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I. Fibroblast growth factor increases the number of induced cartilage cells in demineralized bone matrix in rats

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Implantation of demineralized bone matrix in rodents elicits a series of cellular events leading to the formation of new bone inside and adjacent to the implant. This process is initiated by inductive proteins present in bone matrix, and local growth factors further regulate the process. We have previously shown that local application of recombinant human basic FGF in a carboxymethyl cellulose gel to demineralized bone matrix implants increases the bone yield, as measured by calcium content. In the present experiments, the effects of FGF were evaluated with histomorphometry. The number of cartilage cells at 2 weeks was increased by 15 ng of bFGF. At 3 weeks, the number of cartilage cells was reduced and there was instead an increased area of calcified (bone) tissue as compared to controls. Thus, cartilage formation was increased by bFGF and its replacement by bone came earlier. However, cartilage and bone formation was inhibited in samples treated with 1900 ng of bFGF, but no other effects were seen.

II. Bone morphogenetic protein induces bone in the squirrel monkey, but bone matrix does not

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Demineralized bone matrix (DBM) reproducibly induces bone formation in rodents, but its effects in dogs and primates are uncertain. In previous studies on the squirrel monkey, DBM did not induce bone, although the same implants were effective in nude rats. In the present study, the DBM was augmented with recombinant human bone morphogenetic protein-2. Bone was formed in 10 of 12 monkeys at 6 weeks, as verified by histology and calcium content. In only half of the monkeys, the amount of induced bone was large enough to mimic a clinically useful effect. DBM controls (without extra BMP) did not induce bone in the monkeys. Both DBM controls and augmented implants induced bone in the nude rats. The difficulties to achieve bone induction in "higher" animals, may be overcome, at least partially, by using a higher concentration of the inductive protein, than is present in DBM.

III. Enhancement of new bone formation with Transforming Growth Factor-ß 1

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Purpose: The purpose of this study was to investigate the effect of the growth factor Transforming Growth Factor-beta (TGF-beta) on local bone formation. Two experimental models were used: A) Local application into tibial periostal defect. B) Local application into osteotomy.

Material and methods: TGF-beta 1 was produced from human platelets. Purity was tested by means of SDS-page electrophoresis and HPLC reverse-face electrophoresis. The biological activity of hTGF-beta 1 was tested in a fibroblast in vitro assay. *Study A:* A dose-response study was performed on 6 mature rabbits in order to determine the effect of TGF-beta on bone formation in tibial periostal defects. Operatively a 1x2 cm piece of periost was removed. hTGF-beta 1 was applied to the area of periostal defect during six weeks via a catheter connected to a miniosmotic pump. Three concentrations (0.02, 1.0, and 10.0 µg TGF-beta/day) were studied. *Study B:* Stimulation of fracture healing by local TGF-beta application in a rabbit tibial osteotomy model. Unilateral tibial osteotomies were performed in 14 adult rabbits. TGF-beta was applied continuously to the osteotomy site via a subcutaneously placed miniosmotic pump with a dose of 1 ug TGF-beta/day for six weeks until termination. Fracture healing was evaluated by means of X-ray examination, mechanical testing, histomorphometry and bone densitometry.

Results: Study A: Local TGF-beta application revealed macroscopically and roentgenologically enhanced bone formation in the periostal defect at concentrations of 1 and 10 µg TGF-beta/day. *Study B:* In the osteotomy study, TGF-beta was found to increase callus formation by histomorphometry. The mechanical test showed equal strength of osteotomies with or without TGF-beta application.

Conclusion: The results indicate that local TGF-beta application is able to enhance new bone formation in adult rabbits both in periostal defects and in fracture healing.

More research is required to describe the complex interactions between TGF-beta and other growth factors during stimulation of bone formation.

IV. Staphylococci bacteria fragments stimulate bone resorption in vitro

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Periprosthetic bone resorption is a common feature in most cases of joint prosthetic loosening. We previously have shown, in cases of aseptic loosening of total hip arthroplasty, that the capsule, when compared with other periprosthetic tissues, is the main producer of factors capable of stimulating bone resorption in vitro. In these cases, mediators released by macrophages seem to be involved in osteoclast activation promoting bone resorption. However, the events leading to loosening of infected implants are not known. Therefore, we have in an initial investigation studied the direct effects on bone in vitro of fragments of two bacteria frequently isolated from infected joint implants.

Materials and methods: Nonvital, sonificated fragments of staph. aureus (SA) and staph. epidermidis (SE) were dissolved in CMRL 1066 culture medium in which explants of neonatal mouse calvariae prelabelled with ^{45}Ca and ^{3}H-proline, respectively, were incubated. The bone resorption activity was assessed as percentage release of ^{45}Ca and ^{3}H respectively, into culture medium with and without the presence of inhibitors of prostaglandin synthesis. Also, the production of prostaglandin E_2 (PGE_2) in cultures, was assessed. To reveal osteoclast activity, calcitonin (CT) was added in control experiments. For comparison, parathyroid hormone (PTH) was added to one group of bones in each experiment.

