FRACTIONAL NECROSIS OF THE FEMORAL HEAD EPIPHYSIS
AFTER TRANSIENT INCREASE IN JOINT PRESSURE
AN EXPERIMENTAL STUDY IN JUVENILE RABBITS

J. VEGTER, CH. C. LUBSEN

From the Regional Hospital Helmond-Deurne and the University of Amsterdam

Ischaemia resulting from increased joint pressure may play a role in the pathogenesis of necrosis of the femoral head epiphysis. We studied the effect of temporary vascular occlusion on this epiphysis in young rabbits. Occlusion for six hours resulted in necrosis of trabecular bone and of intertrabecular marrow and vascular tissue, later followed by revascularisation and repair, as has been demonstrated previously.

In contrast, raised intra-articular pressure lasting for only two hours resulted in a more complex picture: trabecular osteocytes were dead, whereas the bone-forming marrow was shown by fluorochrome labelling to remain viable, and to form appositional repair bone throughout the epiphysis. We concluded that transient vascular occlusion may cause the death of trabeculae despite intact perfusion of the bone. This type of change may be important in the pathogenesis of Perthes’ disease.

It is generally accepted that the initial changes in ischaemic necrosis of the femoral head include death of both trabeculae and of intertrabecular bone marrow and blood vessels. The direct cause of this necrosis is vascular occlusion or disruption (Trueta and Harrison 1953; Trueta 1957).

Repair of dead cancellous bone is considered to be heralded by vascular ingrowth from the surrounding living tissue. Appositional new bone is laid down against the dead trabeculae (Phemister 1930; Catto 1965; Rösingh et al. 1969) and this is followed by remodelling (Kenzora et al. 1978). The process is identical to the revascularisation of free autogenous bone grafts.

Temporary ischaemia of the epiphysis of the femoral head, caused by raised intra-articular pressure or an extreme position of the joint, may also result in damage and repair which follows the same sequence of events unless circulation is restored quickly (Woodhouse 1964; Tachdjian and Grana 1968; Henard 1971; Calvert et al. 1984).

In contrast, partial occlusion of the circulation to bone and bone marrow in the rabbit has been shown to result in different histopathological changes suggestive of ischaemia of varying degrees of severity (Rutishauser, Rohner and Held 1960). Reviews of the morphology of Perthes’ disease have shown findings which ranged from an ischaemic arrest of ossification without infarction to multiple complete infarctions of the epiphyseal bone (Inoue et al. 1976; Catterall et al. 1982); these suggested not only variable severity, but also repeated episodes of the ischaemia.

We report an experimental investigation of the effect of temporary occlusion of the blood supply to the epiphysis of the femoral head in juvenile rabbits. This has shown that two different forms of bone necrosis may occur, depending on the duration of the ischaemia.

MATERIAL AND METHODS

The experimental animals were six-week-old female New Zealand White rabbits. Eight rabbits were used to investigate the effect of tamponade by increased joint pressure on the perfusion of the femoral head; these animals were killed immediately after the period of tamponade. Another 34 rabbits were killed three weeks after tamponade for the histological determination of the early consequences of temporary ischaemia on the vitality of the trabecular bone and marrow tissue.

Experimental model. Temporary occlusion of the blood supply of the femoral head epiphysis was achieved by raising the hydrostatic pressure within the joint (Woodhouse 1964; Tachdjian and Grana 1968; Ganz et al. 1981). Each animal was anaesthetised with intramuscular Hypnorm (10 mg fluanisone/0.2 mg fen-
minutes after the application of tamponade pressure, 5 ml of Disulphine Blue was injected into an ear vein.

Three minutes later the animals were killed, and the proximal femora were sectioned in the frontal plane to reveal the extent of dye perfusion (Fig. 2).

**Viability study.** The increased joint pressure was maintained for one, two, four or six hours in different animals, and then the needle was removed and the wound closed. Five successful experiments were performed in each of the four tamponade groups, but another 14 animals were discarded because of leakage or blocking of the system.

**Assessment of bone viability.** On conventional microscopy death of bone cells is shown by the absence of cellular elements in the osteocyte lacunae. After interruption of the blood supply to the femoral head of dogs (Bonfiglio 1954) and in avascular necrosis of the femoral head after transcrural fracture (Catto 1965) it has been shown that the loss of osteocytes is rarely complete until three or four weeks later. Loss of viability of osteocytes can be demonstrated histologically two weeks after vascular interruption. In our experiments the animals were sacrificed three weeks after the temporary increase in joint pressure.

The integrity of the blood supply and the viability of bone forming in marrow can be studied in vivo by fluorochrome labelling. Fluorescence will not be found in bone if the blood supply is absent or markedly diminished (Milch 1963). In viable tissue fluorochromes are deposited in newly formed bone during the phase of rapid mineralisation (Milch et al. 1958), and sequential fluorochrome labelling provides a means of studying the dynamics of bone formation over a period in a single animal.

