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Haemodynamics in acute arthritis of the knee in puppies

In order to study the haemodynamic changes of the juvenile knee in acute arthritis, an experimental model was developed in puppies by unilateral intra-articular injections of Carragheenin solution into the knee. Tissue blood flow was studied by the tracer microsphere technique in eight dogs and simultaneous intra-articular and intraosseous pressure recordings were performed in seven other dogs. The intra-articular pressure was elevated in all arthritic knees. Hyperaemia was found in the knee-joint capsule and distal femoral metaphysis, whereas juxta-articular epiphyseal blood flow rates were not significantly changed. A decrease of femoral muscle blood flow was encountered. Intraosseous pressure recordings during venous tamponade of the knee-joint capsules suggested a qualitative change of bone vasculature in acute arthritis. The juxta-articular bone blood flow in arthritis appears to be influenced by synovial hyperaemia, synovial effusion pressure, an "inflammatory resistance factor" and the anatomical relationship of the epiphyseal vessels to the knee-joint capsule.

Key words: arthritis; bone blood flow; Carragheenin; haemophilic arthropathy; intraosseous pressure; joint pressure; juvenile chronic arthritis; microspheres

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In both juvenile chronic arthritis (JCA) and haemophilic arthropathy (HA), chronic involvement of the knee often leads to bone atrophy and epiphyseal enlargement (Fiszman et al. 1981, Duthie et al. 1972). These growth disturbances have been reproduced in Carragheenin arthritis of the immature dog knee (Bünger et al. 1983c). It could be anticipated that the presence of unspecific chronic synovial inflammation and joint effusion would be important pathogenetic factors of the growth disturbances observed.

Haemodynamic investigations of chronic Carragheenin arthritis of the knee showed evidence of femoral epiphyseal venous engorgement and qualitative changes of the epiphyseal bone vasculature (Bünger et al. 1983b). Regional blood flow measurements demonstrated synovial hyperaemia, increased metaphyseal bone blood flow, but unchanged epiphyseal bone blood flow, which showed increased susceptibility to elevated joint pressure (Bünger 1984). The development of these haemodynamic changes in relation to time is unknown.

The aim of the present study was thus to investigate the synovial inflammation, the intraosseous pressure and the regional blood flow changes during acute Carragheenin arthritis of the immature knee. Furthermore, the relationship between intraosseous pressure and changes of joint pressure was examined.

Material and methods

Fifteen mongrel puppies, 4–5 months of age, with a mean weight of 14.0 kg (10.0–22.0), comprised the material. Eight animals scheduled for flow measurements were subjected to 11–14 days of arthritis, and seven animals scheduled for pressure measurements had arthritis from 13 to 18 days.

Induction of arthritis. In a short Brietal® (methohexital) anaesthesia twice a week, 2–4 ml Carragheenin solution (1 per cent) prepared as previously described (Bünger et al. 1983c) were instilled into the right knee joint. In order to avoid infection, the skin was shaved and disinfected with chlorhexidine solution 0.5 per cent prior to the instillations.

Haemodynamic investigations. Regional blood flow rates (RBF) and intra-articular pressures were determined in eight puppies, while the relationship between intraosseous pressures and intra-articular

pressure was measured in seven puppies. The haemodynamic investigations were performed in general anaesthesia 4 days after the last instillation of Carragheenin.

General procedure. Anaesthesia was induced with intravenous Brietal and maintained with halothane (0.5–2.0 per cent) after orotracheal intubation, via a constant volume ventilator and a gas mixture of atmospheric air and oxygen (50/50). Muscle relaxation was obtained with intermittent doses of Pavulon® (pancuronium bromide). A detailed description of instrumentation and positioning of the dogs has been presented in a previous paper (Bünger et al. 1983a).

Intraosseous pressure recording. A liquid-filled 5-channel electromanometric pressure recording system with constant rate perfusion of heparinized (10,000 U/l) saline (Bünger et al. 1983b) was used to measure intraosseous and intra-articular pressures, mean arterial pressure (MAP) alternating with central venous pressure (CVP). The intraosseous pressure recordings were performed simultaneously and bilaterally in the distal femoral epiphyses (P_{FE}) and the proximal tibial epiphyses (P_{TE}). The position of the pressure cannulae was secured by fluoroscopy and pressure wave recording. Initial intraosseous pressures were measured before cannulation of the knee-joint cavities. Intra-articular pressures (P_J) could be raised by intra-articular infusion of normo-saline solution. P_{FE} and P_{TE} were recorded during bilateral knee-joint tamponade of 50, 75 and 100 cm H_2O and after evacuation of the joints. An equilibration period of 10 min was used.

