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Bone perfusion and oxygenation

Animal experiments and clinical observations

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Introduction

Vascular anatomy of bone

The vascular tree of bone tissue must serve several different functional demands. The bone tissue itself has complex functions, but in addition to this it contains the medullary tissue in the marrow canal. This separate organ has relation both to the hematological system and to the bone system and provides the macrocyte-macrophage progenitor cell-line of bone (Nijweide et al. 1986). Bone functions as the structural framework of the gross body form, and has the ability to remodel and reorganize as a result of increased/decreased load, and it heals and grows new bone under suitable conditions. Furthermore, bone has an intense role in the biochemical homeostasis of different inorganic ions entrapped in the inorganic structure of the bone (Posner 1985). The largest Ca^{++} reservoir in the body is contained in bone and there is a rapid interchange between the bone tissue and the blood (Biltz and Pellegrino 1977).

The vascular tree supplying the bone can be divided according to size, anatomical localization, and function. Traditionally, it has been divided in; 1) heart and great vessels; 2) conduit vessels; 3) bone capillaries; and 4) microcanalicular system (Figure 1, Table 1).

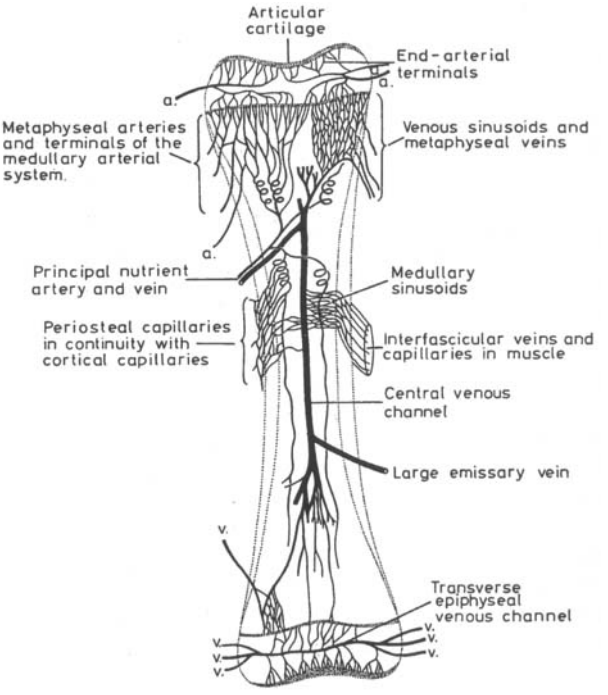

Figure 1. The organization of the vascular supply of a long bone with epiphyseal, metaphyseal and diaphyseal blood supply. (From Brookes 1971 with permission).

The conduit supply to bone is rich and diverse. Four different afferent systems of arteries are seen. The diaphyseal artery has a well-defined and constant anatomical course for each long bone (Trueta and Caladias 1964). The nutrient canal is usually located nearest the most rapidly growing end and directed towards the slowest growing. The artery divides with branches directed towards the epiphyses, and again these main branches divide into a parallel, independent set of arterioles with terminal capillary beds (Lopez-Curto et al. 1980). In the ends of the long bone, the diaphyseal arterial trees anastomose with the metaphyseal system. But the systems have their own vascular territories and according to Brookes (1971) it is only when one vascu-

Table 1. The classification of vessels supplying bone tissue

Afferents	Intraosseous circulation	Venous pathways
epiphyseal arteries metaphyseal arteries	medullary sinusoids	collecting sinuses
		epiphyseal-metaphyseal veins nutrient and emissary veins
nutrient artery	cortical sinusoids	interfascicular venules intramuscular veins
periosteal arteries	periosteal capillaries	periosteal veins

lar system becomes deficient can the other substitute, after a latency period.

The nutrient system supplies the endosteal network, giving rise to the cortical branches supplying the inner third of the cortex and anastomosing with the periosteal network. The intracortical supply travels through the Haversian and Volkmann canals. It has been possible to demonstrate the presence of both arteries and veins in the canals (Brookes et al. 1961).

The veins are more abundant but the anatomical pattern is similar to the arterial system. Usually two nutrient veins drain a central venous sinus (Nelson et al. 1960). Supplementary emissary veins cross the cortex associated with the incoming arteries in the end of long bones (Cuthbertson et al. 1965). The very large caliber of the venous system is in contrast to the thin arterial conduit system. The central sinuses usually drains through the nutrient vein.

The metaphyseal–epiphyseal afferent vessels are numerous and enter the long bones at both sides of the epiphyseal plate. The growth plate represents a barrier between the systems during growth, but in the adult small arteries cross the scar line connecting the vascular territories (Crock 1967). Around the articular joints a vascular unity exists. Two perimetaphyseal arterial rings are united by longitudinal anastomoses crossing the joint lines giving rise to a vascular unity. On each side a deeper network with end nutritive branches arises from the first system (Brookes 1971). The emissary veins are without valves and drain the sinusoids (Harrison and Gossman 1955). Only 20% of the mature bone is cancellous but 80% of the remodeling activity occurs in this part of the bone (Lloyd and Hodges 1971).

The periosteal system is a vascular network branching in the deeper layer of the periost, and connected to capillaries in the osteogenic cellular layer of the periost. Some disagreement exists regarding the role of the periosteal supply. Brookes (1971) advocates that the main flow is centrifugal and the periosteal supply is only compensatory in pathological conditions. In the traditional description, the cortex was believed to be partly nourished from the periosteal system (Rhinelander 1968)

The sinusoids distribute in the marrow circulation and in the spaces of cancellous bone (Bränemark 1968). The sinusoids are capacitans vessels and possess no adventitial layer. The endothelial cells cover the surrounding structures and a large volume of blood can be contained in this large dilated lattice (Yoeffey 1965). Sluggish flow may be connected to the hematopoietic function in the sinusoids.

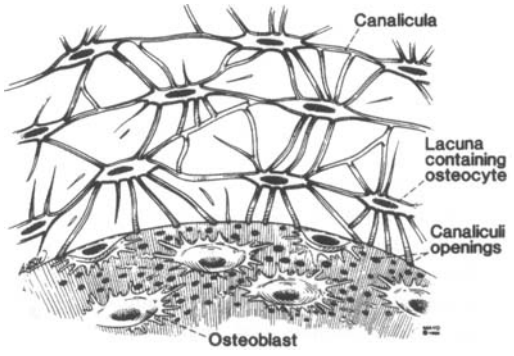

Figure 2. The lacunar–canalicular organization of the bone tissue. The cells are situated in the lacunae of the inorganic matrix and transport of nutrients to and from the cells is via the canaliculi. (From Renkin 1984 with permission).

Lacunar–canalicular system

The cells of bone tissue are embedded in the bone matrix formed by the organic and inorganic constituents. The cells are interconnected by the lacunar canalicular system. This is an important pathway for influx and efflux of ions and larger molecules of the bone tissue. The cells in the lacuna of mature bone are thought to be the controlling unit in the regulation of exchange of solutes, ions and larger molecules such as proteins (Figure 2). A pathway from the capillaries to the lacunae via the canalicular system has been demonstrated with injection studies with thorotrast (Seliger 1970). A passage through the extravascular tissue and the interstitial fluid phase into the lymphatics, has also been demonstrated for larger proteins (Owen and Triffith 1976). Several studies have shown that hydrophilic ions and molecules such as Ca^{++} and Sr^{++}, pass into the bone interstitial fluid by passive diffusion (Hughes et al. 1977, Kelly and Bassingthwaigthe 1977).

Transport of oxygen to bone tissue

Oxygen is transported to the tissue and carbon dioxide from the tissue via the blood stream. For oxygen, this has been called the oxygen cascade, because of the successive drop of oxygen partial pressure along the route from the lungs to tissue capillary blood and to the tissue. The conductance (G) of oxygen flow from tissue capillary blood to tissue is defined as the O_2 flow divided by the difference between the mean partial pressures in the capillary and in the tissue. The principal variables determining the transport of oxygen to the tissue are the oxyhemoglobin dissociation kinetics and the local factors of the capillaries of the tissue. The oxyhemoglobin dissociation can be changed by the Bohr effect (the

dependence of oxygen binding on the proton concentration), concentration of 2,3-diphosphoglycerate, and the temperature. The local factors determining transport are changes in capillary geometry and changes in blood flow modulated by neural or humoral mechanisms.

Gases move freely in and out of the tissue by diffusion (Renkin 1984). The factors affecting diffusion include 1) intercapillary distance, 2) blood flow, 3) concentration gradients, 4) capillary permeability, and 5) capillary surface area. The concentration of oxygen decreases exponentially along the capillary from the arterial to the venous end (longitudinal gradient). Radial gradients are usually physiologically analyzed using the Krogh cylinder concept (Krogh 1919). This can be used to calculate the partial pressure at any given distance from a capillary. If the distance between the capillaries is large (as in bone tissue) the tissue PO_2 can fall to very low values.

The role of blood flow in bone remodeling

The blood flow has profound influence on bone growth and remodeling. The effect of different experimental and clinical procedures and conditions on growth and remodeling is of relevance in this context.

Effect of hyperemia

The blood flow of bone influences the growth and remodeling of the tissue. In studies of leg lengthening, vascular factors have been demonstrated to affect growth. Conditions associated with changed circulation (aneurysms, fractures, tumors) around the growth plate may be associated with increased bone length (Paget 1863). The underlying cause of the overgrowth is considered to be hyperemia around the growth plate (Vanderhoeft et al. 1963). Arteriovenous fistulas have been shown to increase leg length, experimentally (Kelly et al. 1959) and clinically (Horton 1932). The flow distal to an A-V fistula will be sluggish due to the raised venous pressure (Ingebrigtsen et al. 1963) and this creates a chronic hyperemia (Weinmann et al 1964, Keck and Kelly 1964). It has been suggested that the accelerated growth seen in this condition may be due to the increased intraosseous pressure (Keck and Kelly 1964, Vanderhoefft et al 1963). The general vascular engorgement of the circulation of the growth cartilage, and changes in oxygen tension and pH are other suspected triggering mechanisms (Brookes 1971). On the other hand, experimental sympathectomy has been shown to increase blood flow to a limb but fails to increase rate of growth.

Venous stasis

Stasis in the venous system of an extremity has long been known to induce growth (Peck 1957, Keck and Kelly 1964). Experimentally, unilateral treatment with a tourniquet has induced overgrowth (Hutchinson and Burdeaux 1954). Some researchers have suggested that the venous stasis alone is responsible for overgrowth. Femoral vein ligation results in a small increase in bone length and weight (Brookes 1971). The histological effects of prolonged venous stasis have been examined; a decrease in bone matrix, enlargement of vascular channels, increased cellularity of the cortex and deposition of new layers on the outer side of the cortex were found (Abdallah and Harrison 1966, Brookes 1971).

Overgrowth of an extremity can be induced by periosteal stripping (Langenskiöld 1957, Sola et al. 1963). The mechanism has been ascribed to interference with epiphyseal venous drainage and congestion of the metaphysis or centripetal arterialization of the metaphyseal cortex.

Measurement of bone blood flow in conditions with increased remodeling

Only a few studies of the relationship between bone remodeling and blood flow exists. Clinically, increased blood flow is found in several conditions, such as inflammatory joint disorders, Pagets disease and reflex dystrophia. However, most of the conclusions have been drawn on the basis of labelling of the bone tissue with radiotracer. Since many factors other than perfusion determine the accumulation of tracer in bone, the basis of these conclusions is somewhat uncertain. Recently, the blood flow rate was evaluated with microspheres under various conditions, resulting in loss of bone mass (Verhas et al. 1990, Schoutens et al. 1990). Denervation of limbs and ovariectomia/orchidectomia and arthritis, all conditions with increased remodeling, resulted in significantly increased bone blood flow. Likewise, the blood flow increased in anemia, and it was concluded that hypoxia was the most probable factor responsible for the increase in flow.

Oxygen and bone remodeling

In vitro studies

Multiple studies have been performed in cell cultures to examine the influence of oxygen upon bone metabolism. Goldhaber (1958) found progressive resorption of the bone when the oxygen tension was increased to 95% in the gas phase of cell cultures. The resorptive effect of oxygen has been demonstrated even at subatmospheric values (Slegde and Dingle 1965). Brighton

et al. (1969), in contrast, found maximal metaphyseal bone formation in costochondral junctions occurred with 5 percent oxygen in the gas phase, and considered a low oxygen tension or particularly a steep gradient in the metaphyseal area to be an important inductor of growth. This was supported by the finding of mitochondrial release of calcium induced by hypoxia (Brighton and Hunt 1974). Electric currents also induce bone formation, possibly by lowering the oxygen tension in the vicinity of the cathode (Renoij et al. 1983).

Elevation of the CO_2 content of the gas phase in cultures has been shown to increase osteogenesis and calcification (Willmer 1965). Carbon anhydrase inhibitors inhibit bone resorption in cultures, indicating the participation of carbonate and carbon dioxide in bone remodeling (Minkin and Jennings 1972). The bones have the largest stores of CO_2 in the body, but the major part is bound as carbonate, with only limited exchange of bone CO_2 with plasma CO_2 (Poyart et al. 1975). Carbon anhydrase has significant influence on osteoclastic bone resorption and inhibitors of this enzyme affect this process (Kenny 1985).

In vivo studies

Investigation of the effect of oxygen on bone metabolism *in vivo* is hampered by the difficulty of measuring tissue parameters without disturbing the cellular milieu. The majority of investigations have been concerned with the partial pressures in fractures. In areas of osseous repair, low oxygen tension initially has been found (Brighton and Krebs 1972) with simultaneous elevated blood flow (Laurnen and Kelly 1969). General arterial hypoxia, however, decreased bone repair (Heppenstall et al. 1976), but the gradient of oxygen at the fracture site was also affected. It is thus likely that the gradient of oxygen between fracture site and normal surrounding bone is an important stimulator.

Elevation of ambient oxygen tension was found to increase bone growth during exposure, while reduction of the oxygen tension decreased the growth in young rabbits. The cartilage cells of the growth plate are also sensitive to changes in oxygen tension (Persson 1968).

In vivo studies in rabbits have shown high CO_2 tensions in bone in the repair zone of fractures (Kivisarii and Niinikoski 1975). This, and low pH, were also demonstrated in samples of blood taken from bone with healing fractures or arthrosis (Brookes 1971).

Cellular origin of bone; remodeling and CO_2

Bone tissue in the living organism is, like in other tissues, subject to constant changes—the remodeling process. The cells of the tissue are of prime interest in this context. In mature bone, both the osteoclasts and the osteoblasts participate in the process. Osteoblasts are differentiated from the osteoprogenitor cells of the hematopoietic stroma of the bone. The osteoclasts of the bone are derived from the mononuclear hematopoietic precursors, the so called monocyte macrophage progenitor cell line from the multipotential hematopoietic stem cell (Scheven et al 1986). The cells are thus delivered to the bone via the bone vasculature (Kahn and Simmonds 1975). The osteoblast is essential in the formation of the bone matrix and controls the calcification process.

