OSTEOARTHRITIS:

AN EXPERIMENTAL STUDY IN THE DOG

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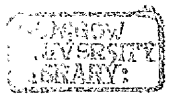

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SUMMARY

The incidence, importance and pathology of osteoarthritis (O.A.) is reviewed, with particular reference to the dog. The main theories of pathogenesis have been outlined. Much of the information on which these hypotheses are based has been gleaned from study of post mortem or surgical specimens, and, notwithstanding extensive current research into the biochemical changes of articular cartilage, the pathogenesis of the disease remains uncertain.

A study of experimentally induced O.A. was carried out in an attempt to elucidate further the early developmental stages of the disease process. O.A. was induced by transection of the anterior cruciate ligament in the canine stifle joint. A total of 52 dogs were used, survival time after surgery ranging from 1 to 48 weeks. A further 3 dogs were subjected to a sham operation to provide a control.

The development of bone remodelling in the unstable joint was studied in detail, using techniques of fluorescent bone labelling, microradiography and vascular perfusion as well as routine histopathological examination. The relevant literature on fluorochrome labelling and microradiography has been reviewed in depth.

Osteophyte formation began as early as 3 days after cruciate section in the marginal zone of the femoral trochlea. Initial fibrous metaplasia at this site was followed by florid deposition of woven bone and later, remodelling produced a mature osteophyte with a trabecular structure covered by fibrocartilage. Endochondral ossification contributed to the development of the osteophyte in the later stages. New bone deposition was still occurring 48 weeks after ligament section. Bone remodelling also occurred in the subchondral zone of the outer face/

face of the trochlear ridge, the periosteal surface of the sub-synovial femoral cortex and the epiphyseal trabeculae, resulting ultimately in recontouring of the joint surface.

Other pathological changes recorded in the joint included erosions of articular cartilage, synovitis, meniscal splitting and fibrous thickening of the joint capsule. The histopathological changes observed in articular cartilage and synovial membrane have been described briefly.

The relationship between clinical and radiographic assessments and the type or degree of pathological change is examined.

No significant pathological change was observed in the control, sham-operated joints. Increased bone remodelling and minor histological changes, possibly representing early stages of spontaneous O.A., were recorded in the contralateral stifle joint from the longer surviving experimental dogs.

The remarkably early development of bone changes in the joint conflicts with the view that osteophytes are a late manifestation of the disease; joint instability may, however, influence osteophyte formation.

Vascular proliferation was associated with each stage of development of the osteophyte and with other areas undergoing bone remodelling. Vascular factors probably play a significant role in the pathogenesis of this condition, which bears a close resemblance to that arising spontaneously in the joint. It is suggested that "osteoarthritis" is the most accurate term to describe both the experimental and naturally occurring condition in the canine stifle joint.

INTRODUCTION

Osteoarthritis (osteoarthrosis, degenerative joint disease) is a very common clinical finding in the stifle joint in the dog. This is often associated with rupture of the anterior cruciate ligament. In spite of the prevalence and importance of this condition in the dog, little is known about its exact nature or pathogenesis.

In this investigation the development of joint changes following experimental section of the anterior cruciate ligament was studied. The purpose of the experiment was: to describe the changes which occur following anterior cruciate section; to compare these changes produced by instability, with those described in osteoarthritis in dog and man; to investigate in detail the development of bone remodelling with particular reference to osteophytes at the margins of the femoral trochlea.

The experimental study was divided into several sections:

1) Assessment of clinical changes following anterior cruciate section.
2) Radiographical evaluation of joint changes.
3) Macroscopical evaluation of bone, cartilage and soft tissue pathology.
4) Investigation of changes in local vascularisation by dye perfusion studies.
5) Microscopic investigation of bone remodelling using fluorochrome labelling, microradiography and routine histology.

Suitable control material was subjected to the same investigations.

Concurrent investigations into the changes taking place in articular cartilage of the femoral and tibial condyles were carried out by other workers and are not reported in this thesis.

A number/

A number of specialised techniques were employed in this study. To evaluate alteration in vascularity a simple technique of vascular perfusion was adopted, this enabled a correlation to be made of the vascular changes in a region with the tissue changes which were developing. Fluorochrome bone labelling was used to demonstrate clearly the sequence and timing of new bone deposition. Contact microradiography was employed to differentiate areas of mineralisation not labelled by fluorochromes and to assist interpretation of fluorochrome label distribution. Routine histology in conjunction with these techniques allowed accurate identification of tissue types and cellular changes.

PART I

REVIEW OF LITERATURE

INTRODUCTION

a) Definitions and Terminology

Osteoarthritis is a disorder affecting many species of animal and man. It has been defined as a "non-inflammatory disorder of movable joints characterised by deterioration and abrasion of articular cartilage, and also by formation of new bone at the articular surface" (Sokoloff 1969). Most authors agree with Collins (1949) that the initial lesion is a destruction of articular cartilage; however all the tissues which comprise a diarthrodial joint may be affected by the disease process. The condition has been associated with ageing, many surveys showing the increasing incidence of osteoarthritis with age (Heine 1926, Bennett, Waine and Bauer 1942, Lawrence, Bremner and Bier 1966), and it has also been widely regarded as a "wear-and-tear" phenomenon. The uncritical acceptance of this hypothesis has been challenged by Dick and Buchanan (1971).

There are many synonyms for the condition. Those most commonly used are:

degenerative joint disease; osteoarthrosis; osteoarthritis; hypertrophic arthritis; arthritis deformans; arthrosis deformans; arthropathy.

The terms degenerative joint disease or osteoarthritis are commonly used in America, but British workers prefer the term osteoarthrosis, since "osteoarthritis" tends to imply an inflammatory disease. Throughout this thesis, the term osteoarthritis, abbreviation O.A., will be used.

b) History

O.A. is a condition which has probably existed for many centuries. There/

There is evidence that skeletons of prehistoric animals, and Egyptian mummies, show changes typical of advanced degenerative joint disease. (Fisher 1922). The first descriptions of the pathology of O.A. in man appeared in the early nineteenth century, but the term "osteoarthritis" was not used until 1890 (Garrod, quoted by Collins 1949). It was not until the early twentieth century that the disease was clearly differentiated from the other chronic deforming arthritis in humans, now known as rheumatoid arthritis (Nichols and Richardson 1909).

In animals, the first descriptions of O.A. appear some years later. Bennett and Bauer (1931) described O.A. in cattle, Callender and Kelser (1938) provided an account of the pathology of equine O.A., and Fox (1939) reported that O.A. was a common occurrence in his survey of a wide variety of captive and wild mammals. O.A. of the canine stifle joint was first reported in association with meniscal injuries by Nilsson in 1949; in some of these cases the anterior cruciate ligament was noted to be totally or partially ruptured. Some years earlier, degenerative changes had been induced experimentally in dogs by patellar displacement (Bennett, Bauer and Maddock 1932).

c) Incidence and importance in man and animals

The high incidence of O.A. in man has been recognised for many years (Heine 1926, Key 1930, Keefer and Myers 1934). Recent surveys carried out in Britain and other countries disclosed not only the high morbidity of O.A. but also the serious economic loss attributable to this condition. Dick and Buchanan (1971) reported that O.A. alone accounts for 40% of loss of working time in both the United Kingdom and the United States of America.

There/

There is much less information concerning the incidence and importance of O.A. in animals. The occurrence of O.A. in many different animal species has been reviewed by Sokoloff (1960, 1969) but little information on the incidence of the condition is available. It is widely recognised, however, that O.A., often following trauma to a joint, is common in the horse, especially in the fore-limb of racehorses. It may also be a problem in both beef and dairy breeds of cattle, particularly in hip, stifle and hock joints (Greenough, MacCallum and Weaver 1972).

d) Incidence and Importance in the dog.

a) General Review

O. A. is common in the dog and may affect any joint, often developing following an injury (e.g. fracture or ligament injury) to the joint. So-called "primary" O.A., i.e. that form of the condition in which the initiating cause is unknown, has also been recorded in the dog.

It is well recognised that there is a particularly high incidence of O.A. in the canine hip joint, especially in certain breeds of dogs. This can be related to the high incidence of hip dysplasia in these breeds, since O.A. is the inevitable consequence of hip dysplasia (Henricson, Norberg and Olsson 1966). Occasionally, O.A.of the hip can occur without any evidence of prior dysplastic changes.(Paatsama and Rokkanen 1974). Bellars and Godsal (1969) reported a high incidence of O.A. of the hip and shoulder joints in their survey of Antarctic sledge dogs and attributed this to abnormal stresses on these joints due to sledge-pulling and abnormal posture. There are a number of other reports of O.A. affecting the shoulder joint (Campbell 1968, Tirgari and Vaughan 1973) and the elbow joint (Campbell 1971, Tirgari 1974), but in general, the incidence/

incidence of the condition in the dog's forelimb is thought to be low.

b) Stifle joint

O.A. of the stifle joint of dogs is a common clinical entity in veterinary practice. It is often associated with rupture of the anterior cruciate ligament, which is widely recognised as one of the major causes of hind-limb lameness in the dog. An incidence of 32% of anterior cruciate rupture and osteoarthritis was reported in one study of hind-limb lameness in Alsatians (Henricson and Olsson 1959).

Many reports have described osteoarthritic changes in the joint developing several weeks after ligament rupture (Paatsama 1952, Pond 1971, Singleton 1960/61, Strande 1967) and some authors have stated that O.A. is the inevitable sequel of anterior cruciate ligament rupture, (Hickman 1964, Pond and Campbell 1972). Others, however, have reported degenerative changes occurring in the ligament and other joint tissues prior to rupture of the cruciate ligament (Paatsama 1952, Shebitz 1961, Strande 1967, Zahm 1965). Thus, in a series of 84 cases of O.A. of the stifle joint, Zahm (1965) found "mild" to "moderately severe" degrees of O.A. in joints in which the anterior cruciate ligament was intact, although histologically, degenerative changes were evident in the ligament. In those joints in which the anterior cruciate ligament had ruptured, she found that severe pathological changes with gross exostoses had developed. Leonard (1971) has also suggested that preliminary degeneration of the anterior cruciate ligament, due perhaps to ageing or to repeated mild trauma, precedes rupture of the ligament.

As Strande (1967) pointed out, if a dog is presented with a torn anterior cruciate ligament several weeks after the onset of lameness, the difficulty lies in establishing whether O.A. was present in the stifle joint/

joint when lameness began, or whether it developed as a secondary phenomenon. Nevertheless, a definite association exists between anterior cruciate ligament rupture and O.A., since degenerative changes will develop in the stifle joint following cruciate rupture, and have been produced experimentally by section of the ligament (Paatsama 1952, Pond 1971). Olsson (1971) however, does not agree that the changes which take place in the stifle joint after rupture of the cruciate ligament are those of true O.A.

A recent survey by Tirgari and Vaughan (1975) of 150 dog cadavers revealed a total of 30 dogs (20%) with O.A. of the stifle joint; of these 30 dogs, only 5 (16%) had ruptured anterior cruciate ligaments. No obvious predisposing cause was noted in the other 25 cases.

Apart from anterior cruciate ligament rupture, other internal derangements of the stifle joint, such as patellar luxation (Bennett, Bauer and Maddock 1932, DeAngelis 1971, Kodituwakku 1962, Leonard 1971) or meniscal tears (Hickman 1964, Nilsson 1949) may give rise to O.A.

Ajmal and Hayward (1970) provided a somewhat scanty description of the histopathology of 3 cases of O.A. of the stifle joint, the state of the anterior cruciate ligament in these joints was not mentioned.

The influence of age, breed, weight or sex of the dog on the incidence of O.A. of the stifle joint is not easy to assess, few of the reports of the condition record sufficient numbers on which to base this information.

Putnam and Archibald (1968) stated that age is the most important predisposing factor of O.A. in the dog, suggesting that changes develop in dogs 8 years of age or older, with the exception of larger breeds in which/

which O.A. may develop at a relatively early age. Singleton (1960/61) has also observed that O.A. is most frequently seen in the older dog, but added that changes may occur at 4 to 5 years of age, especially following trauma to the joint. Putnam and Archibald (1968) have suggested that larger breeds of dogs are more often affected by O.A., and that obesity is an important factor with respect to O.A. of the stifle joint. No relationship between the sex of the animal and the incidence of O.A. has been suggested.

If the relationship between rupture of the anterior cruciate ligament and the development of O.A. is accepted, further information with respect to the influence of age or breed on the incidence of stifle O.A. may be extracted from several detailed surveys on anterior cruciate rupture. Thus Paatsama (1952) in his investigation of anterior cruciate ligament rupture reported a mean age of 6 years at time of onset, while Loeffler (1964) found a mean age of 4.8 years (survey of 61 dogs). Strande (1967) in his survey of 117 dogs with anterior cruciate ligament rupture, recorded an unusually high incidence in dogs less than 2 years old, but most of these were of one particular breed (Chow Chow). If these were excluded, a peak incidence occurred between 4 and 6 years old. Pond and Campbell (1972) in an assessment of 107 cases of rupture of the anterior cruciate reported that it occurred only rarely in dogs under 2 years of age, and most commonly between 4 and 8 years of age. It may be assumed from this data that O.A. occurring as a sequel to cruciate rupture will be most common in middle-aged and elderly dogs.

Anterior cruciate rupture occurs in both large and small breeds of dogs and Pond and Campbell (1972) reported no significant breed involvement. The other three surveys, however, (Loeffler 1964, Paatsama 1952, Strande 1967)/

1967) showed that there was a tendency for larger breeds of dogs to be involved more frequently than the smaller breeds.

PATHOLOGY OF OSTEOARTHRITIS

Although there are many descriptions of the pathological changes occurring in human O.A., and also in various experimental models of this condition, detailed information relating to the pathology of O.A. in the dog is scarce. It is clear that a close resemblance exists between the pathological changes of O.A. in man and in the dog; nevertheless it is also obvious that there are differences in tissue response and therefore it may not be accurate to ascribe the histological features of O.A. in man directly to the condition in the dog. Some reports do provide histopathological descriptions relating specifically to the dog, but commonly, only radiological and macroscopical features are given. For this reason, in the following general literature review, most descriptions refer either to O.A. in man or to experimentally induced lesions in laboratory animals, and where the tissue changes have been noted to occur in the dog (whether in experimentally induced or naturally occurring O.A.) this has been stated.

All the tissues which comprise a synovial joint may be affected by the osteoarthritic disease process, namely:- articular cartilage, bone, synovial membrane, joint capsule, ligaments, menisci and blood vessels. Numerous publications have emphasised this fact, (Garrod 1910, Heine 1926, Pommer 1927, Bennett, Waine and Bauer 1942, Collins 1949, Sokoloff 1969), but in recent years, because of the importance placed on articular cartilage as the primary tissue affected in the disease process, most workers have concentrated solely on changes in articular cartilage, neglecting to consider the joint as a complete unit.

The following review covers each aspect of the pathology of O.A., dealing with cartilage and soft tissue changes briefly and with bone pathology/

pathology in detail.

a) Articular Cartilage

In recent years, degeneration of the articular cartilage has been widely regarded as the primary change of O.A., but exactly what happens first in the degenerate cartilage is still subject to debate.

i) loss of glycosaminoglycan (mucopolysaccharide) ground substance.

Biochemical analyses (McDevitt 1973) and histochemical staining of cartilage using dyes such as Alcian Blue (Stockwell and Scott 1965). Toluidine Blue (Meachim, Ghadially and Collins 1965) or Safranin-O (Rosenberg 1971), have indicated that depletion of glycosaminoglycans is an early event in O.A. Meachim and Stockwell (1973), emphasised, however, that this may not necessarily be the primary event which leads on to fibrillation of cartilage.

ii) loss of surface chondrocytes.

A loss of chondrocytes from the superficial layers of hyaline cartilage has been described as one of the earliest changes in O.A. (Meachim and Collins 1962, Meachim, Ghadially and Collins 1965). Pond (1971) reported loss of the superficial cell layer of articular cartilage is osteoarthritic canine stifle joints.

iii) flaking and fibrillation of cartilage.

Horizontal flaking of the surface layers of cartilage was regarded as the primary event of O.A. by Collins (1949), followed by deeper splits extending vertically into the transitional and deep zones, and eventually reaching the calcified zone (Meachim and Stockwell 1973). Ultimately the cartilage may be sheared off, exposing underlying bone. Some workers have, however, described a benign, age-related form of fibrillation of articular cartilage which does not progress to O.A. (Byers, Contepomi, Farkas 1970)./

1970).

Fibrillation of cartilage involves fragmentation of the collagen network and is associated with proteoglycan depletion (Mankin and Lippiello 1970) and with mechanical softening of the cartilage (Kempson 1973).

Fibrillation and erosion of cartilage, often through to the subchondral bone, has been recorded in natural and experimentally induced O.A. in dogs (Pond 1971).

iv) chondrocyte clumping.

The formation of "cell nests" or clones of chondrocytes in osteoarthritic cartilage has been described by Heine (1926), Bennett, Waine and Bauer (1942), Collins (1949), Sokoloff (1969), Stockwell and Meachim (1973). The association of these abnormal cell clusters with deep vertical clefts in fibrillated cartilage has led to speculation that they represent attempted repair or reactive remodelling in the damaged cartilage.

v) other cartilage changes.

Lacunar resorption, fatty degeneration of cells or matrix, and alteration ("unmasking") of the collagen fibres have been described (Sokoloff 1969).

b) Bone

i) periarticular osteophytes

The presence of marginal proliferations of bone, normally termed osteophytes (synonyms: exostoses, lipping, spurs), has been recognised as an integral part of O.A. since the earliest descriptions of the disease. Bennett, Waine and Bauer (1942) in their monograph on changes in human knee joints, have given an excellent review of the early literature/

literature and early theories concerning osteophyte development.

Bennett and colleagues also described briefly the history of the osteophyte from its earliest appearance as proliferating fibrous tissue in the transitional zone between synovial membrane and articular cartilage, to the well-defined lipping of bone and cartilage in later years. Weichselbaum in 1877 (cited by Bennett, Waine and Bauer 1942) was apparently the first to draw attention to what he called the "zone of proliferation". He described the origin of osteophytes as fibrocartilaginous structures in this zone, and emphasised the importance of the structural and functional characteristics of this marginal or transitional zone which consists of primitive mesenchymal tissue with numerous blood vessels and a marked reparative and proliferative ability.

Collins (1949) described a mature osteophyte as a "true exostosis consisting of cancellous bone whose marrow spaces are continuous with the epiphyseal marrow; and which is covered by fibrocartilage or fibrous periosteum and not by hyaline cartilage".

Trueta (1968) defined an osteophyte as new bone and marrow formed within a degenerate articular cartilage, developing first at the junction of articular cartilage and synovial membrane.

Bennett et al (1942) noted that the development of osteophytes in human knee joints always followed change in the articular cartilage, and this is also emphasised by Collins (1949), who stated "(osteophytes) are not essential to the diagnosis of osteoarthritis and they are relatively late manifestations. Osteophytes always indicate the presence of advanced cartilage destruction in the more central parts of the articular facet which they border". Gardner (1965) and Sokoloff (1974) also echoed this view, and Trueta (1968) stated categorically that degeneration of articular/

articular cartilage precedes osteophyte formation.

In contrast to this, a number of authors have reported the existence of osteophytes without obvious or marked cartilage abnormality. Byers and co-workers in a study of post-mortem human hips described the common occurrence of osteophytes in the presence of limited cartilage degeneration; but they suggested that this type of change should be regarded as "non-progressive" and age-related, and classified separately from "progressive" changes of true O.A. (Byers, Contepomi and Farkas 1970). This hypothesis is supported by a radiological survey of the hip joint undertaken in Sweden, revealing a high radiological incidence of osteophytes in asymptomatic joints (Danielsson 1964).

Marshall and Olsson (1971) concluded, on the basis of experimental investigations in dogs' knee joints, that the diagnosis of O.A. should not be made solely on the presence of osteophytes, since they noted marked osteophyte development in the joints but only minor cartilage changes such as decreased metachromasia, cell clusters and a few clefts. Some earlier experimental work by Magnuson (1941) using a similar experimental model to that of Marshall and Olsson, (resection of the anterior cruciate ligament of the canine stifle joint), showed that exercise was an important factor in the development of osteoarthritic change. Magnuson (1941) reported that changes typical of degenerative arthritis were induced in the unstable joint and described the appearance of exostoses before "any considerable amount of degeneration of cartilage was observed". Pond (1971) also described osteoarthritic changes, including osteophyte proliferation and characteristic cartilage erosions and fibrillation, in canine stifle joints following experimentally induced and naturally occurring/

occurring anterior cruciate rupture.

Recently Tirgari and Vaughan reported osteoarthritic lesions in canine shoulder joints, and commented that while large osteophytes were usually associated with cartilage erosion, some osteophyte development accompanied merely small areas of cartilage fibrillation (Tirgari and Vaughan 1973). Tirgari (1974) has also recorded two dogs with ossicle formation in the shoulder joints, and noted obvious osteophyte formation at the articular margins while the cartilage showed discoloration and loss of sheen but no marked erosions. Sokoloff (1969) commented that while it is normal for the degree of osteophytosis to be proportional to the erosive changes in the articular surface, sometimes marked osteophyte formation may occur with little loss of cartilage, or severe erosions may be present without osteophyte formation. Boyle and Buchanan (1971) described osteophyte formation in a patient with "early" O.A., but pointed out that changes in bone are probably secondary to articular cartilage change.

It is generally accepted that osteophytes develop by a process of endochondral ossification which advances into the under side of articular cartilage or into newly proliferated fibrous tissue or fibrocartilage (Nichols and Richardson 1909, Collins 1949, Trueta 1968, Marshall 1969, Boyle and Buchanan 1971, Telhag and Lindberg 1972, Jeffery 1973).

Bennett and colleagues, however, outlined more than one type of new bone formation at the joint periphery, suggesting that as well as by endochondral ossification into marginal articular cartilage, osteophytes may be produced by periosteal bone formation or by metaplasia from proliferating synovial membrane. The earliest sign of marginal proliferation noted by these workers comprised a proliferation of fibrous tissue/

tissue in the so-called transitional zone, producing a marginal ridging discernible before any subchondral bone reaction was noted and in the absence of any significant cartilage abnormality other than "primary lesions" of the articular surface (Bennett, Waine and Bauer 1942). Similarly, fibrous proliferation in the transitional zone was also the earliest sign of osteophyte formation in experimentally induced arthropathies in the dog (Bennett, Bauer and Maddock 1932) and in rabbits (Bennett and Bauer 1937).

A similar description of the early development of osteophytes in the canine stifle joint following transection of the anterior cruciate ligament is given by Marshall (1969). Initially, there was a fibrous proliferation with slight chondroid metamorphosis situated in the transitional (marginal) zone; later a true spur "with cartilage islands and osseous metamorphosis close to the pre-existing bone" was formed. Further investigation with the same experimental model but with the dogs surviving for longer periods (Marshall and Olsson 1971) emphasised the invasion of joint cartilage by vessels from the bone marrow of the osteophyte (although precisely how this was achieved is not clear) with the subsequent formation of new bone in the deeper layers of the articular cartilage. Telhag and Lindberg (1972) in an account of the development of O.A. in rabbit knee joints following massive surgical intervention concluded that osteophyte development was the result of endochondral ossification of preformed cartilage, and commented on the similarity of their results to those of Marshall.

Keefer and Myers (1934), on the basis of histological examination of osteoarthritic human knee joints, did not agree with the opinion that osteophytes were bony outgrowths but suggested that their formation was

due/

due to depression of the normal joint contours or to remnants of joint margin being forced outward by constant pressure. Heine (1926) also claimed that, in addition to endochondral ossification of articular cartilage and periosteal bone formation, mechanical displacement of undermined articular margins might account for joint deformity.

Collins (1949) suggested that marginal hyperplasia is one of the earliest manifestations of a slow remodelling process which ultimately alters the whole outline of the joint surfaces. He added that osteophytic lipping should not be regarded as an isolated phenomenon but as a part of the whole recasting of the joint under the influence of altered mechanical forces.

ii) subchondral bone sclerosis

Subchondral bone may show striking abnormalities in advanced O.A., and, as Bennett and colleagues pointed out, such changes accompanying degeneration of cartilage were considered by some workers at the beginning of this century as indispensable criteria for the histological diagnosis of the disease (Bennett, Waine and Bauer 1942). Since that time until very recently, less emphasis has been placed on these bone changes.

Bennett, Waine and Bauer (1942) found that the subchondral bone appeared normal unless the overlying cartilage was markedly altered, but that conspicuous change was invariably present when the cartilage had been denuded. Denuded articular surfaces apparently demonstrated pronounced sclerosis of bone, and, in the most severely affected joints, a dense surface of "devitalised and eburnated bone".

Collins (1949) stated that "a cellular reaction in subchondral bone prepares a substantial sclerotic bone surface prior to stripping of articular cartilage". He described the remodelling process which gives rise/

rise to the addition of new bone lamellae to the subchondral plate and adjacent trabeculae, and added that the radiographic appearance of sclerosis is due to thickening of the subchondral plate, epiphyseal cortex and immediately subjacent trabeculae. Gardner (1965) described growth of appositional bone in the region of the bony end plate of an affected joint, resulting in progressive thickening of this bone, and also stated that this bone change accompanies or often precedes cartilage fibrillation.

In recent years, Radin and colleagues have put forward the theory that subchondral bone sclerosis or rather, increased stiffness of subchondral bone, may be important in the early stages of O.A. (Radin, Paul and Rose 1972). Apparently, evidence of healing or healed trabecular microfractures in subchondral bone of patients with relatively early joint degeneration has been noted, and it is suggested that this leads to bone remodelling with resultant increased stiffness. Experimentally, rabbits subjected to repetitive impulse loading developed subchondral bone sclerosis which was manifest before the earliest biochemical abnormality of the articular cartilage, (Radin 1973).

Trueta (1968) described bone sclerosis occurring in areas of maximum osteogenic activity in weight-bearing areas of the joint and associated this with a vascular response which he suggested was elicited by degeneration of joint cartilage. Rutishauser (1956) also described "osteosclerosis" in pressure areas of osteoarthritic human femoral heads, vascular proliferation (of arterioles and venules) was noted in association with this.

Few references can be found to the occurrence of subchondral bone sclerosis in canine O.A. Olsson (1971) stated that subchondral sclerosis is one of the underlying changes of degenerative joint disease but it is not/

not clear from his review article if he is referring specifically to the dog. Bellars and Godsal (1969), reporting on osteoarthritic changes in the hip and shoulder joints of husky sledge dogs, did not give any histopathology except for a very brief description: thickening of the subchondral bone trabeculae was noted. Riser(1973) described thickening of the trabeculae in the subchondral region of dysplastic femoral heads in which secondary O.A. had developed. Bennett and Bauer (1937) induced O.A. in dogs by displacement of the patella and one photomicrograph clearly shows sclerosis and eburnation of bone. Paatsama and Sittnikow (1972) reported trabecular thickening in their series of dogs with experimental anterior cruciate section. On the other hand, Marshall (1969) recorded normal subchondral bone in his series of experimental O.A. following cruciate section.

Hickman (1964) commented that the pathology of O.A. in the dog conforms to the accepted pattern, and that sclerosis and eburnation of subchondral bone follows erosion of the articular cartilage. Putnam and Archibald (1968) also described sclerosis of subchondral bone, evident on x-ray of an osteoarthritic canine stifle joint.

iii) subchondral bone cysts

Sokoloff (1969) has stated that these are found in a large proportion of severely osteoarthritic joints, and that they are particularly common in the hip joint. They are not true cysts since trabeculae and marrow are replaced by mixed connective tissue; their pathogenesis is uncertain. Subchondral cysts have been recorded in the earliest descriptions of O.A., and Bennett, Waine and Bauer (1942) point out that they are not specific for O.A.

Riser (1973) reported that subchondral cysts were found in osteoarthritic/

osteoarthritic hip joints of only two dogs, (the total number examined is not given), he considers them to be a rare occurrence in the dog.

iv) bone remodelling

Osteophyte proliferation, subchondral sclerosis and cyst formation are all part of the process of bone remodelling which occurs in osteoarthritic joints. In addition to these aspects which have already been reviewed, there is a general remodelling process of the internal architecture of the epiphysis which, together with osteophyte formation, alters the bony contours of the severely affected joint.

In some of the early descriptions of the nineteenth and early twentieth century (cited by Bennett, Waine and Bauer 1942) "atrophy" of the epiphyseal bone was regarded as an important, even primary, event of degenerative joint disease. Bennett and his colleagues, however, found no evidence in their survey which supported this, and suggested that bone resorption was enhanced in the "process of trabecular rearrangement incident to severe degenerative joint disease". They described hyperaemia of the bone marrow and concomitant cellular proliferation, osteoblasts lining the trabecular surfaces and producing abundant new bone matrix. In adjacent areas osteoclast activity was evident. They concluded that a process of intense structural readjustment was being carried out. Collins (1949) also described bony remodelling in human knee joints affected by O.A. A cellular reaction in the bone marrow was accompanied by both resorption of bone and the deposition of new lamellar bone, and he also noted an irregular advance of ossification into the calcified cartilage. He stated that this slow alteration of the internal architecture of the entire epiphysis causes many changes in shape of the joint surface, changes in the contour of one face being roughly adapted/

adapted to changes in contour of the opposing face.

Sokoloff (1969) emphasised that change in the contour of joint surfaces, through both erosion and proliferation of tissues, is an important aspect of the osteoarthritic lesion.

Bauer and Smith (1969), Batra and Charnley (1969) and Jeffery (1973) have shown by different techniques that there is considerable osteogenesis or bone turn-over in advanced osteoarthritis of the hip and knee joint in man.

Again, little reference to this process of bone remodelling can be found in literature describing the pathology of canine O.A. Riser (1973) outlined the extensive bone remodelling which is an integral part of canine hip dysplasia and the ensuing degenerative joint disease. Ajmal and Hayward (1970) reported three cases of O.A. in dogs, but did not mention changes in the bone except to comment that in one dog there was "distortion" of the femoral condyles. Presumably this was the result of bone remodelling.

c) Soft Tissue

i) Synovial Membrane

Relatively little emphasis has been placed on the importance of synovial membrane changes in consideration of the pathology or pathogenesis of O.A. In some of the earliest descriptions of the 19th century, however, inflammatory changes in the "synovialis" were put forward as the primary cause of cartilage degeneration (quoted by Bennett, Waine and Bauer 1942). Majority opinion in more recent years has regarded the synovial membrane changes as secondary.

Thus, Bennett, Waine and Bauer (1942) reported first an increased thickness/

thickness of the sub-intimal connective tissue and later proliferation of hyperplastic synovial membrane, occasionally with reddening due to many dilated blood vessels. The cellular proliferation was usually slight, with occasional small perivascular accumulations of lymphocytes. Collins (1949) provided a similar description, and commented that the appearance did not suggest an active inflammatory process. Sokoloff (1972) also stated that "inflammatory changes in the synovium are not characteristic of osteoarthritis", although synovial hypertrophy and fibrosis is seen. Earlier, in his monograph on degenerative joint disease, Sokoloff (1969) pointed out that synovitis may be considerable in symptomatic or advanced cases of O.A.

Lloyd-Roberts (1953) suggested that hyperplasia and fibrosis of the synovial membrane resulted from phagocytosis of fragments of bone and cartilage in an osteoarthritic joint; a similar theory was put forward by Dick and Buchanan (1971). Boyle and Buchanan (1971) commented that it is insufficiently realised that inflammatory changes do occur in O.A., and that these, although not a constant feature, are occasionally severe.

Gardner (1965) while reiterating that O.A. is not inflammatory in nature, stated that a low-grade synovitis often develops and proposed that this is secondary to "mechanical changes in the articular surface" and "indirect trauma" to the synovial tissues.

Several authors have recorded the presence of a fibrous thickening of the synovial membrane in dogs with O.A. (Ajmal and Hayward 1970, Marshall 1969, Olsson 1971, Pond 1971, Riser 1973). In addition, Pond (1971) and Marshall (1969) both commented on an increase in vascularity of the synovial membrane, and Marshall (1969) and Riser (1973) noted villous hypertrophy/

hypertrophy or proliferation. Pond (1971) found that synovial membrane changes developed very early in the disease process, prior to any visible change in the articular cartilage. Tirgari (1972) in a survey of arthritis of the canine stifle joint described an early lymphocytic infiltration near capillaries, and in more advanced cases he noted villi of hyperplastic synovial lining cells, blood vessels and lymphocytes.

ii) Joint Capsule

Pathological change in the joint capsule of an osteoarthritic joint has received little comment. Collins (1949) stated that no abnormality of the soft tissues can be detected in the early stages of O.A., but that in later stages, capsular fibrosis is the most constant of the changes which occur. Lloyd-Roberts (1953) provided a detailed description of capsular changes in O.A. of the hip joint.

Jubb and Kennedy (1970), Marshall and Olsson (1971) and Pond (1971) described fibrous thickening of the joint capsule as a prominent feature of an affected canine joint.

iii) Menisci

Fibrillation and calcification of the menisci from knee joints of elderly people were observed by Bennett, Waine and Bauer (1942). However, Collins (1949) maintained that consistent degenerative changes of menisci in association with O.A. may be observed only in advanced cases.

In O.A. of the canine stifle joint, Marshall and Olsson (1971), Pond (1971), Tirgari (1972) and Tirgari and Vaughan (1975) recorded degenerative changes of the menisci such as splitting or shredding, and in one case even osseous metaplasia.

iv) Vascular Changes

Apart from vascular changes noted in the synovial membrane little mention/

mention is made of vascular abnormalities in the classical descriptions of the pathology of O.A. Bennett, Waine and Bauer (1942) noted hyperaemia of the bone marrow and associated extravasation of red blood corpuscles and vascular tufts penetrating the bone-cartilage interface. Collins (1949) also commented briefly on the presence of fibroblastic and vascular proliferation within the marrow.

Injection studies on normal and osteoarthritic hip joints from human cadavers by Harrison and colleagues (Harrison, Schajowicz and Trueta 1953), demonstated hyperplasia of the intra-osseous arteries of the femoral head in O.A. Other workers have also commented on the vascular proliferation which may be observed within the bone in osteoarthritic joints (Sell 1960, Trias, quoted by Moskowitz, Klein and Mast 1967, Sokoloff 1969).

Abnormal venous drainage has also been demonstated clinically in osteoarthritic joints, notably in the hip joint, by means of intraosseous phlebography, (Phillips 1966, Hulth and Hernborg 1968, Arnoldi, Linderholm and Müssbichler 1972). Helal (1965) described distension of medullary venous sinusoids in knee joints with O.A. Hulth and Hernborg (1968) and Hernborg (1969) demonstrated reduced radioisotope clearance from osteoarthritic femoral heads, and claimed that this indicates impairment of the capillary circulation.

Some early investigators noted an association between arteriosclerosis and O.A., but this was later dismissed as an incidental finding (Bennett, Waine and Bauer 1942, Sokoloff 1969).

PATHOGENESIS OF OSTEOARTHRITIS

Since the first descriptions of O.A., a number of different theories of pathogenesis have been proposed. Some of these theories have subsequently been discarded as untenable, and have not been reviewed here. The three major schools of thought regarding the pathogenesis of O.A. have been outlined below.

1. The initial lesion occurs in the articular cartilage

Currently, the most widely accepted view is that the initial abnormality of O.A. lies in the articular cartilage. In recent years, therefore, much research has been directed at defining the changes that take place in cartilage, and the sequence of these events. As yet, however, the initiating factor (or factors) is still unknown.

Two schools of thought exist: 1) there is a primary depletion of proteoglycan, due to failure of synthesis, excessive degradation or synthesis of abnormal proteoglycan; 2) there is a primary failure of the collagen network due to fatigue, and hence fragmentation of collagen (Editorial: Lancet Nov. 17, 1973).

2) The initial lesion occurs in bone

In contrast to all the work carried out on cartilage, relatively little investigation into the development of bone changes has been undertaken. It is generally accepted that, while gross abnormality of bone can cause O.A., changes in bone are usually secondary to those occurring in cartilage. Radin and colleagues, (Radin, Paul and Rose 1972) have, however, proposed that subchondral bone resonds to repeated impulse loading by remodelling, thus increasing in stiffness and becoming less effective as a shock-absorber. The articular cartilage is thereby subjected/

subjected to increased stress and degenerative changes ensue. This theory is supported by the observations of Foss and Byers (1972) who have demonstated above-average bone density in association with O.A.

3) The initial lesion occurs in osseous vasculature

A variety of vascular disorders have been suggested as factors responsible for the development of O.A.; currently these are widely regarded as secondary rather than primary changes.

Venous congestion was produced experimentally in dogs (Bernstein 1933) and in rats (Brookes and Helal 1968), resulting in minor joint changes in these animals which did not, however, resemble those of O.A. very closely. Brookes and Helal (1968) suggested that such changes, together with the abnormality of venous drainage observed in O.A., strongly implicated a change in the microcirculation as a causal factor in O.A.

Harrison and colleagues (Harrison, Schajowicz and Trueta 1953) maintain that arteriolar hyperplasia is responsible for much of the pathological change observed in O.A., but postulate a "metabolic disorder taking place in situ" as the initial stimulus of the vascular invasion. Phillips (1968) considers that hyperaemia precedes venous congestion in O.A. of the hip joint, and does not regard the vascular changes as secondary to osteoarthritic change.

EXPERIMENTAL MODELS OF OSTEOARTHRITIS

To obtain a clearer understanding of the pathogenesis of O.A. it is necessary to investigate the early stages of development of the disease. This is difficult in naturally occurring O.A. since recognition of the precise time of onset is unlikely, and specimens of affected joints, obtained either at post mortem or at surgery, have usually progressed to show advanced changes.

To overcome this problem, many experimental models of O.A. have been devised; these have been reviewed by Key (1930) and by Sokoloff (1969). In most systems, either a chemical or a physical insult is applied to a joint of an animal, and the ensuing pathological changes determined. Sometimes, however, the pathological changes induced do not resemble the naturally occurring disease, for example, the lesions described in the stifle joint of a dog following femoral vein ligation (Bernstein 1933). One dog (after 9 months' survival) showed discrete "punched-out" cartilage lesions, hyperplasia and hyperaemia of the synovial membrane but no bone changes; and repetition of the experiment with survival for 2 months did not produce the same changes. This is obviously not a good model of O.A. Similarly, there are limitations to a model in which the "insulted" joint develops some but not all of the pathological features of O.A., and in which progression to the state typical of advanced O.A. does not occur. Thus, scarification of articular cartilage may result in histological and biochemical changes in the cartilage but bone lesions do not develop (Meachim 1963).

The various experimental models may be grouped into 5 categories, according to the method used to induce the lesion. They have been outlined below, but only literature relevant to the model used in this investigation/

investigation will be reviewed in detail.

1) Surgical intervention: e.g. section of articular ligaments, meniscectomy, surgical defects of articular cartilage.
2) Mechanical insult: e.g. prolonged joint compression, repeated forced trauma, altered load-bearing.
3) Intra-articular injections of noxious agent: e.g. proteolytic enzymes, inorganic foreign bodies.
4) Genetically influenced: e.g. particular strains of laboratory mice.
5) Local or systemic alterations of blood circulation: e.g. venous ligation producing venous congestion.

Experimental O.A. induced by severing the anterior cruciate ligament in dogs

The first description of "the typical picture of degenerative arthritis" induced by sectioning the dog's anterior cruciate ligament is given by Magnuson (1941), from his description he appears to have cut the medial (medial collateral?) ligament as well as both cruciate ligaments. He stipulated that the dogs should be given ample exercise since neither proliferation of bone nor cartilage degeneration occurred if they were kept confined. Furthermore, he commented that bony exostoses appeared before "any considerable amount of degeneration of cartilage was observed".

Section of the anterior cruciate alone was performed in experimental dogs by Paatsama (1952). The O.A. which developed in these joints was stated to "correspond completely to the clinical and operative picture of dogs operated for long standing injuries" (i.e. to those dogs with spontaneous cruciate rupture and natural O.A.).

Similar experiments were carried out by Marshall (1969) in which section/

section of the anterior cruciate ligament was performed through an arthrotomy. The resulting pathological changes included variable cartilage changes such as irregular thickening, loss of metachromasia and surface erosions which later healed. There were marked marginal proliferations of osteophytes but no changes in the subchondral bone, and the synovial membrane showed thickening, villous hypertrophy and increased numbers of blood vessels. This investigation was repeated as a long-term study by Marshall and Olsson (1971); again slight degenerative changes in the articular cartilage were noted, characterised histologically by decreased metachromasia, cell clustering and formation of clefts, and there was also prominent osteophyte development and remodelling of the condyles. Nevertheless, the authors inferred that the lesion produced was not that of O.A., since "the osteophytes were usually formed and continued to grow even in the absence of changes in the joint cartilage".

Pond (1971) and Pond and Nuki (1973) however, demonstrated clearly that both articular cartilage lesions and periarticular osteophyte formation were produced by experimental section of the canine anterior cruciate ligament. The technique adopted was that of severance of the ligament through a small stab incision rather than by arthrotomy as previously described. This method had the obvious advantage of producing minimum interference with other joint structures. There was a close resemblance between the experimentally induced lesions and those of naturally occurring O.A. in the dog.

On the basis of this work, it was decided to investigate the early developmental stages of experimentally induced O.A., using the method described by Pond and Nuki (1973) to section the anterior cruciate ligament in/

in the canine stifle joint. The project was divided into three parts: 1) investigation of cartilage changes by histology and scanning electron microscopy, to be carried out by M.J. Pond; 2) investigation of biochemical changes in the cartilage, to be carried out at the Kennedy Institute of Rheumatology; and 3) investigation of bone remodelling, which forms the major part of this thesis. Some changes observed in cartilage and soft tissues of the joint have, however, been included in the thesis in an attempt to provide a comprehensive picture of experimental O.A.

PART II

MATERIALS & METHODS

1. Experimental Animal

A total of 55 adult dogs was used in this study. The exact ages of each dog was unknown, but skeletal maturity was ensured by X-ray. The dogs were of no particular breed, but Foxhounds, Collies and Alsatian crosses were the three most common types used in this experiment.

All dogs weighed between 12 kgs and 30 kgs. Twenty one dogs were female, thirty four were male dogs.

The dogs were divided into 2 groups:- 52 dogs of group A were subjected to section of the anterior cruciate ligament of the right stifle (knee) joint, the left stifle remaining unoperated and providing a control; group B consisted of 3 dogs which were subjected to a "sham" operation.

2. Management

The dogs were housed in individual kennels and allowed exercise in concrete runs twice daily for periods varying from $\frac{1}{2}$ hr to 1 hr. All dogs were vaccinated against distemper (Epivax - Burroughs Wellcome) prior to the start of the experiment, and regular treatment with anthelmintics and insecticidal dusting powders was employed during the course of the experiment. The diet was supplemented by weekly addition of bone meal and yeast tablets.

3. Experimental Procedure

a) Section of the anterior cruciate ligament (Group A)

Induction of experimental osteoarthritis in dogs by section of the anterior cruciate ligament through a stab incision was first described by Pond and Nuki in 1973. The same procedure was employed in this series. Each dog was screened before surgery by clinical and radiographic means, to/

to ensure that there was no deformity or osteoarthritis of the knee joint. The dogs were premedicated with 1 mg acepromazine (Acetylpromazine Crookes Veterinary Limited) and then subjected to general anaesthesia, using sodium thiopentone (Intraval, May & Baker Limited) to induce anaesthesia and a mixture of oxygen, nitrous oxide and halothane (Fluothane, I.C.I. Limited) administered by closed circuit, for maintenance. The animal was placed in left lateral recumbency and the right hind limb clipped and washed in the region of the stifle joint for aseptic surgery. The right anterior cruciate ligament was sectioned using a sharp-pointed (Gillette No. E11) scalpel blade. To facilitate section of the ligament, the stifle joint was held in partial flexion. The scalpel blade was inserted into the joint in a postero-medial direction through a stab hole made just lateral to the straight patellar ligament, and the anterior cruciate ligament was cut by turning the blade and withdrawing it in a lateral direction. In the majority of dogs, sectioning of the ligament was achieved with minimal joint trauma; in a few dogs joint cartilage was incised, and in one case (9/2) the scalpel blade broke and the tip of the blade remained within the joint capsule. Penicillin (Penidural, Wyeth Limited) was given routinely post-operatively.

b) "Sham Operation" (Group B)

The procedure employed for anaesthesia and preparation for surgery was the same as that described for Group A dogs. At operation, the anterior cruciate ligament was not sectioned. The scalpel blade (Gillette No. E11) was inserted into the joint through a lateral, parapatellar stab incision and was then rotated to inflict minor trauma to the synovial membrane and in 2 dogs (dogs 1 and 3) minor scarification of the condylar cartilage was also produced. The blade was then withdrawn and the skin

wound/

wound sutured.

c) Post-operative procedure.

Post-operative survival of the dogs ranged from 1 week up to 48 weeks after cruciate section for Group A dogs, and for 2 to 8 weeks for Group B dogs. During their experimental life all dogs were examined at regular intervals, every 3-4 days in the short term survival dogs, and at weekly, fortnightly or longer intervals in the longer surviving animals. On each occasion the degree of lameness was noted and the clinical features were assessed and recorded. In addition, each dog was injected with fluorochrome bone labels at specific time intervals (Table 8) Before euthanasia a plain lateral radiograph was taken of the right stifle joint.

d) Procedure at euthanasia

The dogs were killed by intravenous injection of sodium pentobarbitone (Euthatal, May and Baker Limited). In 26 dogs of group A and 2 dogs of Group B, the femoral arteries of both hind limbs were cannulated immediately following euthanasia, and a suspension of barium sulphate in Prussian Blue dye was injected. These dogs were left for 30 minutes before dissection of the limbs was carried out; in all other cases the left and right stifle joints were excised immediately after euthanasia. Synovial fluid was aspirated from the operated joint and an estimate of volume and character of the fluid was recorded. The pathological features of each joint were recorded in detail, comparing the appearance of operated and control joints. In addition photographic record was obtained in every case. Each stifle joint was then sectioned and the femoral trochlear region (fig. 2), from both left and right stifles, was set aside for investigation of bone changes. The femoral condyles and tibial plateau

were/

were used in the study of cartilage changes. One of the Group B dogs died 19 days after surgery and the delayed post mortem examination precluded biochemical analysis of cartilage from the femoral condyles and tibial plateaux. However, tissue from both stifle joints of this animal was processed routinely for microscopic examination.

4. Vascular Perfusion

Demonstration of blood vessels by perfusion with contrast media is a well recognised technique (Trueta 1968). A very simple method was employed in 28 dogs (26 of Group A and 2 of Group B) in this experiment, using a suspension of barium sulphate (Micropaque, Damancy & Company Limited) in Prussian Blue dye (Berlin Blue, potassium ferrocyanide). Barium sulphate within the vessels was demonstrable on microradiographs, whilst Prussian Blue dye demonstrated vessels on fluorescent microscopy, in standard (decalcified) histological sections and also macroscopically.

After death of the animal, both left and right femoral arteries were cannulated, and the same volume of Micropaque-Prussian Blue suspension injected into each limb under digital pressure. A 2% solution of Prussian Blue dye was employed, in which 10 gms per 100 ml of Micropaque powder was suspended by constant stirring. The volume injected varied with the size of the dog, from 50 mls for the smaller animals up to 80 mls in the larger dogs. Exsanguination or heparinisation procedures were not carried out prior to perfusion.

5. Preparation of Undecalcified Bone Sections

Undecalcified bone sections are essential both for fluorescent bone labelling and for microradiographic investigations. Due to the nature of bone, however, suitable undecalcified sections are difficult to prepare; many of the methods described in the literature are laborious and/

and time-consuming.

Bergendahl and Engfeldt (1960) outlined methods of preparation of mineralised tissues for microradiography. They described a technique of careful grinding of the bone specimen until the appropriate thickness was achieved; cancellous bone was embedded in plastic prior to grinding. Jowsey and colleagues (1965) described a method of cutting thin (approximately 100 μ) sections of undecalcified bone on a milling machine with subsequent grinding of the sections to produce an accurate thickness of 100 μ. Embedding of the bone specimen in plastic was essential for this method. A similar technique was employed by Olsson and Reitz (1966).

Frost (1958) developed a technique of manual grinding of fresh un-embedded bone, by which he claimed to produce undecalcified sections of 10 μ thickness. Johnstone and Tam (1973), however, reported that only 100 μ thick sections could be produced by this method, and that bone dust resulting from the grinding process interfered with structural detail of the section. These authors used an epoxy-resin embedding medium, cut 150 μ sections by milling machine and finally ground the sections by hand to a thickness of 7 μ.

McQueen, Monk, Horton & Smith (1972) reported in detail a method of obtaining undecalcified bone sections using a low speed rotary saw. They recommended embedding of the bone specimen prior to cutting, and employed a grinding technique to achieve the flatness and parallelism required for autoradiography and quantitative microradiography. Bard, Dickens, Edwards and Smith (1974) used the same low speed rotary saw to cut 100 μ sections of fresh undecalcified bone without prior embedding of the specimen. Sections suitable for qualitative work were obtained by using a lower blade speed than that recommended by McQueen and colleagues; grinding/

grinding of the section was not necessary.

A "Microslice 2" machine (Metals Research Limited, Cambridge) was employed (fig. 1). This machine has a tensioned, annular saw blade with an electrometallic diamond cutting surface on its inner edge; the specimen to be sectioned is rigidly mounted on one end of a damped, counterbalanced arm and allowed to contact the rotating blade by adjusting the counterbalance load. By this means the specimen is not forced against the cutting edge and therefore the delicate trabecular structure of the bone is preserved.

Preparation time per section using this machine was kept to a minimum because there was no need to embed the tissue in an epoxy-resin prior to sectioning. The block of fresh bone was simply embedded in hard, low-melting point dental wax* on a glass strip which was then mounted by means of a double-sided pressure-sensitive adhesive tape to the work-table.

With the specimen suitably counterbalanced on the pivot arm and the blade rotating at a slow controlled speed (approximately 200-300 revs per min.), sections of 80-100 μ of the undecalcified bone were obtained. Usually between 3 and 6 sections of 100 μ thickness were taken from the different levels of the femoral trochlea (fig. 2), and the thicker intervening peices of bone (approximately 5 mms thick) were retained for decalcification and routine histological processing.

The thin undecalcified trochlear sections were stored deep frozen. The same sections were used for fluorescent microscopy and for microradiography.

6. Fluorescent Bone Labelling

The rationale of this technique has been described in detail in Part IV. Briefly,/

* Dental Fillings Limited

Briefly, the system affords an excellent record of the dynamic sequence of new bone deposition because the fluorochrome labels become incorporated into bone mineralising at the time of administration of the label, and the labelled bone subsequently fluoresces characteristic colours on exposure to ultra-violet (U.V.) light.

Each dog was given between 2 and 5 different fluorochrome labels at recorded intervals during the experimental period, and following death the distribution of labels was recorded. Full details of materials and method used and results obtained are given in Part IV.

