

Third Scandinavian Meeting on Orthopedic Research

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The First Scandinavian Meeting on Orthopedic Research was arranged in Stockholm in 1987. The participants also discussed the conditions for experimental orthopedics in the Nordic countries, and the need for a separate forum for orthopedic research was stressed. The second meeting took place in Århus, Denmark, the following year, preceding the biennial assembly of the Scandinavian Orthopedic Association.

The present meeting in Oslo was a separate meeting. The papers covered cytokins and growth factors, fracture healing, biomechanics, biocompatibility of implants, leg lengthening and diaphyseal defects, bone mineral analyses, scoliosis, and some other topics.

The Fourth Scandinavian Meeting on Orthopedic Research will occur in Helsinki in June 1990 preceding the 45th Assembly of the Scandinavian Orthopedic Association.

Cytokins and growth factors

Cytokins, possible mediators of bone resorption in prosthetic loosening

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Cytokins are locally produced hormonal substances by inflammatory cells. In a pilot study, large amounts of cytokin has been found in membranes removed from the interface during reoperations.

A mouse-calvaria *in vitro* system was used in which paired bone pieces were cultured for 2 days, and the release of previously incorporated calcium 45 was recorded. The results were expressed as a ratio between the control and the treated bone.

A dose-dependent increase in calcium-45 release was re-

corded in response to $11-1 \beta$ with blocking of the effect by calcitonin. The rise in relative calcium release could be enhanced by incubation with indomethacin, removing the effect of internally produced prostaglandin as was seen with $11-1 \alpha$. This also indicates that $11-1$ does not function through prostaglandin. By culturing for 3 days, a 6-fold increase in calcium release could be calculated in response to $11-1 \alpha$. The maximum effect obtained with 11 closely matched the effect possible with 1.25 vitamin D. Tumor necrosis factor (TNF) alone had only a small increasing effect on the calcium release; but combined with $11-1 \alpha$ or $11-1 \beta$, a significant rise compared with the substances alone was recorded, whereas the effect of 1.25 vitamin D was not significantly increased by TNF.

In vitro $11-1$ alone or combined with TNF has a bone-resorbing effect, which is as powerful as 1.25 vitamin D.

Bone induction causes resorption of bone matrix

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Background: Recent research on bone metabolism has focused on the importance of locally produced and locally acting factors in bone. Substances such as prostaglandins, cytokins, growth factors, and morphogens appear to play important roles in the regulation of growth, turnover, and healing of bone. Bone matrix contains an abundance of such factors, many of which appear to be specific for this tissue, i.e., skeletal growth factor and bone morphogenetic protein.

We wanted to study if inductive demineralized bone matrix (DMB) is resorbed during bone induction to allow release of inductive and growth factors. Utilizing the model of DBM-induced heterotopic bone formation in rats, we followed the release of isotopes from prelabeled DBM during new bone formation and studied the effects of inhibition of new bone formation by indomethacin on the resorption of DBM.

Methods: Growing (90 g) Sprague-Dawley rats were injected with ^3H -proline (10 $\mu\text{Ci}/\text{kg}$) every second day for 10 days. DBM was prepared from the long bones by demineralization, defatting, and lyophilization. The implants weighing 5–7 mg contained about 500 CPM/mg dry wt. Four pieces of DBM were implanted in the abdominal wall in each of 42 rats. The animals were divided into 6 groups of 7 rats; half were treated with daily s.c. injections of indomethacin (2 mg/kg) and half were treated with saline. The animals were killed 10, 20, and 30 days after implantation. The implants were demineralized, lyophilized, weighed, hydrolyzed, and analyzed for ^3H activity. Calcium content of the implants was determined by atomic absorption flame photometry. The ^3H values were calculated as a percentage of the values at implantation.

Results: Histologic studies revealed cartilage formation in and around the implant at 10 days. At 20 and 30 days, the cartilage was replaced by small areas of new bone. There were no qualitative differences between controls and indomethacin-treated rats.

The implants of DBM contained no calcium at implantation, but mineralization started within 10 days, evidencing new bone formation. Mineral accretion was lower in implants from rats treated with indomethacin. At 20 and 30 days, implants from indomethacin-treated rats contained 25 percent less calcium than implants from controls ($P < 0.05$).

Discussion: New bone formation in heterotopic implants of DBM is accompanied by release of ^3H from prelabeled DBM. When new bone formation is inhibited by treatment with indomethacin, the release of ^3H decreases to a similar extent. Furthermore, induced cartilage and new bone formation occurs in and around the implant of DBM. This implies that DBM is resorbed during induced new bone formation. In this process regulatory factors may be released from the matrix to cause further bone induction.

Immune effects on yield of new bone from implants of partially purified bovine BMP in dogs

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Bone grafting is commonly applied in orthopedic surgery. The preferred graft is autologous cancellous bone, but in many instances the sources of autologous bone are not enough to meet the demands, and the additional trauma of procurement of autologous bone is disadvantageous for the patient. Recent experimental and clinical studies indicate that bone-inductive substances extracted from bone matrix are highly effective in the treatment of bone defects. However, immunologic reactions appear to be of great importance for

all grafting procedures.