Results: Extracts of sonificated nonvital SA and SE bacteria added to neonatal mouse calvariae cultures resulted in a dose- and time-dependent enhanced release of ^{45}Ca. The maximum activity was comparable with the effect obtained by the addition of PTH in an optimal concentration. A significant release of ^{3}H, revealing bone matrix degradation, was also obtained by addition of SA and SE extracts to cultures. No stimulation of ^{45}Ca release was observed when nonvital bones were cultured in the presence of bacterial extracts. CT significantly inhibited the stimulatory effect of bacterial extracts. A dose- and time-dependent enhanced production of PGE_2 was also observed with the two different extracts. Addition of different cyclo-oxygenase inhibitors, indomethacin, flubiprufen, meclophenamat and corticosteroids (hydrocortisone and dexamethasone) abolished the bacterial induced production of PGE_2. The bone resorption activity of the bacterial extracts, however, was not reduced by the cyclo-oxygenase inhibitors.

Conclusion: Fragments of Gram-positive bacteria can directly, in the absence of inflammatory cells, stimulate osteoclastic bone resorption by prostaglandin-independent mechanisms. The mechanisms of bone resorption stimulating activity by bacteria fragments on bone in vitro are not understood and further studies are in progress.

V. Biomaterial-centered infections—an experimental study in the rabbit

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Infections in the vicinity of an orthopedic implant still cause serious problems in clinical practice. Understanding of the local milieu of an implant site is of vital importance. The employment of foreign bodies may favour bacterial growth due to damaged circulation or implant material properties. The present study focused on the role of implant design, implant material and surgical technique in biomaterial-related orthopedic infections.

A rabbit model of hematogenous osteomyelitis using Staphylococcus aureus was employed to compare conventional stainless steel plates to titanium plates of either traditional design or of a recently developed low contact concept. In general anesthesia the right tibia of 51 rabbits was exposed and a 4-hole AO-miniplate was screwed to the posterolateral aspect of the distal third of the bone. In one group no plate was used: only the preparation of the drill holes was made, serving as control animals. A bacterial suspension of *Staphylococcus aureus* was injected into an auricular vein 2-10 days postoperatively. Radiograms were taken before inoculation and at killing time. Regular blood samples were collected for serological examination against the injected bacterial strain. Postmortem bacterial cultures were performed from the operated sites, lungs, kidneys and blood. Samples were taken for histological examination of the operated locations. The results suggest a decreased susceptibility to bacterial challenge in the low contact titanium plate compared to the stainless steel. Furthermore, there was no obvious difference in bacterial growth between the low contact plate group and a control group with no plate.

VI. Tensile strain properties of lumbar anterior longitudinal ligament—influence of vertebral bone mineral content

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Introduction: The bone mineral content (BMC) of the human lumbar vertebrae as determined with dual photon absorptiometry (DPA) has proven to be an accurate predictor of biomechanical properties of bone and soft tissues of the human spine (Hansson et al. 1980, Liu et al. 1983, Keller et al. 1987; 1988, Neumann et al. 1989). Bone-ligament-bone (B-L-B) preparations together with use of optical videotechnique enable measurement of tensile strains of both ligament substance and its insertions (Woo et al. 1983). The aim of this study was to examine the regional strain variation along the length and width of the ALL bone-ligament complex using a videoband motion analysis system and to see if this variation was correlated to BMC.

Material and methods: 15 ALL B-L-B specimens were dissected from lumbar motion segments in patients 21-68 years of age. The BMC of each vertebra was determined using dual photon absorptiometry. 12 plastic beads (1.5 mm in diameter) were then sewn to the ligament and its insertions by using 5-0 nylon suture, to build a 4x3 (column x row) grid. The B-L-B complex was attached to the crosshead of a custom mechanical testing apparatus. Ligament thickness and width were measured with vernier calipers with the ligament preloaded to 100 N. The B-L-B preparations were then loaded to failure at a constant crosshead speed of 1 mm/sec. Load and deformation were recorded on a digital oscilloscope and stored in a PC-AT computer. Displacement of the 12 markers were collected from a CCD camera. A second camera collected the clamp-to-clamp deformation for comparison with the crosshead deformation recorded using an LVDT. Tensile strains of the ligament substance (3 total) and insertions (6 total) as well as "total strains" from the outermost pairs of markers were computed from a longitudinal path. Stress-strain curves for the substance insertions and total B-L-B were used to compute ALL yield and ultimate mechanical properties.

Results: The average load and deformation of the ALL failure were 802 N (SD 317) and 12.5 mm (SD 5.2), respectively. The stress at failure and "total strain" at failure were 20.9 MPa (SD 7.3) and 4.86% (SD 1.28), respectively. The average "total" tangent modulus of the 15 B-ALL-B complexes was 743 MPa (SD 281). Comparison of the "total strain" indicated that the strains in the outer portion of the ligament were significantly greater (37%) than in the central region. Three distinct types of ligament failure were observed: Type I: failure in the ligament insertion, type II: failure in the ligament substance, and type III: failure as a combination of type I and II. Specimens with type II failures had a significantly higher BMC than type I. Failure loads, stresses and "total strains" were also higher in the specimens with type II failures. In all failure types the strains were consistently higher in the insertion B (the vertebra with lower BMC) of the B-ALL-B specimens. Insertion strains (EB) were significantly greater for the type I failures in comparison with the type II failures. Substance strains (ESUB) were significantly higher in the type II failures in comparison to type I failures. A relatively uniform strain distribution was found in preparations in the type III group.

