

Symposium on experimental musculoskeletal research

Copenhagen, May 10–11, 1991

Editor: Olle Nilsson

Department of Orthopedics
University Hospital
S-751 85 Uppsala, Sweden

On bone metabolism in paraplegic patients

H. H. Bohr

BMC lab., Med. dept. TTA, 2002, Rigshospitalet,
Copenhagen, Denmark

It is well known that the Bone Mineral Content (BMC) decreases during immobilization as seen during bedrest, space flight and disease. In patients with paraplegia after spinal injury it was shown that BMC of the bones in the lower extremities decreased, while BMC of the lumbar spine and the distal radius remained almost unchanged (Biering-Sørensen et al. 1988). For the femoral neck and the proximal tibia BMC decreased exponentially with a rapid loss of bone during the first 2 years amounting to about 30 and 50 percent respectively and then at a slower rate, reaching almost constant values about 3 years after injury. For the femoral shaft the fall in BMC was relatively smaller, but seemed to continue almost linearly amounting to about 25 percent 4 years after injury (Biering-Sørensen et al. 1990).

Measurements of BMC were made by dual photon absorptiometry, using Gd-153, 1 Ge.. Scanning of the femoral shaft was performed transversely to the bone axis around the middle. From the attenuation curves the external, D , and internal diameter, d , could be determined as previously described (Bohr and Schaadt, 1990). The density of the cortical bone was derived from BMC and the cross sectional area. Eight patients with paraplegia were followed from 1 month to about 5 years after spinal injury. The result shows that the internal diameter of the femoral shaft increased significantly and almost linearly with time. The external diameter showed a small but not significant increase and the density of the cortical bone seemed to decrease with about 20 percent.

These findings indicate that for the femoral shaft, the increased endosteal resorption continues for at least 4 years of immobilization corresponding to the decrease in BMC. For the femoral neck and the proximal tibia the increased bone resorption seems gradually to decline and after 3 years of immobilization a new equilibrium between bone formation and bone resorption is established corresponding to the almost constant values of BMC.

A comparative study of histomorphometry and ^{85}Sr uptake of heterotopic bone induction by demineralized bone in rats

Eirik Solheim, Else Pinholt, Gisle Bang¹ and Einar Sudmann²

Institute for Surgical Research, Rikshospitalet, University of Oslo, ¹Department of Oral Pathology and Forensic Odontology and ²Hagavik Orthopedic Hospital, University of Bergen, Norway

The purpose of the study was to investigate the correlation between ^{85}Sr uptake and histomorphometry of demineralized allogeneic bone powder (DBM)-induced heterotopic bone in rats.

Materials and methods: 5, 10 and 15 mg DBM was implanted in the abdominal muscle of 45 male 8-weeks-old Wistar rats, which had been randomized in 3 groups of 15 rats each. At week 4 the implants were evaluated by ^{85}Sr uptake and histomorphometry of the area of the induced bone. Two indices of ^{85}Sr uptake were calculated; the osteogenic index [(counts/min/mg implant)/(counts/min/mg ilium)] and an index we have called the osteoquantum index in which the weight of the implant has been invalidated [(counts/min implant)/(counts/min/mg ilium)].

Results: The osteoquantum index was found to have a linear relationship to the area of the induced bone with a correlation coefficient (r) of 0.910 and high statistical significance ($p < 0.0001$). No significant correlation was found between the osteogenic index and the area of the bone ($r = 0.272$, $p = 0.09$) or between the osteogenic index and the osteoquantum index ($r = 0.276$, $p = 0.09$). The osteoquantum index and the area of the bone both increased proportionally to the quantity of implanted DBM, while the osteogenic index did not differ significantly between the 3 groups.

Conclusion: The results of the study indicate that the osteoquantum index gives more valuable information about bone formation than the osteogenic index.