7. Microradiography

Contact microradiographs were prepared from undecalcified femoral trochlear sections from left and right stifle joints. Full details of materials and method used and results obtained may be found in Part V.

8. Decalcified Sections

The thicker blocks of bone from the femoral trochleas (fig. 2) were subjected to routine decalcification in 10% formic acid in formalin, double embedded in celloidin and paraffin wax and sectioned on a Spencer rotary microtome to a thickness of 7 μ. For the blocks of bone from the later experimental dogs the double embedding was executed using an automatic tissue processor (Shandon-Elliott). Care was exercised when cutting the sections to take them as close as possible to the cut surface of the block, so that the decalcified sections would correspond to the adjacent undecalcified section. Haematoxylin and eosin, Van Giesson and Toluidine Blue staining techniques were used routinely.

9. Radio-isotope Labelling of Articular Cartilage

Biochemical analyses of articular cartilage were carried out at the Kennedy/

Kennedy Institute of Rheumatology as a separate part of this study. In 29 dogs, intra-articular injection of ^{35}S sulphate in isotonic saline (Amersham) was administered into both operated and control stifle joints at varying intervals between surgery and euthanasia. In 8 dogs, proline was injected intra-articularly. The volume of solution injected was small (0.8 - 1.0ml) in every case, the injection was performed with the animal under general anaesthesia (i/v sodium pentobarbitone) and strict aseptic precautions were taken. A 21-g needle was inserted into the joint capsule at the lateral aspect of the patellar ligament, a quantity of synovial fluid was aspirated (variable, depending on whether right or left joint, degree of synovial effusion present etc.) and the sterile isotope solution then injected into the joint. At death, the femoral condyles and tibial plateau from both left and right stifle joints were deep-frozen as soon as possible after removal and subseqently dispatched to the Kennedy Institute.

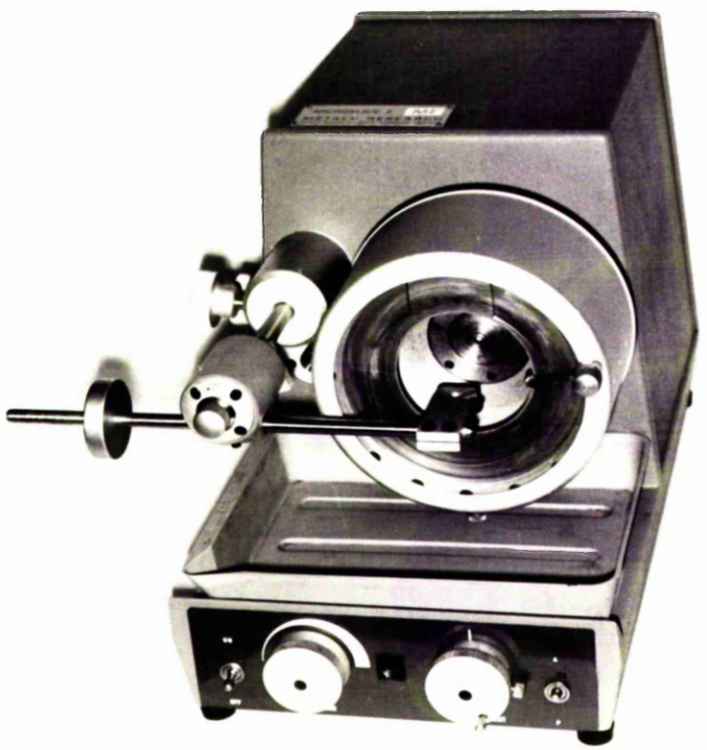
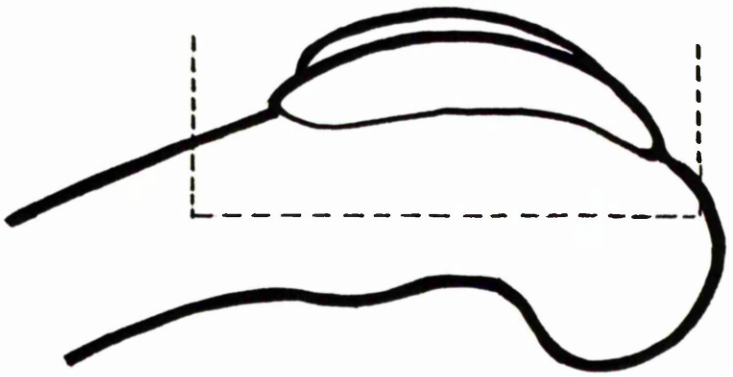


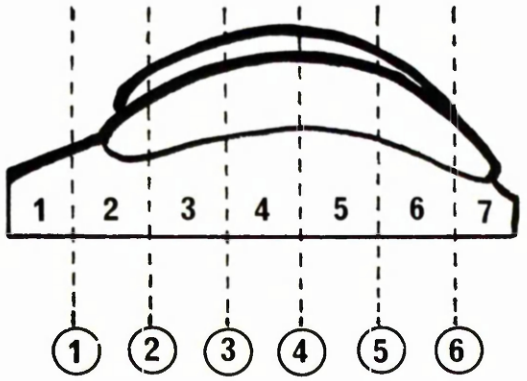
Fig. 1: "Microslice II" machine used to cut undecalcified bone sections.

FIG. 2: DIAGRAMMATIC REPRESENTATION OF METHOD OF OBTAINING TRANSVERSE SECTIONS OF FEMORAL TROCHLEA

① Femoral trochlear block excised



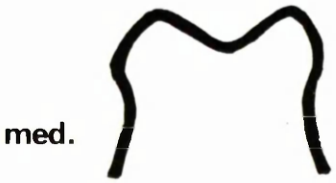
② Trochlea sectioned transversely



thick blocks for decalcification

thin undecalcified sections

③ Transverse section of femoral trochlea



PART III

RESULTS

The 52 dogs in the experimental O-A group (Group A) were given numbers which indicated their duration of survival after ligament section, and also identified them individually within each sub-group. Thus the four dogs surviving for 1 week after surgery were denoted 1/1, 1/2, 1/3 and 1/4 respectively, the prefix number indicating the duration of survival in weeks, and the second number merely distinguishing one dog from another. There were four dogs in each group surviving for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 16 weeks after surgery, and two dogs in each of the 24-week and 48-week survival group.

The 3 sham-operated dogs of Group B were designated dog 1 (2 weeks survival), dog 2 (died at 19 days) and dog 3 (8 weeks survival).

CLINICAL EVALUATION

Periodic clinical assessments were made for each dog following surgery until time of death. The time interval between these assessments varied with the duration of survival of the dog, every 3-4 days in the "short-term" survival animals but perhaps monthly, or even longer, in those surviving 16-48 weeks. The results of the clinical examinations are summarised below and in histograms in figs. 3-7, and have also been tabulated in Appendix 1. In many instances the clinical examination may not have been carried out at the precise time interval designated in the tables, however a close approximation has been made where necessary.

GROUP A DOGS

1. Lameness

The degree of lameness of each dog was assessed while the animal was led at a fast walking pace over a distance of approximately 10 yards. The results have been summarised in fig. 3 and table 1 of Appendix 1. An attempt was made to evaluate the degree of lameness shown by each animal by a system of grading. This proved to be useful in spite of the obvious limitations of such a system. The assessment was entirely subjective, however, and the grades recorded should not be taken as accurate measurements. Considerable variation was sometimes shown by individual dogs at a single examination.

The grading was as follows:

N	=	no lameness detected
(+)	=	slight lameness present, but only as slight abnormality of gait.
+	=	lameness obvious, possibly some reduction in length of limb stride but leg bearing weight at each step.

++/

++ = moderate lameness, dog holding leg up intermittently.

+++ = marked lameness, weight-bearing approximately 1 step in 3.

++++ = 100% lameness, i.e. dog not using leg at all during walking or running.

Of the 27 dogs examined between 1 and 3 days after cruciate section, 5 animals showed 100% lameness, i.e. not weight-bearing on the operated leg, a further 9 dogs showed marked lameness (+++), and 9 were recorded as moderately lame (++). 4 dogs, however were beginning to use the leg well, putting the foot to the ground at each step (+ lameness).

At 1 week, 34 dogs were examined, of which 3 showed ++++ lameness, and 9 showed +++ lameness; of the other 22 dogs, 12 were using the operated leg all the time (+ lameness) and 10 were holding the leg up intermittently (++ lameness).

At 4 to 5 weeks after cruciate section the majority of animals had remarkably good limb function, in spite of continuing instability of the stifle joint. Of 33 dogs examined at this stage, 1 dog was not weight-bearing (++++ lameness), 1 had marked lameness (+++), 9 were moderately lame (++) and 22 dogs were using the leg all the time (+ lameness).

By 7 to 12 weeks after cruciate section, most of the dogs showed merely an obvious abnormality of gait classified as + lameness, whilst 6 dogs were only slightly lame ((+) lameness).

This improvement was maintained so that by 16 weeks, when 5 dogs were examined, 2 were completely sound and the other 3 animals showed only (+) lameness.

No lameness was detected in either dog at 48 weeks after ligament section./

section.

Thus, allowing for individual variation, the general pattern of lameness demonstated in these experimental O-A dogs was as follows. There was an early marked lameness of the operated leg which was either non-weight bearing or taking much less weight than normal. Improvement quickly occurred so that limb function was good within a few (2-4) weeks of surgery, although an abnormality of gait was obvious, the limb-stride being shortened and occasionally the foot slightly medially rotated. Steady improvement in limb function was maintained and in the longer surviving animals soundness of limb was achieved.

A number of dogs showed interesting and usually unexplained variations to this pattern of general improvement with time. It is possible that some of the dogs sustained some unobserved minor trauma to the joint which caused temporary exacerbation of the lameness. For example, dog 2/3 was using the right leg reasonably well 3 days after surgery, and was then observed to slip and fall awkwardly on the concrete floor, and thereafter held the foot off the ground remaining markedly lame until euthanasia at 2 weeks. Dogs 5/1 and 6/4 showed increased lameness a few days after intra-articular injection (of radio-isotope S^{35} in isotonic saline), and in dog 8/2 gross synovial fluid swelling 1 week after intra-articular injection was associated with increased lameness at this time. However, 26 other dogs were given intra-articular injections and were not observed to develop either synovial effusion or increased lameness afterwards. In dog 7/3, synovial fluid swelling subsided 4 weeks after surgery when slight improvement in limb function was noted, but the swelling and lameness were again increased at the sixth week.

2. Muscle Atrophy

At/

At each clinical examination the quadriceps muscle volume of the right hind limb was compared with that of the left hind limb. Because of the subjective nature of this examination, it was difficult in some cases to state with accuracy whether or not muscle atrophy had occurred, especially in the earliest weeks after cruciate section. Often at a very early stage (1-2 weeks) there was an apparent atrophy which may have been due merely to a loss of muscle tone in the right leg; however, no attempt has been made to distinguish this early "apparent atrophy" from that which was obvious at later stages. Any appreciable difference between the muscle volume of the two limbs was recorded as atrophy.

Muscle atrophy was recorded in a total of 8 dogs at 1 week after cruciate section; five of these animals were bearing weight on the operated limb (+ lameness), whilst more marked lameness (grades ++++, +++ and ++) was recorded in the other 3 dogs.

A further 15 dogs examined at 2 weeks after surgery were found to have developed quadriceps muscle atrophy. Of these, 9 dogs although obviously lame were weight-bearing all the time on the operated limb, 4 dogs were moderately lame (++ lameness) and 2 were markedly lame (+++ lameness).

Three weeks after cruciate section 5 more dogs were recorded with muscle atrophy (4 dogs with + lameness and 1 dog with ++++ lameness); and at 4 and 5 weeks a total of 15 more dogs were noted to show appreciable atrophy for the first time. Of these, 10 were bearing weight all the time (+ lameness) 4 were showing moderate (++) lameness and 1 was markedly lame (+++). Thus, in the majority of dogs (82.7%) atrophy was detected within 5 weeks of ligament section.

There did not appear to be any correlation between muscle atrophy and limb function. Even those dogs using the operated limb immediately post-operatively/

operatively developed an obvious atrophy of the quadriceps muscles.

In 11 dogs, the earliest appreciation of quadriceps atrophy was recorded 5 or more weeks after operation, but for these dogs it was possible that the onset of atrophy had occurred some weeks earlier, since the previous clinical examination had been made 2 to 5 weeks before.

Only 5 dogs did not show any appreciable atrophy or loss of muscle tone, of which 4 were short-term survival dogs (three 1-week dogs and one 2-week dog) and 1 was a dog surviving to 16 weeks. In this dog a probable explanation of the lack of difference in muscle volume between left and right thigh was the post-mortem finding of bilateral osteoarthritis of the hip joints, the left hip showing the most severe changes.

In dogs 24/2, 48/1, and 48/2, muscle atrophy detectable in the early stages was no longer apparent at the time of euthanasia. These dogs were sound at this time.

3. Pain

Assessment of pain was based on a cranial response by the dog to manipulation of the right stifle joint, particularly flexion-extension and attempted forward-drawer movements. The varying temperements of the dogs made this assessment extremely difficult to standardise or quantitate. Some dogs were difficult to handle and reacted to any procedure whether or not this was painful. Any doubtful response was recorded in the category of "possible pain". A number of animals became tense on attempted forward drawer movement; since it was possible this reaction was due to pain, these dogs have been recorded as a separate group in fig. 4.

The dogs were examined for the presence of pain at different time intervals/

intervals after cruciate section, and grouped according to their response in 1 of 4 categories:

1) definite pain

2) possible pain

3) tenseness on manipulation

4) no pain recorded

A total of 16 dogs showed a definite pain response on at least 1 occasion; these 16 dogs were subjected to a total of 68 clinical examinations in which pain was demonstrated on 29 occasions. Only 1 dog had a painful stifle at every examination, this was the dog (9/2) in which the scalpel blade had broken at surgery and the tip of the blade was left in the joint; however in spite of continued pain on manipulation the dog was only slightly lame by 5 weeks after surgery. In 1 dog 8/2, pain in the stifle joint was noted to be associated with a gross distension of the joint capsule by synovial fluid, when this subsided the joint was no longer painful. In most instances, however, it was not possible to attribute the presence of pain to any specific factor.

4. "Forward-drawer" Movement

Instability of the operated stifle joint was assessed by producing a "forward drawer" movement of the tibia relative to the femur. In most instances this was performed with the dog held in lateral recumbency with the right leg uppermost. The left hand was placed over the anterior aspect of the distal femur so that medial and lateral femoral condyles were held firmly between thumb and fingers and the index finger rested lightly on the patella. With the thumb of the right hand resting on the caudal edge of the tibial plateau and the index finger on the tibial crest, and with the stifle joint slightly flexed, the tibia was pushed anteriorly in a plane perpendicular/

perpendicular to its long axis. Attempts were made to grade the instability of the joint, but in general the results were unreliable owing to a large number of variable factors, such as variation in size of dog, awkward temperament and difficulty in achieving relaxation of the dog during examination, presence of pain or tenseness on joint manipulation, observer inconsistency. Fig. 5 summarises the results of examination for each experimental dog.

It is well known that the presence of instability as demonstrated by a "forward-drawer" sign may be masked in the conscious dog if it becomes tense on handling. This is borne out by the results obtained in this series; in 10 dogs there were one or more occasions at which no "forward-drawer" movement could be elicited, but this was attributed to pain and tenseness, or to difficulty in handling, since in all cases subsequent examination (sometimes under general anaesthesia) revealed obvious instability of the joint.

Instability of the joint persisted up to time of euthanasia in all dogs except one of the 48-week dogs (48/1) in which no movement could be produced even when the animal was anaesthetised. The tendency, however, was that of a gradual reduction in degree of instability as the duration of the disease increased, and as periarticular fibrosis developed. Only slight "forward-drawer" movement of the tibia was found in dogs 9/4, 10/2, 16/1, 24/1, 24/2 and 48/1 when this was tested under anaesthesia just prior to sacrifice. In dogs 2/1, 3/3, 10/1 gross instability of the stifle was recorded; two of these dogs (2/1, 3/3) were found to have complete section of both anterior and posterior cruciate ligaments.

5. Synovial Fluid Swelling

The presence of synovial fluid swelling of the right stifle joint was/

was detected by palpation at either side of the straight patellar ligament just distal to the patella. Where there was gross excess of fluid within the joint, capsular distension was also obvious proximal to the patella and occasionally laterally at the bursa of the long digital extensor tendon.

The results of this examination have been shown in fig. 6, which may be compared with fig. 7, a histogram of average quantity of synovial fluid aspirated from the joints at the time of death. There was only a slight excess of synovial fluid in dogs killed at 1 and 2 weeks, (with the exception of 2/4), between $1\frac{1}{2}$-3 mls being aspirated at this time compared to a normal quantity of $< \frac{1}{2}$-1 ml. In a number of dogs an excess of synovial fluid could be detected clinically within a week of surgery, in others this only became apparent after 2 weeks, and in 1 dog (10/1) only at the eighth week. The observed swelling of the joint varied from week to week in some dogs, for example dog 7/3, in which fluid swelling was marked at 2 weeks, not detected at 4 weeks and then increased to moderate and marked degree in the sixth and seventh week respectively. Four other dogs 7/1, 10/3, 10/4 and 12/2 showed similar variability. In general, however, the pattern was one of slight to moderate excess of fluid in the first 3 weeks after surgery, increasing in volume in the next few weeks and occasionally becoming markedly excessive (18-22 mls was present in 5 dogs between 5 and 12 weeks). By 16-24 weeks after cruciate section the joint effusion had subsided (average 3 mls) and by 48 weeks the joint fluid appeared normal in colour and viscosity as well as volume.

6. Firm thickening

Periarticular fibrous thickening was appreciated on palpation of the femoral condyles and tibial plateau in the region of the medial and/

and lateral collateral ligaments of the stifle joint. Initially, the fibrosis of the soft tissues around the joint merely reduced the prominence of the bony condyles to palpation, but later the operated joint became obviously thicker than the control stifle. Often the fibrosis and thickening was much more pronounced on the medial aspect of the stifle in the region of the medial collateral ligament.

Fig. 8 records the findings for all the experimental dogs and attempts to grade the degree of firm thickening of the joint. All dogs (with the exception of the 1-week dogs) developed a palpable fibrous thickening of the right stifle, and in 33 dogs this was first detected between 2 and 4 weeks after cruciate section. In 15 dogs it was noted that the fibrous thickening was much more pronounced on the medial aspect of the joint.

7. Click or Crepitus

A total of 24 dogs were reported to have a "click" (presumed to be a meniscal click) on flexion-extension manipulation of the right stifle joint. Commonly this was present in the first few weeks after surgery, sometimes it was elicited on only one examination but in a few cases was persistent.

In 12 dogs mild crepitus was elicited on manipulation, especially on full flexion and extension movement of the joint. This was noted 5 weeks or more after section of the ligament, when osteophyte development was obvious. Many dogs, however, had large osteophytes around the joint but no crepitus on clinical examination.

GROUP B DOGS

All 3 dogs in this group were using the right (sham-operated) hind leg without detectable lameness 2 days after surgery. At no time was lameness/

lameness observed in any of these control dogs.

No fluid or firm swelling of the stifle joint, and no atrophy of the thigh muscles was detected in any dog.

There was no instability of the joint and no clicking or crepitus or pain on manipulation, although dog 1 of this group (which was of notably awkward temperament) did show some resentment to manipulation of the stifle joint.

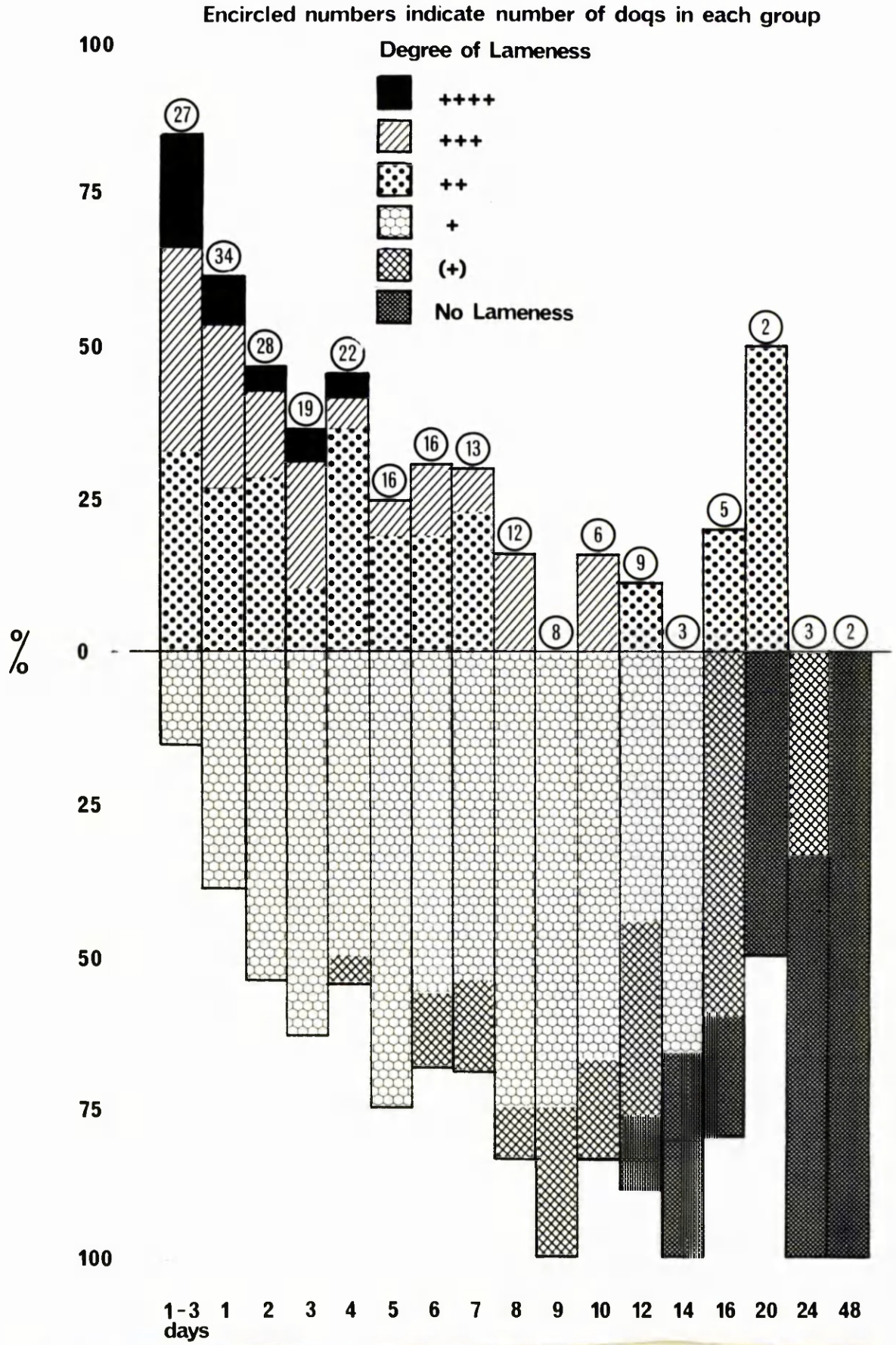


FIG. 3: HISTOGRAM OF RELATIONSHIP BETWEEN DEGREE OF LAMENESS AND DURATION OF SURVIVAL AFTER CRUCIATE SECTION

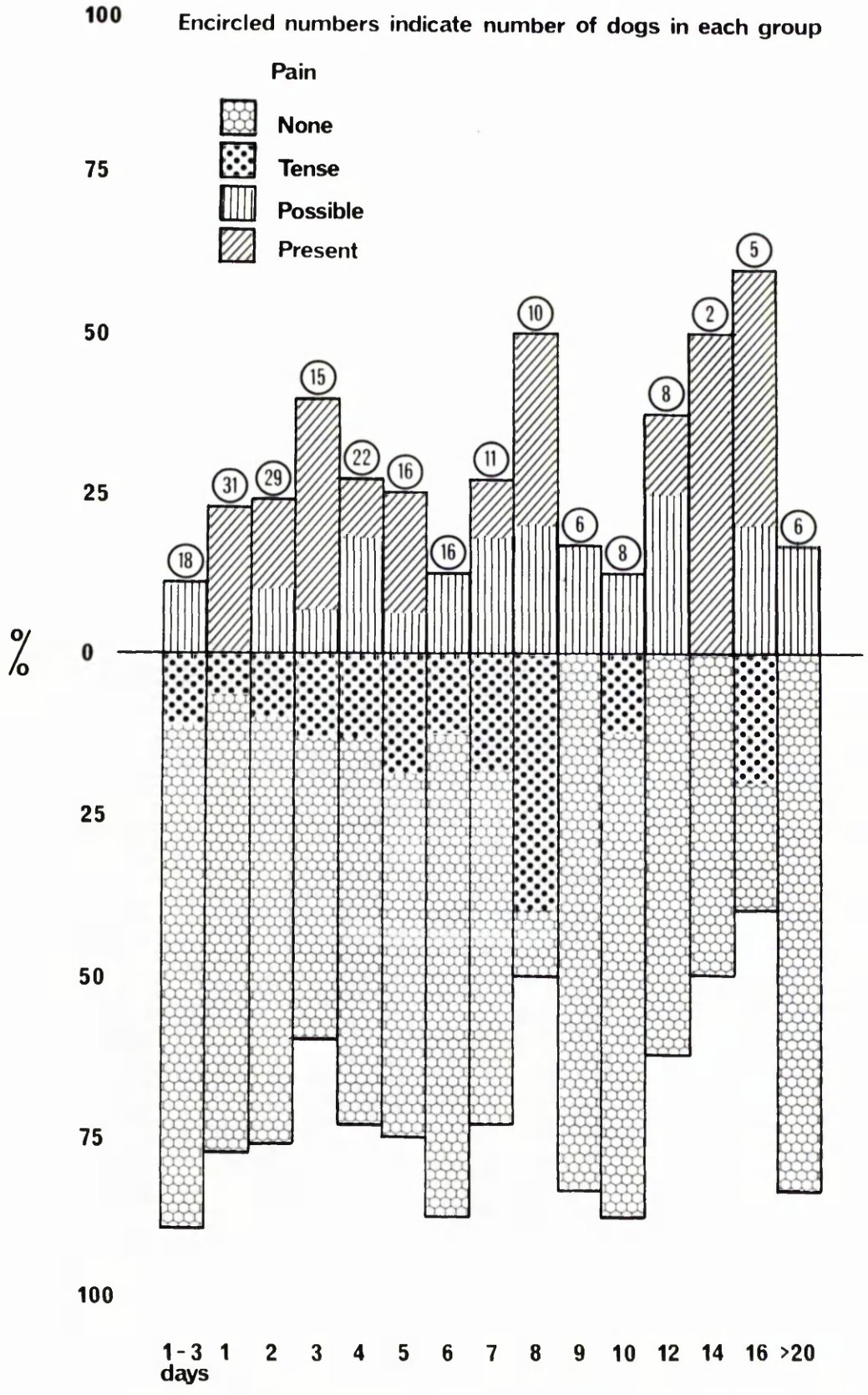


FIG. 4: HISTOGRAM OF RELATIONSHIP BETWEEN PRESENCE OF PAIN AND DURATION OF SURVIVAL AFTER CRUCIATE SECTION

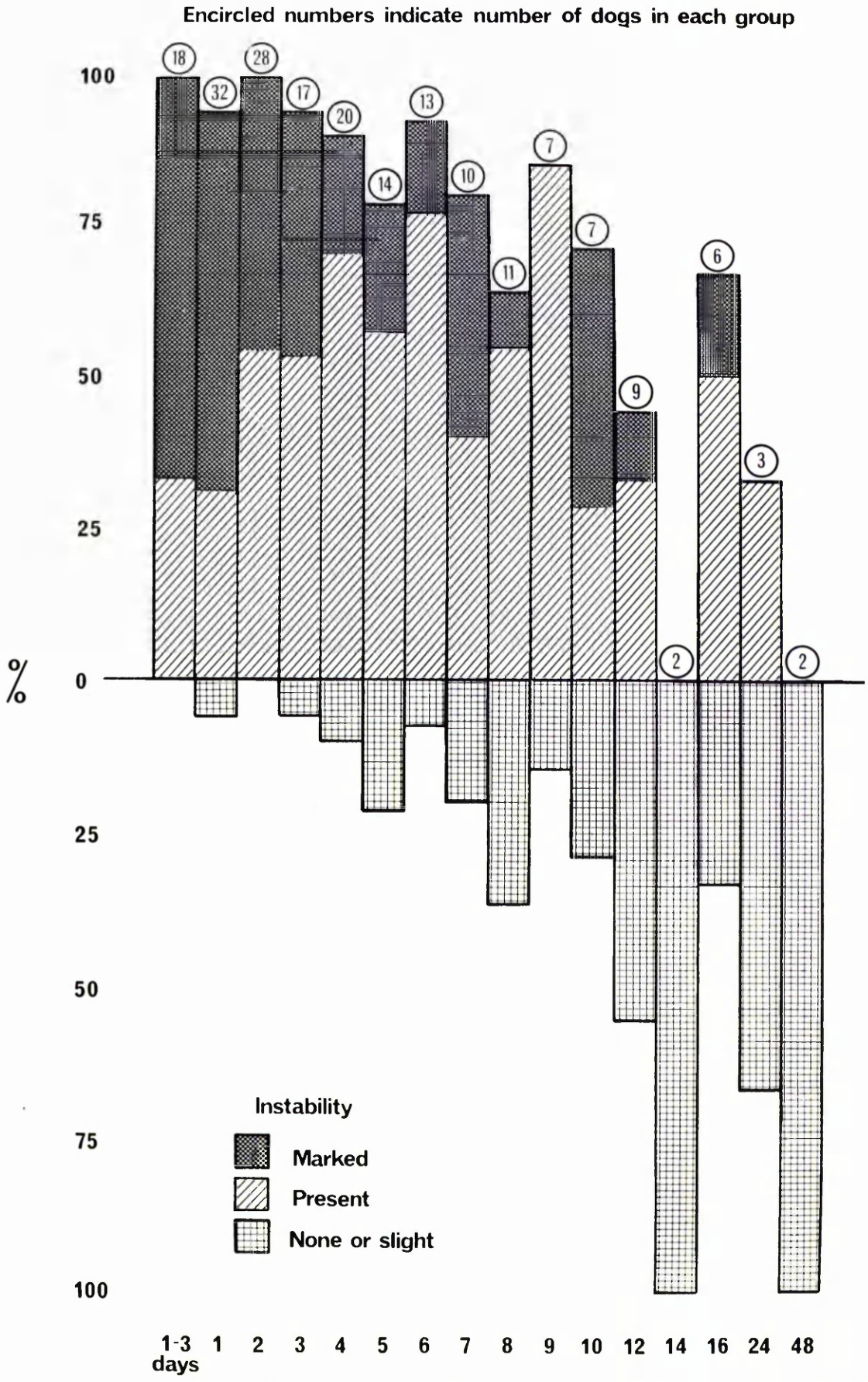


FIG. 5: HISTOGRAM OF RELATIONSHIP BETWEEN DEGREE OF INSTABILITY AND DURATION OF SURVIVAL AFTER CRUCIATE SECTION

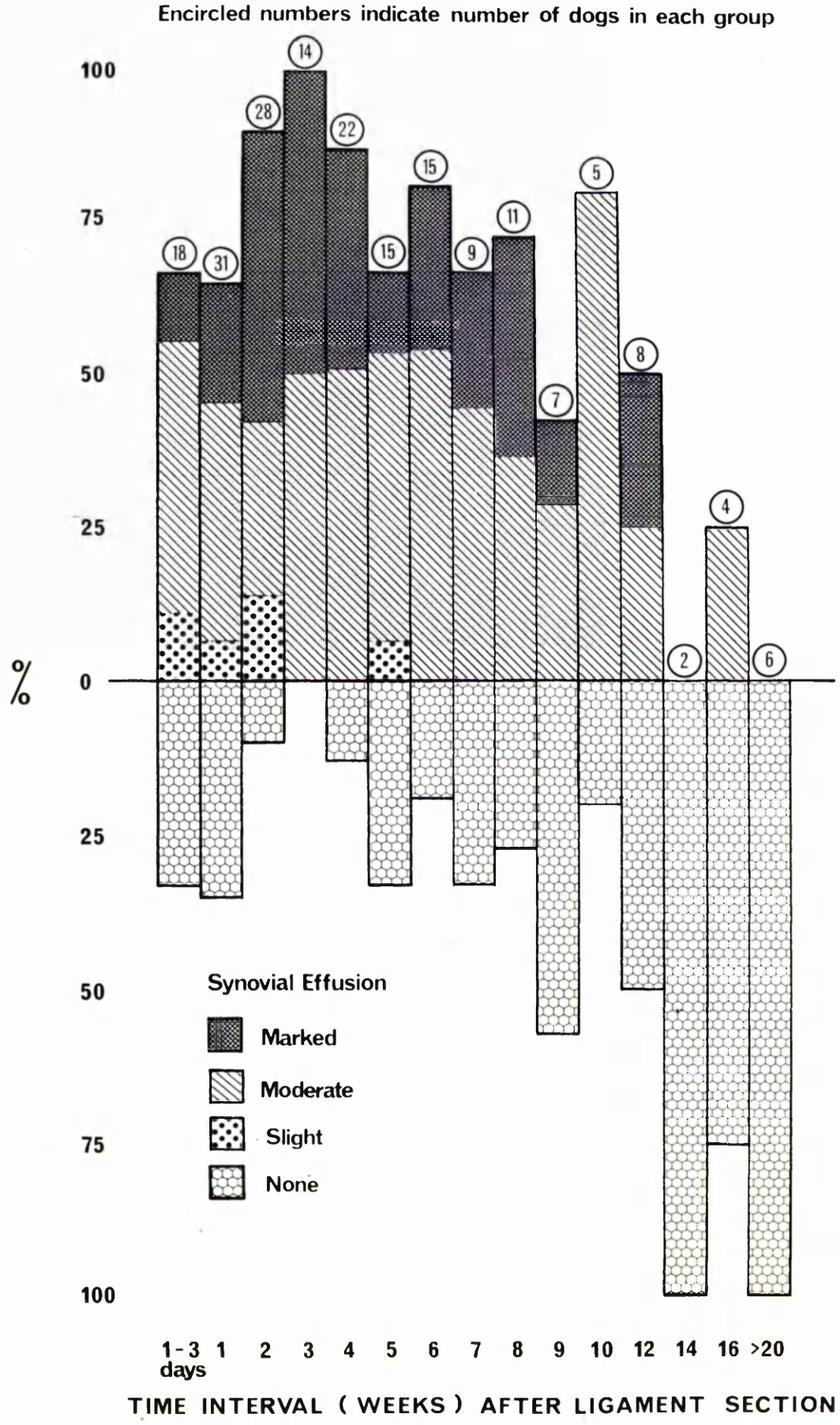


FIG. 6: HISTOGRAM OF RELATIONSHIP BETWEEN DEGREE OF SYNOVIAL EFFUSION AND DURATION OF SURVIVAL AFTER CRUCIATE SECTION

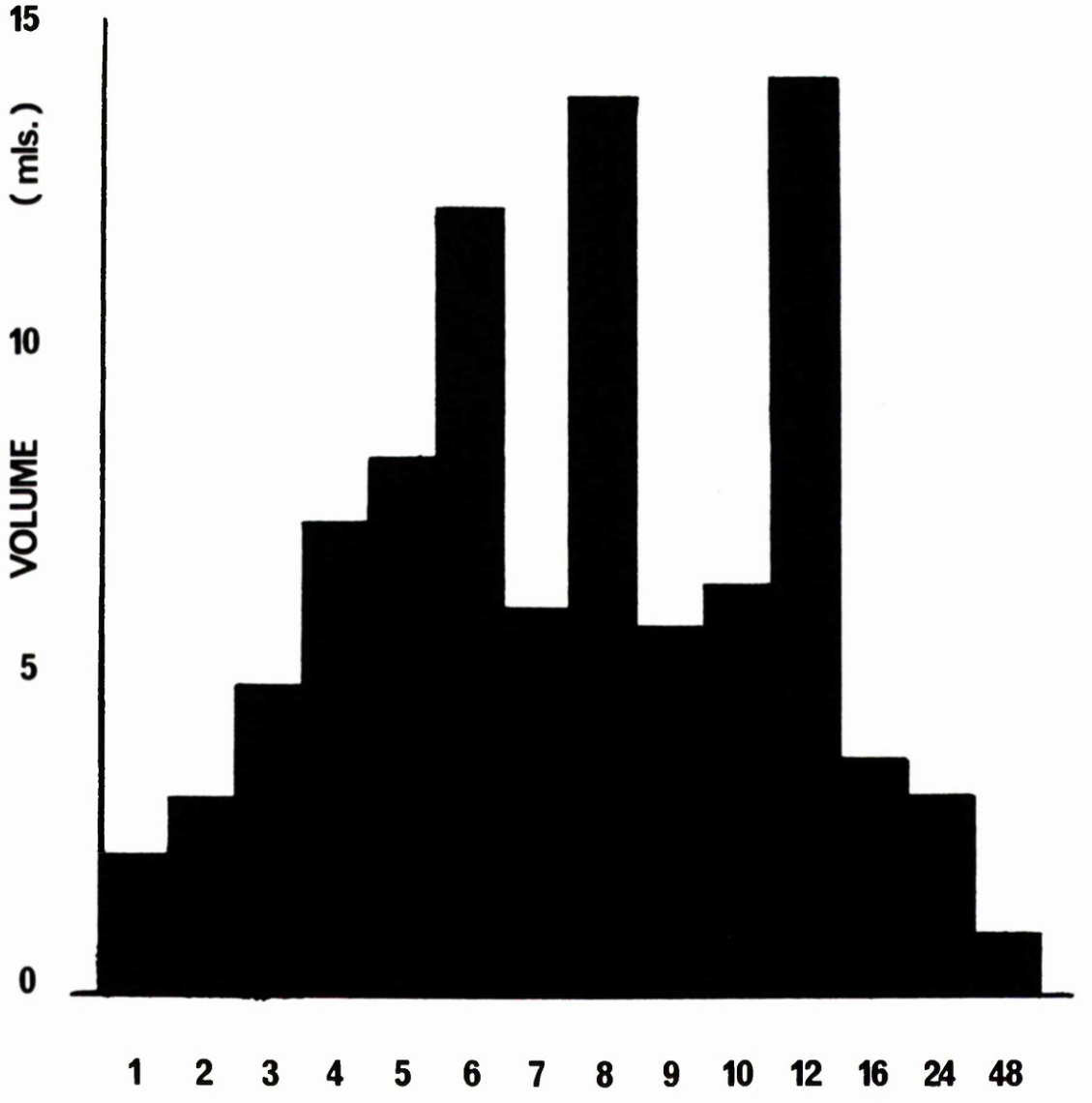


FIG. 7: HISTOGRAM OF QUANTITY OF SYNOVIAL FLUID ASPIRATED FROM THE RIGHT STIFLE AT DEATH (average volumes from total numbers of dogs killed at each time interval)

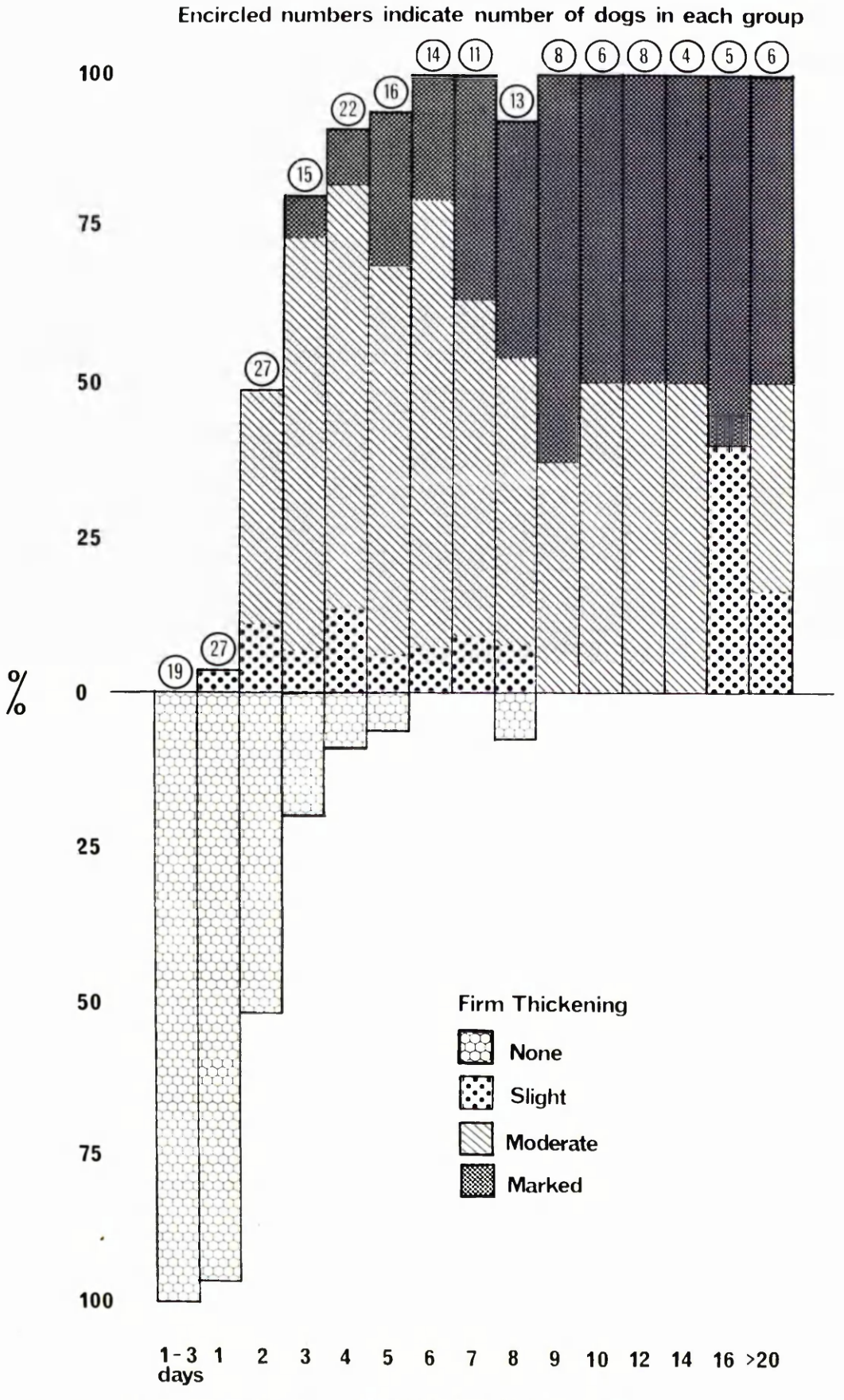


FIG. 8: HISTOGRAM SHOWING THE DEGREE OF PERI-ARTICULAR FIBROSIS RELATED TO THE DURATION OF SURVIVAL AFTER CRUCIATE SECTION

RADIOGRAPHICAL EVALUATION

A total of 47 dogs, 46 of group A and 1 of group B,were subjected to plain lateral radiography of the stifle joint at the time of euthanasia. In one case (10/2) the joint was excised prior to x-ray. No attempt was made to hold the joint in a drawn forward position while the x-ray was taken. The results have been tabulated in table 1.

In 35 dogs, new bone formation was noted at some or all of the following sites:- proximal region of the femoral trochlea, proximal and distal ends of the patella, caudal edge of the tibial plateau, and cranial to the tibial spine; involvement of the fabellae was also recorded in 3 dogs. In some radiographs, osteophyte formation at the margins of the trochlear ridges gave a mottled appearance of the trochlear region of the femur due to an irregular variation in radiotranslucency.

No radiographic change was observed in dogs killed 1 or 2 weeks after cruciate section, or in the "sham-operated" (group B) dog, killed at 8 weeks.

The earliest detected change was a roughening just proximal to the femoral trochlea. This was noted in 1 dog killed at 3 weeks and in 3 dogs killed at 4 weeks. A small amount of new bone formation at the proximal end of the trochlea was detected at 5 weeks after cruciate section (fig. 9). New bone formation was noted most frequently at this site; 27 dogs showed definite evidence of exostoses in the supratrochlear region on x-ray (fig. 10), and in a further 7 dogs there was a minor degree of change, either a slight roughening of the cortex or a very small peak of new bone. In 2 dogs (5/2 and 12/2) the area of new bone formation extended proximally along the femoral shaft. In most cases, new bone formation demonstated radiographically in this proximal trochlear/

trochlear region corresponded to osteophytes in the supratrochlear fossa as recorded macroscopically. However, 5 dogs (8/3, 9/4, 10/4, 12/3 and 16/1) showing obvious x-ray appearance of new bone in this region did not have any osteophytes in the supratrochlear fossa at post mortem. In these dogs there was prominent osteophyte formation on the proximal tips of the trochlear ridges, which accounted for the radiographic appearance. In one dog (5/4) osteophytes were present in the supratrochlear fossa but were not demonstrated on x-ray; this was also true for another dog (10/2) but in this case the radiograph had been taken of the dissected joint and interpretation was difficult.

Osteophyte formation on the caudal edge of the tibial plateau was readily shown on lateral projection of the stifle joint; 18 dogs showed obvious new bone at this site, and in 5 of these animals an extensive amount of new bone was demonstrated(fig. 11). A further 6 dogs had a minor degree of roughening of the caudal aspect of the tibial plateau (fig. 9). There was good correlation with the macroscopic appearance of osteophytes in this region, although 2 dogs (6/3, 7/3) were reported to show roughening of the area on x-ray but no gross evidence of osteophytes and another dog (10/3) appeared to have new bone formation on the caudal aspect of the tibial plateau on x-ray but none macroscopically.

Changes on the patella were located at proximal or distal end and varied from a small amount of roughening or a small peak of new bone difficult to detect on x-ray, to a large mass of new bone readily visualised on lateral projection (figs. 10 and 11). New bone was more frequently seen at the distal than at the proximal end of the patella; 18 dogs had evidence of osteophyte formation at the distal end, while 7 dogs had proximal osteophyte formation. Again there was good correlation with the macroscopic appearance of osteophytes on the patella. In a few cases osteophyte formation/

formation was noted at post mortem but was not visible on x-ray. Two dogs (10/4, 12/3) had radiographic evidence of new bone formation at the distal end of the patella, but none was recorded at this site at post mortem. In one dog (12/3) there was a small radiolucent area on the cranial aspect of the proximal end of the patella.

Roughening of the cortex or small peaks due to new bone formation were noted on the tibial plateau in front of the tibial spine, (approximately at the site of attachment of the anterior cruciate ligament) in a total of 18 radiographs (figs. 10 and 11). Roughening of the borders of the fabellae was seen in only 3 cases (8/1, 16/2, and 24/2) (fig. 11). In dog 10/3 osteophyte formation on the fabellae was recorded at post mortem but was not detected radiographically.

Osteophyte formation on lateral or medial trochlear ridges of the femur was not readily identified on x-ray or the stifle joint because of the lateral projection of the radiograph. A mottled appearance of the trochlear region of the femur was observed in 15 radiographs of dogs surviving for 8 weeks or longer (figs. 10 and 11). This was due to superimposition of osteophytes on the distal end of the femur and hence a variation in radiotranslucency.

Radiographs of 11 dogs killed between 1 and 10 weeks after cruciate section did not show any abnormality (table 1).

In 25 radiographs there was no forward displacement of tibia relative to femur. The other 21 radiographs showed slight to marked forward displacement. Since no attempt had been made to draw the tibia forward during x-ray, no significance can be attributed to the presence or absence of displacement. There was no apparent relationship between the duration of suvival of the dog and the presence or absence of forward displacement/

displacement on the radiograph. For example, none of the stifle joints radiographed 1 or 2 weeks after surgery showed any "drawer-forward" although instability was obvious clinically. It is worth noting, however, that both 48 week dogs (in which joint instability was minimal or absent) showed no "drawer-forward", indicating that stabilisation of the joint in a normal position had occurred.

RADIOGRAPHIC APPEARANCE OF OSTEOPHYTES

Dog	Final X-ray	Proximal to Trochlear ridge	Proximal End Patella	Distal End Patella	Anterior to Tibial Spine	Caudal edge Tibial Plateau	Fabellae	Trochlear Region (Mottling)
1/1	No x-ray							
1/2	No x-ray							
1/3	N.A.D.							
1/4	N.A.D.							
2/1	N.A.D.							
2/2	N.A.D.							
2/3	N.A.D.							
2/4	No x-ray							
3/1		(+)						
3/2	N.A.D.							
3/3	N.A.D.							
3/4	N.A.D.							
4/1		(+)						
4/2	No x-ray							
4/3		(+)						
4/4		(+)						
5/1		+		+				
5/2		+				(+)		
5/3	Poor x-ray	(+)						
5/4	N.A.D.							
6/1	No x-ray							
6/2	N.A.D.							
6/3		+				(+)		

TABLE 1: The appearance of osteophytes on plain lateral radiographs (Rt. stifle, Group A dogs)

Dog	Final X-ray	Proximal to Trochlear ridge	Proximal End Patella	Distal End Patella	Anterior to Tibial Spine	Caudal edge Tibial Plateau	Fabellae	Trochlear Region (Mottling)
6/4		+						
7/1		+		+	+			
7/2		(+)				(+)		
7/3		+		+	+	(+)		
7/4		+				+		
8/1		+	+	(+)		+	(+)	+
8/2		+		+	(+)	+		+
8/3		+						
8/4	No x-ray							
9/1		(+) very small peak						
9/2		+	+	+	+	+		+
9/3		+	+	+	+	+		
9/4		+						
10/1		+	+	+	+	+		+
10/2	P.M.x-ray N.A.D.							
10/3		+			(+)	+		+
10/4		+		(+)	(+)	(+)		+
12/1		+			(+)	+		(+)
12/2		+				(+)		
12/3		+	translucent area	+	+	++		+
12/4		+	+	+	+	+		+

Table 1 continued

Dog	Final X-ray	Proximal to Trochlear Ridge	Proximal End Patella	Distal End Patella	Anterior to Tibial Spine	Caudal edge Tibial Plateau	Fabellae	Trochlear Region (Mottling)
16/1		+		+		+		(+)
16/2		+		+	+	+	(+)	
16/3		++		+	+	+		+
16/4		+		(+)	(+)	+		+
24/1		+	+	+	+	++		+
24/2		+	+	+	+	++	+	+
48/1		+		+	+	++		+
48/2					+	++		

Table 1 continued

KEY

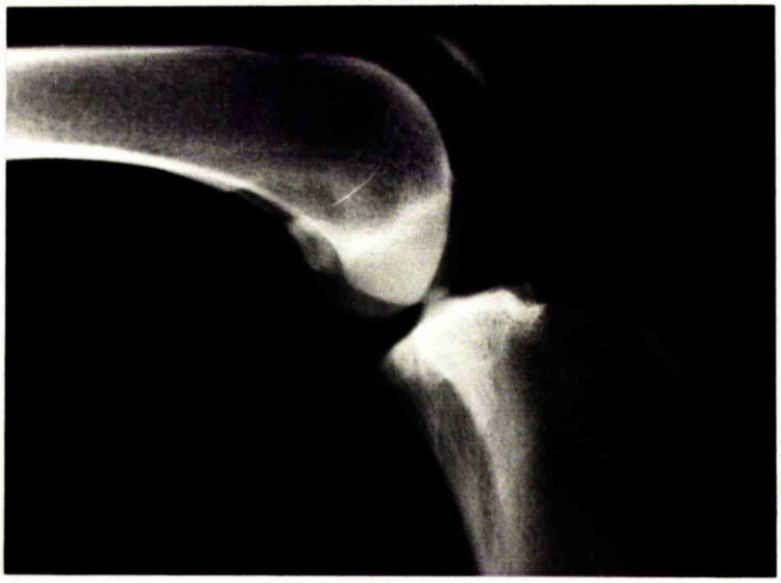
(+) slight roughening

\+ obvious new bone

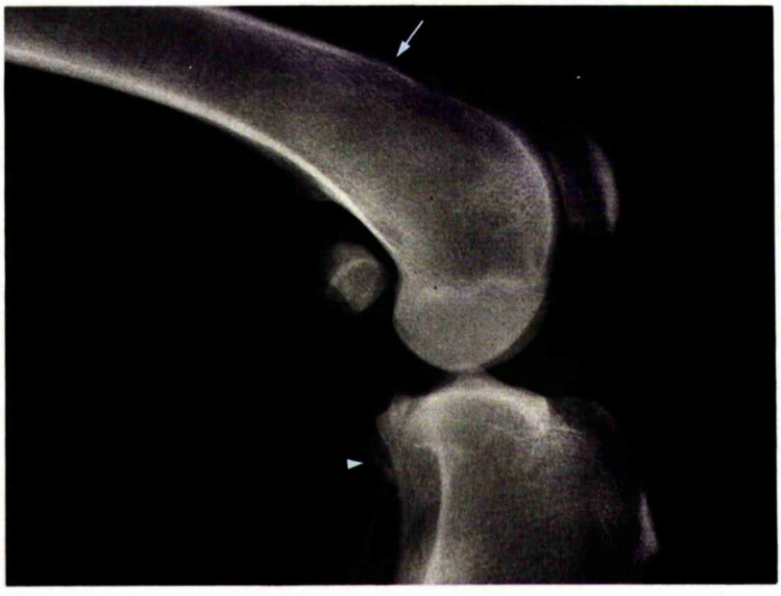
++ marked new bone

N.A.D. no abnormality detected

Fig. 9: Lateral radiographs of right stifle of dog 5/2,
(a) before surgery and (b) at time of euthanasia,
showing early osteophyte development (arrowed).



a



b

Fig. 10: Lateral radiograph of right stifle from dog 10/1, showing obvious osteophytes (arrows). Note also the mottling of the trochlear region.