Discussion: We noted large variations in regional strain which was consistent with the observations of Woo (1983). The results indicated that strains were greater on the outer part of the ligament. The "total strains" were consistently higher in specimens with higher mean BMC while "insertion strains" were consistently higher at the vertebra (of the two) with lower BMC. A spine with higher BMC (reflecting for example a higher degree of previous loading, activity etc.) will withstand a more "total strain" at the injury limit. The weakest region in this spine will be the ligament substance. A spine with lower BMC, on the contrary, will have its weakest region in the ligament insertion but with a lower "total strain" at the failure. The close correlation between the mode of B-L-B complex failure and BMC of the vertebrae in our study, suggested that ligament structure and function are closely related to the bone structure and function, which might have important implications for clinical assessment of spinal trauma, instability etc.

VII. The use of bone metabolic markers in relation to fractures

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To clinically evaluate the bone metabolic activity and to judge the quality of the bone stock, different methods have been developed. Scintimetry and assessment of bone mineral content by photon absorptiometry do provide valuable data as does histomorphometry, although being an invasive technique it is not applicable in regular practise. Thus these and other methods have various limitations. As the demand for the diagnosis and treatment of bone metabolic disorders, especially osteoporosis, has increased, a need to more closely follow changes in bone turn-over have necessitated the development of biochemical markers of bone metabolism.

Markers of bone formation: Serum osteocalcin is the most abundant noncollagenous protein specific for bone and dentin. It is synthesized by the osteoblasts but its precise function remains unclear. However, for the evaluation of bone formation, it has been found superior in both sensitivity and specificity to the most commonly used marker—alkaline phosphatase (ALP)—particularly in osteoporotic subjects. Procollagen I extension peptides and other protein are being investigated for possible use.

Markers of bone resorption: Urinary hydroxyproline is released during collagen degradation but as it originates from other collagen sources than bone tissue as well, there is not a constant correlation to bone resorption. New and sensitive markers of bone resorption are available through the use of pyridinium derivatives, pyridinoline (Pyr) and deoxypyridinoline (D-pyr), collagen cross-links excreted through the urine during degradation of mature collagen.

Osteoporosis may lead to fragility fractures and an increased fracture incidence, both general and age-specific, has occurred during the last decades. Apart from monitoring treatment, it would be of special interest to acquire a possibility to identify patients at risk of sustaining hip fractures as well as other fragility fractures. In order to evaluate the discriminative properties of biomarkers in relation to fracture patients we have conducted a study of 174 women, 80 years of age, with hip fracture. The fracture patients were found to have lower levels of osteocalcin and increased levels of Pyr and D-pyr at the initial sampling within a few hours from fracture, compared with 77 age-matched controls. These levels were not correlated to cortisol response. In 15 patients followed for one week an initial decrease of osteocalcin was noted with a nadir at day 2-3, thereafter an increase was evident. This was also true for ALP, while Pyr and D-pyr remained unchanged. At follow-up 4.5 months after fracture, in 58 women osteocalcin and ALP had significantly increased to reach the same level as was found in the controls. In another study of 26 women with different types of fractures at a mean time of 2.8 years earlier, osteocalcin again was found to be lower compared with controls matched for bone mineral content and age, but without previous fractures.

It appears that elderly women with a hip fracture or general fracture susceptibility have a decreased bone formation prior to fracture and an increased bone resorption. However, they are still able to adequately respond with an increased bone forming activity during fracture healing. When this phase is passed, presumably a return to a lower formation level occurs.

The ultimate goal is of course to find out if the analysis of osteocalcin and pyridinolines may identify the individual fracture susceptible woman.

VIII. In vitro assessment of proximal resistance arteries isolated from cancellous bone

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Introduction: Present knowledge of vascular physiology in bone tissue has been derived from in vivo perfusion studies. However, in such experiments it may be difficult to distinguish direct effects of vascular reactivity in bone from hemodynamic changes in bone secondary from effects in other organ systems. The perfused tibial nutrient artery model is the only isolated bone perfusion model currently available. This model allows direct assessment of normal vascular physiology in the tibia. But information on the vascular pathophysiology in localized bone disease is difficult to obtain. We have therefore established an in vitro method for direct investigation of vascular reactivity in resistance vessels isolated from cancellous bone.

Material and methods: The distal femur was removed from anesthetized pigs (approximately 80 kg). The condyles were sliced sagittally and stored in a physiological saline solution. Proximal resistance arteries were dissected from cancellous bone (1 to 4 vessels per pig) under a stereomicroscope and mounted as ring preparations on a small vessel myograph (1, 2). The diameter (1_{100}) the vessels would have had in vivo when relaxed and exposed to a pressure of 100 mmHg (13.3 kPa) was determined. The vessels were stimulated isometrically with a solution containing 125mM potassium and 10 µM noradrenaline (NAK), or with increasing concentrations of noradrenaline, vasopressin or potassium. From the force development measured, active tension and active pressure were calculated. The active pressure represents the pressure against which the vessel would have been able to contract under in vivo circumstances (1).