Aim: The aim of the present study was to analyze the effects of repeated implants of partially purified xenogeneic bone morphogenetic protein (BMP) on the yield of new bone in an orthotopic site. Repeated trephine defects in mongrel dogs were implanted with bovine BMP and a graded bone inductive, and antibody response was monitored.

Methods: Bovine BMP was prepared from diaphyseal bovine bone and partially purified. The preparation contained several noncollagenous proteins as determined from PAGE. The bone inductive capacity of the preparation was ascertained by heterotopic implantation in mice. Samples of 50 mg bBMP were weighed and packed into gelatin capsules. Trephine skull defects (14-mm diameter) were created in 9 mongrel dogs. In 6 dogs (Group A) the first trephine defect was implanted with 50 mg bBMP followed by an identical operation on the contralateral side 3 weeks later. In 3 dogs (Group B) the first defect was implanted with bovine serum albumin (BSA) and the second with bBMP. The animals were killed 4 to 5 months after the second trephine. The healing of the defects was observed by radiography and histomorphometry. Serum antibodies to BMP were determined by RIA preoperatively, at the second defect, and again 12 weeks later.

Results: Group A was designed to study the effects of a previous implant of bBMP on the healing of a second defect implanted with bBMP. The healing on the first trephine ranged from 100 percent to 88 percent, while the second ranged from 71 percent to 56 percent. The mean difference was – 34 percent.

In Group B the first implant of BSA showed the amount of spontaneous healing and averaged 43 percent. The healing of the bBMP-implanted second trephine ranged from 100 percent to 72 percent.

The titers of serum bBMP antibody were significantly elevated after implants of bBMP, but not after implants of BSA.

Conclusions: The present study shows that an immune response directed towards implants of partially purified bovine BMP affects BMP-induced bone formation in dogs. While xenogeneic bBMP increased the yield of new bone to cause a high percentage of healing of trephine defects in dogs when implanted without previous immunization, the inductive properties of a second set implant were partially inhibited by an immune response.

Local application of IGF-1 to healing bone inside a titanium chamber in vivo

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In order to study the effects on bone healing, insulin-like growth factor I (IGF-I) was continuously applied with a mini-

pump to a healing bone callus located inside a titanium chamber in rabbit tibiae. After 2 weeks, the amount of new bone formed in the chambers was measured as calcium content. Also, its ^{99m}Tc -MDP uptake was measured.

Result: The IGF-I treated side had lower ^{99m}Tc -MDP uptake than the control side. Thus, the mineral turnover rate was diminished ($P < 0.001$), but the total amount of new bone mineral was unchanged.

Discussion: IGF-I plays an important role in the regeneration of other tissues (nerves, muscle) and stimulates bone cells in vitro. The effect on ^{99m}Tc -MDP uptake indicates that IGF-I has reached the bone in sufficient concentrations to exert some effect on bone healing, however without increasing the total bone yield. It would seem that both bone formation and resorption were decreased, leaving the net effect unchanged, or that the IGF-I had first exerted a stimulatory effect, followed by inhibition. Probably, endogenous paracrine production of IGF-I during 2 weeks is already optimal for healing in this model.

Fracture healing

Effects of intramedullary reaming on experimental bone healing

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Reaming in connection with intramedullary (IM) nailing is followed by destruction of all the vessels in the medullary canal. Nonreamed IM nailing has a less dramatic effect on medullary circulation, and revascularization of bone marrow is completed earlier in the nonreamed situation. The aim of the present study was to investigate the speed and quality of bone healing after reamed and nonreamed IM fixation.

Materials and methods: Multiple Kirschner pins with a diameter of 1.1 or 1.6 mm were used for IM fixation. Unilateral transverse midshaft tibial osteotomy was performed on 16 rabbits. Before IM fixation, reaming was performed in 8 animals, whereas no reaming was done in the other group. The animals were killed after 6 weeks, and bone healing was evaluated by radiographs, estimation of the amount of peripheral callus, and mechanical testing in four-point bending.

Results: After 6 weeks, radiographic healing had taken place in all the osteotomized bones except for one in the reamed group. Healing with abundant peripheral callus took place in both groups, and no significant difference occurred. The mean strength of healed bones given as percentages of corresponding control bones was 97 percent in the nonreamed group and 58 percent in the reamed group. This difference is significant. There was also a trend towards greater elastic stiffness in the nonreamed group.

Conclusions: There was no difference in the quality of

bone healing following reamed and nonreamed IM nailing, as both groups healed with abundant peripheral callus. Bone healing proceeded more rapidly in the nonreamed group.

The polymorphonuclear leukocyte, an important factor in fracture healing?

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The role of polymorphonuclear leukocytes (PMNLs) in fracture repair in rats was investigated.

Materials and methods: A transverse osteotomy at the midshaft of the left femur was immediately stabilized by intramedullary nailing. Nine rats were made neutropenic prior to the operation and for 3 days postoperatively by intraperitoneal (i.p.) injections of a specific antiserum against rat PMNLs (antineutrophilic serum, ANS) raised in sheep. All the animals in this group had less than 0.2×10^9 circulating PMNLs/L at the operation. Control rats were injected with the same amount of normal sheep serum (NSS, $n = 8$). All the animals received cerfuroxim (100 mg/kg/day i.p.) together with the serum injections. Six weeks after the operation, the animals were killed. The femurs were dissected free and the amount of callus was measured. The fractures were radiographically evaluated and mechanically tested.