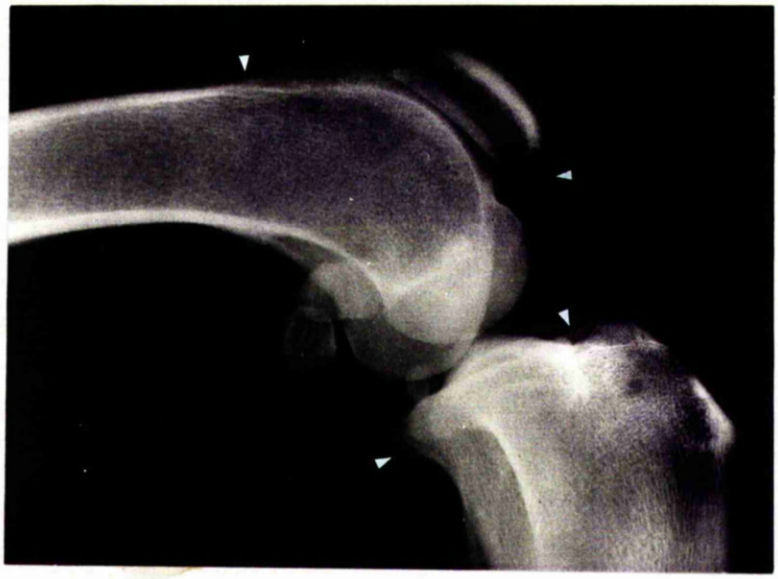
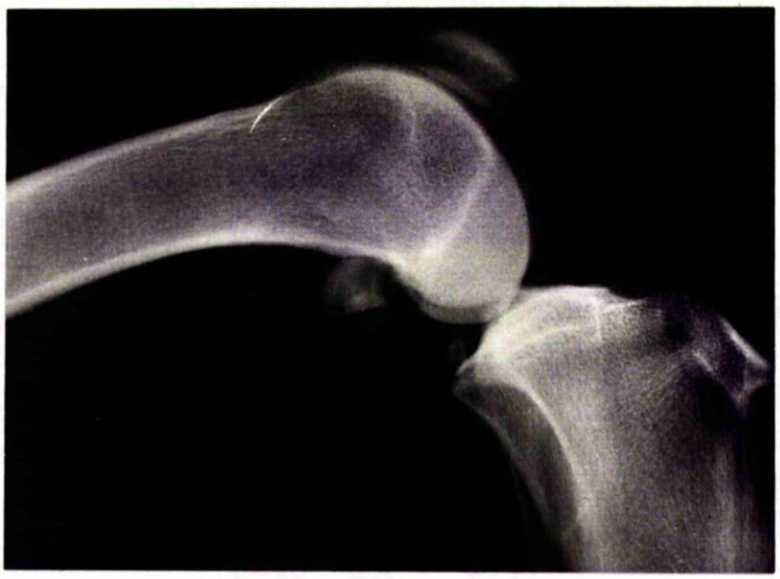


Fig. 11: Lateral radiographs of right stifle of dog 24/2, (a) before surgery and (b) 24 weeks after cruciate section. Osteophytes (arrows) have developed at the margins of the joint. Note also the mottled appearance of the trochlear region.



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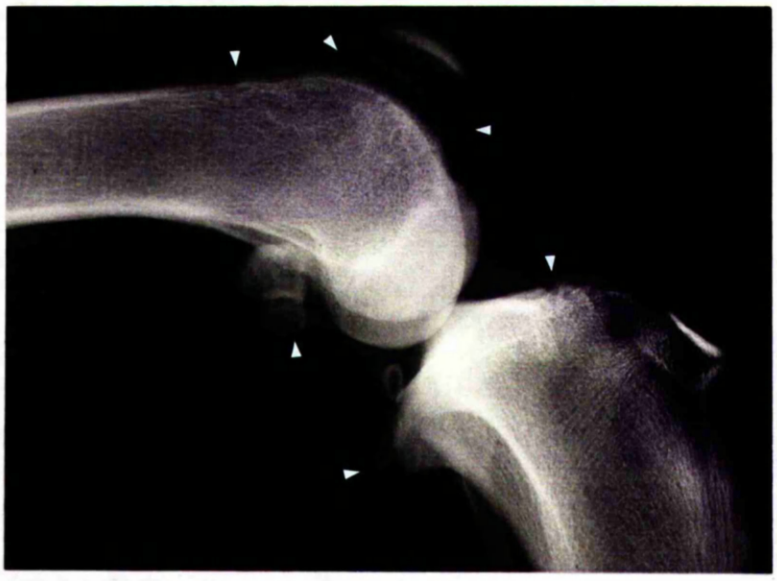


b

MACROSCOPICAL CHANGES

GROUP A DOGS

The pathological changes noted in the right stifle joints have been divided into 3 categories: those affecting bone, those affecting cartilage, and those affecting soft tissues of the joint. The results have been tabulated so that the changes in different tissues from each dog have been recorded (tables 2, 3 and 4). In addition, a general description of the sequence of gross pathological changes which follow section of the anterior cruciate ligament is given below.

1. Macroscopical appearance of osteophytes

Osteophytes were located at the following sites, which may be identified in fig. 12.

1. Supratrochlear fossa of the femur.
2. Margins of medial and lateral trochlear ridges.
3. Intercondyloid fossa of femur, on the lateral aspect near site of origin of anterior cruciate ligament.
4. Edges of medial and lateral femoral condyles.
5. Tibial spine (Intercondyloid eminences of tibial plateau).
6. Edges of tibial plateau on the anterior, medial, lateral or caudal aspect, often most pronounced caudo-medially.
7. Fabellae.
8. Proximal and distal ends of patella.

Osteophyte formation was first noted at the proximal tip of the femoral trochlear ridges and in the supratrochlear fossa 2 weeks after cruciate section. At this stage the osteophyte was an extremely small (less/

(less than 1mm width), slightly raised nodule or ridge at the margin of the trochlear ridge at the point of attachment of the synovial membrane. It produced an irregularity of the surface which was scarcely discernible and only detected by careful scrutiny under optimum conditions of lighting (fig. 17). This type of very small nodule was also noted in one dog killed at 4 weeks, but more often osteophyte growth was rapid, and by 3 or 4 weeks after cruciate section there were a few (or single) small nodules of about 2 mms diameter usually located in the supratrochlear fossa and at the margins of the proximal two-thirds of the trochlear ridges.

From 4 to 7 weeks there was an increase in the number of small nodules (figs. 18 and 20). These were located mainly at the margins of the trochlear ridges, in the supratrochlear fossa and intercondyloid fossa, on the tibial spine and edges of the tibial plateau and in a few dogs also at proximal or distal end of the patella and on the edge of the femoral condyles.

Osteophyte formation between 8 and 10 weeks after cruciate section was usually extensive and readily observed on dissection of the joint. The appearance of osteophytes on the trochlear ridges varied from discrete, slightly raised nodules of varying size (from 2 mms diameter to over 3 mms diameter), extending the full length of the trochlear ridge, to an irregular ridge of new bone, or semi-confluent nodules forming a "mosaic pattern". The majority of dogs also had prominent osteophytes at other locations, such as in the supratrochlear fossa, at the edges of the tibial plateau and in the intercondyloid fossa. Figs. 21, 22 and 23 illustrate the type of change which may be seen at this stage. One dog (9/1) was atypical in that only small osteophytes had developed on the proximal lateral trochlear ridge and intercondyloid fossa. One dog (10/3) was/

was noted to have osteophyte formation on both fabellae of the right stifle joint.

By 12 weeks after cruciate section there was usually a complete but irregular or "knobbly" ridge of new bone on lateral and medial trochlear ridges (fig. 24). All 4 dogs killed at this stage had some degree of new bone formation at the edges of the femoral condyles and on the tibial spine (see table 2), and in 3 out of 4 dogs, osteophytes had developed in the supratrochlear fossa, intercondyloid fossa, edges of the tibial plateau and on the patella.

The appearance of the osteophytes in dogs killed at 16 weeks was very similar to that at 12 weeks, although in 2 dogs at this stage there was a smooth, confluent ridge of new bone covered by cartilage on the lateral margin of the lateral trochlear ridge. This is shown in fig. 25. It can be seen that this new ridge of bone is still distinct from the original trochlear ridge at this stage. A similar appearance was noted in one of the dogs killed at 24 weeks; in the other dog the lateral trochlear ridge had been remodelled, i.e. the new ridge of bone was continuous with the original trochlear ridge and the whole trochlea was effectively widened (fig. 26). Several large masses of new bone were present on the medial trochlear ridge of this dog. Pronounced new bone proliferation on the caudo-medial edge of the tibial plateau was observed in each dog surviving 16 weeks or 24 weeks after cruciate section. Osteophyte formation was also evident in the intercondyloid fossa and on the tibial spine and distal end of patella in each of these dogs (fig. 27). Large osteophytes in the supratrochlear fossa were noted in 4 dogs.

In the longest surviving dogs, killed 48 weeks after ligament section, both medial and lateral trochlear ridges were remodelled, each ridge/

ridge being widened by a smooth, confluent ridge of new bone continuous with the original trochlear ridge (fig. 28). Thus the overall width of the trochlea was markedly increased. The tibial plateau of both dogs had also undergone extensive remodelling with the result that the surface appeared undulating and irregular instead of the normal smooth shape. Recontouring of both femoral condyles had taken place in one dog (fig. 29) and this animal also had osteophyte formation on the fabellae. Remodelling of the supratrochlear region in the other 48 week dog resulted in the extension proximally of the trochlear groove, new bone covered by cartilage being formed within the supratrochlear fossa (fig. 30).

2. Macroscopical appearance of cartilage lesions

Erosions of the articular cartilage of the right stifle joint were recorded in the following areas:- (see fig. 13).

1. Distal end of medial and lateral trochlear ridges in the area of cartilage adjoining the femoral condyles (Area A, fig. 13).
2. Central (weight-bearing) area of medial and lateral femoral condyles (Area B, fig. 13).
3. Central area of medial and lateral tibial condyle, corresponding approximately to the area of cartilage not covered by meniscus (Area C, fig. 13).
4. Peripheral area of medial and lateral tibial condyle, corresponding approximately to the area covered by meniscus.
5. Patella.

In addition to discrete erosions of articular cartilage in these locations, other abnormalities were observed. Loss of sheen, a roughened granular texture of the surface, or the appearance of small linear splits

or/

or fissures in the cartilage were noted, both in the areas of cartilage described above and also in the trochlear groove and peripheral region of the femoral condyles.

Table 3 records the distribution of cartilage lesions in each experimental dog.

Erosion of the central area of the tibial plateau was present in the majority of animals. This was the most common site of cartilage erosion (figs. 23, 24, and 27). It should be noted, however, that difficulty was experienced in interpretation of this appearance since markedly roughened or eroded articular cartilage was often seen in this region of the control (left) stifle joint. Nevertheless, following cruciate section, the extent and degree of erosion observed in this area became more marked with time. In 2 dogs (5/4 and 16/4) exposure of underlying bone and resultant eburnation had developed in the central area of the lateral tibial condyle.

The significance of small erosions at the junction of trochlear ridge and femoral condyle was not known, they were recorded on medial and/or lateral trochlear ridges of 21 dogs, from 3 weeks to 48 weeks after ligament section.

Perhaps the first significant lesion of cartilage was seen 3 weeks following surgery: a small erosion on the posterior aspect of the medial tibial plateau was recorded in dog 3/1. A similar small erosion at the same site was noted in dog 8/1. Neither of these dogs showed distinct erosions of the articular cartilage of the corresponding femoral condyle. In 7 other dogs however, ranging from 4 to 24 weeks after cruciate section, erosion of the cartilage of the peripheral tibial plateau was accompanied by erosion of the corresponding femoral condyle. Of these 7 dogs, the medial compartment of the joint was involved in 5 dogs, the lateral/

lateral compartment in 1 dog, and both medial and lateral in another dog.

Large, oval erosions of articular cartilage were present in the centre of the medial femoral condyle in 9 dogs (fig. 19) and on the lateral femoral condyle in 7 dogs, (total 14 dogs, bilateral lesions present in 2 dogs). The size of erosion varied from 2 x 2 mms to 20 x 10 mms. In one dog (12/2) the underlying bone was exposed and eburnated (fig. 24).

Cartilage erosions were located on the patella in 2 dogs (9/3, 16/3).

In 12 dogs, from 1 week to 16 weeks after cruciate section, the cartilage of the lateral femoral condyle had been incised by the scalpel blade at the time of surgery (fig. 24). Recognition of these "scars" was possible because the area incised was often slightly discoloured and the location was characteristic, adjacent to the intercondyloid fossa where the scalpel blade had been inserted to incise the anterior cruciate ligament. In 3 dogs cartilage on the intercondyloid eminences of the tibia had been incised at the time of cruciate section.

3. Macroscopical appearance of synovial membrane, joint capsule, ligaments and menisci

Pathological changes in the soft tissues of the stifle joint have been recorded in table 4, for each experimental dog.

An assessment of volume and character of synovial fluid, was made after euthanasia had been carried out. Synovial fluid was aspirated from the joint with syringe and its viscosity tested crudely between thumb and forefinger. Results have been shown in table 5.

The first pathological change noted after section of the anterior cruciate ligament was a mild, yellow discoloration of the synovial membrane, together with a small fan of proliferating synovial blood vessels/

vessels at the proximal part of the femoral trochlea shown up by vascular perfusion with Prussian Blue (fig. 15). The increase in blood vessels is readily seen if the appearance at 1 week is compared with that of a normal (left) trochlea in which perfusion with dye has also been carried out (fig. 14). At 2 weeks, 3 dogs showed slight increase in thickness of synovial membrane as well as discoloration, and in perfused joints the vascular proliferation was more extensive than at 1 week (fig.16).

During the first 8 weeks after cruciate section, these changes in the synovial membrane tended to become more pronounced with time (figs. 20 and 22). By 5 weeks there was usually a markedly thickened, yellow or brown-tinged synovial membrane, and in joints which had been perfused, an intense vascular proliferation was noted at all points of synovial reflection. In many dogs it was observed that this vascularisation was most intense in the supratrochlear fossa, at the margins of the trochlear ridges and in the posterior pouches of the joint capsule. Marked vascularity was also noted at the proximal or distal margin of the patella and the cranial attachment of the severed anterior cruciate ligament.

From 9 weeks after ligament section the intensity of vascular proliferation in the synovial membrane appeared to subside a little, although comparison with the control (left) stifle joint showed an obvious increase in the vascularity of the operated joint. Dogs 9/2, 12/3, and 24/2 did, however, still show intense synovial vascularity (fig. 27).

An interesting feature was observed in dog 48/2, the right stifle was noticeably a deeper blue colour than the left, but there was only slight superficial synovial vascularity and no encroachment of proliferating blood vessels at the margins of the articular cartilage as at earlier stages. Rather, it appeared that a greater uptake of blue dye in subchondral blood/

blood vessels of the right stifle was responsible for the difference in colour, and this was especially obvious in the remodelled trochlear ridges of the femur (fig. 28). In the other 48 week dog, it was noted that the synovial membrane in the pouch proximal to the trochlea was of pronounced granular texture (fig. 30).

Villous folds of the synovial membrane may be observed in normal stifle joints particularly in the posterior pouches of the femoro-tibial joint capsule, but in 25 dogs an increase in the number, distribution and size of the villi was considered to be significant. This villous proliferation was seen in the posterior pouches of the joint capsule (13 dogs), at the distal end of the patella (11 dogs), in the intercondyloid fossa near the cranial attachment of the anterior cruciate ligament (6 dogs), at the margins of the tibial plateau near the distal stump of the anterior cruciate ligament (2 dogs), bordering the outer edges of the menisci (1 dog), and in the supratrochlear fossa (1 dog). In 9 dogs between 7 and 48 weeks after cruciate section villous proliferation was recorded as severe. In several dogs a distinct "fan" of blood vessels was observed encroaching on to the proximal limit of the trochlear groove or overlying osteophytes at the trochlear margins (fig. 22).

Fibrinoid strands or plaques adherent to the synovial membrane, or adhesions between adjacent folds of the synovial membrane were present in 31 dogs between 2 and 48 weeks after surgery (figs. 22 and 24). In table 4, these changes have been classed together under the heading "adhesions"; also included in this category was the adhesion between synovial membrane and the tendon of the long digital extensor muscle located within its synovial bursa on the antero-lateral aspect of the stifle joint. Synovial adhesions of the long digital extensor tendon were common, occurring in

a/

a total of 28 dogs. Other adhesions were noted distal to the patella (10 dogs), in the suprapatella pouch of the joint capsule (8 dogs), in the posterior pouches of the femoro-tibial joint (6 dogs), and within the intercondyloid fossa associated with the posterior cruciate ligament (2 dogs).

Thickening of the joint capsule was noted in every case after 3 weeks duration, and a slight increase in periarticular fibrous tissue was even recorded in one dog killed 1 week after cruciate section. The amount and the extent of fibrous thickening of the joint capsule tended to increase with time. There was individual variation however; in 48/2 the joint capsule was extremely firm to cut but only slightly thickened (approximately 0.25 cm) on the medial aspect (fig. 29). Figs. 23, 24, and 27 show obvious capsular thickening at 9, 12, and 24 weeks after cruciate section.

In 15 dogs, it was observed that thickening of the joint capsule was greatest on the medial aspect of the femoro-tibial articulation in the region of the medial collateral ligament.

In 22 dogs, measurement of the thickened joint capsule was made, and in 11 animals it was found to be approximately 0.5 cm thick. In dogs 5/1 and 48/1 the thickness was 0.75 cm. In 7 dogs a capsule thickness of 1 cm was recorded, the duration of survival in these cases ranging from 5 to 16 weeks. In both dogs killed at 24 weeks the joint capsule on the medial aspect of the stifle was more than 1 cm thick (fig. 27).

In addition to becoming thicker the joint capsule became extremely firm on sectioning in some of the longer surviving dogs. No calcification of the capsule was observed.

In association with the increasing thickness of the periarticular soft tissue, the long digital extensor tendon (which originates above the/

the lateral femoral condyle and is located in a small groove on the antero-lateral aspect of the stifle joint), was noted to have increased in thickness in comparison with the tendon of the contralateral limb in 38 dogs.

Meniscal fibrillation or splitting was recorded in a total of 43 dogs; involvement of both medial and lateral meniscus was seen in 4 stifle joints, of lateral meniscus only in 1 joint, and in 38 dogs the medial meniscus alone was affected. Pathological change was recorded as early as 1 week after cruciate section, but at this stage only very mild, superficial fibrillation of the femoral face of the meniscus was seen. By 5 weeks, one dog (5/2) was showing gross fibrillation and tearing ("bucket-handle" tear) of the medial meniscus, and from 7 weeks or longer after cruciate section, severe splitting or tearing of the meniscus was usual (fig. 23). In 1 dog at 7 weeks, and 6 dogs killed 12 weeks or more after surgery, there was very severe shredding or complete disintegration of the medial meniscus (figs. 24, 27 and 29). Calcification of the posterior horn of the meniscus was noted in 2 dogs (10/1, 10/2).

In 5 dogs the posterior cruciate ligament had been sectioned at the time of surgery; in 3 of these the ligament was completely sectioned but in the other 2 dogs a small portion was still intact. In a further 10 dogs there was some haemorrhage into the posterior cruciate ligament, or slight partial section of it, evidence that surgical trauma had been inflicted at the time of sectioning the anterior cruciate ligament. The presence of even complete section of the posterior cruciate ligament did not, however, appear to influence the rate of development of pathological change in the other joint tissues, although it should be noted that section of this ligament was observed only in dogs surviving

up/

up to 5 weeks.

Control (left) Stifle Joints

No macroscopical abnormalities were recorded in the left stifle joints of any experimental dog with the exception of dog 10/3, and possibly dog 48/1.

a) 10/3

This dog was found to have natural O.A. of the left stifle (fig. 31). The pathological features recorded were ;

1. Osteophytes

These were located on lateral and medial trochlear ridges, intercondyloid notch and outer edges of medial and lateral femoral condyles, caudal and medial edges of tibial plateau and tibial spine, distal end of patella and on both fabellae with their corresponding femoral surfaces. The osteophytes consisted of smooth, raised ridges or large nodules of new bone covered by cartilage.

2. Cartilage lesions

A large erosion (15 x 3 mms) was present in the central part of the lateral femoral condyle. Several small "linear" erosions were present on the medial femoral condyle and the central regions of medial and lateral tibial plateau were also eroded. Other areas of articular cartilage appeared dull and slightly roughened.

3. Soft tissue changes

Approximately 0.5 ml of straw-coloured viscous synovial fluid was aspirated from the joint. The synovial membrane was of a creamy-yellow colour, with evidence of slight thickening and granularity. No adhesions or villous folds were observed. The medial meniscus was grossly shredded./

shredded. The anterior cruciate ligament was ruptured.

b) 48/1

The proximal trochlear groove and medial trochlear ridge were observed to be roughened and slightly discoloured. No other abnormalities were recorded.

GROUP B DOGS

a) Dog 1 (killed at 2 weeks)

There was a mild synovitis in the right stifle joint, shown by slight yellow discoloration and an increase in vascularity as demonstrated by greater uptake of blue dye in this joint than in the left stifle. Less than 1 ml synovial fluid of normal colour and consistency was aspirated from the joint. There were no nodules at the reflection of the synovial membrane. The articular cartilage of both left and right joints appeared similar, being smooth and shiny, with the exception of the central tibial plateau and two scalpel scars on the lateral femoral condyle. Anterior and posterior cruciate ligaments and both menisci appeared normal.

b) Dog 2 (died at 19 days)

This dog died within 36 hours of receiving alizarine complexone by intravenous injection and the soft tissues were discoloured purple by the dye. The presence or absence of synovial inflammation was therefore not assessed. No macroscopical changes in articular cartilage, cruciate ligaments or menisci were observed and there was no periarticular osteophyte formation.

c) Dog 3 (killed at 8 weeks)

There were no synovial membrane changes, and no lesions of cruciate ligaments or menisci observed grossly. The articular cartilage appeared normal. No osteophyte formation was present.

Dog	LOCATION OF OSTEOPHYTES								
	Supra - Trochlear Fossa	Lateral Trochlear Ridge	Medial Trochlear Ridge	Inter-Condyloid Fossa	Femoral Condyle	Tibial Spine	Tibial Plateau	Patella Proximal End	Patella Distal End
1/1									
1/2									
1/3									
1/4									
2/1	(+)	(+)	(+)			(+)			
2/2	+	(+)	(+)				(+)		
2/3	(+)		(+)						
2/4	+	(+)	(+)					(+)	
3/1	(+)	+	+	(+)			+		(+)
3/2	+			(+)					
3/3	+	+	(+)			(+)			
3/4	+	+	(+)	(+)					
4/1	+	+	+			(+)	+		
4/2	(+)	(+)	(+)					(+)	
4/3	++	++	++	++	+	(+)	+	+	+
4/4	++		+	+		(+)			(+)
5/1	+	++	+	(+)			+		(+)
5/2	++	+++	+++	++	+	+	++	+	+
5/3	++	+	+	+	+	+	+		
5/4	++	++	+	+	++	(+)		(+)	
6/1		++	++	++		+	(+)	+	+
6/2		+	(+)	+		(+)			+

TABLE 2. POST MORTEM LOCATION OF OSTEOPHYTES (RT. STIFLE. GROUP A DOGS)

Dog	Supra - Trochlear Fossa	Lateral Trochlear Ridge	Medial Trochlear Ridge	Inter-Condyloid Fossa	Femoral Condyle	Tibial Spine	Tibial Plateau	Patella Proximal End	Patella Dist En
6/3	+	+	(+)	+		+		(+)	(+)
6/4	++	+	++	++		+	+		
7/1	++	++	+	++		+	+		++
7/2	+	++	+	++		+	+	(+)	(+)
7/3	++	++	++	+		+			(+)
7/4	+	++	+			+	+		
8/1	++	+++	++		+	+	+	+	++
8/2	++	+++	+	+		+	++		++
8/3		+++	++	++	+	++		+	+
8/4	++	+	++	+		+	++	+	++
9/1		+	(+)	+		(+)			
9/2	+++	++++	++	++	++	++	++	++	++
9/3	++	+++	+++	++++	++	++	+	+	+
9/4		+++	++	+	+	++	++		(+)
10/1	+++	+++	++	++++	++	++	+++	+	++
10/2	+	+++	++	+++	+	+++	+++		
10/3	++	++++	++	++	+	++			+
10/4		+++	+++	+	++				
12/1	++	++	+++	+	++	+	+		
12/2	+++	+++	+++		+	++		+	+
12/3		+++	+++	+	+	++	++	(+)	
12/4	++	+++	++	+	+	+	+++	+	+

Table 2 continued

Dog	Supra - Trochlear Fossa	Lateral Trochlear Ridge	Medial Trochlear Ridge	Inter-Condyloid Fossa	Femoral Condyle	Tibial Spine	Tibial Plateau	Patella Proximal End	Patella Distal End
16/1		+++	++	++	(+)	++	+++		+
16/2	+++	+++	+++	++	+++	+++	+++		++
16/3	+++	++++	+++	+	++	+	+++		++
16/4	+++	++++	+++	++	++	++	+++	+	+
24/1	+++	++++	+++	+++	++	++	+++	+	++
24/2	+	R	+++	+	++	++	+++	+	+
48/1	R	R	R	+++	++	+	R		++
48/2		R	R	+++	R	++	R	+	+

Table 2 continued.

KEY:

(+) Very small nodules or ridges (< 1 mm)

\+ Single, or few, small discrete nodules, or small ridge (~2 mms)

++ Several discrete nodules (~2 mms), or single or few large nodules (>3 mms)

+++ Semi-confluent "mosaic-pattern" nodules, or irregular ridge

++++ Large masses, or smooth confluent ridge

R Remodelled trochlear ridge or articular surface

GROSS CARTILAGE CHANGES

Dog	Junction of Trochlear Ridge and Femoral Condyle		Trochlear Groove	Femoral Condyle		Central Tibial Plateau		Peripheral Tibial Plateau		Patella	
	M	L		M	L	M	L	M	L	Prox.	Dist
1/1						+++	+++				
1/2						+	+				
1/3				+	(+)	+++	+				
1/4				(+)	S	+					
2/1					S	+	S				
2/2					S	+					
2/3				(+)	(+)	+++	+++				
2/4				(+)	(+)	+++	+++	(+)	(+)		
3/1	+++	+++		(+)	+	+++	+++	+++	+	(+)	+
3/2	+++	+++		++		+++	+++			(+)	+
3/3			(+)	(+)	(+)	+++	+++	+	+	(+)	(+)
3/4		+++	(+)	(+)	(+)	+++	+++				
4/1	+++		(+)	+	+	+	+++	(+)	(+)	(+)	(+)
4/2				+	S	+	+			(+)	+
4/3			(+)	+++	(+)	+	+	+++		(+)	+
4/4				++		+	+++				
5/1				+		+++	+++				(+)
5/2			(+)	+		+++	+++	(+)	(+)		
5/3	+++		+	+	S	+	+++			(+)	(+)
5/4				+	S	+	++++		(+)	(+)	+
6/1		+++	+	+	++	+	+++	(+)	(+)	+	(+)

TABLE 3: MACROSCOPICAL APPEARANCE OF CARTILAGE LESIONS (RT. STIFLE, GROUP A DOGS)

Dog	Junction of Trochlear Ridge and Femoral Condyle		Trochlear Groove	Femoral Condyle		Central Tibial Plateau		Peripheral Tibial Plateau		Patella	
	M	L		M	L	M	L	M	L	Prox.	Dist
6/2			(+)	(+)		+	+			(+)	(+)
6/3			++	+	+	+++	+++	+	+		
6/4	+++	+++		+	S	+	+		++		
7/1	+++	+++		+++	(+)	+++	+++	+++		(+)	(+)
7/2			(+)	(+)	(+)	+++	S			(+)	(+)
7/3	+++	+++	(+)	+++	+++	+	+++	+++	+++	(+)	+
7/4	+++			+	+	+	+++	+	+	+	+
8/1	+++		+	++	+	+++	+++	+++	+	+	(+)
8/2			+	+	+	+++	+++	(+)	(+)	(+)	+
8/3			(+)	+	S	+	+++	(+)	(+)	(+)	(+)
8/4			(+)	+++	++	+++	+++	+++	(+)	(+)	(+)
9/1		+++	(+)	(+)		+++	+++			+	+
9/2			+	+++	+++S	S	+++	++	+	+	(+)
9/3			+	+	+++	+++	+++	+	+	+++	+
9/4	+++	+++		++	+++	+++	+++		+++	(+)	+
10/1	+++	+++	(+)	+++	+	+	+++			(+)	(+)
10/2			(+)	+	+	+	+++	+	+	(+)	(+)
10/3		+++	(+)	+	++	+++	+++	+	(+)	(+)	(+)
10/4	+++		+	+	S	+++	+++			(+)	+
12/1			(+)	+++	+	+++	+	(+)		(+)	+
12/2			+	++++	S	+	+++	++++	+	+	+
12/3	+++	S	+	+	+	+++	+++			(+)	+
12/4	+++		+	++		+++	+++	+	+	+	+

Table 3 continued

Dog	Junction of Trochlear Ridge and Femoral Condyle		Trochlear Groove	Femoral Condyle		Central Tibial Plateau		Peripheral Tibial Plateau		Patella	
	M	L		M	L	M	L	M	L	Prox.	Di
16/1	+++		(+)	++	+	+++	+++	+	(+)	(+)	+
16/2			(+)	+	+++	+++	+++	+	+	(+)	+
16/3			+	+	S	+++	+++	(+)	(+)	+	++
16/4			?+++	(+)	+++	+++	++++			(+)	(-
24/1		+++	+	+	+++	+++	+++	(+)	(+)	(+)	+
24/2			+	+++	+	+++	+++	+++	+	(+)	(-
48/1		+++	+	+	+	+++	+++	+	+	(+)	+
48/2				++	++	+++	+++	(+)	(+)		(-

Table 3 continued.

KEY:

(+)	Loss of sheen
+	Rough, granular
++	Fissures or splits
+++	Erosion
++++	Erosion with eburnation of bone
S	Cartilage incised by scalpel at time of cruciate section
?	Possible erosion

SOFT TISSUE CHANGES

Dog	WT (kgs.)	Synovial Membrane D.	T.	V.F.	A.	B.V.	Joint Capsule	Long Digital Extensor Tendon	Menisci Med.	Lat.	Post X
1/1	28.5	(+)				+					
1/2	19.5					(+)					+
1/3	18.5	(+)				(+)	(+)		(+)		
1/4	27	(+)	(+)			(+)			(+)		+
2/1	17	+	+								+
2/2	19	+	+	(+)		+			+		
2/3	17	+					+				+
2/4	24	+	+	(+)	+	+	(+)	+	(+)		(+)
3/1	15	+	+				+	+	+		
3/2	15	+	+				+	+	+		
3/3	18.5	+	+	+	+	+	+		+		(+)
3/4	15	+	+	+	+		+		+		
4/1	24	+	+		+	++	+	+			(+)
4/2	29	+	(+)		++		+	+	(+)		(+)
4/3	22.5	+	+	+	+		+	+			
4/4	18.5	+	+		+	+	+				+
5/1	19.5	+	+	+	+	+	+	+	+		
5/2	21.5	+	+		+		++	+	++	(+)	(+)
5/3	28	+	++	+	+	++	+		+		(+)
5/4	29	+	+	+	+		+	+	(+)		
6/1	21	+	++				+	+	+		
6/2	24	+	++			++	++	+	+		

TABLE 4: MACROSCOPICAL APPEARANCE OF SOFT TISSUE CHANGES (RT. STIFLE, GROUP A DOGS)

Dog	WT (Kgs.)	Synovial Membrane D.	T.	V.F.	A.	B.V.	Joint Capsule	Long Digital Extensor Tendon	Menisci Med.	Lat.	Post X
6/3	21	+	++		+		+	+			
6/4	15	+	++	+		++	+	+	+		
7/1	16.5	+	++	++		++	++	+	++		
7/2	19	+	+	++	+	(+)	(+)	+	(+)		
7/3	27	+	++		+	++	+	+	+++		
7/4	24	+	++				+	(+)	++		
8/1	22	+	++	++	++	++	++	+	+		
8/2	25	+	++		++		+	+	+		
8/3	27	+	++		+		+	+	+		(+)
8/4	12	+	+				+	+	(+)		(+)
9/1	15	+	+			+	+	+	++	+	
9/2	30	+	+	++		++	++	+	++		
9/3	18	+	++	+	+	+	+	(+)	++		
9/4	18	+	++				+	+		(+)	
10/1	20.5	+	+	++	+	+	+	+	++ (c)		
10/2	14.5	+	++	+	+		+	(+)	+ (c)		
10/3	21.5	+	++		+		+		++		
10/4	24	+	++	++	+	+	(+)	+			(+)
12/1	18	+	+		+		++	+	++		
12/2	25	+	++	+	++	+	++	+	+++		
12/3	20	+	+		+	++	+	(+)	+		
12/4	29	+	++	+	++		++	+	+++		
16/1	18	+	++			+	+		++		
16/2	14	+	++	(+)	+		(+)	+	(+)	(+)	

Table 4 continued

Dog	WT (Kgs.)	Synovial Membrane D.	T.	V.F.	A.	B.V.	Joint Capsule	Long Digital Extensor Tendon	Menisci Med.	Lat.	Post X
16/3	18.5	+	++	++			++	+	+++		
16/4	15.0	+	++	(+)		+	++		++		
24/1	23	+	++		++		++	+	++	++	
24/2	22	+	++	(+)	+	++	++	+	+++		
48/1	20	+	+	++	+		++	+	+++		
48/2	15.5	+	(+)	++	+	+	(+)	+	+++		(+)

Table 4 continued

KEY

Synovial Membrane

D - Discoloration
T - Increased Thickness
VF - Villous folds
A - Adhesions
BV - Vascular proliferation

Graded
(+) slight
+ obvious
++ severe

Joint Capsule

(+) Possible or slight fibrous thickening
+ Obvious " "
++ Severe " "

Long Digital Extensor Tendon

(+) Slight increase in thickness
+ Obvious increase in thickness

Menisci (Medial and Lateral)

(+) Slight surface fibrillation
+ Mild splitting
++ Severe splitting or tearing (bucket-handle tear)
+++ Very severe shredding, disintegration
(c) Calcification in meniscus

Posterior Cruciate Ligament

(+) Slight haemorrhage into ligament, or slight partial section.
+ Ligament sectioned

		Quantity Syn. Fluid (mls)	Colour	Blood in Syn. Fluid	Character
1 Week	1	1.5	straw	no	slightly viscous
	2	2	blue (dye)	no	?
	3	1.5	orange	no	viscous
	4	3.5	pink	no	viscous
2 Weeks	1	1.5	orange	no	viscous
	2	1.5	pink	blood-stained	slightly viscous
	3	3	pink	no	fluid
	4	6	pink	no	slightly viscous
3 Weeks	1	5	red-brown	no	fluid
	2	4	pink	blood-tinged	fluid
	3	7	orange	no	fluid
	4	3	orange	no	fluid
4 Weeks	1	8	pink	no	fluid
	2	10	pink/straw	no	slightly viscous
	3	5	orange	no	slightly viscous
	4	6	orange	no	slightly viscous
5 Weeks	1	1	straw	no	viscous
	2	18	red	no	very fluid
	3	4	pink	no	fluid
	4	10	pink/straw	no	fluid
6 Weeks	1	15	brown-tinged	no	fluid
	2	18	brown	blood-tinged	very fluid
	3	9	pink	no	fluid
	4	5	pink	no	slightly viscous
7 Weeks	1	7	orange	no	fluid
	2	1.5	orange	no	viscous
	3	8	straw	no	slightly viscous
	4	7	pink	no	slightly viscous
8 Weeks	1	14*	red	no	very fluid
	2	22	pink	no	very fluid
	3	-**	yellow	no	?
	4	5	straw	no	slightly viscous

TABLE 5: VOLUME AND CHARACTER OF SYNOVIAL FLUID ASPIRATED FROM RIGHT STIFLE JOINT AFTER DEATH.

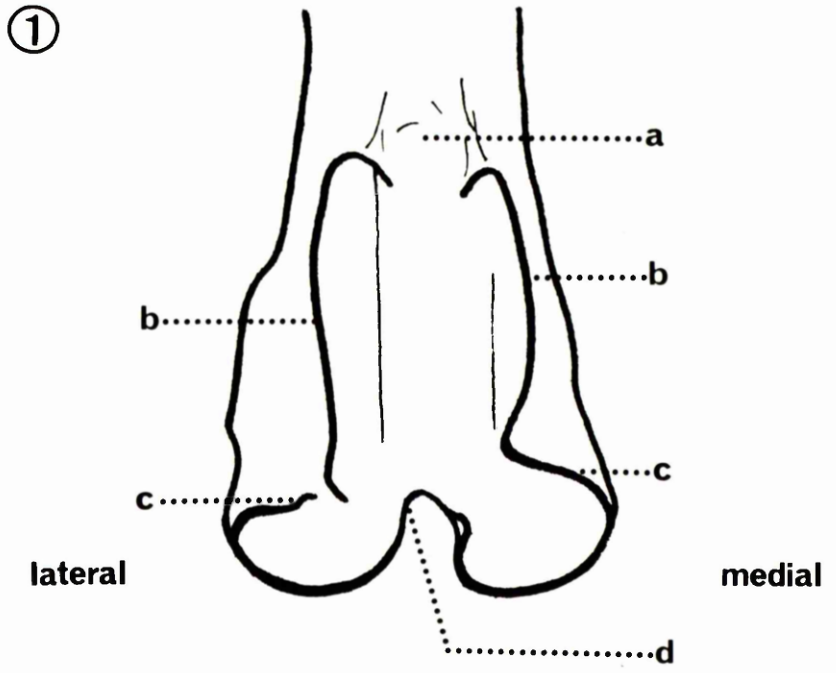
		Quantity Syn. Fluid (mls)	Colour	Blood in Syn. Fluid	Character
9 Weeks	1	4	pink	blood-tinged	slightly viscous
	2	13	straw	no	fluid
	3	4	red	blood-tinged	fluid
	4	1.5	pink	no	slightly viscous
10 Weeks	1	5	orange	no	slightly viscous
	2	5	pink	no	fluid
	3	10	red	no	slightly viscous
	4	5	pink	no	slightly viscous
12 Weeks	1	3	clear	no	viscous
	2	21	straw	no	fluid
	3	-***	-	-	-
	4	18	red	no	fluid
16 Weeks	1	1.5	straw	no	viscous
	2	2	clear	no	viscous
	3	9	straw	no	fluid
	4	2	straw	no	viscous
24 Weeks	1	4	clear	no	viscous
	2	2	pink	no	viscous
48 Weeks	1	<1	clear (yellow tinged)	no	viscous
	2	<1	clear	no	viscous

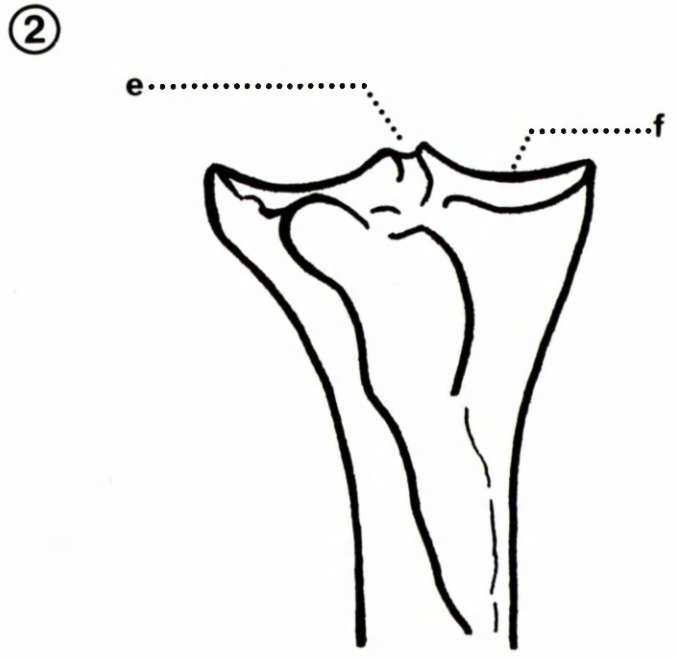
* This dog also had >14 mls aspirated 1 day before

** Synovial fluid not measured

*** Record lost

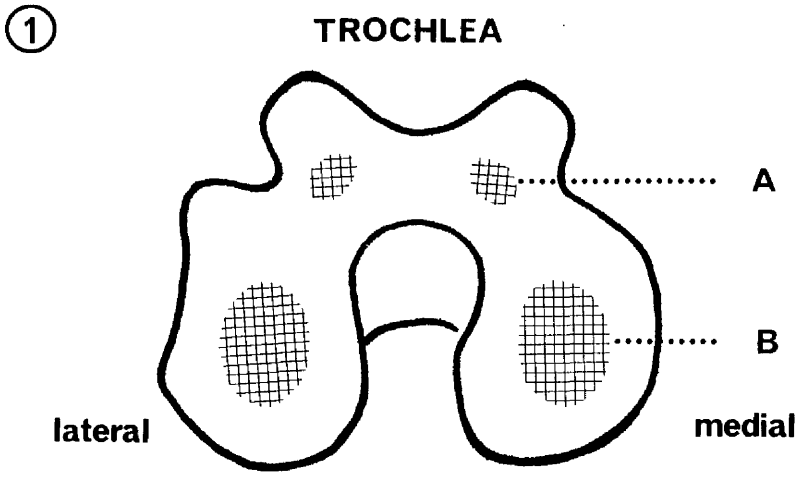
Table 5 continued.





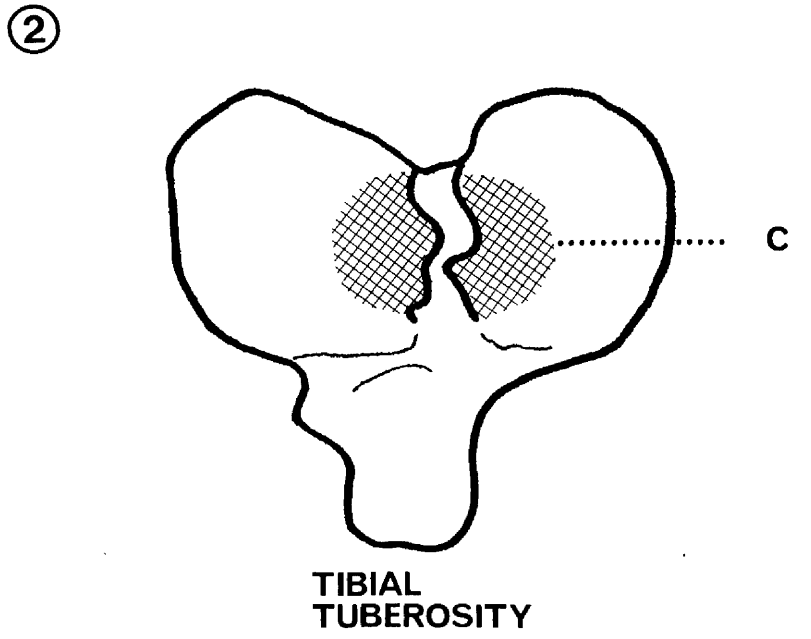
a - supratrochlear fossa
b - trochlear ridge
c - rim of condyle
d - intercondyloid fossa
e - tibial spine
f - caudal tibial plateau

FIG. 12: DIAGRAM TO SHOW SITES OF OSTEOPHYTE FORMATION ON FEMUR AND TIBIA



A - junction of trochlear ridge and femoral condyle

B - central femoral condyle

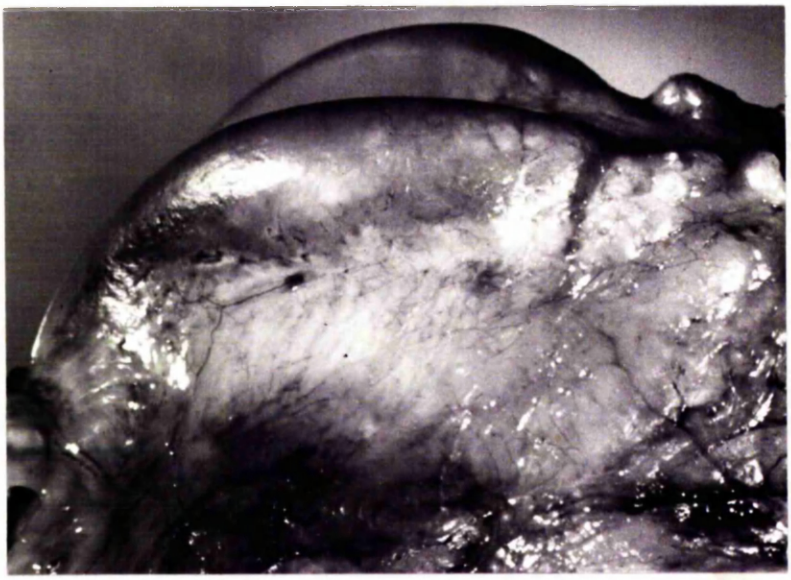


C - central tibial plateau

FIG. 13: DIAGRAM OF FEMORAL CONDYLES AND TIBIAL PLATEAU SHOWING SITES OF CARTILAGE EROSION

Fig. 14: Lateral femoral trochlear ridge from control (left) stifle joint (dog 1/1) to show the normal appearance following perfusion with dye mixture (compare fig. 15).

Fig. 15: Lateral trochlear ridge from right stifle 1 week after cruciate section (dog 1/1). Early vascular proliferation at the margin of the trochlea is demonstrated by perfusion with dye mixture (compare fig. 14, trochlear ridge left stifle of same dog).



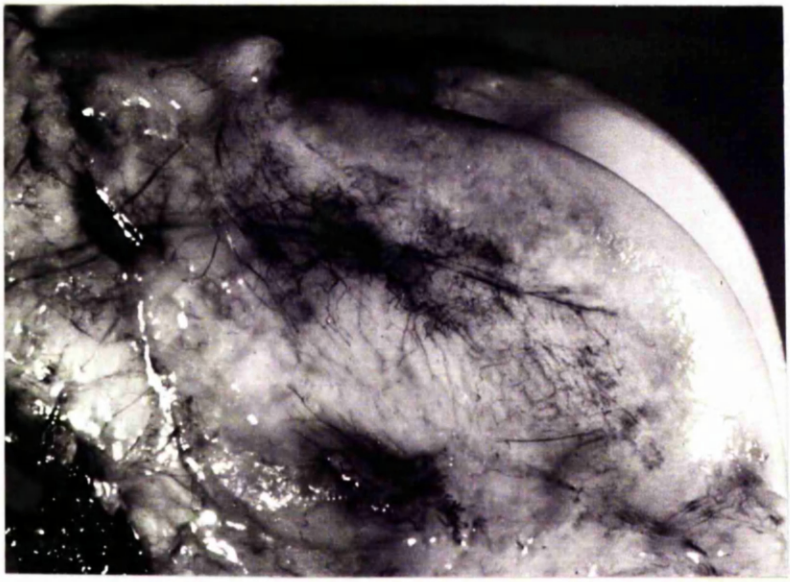
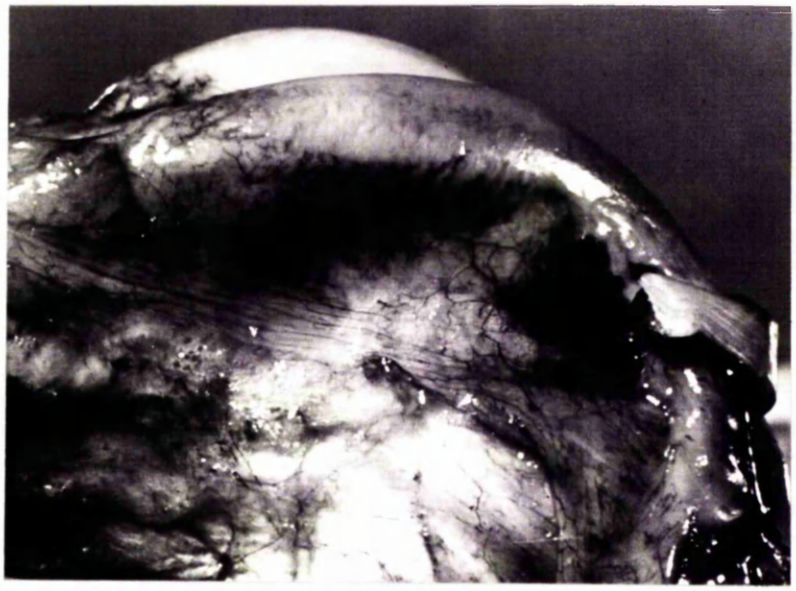


Fig. 16: Lateral trochlear ridge from right stifle 2 weeks after cruciate section. Perfusion with dye mixture shows the increase in vascular proliferation (compare fig. 15).