Results: The success rate of dissection and mounting was about 95%. Thirty-nine viable vessels (1100=250 (159–401) µm) were studied. The force development was maximal at 0.9 x 1_{100}, and this setting was used in all subsequent experiments. When activated with NAK, the maximal tension was (mean±SEM, n=9) 2.92±0.33 N/m and the active pressure 198±19 mmHg (26.4±2.5 kPa) (25 vessels, 9 pigs). This is consistent with values obtained from vessels isolated from other vascular beds (2) and suggests that bone vessels are fully viable using this technique. When stimulated with noradrenaline, vasopressin, and potassium dose-dependent responses were found. There was no difference in force development, agonist sensitivity, or reproducibility between vessels investigated on the first and the second day after dissection. Contractile responses remained constant for 10 hours after mounting.

Conclusion: With the establishment of this technique, controlled in vitro investigations of small trabecular arteries have been made possible, allowing direct assessment of vascular physiology and pathophysiology in bone tissue.

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IX. Strength of cancellous bone in relation to bone mineral as determined by QCT and DEXA

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To study the predictive value of bone mineral for bone strength, we related the results of mechanical tests to quantitative computed tomography and dual energy x-ray absorptiometry.

Materials and methods: 14 pairs of femurs were retrieved at autopsy from 10 men and 4 women, age 50–92 years, weight 56–76 kg, and height 158–178 cm. Bone mineral was studied by QCT and DEXA in femoral neck, shaft and condyles. In punch test, the lateral condyle was compressed in sagittal plane by a piston, 24 mm in diameter. Cubes, 2x2x2 cm, sawed from the medial condyle of the opposite femur were suscepted to axial loading (Schenck Trebel RM 100 Universal Material Testing Machine).

Results: The energy expenditures in the punch tests varied from 1.9 to 22.1 J and in the compression tests from 1.6 to 25.4 J. They were unrelated to the demographic parameters. The maximal loads and energy expenditures in the two types of tests showed pairwise correlations (r=0.81 and 0.84, resp.). The mechanical properties correlated better with the bone mineral in the condyles and femoral heads than in the shafts.

Discussion: Bone implant constructs often failure in osteoporotic bone. Large variations occur in bone strength in the elderly making the individuals differently susceptible to fractures and affecting the results of fracture treatment. Studies are needed to relate different osteosynthesis methods to the fragility of bone.

X. Dispersion of polyglycolide particles after decomposition of absorbable fracture fixation screws and pins

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Introduction: A clinical complication unique to absorbable fracture fixation devices has emerged when these implants have been increasingly used (Böstman 1991). Two to four months after the operation, remnants of polyclycolide implants have spontaneously extruded through the skin in 8% of the patients, on an average. The present experimental study was an attempt to elucidate the pathogenesis of this tissue response.

Materials and methods: A transverse osteotomy in the cancellous bone area of the distal rabbit femur was fixed with a 4.5 x 25 mm screw or with two 1.5 x 30 mm pins made of polyglycolide. Histological sections obtained after a follow-up time of 3, 6, 12, or 36 weeks from 24 animals were analyzed morphometrically under polarized light to examine the behaviour of the birefringent polymeric debris during degradation of the implants.

Results: Two different patterns of dispersion of the polymeric particles into the host-tissues were seen. From 6 weeks on, in all specimens polymeric debris was seen lying intracellularly in phagocytic cells in a regular front not more than 0.85 mm from the original tissue implant boundary. In 6 specimens, however, there occurred bulging expansions filled with largely extracellular polymeric particles at distances of up to 2.8 mm from the implant cavity.

Conclusion: In contrast with the regular front pattern, the particle expulsion observed over long distances cannot be explained by cellular transport by macrophages. Although no external sinuses emerged in the animals, the morphology of the bulging expansions filled with polymeric debris was suggestive of an increased osmotic pressure that probably developed within the implant cavity during degration of polyglycolide.

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XI. Training increases the in vivo fracture strength of the lower leg of rats

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Introduction: Nordsletten and Ekeland (3) found the strength of intact tibias to be nearly doubled tested in vivo during muscle contraction compared to testing after removal of all soft tissues. The aim of the present study was to measure the effect of training and inactivity on the in vivo strength of the lower legs of rats with normal bone mass.

Material and methods: 30 male Wistar rats (weight 317 g) were randomized to three groups. Group A (training) exercised on a treadmill (speed of 27 m/min) for 1 hour, 5 days a week in four weeks. Group B (normal control) were housed under normal conditions without any intervention. Group C (inactivity) underwent right-sided patellarectomy four weeks prior to testing. At in vivo testing the right lower leg of the anesthetized animals was loaded in three-point ventral bending until fracture. During testing a nerve stimulator induced tetanic muscle contraction via an electrode mounted on the ischiadic nerve on the thigh.

Results: The weight gain was higher in Group B (37%) and C (57%) compared with the training group (p<0.05), indicating effect of the training. Ultimate bending moment was 12% higher in Group A than in Groups B and C (p<0.05). Energy absorption was 11% (versus B) and 15% (versus C) higher in Group A (ns). Bending stiffness was 7% and 16% higher in Group A compared with B and C (p<0.05). Deformation at fracture was 1.5% (versus B) and 3% (versus C) lower in Group A (ns).

Discusson: The training intensity was chosen because earlier studies have found no increase in the strength of tibia neither in 8 week-old female rats (2), nor in male rats of the same strain and age (1). The increase in fracture strength in the present study is therefore probably the result of stronger contraction of muscles with an "protective arm of momentum" (as the calf muscles in this test). The same

mechanism may protect against fracture also in the hip, where several strong muscles have long arms of momentum on the femoral neck.

