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Hemodynamics of the juvenile knee

Joint effusion and synovial inflammation studied in dogs

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ACTA ORTHOPAEDICA SCANDINAVICA SUPPLEMENTUM NO. 222, VOL. 58. 1987

MUNKSGAARD · COPENHAGEN

ISBN: 87-16-06376-7
ISSN: 0300-8827

Printed in Sweden
Studentlitteratur
Lund 1987

Index

For every complicated question there is a simple answer and its wrong.

H L Mencken

The present survey is based upon the following papers, which will be referred to by their roman numerals:

I Bünger C, Sörensen SS, Djurhuus JC and Lucht U: Intraosseous pressures in the knee in relation to simulated joint effusion, joint position, and venous obstruction. Scand J Rheumatology 10:283–288, 1981

II Bünger C, Harving S and Bünger E H. Intraosseous pressure in the patella in relation to simulated joint effusion and knee position. Acta Orthop Scand 53: 745–751, 1982

III Bünger C, Hjermind J and Bülow J. Haemodynamics of the juvenile knee in relation to increasing intraarticular pressure. Acta Orthop Scand 54: 80–87, 1983

IV Bünger C, Harving S, Hjermind J and Bünger EH. Relationship between intraosseous pressures and intraarticular pressure in arthritis of the knee. Acta Orthop Scand 54: 188–193, 1984

V Bünger C, Bünger E H, Harving S, Djurhuus J C and Myhre Jensen O. Growth disturbances in experimental juvenile arthritis of the dog knee. Clinical Rheumatology 3: 181–188, 1984

VI Bünger C, Bülow J, Hjermind J and Harving S. Hemodynamics of the juvenile dog knee in relation to increased venous outlet resistance. Pflügers Arch 399: 129–133, 1983

VII Bünger C. Regional blood flow and intraosseous pressure changes of the juvenile knee in experimental arthritis. In Bone Circulation (eds Arlet J, Ficat RP and Hungerford DS) Williams & Wilkins, Baltimore/London p 216–221, 1984

VIII Bünger C, Hjermind J, Bach P, Bünger E H and Myhre Jensen O. Haemodynamics in acute arthritis of the knee in puppies. Acta Orthop Scand 55: 197–202, 1984

IX Bünger C, Tøndevold E, Bülow J and Hjermind J. Microcirculation of the juvenile knee in chronic arthritis. Clin Orthop 204: 294–302, 1986

X Bünger C, Bülow J, Bach P and Sølund K. Blood supply of the juvenile knee in arthritis at rest and during exercise. Trans Orthop Res Soc 9: 44, 1984

Introduction

Thorough understanding of the function of the juvenile knee necessitates not only knowledge of the morphology, biochemistry, and biomechanics, but also comprehension of the knee as a physiological unit. The relationship between the metabolic milieu of the joint capsule, articular cartilage, and bone tissue plays an important role in this respect, and is decisive with regard to such well known functions as bone growth and haematopoiesis occurring parallel to demands for strength, stability, and movement.

Physiological thinking may thus provide a rational basis for research into pathophysiology, symptoms, and therapy of both traumatological, medical, and rheumatological joint diseases.

The anatomical background for the particular interest shown in the haemodynamics of the juvenile joint in the surveyed studies, lies in the separate blood supply of the epiphyses and its medullary separation from the metaphyseal vascular bed until growth ceases. Due to the intracapsular positioning of the epiphyses, as seen in the hip joint and to some extent in the knee joint, changes in joint pressure as well as synovial inflammation may influence the vascularization of the juxta-articular epiphyses.

The clinical impetus of the studies on which the present survey is based has primarily sprung from a wish to investigate pathogenetic mechanisms in the development of bone lesions in the knee joint as found in hemophilic arthropathy (HA) and juvenile rheumatoid arthritis (JRA). Despite a different aetiology, there are common features in the growth disturbances seen in HA and JRA, e.g. epiphyseal overgrowth, juxtaarticular osteoporosis, squaring of the patella, and altered longitudinal growth (Ahlberg 1979, Duthie et al. 1972, Goel et al. 1974, Arnold & Hilgartner 1977, Stein & Duthie 1981, Shapiro 1982). These bone changes are generally regarded as being due to inflammatory hyperaemia and disuse (Brattström 1963, Stein & Duthie 1981, Speer 1984).

Quantitative data on the haemodynamic changes in the knee following JRA and HA are not yet available, nor has the influence of the common pathogenetic factors, the chronic synovial inflammation, and the elevated joint pressure on the haemodynamics of the juxtaarticular bones been quantitatively investigated clinically or experimentally.

Until recently, knowledge of the regulation of blood perfusion of the knee joint, both in man and animals, has been confined to intraosseous pressure measurements (Arnoldi & Reimann 1980) and evaluation of the synovial blood blood flow by means of the 133-Xe clearance technique (St Onge et al. 1971).

A number of clinical and experimental studies have focused on the impact of elevated hip joint pressure on the haemodynamics of the femoral head epiphysis in relation to Perthes' disease, pyogenic arthritis and fractures of the femoral neck in children (Tachjian & Grana 1968, Woodhouse 1970, Launder et al. 1981, Sutherland et al. 1980, Lucht et al. 1983, Wingstrand 1986). The quantitative results of these studies cannot be transferred directly to the knee owing to differences in the anatomical relationship between joint capsule and blood supply of epiphysis.

The first step in the study of bone lesions of the knee in JRA and HA, taken in 1980, focused on the haemodynamics of the juxta-articular tissues, in particular the influence of increased intra-articular pressure and chronic synovial inflammation. After developing a method permitting simultaneous multiple intraosseous pressure measurements in the normal immature dog knee, it was considered expedient to investigate the following aspects (I, II):

- The juxta-articular intraosseous pressure and its change during increasing joint pressure.
- The juxta-articular intraosseous pressure reaction to segmental inhibition of the venous drainage.
- The impact of changing joint position on the juxta-articular intraosseous pressure.

After further refinement of the model for simultaneous regional blood flow (RBF) and intraosseous pressure (IOP) measurement (III), it was endeavoured also to analyse:

- The influence of IOP measurements on the RBF in the juxta-articular bones.
- The relationship between IOP and RBF in steady state and during change of joint pressure.
- The effect of knee joint tamponade on RBF of the extremity as a whole.

Evidence of regulatory mechanisms of the knee blood perfusion during tamponade was revealed in III, which called for the design of a new "acute" model (VI), in which it was possible selectively to increase the venous drainage resistance of the distal femoral epiphysis. Employing this model we studied:

- Regulatory mechanisms of RBF in the distal femoral epiphysis in relation to isolated increase of venous outlet resistance.

Pathological conditions may alter the regulation of the individual organ blood perfusion (Nielsen 1984). The design of an arthropathy model was therefore imperative in order to study the haemodynamics in arthritis under various pathophysiological loadings. Importance was attached to the presence of chronic synovial imflammation and elevated resting joint cavity pressure, which was achieved by Carrageenan-induced arthritis (V). The resultant growth disturbances of the knee bear resemblance to those seen in JRA and HA. The primary studies in the arthropathy model (IV, VII) focused on:

- The regional changes in IOP and blood flow in arthritis.
- The impact of changing joint pressure on the haemodynamics of the arthritic joint.

As qualitative changes of joint vasculature were found following chronic arthritis, a supplementary study of:

- The haemodynamic changes in acute arthritis.

was undertaken (VIII) to determine whether these qualitative changes were a late or an early event. Thereafter simultaneous measurement of RBF and estimation of microvascular volumes (IX) permitted analysis of:

- Microcirculatory changes of juxta-articular tissues in chronic arthritis.

Finally a study in awake non-medicated, but chronically instrumented dogs was conducted (X) to examine:

- Regional blood flow of the immature arthritic knee at rest, during and after exercise.

The following survey, which is based on the above previously published papers, gives a description of the embryology and development of the knee joint, its vascular anatomy and growth patterns with the prime purpose of assisting the understanding of the physiology and pathophysiology of the immature knee here reported. Similarly a brief survey of the literature on the vascular contribution to growth is presented.

I Biological background

A. The embryology of the knee joint

Chondrification and cavitation

The individual constituent of the skeleton begins as mesenchymal condensations during the embryonic period (Gray & Gardner 1950).

Some of these condensations ossify directly to form the membrane derived of intramembranous bones like cranial and facial skeleton. However, most of the postcranial skeleton is derived from a cartilage model, transformed from a mesenchymal frame. During this chondrification the mesenchymal tissue at the sites of future joints remains as homogenous interzones, but contributes by appositional growth to the increase in length and width of the cartilaginous elements. Three layers of cells can be identified, two chondrogenic layers and an intermediate less dense layer. The primitive joint capsule begins to form at the peripheral portion of the interzone from the interface of the intermediate and deep mesoderm (Andersen 1964)(Fig. 1.1), which gives rise to the perichondrium and periosteum. Blood vessels penetrate the peripherally evolving joint capsule, but do not penetrate the central future joint regions. Menisci and cruciate ligaments appear as further joint cellular condensations in the intermediate mesenchymal layer between the condrogenic zones.

Minute spaces now begin to appear in the intermediate zone. These spaces coalesce and form the joint cavity (Fig. 1.2). Cavitation may be an enzymatic process (Wassiley 1972). By the 10th week of gestation (31 mm embryo) cavitation is ended, and the knee joint spaces are firmly established (Bauman & Lahlaidi 1973).

Joints are relatively self-differentiating structures that can attain their basic form even when grown in culture, provided small amounts of adjacent mesenchymal skeleton are included in the explant (Ogden 1980)

Formation of primary ossification center

The primary ossification center in the major long bones is established towards the end of the second gestational month, prior to which a midshaft narrowing with perichondral formation of a primary bone collar has taken place. Within this bone collar a capillary network develops, and replacement of cartilage by bone is initiated. Several vessels

may enter and initiate ossification, but one will usually become dominant giving rise to the nutrient artery. The sequential replacement of cartilage by bone, i.e. the endochondral ossification, progresses rapidly towards each end of bone, while new cartilage formations also occur at approximately the same rate for each end (Fig 1.3). Significant differences in rate of growth for each epiphysis and physis apparently only arise postnatally (Ogden 1980).

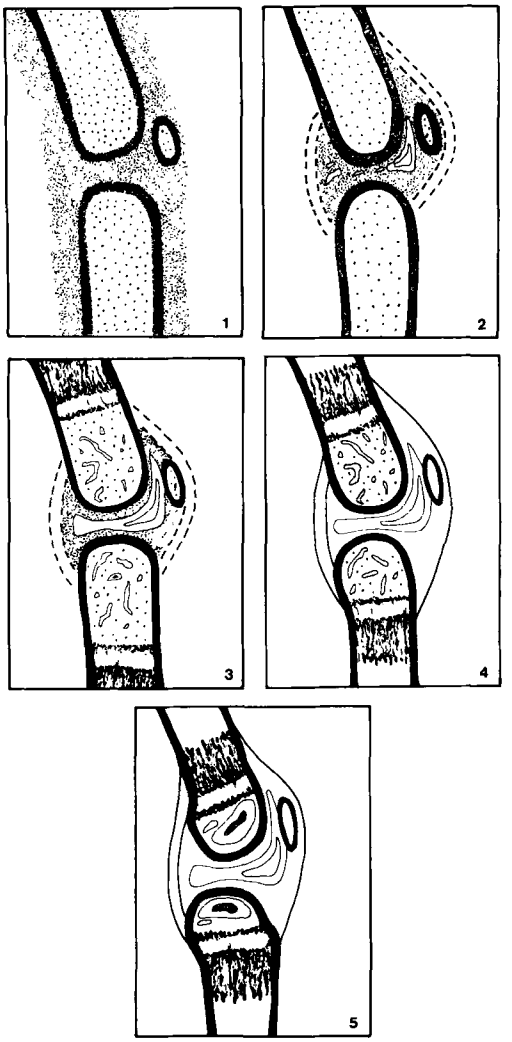


Figure 1. Diagram of the development of the human knee joint. 1. Embryonic period at 7 weeks (21 mm stage): Chondrification of the mesenchymal anlage has occurred. The future joint space remains as a homogenous interzone of mesenchyme. 2. Embryonic period at 8 weeks (31 mm stage): Cavitation, formation of joint capsule and synovial membrane begin at about the same time. 3 and 4. Fetal period second to fourth gestational month (from 130 mm): The cartilage canals containing vessels are present, the primary ossification centers are developed and the endochondral ossification progresses towards the epiphyses. 5. Postnatal period at 1 year. Secondary ossification centers are developing.

Early in the fourth gestational month, the cartilage at the ends of bone begins to resemble growth plates (Fig 1.4). Concomitantly with the extension of the primary ossification center to the cartilaginous ends, the primary periosteal collar also progresses towards the epiphyses, but stops at the growth plate giving rise to the development of the zone of Ranvier (Ranvier 1875).

As the bone enlarges, an extensive remodelling is taking place in the metaphyseal region. Bone is removed from the peripheral cortical surface, and new bone is added to the endosteal surface. Late prenatally, the woven bone begins to be replaced by lamellar bone.

Formation of secondary ossification center

Early postnatally, the secondary ossification centers begin to form. Initially, vessels enter and are distributed to the chondroepiphysis within specialized structures termed cartilage canals, which course throughout the epiphysis and occasionally communicate with the metaphyseal circulation in particular through the peripheral parts of the growth plate. Initially, the cartilage canals supply discrete regions within the epiphyses with largely no intra-epiphyseal anastomoses (Haines 1933, Trueta 1968, Wilsmann & Van Sickle 1970). The canals which contain both artery and veins (Teot et al 1982) serve main functions as internal structural support, source of chondroblastic cells for the continued enlargement of the epiphyses and are of main importance in the genesis of the secondary ossification center (Ogden 1980). By the development of the secondary ossification center, more anastomosing vascular patterns appear, but the gross intraosseous vascular pattern remains unchanged, even throughout life (Brookes 1971).

The secondary ossification centers of the human knee are fully developed by the age of 2 years. In the dog they appear during the first postnatal month and are fully developed at the age of 3 months (Hare 1961, Chapman 1965).

B. The vascular anatomy of the immature knee

In 1743 William Hunter in his classic paper wrote, "All around the neck of bone there is a great number of arteries and veins, which ramify into smaller branches and communicate with one and another by frequent anastomoses like those of the mesentery. He designed this the "Circulus Articuli Vasculosus, the Vascular Border of the Joint". Since then a number of authors have thoroughly studied the vasculature of the immature knee (Gardner 1954, Trueta & Morgan 1960, Crock 1967, Scapinelli 1968, Brookes 1971, Teot et al. 1982).

Metaphyses

Three principal routes of blood supply are found in the metaphyses: 1. the nutrient artery, 2. the metaphyseal penetrating arteries, and 3. the perichondro-osseous arteries.

The nutrient artery supplies central areas of the metaphyses. Peripheral branches give off small vessels to the inner aspects of both cancellous and cortical bone of the metaphyses. Central longitudinal branches reach the growth plate in an arborising vascular pattern.

A considerable number of *metaphyseal penetrating vessels* deriving from branches of the supreme genicular artery and the superior genicular arteries also contribute to the nourishment of the distal femoral metaphysis and the growth plate. In the proximal tibial metaphysis, the supplying vessels mainly originate from the middle and inferior genicular arteries and from the posterior and anterior recurrent tibial arteries. Terminal branches from these vessels and the nutrient artery pass vertically towards the bone-cartilage junction of the growth plate and end in vascular loops or capillary tufts just below the last intact transverse septum at the base of the cartilaginous portion of the plate (Brighton 1978). It is believed that the nutrient artery supplies as much as four-fifths of the metaphyses and mainly the central regions.

The most peripheral regions of the metaphyses are supplied by the *perichondro-osseous arteries*, which also originate from the genicular arteries and form an intercommunicating vascular network. They supply the fibrous peripheral structures of the growth plate, the groove of Ranvier, and the perichondral ring of Lacroix (1951).

There have been some points of disagreement with respect to the *venous drainage*. Nelson et al.(1960) observed that the veins follow the arteries and are equal in number. Tilling (1958), on the other hand, found that veins leave bone through different foramina than arteries. Brookes (1971) and Post and Shoemaker (1964) have pointed out that the venous outflow canals are more numerous than the entering arterial vessels. On intraosseous phlebographies in dogs we found the venous drainage to take place through the same main routes as the arterial supply. A large calibered medullary venous sinus was always visualized along with multiple perforating metaphyseal veins in 8–12 week old puppies (Fig. 2) (I). The major venous drainage of the metaphyses takes place through the latter vessels in older dogs (Post & Shoemaker 1964). Around the metaphyseal regions in a growing bone they form a "veritable spongy work of canals" (Morgan 1959), which is intimately anastomosing and draining into both the superficial and deep veins of the limb.

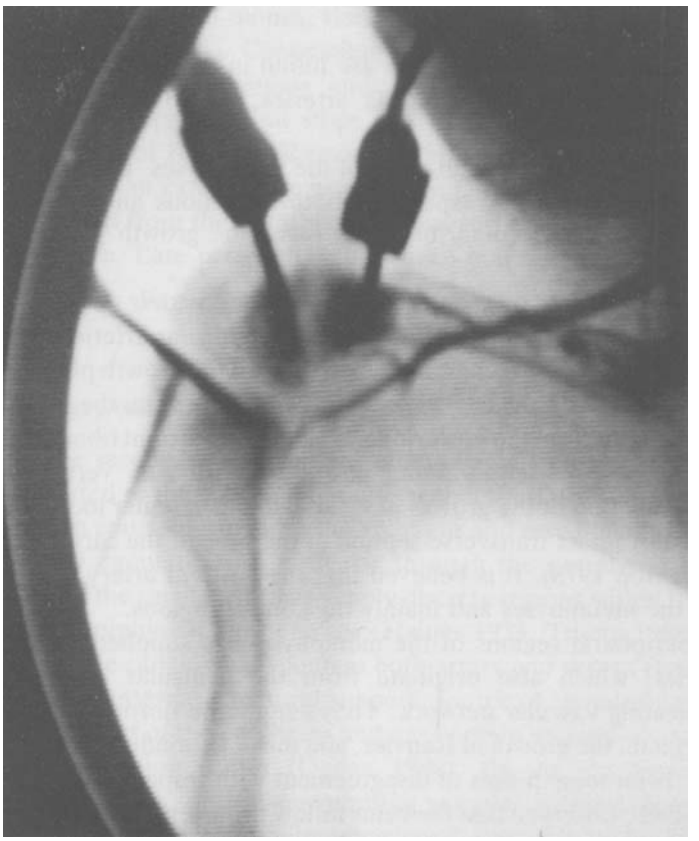


Figure 2. Image intensifier visualization of the knee of an 8-week puppy. Pressure recording cannulae are situated in the epiphyses of the knee and in the distal femoral metaphysis. X-ray contrast injection into the bone through the cannulae shows the main venous drainage. A medullary sinus drain the metaphyseal bone compartment.

Epiphyses

The femoral and tibial epiphyses of the knee receive blood from three groups of arteries: 1. the juxta-metaphyseal arteries, 2. the intercondylar arteries, and 3. condylar arteries.

Extraepiphyseal blood supply

In the neonate the developing secondary ossification centers of the knee are mainly supplied by the juxta-metaphyseal arteries and the intercondylar arteries from the middle genicular artery. *The intercondylar arteries* penetrate the posterior joint capsule, have an intracapsular course, and give off branches to the cruciate ligaments

and to the fat pad, where it anastomoses with branches of the inferior genicular artery. The site of entrance into both epiphyses is closely related to the cruciate ligaments. The *juxta-metaphyseal arteries* reach the epiphyses through the anastomosing perichondro-osseous vascular network. The third group of supplying vessels, the *condylar arteries* are branches of all the genicular arteries except the middle genicular artery. In the newborn they converge radially towards the nucleus of the secondary ossification center through the cartilage canals. By the development of the center these arteries form an intraepiphyseal anastomosing vascular pattern, but are initially independent reaching the epiphyses next to the capsular attachments.

Intraepiphyseal blood supply

The epiphyseal blood supply of *the growth plate* is characterized by small arterial branches derived from intraepiphyseal arteries, which arborize in a rake-like fashion to supply the proliferative zone of the growth plate. However, none of the arterial branches from the epiphyseal vessels penetrate the cartilage portion of the growth plate beyond the uppermost part of the proliferative zone (Brighton 1978), leaving the hypertrophic zone of the growth plate avascular.

The blood supply supply of *the subchondral bone*, where the appositional endochondral bone growth takes place, is also characterized by fine arterial terminals radiating towards the subchondral bone plate ending in sinusoids, which are quite regular in caliber and empty into larger anastomosing venous sinuses. These sinuses drain into the circulus articuli vasculosus close to the articular margin (Crock 1967).

The microvascular bed of the epiphysis is supplied by arterioles, which are muscle coated vessels, capable of being resistance vessels (Yoffey 1962). These vessels suddenly loose their muscle coat and continue as thin walled vessels, capillaries which consist of only endothelial and adventitial layers, before they empty into venous sinusoids. By vital microscopy Branemark (1959) was able to improve the understanding of the microvascular bed of bone. In the living rabbit fibula he observed rhythmic changes of volume and flow of the sinusoids, which generally emptied into collecting venules, but sometimes the sinusoids were by-passed by shunts, indicating a possible presence of resting capillaries.

The lack of muscular coats of the sinusoids, venules and the veins is a significant anatomical characteristic of bone marrow vasculature (Nelson et al. 1960, Burkhardt 1962, Yoffey 1965, Brookes 1971). Yoffey (1965) found the walls of sinusoids and venules to be identical, composed of a single endothelial layer. Even larger intraosseous veins such as the nutrient vein have no muscle coat and are only two or three cell-layers thick. The lack of muscle coat on intraosseous veins is of physiological significance as bone arteries and arterioles are the only intraosseous vessels that can actively regulate blood flow. However, the absence of vein valves in intraosseous veins (Brookes 1971) also implies that the microvascular bed of bone is very vulnerable to changes in extraosseous venous resistance.

At the ultrastructural level the capillary bed is believed to be open (Brookes 1971). By electronmicroscopy approx. 60 Å sized spaces have been demonstrated at the cell

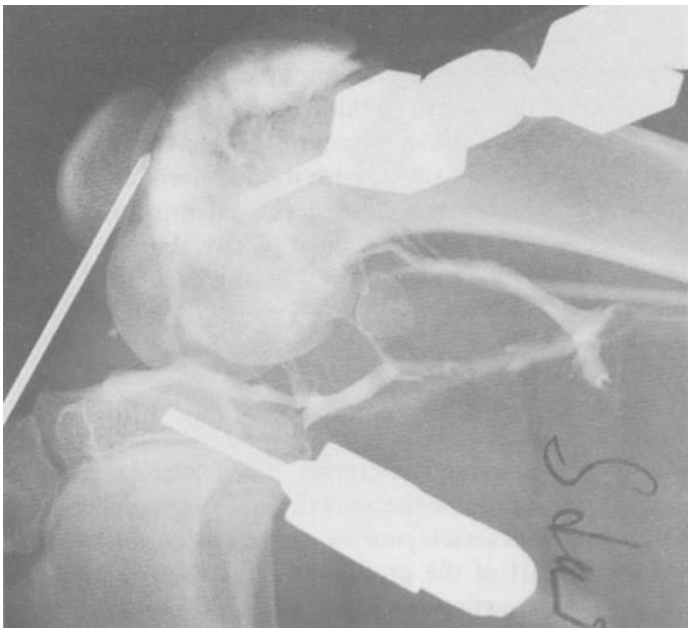


Figure 3. Intraosseous phlebography in the distal femoral epiphysis. Multiple effluent sites are present. The main drainage takes place into the popliteal and distal femoral veins.

borders of the endothelial cells. A third characteristic feature of the capillary and venous vascular bed of bone is its very large capacity compared with that of the arterial vascular bed (Brookes 1971).

The venous drainage of the epiphyses of the knee largely follows the arterial supply. The larger intraepiphyseal veins, the so-called radiate veins, converge towards the fossa intercondylaris, where the main venous drainage of the distal femoral epiphysis is located. Also, the intraepiphyseal veins form an anastomosing network which presents as multiple venous effluent sites following intraosseous phlebography (Fig 3). The draining veins in the intercondylar posterior part of the knee, empty either directly into the popliteal vein or indirectly via larger veins. A particular branching pattern is composed by the epiphysio-metaphyseal veins, which drain into the femoral vein at the level of the adductor canal.

Dissection of the plastic-embedded veins showed an intimate relationship of the draining veins to the joint capsule. The larger intercondylar veins penetrated the posterior capsule. Both superior and inferior major veins were located in the capsule on both sides of the joint and followed the superior and inferior genicular arteries. Even the recurrent tibial veins were visualized on phlebograms from the distal femoral epiphysis. This confirms the presence of an intimate anastomosing venous drainage system around the knee joint, but may also be a result of retrograde filling of the vessels. However, the abundant filling of tiny veins in the joint capsule supports the concept of an anastomosing venous drainage.

An important venous drainage is that of the subchondral bone of the articular surfaces. As pointed out by Crock (1967) and Jaffe (1929), the subarticular collecting veins drain off around the margins of the articular cartilage, merging into collecting trunks, which join the lowest radiate veins as they pass through to the surface of the posterior part of the lower femur. However, on the medial and lateral aspect of the joint they join the perichondro-osseous veins close to the capsular attachment and may as such be influenced by changes in intra-articular pressure as the majority of the femoral epiphyseal effluent. As demonstrated in Fig 4, only a small part of the distal femoral epiphysis is extra-articularly located. On the medial and lateral side of the knee, the capsular attachment is located anteriorly on the epiphysis. The perichondro-osseous vasculature and emerging vessels are therefore located extra-articularly at this site.

The main drainage from the proximal tibial epiphysis passes through the recurrent tibial veins and through branches of the medial genicular veins, some of which are intra-articularly located, in particular in relation to the ligamentous attachment at the eminentia intercondyloidea. However, the attachment of the knee joint capsule does not pass beyond the proximal tibial growth plate.