Fig. 17: Lateral aspect of right femoral trochlea from dog 2/1, showing the earliest osteophyte formation detected macroscopically - a tiny ridge (arrowed) is just discernible at the proximal limit of the trochlea.



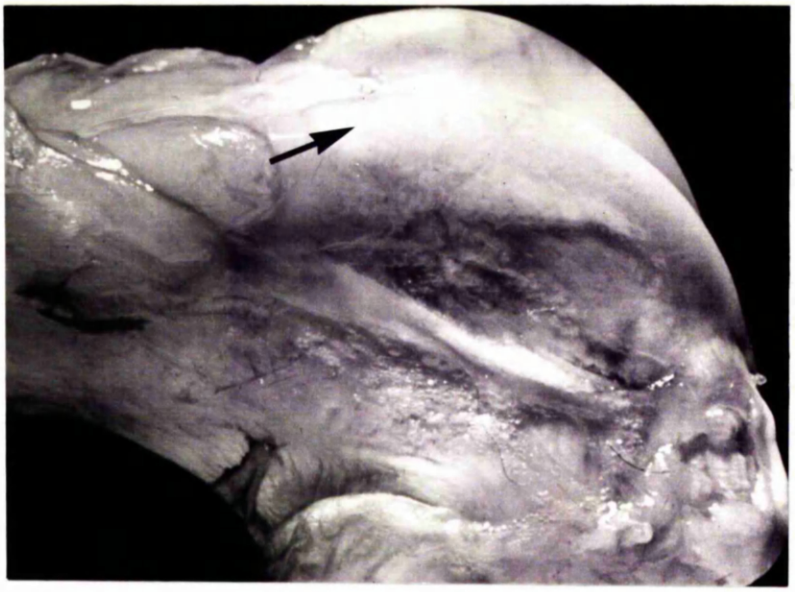
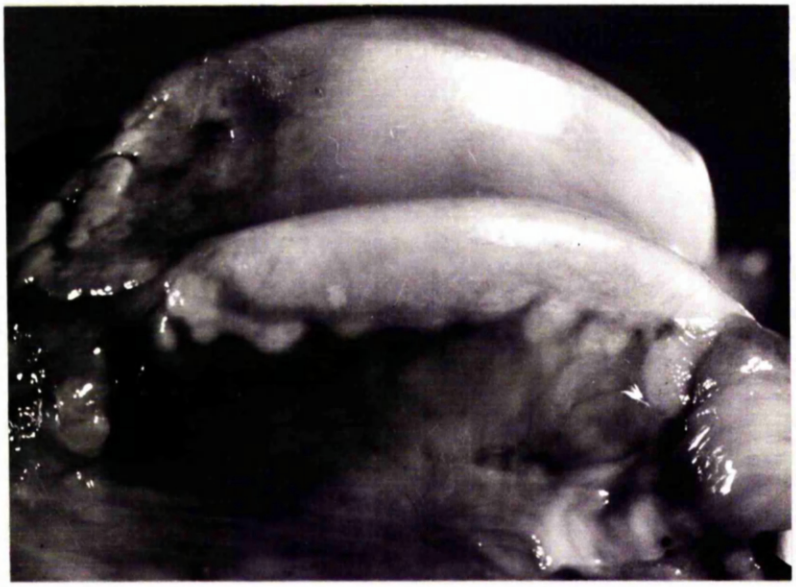


Fig. 18: Lateral view of right femoral trochlea from dog 5/4, to show irregular nodular osteophyte formation in the supratrochlear fossa and on margins of lateral ridge. Discoloration of the synovial membrane is also evident.

Fig. 19: Femoral condyles and tibial plateau from right stifle 4 weeks after cruciate section (dog 4/3) showing a large, shallow, oval cartilage erosion in the central region of the condyle. The intercondyloid fossa is filled with granulation tissue; a few small scalpel scars may be seen on the lateral aspect.



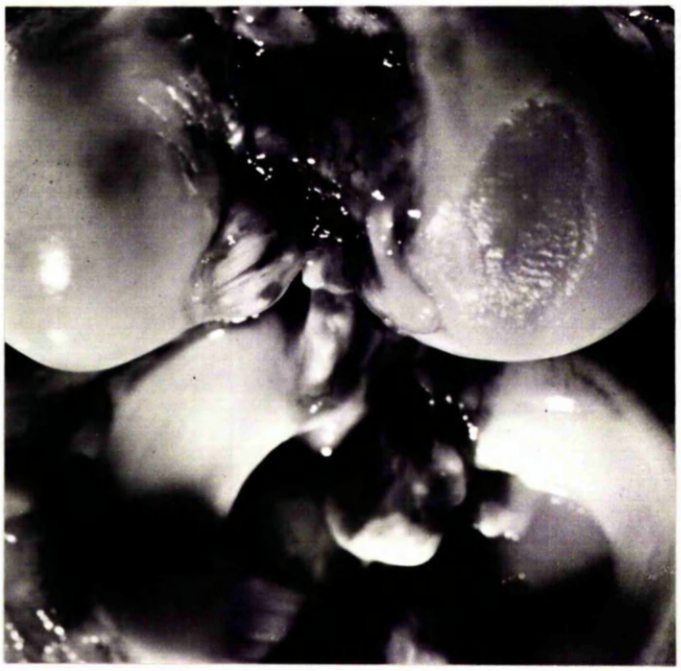
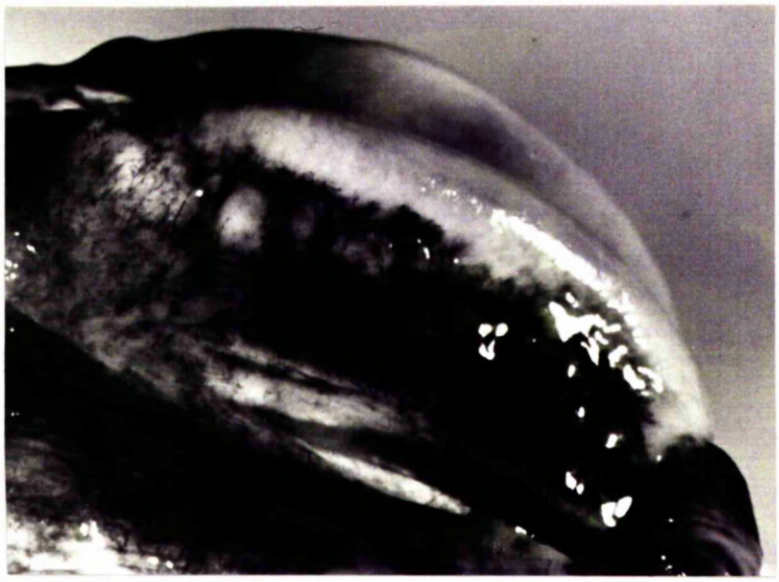


Fig. 20: Lateral trochlear ridge 7 weeks after ligament section (dog. 7/1). Marked synovial vascularity is shown by the uptake of dye mixture, small branching blood vessels may be seen overlying the osteophyte nodules and encroaching on the trochlear ridge.

Fig. 21: A slight raised and uneven ridge of new bone at the margin of the lateral trochlear ridge at 8 weeks after cruciate section (dog 8/3).



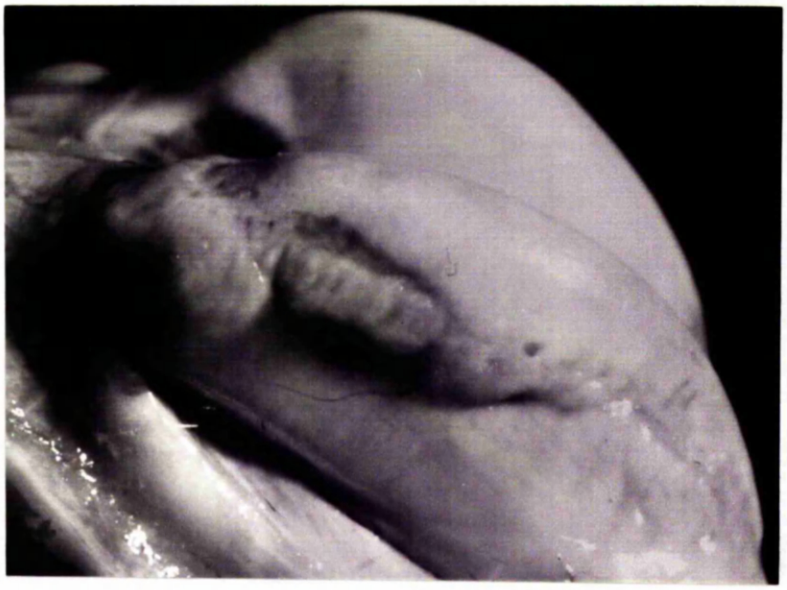
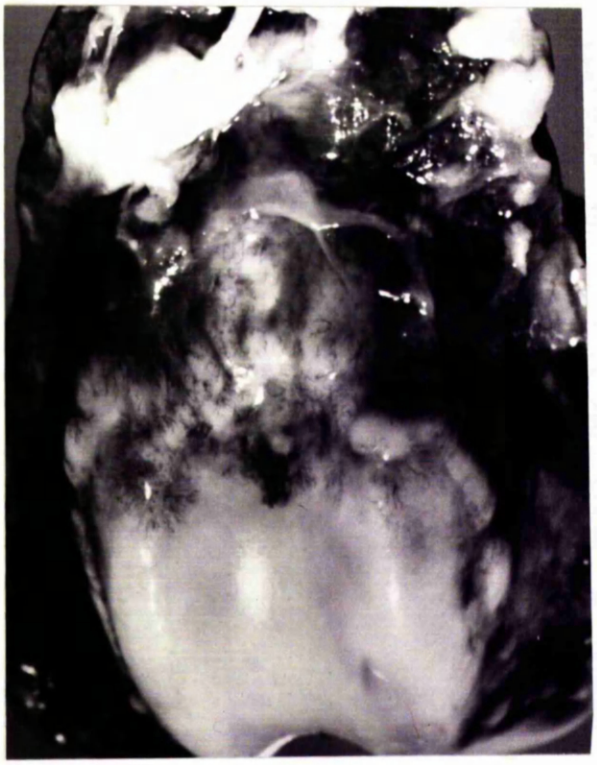


Fig. 22: Femoral trochlea from dog 8/1, showing osteophyte formation, synovial vascular proliferation (perfused specimen), and synovial adhesions in proximal joint pouch.

Fig. 23: Femoral condyles and tibial plateau 9 weeks after cruciate section (dog 9/3), showing large mass of new bone in intercondyloid fossa, splitting of medial meniscus, central erosion on lateral tibial plateau (arrowed), synovial vascularity and thickening of joint capsule.



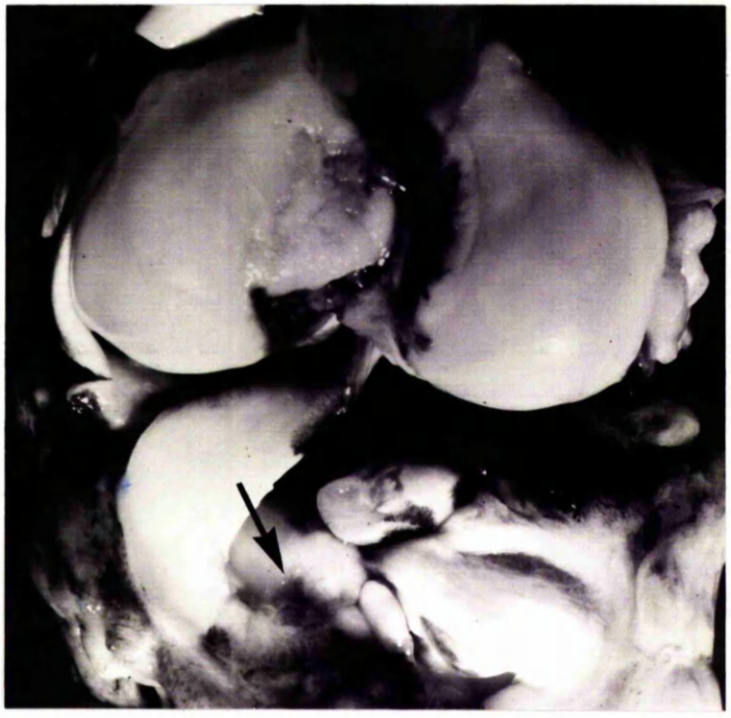


Fig. 24: Right femoral trochlea and condyles and tibial plateau from dog 12/2. Osteophytes may be seen on medial and lateral trochlear margins and on the patella. A large erosion with central area of eburnation is present on the medial femoral condyle and an erosion of the central tibial plateau on the lateral side. The medial meniscus is almost completely disintegrated, the joint capsule is thickened and the synovial membrane shows marked vascularity and a large infrapatellar adhesion.

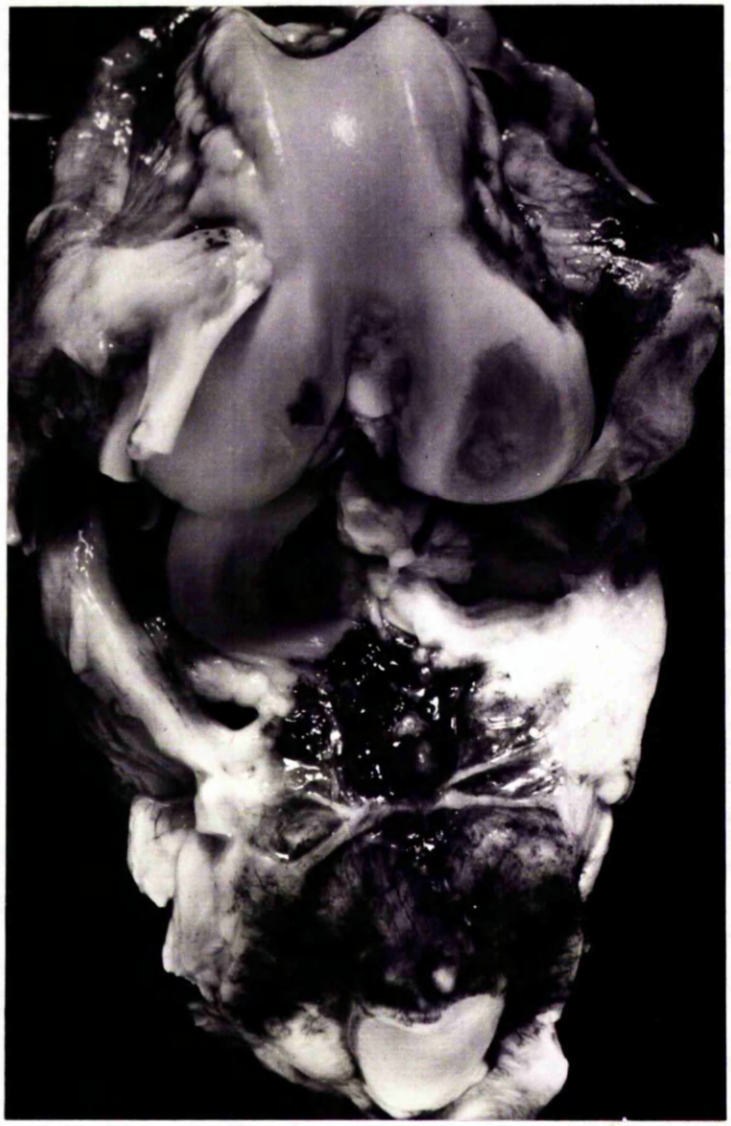
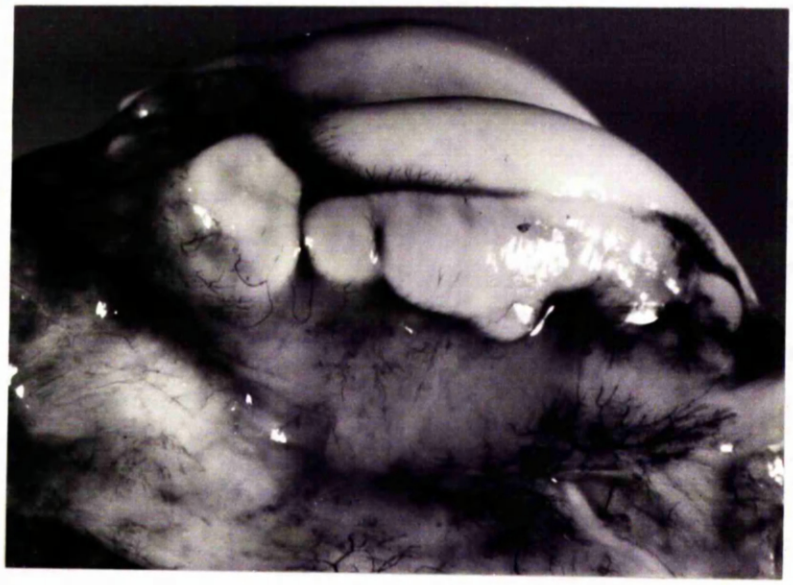


Fig. 25: Lateral aspect of femoral trochlea from dog 16/4 showing the prominent, smooth ridge of new bone covered by cartilage at the lateral margin. Note also the synovial vascularity (perfused specimen).

Fig. 26: Lateral trochlear ridge from dog 24/2, showing confluence of the osteophytes with the trochlear ridge, resulting in widening of the ridge (see also fig. 27). There is intense synovial vascularity and a few vessels encroach on to the new bone (perfused specimen).



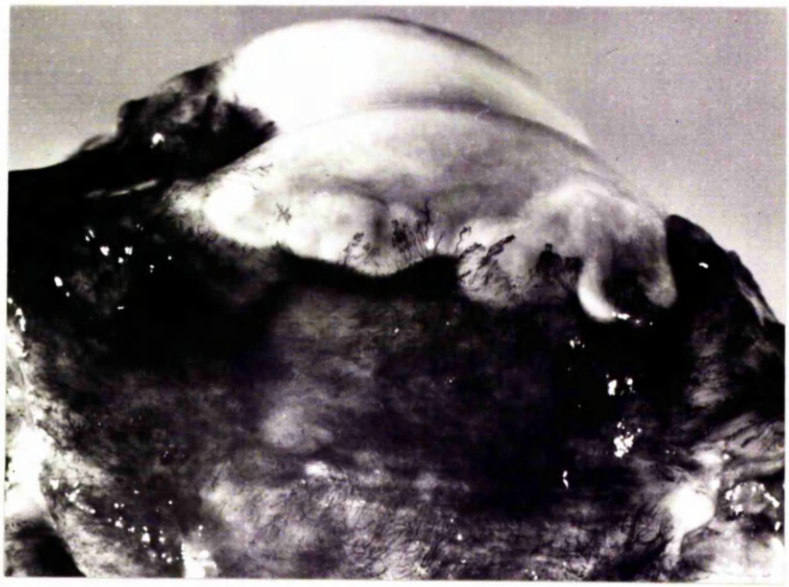
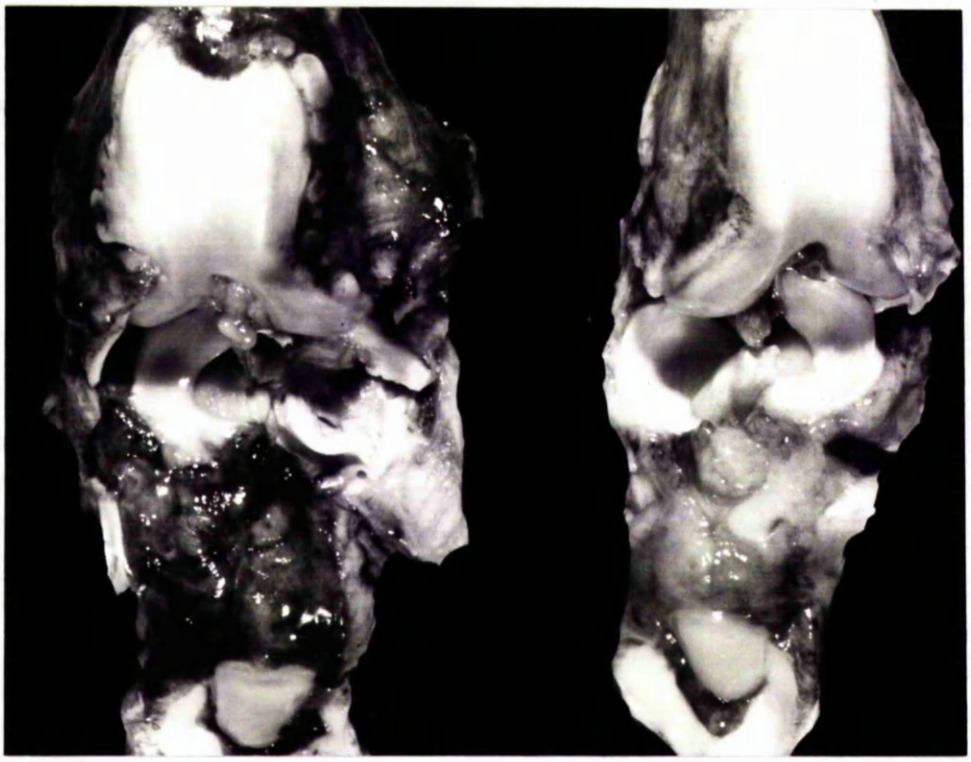


Fig. 27: Comparison of right and left stifle joints from a dog 24 weeks after transection of the right anterior cruciate ligament (dog 24/2). Note the marked increase in width of the right trochlear region compared to left and the difference in vascularity (perfused specimen). Also obvious are the marked splitting of the medial meniscus, increased thickness of the joint capsule, roughening of the cartilage of the central tibial plateau and osteophytes on the tibial spine.

Fig. 28: Lateral femoral trochlea from dog 48/2, showing the remodelled trochlear ridge, with subchondral vascularity causing discoloration of the new bone. (perfused specimen).



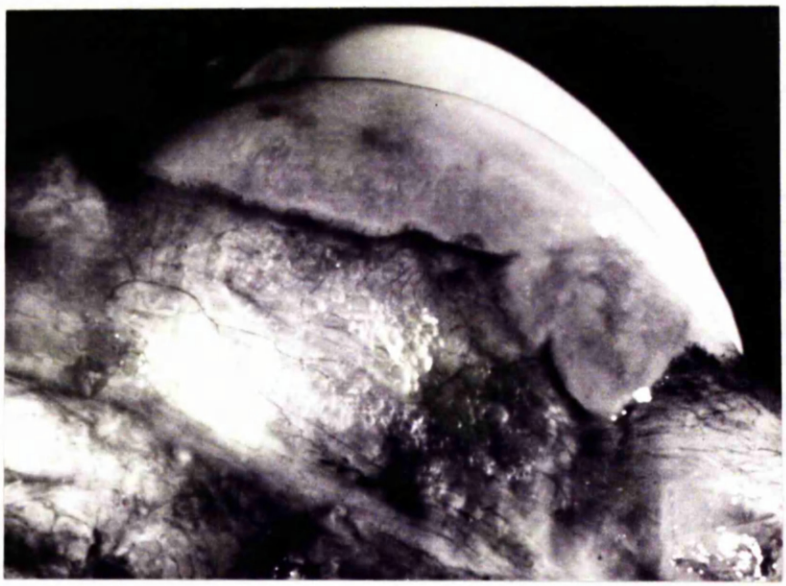
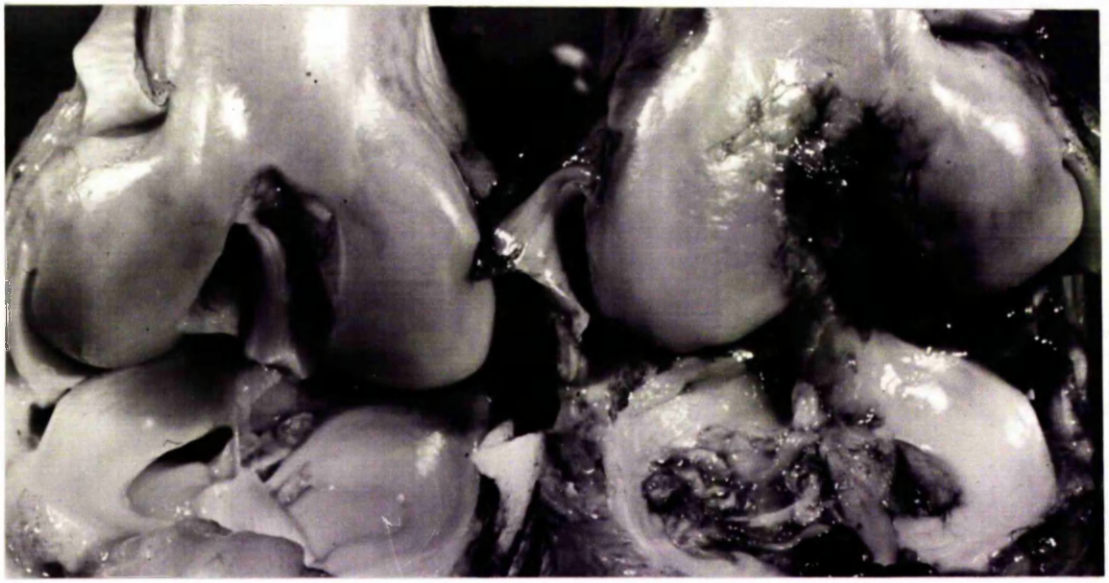


Fig. 29: Comparison of left and right femoral condyles and tibial plateaux 48 weeks after ligament section, (dog 48/2). The right stifle joint shows obvious recontouring of the femoral condyles if compared with left. Note also the medial meniscus, synovial vascularity, and roughness of articular cartilage.

Fig. 30: Right femoral trochlea from dog 48/1, showing prominent smooth osteophyte nodules, remodelling of the proximal limit of the trochlear groove and the thickened, granular synovial membrane.



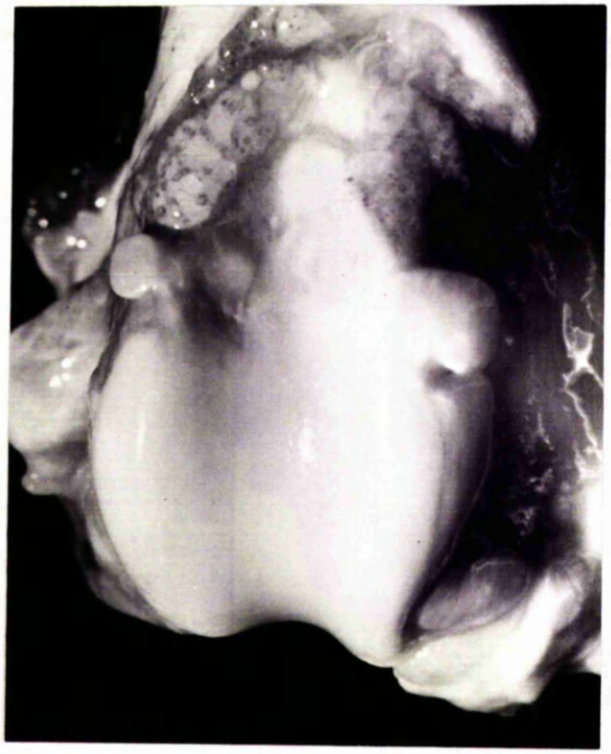
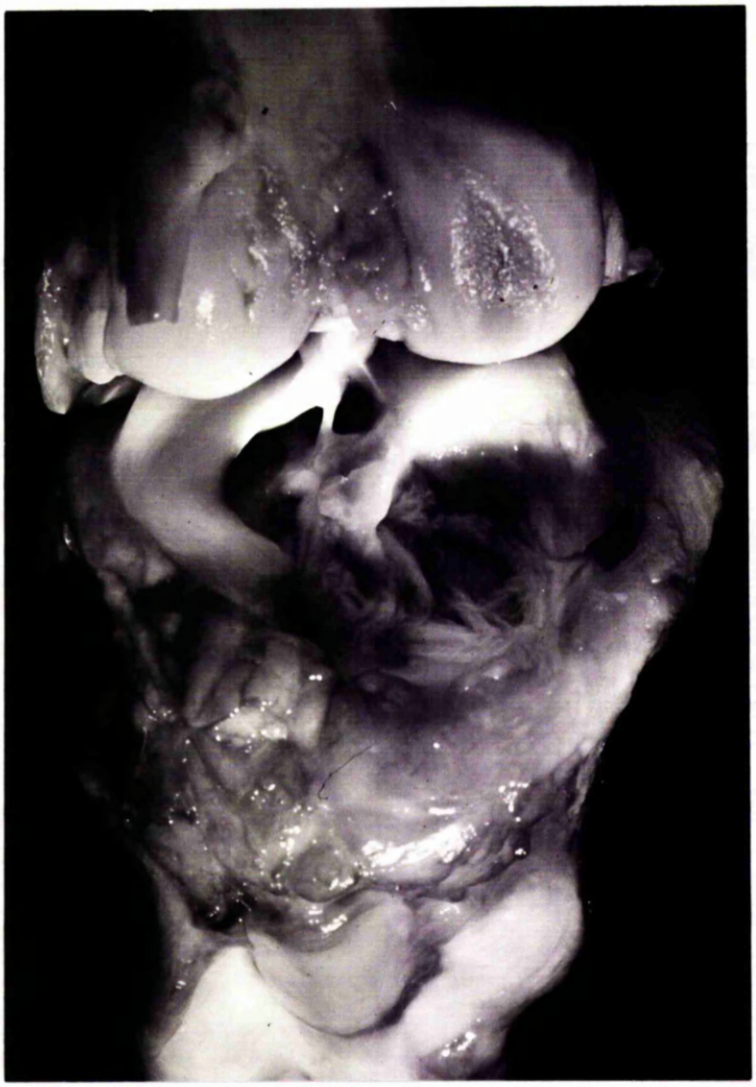


Fig. 31: Natural O.A. in the left stifle joint of dog 10/3. Note the femoral condyle erosions, disintegration of medial meniscus and capsular thickening and remodelling of the femoral condyles. Granulation tissue and new bone in the intercondyloid fossa obscure the site of origin of the anterior cruciate ligament (ruptured).



DISCUSSION

It is important to establish whether the changes produced by surgical transection of the anterior cruciate ligament in the canine stifle joint resemble those of naturally occurring O.A. in the stifle, or whether there are significant differences from the spontaneous condition. The pathological features of natural O.A. have been described elsewhere and reviewed in detail in part I.

Olsson (1971) does not consider that the changes in the canine stifle joint caused by instability after rupture of the cruciate ligaments are those of true O.A. This comment is based on the observation that only "minimal involvement" of joint cartilage occurred in spite of osteophyte formation, thickening of the synovium and derangement of menisci. On the other hand, Tirgari and Vaughan (1975) in their report on osteoarthritic stifle joints, do not record any difference in the degree or distribution of cartilage defects in those joints with ruptured cruciate ligaments compared to those with intact cruciate ligaments. These authors found varying degrees of articular cartilage erosion, from superficial areas of roughening to deep erosions exposing subchondral bone.

In this experimental series, discrete cartilage erosions were recorded less consistently than the presence of osteophytes or meniscal fibrillation or other soft tissue changes. Nevertheless, distinct cartilage erosions at one or more locations were recorded in approximately half the dogs surviving longer than 6 weeks, and occasionally eburnation of subchondral bone was noted.

The progressive development of osteophytes, meniscal fibrillation, joint capsular fibrosis, and synovial membrane changes, may be readily discerned/

discerned by comparison of the macroscopic appearance of the operated joints at different time intervals after cruciate section. The progressive nature of cartilage degeneration was less obvious however, although there was a definite increase in size and distribution of cartilage erosions and in areas of dull or roughened cartilage in those animals killed 6 weeks or more after surgery, compared to those killed before 6 weeks. Improvement of the subjective assessment of the macroscopical appearance of cartilage could have been achieved by using a grading system such as that devised by Meachim (1972), whereby the degree of cartilage fibrillation is graded according to the degree of retention of indian ink by the cartilage. This technique was however performed routinely only on specimens of cartilage subjected to biochemical analysis.

The early detection on macroscopical examination of osteophyte nodules at 2 weeks after cruciate section is interesting. It is in general agreement with the report of Marshall (1969) who investigated the development of periarticular osteophytes in a similar experimental model, and recorded the presence of nodular proliferations at the point of reflection of the synovial membrane 26 days after ligament section. Paatsama and Sittnikow (1972) also reported early osteophyte development following transection of the cruciate ligament; in their series the earliest changes were noted 3 weeks post-operatively.

It is also interesting to note that vascular perfusion clearly demonstrated the proliferation of small finely branching blood vessels in the synovial membrane 1 week after cruciate section. The location of this vascular proliferation at the margin of the trochlear ridge corresponded to the area where the first sign of osteophyte formation

was/

was recorded. Later, the osteophyte nodules or ridges in some perfused specimens had an obvious blueish tinge due to the presence of blue dye in subchondral blood vessels. The association between vascular proliferation and bone development and the possible significance of this observation will be discussed further (part VI).

As well as the increased synovial vascularity, other changes such as increased thickness, discoloration, villous proliferation were recorded in the synovial membrane of the operated joint. This is in agreement with the findings of Pond (1971) who commented on the "very early" appearance of synovial membrane changes in the disease process, and with the report by Tirgari and Vaughan (1975) who found that the synovial membrane was "invariably hyperaemic and in patches had a velvety appearance due to villous proliferation".

The association between meniscal damage and osteoarthritic change in the stifle joint is well documented (Nilsson 1949, Paatsama 1952, Strande 1967, Pond 1971, Tirgari and Vaughan 1975). The medial meniscus is reported as being affected more frequently than the lateral meniscus. The findings of the present study confirm these earlier reports. It was not possible however to correlate the appearance of meniscal fibrillation with the presence or absence of cartilage erosions of the corresponding condyle, or with the degree of lameness shown by the dog prior to death.

As might be expected, the changes which develop after section of the anterior cruciate ligament closely resemble those which follow spontaneous cruciate rupture and, macroscopically at least, also resemble changes typical of O.A. Furthermore, no such changes were produced in joints subjected to stab-incision without ligament section. In other words, the macroscopical appearance and distribution of osteophytes, cartilage lesions/

lesions, meniscal fibrillation, joint capsule and synovial membrane changes are very similar in this experimental model and in spontaneous O.A. of the stifle joint.

HISTOPATHOLOGY

GROUP A DOGS

Femoral trochlear blocks from both left and right stifle joints of each experimental dog were sectioned transversely as described previously (p. 37; Fig. 2), to produce alternate undecalcified sections and 5 mm thick blocks for routine decalcification and histological examination. As far as possible, histological sections adjacent to, and therefore comparable with, the undecalcified sections were prepared.

The left femoral trochlear sections were used essentially to provide a basis of comparison with the diseased tissue of the contralateral joint, so that the presence and degree of pathological change in the right stifle joint could more readily be assessed. The histological appearance of these left sections has not been described for each dog; a representative normal trochlear section is illustrated in fig. 32. In 4 dogs, however, pathological changes were noted in sections from the left femoral trochlea. These dogs comprised one case of naturally occurring O.A. of the left stifle (10/3), and 3 dogs surviving to 24 and 48 weeks after ligament section. These results have been presented separately (p 99).

For the right stifle joints, the results have been presented in table 6 by describing a representative mid-trochlear section from each dog. This method of tabulation of results from a single section allows assessment of the pathological changes observed in each dog, and also shows the progressive development of these changes from 1 to 48 weeks after cruciate ligament section.

The selection of a single transverse section from each femoral trochlea does not, however, mean that there is uniformity of pathological change from supratrochlear fossa to femoral condyles: variation in the histological appearance at the different levels was observed in every case. Different/

Different features were noted particularly in the proximal sections taken through, or close to, the supratrochlear fossa, and also in the distal section if this was adjacent to the femoral condyles. However, the variation in the degree and type of pathology observed in these particular sections was explained by the different distribution of tissues at these levels, i.e. the proximal and distal sections were not truly representative of the femoral trochlea. The histological appearance of each transverse section from the right femoral trochlea has been presented in tabulated form in appendix 2.

The results have been sub-divided into three categories; namely, changes affecting (A) Bone, (B) Cartilage and (C) Synovial membrane. Osteophyte development and other bony remodelling processes have been recorded in greatest detail, but pathological changes in the articular cartilage and synovial membrane have also been included in an attempt to provide a comprehensive picture of the disease process.

A general description of the histopathological changes is given below.

(A) BONE

A number of different areas of bone remodelling were recognised on examination of femoral trochlear sections, and to facilitate description these have been classified as follows:-

i) Marginal osteophyte

This designates the localised development of new bone situated at the chondro-synovial junction in the marginal or transitional zone.

ii) Subchondral zone

Although any bone subtending cartilage is "subchondral bone" the term/

term has been specifically applied to bone beneath the hyaline cartilage and fibrocartilage on the outer non-articulating faces of the trochlear ridges.

iii) Periosteal zone

Bone remodelling involved the outer, periosteal, surface of the femoral cortex caudal to the synovial membrane attachment extending for some distance from the articular surfaces. This has been called the periosteal zone.

iv) Trabecular bone

The trabeculae of the distal epiphysis of the femur also underwent remodelling.

The location of these areas may be identified in fig. 32. It should be noted that the distinction between the different areas of bone remodelling was not always possible; for example, in the longer surviving dogs, subchondral bone remodelling and marginal osteophyte together constituted one large "osteophyte" or remodelled trochlear ridge. Nevertheless, the classification was useful, and emphasised the fact that remodelling of the joint contours was due to a number of concurrent processes of bone remodelling.

i) Marginal Osteophyte

Histologically, marginal osteophyte formation was noted in a total of 50 dogs. The appearance was graded into seven categories.

Grade 1

This was the earliest recognisable change, and consisted of a small focal collection of mainly spindley fibroblast-like cells on the outer aspect of the femoral cortex in the marginal zone (figs. 33 and 40).

At/

At the centre, the cells were rounder or ovoid, with less densely staining nuclei and prominent nucleoli (fig. 41). Sections stained with Toluidine Blue showed either traces of metachromasia or none at all. A few small blood vessels were present within, or adjacent to, the clump of cells.

Grade 2

Grade 2 osteophytes were small focal deposits of cellular woven bone, lying outside the femoral cortex at the chondro-synovial junction, and covered on the outer edge by a thin layer of very cellular fibrous tissue (figs. 34 and 42). Chondrocytes were sometimes present within the osteophyte and varied from a very few with a scattered distribution to focal accumulations towards the periphery of the osteophyte. Small spaces, containing blood vessels and cells presumed to be osteoblasts, were present within the woven bone, blood vessels were also noted at the periphery of the osteophyte(fig. 42).

Grade 3

This osteophyte was similar in structure to grade 2, but was generally larger in size (fig. 35). The spaces or cavities within the woven bone were not only larger at this stage but more numerous, and osteoclasts as well as osteoblasts were recorded within them. The presence of chondrocytes (identified by Toluidine Blue staining) varied from none present to a few either sparsely distributed or more often in focal clumps usually at the outer edge of the osteophyte (fig. 43).

Grade 4

At this stage the osteophyte was normally considerably larger than a grade 3 osteophyte and composed partly of woven bone but with early trabecular formation within the centre of the osteophyte and wide inter-/

inter-trabecular spaces containing many cells and blood vessels (figs. 36, 44 and 45). The trabeculae were fragmented and irregular in shape and distribution, and osteoblasts were identified on the surface or adjacent to them (fig. 46). Large multinucleate osteoclasts were also present, especially towards the outer margin of the osteophyte (figs. 45 and 47). In some sections, resorption of the pre-existing femoral cortex had occurred and communication established between osteophyte and bone marrow spaces of the distal femoral epiphysis. On the outer edge, the osteophyte was bounded by cellular fibrous tissue and focal fibrocartilage (fig. 44).

Grade 5

Grade 5 osteophytes were composed of both cancellous bone, forming uneven and haphazardly arranged trabeculae with wide inter-trabecular spaces (figs. 37 and 48), and cellular woven bone which was usually located at the outer and lower margins of the osteophyte but also in small amounts in the trabeculae. Free communication was usually evident between the bone marrow spaces of osteophyte and distal femur (fig. 37). The outer limit of the osteophyte was composed of a thin shell of bone outside which was usually a thick layer of fibrocartilage (figs. 37 and 48). The bone-cartilage interface was studded with blood vessels budding into the cartilage from the cellular bone marrow of the osteophyte (fig. 48).

Grade 6

At this stage the osteophyte was not as well defined as at earlier stages, remodelling processes having destroyed the original cortical demarcation between osteophyte and bone marrow, with continuity between the bone marrow spaces and the trabeculae of osteophyte and femur (fig. 38). In addition, the area of bone remodelling in the subchondral zone was confluent/

confluent with the marginal osteophyte in some sections (fig. 38). The trabeculae of the osteophyte were more smooth and regular than at earlier stages, and were composed mostly of lamellar bone (fig. 49). The cellularity and vascularity of the marrow spaces, with plump osteoblasts lying on the surface of trabeculae and occasional osteoclasts at the outer edges of the osteophyte, showed that active remodelling was still going on (fig. 49). Furthermore, abundant vascular budding into the fibrocartilage overlying the osteophyte was evident at this stage (fig. 49).

Grade 7

Grade 7 osteophytes were "mature" osteophytes, composed of a smooth ridge of cancellous bone with regularly orientated trabeculae and in some cases extending from the tip of the trochlear ridge to the synovial membrane attachment (fig. 39). There was relatively little evidence of trabecular remodelling, but at the outer edge of the osteophytic ridge, some vascular budding with new bone deposition was still evident (fig. 50). The osteophyte was bounded by a thin plate of cortical bone and fibrocartilage (figs. 39 and 50).

Using this system of grading, the osteophyte appearance of each trochlear section was recorded in table 6 and appendix 2.

ii) Subchondral Zone

Many dogs showed no evidence of histological change in this region of the femoral trochlea. The earliest recorded change occurred at 3 weeks (see Appendix 2).

Initially, a few vascular buds penetrated the subchondral bone plate into/

into the overlying fibrocartilage (fig. 51). In some cases this was accompanied by remodelling of adjacent trabeculae shown by focal bone resorption and obvious osteoblasts lining the trabeculae. A small number of vascular buds may be present in this region in a normal joint, and the presence of two or three such buds was not considered pathological unless accompanied by obvious bone remodelling.

In some sections extensive vascular budding was observed from tip of trochlear ridge to chondro-synovial junction, but more often only part of the subchondral zone was involved. As the number and extent of vascular tufts increased, deposition of woven bone occurred in the deep face of the fibrocartilage, together with obvious remodelling of subjacent trabeculae (fig. 52). The process of endochondral ossification and adjacent remodelling continued, (fig. 53), giving rise to an appreciable thickness of cancellous bone with consequent widening of the trochlear ridge (fig. 38). Ultimately, subchondral remodelling and osteophyte proliferation together resulted in recontouring of the entire ridge (fig. 39).

Lateral, medial, or both trochlear ridges were affected by subchondral remodelling. Almost invariably, vascular budding was restricted to the outer non-articulating face of the trochlear ridge, although it was observed within the trochlear groove in 2 dogs (16/4, 48/1) in sections taken from the proximal third of the femoral trochlea (fig. 39). Subchondral trabecular bony remodelling in the trochlear groove was, however, relatively common (recorded in 19 dogs). It was shown by resorption and disorganisation of trabecular structure, together with localised hypercellularity including the presence of numbers of osteoblasts and osteoclasts (figs. 54 and 55).

iii)/

iii) Periosteal Zone

The first change recorded on the periosteal surface of the femoral cortex was an increased cellularity and thickness of the fibrous connective tissue layer of the periosteum. In the tabulated results (Table 6 and Appendix 2) this was denoted by the letter "F", (fibroplasia). The fibroblast proliferation was either diffuse or multiple and focal, and sometimes areas of fibroplasia and new bone deposition occurred adjacent to each other.

Histologically, woven bone deposition on the periosteal surface was recorded first at 3 weeks. The initially thin and irregular layer of woven bone increased in thickness with time following surgery, and by 5 to 8 weeks there was usually a well defined layer of periosteal new bone on medial and lateral femoral cortex (figs. 35 and 36). The highly cellular woven bone was studded with many small vascular channels. Occasionally larger irregular cavities alsocontaining blood vessels were noted in the deeper layers of the new bone and sometimes also within the original femoral cortex (figs. 36 and 56).

At later stages, continued deposition of woven bone was apparent on the outer surface, while a process of remodelling of the deeper layers and femoral cortex gave rise to an irregular network of lamellar bone. By 24 weeks, the periosteal zone comprised a broad band of irregular and somewhat fragmented trabeculae, with a fairly thick layer of compact bone at the outer edge in which a few developing Haversian systems could be seen (figs. 38 and 57). The original femoral cortex was no longer discernible.

At 48 weeks, the bone remodelling had resulted in a greatly thickened femoral cortex composed largely of mature compact bone (fig. 39). There/

There were fewer signs of active remodelling than at earlier stages, although some of the osteons showed evidence of continued new bone deposition (fig. 58).

iv) Trabeculae

Histological assessment of trabecular remodelling was not easy in the early stages, partly because of the subtle nature of the changes and the extensive surface area to be examined, and also because there was less consistency in the "normal" trabecular pattern than, for example, in a"normal" marginal or subchondral zone. Furthermore, the trabeculae were more likely to be damaged and fragmented by sectioning processes than the other areas. Trabecular remodelling was first recognised histologically at 7 weeks (Table 6) with an apparent thinning of the trabeculae. The fact that the trabeculae had become thinner due to resorption was appreciated if the appearance of sections from the operated joint were compared with those from approximately the same level of the control (left) femoral trochlea (figs. 59 and 60). In addition, the trabeculae were sometimes somewhat fragmented and the bone marrow showed a slightly increased cellularity. Occasionally the presence of numbers of plump osteoblasts lining the trabeculae and irregular areas of bone resorption indicated remodelling was going on (figs. 54 and 55), although no difference was observed in trabecular thickness.

Extensive trabecular remodelling resulted in an increased width of the femoral trochlea. Again this was assessed by comparison of left and right trochlear sections from the same dog.

Subchondral bone remodelling contributed to this increased overall width, especially in the longer suviving animals (e.g. 24 and 48 weeks, see/

see figs 38 and 39) but it was also recorded in sections from dogs where there was little or no subchondral remodelling. (The increase in width due to swelling of cartilage was discounted in this assessment).

B) CARTILAGE

The histopathological changes recorded in the hyaline cartilage of the trochlear groove and in the hyaline and fibrocartilage of the trochlear ridges were subdivided into 3 categories in Table 6. In summary, the changes observed were 1) swelling of cartilage, 2) loss of metachromasia or increased metachromasia with Toluidine Blue stain and 3) degenerative changes, e.g. flaking of the surface, zones of acellularity, and clumping of chondrocytes.

i) Swelling of Cartilage

The outer face of each trochlear ridge is covered normally by a fairly thin layer of fibrocartilage which merges with hyaline cartilage at the tip of the ridge and with the synovial membrane attachment distally. All dogs killed 6 weeks or more after ligament section (with the exception of dog 7/1) showed some degree of swelling of this fibrocartilage on medial and/or lateral trochlear ridge (see Appendix 2). In the early stages the increase in thickness was usually slight (fig. 36) and often was observed only in one or two of the several sections cut from the trochlear block. The swelling of the cartilage became more pronounced in the longer surviving dogs, the trochlear ridge and trochlear groove being covered by what appeared to be hyperplastic cartilage (figs. 37-39 and 64). The adjacent osteophyte was also often covered by a markedly thick layer of fibrocartilage (fig. 37). A possible association between the increased thickness of this cartilage and underlying/

underlying vascular budding was suggested by a relatively greater degree of swelling in those areas where budding had occurred. Swelling of the hyaline cartilage in the trochlear groove was also recorded in 15 dogs, notably occurring in dogs killed 16 weeks or more after cruciate section.

ii) Metachromasia

Increased metachromasia with Toluidine Blue stain was recognised when the cartilage (usually fibrocartilage on the outer non-articulating face of the trochlear ridge) showed an obvious increased intensity of staining which was purple rather than the normal blue colour. Often the staining was most intense around chondrocytes. A pronounced fibre-network within the cartilage was evident.

Increase in metachromasia was almost invariably associated with swelling of the cartilage on the medial or lateral trochlear ridge, although swelling of the cartilage appeared to develop slightly earlier than the alteration in staining, since increased thickness was occasionally recorded without increased metachromasia.

Reduction in metachromasia, loss of blue staining of the cartilage with Toluidine Blue, occurred in 33 dogs between 2 and 48 weeks after cruciate section. Obvious loss of metachromasia occurred in association with pannus formation in sections obtained from the proximal limit of the femoral trochlea (fig. 61). Discounting the appearance of these proximal sections, 16 dogs showed merely a loss of metachromasia of the superficial layer of hyaline cartilage in the trochlear groove (fig. 62); 13 of these animals were killed 2 to 6 weeks after surgery. A more pronounced loss of metachromasia of the trochlear groove cartilage (not associated with pannus) was recorded in 14 dogs killed 7 weeks or more after surgery. In 3 dogs, loss of metachromasia was recorded only

in/

in association with pannus.

iii) Degeneration

A variety of histological features thought to be representative of early degenerative changes of hyaline cartilage have been grouped together and recorded in a single category in Table 6 and Appendix 2.

The following features were noted:

- flaking of the surface layer of cartilage associated with small zones of acellularity in the superficial layer (fig. 63)

- small vertical splits in the superficial layer of the cartilage, with associated zones of acellularity and clumping of chondrocytes (fig. 64)

- round, acellular areas resembling cysts in the cartilage, usually adjacent to cartilage showing flaking or splitting

Less obvious changes, such as irregularity of chondrocyte distribution with possible clumping of cells, were recorded in table 6 as "possible degeneration". These may not have been significant pathological changes. Degenerative changes in the cartilage of the trochlear groove were recorded in 25 dogs, killed 6 weeks or more after ligament section (Appendix 2).

C) SYNOVIAL MEMBRANE

In Table 6 and Appendix 2 histological changes recorded in the synovial membrane of each dog have been divided into 6 categories. These are explained below.

1) Thickness

The increased thickness of the synovial membrane was due to increased cellularity of the intimal and subintimal layers and/or fibrosis of the subsynovial tissue. The increase in thickness ranged from very slight

1-2/

1-2 weeks after surgery, to very marked at 12 to 24 weeks.