Conclusion: Training increased the in vivo fracture strength during contraction of the lower leg muscles of rats.

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XII. Postischemic muscle necrosis after graded periods of reperfusion

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Introduction: Several studies indicate that after muscle ischemia part of the damage occurs during reperfusion, and that the granulocytes play a major role in this injury (1). The aim of this study was to determine the exact time of appearance of the granulocytes and to measure the size of the necrotic lesions at various periods of reperfusion after 4.5 hours of complete vascular occlusion.

Materials and methods: A modified tourniquet model was used to induce complete ischemia for 4.5 hours in the left hindlimb of male Wistar rats (2). The animals were killed after survival periods of 2, 4, 5, 6, 8, 16, 24, 48 and 72 hours, and cross-sections of the anterior tibial muscle were prepared for histological examination. The sections were stained with H+E for conventional histology. Immunostaining for albumin was used as a marker of irreversible cell injury. The size of the lesions was measured by morphometry. Granulocytes were counted on toluidine blue stained sections on historecin embedded material.

Results: In the immunostained sections, the area of necrosis could be measured even at the shortest survival periods. All sections showed a central area of complete necrosis (41% of the cross-sectional area) which was interpreted to be a no-reflow zone. This area was surrounded by a zone with reflow and partial necrosis (37%). There was no significant increase in size of these areas from 2 to 72 hours postoperatively. Granulocytes appeared after 6 hours and increased to 24 hours. They were present only in the zone of partial necrosis.

In H+E stained sections the muscle fibre changes were vague after reperfusion periods shorter than 24 hours. After longer survival periods zones of necrosis with and without resorption could be separated and measured (2), and the areas of necrosis were in accordance with those found with immunostaining.

Discussion: Two types of necrotic areas could be measured in the anterior tibial muscle after acute hindlimb ischemia in the rat. By using immunostaining with rat antisera against albumin these zones could be discerned two hours after the ischemic period. The central, most damaged zone probably represented a no-reflow area. A possible harmful effect of granulocytes after 4.5 hours of ischemia will not occur until 5–6 hours of reperfusion and only in the penumbra of the lesions.

Conclusion: The present model showed no significant increase in size of the ischemic damage during 2–72 hours of reperfusion. Granulocytes appeared after 6 hours and did not seem to change the damage.

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XIII. Differential effects of exercise on blood flow in the spinal cord, nerve roots, and the dural sac. An experimental study in minipigs

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Introduction: The pathophysiology of motor and sensory dysfunction associated with spinal stenosis and nerve root entrapment syndromes has been related to activity induced ischemia or a decrease in regional blood flow in cauda equina or nerve roots. However, normal hemodynamic reactions of the spinal cord and associated structures during physical activity are unknown. Baseline hemodynamics of the spinal cord, nerve roots, and the dural sac have previously been reported (1). The aim of the present study was to determine the blood flow response of the spinal cord, nerve roots, and the dural sac during exercise.

Material and methods: The material comprised 10 Göttingen minipigs 18 months old, weighing 38–43 kg. Two bacon pigs of similar weight were used as pilots. The pigs were trained daily for 6 months on a treadmill. Chronic catheterization of the left cardiac ventricle and the descending aorta was established for blood flow measurement with microspheres. Both catheters were introduced through the right carotid artery and were tunnelled subcutaneously to the back. Catheters were flushed three times daily with heparinized saline. Blood flow was measured 3–4 days after catheterization at rest, after 15 min exercise at 3 km/hour, and at rest again 30 min post exercise using three different microsphere labels and reference sampling from aorta. The entire spine was removed segmentally, frozen in liquid nitrogen, and meticulously separated in white and grey

matter. Nerve roots, cauda equina, and the dural sac also were collected. Isotope counts were corrected for background, cross talk, and decay during counting. Regional blood flow data (RBF, ml/min/100 g) were analyzed using ANOVA and paired *t*-test. Four minipigs were excluded: One resisted training, three were lost due to complications secondary to chronic instrumentation.

Results: Selected RBF values (mean, SEM, n=6) are shown below. Grey matter had approx. 8 times higher RBF than white matter ($p<0.001$). The spinal cord blood flow distribution exhibited a characteristic pattern with higher flow values in motoractive segments at the cervico-thoracic and lumbo-sacral levels (ANOVA, $p<0.001$). Blood flow increased significantly in both grey matter ($p<0.05$), white matter ($p<0.01$) and the total spine ($p<0.01$) during exercise, most markedly in motoractive segments, but the characteristic RBF pattern along the spinal cord and the RBF gradient between grey and white matter prevailed. No blood flow changes were found during exercise in nerve roots (incl. cauda equina) or the dural sac. All blood flow values returned to baseline post exercise.