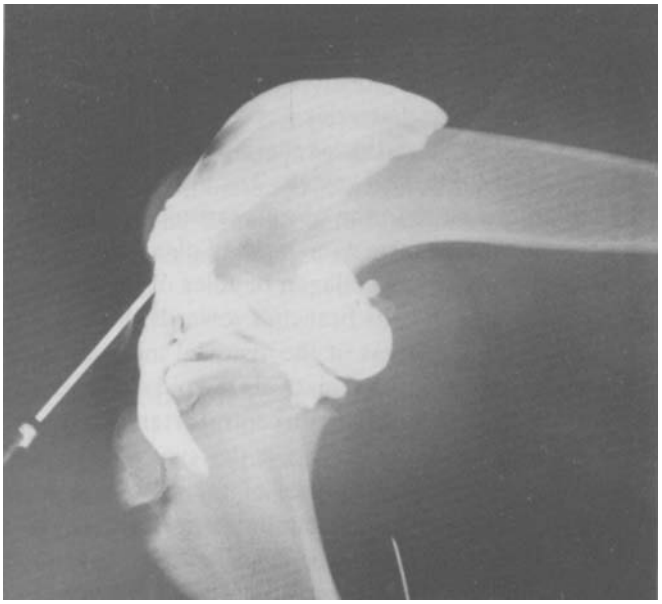


Figure 4. Arthrogram of the immature dog knee. The distal femoral epiphysis is almost enveloped by the large femoro-patellar and popliteal sacs.

Patella

The arterial supply of the patella has been described in detail (Crock 1967, Scapinelli 1967, Björkström & Goldie 1980). The cartilage anlage of the patella is supplied by

two groups of arteries. One midpatellar group enters the central anterior surface and one group enters the deep surface of the lower pole. A dual supply of the lower half of the patella is thus provided leaving the upper half more susceptible to ischaemia in the case of fracture (Scapinelli 1967).

The venous drainage largely parallels the arterial supply (II). The most prominent venous drainage took place through the lower pole behind the inferior patellar ligament. A circumferential venous system was consistently found around the patella, supplied by radiating veins, which emerge through the anterior patellar surface. The peripatellar network emptied mainly through the genicular veins.

Joint capsule

The sources of origin of the *arterial blood supply* are multiple. Vessels from all the genicular arteries divide and anastomose freely to form a complex network around the joint between the capsule and the synovial membrane at their attachment to the epiphyseal surface (Hunter 1743, Davies & Edwards 1948, Gardner 1960, Scapinelli 1968, Brookes 1971). From this network branches arise to supply ligaments, tendons, and menisci.

The microvascular bed of the synovial membrane is complex and reflects its complicated function, which has latest been reviewed by McCarty (1980) and Simkin & Nilson (1981). The larger synovial arteries directly enter the synovial membrane in the base of the fat pads and at the articular margins, where they communicate with the perichondro-osseous and epiphyseal vessels. The larger synovial arteries run in the deeper layer of the membrane close to the fibrous capsule and parallel to the synovial surface. At irregular intervals they form wide-meshed plexuses, from which secondary plexuses are formed, located between collagen bundles of the more superficial layers of the membrane. This plexus sends its branches towards the synovial lining.

According to Scapinelli (1968), areas of the synovial membrane, subjected to the highest degrees of mechanical forces, are relatively devoid of vessels. The synovial villi and fringes are all incompletely penetrated by a central arteriole ending in a convoluted loop, thus extending the surface area between the synovial capillary bed and the synovial surface. Electron microscope studies of the synovium have shown spaces between synovial lining cells that freely communicate with the joint space (Barland et al. 1962, Schumacher 1969, Dryll et al. 1977). The subsynovial capillaries appear to be fenestrated. The anatomical barrier of molecules between blood and synovial fluid is thus determined by the endothelial pore size and the transport or diffusion through the interstitial space between the synovial lining cells.

The venous drainage takes place through a subsynovial anastomosing net of veins generally more numerous and voluminous than the arteries. Valves occur frequently in all, even in the smallest veins, and thus provide the organ with an excellent transport mechanism, which is facilitated by cyclic changes of tension and pressure as seen during continuous passive joint motion (O'Driscoll et al. 1983).

The possible presence of *arteriovenous shunts* constitutes a particular feature of the joint capsule vasculature (Gardner 1954, Clara 1956, Scapinelli 1968). Located at the

junction between capsule and bone they have been suggested to play a major role in the redistribution of blood between capsule, synovium, and epiphyseal bone. However, quantitative evidence of a-v-shunts has so far only been documented in the skin and coronary heart vasculature (Marschall et al. 1979, Foldes & Hales 1980).

C. Growth patterns of the knee

Traditionally, the growth of long bone is considered to be longitudinal. However, enlargement and growth of the metaphyses and epiphyses also require expansion in diametric or latitudinal direction (Rang 1969, Ogden 1980).

Within the epiphyses of the knee the latitudinal growth is provided by endochondral bone apposition. However, the major contribution to latitudinal growth of the epiphyses is from the perichondral ring, the zone of Ranvier, where a cellular apposition governs the widening of the growth plate (Lacroix 1951, Shapiro et al. 1977). The apparent progressive decrease of the metaphyseal diameter towards the diaphysis, requires considerable remodelling of the primary metaphyseal woven bone. At the metaphyses the peripheral primary spongiosa is resorped together with periostal bone deposition and endosteal bone resorption. The periosteal bone typically contains longitudinal lamellae. Towards the diaphysis the latitudinal growth is accomplished by periosteal apposition and endosteal bone resorption (Ogden 1980).

The longitudinal growth of the knee provided by the tibial and femoral growth plate is endochondral and dependent on the production of chondrocytes in the proliferating zone of the growth plate. The structure and function of the growth plate have been studied in detail and most recently reviewed by Brighton (1978).

D. Vascular contribution to growth

The control of bone growth is complex being influenced by both genetic, nutritional, metabolic, hormonal, neurogenic, physical, and mechanical factors. The vascular supply is of fundamental importance for bone growth and bone homeostasis, whereas the cartilage is less vulnerable to disturbances in blood flow (Brookes 1971, Ray 1972).

The primary function of the bone vasculature is to serve as transport route for nutritional, metabolic, and hormonal compounds necessary for bone growth, bone homeostasis, and haematopoisis. Nevertheless, both immature and mature bone is capable of differentiation and growth in itself as evidenced by tissue culture techniques (Brighton 1978) and isolation of the bone morphogenic protein (BMP) from bone matrix (Urist 1979).

Observations on parabiosed rats (Buring 1975) have affirmed the theory that preosteoclasts and osteoclasts are derived from the monocyte-macrophage cell lines. The transport of these precursor cells to the sites of remodelling is another function of bone vasculature. Transplantation experiments have evidenced that the bone

resorption process itself not only requires presence of living osteoclasts, but also depends on revascularization, which probably supplies the activating substances such as parathyroid hormone and monocytes, which produce osteoclast activating factor (Yoneda & Mondy, 1979).

The vascular density may thus to some degree reflect the metabolic demands and bone turn over (Whiteside et al. 1977, Schnitzer et al. 1983).

By experimental reduction of the blood supply to either side of the epiphyseal cartilage, Trueta and Amato (1960), in their classical paper showed that the only source of nourishment of the growth cartilage was the supply from the epiphyseal vessels, whereas the metaphyseal blood supply was necessary to obtain disintegration of cartilage cells of the growth plate and to obtain calcification.

Indirect evidence of vascular contribution to growth could be expected in pathological conditions. Impairment of vascular supply may lead to necrosis or segmentation of the distal femoral epiphysis, resulting in severe deformity of the knee (Ahlback et al. 1968). Osteochondritis dissecans might be a result of vascular occlusion of the subchondral arcuate arteries. Osteomyelitis or trauma of the distal femur or proximal tibia may lead to increased length of the affected leg, whereas fractures such as Salter-Harris type IV (Salter & Harris, 1963) may cause segmental growth inhibition secondary to ischaemic lesions of the growth plate.

Congenital and traumatic arterio-venous fistulae is a common cause of overgrowth of the bones distal to it (Ejrup & Hierton 1961, Goidanich & Campanacci 1963). The overgrowth is in particular latitudinal but may also be longitudinal with increased cortical thickness. These growth disturbances have been reproduced experimentally by anastomosing the femoral artery to the femoral vein (Keck & Kelly 1965, Vanderhoeft et al. 1963). The growth changes were most pronounced in the tibiae and metatarsals. Initially, the haemodynamic changes of bone showed depression of blood flow distal to the fistulae, but after some weeks slight hyperaemia was observed by ^{85}Sr clearance technique (Weinmann et al. 1964), whereas flow in skin and muscles was significantly increased in chronic fistulae after 4–6 months duration (Rogers et al. 1963). The arteriovenous oxygen saturation difference was found to be increased distal to the fistulae after 1–12 weeks (Kelly 1966).

The influence of stasis alone following long term tourniques (Hutchison & Burdeaux 1954) on immature bone was also significant producing increased notably latitudinal growth.

It is difficult to evaluate the actual stimulative compound on bone growth from these studies, as simultaneous pressure and blood flow measurements were not performed. Furthermore different observation periods following creation of the fistulae have been employed to evaluate a biologic phenomenon where considerable changes related to time can be expected.

The origin of growth stimulation may be different in respect of periosteal apposition and endochondral growth. It could be suggested that the relatively low pO_2 and/or increased pCO_2 in the microvascular bed had a stimulatory effect on osteogenesis as found in in vitro studies (Wilmer 1965). Other metabolic factors, e.g. PG's and tromboxanes, could also be involved. Changes of the bone perfusion surface area and

its permeability, as found during fracture healing (Paradis & Kelly 1975) and during electrical stimulation may play a role too (Nannmark et al. 1984).

Medullary plugging i.e. application of various materials in the medullary canal is also known to stimulate bone growth, in particular endochondral growth (Trueta 1953, Langenskiöld 1957, Taillart & Morscher 1965). The cause of this growth increase was believed to be due to an increase in metaphyseal blood supply to the marrow cavity. However, in rabbits Hansson (1967) found that during plugging of the proximal part of the medullary canal, leaving the distal vessels intact, the growth took place in the distal epiphysis and not in the proximal epiphysis. Thus, it was concluded that the stimulatory effect most likely was the result of increased blood flow in the epiphyseal, metaphyseal and perichondral vessels of the distal tibiae. However, non-invasive measurements of the influence of marrow plugging on the haemodynamics of bone has not yet been published.

Other growth stimulatory procedures believed to act via hyperaemia are *periosteal stripping* (Langenskiöld 1957, Sola et al. 1963) and induction of diaphyseal fractures (Wray & Goodman 1961, Hansson 1967). The latter authors also considered that liberation of growth stimulating substances played a role. However, neither of the theories have been experimentally supported (Shim 1968, Nickodem et al. 1984). An appropriate explanation is that the increased blood flow and hypervascularity observed in relation to enhanced bone growth or bone healing, is merely secondary to increased metabolism.

Whether increased blood flow alone can induce an increased formation of bone seems still to be an open question. Procedures such as internal and external heating have been reported to increase bone blood flow (Shoutens 1979) and stimulate bone growth in the rat and dog (Richards & Stofer 1959). However, growth and blood flow were not measured simultaneously. A number of pathological conditions such as Paget's disease and algodystrophia are not only related to increased bone blood flow, but also to increased bone resorption (Driessens 1984).

Thus the above mentioned studies have predominantly provided descriptive data, which does not allow a conclusion framework for predicting the actual growth stimulating compound following changed haemodynamics.

A number of *inflammatory joint diseases* in childhood, such as hemophilic arthropathy, juvenile chronic arthritis, tuberculous synovitis of the knee, and septic arthritis, result in increase leg length and epiphyseal enlargement (Schapiro 1982). Hyperaemia of joint and adjacent tissues has generally been conceived as a causative factor in these growth changes (Brattström 1963, Arnold & Hilgartner 1979, Stein & Duthie 1981, Speer 1984). However, quantitative data on blood flow or other haemodynamic changes in these juvenile arthropathies have not so far been reported. The experimental evidence of the surveyed studies and discussions in relation to clinical experience shall be dealt with in detail in chapters II and V.

II Methodological considerations

A. Choice of experimental animal

A canine model was used (I-X), because haemodynamic investigations utilizing the tracer microsphere technique with chronic instrumentation (X) and intraosseous pressure registration in juxta-articular epiphyses (I, II, III, IV, VI, VII) demand a certain size of the experimental animal as well as good cooperation. Animals with a major growth potential such as Labrador breeds were preferred to facilitate growth studies (V). Furthermore the dog has a chondroosseous structure and circulation which is close to the human (Rhinelander 1976, Eitel et al. 1981), and the circulation in the adult and immature dog bone is well elucidated (Morris & Kelly 1980, Schnitzer et al. 1982, Tøndevold 1983). In order to minimize interindividual variation, puppies from the same litter were used ignoring sex differences.

B. Haemodynamic parameters

Intraosseous pressure registration

Schultze and Behau (1912) and Rothmann (1913) were the first to report measurements of intraosseous pressure (IOP) in the dog femur. They found an IOP between 2.7 and 4.0 kPa. A number of authors have later discussed the methodological problems in IOP registration (Larsen 1938, Herzig & Root 1959, Azuma 1964, Michelsen 1967, Wilkes 1969, Polster 1970, Lemperg & Arnoldi 1978, Tøndevold 1983). The sources of error so far recognized vary with animal age and size, the site of measurement and the pressure registration technique.

The physiological intraosseous pressure level is determined by the perfusion pressure, the resistance in arterial and capillary bed, the net capillary filtration, possible lymphatic drainage, and the venous resistance. Lymphatic drainage in bone has not been demonstrated, and some simple equations may therefore serve to describe the IOP provided the Hagen Poiseuille Law is valid in bone.

Under normal circumstances the arterial influx

$$Q_a = \frac{P_a - IOP}{R_a}$$

equals the venous outflux:

$$Q_v = \frac{IOP - P_v}{R_v}$$

where P_a and P_v are the arterial pressure and the draining vein pressure, respectively, and R_a and R_v the arterial and venous resistances. As calculated in (VI), and as a logical consequence of the lack of muscular coats in intraosseous veins, the arterial inlet resistance is much greater than the venous outlet resistance, i.e. $R_a >> R_v$ at rest.

It can also be derived from the equations that evaluation of IOP levels necessitates monitoring of the systemic pressures as well as the central venous pressure. The parallelity between blood flow and IOP, which has been found in the bone marrow (Held & Thron 1962, Shaw 1963, Azuma 1964), is dependent on a constant R_v. Thus, the use of IOP alone as an estimate of bone blood flow may be feasible, but requires monitoring of other parameters. The relationship between pressure and flow is probably also dependent on the anatomical location considered, and it may change profoundly in pathological bone conditions.

Technical problems related to site of IOP measurement

Substantial interindividual variation in IOP even in the same species has been reported (Polster 1970), data which seriously question the methodological reliability. The greatest variations in pressure are found in the IOP of the medullary canal of the diaphyses (Stein et al. 1964). Following introduction of a pressure recording cannula into the marrow, lesion of a larger artery may alter the arterial inlet resistance. The subsequent haemorrhage, which more or less always is present around the tip of the cannula (Azuma 1964), may compress the draining veins. This effect is enhanced by the incompressibility of bone and may thereby create an artificially high IOP. IOP measurement may also encounter problems such as leakage around the cannulae leading to a false low IOP. Various procedures such as the use of threaded cannulae (Polster 1970), conical cannulae (Lemperg & Arnoldi 1978) and dental cement (Azuma 1964) have been applied in an effort to counteract leakage. Wilkes and Wisscher (1975) avoided bone marrow cannulation by developing a tonometric technique, by which they removed the cortical bone and left the endosteal membrane intact. However, this method is rather time consuming, has a 50% failure rate and cannot be used in cancellous bone.

Due to the homogeneous structure of normal immature bones adjacent to joints and their increased deformeability, the pressure measured here by cannulae would not necessarily meet the same problems.

Pressure measurements in our studies were performed in epiphyseal, metaphyseal, and patellar bone. In very young puppies (I) the cannulae could be pushed by hand into the desired location. In older puppies we used a light hammer, but probably due to the high deformeability of immature bone (Torzilli et al. 1982), leakages never appeared provided the selfcarrying Radner cannulae were driven more than 0.5 cm into the bone in order to obtain sufficient support. A uniform position of the measuring cannulae tips in the different series was secured by fluoroscopy. All knees were transcutaneously cannulated from the medial side (Fig. 5). The femoral metaphyseal cannulae were placed 1 cm proximal to the epiphyseal plate. The femoral metaphyseal cannulae were located 0.5 cm distal to the growth plate midsagittally and the tibial epiphyseal cannulae in the middle of the epiphysis. Tibial location was sometimes hampered due to the narrow compartment. After introduction of the cannulae the correct nonobstructed position of the 1.5 mm inner diameter cannulae was checked by a nonresistant infusion of a few ml heparinized saline (I). An obstructed intraosseous cannula was never withdrawn, but was rotated slightly, which often brought the cannula tip into a nonobstructed position. Clotting at the measuring sites was prevented by a low constant rate perfusion of 3.75 to 5 ml heparinized saline per hour (I).

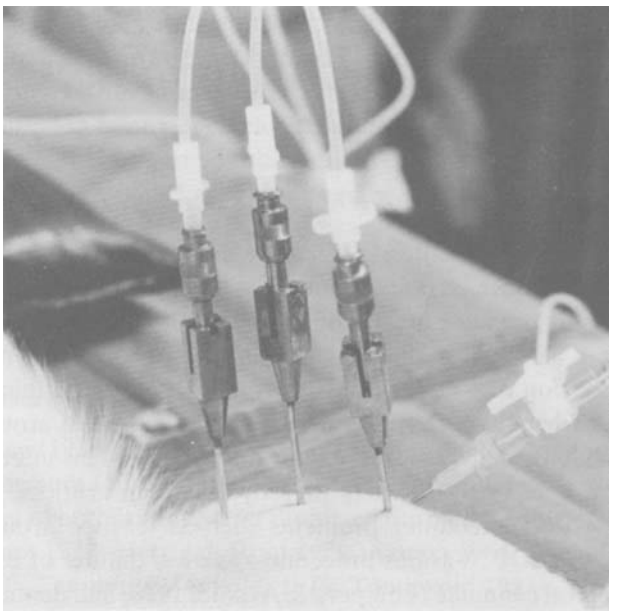


Figure 5. Pressure recording cannulae transcutaneously placed in the juxtaarticular bones of the knee. The cannulae are connected to liquid-filled manometer tubes.

The atmospheric pressure at the heart level was chosen as reference zero pressure. The correlation between pressure units used was:

1 mmHg = 133 Pascal (Pa) = 13.6 mm H_2O

the limbs were investigated in randomized order in (I, II, IV), whereas simultaneous pressure measurements in right and left limbs were performed in VI and VIII. The

latter is preferable when right and left measurements are to be compared. Though central haemodynamics are kept constant, a change in local vascular reactivity due to different anaesthetic levels may increase the variation between right and left values measured consecutively.

Technical problems related to pressure recording system

Static and dynamic characteristics

An electromanometric pressure recording system (I) was applied incorporating transduction of hydrodynamic pressure into electric signals by means of strain gauges. The pressure components recorded were both fast, i.e. pulsatile, and slow, i.e. baseline IOP during 2 to 4 hours. This required certain dynamic and static characteristics of the measurement system. The *static characteristics* are largely determined by the transducer quality. The zero drift at a constant temperature for a Siemens 746 strain gauge transducer is less than 6.65 Pa per 24 h (Bünger & Hjermind, unpublished data). The temperature stability is better than 0.03 kPa per C°. The relationship between the pressure and the electric signal was found to be close to linear deviating less than 1% from – 1.3 kPa to 39.9 kPa (manufacturer's data), which was well beyond the pressure ranges recorded.

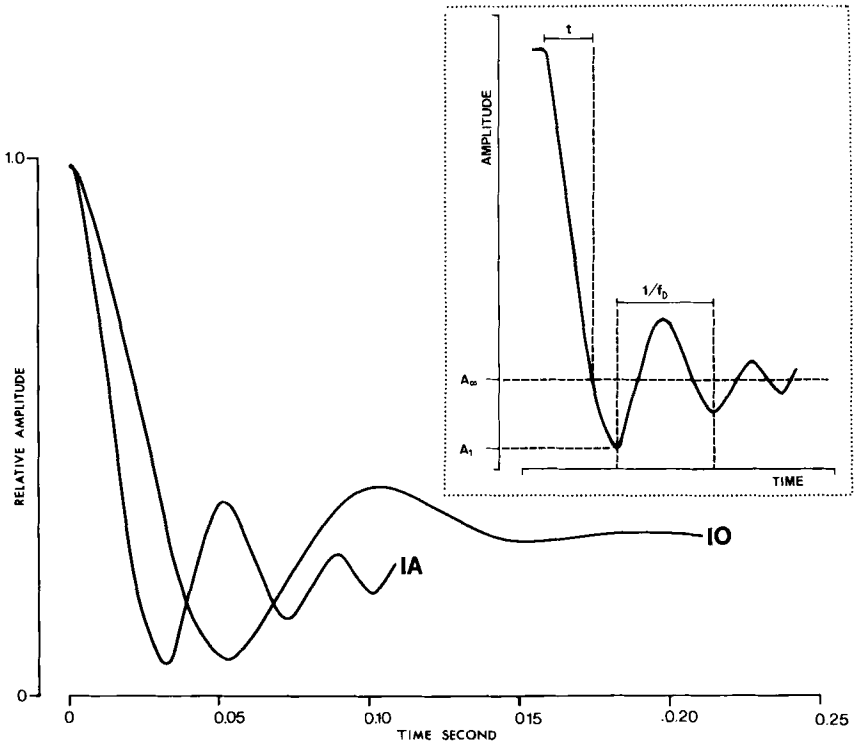


Figure 6. Step function test of pressure recording system as used for intraosseous pressure (IO) and intraarticular pressure (IA). The calculation of damping factor and resonance frequency from the curves are shown in Table 1.

Table 1. Dynamic characteristics of pressure recording system (Siemens strain gauge transducers 746, Siemens pressure amplifier 863 Portex manometer tubes). System used for intraosseous pressure registration (IOP) included a perfusion system, which was excluded when measuring intraarticular pressure (IAP).

	Modifications of pressure recording system	
	IOP	IAP
Rise time t	0.033 sec	0.018 sec
Logaritmic decrements: $k=-\ln \frac{A_1-A_\infty}{A_\infty}$	3.624	1.567
Damped resonance frequency: $f_D=\frac{1}{T_D}$	10.87 Hz	23.81 Hz
Damping Factor: $d=\frac{k}{\sqrt{4\pi^2+k^2}}$	0.38	0.22
Natural undamped frequency: $f_o=\frac{f_D}{\sqrt{1-d^2}}$	16.58 Hz	26.62 Hz
Overshoot: $\frac{A_1-A_\infty}{A_\infty}\times 100$	33%	21%

The dynamic characteristics are determined by all components of the recording system from the Radner biopsy cannulae, connector, Portex^R tubes, stop-cocks, transducer chamber and perfusion system. This pressure recording system was tested in toto as used for IOP recordings and without perfusion system as used for intra-articular pressure (IAP) recordings. An explosion chamber technique was applied to perform the step function test (Fig.6) from which the dynamic characteristics were calculated (Table 1) (Tybjerg Hansen 1949, Cerni & Foster 1962). Inclusion of the perfusion system increases the damping factor from 0.22 to 0.38, whereas the undamped resonance frequency decreased from 26 Hz to 17 Hz. The characteristics show that the system allows satisfactory registration of IOP and IAP baseline pressure.

Biological preparation

To obtain a comprehensive picture of the haemodynamic changes of the knee during various physiologic and pathophysiologic conditions, multiple simultaneous intraosseous pressure measurements were used in I, II, III, IV and VIII. This was

accomplished by the use of a four or five channel pressure recording system. To test the stability of the system, bilateral IOP recordings from the tibial and femoral epiphyses were continued during steady state for 360 min in Halothane anaesthesia. No change of intraosseous pressure level was recorded, neither during perfusion of the cannulae nor when perfusion was abandoned. Thus no clotting had occurred around the pressure cannulae. The duration of IOP measurement never exceeded 4 hours within the single experiment.

The application of perfusion in the system inevitably affects the IOP level, but the influence of a constant rate perfusion on the IOP level was found to be negligible, provided a perfusion rate below 9 ml/h. The intrinsic resistance in the system is very small, causing a 0.7 kPa increase of IOP at a perfusion rate of 200 ml/min (Bünger et al. 1984). Likewise, the juxtaarticular pressure recording in the juvenile knee during venous knee joint tamponade was unaffected by the presence of a constant rate perfusion of 5 ml/h.

Influence on bone blood flow

It is uncertain to which extent the pressure measurement trauma would inflict on the regional blood flow in relatively small juxta-articular bone compartments of the knee. In (III) and (VI), bone cannulation was found to cause insignificant hyperaemia in the cannulated compartment. This is in accordance with the observations of Bouteiller et al. (1979).

Positioning of the limb

The IOP is naturally influenced by the position of the measuring site in relation to the heart level (Polster 1970). The change in IOP follows the change in hydrostatic pressure when the site is lowered (0–40 cm), but when raised e.g. 40 cm the fall in IOP is less (50%) probably due to activation of autoregulatory mechanisms.

In measurement of static IOP in the juxta-articular bones and intra-articular pressure, it is important to use a standardized positioning of both the hip (Krebs et al. 1982) and the knee joint (I, II). Therefore, the dogs were generally placed in supine position with the hips in 90 degrees flexion, 45 degrees abduction, and neutral rotation. The knees were examined in 90 degrees flexion although the position of minimal IAP during joint effusion was found to be at approximately 60 degrees flexion.

