Acta Orthop Scand 56, 167–178, 1985

Bone circulation and bone metabolism

Symposium organised by the Scandinavian Orthopaedic Association

Copenhagen, October 26–27, 1984

Editor: Cody Bünger

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Microvascular anatomy and function of bone

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Bone is a richly vascularized tissue, which at rest receives more than 10% of the cardiac output. Various regions of the same long bone may receive very different amounts of blood. Töndevold (1983) used the microsphere technique in an evaluation of the blood flow in the canine femur and tibia, and found values of around 20–30 ml/min per 100 g tissue in the metaphysary regions, whereas the diaphyses were only supplied with around 3 ml/min per 100 g tissue. The long bone is supplied via three different afferent pathways: the nutrient artery, the epi-metaphyseal arteries and the periosteal arteries. The relative importance of each of these vascular systems is not known in detail, although there is a general tendency to regard the periosteally mediated supply to be of less importance than the others, at least where adult bone is concerned. The theory of cortical blood supply presented by Trueta & Carvadias, that the inner two thirds of the cortex is nourished from the marrow and the outer one third of the cortex from periosteal vessels, has today been largely replaced by the concept of Brookes et al. that the blood flow of the cortex is mainly centrifugally directed, i.e. originating from medullary vessels, whereas the periosteal cortical supply under normal conditions is significant only in the vicinity of fascial attachments to the bone. An important difference between the juvenile and adult has been emphasized, and is probably valid for other species as well. There is, mainly, a longitudinal capillary network in juveniles, while in adults a transverse, anastomosing vascular system develops. Lopez-Curto et al. (1980) in a microfil study in the canine tibia, demonstrated that separate capillary networks seemed to supply the bone tissue and the marrow, respectively. The authors claimed the existence of parallel capillary systems, not serial ones with capillary division first in the marrow and then in the bone as had been postulated previously.

In the case of a bone fracture, several vascular changes from the normal state occur. In the fracture region, there is formation of a haematoma, previously believed to constitute an important source of osteoprogenitor stem cells. Even if this assumption is not quite correct, there is experimental evidence that the presence of a blood clot is an important stimulus for vascular ingrowth.

Ficat et al. (1984) describe three further vascular stages in the fracture repair response: 1) vascular occlusion during the formation of fibrous callus, 2) vascular proliferation when vessels from the outside (not necessarily periosteal vessels) supply the fracture area and the bone blood flow is mainly centripetally directed, and 3) vascular union over the fracture gap with the vessel pattern being subsequently increasingly ordered. In cortical bone, a special type of vascularly directed bone repair, named creeping substitution, is responsible for this gradually normalised vascular arrangement.

Reaming and nailing of a fracture results in a primary avascularity of ⅔–⅙ of the inner cortex, with revascularization occurring primarily from the outside (Pfister 1984). Internal fracture fixation with a plate is followed by rapid repair of the disrupted blood flow caused by the screw, and a cortical avascularity localized beneath the plate that is repaired from medullary vessels (Gunst et al. 1984).

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Regulating mechanisms of bone blood flow

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Neural control of bone blood flow

Blood vessels in bone are richly supplied with sympathetic fibers. Increased sympathetic activity is achieved by general hypoxemia, by hemorrhage (to a mean arterial pressure of 50 mm Hg) and by acute denervation of carotid and aortic baroreceptors. These maneuvers lead in the chloralose/urethane-anesthetized dog to an increase in vascular resistance in cortical as well as in cancellous bone. Similarly, a decrease in the central sympathetic outflow induced by stimulation of the carotid baroreceptors leads to a decrease in vascular resistance in bone in the chloralose/urethane-anesthetized dog. If the sciatic nerve in dogs is sectioned, bone blood flow increases 5–45 per cent, and similarly lumbar sympathectomy is followed by a 25–30 per cent increase in bone blood flow. Stimulation of the lumbar sympathetic chain, on the other hand, causes vasoconstriction in bone.

Thus, during resting conditions there is a certain centrally elicited sympathetic tone in the blood vessels of bone. This tone may be modified during different pathophysiological conditions such as hemorrhage and hypoxemia.

It is unlikely that the local sympathetic venoarteriolar axon reflex described in other tissues is active in bone, since the transmural pressure in the veins layered in the osseous structure cannot be changed during venous stasis. However, spreading of the reflex from extraosseously located veins cannot be excluded.

Humoral regulation of bone blood flow

Noradrenaline induces vasoconstriction in canine bone when given i.v. in a dose of about 5 nmol/(kg·min). However, this dose is unphysiologically high compared to the doses needed in adipose tissue and skeletal muscle to elicit vascular responses. Thus it cannot be concluded that noradrenaline plays a role as circulating hormone in the regulation of bone blood flow. Against a significant role, at least during rest, are the findings that resting blood flows in bone in awake dogs and in dogs anesthetized with halothane, which severely depresses the concentrations of circulating catecholamines, are the same (Skovsted 1982).