	Rest	Exercise	Post exercise
Total spine	17.0 (0.7)	22.7 (1.3)**	17.9 (0.8)
White matter	7.7 (0.5)	9.8 (0.3)**	8.3 (0.4)
Grey matter	40.6 (1.9)	55.5 (3.8)*	42.5 (2.1)
Nerve roots	10.1 (1.4)	9.3 (1.3)	7.9 (0.5)
Dural sac	1.3 (0.1)	1.4 (0.2)	1.4 (0.2)

Discussion: From this study it may be suggested that the motor active segments of the spinal cord are more susceptible to ischemia because of higher nutritional demands during exercise. Nutrition of nerve roots might not be directly dependent on RBF during exercise. Nerve roots may in part be nourished by diffusion (1), which might represent a considerable nutritional buffering capacity during conditions with increased demands.

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XIV. Rapid increase in CGRP-containing nerve fibres follows experimental fracture of rat tibia

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We studied the distribution and nature of innervation and analysed quantitative periosteal neural changes during healing of experimental fractures of rat tibiae at 7 and 21 days (fracture simulating standardized osteotomy model was used). For this purpose, we used neuroimmunohistochemical methods and image analysis quantification for protein gene product 9.5 (PGP 9.5) and calcitonin-gene related peptide (CGRP). The results show that a rapid terminal sprouting and reinnervation both in length and density of nerves occur after experimental fracture; in the periosteal reaction close to the callus surface both PGP 0.5- and CGRP-containing fibres were found in soft tissue pockets. At day 21, numerous PGP 0.5- and CGRP-immunoreactive fibres with convoluted course were found throughout the periosteum both in perivascular location and as individual free-ending terminals. CGRP-containing sensory afferent nerve fibres comprise the majority of periosteal neural population after such experimental fracture. Further, in this experimental model no nerves appeared to penetrate into the callus tissue from bone marrow. The most remarkable finding was the rapid increase in CGRP-containing nerve fibres in the periosteal reaction which follows the experimental fracture of rat tibia during the first 21 days.

XV. Effect of synovial effusion on juxtaarticular hemodynamics—knee joint tamponade studied in immature pigs and dogs

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Introduction: Increased intraosseous pressue (IOP) is a painful adjunct of inflammatory and degenerative diseases of joints. Intraosseous hypertension also may occur in the intraosseous engorgment-pain syndrome. The relation of this condition to early osteoarthrosis is uncertain. The pathomechanism appears to be impaired venous outflow from bone due to either extraosseous venous compression by synovial effusion, capsular oedema and subsynovial fibrosis, or intraosseous vascular pathology at the outlet from bone. Knee joint tamponade in young dogs causes increased intraosseous pressure in the distal femoral epiphysis. As part of an experimental series designed to elucidate the hemodynamic mechanisms in bone during knee joint tamponade, we measured regional bone blood flow (RBF), intraosseous vascular volume (VV) and mean transit time of blood (MTT) in two species.

Material and methods: Identical procedures were performed in 8 dogs and 8 pigs. One knee joint was cannulated, and the intraarticular pressure was elevated to 75% of mean arterial pressure by intraarticular infusion of dextran. IOP was monitored bilaterally by bone cannulation. RBF was measured after 30 min steady state with 15-µm ^{141}Ce-microspheres. Plasma volume (PV) and red cell volume (RCV) were determined by the distribution volumes of ^{125}I-

fibrinogen and ^{51}Cr-erythrocytes, respectively. VV was obtained by RCV+PV, and MTT by VV/RBF.

Results and discussion: IOP was elevated by 317±21% ($p<0.001$) in the distal femoral epiphysis of dogs, but not pigs, indicating differences in the relation between the knee joint capsule and epiphyseal drainage routes between these two species. Pig bone generally was more vascular with higher RBF and VV values. Pigs had unchanged RBF and MTT during knee joint tamponade, but subtle changes in the PV and RCV distributions were detected, and VV of pigs decreased significantly in patella (16±5%, $p<0.05$) and subchondral bone (12±4%, $p<0.05$). Precapillary vasoconstriction in bone is an unlikely explanation because RBF was unchanged. Intraosseous veins have no muscularis and cannot contract. The only possible explanation seems to be that subchondral bone in pigs is sufficiently compliant to yield to mechanical compression during joint tamponade. Dogs exhibited decreased RBF ($p<0.05$) and increased MTT ($p<0.05$) in the medial femoral condyle, but PV, RCV and VV remained unchanged. The increased intraosseous drainage time known from human studies of bone with increased intraosseous pressure thus may be reproduced experimentally in dog knees. The intraosseous vascular system, encaged in hard tissue, appears unable to expand during acute intraosseous venous stasis.

XVI. Prevention of arthritic bone loss by naproxen—a histomorphometric study in carragheenan induced juvenile gonarthritis

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Chronic arthritis is associated with juxtaarticular osteopenia, but our knowledge of the dynamics of arthritic bone loss is scarce. Prostaglandins of the E series, which are generated in arthritic joints, activate bone remodelling, but the effect of antiinflammatory treatment with prostaglandin synthesis inhibitors on juxtaarticular bone in chronic arthritis remains controversial. We studied the structure and dynamics of juxtaarticular bone in carragheenan induced experimental arthritis during naproxen treatment by bone histomorphometry.