We started fluorochrome labelling the day after operation and, because of the regular cyclic pattern of

![Image](image-url)

**Fig. 1**

Method of raising intra-articular pressure. A threaded needle is placed in the dorsolateral part of the joint and sealed with cyano-acrylate (1). The needle tip has a closed end with side openings to prevent blockage by the femoral head (inset). The dextran reservoir is 200 cm above the joint. Continuity with the joint is checked by movement of the limb (2), which should cause displacement of an air bubble in the horizontal part of the tubing (3).

tanyl) 0.5 ml per kg body weight, and by a lateral approach the dorso-lateral rim of the acetabulum and the capsule of the left hip were exposed. By traction on the limb the femoral head was partially subluxated from its socket. A 2 mm hole was drilled through the acetabular wall to the weight-bearing area of the socket (Fig. 1) and a specially designed 18-gauge needle with two side apertures and a self-tapping thread was screwed into the hole. When the needle was properly placed, the contact zone between needle and bone was dried and a water-tight seal made with cyano-acrylate. A fine epidural cannula passed through the needle was used to fill the joint and the needle lumen with dextran 70. The cannula was then withdrawn and the needle connected by tubing to a reservoir. An arterial tamponade was created by maintaining the infusion bottle 200 cm above the level of the hip. The continuity of the hydrostatic system was verified by observing that displacement of a bubble in a horizontal part of the intravenous tubing was produced by any change in the position of the limb.

**Perfusion study.** The hydrostatic pressure system functioned well in six of the eight rabbits. Two in which the needle to bone junction leaked were excluded. Ten

![Image](image-url)

**Fig. 2**

Result of Disulphine Blue perfusion. On the right, perfusion of the epiphysis has been abolished by tamponade. On the left, the control hip is perfused normally with the dye.
bone growth in the young rabbit (Tam et al. 1974), we repeated this labelling every four days. Six fluorochromes were given by subcutaneous injection in the following sequence and dosage: xylenol orange 90 mg/kg twice, calcein green 10 mg/kg, oxytetracycline 37.5 mg/kg and alizarin complexon 30 mg/kg twice. The animals were killed two days after the last injection, the proximal ends of the right and left femora were removed and these were sectioned in the frontal plane for microscopic study.

Decalcified material. The dorsal parts of the heads were fixed in 10% formalin in physiological saline and then the specimens were decalcified under continuous movement in 2.5% nitric acid in a saturated solution of picric acid in 70% alcohol for about six days. Complete decalcification was checked by x-ray examination and the decalcified specimens were then dehydrated and embedded in Paraffin.

Sections (5 μm) were made of tissue 2 mm deep to the cut surface, and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) or by the Masson-Goldner trichrome method.

Undecalcified material. The ventral parts of the heads were fixed in 80% alcohol. After dehydration the material was embedded in methylmethacrylate. Sections (120 μm) were prepared with a rotary saw in a direction parallel to the original plane of cleavage. These sections were ground down to a thickness of 100 μm between roughened glass plates and contact microradiographs were made on Kodak 649-0 spectroscopic film.

Finally the sections were mounted and the pattern of uptake of the fluorochromes was studied with a fluorescence microscope.

RESULTS

Perfusion study. In all six rabbits with proper continuity between the pressure system and the joint cavity, there was no Disulphine Blue in the treated capital epiphysis of the femoral head, proving loss of blood supply (Fig. 2). The metaphysis of the femur and the ossification centre of the greater trochanter had been normally perfused, as shown by the blue colour in these and throughout the control femur. These findings confirm that tamponade had resulted in complete and reproducible occlusion of the vascular supply of the femoral head epiphysis.

Viability study. In each of the four time groups, tamponade was successful in five animals. Two of these 20 animals were lost from the study: one from the four-hour group died at 10 days probably from an overdose of Hypnorm given before a fluorochrome injection, and one from the six-hour group had a joint infection.

Occlusion for one hour. The cartilage of the femoral head and the epiphysis in cross-section was the same colour as that of the control hip. On histology, the trabecular osteocytes were alive and the trabeculae were covered with a layer of mostly flat, inactive endosteal cells. The inter trabecular spaces were filled with haemopoietic and fat cells (Fig. 3). All six fluorochrome labels were equally present in both the treated and the control femoral head epiphyses.

Occlusion for two hours. Again there was no change in the colour of the cartilage of the femoral head or of the epiphyses. Microscopy, however, showed that in four of the five epiphyses there were signs of cell decay and of regeneration. These changes were most prominent on the articular side of the epiphysis and there were local areas of advanced osteocyte degeneration, shown by empty lacunae within some trabeculae (Fig. 4).