Fibroblast growth factor increases or inhibits induced bone formation, depending on dose

Per Aspenberg, L Stefan Lohmander and Karl-Göran Thorngren

Department of Orthopedics, Lund University Hospital, S-221 85 Lund, Sweden

Aim: The implantation of demineralized bone matrix (DBM) in rodents elicits a series of cellular events leading to the formation of new bone inside and adjacent to the implant. This process is believed to be initiated by an inductive matrix protein, but local growth factors may further regulate the process, once it has been initiated. We have previously shown that local application of recombinant human basic FGF in a carboxymethyl cellulose (cmc) gel to DBM implants increases the bone yield as measured by calcium content 3 weeks after implantation in rats. We now studied dose-response and time effects.

Methods: Pairs of demineralized rat femoral diaphyses were implanted intramuscularly in rats. The marrow canal of one implant in each pair was filled with a cmc gel containing various doses of bFGF, and the amount of calcium deposited in the implant was measured at 3 weeks as an estimate of bone yield. With an bFGF dose 15 ng, calcium yield was also measured at 2, 3, 4, 5 and 6 weeks

Result: The calcium content was increased by 75 ng of bFGF at 3 and 4 weeks (by 31 and 27 percent), but not at 2, 5 or 6 weeks. Doses of 3, 15 and 75 ng of bFGF increased the calcium content at 3 weeks, with an optimum at 15 ng (78 percent increase). No effect was seen with 0.6 or 380 ng, whereas 1900 ng had a profound inhibitory effect (69 percent decrease).

Conclusion: Bone formation in vivo may be both stimulated and inhibited by bFGF, depending on dosage. In future attempts to stimulate bone formation with bFGF, dose and time regimens must be well controlled.

Effects of NSAIDs on heterotopic bone formation in rats and man—comparison of experimental and clinical results

Olle Nilsson

Department of Orthopedics, Karolinska Hospital, Stockholm, Sweden

Nonsteroid antiinflammatory drugs (NSAIDs) affect fracture healing and new bone formation. We compared the effects of NSAIDs on experimental heterotopic new bone formation in rats to the effects on clinical periarticular heterotopic bone formation following total hip arthroplasty (THA).

Animal model of heterotopic ossification (HO): HO was induced in the abdominal wall of growing rats by implanting

pieces of demineralized allogeneic bone matrix (DABM). Groups of rats were treated with indomethacin (2 mg/kg and day) for different periods during the induction process. The amount of new formed bone was analyzed at 3 weeks.

Clinical heterotopic ossification after THA: Periarticular heterotopic ossification (PHO) occurs in 20–90% of patients operated with THA. Prophylactic treatment with NSAIDs for 3 to 6 weeks has been shown to effectively prevent HO. A double-blind investigation was performed to analyze if 8 days of postoperative treatment with NSAIDs is equally effective as 3 weeks treatment in preventing HO after THA.

Results: In the experimental model DABM provided a strong stimulus to bone formation. Indomethacin caused a 20% decrease in net bone formation (ash content of implants) when given in conjunction with the implantation procedure, while treatment in other phases of the induction procedure did not affect bone formation. Similarly, short-term treatment with ibuprofen after THA was equally effective in preventing HO as a longer period of treatment (Table).

Table. Radiographic occurrence of PHO in patients operated on with THA

	Grade of PHO					
	0	I	II	III	IV	N
(1) Ibuprofen 21d	44	3	1	0	0	48
(2) Ibuprofen 8d	41	5	1	1	0	48
(3) Placebo	29	9	6	3	1	48

PHO was graded from AP radiographs. Grades I–IV denotes increasing heterotopic bone, with IV being virtual ankylosis of the hip joint. The patients were treated with ibuprofen or placebo from the day of surgery.

Conclusions: In both the experimental and clinical HO a strong correlation between the inhibitory effect of NSAIDs and the timing of treatment was noted; the drug was effective in preventing HO only when given in conjunction with the surgical procedure. Further, in both the clinical and experimental model the formation of HO was highly dependent on the individual, some individuals seem more prone to react with a bone forming response to induction with DABM or the surgical procedure of THA. Thus, there are several similarities between the effects of NSAIDs on experimental heterotopic bone formation in response to DABM and clinical heterotopic bone formation following THA.