2) Cellularity

The initial change appeared to be proliferation of surface cells, detected as early as 1 week after surgery (on comparison with the contralateral control joint). By 4 weeks (fig. 65) the synovial layer was many times thicker than normal, the increased cellularity being due to synovial lining cell proliferation and infiltration by chronic inflammatory cells such as fibroblasts and lymphocytes. In later stages of the disease process, small focal accumulations of cells in the sub-intimal layer were sometimes seen.

3) Fibrosis

By 3 - 4 weeks after cruciate section an increase in subsynovial fibrous tissue was evident histologically (fig. 65) in the majority of sections examined. Again the degree of fibrosis generally increased with time following surgery, a marked increase in fibrous thickening (fig. 66) was recorded in each dog killed between 12 and 24 weeks (Table 6). However, both 48-week dogs showed less fibrosis. (Some sections from dog 48/2 showed no apparent increase in fibrous tissue)

4) Vascularity

An increase in the number of capillaries and small blood vessels was evident even at 1 week after cruciate section. In the early stages (1-2 weeks) comparison with sections from the control stifle joint was necessary to establish the presence of increased vascularity. Blood vessels were shown clearly by perfusion with dye mixture (fig. 65). By 6 to 8 weeks after surgery the vascular proliferation in most dogs was moderate or severe, and this state of hypervascularity was generally maintained up to 24 weeks' survival (fig. 66). One of the longest surviving/

surviving dogs (48/1) did not show increased vascularity of the synovial membrane of the operated joint (vascular perfusion was not carried out in this dog).

5) Villous Proliferation

In 15 dogs, the thickened synovial membrane had been thrown up into small folds or finger-like projections (fig. 66). This villous proliferation was recorded mainly in the synovial membrane of the supra-trochlear region except for the longer surviving dogs (especially 24-week dogs) when it occurred at each level of the trochlea (Appendix 2).

6) Pannus

A thin layer of vascular granulation tissue in the trochlear groove was noted in sections cut from the proximal end of the femoral trochlea (close to the supratrochlear fossa) in a total of 35 dogs from 4 to 48 weeks after surgery (Appendix 2). The presence of this "pannus" was restricted to sections cut through the supratrochlear fossa, or those from the proximal region of the femoral trochlea. Where the granulation tissue encroached on to the proximal limit of the trochlea, hyaline cartilage in the trochlear groove beneath the pannus had been transformed into fibrocartilage (fig. 61). Occasionally a thin layer of vascular granulation tissue was noted overlying the osteophyte and encroaching on to the outer face of medial or lateral trochlear ridge (fig. 37). The cartilage beneath this tissue was hyperplastic rather than degenerate.

Left Femoral Trochlear Sections

Sections of the control (left) femoral trochlea from each dog were regarded as normal, providing a basis for comparison with the diseased tissue of the right stifle. However, histopathological changes were noted in left trochlear sections from dogs 10/3, 24/2, 48/1 and 48/2.

Dog 10/3

Spontaneous O.A. was present in the left stifle joint of this dog. Histologically the following features were recorded:

1. Grade 7 osteophytes (remodelling of medial and lateral trochlear ridges); 2. remodelling of femoral cortex (periosteal zone) and trabeculae; 3. swelling of articular cartilage with small clefts, surface flaking and acellular areas; 4. superficial loss of metachromasia; 5. thickening of synovial membrane with marked sub-intimal fibrosis, slight increase in cellularity and numerous small blood vessels.

Dog 24/2

Mild surface flaking of the hyaline cartilage of the trochlear groove was noted, and there was a loss of metachromasia with Toluidine Blue stain.

Dog 48/1

Appositional bone formation on the subchondral trabeculae had resulted in increased thickness of the trabeculae. A few superficial vertical splits were noted in the articular cartilage of the trochlear groove.

Dog 48/2

Flaking of the superficial layer of articular cartilage in the trochlear groove and loss of metachromasia were recorded.

GROUP B DOGS

Routine, decalcified transverse sections from both left and right femoral trochleas were examined for dogs 1 and 2, and from left trochlea only for dog 3.

Dog 1 (killed at 2 weeks)

There was a slight increase in cellularity and in the number of blood vessels (perfusion with dye mixture had been carried out) in the synovial membrane of the right femoral trochlea, indicating a mild synovitis. No abnormality was noted in bone or cartilage.

Dogs 2 (19 days) and 3 (8 weeks)

No abnormality of synovial membrane, bone or cartilage was detected in sections from these dogs.

Key to Table 6

Osteophyte - Grades 1 - 7 (see text, p 87-90)

Suffix 'c' denotes presence of chondrocytes (invariably present Grades 4 - 7)

Periosteal Zone

F	Fibroplasia
B B+ B++ B+++	Bone deposition (graded according to extent of involvement and amount of new bone deposited)
R	Remodelled cortex

Other histological changes

±	possible
(+)	slight
+	some
++	moderate to marked
+++	very marked
-	reduced
(-)	superficial loss

	1/1	1/2	1/3	1/4	2/1	2/2	2/3	2/4
A) BONE								
i) Osteophyte								
Medial		1			2c	1	1	2
Lateral					1	?1	1	1
ii) Subchondral Zone								
a. Vascular Buds								
Medial								
Lateral								
b. Bone Remodelling								
Medial								
Lateral								
T. groove								
iii) Periosteal Zone								
Medial		F			F	F		
Lateral		F						
iv) Trabeculae								
Thickness								
Trochlear width								
B) CARTILAGE								
i) Swollen								
Medial								
Lateral								
T. groove								
ii) Metachromesia								
Medial								
Lateral								
T. groove								
iii) Degeneration								
T. groove								
C) SYNOVIAL MEMBRANE								
Thick				(+)		(+)	(+)	
Cellular		±		+		(+)	+	(+)
Fibrous								
Vascular		+	+	(+)		+		+
Villous								
Pannus								

TABLE 6: HISTOPATHOLOGICAL CHANGES FOLLOWING CRUCIATE SECTION

(MID-TROCHLEAR SECTIONS FROM RT. FEMUR, GROUP A DOGS)

	3/1	3/2	3/3	3/4	4/1	4/2	4/3	4/4
A) BONE								
i) Osteophyte								
Medial	2c		2	1	2c	2c	3c	2
Lateral	1,2	?1	2c	1	2c	2c	3c	2
ii) Subchondral Zone								
a. Vascular Buds								
Medial								
Lateral					+			
b. Bone Remodelling								
Medial					+			
Lateral					+			
T. groove								
iii) Periosteal Zone								
Medial	B		F		F			F
Lateral	B				F			F
iv) Trabeculae								
Thickness								
Trochlear width								
B) CARTILAGE								
i) Swollen								
Medial								
Lateral								
T. groove								
ii) Metachromasia								
Medial	(-)	(-)						
Lateral								
T. groove						(-)	(-)	-
iii) Degeneration								
T. groove								
C) SYNOVIAL MEMBRANE								
Thick	(+)	+		+	+	+	+	+
Cellular	+	+	++	+	+	+	+	+
Fibrous			+	+			+	
Vascular	+		++	+	++	+		+
Villous				(+)				
Pannus								+

Table 6 continued

	5/1	5/2	5/3	5/4	6/1	6/2	6/3	6/4
A) BONE								
i) Osteophyte								
Medial	3	3	3	2	2	2c	2c	2
Lateral	3	4	3	2c	3	3c	2c	2
ii) Subchondral Zone								
a. Vascular Buds								
Medial			+		+			
Lateral			+		+	+		
b. Bone Remodelling								
Medial					+	+		
Lateral		+			+			
T. groove		+			+			
iii) Periosteal Zone								
Medial	F	B	F	F,B	B	F	F	B+
Lateral	F	B+	F		B+	B	F	B+
iv) Trabeculae								
Thickness								
Trochlear width								
B) CARTILAGE								
i) Swollen								
Medial					+		+	
Lateral					+		+	
T. groove								
ii) Metachromasia								
Medial					+			
Lateral			(+)		+			
T. groove			(-)	(-)				(-)
iii) Degeneration								
T. groove								
C) SYNOVIAL MEMBRANE								
Thick	+	++			++	++	+	
Cellular	+	+	+	+		+	+	+
Fibrous	+	++			++	++	+	
Vascular	+	+	+		+	++	+	+
Villous								
Pannus	+	+						

Table 6 continued

	7/1	7/2	7/3	7/4	8/1	8/2	8/3	8/4
A) BONE								
i) Osteophyte								
Medial	3	3	4	3	4	4	3	4
Lateral	3	4	3c	3	4	4	3	4
ii) Subchondral Zone								
a. Vascular Buds								
Medial		+			+			
Lateral	+		+			+	+	
b. Bone Remodelling								
Medial			+		+	+		
Lateral			+			+	+	
T. groove						+		
iii) Periosteal Zone								
Medial		B+	F	B+	B++	B+	3	B
Lateral		B+	B+	3	B++	B++	B	
iv) Trabeculae								
Thickness		-	-		--	-		-
Trochlear width						+		
B) CARTILAGE								
i) Swollen								
Medial				+			+	
Lateral				+	+	+		
T. groove								
ii) Metachromasia								
Medial				+			+	
Lateral	(+)			+			(+)	
T. groove		-						
iii) Degeneration								
T. groove		+				+	+	+
C) SYNOVIAL MEMBRANE								
Thick	+	++	++	+	++	++	+	+
Cellular	+	++	+	+	++	+	+	+
Fibrous		+	++		+	++	+	+
Vascular		+	++	+	++	++		+
Villous								
Pannus								+

Table 6 continued

	9/1	9/2	9/3	9/4	10/1	10/2	10/3	10/4
A) BONE								
i) Osteophyte								
Medial	2	2	5	4	4	4	5	4
Lateral	2	4	5	4	5	3c	5	4
ii) Subchondral Zone								
a. Vascular Buds								
Medial			(+)	+		+		+
Lateral				++		++		+
b. Bone Remodelling								
Medial			+	+				+
Lateral				+				+
T. groove			(+)					
iii) Periosteal Zone								
Medial	B	F,B	B	B+	F		B++	F
Lateral	B+	B+	B	B++			B+	B+
iv) Trabeculae								
Thickness		-	-	-				
Trochlear width		+	+					
B) CARTILAGE								
i) Swollen								
Medial	(+)	+	(+)	+		+	++	
Lateral	(+)	+	(+)	+	+	+	++	+
T. groove								
ii) Metachromasia								
Medial	(+)	(+)	+	+			+	
Lateral	(+)	(+)	+	+	+		+	
T. groove	-	(+)		(-)	-			
iii) Degeneration								
T. groove		+		+			+	
C) SYNOVIAL MEMBRANE								
Thick	+	++	++	++	+	+	++	+
Cellular	+	+	++	+	+	+	+	
Fibrous		++	++	++	++	+	++	+
Vascular	+	+	+	++	+	+	++	+
Villous		+						
Pannus								

Table 6 continued

	12/1	12/2	12/3	12/4	16/1	16/2	16/3	16/4
A) BONE								
i) Osteophyte								
Medial	4	4	5	4	5	4	4	5
Lateral	4	3,4	5	5	5	4	5	5
ii) Subchondral Zone								
a. Vascular Buds								
Medial		+			+	+		++
Lateral		+	+		++	+		+
b. Bone Remodelling								
Medial		+			+	+	+	+
Lateral			+		+		+	
T. groove							+	
iii) Periosteal Zone								
Medial	B+	B++	B++	B	B++	B+	F,B	
Lateral	B+	B++	B++	B+	B++	B+	B++	B+
iv) Trabeculae								
Thickness	-			-				
Trochlear width			+	+				
B) CARTILAGE								
i) Swollen								
Medial	+				+		+	+
Lateral	+	+	+	+	+	+	+	+
T. groove			+		+		+	
ii) Metachromasia								
Medial	(+)						+	
Lateral	(+)	(+)	(+)	+		(+)	+	+
T. groove	(-)					(-)	-	
iii) Degeneration								
T. groove		+	±	+	+		++	+
C) SYNOVIAL MEMBRANE								
Thick	++	++	++	++	++	++	++	++
Cellular	+	++	+	+	+	+	++	+
Fibrous	++	++	++	++	++	++	++	++
Vascular	++	++	++	++	+	+	++	+
Villous							+	
Pannus						+	+	

Table 6 continued

	24/1	24/2	48/1	48/2				
A) BONE								
i) Osteophyte								
Medial	5	6	7	7				
Lateral	5	6	7	7				
ii) Subchondral Zone								
a. Vascular Buds								
Medial		++	+	+				
Lateral		++		+				
b. Bone Remodelling								
Medial		++	++	+				
Lateral		++	++	+				
T. groove		+	+					
iii) Periosteal Zone								
Medial	B++	B+	R	R				
Lateral	B+++	B+++	R	?R				
iv) Trabeculae								
Thickness	-	-		-				
Trochlear width		++	++	+				
B) CARTILAGE								
i) Swollen								
Medial	++	+	(+)					
Lateral	++	+	+					
T. groove	+	+	+	+				
ii) Metachromasia								
Medial			+					
Lateral			+					
T. groove				-				
iii) Degeneration								
T. groove	+	+	++	+				
C) SYNOVIAL MEMBRANE								
Thick	++	+++	+	+				
Cellular	++	++	+	+				
Fibrous	++	++	+					
Vascular	++	++						
Villous	+	+	(+)	+				
Pannus								

Table 6 continued

Fig. 32: Photomicrograph of transverse section from normal (left) femoral trochlea, showing articular cartilage in trochlear groove (TG) extending over the trochlear ridge (TR) and becoming fibrocartilage.

a = marginal or transitional zone
b = subchondral zone
c = periosteal zone
d = trabeculae

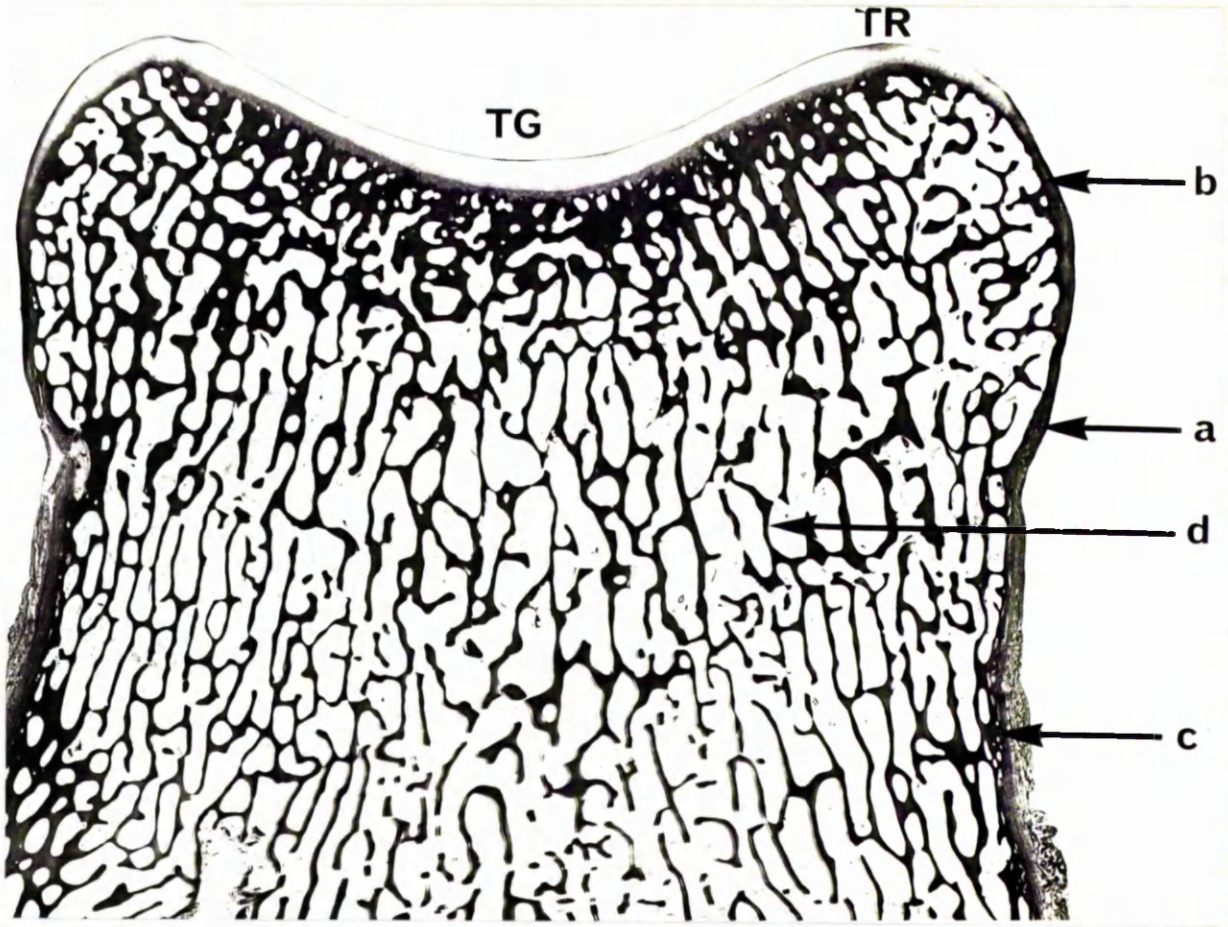
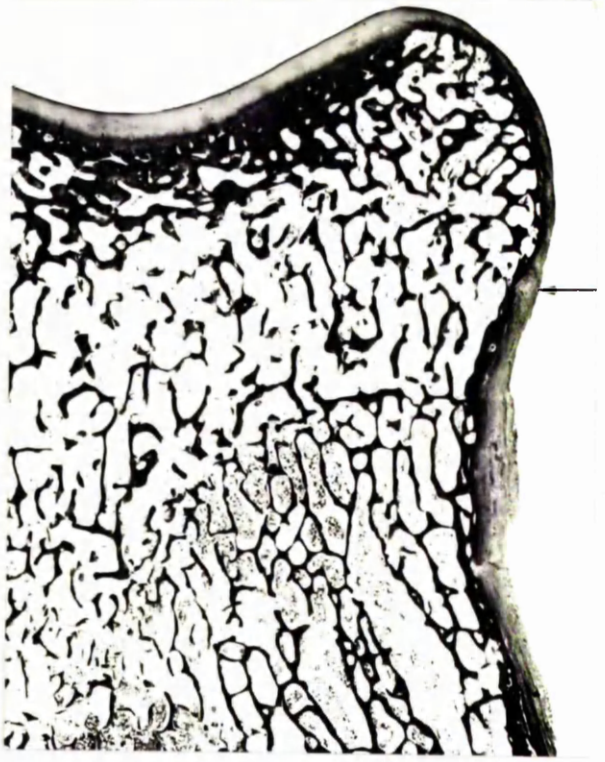

TR
TG
b
a
d
c

Fig. 33: Femoral trochlear ridge 2 weeks after cruciate section (dog 2/2). A grade 1 osteophyte (arrowed) is just discernible. No subchondral zone budding and no periosteal new bone is present.
(H. & E. X 8)

Fig. 34: Femoral trochlear ridge from dog 4/1, showing a grade 2 osteophyte at the marginal zone (large arrow) and very small foci of periosteal new bone (small arrows). Slight cellularity of the synovial membrane is evident (most of the synovial membrane has been cut away).
(H. & E. X 8)



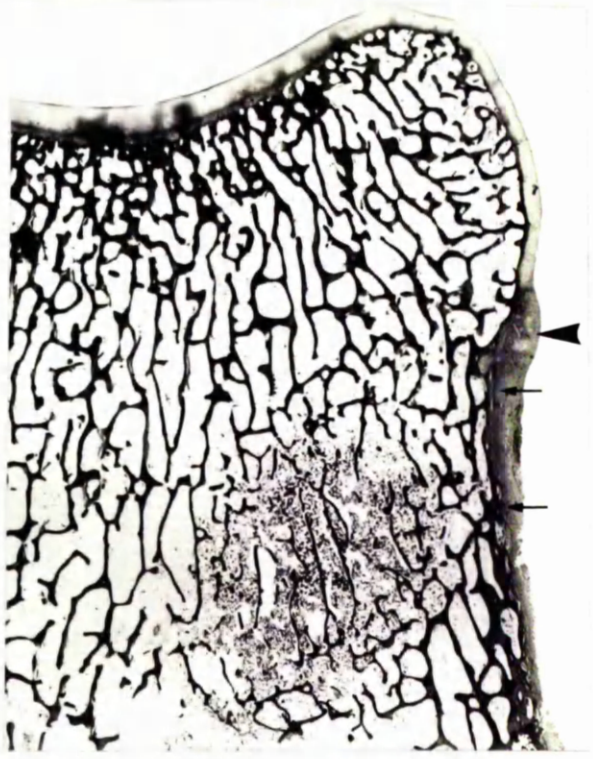
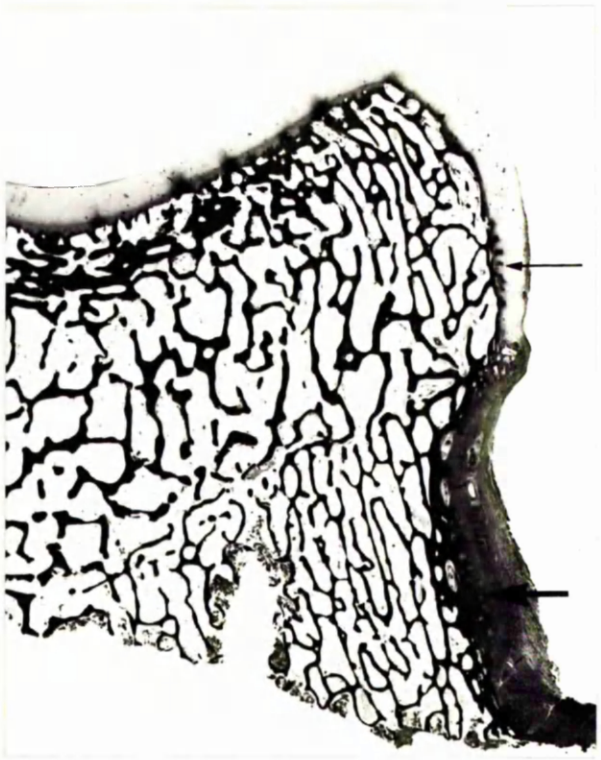


Fig. 35: Femoral trochlear ridge 6 weeks after cruciate section (dog 6/2) showing grade 3 osteophyte, an area of subchondral budding (small arrow) and increased thickness of cartilage on the outer face of the trochlear ridge. Periosteal new bone has been laid down on the femoral cortex (large arrow) beneath sub-synovial fibrous thickening.

(H. & E. X 8)

Fig. 36: Femoral trochlear ridge at 8 weeks after cruciate section (dog 8/1). Note the grade 4 osteophyte at the marginal zone, a few vascular buds in the subchondral zone (arrowed) and a thick layer of woven bone on the periosteal surface of the femoral cortex. The epiphyseal trabeculae are thin and uneven.

(H. & E. X 8)



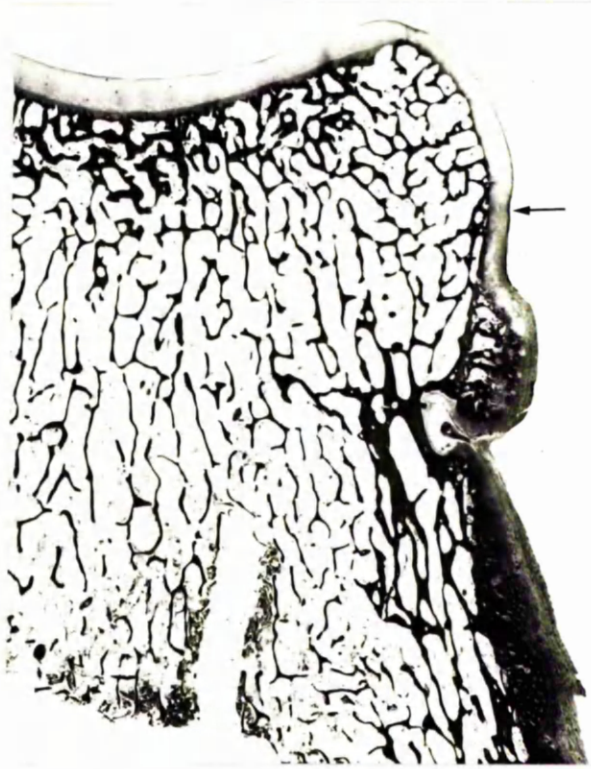
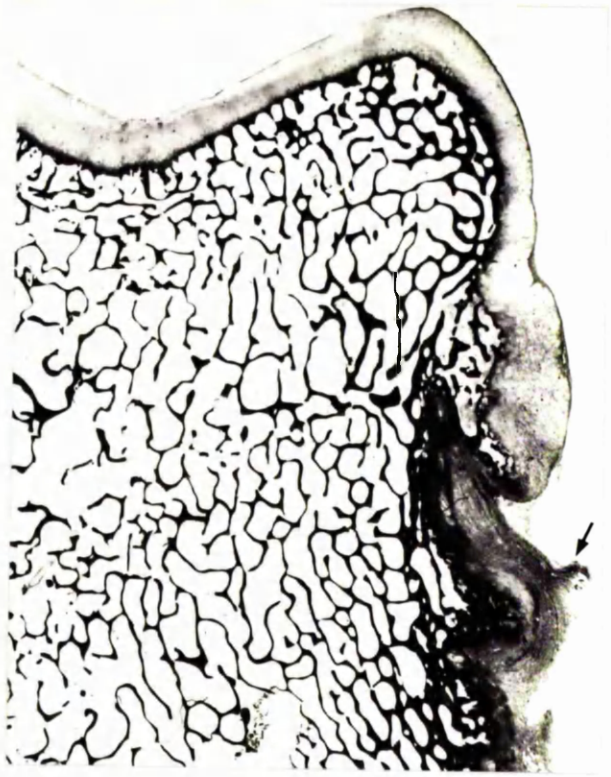


Fig. 37: Femoral trochlear ridge 16 weeks after cruciate section (dog 16/3). The grade 5 osteophyte is covered by thick cartilage, and below the osteophyte is a wide band of periosteal new bone. A small tag of thickened, vascular synovial membrane may be seen (arrowed). No subchondral vascular budding is present in this section. The cartilage of the trochlear groove shows degenerative changes.

(H. & E. X 8)

Fig. 38: Femoral trochlear ridge 24 weeks after cruciate section (dog. 24/2). A grade 6 osteophyte is confluent with an area of subchondral bone remodelling. The articular cartilage appears slightly thickened. Periosteal new bone deposition is extensive. The small amount of synovial membrane present on the section is thickened and thrown up into small folds. Areas (a), (b) and (c) have been shown at higher magnification in figs 49, 53 and 57 respectively.

(H. & E. X 8)



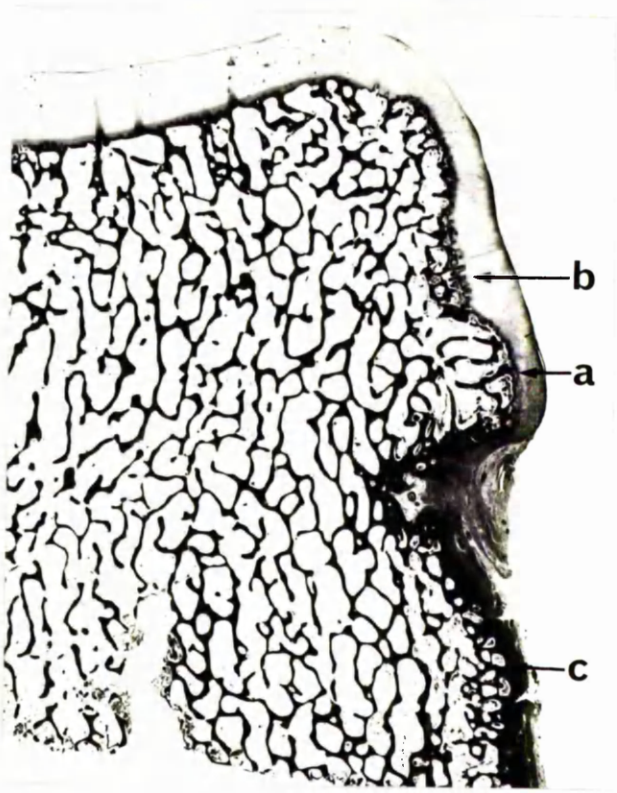

b
a
c

Fig. 39: Femoral trochlear ridge 48 weeks after cruciate section (dog 48/1). The grade 7 osteophyte and subchondral zone remodelling have together resulted in recontouring and widening of the trochlear ridge. Remodelling of the femoral cortex has also occurred (Area (a) corresponds to fig. 58). Vascular budding into the trochlear groove cartilage may be seen; the section was cut from the proximal region of the femoral trochlea, see fig. 30.

(H. & E. X 8)

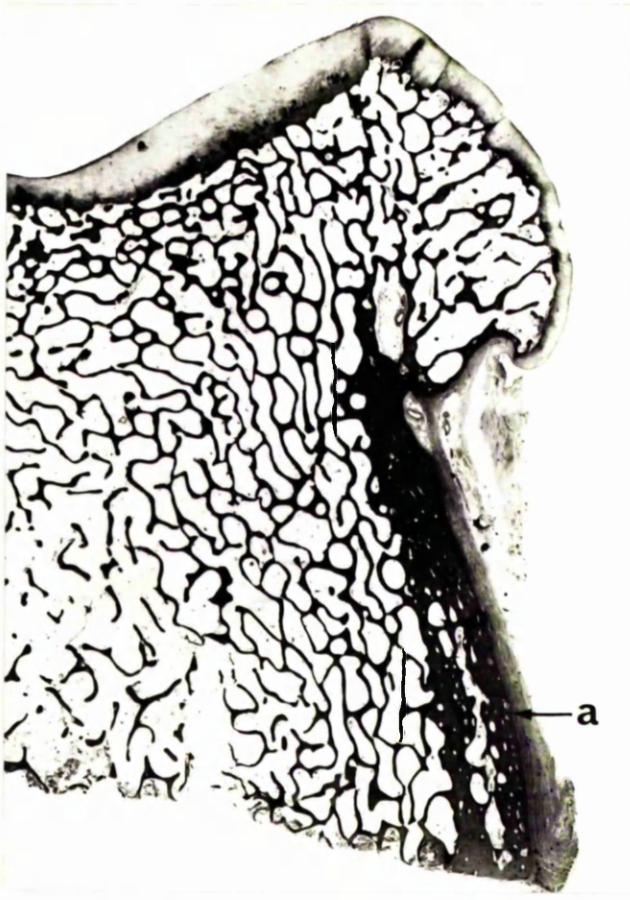
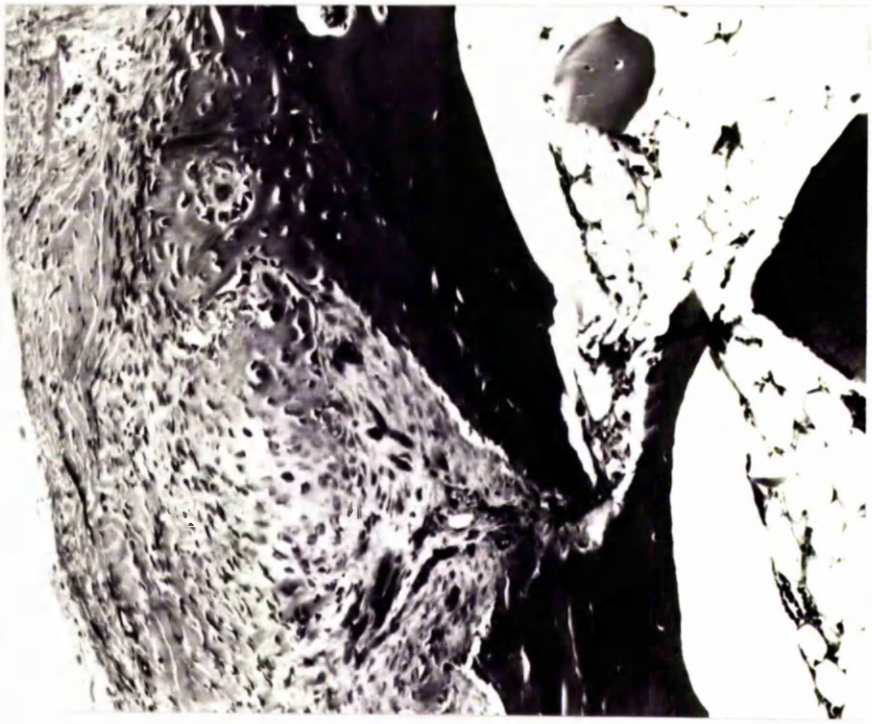

a

Fig. 40: Grade 1 osteophyte (from dog 2/2) at the marginal zone of medial femoral trochlear ridge. A focal accumulation of proliferating mesenchymal cells is situated outside the intact femoral cortex.

(H. & E. X 100)

Fig. 41: Same osteophyte as fig. 40, at higher magnification showing the central collection of metaplastic mesenchymal cells with pale staining nuclei and prominent nucleoli and an outer rim of spindley fibroblasts.

(H. & E. X 400)



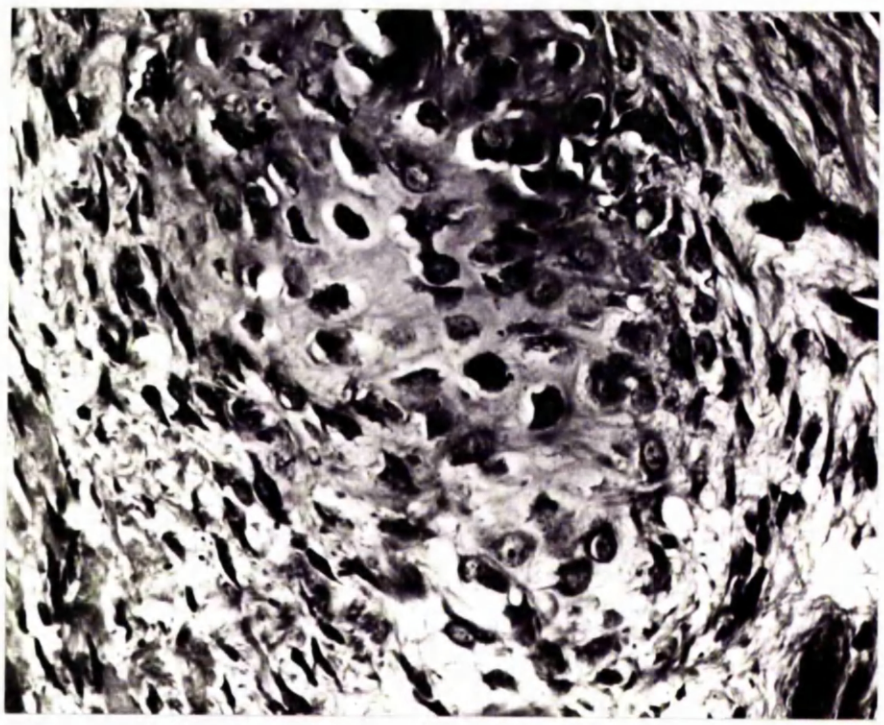
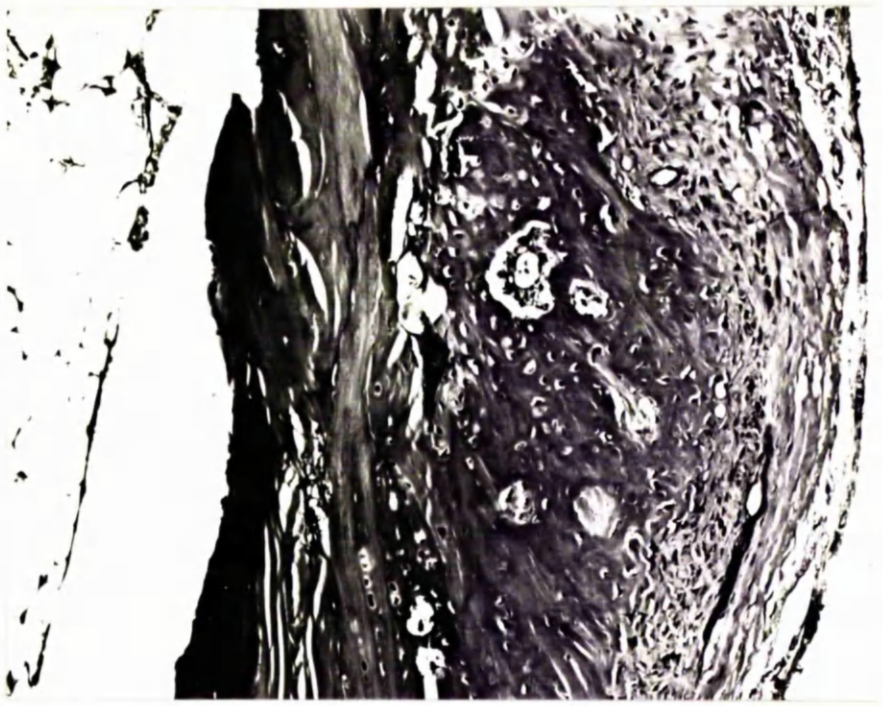


Fig. 42: Grade 2 osteophyte on lateral trochlear ridge (dog 6/4) consisting of a small focal deposition of highly cellular woven bone outside the intact femoral cortex. The osteophyte is bounded by cellular, vascular fibrous tissue and contains a number of spaces in which blood vessels may be identified.

(H. & E. X 100)

Fig. 43: Grade 3 osteophyte on lateral trochlear ridge (dog 7/3). The section shows focal clumps of chondrocytes at the outer edge of the osteophyte as well as a few scattered chondrocytes. Blood vessels filled with dye mixture are visible within the osteophyte.

(Tol. Blue X 100)



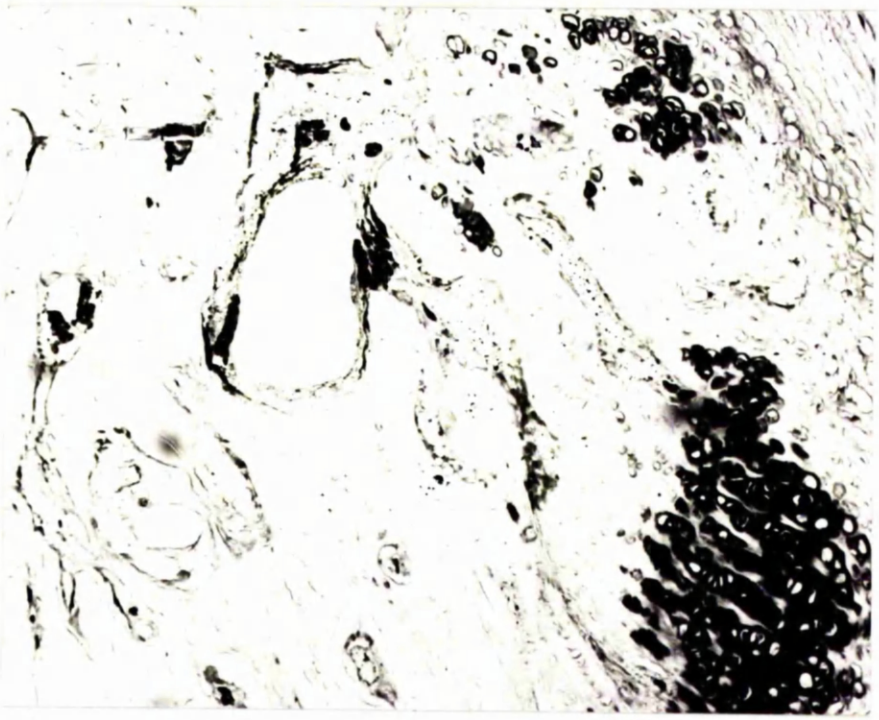
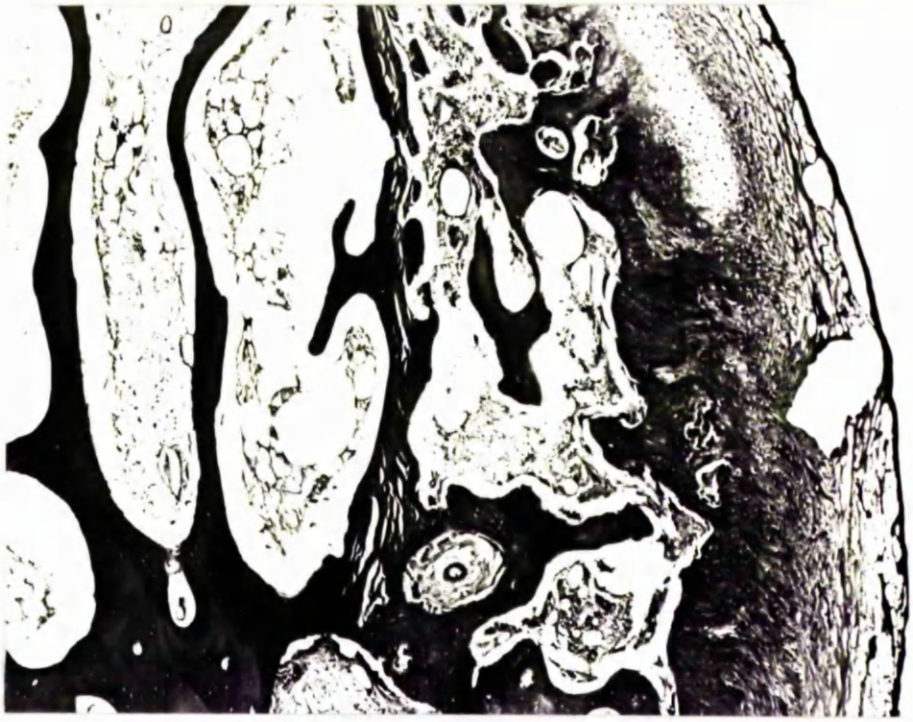


Fig. 44: Grade 4 osteophyte on lateral trochlear ridge (dog 8/1), composed mainly of cellular woven bone but with an early trabecular arrangement in the centre. Resorption of the pre-existing femoral cortex has occurred although it is still intact in this section. Focal cartilage is present on the outer margin of the osteophyte.

(H. & E. X 25)

Fig. 45: Higher magnification of another grade 4 osteophyte (8/1). Note the fragmented, haphazardly arranged trabeculae and spaces containing many cells and dark (dye-filled) blood vessels. At the outer margin a number of osteoclasts and blood vessels budding into overlying fibrocartilage may be seen.

(H. & E. X 100)



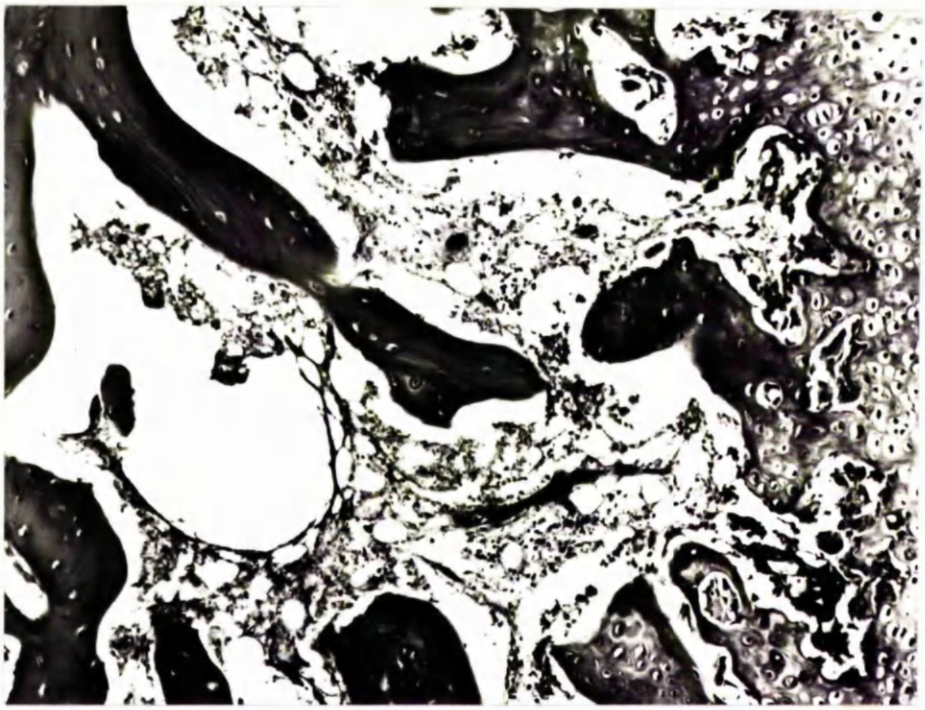


Fig. 46: Outer edge of grade 4 osteophyte showing plump, active osteoblasts laying down new bone on a trabecular surface (to left of picture) and also at the lateral margin, below fibrocartilage (to right of picture).

(H. & E. X 400)

Fig. 47: An area of bone undergoing active remodelling from a Grade 4 osteophyte. In the centre of the photomicrograph is a large multinucleate osteoclast, 2 smaller osteoclasts may be seen to the right of this.

(H. & E. X 400)

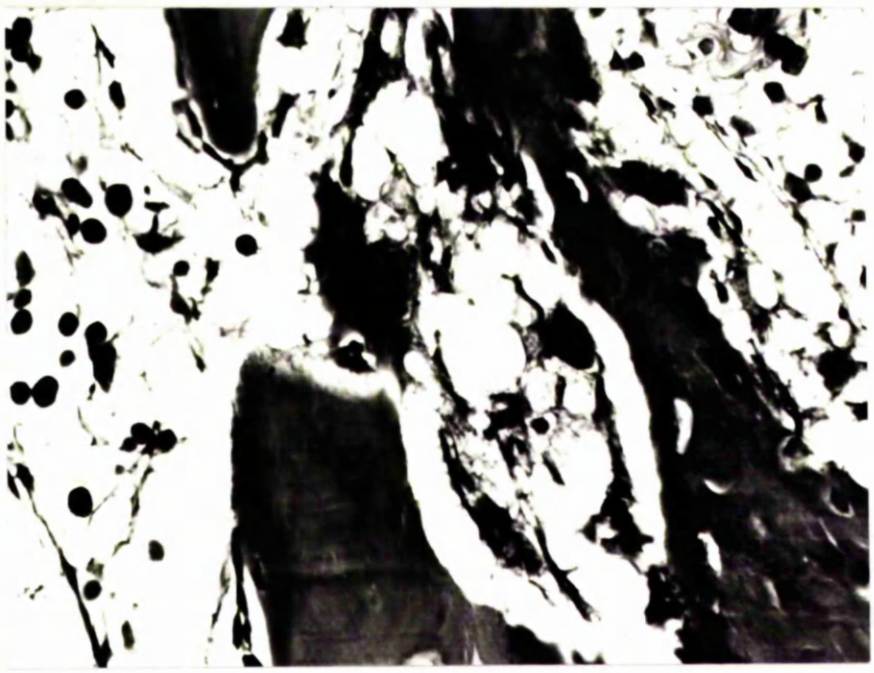
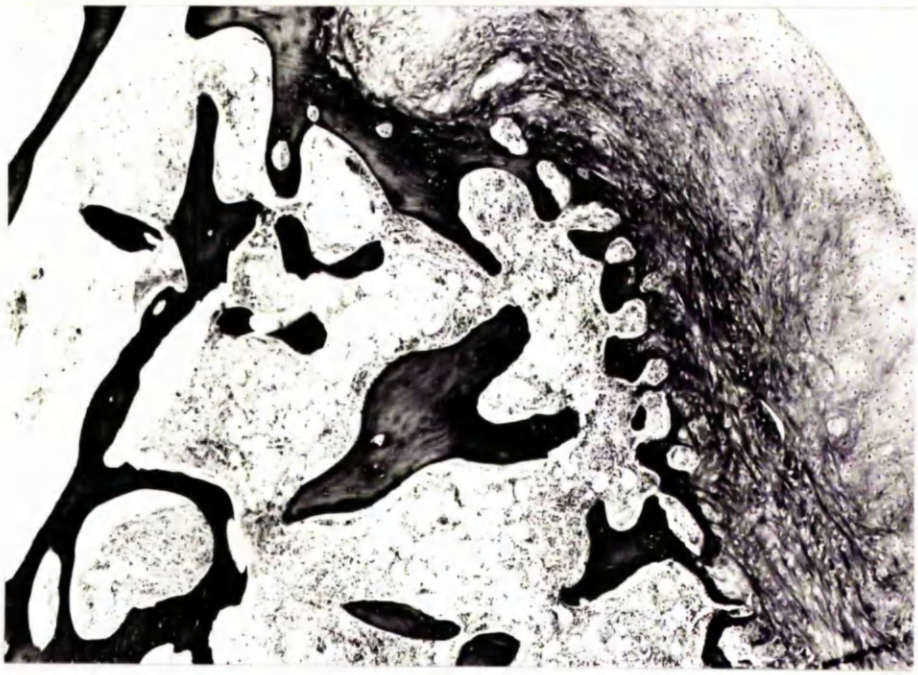


Fig. 48: Grade 5 osteophyte on lateral trochlear ridge (dog 16/3). The osteophyte consists of haphazard, fragmented trabeculae, composed partly of cancellous and partly of woven bone, highly cellular bone marrow spaces in the osteophyte having free communication with the bone marrow of the femur, and a thick layer of fibrocartilage on the outer margin. Vascular budding is present at the bone-cartilage interface.

(H. & E. X 25)

Fig. 49: Grade 6 osteophyte on lateral trochlear ridge (dog 24/2). The trabeculae are well formed and composed largely of cancellous bone although woven bone is still present. Active remodelling is shown by the cellular bone marrow and osteoblasts lining the trabecular surface (top right and lower left). Endochondral ossification is occurring at the outer border. Blood vessels appear dark due to perfusion with dye mixture (see fig. 38, low power photomicrograph).