In bone remodeling, the initial event is the resorption of bone carried out by the osteoclast. The osteoclasts are activated from a resting state by stimulators of bone resorption via modulation from the osteoblast. The activated osteoclast removes the unmineralized collagen layer and releases lysozymes solubilizing the inorganic phase of the bone matrix. The bone mineral can be solubilized under the action of acid, and an increased production of lactate and citrate (Mecca et al 1963, Vaes 1968) has been found. The secretion of acid by the osteoclast has also been demonstrated *in situ* (Baron et al 1985) and evidence of an active proton pump in the osteoclast has accumulated (Ghiselli et al 1987). The hydration of CO_2 into H_2CO_3 appears to be the mode of action of the proton pump. It was earlier shown that acetazolamide inhibits bone resorption (Siegmund et al 1960). This inhibition has been shown both *in vivo* (Kenny 1985) and *in vitro* (Minkin and Kenny 1972). Furthermore, it has been shown that bone resorption appears to be dependent on the ambient CO_2 concentration (Mahgoub and Stern 1974).

Intention with the present studies of bone circulation

From these data it is clear that maturation of bone and some pathological processes are closely associated to the function of the circulation. Since evidence exist that both increased and decreased blood flow may trigger growth or degenerative changes in bone tissue a regulating factor is likely to be present in the blood. The most important role of the circulation is to transport oxygen to the tissue and consequently, it is likely that this substance has an active regulatory role. This was supported by the experiments showing changes in bone structure following perturbations in oxygen concentration.

Degenerative diseases of bone and joints have been examined in detail and indirect studies (scintigraphy,

pressure studies, histology) have suggested that impaired circulation and hypoxia contribute to the pathogenesis of arthrosis and osteonecrosis. If an impaired circulation plays a role in the pathogenesis of these diseases, tissue hypoxia may be a central factor among the mechanisms leading to the pathology.

In the present series of experiments, we aimed to show that the oxygen supply plays a significant role in the function of bone tissue and that a decrease in its supply could be associated with deleterious conditions.

As a consequence of this a number of questions arise:

1. Can a membrane covered inlet for a mass spectrometer be used to measure intraosseous PO_2 and PCO_2 reliably.
2. Does acute changes in bone perfusion affect intraosseous partial pressures of oxygen and carbon dioxide.
3. Can pathophysiologically mechanisms leading to a decrease in supply of oxygen to the bone tissue be demonstrated.
4. Does intraosseous hypoxia exist in the early phases of osteonecrosis and degenerative joint disease, and is it possible to demonstrate this hypoxia in diseased bone.
5. Are the development of radiological, scintigraphical, histological, biochemical and hydrodynamic changes of degenerative bone changes associated with intraosseous hypoxia.
6. Does there exist procedures which can abolish the pathogenetic effect of decreased blood flow or hypoxia on bone tissue and can this effect be registered.

The objective of this series of experiments was to answer these questions.

Methodological considerations

A number of methods have been used to evaluate the intraosseous circulation in this series of experiments. Most emphasis is laid on the series of investigations using mass spectrometry. Partial pressures of gases in bone tissue were assessed by mass spectrometry, the blood flow with mass spectrometry and inert gas analysis or the tracer microsphere technique. The intraosseous pressure was measured with a transducer and intraosseous lactate concentration and histology were examined using biochemical and histological methods.

The methodological aspects of the techniques used, especially mass spectrometry, are of fundamental importance in this context.

Mass spectrometry

With mass spectrometry it is possible to measure concentrations of gases in aqueous solutions. Mass spectrometers separate ions in vacuum. The principles for this can be different but in this context the quadropole mass spectrometer has been utilized. The quadropole mass spectrometer (Figure 3) has four stainless steel rods placed parallel to each other to form a passageway for the ions. Radiofrequency (rf) and DC fields are applied to the rods and at any combination of these fields only ions of one mass to charge ratio can travel through the passage way (Dawson 1976).

Sample molecules are ionized by electrons ejected from a hot filament. A mass spectrometer responds so rapidly that the response time of the measuring system is determined by the inlet. In membrane inlet mass spectrometry, the response time is determined by the membrane and the unstirred layer outside the membrane exactly as it is in measurements with a membrane covered oxygen electrode.

The quadropole mass spectrometer can be operated in two ways. The fields on the rods can be scanned either continuously to produce a mass spectrum or discontinuously to monitor the evolution of selected peaks (Figure 5). At the normal time scale for measurement in tissue, one can, in practice, achieve a continuous reading of the different peaks by switching between the masses.

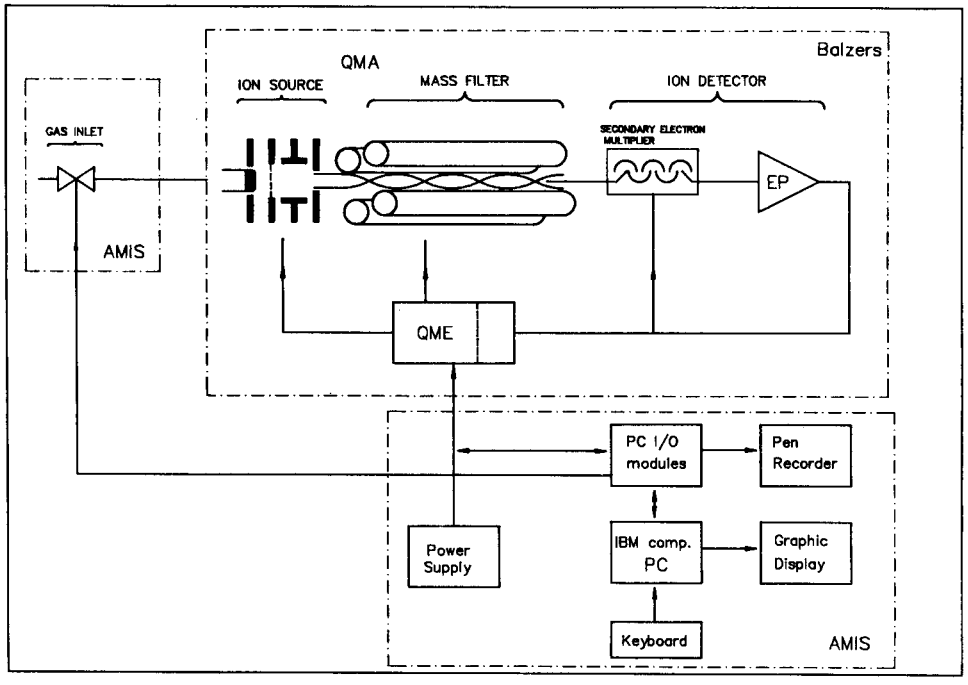

Figure 3. The quadropole mass spectrometer with sampling system.

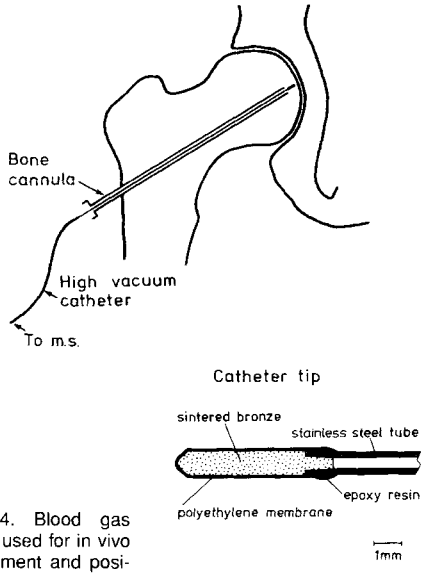

Figure 4. Blood gas catheter used for in vivo measurement and positioning in bone (VI).

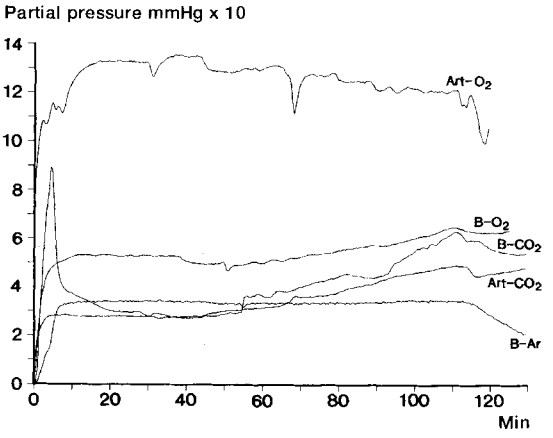

Figure 5. Tracing of measurement of intraosseous and arterial PO_2 and PCO_2. The argon curve was used to test the signal of oxygen for any flow dependence (I).

Inlet system

The inlet system of the mass spectrometer can be individually designed according to specific requirements for use. We used a specially designed blood gas catheter (Figure 4) (Lundsgaard et al. 1980). Since the mass spectrometer responds linearly to the rate of entry of the gas, it follows that the mass spectrometer responds linearly to the concentration of the gas in the stirred bulk of liquid (Woldring et al. 1966, Lundsgaard et al. 1980). The inlet used in this study consisted of a membrane inlet placed on the tip of a thin stainless steel tube catheter. Analytical, expressions have been derived for response time, flow dependency and signal to background ratios for the blood gas catheter (Lundsgaard et. al 1980). The expressions have been used to optimize the design of the catheter and to obtain very short response time and a very low flow dependence.

The flow dependency of the catheter arises from the obstacles of a gas molecule passing from a stirred liquid into the mass spectrometer. One obstacle is the unstirred layer of liquid adjacent to the membrane. The steady state rate of diffusion of a gas through an unstirred layer is proportional to the difference in partial pressure of the gas across the layer (Lundsgaard et al. 1978). Rapidly responding catheters with thin membranes have quantitatively important flow dependency due to the high consumption of gas. Flow dependency on the other hand is also determined by the size of the diffusion area. In the manufacturing of the catheter, a thin membrane was used to reduce the response time and flow independence was obtained by choosing a very small diffusion area with multiple, separate diffusion areas to ensure proper signal to background ratio (Lundsgaard et al. 1980).

The stirring effect was further evaluated by an *in situ* technique based on the measurement of an inert reference gas. The partial pressures of respiratory gases can be measured together with an inert reference gas, e.g. argon. The principle is to test for flow dependence through simultaneous measurement on the inspired, inert reference gas of known partial pressure (Lundsgaard et al. 1978). Any depression in signal of the reference gas must be due to stirring effect, if the tissues and the blood are in thermodynamic equilibrium with the inspiratory gas. This permits continuous *in situ* control of calibration, since the ratio between measured and actual partial pressure will be the same for oxygen and argon. The catheters used in the present studies were slightly modified compared to the original version. The surface of the sintered metal supporting the membrane was ground using a new technique which ensured a large number of very small and separate diffusion areas. This geometry of the diffusion area ensured a negligible stirring effect on the catheter (Svalastoga and Grønlund 1985).

Advantages of mass spectrometry

Mass spectrometry offers several benefits compared to traditional methods of measurement of concentrations of partial pressures: 1) The sensitivity of the method is

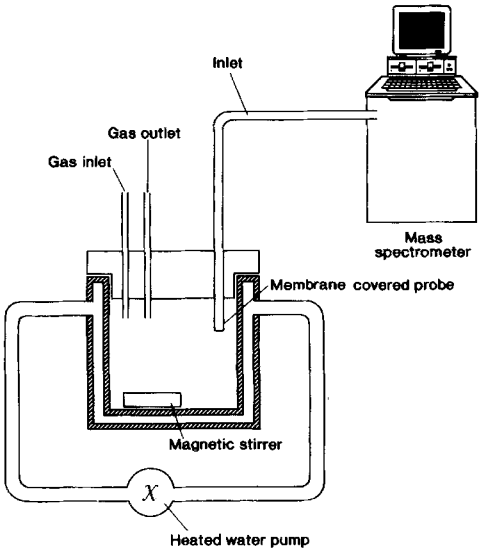

Figure 6. The chamber for *in vitro* measurement.

high, enabling registration of small differences in concentration; the sensitivity was found to be far better than necessary to fulfill the demands to the sensor under the *in vivo* conditions. 2) The response time of the mass spectrometer is short and is only limited by the membrane and inlet system (Woldring et al. 1970); this is important when measuring washout kinetics and in registration of changes occurring after perturbation of physiological parameters. 3) The mass spectrometer signal is very stable and the mass spectrometer can operate for several hours after calibration without drift in the background signal; this is important in clinical context, where it can be difficult to obtain a calibration within a short time interval from the measurement, and maintain sterility simultaneously. Stability is also imperative *in situ* physiological experiments, where changes in blood gases are followed for hours. 4) The mass spectrometer allows registration of several gases simultaneously with only one probe and one analyzing system and based upon one calibration; this is in contrast to all other probes that measure partial pressures of gases; due to this option, it is possible to measure concentrations of respiratory gases and inert gas washout simultaneously. 5) With the mass spectrometer it is possible to perform an *in situ* calibration by measuring an inert reference gas simultaneously with registration of PO_2 and PCO_2 (Lundsgaard et al 1978).

Mass spectrometry in measurement of bone PO_2, PCO_2, and perfusion

In vitro test of membrane inlet

The membrane inlet was tested in a specially designed chamber (Dahl et al 1992) (Figure 6). The catheter was placed inside a closed chamber with inlet and outlet pipes used to regulate the partial pressures of the fluids in the chamber. The chamber had thermostatically controlled temperature, a magnetic stirrer, and the catheter was placed in the periphery to ensure proper stirring. The inflow and outflow of gases of the chamber were controlled.

The partial pressure of gases was followed continuously by the mass spectrometer, and from the slopes of the curves obtained it was possible to calculate molar changes of gases in the solution from

$$n = (slope_c \times S \times V_{ch}) / 25.42$$

where $slope_c$ represents the time related corrected slope, S the solubility constant of the gas and Vch volume of the measuring chamber. In this chamber we measured:

1) Gas consumption by the probe with different membrane thicknesses (Table 2).

2) Reproducibility, by repeated measurements in a medium perfused with the same mixture of gases (9.96% oxygen and 2.99% carbon dioxide, 1.01% argon, and 86.04% nitrogen).

3) Reproducibility, by leading a known gas mixture through the chamber, disconnecting the gas supply for a period of 3 min. and then repeating the procedure 8 times. The steady state value of measurement was for oxygen 22.34 ± 0.008% (MEAN ± SD) and for carbon dioxide 0.046 ± 0.0003%

4) Sensitivity, by changing the mixture of two gases, one with atmospheric air and one with nitrogen. The mixture was changed with 5% steps which caused changes in the oxygen concentration in the range 1%, and it was found that the sensitivity was in the range below 0.05%. Another curve of changing gas mixture in an aqueous solution is shown in Figure 7.