Effect of platelet derived growth factor (PDGF) on heterotopic bone formation in rats

Olof Risto, Ola Wahlström, Avni Abdiu and Thomas Walz

Departments of Orthopedics and Cellbiology, University Hospital, S-58185 Linköping, Sweden

Aim: To study the effect of platelet derived growth factor (PDGF) on the amounts of induced new woven bone.

Methods: PDGF (20ng/ml) was administered continuously for two weeks via microosmotic pumps to 6mm long pieces of demineralized rat femur inserted in muscle pouches. Each rat had a control piece inserted in the contralateral gluteal muscle. The samples were collected after 4 weeks for measuring ash and wet weights. Fourteen rats were evaluated. In order to obtain samples for histological examinations another four rats, treated in the same way, were killed after one, two, three and four weeks respectively.

Results: The mean ash weights of the PDGF treated samples were (7.8 ± 4.9 mg) and of the control samples (5.2 ± 1.7 mg) ($p < 0.05$). Histological examination showed considerably more connective tissue proliferation after one week in the PDGF treated samples.

Conclusions: The results support the theory of PDGF stimulating new bone formation. Histological examinations point out that one mechanism can be increased connective tissue outgrowth.

Tissue replacement of biodegradable polyglycolide screw in fixation of rabbit distal femoral osteotomy

O. Böstman, U. Päiväranta, E. Partio, M. Manninen, A. Majola, J. Vasenius and P. Rokkanen

The Department of Orthopedics and Traumatology, University Central Hospital, SF-00260 Helsinki, Finland

Aim: With the increasing clinical use of absorbable internal fracture fixation devices, detailed knowledge about the degradation and tissue replacement of these implants is required. To examine these processes an experimental study was designed.

Methods: Totally biodegradable screws, 4.5 mm in thread diameter and 30 mm in length, made of polyglycolide were used to fix a transverse distal femoral osteotomy simulating fracture in cancellous bone in 22 mature rabbits. Degradation and tissue replacement of the implant were studied histologically, histomorphometrically and micro-radiographically at sequential stages of absorption 3, 6, 12, and 36 weeks after operation.

Results: At 3 weeks the physical appearance of the screw was unchanged. At 6 weeks the first microscopic signs of degradation could be seen along the thread ridge. At 12 weeks the mean area fraction of retained polyglycolide

under polarized light was 26.3% of the sample fields at the periphery of the implant and 72.0% in the core area. At 36 weeks no polymeric material could be discerned, the predominant tissue component within the implant cavity being loose connective tissue. The pooled mean fractional occurrence of trabecular bone and hematopoietic bone marrow in the cavity at this time was still less ($p < 0.01$) than at the corresponding site on the intact control side but the degree of tissue restoration varied greatly from animal to animal. The signs of foreign-body reaction were mild throughout the study.

Conclusions: The degradation behavior of polyglycolide fracture fixation screws was uniform and well predictable. In contrast, the tissue replacement accompanying absorption of the implant was found to be inconsistent, a finding that may deserve attention if large absorbable implants are to be clinically introduced.

Bone grafts in T-cell deficient rats

O. J. Kirkeby, T. Berg Larsen and P. Lereim

Institute for Surgical Research and Sophies Minde Orthopedic Hospital, University of Oslo, Oslo, Norway

Aim: The T-lymphocyte immune system might be important for the inferior biological function of bone allografts as compared to autografts. Athymic rats have no functioning T-lymphocyte system. The revascularization, the formation of new bone, and the resorption of fresh syn- and allogeneic cancellous bone which was transplanted to an intramuscular pouch has been studied in athymic and normal rats.

Methods: Revascularization was evaluated with radioactive microspheres, formation of new bone was assessed with strontium incorporation, and resorption was assessed by measuring the graft weight reduction. Animals were killed at 3, 6, and 12 weeks after transplantation.