(H. & E. X 100)



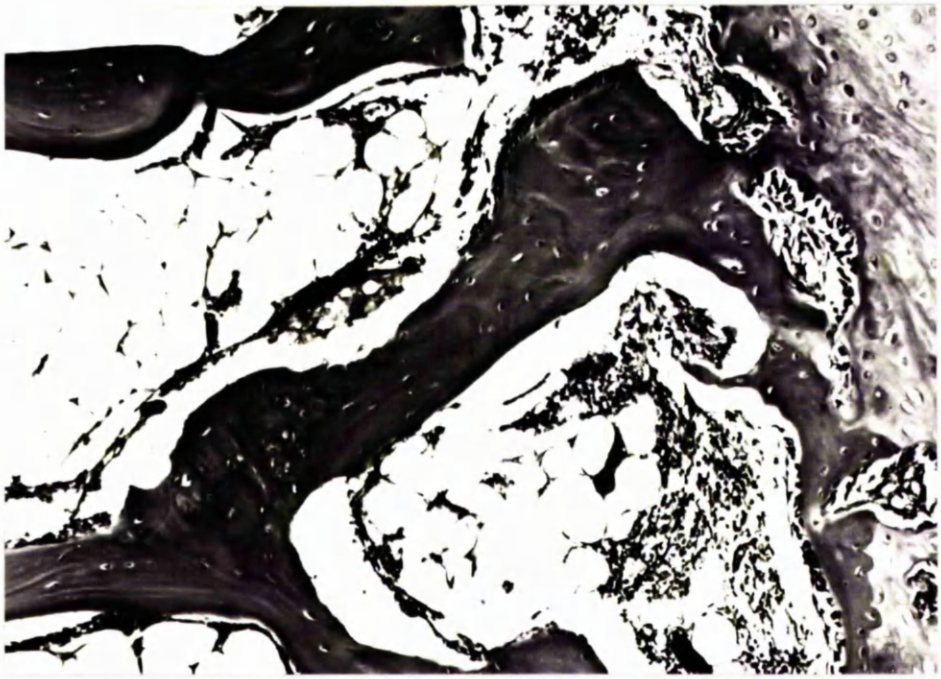
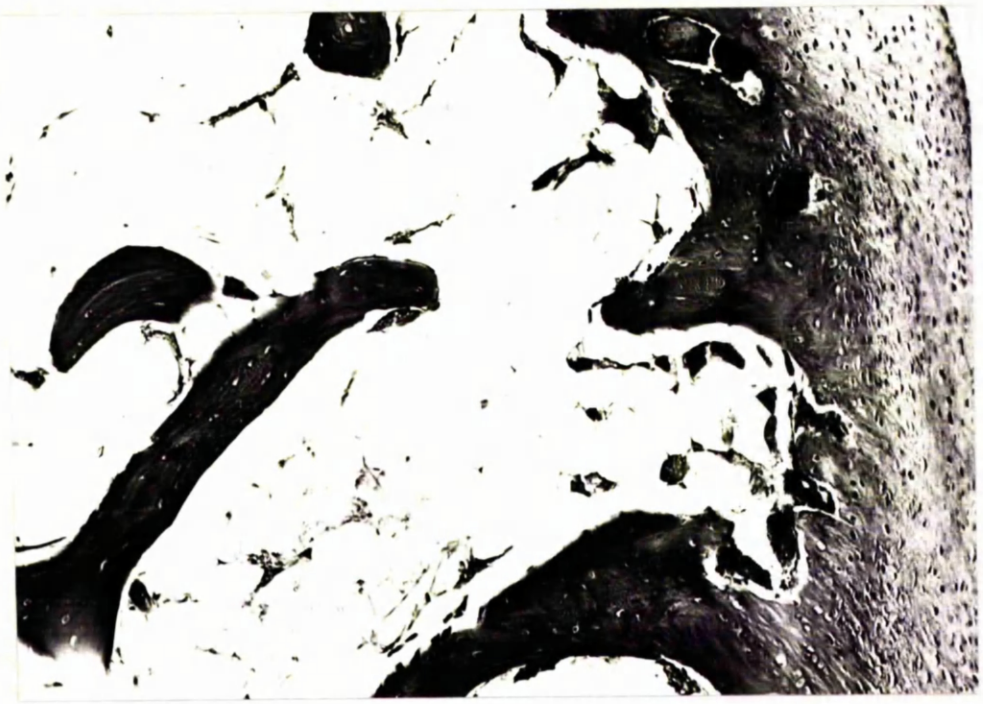


Fig. 50: Grade 7 osteophyte from dog 48/2 (lateral trochlear ridge). The trabeculae are fairly smooth and regularly arranged and there is little evidence of bone remodelling except at the outer border beneath the thin layer of fibrocartilage, where a number of dark blood vessels may be seen.

(H. & E. X 100)

Fig. 51: Lateral subchondral zone (outer face of trochlear ridge) showing a single vascular tuft penetrating the subchondral bone plate into the overlying fibrocartilage.

(H. & E. X 100)



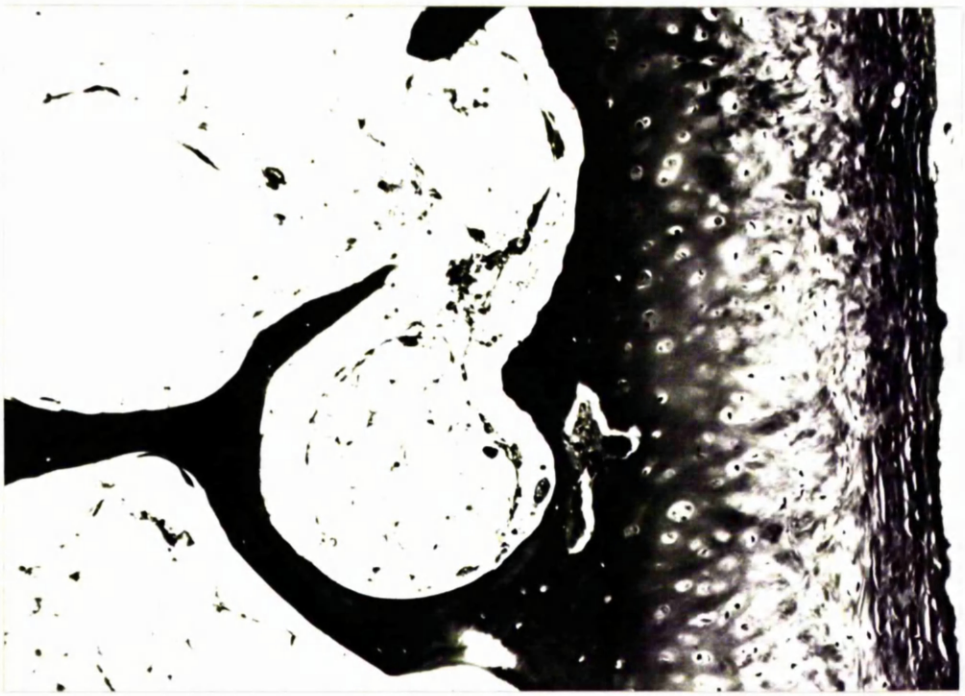
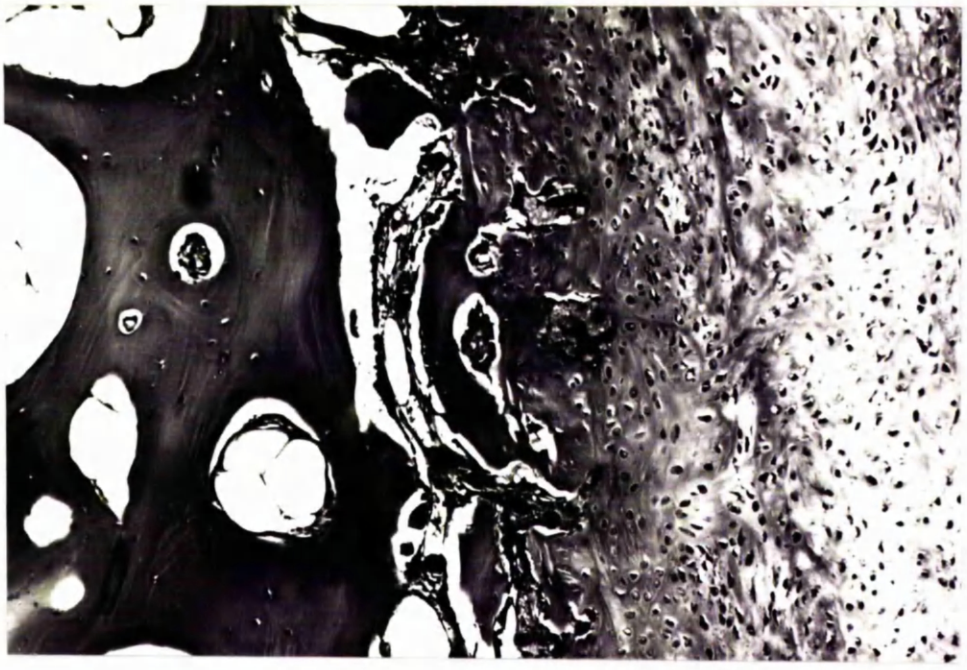


Fig. 52: Subchondral zone of lateral trochlear ridge of dog 7/3 showing numerous blood vessels (perfused with dye) budding into the fibrocartilage, with associated woven bone deposition and remodelling of subjacent trabeculae. (H. & E. X 100)

Fig. 53: Subchondral remodelling of the lateral trochlear ridge of dog 24/2. Blood vessels (perfused with dye) may be seen budding into the fibrocartilage and in the cellular marrow spaces. There is deposition of bone in association with the vascular tufts, Osteoblasts lining the trabeculae and irregular resorption surfaces are evidence of continued remodelling of the trabeculae. (see fig. 38, lower magnification) (H. & E. X 100)



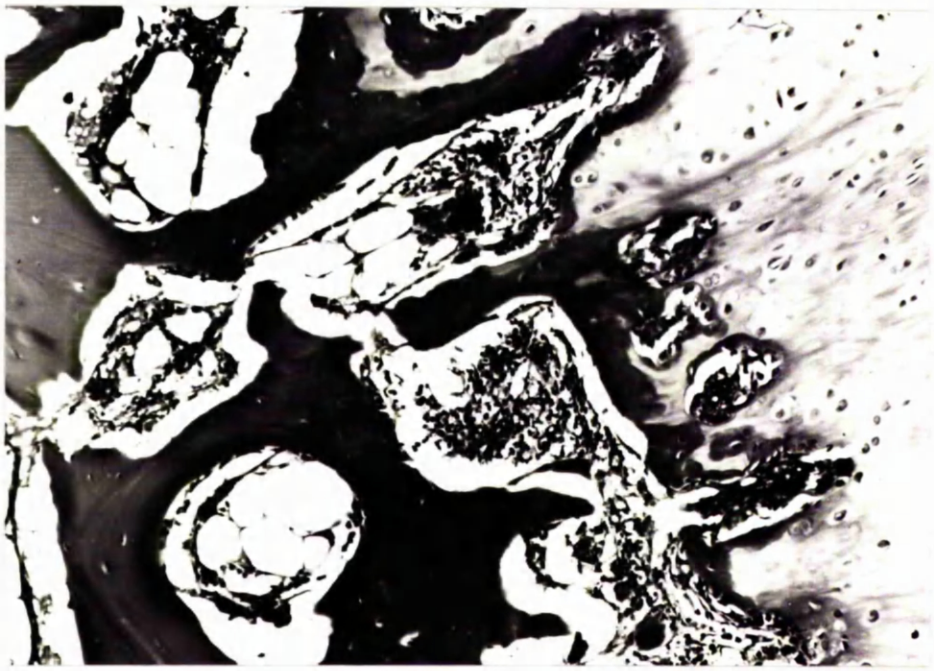
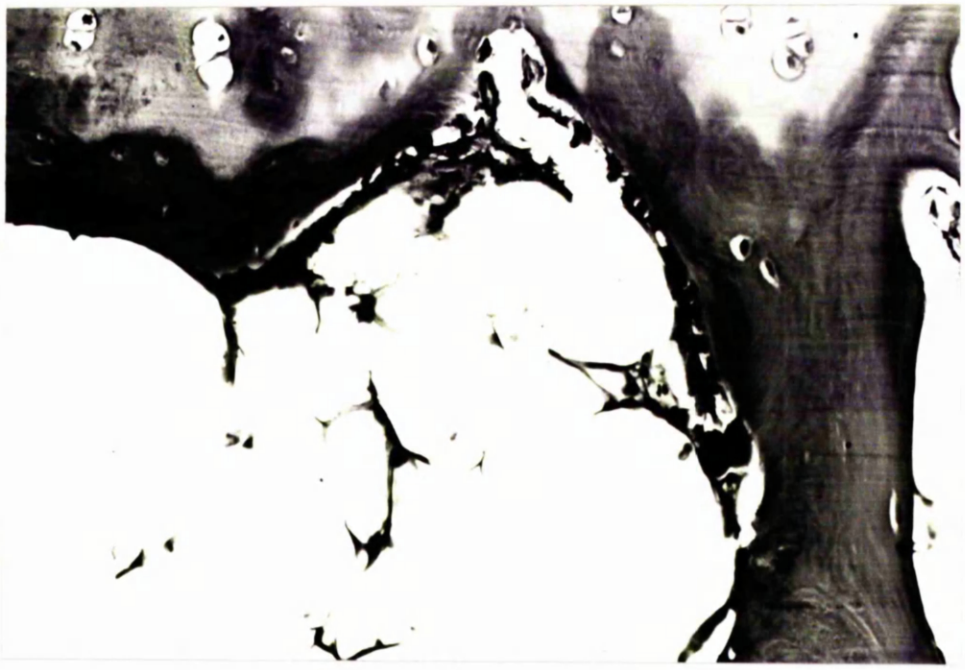


Fig. 54: An area of subchondral and trabecular remodelling beneath the articular cartilage of the trochlear groove (dog 9/3). Plump osteoblasts and small blood vessels lie on the surface of the bone.
(H. & E. X 250)

Fig. 55: An area of active trabecular resorption shown by the presence of 5 multinucleate osteoclasts (processing of the tissue has caused detachment of the cells from the bone surface)
(H. & E. X 400)



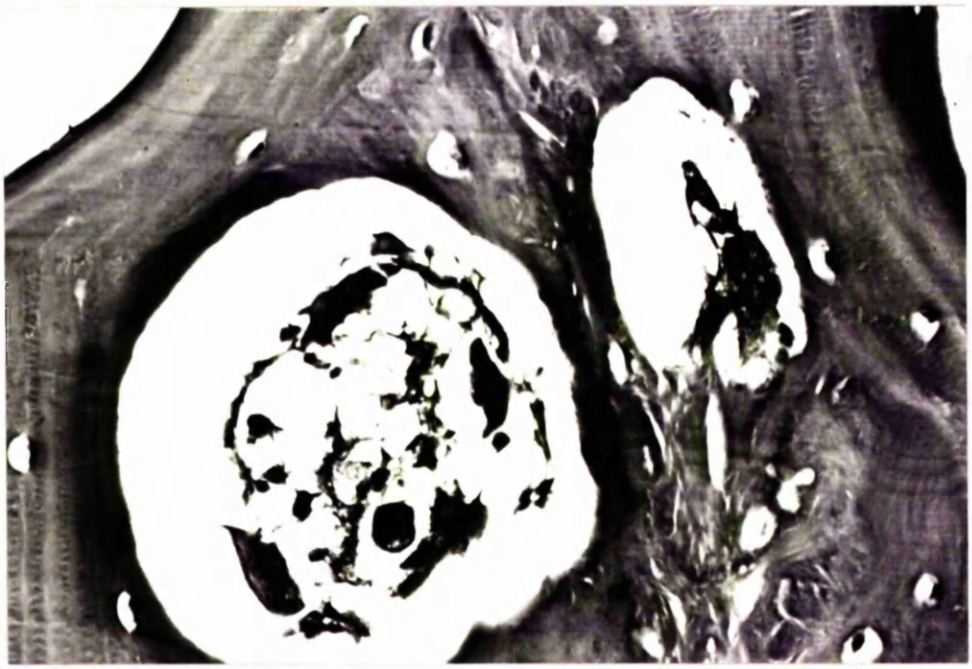
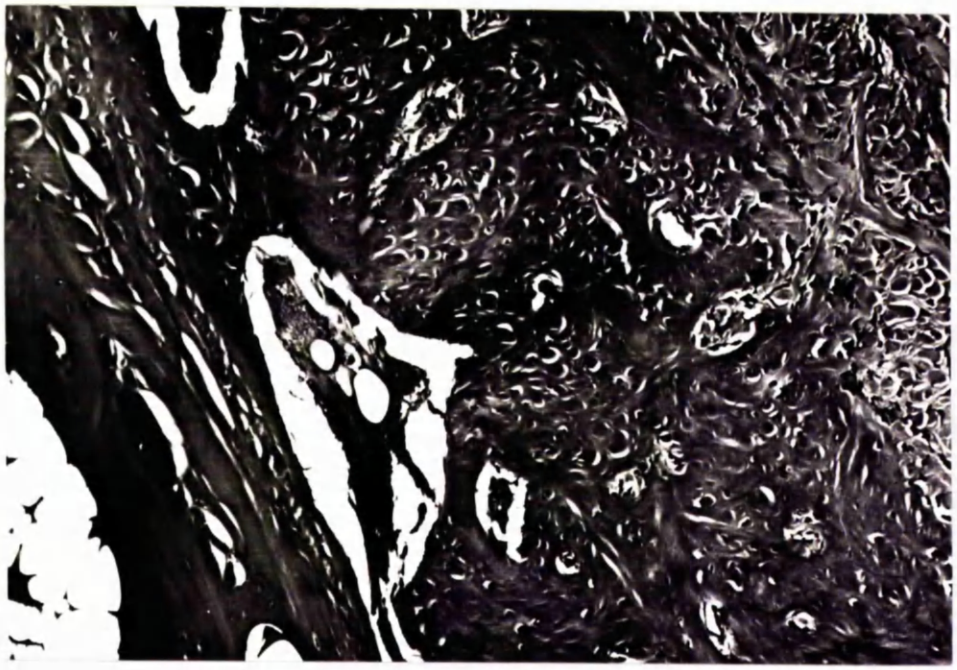


Fig. 56: Lateral periosteal zone of femoral trochlea 8 weeks after cruciate section (dog 8/2). A thick layer of cellular, vascular woven bone has been deposited on the periosteal surface of the femoral cortex (on the left of the photograph).

(H. & E. X 100)

Fig. 57: Part of the periosteal zone remodelling from the lateral femoral cortex 24 weeks after cruciate section (dog 24/2). Fragmented and irregular trabeculae form the inner layer of the new bone while at the outer edge (to the right of the photograph) osteon formation is beginning. (see fig. 38).

(H. & E. X 100)



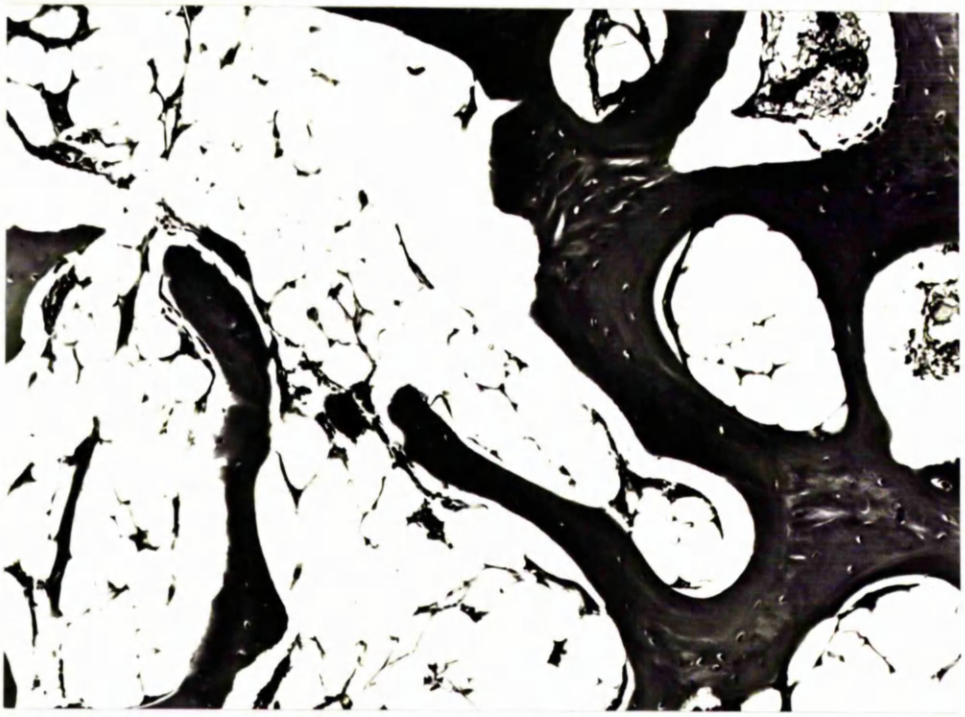


Fig. 58: The femoral cortex 48 weeks after ligament section (dog 48/1), greatly thickened and consisting largely of compact bone. Developing osteons may be seen towards the outer edge of the cortex (to the right of the photograph). (see fig. 39)

(H. & E. X 100)

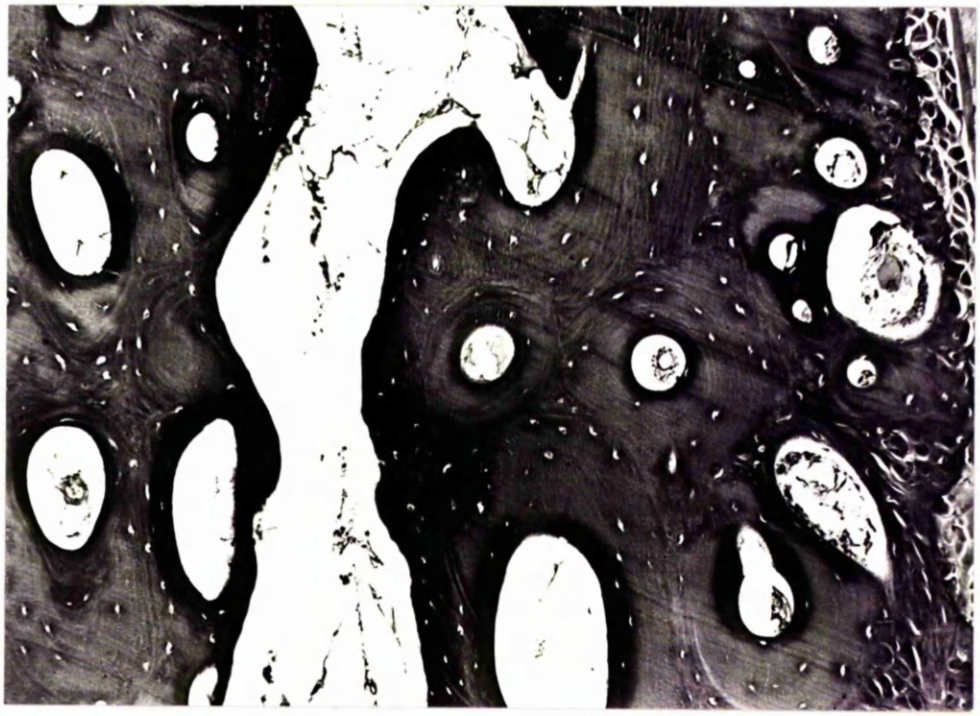
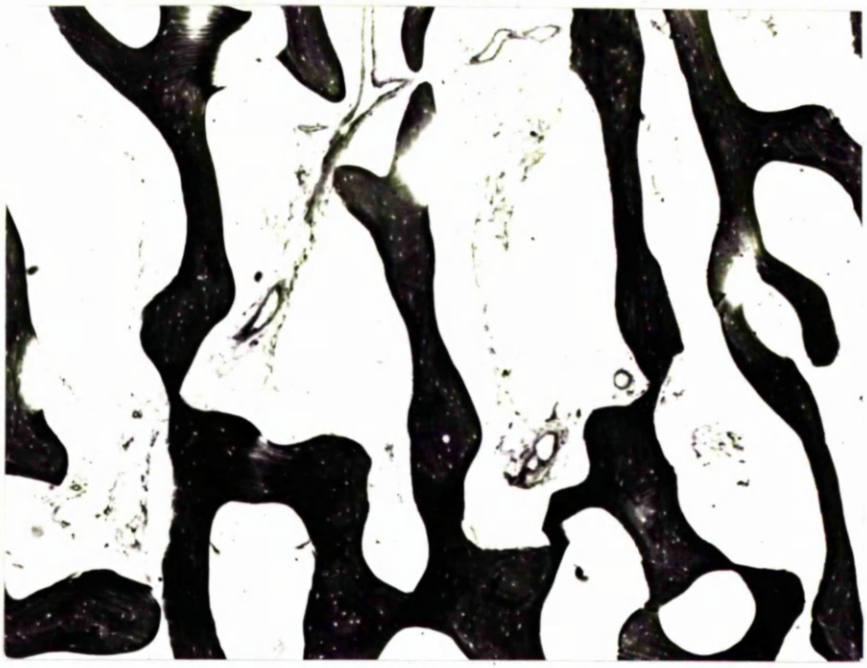


Fig. 59: Epiphyseal trabeculae from the trochlear region of the control (left) stifle joint 8 weeks after cruciate section of the right stifle (dog 8/2). Compare with fig. 60.

(H. & E. X 25)

Fig. 60: Epiphyseal trabeculae from the right femoral trochlear region 8 weeks after cruciate section (dog 8/2). On comparison with the control joint, it is evident that the trabeculae are much thinner and have a slightly fragmented appearance. The bone marrow shows increased cellularity.

(H. & E. X 25)



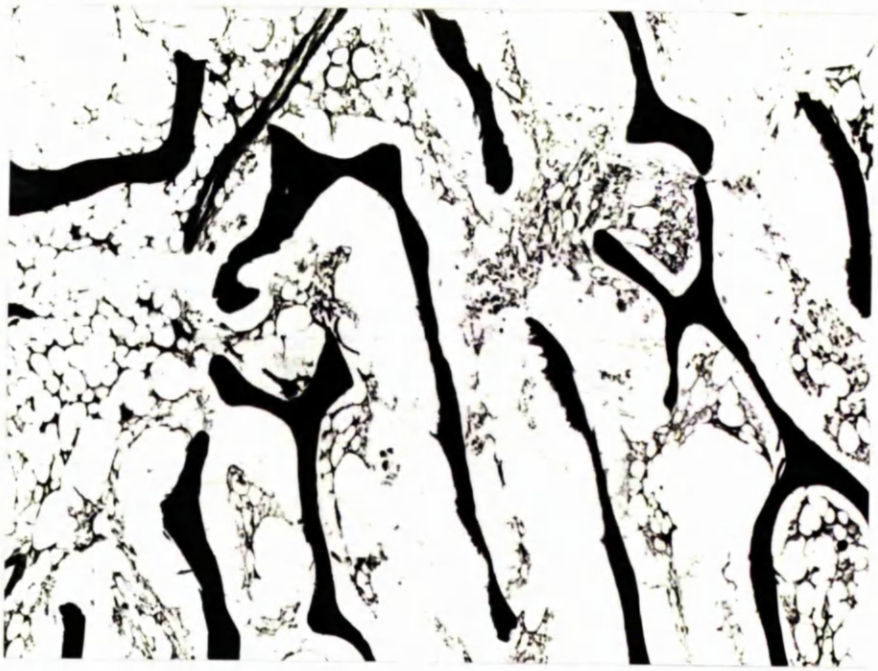
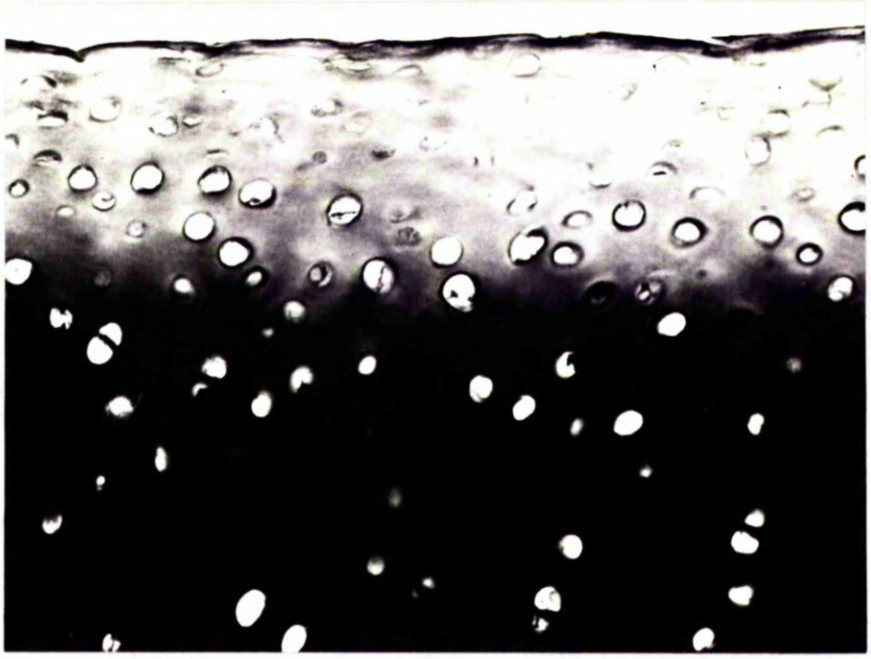


Fig. 61: Articular cartilage from trochlear groove of dog killed 4 weeks after cruciate section (dog 4/1), showing loss of Toluidine Blue staining in the superficial layer of cartilage.

(Tol. Blue X 250)

Fig. 62: Section through the proximal trochlear groove of dog 16/2, stained with Toluidine blue. A layer of pannus overlies fibrous tissue and fibrocartilage which has replaced the articular cartilage. A thin layer of articular cartilage remains and is darkly stained.

(Tol. Blue X 250)



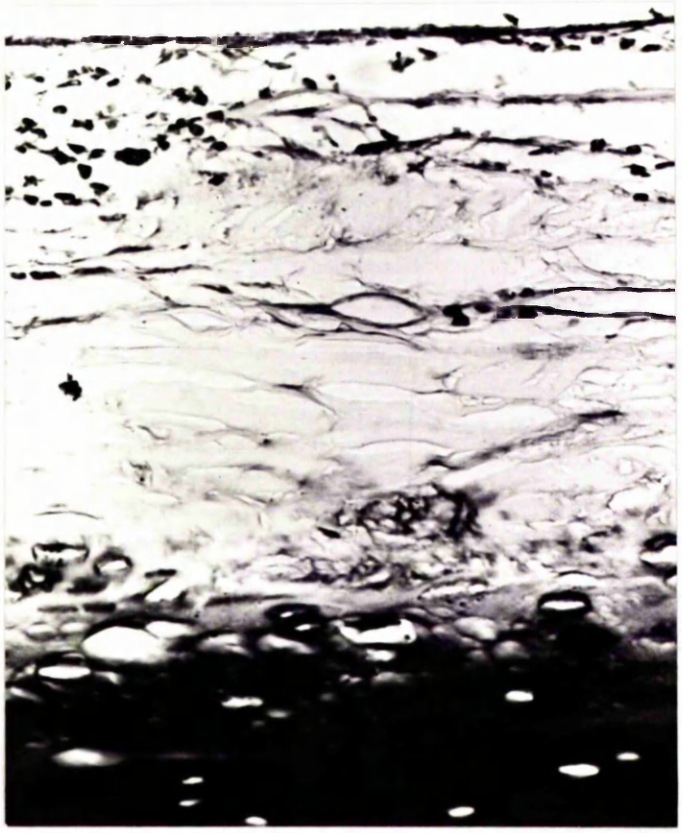
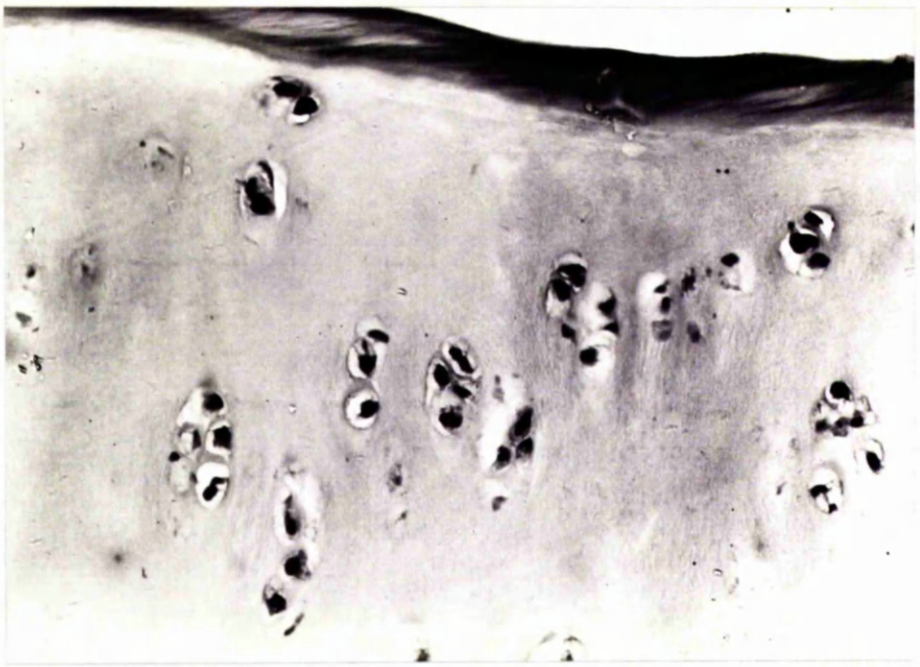


Fig. 63: Superficial zone of articular cartilage from the trochlear groove, showing zones of acellularity and clumping of chondrocytes beneath an irregular eosinophilic surface layer (dark grey). (Mid-trochlear section from right stifle of dog 48/1).

(H. & E. X 400)

Fig. 64: Articular cartilage from dog 24/2 showing superficial flaking, small vertical splits and irregular distribution of chondrocytes.

(H. & E. X 250)



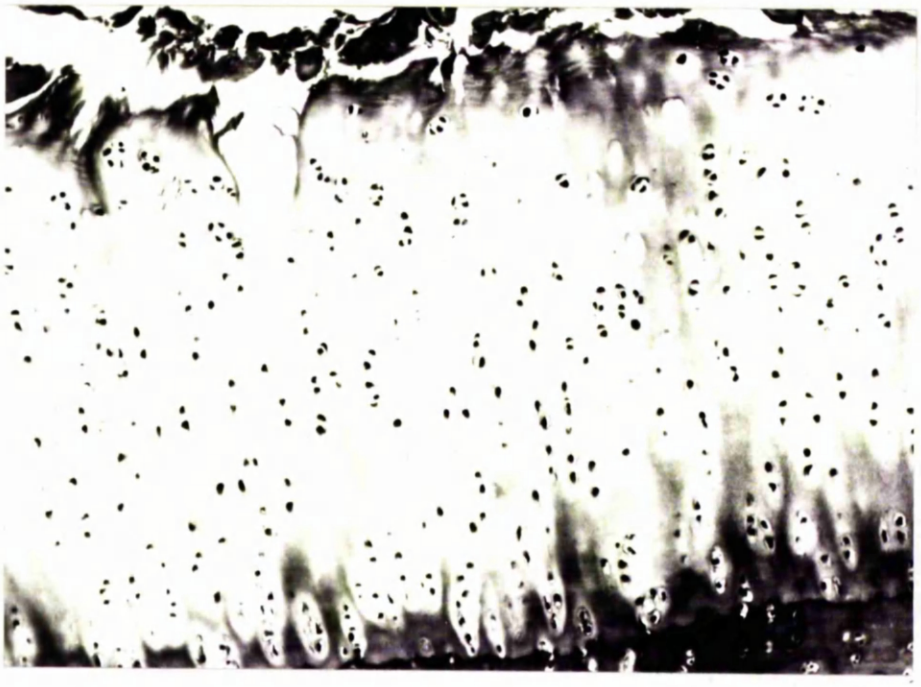
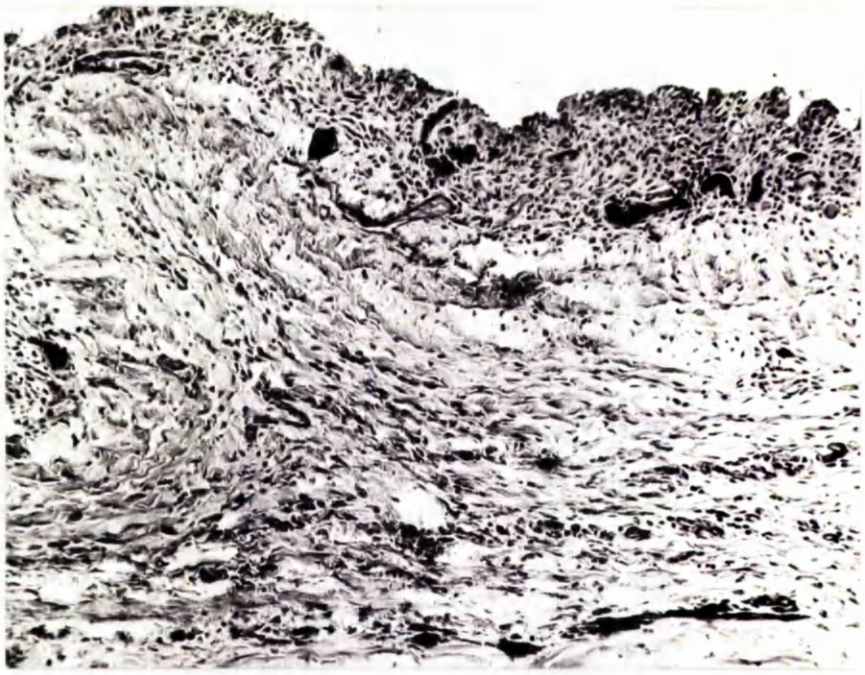


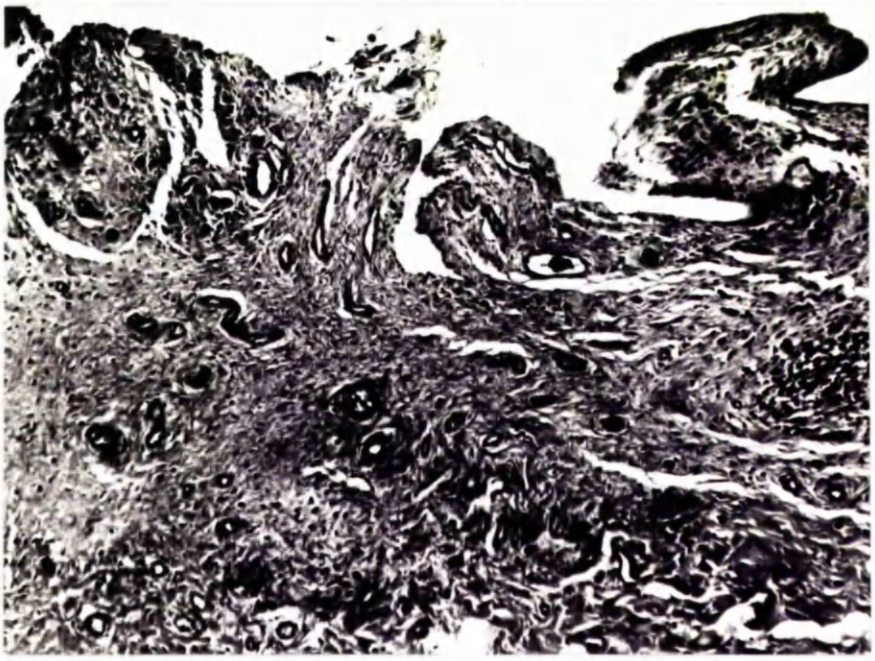
Fig. 65: Synovial membrane from dog 4/1, showing cellularity of the superficial synovial intima and subsynovial fibrosis. Small blood vessels filled with dye mixture may be seen both in the superficial layer and deep to the synovial lining.

(H. & E. X 100)

Fig. 66: Synovial membrane from dog 24/1, there is marked subintimal fibrosis, with many blood vessels throughout the thickened tissue. The superficial layer of the synovial membrane has been thrown up into small folds.

(H. & E. X 100)





DISCUSSION

The histopathological features outlined in the preceding section clearly show the progressive development of a condition which in many respects resembled naturally occurring O.A. The basis for comparison of experimental and spontaneous O.A. at the histological level is however somewhat limited owing to a scarcity of detailed reports of the histopathology of the spontaneous condition in the dog.

The most prominent and consistent change observed was that of osteophyte formation at the marginal zone. This appeared first as a collection of fibroblast-like cells and matrix and rapidly developed into a small deposit of woven bone, with or without foci of chondrocytes at the outer margin. This is in general agreement with Weichselbaum (1877, cited by Bennett et al 1942) and Bennett, Waine and Bauer (1942), who described the origin of osteophytes in the marginal zone as proliferating fibrous or fibrocartilagenous tissue.

Continued deposition of woven bone and concurrent resorption and remodelling of the osteophyte occurred. In later stages (48 weeks) the osteophyte consisted of mature cancellous bone in an organised trabecular pattern with an outer border of fibrocartilage or fibrous tissue overlying bone, the marrow spaces of the osteophyte were continuous with those of the epiphysis. This description is very similar to that of a mature osteophyte given by Collins (1949) in his account of osteoarthritis of the human knee joint.

Vascular budding into the fibrocartilage adjacent to the developing osteophyte was noted in many dogs. As pointed out by Woods, Greenwald and Haynes (1970) comparison with sections from control normal joints was sometimes necessary to appreciate whether the degree of vascular invasion observed/

observed in the diseased joint was abnormal. As the disease progressed however the vascular budding and associated bone remodelling became more extensive, and ultimately this area of subchondral remodelling became confluent with the osteophyte to form a remodelled joint surface in the longest surviving dogs. In addition, woven bone was deposited on the periosteal surface of the femoral cortex and underwent remodelling to produce mature cancellous or compact bone. These findings are in agreement with the observations of Bennett and colleagues (1942) who indicated that endochondral ossification of marginal articular cartilage, periosteal bone formation and metaplasia of proliferating synovial membrane may all contribute to the formation of new bone at the joint periphery..

It is generally accepted that osteophytes are formed by a process of endochondral ossification of marginal articular cartilage. Endochondral ossification certainly contributed to the development of the osteophyte in the experimental model described in this thesis, occurring in the so-called "subchondral zone" adjacent to the margins of the joint and also in the developing marginal osteophyte when this was covered by a layer of fibro-cartilage. However, it was not observed in every case, nor was it recorded in the early stages of osteophyte formation when the osteophyte consisted of deposits of woven bone, sometimes with foci of cartilage but often with few or no chondrocytes present. Trueta (1968) stated that degeneration of the cartilage preceded endochondral ossification, but in many cases repoited in this series, there was an apparent hyperplasia (increase in thickness and increase in metachromasia) of the cartilage on the outer non-articulating face of the trochlear ridges, which was observed usually in association with vascular budding in this region. Endochondral/

Endochondral ossification of weight-bearing areas of articular cartilage was not observed, and these were the only areas of cartilage in which degenerative changes were recorded.

Bennett, Waine and Bauer (1942) and Collins (1949) described a cellular reaction in the epiphyseal bone marrow accompanied by both resorption of trabecular bone and deposition of new lamellar bone. Similarly, Jeffery (1973) in a study of femoral heads with advanced O.A., reported that there was appositional bone formation on trabeculae, while Batra and Charnley (1969) recorded the presence of osteoid in osteoarthritic femoral heads. The present study has also shown trabecular remodelling, including resorption and new bone deposition, to be a feature of this experimental O.A. model. In earlier studies on experimental O.A. by Marshall (1969) and Marshall and Olsson (1971), remodelling of trabeculae was not recorded. Paatsama and Sittnikow (1972), however, reported thickening of bone trabeculae in their series.

It is possible that some of the trabecular thinning observed in the right stifle joint was "disuse osteoporosis" because of lameness and relative disuse of the limb after cruciate section. However, persistent severe lameness was not a feature of the condition and by 7 - 12 weeks the majority of dogs were weight-bearing on the operated limb at each stride, although there was still an obvious gait abnormality. There was no correlation between the duration and degree of lameness shown by an individual dog and the presence or absence of trabecular thickening on histological examination.

Subchondral bone cysts have not been observed following cruciate section, either in the present experimental study or in the series carried out by Marshall (1969) and Marshall and Olsson (1971). Paatsama and/

and Sittnikow (1972) described a "cyst-like formation" in association with an osteophyte in one of their experimental dogs. As already indicated (p 20) subchondral bone cysts in association with naturally occurring O.A. appear to be a rare occurrence in the dog.

Histological changes in the articular cartilage following cruciate section have not been described in any detail in this thesis, since this part of the study has been carried out by a colleague (M.J. Pond). Nevertheless, in examination of the femoral trochlear sections, certain cartilage changes were observed and have been included in the results. Early degenerative changes were recorded in the hyaline cartilage of the trochlear groove in some dogs, notably in the longer surviving animals (e.g. dogs surviving more than 12 weeks after surgery). These changes included loss of metachromasia, zones of acellularity and chondrocytes clumping and small flakes or splits in the surface of the cartilage. Much more marked degenerative changes were noted in sections of the femoral condylar (weight-bearing) cartilage from these dogs. These findings are in agreement with those reported by Pond (1971) and Tirgari and Vaughan (1975) in cases of natural O.A. of the stifle joint.

Proliferation of cartilage was observed in association with areas of bone remodelling, i.e. on the outer, non-articulating surfaces of the trochlear ridge where there is normally only a thin layer of fibro-cartilage, and on the outer edge of more mature osteophytes. Sokoloff (1974) pointed out that production of new cartilage is inevitable with osteophyte development; he also noted that discrepancies may occur in analysis of chemical composition of osteoarthritic cartilage if the existence of such newly formed cartilage is not taken into account.

The/

The possible association between increased cartilage thickness and vascular budding is interesting, in view of the fact that Woods and colleagues (1970) noted similar findings in human femoral head articular cartilage and postulated that cartilage nutrition is influenced by the number of points of contact existing between cartilage and soft tissue. In some dogs soft tissue "pannus" was noted to overlie hyperplastic cartilage on the outer face of the trochlear ridge: perhaps the hyperplasia of the cartilage in these animals could be attributed to increased nutrition from the vascular granulation tissue.

Hyperaemia of the synovial membrane and associated cellular proliferation and, later, sub-intimal fibrosis and occasionally villous proliferation were recorded following cruciate section. Thus, a varying degree of synovitis was an inevitable sequel to section of the anterior cruciate ligament, and was a constant feature in this model of O.A. These findings are similar to synovial membrane changes described by Pond (1971), Tirgari (1972), Riser (1973), and Tirgari and Vaughan (1975) in cases of naturally occurring O.A. in dogs. Descriptions of histopathological changes in the synovial membrane in human O.A. are also similar (p 22).

In some animals a thin layer of granulation tissue encroaching on to the articular cartilage was noted in sections obtained from the proximal limit of the femoral trochlea. Similar tissue was occasionally recorded on the outer face of lateral and medial trochlear ridges. The significance of this synovial proliferation was not known; no other descriptions of the same experimental model or of spontaneous O.A. report similar histological findings, although Tirgari and Vaughan (1975) observed, macroscopically, encroachment of synovial membrane on the ridges of the femoral trochlea. It should be noted that although the

term/

term "pannus" was used as a convenient abbreviation in the tables of histopathology results, it is not known whether this synovial proliferation should be considered the same as the pannus of an "inflammatory arthritis". Both tissues are essentially vascular granulation tissue developed from synovial membrane and covering an articular surface. Pannus formation in an "inflammatory arthritis" is accompanied by invasion or erosion of subtending hyaline cartilage. While the cartilage in the proximal trochlear groove was invariably transformed into fibrocartilage beneath the granulation tissue, on the medial or lateral trochlear ridges the cartilage appeared hyperplastic rather than degenerated or eroded. In addition, the distribution of the so-called "pannus" within the stifle joint was confined to non-articulating surfaces, especially the proximal limit of the trochlea. The presence of this tissue was not therefore considered to be important in the pathogenesis of the cartilage degeneration recorded in other areas of the joint.

PART IV

FLUORESCENT BONE LABELLING

REVIEW OF LITERATURE

Introduction

Milch, Rall and Tobie (1957, 1958) were the first to report the use of tetracycline antibiotics as fluorescent bone labels. They found that tetracycline was retained specifically by bone and was readily visualised by the characteristic yellow fluorescent colour emitted on exposure of the bone to ultraviolet light. Earlier studies of bone growth measurement had been carried out by Schour (1936), using Alizarin Red S as a label, but the toxicity of this substance precluded its widespread use. The tetracyclines however were suitable not only for the study of bone growth in experimental animals but could be used safely in human subjects. (e.g. Frost 1960, Frost, Villaneuva and Roth 1960, Rush, Pirok and Frost 1966, Jeffery 1973).

The use of more than one label soon followed, thus enabling the process of bone remodelling and the sequence of new bone deposition to be assessed. Whilst some of the tetracyclines may be distinguished by a variation in the colour of fluorescence (Harris, Haywood, Lavorgna and Hamblen 1968; Bohr, Ravn and Werner 1968) this is not always easy since the colours are very similar. A number of other compounds, however, were found to label bone in a manner similar to that of the tetracyclines but possessed the advantage of distinct and different fluorescent colours. So far these fluorochrome labels have been used only in experimental animals. Alizarin Red S and haematoporphyrine have each been used in conjunction with a tetracycline to provide a double label, (Harris 1960, Coutelier 1964). Another combination, that of tetracycline and 2,4 bis N,N' - di (carbomethyl) aminomethyl fluorescein (DCAF) has been reported by Suzuki and Mathews (1966). Similarly, trichrome labelling

was/

was achieved using oxytetracycline and DCAF with either Alizarin Red S (Olsson 1968, Rietz 1968) or with haematoporphyrine (Olerud and Lorenzi 1970).

There are, however, disadvantages in the use of some of these substances. Alizarin Red S is not only toxic but also inhibits further bone formation (Harris, Travis, Friberg and Radin 1964) and is therefore restricted to use just before the animal is killed. The toxicity of haematoporphyrine also limits its use: high doses are required to obtain distinct labelling and these may lead to lethal intestinal complications (Rahn and Perren 1970). Furthermore, Alizarin Red S and haematoporphyrine have been observed by Rahn and Perren (1970) to be deposited at "somewhat different sites" from that of tetracycline and DCAF.

These difficulties prompted the search for other compounds suitable for use as fluorescent labels. Such a label should be deposited, and remain, at sites of new mineralisation of bone, should produce a clearly outlined fluorescence which contrasts with that of other labels, and should have low general toxicity and little or no interference with calcification or deposition of bone. Calcein-blue, xylenol orange and alizarine complexone were found to fulfil these criteria, producing distinctive blue, orange and red fluorescence respectively. (Rahn and Perren 1970, 1971 and 1972). All these substances were of low toxicity and had little effect on bone deposition as assessed on embryonic bone growth in tissue culture (Rahn, Fleisch, Moor and Perren 1970).

Mechanism of fluorescence

Fluorescence occurs when electrons displaced from their normal orbit by absorption of light energy, return to that orbit in a stepwise fashion,/

fashion, thereby generally emitting light of a lower energy level than that absorbed. The molecule must be able to absorb light and also requires at least one electron-donating substance on a resonating nucleus.

Mechanism of binding of label

Being the most widely used of the fluorochrome labels, tetracyclines are the only compounds for which detailed information is available regarding the mechanism of binding to bone. Even for these drugs, however, there is still some uncertainty about the nature of the physico-chemical bond between label and tissue. This is not surprising since the precise mechanism of calcification of bone and even the exact chemical character of bone mineral have yet to be defined.

It has been shown that the fluorophore in bone is likely to be chemically unaltered tetracycline (Titus, Loo and Rall 1957-58). Tetracyclines are known to form complexes with calcium salts, and Ibsen and Urist (1964) in their detailed review of the biochemistry and physiology of the tetracyclines, propounded the theory that chelation with calcium associated with the apatite molecule was the major mode of skeletal tetracycline binding. To explain the observed concentration of tetracycline in newly proliferated bone and not in pre-existing mature bone, these authors suggested that calcium within new bone was relatively more available, the apatite crystals having a greater hydration shell and a relatively greater surface area for binding tetracycline. They also suggested that the large molecules of tetracycline would not readily diffuse through older, denser bone.