Material and methods: Sixteen dogs, initially 3 months old, were matched pairwise by sex, weight and litter. Unilateral gonarthritis was induced in all dogs by weekly intraarticular injection of 2–3 ml carragheenan 1% for 12 weeks. One from each pair was randomly treated with p.o. naproxen (dosage initially 5 mg/kg, maintenance 2 mg/kg, serum-naproxen approx. 30 µg/ml), the other served as control. Bone was double labelled with i.v. tetracycline (15 mg/kg) prior to termination. An undecalcified 3 mm thick sagittal section of the distal femoral epiphysis was embedded in methylmethacrylate. Five um sections were stained with Goldner trichrome for light microscopy, and 20-µm unstained sections were prepared for fluorescence microscopy of cancellous bone centrally in the epiphysis.

Results: Untreated arthritis was associated with 25% reduced trabecular bone volume ($p=0.01$), an increased fraction of mineralizing surfaces ($p=0.02$), and an increased mineral appositional rate ($p=0.04$) as compared with uninflamed control knees. Naproxen treatment reduced the decline in trabecular bone volume following arthritis induction ($p=0.05$). The increase in the fraction of mineralizing surfaces in arthritis was reduced by naproxen treatment ($p=0.03$). There was a tendency to lower adjusted appositional rate ($p=0.10$) and higher mineralization lag time ($p=0.08$). No differences were found between groups with respect to fractional osteoid surface, trabecular thickness, trabecular plate separation, trabecular plate density, and mineral appositional rate.

Conclusions: Canine carragheenan induced gonarthritis is associated with trabecular bone loss and increased formative capacity in juxtaarticular bone. Naproxen treatment partially prevents the trabecular bone loss and reduces the development of a higher formative capacity in arthritis. These effects might be due to preventive actions of naproxen treatment on either the negative remodelling balance or the accelerated activation frequency in arthrits, but our data cannot distinguish between these effects. However, a reduction in trabecular bone volume in untreated arthritic knees of as much as 25% cannot be explained solely by a change in activation frequency.

XVII. Articular cartilage changes following experimental meniscal lesions, meniscectomy and meniscal repair

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We assessed the reaction of the knee joint cartilage in rabbits to a well-defined meniscal lesion in the avascular part of the fibrocartilage and compared the outcome after varying periods of time following nontreatment, meniscectomy and meniscal repair.

A longitudinal and stable lesion of the medial meniscus of the right knee was made in 30 rabbits. After three months a repair was performed through a full thickness radial cut from the lesion to the capsular insertion in 12 rabbits. Six animals had a meniscectomy, while the lesion was untreated in 12. The left knee joints underwent a sham procedure and

served as controls. The articular cartilage was studied at three-month intervalls. The femoral and tibial condyles were examined under a stereo-microscope and the cartilage of each condyle classified according to Shapiro and Glimcher. Microscopically, semiquantitative histologic-histochemical grading according to Mankin was performed.

None of the untreated lesions had healed. After three and six months the tears were unchanged but in all three menisci inspected after nine months lengthening of the lesion was seen. In all repaired menisci lesions had healed. Cartilage changes three months after meniscectomy were more pronounced than after meniscal repair or in untreated lesions. Meniscal repair did not reverse cartilage changes but prevented severe joint degeneration due to further splitting of the unrepaired lesion with destabilizing of its central part.

XVIII. Proteoglycan fragments in knee synovial fluid—the role of trauma

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Osteoarthrosis (OA) is a disabling disease of great socio-economic impact. Our inability to monitor early joint cartilage changes in OA hinder the development of new medical treatments, e.g. drugs capable of delaying the disease progress. However, it has been suggested that fragments from the joint cartilage matrix, like proteoglycans, which are released into the synovial fluid in both normal and diseased cartilage may serve as markers to monitor the development of posttraumatic OA (1). We have previously demonstrated that: 1. Proteoglycan fragments are massively released from the joint cartilage into the synovial fluid as a result of trauma to the knee with injury to meniscus or cruciate ligament (2). 2. The concentration of proteoglycan fragments in joint fluid in these patients is significantly elevated over that of a normal reference group for several years after injury (2). 3. There is an inverse relationship between the OA grade and the concentration of proteoglycan fragments in joint fluid (3). 4. Acute pyrophosphate synovitis in a joint with OA increases the release of proteoglycan fragments into the synovial fluid (3). 5. Exercise will moderately increase the concentration of proteoglycan fragments in knee synovial fluid in knee healthy athletes (4). Our continued collection of samples from patients with knee injury now allows us to examine differences in the temporal patterns of release of proteoglycan fragments from the cartilage matrix in patients with meniscus or cruciate ligament injury.

Patients and methods: Joint fluid samples were obtained from patients with previous knee injury and pain or instability. For all of these patients, the diagnosis of meniscus injury (A) or cruciate ligament injury isolated or in combination with meniscus injury (B) was made through arthroscopy. Samples from knee healthy athletes (C) before exercise were used as controls (4). Joint fluid was centrifuged and frozen in aliquots at –70 °C. The concentration of cartilage proteoglycan fragments was determined by immunoassay (5).

	Group	% Male	Age
A	274	77	38
B	364	69	29
C	16	63	28

Results: In groups A and B the highest average concentration was observed 5–7 days after injury, with sustained and significantly elevated levels for up to 20 years after injury, when compared with the healthy control group. In the separate analysis of the temporal patterns of proteoglycan release of groups A and B, we found no difference between the two groups either in the acute (0–5 weeks) or the late chronic phase (>2 years). However, in the time period between 5 weeks and 1–2 years, patients with cruciate ligament injury had higher concentrations of proteoglycan fragments in the joint fluid compared with those with meniscus injury.