Intra-articular pressure measurement. The atmospheric pressure at the level of the knee was chosen as reference zero pressure. The knee-joint puncture was performed with a liquid-filled Terumo 18G cannula connected to a three-way stop-cock, which was closed during penetration of the skin. By opening the stop-cock, an immediate connection to the pressure recording system (with no perfusion) could be obtained without leakage from the knee-joint cavity.

Blood flow measurements. The microsphere method with reference blood sampling was used to measure RBF. NEN-Trac microspheres (New England Nuclear) 15 μm ± 3 μm labelled with ^{113}Sn and suspended in 10 per cent Dextran with 1 per cent Tween 80 added were used (Bünger et al. 1983a). Steady state was controlled before and after flow determinations by means of MAP, CVP, cardiac output, core temperature and blood gases, including pH. A steady-state period of 0.5 h preceded the haemodynamic investigations. All dogs were killed with a saturated dose of potassium chloride and bacterial cultures taken from the synovial fluid. Tissue biopsies were taken immediately post-mortem and gamma radiation counting was performed (Bünger et al. 1983a).

Histological examinations. Immmediately after the animals had been killed, specimens were taken from the knee-joint capsules bilaterally at the level of the inferior patellar pole and fixed in formalin. The specimens were stained with haemotoxin-eosin and van Gieson.

Statistics

Student's *t*-test for paired data was used to compare pressures in the experimental and contralateral knees. All flow data were $\log_{10}$ transformed and examined using a two-way analysis of variance with random effects (Searle 1971). An F-test was used to compare the flow rates in symmetrical locations. Ninety-five per cent confidence limits of RBF rates in the single limb were calculated by: $\text{antilog}_{10}(\bar{x} \pm 1.96 \sqrt{1/8\,\sigma^2_{bL} + \sigma^2_b})$, where σ^2_{bL} was the stochastic variation between limbs and dogs and σ^2_b the stochastic variation between dogs. The relationship between RBF rates in one limb and between joint pressure and RBf rates was analysed using the "maximum likelihood" estimate of the coefficient of correlation. A P-value < 0.05 was considered statistically significant.

Results

Pathoanatomic changes. Joint effusion, increased external temperature and swelling of the knee-joint capsule were present in all experimental knees after two Carragheenin instillations. At dissection, after 11–18 days, the synovium was yellow and oedematous with translucent yellow synovial fluid. Bacterial cultures were negative in 11 cases, while one arthritic knee in the flow group contained *Bacillus anitratum* and non-haemolytic streptococcus. Macroscopically, this knee did not differ from the others.

Histology. All cases showed acute inflammation of the synovial membrane of the arthritic knees. The synovial lining was thickened with focal ulcerations, fibrinous exudate and pseudomembrane formation. The hypertrophied synovium

was characterized by granulation tissue with fibroblast and endothelial proliferation. The inflammatory cells were mainly macrophages along with neutrophils and a few eosinophils. In some dogs with 14–18 days of arthritis, the inflammation of the knee joint capsules was subacute. Villous hypertrophy was encountered, and the synovial lining partly covered the fibrin deposits. Numerous haematoxyphil granules, probably fragments of disintegrated nuclei, were observed in the vicinity of the organizing fibrin deposits. Lymphocytes were the prominent inflammatory cell component, often with follicular accumulations. The histology of the case with positive bacterial culture could not be distinguished from the early inflammatory changes of the other knees.

Regional blood flow measurements (Figures 1 and 2). Acute arthritis of the knee resulted in elevation of RBF in the joint capsule and in the menisci ($P < 0.0001$). The blood perfusion in the femoral muscles was low in the arthritic limb ($P < 0.005$).