In the isolated, perfused dog tibia, it has been demonstrated that porcine calcitonin infused arterially caused vasoconstriction, and this could not be blocked by the α-adrenergic antagonist phentolamine. Thus calcitonin may exert direct vasoconstrictor effects in bone (Driessens et al. 1981). In the same preparation hydrocortisone at low concentration augmented noradrenaline-elicited vasoconstriction, while at high concentration it abolished the vasoconstriction in response to nerve stimulation and noradrenaline infusion, probably by depression of vascular smooth muscle responsiveness. In this *in vitro* preparation parathyroid hormone did not possess any vascular activity. However, it has been found that circulating parathyroid hormone may dilate bone blood vessels. Similarly, acetylcholine given intra-arterially may increase bone blood flow.

Local control of bone blood flow

Bone blood flow measured as medullary pressure shows autoregulation down to a mean arterial pressure of about 80 mm Hg. Below this limit, perfusion in bone is reduced when mean arterial blood pressure is further lowered.

Evidence for a local metabolic regulation of bone blood flow has been obtained in dogs during pro-

longed exercise (Tøndevold & Bülow 1983) and in anesthetized dogs during prolonged venous stasis (Bünger et al. 1983). During prolonged exercise in adult dogs, a significant increase in blood flow in cortical as well as in cancellous bone was found after 2 h of exercise. The flow remained elevated for at least 1 h after the exercise bout. In contrast to this, it has been found that a short period of exercise in adult dogs decreased blood flow to bone, and likewise in puppies it has been found that blood flow is reduced in bone after 30 min of exercise on a treadmill (Bünger et al. 1984). Thus the "active hyperemia" in bone during exercise is a very delayed phenomenon, only compatible with a metabolic regulating mechanism.

Similarly, when venous stasis is induced by ligation of the medial genicular vein in dogs, increasing the venous outlet resistance from the distal femoral epiphysis 3-fold, the arterial inlet resistance remained unchanged after 5 min stasis. However, after 1 h stasis the arterial inlet, as well as the venous outlet resistances, were found to be significantly reduced. Again, this very slowly induced vasodilation can only be elicited by local metabolic events.

Some local metabolites have been found to influence bone blood flow. An increase in arterial CO_2 tension and a reduction in pH induces vasodilatation in bone from baboons anesthetized with chloralose and urethane. A normal local tissue metabolite like adenosine can, when infused systemically, induce vasodilatation in bone, but a significant effect during physiological or pathophysiological conditions remains to be demonstrated. Likewise, the vasodilating effect of locally produced prostaglandins, especially of the E series, remains to be demonstrated experimentally. However, indirect evidence points to an effect in the early vasodilation found after experimental fractures, as proposed by Gregg et al. (1984).

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Bone blood flow measurement by tracer microsphere technique

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The microsphere method is widely used in quantitative measurements of regional blood flow to bone and marrow. The current concepts of the technique applied to bone were recently described by Gross et al. (1981). The method provides noninvasive, repetitive flow measurements in all regions of bone in both anaesthetized animals and in awake, ambulant animals. A number of assumptions in applying the method to measure blood flow have been outlined. The most important are that the spheres must be uniformly mixed with blood at the site of injection, that the spheres should have no effect on systemic or local haemodynamics, and that the spheres should be entrapped in the first passage of microvasculature. To obtain statistical accuracy a sufficient number of spheres must be injected. The main disadvantages of the method are that flow can only be measured discontinuously and a limited number of times in each experimental animal. The instrumentation often includes ligation and cannulation of the carotid arteries, which may lead to changed sympathetic nervous system activity and thus influence peripheral haemodynamics. Only a few studies have dealt with the reproducibility of the technique in measuring bone blood flow (Jones et al. 1982). The purpose of the present study was therefore to measure the variation of bone blood flow in steady state and to evaluate the sources of stochastic variation in relation to circulating adrenalin and noradrenalin.

Materials and methods

In each of four mongrel puppies, 10–13 kg, aged 4 months, 4 repetitive flow measurements were performed during steady state using 6.0×10^6 NENTRAC 15 μm spheres in each flow measurement. In two dogs flow was measured at 15-min intervals and

in the other two dogs at 45-min intervals. Flow was evaluated in 24 symmetrical tissue biopsies, using a three-way variance components model. Plasma catecholamines were analysed prior to anaesthesia (halothane), before instrumentation, and before and after each flow determination. In two other puppies, which were chronically instrumented (Bünger et al. 1984), flow was measured in the awake state and catecholamines were monitored. A control group of two puppies was anaesthetized and instrumented only in the brachial vessels. Plasma catecholamines were measured in steady state for 3 h.