Discussion: The mechanisms responsible for the release of cartilage matrix fragments in OA and inflammatory joint disease are not well known. A role for enzymes and cytokines had been proposed (1). In the present study we confirm our earlier finding that large amounts of cartilage proteoglycan fragments are released into the joint fluid within the first 24h after injury. The peak concentration is reached at about 1 week after injury for both meniscus and cruciate ligament injury. However, the proteoglycan release patterns in the time period from 5 weeks to 1–2 years differs between the two groups. After 2 years, the concentrations are again comparable. The hemarthrosis commonly associated with cruciate ligament injury and also the magnitude of the trauma are two possible reasons for the increased release of proteoglycan fragments in patients with cruciate ligament injury. Prospective studies with additional markers, enzymes and inhibitors are, however, required to explain these patterns and their possible relationship to the development of posttraumatic OA in these patients.

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XIX. Immunocytochemical studies of bone sialoprotein distribution between articular cartilage and subchondral bone of young rats

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Introduction: Like other types of connective tissues, cartilage and bone are composite materials. The major structural elements in articular cartilage are proteoglycans and collagen type II, while in bone a mineral phase of hydroxyapatite and collagen type I are dominating. In addition, both tissues contain a number of other matrix molecules which are involved in interactions with other matrix constituents. Bone sialoprotein (BSP) is an acidic glycoprotein associated with the mineral matrix in bone and dentin. By ultrastructural immunocytochemistry we studied the distribution of BSP in articular cartilage and bone especially focusing on the interface between the two tissues.

Materials and methods: Proximal epiphyses of 21 and 84-day-old rats were studied. The tissues were fixed by vascular perfusion using a fixative combination of phosphate buffered glutaraldehyde and paraformaldehyde followed by low temperature dehydration and embedding. Ultrathin sections were incubated with a polyclonal antibody to rat BSP and protein A gold was applied for detection. Electron micrographs were taken with a stratified sampling technique and the number of gold particles per unit area was estimated in the different compartments according to conventional stereological principles.

Results: Histologically the youngest rats had a growth zone at the lower end of the articular cartilage, and the oldest rats had an established subchondral bone plate. The concentration of BSP was highest in the interface betwen mineralized cartilage and bone in both younger and older rats: the ratio for the three different compartments, osteocartilaginar interface, bone and cartilage was: 1: 0.34: 0.07 for the 21-day-old rats and: 1: 0.33: 0.02 for the 84-day-old rats. Control sections incubated with rabbit serum showed very low levels of immunoreactivity.

Discussion: The strikingly high concentration of BSP in the osteocartilaginar interface in this study supports the theory that BSP is important in the process of bone formation. However, this high concentration was not only observed in the young growing animals, but was also found in the older animals with an established subchondral bone plate. Thus the accumulation of BSP at the interface between mineralized cartilage and bone is not a phenomenon restricted to growth cartilage. The present results give further support to the notion that BSP is a factor produced by osteoblasts for anchoring components of bone to the surface of calcified cartilage or for regulating processes at the osteocartilaginar interface.

XX. Collagen volume and surface densities and fibril diameter in bovine articular cartilage

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Introduction: Articular cartilage is the weakest link in the locomotor system. In joint cartilage collagen type II forms a fine-fibrillary framework, responsible for the tissue's tensile strength. Collagen architecture in terms of fibril diameter and density differs between the zones and compartments of the tissue, and this variation is undoubtedly of importance for the mechanical properties.

Material and methods: Bovine fetlock joint cartilage was short-term fixed in a phosphate-buffered mixture of glutaraldehyde and paraformaldehyde and subsequently low temperature-embedded in a polar resin. Ultrathin sections were cut both parallel and perpendicular to the joint surface. Electronmicrographs were taken from the superficial, radial and calcified zones using a stratified random sampling technique. Collagen surface density (Sv), volume density (Vv) and collagen fibril diameters were estimated according to conventional stereological principles.

Results: Collagen Vv increased with increasing distance from the articular surface and also with increasing distance from the chondrocytes. Collagen Sv increased with increasing distance from the cells, but was of a similar magnitude in the different zones, despite striking differences in collagen diameter. Moreover, in the territorial and interterritorial compartments of the radial and calcified zones there is a substantial amount of thin fibrils intermingled with coarser ones.

Discussion: The present investigation provides quantitative data regarding collagen architectures in articular cartilage. The increasing collagen Vv and fibril diameter from the surface to the calcified zone parallels the concentration gradient of the large aggregating proteoglycan, and our results are in harmony with previous mechanical experiments showing an increasing stiffness of the lowermost part of the cartilage. The large variability in collagen fibril diameter even in the lowermost interterritorial zone, indicates that collagen fibrils may have other roles in addition to taking up static loads, since such forces are generally believed to be handled by the coarser fibrils. Thus, the slender fibrils may have a role in distributing load, thereby reducing peak stresses and risks of tissue damage. However, empiric mechanical laws are not necessarily applicable on the ultrastructural level where other factors like osmotic and electrostatic forces are operating. Interestingly, collagen Sv is in same order of magnitude throughout the tissue. This observation is highly important in discussions regarding the molecular interactions responsible for the mechanical properties of the tissues.