The juxta-articular bone blood flow showed a characteristic pattern of changes in the arthritic knees. The RBF in the femoral metaphysis was increased ($P < 0.0001$), whereas the femoral and tibial epiphyseal RBF was insignificantly changed. In the medial condyle an insignificant decrease of RBF was encountered, in contrast to a substantial but not significant increase of RBF in the proximal tibial epiphysis. In the proximal femur the RBF was moderately elevated, whereas the acetabular bone blood flow was decreased. The flow changes in the one infected knee followed to some degree the total material. The relative flow changes were 582 per cent in the joint capsule, 356 per cent in the distal femoral metaphysis, 16 per cent in the medial femoral condyle but −28 per cent in the proximal tibial epiphysis, which exceeds the 95 per cent confidence limits in the total material (−8 to 153 per cent).

In the control limbs, the RBF rates in the medial condyles were always higher than the RBF rates in the lateral condyles of the femur. The metaphyseal flow rates in the distal femur and distal tibia were low (1–8 ml × 100 g^{-1} × min^{-1}) compared to the epiphyseal blood flow rates (10–26 ml × 100 g^{-1} × min^{-1}). In both the arthritic and control limbs the RBF of the medial acetabulum (os pubis) was significantly low compared to the lateral acetabular roof (os ilium) ($P < 0.05$).

Knee blood flow relation to joint pressure. In acute arthritis, no correlation between joint pressure and the RBF in the knee-joint capsule

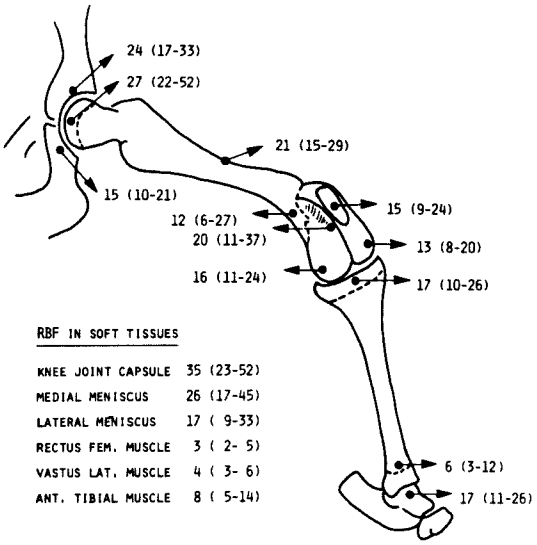

Figure 1. Mean blood flow rates (ml · 100 g^{-1} · min^{-1}) in the hindlimb during acute Carragheenin-induced arthritis of the knee; 95% confidence limits are given in brackets (N = 8).

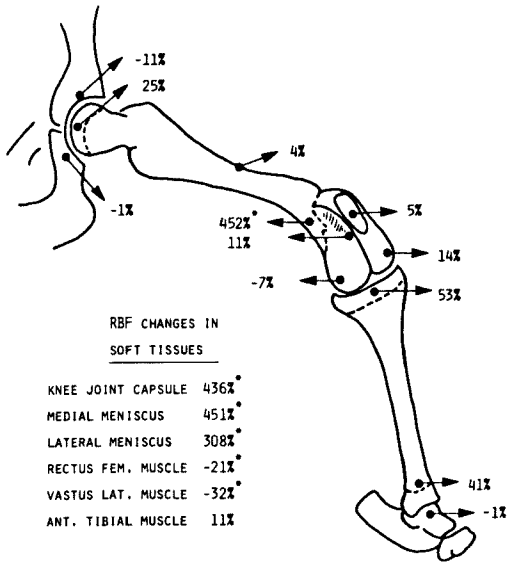

Figure 2. Relative change of blood flow during acute arthritis of the knee compared to the contralateral limb. Mean change $= \sum_{1}^{8} C \cdot N^{-1}$, where C is the relative change in the single dog. Significantly different compared to control limb ($P < 0.05$).

or tibial epiphysis could be demonstrated. However, a positive, but not significant, correlation between joint pressure and the RBF in the femoral metaphysis and distal femoral epiphysis was present. The metaphyseal bone blood flow showed the highest correlation to the joint pressure ($R = 0.633$ $P < 0.10$).