Results and comments

No systematic change of regional blood flow in relation to time or between right and left tissue biopsies was found. Stochastic variation between dogs, between dogs and limbs and between dogs and successive flow determinations was significant in most locations. A considerable residual stochastic variation, representing "measuring error", was found in all biopsies. The corresponding coefficients of variation for flow measurements in a number of locations are given in Table 1. The greatest stochastic variation could be ascribed to the difference between dogs. The high variation between symmetrical biopsies in immature metaphyseal bone probably reflects difficulties in obtaining real symmetrical biopsies in a compartment, where major flow gradients exist. The residual coefficient of variation was about 1.5 to 2 times higher than theoretically expected from the number of spheres in blood and tissue samples. Differences in spatial distribution of spheres in reference blood samples and tissue samples during counting, as well as errors introduced during computation of the different microsphere activities from raw data, probably contribute to this greater variation. In most of the tissues the time-dependent variation was significantly reduced when the time interval between measurements was short. The time-dependent variance component was thus reduced by 43 per cent. The flow values in the two groups (15 min versus 45 min) differed by only 3 per cent.

Injection of microspheres did not change the level of circulating catecholamines, nor the level of suprarenal blood flow. Halothane significantly depressed both adrenalin and noradrenalin. The surgical preparation including ligation of carotid arteries caused a significant increase of both adrenalin and noradrenalin, but the level never exceeded the preanaesthetic levels. The chronically instrumented dogs showed significantly higher circulating adrenalin compared to the awake, non-instrumented controls.

Table 1. Regional blood flow (RBF), 95 per cent confidence limits in four flow measurements in each of four mongrel puppies in relation to four components of stochastic variation: variation between dogs (D), variation between limbs (L), variation in relation to time (T), and residual variation (R).

Tissue	RBF ml×100 g^{-1}×min^{-1}	Coefficients of variation per cent			
		D	L	T	R
Femoral head	11–30	46	13	28	17
Femoral diaphysis	11–24	35	12	32	11
Femoral metaphysis	1–13	125	109	0	87
Patella	10–27	50	9	29	8
Medial femoral condyle	5–35	92	6	32	15
Anterior tibial muscle	5–15	51	32	40	20
Suprarenal glands	508–699	12	13	13	7

Conclusion

The tracer microsphere method gives reproducible determinations of regional blood flow, especially when the time interval between microsphere injections is short. The smallest variation between flow measurements in an experimental situation is achieved when using the animal as its own control within the single flow measurement. Injection of microspheres does not influence concentrations of circulating catecholamines.

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Mass spectrometry for continuous *in vivo* measurement of subchondral pO_2, pCO_2 and qualitative bone blood flow

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Knowledge of normal gas tensions in subchondral bone is of potential interest for the understanding of both normal metabolism and the metabolism in pathological bone conditions. Measurements have been tried, including *in vitro* analyses on extracted bone blood, a method which may introduce serious changes in the flow characteristics of bone and thereby changes in the environmental gases, or *in vivo* by polarographic methods, which are hampered by the lack of an *in situ* calibration technique.

Mass spectrometry is a method which makes possible the *continuous and simultaneous in vivo* registration of partial pressures of several gases. We have measured gas tensions with a mass spectrometer (SX-200, VG Gas Analysis, Winsford, UK) via a blood gas catheter. In essence, it is a stainless steel tube, the lumen of which is connected to the high vacuum (10^{-7} atm.) chamber of the mass spectrometer. The catheter tip is covered by a polyethylene membrane supported by a sintered bronze plug. When the membrane is in contact with the bone tissue, gases in the tissue diffuse through it and are drawn towards the vacuum chamber and detector.

The mass spectrometer signal is a linear function of the partial pressure of the measured gas at the membrane surface, but the signal is not necessarily equal to the partial pressure in the bulk of the tissue, because the mass spectrometer removes gases, and thereby creates a diffusion gradient extending from the membrane surface and into the medium. The magnitude of this gradient is unknown because it depends on the tissue perfusion. We have solved this problem by measuring, simultaneously, the signal of an inert reference gas, argon, of known partial pressure. Argon is a normal constituent of the atmosphere, and its partial pressure in the tissue is equal to that of the atmosphere (0.93 per cent of the barometric pressure). The relationship between the bulk oxygen partial pressure, pBO_2, and the oxygen and argon signals (SO_2 and SAr) is given by:

$$pBO_2 = pBAr \times kAr\,(SO_2 - SoO_2) / kO_2 \times (SAr - SoAr)$$

(Kofoed et al. 1983), where pBAr is the bulk partial pressure of argon, SoO_2 and SoAr are the background signals of oxygen and argon, kO_2 and kAr are the proportionality constants between the mass spectrometer signals of oxygen and argon and the partial pressures of these gases at the membrane surface. The background signals were measured by closing a valve, which separates the mass spectrometer from the catheter. The two constants, kAr and kO_2, were obtained by measuring the argon and oxygen signals in a well-stirred blood sample where the partial pressures of oxygen and argon at the membrane surface are equal to the known bulk tensions.

