nondestructively in the following modes: axial compression, mediolateral (ML) and craniocaudal (CrCa) bend-

ing, and torsion. Torsion to failure was then performed. Stiffness was calculated and the torque and rotation at failure recorded.

In Vivo Study. Twenty-four mature beagle dogs received bilateral frozen allograft replacement of the femur. Interlocking nail diameter was chosen to fit the medullary canal. Treatments were randomly assigned: 'N' = ILN alone (n = 10), 'NC' = ILN plus intramedullary cement (n = 10), 'NCP' = ILN plus cement and periosteal autogenous cancellous bone (n = 7), 'NP' = ILN plus periosteal cancellous bone (n = 7), 'NE' = ILN plus endosteal cancellous bone (n = 7), and 'NPE' = ILN plus periosteal and endosteal cancellous bone (n = 7). Dogs were euthanatized 6 months after surgery, the bones harvested, the ILN removed, and mechanically tested as above. Statistical analysis was performed using ANOVA on raw data and data normalized to intact bone. Significance was set at $P < .05$.

In vitro bones with 6-mm nails or bone cement had increased global stiffness. These results may have importance during the early stages of bone incorporation and later during allograft remodeling. For the in vivo study, overall biomechanic analysis indicated that groups N, NCP, and NPE had significantly greater global stiffness than groups NC, NE, and NP. The results suggest that optimal mechanical properties at 6 months are achieved with ILN alone, ILN plus intramedullary cement and periosteal cancellous bone graft, or ILN plus periosteal and endosteal cancellous bone graft.

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SURGICAL REDUCTION OF ILEAL IMPACTIONS IN THE HORSE: 29 CASES. RR Hanson,* JC Wright, AN Baird,* J Schumacher, J Humburg, DG Pugh. Auburn University, Auburn, AL.

Ileal impaction is the most frequently reported cause of small intestinal nonstrangulating obstruction in adult horses. This study evaluates time of year, signalment, clinical signs, physical examination findings, and surgical outcome for ileal manipulation and/or jejunocecostomy for horses with ileal impaction.

Medical records from 449 horses with colic between January 1988 and December 1993 were examined. Ileal impaction requiring surgical intervention was diagnosed in 29 of 449 (6.5%) horses. Mean duration of clinical signs of abdominal pain prior to referral was 16 hours. At admittance, signs of abdominal pain were mild in 4 (14%) horses, moderate in 19 (66%), and severe in 6 (21%). Transrectal palpation was used to localize the impaction in 11 of the horses (38%). Generalized moderate distention of the small intestines was present in 20 (69%) horses and nasogastric reflux was present in 15 (52%) of the horses. There was an increased risk for ileal impaction during the fall of the year (95% confidence interval on odds ratio = 1.11 – 4.37).

Thirty celiotomies were performed in 28 horses. Twenty-six horses were recovered from surgery, of which 23 (88%) experienced no postoperative complications. The impaction of the ileum was reduced by extraluminal massage alone in 23 of 26 horses (88%). Saline solution (1,500 mL) containing 60 mL of dioctyl sodium sulfosuccinate was infused into the mass in two horses in which dilution and massage alone were unsuccessful in reducing the impaction. All surviving horses returned to previous use. Analysis of results of examinations performed at the time of admission indicated no significant differences ($P > .05$) for temperature, heart rate, respiratory rate, nasogastric reflux, distention of the small intestine, palpable ileal impaction, hematocrit, and total protein concentration between horses that lived versus horses that died or were euthanized.

In this study, 23 of 26 horses (88%) were treated successfully with extraluminal massage for reduction of the impaction. A jejunocecostomy is not necessary as treatment of most cases of simple ileal impaction because of the possibility of complications associated with the anastomosis.

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PRESSURE-VOLUME RELATIONSHIPS IN NORMAL EQUINE MIDCARPAL JOINTS. J Hardy,* AL Bertone,* WW Muir. The Ohio State University, Columbus, OH.

Joint effusion and increased intraarticular pressure (IAP) is a common finding in joint disease and can contribute to articular damage by decreasing blood flow and increasing pain. This study investigated the pressure-volume relationships of normal equine midcarpal joints and determined the influence of synovial fluid as compared to saline on these relationships.

Horses (n = 24, 48 midcarpal joints) were placed in dorsal recumbency and the four midcarpal joint compartments were cannulated; fluid (saline or synovial fluid) was infused or withdrawn through the dorsal joint pouch. PV relationships were described for IAP up to 80 mm Hg, and the effects of joint angle, nature of infusate, compartmentation, time-dependence and history-dependence were investigated.