Results: The circulation and strontium incorporation in allogeneic grafts in normal rats were greatly impaired as compared to the athymic group and the syngeneic grafts. The allografts in normal rats had a smaller weight reduction than the allografts in athymic rats, suggesting impaired resorption.

Conclusion: We conclude that the T-lymphocyte system is at least partly responsible for the difference between syngeneic and allogeneic bone grafts, and that the thymus-dependant primary rejection mechanism probably is important for the biological function of allogeneic bone grafts.

An experimental analysis of partial ACL lesions and ligamentous and capsular restraints in the lateral knee compartment in relation to anterolateral knee instability

Svend E. Østgaard, Peter Helmig, Peter Suder, Strange Nielsen and Ivan Hvid

Biomechanics Laboratory, Orthopedic Hospital, University of Aarhus, DK-8200 Aarhus N, Denmark

Aim and methods: Anterolateral knee instability (1) was evaluated in an experimental setting using 12 normal osteoligamentous knee preparations. Abduction-adduction rotation, coupled tibial translatory movement and coupled tibial axial rotation were recorded continuously and simultaneously during flexion or extension while applying a well defined valgus directed moment.

Results: Both during flexion and extension a small abduction rotation and a small coupled anterior tibial translation were found after cutting the AMP but not after cutting the PLP. During extension a small coupled internal rotation was observed after transection of AMP or PLP, but during flexion no significant coupled internal rotation was found until after complete transection of the ACL. In the ACL deficient knee a small abduction rotation, internal rotation and anterior translation were found both during flexion or extension, somewhat larger after cutting the anterolateral capsula as compared to cutting the posterolateral capsula and the popliteus tendon, but only after cutting both the ALC and PLC including the PT a consistent significant increase was found in abduction rotation, internal rotation and anterior translation both during flexion or extension.

Conclusion: The study demonstrates the importance of realizing partial ACL lesions (primary constraints) and capsular lesions in the lateral compartment (secondary constraints) when evaluating patients with ACL-ruptures/reconstructions as these lesions will add to the interindividual variability of anterolateral knee instability and thus influence the grading of the instability.

Reference

1. Østgaard SE, Helmig P, Nielsen S, Hvid I. Anterolateral instability in the anterior cruciate ligament deficient knee. *Acta Orthop Scand* 1991; 1: 1-5.

Screw fixation of femoral neck fractures: Four methods tested by cyclic bending loading on osteotomized cadaver femurs

Jan G. Benterud, Arne Høiseth, Knut Strömsö and Antti Alho

The Orthopedic Department and the Department of Radiology, Ullevaal Hospital, University of Oslo, Norway

The aim of this study was to compare a new screw for fixation

of femoral neck fractures to a well known, successful implant. A transverse osteotomy was performed in the middle of the femoral neck on 24 autopsy specimens. The osteotomies were fixed with Olmed® screws and with an own construction, the Ullevaal Hip Screw, using 2 and 3 screws of each type in 4 experimental groups. A theoretical breakdown vertical loading force of the osteotomy, if it had been fixed with 3 Ullevaal screws and receiving a static load, was calculated, based on measurements of CT related mass in the trochanteric region. 40% of this force was given in dynamic cycles. Deformations of the osteotomies were recorded.

Results: The osteotomies fixed with 3 Ullevaal screws showed significantly less deformation than all the other combinations after 1 minute, after 10 minutes less than 2 Ullevaal screws, after 30 and 60 minutes less deformation than 2 Ullevaal and 3 Olmed screws. There were no significant differences between the other groups.

Conclusions: 3 Ullevaal screws give a stable fixation in our experimental model. It is not obvious that 3 screws give a more stable fixation than 2, the screw design probably decides. The cyclic tests may simulate a patient's first stage of mobilisation.