Qualitative estimation of regional blood flow

The argon signal is a unique function of the following parameters: (1) the bulk partial pressure of argon, (2) the permeability coefficient of argon in the tissue, (3) the permeability coefficient of argon in the membrane, (4) the geometry of the catheter tip, and (5) the blood flow in the bone adjacent to the membrane surface.

Since the argon partial pressure, the membrane and tissue permeabilities, and the geometry remain constant in an experiment, a decrease in the argon signal can be explained solely by a reduced blood flow in the bone adjacent to the surface of the catheter membrane. This relationship between the regional blood flow and the argon signal was used to estimate, qualitatively, changes in the regional blood flow (Grønlund et al. 1984).

The application of mass spectrometry has by now been used in several studies concerning environmental changes in the subchondral bone during the osteoarthritic process which have indicated that: 1) Acute joint effusion causes relative hypoxia and diminished regional blood flow in the subchondral bone (Grønlund et al. 1984), 2) No activation of regulatory processes is found within the first half hour of joint effusion as regional blood flow is continuously diminished and hypoxia and hypercapnia are increasing. These changes are reversed within 15 min after normalization of the joint pressure, but are still present after 3 weeks in an experimental model of synovitis. 3) At the 4-month stage of experimental osteoarthritis, no significant changes were recorded in the subchondral bone gas tensions (Svalastoga et al. 1984), whereas 4) In mature experimental osteoarthritis, a significant revascularization must have taken place, as regional subchondral pO_2 was significantly increased in spite of a simultaneous venous congestion demonstrated by intraosseous phlebography.

These and further observations might clarify some of the obscure points in the osteoarthritic process. They seem at least to indicate significant changes in the metabolic environment, which may influence enzymatic reactions and protein synthesis.

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In vivo effects of bone cement on circulation, fat cells and bone

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There are several potentially negative biological side effects of bone cement, such as: heat at polymerization (1), monomer leakage (2), and poor biocompatibility (3). In addition, there is an inevitable surgical trauma (4) at reaming of the medullary cavity when preparing the bed for a joint replacement. In the clinical situation, these various side-effects may combine to result in a greater tissue injury than would be expected from a separate evaluation of the tissue impact of each of them.

1. Heat at cement polymerization

A heat impact between 45°C and 70°C is found with the use of modern bone cements. The critical temperature for bone necrosis is around 47°C, application time 1 min. The same temperature-time level was demonstrated to disturb incorporation of a bone implant (Eriksson & Albrektsson 1984).

2. Monomer leakage

A test chamber for vital microscopy of bone was constructed to test the effects of bone cement monomer leakage. The method allows for observations of the same bone tissue compartment before, during and in repeated sessions after insertion of curing bone cement in a canal which leads down to the tissue that is observed in the vital microscope. As only a small amount of curing cement is introduced into the canal and only 0.15 mm^2 comes in contact with the tissues, the temperature effects are negligible. Therefore, the test chamber measures the impact of monomer leakage and, if the animals are followed up for long enough, it also measures possible negative side-effects due to poor biocompatibility of the bone cement. The acutely observed effects at the insertion of curing bone cement consisted of circulatory arrest, haemolysis and the formation of emboli. If the observed tissue was rich in fat cells, the tissue injury was widespread; otherwise, the zone of injury was limited to a few hundred microns immediately adjacent to the bone cement. In several cases, droplets, probably consisting of a mixture of air, monomer and fat globules, were formed and actually seen being embolized in larger veins of the area. During the weeks following cement insertion, there was a widespread resorption of fat cells, whereas the bone resorptive response generally was rather limited. The reason for the more severe tissue injury in the case of a fat cell-rich tissue is presumably the fat solubility of the monomer. A direct bone-to-cement interface was not observed in any case. An inflammatory response was seen in some cases of cement insertion and in all three cases where pure monomer was infused as a test of the method. Pure monomer gave rise to more severe tissue reactions than did curing bone cement, while insertion of silicone into the chamber did not result in noticeable tissue injury.

3. Biocompatibility of bone cement

Studies by Albrektsson (1984) and Pedersen et al. (1983) have investigated the tissue reactions to previously cured bone cement where temperature elevation, monomer leakage and surgical trauma were controlled. These studies indicated negative side-effects of cured bone cement on the bone-healing response.

4. Surgical trauma

Eriksson et al. (1984) measured the temperature at the insertion of Richards plates under clinical conditions and found, at a distance of 0.5 mm from the drill periphery, a temperature of 89°C (average), in spite of profuse saline cooling during surgery. To my knowledge, no studies have been published on the temperatures at reaming of the medullary cavity, but the surgical trauma is substantial.