The sigmoid PV curves were best described by $IAP = A \times e^{(B \times volume)} - C$, where B is the fractional change in pressure per unit of volume (Elastance), and A and C are constants. Compartmentation was not observed. Elastance was greater at sub- than supra-atmospheric pressures, at 90° than 135° angles and with synovial fluid than saline. Hysteresis (area under the infusion-withdrawal curves) was greater at 90° than 135° angles and with synovial fluid than saline. Elastance increased with sequential distentions at high IAP. Stress relaxation was a positive logarithmic relationship to IAP.

These findings illustrate that the joint is more compliant with synovial fluid than saline and emphasizes the role of joint angle, prior distention cycles and decay of IAP with time in normal articular PV relationships. The greater hysteresis present with synovial fluid may help maintain the normal negative IAP, which favors fluid exchanges.

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BLOOD FLOW, PERMEABILITY, O_2 METABOLISM AND CELLULAR RESPONSE OF INNERVATED OR DENERVATED ISOLATED JOINTS IN AN IL-1 MODEL. J Hardy,* AL Bertone,* WW Muir, SE Weisbrode. The Ohio State University, Columbus, OH.

Articular inflammation may increase oxygen demand, resulting in articular hypoxia, thus contributing to tissue injury. Altered permeability may modify transarticular fluid exchanges. Joint innervation may modulate the inflammatory response. This study investigated oxygen metabolism, permeability, blood flow and cellular responses in an Il-1 model of acute articular inflammation.

An isolated auto-oxygenated pump-perfused metacarpophalangeal joint preparation was performed in one metacarpophalangeal joint of 24 horses and assigned to one group (n = 6/group): control; control-denervated; inflamed; inflamed-denervated. Inflammation was induced by intra-articular injection of 0.35 ng/kg of rhIl-1β. Oxygen delivery (DO_2), consumption (VO_2), and extraction ratio (ER) were calculated from arterial and venous blood gases. Blood flow (BF) to the synovial membrane was determined by colored microspheres at 0, 1, and 5.5 hours. Permeability to macromolecules was determined by intra-articular injection of Evans blue albumin (MW:68,000) and FITC-Dextran (MW:144,000) Synovial fluid WBC was determined at 0, 4, 5 and 5.5 hours and synovial membrane inflammation was determined by scoring of histopathology specimens.

Synovial fluid (SF) WBC ($P = .001$) and neutrophilic vasculitis scores ($P = .017$) were significantly higher in inflamed groups. DO_2 and synovial membrane BF progressively increased but were not different among groups. VO_2 ($P = .012$) and ER ($P = .049$) were significantly increased in inflamed groups. Permeability to albumin was significantly increased in the villous synovial membrane of the inflamed groups ($P = .025$), and dextran permeability was increased in the innervated groups ($P = .024$), with a trend to increased permeability in inflamed groups ($P = .082$).

Interleukin 1 caused increased SF and synovial membrane WBC trafficking. Permeability to small molecules was increased with inflammation, whereas denervation decreased permeability to large molecules. This study documented increased articular oxygen demand with inflammation, which may not be met if joint effusion results in decreased blood flow.

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CHANGES IN MATERIAL PROPERTIES OF THIRD METACARPAL CORTICAL BONE DURING PREGNANCY IN THE MARE. DL Hawkins,* SM Stover.* University of Florida, Gainesville, FL.

Introduction: Although there do not appear to be large changes in skeletal mass or density during normal pregnancy, pregnancy-associated changes in bone turnover or mass occur and may be associated with biomechanical failure. The objective of this study was to investigate the effect of late gestation, age, and parity (number of live foals) on material properties of third metacarpal cortical bone in the mare and to determine whether these properties may increase the risk of fracture in mares in late gestation compared to nonpregnant mares.

Methods: Mechanical and microradiographic properties of third metacarpal (MCIII) dorsal cortical bone specimens from 8 healthy, postpartum mares (treatment group) that died or were euthanatized within 24 hours after parturition due to foaling complications were compared with those of 6 age-matched, healthy, nonpregnant mares (control group). The dorsal half of transverse mid-diaphyseal sections of MCIII bones was divided into lateral, dorsal, and medial regions for all testing and analyses.

Results: Significant differences were not found for any mechanical testing variable between control and postpartum mares when all regions were consid-

AN *IN VIVO* STUDY ON THE EFFECT OF INTRAMEDULLARY BONE CEMENT AND AUTOGENOUS CANCELLOUS BONE ON SEGMENTAL ALLOGRAFT/HOST BONE STRENGTH

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Relevance to Musculoskeletal Conditions: Complications of segmental allograft bone replacement include fracture of the allograft and non-union. This study addresses the outcome 6 months after implantation of methods to improve construct stability and strength.