Intra-articular pressure (IAP) measurement

This procedure was performed with the knee in 60 degrees flexion. A liquid filled Terumo 18G cannula connected to a three-way stop cock, which was closed during skin penetration, was used to measure joint pressure. By opening the stop cock, an immediate connection to the joint cavity was achieved without leakage, and pressure

was measured. The knee joints were always cannulated from the medial side of the knee. The tip of the cannula was placed in the intercondylar compartment. Placing the cannula in the femoropatellar compartment, frequently produced abnormally low pressures secondary to a suction effect by patellar dislocation.

Bone blood flow measurement

There is no ideal method for quantitative measurement of the bone blood flow primarily because of the relative inaccessibility of bone and the multitude of supplying and draining vessels. Studies of bone blood flow have been undertaken by collection of venous outflow (Cumming 1962, Shim & Patterson 1967), perfusion of isolated bones (Drinker & Drinker 1916, Driessens & Vanhoutte 1979), thermal clearance (Shaw 1963), fractional distribution of labelled erythrocytes (Brookes 1971), uptake or clearance techniques (Copp & Shim 1965, Kelly et al. 1971, Whiteside 1977) and fractional distribution of diffusible tracers (Kane 1968). These methods have major disadvantages due to extensive surgery for preparation, only one measurement in each animal being possible, or theoretical problems concerning the extraction and partition coefficient of the indicator (Gross et al. 1979). In addition, blood flow to the different components of bone cannot be determined independently, and the methods cannot be used in awake animals.

Tracer microsphere technique

Most of these objections are met by the tracer microsphere technique used for regional blood flow measurements in III, VI, VII, VIII, IX and X. The technique was initially described by Rudolf & Heymann (1967) and was first applied in bone by Lunde & Michelsen (1970).

The principles of the tracer microsphere technique have been reviewed by Hales (1974). The method is based on the bolus fractionation principle. Quantification is achieved by application of an "artificial organ" (reference blood sample) (Domenech et al. 1969) in which the flow is known. The advantages of the method are that it is locally noninvasive during measurements, it allows flow measurements in minute, inaccessible regions, up to six measurements can be performed on the same animal, it can be used in awake, ambulant animals, and the interval between measurements can be extended up to 30 days on use of long life isotopes (Jones 1982).

The main disadvantages of the method are that flow can only be measured discontinuously, costs are high, and only a limited number of flow measurements can be performed in each experimental animal.

Theoretical requirements and considerations

Some important theoretical requirements must be fulfilled to obtain reliable flow

measurements: 1) The microspheres must be well mixed at the site of injection, and evenly distributed in the blood. 2) The injection of spheres must not alter the systemic haemodynamics. 3) A sufficient number of microspheres must be injected to allow at least 400 spheres to embolize in the single biopsy for accurate measurement of blood flow (Buckberg et al. 1972). 4) The spheres must be trapped during the first passage of the region of interest. As discussed in (III) the results obtained suggested that these requirements were fulfilled.

The technique we used included positioning of one catheter in the left heart ventricle and one in the abdominal aorta, as described in detail in (III). Both catheters were inserted via the carotid arteries, which were ligated. This instrumentation has also been used in studies in awake dogs (Morris & Kelly 1980, Tøndevold & Bülow 1983). Carotid arterial ligation might, however, influence the carotid baroreceptors and alter sympathetic nervous system activity (Kirchheim 1976). Likewise, the use of general anaesthesia might modulate the sympathetic nervous activity. Since most vascular beds have a certain sympathetic tone, the model preparation used in (III, VII, VIII and IX) might alter the peripheral haemodynamics and thereby downgrade the validity of the conclusions.

The reproducibility of the tracer microsphere technique has been tested in different animal species by injection of mixtures of microspheres labelled with different isotopes. It has been demonstrated that the method gives reasonably accurate RBF determinations, provided the above mentioned conditions are met (Hales 1974, Fan et al. 1979, Hales et al. 1979, Liard 1981). Only a few studies have dealt with the time dependent variation in flow. Jones et al. (1982) ascribed average flow values dispareties to the variation between dogs. However, the statistical method applied to reach this conclusion was not reported.

Reproducibility

The reproducibility of the tracer microsphere technique as used in the present model (III, VII, VIII and IX) was investigated (Bünger & Bülow 1985).

Four repetitive steady state blood flow measurements were performed in four puppies aged four months. Symmetrical tissue biopsies were taken from 24 different locations, and the flow values were analysed separately using a three-way variance components model (Searle 1971). The systematic and stochastic components of variance due to interindividual variation, intraindividual right to left variation, variation due to time and the residual variation, i.e. measuring error, were evaluated separately. There was no systematic change of RBF in relation to time in any biopsy. Likewise, the systematic discrepancy between right and left tissue was negligible, except between caput and cauda pancreatis ($P < 0.0005$). A stochastic effect was seen in all biopsies and all effects proved significant in eight of 14 biopsies. The corresponding coefficients of variation are given in Table 2. In the two metaphyseal biopsies, variation due to time was insignificant, i.e. the individual time variation was negligible compared with the residual variation. Flow measurements in the joint capsules showed only residual variation.

Very large variations between right and left biopsies were found in the metaphyseal biopsies. This probably is an indicative of the difficulties in obtaining symmetrical biopsies in a compartment with large flow gradients. The problem was solved in X, where the bones were split sagittally before biopsies from the metaphyses were taken. High residual variations were generally found in tissues receiving a low percentage of cardiac output as expected from the theoretically calculated error (Buckberg et al. 1972). The time related variation could be considerably reduced by shortening the interval between flow measurements from 45 min to 15 min. The time related variation was on average reduced to 57% of the values calculated in dogs with long time intervals, while the flow rates only differed by 3% in the two groups. However, compared to the theoretically calculated total experimental error (Buckberg et al. 1972) the values were significantly higher i.e. 1 1/2–2 times the expected value.

Table 2. RBF in four flow measurements in each of four mongrel 10 to 13 kg puppies in relation to four components of variation (D%) Interindividual coefficient of variation, (L%) Variation between limbs, (T%) Variation in relation to time and (R%) Residual variation, which mirrors measuring error within the single flow measurement.

Tissue	RBF $ml \times 100g^{-1} \times min^{-1}$ (95% confidence)	D%	L%	T%	R%
Acetabulum	11–34	53	19	25	11
Femoral head	11–30	46	13	28	17
[a]Femoral diaphysis	11–24	35	12	32	11
Distal femoral metaphysis	1–13	125	109	0	87
Patella	10–27	50	9	29	8
Medial femoral condyle	5–35	92	6	32	15
Proximal tibial epiphysis	6–20	58	27	30	15
[a]Tibial diaphysis	10–26	43	16	29	11
Distal tibial metaphysis	1–10	112	14	15	32
Knee joint capsule	4–5	7	12	16	26
Rectal femoral muscle	4–8	34	<1	33	20
Anterior tibial muscle	5–15	51	32	40	20
Suprarenal glands	508–699	12	13	13	7
Pancreas	35–63	19	18	38	12

(a) Compact diaphyseal bone, (b) Variation between caput and cauda pancreatis.

In the present experiments the influence of spatial distribution of microspheres in reference blood samples and tissue biopsies was not considered, though it probably plays a significant role (Hales 1974). Another source of error which is of increasing importance in measuring low flow rates is the rounding errors introduced during computation of the different microsphere activities, e.g. correction for cross talk etc.

Influence on sympathetic tone

There are two possibilities of modulating the sympathetic tone using microsphere technique: One is the manipulation of carotid baroreceptor. The other is embolization by spheres of the adrenal gland. We monitored the change of sympathetic nervous system activity during the experimental procedure by repeated determination of plasma catecholamines. Halothane anaesthesia 0.5–2% caused a significant reduction of the circulating noradrenalin and adrenalin level compared with the preanaesthetic catecholamine level. After surgical preparation which included exposure and ligation of both carotid arteries, a rise in circulating catecholamines was found. However, the level did not exceed that measured in the preoperative awake state. No effect on systemic haemodynamics, adrenal blood flow or circulating catecholamines was found following injection of microspheres. Thus, the changes in the catecholamine level during the experimental procedure were considered too small to cause significant changes in peripheral circulation. This was strongly supported by the absence of any systematic change in RBF in 24 different tissue biopsies in the same study, although flow was not measured prior to surgical preparation.

It is concluded that the applied flow measurement technique provided reproducible determination of regional blood flow rates, especially with short intervals between measurements.

Estimation of microvascular volumes

Fluid spaces exist, through which solutes have to pass to reach the bone tissue (Cooper et al. 1966, Owen et al. 1973, Lopez-Curto et al. 1977). Hughes et al. (1978) have measured the fluid spaces in bone by means of compartmental analyses of wash-out curves after injection of ^{99m}Tc-MDP into the nutrient artery. They were able to identify four components with different half-lives, from which it was suggested that at least the haversian system of cortical bone consists of four clear compartments: the capillaries, the perivascular fluid space, the bone fluid space and the bone itself. Recently, Morris et al. (1982) have shown that there is no evidence that bone fluid has a composition different from perivascular fluid. They simply defined the perivascular fluid space by subtracting the estimated plasma space measured by ^{111}In-labelled transferrin from the estimated extracellular space measured by tritiated water.

The principal transport mechanism for ions and molecules across the endothelial layer of bone capillaries is believed to be passive diffusion (Cofield et al. 1975, Davies et al. 1976, Hughes et al. 1977). However, controversy still prevails regarding the physiological interpretation of bone capillary structure (P.J. Kelly 1984), though there is general agreement that molecular selection does occur (Kaley and Altura 1977). Capillary porosity, measured using tracers of different molecular size, varies among normal tissues (Bell et al. 1980), and the vascular permeability is probably profoundly changed in pathological states such as during fracture healing (Paradis and Kelly 1975) and in arthritis (IX). It is probably only the vascular space, estimated with ^{51}Cr-tagged red cells and assuming constant haematocrit, and the water space, using tritiated water, that can be determined with certainty (Kelly 1984).

Methodological speculations on the estimation of microvascular space as performed in (IX) consequently include: 1. selection of tracers, 2. duration of equilibration periods, 3. stability of labelled tracers, 4. tissue sampling technique and radiation counting procedures.

Red cell volume

The microvascular volume was defined as red cell volume + plasma volume. From the values of these two parameters it was also possible to determine variation in tissue haematocrit defined as:

$$\frac{\text{red cell volume}}{\text{red cell vol.} + \text{plasma vol.}}$$

The use of ^{51}Cr labelled red cells to estimate red cell volume is safe provided 1) that no capture of the labelled cells takes place in the spleen, 2) that no haemolysis occurs, and 3) that no leak of ^{51}Cr takes place. The recommendation by Tøndevold (1983) were observed, and splenectomy was performed to avoid loss of erythrocytes in the spleen, which may change the arterial haematocrit (Dietz et al. 1979).

After labelling, the configuration of the resuspended red cells was checked by microscope, and plasma was analysed for free ^{51}Cr. The non-bound ^{51}Cr fraction amounted to less than 0.1%. An equilibrium period of 10 min was chosen. The data in (IX) showed that the mean transit time of red cells in the metaphysis - the most sluggishly perfused areas of the immature bones measured - ranged from 15 to 35 seconds, which suggests that a steady state concentration of tracer in arterial blood was obtained.

Red blood cell space (V_{rbc}) was calculated from the ^{51}Cr activities as:

$$V_{rbc} = \frac{\text{cpm of biopsy}}{\text{mass of biopsy}} \times \frac{\frac{M\ ref}{100 \times d \times Hct}}{\text{cpm of ref. sample}}$$

where cpm = counts per minute
M ref = mass of reference sample
d = specific gravity of blood (1.063 g per ml)
Hct = Haematocrit of reference sample from the the left heart ventricle.

No correction was made for trapped plasma in measuring Hct, nor was the specific gravity concentration of the blood corrected for variation in plasma proteins. V_{rbc} in the inferior metaphysis of the femur in (IX) was 2.78±0.58 (SD) ml×100g comparable with values measured in immature rats, 1.87±0.37 ml×100 g^{-1} (Brookes 1965) and in adult dogs, 3.22±2.62 ml×100 g^{-1} (Tøndevold 1983), considering differences in maturity and species.

Plasma space

Traditionally labelled albumin has been used for measurements of the plasma space (V_p). However, as albumin with a mol wt of 69.000 g/mol and a Stoke Einstein radius of 36 Å (Studer & Potchen 1974) is small enough to leak out of the plasma space in bone (Owen and Triffitt 1976, Owen et al. 1973, Morris et al. 1982, Tøndevold 1983), we therefore used ^{125}I-human fibrinogen, mol wt 3.4×10^5 and a Stokes-Einstein radius of 106 Å (Studer and Potchen 1971). In an analysis of plasma tracers Eliasen et al. (1982) found no change in the distribution volume from 5 min to 30 min after injection when they used ^{125}I-human fibrinogen, whereas the ^{99m}Tc-Albumin space changed significantly. In the same material analysing the bones Tøndevold & Eliasen (1982) after an equilibration period of 10 min found a more than two-fold increase of ^{99m}Tc albumin space as compared with the ^{125}I-human fibrinogen space. This is in contrast to observations of Watson et al. (1980), who found that the plasma concentration of tracer albumin (^{131}I-labelled human serum albumin) and ^{125}I-human fibrinogen remained constant within 7% of the initial level during the first five hours after the start of tracer infusion.

Using ^{125}I-human fibrinogen and ^{131}I-albumin simultaneously as plasma tracers in dogs we have lately measured the overestimation of V_p by albumin after 10 min equilibration. In mature cortical bone the mean ratio of albumin/fibrinogen space was 1.61 ± 0.11 (SD), in bone marrow 1.23 ± 0.14, and in cancellous bone 1.32 ± 0.15. The larger albumin space reported by Tøndevold & Eliasen might be due to unstable bindings between Tc and albumin.

The use of ^{125}I-human fibrinogen as plasma tracer creates other errors such as overestimation of V_p due to free iodine, which makes up about 5% of total radioactive iodine. Ultrafiltration through Millipore^R filters reduces free iodine significantly (Eliasen et al. 1982).

Ultrafiltration was not performed in the present studies and we may therefore have overestimated V_p of cortical bone by approximately 4.1% and the V_p of bone marrow by 3.1%. These estimates are based on the assumption that the ratio between plasma volume and extracellular volume (transferrin/sucrose space, Morris et al. 1982) is 0.19 in cortical bone and 0.38 in marrow.

Based on the above observations on ^{125}I-fibrinogen, and cosidering the unknown but probably significantly increased permeable surface area in the tissue compartments of the arthritic knee, we chose an equilibration period of 10 min.

V_p was calculated according to the calculation of red blood cell volume from the ^{125}I activities by replacing Hct in the formula above with 1 – Hct.

Biopsy taking technique

Variation in biopsy taking constitutes a considerable source of error in the estimation of microvascular volumes in bone and joint tissue and also in the measurement of blood flow. Flow gradients in immature bone are large (X), and if not accounted for the biopsy procedure may introduce an "averaging error". As demonstrated in the studies on the reproducibility of the flow measurement technique (Table 2), this probably caused high coefficients of variation between symmetrical tissues notably in the metaphyseal bone regions.

In III, VI, VII, VIII, and IX a Bordier^{R} trepine (8 mm in diameter) was used for epiphyseal and metaphyseal bone sampling (Fig 7), whereas in X the subchondral bone (0.5 cm) was separated from the more centrally located bone. Likewise, biopsies were taken from metaphyseal bone at two levels. Prior to tissue sampling for estimation of the microvascular volume and immediately after sacrifice, the iliac veins were ligated to avoid loss of blood during manipulation of the hindlimbs. Morris et al. (1982) compared the vascular volumes in samples of frozen and unfrozen bone and found no essential difference. We preferred sharp sampling technique in unfrozen tissue.

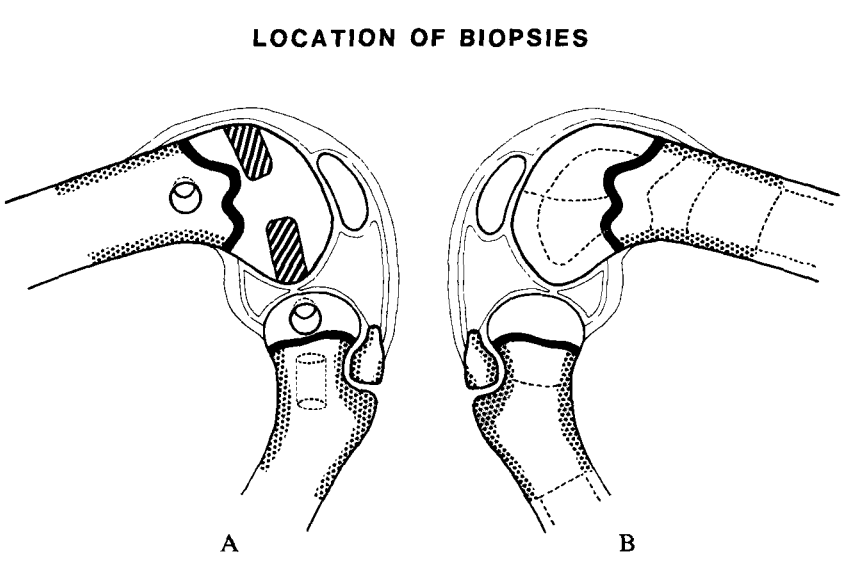


Figure 7. Schematic presentation of bone sampling of the knee as first used (III, VI, VII, VIII, IX) applying a 8 mm Bordier trepine (A). In the latest studies (X) the subchondral bone of the femoral epiphysis was separated from central bone with an osteotome (B).

C. Induction of arthropathy

A number of factors determined the selection of the arthritogenic agent. First, we wanted to induce a monoarthritis of the knee in order to have a contralateral control. Second, nonsuppurative chronic synovial inflammation and joint effusion had to be main features of the arthritic joint in order to mimic common pathological characteristics of haemophilic arthropathy (HA) and juvenile chronic arthritis (JCA). Third, the investigational experience from the normal dog knee (I, II, III) should be utilized. Thus, no attempt has been made to clarify the complex aetiology of HA or the aetiology of the group of probably different diseases included in JCA as proposed by the European League Against Rheumatism (EULAR) (Wood 1977).

Carrageenan mode of actions

Carrageenan, the arthritogenic agent here used (IV, V, VII, VIII, IX, X), has traditionally been employed for induction of experimental inflammation. Carrageenan-induced footpad oedema in the rat (Winter et al. 1962) was utilized in particular for the evaluation of prospective antiinflammatory drugs. The biological effects of Carrageenan, reviewed by Anderson (1967) and Di Rosa (1972), include the ability to induce acute and chronic inflammation, an anticoagulant activity by activation of the plasmin system via the Hageman factor and induction of disseminated intravascular coagulation (DIC). A review of the effects of Carrageenan on the immune system was published by Thomson and Fowler (1981), who concluded that Carrageenan impairs immune responses, that are T-cell dependent and involve participation of macrophages towards which it appears to be selectively cytotoxic. Carrageenan also demonstrates anticomplementary activity against the early complement components. Efforts have been made to isolate groups of Carrageenans, that could be used clinically as immune suppressants, but hitherto side effects like DIC, hepatotoxity and nephrotoxity have precluded this purpose.

The mode of action of Carrageenan in the induction of arthritis, discussed in V and VIII is summarized in Fig. 8. It is believed that the inflammatory process is initiated by release of lysosomal enzymes and prostaglandines. The cytotoxic effects of Carrageenan on macrophages are not specific. Other polysaccharide macromolecules have been shown to release lysosomal enzymes in vitro from macrophages, depending on their size and the degree of sulphatation (Schorlemmer et al. 1977) and from embryonic cartilage (Sledge & Blackburn 1968). Florid synovitis as produced in rabbit knees by repeated intra-articular injections of dextran sulphate was associated with high synovial acid phosphatase and beta-glucuronidase activities (Steinberg et al. 1980).

Gardner (1960) first introduced Carrageenan as an arthritogenic agent producing arthritis in the rabbit knee by periarticular injections. The intra-articular route of administration was used in later investigations (Lowther & Gillard 1976, Gillard & Lowther 1976, Lowther et al. 1976). In rabbit knees it was demonstrated, that

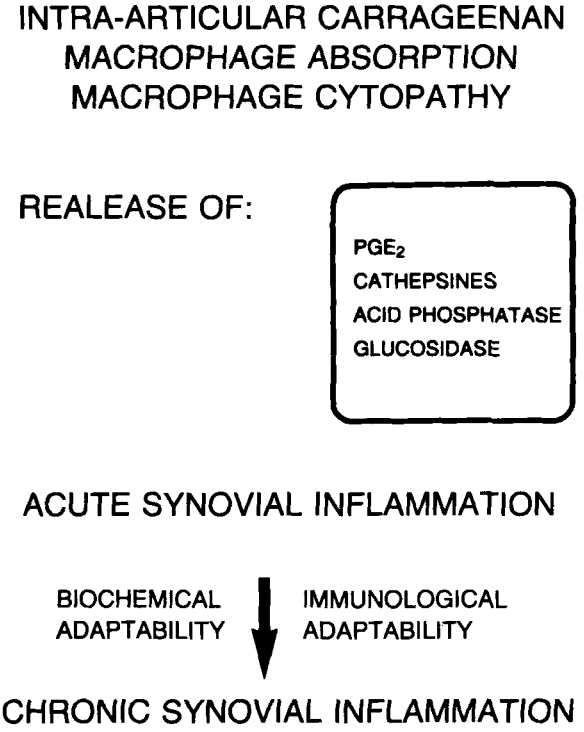


Figure 8. Mode of action of Carrageenan, when injected into a synovial joint.

Carrageenan induced arthritis was accompanied by high synovial acid phosphatase activity, high beta-glucuronidase activity and loss of cartilage proteoglycan (Lowther et al. 1976, Lowther et Gillard 1976). The present studies are the first to report on polysaccharide induced juvenile arthritis and its influence on juxta-articular bone growth, bone metabolism, and blood circulation.

Carrageenan-induced arthropathy

Experimental arthropathy as presented in V and VIII passes through a number of stages. The synovial inflammation is initially characterized by ulcerations, fibrinous exudate and formation of granulation tissue. It gradually turns into a subacute stage after two to three weeks, and at eight weeks the chronic synovial inflammation with villous hypertrophy is present (Bünger et al. 1981). In later studies (IV, VII, IX and X) we selected a 10 to 12 weeks duration of arthritis in our haemodynamic studies according to the definitions of JRA by the American Rheumatism Association and JCA by the EULAR.

The joint changes following three months of Carrageenan-induced arthritis (Fig. 9a,b,c,d) are largely irreversible. In five dogs with a follow-up of three to 12 months progressive degenerative changes were observed (Fig 10). The articular cartilage was

pale and fibrillated and in areas of the patello-femoral groove and on the tibial plateaus, the articular surfaces were denuded of cartilage. Epiphyseal overgrowth was still present. Histology of the joint capsule showed persistent chronic synovial inflammation and fibrosis. Bone cyst formation and bone erosion at the osteochondral junction were frequently encountered.

Some requirements regarding the manufacturing of the arthritogenic Carrageenan agent, the injection procedure, the severity of the arthritis, and comparability to JRA and HA had to be fulfilled to give reproducible and relevant information on the microcirculation of the knee in arthritis.

The applied Carrageenan solution was manufactured from raw seaweed Chondrus crispus. A uniform processing was used as described in V. The processed lot amounting to 2 L was dispersed into 20 cc vials, providing uniform Carrageenan concentration for all experiments. However, minor variations in concentration within the individual lots could not be avoided. Gross evaluation of colour and viscosity of the solutions prevented major variations, but small differences within the individual lots were unavoidable. We did not measure the arthritogenic activity of the individual Carrageenans, kappa, lambda or iota Carrageenan reported to show different activity towards the reticuloendothelial system (Davidson et al. 1981). When adding our Carrageenan solution to the growth medium of synovial tissue cultures no growth stimulatory nor inhibitory effect was measured (Andreasen 1984).

The unfractioned extract of Chondrus crispus contains about 99% of the most active kappa and lambda Carrageenans (Di Rosa 1972), which may explain why we were generally successful in producing arthritis.

During the experimental periods, the severity of arthritis was adjusted according to the Danish Law concerning Experimental Animals of 1977, chapter 1, part 2, implicating that the dogs did not suffer chronic pain. Intraarticular injections were always performed in general anaesthesia. The volume of Carrageenan solution and frequency of injections were adjusted to the individual animal to preserve limb use, also of the experimental limb. Weekly intra-articular injections were generally well tolerated. Reduced weight bearing on the post injection day was accepted. The weight gain of the experimental animals followed that of controls injected with saline. Signs of systemic weakening were not encountered.

The requirements to the model as outlined above concerning synovial inflammation and presence of joint effusion were fulfilled. The intra-articular pressure in the acute arthritis and in the chronic stages was always above the slightly subatmospheric normal knee joint pressure (IV, VII, VIII, X). As concluded in V, we found Carrageenan induced arthritis to be a reproducible model of non-specific gonarthritis.