Table 2. The gas consumption of the blood gas catheter with different thickness of polyethylene membrane

Thickness of membrane	O_2 consumption (%/min)
20 µm	0.053
30 µm	0.023
40 µm	0.013

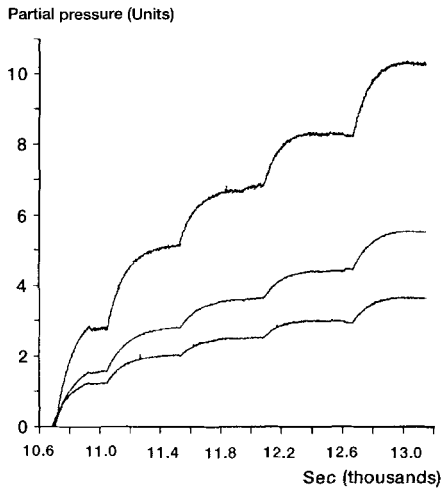

Figure 7. Partial pressures of O_2 (upper), CO_2 (middle) and Ar (lower). O_2 tension was changed from 0–13%, step 2.6%, CO_2 from 0–6%, step 1.2%, Ar from 0–4% step 0.8%.

In vivo measurement with mass spectrometry

To measure intraosseous PO_2, PCO_2, P_{fr} (partial pressure of freon) and P_{Ar} (partial pressure of argon) the blood gas catheter (Lundsgaard et. al 1980, Kofoed et al. 1983) was inserted into the bone tissue through a cannula (Figure 4). The measurement was performed inside the cancellous bone compartment in the blood filled spaces between the lattice of spongious bone. These spaces are filled with the blood of the sinusoids and the walls are endothelial surfaces.

In the last version of the system used, the mass spectrometer was equipped with a specially developed multi inlet system (Figure 3) which enabled simultaneous measurement at three different sites. The system was controlled by a computer switching between the three inlets with a frequency of 0.33 Hz.

The output from the mass spectrometer was sampled in a computer and displayed on-line on a screen with gases from same inlet tracked on the same screen (Figure 5). The system allowed on line switching between the screen of the three inlets. All inlets of the mass spectrometer were calibrated before and after the experiments using a calibration chamber permitting saturation of saline with reference gases under thermistor control of temperature.

In vivo reliability (I)

In vivo reliability of intraosseous mass spectrometer measurements and the correlation to conventional analysis of bone blood samples were tested in a series on anesthetized pigs. The partial pressure of oxygen and carbon dioxide were determined bilaterally and simultaneously in the femoral condyle by mass spectrometry (Figure 8). Immediately after this the blood gas catheter was temporarily withdrawn from the cannula and a small sample of bone blood (0.5 mL) was slowly aspirated from the bone. The PO_2 and PCO_2 of the blood samples were subsequently measured with a conventional blood gas analyzer (ABL-3, Radiometer, Copenhagen, Denmark).

The values of bone PO_2 and PCO_2 measured *in situ* with mass spectrometry, and in blood samples with the conventional Clark electrode (ABL-3) were not significantly different (Figure 9). On average, the partial pressure of oxygen in bone measured with the on line

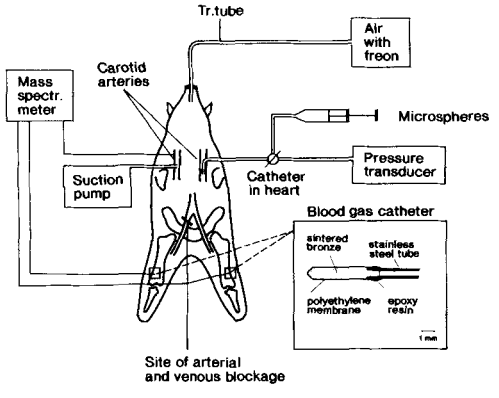

Figure 8. Experimental set-up of one series of animal experiments. The carotid arteries was cannulated bilaterally and the blood gas catheter was inserted in the cancellous bone of the femoral condyle (I).

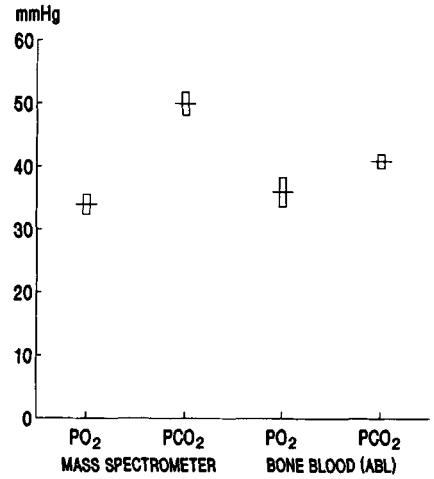

Figure 9. Comparison of measurement with mass spectrometer and ABL.

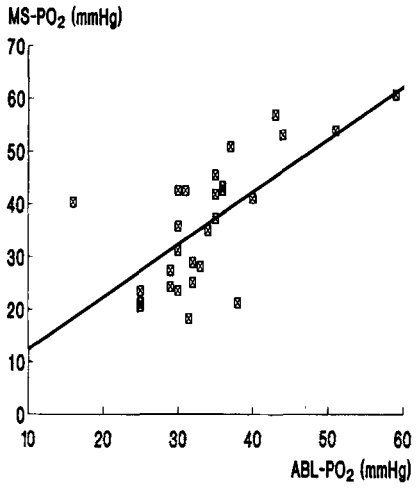

Figure 10. Correlation between PO_2 measured with mass spectrometer and ABL in the lateral femoral condyle. From Kiær et al. 1992 (I)

mass spectrometer and with the ABL-3 were 34 ± 1.6 mmHg and 36.3 ± 2.3 mmHg, respectively. The results of the two methods were correlated (Figure 10; r = 0.68, $p < 0.01$). The average partial pressure of carbon dioxide was higher measured *in situ* compared to the result of the blood samples (50 ± 1.8 mmHg and 40.5 ± 1.1 mmHg, respectively), but again the data were correlated ($r = 0.46$, $p < 0.013$).

In this reliability study, where the *in situ* technique was compared with a conventional method analyzing blood samples, a good correlation was thus found between the results of the methods. The difference in real values of PCO_2 may be explained by the unphysiological nature of the aspiration technique, because the bone tissue is flushed during this procedure. *In situ* measurements are more physiologically correct and allow on-line monitoring of changes in partial pressures.

Reproducibility of local measurement in bone (I)

The reproducibility of measurement with mass spectrometry at the same local area of bone was also tested in the same series of experiments. Differences between the same anatomical area of bone in bilateral measurements and repeatability were tested by repeated measurement within 15 min. Under normal conditions, no significant difference was seen between the partial pressure of oxygen and carbon dioxide of the left and right lateral femoral condyle (PO_2 $p = 0.3$; PCO_2 $p = 0.53$). Repetition of the measurement showed no difference between first and second measurement (PO_2 35.6 ± 3.9 and 38.2 ± 3.4 mmHg; PCO_2 49.6 ±2.8 and 49.2 ± 2.8 mmHg). Similarly, no differences between the left and right femoral condyle, or between two separate measurements at the same location, were found in the blood samples taken from bone.

The reproducibility of measurement with mass spectrometry in animal bone was thus found acceptable. In another study (VI) no difference in partial pressure was found between two close locations of normal human bone. This indicates good accuracy of the method and that found differences are true.

Consumption of gases by measuring probe

One problem in measuring gases with membrane covered sensors is the gas consumption of the probe (Lundsgaard et. al 1980). Effort has been employed to reduce this factor (Svalastoga and Grønlund 1985). The version of the blood gas catheter used in this study was tested in the closed chamber (Figure 6). The consumption of oxygen and carbon dioxide by the catheter was 2.36×10^{-10} and 6.67×10^{-10} mol/min. This amount is negligible compared to the amount of gas transported to tissue by the blood stream. If the mean whole body arteriovenous oxygen difference is set to 46 mL/L blood (Ganong 1975), the oxygen delivery to the tissue will be on average 2×10^{-6} mol/mL blood. With an average flow about 0.1 mL/g bone/min it is thus obvious that the consumption of the catheter could hardly affect the partial pressures of the intraosseous compartment.

Determination of bone blood flow

Blood flow in bone regulates oxygen delivery to the tissue. Most methods used to determine variations in bone blood flow are indirect. Presently, the most widely used method (Morris and Kelly 1980, Strachan et al. 1990) is based on injection of radioactive isotopes bound to particles which become trapped in the arteriolar system (microspheres). Other earlier methods included collection of effluent blood from veins (Cumming 1962), thermocouple methods (Shaw 1963), phletysmography (Holling et al. 1961), clearance of bone seeking isotopes (Copp and Shim 1965, Kane and Grim 1965), fractional distribution of labelled erythrocytes (Brookes 1971), and washout of injected tracers (Whiteside et al. 1977). In this study (II), mass spectrometry has been used to assess the local bone perfusion by measurement of inert gas washout (Lassen 1967, Kety 1951). The principle is to load the bone tissue with an inert gas, and then measure the washout curve (Figure 11) in the bone compartment by a blood

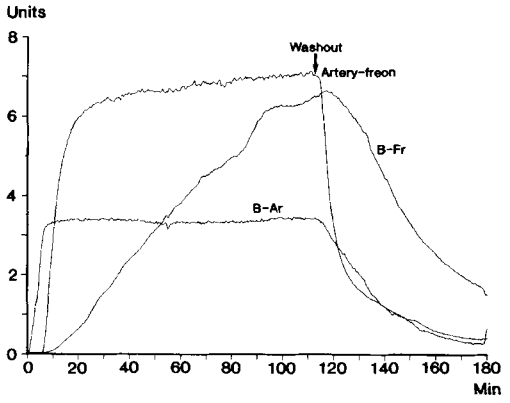

Figure 11. Registration of intraosseous gas tensions and the washout curve of freon from bone. B-Ar = bone argon, B-Fr = bone freon. From Kiær et al. 1993 (II).

gas catheter connected to a mass spectrometer (Lundsgaard 1980). The flow measurement can be repeated since it does not require removal of a bone specimen, as labelling techniques do (Copp and Shim 1965, Buckberg 1971). Washout registration and measurement of respiratory gases in solution can be performed simultaneously by one probe.

Inert gas washout

Bone blood flow was determined by analysis of washout kinetics of an inert gas (Lassen 1967). Tissues were loaded with the inert gas, freon, mixed to inspiratory air. The mass spectrometer signal of freon in the arterial blood and the bone tissue was then used to follow wash-in (Figure 11) until steady state was reached. At this point, a mixture of 20% oxygen and 80% nitrogen was led to the respirator and the freon was washed out.

Using the mass balance equation, and if m(t) describes the measured washout of inert gas from a monoexponential compartment of bone, the perfusion coefficient is given by

$$m(t) = m_0 e^{-kt} \text{ with } k = Q/V$$

where m_0 is the concentration of inert gas at the time 0, Q is the perfusion coefficient and V the volume of distribution (partition coefficient). This equation is valid for the pure washout situation in a system without recirculation. After correction for background and response time, k was found by the logarithmic derived of m(t) since

$$\ln(m(t)) = \ln m_0 - kt$$

The slope of the regression line to this equation was found. The numerical value 2 was used for the blood/tissue partition coefficient of freon in mixed bone.

Methodological problems in the inert gas washout technique

The use of the indicator technique necessitates some assumptions (Lassen and Pearl 1979). It must be assumed that the vascular pattern of the bone tissue is organized in parallel and not serially. The vascular organization of the bone blood supply (see above) indicates separation of the vascular territories. Dye perfusion experiments have shown that diaphyseal and metaphyseal arterial networks only anastomoses, if at all, at the precapillary level (Brookes 1971).

Another assumption is that there is no recirculation (feedback shunting) of the tracer. Indeed, freon is rapidly eliminated by the lungs (Wagner et al. 1974, Grønlund et al. 1989). The effect of recirculation on the bone compartment is the solution of the convolution integral of the washout in the compartment and the function describing recirculation (Lassen and Pearl 1981, Drayer et al. 1979). The solution can be expressed as

$$P_{i,fr}(t) = P_{i,fr}(0)\exp(-k_i t) + \lambda P_a(0)[(k_i/(k_a - k_i)] \times [\exp(-k_i t) - \exp(-k_a t)]$$

where $P_{i,fr}(0)$ is the initial value of freon in the bone compartment, $-k_i$ is the slope of i^{th} compartment, $P_a(0)$ is the arterial concentration at $t = 0$, k_a is the slope of washout in arterial blood and λ the partition coefficient. In the experimental series the washout curve of the arterial blood was followed simultaneously with the sampling in bone. The k_a of the arterial blood was calculated in one experiment and the washout curve from bone was corrected for the effect of recirculation. The perfusion coefficient was underestimated by 8%, without correction for recirculation if k_i was 0.074 and ka 0.30. This finding was expected since washout in the lungs is very fast compared to the slow bone compartment. The value added to the washout curve by the second term in the equation will thus have negligible influence.

Another way to check indicator washout curves is to use multiple indicators and examine possible differences between indicator curves. Argon is a normal constituent of the atmosphere and can be used as a inert reference gas. Washout is accomplished by leading an argon free gas mixture to the experimental animal or human and following the washout curve. Argon was used as a control for freon washout (Figure 11). It was seen that bone argon is washed out more quickly than freon

because of a shorter response time of the mass spectrometer. The flow obtained with freon and argon showed no significant difference. This reduces the risk of any systematic error due to improper dispersion of the indicator.

The radioactive tracer microsphere method

The RTM method is based on the principle that microspheres are supplied by the blood stream and become trapped in the tissue capillary lattice. In this study, microspheres (15 μ) labelled with 46-Scandium or 103-Ruthenium were used. The microspheres were injected via a pig tail catheter into the left ventricle and reference samples were obtained from the abdominal aorta (Tøndevold et al. 1982a). Biopsies from the bone were taken from the points where measurements with the mass spectrometer were performed during the experiment. The activity in an individual sample was corrected for cross talk, physical decay and background during the counting procedure. The blood flow in the samples was calculated from the formula

$$Q/V = \frac{\text{Activity (100 g tissue)}^{-1} \times \text{reference pump flow}}{\text{total activity of reference sample}}$$

Methodological problems of the microsphere technique

There are several limitations of the microsphere technique. The method is discontinuous, repetition can be done a limited number of times, and it is necessary to obtain tissue samples. The method is insufficient for investigation of the microcirculation due to a lower limit of biopsy size, and the method is unable to detect arterio-venous shunting (Morris and Kelly 1980). The flow obtained with microspheres has a high variability (Tothill et al. 1987). It is well recognized (Tøndevold 1983, Bünger et al. 1986) that the variability increases with increasing blood flow, and this has been ascribed to so called plasma skimming and axial accumulation of spheres in the central stream of the arterial vessels (Tøndevold 1983). This variability has been acquiesced to, because the method offers the information most easily, is locally noninvasive, allows measurements in awake animals and inaccessible regions. Therefore the method was chosen as the reference method in the evaluation of reliability of the washout method.