In vitro experiments by the same authors (Urist and Ibsen 1963) showed that the growing crystals during precipitation of apatite had

a/

a much higher capacity for binding oxytetracycline than pre-formed apatite, indicating that the calcium salt may be highly reactive to oxytetracycline during the initial stage of mineralisation. Recently, Tam, Reed and Cruickshank (1974), using a sophisticated modification of tetracycline labelling, clearly demonstrated that tetracycline was deposited during the earliest phase of mineralisation of new bone (in the "zone of rapid mineralisation"). There is evidence to suggest that the mineral deposited at this early stage is amorphous calcium phosphate and not crystalline apatite. (Fitton Jackson 1957, Vaughan 1970). Such a difference in mineral structure of newly calcified bone might explain its avidity for tetracycline.

It is possible that the organic matrix plays a role in tetracycline binding, as suggested by Milch et al (1958). These workers demonstrated that, in experimentally created bone defects, where callus and established bone were in intimate anatomical relationship, only the new bone showed fluorescence following tetracycline administration. Furthermore, calcifying cartilage at the epiphyseal plate and in subchondral areas did not fluoresce, whilst adjacent newly formed trabecular bone was clearly labelled. They therefore proposed that there was a complex interfacial reaction between tetracycline, calcium and the protein matrix of newly formed bone. Later, this theory was elaborated, to suggest that tetracyclines were bound to the calcium of "seeded" crystal nucleation sites on collagen fibrils (Milch, Tobie, and Robinson 1961).

This was discounted by Ibsen and Urist (1963), who found that, although oxytetracycline was bound to collagen in vitro, the complex formed did not fluoresce; they also demonstated that experimentally induced/

induced rachitic osteoid failed to bind oxytetracycline. It seems likely, however, that the organic matrix of bone is implicated, at least indirectly, in the binding of tetracycline, since there is a great deal of evidence to suggest that the collagen fibres of the matrix play an important part in the actual mechanism of calcification (Vaughan 1970). Indeed, many electron microscope studies have shown that the earliest demonstrable crystals are associated with the periodic banding along the collagen fibre (Fitton Jackson and Randall 1956, Fitton Jackson 1957, Fernandez-Moran and Engstrom 1957), and most workers agree that the crystals are probably within the collagen fibre.

Another explanation of the mechanism of binding of tetracyclines within bone was put forward by Frost and Villaneuva (1960) who suggested that following the formation of a complex with calcium, the label was merely 'cemented in' by further mineralisation.

No detailed information is available with respect to binding sites for other fluorochrome labels, although Milch et al (1961) commented on the structural similarity of the tetracycline and alizarin molecules and suggested that they were bound to bone by similar mechanisms. They also noted that alizarin attaches to physiologically available apatite crystal surfaces. Rahn and Perren (1971) stated that the sites of fluorescence of tetracycline, fluoresceins and xylenol orange were identical, and commented that the usefulness of polychrome labelling was not impaired by the lack of understanding of the exact physicochemical bonding mechanisms involved.

Sites of deposition of label

i) New bone -

It has been clearly demonstated that the tetracyclines become incorporated/

incorporated into bone which is calcifying at the time of administration and are retained indefinitely, thus providing a reliable label of every site of active new-bone formation. (Milch et al 1957, 1958, Harris, Jackson and Jowsey 1962, Urist and Ibsen 1963). This is also true for the other fluorochrome bone labels used in this study, fluorescein complexone (DCAF), xylenol orange and alizarin complexone, (Suzuki and Mathews 1966, Rahn and Perren 1971, 1972).

ii) Pre-existing bone -

A diffuse fluorescence due to a low concentration of tetracycline in areas of bone already mineralised has been described by Harris et al (1962) and by Urist and Ibsen (1963). This diffuse component, however, was readily distinguished from the intense fluorescence due to uptake of label in newly formed bone, and was found only at high dosage levels (> 150 mgs/kg Harris et al 1962). It was not found with Alizarin Red S (Harris et al 1964). A peri-lacunar fluorescence surrounding some of the osteocyte lacunae has also been observed with both the tetracyclines and Alizarin Red S (Harris et al 1962, Harris et al 1964). This fluorescence may be an artefact. Harris and his colleagues also describe a third site of localisation of label in non-growing bone, in association with the phenomenon of "edge sclerosis", this produces discrete foci of low intensity fluorescence.

iii) Bone resorption cavities -

Olerud and Lorenzi (1970) described labelling of resorption cavities in bone, stating that resorption lines were actively labelled and that triple fluorochrome labelling was especially useful in the study of bone resorption. These authors do not, however, state the time interval between fluorochrome administration and death of the animal, and it is possible/

possible the phenomenon described was a transient adsorption of label. Harris and colleagues (1962), reported that both resorption cavities and inactive bone surfaces showed fluorescence immediately after intravenous administration of tetracycline, but that this fluorescence rapidly disappeared. Similarly, red fluorescence was demonstrated on resorbing, depositing and inactive bone surfaces within a few days of injection of Alizarin Red S, but later, only sites of new bone formation were labelled (Harris et al 1964).

iv) Calcifying cartilage -

There are conflicting reports about fluorescent labelling of calcifying cartilage.

Milch and colleagues (1958) and Holmes (1963) report absence of fluorescence in the zone of provisional calcification of cartilage at the epiphysis, although adjacent newly-formed trabecular bone in the metaphysis was clearly labelled. Similarly, subchondral calcifying cartilage at the articular surface did not show tetracycline fluorescence, (Milch et al 1958). In a later publication, however, Milch and his associates reported fluorescence in areas of calcified cartilage in skeletal neoplasms(Milch, Tobie and Robinson 1961). Urist and Ibsen (1963) described tetracycline fluorescence of calcifying cartilage in fracture callus in rats, and Hansson (1967) used oxytetracycline labelling to measure the rate of endochondral calcification in rabbit long bones.

v) Other tissues -

Immediately followingtetracycline administration, all soft tissues except brain show yellow fluorescence, but this disappears as the blood level drops (Milch et al 1958, Ibsen and Urist 1964).

Duration/

Duration of retention of label

Tetracycline fluorescence persists for approximately six hours in soft tissues, and thereafter progressively diminishes, being undetectable twelve to twenty-four hours after injection. In bones and teeth, however, the fluorescence persists for longer, at least ten weeks according to Milch and colleagues (1957). Frost (1961) reported tetracycline retention in the skeleton for at least nine years.

It is possible that once tetracycline, or another label,has been incorporated into bone, it remains permanently until that bone is resorbed.

MATERIALS AND METHOD

Five different fluorochromes were used in this study, namely, oxytetracycline, tetracycline, alizarine complexone, xylenol orange, and calcein (fluorescein complexone). One label, tetracycline, was employed in only one dog and discontinued because of the similarity of the fluorescent colour to that of oxytetracycline. The fluorochromes have been listed in table 7, which also shows the colour of fluorescence and the dose rate employed for each substance.

The labels were given to each Group A dog at recorded time intervals after section of the cruciate ligament. The interval between injections varied from 3days to 32 weeks, depending on the duration of survival of the animal. 28 dogs received a total of 3 labels, 15 were given 4 labels, while 9 dogs received only 2 fluorochromes. The number of fluorochromes administered and the time of administration has been shown in table 8 for each experimental dog.

Each fluorochrome was administered by the intravenous route; where practicable, the injection was given slowly (10-20 mls per min.). No serious side effects were observed after the administration of any of these substances in the experimental O.A. group of dogs (Group A). If, however, the dyes dissolved in bicarbonate solution were injected too rapidly the dogs vomited immediately after injection. Some excitable dogs became docile and easier to handle after administration of the dyes, and in all dogs the sclera, conjunctival and oral mucous membranes became discoloured by the dye substances. Urine discoloration was also noted for a period of up to 2 days.

Following the death of the animal, undecalcified transverse sections of the femoral trochlea were obtained (see p. 35 and fig. 2) and examined/

examined by transmitted ultraviolet (U.V.) light of 530 λ wavelength. A Zeiss R.A. microscope with a fluorescent illuminator (No. II) was used, a BG12 exciter filter and 53-50-44 barrier filter combination giving the best results. The sections were simply mounted in saline on a glass slide.

To obtain a permanent record of the fluorochrome distribution, diagrams were made from each trochlear section in 27 dogs. In addition, photographic record on Kodak H.S. Ektachrome film was also obtained for most of these sections, and for the sections cut from the other 25 dogs.

A similar procedure was employed for the 3 sham-operated (Group B) dogs. Dog 1 was given oxytetracycline, alizarine complexone and xylenol orange at 4, 9 and 13 days respectively. Dog 2 reacted adversely to the intravenous injection of oxytetracycline but recovered within 48 hours and 1 week later was given alizarine complexone intravenously. A state of collapse followed this injection and the dog died within 36 hours. Dog 3 received 4 fluorochrome labels, given at 2, 4, 6 and 8 weeks after surgery.

FLUOROCHROME	COLOUR OF FLUORESCENCE	DOSE RATE mg/kg IV	PREPARATION
1. Oxytetracycline *	Yellow	20	50 mg/ml Aqueous Solution
2. Alizarine Complexone	Red	30	3% solution in 2% Na H CO_3
3. Xylenol Orange	Orange	90	3% solution in 2% Na H CO_3
4. Fluorescein Complexone (Calcein)	Lime-Green	30	3% solution in 2% Na H CO_3
5. Tetracycline **	Yellow	20	500 mg/ml Aqueous Solution

* Oxytetracycline hydrochloride - Terramycin injection, kindly supplied by Pfizer Limited, Sandwich, Kent.

** Tetracycline - Tetracyn " " " " " " " "

TABLE 7: DOSE RATE AND PREPARATION OF EACH FLUOROCHROME LABEL, AND COLOUR OF FLUORESCENCE EMITTED.

RESULTS

GROUP A DOGS

Undecalcified transverse sections from both right and left femoral trochleas were examined.

Fluorochrome uptake in the trochlear region of each right stifle joint is summarised in table 8. The presence of fluorescent labels is recorded separately for the following areas: 1) osteophyte (marginal zone), 2) subchondral zone, 3) periosteal zone (femoral cortex) and 4) trabeculae. These areas may be identified in fig. 32.

Variation occurred in the amount of fluorescent labelling and in the pattern of distribution in the sections taken from different levels of the same femoral trochlea. Table 8 records the distribution of labels in a representative mid-trochlear section for each dog and these results may be compared with the histological appearance of adjacent decalcified sections, as recorded in table 5.

No record is available for 3 dogs; in dog 1/1, the femoral trochlea was eaten by another dog before sections were obtained; in dogs 6/2 and 8/4 the sections were stored in absolute alcohol and fading of fluorescence had occurred. In dogs 5/2, 6/1 and 10/1, orange fluorescence from the xylenol orange label had either faded or was of low intensity, making interpretation of the complete fluorochrome distribution pattern difficult. In 9 dogs (2/2, 3/3, 3/4, 4/4, 7/1, 10/1, 16/3, 16/4, 48/1), the photographic record of fluorochrome distribution was incomplete, in some cases because of technical faults photographically, in others because no photographs were taken due to the close resemblance of the pattern of fluorochrome uptake to other sections already photographed.

Sections from the control (left) stifle joints showed a variable amount/

amount of labelling of trabecular bone and occasionally of cortical bone. In general, however, sections from the control joint showed only small traces of fluorochrome label, which were sparsely distributed throughout the trabecular bone of the section and usually consisted of only one label or several labels very closely spaced. In some dogs, left trochlear sections did not show any fluorochrome uptake at all. Moderate or marked amounts of fluorochrome deposition were noted in left trochlear sections from 9 dogs, and these results have been described separately (p. 136).

1) Osteophyte - marginal zone

Uptake of fluorochrome labels in the marginal zone of the right femoral trochlea was observed in every dog examined. A small amount of red fluorescence was noted at the marginal zone of the proximal section obtained from dogs 1/4 and 2/2, but this does not appear on table 8 since results recorded in the table are confined to description of mid-trochlear sections.

Seven dogs were injected with oxytetracycline 2 days after cruciate section, but no uptake of this label in the marginal zone was seen in any sections obtained from these animals.

The earliest deposition of new bone at the marginal zone was noted 3 days after cruciate section, and consisted of a thin line of oxytetracycline-labelled mineral outside the pre-existing femoral cortex (fig. 67).

By 2 to 3 weeks after surgery, small, discrete deposits of fluorochrome-labelled mineral were readily observed at the marginal zone. These early osteophytes were noted in sections cut from the proximal part of the femoral trochlea, and in dogs 2/1, 2/4 and 3/1 were larger on the medial trochlear ridge than on the lateral ridge.

At/

At this stage the pattern of fluorochrome distribution showed that there was an irregular accretion of mineral on the outer surface of the existing, intact femoral cortex, with new bone being laid down on the outer edge in successive "waves", each irregular band of label having a slightly hazy appearance (fig. 67). The shape of this small osteophyte was variable, but round, ovoid or half-moon outlines were most common. In perfused specimens small blood vessels were seen in the overlying synovial membrane or closely associated with the new bone deposition, they appeared dark blue or black (fig. 68).

Between 4 and 6 weeks after cruciate section, the pattern of the successive band of labelling in the osteophyte was usually disrupted by the development of central cavities within the nodules of bone. The appearance of fluorochrome labelling of a 5 week osteophyte is shown in fig. 70. Another fluorescent photomicrograph of a 5 week osteophyte in fig. 69 shows a slightly unusual appearance due to the presence of cartilage at the edge of the osteophyte which has not taken up any label. Communication between bone marrow spaces of the distal end of the femur and resorption cavities within the osteophyte was established as early as 4 weeks in one dog (4/3), and a small blood vessel filled with dye mixture was visible within a narrow channel penetrating the femoral cortex adjoining the osteophyte. Resorption of the femoral cortex was also observed in sections from dogs 5/1 and 5/2 (fig. 70), but more often in dogs killed at this stage the osteophyte was still a discrete deposition of new bone, located on the outer surface of the femoral cortex and without any apparent communication between the marrow spaces of the femoral epiphysis and the osteophyte.

From 7 to 10 weeks after ligament section the pattern of fluorochrome distribution/

distribution tended to become more complex than the irregular bands of label typical at earlier stages. Thus, deposition of the most recently administered fluorochrome was not confined to the outer margins of the osteophyte, but also could be seen in the central part. This indicated that, as well as increasing in size by accretion of woven bone at the periphery, remodelling of the internal architecture by bone resorption and further deposition of new bone was occurring. In many sections there was the hint of an early trabecular arrangement of bone within the substance of the osteophyte (figs. 71 and 72) and often (though not in all cases) free communication between bone marrow spaces of epiphysis and osteophyte was apparent (fig. 71). The pattern of fluorochrome uptake in the central region of the osteophyte also differed from that at the periphery: instead of the slightly hazy, broad bands of label characteristic of woven bone, the fluorochrome was deposited in a clearly-defined linear pattern, indicating that appositional bone was being laid down (fig. 72). In some sections from dogs in which the femoral artery had been perfused with dye mixture, small dark blood vessels were observed lying within regular vascular channels within bone labelled by the most recently administered fluorochrome (fig. 71).

There was a considerable range of appearances within the group of dogs killed 7 to 10 weeks after cruciate section. For example, dog 9/1 was unusual in showing relatively small osteophytes with a pattern of labelling resembling that of dogs killed 3 weeks after surgery. On the other hand, osteophytes in dogs 9/3 and 10/3 were large with a well developed trabecular pattern.

Dogs killed at 12 weeks also showed a somewhat variable picture with respect to fluorochrome labelling of osteophytes. In dog 12/1, distribution/

distribution was basically that of irregular broad bands of label with the recently administered fluorochrome at the outer edge, distinct resorption cavities were present within the osteophyte and there was communication with the marrow spaces of the distal end of the femur. In dogs 12/2 and 12/3, the pattern was similar; a less regular distribution of fluorochrome was recorded, marked bone resorption having taken place in the centre of the osteophyte and in some sections evidence of a developing trabecular structure being obvious (fig. 74). In dog 12/4, the osteophytes were large, resorption of bone had removed most, or all, of that which had been labelled with oxytetracycline (administered at day 30), and a regular trabecular structure was evident in some sections.

In dogs 12/2 and 12/3 many blood vessels were noted within the substance of the osteophyte (fig. 73), and in the overlying synovial membrane.

In the mid-trochlear sections of both 12/2 and 12/3, the osteophyte was continuous with the area of bone remodelling in the adjacent subchondral zone of the trochlear ridge.

The pattern of fluorochrome labelling was remarkably similar in all the dogs killed 16 weeks after cruciate section (fig. 75). There was a large protuberant ridge of new bone, covered by cartilage, which was continuous with either medial or lateral trochlear ridge, i.e. the original line of the femoral cortex could still be discerned but resorption and remodelling had resulted in continuity between trabeculae of the distal end of the femur and those of the osteophyte. Fluorochrome labelling of the trabeculae of the osteophyte was somewhat haphazard in appearance. Only traces of the first-administered fluorochrome (oxytetracycline) were discernible, larger amounts of the second label (alizarine/

(alizarine complexone) were present in an irregular fashion in the trabeculae, and in addition there was some unlabelled bone (fig. 76). The last fluorochrome administered (given either 2 or 4 weeks before death) had also labelled the trabeculae, and a diffuse, hazy appearance of this label was observed at the outer margin of the osteophyte where woven, cellular bone was still being formed (fig. 75). In dogs 16/1, 16/2, and 16/3, the osteophyte ridge was continuous with bone remodelling in the subchondral zone of the trochlear ridge. In sections from 16/1 and 16/4 perfused blood vessels were noted in association with the osteophyte development (fig. 76).

At 24 weeks, one dog 24/1 showed a pattern of fluorochrome uptake which was similar to that at 16 weeks. In the other dog, 24/2, the osteophyte ridge was even more extensive than at 16 weeks: a large ridge of new bone covered by cartilage extended from the tips of the trochlear ridges to the attachment of the synovial membrane.

The mature trabecular structure of this ridge of new bone was irregularly labelled by thin, clearly-defined lines of alizarine complexone (administered at 13 weeks, day 91). Much of the trabecular bone was unlabelled. No oxytetracycline uptake (administered at 5 weeks, day 36) was seen. Fluorescence due to xylenol orange (administered 2 days prior to euthanasia) was observed in linear pattern on the surface of some of the osteophyte trabeculae, particularly towards the outer edges of the osteophyte ridge. A hazy, ill-defined distribution of orange fluorescence was also present on the outer border of the osteophyte beneath the cartilage, especially at the upper or lower limits of the osteophyte (fig. 77).

Sections from dogs killed 48 weeks after cruciate section showed relatively/

relatively little fluorochrome uptake within the "osteophyte", which at this stage was no longer a distinct separate ridge of new bone but rather was an integral part of the remodelled trochlear ridge. In dog 48/1 the remodelling had resulted in a very marked increase in width of the trochlear ridge, especially on the lateral aspect (fig. 78). In dog 48/2 the increase in width was less pronounced. The structure of the remodelled ridge and the pattern of fluorochrome distribution was similar in both dogs. A regular trabecular arrangement was present, most of the trabecular bone being unlabelled but small irregular deposits of the different labels being identified within, or on the surface of, these trabeculae (figs. 78 and 79). In dog 48/1, clearly defined linear depositions of the fourth fluorochrome (fluorescein complexone, administered 46 weeks, day 322) were present at the outer edge beneath the cartilage (fig. 78). In dog 48/2, the final fluorochrome (xylenol orange, administered day 334, 2 days before death) was located mainly as a slightly irregular fringe at the outer edges of the remodelled ridge. In this dog, vascular perfusion had demarcated a number of small blood vessels which were associated with the outer fringe of orange fluorescence (fig. 79), and in one section a fairly large blood vessel filled with dye was observed in the inter-trabecular spaces at the outer edge of the new bone (fig. 79b).

2) Subchondral zone

The subchondral bone of the outer, non-articulating face of the trochlear ridges was not labelled by fluorochrome uptake in dogs killed from 1 to 3 weeks after cruciate section. However, sections from dog 4/1 and 5/3 did show small deposits of fluorochrome which had been/

been administered 13 days after surgery, and in dogs 6/1 and 8/2, fluorochrome given on day 15 was present in this subchondral region. Thus in only four dogs, of the 24 which had received fluorochrome labels, was there evidence of new bone deposition at this location within 15 days of cruciate section. In a further 9 dogs, fluorochrome label administered between 3 and 5 weeks was deposited in the subchondral zone.

In 2 dogs killed more than 8 weeks after surgery (9/1 and 12/1) no significant fluorochrome labelling was recorded in the subchondral region at any level of the femoral trochlea. In another dog (12/4) there was relatively little evidence of new bone deposition at this site; fluorochrome deposition was present in the medial subchondral zone of the distal section only.

In all other dogs killed and examined 8 weeks or more after cruciate section, fluorescent labelling was evident on medial and/or lateral trochlear ridge in one or several of the transverse sections.

In 3 dogs, 24/2, 48/1 and 48/2 there was confluence of the subchondral zone with the osteophyte and the distinction between the two areas of remodelling was no longer valid. Nevertheless, this remodelled ridge was covered by cartilage and "subchondral" deposition of fluorochrome was evident. In dogs 48/1 and 48/2 only a small fringe of the fluorochrome administered prior to euthanasia was present at the outer margins of the remodelled ridge, as illustrated in figs. 78 and 79.

In 6 dogs, (7/3, 8/1, 9/2, 10/3, 12/2 and 12/4) a greater uptake of fluorochrome labels in the subchondral zone was noted in sections cut from the distal end of the femoral trochlea (i.e. adjacent to the femoral condyles) than in sections obtained from the more proximal part.

The pattern of fluorochrome labelling in the subchondral region consisted/

consisted of varying amounts of label deposited in an irregular fashion on the cortex and adjacent trabeculae directly beneath the hyaline or fibrocartilage covering the outer surfaces of the trochlear ridges. Sometimes there was sequential apposition of new bone, with a clearly defined linear deposition of one label upon another in a regular pattern, the first label being laid down at the outer edge immediately subjacent to the cartilage, with subsequent labels being deposited on the surface nearest to the bone marrow. Often no such regular arrangement of labelling was observed, and where marked bony remodelling was taking place in this region, a hazy, ill-defined deposition of fluorochrome label was observed, (figs. 80 and 81).

The extent of fluorochrome uptake varied from small focal areas to involvement of the entire trochlear ridge from the tip of the ridge to the level of the osteophyte at the marginal zone. With the exception of sections from the proximal (supratrochlear) region of the trochlea from some dogs,and mid-trochlear sections from dogs 6/1, 8/2, 9/3, 16/1, 24/2, 48/1, little fluorochrome labelling was observed subchondrally in the trochlear groove.

In dogs in which vascular perfusion had been carried out, some sections showed small vessels filled with dye in association with the subchondral fluorochrome uptake (figs. 80 and 81).

3) Periosteal Zone (Femoral Cortex)

Traces of fluorochrome label were detected on the outer periosteal surface of the femoral cortex (caudal to the synovial membrane attachment, see fig. 32) as early as 3 days after cruciate section. (dog 2/3), and in dogs 5/2 and 6/4, small amounts of label had been deposited at this site 7 days after surgery.

Generally/

Generally, between 3 and 7 weeks after cruciate section there was some deposition of fluorochrome label on the periosteal surface. The distribution varied from focal to diffuse, and typically consisted of a series of "wavy" lines of fluorescent labels with the first label adjacent to the periosteal surface of the cortex and subsequent labels arranged parallel to and on the outer face of the first (figs.70a and 82). This crenellated lamellar distribution of fluorochrome labels was recorded in sections from 23 dogs, ranging from 4 to 16 weeks after cruciate section.

Labelling in the femoral cortex of dogs killed at 16 or 24 weeks had, in most of the sections, a multi-layered "sandwich" appearance (fig. 83). In other words, there were successive layers of differently labelled bone throughout the thickness of the femoral cortex, indicating that there was active remodelling of both the original cortical bone and of new bone which had been deposited earlier on the outer aspect of the cortex. Not only was intense labelling of this region evident, but the cortex was thicker than that of comparable sections from the left trochlea.

In the longer surviving dogs (24 and 48 weeks), the pattern of fluorochrome uptake clearly demonstrates the development of Haversian systems within the thickened femoral cortex. This has been shown in figs. 84 and 85. In fig. 84, the medial femoral cortex from dog 24/2, there are basically three distinct layers of Haversian systems, each labelled by a different fluorochrome. Thus, on the inner aspect of the cortex is a band of oxytetracycline-labelled Haversian canals (5 weeks, 36 days), then a band of predominantly alizarine-labelled Haversian canals (13 weeks) and, towards the outer edge of the cortex, some labelling by xylenol/

xylenol orange (given just before death at 24 weeks) may be seen. This shows clearly the increase in thickness of the cortex which has occurred between 5 and 24 weeks after section of the anterior cruciate.

A small amount of fluorochrome labelling of the Haversian systems of the femoral cortex was observed in sections from some of the control (left) stifle joints.

4) Trabeculae

Since it was usual to find small amounts of trabecular fluorochrome deposition in the control trochlear sections, there was some difficulty in determining the onset of significant trabecular uptake in sections from the right stifle joint. By 5 or 6 weeks there was a significant increase in labelling of the trabeculae in 2 dogs (5/2, 6/3), and in a further 7 dogs a small amount of trabecular fluorochrome uptake was recorded. From 8 weeks after cruciate section, all dogs, (with the exception of 9/1), showed moderate to marked labelling of the trabeculae. In general, there was a linear distribution of fluorochrome labels in the trabeculae. Thus the label was present either on the surface or within the substance of a trabecula, and a parallel arrangement of two or three labels was often observed (fig. 86).

The distribution of fluorochrome throughout the trochlear region of the femoral epiphysis was variable. From 4 to 7 weeks it was often sparse, and from 8 weeks the whole of the trochlear region appeared to be involved. In some cases, labelling appeared to be more intense in trabeculae in the outer areas of the trochlear ridges, (adjacent to the subchondral zone and osteophyte) rather than in the area subjacent to the trochlear groove.

5) Labelling of the left trochlear sections

Trochlear/

Trochlear sections from the left stifle of 34 dogs were examined for fluorescence.

No fluorochrome labelling was seen in 2 dogs (1/4, 2/4).

Very small amounts of label were found on trabeculae in sections from 16 dogs (1/3, 3/1, 4/2, 5/2, 6/1, 6/4, 8/2, 8/4, 9/1, 9/2, 10/1, 10/4, 12/2, 12/3, 16/2, 16/3). Slightly more uptake of trabecular label was observed in 6 dogs (4/1, 4/3, 7/1, 9/3, 12/1, 12/4) but this was of single-label distribution, or the labels had been deposited very close to each other.

Moderate amounts of trabecular fluorochrome labelling were noted in sections from 7 dogs (6/3, 8/3, 9/4, 12/1, 24/1, 24/2, 48/2). In addition, subchondral fluorochrome deposition was recorded in 3 of these dogs (8/3, 9/4, 48/2).

Left trochlear sections from 16/4 and 48/1 were found to be labelled with a lot of fluorochrome deposition throughout the trabeculae. In 16/4 an area of fluorochrome deposition at the marginal zone of the proximal trochlear section was recorded, and in 48/1 there was a lot of fluorochrome labelling of the femoral cortex.

GROUP B DOGS

a) Dog 1 (killed at 2 weeks)

Traces of oxytetracycline and alizarine fluorescence on the trabeculae were noted in sections from both left and right femoral trochleas.

b) Dog 2 (died at 19 days)

Moderate amounts of alizarine complexone uptake on both trabeculae and femoral cortex of left and right femoral trochlear sections were observed in this dog (Death had occurred within 36 hours of injection of dye and the dog's tissues were still discoloured).

c) Dog 3 (killed at 8 weeks)

No fluorescent microscopy was carried out for this animal.

Dog	Admin. of Fluorochrome (days)	Osteophyte (Marginal Zone) Med.	Lat.	Subchondral Zone Med.	Lat.	T.G.	Periosteal Zone Med.	Lat.	Trabeculae
1/1		NO RESULTS							
1/2	2								
	6	+							
1/3	2								
	5	(+)							
1/4	2								
	5								
2/1	7	+	(+)						
	13	+	+						
2/2	7								
	13								
2/3	3						(+)		
	7		(+)				(+)		
	12	+	(+)				(+)		
2/4	3	+							
	7	+							
	12	+	(+)						
3/1	6	+							
	13	+	(+)						
	20	+	(+)				+	+	
3/2	6								
	13								
	20	(+)	(+)					(+)	
3/3	5								
	9								
	13	NR	+						
	19		+						
3/4	5								
	9								
	13	(+)	NR						
	19	(+)							
4/1	2								
	13	+	+	+				(+)	
	20	+	+	+			(+)	(+)	
	26	+	+	+			(+)	(+)	

TABLE 8: DISTRIBUTION OF FLUOROCHROME LABELS IN MID-TROCHLEAR SECTIONS FROM RIGHT STIFLE JOINT

Dog	Admin. of Fluorochrome (days)	Osteophyte (Marginal Zone) Med.	Lat.	Subchondral Zone Med.	Lat.	T.G.	Periosteal Zone Med.	Lat.	Trabeculae
4/2	2								
	13								
	20	(+)	(+)					(+)	(+)
	26	+	+					(+)	(+)
4/3	5	(+)	+						
	12	+	+						
	19	+	+						
	26	+	+				+		(+)
4/4	5								
	12	NR							
	19		+						
	26		+					+	(+)
5/1	7							(+)	(+)
	27	+	+				(+)	(+)	(+)
	33	+	+				(+)	(+)	
5/2	7	+					+	+	+
	27	+	++				+	+	+
	33		+						(+)
5/3	2								
	13				(+)		+		(+)
	20	+	+		(+)		+	(+)	(+)
	27	+	+		(+)		+	(+)	(+)
5/4	2								
	13		+						
	20	(+)	+				+		
	27	(+)	+				+		
6/1	15	(+)	+	+	+	+	+	+	
	30	(+)	+	+	+	+	+	+	+
	41	(Xylenol orange faded)							
6/2	NO RESULTS - Fluorochromes faded								
6/3	7								
	21				+		(+)	(+)	+
	35	+	+		+		(+)	(+)	+

Table 8 continued

Dog	Admin. of Fluorochrome (days)	Osteophyte (Marginal Zone) Med.	Lat.	Subchondral Zone Med.	Lat.	T.G.	Periosteal Zone Med.	Lat.	Trabeculae
6/4	7						+		
	21	+	+				+	+	
	35	+	+				+	+	
7/1	8								
	22	+	NR						
	35	+							
	47	+							
7/2	8	(+)							
	22		(+)						
	35	+	+						(+)
	47	+	++					+	(+)
7/3	15		(+)					+	
	30	+	+		+			+	(+)
	42	+	+		+			+	(+)
7/4	15								
	30	+	+						
	42	+	+						
8/1	15	(+)	+				+	+	(+)
	30	+	+	(+)			+	+	+
	43	++	++	+	+		+	+	++
8/2	15	+	+	+	+	+	+	+	+
	30	+	+	+	+	+		+	+
	43	+	++	+	+	+	+	+	++
8/3	7		(+)					+	
	20	+	(+)	(+)	(+)			+	
	34	+	+	(+)	(+)			+	+
	48	+	++	(+)	(+)		(+)	+	+
8/4	NO RESULTS								
9/1	22	+	+				+	+	
	37	+	+				+	+	
	49		+					+	
9/2	22	+	+					+	(+)
	37	+	+				+	+	(+)
	49	+	+				+		(+)

Table 8 continued

Dog	Admin. of Fluorochrome (days)	Osteophyte (Marginal Zone) Med.	Lat.	Subchondral Zone Med.	Lat.	T.G.	Periosteal Zone Med.	Lat.	Trabeculae
9/3	28	+	+	+		(+)	+	+	+
	35	+	+	+		(+)	+	+	+
	43	+	+		(+)	(+)	+	+	+
	56	+	+		+	+	+	+	+
9/4	28	(+)	+	+	+		+	+	+
	43	+	+	+	+		+	+	+
	56	+	+	+	+		+	+	+
10/1	14	NR	(+)				NR		
	42		+	(+)	+			+	+
	56		?+	(+)	+				
	68		+	(+)	+			+	+
10/2	14	+	(+)				+	+	+
	42	+	(+)		NR		+	+	+
	56	+	(+)	+			+	+	+
	68	+	+	+			+	+	+
10/3	36	+	+				+	+	(+)
	48	+	+				+	+	(+)
10/4	36	+	+	(+)	(+)		+	+	+
	48	+	+	(+)	(+)		+	+	+
12/1	49	(+)	+				+	+	+
	63	+	+				+	+	+
	77	+	+				+	+	+
12/2	63	+	+				+	+	+
	77	+	+	+			+	+	+
12/3	30	(+)	+		+		+	+	+
	56	+	+		+		+	+	+
	84	+	+		+		+	+	+
12/4	30	+					+	+	+
	56	+	+				+	+	+
	84	+	+				+	+	
16/1	28				+				+
	55	+	+	+	+	(+)	+	+	+
	83	+	+	+		+	++	++	+

Table 8 continued

Dog	Admin. of Fluorochrome (days)	Osteophyte (Marginal Zone) Med.	Lat.	Subchondral Zone Med.	Lat.	T.G.	Periosteal Zone Med.	Lat.	Trabeculae
16/2	28	(+)	(+)	+			+	+	+
	55	+	+	+	+		+	+	++
	83	+	+				+	+	++
16/3	42	+	+				+	+	(+)
	70	+	+	+	+	NR	+	+	(+)
	98	+	+	+	+		+	+	(+)
16/4	42	NR	(+)	NR		NR	NR	(+)	+
	70		+		+			+	+
	98		+		+			+	+
24/1	29							(+)	+
	84	+	+	+			+	+	+
	139	++	++		+		++	++	+
24/2	36	(+)		+	+		+	+	+
	91	+	+	+	+	(+)	+	++	+
	167	+	++	+	+	(+)	+	+	+
48/1	29	NR	(+)	NR			NR	+	+
	90		+					+	+
	169		+			+		+	+
	322		+		+	+		+	+
48/2	56	(+)	(+)					+	+
	112	+	+				NR	+	+
	334	(+)	+		+			+	+

KEY

(+) Small amounts fluorochrome

\+ Obvious labelling

++ Marked labelling

NR No record (for this area of mid-trochlea section)

med Medial

lat Lateral

TG Trochlear groove

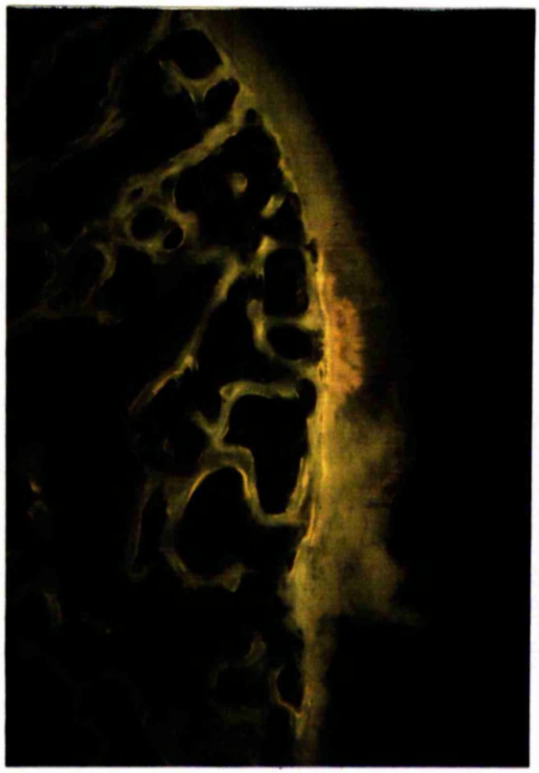
Table 8 continued.

Fig. 67a: Fluorescent photomicrograph of trochlear ridge from dog 2/4. Note position of osteophyte at marginal zone.

(X10)

Fig. 67b: Enlargement of osteophyte from fig. 67a.
Oxytetracycline administered at 3 days
Alizarine complexone administered at 7 days
Xylenol orange administered at 12 days
Compare fig. 88

(X 35)



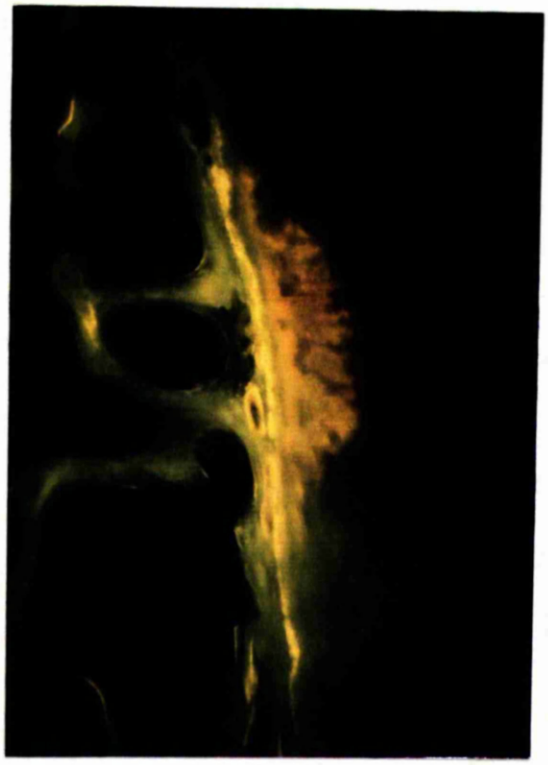
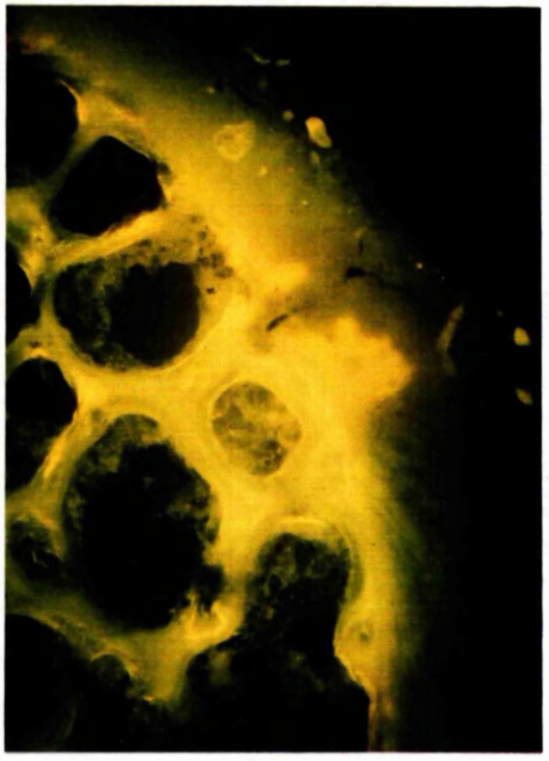


Fig. 68: Small osteophyte from dog 2/4, showing associated blood vessel (perfused with dye)

(X 35)

Fig. 69: Lateral osteophyte from dog 5/2.
Oxytetracycline administered at 1 week
Alizarine complexone administered at 4 weeks
Xylenol orange administered at 5 weeks (day 33)
Note area of unlabelled cartilage at periphery.
Compare fig. 91

(X 35)



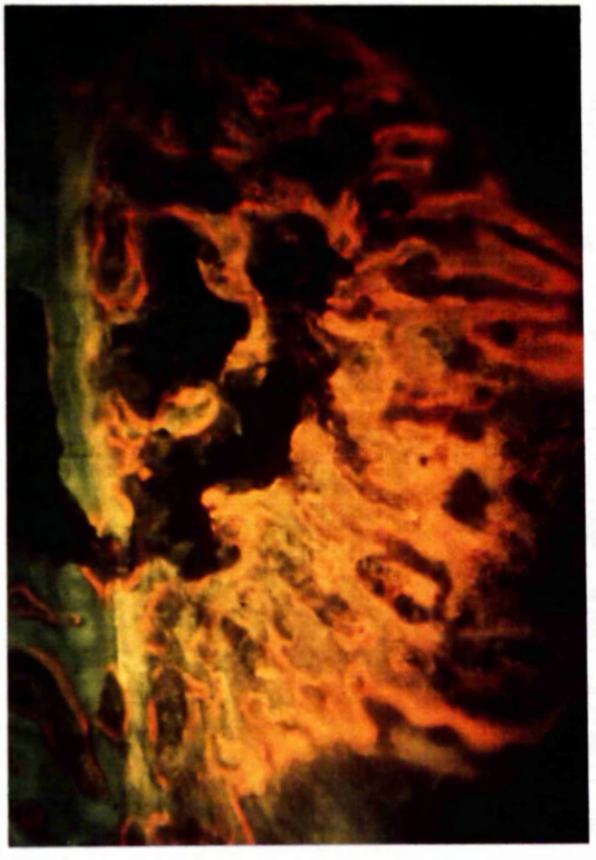
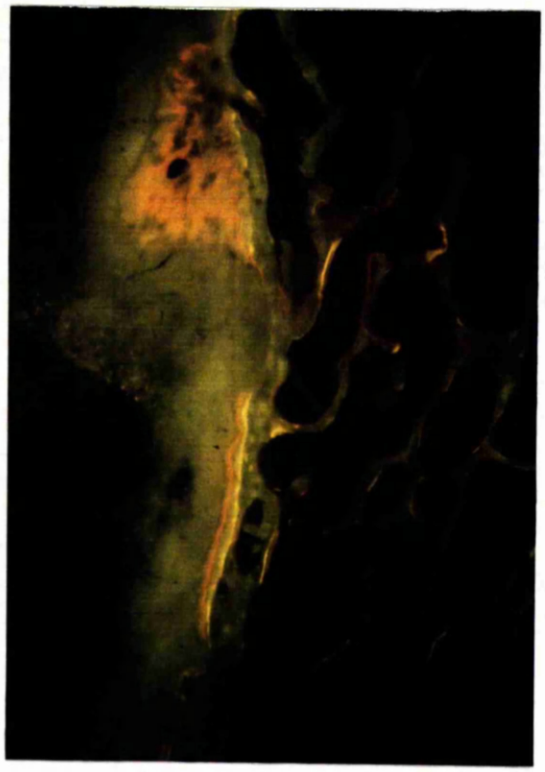


Fig. 70a: Medial trochlear ridge from dog 5/1. Note marginal osteophyte, periosteal new bone, small amount of trabecular labelling and synovial blood vessel (perfused specimen)

(X 10)

Fig. 70b: Higher magnification of osteophyte of fig. 70a.
Oxytetracycline administered at 1 week
Alizarine complexone administered at 4 weeks
Xylenol orange administered at 5 weeks (day 33)
Compare fig. 90.

(X 35)



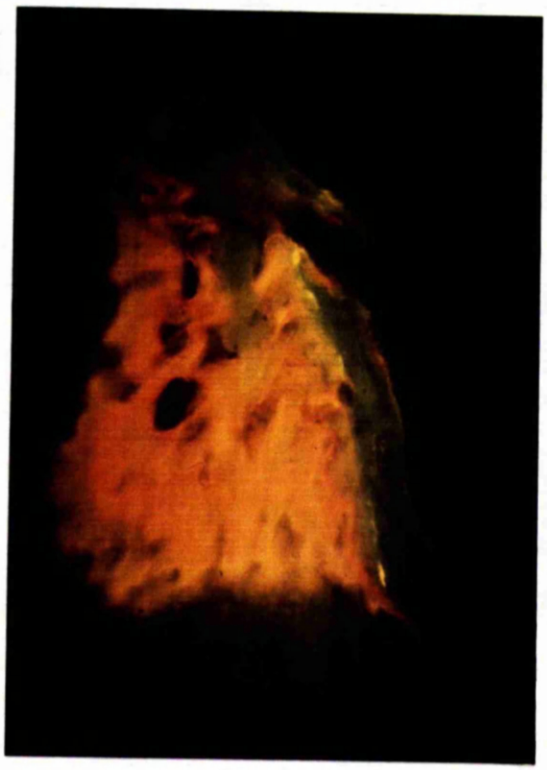


Fig. 71: Lateral osteophyte from dog 8/1.
Oxytetracycline given at 2 weeks
Alizarine complexone given at 4 weeks
Xylenol orange given at 6 weeks
Note perfused blood vessel (arrowed) and trabecular labelling.
(X 35)

Fig. 72: Medial osteophyte from dog 10/2.
Oxytetracycline given at 2 weeks
Alizarine complexone given at 6 weeks
Xylenol orange given at 8 weeks
D.C.A.F. given at 10 weeks
(X 35)

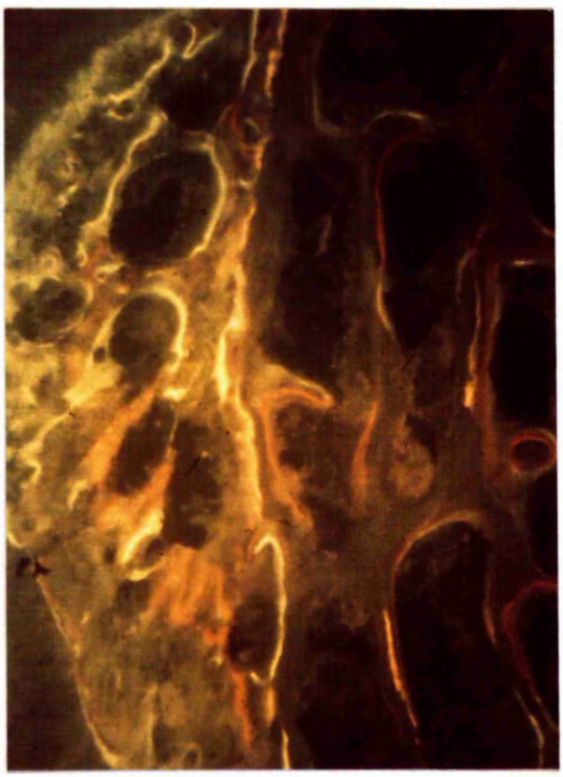


Fig. 73: Lateral osteophyte from dog 12/3, showing numerous perfused blood vessels in association with new bone deposition.

(X 120)

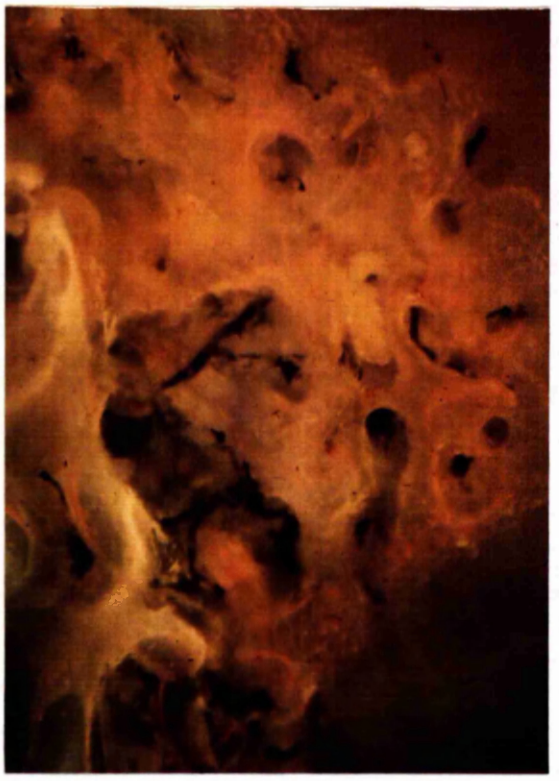
Fig. 74: Another lateral osteophyte from 12/3. Note blood vessels and early trabecular arrangement.

Oxytetracycline given at 4 weeks

Alizarine complexone given at 8 weeks

Xylenol orange given at 12 weeks

(X 35)



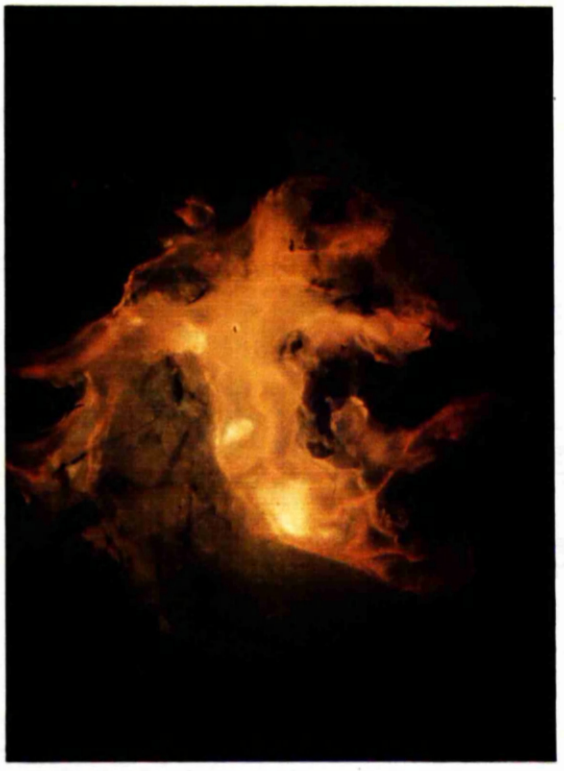
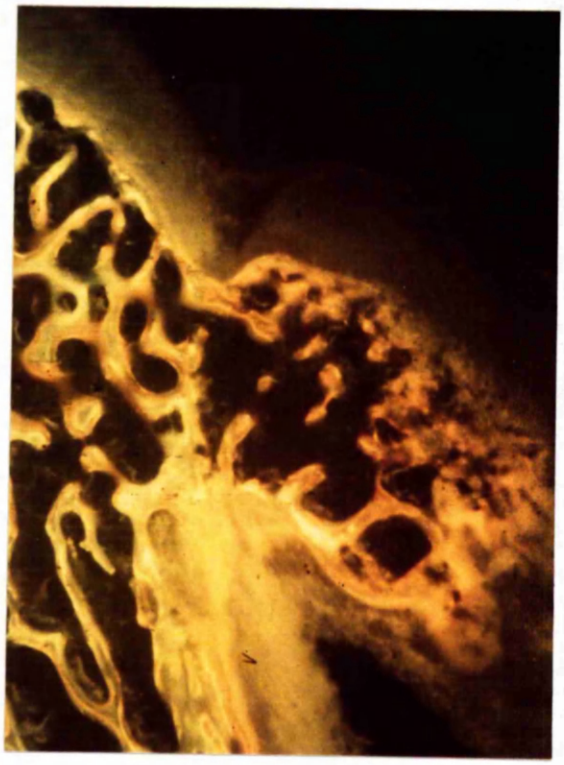


Fig. 75: Lateral trochlear ridge from dog 16/3.
Oxytetracycline given at 6 weeks
Alizarine complexone given at 10 weeks
Xylenol orange given at 14 weeks
An unlabelled zone of calcified cartilage is present at the outer edge of the osteophyte (compare fig. 95).