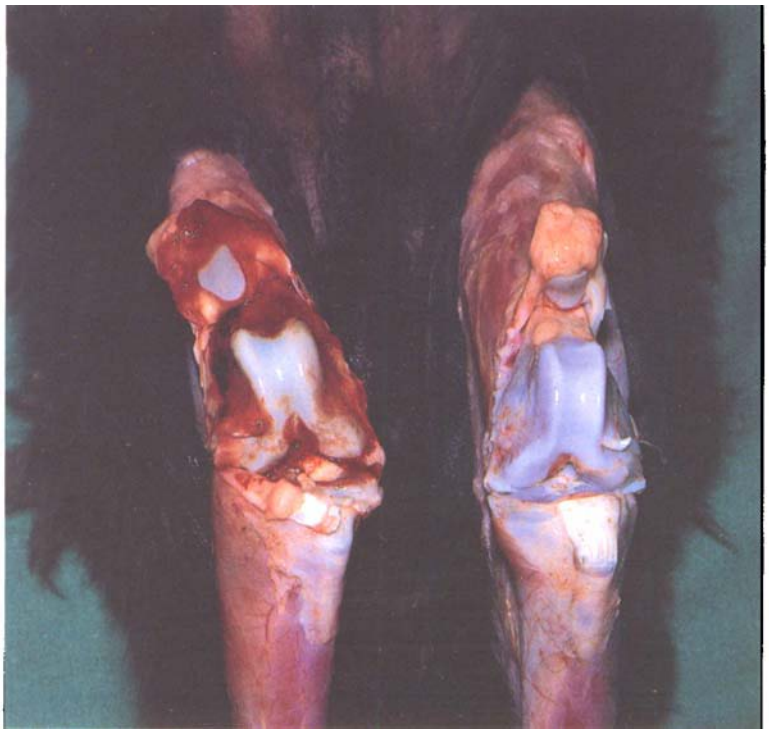


Figure 9a. Gross appearance of the knees of a mongrel puppy with Carrageenan-induced unilateral arthritis (A). C=Contralateral knee.

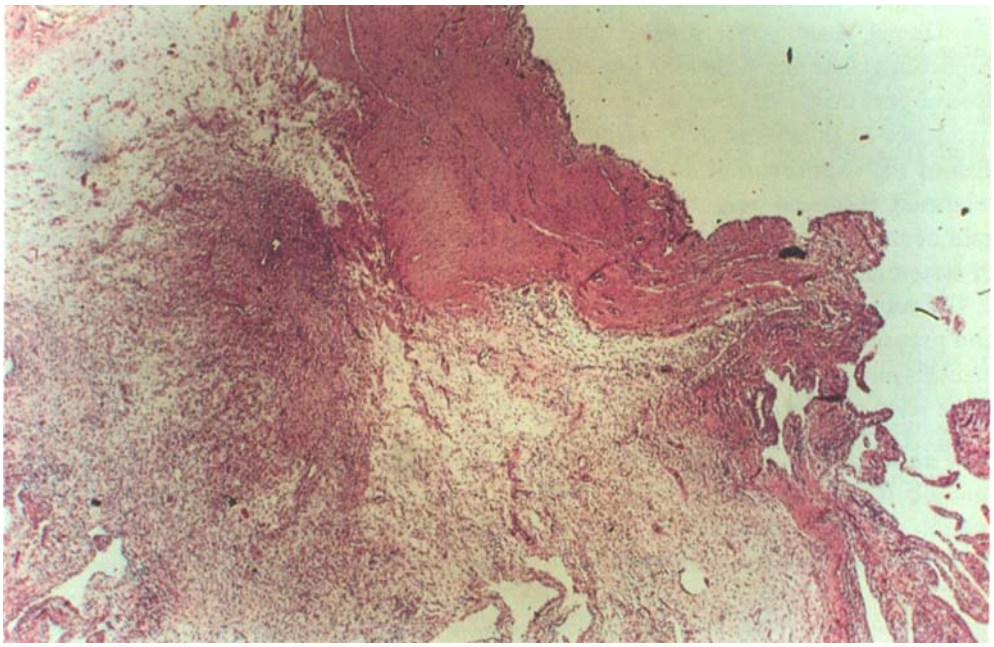


Figure 9b. Microscopic appearance of the arthritic synovial membrane. Villous hypertrophy, organized fibrin deposits and lymphocyte aggregates are prominent features. HE, × 100.

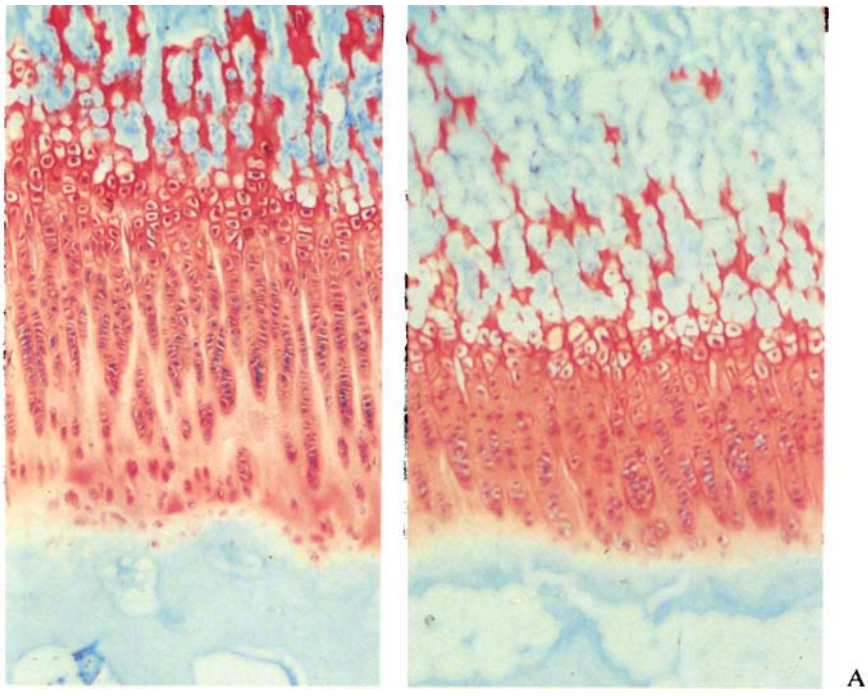


Figure 9c. Histological appearance of the femoral growth plate of the knee in unilateral arthritis (A). A marked narrowing in the cartilaginous part of the plate is found on the arthritic side. C=Controlateral knee: this means that something has to se inserted. Ironhematoxylin, Safranin –O stain, × 10.

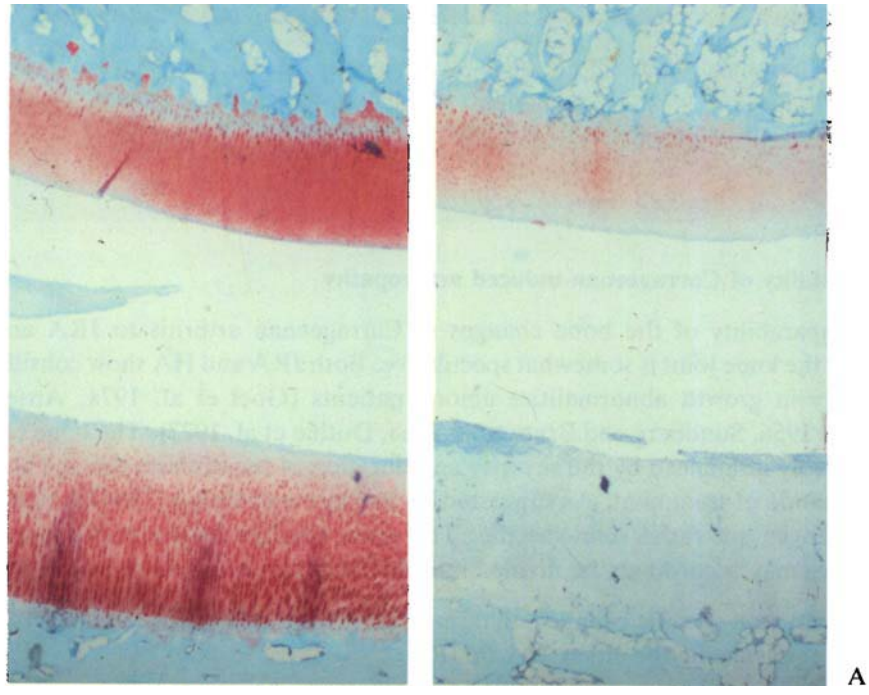


Figure 9d. Histological section of the femoro-tibial joint space of arthritic (A) and control (C) knee, Loss of proteoglycans is prominent in the articular cartilage of the arthritic knee. Subchondral bone is osteopenic. Ironhematoxylin, Safranin – O stain, × 24.

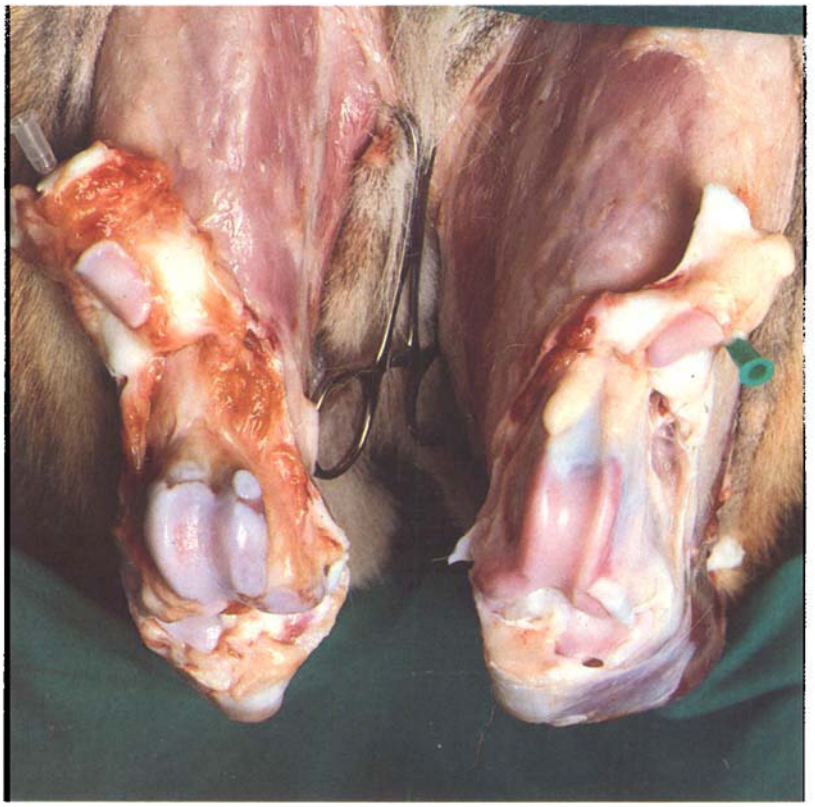


Figure 10. Gross appearance of the knees 8 months after induction of unilateral arthritis (A). Severe degeneration of articular cartilage, erosions at the epiphysio-synovial junctions and pigmented hypertrophic synovium are present. C=Contralateral knee.

Comparability of Carrageenan-induced arthropathy

The comparability of the bone changes in Carrageenan arthritis to JRA and HA affecting the knee joint is somewhat speculative. Both JRA and HA show considerable variability in growth abnormalities among patients (Goel et al. 1974, Ansell and Bywaters 1956, Sundberg and Brattström 1965, Duthie et al. 1972). The bone changes are probably influenced by the severity and duration of the disease, the age at onset, and the mode of treatment. As suggested by Boldero and Kemp (1966), a number of bone changes are rather non-specific. The gross changes of the joints in juvenile arthritides may accordingly be divided into different categories as shown in Table 3:

I. Largely non-specific changes, that may result from relative disuse and synovitis.
II. Changes specific for either HA or JRA.
III. Non-specific changes attributable to degenerative processes.

Table 3. Categories of gross changes of the knee in juvenile arthritides in relation to specificity and aetiology.

Group I nonspecific	Soft tissue	Joint effusion Synovial hypertrophy Capsular fibrosis Muscle vasting Joint cartilage overgrowth
	Bone	Juxta-articular osteoporosis Epiphyseal overgrowth Squaring of inferior patellar pole Abnormal joint alignment Atrophy of metaphyseal bone Endochondral growth stimulation or inhibition Premature epiphyseal closure
Group II specific	Soft tissue	Aggressive pannus (JRA) Hemarthrosis (HA)
	Bone	Subchondral hemorrhagic cysts (HA) Epiphyseal infarction (HA) Erosions at osteochondral junctions (JRA) Metaphyseal periosteal ossification (JRA).
Group III degenerative	Soft tissue	Loss of joint cartilage Capsular fibrosis
	Bone	Osteophytes Subchondral radiolucent cysts Subchondral bone sclerosis and collapse. Loss of joint alignment

The growth abnormalities following Carrageenan arthritis (V) (Fig 11) and experimental hemarthrosis of the immature dog knee (Hoaglund 1967) consequently belong to group I. The great majority of bone changes in HA and JRA also belong to group I.

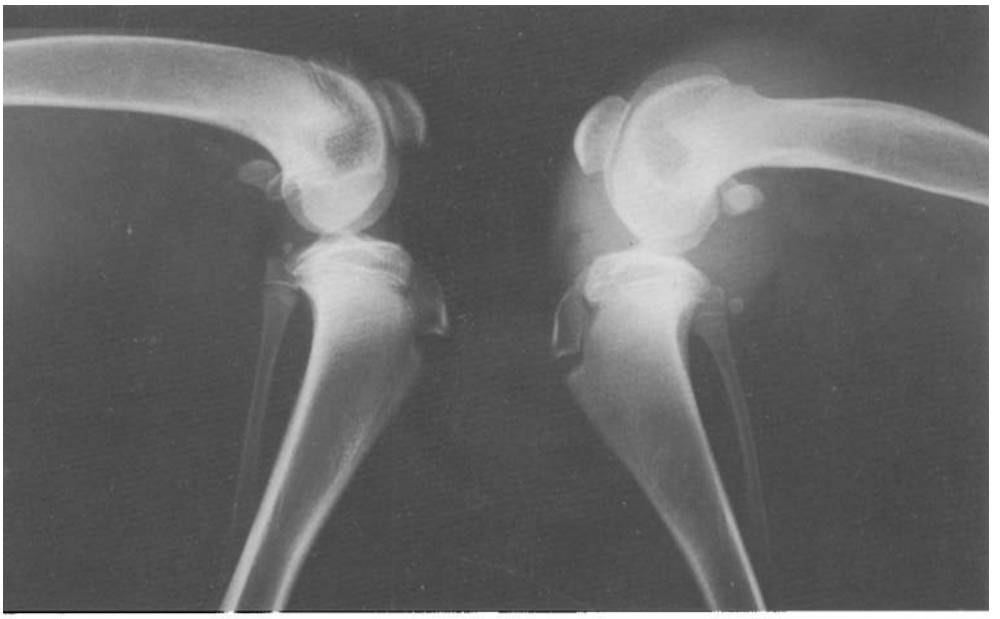
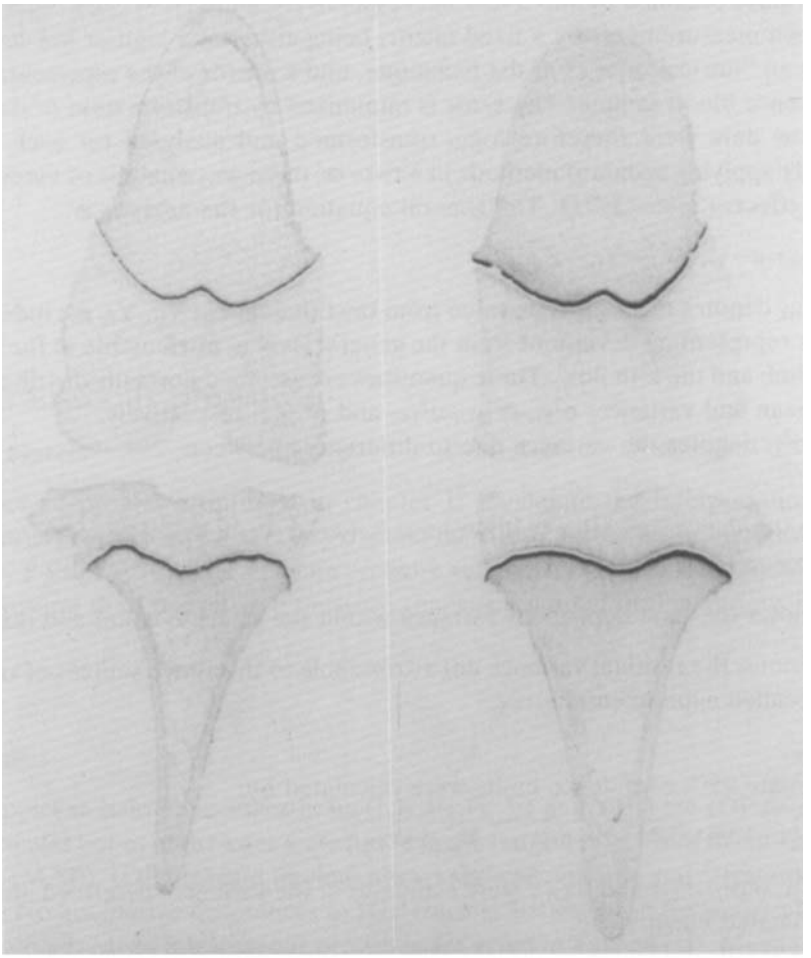


Figure 11. Radiological appearance of the knees following 3 months of Carragheenan-induced arthritis. The arthritic knee (A) is characterized by epiphyseal enlargement of the distal femoral epiphysis, metaphyseal hypotrophy, and increased soft tissue shadow. C=Contralateral knee.

Carrageenan synovitis significantly hampers longitudinal growth of the distal femoral growth plate. Whether this is a specific effect exerted by Carrageenan on the growth plate or due to severe unspecific joint inflammation cannot be established from the surveyed studies. The additional finding of narrowed or decreased height of the distal femoral growth plate (V) suggests a decreased proliferation rate of chondrocytes in the proliferative zone of the growth plate (Fig 9c).

Using contact autoradiography we have lately demonstrated a decreased deposition of ^{99m}Tc-MDP in the growth plates of the arthritic knee suggesting decreased calcification (Fig 12). In contrast to the chondrocytes in the growth plate, we found no evidence of dysfunction of chondrocytes in the basal layers of the articular cartilage, which during immaturity is believed to receive nutrition via diffusion from the epiphyseal vasculature (McKibbin and Holdsworth 1966, Hough et al. 1974). In fact, in the early stages of arthritis (10-12 weeks) an increased thickness of articular cartilage on the femoral condyles was found (Holm et al. 1985). In the superficial layers dead chondrocytes and loss of proteoglycans were prominent findings probably secondary to the influence of degradative substances in the synovial fluid.



A C

Figure 12. Contact autoradiography of deposition of ^{99m}Tc – MDP in arthritic (A) and control knee (C) following 3 months of arthritis. In the arthritic knee the uptake of ^{99m}Tc – MDP is decreased in the growth plates, most marked in the femoral growth plate, whereas at the margins of the epiphyses the uptake seems to be increased.

D. Statistical methods

In the analysis of pressure values and bone growth parameters Student's t-test for paired samples was used to test significant differences. The variation of the data could be described by a normal distribution, which was checked by a probit analysis.

Evaluation of flow and volume data in multiple locations with a time component as in III, VI and X necessitates a more complex statistical analysis.

Flow values obtained by the tracer microsphere technique may vary between dogs or between measurements by a fixed factor, being either at a high or low level. This is due to an "intrinsic error" of the technique, and a mirror of the representativity of the reference blood sample. The error is minimized by transformation of data.

All flow data were therefore $\log_{10}$ transformed and analysed for each location separately applying standard methods in a two- or three way analysis of variance with random effects (Searle 1971). The general equation for the analysis is:

$$X_{ijk}=u+U_i+V_{ij}+Y_{ik}+Z_{ijk}$$

where X_{ijk} denotes the $\log_{10}$ flow value from the i'th dog. U_i, V_{ij}, Y_{ik} are independent variables representing deviations from the general level u, attributable to the i'th dog, the j'th limb and the k'th flow. These quantities are assumed normally distributed with a zero mean and variances σ^2_D, σ^2_{DL}, σ^2_{DT} and σ^2_{DLT}, respectively.

Thus σ^2_D denotes the variance due to differences between dogs.

σ^2_{DL} denotes the variance due to differences between symmetrical flow determinations within each dog

σ^2_{DT} denotes the time dependent variance within the single location and dogs.

σ^2_{DLT} denotes the residual variance not attributable to the above sources of variation, the socalled experimental error.

Approximate 95% confidence limits were calculated by:

$$\text{antilog}_{10}(\bar{x}+1.96\sqrt{1/N\ (\hat{\sigma}^2_D+\hat{\sigma}^2_{DL}+\hat{\sigma}^2_{DT}+\hat{\sigma}^2_{DLT}}$$

where $\hat{\sigma}^2_D$, $\hat{\sigma}^2_{DL}$, $\hat{\sigma}^2_{DT}$ and $\hat{\sigma}^2_{DLT}$ were estimates of the variances described above, and $\bar{x}$ the estimated mean value.

Comparison between flow rates in a test limb ($x_{\cdot 1T}$) and control limb ($x_{\cdot 2T}$) at a given time T was performed using the test statistic:

$$U_T=\frac{x_{\cdot 1T}-x_{\cdot 2T}}{\sqrt{\frac{1}{N}(\hat{\sigma}^2_{DL}+\hat{\sigma}^2_{DLT})}}$$

The distribution of U_T was approximated by a Student t-distribution. A P-value < 0.05 was chosen as the level of significance.

III The normal knee

A. Intraosseous pressures

General aspects

A number of problems outlined in chapter II invalidate the interpretation of the intraosseous pressure levels and may explain the considerable variation reported (see review by Polster 1970). The absolute pressure values may therefore be physiologically less interesting than the relative pressure changes recorded during various interventions.

Own studies

In 47 puppies in halothane anaesthesia (I,II,III,IV,VI and VIII) the IOP levels in the juxta-articular bones of the knee were found in the range 6% - 24% of the mean arterial pressure (MAP). IOP pressure tracings always showed pulsatile and respiratory waves (Fig 13). No qualitative differences in IOP tracings between the juxta-articular bones were observed. Mean intraosseous pressures are given in Table 4 (II). Mean pressures of the knee recorded in 90° flexion were in the range: P_{FM} 0.7–1.3 kPa, P_{FE} 0.7–2.6 kPa, P_{TE} 1.1–2.9 kPa (I, III, IV, VI, and VIII). The highest IOP s were measured in VIII, where premedication with Combelin^R, which exerts a vasodilating effect on the peripheral vasculature (VI) was omitted and relatively older dogs were used. The $P_{patella}$ was significantly higher than P_{FE} and P_{TE} (II), and P_{FE} showed the lowest values (III, IV). There was no difference in pressure level between right and left tissues. We

Table 4. Intraosseous pressures (P) in the patella, the femoral epiphysis (FE) and the tibial epiphysis (TE) of 6 dogs aged 10 weeks. Mean ± SEM pressure values in kPa are given.

$P_{Patella}$		P_{FE}		P_{TE}	
Right	Left	Right	Left	Right	Left
1.64	1.60	1.22	1.04	1.37	1.40
±0.11	±0.13	±0.25	±0.13	±0.20	±0.16

were not able to demonstrate any age dependent changes in IOP from eight weeks to five months, though the MAP in halothane anaesthesia was lowest in the youngest puppies (I). In recent studies using chloralose anaesthesia P_{FE} was stable at 2.8±0.4 kPa (18% of MAP) in 12 weeks puppies (Harving et al. 1985).

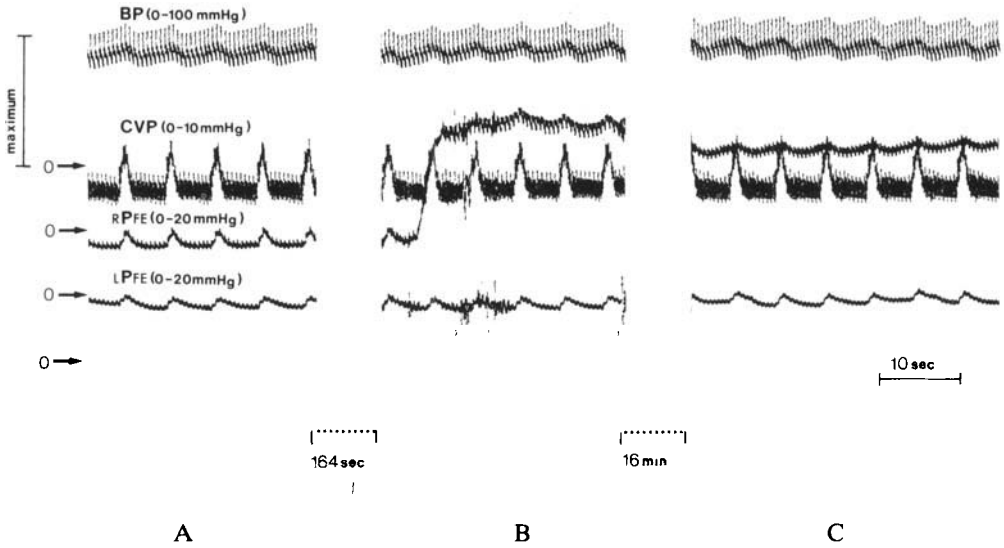


Figure 13. Intraosseous pressure tracings from the right (RP_{FE})and the left (LP_{FE}) femoral epiphysis in relation to systemic arterial (BP) central venous (CVP) pressures. Pressure tracings are given during steady state (A), during ligation of the middle genicular vein (B), and 16 min after ligation (C). From Bünger et al. 1983 (VI).

At first glance the IOP values measured in the resting knee are very low, which may be explained by a number of factors: First, the very young animals have a low MAP (7.4 kPa in I) compared with older animals (10.9 kPa in VIII). Second, the use of Combelen premedication and halothane, which both may decrease blood pressure (Goodman & Gilman 1980). Third, the knees were situated 10 to 20 cm above heart level in order to obtain resting position of both the hip and knee joint.

Most previously published IOP measurements have been performed in barbiturate anaesthesia. However, the use of barbiturates may account for the relatively high values of IOP reported, since repeated barbiturate injection increases bone blood flow as much as twice the previous value (Niv et al. 1981). The effect of halothane on bone blood flow has not been specifically studied. There is no significant difference between flow rates obtained in awake dogs (X) and the rates obtained during halothane anaesthesia either with (VI) or without (VIII) Combelen premedication (Table 5). Furthermore, the presence of regulatory mechanisms for regional blood flow was established (III,VI). Combination of Combelene and halothane did therefore not invalidate the present experiments, although such a combination cannot be recommended for future physiological haemodynamic studies.