Joint capsular RBF relation to RBF in juxta-articular bone. In the arthritic knees, significant correlations were observed between capsular blood perfusion and RBF in the tibial epiphysis and in the trochlea of the distal femoral epiphysis. There was no correlation between capsular flow and RBF in the medial femoral condyle, lateral condyle and femoral metaphysis.

Intraosseous pressures (Figure 3). Acute arthritis gave an insignificant pressure elevation in the femoral epiphysis and an insignificant pressure reduction in the tibial epiphysis. Joint tamponade increased the P_{FE} in the control knees while there was only an insignificant increase in the P_{EE} on the arthritic side (Figure 3). This difference was significant at venous tamponade of 75 and 100 cm H_2O ($P < 0.05$). No major changes of the P_{TE} levels were measured during knee-joint tamponade. After evacuation of the joints the intraosseous pressures returned to preload values.

Intra-articular pressures (Table 1). The resting joint pressures during acute arthritis ranged from 3 mm Hg to 23 mm Hg, consistently increased compared to the slightly negative pressures in the contralateral knees, ranging from 3 mm Hg to –5.5 mm Hg. There was no difference in P_J between flow and pressure group. The joint pressure in the one knee with positive bacterial culture was 23 mm Hg, the highest of all knees.

Table 1. Intra-articular pressures (P_J) in unilateral acute arthritis of the knee in puppies. Mean values ± standard error of the mean (SE) are given

	Flow group N = 8 P_J mmHg mean ± SE	Pressure group N = 7 P_J mmHg mean ± SE
Arthritic knees	*10.2 ± 2.4	*10.3 ± 1.3
Control knees	–0.8 ± 0.9	–0.2 ± 0.7

* $P < 0.01$ compared to control knees

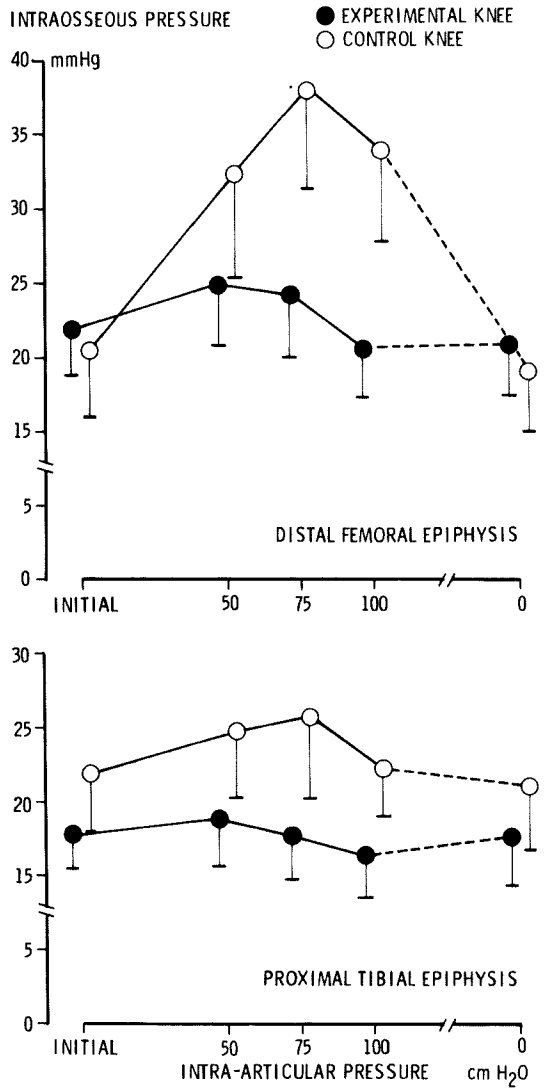

Figure 3. Intraosseous pressures during venous tamponade of the knee joint followed by evacuation of the joint effusion in seven dogs with unilateral acute arthritis of the knee. Mean values and standard error of the mean are indicated.

Central haemodynamics during bone blood flow and pressure measurements showed a MAP of 82 ± 5 mm Hg. CVP was 2.8 ± 0.5 mm Hg and cardiac output 2.9 ± 0.2 l × min $^{-1}$.