Introduction: Complications of segmental allograft bone replacement, including fracture of the allograft and non-union at the host/allograft interface, occur in 10 to 15% of cases. The aim of this study was to examine the biomechanical effects of adding bone cement to the allograft medullary canal and 3 supplementary autogenous cancellous bone graft methods in an *in vivo* segmental replacement model in the dog.

Methods: Under an institutional review board approved protocol, 24 mature beagle dogs, weighing 10-15 kg each, were randomly divided for bilateral frozen allograft replacement of the mid-diaphysis (2.5-cm) of the femur. In all cases, allografts were from immunologically mismatched donors. Allograft stabilization was performed with an interlocking nail (ILN) technique, using two 2.7-mm screws proximal and distal to the allograft. Nail diameter (5 or 6 mm) was chosen to fit the medullary canal. Treatments were randomly assigned to left and right legs as follows: group 'n' = ILN alone (n=10), 'nc' = ILN plus intramedullary cement (n=10), 'ncp' = ILN plus intramedullary cement and autogenous cancellous bone placed periosteally (n=7), 'np' = ILN plus cancellous bone placed periosteally (n=7), 'ne' = ILN plus cancellous bone placed within the allograft (n=7), and 'npe' = ILN plus cancellous bone placed periosteally and within the allograft (n=7). In all groups, dogs were euthanatized 6 months after surgery. The bones were harvested immediately, the ILN removed, and potted for mechanical testing. The bones were tested in an MTS 858 Bionix Test System with flex control grips. Extensometers were placed across opposite sides of each osteotomy to measure local deformation. The bones were tested non-destructively through 5 cycles under load control in each of the following modes: axial compression (50 N load), mediolateral and craniocaudal bending (±2.0 N•m moment), and torsion (±2.5 N•m torque). Torque to failure was then performed at a rate of 1.5°/sec. Stiffness was calculated for each test and the torque and rotation at failure recorded.

Statistical analysis was performed using ANOVA. Significance was set at $P<0.05$. When significance was present, means were separated by Duncan's multiple range test.

Results: To allow comparison between test modes, stiffness was expressed as the percent of intact bone. Constructs were significantly weaker than intact bone, and torsion was weaker than other test modes. Overall global stiffness, comparing across all test modes, resulted in groups ncp, npe, and n having significantly greater stiffness than groups nc, ne, or np. (Figure 1).

Proximal and distal osteotomy stiffness was normalized to intact bone at the same location and expressed as the percent of intact bone. Overall, group ncp was significantly stiffer than all groups except group npe (Figure 2). Evaluated by test mode, a significant difference between treatments was present in axial compression and craniocaudal bending that corresponded to the overall results.

Discussion: At 6 months post-implantation, the addition of cement by itself did not improve stiffness. However, combined with periosteal cancellous bone graft, a significant increase in local stiffness was achieved. Adding cancellous bone to both the periosteal and endosteal surface was also beneficial, but had no increase in stiffness over an ILN without adjunct treatment. Histologic results of the treatments studied will be presented at a future date to provide further insight into the effects of bone cement and cancellous bone on allograft incorporation.

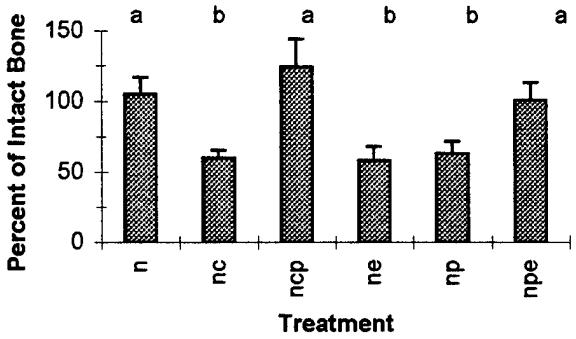

Figure 1. Overall global stiffness of bone constructs (with ILN removed) 6 months after implantation (mean ± sem). See 'Methods' for treatment abbreviations. Letters above columns that differ indicate significance ($P<0.05$).

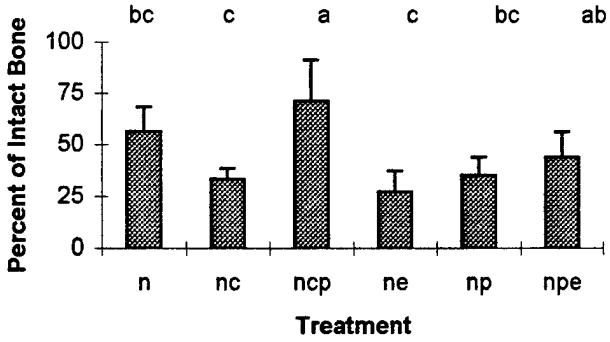

Figure 2. Overall local (osteotomy) stiffness of bone constructs (with ILN removed) 6 months after implantation (mean ± sem).

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