Results: Treatment with ANS caused no improvement in radiographic healing. No difference in the amount of callus was observed between the groups. The bending moment, however, was significantly higher in ANS-treated animals ($P < 0.02$), but no difference was observed in rigidity or total energy absorption.

Conclusion: In the present model, PMNLs seem to have a negative effect on fracture healing.

Accessory immunologic cells in fracture healing and bone induction

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The differentiating tissues in fracture healing and in demineralized bone matrix power-induced (DBM) bone formation were investigated with monoclonal antibodies with respect to the occurrence of Ia-presenting cells, macrophages, and lym-

phocytes with interleukin-2 receptors (IL-2). Ia-antigen is normally presented by B lymphocytes and by dendritic cells (DC), which are macrophage-related. DC is the most important cell presenting foreign antigen to the lymphocytes. When this has occurred, the T-lymphocytes become activated producing IL-2 and gamma-interferon, with the result that many other cells such as macrophages, endothelial cells, and mesenchymal cells begin to present Ia-antigen, i.e., the effector phase of the immunologic defence has started. Ia consists of molecules on the cell surfaces belonging to class II of the major histocompatibility complex (MHC), and Ia-antigen is specific for the species and the individual.

In both types of granulation tissues, the mesenchymal cells from the surrounding musculature were accompanied by great numbers of Ia-positive cells and common macrophages. The occurrence of IL-2 receptors was sparse in fracture healing, but rich in DBM induction, probably due to its immunogenic property, though weak. On day 7, almost all the existing cells in DBM induction were Ia-positive, signifying an immunologic effector phase apparently directed to the remaining, still passive bone matrix in the periphery while the central part of the inserted bone powder was producing cartilaginous cells and matrix. The Ia-positive cells were numerous in fracture healing, but spotwise abundant at about 7 to 10 days after DBM induction. The presence of accessory immunologic cells in fracture healing is perhaps due to a surveillance function (a standard response upon injury), but the cell differentiation might to some extent be dependent on the active mediators emitted from Ia-presenting cells and macrophages.

The investigation strengthens the conception that induction also occurs in experimental fracture healing and that the origin of the mesenchymal cells is the surrounding musculature.

Biomechanics

Quantification of structural anisotropy based on volume orientations—a new concept

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In the investigation of the mechanical properties of trabecular bone, attention has turned to the structural (architectural) properties. This is motivated by systematic errors, which make mechanical testing of trabecular bone difficult, and by the hope that a complete structural description will explain all the variation of mechanical parameters. In some recent works, the tensorial mean intercept length (MIL) method suggested by Harrigan and Mann (1) or a modification of this method (2) has been used for evaluating the structural properties of tra-

becular bone. The MIL methods are based on the spatial distribution of the directions of two-dimensional surface elements of the trabeculae.

It can, however, be postulated that what we observe when we perceive anisotropy is not the surface distribution, but the distribution of volume in space, and the distribution of surface may not be relevant in an examination of the influence of structure on mechanical parameters. This has led to the development of the concept of "volume orientation" (3), which at first sight seems to be a semantic paradox.

In a three-dimensional structure, local volume orientation is defined for each point within the phase of interest as the hemispheric direction of the longest intercept. This allows the definition of mean volume orientation and the application of parametric directional statistics. Nonparametric methods will be applicable as well.

A consequence of the definition of volume orientation is that elements of larger volume weigh more than others in the distribution. This may well be considered an advantage if the well-oriented elements are thicker than the more off-axially oriented ones, which usually is seen in weight-bearing trabecular bone.

Using the concept of volume orientation, the degree of anisotropy in a cube of trabecular bone has been estimated using information from the surface of the cube. In order to relate the structural information from the surface of the cube to the three-dimensional structure, a geometric model is mandatory. We used a model based on a local platelike trabecular structure, and we used the Dimroth-Watson distribution for statistical modeling of the local volume orientation. Three-dimensional reconstruction of the trabecular structure seems an attractive alternative, which at the moment is being investigated at our laboratory.

References

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Muscle contribution to tibial fracture strength in rats

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Telemetric studies of the human lower leg in vivo suggest the loading capacity to be substantially higher than the loading

limits of tibia from cadavers. A method to study the muscle contribution to the loading capacity of the tibia more directly has been developed for anesthetized rats.

Material and methods: The lower legs of anesthetized rats were deflected ventrally to fracture in a test jig. The exact location of the resulting tibial fracture was determined, and the contralateral tibia was tested correspondingly after free dissection. Three groups of rats were used. The first group was killed by neck elongation while under ketamin anesthesia, and the lower legs were tested for 30 min to evaluate any effect of dead musculature. The second and third groups were tested *in vivo* under fluanisone-fentanyl/ketamin and fluanisone/fentanyl/midazolam anesthesia, respectively.