We used the titanium bone chamber to investigate the combined influence of temperature elevation, monomer leakage and tissue biocompatibility, but with simultaneous control of the surgical trauma. In this experiment, we were able to ream out the tibial medullary cavity with a saline infusion, thereby minimizing the surgical trauma, and then insert cur-

ing bone cement into the medullary cavity of the rabbit tibia. Severe vascular damage and bone injury were observed by vital microscopy, which suggests that substantial tissue injury is caused by bone cement even with control of the surgical trauma.

In summary, curing bone cement leads to tissue injury with signs of permanent bone tissue damage. The clinically good results reported with, for example, hip arthroplasties, seem to indicate that the body defense mechanisms can nevertheless cope with the situation in most cases, provided there is no overloading of the injured bone or bone-to-implant interface.

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Bone blood flow during short-term, asymmetrical pulsating current stimulation

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To investigate the circulatory effects of electrical stimulation on bone, we measured blood flow in the long bones of dogs during stimulation with asymmetrical, pulsating direct current. The canine tibias were stimulated for a period of 3 h, using Jørgensen's apparatus connected to the bones with the aid of Hoffman's external fixation pins. Six mongrel dogs with closed epiphyseal plates were used. The dogs were chronically instrumented for blood flow measurements with tracer microsphere technique.

The intact bones were stimulated with 1.5 mA for 3 h. The contralateral leg was used as an unstimulated biological control. The perfusion was measured prior to the stimulation, after half an hour, and after 3 h of continuous stimulation in the dogs. The microspheres used were NEN-TRAC microspheres with a diameter of 15 μm, labelled with ^{141}Cerium, ^{46}Scandium and ^{103}Ruthenium. A single dose contained 10^7 microspheres and carried approximately 300 μCi. The spheres were suspended in 0.9 percent saline, and 0.01 percent Tween 80 was added. After the last injection of spheres, the dogs were killed with an overdose of saturated KCl. The long bones and other regions of interest were dissected free. In the tibias, the biopsies were taken as circular discs of 1 cm thickness, and the bone between the electrodes was taken as 8 biopsies. The marrow was counted separately. All biopsies were placed in pre-weighed plastic vials and gamma radiation was performed. The perfusion rates in all parts of the tibias were identical in the stimulated and in the non-stimulated control. The values were identical to values and flow patterns obtained in earlier studies.

In this study, we could not demonstrate any effect of the asymmetrical, pulsating direct current on the blood flow rate in stimulated bone.

Electrical stimulation of bone

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There are no published studies in which a possible electrical influence on the bone blood flow rate has been investigated. Nannmark et al. (1984) were able to describe a vascular leakage after stimulation with alternating currents (AC) or direct currents (DC), but not after stimulation with pulsed electromagnetical fields. This was a vital microscopic study and the authors observed no obvious alterations in blood flow rate during the 3-h electrical stimulation that was applied.

We have used the previously described vital microscopy bone chamber in combination with Laser-Doppler flowmetry to investigate possible (3 h) changes in blood flow rates after DC-stimulation of 5 μA, continuous current, or after stimulation with pulsed electro-magnetical fields repeating at 15 Hz. For DC-stimulation the equipment described by Herbst (1982) was used, while the electromagnetical experiment was performed with a stimulator of the Bassett-type, manufactured by Electro-Biology Inc, NJ, USA. During the 3-h electrical stimulation, the animals were checked with respect to blood pressure, body temperature and blood pH, pO_2 and pCO_2-levels. The Laser-Doppler technique introduced by Öberg et al. (1979) was used for blood flow estimations.

The present experiment started with the insertion of a vital microscopic chamber into the adult rabbit tibial metaphysis. After about 8 weeks, the tissue that had grown into the chamber space was analysed by vital microscopy and registered with photo and 16-mm ciné film. Thereafter, the probe of the Laser-Doppler flowmeter was attached to the bone chamber and left there during the entire experiment when electrical or electromagnetical stimulation was used (2 h).

The results are based on five animals stimulated with DC, five animals stimulated with pulsed electromagnetical fields and five control animals, in which no electrical stimulation was applied. The control animals demonstrated a fairly even blood flow rate. The control curves did not significantly differ from those obtained after stimulation with 5 μA DC. In the latter cases there were no reliable indications of any systematic blood-flow alterations in the form of blood-flow increases or decreases. However, the blood flow seemed to be more unstable than in the control measurements. Electromagnetic stimulation with a repeated pulse of 15 Hz seemed to lead to a decrease of blood flow. This slower blood flow was quite evident in four cases, while the fifth showed a fairly unaltered flow.

This study has investigated blood flow changes within a short period of time. It must be emphasized that a slow increase or decrease of flow over a longer stimulation is quite possible also with DC-stimulation, although it has not been evaluated in this study.