Results of studies with microspheres and the washout technique (II)

The correlation between measurement of bone blood flow with microspheres and the washout technique was evaluated. Totally, a data pair of 22 samples obtained with both methods was available for comparison of blood flow at the same location of the femoral condyle. There was no statistically significant difference in blood flow measured with inert gas washout and microspheres (washout: 7 ± 0.7 mL/min/100 g, microspheres: 9 ±2.9 mL/min/100 g). Regression analysis showed significant correlation between the methods ($r = 0.53$, $p < 0.013$; Figure 12).

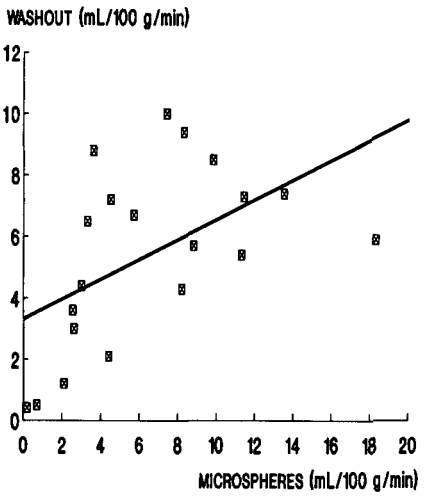

Figure 12. The correlation of bone blood flow measured with the washout technique and with microspheres in the femoral condyle (II).

Correlation between PO_2, PCO_2 and blood flow

The correlation between intraosseous PO_2, PCO_2 and blood flow was evaluated by obstructing; 1) the arterial supply or 2) the venous outlet of the bone and measuring the parameters before and after (II). Correlation analysis (Spearman's rank) showed statistically significantly positive correlation between blood flow and PO_2 ($\rho = 0.64$, $p < 0.006$) and negative correlation between blood flow and PCO_2 ($\rho = 0.58$, $p < 0.0012$). From this, we can conclude that intraosseous oxygen and carbon dioxide tensions are valuable indicators of the condition of the microcirculation and the blood flow since circulatory perturbations were reflected on the intraosseous values.

Measurement of intraosseous pressure

The intraosseous pressure was measured by connecting a bone cannula (inner and outer diameter 1.4 and 2.0 mm) to a pressure transducer (Dameca, Copenhagen,

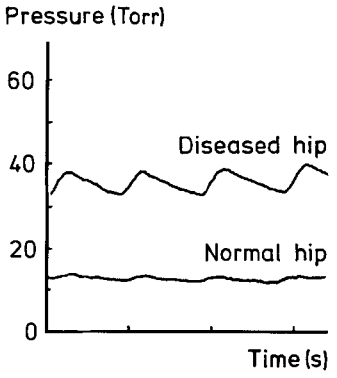

Figure 13. Measurement of intraosseous pressure in the hips of the same patient, one hip with osteoarthritis. Presence of the pulse curve is seen (VI)

Denmark) via a polyethylene tubing filled with saline. The vertical position of the pressure transducer was at the level of heart. The pressure registration set was delivered for disposal use and had a continuous low grade infusion system to prevent clotting and obstruction (Lemberg and Arnoldi 1978). The signals from the transducers were recorded on a strip chart recorder (Figure 13). Pulsatile waves were required for acceptance of the recordings. The technical problems related to pressure recording has been extensively reviewed in recent studies (Lemberg and Arnoldi 1978, Tøndevold 1983).

Measurement of lactate concentration and pH

In the clinical series of experiments (VI, VII) the levels of lactate in the intraosseous blood were determined with a standard, clinical chemistry method. Two mL of a 2.5-mL blood sample, obtained via the bone cannula, was immediately exposed to Long's mixture and the lactate concentration was measured by spectrophotometry using the NAD-LDH method (Gutman et al. 1974). pH was measured in the remaining 0.5 mL of the blood sample by a conventional glass electrode (BMS, Radiometer, Copenhagen). There exist no methods for examination of lactate *in situ*. The aspiration of blood may change the lactate concentration and the quantitative importance is difficult to evaluate. The paired design of the study eliminated the effect of this systematic error.

Histological examinations of bone tissue

Bone biopsies were examined for the presence of osteonecrosis (VII,VIII). The biopsies were placed in 4% buffered formal aldehyde for 48 hours in order to make sure that fixation was complete before decalcification in formic acid, 4 M, with 0.5 M sodiumformiate. As soon as the tissue was judged suitable for cutting, it was embedded in paraffinwax. Sections were stained with hematoxylin-eosin. The slides were evaluated by the same pathologist using the classification system proposed by Arlet et al. (1984). Four grades of necrosis were used. Grade 1 is characterized by necrosis of hemopoietic tissue if present. If not, the markers are exudation of plasma constituents, acute stasis and hemorrhage. Grade 2 shows additional necrosis of fat followed by "oil cysts" formation and groups of lipophages. In Grade 3 necrosis of bone is added. Trabecular necrosis is diagnosed when 50% or more of the osteocyte lacunae are devoid of nuclei. Grade 4 exhibits additional signs of regeneration of bone and fibrosis. Figure 19 shows a grade 2 necrosis.

Physiological and pathophysiological models of bone circulation

Heterogeneity of the bone circulation

Recent studies have suggested that a wide range of flow heterogeneity exists in bone tissue (Tøndevold 1983, Tothill et al. 1987, Villans et al. 1990). Tøndevold and Eliasen (1982a) found a flow rate of 14.3 ± 1.9 (mL/min/100 g) in cancellous bone of the femoral head in dogs and a rate of 1.2 ± 0.1 in diaphyseal bone and 8.3 ± 2.1 in the distal epiphysis. Gross et al. (1979) have demonstrated a difference in flow between epiphyseal and diaphyseal bone with flow rates of 2 ± 0.5 (diaphyseal) and 26 ± 6 (inf. epiphyseal) respectively (Table 3).

This indicates a considerable flow-related complexity in bone gas exchange. The microsphere technique has a low sensitivity to flow heterogeneity due to the resolution limit of 0.5–1.0 g tissue, since at least 383 microspheres have to be trapped in the biopsy (Buckberg et al. 1971) to ensure a statistically valid measurement. This may be inadequate to describe the microcirculation because many functionally important tissue units may be much less than 0.5 g.

Analysis of wash out curves have previously been performed on a single or dual compartmental basis, where "anatomically" different parts of the tissue has been defined as uniform compartments (Lassen and Pearl 1979). However, differences in flow rates in bone based on heterogeneity in anatomically similar compartments have not yet been examined. In a study of the rabbit femur in 6 animals (Kiær and Grønlund 1991, Kiær 1992), the washout curve of freon was measured in the venous blood from the nutricial vein by a blood gas catheter connected to a mass spectrometer and the flow rates of the tissue were calculated. The washout curve was analyzed using a 50 compartment model, which is assumed to allow for any degree of inhomogeneity that can possibly occur in the bone (perfusion coefficients from 0.1–1000 mL blood/100 mL tissue/min). The algorithm used to analyze the data determines the distribution of blood flow in the 50 compartments of the bone model giving the best fit of the model to the experimental data (Lewis et al. 1978, Grønlund et al. 1989). A heterogeneity was demonstrated in the bone perfusion with two main flow/volume modes centered around 0.05 and 0.5 mL/min/g tissue and a considerable heterogeneity inside the modes.

The effect of vascular obstruction of bone blood flow

Arterial obstruction

The pathophysiology of several bone disorders, e.g. osteonecrosis, are closely related to impaired circulation of the arterial branches. The effect on bone of occlusion of arterial inflow to the lower limb has previously been examined in physiological studies. Most of

Table 3. Earlier findings on bone blood flow in experimental animals using different methodological principles

Authors	Animal	Type of bone	Methods	Flow value mL/min/100g
Copp & Shim 1965	Rabbit	Femur	Isotope clearance	9–12
Shim 1967	Rabbit	Femur	Isotope clearance	12.5
Tøndevold & Eliasen 1982	Dog	Femoral head	Microspheres	14.3 ± 1.9
		Diaphysis	Microspheres	1.2 ± 0.1
		Distal epiphysis	Microspheres	8.3 ± 2.1
Gross et al. 1979	Dog	Femoral diaphysis	Microspheres	2 ± 0.5
		Distal Epiphysis	Microspheres	26 ± 6
Weiland et al. 1980	Dog	Rib	Hydrogen washout	8.4 ± 3.3
Bünger et al. 1986	Dog	Femoral condyle	Microspheres	10.0
Jurvelin et al. 1988	Dog	Trochanter	Hydrogen washout	11.1 ± 4.3
Li et al. 1989	Dog	Endosteal cortex (femur)	Microspheres	2.89
Davis et al. 1990	Rabbit	Femoral head	Microspheres	13.0

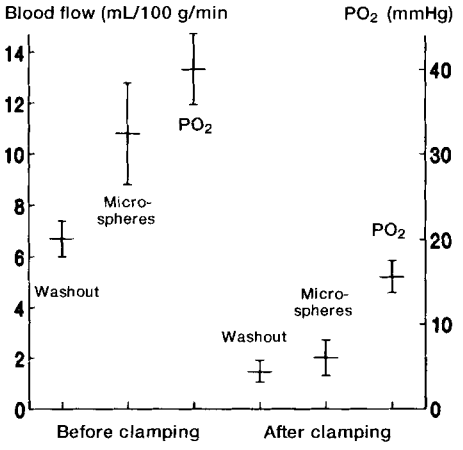

Figure 14. The PO_2 and blood flow of the pig femoral condyle before and after occlusion of the arterial supply.

these studies have used intraosseous pressure as the hemodynamic parameter of interest. Azuma (1964) found a decrease in pressure from 30 mmHg to near 0 following arterial occlusion. The same effect was demonstrated by Shim et al. (1972). This result was ascribed to the close relation between pressure and flow. The relation between vascular occlusion and bone blood flow, PO_2 and PCO_2 has not been studied before.

Own studies (I, II)

We investigated the effect of obstruction of the greater vessels on bone blood flow, PO_2 and PCO_2. The blood flow of the cancellous bone of the femoral condyles was measured using the washout technique and microspheres. Blood flow was first measured undisturbed and then following clamping of the external iliac artery above the inguinal ligament.

The blood flow of the femoral condyle (Figure 14) was significantly reduced to 20% of the original value following arterial occlusion (washout: 6.7 ± 0.7 to 1.45 ± 0.4, $p < 0.025$; microspheres: 10.8 ± 2 to 2 ± 0.7, $p < 0.015$). This was associated with severe hypoxia (difference: 24.5 mmHg; $p < 0.001$) and the bone PCO_2 increased significantly (difference: 33 mmHg; $p = 0.04$).

Venous obstruction

It has been demonstrated that ligation of the femoral vein resulted in venous congestion, increased CO_2 tension and low pH in bone medullary blood (Brookes and Helal 1968). The histological picture was changed and the differentiation into organized bone tissue in fracture repair, was depressed. Occlusion of the femoral vein results in a prompt rise in intraosseous pressure (Azuma 1964, Shim et al. 1972). The long term effect of venous obstruction on bone tissue was tested in rats (Abdallah and Harrison 1966). Histologically, signs of vascular congestion were found and thickening and enlargement of the periost, with dedifferentiation of the cortical areas and ossification of joint cartilage gradually developed. Obstruction of venous drainage has been associated with development of arthrosis in several studies (Arnoldi et al. 1972).

Own studies (I, II)

In an animal model of venous congestion the PO_2, PCO_2 and the blood flow of the femoral condyles were measured using the washout and microsphere technique. The blood flow was first measured, and then after clamping the external iliac vein above the inguinal ligament.

Venous occlusion gave no significant difference in blood flow as measured with the washout method, whereas there was a statistically significant decrease when measured with microspheres ($p < 0.025$). Venous occlusion resulted in a lowering of the intraosseous PO_2 (difference: 17 mmHg; $p < 0.002$) and an insignificant increase in PCO_2 (difference: 11.7 mmHg; $p = 0.23$).

Interpretation of results of arterial and venous occlusion

Maintenance of tissue PO_2 is closely related to the blood flow. In our model we aimed at decreasing the blood flow moderately and significantly by increasing the outlet resistance or obstructing the arterial supply, respectively. The bone blood flow was reduced 2 fold after venous obstruction and 5 fold after arterial occlusion. The consequence of this was a 1.5 and 2.6 fold reduction in bone PO_2. Major perturbations of the blood flow were thus reflected on intraosseous oxygen tension. Intraosseous PO_2 and PCO_2 can thus be used as indicators of the condition of microcirculation. There are, however, some constraints for this use of the parameters. Since the diffusion rate depends on the blood flow, the surface area, the gradient of oxygen and the permeability, all these other parameters must be assumed constant.

These experiments also suggest that intraosseous pressure may be a reliable indicator of blood flow in cases with reduced arterial supply, whereas venous congestion results in an inverse relationship between intraosseous pressure and blood flow. The intraosseous pressure alone is thus insufficient for studies of the pathologically changed bone circulation.

Physiological regulation of bone blood flow

The total bone blood flow constitutes approximately 9% of cardiac output (Brookes 1971, Folkow and Neil 1971). Little is known about mechanisms regulating bone perfusion but previous studies have indicated that both metabolic, humoral and neural factors are involved (Shim et al. 1966, Davis et al. 1981, Tøndevold 1983, Bünger 1987). Morphological studies have demonstrated nerve fibers adjacent to the blood vessels of bone (Duncan and Shim 1977) and pharmacological studies have shown profound changes in bone perfusion following infusion of vasoactive drugs (Shaw 1964, Gross et al. 1979, Brinker et al. 1990). Flow resistance in bone vessels increases after onset of exercise possibly due to metabolic regulation (Gross et al. 1979). Finally, calcitonin, parathormone and glucocorticoids have been shown to affect the flow resistance in blood vessels of bone, indicating humoral regulation (Dreissens et al. 1981)

The influence of arterial blood pressure on intraosseous pressure has been studied before. Tøndevold et al. (1979) varied the blood pressure in dogs by a combination of graded halothane anesthesia and intravenous loading with saline. In this way, arterial pressures lying both below and above the normal pressure were obtained. Decreasing arterial pressure resulted in a rapid fall in intraosseous pressure but it remained at the normal level (Table 4) at arterial pressures above normal. Kita et al. (1972) studied the effect of acute hypovolemic shock in dogs on the intraosseous pressure and found a relatively larger fall in the intraosseous pressure than in the arterial pressure. The conclusion of both these studies was that the bone blood flow is not preserved under acute hypotension. Instead, the flow is reduced possibly to allow a better perfusion of more vital organs. This conclusion does, however, rely on the assumption that intraosseous pressure and blood flow are proportional. Wilkes and Visscher (1975) studied the perfusion rate – pressure changes in an experimental preparation of the canine tibia and found a nearly linear relationship between perfusion and intraosseous pressure. Their results indicated a regulatory function of the effluent veins from the bone. Since the bone pressure is relatively far from arterial and venous pressures, both inflow and outflow resistances are of physiological importance. Therefore, autoregulatory mechanisms might maintain the perfusion despite a fall in bone pressure by a balanced decrease in both flow resistances.