Against this dead lamellar bone, separated by a pronounced cement line, woven bone had been deposited, and in most places this living repair bone was gradually being changed into lamellar bone. The trabeculae were covered with mostly cylindrical, that is active, osteoblasts. The haemopoietic cells in the marrow spaces had almost disappeared. In the one exception, the histology was completely normal.

All six fluorochrome labels were equally present in the treated and the control femoral head (Fig. 5). It may be concluded that in contrast to the trabecular osteocytes, the bone-forming marrow tissue remained alive during the two hours ischaemia and bone vascularisation remained intact.

Occlusion for four hours. Again, no colour change was found in the cartilage, and thus no indication of a permanent disorder in blood supply. Microscopy revealed the same abnormalities as after two hours' tamponade, with woven bone diffusely deposited on dead trabeculae and gradually changing into lamellar bone (Fig. 6). The living bone was largely bounded by active osteoblasts. In the rabbit that died 10 days after the ischaemic episode the layer of new bone was clearly narrower. The haemopoietic marrow had largely disappeared. The decay of osteocytes was not limited to the articular side of the epiphysis, but had spread towards the epiphyseal plate.

In contrast with the findings in the two-hour occlusion group the first fluorochrome label was absent in two specimens and the first and second fluorochrome labels were absent in three specimens (Fig. 7).

Occlusion for six hours. In three of the four specimens the normal pink-blue transparency of the articular cartilage had vanished and the epiphyseal cartilage was whiter than in the control hip. Histologically there was complete necrosis of both trabecular bone and marrow tissue in these three rabbits. At the periphery of the epiphysis there was ingrowth of granulation tissue, indicating revascularisation and the beginning of new bone formation (Fig. 8). In these three rabbits, all six fluorochrome labels were absent. In the fourth rabbit, which had a macroscopically normal epiphysis, the histological picture was the same as in the four-hour occlusion group, but at fluorescence microscopy only the last three labels were present.
Results three weeks after varying periods of occlusion.

**Fig. 3**
One-hour occlusion group. Section from the epiphysis is normal, showing viable trabeculae of lamellar bone (LB), marrow tissue (LM) and flat osteoblasts (arrow) (PAS, × 200).

**Fig. 4**
Two-hour occlusion group. There is dead trabecular bone (DB) with many empty lacunae. Viable appositional repair bone (LB) is darker staining, and the osteoblasts (arrows) are cylindrical, indicating activity (PAS, × 300).

**Fig. 5**
Two-hour occlusion group. Undecalcified section under fluoroscopy. All six fluorochrome labels, given at four-day intervals, are seen. This indicates intact perfusion and persistently viable osteoblasts (× 400).

**Fig. 6**
Four-hour occlusion group. Dead bone (DB) shows empty osteocyte lacunae. There is appositional woven bone (*) throughout the epiphysis but this becomes mature lamellar bone (LB) (Masson–Goldner trichrome, × 400).

**Fig. 7**
Four-hour occlusion group. Undecalcified section under fluoroscopy. Absence of the first two fluorochrome labels indicates either transient metabolic inactivity or death followed by early replacement of the bone-forming cells (× 400).

**Fig. 8**
Six-hour occlusion group. Section of the epiphysis near the perichondral ring. Both trabecular bone (DB) and marrow tissue (DM) of the whole epiphysis are dead. There is ingrowth of vascular tissue (*) into the intertrabecular spaces and this is followed by a front of appositional new bone formation (arrows) (PAS, × 400).
DISCUSSION

In man, as well as the rabbit, the juvenile femoral head epiphysis is supplied solely by intracapsular, extraosseous vessels (Brookes and Harrison 1957; Trueta 1957), which may be compressed and obstructed by raised intra-articular pressure. Experimental tamponade of the hip in puppies has resulted in conflicting views; there is controversy whether venous or arterial pressures are required to produce diminished perfusion (Borgs-miller et al. 1980; Launder, Hungerford and Jones 1981; Lucht et al. 1983) and, more importantly, necrosis of the epiphysis (Kemp 1973; Woodhouse 1964; Tachdjian and Grana 1968). Because of our clinical observation that in transient synovitis a tamponade pressure greater than arterial pressure can occur in certain "normal" positions of the hip (Vegter 1987), we chose an intra-articular pressure higher than the arterial blood pressure of the rabbit.

Rössingh and James (1969) found in rabbits that a significantly reduced Feulgen-DNA content in the osteocyte nuclei, indicating cell death, occurred only after six hours' ischaemia, but it is possible that before the Feulgen-DNA content is reduced the "point of no return" of the osteocytes has already been reached. Our in-vivo results agree with the in-vitro experiments described by James and Steij-n-Myagkaya (1986). They also concluded that osteocytes suffer irreversible damage after ischaemia of about two hours.