Results: The ultimate bending moment increased by about 40 percent and the ultimate absorbed energy by about 85 percent when the tibia was tested *in situ* as compared with in the dissected state. The bending stiffness decreased by about 20 percent, and the ultimate deflection angle increased by about 50 percent. No significant differences were observed between the three groups of rats.

Conclusion: Relaxed (anesthetized) or dead musculature increased the tibial bending strength by 40 percent and the absorbed energy by 85 percent. The contribution of activated musculature to lower leg loading capacity is probably substantially higher.

In vivo strain of the rat femur related to its biochemical composition

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This study was designed to find an explanation for reduced strain recorded *in vivo* from the surface of the femur of old rats compared with younger ones. The ratio between calculated stress and corresponding recorded strain during the 3-point bending test *in vitro* had revealed that older animals had higher values (greater modulus of elasticity) than younger rats ($P < 0.01$).

Material and methods: Peak strain was recorded from strain gauges on the anterior surface of the femoral diaphysis of 6-, 12-, and 52-week-old rats running on a treadmill. Collagen content in the bones was measured by amino-acid analysis. To estimate the rate of collagen turnover, radioactive proline was injected and the proportion of radioactive hydroxyproline was measured. Total content of minerals (calcium, phosphorus) was estimated by means of continuous flow spectrophotometry.

Results: The total amount of collagen and minerals increased with age ($P < 0.001$). Collagen synthesis decreased ($P < 0.001$), while mineralization (calcium/hydroxyproline ratio) increased with age ($P < 0.001$).

Conclusions: Chemical alterations like decreased turnover rate of collagen and increased mineralization may support the finding of reduced strain in femora of 52-week-

old rats. Both findings may be interpreted as reflecting stiffer bone tissue (greater modulus of elasticity) in the older animals.

Changes in femur after tibial fracture

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Changes in femur after tibial fractures were studied in 34 rats divided into three groups. One group received an intramedullary nail with a distal lock; one group received an intramedullary nail that only provided stability in the longitudinal axis; finally, the last group received no fixation. The animals were killed after 3 weeks. The femora were weighed; and the strontium-85 incorporation, the strength, and the stiffness were measured.

The reduction in weight of femur on the fractured side was approximately 10 percent for all the groups.

Incorporation of strontium was 12 percent higher on the fractured side. This means that new bone formation was higher on the fractured side.

To study the mechanical strength in femur, we tested the bone in torsion. The reduction in mechanical strength was approximately 30 percent on the fractured side after 3 weeks. The stiffness decreased by approximately 50 percent on the fractured side. The stiffness expresses the degree of calcium content in the bone. The less calcium, the softer the bone becomes. A relatively minor loss of calcium gives a great reduction in stiffness.

There was no difference in weight reduction, strontium incorporation, loss of strength or stiffness between the groups.

It appears that different ways of treatment had minor influence on the osteopenia in femur. The osteopenia in femur might be more related to the fracture than to the level of activity.

Biocompatibility of implants

Preliminary results of experimental cementless press-fit resurfacing of the cat hip joint

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Cemented double cups have shown unacceptably high failure rates. We have therefore designed a new cementless double cup for the hip joint. A similar prosthesis has been tested on a cat model.

Material and methods: Twelve cats weighing 2.9-4.9 kg underwent a surface-replacement procedure in the right hip. The acetabular component was made from UHMW polyethylene and had a cylindrical and threaded outer surface. The femoral component was made from commercially pure titanium. The internal surface was cylindrical and threaded. The whole titanium surface was oxidized. The acetabulum and femoral head were prepared by using special reamers. The prosthetic components were screwed into position. Unrestricted post-operative weight bearing was allowed. One animal was killed after 1.5, 3, 4.5, and 6 months. Further killing is planned every 6 months. The prosthetic component, together with the adjacent bone, was removed and prepared for sectioning in the undecalcified state. The cut slices were examined with light microscopy.

Results: All the cats were fully weight bearing a few days after the operation. One cat dislocated the hip 3-4 weeks post-operatively, and this animal was killed at 1.5 months. All the other cats functioned well without limping throughout the observation period. No radiographic or macroscopic signs of loosening were seen in the removed components. Histologic examination of the femoral head revealed contact between bone and titanium in all the specimens. At 1.5 months, the contact was limited to some of the outer edges of the threads. At 3, 4.5, and 6 months, there was an increasing bone filling in the threads. At 6 months, several areas had positive, direct bone-metal contact. The contact between bone and the acetabular polyethylene prosthesis was much poorer. In all the specimens the polyethylene cup was surrounded by a continuous layer of connective tissue.

Strength retention of self-reinforced polyglycolide (SR-PGA) and SR-poly(lactic acid (PLA) composite rods in vitro and in vivo

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The strength retention of SR-PGA and SR-PLA rods was not previously studied under vitro and in vivo conditions.