Table 5. Regional blood flow ($ml \times 100g^{-1} \times min^{-1}$) of the immature dog knee related to different types of anaesthesia.

	Control awake N=9	Halothane N=8	Halothane +Combelen N=6
Patella	15 (12–20)	14 (9–23)	16 (9–26)
Femoral epiphysis	16 (11–25)	17 (12–26)	16 (11–24)
Tibial epihysis	20 11–37)	11 (7–17)	11 (6–19)

Mean values, 95% confidence limits in brackets

Investigations by others

Experimental studies

Most pressures within bones reported are diaphyseal bone marrow pressures of the tibia in dogs (Rothmann 1913, Kalser et al. 1952, Larsen 1938, Bloomenthal et al. 1952, Stein et al. 1957). They describe a wide range of pressures (1.6 to 6.7 kPa in the adult dog). Most authors suggest that the marrow pressure is related to the medullary venous pressure (Held & Thron 1962, Michelsen 1967). Wilkes & Wisscher (1975) who measured on the exposed endosteal membrane in Nembutal anaesthesia found a marrow pressure of 3.1±0.7 kPa (17% of MAP) and nearly equal to the nutrient vein end pressure averaging 2.5±0.8 kPa. In Nembutal anaesthesia Polster 1970 reported from multiple pressure registration in the rabbit femur, that the epiphyseal pressure in 86% of the cases was lower than the diaphyseal marrow pressure. He was not able to demonstrate any relationship between arterial blood pressure and IOP, and reported 70% of IOP values in the range 11–30% of MAP. He concluded that the variation in IOP between different regions of the long bone most probably depended on the risk of penetrating intraosseous vessels. This risk was considered highest in diaphyseal marrow.

In adult dogs Tøndevold et al.(1979) examined the relationship between IOP and MAP. The change of MAP was induced by different levels of halothane anaesthesia. At a MAP above 10.6 kPa, the IOP's in diaphyseal, metaphyseal and epiphyseal bone of the femur ranged from 18 to 27% of MAP, whereas at a MAP below 10.6 kPa the IOP was lower ranging from 11 to 17% of MAP. There was no significant difference in IOP among the three sites of pressure registration. The author suggested that a "threshold value" of MAP exists, below which autoregulatory mechanisms direct blood to more vital organs. However, the lack of simultaneous information on blood flow makes the results inconclusive.

Only a few observations on IOP in immature bones have been reported, and no data on IOP around the juvenile knee were available before the present series of studies were initiated.

Human studies

In adult volunteers Arnoldi et al. (1975) by simultaneous measurements found a P_{FE} of 1.1±0.2 (S.E.) kPa and a P_{TE} of 1.3±0.3 kPa (N=16). Lynch (1974) reported a higher P_{TE} (3.9 kPa, range 2.3–4.5 kPa) while Lemperg and Arnoldi (1978) in a large series measured a mean P_{TE} of 3.6 kPa (0.9–9.5 kPa) and a mean P_{FE} of 3.8 kPa (0.4–10.6 kPa). In the patella in normal, painless patellofemoral joints the mean IOP has been recorded to 2.0 kPa (0.5–5.6 kPa) (Ficat & Hungerford 1977, Björkström et al. 1980). Thus, in the human knee there seems to be an IOP variation comparable to that obtained experimentally. The variation cannot be explained by different levels of blood pressure, nor by different levels of venous pressure. It is suggested that the variation is due to lesion of intratrabecular vessels during insertion of cannulae combined with local intraosseous obstruction of outflow.

B. Regional blood flow

The immature skeleton receives approximately 10% of the cardiac output at rest (Gross et al. 1979, Morris & Kelly 1980). Immature bone exhibits internal structural and circulatory differences, which change by development and growth (Schnitzer et al. 1982). The regional blood flow appears to be closely related to the metabolic activities associated with haematopoiesis, bone formation, and bone remodelling (Whiteside et al. 1977, McKinstry et al. 1982, Kelly 1984).

Juxta-articular bones

The normal knee has a constant pattern of RBF distribution. Investigations of 46 mongrel puppies in halothane anaesthesia showed relatively low perfusion rates of the soft tissues compared with that of the adjacent bones (III, VI, VII, VIII, IX, Bünger & Bülow 1985) (Table 6). The highest RBF rates in the juxta-articular bones were measured in the trochlear area and the medial femoral condyle, whereas lower flow rates were found in the metaphyses. However, the largest variation in RBF was obtained in the metaphyses. As discussed in chapter II, this probably reflects difficulties in obtaining identical biopsies. Immature cortical bones are richly perfused when compared with the cortical RBF rates in mature bones of approx. $2\ ml \times 100\ g^{-1} \times min^{-1}$ (Tøndevold 1983, Morris & Kelly 1980, Schnitzer et al. 1982). The microvascular volumes are very small, giving rise to the shortest transit time of blood and the highest tissue haematocrit within immature bone (IX) (Table 7).

Table 6. Regional blood flow rates ($ml \times 100g^{-1} \times min^{-1}$) in the immature knee of the anaesthetized supine dog. Range of mean values in brackets from observations in surveyed studies. N=46.

Femoral diaphysis cortical bone	7 (6–20)
Femoral metaphysis	7 (2–16)
Femoral epiphysis Trochlea	17 (9–24)
Femoral epiphysis Medial condyle	14 (11–18)
Femoral epiphysis Lateral condyle	10 (8–11)
Tibial epiphysis	12 (11–13)
Patella	13 (6–15)
Knee joint capsule	3 (1– 6)
Medial meniscus	2 (2– 3)
Lateral meniscus	3 (2– 4)

Table 7. Microvascular volumes (VV), tissue haematocrits (Hct) and transit time of red cells (ETT = $\frac{\text{red cell volume}}{\text{red cell flow}} \times 60$) in the immature knee. (N=8, 95% confidence limits in brackets).

	VV $ml \times 100\ g^{-1}$	Hct %	ETT seconds
Femoral diaphysis	1.3 (1– 2)	23 (20–27)	8 (7–13)
Femoral metaphysis	14.0 (11–17)	20 (17–23)	27 (21–33)
Femoral epihysis trochlea	5.3 (4– 7	19 (16–22)	16 (9–18)
Patella	3.0 (2– 4)	26 23–30)	19 (15–23)
Tibial epiphysis	5.5 (4– 7)	19 (17–20)	13 (11–17)
Tibial metaphysis	12.0 (13–19)	16 (13–19)	30 (20–74)
Tibial diaphysis	1.3 (1– 2)	24 (20–29)	11 (5–16)
Joint capsule	0.9 (0.6–1.3)	18 (13–24)	11 (5–19)

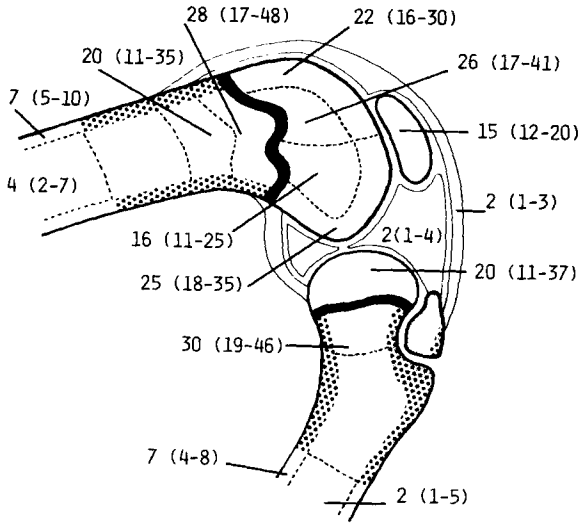


Figure 14. Regional blood flow rates of the normal knee in awake dogs. From Bünger et al. 1984, X.

Flow gradients exceeding the above differences in blood supply have been reported within the separate bone compartments (X, Light et al. 1984) (Fig. 14). In *the metaphyses* of the knee the most rich perfusion was documented in the metaphyseal segment of the growth plates at a zone of 1.5 mm in depth, which bordered on the physis. No change between the flow rates was observed when peripheral and central segments at the same metaphyseal level were compared. Light et al. 1984 measured mean flow rates up to 119 $ml \times 100g^{-1} \times min^{-1}$ adjacent to the femoral growth plate.

The highest flow rates we found were 46 $ml \times 100g^{-1} \times min^{-1}$ in an 8 mm segment adjacent to the plate (X). This zone also had the most prominent uptake of ^{99m}Tc-MDP in contact autoradiographic studies (Fig. 15). The vasculature here is dominated by capillary loops. Osteoblasts are forming osteoid on the remnants of the already calcified cartilage matrix. This abundant density of capillaries is reflected by an overall increase in microvascular volumes and a prolonged transit of red cells and plasma (IX).

In the *juxta-articular epiphyses* of the knee there are significant flow gradients between subchondral bone beneath the articular cartilage and the central epiphyseal bone (X, Light et al. 1984) (Fig. 14). We were not able to show this relationship in the patellar femoral groove, where the growth plate in dogs approaches the articular surface and leaves the interlining epiphyseal bone very narrow. Whether the segment of the epiphysis close to the growth plate is more richly vascularized than the central epiphyseal bone, has not been studied in detail. The peripheral zone of the epiphyses with high RBF rates corresponds to the area of appositional bone growth, which can be visualized on autoradiography as an approximately 1 mm subchondral zone of increased ^{99m}Tc-MDP uptake (Fig. 15). The microvascular volume of the epiphyses is approximately half of that of the metaphyses. Thus, the mean transit time of blood is shorter (Table 7).

In the patella the microcirculation is close to that of the juxta-articular epiphyses, except for a relatively high Hct (IX).

The soft tissues of the knee joint receive a fairly even blood supply. No differences in RBF rates were found among meniscii, fat pad, and joint capsule, and they were not affected by anaesthesia (VIII, X). The blood flow rates approach the values found in adipose tissue and ligamentous tissue elsewhere in the body (Bülow 1983).

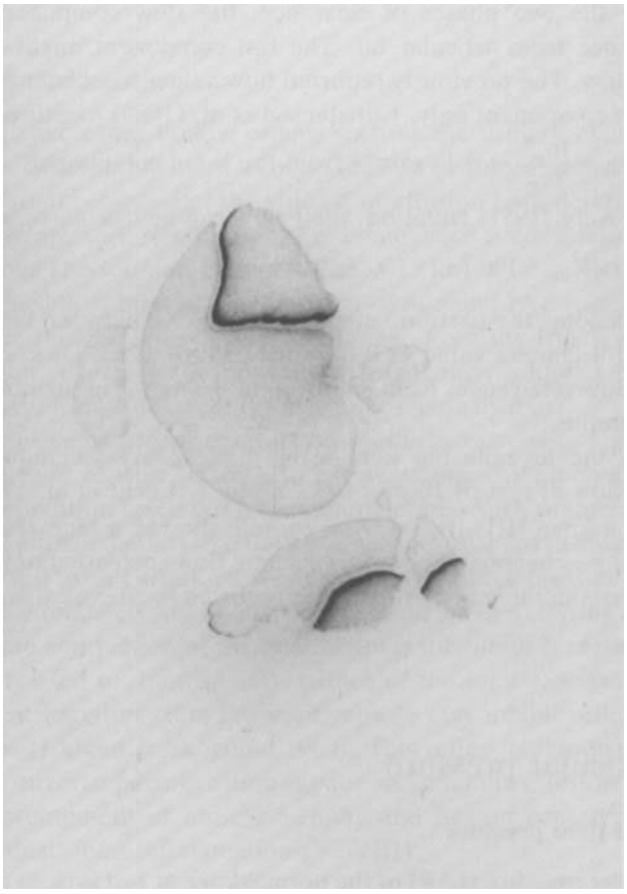


Figure 15. Sagittal contact autoradiography of the normal immature dog knee 2 hours after intravenous injection of 20mCi ^{99m}Tc-MDP The deposition of isotope is located in the calcification zone of the growth plates and at the periphery of the epiphyses.

In the *knee joint capsule* RBF is probably higher in the luminal part of the synovial membrane. We were not able to separate these layers during biopsy processing, but we generally managed to separate the synovial capsule from the fibrous capsule, and the values reported are thus from the synovial capsule and the first reported using the tracer microsphere technique.

In studies using ^{133}Xe clearance the synovial perfusion in normal human knees has been recorded from 0.11–0.73×100 $ml^{-1}\times min^{-1}$ (Porter et al. 1970). Dick et al. (1970c) found higher flow rates (1–2 ml×100 ml^{-1} min) in canine and human synovial joints. However, major methodological sources of error are associated with the ^{133}Xe clearance method, which may account for the low level of flow values reported. Phelps et al. 1972 showed that the ^{133}Xe clearance from both canine and human joints consisted of two components, a rapid one and a slow one. By direct analysis of tissue ^{133}Xe contents during the two phases of clearance, the slow component was found to represent clearance from articular fat. The fast component might represent actual synovial blood flow. The previously reported flow values have been calculated on the basis of the slow component only. Christensen et al. (1980) measured an initial wash out constant $K_{init} = \frac{\ln 2}{T_{1/2}} = 0.14\ min^{-1}$ from the rapid component.

Applying the Kety (1951) equation, their flow can be calculated as:

$$F = lambda \times K_{init} \times 100\ (ml \times 100\ ml^{-1} \times min^{-1})$$

where lambda denotes the partition coefficient of ^{133}Xe between blood and synovial tissue. Provided a lambda value of 0.6 to 1.0 (Bülow 1983, Dick et al. 1970c), the synovial blood flow rate ranges from 8.4 to 14 $ml^{-1}\times min^{-1}$ in the normal rabbit knee in the given example.

In studies on the juvenile hip joint using microsphere technique, we measured capsular blood flow at rest of 10 $ml\times 100^{-1}\times min^{-1}$ (Lucht et al. 1983). Both in the hip joint and the knee (III) the synovial capsule showed a high residual blood flow capacity following ischaemia. The postischaemic flow increase amounted a factor 20 to 80, which is considerably higher than that reported in adipose tissue (Bülow 1983).

C. Intra-articular pressure

Resting synovial fluid pressure

The intra-articular pressure (IAP) of the normal knee at rest is close to zero or slightly subatmospheric (II, IV, VII, VIII, X, Jayson & Dixon 1970a, Levick 1979). With quadriceps contraction and footstance during walking it becomes markedly negative (Jayson & Dixon 1970b). In our experience a similar "vacuum" effect resulting in an abnormal negative IAP can be obtained by retropatellar positioning of the pressure recording cannulae. This may account for the lowest IAP values reported by Levick (1979), who found a mean pressure of −0.6 kPa (range 0 to −1.6 kPa) in the rabbit knee.

Relation to the joint position

The IAP is dependent on the joint angle. It increases when the knee is both actively and passively flexed (Levick 1979, Caughey & Bywaters 1963, Jayson & Dixon 1970b, III). The IAP of the normal dog knee is at a minimum, when the knee is in 90 degrees of flexion (III), whereas in the human knee the lowest IAP is measured at 20 to 60 degrees of flexion (Eyring & Murray 1964).

Influence of joint effusion

The joint cavity, like other hollow organs such as the urinary bladder, follows the pressure-volume relationship of a rubber balloon within certain limitations. The elastance of the joint capsule and the volume of effusion both determine the level of IAP. The volume/pressure relationship, i.e. compliance is considerably decreased in arthritic conditions (Jayson and Dixon 1970a).

When joint effusion is present, the position of minimal IAP is between 30 and 70 degrees flexion in the dog knee (II). In the human knee the IAP is least at about 30 degrees of flexion, which is the position spontaneously adopted by patients with effusions and that of least discomfort (Jayson & Dixon 1970c).

Further flexion or quadriceps contraction produces extremely high pressures that may rupture the joint capsule, leading to extravasation of joint content into the calf with a resulting condition resembling thrombophlebitis. Such rupture is most likely to occur in recently inflamed joints that have not become adapted to the effects of chronic effusion (Jayson & Dixon 1970a). However, the threshold of pressure elevation which causes symptoms is relatively low i.e. between 2.6 and 5.2 kPa (Eyring & Murray 1964). At a high IAP, the contraction of the quadriceps is inhibited. It seems likely that this phenomenon is related to the degree of stretch of the joint capsule. Studies on cats show that afferent impulses from the joint capsule may inhibit reflex contraction of of the quadriceps (Eklund & Skoglund 1960). This reflex inhibition may account for the difficulties in quadriceps contraction in patients with joint effusion. It may also play a role in the development of muscle atrophy and flexion contractures, that often accompany sustained joint inflammation (V, VIII).

D. Relationship between intra-articular pressure and haemodynamics of the juxta-articular tissues of the normal knee

The interdependency between the intra-articular pressure and the intraosseous pressure of the knee was first documented by Arnoldi et al.(1979) in rabbits. Artificial IAP elevation caused an increase of the IOP of the distal femur. The increase was close to the diastolic blood pressure. These findings suggested, that chronic joint effusion with elevated IAP might create a state of chronic venous engorgement in bone, accompanied by pain and destruction of subchondral bone.

Later we found that the influence of joint pressure elevation was most marked in the immature knee joint with substantial regional variation (Lucht et al. 1981, I). Therefore, in the following description emphasis is given to a review of the regional impact of joint tamponade.

Joint capsule

In the knee the haemodynamics of the joint capsule is most susceptible to increases of IAP. In normal canine joints distended by saline, pressure waves corresponding to the arterial pulse can be recorded (McCarty 1980). As IAP approached the systolic blood pressure, a progressive elimination of IAP pulse waves was noted and interpreted as an obstruction of synovial blood flow. Using the ^{133}Xe clearance technique, Phelps et al. (1972) found that small changes in IAP caused significant decrease in clearance.

According to our own observation in III and VII (Tables 8 and 9), cannulation of the knee joint cavity has no effect on the synovial blood flow. Knee joint tamponade of 3.9 kPa (III) or 6.5 kPa (VII), which can be considered as venous tamponade, cause

Table 8. Regional blood flow rates (ml×100 g^{-1}× min^{-1}) during control conditions (Initial), after knee joint cannulation bilaterally and evacuation of knee joint effusion in arthritic knee, at knee joint tamponade of 13.0 kPa and 1/2h after reevacuation of the knee joint. Arthritic knees (A). Control knees (C). Mean values and 95% confidence limits in brackets.

	Initial		0 kPa		13.0 kPa		0 kPa	
C	A	C	A	C	A	C	A	C
Femoral metaphysis	*16 (8–34)	6 (3–12)	*19 (9–40)	7 (3–16)	13 (6–27)	5 (2–11)	20 (9–42)	9 (4–18)
Trochlea patella	14 (7–28)	15 (7–29)	16 (8–32)	17 (9–35)	3 (1– 6)	4 (2– 7)	15 (7–30)	17 (8–35)
Medial condyle	12 (6–24)	12 (6–23)	15 (8–29)	16 (8–31)	*2 (1– 4)	4 (2– 8)	15 (8–29)	19 (10–37)
Lateral condyle	11 (6–24)	13 (6–27)	15 (7–30)	15 (7–31)	*4 (2– 8)	8 (4–17)	16 (8–33)	15 (8–32)
Patella	11 (5–26)	11 (5–26)	15 (6–34)	14 (6–34)	6 (3–15)	5 (2–12)	14 (6–33)	17 (7–39)
Tibial epiphysis	10 (4–23)	10 (4–23)	11 (5–25)	11 (5–26)	8 (4–18)	9 (4–21)	14 (6–32)	14 (6–31)
Knee joint capsule	*17 (4–75)	1 (0.3– 5)	*18 (4–77)	1 (0.2– 4)	*1 (0.2– 3)	6 (1–24)	18 (4–79)	14 (3–61)

*$p<0.05$ compared to control knee

Table 9. Regional blood rates ($ml \times 100g^{-1} \times min^{-1}$) during control conditions, at knee joint tamponade of 6.5 kPa, 13.0 kPa and 26.0 kPa (IV). Arthritic knees (A). Control knees (C). Mean values and 95% confidence limits in brackets.

	Initial		6.5 kPa		13.0 kPa		26.0 kPa	
	A	C	A	C	A	C	A	C
Femoral metaphysis	9 (2–38)	5 (1–18)	22 (5–88)	13 (3–54)	*15 (4–62)	2 (0.4– 7)	21 (5–84)	9 (2–36)
Trochlea patella	14 (7–29)	11 (5–22)	26 (12–53)	19 (9–39)	8 (4–16)	10 (5–21)	3 (1– 6)	6 (3–13)
Medial condyle	14 (7–30)	14 (7–30)	27 (12–56)	25 (12–54)	7 (3–15)	8 (4–17)	*2 (1– 4)	6 (3–12)
Lateral condyle	12 (6–28)	11 (5–25)	29 (11–54)	21 (10–48)	10 (5–23)	12 (5–27)	*3 (1– 6)	10 (5–23)
Patella	12 (3–49)	11 (3–45)	24 (6–101)	17 (4–69)	*1 (0.2– 4)	4 (1–18)	1 (0.2– 4	2 (0.4– 7)
Tibial epiphysis	15 (8–30)	11 (6–22)	34 (17–67)	26 (13–51)	28 (14–55)	19 (10–38)	32 (16–62)	25 (13–49)
Knee joint capsule	*9 (2–36)	2 (1– 8)	9 (2–31)	5 (1–18)	1 (0.2– 4)	1 (0.4– 5)	*4 (1–14)	10 (3–40)

*P<0.05 compared to control knee

a significant increase of capsular RBF. At mixed arterial and venous tamponade (13.0 kPa) this flow increase was obliterated in VII (Table 9), whereas in III the flow further increased. The discrepancy may be due to a higher relative tamponade in VII. In both studies a profound hyperaemia was present 30 min after evacuation of the joint effusion, suggesting the presence of ischaemia of the synovial capsule during tamponade and secondary release of vasodilating substances.

Distal femoral metaphysis

Knee joint tamponade has no effect on the IOP in the distal femoral metaphysis (I, IV), probably owing to the extracapsular location of the metaphyseal vascular supply and venous drainage. The lack of pressure response does not preclude an effect on the other haemodynamic parameters of the metaphysis. In III an increased RBF of the bones on the entire hindlimb was observed during knee joint tamponade in halothane anaesthesia. The increase might be secondary to a decrease in the sympathetic tone in bone vasculature. Halothane induced vasodilation should also be considered, although it seems unlikely facing the slight increase in total peripheral resistance towards the end of the study.

The mechanism that triggers the vasodilation in bone secondary to tamponade is still unknown. An isolated increase of venous outlet resistance from the distal femoral epiphysis does not influence the RBF of the distal femoral metaphysis (VI).

Our later observations in the normal knee during tamponade (VII) are not fully comparable with those in III, since the tamponade level in relation to arterial blood pressure was higher in VII. However, at an IAP of 6.5 kPa a two-fold increase of RBF was found, whereas at 13.0 kPa joint pressure the RBF of the metaphysis was low compared with the initial values (Table 9). In the other subgroup of dogs in VII, where abruptly introduced tamponade of 13.0 kPa was applied, no significant change of RBF was noted (Table 8).

In the immature hip joint we found no effect of venous tamponade (61% of MAP) on RBF of the proximal femoral metaphysis (Lucht et al. 1983). In contrast to the findings in the knee, a significant increase of RBF in the proximal metaphysis was measured during arterial tamponade (163% of MAP).

To conclude knee joint tamponade does not affect the intraosseous pressure or the venous outlet resistance in the distal femoral metaphysis. RBF may be either increased or decreased probably depending on the change in the sympathetic vascular tone of the extremity. In series of studies, hyperaemia was released by venous tamponade. At higher joint pressure levels no constant pattern of RBF change has been documented.

Distal femoral epiphysis

Three areas, the trochlear area, the medial and the lateral condyles were investigated separately in our flow studies (III, VII) as indicated in Fig. 7A. Intraosseous pressure registration in the distal femoral epiphysis (P-FE) was taken from the central epiphyseal bone.

Venous tamponade causes a reproducible increase of P-FE (I, II, III, IV, and VIII). Some variability in pressure increase among the knees is found in all studies. A few *"nonresponders"* may be encountered, (up to 1 in 15 dogs), and they occur in a nonpredictable pattern being siblings to "responders"..

Both knees of the individual dog generally show the same reaction to joint pressure elevation. Although undocumented it is suggested, that " responsiveness" might be attributed to variability in location of venous drainage from the distal femoral epiphysis. Attention was not made to this phenomenon in the surveyed studies.

The effect of joint tamponade on P-FE and RBF commences at joint pressure between 1.3 and 3.9 kPa (I, II, III). The most marked increase of P-FE is observed at joint pressures between 7.8 and 14.3 kPa. The increase of P-FE is highest (312%) in the youngest dogs (I). In older immature dogs the average maximal increase in halothane anaesthesia is seen in the range 170%–225% of the resting P-FE (II, III, IV, VIII). The elevation of baseline P-FE pressure is accompanied by increased pulse amplitude, as found during selective ligation of the medial genicular vein (VI) (Fig. 13). At joint pressures approaching the systemic arterial pressure, the pulse amplitude decreases and the baseline pressure often declines. In chloralose anaesthesia and

Haldid anaesthesia we have found higher maximal increases in P-FE, up to three fold in older puppies during tamponade (Harving & Nielsen 1983, Holm et al. 1986).

The changes of RBF in the distal femoral epiphysis during tamponade are described in III and VII. In relation to the flow increase in "nonloaded" bone compartments, i.e. compartments where the supplying and draining vessles supposedly are not affected, a decrease of RBF was found in the femoral epiphysis in III, although a significant increase in RBF was measured during venous tamponade, as compared with the initial value.

However, a distinction must be made between the effect of joint cannulation and that of the tamponade when dealing with actions of knee joint tamponade on the juxta-articular vasculature.

Cannulation of the knee cavity was followed by an insignificant increase of RBF in the distal femoral epiphysis of the control knees in VII (Table 8). Continuous intraosseous monitoring of pO_2, pCO_2 and p-Argon by mass spectrometry showed a significant increase of pO_2 secondary to joint cannulation, suggesting an increase of RBF (Harving & Nielsen 1983).