From our results it appears that different durations of temporary vascular occlusion can produce two different forms of necrosis of the femoral head epiphysis. Occlusion for six hours resulted in the classical sequence of events seen in avascular necrosis (Phemister 1930; Catto 1965; Rössingh et al. 1969; Kenzora et al. 1978) in three of the four animals. Necrosis of osteocytes and of marrow and vascular tissue was followed by revascularisation which appeared first in the dorso-cranial part of the epiphysis, and had not reached the ventral part of the epiphysis in this early phase.

By contrast, occlusion for only two hours produced a fractional osteonecrosis, characterised by necrosis of some trabecular osteocytes, while vascular and bone-forming marrow tissues seemed to remain alive. Moreover, repair starts immediately after the ischaemic episode. This type of necrosis has not, to the best of our knowledge, been observed previously after temporary ischaemia. This is probably because in-vivo estimation of bone viability after ischaemia has not included fluorochrome labelling or scintimetry.

Fractional necrosis may be explained by a difference in susceptibility to ischaemia of the different cell types of the epiphysis or it may be due to differences between the microcirculation of trabeculae and bone marrow.

In this context Spencer et al. (1986) recently reported an early case of avascular necrosis of the femoral head following renal transplantation, and demonstrated completely dead osteons within otherwise normal areas. These authors suggested that the initial lesion might have occurred in the bone itself rather than in the marrow space, either by a direct cytotoxic effect on the osteocytes or by interference with the microcirculation.

Micrographs of the femoral head epiphysis of juvenile dogs (Henard 1971) after six hours of vascular occlusion produced by an extreme joint position showed a large amount of repair bone against the dead trabeculae. This bone was deposited as early as 7 and 12 days after the ischaemia. Rössingh, Steendijk and van den Hooff (1972) also found repair bone in the femoral head only eight days after fracture of the femoral neck. These observations of very early repair after ischaemia provide further evidence of fractional rather than total osteonecrosis.

Gregg and Walder (1980), studying caisson disease in rabbits, also observed dead cortical and trabecular bone in combination with appositional woven bone; these lesions appeared early on scintigraphy, again suggesting fractional necrosis. Further confirmation can be found in studies of free vascularised bone transplants. Some fibular (Bos 1980) and rib grafts (Berggren et al. 1982) showed dead osteocytes while postoperative fluorochrome labels were normal throughout the cortex.

The absence of the first or the first two fluorochrome labels after four hours' occlusion suggests either temporary metabolic inactivity of the osteoblasts or degeneration of these cells followed by their early replacement from the vascular endothelium (Trueta 1963). This last sequence of events was observed in puppies by Kemp (1973) after tamponade to venous pressure for six hours. Damage to osteoblasts, despite normal vascularisation, was also suggested by Strömqvist (1983) to occur after femoral neck fractures. Of 10 fractures with reduced technetium uptake in the femoral head in the metabolic phase of scintimetry, three had no abnormality in the preceding vascular phase of the bone scan.

The repair process after fractional necrosis differs in two ways from that after total necrosis. In fractional necrosis the inter trabecular vascular tissue has full function, so that repair and remodelling not only follow directly after the ischaemic episode, but occur throughout the epiphysis. In contrast, repair after total necrosis occurs much later after the ischaemia and is characterised by a revascularisation front, appositional new bone formation and remodelling (Rössingh et al. 1969; Kenzora et al. 1978; Takaoka et al. 1981).

Deformation of the femoral head after avascular necrosis may result from mechanical weakening caused by bone remodelling during revascularisation and new bone formation (Sherman and Phemister 1947; Rössingh et al. 1969, 1972). Our investigation indicates that this mechanism of bone weakening may result from the far shorter period of ischaemia (two hours), which can produce fractional bone necrosis. We suggest that this
type of bone necrosis corresponds to the initial phase of Perthes’ disease; ensuing repair and remodelling may then result in resolution of the epiphysis and fully developed Perthes’ disease. It is concluded that after increasing the joint pressure for at least two hours the osteocytes of the femoral head epiphysis in rabbits die, while the vascular perfusion of the bone remains intact. This differs from the total necrosis which follows six hours of tamponade.

It is a pleasure to thank Professor A. van den Hooff for his encouragement to perform this study, providing laboratory facilities for the undecalcified material and correcting the manuscript. Thanks are also due to the Department of Pathology of the Regional Hospital Helmond-Deurne, and particularly Dr V. Noordhoek Hegt, for preparation of the decalcified material. We are also most grateful to Miss W. Tijgelaar-Gutter and Mr L. Chr. J. Van de Ven for the histological preparations. Finally, we would like to thank Mr Th. M. G. van Hout for photography.

REFERENCES