Discussion

The acute synovial inflammation in the present study can most probably be attributed to a selective cytophatic effect of Carragheenin on macrophages (Thomsen et al. 1976). Car-

ragheenin is resistant to biochemical degradation by lysosomal glycosidases, and after ingestion by macrophages it persists within secondary lysosomes (Thomsen & Fowler 1981) and causes lysosomal dysfunction. Osmotic swelling and rupture of lysosomes may result in release of hydrolytic enzymes into the cytoplasm and subsequent cell lysis can be anticipated (Velo et al. 1973), along with the release of degradative enzymes and prostaglandins into the extracellular substance of the synovium and into the joint cavity. In the present study, evidence of cell lysis was found after 2 weeks by the abundant presence of cell debris in the extracellular substance of the synovial membrane and in the fibrinous exudate.

The induced inflammatory changes of the knee-joint capsule in the present study bear a resemblance to the early arthritic findings in haemophilic dog knees (Swanton 1959), in which fibrinoid inflammation and hyperplastic synovial lining were characteristic features. Knowledge of the early synovial changes in haemophilic arthropathy and juvenile chronic arthritis is limited, since few specimens from patients have been available. However, there is general agreement on a striking similarity between rheumatoid arthritis, pigmented villonodular synovitis and HA in the subacute stage, which is characterized by synovial hypertrophy, focal aggregations of inflammatory cells and subsynovial fibrosis (Arnold & Hilgartner 1977). Such changes were observed after 3 months of injections of Carragheenin into the knee joint of mongrel puppies (Bünger et al. 1983c), but were also seen in baby rabbits after injection of whole blood or its components (Wolf & Mankin 1965), as well as in immature dogs (Haeglund 1967).

In the present study, the acute synovial inflammation was reflected by a high blood flow in the knee-joint capsule. The relative flow increase was higher than in 12 weeks Carragheenin-induced gonarthritis (Bünger 1984). In the normal juvenile knee the anatomical relationship of the juxtaarticular bone blood vessels to the joint capsule is a major determinant of the venous outlet resistance from juxta-articular bone during knee-joint tamponade (Bünger et al. 1981, 1982, 1983a). It is likely that the haemodynamic effect of synovial inflammation and effusion is influenced by the same relationship. The presence of hyperaemia in the distal femoral metaphysis could be explained by increased osteoblastic activity (Whiteside et al. 1977), by release of vasodilatating substances from the inflamed synovium (McCarty 1974) or simply by vasoactivity secondary to increased heat production (Kennedy 1981). The development of metaphyseal hypotrophy at later stages of Carrageheenin arthritis makes the last two mechanisms most probable. In the distal femoral epiphysis, where the appositional growth is increased (Bünger et al. 1983c), the presence of hyperaemia should also be expected; however, the flow changes observed were insignificant. Despite release of vasodilatating substances, both inflammatory oedema and elevated intraarticular pressure could account for the relatively unchanged flow levels in the distal femoral epiphysis and in the patella. The return of intraosseous pressures to preload values after removal of knee joint effusion (Figure 3) suggests that the inflammatory factor is of importance. However, a complementary influence of perivascular oedema and hydrostatic joint pressure must be considered, and it is thus difficult to separate the two factors. From the knee-joint tamponade experiment, it can be hypothesized that the low increase of P_{FE} and earlier decrease in the arthritic knee is a result of relative arterial insufficiency, i.e. incapacity of further vasodilatation compared to the control knee during increased venous outlet resistance.

In flow studies in 12 weeks arthritis (Bünger 1984), a significant decrease of blood supply was measured in the arthritic distal femoral epiphysis compared to the control knee at a knee joint tamponade of 100 cm H_2O. Already at 50 cm H_2O, the RBF in the arthritic joint capsule was significantly decreased. As the recorded intra-articular pressures in the present study were resting pressures, which are raised during joint motion, in particular knee flexion (Bünger et al. 1982, Agudelo et al. 1972), the increased intra-articular pressure in acute arthritis must be considered to be of pathophysiological importance. The significant decrease of blood flow in the vastus lateralis and rectus femoris muscle might also be secondary to elevated knee joint pressure, which may cause a reflex inhibition of

quadricips femoris muscle activity (Newton 1982), and thus a decrease of blood flow. Progressive atrophy of the quadriceps developed in chronic Carragheenin-induced gonarthritis (Bünger et al. 1983c).

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