Effect of induced hypovolemia upon bone PO_2, PCO_2 and pressure (III)

The influence of general hypovolemia on bone circulation and possible regulatory effects were investigated by recording the intraosseous pressure and PO_2 and PCO_2 continuously by mass spectrometry in rabbits. Hypovolemia was induced by repeated bleedings. Since the partial pressures of oxygen and carbon dioxide depend on local circulation, a qualitative estimate of the bone blood flow is obtained by measuring these parameters. Furthermore, the relationship between intraosseous pressure and oxygen tension was studied. The arterial pressure fell already after the first 10 ml of bleeding. A transient drop in blood pressure with partial recovery was seen in all animals immediately after each blood loss indicating intact system pressure regulation. The intraosseous pressure followed the arterial pressures, but was 3.5 fold lower. The relationship between the total blood loss and the normalized arterial blood pressure and the normalized intraosseous pressures as a function of the blood loss in 7 experiments is shown (Figure 15). The slope of the curve reflecting the fall in normalized intraosseous pressure was slightly larger than that of the fall in normalized arterial pressure.

The partial pressures of oxygen and carbon dioxide were also affected immediately after the first 10 mL bleeding. Figure 15 shows the intraosseous PO_2 normalized with respect to the initial value as a function of

Table 4. Earlier findings on bone intraosseous pressure in animals (mmHg)

Author	Bone	Region	IOP mean (range)
Bloomenthal 1952	Dog femur	Diaphysis	50 (20–115)
Shaw 1963	Cat femur	Diaphysis	37
Shim et al. 1972	Rabbit tibia	Diaphysis	(20–60)
Kita et al. 1972	Dog tibia	Diaphysis	33.4
Tøndevold et al. 1979	Dog femur	Epiphysis	27.6 (5)
		Metaphysis	23.5 (2.0)
		Diaphysis	27.7 (3.9)
Bünger et al. 1986	Dog femur	Epiphysis dist.	9 (5.3–19.5)

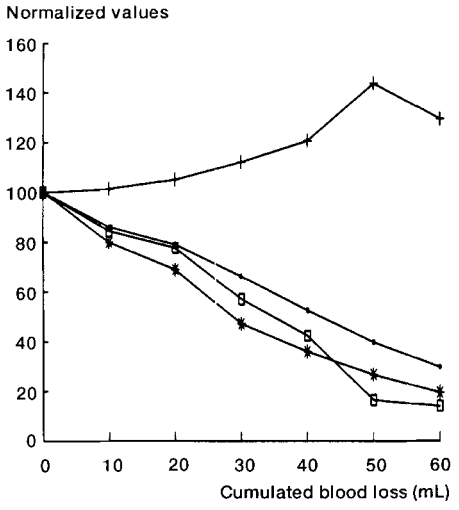

Figure 15. The mean normalized values of bone pO_2, PCO_2, IOP and the arterial pressure as a function of blood loss in 7 experiments. ▪ arterial pressure, + bone CO_2, * bone pressure, and □ bone O_2.

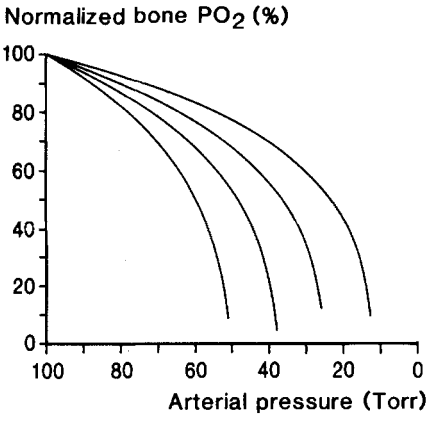

Figure 16. The relationship between PO_2 and intraosseus perfusion, with four assumed oxygen consumption rates. The curve with the highest consumption rate (4 µmol of O_2/min) is shown to the left, and the lowest rate to the right (III).

the cumulated blood loss. The response in PO_2 was slightly delayed compared to the response in the intraosseous pressure.

The influence of the non-linearity of the oxygen binding curve

The changes in intraosseous PO_2 and PCO_2 may provide data for a more precise evaluation of the perfusion. However, the interpretation of the relationship between PO_2 and blood loss is complicated by the non-linearity of the oxygen binding curve of blood. At the steep part of the oxygen binding curve (in the area around P_{50}), tissue PO_2 should be relatively independent of variations in the tissue perfusion. Even in a non-regulated system one would expect a non-linear relationship between PO_2 and flow. To estimate the effect of this a computer simulation was made to study the relationship between intraosseous PO_2 and pressure. The simulation assumes that 1) the intraosseous PO_2 is the arithmetic mean of arterial and venous PO_2, 2) the hemoglobin oxygen binding curves of human and rabbit blood are similar, and 3) the intraosseous pressure is proportional to the blood flow (Wilkes and Visscher 1975). Under these assumptions the relationship between intraosseous PO_2 and pressure is shown in Figure 16. The four curves are calculated at assumed oxygen consumption rates of 1, 2, 3, and 4 µmol/O_2/min. The shape of the theoretical curves is similar to the shape of the curves describing the relationship between PO_2 and cumulated blood loss. Since the intraosseous pressure is linearly related to the blood loss, the simulation leads us to the conclusion that perfusion was directly related to the changes in intraosseous pressure.

Interpretation of results

The results of our measurements of PO_2 and PCO_2 and the computer simulation indicate that pressure is not preserved under hypovolemia. It thus appears that the perfusion of bones in an acute hypovolemic shock is limited by the pressure of the greater arteries. The present study indicates that the regulation of bone blood flow does not counteract the local effects of hypovolemia. It may, however, be indicated that neurogenic mechanisms increasing the perfusion pressure in the conduit vessels exert regulation on the bone perfusion following hypovolemia. It can further be concluded that PO_2 and PCO_2 are closely correlated to the intraosseous pressure during hypovolemia and sensitive indicators of the state of the perfusion.

Relation of increased intraarticular pressure to subchondral perfusion

Effect of hip joint tamponade

Transient synovitis of the hip joint is often related to later development of Legg–Calve–Perthes disease, coxae magna and arthrosis (Valdemarra 1963, Kemb 1973, Kloiber et al. 1983). It has been suggested that articular inflammation has secondary effects upon vas-

cularity of bone, causing segmentary collapse after a time interval (Borgsmiller 1980). The epiphysis is particularly susceptible because the demands of the immature bone have to be met by the epiphyseal vessels alone since the growth plate cartilage present a vascular barrier. The intracapsular course of the vessels makes them vulnerable to obstruction by raised intra-articular pressure.

It has been shown that it is possible to produce a localized necrosis of the femoral head by increasing the joint pressure (Woodhouse 1964, Tachdjian and Grana 1968, Ogden 1974, Kemb 1981). The level and duration of the increased pressure seem to be the major determining factors, and it has been shown that this pressure in joints with an effusion is influenced by posture. In the hip the rise in pressure is most pronounced in forced extension (Eyring and Murray 1964, Kallio and Ryöppy 1985, Wingstrand 1986).

A prolonged tamponade at 150 mmHg for more than 10 hours results in an avascular necrosis of the femoral head (Tachdjian and Grana 1968). In another study, total necrosis of the femoral head of rabbits has been shown after six hours of tamponade with trabecular necrosis after only 2 hours (Vegter and Lubsen 1987). Intracapsular tamponade results in increased intraosseous pressure and decreased trapping of microspheres in puppies, but the effect on bone tissue PO_2 and PCO_2 has not been evaluated previously.

Experimental joint effusion (IV)

In a study of simulated joint tamponade the PO_2 and PCO_2 were measured in the femoral head of juvenile goats. Macrodex was infused in the joint cavity through a cannula passing the acetabulum. The measurements were performed at rest, after infusion of macrodex and with macrodex and 1.5 kg traction in extension. Tamponade of 75 mmHg caused the oxygen tension in the femoral head to drop (Table 5), and traction further deceased the oxygen tension. The PCO_2 increased, but to a lesser extent and only in combination with traction gave statistically significant effect. Traction resulted in a profound increase in intraarticular pressure when a tamponade was priorly present in the hip joint (from 75 mmHg to 360 mmHg).

Table 5. The intraosseous PO_2 and PCO_2 of the femoral head before and after joint effusion of the hip joint and traction (mmHg)

	Subchondral PO_2		Arterial PO_2
Initial	Joint tamponade (75mmHg)	Tamponade and traction	
48 (4)	29 (3)	17 (2)	80 (6)
50 (5)	51 (5)	68 (5)	38 (2)

Injection of fluid has been used in several studies to simulate joint effusion. Saline (Launder et al. 1981, Bünger et al. 1982, Kofoed and Lindenberg 1986), silicone oil (Tachdjian and Grana 1968) and macromolecular plasmasubstitutes (Lucht et al. 1983) have been used. In our study we showed that the choice of fluid is important because of the permeability of the synovial membrane. Saline leaves the joint by effusion of fluid into the periarticular tissues when the pressure rises (Levick 1980) and continuous infusion is necessary to maintain an elevated pressure. In our experiments a temperature sensor was placed in the bone adjacent to the joint surface and saline was infused. This lowered temperature from 37.8 to 33.8 °C. This hinders reliable measurements with the temperature sensitive mass spectrometer and may seriously disturb hemodynamic and metabolic parameters in the subchondral bone. On the basis of these experiments we suggest to use a fluid to which synovium is impermeable in joint tamponade studies.

Clinical studies of joint tamponade (V)

On the basis of the theories advanced above, it has been proposed that the necrosis of the femoral head seen after undisplaced fractures may be due to hemarthrosis and tamponade (Strömquist et al. 1988). Therefore, a study of patients with undisplaced fractures of the hip was performed where the PO_2 was measured in the femoral head before and after aspiration of the joint cavity in relation to surgery.

In the undisplaced fracture of the femoral neck, the capsule is intact. The fracture is intracapsular and bleeding from the fracture will accumulate in the hip joint. The pressure in the joint may rise to a level near the arteriolar pressure. Increasing evidence has accumulated that this may harm the vascular supply of the femoral head (Crawfurd et al. 1988) and this was also supported by the experimental study (IV). Therefore, it has been advocated to aspirate the hemarthrosis of the hip joint in connection to the operation for the undisplaced fracture. In this study we examined the PO_2 of the femoral head in 9 patients with Garden stage 1 fractures. After measurement of the PO_2, an aspiration of the hip joint was performed.

The median volume of the aspirate was 4 ml. In two patients the PO_2 increased substantially, indicating restoration of the blood flow of the femoral head. These two patients had an aspirate of 3 and 7 mL removed from their joint cavity. This indicates that the pathophy-

siological effect of tamponade may be important. These results are also supported by scintimetric studies demonstrating an increased uptake in the femoral head scintrimetrically (Strömquist et al. 1988), after joint aspiration.

Interpretation of results

These studies show that joint effusion can produce hypoxia in the subchondral bone compartment and that traction enhances the effect. This was interpreted to be the result of reduced blood flow in the bone, but compensatory mechanisms may reverse the effect after a longer period. The result found in the animal model could thus be reproduced in this clinical situation with possible hip tamponade. It is thus likely that the bone necrosis found following experimental tamponade (Vegter and Lubsen 1987) and fracture of the femoral neck may be caused by reduced blood flow and ischemia due to obstruction of the vessels with an intraarticular course.

The intraosseous circulation and pathogenesis of arthrosis

Arthrosis has previously been ascribed to the process of wear and tear. However, the disease has a different distribution in different ethnological groups, which is not consistent with this theory (Hoaglund et al. 1985. Additionally, a morphological difference exists between the limited degenerative changes related to aging and the progressive changes of the disease process (Byers et al. 1970). Although wear and tear may not be responsible for initiation of the disease, since the normal joint has great capabilities for repair, it may have great importance in the joint weakened by microstructural changes.

Directions in arthrosis research

Many hypotheses, as yet unproven, have been proposed to explain the pathogenesis of arthrosis. Research has assessed biomechanical, biochemical, and pathophysiological theories. Cartilage and subchondral bone have both been ascribed a principal role in the initiation of the disease and research has alternated between the cartilage changes and the subchondral bony changes.

From the biomechanical point of view, changes in extent and direction of the load on a joint are supposed to be the main trigger-mechanism. According to one biomechanical hypothesis (Radin et al. 1970, Radin et al. 1982), damage to the cartilage is incurred by callus formation secondary to fatigue fractures of the subchondral bone, induced by excessive loading. The callus formation decreases the elasticity of the subchondral bone thus reducing the energy absorbing capacity followed by lesser resistance of the cartilage to mechanical stress.

According to theories proposed by cartilage researchers, the damage is believed to be initiated by biochemical changes in the glycosaminoglycans of the intercellular matrix of the cartilage (Mankin and Lipiello 1971). An increase in proteoglycan and collagen degrading enzymes in the cartilage is suspected to induce the deterioration (Erlich et al. 1986).

Several studies have shown that biochemical changes correlate well with cartilage damage but it is still uncertain whether they occur first in the pathogenesis of arthrosis (Ali and Bailis 1974, Mankin and Brandt 1984, Martell-Pelletier et al 1984).

In the opinion of others, the bone changes of the epiphyses are of major importance. The focal osteocytic necrosis found has led to more focus on the metabolism of bone cells.

Pathomorphology and pathophysiology of arthrosis

The morphological picture of arthrosis varies depending on the stage of the disease and the changes include all the main anatomical structures of the joint.

Cartilage

Early in the disease, cartilage shows lesions with fibrillation, loss of organization and proliferation of chondrocytes to form cell clusters (Solokoff 1979). Chondrocyte type shifts towards immature chondroblasts. The matrix shows structural changes with different patterns of metachromasia. In the advanced stages of the disease, the cartilage is totally deteriorated, with spots of denuded bone.