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Scintimetric evaluation of bone metabolism

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When introduced, bone scintimetry with technetium-labelled phosphate compounds was mainly used for the detection of malignant or infectious bone lesions. The field of indications has widened, and today some 50 per cent of isotope investigations concern the musculo-skeletal system.

One of many contributory reasons is the development of numerical evaluation. Numerical evaluation of isotope uptake in standardized regions of interest yields a possibility of following the course of a disease in consecutive investigations, and also of statistical treatment of obtained data. This presentation confirms these statements when applied to femoral neck fracture. The femoral head ratio refers to an isotope uptake comparison fractured/intact side.

Prognostic value of scintimetry

In a 3-year study of 40 fractures, the femoral head ratio was significantly lower (0.80 ± 0.11) in fractures with healing complications (redisplacement, non-union, segmental femoral head collapse) than in normal healing (1.50 ± 0.54) at 2 weeks after surgery (Figure 1), whereas in investigations performed at 12 months or later the reverse was true. In a 2-year follow-up of 176 consecutive scintimetries, these results were confirmed: 92 of 101 with a favourable prognosis (ratio > 1.0) at 2 weeks had healed uneventfully and the remaining nine showed healing complications. Sixty-five of 75 with an unfavourable prognosis developed complications within

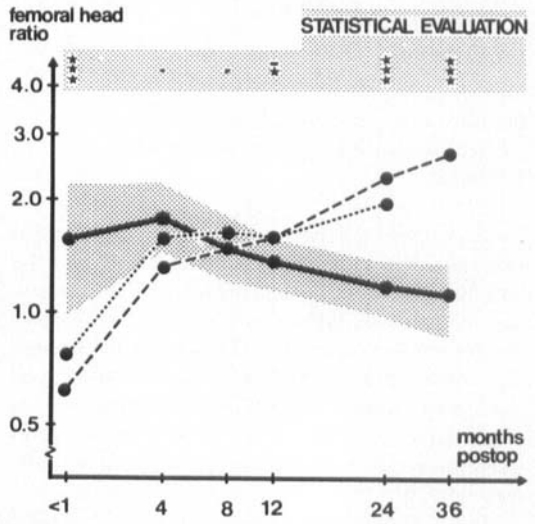

Figure 1. Long-term follow-up regarding the scintimetric pattern in femoral neck fracture healing.
▩ = uncomplicated healing (mean ± SD).
. . . = non-union/redisplacement.
– – – = segmental femoral head collapse.

2 years of fracture, and ten healed uneventfully ($p < 0.0001$).

Pre- versus postoperative scintimetry

Twenty-two cases had scintimetry performed prior to and 2 weeks after femoral neck fracture nailing with a 4-flanged nail. Six cases with a preoperative femoral head ratio $\geqq 1.10$ showed an increased isotope uptake after surgery. Eight cases with a preoperative ratio $\leqq 0.90$ showed the same pattern after surgery. The remaining eight had borderline preoperative ratios (between 0.90 and 1.10) and of these, two showed increased and six decreased uptake after nailing, indicating varying effects of the nailing procedure. The fracture diastasis often produced by flanged nails was regarded as a possible course of capsular vessel injury.

Comparison of different types of osteosynthesis

With the background described, a comparison regarding postoperative femoral head scintimetry was performed in femoral neck fractures operated with either a 4-flanged nail or two hook-pins; the insertion mechanism for the latter is low-traumatic. In undisplaced fractures, no difference in femoral head ratio between the two groups was seen. In 182 consecutive displaced fractures, the following figures were seen: 60 of 86 operated on with the nail had a ratio <1.0 and $26 > 1.0$, while 32 of 96 operated on with hook-pins had a ratio <1.0 and $64 > 1.0$ ($P < 0.0001$). These results were also reflected in a 2-year radiographic follow-up.

Effect of intracapsular hematoma on femoral head vascularization

Single cases with undisplaced femoral neck fractures and hip joint effusion verified by computed tomography showed an uptake defect over the femoral head at scintimetry (ratio <1.0). Hip joint puncture revealed an intracapsular pressure of >200 mm Hg and hematomas were aspirated. After this procedure, the femoral head ischemia was reversed according to scintimetry and the patients became asymptomatic. Whether these cases are unique or less uncommon is being evaluated currently, but it is evident that at least in single cases, hip joint effusion might reduce femoral head vascularization.

Aspects of microcirculation and bone metabolism of the juvenile knee in arthritis

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The bone lesions and growth abnormalities seen in children with juvenile rheumatoid arthritis (JRA) and haemophilic arthropathy (HA) were the clinical stimulus for our recent studies. Although multifactorial, they bear some major resemblance. Juxta-articular osteoporosis and epiphyseal enlargement may be found in both diseases; the knees are often affected; and in spite of current therapy, both JRA and HA may lead to severe functional impairment, deformity and pain.