Restoration of hormonal anabolic deficit and muscle glycogen consumption in competitive orienteering

L. Tsai¹, C. Johansson¹, E. Hultman², R. Tegelman² and Å. Pousette²

¹Department of Orthopedic Surgery and ²Department of Clinical Chemistry, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden

Aim: To study the consumption and restoration of muscle glycogen and the changes in anabolic and catabolic steroid hormones in male elite orienteers during and after an orienteering competition.

Methods: 5 male elite orienteers with similar performance capacity and training background volunteered to participate. Venous blood samples and muscle biopsies from m. vastus lateralis were collected at 08.00 (test 1), 11.00 (test 2), 15.00 (test 3) on day 1, and at 08.00 (test 4) on day 2. Following test 1, all subjects performed a 14-km orienteering race with 700 meters of altitude variation, in accordance with ordinary international competition courses. Immediately after the race, liquid carbohydrates were ingested. At 17.00 on day 1, a dinner consisting of 300 g carbohydrates was served. Serum levels of cortisol and testosterone were analyzed by radioimmunoassay (RIA), and sex hormone binding globuline by immunoradiometric assay (IRMA). Albumin was analyzed according to Pinell et al and adrenaline and noradrenaline by high pressure liquid chromatography (HPLC). The muscle biopsies were analyzed for glycogen content according to the method described by Harris et al.

Results: The average muscle glycogen depletion following the race was 66%. During the initial four post-exercise hours, approximately 25% was restored. After 24 hours, an almost complete restoration was observed in 4/5 orienteers. However, one subject only restored 60% of the actual loss. Serum cortisol increased significantly during the race and was normalized after 24 hours. A synchronous increase in adrenaline and noradrenaline was observed. The magnitude of the increment implied a running activity close to $\dot{V}O_{2max}$. Free-testosterone did not change during the race, but decreased during the first four post-exercise hours. The free testosterone/cortisol ratio which has been proposed as an indicator of anabolic/catabolic hormone balance, decreased during the race and was normalized in 4/5 subjects during the first four post-exercise hours. No significant correlation was found between the relative changes in muscle glycogen versus the free testosterone/cortisol ratio.

Conclusion: In the present study, the anabolic/catabolic hormone balance was normalized within four hours post-exercise. Simultaneously, only 25% of muscle glycogen was restored. The decreased levels of testosterone observed four hours after the race may explain the relatively low rate of glycogen restoration, since testosterone has been shown to stimulate the glycogen synthetase I enzyme.

Muscle contraction increases tibial strength in rats

Lars Nordsletten¹ and Arne Ekeland^{1,2}

¹Institute for Surgical Research, ²Rikshospitalet, and Surgical Clinic Ullevål Hospital, University of Oslo, Norway

Binding release values for alpine skiers recommended by the International Association for Safety in Skiing (IAS) are partly based on testing of tibia cadaver specimens. Telemetric studies have revealed that the musculature may increase the fracture strength substantially. It is, however, difficult to measure the magnitude of muscle contribution to the leg loading capacity in humans.

Aim: To determine the effect of muscle contraction on leg loading capacity in young and adult rats.

Material: Male Wistar rats were classified as young; mean weight 231g (8 weeks) and adult; mean weight 341g (13 weeks).

Method: Tetanic contraction in lower leg musculature was electrically induced with a bipolar electrode ligated to the sciatic nerve. The stimulation frequency was 80 Hz. During stimulation the right lower leg was deflected ventrally to fracture in a test jig. The load was applied with a deformation rate of 0.08 radians (5.0 degrees) per second. The left tibia was tested after removal of all soft tissues, and served as control.

Results: A transverse fracture was induced 17.5 mm proximal to the malleolar plane. The increase in average ultimate bending moment during muscle contraction was

73% in young and 84% in adult rats (ns). Ultimate (absorbed) energy increased 150% in young rats versus 300% in adult rats ($p = 0.005$). The mean ultimate bending stiffness decreased by 17% in young and by 24% in adult (ns). The ultimate deflection angle increased by an average of 70% in young rats and 140% in adult ($p = 0.03$).