On beforehand the change in RBF of the distal femoral epiphysis during tamponade might depend on the pressure of the tamponade, the duration of the tamponade, whether the tamponade is abruptly applied or gradually introduced, and on the location in the epiphysis.

During *venous tamponade,* i.e. joint pressure elevation between CVP and diastolic arterial pressure, an increase of RBF in the distal femoral epiphysis is observed (III, VII, Table 9). At higher pressures (13.0 kPa), a significant drop in RBF was observed in VII, while flow was still elevated in III, probably due to the factors outlined above. A difference between the two groups investigated in VII at a tamponade of 13.0 kPa was noted. RBF was significantly lower in the group of dogs, where abrupt tamponade was applied than in the group, where there was an intermediate pressure of 6.5 kPa. This may reflect the presence of activated regulatory mechanisms for epiphyseal bone blood flow in the latter group.

The different areas of the distal femoral epiphysis investigated are not equally vulnerable to joint tamponade. The lateral condyle is clearly the least vulnerable (III, VII) (Tables 8 and 9), but so far no anatomical explanation has been offered. It may be, that the venous drainage from the lateral condyle is mainly directed via the epiphysio-metaphyseal venous network, which to some extent is extra-articularly located.

The intraosseous gas measurements in the distal femoral epiphysis showed that venous tamponade caused an immediate fall in pO_2 followed by a rise. This U-shaped change in pO_2 disappeared at tamponade pressures close to MAP, which indicates that the regulatory mechanisms cannot overcome the loading (Harving et al. 1986). The pO_2 showed irreversibly lower values from at tamponade of 75% of MAP and remained significantly decreased from initially 6.6 ± 0.7 kPa to 3.6 ± 0.5 kPa at a tamponade of 100% MAP. This might implicate, that the epiphyseal bone vasculature of the knee possesses regulatory mechanisms for bone blood flow, which within periods of up to an hour are capable of overcoming knee joint tamponade up to 75% of MAP. This

is in accordance with equivalent observations in rabbits, where a tamponade of 85% MAP resulted in a significant depression of epiphyseal pO_2 from 5.1 kPa to 4.0 kPa (Grønlund et al. 1984).

In the normal hip joint of puppies acute venous tamponade may significantly depress the RBF of the caput femoris epiphysis (Launder et al. 1981, Lucht et al. 1983). This points out a difference in the susceptibility of femoral head blood flow to joint tamponade from that of the distal femoral epiphysis of the knee. Woodhouse (1964) showed that puppies developed complete avascular necrosis of the femoral head after venous tamponade of 6.7 kPa for 12 hours.

During arterial tamponade of the knee, i.e. joint pressure elevation above MAP, the RBF of the distal femoral epiphysis is significantly decreased in the medial femoral condyle and in the trochlear area, whereas the RBF in the lateral condyle is less vulnerable, being only significantly depressed when abrupt tamponade is applied (VII, Table 8). RBF further decreases when the joint pressure is raised from 13.0 kPa to 26.0 kPa (Table 9), but probably only when the tamponade of 13.0 kPa has been introduced gradually.

Intraosseous gas tensions during arterial tamponade show unchanged values compared with those measured at 100% MAP. Abruptly introduced tamponade of 150% MAP is followed by hyperaemia of a few minutes' duration, as judged by initially elevated pO_2, and decreased pCO_2. Thereafter pO_2 decreased significantly from an initial value of 7.1 ± 1.1 kPa to 4.8 ± 1.2 kPa, whereas pCO_2 insignificantly increased from 6.0 ± 0.7 kPa to 6.7 ± 0.8 kPa. (Harving et al. 1986).

In the hip joint arterial tamponade of 20.0 kPa, i.e. 163% of MAP causes cessation of the femoral head blood flow (Lucht et al 1983). Arterial tamponade for 10 hours leads to irreversible necrosis of the femoral head epiphysis in puppies (Tachdjian & Grana 1968). It has not been investigated whether long term arterial tamponade of the knee will lead to necrosis of the distal femoral epiphysis. However we have demonstrated suppurative arthritis to cause avascularity of the distal femoral epiphysis judged by absence of uptake of ^{99m}Tc-MDP on bone scan after 48 hours of arthritis (Knudsen et al. 1986).

Evacuation of the simulated joint effusions from the normal knees is accompanied by qualitative changes of IOP pressure tracings dependent on the level of joint pressure introduced, and the effluent flow velocity from the joint cavity. If IAP surmounts the systemic pressure, a short period of elevated IOP and increased pulse wave amplitude is usually found before normalization of the IOP level. This takes place instantly when emptying the joint cavity. pO_2 normalizes within 3 minutes (Harving et al. 1986), whereas p-Argon is increased up to 30 min posttamponade, supporting the findings of hyperaemia 1/2 hour posttamponade in the distal femoral epiphysis (III, VII).

In conclusion, tamponade of the knee joint cavity influences the haemodynamics of the distal femoral epiphysis. The impact depends on the below factors.

1. The degree of tamponade. IAP increases up to 75% of MAP are tolerated for up to one hour probably due to activation of regulatory mechanisms of epiphyseal bone blood flow.

2. The rate of introduction. Abruptly introduced tamponade close to MAP impairs the blood supply to the epiphysis significantly more than gradually introduced tamponade at the same level.
3. Duration of the tamponade. Below an IAP of 100% of MAP, changes of IAP are followed by tardive, adaptive shifts in the intraosseous pO_2.
4. Location in the epiphysis. The regional blood flow of the lateral condyle of the distal femoral epiphysis is less susceptible to joint pressure increase than the medial condyle and the trochlea patellar area.

Joint effusion pressures at 0.9–2.9 kPa may cause a significant elevation of IOP in the femoral epiphysis. Joint tamponade up to 75% of MAP results in an increase of IOP up to 300% of its initial value. RBF rates are increased at low tamponade levels, whereas the intraosseous pO_2 measured remains unchanged. Above 75% of MAP, IOP may decline, and RBF and pO_2 are depressed. Evacuation of the joint cavity following tamponade results in immediate return of IOP, while reactive hyperaemia up to 30 min posttamponade prevails.

Patella

The changes of the patellar haemodynamics during simulated joint effusion parallel the findings in the distal femoral epiphysis (II, III, VII Tables 8 and 9). The increase of IOP during venous tamponade is more pronounced than the IOP increase in the distal femoral epiphysis. No plateau of increase is reached. This could be secondary to the use of perfusion in the pressure recording system in the relatively small bone compartment. RBF measurements and X-ray contrast clearance rates revealed hyperaemia in the patella during venous tamponade. At higher tamponade levels, RBF was profoundly decreased, suggesting a more pronounced vulnerability of the patellar blood supply than the supply of the distal femoral epiphysis in the presence of high pressure joint effusions. Posttamponade hyperaemia is also present in the patella.

Proximal tibial epiphysis

In contrast to the distal femoral epiphysis and the patella, the supplying and draining vessels of the proximal tibial epiphysis are largely extra-articularly located (Lucht et al. 1981). The IOP of the proximal tibial epiphysis is therefore unaffected by joint tamponade (Lucht et al. 1981, I, II, III, IV, VIII).

However, considerable hyperaemia may be found during and after venous tamponade (III, VII Table 9) corresponding to the hyperaemia in the distal femoral metaphysis. The hyperaemia found in the "nonloaded" bone compartments during tamponade may reflect the attempt of the regulatory mechanisms of blood flow to overcome the load. Abruptly introduced tamponade is not accompanied by this effect (Table 8). The trigger mechanism for this vasodilation is inknown. Isolated increase of venous outlet resistance from the distal femoral epiphysis does not release hyperaemia of the juxta-articular bones of the knee (VI).

E. Effect of exercise training on RBF of the knee

The most significant change of systemic haemodynamics during exercise is an increase in cardiac output. At the same time cardiac output is redistributed in favour of exercising muscles. Blood flow in working muscles may increase ten-fold or more. The vasodilator mechanisms involved in exercise hyperaemia are multiple and depend on 1) the exercise pattern, 2) the duration of exercise, and 3) the relationship between O_2 supply and use (Sparks 1978). The vasodilation in muscles is not affected by sympathectomy or by chronic somatic denervation (Heistad & Abboud 1974). the proposed vasodilator mechanisms are pO_2, interstitial K^+, tissue hyperosmolality, pH, and a myogenic response. In nonexercising muscles a three-fold increase in vascular resistance is found during exercise (Gross et al. 1979).

The concurrent effect of exercise on the haemodynamics of the knee depends on the tissue concerned. Both in mature and immature bone, exercise results in vasoconstriction (Gross et al. 1979, Tøndevold & Bülow 1983, X, Table 10). In the immature knee, we found a significant decrease of regional blood flow in all juxtaarticular bone compartments during exercise, whereas cortical bone blood flow was unchanged (X). The RBF of the knee joint capsule increased two-fold.

The induction of vasoconstriction in bone during exercise is most likely explained by increased adrenergic activity. An alpha-adrenergic vasoconstriction in bone and a beta-adrenergic relaxation in the synovial vascular bed during exercise may explain the different vascular responses of the normal knee during exercise. During prolonged exercise both plasma noradrenalin and adrenalin will increase (Christensen et al. 1979). A neurogenic element is probably working in concert, since sympathectomy inhibits exercise-induced vasoconstriction, in particular in the distal part of bone (Rutherford & Valenta 1971).

F. Conclusions

The haemodynamics of the normal knee appears closely related to the metabolic activity necessary for appositional growth of the epiphyseal bone and endochondral growth of the epiphyseal plates.

The intraosseous pressures

The intraosseous pressures in the juxta-articular bones range from 6% to 24% of the mean arterial pressure depending on medication and anaesthesia applied during measurement. The highest pressures in the knee are found in the patella, the lowest in the distal femoral epiphysis.

Table 10. Regional blood flow rates ($ml \times 100g^{-1} \times min^{-1}$) in 5 months mongrel puppies at rest, during exercise training at 4 km per h and 30 min post exercise. Mean values and 95% confidence limits in brackets. N=9.

	Rest	Exercise	Post exercise
Acetabulum	16 (12–22)	10 (7–13)	17 (13–23)
Caput femoris epiphysis	27 (19–40)	14 (10–20)	19 (13–27)
Proximal femoral metaphysis	24 (17–33)	17 (12–24)	20 (15–28)
Cortical bone femoral diaphysis	7 (5–10)	8 (5–11)	11 (8–16)
Distal femoral metaphysis	28 (17–48)	11 (7–19)	15 (9–25)
Peripheral femoral epiphysis	27 (21–36)	15 (11–20)	20 (15–27)
Patella	15 (12–20)	11 (9–15)	16 (12–21)
Proximal tibial epiphysis	20 (11–37)	15 (8–28)	23 (12–43)
Proximal tibial metaphysis	30 (19–46)	20 (13–31)	29 (18–44)
Cortical bone tibial diaphysis	6 (4– 8)	6 (4– 9)	10 (7–14)
Rectus femoris muscle	8 (5–15)	98 (54–178)	7 (4–14)
Vastus lateralis muscle	27 (17–45)	64 (39–105)	32 (20–53)
Anterior tibial muscle	8 (4–13)	120 (68–213)	9 (5–17)
Knee joint capsule	2 (1– 3)	5 (3– 8)	3 (2– 5)

Haemodynamics of the knee joint capsule

At rest the knee joint capsule and menisci receive a fairly equal blood supply, which is less than that of the adjacent bone. The microcirculation of the joint capsule is characterized by small microvascular volumes, a tissue haematocrit close to that of epiphyseal bone, and a short transit time of blood, almost similar to that found in cortical bone.

Microcirculation of the juxta-articular bones

Within the juxta-articular bones the microcirculation differs between the metaphyses and the epiphysis. The blood flow rates in radiate tissue samples show no regional differences, but the microvascular volumes are significantly higher in the metaphysis than in the epiphysis. Accordingly, the transit time of blood components is relatively prolonged reflecting a higher capillary density in the metaphyses. Concentric bone sample analysis provides a more differentiated picture of the haemodynamic milieu and allows recording of high subchondral RBF rates and lower RBF rates in the central part of the epiphyses. Likewise, very high RBF rates may be measured in the calcification zone of the growth plates.

The influence of joint effusion on the juxta-articular tissues

The haemodynamic changes following joint tamponade depend upon the relationship between the joint cavity and the compartment of interest. The level of joint pressure, the rate of pressure increase, and its duration are also of major importance. The joint capsule, the patella and the distal femoral epiphysis are the tissue compartments most prone to experience elevation of the joint pressure, whereas the tibial epiphysis and the juxta-articular metaphyses show no vulnerability, and may react with hyperaemia in response to joint tamponade.

Joint effusion pressures down to 0.9 kPa may cause significant elevation of IOP in the distal femoral epiphysis. At low tamponade levels RBF tends to increase in the susceptible tissue compartments. Tamponade levels up to 75% of MAP are tolerated by the distal femoral epiphysis. Above this level RBF and intraosseous pO_2 decline and ischaemia developes. Posttamponade hyperaemia is most pronounced in the joint capsule, but is also present in the loaded bone compartments as an indicator of accumulating vasodilating substances.

Effect of exercise training on the haemodynamics of the knee

Exercise training cause redistribution of cardiac output from all juxta-articular cancellous bones to the working muscles. This vasoconstriction may reduce RBF by 50% in bone. However, in the joint capsule RBF significantly increase during exercise.

An alpha-adrenergic vasoconstriction is suggested to dominate bone blood flow, and beta-adrenergic vascular relaxation might be responsible for the hyperaemia in the joint capsule during exercise.

IV Regulation of haemodynamics

The regulatory mechanisms of knee joint circulation may count a number of central and local control mechanisms, that serve to maintain both organismic and local tissue homeostasis as in other tissues and organs (Johnson 1978). Studies of the circulatory control of juxtaarticular bones are not available. However, the principles of haemodynamic control of long bones have been elucidated by several authors (Drinker & Drinker 1916, Trotman & Kelly 1963, Michelsen 1967, Shim 1968, Gross et al. 1979, Driessens et al. 1981, Tøndevold 1983).
The three principal control mechanisms are of neural, humoral, or metabolic origin. The central control is basically neural and humoral, whereas the local control is primarily governed by metabolic and myogenic factors. A local neurogenic control mechanism, the so-called veno-arteriolar axon reflex mechanism exists in some organs (Henriksen 1977).

A. Neural control of bone circulation

All types of bone in humans and animals are richly supplied with nerves (Shim 1977). The blood vessels at both intraosseous and extraosseous levels, especially arteries, are abundantly supplied by both myelinated and unmyelinated nerve fibers. Nerve fibers are seen in all parts of bone at the surfaces of small arterioles and capillaries. In larger arteries they are found both at the surfaces and between the tunicae adventitia and media. Histochemical examination has demonstrated the presence of both adrenergic and cholinergic nerve fibers (Shim 1977). Within joints both the synovial and fibrous part of the joint capsule are richly supplied with nerves with both kinesthetic, reflexive, nociceptive, and vasomotor functions (Newton 1982, Freeman & Wyke 1967, Wyke 1981).
Thus, the blood vessels of bones and joints are innervated and may participate in the overall body homeostasis.

Central control

During rest there is a certain centrally elicited sympathetic tone in the peripheral blood vessels. The neural control mechanisms that alter this vasomotor tone are located at

various sites in the central nervous system. The cholinergic dilator mechanisms arise from the motor cortex, the hypothalamic defence area, and from the midbrain (Folkow & Neil 1971). The adrenergic vasoconstrictor activity is predominantly elicited from the vasomotor center in medulla oblongata, but areas in the midbrain and the hypothalamus may also contribute (Sagawa et al. 1974). Adjustments of the vasomotor tone are constantly taking place both via central inhibiting areas in the gyrus cinguli and via peripheral reflex mechanisms. The latter are mainly arterial baroreceptors in the aortic arch and carotid sinus. Chemoreceptors at the same locations may also alter the vasomotor tone, provided the carotid sinus pressure is not too high (Mancia et al. 1976).

Sympathetic nervous control

In bone the role of the sympathetic innervation of blood vessels has been studied by Gross et al. (1979). Increased sympathetic activity achieved either by general hypoxemia, by severe hypotension secondary to haemorrhage (MAP < 6.7 kPa) or by acute denervation of carotid baroreceptors raises the vascular resistance in cortical cortical as well as cancellous bone in chloralose/urethane anaesthetized dogs. Decrease of the central sympathetic outflow obtained by stimulation of the carotid sinus baroreceptors resulted in a decrease of vascular resistance in bone by 30% and by 80% in skeletal muscle in the same model (Gross et al. 1979)

Lumbar sympathectomy and transsection of the sciatic nerve is followed by a fall in vascular resistance in bone resulting in an increased RBF rate (Trotman & Kelly 1963, Shim et al. 1966). In dogs the effect on RBF in bone is a 25–35% increase in the proximal part of the extremity and 30-60% in the distal part. The effect subsides after 1–3 weeks (Davis et al. 1981).

Stimulation of the sympathetic trunk on the other hand causes vasoconstriction and subsequently a decreased RBF (Drinker & Drinker 1916, Weis & Root 1959, Shim et al. 1972, Tran 1980). In the isolated perfused tibia periarterial electrical nerve stimulation caused a frequency dependent increase in perfusion pressure (Driessens & Vanhoutte 1979). Since this response was abolished in the presence of the alpha-adrenolytic drug phentolamine, it was suggested to be secondary to activation of adrenergic nerves (Vanhoutte 1978).

The *sympathetic venoarteriolar axon reflex* is unlikely to be elicited in bone, as the transmural pressure in intraosseous veins is unchangeable. Accordingly, we found no immediate effect on arterial inlet resistance in the distal femoral epiphysis after ligation of the middle genicular vein (VI). However, in both the knee joint capsule and in the proximal tibial epiphysis an insignificant decrease of RBF was noted, but there was no conclusive evidence of a distal spread of the reflex to bone (VI).

B. Humoral control

A number of hormones affect the circulation in bones and joints, when given systemically or into the perfusate of an isolated perfused tibia of the dog (Wilkes 1969, Gross 1979, Driessens & Vanhoutte 1979, Driessens et al. 1981, Shim et al. 1972). The exact role of these substances in the regulation of haemodynamics during normal physiological circumstances has not yet been established.

Vascular bed of bone

Intravenous infusion of *norepinephrine* (25 micrograms per min) raises the MAP in dogs, whereas the bone RBF is largely unchanged indicating a significant increase of vascular resistance (Gross et al. 1979). In the perfused tibia infusion of norepinephrine causes a dose dependent increase in perfusion pressure, a response which is inhibited by phentolamine (Driessen & Vanhoutte 1979). However, the magnitude of the response depended on the perfusion flow rate. This suggests a length-active tension relationship of the vascular smooth muscle cell of the perfused vessels and is thus an indicative of absence of myogenic tone in the perfused tibia. Therefore, the authors found no effect of *acetylcholine* and *isoproterenol* when infused alone. However, both acetylcholin and isoproterenol caused inhibition of the vasoconstrictive response to norepinephrine.

In intact animals infusion of *isoproterenol* is followed by lowering of the systemic arterial pressure and a concomitant drop in the intraosseous pressure and the venous outflow (Shim et al. 1972). *Epinephrine*, on the other hand, both increases the systemic arterial pressure and decreases the bone blood flow by an arterial vasoconstriction (Shim et al. 1972, Wilkes 1969). *Histamine* and *acetylcholine* at low doses decrease the arterial vascular resistance in perfused tibias (Wilkes 1969), while histamine, acetylcholine and *serotonin* consistently increase the venous outlet resistances. Infusion of *adenosine* (4.7 micromoles x kg^{-1} body weight x min^{-1} intravenously) in anaesthetized dogs decreases the vascular resistance in bone. However, the doses reported to reach this conclusion were unphysiologically high compared with those needed to trigger a vascular response in skeletal and adipose tissue (Hjemdahl et al. 1979).

In perfused tibias from adult dogs *calcitonin* causes a dose-dependent increase in the perfusion pressure, suggesting vasoconstriction of bone vessels (Driessens et al. 1981). Since the effect is not significantly affected by phentolamine, and since the hormone does not interfere with the vasoconstrictor response to adrenergic stimuli, it has been concluded that calcitonin exerts a direct effect on the vascular smooth muscle cells in bone. This may explain the reduction in bone blood flow following calcitonin therapy in Paget's disease as found by thermography (Ring et al. 1977) or by dynamic pool imaging using ^{99m}Tc-labelled human serum albumin (Driessens et al. 1984).

The parathyroid hormone showed no effect on the bone blood vessels of the perfused tibias, although during hypercalcaemia secondary to PTH stimulation, bone blood flow is high (Boelkins et al. 1976).

Hydrocortisone, at low concentrations, augments the stimulative effect of norepinephrine, while in higher concentrations, a dose dependent inhibition of the response to adrenergic activation is found (Driessens et al. 1981). The depressant effect of hydrocortisone is antagonized by propranolol, which suggests that the glucocorticoid facilitates beta-adrenergic relaxation of the vascular smooth muscle cells by catecholamines.

Synovial vascular bed

The synovial microcirculation is also a product of an agonist-antagonist balance of adrenergic, cholinergic and a number of other receptor influences. Intra-articular administration of *isoprenaline* results in a marked increase in the clearance rate of 133 Xe from diarthrodial joints (Dick et al. 1971a). In contrast, the clearance rate is markedly decreased, when *norepinephrine* is administered. The effect of beta- and alpha-receptor stimulation can be blocked by propranolol and phentolamine, respectively, confirming the presence of both *beta- and alpha-receptors* in the synovial vascular bed (Dick et al. 1971 a, b). Similar studies have been conducted with *metacholine* and atropine demonstrating the participation of *cholinergic receptors* in synovial blood flow control (St Onge et al. 1971). Likewise Grennan et al. 1975 demonstrated a *H_2 receptor* control of synovial blood flow showing that metiamide, a H_2 receptor antagonist, produced a dose related abolition of the histamine vasodilation response. This may explain the effect of *cimetidine* on adjuvant arthritis reported by Al-Habdubi and Zeitlin (1979).

C. Local control

Autoregulation

Autoregulation is the tendency for blood flow to remain constant in the face of local changes in arterial pressure to the organ (Johnson 1978). It is seen in most organs and tissues of the body (Johnson 1964). The resistance changes predominantly occur at the precapillary segment of circulation. The role of autoregulation is both to maintain adequate nutrient flow and to maintain a constant capillary pressure.

The factors that may influence the tone of resistance vessels in peripheral circulation in generel may be either myogenic or metabolic, i.e. autoregulation can be elicited either by pressure sensitivity of the arterioles or by accumulation or washout of vasodilator metabolites following a change in flow (Johnson 1978). The mechanisms involved in local regulation of bone blood flow have not yet been fully clarified.

Judged from intraosseous pressure measurements in adult dogs respirating increasing concentrations of halothane, bone circulation exhibits autoregulation down to a mean arterial pressure of 10.6 kPa (Tøndevold 1983). Below this level e.g. during

haemorrhagic hypotension, there is a significant decrease in bone blood flow and an increase in the vascular resistance in bone (Gross et al. 1979).
Likewise, systemic moderate hypoxia reduces blood flow and increases vascular resistance in bones and marrow (Gross et al. 1979).

Metabolic control of bone blood flow

Systemic *hypoxia* below 9.9 kPa was found to decrease bone marrow pressure in dogs (Eriksen et al. 1979). Systemic *hypercapnia* in baboons on the other hand causes an increase of blood flow and decreases vascular resistance in bone and marrow (Gross et al. 1979). Eriksen et al. (1979) found no significant influence on bone marrow pressure in the presence of systemic hypercapnia, but this does not exclude the possibility of increased blood flow. The vasodilator response to hypercapnia has also been observed in rabbits during rebreathing of expired air (Cumming 1962, Shim & Patterson 1967), which thus confirms that carbon dioxide and hydrogen ions are important local mediators of vasodilation in bone.

We found evidence of local metabolic control of epiphyseal bone blood flow (VII). During venous stasis, induced by ligation of the middle genicular vein, no significant changes of arterial inlet resistance was measured after a 5 min stasis. However, after one hour the arterial inlet resistance and the venous outlet resistance were significantly reduced, suggesting the presence of a tardive metabolically induced arterial dilation. The decrease of venous resistance is most likely explained by passive distension, as there are no muscular structures in the intraosseous veins (Wilkes 1969).

The mediator of the arterial dilation is still unknown. It is unlikely that any measurable changes in carbon dioxide, lactic acid, oxygen tension, and pH occurred, since the blood flow was largely unchanged. Other local metabolites such as adenosine and prostaglandins, particularly of the E type, might be responsible. In the rabbit administration of indomethacin (3 mg/kg) causes no immediate (15 min) change in resting bone blood flow, suggesting that prostaglandins do not directly influence the resting bone blood flow (Hierton 1981).

The most detailed information on the role of *adenosine* in vasodilation so far has been obtained from the coronary bed (Rubio & Berne 1975, Johnson 1978). Increased amounts of adenosine and its break down products are found during reactive hyperaemia, hypoxia, and increased cardiac activity. A number of stimuli, predominantly hypoxia, cause immediate synthesis of adenosine in tissue cell walls by hydrolysis of AMP through the action of 5'-nucleotidase. The actual trigger mechanism is still unknown.

In bone, infusion of adenosine causes profound vasodilation. The vasodilating effect of adenosine predominates over the vasoconstrictor stimuli released during haemorrhagic hypotension (Gross et al. 1979). However, there have been no reports on release of adenosine in the vascular bed of bone.

Further evidence of local metabolic control was found *post exercise* in cortical bone (Table 10) (X). Postexercise hyperaemia was solely observed in cortical bone and does probably not represent a repayment phenomenon, since the blood flow in cortical bone

remained unchanged during exercise. It is more likely that the hyperaemia reflects a vasodilation secondary to a change in bone metabolism or that it represents arterial vasodilation following increased intraosseous and venous pressure, which accompany muscular contractions (Valderrama & Trueta 1965, Shim et al. 1972). Delayed hyperaemia was also observed by Tøndevold & Bülow (1983) after two hours of exercise and remained present 45 min post exercise.