Materials and methods: The strength retention of SR-PGA and SR-PLA composite rods was evaluated after immersion in 37 °C distilled water (in vitro) and after implantation in the subcutis of rabbits (in vivo). Cylindrical SR-PGA composite rods, 3.2 mm in diameter and 50 mm long, were manufactured using commercial PGA sutures as the raw material. The rods were sterilized in ethylene oxide. Rods made of poly-(L)-lactic acid (SR-PLLA; mol. weight of PLLA was

260,000) and poly-(DL(L)-lactic acid (SR-PDLLA; mol. wt of PDLLA was 100,000) were used. The rods were gamma sterilized (2.8 Mrad). Thirty-seven rabbits were used, and the follow-up times were from 1 to 48 weeks. Bending and shear strength of the rods were measured by the J J Lloyd T5003 tensile testing equipment.

Results: SR-PGA rods had an initial bending strength of 350 MPa \pm 50 MPa and a shear strength of 200 MPa \pm 20 MPa. The bending strength (MPa) of SR-PGA rods in vitro was 45 \pm 8 after 4 weeks, and their shear strength was 137 \pm 7. In vivo the bending strength of SR-PGA rods was 13 \pm 3, and their shear strength was 57 \pm 10; the rods lost their strength in 6 weeks.

SR-PLLA rods had an initial bending strength of 210 \pm 20 and a shear strength of 100 \pm 10. SR-PDLLA rods had an initial bending strength of 180 \pm 20 and a shear strength of 70 \pm 15. In vivo after 12 weeks, the bending strength of SR-PLLA rods was 105 \pm 10 and the bending strength of SR-PDLLA rods 10 \pm 5. After 12 weeks, the shear strength of SR-PLLA rods was 90 \pm 10, and the shear strength of SR-PDLLA was 50 \pm 10. At 24 weeks, the shear strength of SR-PLLA rods was 30 \pm 10, and SR-PDLLA rods had lost their shear strengths totally.

Conclusion: In vivo, SR-PGA rods lost their strength in 6 weeks. The strengths of SR-PLLA rods were at the level of the ones of cortical bone at least during 12 weeks.

Mechanical fixation of cobalt-chromium and stainless steel implants in rats

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Tissue sensitivity to metals has long been proposed as an underlying cause of loosening of implant materials despite the fact that biocompatibility studies revealed no substantial evidence. The aim of this study was to evaluate the mechanical fixation and the histologic reactions around implants made of cobalt-chromium and stainless steel alloys.

Material and methods: Threaded femoral implants measuring 25 mm were made of stainless steel (ASIF) and cobalt-chromium alloy (Howmedica) wires and inserted in the femora of two groups of young growing rats. Screws made of the same metals were inserted transversally in the proximal tibiae of the same animals.

The pull-out strength was tested in different groups immediately after insertion and then every third week up to the 12th week.

Histologic reaction around the implants was evaluated by means of light microscopy, tetracycline labeling, and microradiography.

Results: The stainless steel implants demonstrated continuously increasing pull-out strength with time throughout

the observation period. The cobalt-chromium implants showed a marked progressive reduction of its fixational strength during the second half of the observation period.

No consistent histologic differences in reaction to the implants were found between the two materials.

Conclusion: The cobalt-chromium implants caused a reaction that led to diminished fixation to bone after 6 weeks. Conventional histology and microradiography did not reveal a cause for this diminished fixation. Further studies need to be done to confirm and elucidate these preliminary studies.

Leg lengthening and diaphyseal defects

Growth retardation following experimental limb lengthening by epiphyseal distraction

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Controversy surrounds the effects of epiphyseal distraction on subsequent longitudinal growth in limb lengthening. Any change in growth potential of the growth plate subjected to distraction has not been assessed quantitatively in controlled studies.

Material and methods: Three studies of limb lengthening by epiphyseal distraction was performed in two animal species using the femur or tibia as the experimental bone. After the lengthening procedure, bilateral longitudinal growth was evaluated semiquantitatively until growth had ceased or residual growth was negligible.

Results: The involved growth plate appeared radiolucent after the lengthening procedure, but looked less distinct and had reduced height compared with controls. In 7 of 22 animals, the growth plate of the lengthened bone appeared to close earlier than that of the controls. Growth retardation was found in all the animals, with an average reduction in growth potential of each study ranging from 40 to 70 percent.

Conclusions: The results demonstrate that epiphyseal distraction is a valid method of limb lengthening, but the procedure appears to have a regular harmful effect on the growth plate. Clinical limb lengthening by this method should only be carried out close to maturity if the decreased leg-length discrepancy is not to increase during further growth.

Stress protection in experimental bone lengthening by external fixation

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In clinical limb lengthening, fracture of the elongated bone is a major complication. Fractures may involve the area of intact bone outside the lengthening zone suggesting that stress protection leads to impaired bone strength, the extent of which has not been assessed by evaluation of mechanical strength.

Material and methods: In 4 animal experiments of limb lengthening by gradual external distraction, the effect of stress protection on intact bone was investigated by radiography and evaluation of torsional strength. A total of 20 of 56 elongated bones that failed during testing outside the lengthening zone and without involvement of pin channels were included.

Results: Decreased radiodensity and reduced cortical thickness of intact bone was a prevalent finding. The relative torsional strength was significantly reduced, with average values ranging from 50 to 85 percent. Measurements of strength of the ipsilateral unlengthened bone versus its contralateral control showed average relative values ranging from 67 to 90 percent.