Conclusion: Tetanic contraction of the lower leg muscles increases the fracture strength approximately 80% in young and adult rats. The increase in energy absorption by muscle contraction is twice as high in adult rats.

The protective effect of low grade hypothermia in skeletal muscle ischemia

S. Skjeldal, B. Grøngaard, O. Reikerås, A. Svindland and A. Torvik

Sophies Minde Orthopedic Hospital and Ullevål Hospital, University of Oslo, Norway

Aim: The aim of this study was to induce complete hind limb ischemia in rats, and measure necrosis in the anterior tibial muscles at different environmental temperatures during the ischemia.

Methods: Wistar rats were submitted to complete ischemia in the left hind limb for 4.5 hours. Three days later the animals were killed, and complete sections of the calfs were prepared for histologic investigation. Areas of necrosis within the anterior tibial muscles were measured by morphometry. During the ischemia the animals were kept in an incubator where the temperatures in 5 different experimental groups were 21, 22, 24, 27, and 34 °C, respectively.

Results: The temperature in the ischemic limbs was reduced to one degree above the environmental temperature during the ischemia, and approached the temperature in the control limb during the initial two hours of reperfusion. The necrosis was 9, 29, 80, 96, and 100% in the 5 groups.

Conclusion: Small differences in room temperatures alter the outcome in experimental muscle ischemia.

The quantitative metabolism in bone cells measured in vitro using mass spectrometry

Benny Dahl, Thomas Kiær, Gert R. Andersen and Biarne Lund

Department of Orthopedics, Rigshospitalet, Copenhagen, Denmark

Aim: The metabolism of bone cells plays a major role in the regulation of remodelling and reorganizing of bone tissue. It has been shown that resorption of bone takes place at a relatively low pH obtained through excretion of organic acids from the bone cells. Furthermore human bone cells consume oxygen rather slowly compared to the rapid consumption of

glucose which is transformed into lactic acid. Hence, alterations in the metabolism of the cells is necessary to initiate the basic processes in remodelling.

Until now it has only been possible to assess the metabolism indirectly. The aim of these experiments was to assess the feasibility of mass spectrometry for the registration of bone cell metabolism.

Methods: Registration of bone cell respiration was performed using a mass spectrometer (VG, Middleworth, UK), with a special controlling and sampling system developed for measurements in cell cultures (AMIS, Odense, DK). Measurements were performed in a specially designed cell chamber. With this technique it was possible to measure the oxygen consumption and carbon dioxide production in the chamber directly and continuously. The molar consumption/production of the relevant gases was calculated using the time related decrease/increase of the mass spectrometer signal and the solubility constant for the gases. Human bone cells were obtained from patients undergoing surgical procedures for osteoarthritis and after 5 weeks of cultivation second generation cells were used for experiments.

Results: The oxygen consumption in different pellets of human bone cells varied from 0.09×10^{-9} to 2.09×10^{-9} moles/min and the carbon dioxide production varied from 0.07×10^{-9} to 1.87×10^{-9} moles/min.

Conclusion: On the basis of these preliminary experiments we conclude that direct measurement of human bone cell metabolism is possible using mass spectrometry.

In vivo colocalization between fibromodulin and collagen I & II

Håkan Hedlund, Silwa Mengarelli-Widholm¹,
Dick Heinegård², Finn P Reinholdt¹ and Olle Svensson

Departments of Orthopedics and ¹Pathology, Huddinge University Hospital, Huddinge and ²Department of Physical Chemistry, University of Lund, Lund, Sweden

Aim: There is increasing evidence that connective tissue cells use matrix proteins for communication and for controlling the composition of the intercellular substance. Fibromodulin, an anionic 59-kDa glycoprotein, binds to collagen in vitro, inhibiting fibrillogenesis. It therefore has a potential role regulating fibril dimensions also in vivo. The aim of this investigation was to study the ultrastructural relationship between fibromodulin and collagen I and II.