Chondrocytes are nourished by the synovial capillaries "per diffusionem" through the synovial membrane and the articular space. Transport of nutrients along this path may be impaired due to inflammatory changes in the synovial membrane and due to joint effusion (Arnoldi et al 1980). An alternative pathway for nutrients goes from the capillaries of the subchondral bone plate to the cartilage (Bullough and Jagganath 1983), but it is generally accepted that the synovial membrane is the dominant source of nutrients to the chondrocytes. Normal cartilage is in a steady-state balance, where the intercellular matrix is produced and degraded at the same rate. Several noxious substances can disturb this balance (Fassbender 1986) and stimulate the chondrocytes to produce more digestive enzymes (proteases etc.) (Sapolsky and Howell 1982), a change which results in decomposition of the intercellular matrix. There is evidence (Dingle 1979) to support that such substances leak out from inflamed synovial membrane. Oxygen deprivation may also stimulate the release of these proteolytic enzymes (Ahlquist 1984).

Synovial membrane

Initially, the synovial membrane goes through a stage of acute synovitis with inflammation, cell proliferation, edema and increased enzyme activity (Solokoff 1979,

Arnoldi et al 1980). In this stage considerable joint effusion occurs. In later stages the synovial membrane is replaced by scar tissue which results in contractures of the joint. Animal studies of synovial function in experimental arthrosis have shown prolonged washout of xenon from the joint cavity (Christensen et al. 1982) and this was believed to represent either lowered exchange function or decreased blood flow in the synovial membrane. A decreased exchange function was also indicated by demonstration of a reduction in diffusion capacity by a factor of four, and an increase in synovial metabolism and oxygen use by a factor of two, in another animal model of acute arthrosis (Svalastoga and Grønlund 1985, Svalastoga and Kiær 1989). Using an animal model a new method to calculate the diffusion capacity, oxygen consumption and blood flow of the synovial membrane was used. The principle of this method was to perfuse the joint cavity with saline solutions with different oxygen and nitrogen partial pressures, measuring the partial pressures in the perfusate flowing out of the joint. A set of equations express the relationship between blood flow, diffusion capacity and oxygen consumption and partial pressures of oxygen and nitrogen flowing to and from the joint under different conditions. In a model of chronic arthrosis a statistically significant increase in oxygen consumption was found (Svalastoga et al. 1989). Hypoxia, lowering of pH and accumulation of lactate in synovia withdrawn from diseased joints (Treuhaft and McCarthy 1971) could thus be easily explained.

Bone

The bone tissue of arthrosis is characterized by extensive exophytic growth in the margins of the articular surface and intervening areas of necrosis and cyst formation in the central areas of the subchondral bone (Brookes 1971, Solokoff 1979). Remodeling and disorganization of the epiphyses are commonly found. Microscopically, necrosis of the marrow and replacement with connective tissue is seen. The resorptive and proliferative activities in the bone lamellae indicate active remodeling. In the cysts, the bone lamellae are replaced by connective scar tissue or fluid, and in the periphery, a zone of newly formed bone is found. During the course of the disease, the numbers of empty lacunae increase because of necrosis of osteocytes (Milgram 1983, Wong et al. 1987).

Vascular changes

A prominent feature of arthrosis is the universal dilation of blood vessels and hypervascularity (Figure 17) (Harrison et al. 1953, Hulth 1958, Brookes and Helal 1968). Microangiographic studies have shown invasion of vessels in the cartilage from the subchondral bone plate where a separation is normally found at the level of the tide mark (Solokoff 1979). Ingrowth of vessels

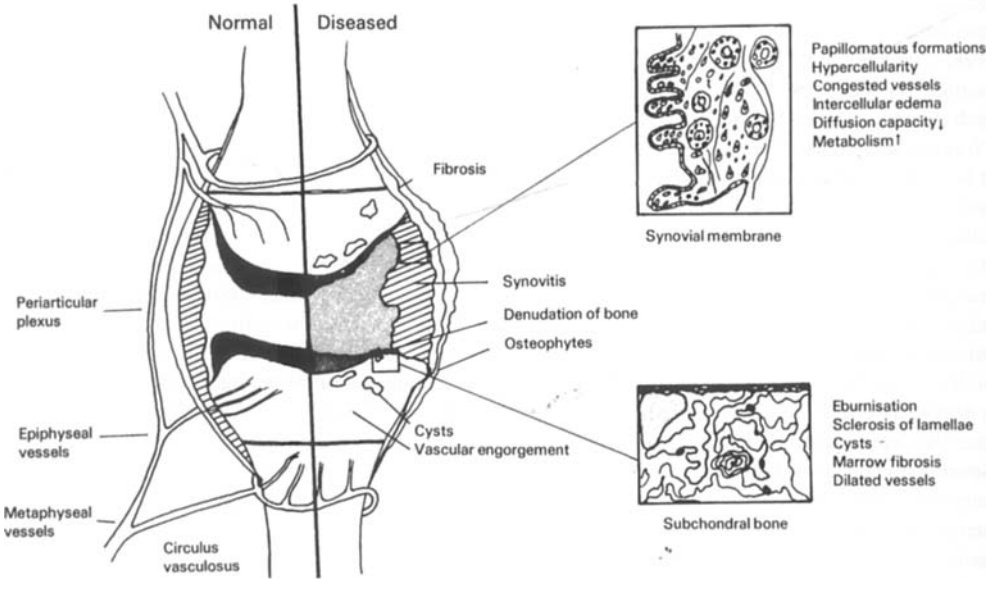

Figure 17. The vascular supply to a joint with metaphyseal and epiphyseal vessels and the periarticular plexus supplying the capsule. The other part of the diagram shows the characteristic morphological lesions in osteoarthritis. The sections of the diseased tissue represent a diagrammatic conception of some of the morphological changes involved.

Table 6. The human normal intraosseous pressure (mmHg)

Author	Bone	Region	IOP mean (range)
Arlet et al. 1968	Femur	Metaphysis	17.2 (12–26)
Arnoldi et al. 1972	Femur	Neck	18.7 (12.9–23.5)
Lynch 1974	Tibia	Epiphysis	29.1 (17–34)
Lemberg & Arnoldi 1978	Tibia	Prox epiphysis	28.3 (±15.2)
Green & Griffin 1982	Femur	Head	30.7 (12–51)
Chigira 1984	Different	–	22.0 (±11)
Kiær et al. 1988 (VI)	Femur	Head	26.0 (±3.2)

near the articular cartilage margin precede formation of osteophytes (Harrison et al. 1953). Another abnormal feature is formation of new vessels arising from the joint capsule and entering the bone just beneath the cartilage border. The synovial membrane also shows dilation and hyperplasia of the vasculature, and the cyst walls are encircled by dilated sinusoids and veins.

Pathophysiologically, the changes correlate with morphological findings. An engorgement of the vascular tree is seen, together with prolonged emptying of contrast material to the extraosseous veins (Hulth 1958, Arnoldi and Reimann 1979). The intraosseous hydrostatic pressure (Table 6) has often been used to evaluate bone circulatory physiology, and several animal and human studies of diseased and normal bone have been performed (Azuma 1964, Arnoldi et al. 1972, Ficat and Arlet 1980), showing increased intraosseous pressure. Interindividual variation of pressure between species is considerable, but an upper limit of 30 mmHg in human bone has been proposed (Lemberg and Arnoldi 1978). Arnoldi et al. found values of 45 mmHg in diseased femoral heads and, in contrast to this, 19 mmHg in normal tissues (Arnoldi et al. 1972). An abnormally high amplitude of the pressure waves normally found in bone was also seen in the diseased bone.

The stress test which records the response of the bone circulation to a injected load of saline shows, in addition, a prolonged response in arthrosis in contrast to the normal quick return to preloading values (Lemberg and Arnoldi 1978, Ficat and Arlet 1980) . Direct measurements of bone blood flow have, however, not been performed in humans, whilst animal studies of bone blood flow showed varying results with prominent regional differences in subchondral bone (Jones et al. 1982, Bünger et al. 1987).

Scintigraphic examination

Scintigraphically, increased uptake is found in osteoarthritic bone. Autoradiography, together with scintigraphy, of specimens of osteoarthritic bone have, however, demonstrated that generally increased activity is due to uptake in localized areas of the subchondral bone (Christensen 1985). These areas correspond to the osteophytes and cyst walls having an increased content of active enzymes, such as alkaline and acid phosphatases (Reimann and Christensen 1979). Scintigraphic tracers, such as ^{99}Tc, bind to the inorganic graphite crystal lattice and have been shown to accumulate in areas with active lay-down of new bone. The uptake, hence, corresponds partly to blood flow and, more importantly, to metabolic activity and especially calcification, indicating active resorption and formation in the osteophytes and cyst walls.

Own studies

Subchondral pressure, PO_2, PCO_2 and lactate concentrations in late stage arthrosis (VI)

In a clinical study of arthrosis, we studied tissue hypoxia in the femoral head of late-stage primary coxarthrosis. Simultaneous *in situ* measurements of PO_2 and PCO_2 were performed in normal and osteoarthritic femoral heads of patients undergoing unilateral, total hip replacement. Furthermore, lactate concentrations and pH were measured in blood samples taken from the bone tissue in order to detect a possible increase in anaerobic metabolism of the diseased bone tissue. In another study (VII) of 31 patients, the concentration of lactate and pH in blood samples from the diseased femoral head were compared to that of the ipsilateral greater trochanter.

Thirteen patients with unilateral coxarthrosis were studied. The measurements were performed bilaterally in the femoral head in connection with Charnley total hip replacement, using the normal hip as a reference. A blood-gas catheter was introduced into the spongious bone through a cannula positioned subchondrally in the femoral head.

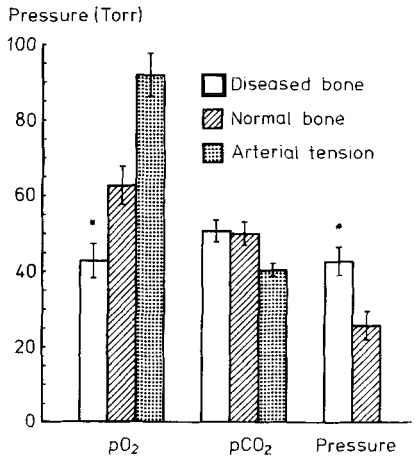

Figure 18. The paired observations of PO_2, PCO_2 and pressure of the femoral head with and without arthrosis. Significant differences were found in pressure and PO_2.

Table 7. The concentration of lactate and pH (*SD*) of blood from the femoral head of diseased and normal hips (Series 1) and from the femoral head and greater trochanter of hips with arthrosis (series 2)

	No. of patients	Lactate conc. µmol/mL	pH
Series 1			
Normal hip	10	1.14 *0.16*	7.353 *0.020*
Diseased hip	10	1.60 *0.22*	7.334 *0.017*
Series 2			
Trochanter	31	1.07 *0.28*	7.357 *0.078*
Femoral head	31	1.67 *1.14*	7.345 *0.063*

The average subchondral PO_2 (Figure 18) was significantly lower in the diseased hips (43 ± 5 and 63 ± 5 mmHg). No significant difference could be demonstrated in the subchondral PCO_2 between normal and diseased hips. The subchondral pressure was significantly higher in the diseased hips (26 ± 12 and 44 ± 4 mmHg) and the amplitude of the pressure wave was higher. The intraosseous lactate concentrations of the normal and diseased hips were significant different (1.1 ± 0.2 µM and 1.6 ± 0.2 µM) and the lactate concentration of the diseased femoral heads was higher than that of the ipsilateral greater trochanter (Table 7). pH was the same in the normal and diseased hips.

Subchondral pressure, PO_2, PCO_2 and the histological picture of the initial painful hip

In another study (VIII) 21 patients with hip pain of short duration (< 1 year) were examined clinically, radiographically and scintigraphically. Intraosseous pressure, PO_2 and PCO_2 were also measured, both in the femoral head and the greater trochanter, and a bone specimen was taken from the femoral head and examined histologically. Radiography showed slight arthrosis (joint space narrowing of less than 2 mm, with osteophytes at one pole and slight sclerosis or cyst formation) in 13 hips, 11 hips showed no radiographic changes and the rest had osteonecrosis (see 'Osteonecrosis' p 28). All hips were treated with a core decompression of the diseased hip and the bone specimens obtained were treated and examined according to the histological methods previously described.

Only half of the patients with slight arthrosis on the radiographs showed increased uptake on the scintigrams. The PO_2 of the femoral head was lower than that of the greater trochanter (58 ± 6 and 68 ± 7 mmHg) and the intraosseous pressure was significantly higher (38 ± 5 and 21 ± 4 mmHg). The PO_2 was significantly lower and pressure was higher in the hips with arthrosis than in the hips with normal radiography and no histological necrosis. All hips with slight arthrosis, examined histologically, showed signs of tissue necrosis, Grade 1–3.

Implications of increased intraosseous pressure and decreased PO_2

Our findings in this study, together with previous reports of intraosseous pressure (Table 6), support the hypothesis that an intraosseous pressures above 30 mmHg should be considered pathological, but the diagnostic sensitivity of the examination is low due to large variability of normal pressure. The result of the pressure measurements found in the study was comparable to the results reported by Arnoldi et al. (1973). They found a mean difference of 30 mmHg between the normal and diseased femoral head, comparable to the mean difference of 18 mmHg reported in the present study. Pressures in the normal hip were similar to those obtained in three previous studies (Table 6) of 19 mmHg, 24 mmHg and 25 mmHg.

The values of PO_2 and pressure of both normal and diseased bone showed considerable variability, with substantial overlap between the two groups. It is thus difficult to set a pressure value above which bone may be considered pathological. If the upper limit of normal pressure is set at 30 mmHg (Lemberg and Arnoldi 1978), 95% of the hips with pressures above this level in this study (VIII) were considered abnormal, using histological examination. But it is possible to find hips having necrosis with lower intraosseous pressure, and hips without histological changes with higher pressure. The same pattern was found in the distribution of values for PO_2, where 95% of the patients with PO_2 below

60 mmHg showed histological necrosis whereas only 6% of 17 patients with necrosis had partial pressures above this level.

The data on intraosseous PO_2, lactate concentration and pressure support the hypothesis that outflow resistance increases in arthrosis (increased pressure) and causes reduced blood flow (decreased PO_2). The increased lactate concentration indicates that the bone tissue has partially switched to anaerobic metabolism which is an indirect sign of tissue hypoxia. Another interpretation of the results is that the decrease in PO_2 is due to an increase in oxygen consumption and that the rise in outflow resistance is compensated by a fall in the inflow resistance. In the absence of flow measurements, the two hypotheses cannot be fully validated. In the series of animal experiments (II) there was, however, no compensation following experimentally induced increased outlet resistance. The demonstration of necrosis of marrow tissue and bone gives further evidence for a relative anoxemia of the subchondral bone. This can explain many of the morphological findings described above with neovascularisation of the subchondral bone and remodeling.