Occlusion of the femoral artery in dogs for one min causes an immediate fall of tibial marrow pressure and venous outflow. By release, these parameters return to normal levels after a short period (a few min) of overshooting, interpreted as an indicative of reactive hyperaemia (Shim et al. 1972).

Inversely, *transient circulatory arrest* in canine long bone marrow and cortex for two hours achieved by an external pneumatic tourniquet does not result in tardive changes (15 min and 24 hour) in bone blood flow (Kennedy et al. 1981). However, these studies are less suitable for analysis of bone blood flow regulation, because of the possible overshadowing of the profound postischaemic hyperaemia in muscles, skin and nerve tissue, and possibly by an increased alpha-adrenergic stimulation in the 15 min posttourniquet measurement.

An equivalent phenomenon of probably metabolically mediated vasodilation is present in the distal femoral epiphysis and patella following *tamponade* (84-100% of MAP) of the knee joint capsule (III, VII, Table 8). However, in these experiments vasodilating substances probably accumulated during tamponade. Our later experiments using mass spectrometric monitoring of intraosseous pO_2 and pCO_2 have confirmed this assumption. During tamponade (100% of MAP) pO_2 decreased from 6.5 kPa to 3.8 kPa whereas pCO_2 increased from 5.7 to 6.5 kPa (Harving et al. 1986). Aspirated intraosseous blood showed a concomitant increase of lactic acid from 3.3 mmol/l to 5.3 mmol/l (Holm et al. 1986). It is not known whether vasodilating substances like pCO_2 and lactic acid act independently or via changes in pH as in the brain (Wahl et al. 1970).

In the femoral epiphysis of the rabbit knee, joint tamponade of 85% of MAP provoked a significant fall of pO_2 from 5.1 to 4.0 kPa measured by mass spectrometry after 10 min of tamponade (Grønlund et al. 1984), posttamponade values were not reported. Their results suggest that the vasodilation secondary to tamponade reported by us (III) could be a delayed phenomenon and thus probably of metabolic origin. Our observations (Harving et al. 1986) utilizing continuous intraosseous registration of pO_2, pCO_2 and p-Argon by mass spectrometry suggest that autoregulation of epiphyseal bone blood flow exists, maintaining unchanged intraosseous gas values during tamponade of the knee from zero to 75% of MAP. At higher levels of tamponade pO_2 and p-Argon subsequently decline. Release of the tamponade is followed by an increase of pO_2 and p-Argon indicating hyperaemia (Harving et al. 1986).

However, complex problems are encountered evaluating and comparing various studies on RBF autoregulation. Surgical preparations and the use of different anaesthetics (Skovsted 1982) may change the sympathetic nervous system activity and the responsiveness of the microvasculature.

Metabolic control of synovial blood flow

In the joint capsule the local control of microcirculation is probably also governed by a number of metabolic substances. As evidenced by *joint tamponade* experiments (III, VIII, Lucht et al. 1983), synovial ischaemia leads to profound vasodilation. A multifold blood flow increase is present 30 min posttamponade. The response in the joint capsule is much more pronounced than the response in bone. The substances, which produced postischaemic vasodilation in the synovium, may include pO_2, pCO_2, H+, lactate and adenosine as outlined for bone, but their exact role in regulation of synovial blood flow under physiological conditions remains to be ascertained.

In contrast to the findings in cortical bone, *exercise* causes no tardive changes of normal synovial blood flow, albeit during exercise a two-fold increase of synovial blood flow is present (X). The vasodilation elicited by exercise is most likely explained by a beta-adrenergic effect, exerted by increased levels of circulating catecholamines (Christensen et al. 1979).

It is well known that joint inflammation is associated with increased levels of pCO_2, H+ and lactic acid (McCarty 1980). A number of mediators of microvascular dilation have been demonstrated during synovitis such as histamine (Al-Habdubi et al. 1979), and prostaglandins of the E series (Blackham et al. 1974, Higgs et al. 1974).

The relative effect of various prostaglandins, bradykinin, histamine and 5-hydroxytryptamine (5-HT) on canine synovial microcirculation were investigated by Dick et al. 1976. Prostaglandin E_1 was the most potent vasodilator tested, whereas prostaglandin $F_{1\ alpha}$ had no effect. Prostaglandin E_2 is also a potent vasodilator, but approximately five to ten times less potent than proglandin E_1. On a molar basis bradykinin is as potent as prostaglandin E_2. The vasodilator effect of histamine and 5-HT lay between that of prostaglandin E_2 and bradykinin on the one side and prostaglandin $F_{2\ alpha}$ on the other. Prostaglandin E_1, bradykinin and histamine have also been demonstrated to increase synovial vascular permeability (Grennan et al. 1977).

In the synovium of the rabbit knee no changes of resting blood flow are encountered following administration of indomethacin (Hierton 1981). As for bone, this may implicate that prostaglandins only play a minor role in the regulation of synovial blood flow during normal circumstances.

D. Conclusions

The regulatory mechanisms of the haemodynamics of the immature knee count a number of both central and local control mechanisms. The blood vessels of both the juxta-articular bones and the joint capsule are richly innervated and participate in the overall body homeostasis. The role of humoral control during normal physiological circumstances is not yet established. However, the circulation of both the bones and the soft tissues is a product of agonist/antagonist balance of adrenergic, cholinergic, and a number of other receptor influences. The adrenergic control in bone and joint

capsule is predominantly alpha-adrenergic and beta-adrenergic, respectively. Autoregulation of the blood flow of the knee exists. The local control of blood flow in the juxta-articular bones and joint capsule is mainly of metabolic origin, the microcirculation of the joint capsule being more reactive to changes in the metabolic environment. The mediators of vasodilation in the autoregulation of subchondral bone blood flow are yet unknown.

V The arthritic knee

Studies of the normal immature knee have demonstrated, that the haemodynamic impact of joint effusion on the juxta-articular tissues depends on the anatomical relationship between the joint capsule and the compartment of interest, the level and the duration of the pressure load. During physiological joint use considerable variations in joint pressure can be expected in concert with a number of local and systemic regulatory mechanisms, which tend to preserve bone blood supply and redistribute blood flow in favour of working muscles, respectively.

The evaluation of haemodynamic changes of the knee in arthritis during physiological circumstances is therefore rather complex. Thus, the present chapter endeavours to give a stepwise description of the reactions of the individual vascular compartments following acute and chronic synovial inflammation, static changes of joint effusion pressure, and exercise training.

A. Microcirculation of the joint capsule

The most significant haemodynamic change in arthritic knees is found in the joint capsule. Acute inflammation of the synovial membrane is accompanied by a local four-fold elevation of RBF at a joint effusion pressure of 1.3 kPa (VIII). In halothane anesthesia we found a RBF of the joint capsule of 35 $ml \times 100g^{-1} \times min^{-1}$ (23–52, 95% confidence limits). No correlation between joint pressure and synovial blood flow was present in our study of acute arthritis, though a positive correlation could be demonstrated between the joint pressure and the metaphyseal RBF of the knee (VIII).

During the first phase of acute arthritis, the synovial membrane is dominated by focal ulcerations, fibrinous exudate, and pseudomembrane formation. The deeper layers of the synovial membrane are characterized by granulation tissue with fibroblasts and endothelial proliferation. After two to three weeks the membrane gradually assumes a histologically more organized appearance with villous hypertrophy, organized fibrin deposits, and chronic inflammation with follicular accumulation of lymphocytes and plasma cells. At this stage the corresponding synovial RBF show some variation. Thus in halothane anesthesia mean RBF rates range from 9 to 17 $ml \times 100g^{-1} \times min^{-1}$ (Tables 8 and 9, IX). In the awake nonmedicated dog, the capsular flow rate is significantly higher ranging from 17 to 44 $ml \times 100g^{-1} \times min^{-1}$ (X).

In chronic arthritis (12 weeks) the microvascular volume is two-fold increased compared with the normal capsule (IX), but the value is clearly less than that measured in the adjacent bone. The tissue haematocrit is low.

The increase of the synovial blood flow in arthritis as measured by us may be due to a relatively higher proportion of synovial tissue in capsular biopsies from the arthritic knee. Other factors such as capillary recruitment, vasodilation, and angioproliferation may also be involved. Carrageenan-induced inflammation causes release of vasodilating substances like PGs, bradykinins and histamines (DiRosa 1972). In addition, low pO_2, elevated pCO_2 and H^+ of the metabolically highly demanding inflamed synovial tissue will contribute to the vasodilating forces (Lund-Olesen 1970, McCarty 1980). Factors tending to obstruct the synovial blood flow are decreased red cell deformability, if present at all, as well as synovial tissue oedema and elevated joint effusion pressure.

The intra-articular pressure of the knee in chronic Carrageenan-induced arthritis at rest is 0.7–1.3 kPa (IV, VII, X). Evacuation of the genuine joint effusion of the arthritic dog knee does not result in a significant elevation of synovial RBF when measured after 30 min (Table 8). However, a complementary influence of oedema and pressure should be considered. In view of the high metabolic demands of the hypertrophic synovial villi, it has been hypothesized, that synovial tissue oedema and synovial fluid volume increases contribute to local hypoxia and inflammation, thus playing a major role in the pathogenesis of rheumatoid arthritis and other nonspecific synovitides (Ahlqvist 1984).

The rheumatoid microvascular alterations include venular and capillary dilation (Branemark 1963, Goldie 1970). The findings of decreased local haemotocrit and a short mean transit time of blood (IX) suggest that hyperaemia of the arthritic knee joint capsule primarily is due to local vasodilation. Inversely Branemark (1963) and Goldie (1970) reported a decrease of the corpuscular flow velocity, which they explained by an increase in blood viscosity due to egress of plasma. The data presented by us (IX) indicate, that the local plasma volume rather increases relatively to the red cell volume, which is to be exspected for rheological reasons, when flow increases due to vasodilation (Cokelet 1978). However considerable microvascular differences may arise between the luminal and the peripheral parts of the hypertrophic synovial membrane, which may account for the different observations reported.

Influence of static joint tamponade on synovial blood flow

The joint effusion pressures in arthritis measured by us were always resting values. Due to the decreased compliance of the inflamed joint capsule secondary to perisynovial fibrosis, higher intra-articular pressures are to be exspected during joint use. In the investigation of the effect of an isolated intra-articular pressure increase on the haemodynamics of the arthritic knee (VII, Tables 8 and 9), venous tamponade of 6.5 kPa, i.e. 37 mmHg, did not alter the synovial RBF. Tamponade of 13.0 kPa i.e. 74 mmHg caused cessation of synovial RBF both when the tamponade was gradually and abruptly introduced. The finding of flow increase in some dogs during tamponade of 26 kPa is most likely a result of rupture of the synovial membrane.

The threshold of intra-articular pressure elevation, at which the synovial RBF is obstructed, is thus below the arterial blood pressure. This value is easily reached during immobilization of the arthritic joint, e.g. traction in extension of the hip in coxitis or casting of the knee in extension. The significance of joint pressure elevation for the transsynovial transport of nutrients, the function of lymphatics, and the proliferation of synovial tissue in synovitis needs further investigation.

Influence of exercise

During joint use the presence of even minor joint effusions creates pressure increases far above MAP, thereby obstructing synovial blood flow (Jayson & Dixon 1970c). On the other hand, joint motion may also create considerable pressure fluctuations, which contribute to transsynovial transport. Continuous passive joint motion (CPM) facilitated the transsynovial transport of blood from the joint cavity of the normal rabbit knee (O'Driscoll et al. 1983). Inversely passive motion of the dog knee following urate crystal-induced inflammation produced larger effusions, higher synovial fluid leukocyte counts, and increased leakage of intravenously injected carbon from synovial vessels (Agudelo et al. 1972). At the same time, clearance of ^{133}Xe is markedly accentuated as a sign of increased synovial blood flow, whereas pH of the synovial fluid was unchanged low compared with immobilized urate injected control knees. Exercise in dogs with Carrageenan-induced unilateral gonarthritis does not, at first glance, change synovial RBF compared with preexercise values (X, Table 12, Fig.19+20). A threefold increase of synovial RBF was observed 30 min after exercise, possibly suggesting, that the nutritive demands of the inflamed synovium are not met during exercise. It can be speculated that the luminal part of the synovium is particularly vulnerable. We observed increasing joint effusion following exercise.

Thus, there is no doubt that joint motion and use accelerate the inflammatory process. Whether this is secondary to local synovial ischaemia or by facilitated transsynovial transport of leukocytes is still an open question. Nevertheless, controlled movement such as continuous passive movement (CPM), as recently introduced in the postoperative treatment of intra-articular fractures (Salter et al. 1984), may reduce synovial joint effusions and inflammation in chronic arthritides.

B. Juxta-articular bones

Intraosseous pressure

The intraosseous pressure of the juxta-articular bones of the immature knee in arthritis varies according to the anatomical relationship to the joint capsule. Acute Carrageenan-induced gonarthritis showed an insignificant pressure elevation in the femoral epiphysis and an insignificant pressure reduction in the tibial epiphysis (VIII). After arthritis for 12 weeks, the IOP of the femoral epiphysis was significantly raised as

opposed to the femoral metaphyseal and tibial epiphyseal pressures (IV). The pressure tracings from the femoral epiphysis may show an increased amplitude of pulse waves, indicative of intraosseous vasodilation (Fig 16) (IV). Intraosseous phlebography with a prolonged clearance time in the femoral epiphysis suggests an increased vascular volume (IV). Phlebographies were only performed in the femoral epiphysis, and they pointed to a state of venous engorgement known from the vascular lesion of other bone and joint disorders (Arnoldi & Reimann 1979, Driessens et al. 1984).

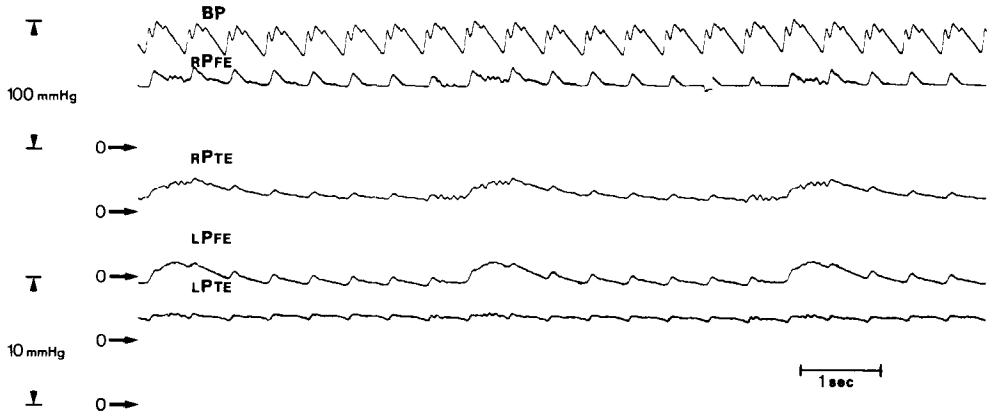


Figure 16. Pressure tracings from the brachial artery (BP), the arthritic femoral epiphysis (RP_{FE}),the arthritic tibial epiphysis (RP_{TE}), the control femoral epiphysis (LP_{FE}), and the control tibial epifysis (LP_{TE}). An increased amplitude of pulse wave is present in the arthritic femoral epiphysis.

Microcirculation of the metaphysis

The regional blood flow of the distal femoral metaphysis is significantly elevated in acute arthritis (VIII). This is the only bone compartment, where a total increase of RBF is found. The blood flow may still be significantly elevated at 10 weeks (Table 8), but after 12 weeks it tends to be at the level of the contralateral knee. During this period, a substantial hypotrophy of the distal femoral metaphysis develops (Fig 11). The initial hyperaemia may reflect an increased osteoclastic activity at the peripheral rim of the metaphysis as indicated by the patchy appearance of this area on contact autoradiographies (Fig 12). The microcirculation of the metaphyses in chronic arthritis is further characterized by an unchanged microvascular volume and a shorter transit time of blood (Table 11, Fig.17+18).

The normal immature metaphyseal bone is very richly supplied with blood at the zone of calcification (Schnitzer et al. 1982). In chronic Carragheenan-induced arthritis the zone of calcification is narrowed and its uptake of ^{99m}Tc-MDP is lower concomitant with a decreased endochondral growth of the epiphyseal plate (V) (Fig.12). The changes in metaphyseal bone haemodynamics measured do not explain this major

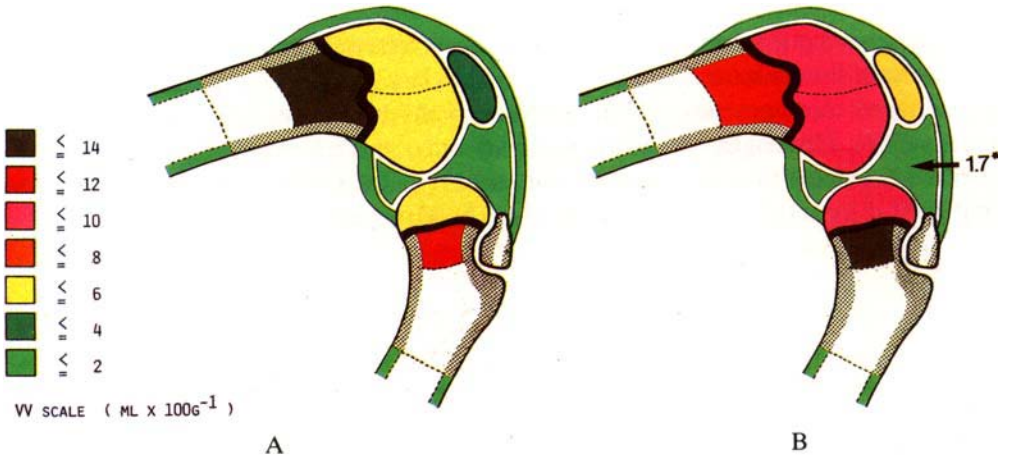


Figure 17. Microvascular volumes of the normal immature dog knee (A) and the arthritic knee (B) estimated from the distribution volumes of ^{125}I-fibrinogen and ^{51}Cr labelled red cells. N=8.

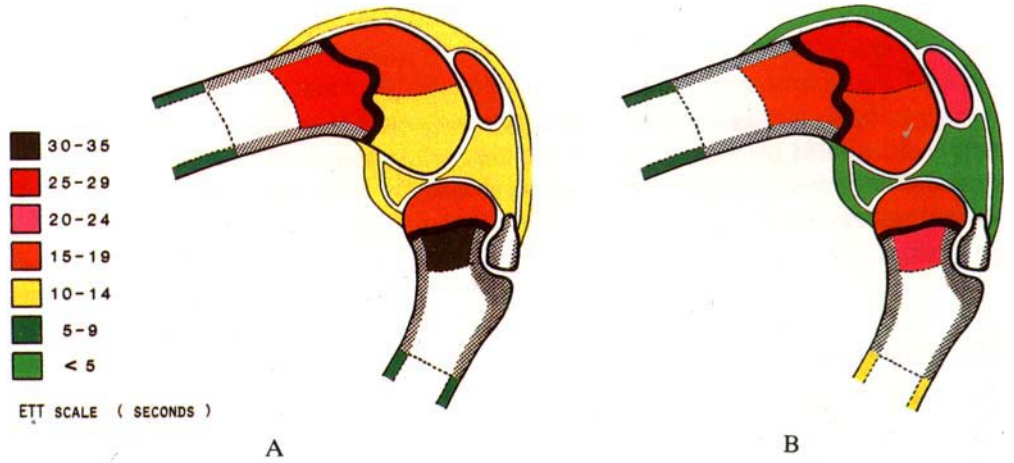


Figure 18. Mean transit time of erythrocytes in the normal knee (A) and in the arthritic knee (B) calculated as the red cell volume/red cell flow ratio. N=8.

change of growth and bone metabolism. The trend of smaller vascular volumes and a shorter transit time of blood is in accordance with a diminished metaphyseal capillary network.

The microcirculation of the juxta-articular epiphyses and the patella

Intraosseous pressure measurement with phlebography in the arthritic knee shows venous engorgement in the femoral epiphysis (IV), but this is not reflected by changes

in total RBF rates (VII, VIII, IX). In all juxta-articular bones of the arthritic knee, the microvascular volumes are increased (IX) (Fig.17). The unchanged RBF and tissue haematocrit act to significantly prolonged transit of both red cells and plasma. The transit time in the trochlea area of the distal femoral epiphysis is longer than in the tibial one. The trochlea area of the femoral epiphysis, which shows the most marked arthritic changes, also experiences the greatest prolongation of transit time (Fig 18),(Table 11). The epiphyseal plates constitute a line beyond which this microvascular change does not extend.

Table 11. Microvascular volumes (VV=Cr^{51} red cell volume+I^{125}fibrinogen volume), tissue haematocrit (HCT) and transit time of red cells (ETT=$\frac{\text{red cell volume}}{\text{red cell flow}} \times 60$) in the arthritic knee. (N=8, 95% confidence limits in brackets)

	VV $ml \times 100g^{-1}$	Hct %	ETT seconds
Femoral diaphysis	1.3 (1- 2)	25 (21–30)	10 (5–14)
Femoral metaphysis	12.0 (9–14)	20 (17–23)	18 (12–28)
Femoral epiphysis trochlea	9.7 (8–12)	21 (19–25)	29 (16–44)
Patella	*5.5 (4- 7)	24 (21–28)	*26 (19–31)
Tibial epiphysis	*8.7 (7–11)	20 (19–21)	18 (10–23)
Tibial metaphysis	14.0 (13–16)	16 (13–19)	**26 (16–31)
Tibial diaphysis	1.4 (1- 2)	25 (21–30)	17 (7–22)
Joint capsule	*1.7 (1- 3)	*11 (9–14)	**2 (1- 3)

*Value significantly increased compared to control knee
**Value significantly decreased compared to control knee

The intimate relationship between the synovial and epiphyseal bone vasculatures point to a possible influence of vasoactive substances from the inflamed synovium on the bone vasculature causing vasodilation. A primary increase of bone metabolism secondary to the influence of PGE_2 and osteoclast activating factor (Yoneda & Mundy 1979) may also cause vasodilation. A third mechanism is the mechanical effect of joint

effusion, which increases the venous outlet resistance from the femoral epiphysis and the patella in particular, and causes vasodilation in normal epiphyseal bone (I,II,III). In the resting knee this effect might be overshadowed by the previously mentioned dilating factors. Accordingly, we found no significant change of epiphyseal and patellar RBF when removing the genuine joint effusion in chronic arthritis (VII, Table 8). The presence of both elevated RBF and VV in ipsilateral hips of the arthritic knees in (IX) lent further credibility to the role of the findings of an increased microvascular volume and a prolonged blood transit time as an indicator in particular of a qualitative change of arthritic subchondral bone vasculature. In the femoral head epiphysis the transit time of blood was unaffected. The vascular change could be caused by vascular recruitment secondary to limb disuse (Semb 1966).

It remains an open question whether the observed vascular change in subchondral bone is primary or secondary to altered bone metabolism. The presence of increased permeable surface area between blood and bone undoubtedly enhances the metabolism and the destruction of bone in juvenile arthritides. In the present model, evaluation of subchondral bone by histomorphometry and bone strength by osteopenetrometry revealed an increased bone formation rate, decreased trabecular bone volume, decreased trabecular thickness, and a 50% reduction of subchondral cancellous bone strength in both the femoral and tibial epiphyses (Holm et al. 1985, Bünger et al. 1983).

Joint scintigraphy yields evidence of a progressive increase of bone metabolism of the epiphyseal bone in chronic arthritis (Stender Hansen et al. 1986). Contact autoradiography confines the increased uptake of ^{99m}Tc-MDP in chronic arthritis to a narrow subchondral zone around bone cysts and at the periphery of osteophytes. The central epiphyseal bone has a decreased uptake. Thus the overall impression is that in "late" arthritis after three months in the present model (V), the repair processes supervene and dominate the metabolic changes of subchondral epiphyseal bone.

The haemodynamic studies so far reviewed do not allow to differentiate between the vascular changes in subchondral and central epiphyseal bone due to the biopsy taking technique (Fig.7). The haemodynamic values obtained from radiate biopsies are average values. By separating these two bone areas some interesting flow changes can be found (X), (Fig.19). In the subchondral bone RBF is significantly higher than that of the central epiphysis. These flow gradients are to some degree also present in the normal epiphysis. A comparison of the RBF rates of the arthritic knee and the contralateral knee at rest reveals that only the decrease of central epiphyseal blood flow is significant (Table 12), which may be due to the limited number of experimental animals. One can speculate that the observed flow gradients both in the normal and arthritic femoral epiphyses probably also exist in the tibial epiphysis and in the patella. However, due to the narrow bone compartments, we were not able to make such separation without damaging the biopsies. The observed flow gradients may also call for a re-evaluation of the measurements of increased juxta-articular vascular volume of bone in arthritis (Table 11, Fig.17). If the increase of VV is confined to the subchondral bone, the increase has been considerably underestimated in IX.