Methods: Bovine articular cartilage, tendon, and cornea, were fixed for 2 h at room temperature in 0.1 M phosphate buffered 0.3% glutaraldehyde and 0.3% paraformaldehyde, pH 7.4; dehydration was performed by stepwise increasing concentrations of methanol at temperatures progressively lowered to 228 °K. The polar resin Lowicryl K11 was mixed with crosslinker and infiltrated in gradually increasing concentrations. Polymerization was initiated by UV-light and the blocks were polymerized overnight at 233 °K. Ultrathin sections were incubated with primary rabbit poly-

clonal antibodies against fibromodulin. For detection, we used protein-A coated with 10-nm gold probes. On high-power electron micrographs, we measured the distributions of markers along the collagen fibrils, using a semiautomatic image analyzer.

Results: Fibromodulin had a similar relationship to collagen I and II, with distribution maxima at the fibrils' d-bands, i.e., the gap areas.

Conclusion: The association between fibromodulin immunoreactivity and the collagen fibrils' transverse bands, corroborates previous in vitro-studies suggesting that this matrix protein may be important in the regulation of fibrillogenesis. However, previous studies have shown that other matrix constituents—e.g., collagen IX and XI, the large aggregating proteoglycans, and minor proteoglycans like decorin—also have a regular d-periodic arrangement along collagen fibrils. Hence, the specific role, if any, of fibromodulin remains yet to be determined.

Effects of gradual physeal distraction on the vascular supply of the growth area—a microangiographic and histologic study in rabbits

Anne Alberty, Jari Peltonen, and Veijo Ritsila

The Orthopaedic Hospital of the Invalid Foundation,
Helsinki, Finland

In many recent experimental studies on physeal distraction some degree of growth disturbances have been observed (2,3). There are, however, also reports where growth after physeal distraction had not been markedly affected (1,4). The clinical use of the method has been limited for fear of growth disturbances. Because physeal growth and its affections are closely related to the blood supply of the growth area, the present study was performed to ascertain the vascular changes after physeal distraction and lengthening.

Materials and methods: A unilateral or a circular external fixation device was used for gradual physeal distraction of the distal femur in 45 rabbits. The contralateral femur was used as non operated or sham operated control (fixation pins only). Microangiography with barium sulfate was performed after distraction of 4–21 days after an interval of 0–4 days or 6 weeks. Microradiographs were made of decalcinated 500 sections and histologic studies after re-sectioning and staining.

Results: Capillary filling in nonoperated control specimens was regular. In the unilateral fixator group filling defects were observed in both epiphyseal and metaphyseal capillaries in sham operated and distracted specimens. In the circular fixator group the sham operated specimens were normal. With the circular fixator, after 4 days of distraction marked epiphyseal capillary enlargement with decreased filling of the metaphyseal capillary end loops occurred, and histologically hyperplasia and separation in the hypertroph-

ic chondrocyte zone of the physis was seen. At 9 and 21 days, the capillary changes persisted and new capillaries had appeared into the physeal space. Anastomoses between the epiphyseal and metaphyseal vasculatures also occurred. Bone formation into the longitudinal septae of the hyperplastic physis and bone bridges across the physis were seen. After six weeks, in most cases, there was a network of anastomosing capillaries between the epiphyseal and metaphyseal vessels and histologically bony ingrowth of the physis occurred. The perichondrial vessels remained intact throughout the distraction.

Conclusions: The epiphyseal capillary enlargement may signify accelerated nutritional demands of the physis, whereas the metaphyseal changes may be related to separation in the hypertrophic zone. A possible relationship between early epiphyseal capillary changes and later growth disturbances may exist.

References

1. Connolly F, Huurman W, Lippiello L, Pankaj R. *Clin Orthop* 1986; 202: 258–268.
2. De Pablos J Jr, Canadell J. *Clin Orthop* 1990; 250: 73–80.
3. Fjeld T O, Steen H. *J Pediatr Orthop* 1990; 10(4): 463–6.
4. Peltonen J, Alitalo I, Karaharju E, Helio H. *Acta Orthop Scand* 1984; 55: 359–62.