Conclusions: In experimental limb lengthening by external gradual distraction, stress protection may reduce mechanical strength of intact bone to about one half the normal value. Reduced weight bearing may account for a decrease in bone strength of about the same magnitude as that of the external device. In addition to a temporary inferior strength of the newly formed bone in the lengthening zone, reduced strength due to stress protection implies a significant risk of fracture outside the elongation area long after a limb-lengthening procedure.

Comparison of bone-inductive procedures in the reconstruction of large diaphyseal defects in sheep

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The discovery of locally active substances in bone with mitogenic and inductive properties has opened new prospects for the treatment of bone defects. Clinically, autogenous cancellous bone marrow has been regarded as the most potent osteogenic graft for skeletal reconstruction. The introduction of the Ilizarov technique for repairing diaphyseal defects offers a

most promising alternative.

The aim of the present study was to compare the bone inductive capability of implanted allogeneic bone matrix to that of a modified Ilizarov procedure for reconstruction of large diaphyseal defects in sheep.

Methods: In 36 sheep the right femur was stabilized with an external fixator using Schanz screws. A 4-cm diaphyseal bone defect was created. The animals were divided into 5 groups according to type of defect substitution: (1) demineralized allogeneic bone cylinder, (2) demineralized allogeneic bone chips, (3) modified Ilizarov procedure, where an osteotomized piece of the proximal femur was moved distally at a rate of 1 mm/d, (4) autogenous bone chips from the iliac crest, (5) defect left empty.

Postoperatively, the sheep were allowed full weight bearing. Bone formation was monitored by radiography at 2 weeks, and then at monthly intervals. The animals were killed after 3 to 5 months, and the femoral specimens were analyzed by bone mineral densitometry, histology, and isotope (^3H -proline and ^{45}Ca) uptake determinations.

Results: Six sheep were lost, 4 because of loosening of the external fixator and/or infection, 1 because of femoral neck fracture, and 1 because of sudden death 2 days after surgery.

The Ilizarov procedure proved to be highly effective in bridging the defects with new bone. A full bridging of the defect was seen in 7 of 8 animals, although in 3 there was incomplete fusion distally. This was due to irregular new bone formation at the distal osteotomy site preventing the movable bone piece to reach the end of the defect. The mineral content of new bone induced by the Ilizarov procedure was similar to that of the contralateral intact femur in all the animals except 1.

The allogeneic bone matrix implants, regardless of whether cylinders or chips, evoked a variable response. Complete bridging of the defect occurred in 4 of 13 animals. In another 2, there was a considerable amount of new bone, although incomplete bridging. In 6 sheep, there was no evidence of new bone formation.

Autogenous bone chips from the iliac crest resulted in complete defect healing in 3 or 4 animals, but only limited amounts of new bone according to densitometry. All the defects left empty led to nonunion.

Conclusion: The Ilizarov procedure is highly effective for bridging large diaphyseal defects in sheep, whereas allogeneic bone matrix gives a more variable osteogenic response.

Bone mineral analyses

Determination of bone mineral content using single-energy computed tomography

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High correlations have been recorded between mechanical properties of bone and CT attenuation values. This study was undertaken to investigate the accuracy of single-energy CT as a measure of bone mineral content.

Material and methods: Twenty cadaver femora (10 women, 10 men, 73 ± 12 years) were harvested and kept at -18°C in sealed plastic bags. Ten-millimeter CT slices were taken through the femoral condyles (G.E. 8.800, 120 Kv, 100 MAs). No distinction was made between cortical and cancellous bone. The lower threshold limit was set to 50 CT number to reduce the soft-tissue problem. The CT volume was calculated by the machine. The marked CT slice was cut, and the volume measured. The calcium content in the slices was assessed with the neutron activation method.

Results: A correlation was noted between CT density and calcium concentration ($r = 0.89$, $P < 0.001$). The strongest correlation was recorded between the calcium mass value (calcium concentration \times volume) and CT bone mass value ($\text{MRM} = \text{CT density} \times \text{CT volume}$, $r = 0.96$, $P < 0.001$). Linear regression analysis yielded the following relationship: $\text{Ca mass} = 0.20 \times \text{MRM} + 857$; $\text{Ca concentration} = 0.20 \times \text{CT density} + 15$.

Conclusion: The present study shows that CT of the femur is significantly correlated with the bone mineral content. It is suggested that CT may become a useful clinical tool in the assessment of bone density and mass.

Bone mineral analysis by computed tomography: Precision of measurements and calibration problems

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Many sources of error have been ascribed to the estimation of the bone mineral content (BMC) by means of computed tomography (CT). Simultaneous scanning of phantoms for calibration/correction has been advocated. The real error of these measurements is, however, not clarified. The precision of these measurements is rarely determined nor has the simultaneous scanning of calibration phantoms been evaluated by practical tests.