Possible pathomechanisms of vascular congestion

Neuropeptide mediators in arthrosis

Several neuromediators may be involved in the pathogenesis of arthrosis. Activation of nociceptive nerve endings by mechanical and chemical irritants related to joint pathology has been demonstrated. Some fibers of somatosensory nerve endings have a neurosecretory function. The most important in this context is substance P, which produces a strong increase in local microcirculation and plasma extravasation. The peptides stimulate mast cells and serotonin and histamine release (Lembeck et al. 1982). This phenomena has been called neurogenic inflammation. There is increasing evidence that joint disease may involve neurogenic peptidergic mechanisms. In animals with experimentally induced polyarthritis, the content of substance P of sensory type C fibers is much enhanced (Levine et al. 1984) and infusion of substance P into joints increases the severity of the disease. Medically, depletion of substance P (Colpaert et al. 1983, Levine et al. 1986) or nerve transection (Courtright and Kuzell 1965) in rats reduces the severity of arthritis. *In vitro* experiments have also shown the stimulatory effect of substance P on synoviocyte release of prostaglandin E2 and collagenase. All these results indicate a connection between neurogenic mediators and the grade of inflammation and vasodilation in the inflammatory stage of arthrosis.

Relationship of joint degeneration to bone circulation

Examination of patients with arthrosis has failed to show any compression of the extraosseous veins. Inside the bone no single factor responsible for the congestion can be demonstrated. The architecture of the venous system inside bone, with thin walled sinusoids without valves, makes the circulation susceptible to any kind of anatomical deterioration and compression. The disintegration of bone structure in arthrosis is likely to cause degradation of the large endothel covered cavities and hindrance of normal flow. The effect of synovitis on the epiphyseal circulation has been discussed in detail in 'Physiological and pathophysiological models on bone circulation.' In the later stages of arthrosis with massive fibrosis of the capsule, the epiphyseal veins may be compressed in their transcapsular course. This is supported by the fact that torsion of the capsule increases the intraosseous pressure and degenerative changes in bone nearby joints and osteocyte death can be produced by bracing the joint with capsular stretching (Henard and Calandruccio et al. 1970, Michelsson 1974, Langenskiøld et al. 1979)

Increased pressure is unlikely to be the direct cause of cell damage, but if the physiological basis for the pressure increase is an augmented outlet resistance the bone blood flow may suffer. Reduced tissue perfusion can cause cell damage either by inadequate oxygenation or by accumulation of tissue metabolites.

It has been shown that the appearance of an intraarticular effusion, on the basis of several etiologies impedes the nutrition of the articular cartilage and concurrently the flow from the epiphyseal veins of the bone ends by compression of the intrasynovial veins. It is likely that cartilage damage occurs early in the course of effusion as a result of interruption of the transport path to the cartilage. Ischemic cell injury in bone is possible as it has been shown that only a period of 4 hours of ischemia is sufficient to harm osteocytes (James and Steijn-Myagkaya 1986). Changes in bone structure are, however, a slowly propagating process, with potential for repair, but recurrent joint effusions due to cartilage suffering may reinforce the degradating process.

Osteonecrosis

The pathological process in bone with cyst formation, sclerosis, flattening, sequestrum formation and secondary arthrosis has been variously termed osteonecrosis, avascular necrosis, ischemic necrosis and aseptic necrosis. It is generally accepted that this condition starts with episodes of bone cell ischemia followed by structural changes of the inorganic elements (Glimcher and Kenzora 1979, Ficat 1985). This is supported by the fact that interruption of the blood supply to the femoral head by a neck fracture generates a picture very similar to avascular necrosis (Strömquist 1983). Bone cell ischemia has not been demonstrated directly but has been suggested by the results obtained with indirect techniques such as bone marrow pressure measurements (Ficat and Arlet 1980), intraosseous venography (Brookes and Helal 1968, Ficat and Arlet 1980), radionucleotide scans (D'Ambrosia et al. 1978) and histological methods (Arlet et al. 1984). Newer studies have shown pathological filling of the bone vessels in specimens taken from the femoral head of osteonecrosis (Spencer and Brookes 1988)

Pathogenesis of osteonecrosis

Avascular necrosis of bone is a result of long standing severe ischemia. A blockage in the microcirculation of the cancellous bone plays a central role in the evolution of the disease.

Congestion of the bone marrow, increased intraosseous pressure (Ficat and Arlet 1980) and histological necrosis (Ilardi and Solokoff 1984, Wong et al. 1987, Arlet et al. 1984) have been demonstrated in several studies. Pathophysiologically, the circulation of the bone in osteonecrosis is marked by hydrodynamic abnormalities, with raised pressure and increased outflow resistance. The bone tissue has no option for expansion if the pressure rises, and increased pressure above the arteriolar level can compress the capillaries and the sinusoids, with further congestion and establishment of a *circulus vitiosus*.

The hindrance of circulation can be intraosseous or extraosseous. Intraosseous conditions may include embolism with air or fat, thrombosis, vasculitis, and extravasal conditions such as hemorrhage, edema, and the increased cellularity in inflammation (Jones 1971, Ficat and Arlet 1980). Extraosseous conditions are probably infrequent but arterial embolism, venous compression and phlebitis have all been suggested as possible activators of the disease. Osteonecrosis is often associated with specific conditions, and in some of these, the cause of the bone ischemia can be justified. In caisson disease (decompression disease), N_2 bubbles form in the end capillaries, in sickle cell anemia microthromboses form in the capillaries and cerebrosides are deposited in the vessels in Gauchers disease.

Treatment with steroids often results in osteonecrosis, and this has been explained by coagulation disturbances (Brettger et al. 1970), vasculitis (Miles 1980), and microfractures of osteoporotic bone. Generally, fat metabolism is changed following long standing steroid therapy, and it has been suggested that hyperlipemia and/or fat embolism may cause the changes. Another possibility is, that the changed cell metabolism or fat deposition in the cells causes cell death. Experimentally, it has, however, only been possible to generate steroid induced osteonecrosis in a single study (Wang et al. 1983). The cause of osteonecrosis may, however, be multifactorial, but it seems documented that it is not a manifestation of an arterial failure, i.e. a true avascular necrosis.

Pathomorphology of osteonecrosis

The histological lesions of osteonecrosis can be examined in specimens from diseased bone. From the femoral head, a core biopsy can be obtained by using a hollow trephine introduced through the neck of the femur. Pathomorphologically, osteonecrosis has been divided into four stages, as previously described (Figure 19).

Clinical stages of osteonecrosis of the hip

In osteonecrosis of the hip, five clinical stages has been identified (Ficat 1985). Stage 0 is the preclinical stage without radiographic changes, the so called silent hip. Some studies have shown development of biopsy proven osteonecrosis in 64.7% of these silent hips if the intraosseous pressure was also found increased at initial examination. Stages 1 to 4 characterize the corresponding to increasing clinical severity of the disease and this has been extensively reviewed by Ficat and others (Hungersford and Zizic 1983). In Stage 1, a normal plain radiograph is found. Stage 2 is characterized by pain, osteopenia, subchondral sclerosis, and occasionally cysts formation. In Stage 3, the hip is symptomatic and, radiographically, with the crescent sign; and during Stage 4, collapse of the femoral head combined with a narrowed joint space and acetabular changes occur.

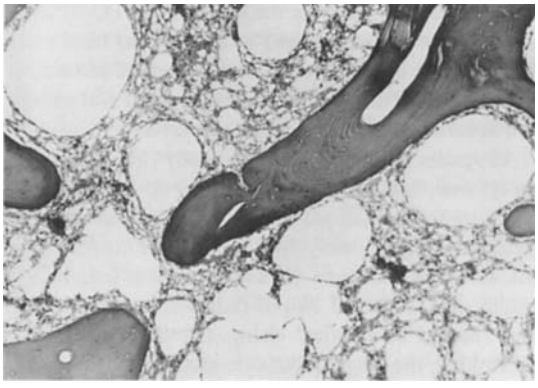

Figure 19. A histological section of bone from a femoral head with osteonecrosis. Histologically there is osteonecrosis—Stage 2—with eosinophile reticulosis between numerous oil cysts.

Recently, therapy has been directed against early intervention with decompression and drainage of the bone compartment. Several studies have shown this treatment justified in the early stages (0 and 1) but having limited value in more advanced stages (Ficat 1985, Tooke et al. 1988). In this study the operation was performed under general anesthesia and under guidance of an image intensifier.

The clinical examination of osteonecrosis

The clinical examinations include plain radiography, bone scintigraphy, CT scan, MRI, intraosseous pressure determination, intramedullary venography, and histological examinations of core biopsy specimens (Arlet and Durroux 1984).

Radiography

The changes in osteonecrosis often appear late on standard radiographs, passing the preradiographic stage (Hungersford and Zizic 1983, Ficat 1985, Pedersen and Kiær 1987).

The radiographic stages are shown in Figure 20. Use of computerized tomography can increase the sensitivity of the radiographic examination in the later stages, but the sensitivity is not much higher than that of normal radiographs in the early preradiographic stages. CT-scanning is a more sensitive method for detection of subchondral fractures (Mitchell et al. 1986 and has therefore provide a better tool for differentiation between Stage 1 and 2 osteonecrosis.

Scintigraphy

A bone scan with ^{99}Tc-MDP shows increased uptake in bone with increased blood flow or in bone with active remodeling (D'Ambrosia et al. 1978, Miki et al. 1987). Normally, the scans may show increased uptake in early necrosis, or rarely decreased uptake. These changes are unspecific and can also be seen in other pathological conditions of bone.

Intraosseous pressure registration

It is possible to detect the circulatory changes of osteonecrosis before the radiographic signs appear on radiographs, using intraosseous pressure registration (Ficat and Arlet 1980, Hungersford and Zizic 1983, Robinson et al. 1989). With this method, osteonecrosis of the hip can be recognized in the early stages where pain and limitation of movement are present but no changes are seen radiographically. Pressure registration can be supplemented with the stress test, where 5 mL of isotonic saline is injected into the bone and the pressure is recorded five minutes following the injection. In osteonecrosis, the normalization of pressure will be prolonged. This indicates severe venous congestion of the bone. When supplemented by intramedullary phlebography, this is illustrated by delayed emptying of the dilated intramedullary sinusoids and a reflux of contrast material to the diaphysis, lasting more than 15 min. This is in contrast to normal quick clearance of contrast from the intramedullary space.

MRI scanning

Magnetic resonance imaging can show changes in the elements of the marrow before the other noninvasive

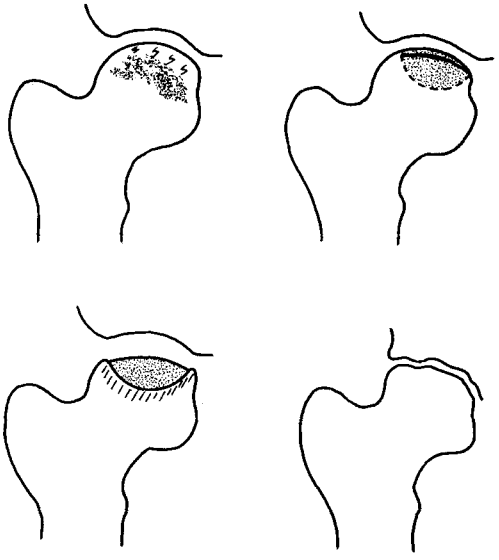

Figure 20. The radiographic appearance of the four stages of osteonecrosis.

Table 8. The distribution of histological findings against radiographic findings in hips with pain of short duration

	Radiography		
	Normal	Osteonecrosis	Osteoarthritis
Histology			
Normal	4	1	0
Necrosis	7	7	9

diagnostic techniques. Several recent studies with this method have shown that it is superior to other noninvasive examination methods. In one study comparing MRI to the histological findings of core biopsies, a good correlation was found between the findings of magnetic resonance imaging and the results of biopsies in Stage 1 and Stage 2 osteonecrosis. In the initial stage, however, no definite correlation was found between the methods (Robinson et al. 1989). Instead, the signal intensity was found to be a function of marrow degeneration and loss of fat content, and not predictive of the particular histological morphological pattern (Simmonds et al. 1989)

Own investigations of osteonecrosis

Patients and experimental procedures (VIII)

In a study of patients with hip pain of short duration (< 1 year), a picture indicating osteonecroses was found radiographically in 8 hips. In 11 hips (4 men and 5 women), no radiological changes were found indicating a Ficat stage 1 osteonecrosis. In 13 hips slight arthrosis was found and these patients were similarly examined but with a successful core biopsy only in 9 cases.

The patients were examined with standing oblique radiography, CT and scintigraphy of the hip and physical examination. All patients complained of hip pain and none of them had known predisposing factors for osteonecrosis. In all cases the intraosseous PO_2, PCO_2 and pressure were measured in the femoral head and, finally, a core decompression was performed and a core biopsy taken. The biopsies were examined and classified according to Arlet and Durroux (1984).

All patients with radiographically osteonecrosis, except one, had histological necrosis in the biopsies. The histological changes seen when osteonecrosis was present radiographically were impossible to discriminate from the ones seen when arthrosis was seen radiographically. Seven of eleven radiographically normal hips showed histological changes from grade 1 to 3 confirming, the diagnosis of osteonecrosis (Table 8).

The results of the intraosseous measurements, in patients with osteonecrosis or normal findings radiographically, were separated according to the histological findings in two groups, one with histological necrosis and one without. The mean intraosseous pressure in the femoral head of patients with histological necrosis and Stage 1–2 osteonecrosis was significantly higher than in patients without histological necrosis (unpaired t -test, $p < 0.05$; Table 9) . The intraosseous pressure was also significantly higher than that of the normal hips in the study of unilateral arthrosis. The intraosseous pressure in hips with radiographic osteonecrosis was 53 ± 6 and this group of patients had the highest intraosseous pressure of all examined hips.

The intraosseous PO_2 was significantly lower in hips with histological necrosis than in hips without histological necrosis (Table 9). The intraosseous PO_2 was lowest in the femoral heads of hips with obvious or suspected radiographic osteonecrosis and histological necrosis. In 11 of the hips the intraosseous pressure and PO_2 were measured again shortly after decompression. The average decrease in pressure in 13 hips with histological necrosis was 19 mmHg compared to 7 mmHg in 4 histologically normal hips; this difference was significant ($p < 0.001$). The partial pressure of oxygen increased 7 mmHg in histological normal hips (NS) and 18 mmHg in hips with histological necrosis following decompression ($p < 0.05$).