Common pathogenetic factors in JRA and HA are the presence of chronic synovial inflammation and elevated joint pressure. We have focused on the influence of these two factors on the haemodynamics of juxta-articular bone in a series of experimental investigations in an animal model in the knee of the immature dog.

Arthritis was induced by repetitive intra-articular injections of Carrageenan solution. The resulting bone changes included juxta-articular osteoporosis and epiphyseal overgrowth. The resting intra-articular pressure in the model was 5–10 mm Hg. Haemodynamic investigations showed that the juxta-articular bone blood flow was influenced by the position of the knee and the anatomical relationship to the joint capsule. In arthritis, the synovial effusion pressure, the degree of synovial hyperaemia and a vascular "inflammatory resistance factor" determined the level of subchondral bone blood flow. In spite of synovial hyperaemia, the epiphyseal and patellar bone blood flow was largely unchanged. The vascular supply of particularly the distal femoral epiphysis and the joint capsule showed increased vulnerability to intra-articular pressure elevation in arthritis compared to control knees, indicating the presence of the vascular resistance factor.

From simultaneous flow/volume determinations using I^{125} fibrinogen and Cr^{51} labelled erythrocytes as tracers in measuring microvascular volumes, the microcirculation was further described. High blood flow rates, an increased microvascular volume and a short transit of blood were the main characteristics of the arthritic joint capsules, while in the subchondral epiphyseal bone, increased microvascular volume and prolonged transit of blood were the main

features. The epiphyseal plates were limits for the extension of these changes. Whether the vascular changes of subchondral bone are primary following synovial inflammation or secondary to changes of bone metabolism is still a matter of investigation. However, the resultant increase of permeability surface area between blood and bone may facilitate the metabolism, resorption and subsequent destruction of subchondral bone in chronic arthritides of childhood. Our most recent studies in the model using microautoradiography suggest that a hypermetabolism of bone is present in a very narrow part of the subchondral bone, whereas the metabolism seems to be decreased in the more central epiphyseal bone.

Bone metabolism of healing fractures

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Review of normal fracture repair

Local blood vessels are ruptured during a bone fracture, and a hematoma is formed between the fragments. Subsequently, thrombosis will occur in the vessels close to the fracture line. Proteolytic enzymes from aggregated platelets and leukocytes will activate the complement system, and inflammatory cells are attracted to the area by chemotaxis. Vitamin A is a prerequisite for this chemotaxis, whereas cortisone acts as an inhibitor. Macrophages activated by phagocytosis and platelets activated by thrombin will thereafter release a substance which stimulates the replication of fibroblasts. Simultaneously, osteogenic cells in the periosteum near the fracture start to proliferate and form new osteoblasts. Both the fibroblasts and the osteoblasts commence to produce collagen. The collagen synthesis is stimulated by hypoxia and lactate, and vitamin C is essential for a normal synthesis. Collagen types I and III are formed during early fracture repair. Later, the types II and I are produced as cartilage, and bone is formed in the callus. Vitamin D is necessary for a normal mineralization of the callus tissue, and fracture healing is impaired in patients with rachitis.

Osteocalcin (Bone-Gla-Protein) is the most abundant non-collagenous protein in bone. This protein has an affinity to hydroxyapatite and may play an important role in bone mineralization. In accordance with this, serum levels of osteocalcin have recently been shown to reflect osteogenesis (Sambrook et al. 1984).

Continuous bone remodelling takes place throughout the healing of a fracture. Dead bone close to the fracture line and woven bone from the early callus are resorbed by osteoclasts and replaced by lamellar bone. The latter contains only type I collagen. The activity of the osteoclasts is stimulated by prostaglandins and by the osteoclast-activating factor from activated lymphocytes. During bone resorption, osteoclasts or bone degradation products will release a coupling factor. This will stimulate osteoblasts to produce more bone, coupling bone resorption with bone formation.

According to McKibbin (1978), the fracture also induces osteogenic precursor cells in the local soft tissues to form bone and contribute to the callus mass produced by periosteal- and endosteal-derived cells. There has been a wide search for responsible inductive factors. These may be the bone morphogenic protein, the coupling factor, relative hypoxia or electrical impulses. Thus, in the early callus, pO_2 is low and the callus surface is electronegative. As the fracture heals, the tissue pO_2 reverts to normal and the surface charge of the fracture site is no longer negative.

Own study

A standard mid-femoral fracture was made in 37 male rats. The rats were 25 days old and had a median body weight of 61 g. At 10, 20, 30 and 40 days after the fracture, groups of rats were killed. Both femora were dissected free, radiographed and subjected to torsional tests and bone metabolic analyses.