The precision of measurements of the bone mineral content as density- (DRV) and mass-related values (MRV) in the femoral condyles has been evaluated as the interpersonal and intrapersonal variation of replicated measurement over time on two different CT equipments. Three measurements were performed on a GE 8800 and two on a Philips Tomoscan 350 using ordinary body-scanning parameters and computing mean density and area of pixels with CT density (CT number) of 100 or more. There was no systematic difference between the two equipments. The precision was found to be high, in the order of 0.2 to 0.3 Z-score units.

Five femoral condyles and three phantoms with known concentrations of K_2HPO_4 were scanned alone and placed inside body phantoms with increasing densities. There was a reduction of the determined DRV by increasing body densities. Calibration by means of a simultaneously scanned calibration phantom placed outside the body substantially and unsystematically underestimated the effect of beam hardening induced on the centrally located objects. While the mean K_2HPO_4 in the three phantoms was approximately 117 mg/mL, the calibrated CT densities (CT numbers) equalled K_2HPO_4 by means of linear interpolation was only 94 and 87 units of K_2HPO_4 in the whole phantoms and in the center of the phantoms, respectively. There was also a significant difference between the measurements in the condyles after calibration. For such a calibration system to be effective, the object and the calibration phantom must be subject to the same variation. By scanning an object and a calibration phantom located respectively in the center and more peripherally in the CT gantry, they are subject to different beam-hardening effects. Furthermore, in clinical work where the object (usually a lumbar vertebra) is located within patients with substantial differences in size, fatness, and muscle mass, the difference in beam-hardening effect is unpredictable.

It is concluded that these measurements have a high precision with negligible fluctuation due to equipment instability. Simultaneous calibration by scanning phantoms placed outside a body is therefore not required due to equipment instability and such a calibration does not correct the effects of beam-hardening effects. Furthermore, in clinical work where the object (usually a lumbar vertebra) is located within patients with substantial differences in size, fatness, and muscle mass, the difference in beam-hardening effect is unpredictable.

It is concluded that these measurements have a high precision with negligible fluctuation due to equipment instability. Simultaneous calibration by scanning phantoms placed outside a body is therefore not required due to equipment instability and such a calibration does not correct the effects of beam hardening.

Scoliosis

Effects of rib elongation on the normal and the scoliotic spine of the rabbit

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Earlier results have provided evidence that idiopathic scoliosis might be connected to asymmetric growth in length of rib pairs. In this work, attempts have been made to influence the normal and scoliotic spine of rabbits by rib elongation.

1. Normal spine: In adult rabbits elongation of one rib by 1

cm after osteotomy and application of a metallic expander resulted in immediate 3-dimensional deviation of the spine with formation of scoliosis and decrease of the normal kyphosis and lordosis. Moreover, vertebral rotation, rib-hump formation, and deviation of the spinous processes occurred in the horizontal plane. The morphometric changes of the spine provoked in the rabbits corresponded closely to those observed in adolescent idiopathic scoliosis with the exception that they were located on the "wrong" side. This might be due to the sudden forced displacement of the vertebrae without possibility of successive muscular adaptation.

II. Scoliotic spine: Growing rabbits were subjected to segmental resection of three intercostal nerves on the right side. One to 3 months later, the animals had developed moderate left convex thoracic scoliosis.

A. In one group of animals, elongation of one rib on the convexity by 1 cm after osteotomy and application of a metallic expander resulted in immediate correction of the scoliotic deformity, which was still maintained 3 weeks later.

B. In 2 groups of scoliotic animals, a repeat resection of three intercostal nerves on the convex side was performed 1 or 2 months after the first operation. The new procedure resulted in slight regression or arrest of the progress of the scoliosis as compared with a third group of rabbits, which did not undergo the second operation.

Conclusions: The results of these studies give further support to a new concept relating asymmetric longitudinal rib growth with the initial pathomechanism of idiopathic scoliosis. They also suggest that regulation of rib growth might be a new way of approach for easy and effective correction of the early progressing scoliosis in humans.

Spinal mobility and posture in children aged 8–16 years

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Spinal mobility and posture were measured in 294 boys and girls, aged 8–16 years. The upper thoracic incline was more vertical in girls, but the postural curves showed no significant age-related differences in either sex. Thoracic extension, rotation, and lateral flexion decreased with age after 10 years, and were most reduced at the age of 13 in both genders. At this age, girls significantly differed from boys, as they had less pronounced kyphosis, smaller thoracic forward and lateral flexion, and larger thoracic rotation to the right than to the left. In the lumbar spine, no such age- and gender-related differences were noted.

Miscellaneous

Experimental anaerobic osteomyelitis: A novel rabbit model

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Effects of anti-estrogen on bone in castrated and intact female rats

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Many researchers have emphasized the effects of estrogen on bone. Ovariectomy is known to cause osteoporosis, which can be prevented, for example, by estrogen replacement. Anti-estrogens are commonly used to treat breast cancer because they block estrogen receptors. They also have a small stimulatory effect, i.e., estrogenic properties.

Material and methods: Totally, 158 female rats were used. They were subjected to an ovariectomy or a sham operation. Anti-estrogens (tamoxifen, keoxifene, and MER 25) were given orally, the controls receiving the vehicle (peanut oil) only.