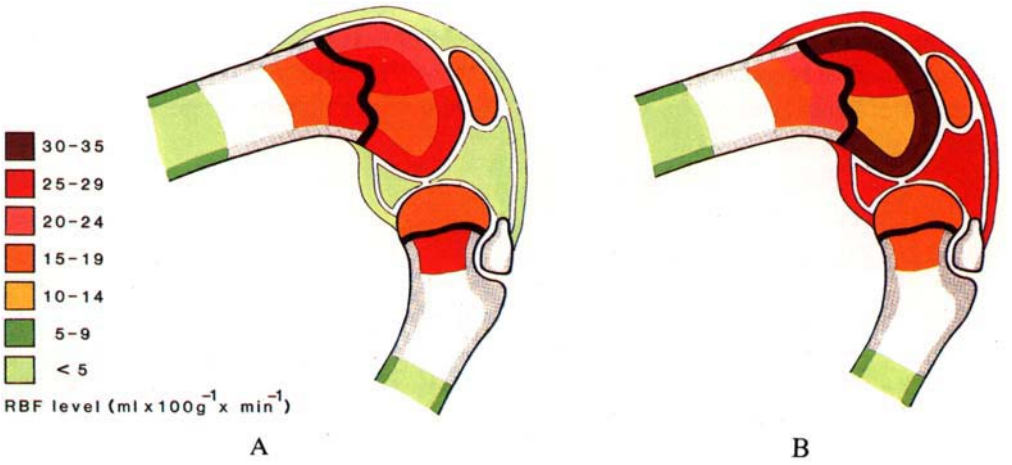


Figure 19. Regional blood flow rates of the normal knee (A) and the arthritic knee (B) in the awake standing puppy, aged 5 months. (N=9) (from Bünger et al. 1984, X).

Influence of static joint tamponade

Distal femoral metaphysis

Tamponade of the joint cavity of the arthritic knee did not affect the IOP of the distal femoral metaphysis (IV). RBF may increase following both venous and arterial tamponade (VII, Table 9), but this was not found in a group of dogs where the initial RBF was already high and probably reflected the presence of vasodilation (Table 8).

Distal femoral epiphysis

Simultaneous intraosseous pressure measurements during knee joint tamponade provided the first evidence of a qualitative change of epiphyseal bone vasculature in chronic arthritis (IV). The arthritic knee is unable to build up IOP in the femoral epiphysis and to maintain an elevated IOP during the application of increasing joint pressure, as the normal knee (I,II,III,IV). The induction of qualitative vascular changes is probably an early event and is present in the model of acute arthritis (VIII). Regional blood flow measurements during bilateral tamponade in puppies with unilateral arthritis show that the fall in IOP during tamponade is most likely to reflect a decrease of blood flow, i.e. an increased vulnerability of the blood supply of the arthritic knee to a joint pressure increase (VII) (Tables 8 and 9). Both femoral condyles are very sensitive to joint tamponade in contrast to the normal knee where the lateral condyle is less sensitive. From these observations it can be hypothesized that joint inflammation is accompanied by a relative arterial insufficiency, i.e. an incapacity of further vasodilation compared with normal vasculature when exposed to increased joint pressure. We have called this an "inflammatory resistance factor".

Gradually introduced tamponade influenced the RBF of the distal femoral epiphysis less than abruptly introduced tamponade, suggesting that some regulatory mechanisms for subchondral bone blood flow apply in arthritis. The reactive hyperaemia following removal of joint tamponade was not significant in the arthritic epiphyses, another indirect sign of vasodilation.

Patella and proximal tibial epiphysis

In arthritis the patella shows an unaffected, high susceptibility to joint pressure increase (Tables 8 and 9). The pattern of reactivity could have been screened by IOP recording and flow measurements at lower tamponade levels. The proximal tibial epiphysis being largely extra-articularly located reacted almost as the normal tibial epiphysis. During tamponade a substantial, but not significant flow increase was observed, suggesting that in this bone compartment a vasodilating capacity is present in spite of synovial inflammation of the knee.

Influence of exercise

The redistribution of blood flow from cancellous bone to functioning muscles during exercise also takes place in the arthritic knee (Fig 20B, Table 12). The decrease of RBF in the arthritic knee and the control knee is not significantly different. No differences between femoral and tibial epiphyseal flow decrease is found in the arthritic knee. This suggests that an arteriolar vasoconstriction is activated during exercise, and that this mechanism is well working in arthritic subchondral bone. The lack of any difference between the blood redistribution of the tibial and femoral epiphyses shows, that the increased venous outlet resistance of the femoral epiphysis in the presence of at least minor joint effusions does not influence the blood flow changes observed during exercise.

The speculated causative role of increased alpha adrenergic activity in the redistribution of bone blood flow calls for an evaluation of the general sympathetic tone in the surveyed study (X). The level of adrenergic activity may have been affected by exercise training of the arthritic knee in addition to the general increase in adrenergic activity as found during exercise (Christensen et al.1979). This may explain why the increase of vascular resistance in cancellous bone exceeds previous data for normal adult dogs (Gross et al. 1979, Tøndevold 1983). On the other hand, the immature skeleton receives a higher percentage of cardiac output, and thus the redistribution level of RBF observed in (X) may be a natural consequence hereof.

The postexercise flow changes in arthritis suggest that during exercise the metabolic needs of the highly demanding subchondral bone are not met (Table 12). The level of postexercise flow rates reported suggests that after 30 min rest there is still some vasoconstriction. Nevertheless, an increase of RBF in the arthritic femoral epiphysis is present resulting in a significant elevation of subchondral bone blood flow. In the central epiphyseal bone the RBF is elevated post exercise.

Table 12. Regional blood flow rates ($ml \times 100g^{-1} \times min^{-1}$) in the arthritic limb of puppies with arthritis of the knee at rest, during exercise training at 4 km per hour and 30 min post exercise. Mean values and 95% confidence limits in brackets, N=9.

	Rest	Exercise	Post exercise
Acetabulum	*26 (20–34)	*19 (14–25)	*26 (20–35)
Caput femoris epiphysis	**17 (11–24)	13 (9–18)	20 (14–29)
Proximal femoral metaphysis	21 (15–29)	14 (10–20)	16 (12–23)
Cortical bone femoral diaphysis	7 (5–10)	7 (5–10)	12 (8–17)
Distal femoral metaphysis	24 (14–40)	9 (5–15)	15 (9–25)
Peropheral femoral epiphysis	35 (27–47)	17 (13–22)	*32 (24–43)
Proximal tibial epiphysis	18 (10–34)	12 (6–22)	22 (11–37)
Proximal tibial metaphysis	20 (13–31)	17 (11–26)	26 (15–36)
Cortical bone tibial diaphysis	*8 (6–12)	8 (6–12)	12 (8–18)
Rectus femoris muscle	10 (5–18)	*92 (50–166)	11 (6–19)
Vastus lateralis muscle	16 (10–26)	*67 (41–110)	21 (13–35)
Anterior tibial muscle	10 (6–18)	*144 (81–254)	19 (11–33)
Knee joint capsule	*27 (17–44)	*28 (18–46)	*61 (38–98)

*significantly increased value,
**significantly decreased value compared to control limb.

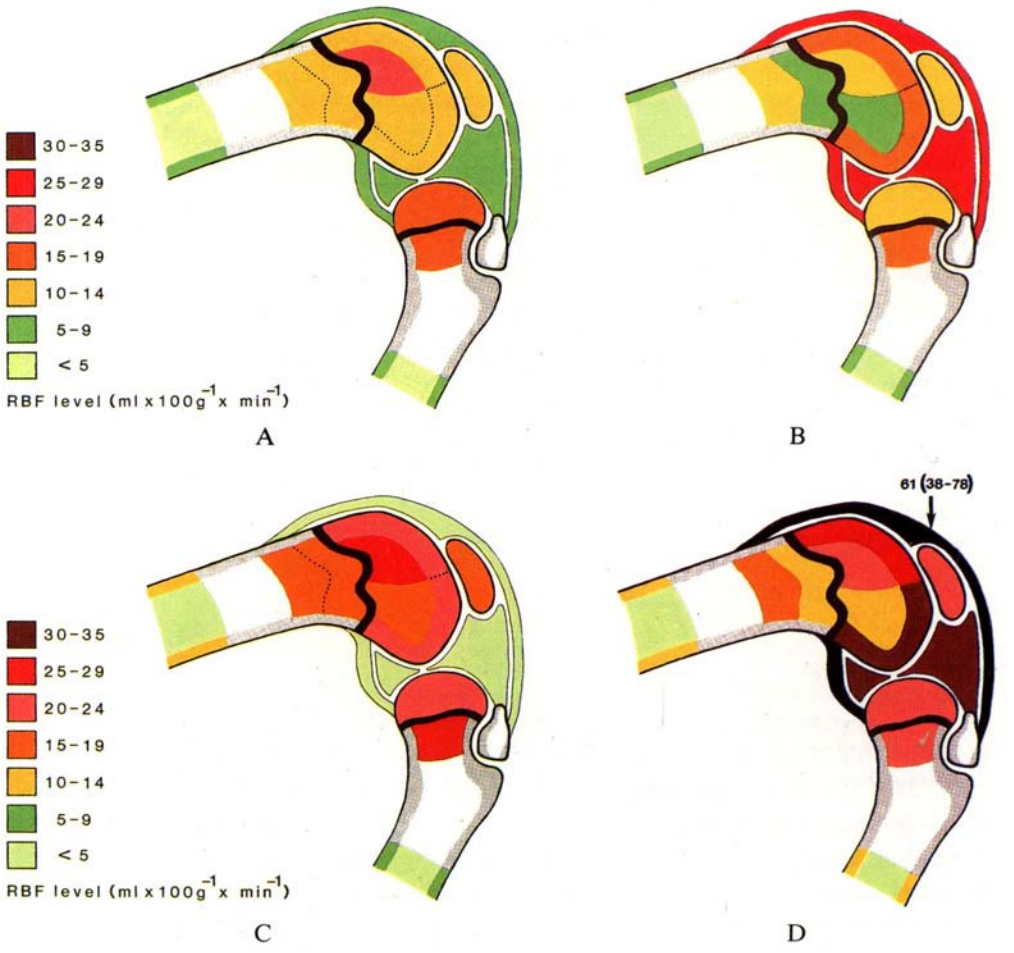


Figure 20. Regional blood flow rates of normal (A+C) and arthritic (B+D) knees during exercise training of 4 km per h. (A+B) and 30 min postexercise (C+D). N=9, from Bünger et al. 1984 (X).

C. Conclusions

At rest the microcirculation of the knee joint capsule is characterized by hyperaemia, increased microvascular volumes, short transit of blood, and a low tissue haematocrit.

The microcirculation of the juxta-articular epiphyses and the patella is dominated by an overall unchanged RBF, increased vascular volumes and prolonged transit of blood. The growth plates are borderlines for the extension of increased microvascular volumes in chronic arthritis. The tissue haematocrits are unchanged. However, considerable differences is the microcirculation within the single bone compartment may exist as RBF in the arthritic femoral epiphysis is both elevated in the subchondral zone and depressed in the central epiphyseal zone.

In arthritis of the knee the haemodynamics of the juxta-articular tissues are influence by.

1. *The degree and duration of the synovial inflammation.* Acute synovial inflammation is followed by the most pronounced synovial hyperaemia and increased metaphyseal blood flow rates.

2. *The anatomical relationship between the joint capsule and the juxtaarticular bone.* Evidence of intraosseous venous engorgement has been demonstrated in the distal femoral epiphysis in chronic arthritis. Due to the intraarticular location of supplying and draining vessels of the femoral epiphysis and the patella, these bone compartments are susceptible to ischaemia when exposed to high joint effusion pressures.

3. *The joint effusion pressure.* Joint effusion pressures in both acute and chronic arthritis between 0.7 and 2.0 kPa does not endanger the blood supply to the juxta-articular bones at rest, but may contribute to induction of chronic venous engorgement in the distal femoral epiphysis. At least for shorter periods static joint effusion pressures up to 50% of the mean arterial pressure are tolerated by the mechanisms regulating the juxta-articular bone blood flow, whereas the RBF in the luminal parts of the synovium probably is insufficient.

Under the influence of higher static joint pressures, the synovium and the distal femoral epiphysis show a larger vulnerability than the normal knee. The presence of a local vascular inflammatory resistance factor is suggested.

4. *Limb use.* Exercise training causes redistribution of RBF from all cancellous bone structures of the normal and arthritic knee in favour of the working muscles. The redistribution is not influenced by the anatomical location, the presence of increased venous outlet resistance or the synovial inflammation. It is suggested, that the metabolic demands of the arthritic subchondral bone are not met during exercise.

Hyperaemia in the synovial vascular bed is not affected by exercise. However, postexercise flow values suggest a severe aggravation of the inflammatory process, which may be due to local synovial ischaemia during exercise.

Summary

Aspects of the haemodynamics of the immature knee with specific reference to joint pressure elevation and synovial inflammation in dogs are described in the surveyed studies.

Simultaneous multiple pressure registrations in the juxta-articular bones of the knee include transcutaneous bone cannulation and pressure recording by means of liquid filled electromanometric pressure recording systems (I).

The intraosseous pressures (IOP's) range between 6% and 24% of mean arterial pressure depending on medication and anaesthesia applied during measurements. The highest pressures are seen in the patella (II), the lowest in the distal femoral epiphysis.

The IOP of the distal femoral epiphysis and the patella increases significantly during artificial elevation of the joint pressure. No reaction is found in the tibial epiphysis and in the femoral metaphysis. The differing presssure response is suggested to reflect the anatomical relationship between the vascular supply and drainage of the compartment and the joint capsule. The IOP increase in the susceptible compartments is present at a joint pressure of 0.9 kPa, and increases until a plateau is reached at a joint tamponade of 75% of the mean arterial pressure. A hypothesis is proposed that a state of chronic venous engorgement could be responsible for the enlargement phenomenon found in the distal femoral epiphysis in chronic juvenile arthritides.

By segmental ligation of the draining veins different levels of IOP elevation can be induced in accordance with the degree of occlusion (I). It is deduced, that during tamponade of the knee joint capsule, not only the drainage through the medial genicular vein, but also the venous drainage of the distal femoral epiphysis passing through the epiphyseo-metaphyseal network is affected.

The joint position affect the IOP's significantly (I,II). During passive knee flexion the IOP of the distal femoral epiphysis and the patella increases, whereas the tibial epiphyseal pressure rises during passive extension. The presence of a moderate joint effusion augments the IOP changes measured during movement of the empty knee.

Uncertainty existed as to the influence of the bone cannulation on the local bone blood flow. By simultaneous flow and pressure measurements this effect was found to be negligible (III).

Further, the increase in IOP of the distal femoral epiphysis during joint tamponade is found mainly to reflect an increase in venous outlet resistance and not only an increase in blood flow, which is released in all bony structures of the test limb.

Therefore, removal of the joint effusions results in an immediate drop in the IOP of the distal femoral epiphysis. However, hyperaemia, which is most pronounced in the distal femoral epiphysis and the knee joint capsule, persists for at least 30 min. (III). The presence of both local and regional hyperaemia during and after tamponade can most likely be explained by activation of both metabolic and neurogenic regulatory mechanisms of the knee blood flow. However, the trigger mechanisms are still unknown.

In order to investigate aspects of the regulation of subchondral blood flow of the knee, the arterial and venous resistances were measured 5 min and 60 min after isolated occlusion of the median genicular vein (VI). Ligation of the vein causes an instantaneous three-fold increase of the venous outlet resistance. After one hour both arterial inlet and venous outlet resistances decrease. Regional blood flow is unchanged. These results further support the concept of autoregulation of epiphyseal bone blood flow, the mechanism triggered being predominantly metabolic.

It can be concluded, that the flow changes found during joint tamponade are hardly released by increased venous outlet resistance only.

A model of nonspecific unilateral juvenile gonarthritis in puppies was developed by repeated intra-articular injections of Carrageenan for 10–12 weeks (V). The main pathophysiological features of the model are chronic synovial inflammation and elevated joint pressure. The resultant bone lesions include epiphyseal overgrowth, reduced endochondral growth of the femoral growth plate, and osteopenia of the juxta-articular bone. The bone lesions are considered comparable with those found in haemophilic arthropathy and chronic rheumatoid arthritis in children.

Simultaneous IOP measurements and intraosseous phlebographies in our arthropathy model reveal a modest, but significant elevation of IOP in the distal femoral epiphysis in arthritis and a dilated vascular network (IV). On observing the IOP in the juxta-articular bones during artificial elevation of IAP, a new qualitative change of bone vasculature have emerged, which is characterized by an inability of the arthritic epiphysis to maintain a high IOP.

Regional blood flow (RBF) measurements using the tracer microsphere technique reveal an unchanged total RBF of the juxta-articular epiphysis of the arthritic knee, whereas RBF of the soft tissues is significantly increased (VII). Static joint pressure elevation up to 50% of MAP for shorter periods is not critical for the epiphyseal and patellar RBF of the arthritic knee, whereas the luminal blood flow of the metabolically highly demanding synovium probably becomes insufficient. At higher static joint pressures the RBF of the synovium and the distal femoral epiphysis shows a larger vulnerability than the normal knee, signifying the presence of an "inflammatory resistance factor".

In acute arthritis (2–3 weeks) the qualitative changes in the vasculature of the knee are present (VIII). Hyperaemia of the distal femoral metaphysis, probably reflecting an increased bone resorption activity, is found in acute arthritis.

The qualitative changes in the knee vasculature in chronic arthritis were analysed by simultaneous estimation of the RBF and the microvascular volume (VV). Calculation of the volume/flow ratios allowed estimation of the transit time of plasma and red blood cells (IX). The microcirculation in the joint capsule in chronic arthritis

is characterized by hyperaemia, increased VV with short transit of blood and a low tissue haematocrit.

The juxta-articular epiphysis and the patella are dominated by a total unchanged RBF, an increased VV and a prolonged transit of blood with unchanged tissue haematocrit. The growth plates constitute a line beyond which these changes do not extend. In the juxta-articular metaphysis, which on the normal side possess the highest VV and the most prolonged transit of blood, the VV tend to be reduced. The demonstrated vascular change in the arthritic epiphyses probably reflects a dilated capillary network and hence an increased permeable surface area between blood and bone, thus facilitating the metabolism and probable destruction of subchondral bone in juvenile arthritis.

In the last study (X), another bone sample technique in flow measurements in awake, erect, nonmedicated dogs with unilateral arthritis was applied to investigate the influence of active joint motion and exercise training on the RBF of the knee. RBF measurements in concentric bone samples reveal significant RBF gradients within the individual bone compartments both in the normal and in the arthritic knee. High flow rates are present subchondrally in the femoral epiphysis. Arthritis is accompanied by increased flow gradients causing a higher subchondral and a lower central epiphyseal RBF.

Exercise training gives a redistribution of RBF to working muscles, amounting up to 50%, from all cancellous bone structures. The normal synovial RBF increases two-fold, while the RBF of the arthritic capsule shows unchanged values. The RBF redistribution is not influenced by the anatomical relationships, the presence of increased venous outlet resistance or synovial inflammation. Thus, in this context, it outweighs the influence of elevated joint pressure on the bones. In the joint capsule and in the subchondral bone of the distal femoral epiphysis a severe hyperaemia prevails post exercise suggesting aggravation of the inflammatory process. The underlying mechanism could be ascribed to synovial ischaemia during exercise.

To sum up we have provided evidence of a differentiated haemodynamic milieu of the normal knee, that correlates with the metabolic activities of the developing knee. Autoregulatory mechanisms for the epiphysial and synovial blood flow exist, their nature still being unknown.

The influence of joint pressure elevation varies with the anatomical relationship to the joint cavity, the level and duration of pressure rise. In the susceptible bone compartments like the distal femoral epiphysis and the patella, joint pressure elevation up to 50% of mean arterial pressure is tolerated for shorter periods.

In arthritis early qualitative changes in the vasculature appear simultaneously, which renders the vascular bed of the joint capsule and femoral epiphysis more vulnerable to joint pressure elevation than in the normal knee. The microcirculation of the juxta-articular epiphyses is characterized by an increased permeable surface area between blood and bone with prolonged transit of blood. Central hypovascularity and a zone of subchondral hypervascularity are prominent features of the epiphyseal haemodynamics in arthritis. Joint use results in a significant redistribution of blood from all bones of both the normal and arthritic knee to the working muscles. The arthritic process of both capsule and subchondral bone seems thereby aggravated

judged by postexercise flow rates. The underlying mechanism may be hypoxia of the metabolically highly demanding arthritic tissue.

Clinical implications

Although conclusions by analogy from experimental investigations to clinical practise can always be questioned, some general implications do spring from the surveyed studies.

The verification of the differentiated haemodynamic milieu of the juxta-articular bones of the knee improves our understanding of normal skeletal growth. It facilitates the evaluation and development of methods, that can be used in the assesment of vascular components of various bone disorders. Thus, the shortcomings of both invasive methods, which include single measurement sites are stressed, as well as methods summarizing the events in larger bone areas, within which considerable variation in vascular supply may exist. On the other hand evidence has been provided for the methodological validity of the widespread use of intraosseous pressure measurements even in small compartments.

We did not provide evidence of a primary vascular stimulus in the genesis of bone lesions of the knee, with the exception of extreme situations such as arterial tamponade of the joint cavity, which may hamper the circulation of the distal femoral epiphysis and the patella in suppurative arthritis. Immobilisation of the knee in full extension during casting or traction may also in the presence of joint effusion cause ischaemia of both epiphyses.

The developed arthropathy model has provided evidence of the importance of the nonspecific inflammatory component in juvenile arthritides. This component seems to be responsible for the development of juxta-articular osteopenia, the overgrowth phenomenon, and the subsequent supervention of osteoarthrosis. Efforts to manage this inflammatory component at an early stage of haemarthrosis and arthritis seems justified and appropiate. Though the presence of mild joint effusions in the resting knee in itself did not measurably influence the haemodynamics of the juxta-articular bones, there were sufficient data, pointing to an aggressive attitude towards evacuation of larger joint effusions in juvenile joints. First the demonstrated higher vulnerability of the arthritic bone vasculature and luminal synovial tissue, second the chronic venous engorgement of the distal femoral epiphysis, that may be one of the factors causing enlargement of the femoral epiphysis, although the actual growth stimulating compound not yet has been found.

The verification of redistribution of blood from cancellous bone to muscles during exercise is of equal or perhaps of even greater importance in the understanding of possible mechanisms in the development of ischaemic lesions in immature bone. In coexistence with a local vascular disease in bone, this factor may have a detrimental effect and may trigger a state of permanent ischaemia. Such a relationship should be considered in pathogenesis of Legg Calvé Perthes' disease. Thus, there seems to be experimental evidence not to deviate from the normal clinical practise and avoid

exercise training in unclear joint conditions, since exercise may also aggravate the inflammatory process both in the joint capsule and adjacent bone.

Suggestions for future research

The surveyed studies have mainly been descriptive in their analysis of the reactions of the haemodynamics of the normal knee to various stimuli. A framework has been set to fill in more data on the intraosseous compartmental haemodynamics and metabolism. Continued studies including development of parameters, that simultaneously reflect the metabolic reactions in bone would improve the understanding of bone circulation. The refinement of computerized scintimetric techniques and the combination of dynamic and static scans may be profitable both experimentally and clinically.

Evidence was provided of autoregulatory mechanisms of subchondral bone blood flow. Knowledge of the nature of these mechanisms, particularly the metabolic components, is incomplete and deserves further studies. Likewise the relationship between autoregulation and capillary function, particularly capillary permeability needs further clarification, as it may be one of the "regulatory tools" by which bone can overcome increased metabolic demands. Active modulation of capillary permeability in bone may be one of the future therapeutic approaches in various problems of fracture treatment, bony ingrowth in implants, and in the healing of arthritic lesions.

The Carrageenan-induced arthropathy model may be a tool for further investigation of the advancing, destructive processes in arthritis. By the combination of analyses from other arthritic models, such as chronic haemarthrosis and septic arthritis, the broad spectrum arthritic states would provide an excellent substratum for the investigation of the pathogenetic role of different mediators of inflammation.

Further, our methods for induction of arthritis might be useful in the development of a model of chronic coxitis. According to the literature, attempts have so far been unsuccesful in producing both chronic synovial inflammation and elevated hip joint pressure. The accomplishment of haemodynamic studies in such a model would clarify important aspects of the pathogenesis of Legg Calvé Perthes' disease.

Our knowledge on the physiology and function of the synovial membrane has so far been mainly been based on studies of normal synovial tissue. The influence of joint pressure on the synovial blood flow only yields limited information on the metabolism and exchange across the synovial blood-joint barrier. The influence of elevated joint pressure on the transport function of the inflamed synovial membrane is another subject for future research. The application of NMR imagining in this context seems profitable.

Acknowledgements

This thesis is based on investigations carried out during my appointment as registrar at the Orthopaedic Hospital in Aarhus 1980–81, and during my research fellowship at the Institute of Experimental Clinical Research, the University of Aarhus, the Orthopaedic Hospital in Aarhus, and the University Department of Pathology, Aarhus Amtssygehus from 1981 to 1983.

I am greatly indebted to Professor, dr. med. O. Sneppen, head of the Orthopaedic Hospital, who initiated this research line, for his trust and enthusiam in all phases of the work and for providing excellent working conditions.

A special gratitude is shown to Professor, dr.med. J.C. Djurhuus, head of the Institute of Experimental Clinical Research for his never failing constructive criticism, for inspiring discussions during the initial studies and also during the preparation of this survey.

Professor dr. med. O. Myhre Jensen provided unique support in the histopathological evaluation of our studies, and is gratefully acknowledged.

My thanks also go to dr. med. U. Lucht for excellent collaboration and initial guidance. Particular gratitude is shown to my co-workers dr. med J. Bülow and dr. med. E. Tøndevold, who came to Aarhus and shared their experience with us. Without the outstanding dignity of the other members of our research group, dr. S. Schwartz Sørensen, dr. S. Harving, dr. J. Hjermind, cand. scient. P. Bach, dr. K. Sølund and my wife, dr. E. Bünger these studies were never completed.

Secretary Mrs Birthe Bjørn is gratefully acknowledged for typing large parts of the articles and the survey. M. Pilegaard, authorized translator, kindly afforded linguistic advises. Scientific artist J.H. Nex is thanked for excellent cooperation.

Last but not least, most gratitude is due to my wife Estrid, and our children Ida, Mathias and Pernille, for their patience and devotion.

This study was kindly supported by the University of Aarhus, the Danish Rheumatism Association grant no. 425–233, the Danish Medical Resarch Council grants no. 512–616, 12–2082, and the professor Thomasen foundation.

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