Table 9. The intraosseous PO_2, PCO_2 and pressure of hips with pain of short duration. NF+ON represents the group of hips with radiographical normal findings or with osteonecrosis, SOA represents the group of patients with slight osteoarthritis on the radiographs, used for comparison

	Pressure		PO_2		PCO_2	
Radiography	NF+ON	SOA	NF+ON	SOA	NF+ON	SOA
Histology						
Normal	26 ±6 (5)	–	71 ±8(5)	–	48 ±2 (5)	–
Necrosis	51 ±4 (14)	38 ±6 (13)	36 ±6 (11)	58 ±6 (10)	48 ±6 (11)	46 ±6 (10)

Long term results of decompression (IX)

The good results of decompression in the early stages of osteonecrosis have ranged from 80–90% (Ficat 1985) to 25–50% (Camp et al. 1986). In this study we have followed the patients prospectively after treatment with decompression. Only patients with Ficat stage 1 osteonecrosis were followed with clinical, radiographic and scintigraphic examinations. The patients were finally reviewed, with a median follow-up period of 33 months (1–5 years). There were 14 cases with totally 18 diseased hips in this series, and 9 had normal radiographs preoperatively.

The follow-up showed that all patients had temporarily relief of pain, but in only six hips did this last for more than 12 months. Four hips showed radiographic progression to a more advanced stage of necrosis. Thus only 33% had a good result in this study.

Interpretation of experimental results

The results of our preoperative examinations confirms that increased scintigraphic uptake and radiographic changes evolve simultaneously. Hips in which the radiographs were normal rarely showed positive scans (2/11) but many of these had histological changes (6/11). Necrosis can thus be seen without any scintigraphic and radiographic changes, indicating that both the grossly morphological (radiography), and the dynamic method (scintigraphy), are insensitive in the diagnosis of osteonecrosis. When radiographic changes occur they always indicate histological changes, but a normal radiograph does not exclude histological changes. This is supported by the findings of other studies showing that histologically morphological changes can exist despite normal plain radiographs (Ficat 1985, Bassett at al 1987, Marcus et al. 1973).

The pathogenesis of osteonecrosis is still unclear. A large effort has been dedicated to reveal the features of the disease, the best diagnostic tools and the treatment of choice. The function of osteocytes depends on normal circulation and supply from the blood stream. If the osseous outflow resistance is elevated the normal vascular flow path of least resistance will be compromised. The flow direction will changed from the normal downstream through the venous system and upstream into the arterial tree, and the blood flow will cease.

The blood flow through bone tissue is described by the equations

$$F = \frac{P_{ART} - P_{IO}}{R_I} \quad \text{and} \quad F = \frac{P_{IO} - P_V}{R_O}$$

where F = blood flow, P_{ART} = mean arterial pressure, P_{IO} = intraosseous pressure, R_I = inflow resistance, P_V = venous pressure and R_O = outflow resistance. Animal studies have indicated weak autoregulation of inflow resistance (II, III). If inflow resistance is assumed to be constant and a qualitative evaluation of blood flow under different conditions is possible. If the mean arterial pressure is set to 80 mmHg, an increase in intraosseous pressure from 26 to 45 mmHg would imply a reduction of the intraosseous blood flow, by a factor 1.6. In the computer simulation study, the relationship between intraosseous pressure, decreasing PO_2 and flow was investigated. At a consumption rate of 1 $\mu mol/O_2/min$ a decrease in flow by a factor 1.6 will reduce intraosseous oxygen partial pressure from 75 mmHg to 50 mmHg, assuming constant consumption. The effect is thus a relative anoxia in the environment of the osteocyte.

Peroperative measurements showed a significant increase in oxygen partial pressure and decrease in pressure following decompression. This has earlier been ascribed to the compartmental nature of bone, where opening of the medullary cavity results in increased drainage (Kantor et al. 1985, Tooke et al. 1986, Ficat 1985). It has been reported that the opening of bone causes revascularisation and this could block further development of disease. This effect is most prominent in early stages of the disease (Levine et al. 1987). If the increase in blood flow was permanent or a transient finding could not be deducted from this study.

The specimens from osteoarthritic and osteonecrotic bone showed the same manifestation of necrosis histologically. This implies that necrosis of the marrow and trabecles is an early manifestation in both conditions. Osteocyte necrosis has previously been described in osteonecrosis (Rutishauser et al. 1960, Inoue and Ono 1979). Sixty four percent of femoral heads with a preoperative diagnosis of arthrosis removed at arthroplasty, showed death of the osteocytes, in a study of human arthrosis (Wong et al. 1987). Irreversible osteocyte death is seen after 2–4 hours ischemia (James and Steijn-Mayagkaya 1986). The degradation of the normal bone architecture in osteonecrosis and arthrosis is due to active resorption and remodeling initiated by osteocyte death. The etiology of the bone ischemia may, however, be different under the two conditions. In osteonecrosis the bony changes are more pronounced and the pathophysiological findings, with very high intraosseous pressure and infarction of segments of the femoral head, indicates a more severe vascular disorder.

Clinical interpretation

Core decompression has been advocated in the treatment of osteonecrosis of the hip. However, newer studies conflict and are less optimistic regarding the effect of this procedure. The natural history of the disease is sparsely documented. The duration of disease in Stages 1 and 2 before progression, has been reported to take from 12 months (Marcus et al. 1973) to 3 years (Lee et al. 1980). There appears to be a general agreement that core decompression in Stage 2 and 3 osteonecrosis will not change the rate of progression to collapse. Proponents for treatment with core decompression have claimed that the treatment shall be initiated in "the silent stage" (Stage 1) to be effective. However, only a few studies have evaluated the effect of core decompression in stage I disease with a reasonable follow up period. This is necessary to evaluate the statements given by Ficat (1985) and Hungersford and Zizic (1983) that core decompression only has effect in Stage 1 disease.

Our study with genuine material of Stage 1 disease, which is unique, gave a 33% rate-of-success of core decompression. This is not significantly better than the indications of healing rate in hips left untreated. On the basis of these results there seems not to be a place for core decompression in the treatment of osteonecrosis. This was also concluded in other recent studies (Camp and Colwell 1986, Seiler et al. 1989) but these studies are more difficult to evaluate since the materials examined represented mixed stages of osteonecrosis

Several other treatments have been proposed. Most important are osteotomy, muscle pedicle grafting, phemeister type bone grafting, vascular grafting and pulse electromagnetic fields. However, the data is very sparse and no conclusion can be drawn about the rate of success in these methods.

General discussion

Studies of tissue oxygenation and blood flow have shown that changes in oxygen exerts a potent control of the flow regulating mechanisms and can induce a metamorphosis of the cellular metabolism (Goldhaber 1958, Brighton et al. 1969). From earlier *in vivo* studies it appears that transition of bone tissue from cartilage to calcified bone is stimulated by low oxygen tension (Brighton et al. 1971) and that the metabolism of regenerating bone is anaerobic. It seems, that low oxygen tension stimulates the pluripotential cells of the bone and that a gradient of oxygen across a barrier between normal and healing bone is essential to induce repair and calcification (Heppenstall et al. 1976).

The basis of these observations has been ascribed to special characteristics of the bone cells. These cells possess the potential for "aerobic glycolysis" in addition to normal anaerobic glycolysis (Neumann 1977). By this, bone cells continue to produce lactate despite a increase in the ambient oxygen level, in contrast to the normal cessation of lactate production—"the Pasteur effect." This has been related to the metabolic control of resorption, where an acidification of the ambient milieu is essential. In a preprogrammed fashion the cells may undergo changes if the ambient oxygen level is changed, with resorption, proliferation of primitive mesenchymal tissue and ingrowth of vessels and formation of collagen.

The exchange of O_2 in bone

The clinical measurements showed that intraosseous hypoxia was found in subchondral bone of degenerative joint disease and osteonecrosis. It has been documented for years that intraosseous hydraulic pressure is increased in parallel with the development of these conditions. Increased tissue pressure may modify the circulation when it rises to values comparable to the microvascular pressure. In bone, this effect is significant because this organ has no compliance, as it is constrained within the container of cortical bone. Vital microscopic studies of skeletal muscle (Reneman et al. 1980) have shown that capillary flow ceased with external pressure 24 mmHg below the arteriolar pressure. The very high intraosseous pressure found in osteonecrosis can be a critical hindrance to the capillary blood flow of bone. This may be compared to the compartment syndrome of skeletal muscle or the cranium. The source of the pressure increase can be distension of arteries, increased capillary filtration or a rise in venous pressure. Edema of the tissue, hemorrhage and swelling of the cells (hypoxia) may aggravate the condition.

In this series of experiments we found no definite pathophysiological explanation for the rise in tissue pressure. Despite years of research the initial cause has not been found. The most likely explanation is increased venous pressure. This may influence the autoregulation of the capillary pressure, as it has been shown in other tissues that an increase in venous resistance results in an increase in precapillary resistance (Nagle et al. 1968). The effect of this response is difficult to evaluate since other regulatory mechanisms (metabolic, neurogenic) may counteract the effect.

Several autoregulatory principles are involved in the maintenance of tissue perfusion. Most important are the metabolic, neurogenic, myogenic and humoral. Oxygen exerts regulatory function on blood flow. Lack of it can induce increases in blood flow by relaxation of arteriolar smooth muscle (Duhling 1972). Metabolic mediators released because of a lack of oxygen may be the main source of the effect, and how large a part of the stimulus which is induced by this is unclarified.

Next to blood flow, the most important limiting factors of the exchange of oxygen and carbon dioxide are diffusion gradients. The penetration of the capillary is determined by the product of permeability and surface area, and for low blood flow rates the exchange will be flow limited. But for lipophilic substances, like O_2 and CO_2, the distribution of perfused capillaries and the extracapillary diffusion gradients are limiting for higher flow rates. This refers to the Krogh Erlang equation, which gives an estimate of the difference across a distance in the tissue, given the diameter of the capillary and the uptake of the gas. The important intercapillary distance and the density of capillaries is very difficult to measure in bone tissue due to the inaccessibility of the tissue. The distance is, however, complicated by the extra transport pathway of the extravascular fluid spaces inserted between the capillaries and cells of bone.

The exchange of CO_2 in bone

From this series of experiments it appears that no difference can be demonstrated in the PCO_2 of normal and diseased bone. The CO_2 content of bone has for a long time been an object of interest. The skeleton contains the main CO_2 reservoir in living organisms. It was shown that at least two fractions exist, with the major fraction bound as carbonate in the lattice crystals.

Another fraction exists as bicarbonate and is exchangeable with the CO_2 of the extra cellular fluid (Poyart et al. 1975). The exchange between the two pools has been shown to be slow. Furthermore, the osteoclasts have an active proton pump with secretion of acid and hydration of CO_2 into carbonic acid (Ghiselli et al. 1987). According to the Henderson/Hasselbach equation, the main part of CO_2 in the living organism under physiologically pH conditions is present as bicarbonate.

It thus appears that the gas exchange of CO_2 in bone is complex and that the gas is involved in processes specific for the function of bone as an organ. If the CO_2 is used in the process of acidification by the hydration reaction, a net consumption of carbon dioxide can be the effect, with a negative RQ, i.e., a greater amount in the arterial blood than venous blood. This was not shown in the measurements on venous blood. The effect may be concealed by the bicarbonate exchange with the inorganic part of the bone. *In situ* registration of the partial pressure in the tissue will not enable any conclusions about the changes in transport from the cells. This requires estimation of A–V differences together with tissue registration.

Conclusion

The results of the present series of measurements of bone tissue oxygenation documented some new findings. It is possible to draw some conclusions from these series of experiments:

1. The data suggest that reliable informations about the intraosseous PO_2 and PCO_2 can be obtained with mass spectrometry with a catheter inlet. This method is useful both for clinical and experimental animal studies.

2. Changes in perfusion rate affect the values of intraosseous PCO_2 and PO_2 and a correlation exist between these values and blood flow. This allows the use of these parameters as indirect indicators of the quality of the bone perfusion.

3. Reduction of the oxygen supply of the epiphyseal end of long bones can be induced by specific perturbations. Hypoxia, a possible stimulus eliciting the response of focal necrosis and new bone formation was seen after: mechanical obstruction of the artery or veins; joint tamponade; and hypovolemia and failure of the microcirculation. Hypothetically, mechanisms leading to reduced regional perfusion and hypoxia may be involved in bone pathology and the mechanism documented here may all be active in clinically conditions.

4. Increased intraosseous pressure, hypoxia and increased lactate concentration are associated with advanced arthrosis, and hypoxia is associated with histological signs of necrosis in early arthrosis. This supported the opinion that a circulatory abnormality is a part of both diseases and may be involved in the pathogenesis.

5. Intraosseus hypertension, scintigraphical abnormalities and grossly/microscopically morphological changes occurs in parallel with a decrease in blood flow and local hypoxia. This indicates a causal relationship between the findings.

6. Decompression results in a decrease in intraosseous pressure and increase in oxygenation. This suggests a effect of this procedure, but a clinical effect could not be proved in the follow up study.

Suggestions for further research

The technique used in these studies was invasive as it was required to cannulate the bone. This technique has earlier been extensively used in measurements of intraosseus pressure and it has been shown that a hemorrhage is present around the tip of the cannula (Azuma 1964). This may produce some error in the registered data. It is also plausible that the trauma of introducing the cannula may elicit a vascular response with effect on the recordings. Until now, it has, however, not been possible to measure these parameters noninvasively, except for one study using tonometry (Wilkes and Wischer 1975). This method is not useful for clinical experiments. There exist, no method of noninvasive oxygen measurement of bone, and except for studies with microplatinium electrodes, no other *in situ* methods have been described. Until the technological development makes noninvasive methods useful, the present method with bone cannulation is the only way to obtain the measurements.

The further research in this field will depend on technological progress. It may be possible that further development in emission scanners detecting at the molecular level will allow noninvasive measurements. The use of MR scanners can supply information to be compared with these data. This can eliminate the need of flow measurements since the flux of metabolites may be determined directly.

In the laboratory, development of new cellular-techniques may allow direct measurement of the respiration of the cells. Data on this will be interesting to compare to other assays describing rate of resorption and formation of new bone. This may clarify the role of the respi-

ration in these processes. *In vitro* studies can also give further insight into the role of oxygen and carbon dioxide in the regulation of cellular determination, the pathway of CO_2 in bone and the role of this substance in homeostasis of pH and calcium.

Recent studies of soft tissues have shown that oxygen has a principal role in wound healing. Many aspects of hard tissue healing resemble the mechanisms employed in the healing of soft tissue. This field is thus open for research on the effect of oxygen as an inductor of the primitive mesenchymal response, the proliferation of vessels, the invasion of the living cells of bone and the role of the vascular response in the abnormal healing of bone, such as delayed healing or pseudarthrosis.

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