Results: Ovariectomy in 3-month-old rats was associated with increased growth resulting in a higher body weight and a larger femur, so that after 2 months the body weight was 34 percent higher, the dry weight of the femur was 9 percent higher, and the ash content of the femur was 10 percent higher compared with intact rats ($P < 0.001$). When 9-month-old rats were ovariectomized, the body weight also increased, but the bone mass and mineral content of the femur became lower than in intact rats. After 4 months, the body weight was 15 percent higher in ovariectomized rats ($P < 0.01$), whereas the dry weight of the femur was 12 percent lower, the femur ash was 14 percent lower, and the ash content of the femur was 12 percent lower than in intact rats ($P < 0.001$). Anti-estrogens, as well as estradiol-3-benzoate, prevented the increase in body weight from ovariectomy in both groups. They also counteracted the increase in femur ash in the younger rats. In the older ovariectomized rats, anti-estrogen caused a significantly higher ash content of the femur than in controls ($P < 0.05$). The mean values of femur ash per volume in ovariectomized rats were the highest when a combination of tamoxifen or keoxifene and estradiol-3-benzoate was given. This demonstrates a lack of antagonistic effect between the estrogen and the anti-estrogen on bone. Neither of the anti-estrogenic

drugs caused significant osteopenia when given to intact rats.

Conclusions: Anti-estrogens and estrogen help to prevent osteoporosis in rats after ovariectomy. This could be caused by their inhibitory effects on estrogen receptors in the mid-brain, but tamoxifen, keoxifene and MER 25 might also have only a stimulatory effect on estrogen receptors in bone. If these results are extrapolated to the human situation tamoxifen could be used alone or in combination with estrogen to prevent osteoporosis due to estrogen deficiency.

Parathyroid cell number and size in hypercalcemic rats: A stereologic study

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Recently developed stereologic methods for unbiased estimations of particle number and size were employed to study parathyroid growth in normal and hypercalcemic young rats. Thus, the parathyroid cell number and size of parathyroid secretory cells were estimated by both the disector method and the volume-weighted mean volume method. The glandular volume was calculated from serial sections, while the volume density of secretory cells was estimated by conventional stereologic techniques. Three groups of animals were studied: normal rats at 3 weeks of age, hypercalcemic rats at 7 weeks of age, and age-matched controls. Hypercalcemia was induced by feeding the animals a purified diet, which was nutritionally adequate except for low amounts of phosphate (0.02 percent), from 3 weeks of age. During the period from 3 to 7 weeks of age, the number of parathyroid secretory cells increased by 100 percent while the mean cell volume increased by 20 percent. However, when calculated per gram body weight, the volume and number of cells were larger in the younger animals. The phosphate-depleted animals grew slowly and developed severe hypercalcemia. Their parathyroid secretory cells were smaller and each gland contained fewer cells than in age-matched controls. The lower cell number and cell volume, however, were proportional to the reduced body weight. Data from the 3-week-old animals indicate that the reduced cell number and size in hypercalcemic rats reflected growth arrest rather than atrophy.

Proteoglycan fragments in joint fluid from patients with arthrosis of the knee

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At the time of radiographic diagnosis of arthrosis, the cartilage changes are already far advanced. During turnover of the cartilage, molecules from the matrix are released to the synovial fluid (1). We have assayed the levels of proteoglycan fragments in knee joint fluid from patients with different stages of gonarthrosis.

Patients and methods: Knee-joint fluid was collected from patients aged 17–75 years. Examination was made at least 0.5 years after beginning of joint pain or trauma (average 2.5 years). Samples were analyzed for cartilage proteoglycan fragments by immunoassay (2).

The patients were grouped as follows:

Control Arthroscopically normal patients with knee pain
 OAA Arthrosis by arthroscopy, no known knee trauma
 OAX Arthrosis by radiographs, no known knee trauma.
 Tr Knee trauma, no arthrosis by arthroscopy or radiographs
 Tr + OAA Previous knee trauma, arthrosis by arthroscopy
 Tr + OAX previous knee trauma, arthrosis by radiographs

Knee injury included cruciate ligament rupture and meniscal tear alone or in combination. Arthrosis was femorotibial, femoropatellar, or both.

Results: With advancing arthrosis stages, a decreasing trend in proteoglycan-fragment concentration was noticed.

Discussion: We have made a preliminary study of the proteoglycan fragment concentration in the knee joint in a limited number of patients. A decreasing trend in proteoglycan epitome concentration was noticed with an increasing degree of arthrosis. This suggests that the joint fluid proteoglycan epitome concentrations in degenerative joint disease may be related to the mass of cartilage present in the joint at each stage, as already proposed for inflammatory joint disease (3). This in turn underscores the fact that cartilage matrix changes in joint disease may already be well advanced by the time the diagnosis is made by either arthroscopy or radiographs.

Table 1.

	<i>n</i>	Sex (percent M)	Trauma Mean age	Sampling Mean age	Proteoglycan Conc. µg/mL
Control	18	50	28.0	30.4	65
OAA	15	58	40.8	43.5	173
OAX	30	48	54.1	59.0	110
Tr	102	80	41.2	44.1	194
Tr + OAA	8	65	43.0	45.6	118
Tr + OAX	13	73	42.9	48.9	106

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