The dry weight of the callus reached the peak value after 20 days, and the fractures were radiographically healed after 30 days. During the first part of the study, the healing fractures had a relatively higher content of hydroxyproline (i.e. collagen) than of calcium. This was also reflected in the specific activities of ^{14}C-hydroxyproline (i.e. collagen synthesis) and ^{85}Strontium (i.e. mineralization). The concentration of calcium and the mechanical strength of the healing fractures matched those of the contralateral intact bones after 40 days. The ratio of calcium to phosphorus in the healing fractures increased from about 1.3 to 1.55 during the experiment, suggesting a gradual transition of amorphous calcium phosphate to hydroxyapatite as the callus was remodelled.

The metabolic activity in the fractured bone (Figure 1) was also high at the end of the study, reflecting bone remodelling. This seems to last at least 1 year after femoral fracture in rats. In humans, increased retention of ^{85}Strontium has been observed in fractured bones several years after the fracture occurred.

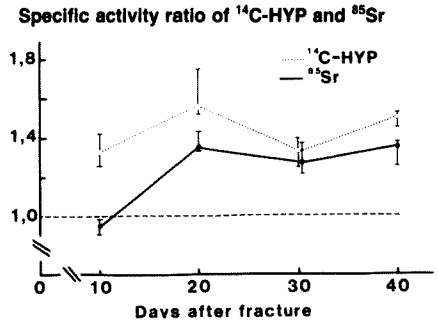

Figure 1. Specific activities of ^{14}C-hydroxyproline (^{14}C-HYP) and ^{85}Strontium (^{85}Sr) in healing femoral fractures as a function of time. The values are expressed as the ratio of fractured to intact femora.

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Bone metabolism after trauma

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From studies using single photon absorptiometry, it is known that after a tibial diaphyseal fracture, on average 45 per cent of the mineral is lost in the proximal metaphysis of the same bone. The morphological basis of this demineralization has so far seldom been described, mostly in animal experiments; there have been few human studies.

Material and methods

Histomorphometric and electron microanalysis techniques were used to examine undecalcified tibial trabecular bone biopsies from 20 cases, who had had a fracture of the same diaphysis 0.5–152 months previously. The distance from the biopsy site to the fracture was 19 ± 6 SD cm, so the callus tissue should not have interfered with the biopsy region. The biopsies were fixed in buffered formalin and embedded undecalcified in methylmethacrylate. For histomorphometric analysis, the specimens were cut into 5 μm sections and stained according to Goldner. Some specimens were also stained according to von Kossa. Unstained sections from five cases were analysed in the scanning electron microscope to evaluate the histochemical findings on these patients. Ground sections of 100 μm were used to quantify the calcium in central parts of randomly chosen bone trabeculae. Biopsies from the same location in 42 cadavers, which had sustained sudden death, served as controls.

Results

The trabecular bone volume was decreased by 20 per cent after fracture, but the difference was not significant. There was an increase of the osteoid volume by a factor >100. The length of the osteoid surfaces was also greatly increased. The highest values for osteoid tissue appeared in those cases where about half a year had passed since the fracture occurred. The number of osteoclasts had also increased, and this was most obvious during the first months after the fracture. The relation between active and inactive osteoblasts was unaffected. However, the total number of osteoblasts increased in proportion to the osteoid surfaces.

The most striking finding was a specific and not previously described pattern of osteoid layers in between layers of mineralized bone. This pattern could sometimes be found throughout the whole width of the trabeculae. A striking non-uniformity of the osteoid localization was also noted. By means of electron microanalysis on unstained sections, these findings were confirmed and hence the possibility that they were stain artefacts could be excluded. On unstained but thicker sections, the concentration of calcium, centrally in the trabeculae, was calculated to be 29 ± 10 SD weight per cent, whereas for the control cases the values were higher and also more uniformly distributed (45 ± 1 SD weight per cent).

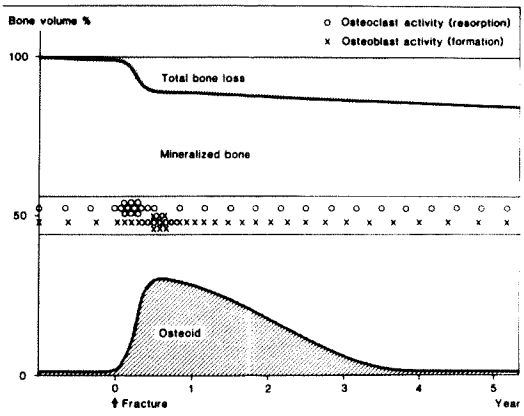

Figure 1. The sequence of morphological events in post-traumatic osteopenia.

Discussion

A schematic presentation of the morphological changes that take place in the skeleton after trauma is shown in Figure 1. A reservation must be made about the trabecular bone volume values, because the approximate 20 per cent loss found in this study is not significant. However, it is known from a roentgen study on diaphyseal bone that after trauma about 10 per cent of the cortical bone is lost (Nilsson & Obrant 1983), and we have no reason to believe that trabecular bone changes would be less.

Reference

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