(X 10)

Fig. 76: Outer margin of a lateral osteophyte from dog 16/1.
Oxytetracycline given at 4 weeks
Alizarine complexone given at 8 weeks
Xylenol orange given at 12 weeks
Note perfused blood vessels (arrowed)

(X 35)



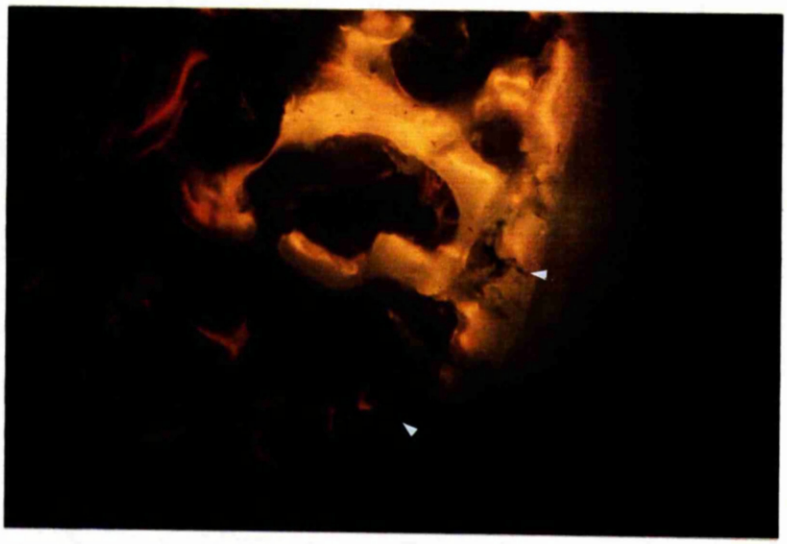
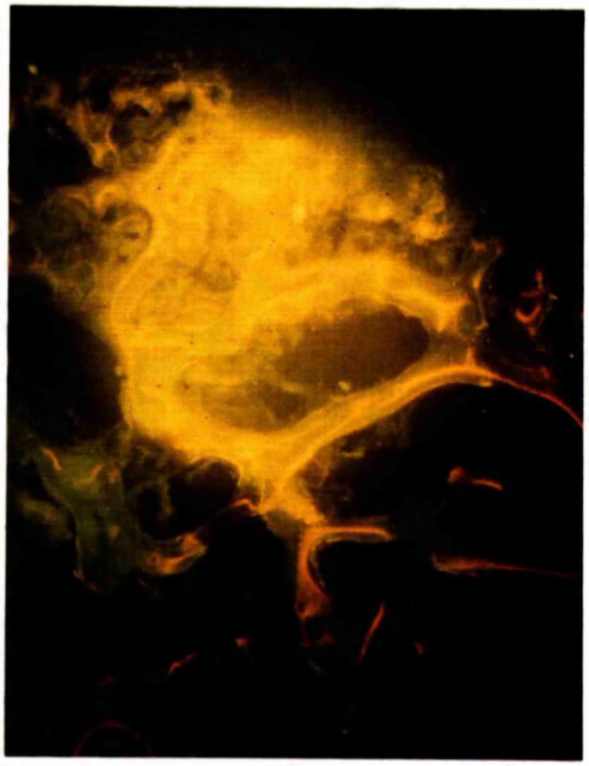


Fig. 77: Part of a lateral osteophyte from dog 24/2, showing trabecular structure with linear deposition of alizarine complexone (13 weeks) and hazy xylenol orange fluorescence (24 weeks) at outer edge.

(X 35)

Fig. 78: Lateral trochlear ridge from dog 48/1. The last-administered label (D.C.A.F.) given 2 weeks before death, is present at the outer edge (arrowed)

(X 10)



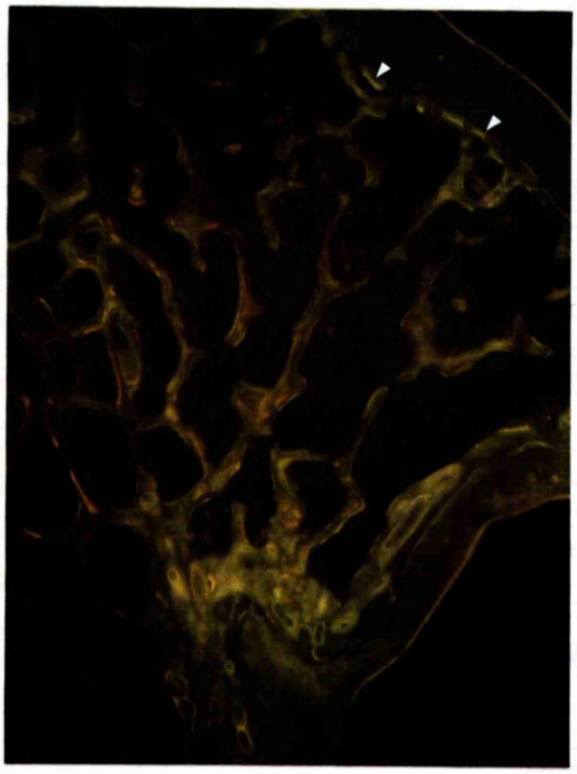
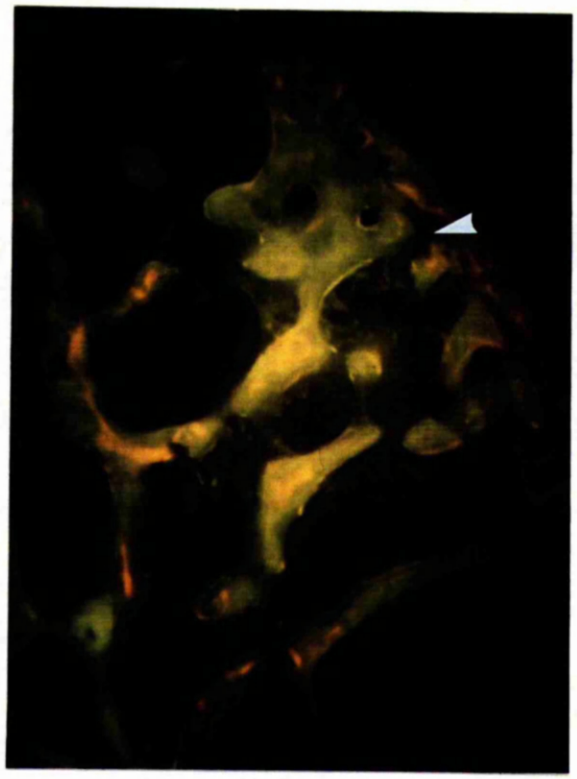
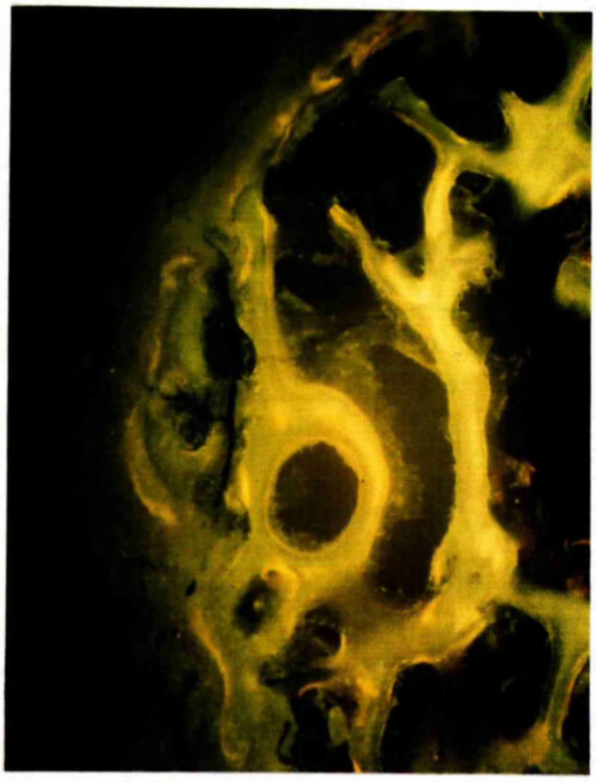


Fig. 79a: Part of the remodelled lateral trochlear ridge from dog 48/2. Small amounts of alizarine complexone (16 weeks) label the trabeculae, xylenol orange (48 weeks) labels the outer edge.
Note small blood vessel (arrowed) compare fig. 101.
(X 35)

Fig. 79b: Medial trochlear ridge from dog 48/2, from the same section as fig. 79a.
Note the large blood vessel (perfused)
(X 35)



a



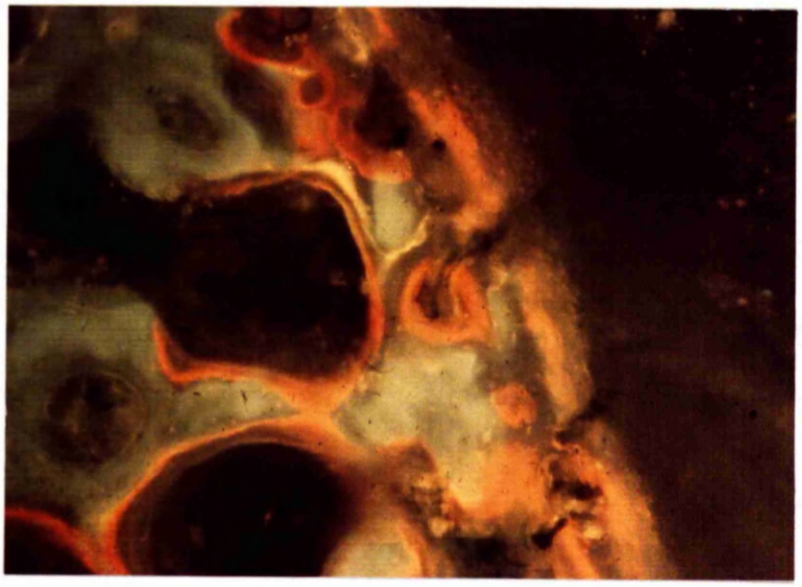
b

Fig. 80: Subchondral remodelling from dog 7/3, (lateral trochlear ridge). Many small blood vessels are present in association with the new bone deposition (perfused with dye)

(X 120)

Fig. 81: Subchondral remodelling of lateral trochlear ridge from dog 16/1. Small blood vessels are arrowed. Note also trabecular labelling.

(X 35)



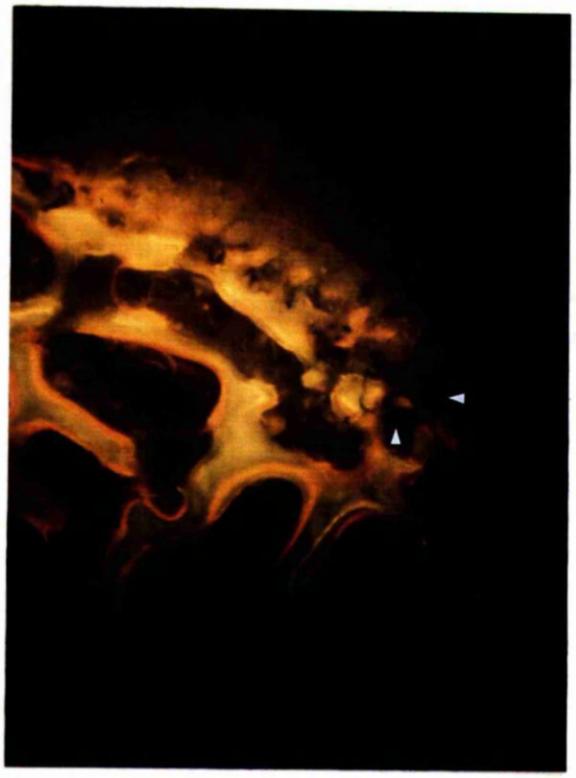
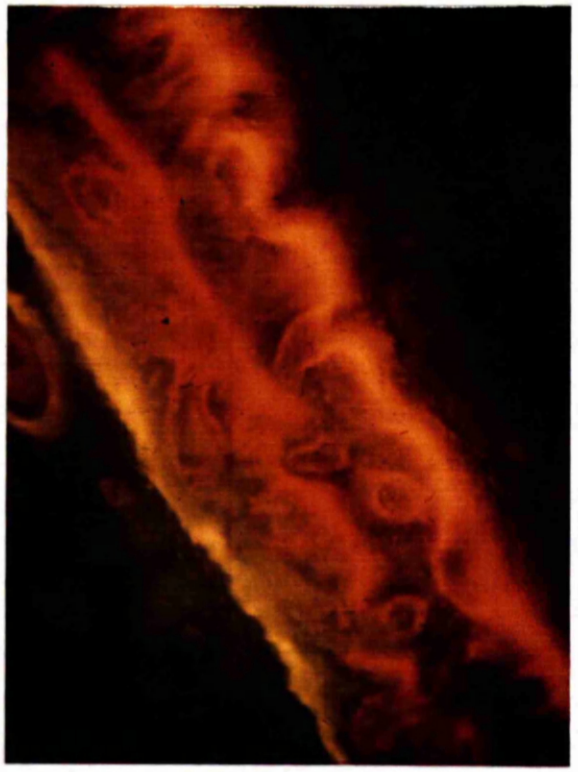


Fig. 82: Periosteal new bone deposition on lateral femoral cortex from dog 8/1.
Oxytetracycline given at 2 weeks
Alizarine complexone given at 4 weeks
Xylenol orange given at 6 weeks
(X 120)

Fig. 83: Periosteal zone remodelling at 16 weeks (dog 16/1)
Small blood vessels are arrowed.
Oxytetracycline given at 4 weeks
Alizarine complexone given at 8 weeks
Xylenol orange given at 12 weeks
(X 35)



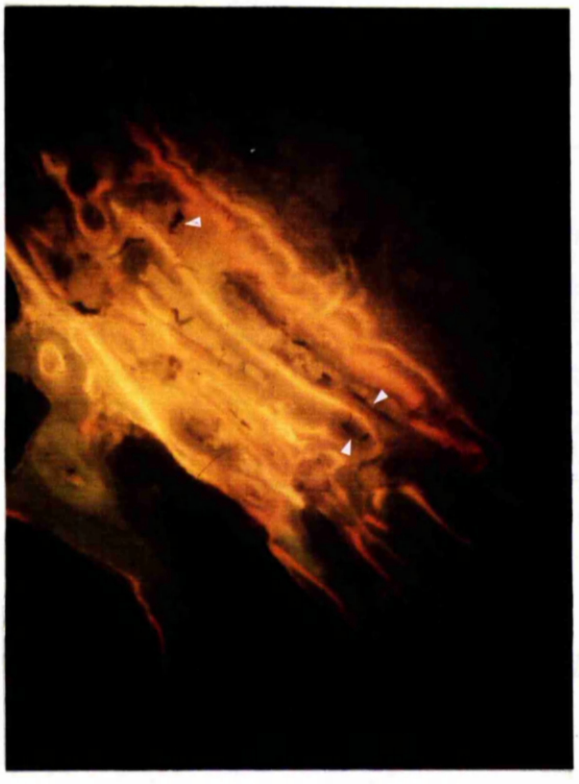
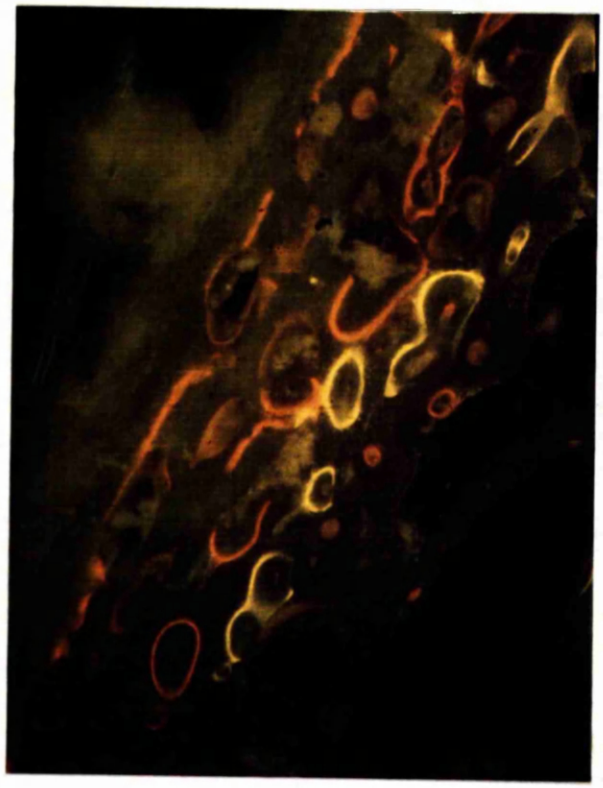


Fig. 84: Medial femoral cortex from dog 24/2. Increased thickness is shown by the three bands of osteons labelled yellow, red and orange.
Oxytetracycline given at 5 weeks
Alizarine complexone given at 13 weeks
Xylenol orange given at 24 weeks
(X 35)

Fig. 85: Remodelled lateral femoral cortex from dog 48/1. Osteons labelled by each of the four different fluorochromes may be identified.
(X 35)



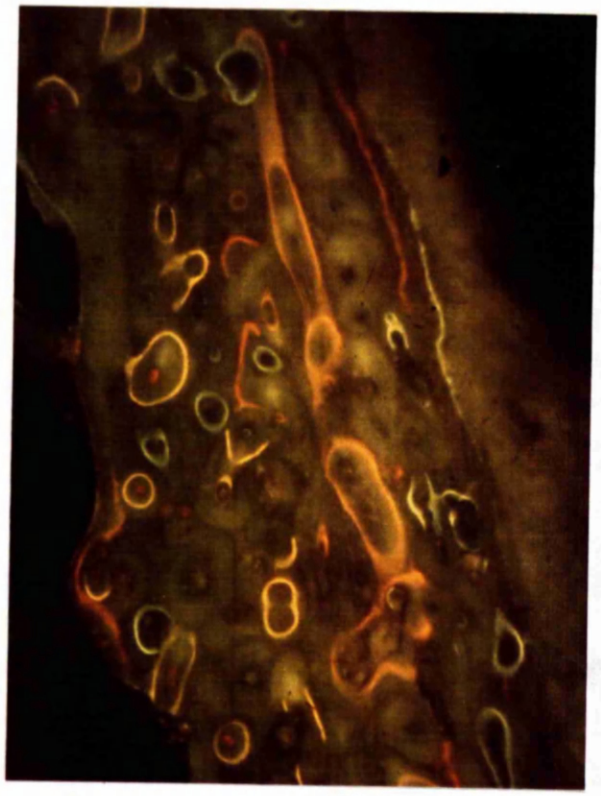
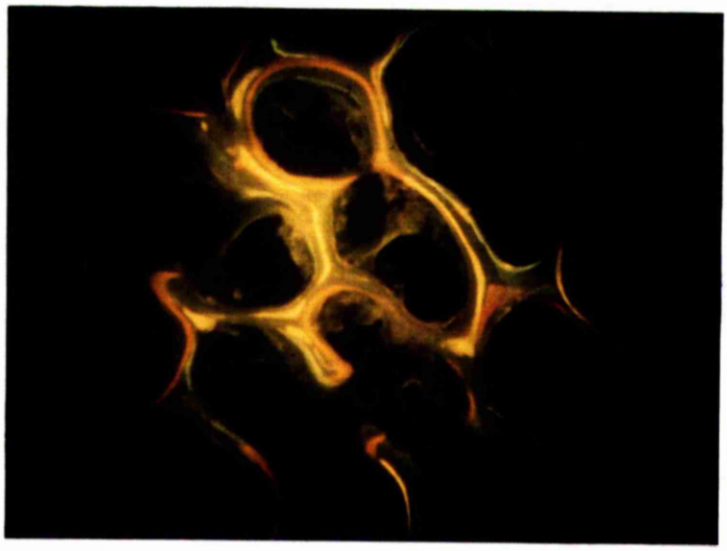


Fig. 86: Linear deposition of fluorochrome labels on the trabeculae of the right femoral epiphysis from dog 48/1.

(X 35)



DISCUSSION

Fluorochrome labelling of bone provides an excellent picture of the sequence and timing of new bone deposition since the labels are incorporated into bone calcifying at the time of administration (Milch et al 1957, 1958; Harris et al 1962; Urist and Ibsen 1963: Suzuki and Mathews 1966; Rahn and Perren 1971, 1972); they probably remain permanently in that bone which is labelled until resorption occurs (Frost 1961); and they may be distinguished from each other by the different fluorescent colours emitted on exposure to U.V. light (Rahn and Perren 1970, 1971, 1972).

Bearing these facts in mind, it is possible to reach the following conclusions from the results obtained with fluorochrome labelling of the experimental dogs.

1) New bone is laid down at the marginal zone remarkably early after cruciate section, the earliest recorded deposition occurring at 3 days. The development of the osteophyte at the marginal zone of the femoral trochlear was therefore the first recorded evidence of bone remodelling in this model of O.A.

2) All dogs killed 2 or more weeks after cruciate section showed some degree of new bone deposition at the marginal zone.

3) Accretion of mineral was progressive, new bone being laid down in successive layers on the outer surface of the osteophyte in a pattern which was suggestive of woven bone formation. Active remodelling of this woven bone soon followed, with resorption of central areas of the osteophyte and successive deposition of new bone so that a trabecular structure was formed.

4) Bone remodelling was still taking place 48 weeks after ligament section.

5) /

5) Development of the osteophyte at the marginal zone was not the only area to undergo bone remodelling. Fluorochrome uptake was recorded in the subchondral bone of the outer non-articulating face of the trochlear ridges. In longer suviving dogs this area of bone remodelling was noted to be confluent with the osteophyte.

6) There was also marked new bone deposition on the periosteal surface of the femoral cortex. Initially the pattern of fluorochrome distribution indicated this was woven bone. Subsequently, remodelling was apparent and in the longer surviving dogs a thickened cortex of compact bone was observed.

7) Fluorochrome labelling also indicated that appositional new bone was laid down on the trabeculae of the femoral epiphysis.

8) Increased fluorochrome uptake was observed in some of the control stifle joints, particularly of the longer surviving dogs. The possible significance of this observation has been discussed (p.

* * *

Reports by Marshall (1969) and by Marshall and Olsson (1971) record the use of fluorochromesin dogs in which transection of the anterior cruciate ligament had been performed.

In the earlier series by Marshall (1969), fluorochrome labels were given to a total of 8 dogs, and the maximum survival after surgery was 58 days. Marshall comments only briefly on the results obtained. He states that bone formation appeared to "reach a peak" 30 to 40 days after surgery but that no definite pattern was recognised. My results, however, indicate that a consistent pattern of fluorochrome labelling, and hence of new bone deposition, was observed in each case. No attempt was made to assess rates of new bone formation in the present study./

study.

Marshall recorded fluorochrome deposition in the osteophyte as early as 7 days after surgery; his finding was substantiated by the results of my experimental study. However, apart from the osteophyte, he did not find any other areas of "similar rapid growth". This statement does not make it clear whether in fact another area of dissimilar or less rapid bone growth was observed. Certainly no mention was made of subchondral or periosteal or trabecular fluorochrome uptake, all of which were noted before 8 weeks in the animals reported in this thesis.

The second series of experimental dogs (Marshall and Olsson 1971) were maintained for longer periods, the interval between surgery and death ranging from 209 to 698 days. Again, the description of fluorochrome distribution is brief: in the "early death" group (surviving 209 to 262 days), trabeculae of the osteophytes were labelled by "more appositional lines of all three fluorochromes than the trabeculae of the preformed bone". The authors accepted this as an indication of continued growth of the osteophytes. It is possible the pattern of labelling was similar to that recorded in the longer surviving (24 and 48 week) dogs of the present series, although no mention was made of other areas or patterns of fluorochrome distribution, such as were recorded in the present study. Marshall and Olsson also commented on the presence of labelling in their "late death" group: the last-administered label was "not found more frequently in the osteophyte than in the preformed bone", indicating cessation of growth at this stage. No experimental dog of the series reported here was maintained for a comparable length of time.

No comment was made on the presence or absence of fluorochrome labelling/

labelling of the control stifle joint in either of these earlier experimental studies.

The question of whether or not calcifying cartilage is labelled by fluorochrome deposition has been the subject of minor controversy. Milch et al (1958) did not find any fluorescence in the zone of provisional calcification at the epiphysis, nor in subchondral (articular) calcifying cartilage. Holmes (1963) recorded similar results. By contrast Hansson (1967) used tetracycline labelling to measure the rate of endochondral calcification in rabbits; his description of fluorochrome uptake in calcifying cartilage was however equivocal. Other workers have described fluorochrome labelling of calcifying cartilage in healing fracture callus (Urist and Ibsen 1963), in skeletal neoplasms (Milch et al 1961) and in non-specified "calcifying cartilage" (Frost et al 1960).

In this study, no fluorescence of calcified cartilage in the osteophyte was observed. A good example was provided by one 5-week dog labelled 24 hours before death, in which a discrete focus shown to be calcified cartilage on the corresponding microradiograph, did not show fluorochrome uptake. Adjacent bone was clearly labelled, and other sections of osteophytes from the same dog had a fringe of the last fluorochrome deposited in new bone on the outer edge. Perhaps this area of cartilage was not calcifying at the time of administration of the label, but an alternative and more acceptable explanation is that calcifying cartilage in this situation is not labelled by fluorochrome deposition.

Fluorochrome labelling proved to be an extremely useful method of determining the sequence of bone deposition. Correlation with the microradiographic appearance and histological findings was good, and, in conjunction with these other examinations, a clear picture of the process/

process of bone remodelling in this experimental model of O.A. was established. This has been discussed further in part VI.

PART V

MICRORADIOGRAPHY

REVIEW OF LITERATURE

Introduction

The earliest form of contact microradiography was attempted in the late 19th century simply by enlarging a radiograph taken with the object close to the photographic plate; since then the use of x-rays to study microscopic detail of tissues has undergone extensive development. Cosslett and Nixon (1960), in their book on x-ray microscopy, have provided a comprehensive account of historical development, basic principles, biological application, and techniques of contact microradiography.

Definition

Microradiography or x-ray microscopy is the examination of microscopic detail of tissues by means of x-rays: of all the available methods, contact microradiography is the simplest. It involves the recording of a one-to-one x-ray image on a photographic plate with subsequent enlargement by high power optical microscopy.

Application to the study of bone

a) qualitative microradiography

Differential absorption of the x-rays by mineralised tissue permits assessment of the state of mineralisation of bone not possible on histological examination of decalcified sections. Early work by Engström and Amprino (1950) showed that there was different absorption by different Haversian systems, and that this contrast was obliterated by decalcification of the sections prior to microradiography. A number of papers subsequently described the microradiographic appearance of normal human bone, and of bone in a variety of pathological conditions such as osteoporosis and Paget's disease, (e.g. Jowsey 1970, Jowsey et al 1965, Kelly/

Kelly, Peterson and Janes 1957, Sissons, Jowsey and Stewart 1960a, and Vincent 1954).

Using microradiography, Rowland and colleagues (Rowland, Jowsey and Marshall 1959) demonstrated clearly the difference in density of bone mineral between different species.

Areas of bone formation in compact bone may be recognised on microradiographs because newly-formed osteons are less mineralised and therefore appear less dense (McLean and Urist 1968, Sissons et al 1960a). Another distinguishing feature of new bone deposition described by Jowsey (1960) is the presence of lamellae of low mineral density arranged concentrically around the central canal of the osteon, forming a smooth surface.

Bone resorption is characterised by an uneven crenated surface of usually highly mineralised bone (Jowsey 1960, Sissons et al 1960a).

Osteocyte lacunae appear as small regular black dots (Vincent 1954, Cosslett and Nixon 1960, Jowsey et al 1965) although occasionally they may be filled with mineral and appear as white specks on the microradiograph (Jowsey 1960).

b) Quantitative microradiography

The mineral density of bone may be measured microradiographically if the method used is made quantitative. This may be achieved by including on the microradiograph an aluminium step-wedge and by employing a plane parallel bone section of known thickness. This has been described by various authors (Jowsey et al 1965, Rowland et al 1959, Sissons et al 1960b), and will not be discussed here since quantitative microradiography was not employed in this study.

c) Combination of microradiography with other techniques

A large number of publications testify to the value of combining microradiography/

microradiography with a variety of other techniques employed to determine bone ultrastructure. Thus, microradiographic examination of bone has been used in conjunction with fluorescent bone labelling (Harris et al 1968, Hulth and Olerud 1964, Jowsey et al 1965, Kelly et al 1963 Olsson and Reitz 1966, Reitz 1968), with conventional histological examination of stained decalcified sections (Kelly et al 1957, Sissons et al 1960a, Vincent 1954), with examination of unstained sections by transmitted or polarised light (Jowsey et al 1965, Kelly et al 1957, Kelly et al 1963, Olsson and Reitz 1966, Vincent 1954), and with autoradiography (Rowland 1966, Vaughan 1970, Vincent 1954).

d) Microangiography

This is a specialised form of microradiography in which the blood vessels are rendered radiopaque by means of a contrast medium which has been injected into them.

Trueta (1968) described a simple technique of perfusion with a Micropaque* (barium sulphate) and Berlin Blue mixture, and achieved good results using 1 mm thick sections which were decalcified prior to microradiography. Peterson and colleagues (Peterson et al 1957) and Kelly and Janes (1968) used either Micropaque alone or a mixture containing barium sulphate and gelatin to delineate blood vessels in cancellous bone, but preferred Thorotrast ** (thorium dioxide) for cortical bone studies. These workers also used decalcified bone sections of 400 μ, 1 mm or 2.5 mms thickness.

Decalcification of the bone prior to microradiography allows optimum visualisation of blood vessels, but obviously not of bone tissue. Microradiographs of undecalcified bone, however, show the relationship between bone and the blood vessels filled with contast medium (Rietz 1968).

Techniques/

* Micropaque - Damancy & Company Limited
** Thorotrast - Fellows Testagar

Techniques of Microradiography

The essential requirements for contact microradiography consist of an x-ray tube to produce a suitable beam of radiation, a fine-grain photographic emulsion to record the image and an optical system of high resolving-power to enlarge the image details to visible size.

a) X-ray source

Ideally, the x-ray tube should have a small focal spot, approximately 0.1 mm has been recommended for high-resolution investigations (Cosslett and Nixon 1960). Using an effective x-ray source of 1mm width, however, good microradiographs may be obtained provided the distance from tube to photographic plate is large so as to reduce geometrical blurring. High resolution was achieved in a study of undecalcified bone sections using a focal spot size of "less than 1mm wide", with a target-to-film distance of 20 cms (Jowsey 1960). Peterson and colleagues (1957) employed an effective focal spot of 0.7 by 0.8 mm, with a target-to-film distance varying from 14 to 22 cms.

Copper, which has a radiation wavelength of 1.54 Å, has been recommended as a suitable material for the x-ray target, (Peterson et al 1957, Jowsey 1960, Jowsey et al 1965) but targets of tungsten (Rowland et al 1959, Cosslett and Nixon 1960, Vincent 1954 and Olsson and Reitz 1966), and of chromium and molybdenum (Cosslett and Nixon 1960) have also frequently been used.

The target requires to be water-cooled because long exposure times are required, (Cosslett and Nixon 1960).

b) Recording of image

A fine-grain photographic emulsion is necessary to achieve good resolution/

resolution and the specimen must be as close as possible to the emulsion to minimise the penumbra (Cosslett and Nixon 1960). Jowsey and colleagues (1965) and Peterson and co-workers (1957) gave details of one method of achieving close specimen to film contact which they used successfully.

Jowsey and associates (1965) reported that sections of 100 μ thickness produce optimum microradiographs of undecalcified human bone. They stated that the use of thinner or thicker sections not only affected the apparent density of the specimen, but also altered the appearance of the bone surfaces.

Standard procedure for developing the photographic plate is described, the resulting microradiograph is an unmagnified image of the specimen.

c) Secondary magnification

Secondary magnification of the original image is obtained by light microscopy and by photomicrography of selected areas. In fact, as Cosslett and Nixon (1960) emphasised, the ultimate limitation of the contact method of microradiography is set by the resolving power of the microscope used for the final enlargement. Nevertheless, resolution approaching 1 μ may be achieved with suitable specimens (Bergendahl and Engfeldt 1960). With microradiographs of undecalcified bone sections, only lower magnifications are possible: Olsson and Reitz (1966) show a microradiograph of magnification 286 times.

MATERIALS AND METHOD

Undecalcified trochlear sections were prepared as described (Part II). Moisture was removed from the bone sections with tissues, and the sections were then positioned on the emulsion surface of a photographic plate (Ilford Maximum Resolution Plates). Latterly, a thin film of polythene ("cling-film" wrap) was used to cover the plate to protect it from any moisture remaining in the section.

Photographic plate and sections were then clamped firmly between two sheets of methyl-methacrylate (Perspex), the upper sheet being 1.5 mms thick and the lower supporting sheet 6 mms thick. This was then placed in the path of the x-ray beam at a distance of 28 cms from the target. A Machlett OEG 50A x-ray tube with a tungsten target and focal spot size of 1.5 mm was used. Exposure factors of 20 KV, 15 mamps, for 40 minutes' duration were necessary to achieve good bone detail from the 100 μ thick sections.

After exposure, the sections were removed and the photographic plate developed for $2\frac{1}{2}$ minutes in Kodak D19 under continuous agitation. The microradiographs were examined with a light microscope and photographs taken of selected areas.

RESULTS

GROUP A DOGS

Microradiographs were obtained from the undecalcified femoral trochlear sections after fluorescent microscopy had been carried out, and were therefore directly comparable with the fluorescent photomicrographs. Description of the microradiographic appearance has been subdivided into 4 categories, to correspond with the 4 areas of bone remodelling described in the histology and fluorescent labelling results, i.e. 1) osteophyte (marginal zone); 2) subchondral zone; 3) periosteal zone (femoral cortex) and 4) trabeculae.

1) Osteophyte - marginal zone

1 - 4 weeks

In those sections from dogs 1/3 and 1/4 in which traces of fluorochrome were present in the marginal zone, there were the merest traces of small, poorly mineralised deposits at this site. A similar appearance was present in some of the sections obtained from dogs killed at 2, 3 and 4 weeks after cruciate section (fig. 87).

Between 1 and 4 weeks the osteophyte consisted of bone which was relatively poorly mineralised compared to the pre-existing femoral cortex, and which had been deposited on the outer aspect of the intact cortex (figs. 88 and 89).

The finely-stippled appearance on the microradiograph was due to the presence of numerous osteoblasts, or osteocytes, within the cellular bone. At the outer edges of some osteophytes a slightly coarser stippling, which was associated with less dense mineral deposition, indicated the presence of chondrocytes within calcifying cartilage (fig. 89). There were many vascular channels within the new bone; these were non-radiopaque,/

radiopaque, smooth, regular channels, considerably larger in diameter than the chondrocyte lacunae (fig. 89). In perfused specimens, small radiopaque markings (blood vessels containing Micropaque) were visible either in the synovial membrane overlying the osteophyte (fig. 89), or lying within the vascular channels. In dog 4/3, a Micropaque-filled blood vessel had perforated the femoral cortex beneath the osteophyte. Larger, irregular cavities indicative of bone resorption were also present and were situated adjacent to the femoral cortex or in the centre of the osteophyte (fig. 89).

5-7 weeks

At this stage, the deposition of new bone at the marginal zone was still a discrete mass of cellular woven bone, clearly distinguishable from the original femoral cortex lying beneath it. Osteocyte lacunae and many vascular channels were identified in the osteophyte (fig. 90) and in some sections, small areas of calcified cartilage were apparent at the outer margin of the osteophyte. Fig.91 illustrates an unusual appearance of an osteophyte 5 weeks after cruciate section, in which a large area of calcified cartilage may be seen; this microradiograph may be compared with the fluorescent photomicrograph of the same section (fig. 70).

The size and number of resorption cavities within the osteophyte were increased, (figs. 90 and 91), in some cases with the suggestion of an early trabecular structure beginning to develop within the osteophyte. Sometimes focal resorption of the femoral cortex had occurred so that communication was established between the bone marrow spaces of the femur and the cavities within the osteophyte (fig. 91).

Where vascular perfusion had been performed, filled blood vessels could be seen lying in vascular channels, resorption cavities and overlying synovial/

synovial membrane.

8 - 10 weeks

In most dogs killed between 8 and 10 weeks after ligament section, the osteophyte at the marginal zone was considerably larger than at earlier stages. In addition the microradiograph demonstrated large resorption cavities within the centre of the osteophyte and early trabecular formation (fig. 92). The outer margin consisted of very cellular bone perforated by many vascular channels and having a honeycomb or sponge-like appearance (fig. 93). The mineral density of the bone was less than that of the pre-existing femoral cortex. In some dogs there appeared to be no communication between bone marrow spaces of the femur and the centre of the osteophyte, the femoral cortex being intact. In others, some communication had been established although the line of the original cortex was still readily identified (fig. 92). Identification of small blood vessels filled with Micropaque was possible in some of the perfused specimens, they were located in particular at the outer margins of the osteophyte in the cellular woven bone (figs. 92 and 93).

12 weeks

In two dogs (12/2 and 12/1) the appearance of the osteophyte resembled that described for dogs killed between 8 and 10 weeks. In dogs 12/3 and 12/4 a more mature ridge of bone was present at the marginal zone, consisting of well mineralised trabeculae with a somewhat haphazard and fragmented arrangement, and large inter-trabecular spaces (fig. 94). The ridge of new bone in some sections had free communication with the distal end of the femur, but in others the femoral cortex appeared intact. The outer border of the osteophyte (beneath the covering/

covering layer of cartilage, a uniform, faintly radiopaque layer on the microradiograph) was a thin plate of very cellular bone perforated by numerous vascular channels.

16 weeks

By 16 weeks after cruciate section, microradiographs showed a prominent ridge of new bone jutting out from the medial or lateral trochlear ridge, consisting largely of smooth, well-developed trabeculae of bone and wide inter-trabecular spaces (figs. 95 and 96). The femoral cortex beneath the osteophyte had been resorbed and remodelled in most sections, so that the trabeculae of the osteophyte appeared to be continuous with those of the femoral epiphysis (fig. 96). Free communication existed between bone marrow spaces of osteophyte and femur (fig. 95).

The mineral density of the trabecular bone of the osteophyte was similar to that of the original trabecular bone, as indicated by similar radiolucency on the microradiograph. However, in many sections there was a broad fringe of very cellular woven bone and a zone of calcified cartilage at the outer edges of the osteophyte, and this was of lower mineral density (fig. 95). In a few sections the outer border of the osteophyte was formed by a thin shell of compact bone with a slightly roughened outer edge (fig. 96) and covered by a thick uniform layer of non-calcified, faintly radiopaque tissue presumed to be cartilage.

In perfused specimens, small blood vessels were located chiefly in vascular channels in the less mature bone. This is well illustrated in fig. 97, which shows the highly vascular bone from the edge of a 16-week osteophyte.

24 weeks

In one dog at this stage (24/1) the microradiographic appearance of the osteophyte was similar to that at 16 weeks, and comprised a ridge of/

of dense, trabecular bone situated at the marginal zone (fig. 98). The outer border of the osteophyte consisted of a thin plate of very cellular bone and calcified cartilage in which there were a number of vascular channels.

In the other 24-week dog, the osteophyte development was continuous with the area of subchondral bony remodelling on the outer face of the trochlear ridge. This ridge of new bone was composed of well-mineralised trabeculae which however had a slightly irregular and fragmented appearance by comparison with the epiphyseal trabeculae. The outer plate of bone was very cellular and perforated by many small vascular channels (fig. 99).

48 weeks

By 48 weeks after cruciate section remodelling of the trochlear ridge was evident from the microradiographic appearance, a smooth ridge of trabecular bone extending from the tip of the trochlear ridge to the synovial membrane attachment (fig. 100). The trabeculae were composed of mature well-mineralised bone and had a regular pattern. The outer edge of the remodelled ridge had a slightly irregular pattern of bone deposition, in which the presence of vascular channels, small irregular resorption cavities and, in dog 48/2, small Micropaque-filled blood vessels, indicated that bone remodelling was still going on (fig. 101).

2) Subchondral Zone

1 - 5 weeks

Very little or no change in the microradiographic appearance of the subchondral zone was observed in the trochlear sections from dogs killed between 1 and 5 weeks after cruciate section. With the knowledge that histological sections and fluorochrome uptake indicated bone remodelling in/

in this region in some dogs, very careful scrutiny revealed possible early changes in sections from dogs 4/1 and 5/3. These changes consisted of very small resorption cavities and slightly enlarged osteocyte lacunae in the subchondral bone plate. The superficial aspect of the bone was possibly slightly more irregular and "fuzzy" in outline than normal, although this surface is normally not completely smooth. One or two small, regular vascular channels were present in subjacent trabeculae, adjacent to which small areas of bone with indistinct edges were identified as possible sites of new bone deposition. It was however very difficult to detect these minor changes on the microradiograph.

6 - 10 weeks

The first obvious evidence of bone remodelling in the subchondral zone was noted at 6 weeks. The appearance was that of an irregular, thin, sponge-like layer of cellular bone or calcified cartilage situated on the outer edge of the subchondral bone plate and continuous with it. The degree of mineralisation of this layer was less than that of pre-existing bone. An occasional focus of bone resorption was identified in the subchondral bone.

A similar microradiographic appearance was observed in femoral trochlear sections from the majority of dogs killed between 6 and 10 weeks after cruciate section (figs. 102 and 103). However, the thickness of the spongey layer, as well as the extent of involvement of the trochlear ridge, varied a little in different animals. It was not always possible to state from the microradiographic appearance whether the new mineral deposition consisted of woven bone or calcified cartilage. Sometimes the appearance resembled calcified cartilage rather than bone, consisting of a poorly mineralised and ill-defined layer with radiolucent holes of comparable/

comparable size to chondrocyte lacunae within the osteophyte. In other sections the appearance was more like that of woven bone.

Occasionally, small Micropaque-filled blood vessels were noted in association with the area of bone remodelling (figs. 103 and 104).

In dog 9/3, remodelling of the trabeculae adjacent to the area of subchondral new bone was observed, the trabeculae appearing irregular in shape and distribution.

12 - 16 weeks

At this stage, sections in which subchondral bone remodelling was observed showed slight to moderate degrees of trabecular remodelling associated in some cases with a distinct deposition of a layer of spongey new bone on the outer face of the trochlear ridge. Occasionally marked trabecular remodelling was recognised by an increase in thickness and an irregularity of width of trabeculae and disorientation of the normal regular pattern (fig. 105).

In some sections from perfused specimens, small blood vessels were noted at the outer edge of the area of remodelling.

24 weeks

In dog 24/1, a localised area of remodelling of subchondral trabeculae associated with a thin outer layer of well-mineralised but "spongey" new bone was present in one section (fig. 106).

The other 24-week dog showed advanced subchondral remodelling, the whole of the outer face of the trochlear ridges being involved. This area of remodelling was confluent with the osteophyte at the marginal zone in most sections. The width of the trochlear ridge was obviously increased, and the newly formed trabeculae were irregular and of slightly variable mineral density (fig. 107). Small Micropaque-filled blood vessels/

vessels were present in the outer edge beneath the cartilage (fig. 107).

48 weeks

In both dogs killed 48 weeks after surgery there was obvious remodelling of the entire trochlear ridge, so that the distinction between "subchondral zone" and "osteophyte" was no longer valid. The microradiographic appearance at this stage has already been described (p.158) and is illustrated in figs.100 and 101.

3) Periosteal Zone

1 - 5 weeks

No periosteal new bone deposition on the femoral cortex was seen in sections from dogs killed 1 and 2 weeks after cruciate section, the earliest deposition of periosteal bone being recorded at 3 weeks.

Between 3 and 5 weeks, the microradiographic appearance of the periosteal surface of the femoral cortex was variable. In many sections the surface was a normal, fairly smooth outline but in others the surface was slightly roughened. Where periosteal new bone had been laid down, the microradiograph showed either an irregular, focal deposit or a thin, diffuse layer of very cellular woven bone having a "wavy" outline (fig. 108). The new bone had a sponge-like or honeycomb texture and was not as well mineralised as the pre-existing femoral cortex. In some cases, a thin radiolucent line separated the layer of new bone from the original cortex.

6 - 10 weeks

The majority of sections from dogs killed between 6 and 10 weeks showed new bone deposition on the femoral cortex. This layer of new bone was of variable thickness and consisted largely of cellular woven bone traversed/-

traversed by regular vascular channels, which resulted in a distinctive sponge-like appearance (fig. 109). Examination at higher magnifications revealed the presence of numerous regularly distributed linear or fibrillar markings within the woven bone. These fine, linear radiolucent marks presented a parallel or herring-bone pattern and were thought to indicate the pattern of collagen fibre distribution; they were noted within woven bone at various stages of development (see fig 111).

By 10 weeks, large resorption cavities were present in some of the sections in the deeper layers of the periosteal new bone both adjacent to and involving the original femoral cortex.

12 - 16 weeks

At this stage after cruciate section most sections showed a fairly thick layer of well-mineralised new bone on the periosteal surface of the femoral cortex. The outer fringe of this layer consisted of cellular woven bone, but, adjacent to the original cortex, there was mature bone with an "open lattice-work" type of distribution (fig. 110) which in some areas resembled an irregular and ill-defined trabecular pattern. Osteons at various stages of development were also noted in some sections, particularly in dogs killed at 16 weeks.

24 weeks

In dog 24/1, the periosteal new bone consisted of a broad layer of largely trabecular bone, the trabeculae being thin and somewhat irregular in shape and distribution but composed of well-mineralised bone. There was still a fringe of cellular woven bone at the outer edge, perforated by many small vascular channels, and in which a number of irregular surfaces undergoing bone resorption could be identified (fig. 111).

The/

The other 24-week dog presented a very similar appearance but the trabeculae were broader and more regular in shape and distribution and a number of developing osteons were located in the outer fringe of woven bone.

48 weeks

At 48 weeks after cruciate section, the periosteal region consisted of a broad layer of compact cortical bone with many Haversian systems in cross-section showing different stages of development (fig. 112), in some sections only the outer edge consisted of compact cortical bone, and deep to this there were well-developed, broad, regular trabeculae.

4) Trabeculae

The microradiographic appearance of normal trabeculae (from control trochlear sections) was not uniform. In general, however, they had a regular distribution, the surface was smooth and the degree of mineralisation even. Sites of new bone deposition and irregular edges undergoing resorption were noted infrequently. The width of the trabeculae appeared to vary a little in different areas of the section, and with different planes of section through the trochlea. Occasionally, sections from control femoral trochleas showed obvious bone remodelling of trabeculae.

Because of the variable normal appearance, the large area of trabeculae in any one section, and the subtle nature of the microradiographic changes, it was not easy to assess the microradiographic appearance of trabeculae from operated joints. However, with increasing time following section of the cruciate ligament there was an increase in bone remodelling, as shown by the number of sites of new bone deposition and bone resorption/

resorption (fig. 103). In some of the longer surviving dogs the trabeculae appeared to be thinner and more fragmented than those of comparable control sections.

GROUP B DOGS

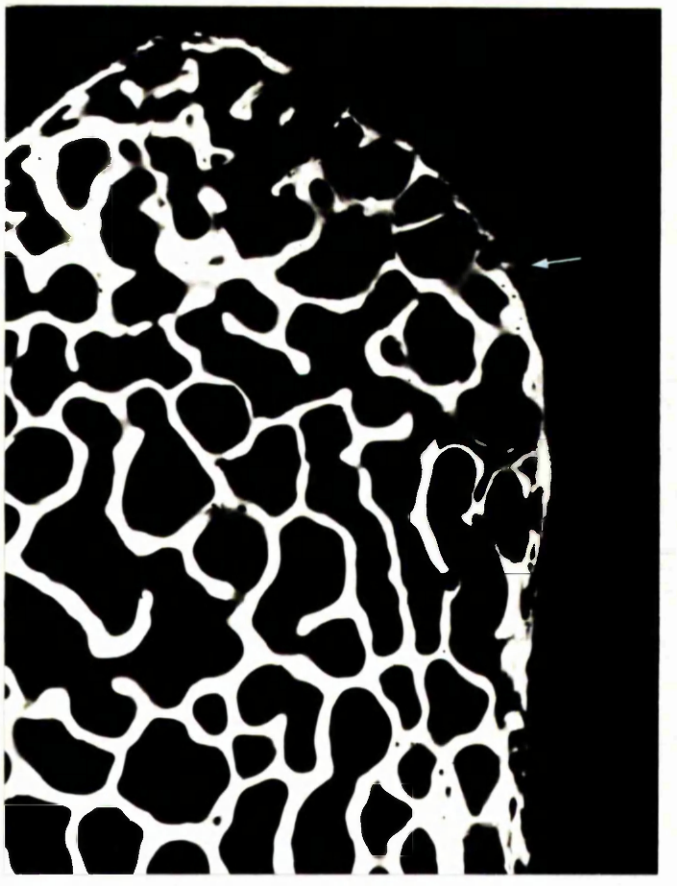
Since there was no evidence of bone remodelling either macroscopically or microscopically (on fluorescent microscopy and histological examination), microradiographs were not obtained from the undecalcified trochlear sections of the sham-operated dogs.

Fig. 87: Lateral trochlear ridge from dog 2/4. The marginal osteophyte is just discernible (arrowed). Compare fig. 68

(X 12)

Fig. 88: Higher magnification of another 2 week osteophyte (dog 2/4). Note areas of bone resorption (*) and synovial membrane (S.M.) Compare fig. 67.

(X 40)



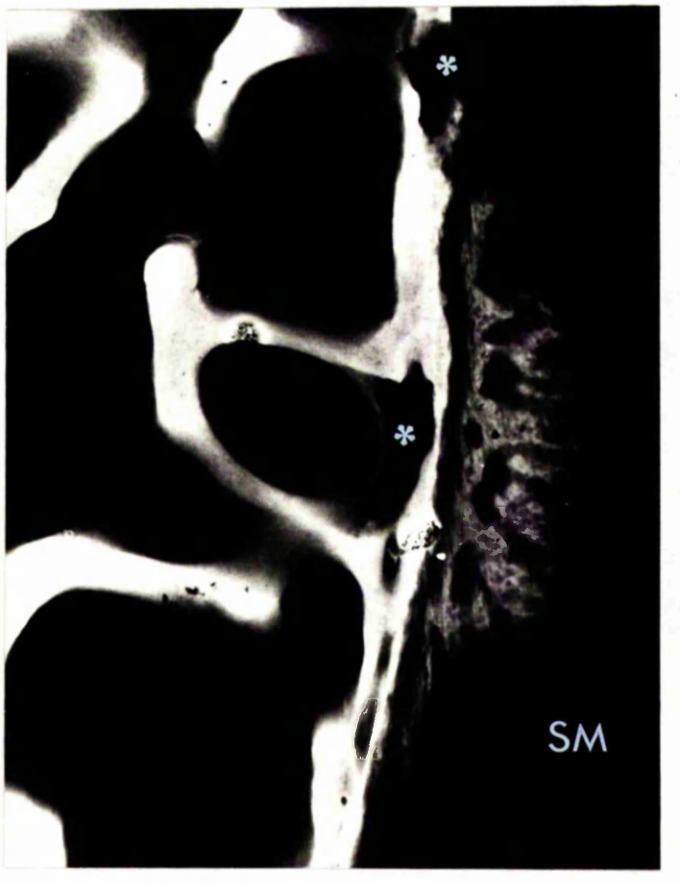
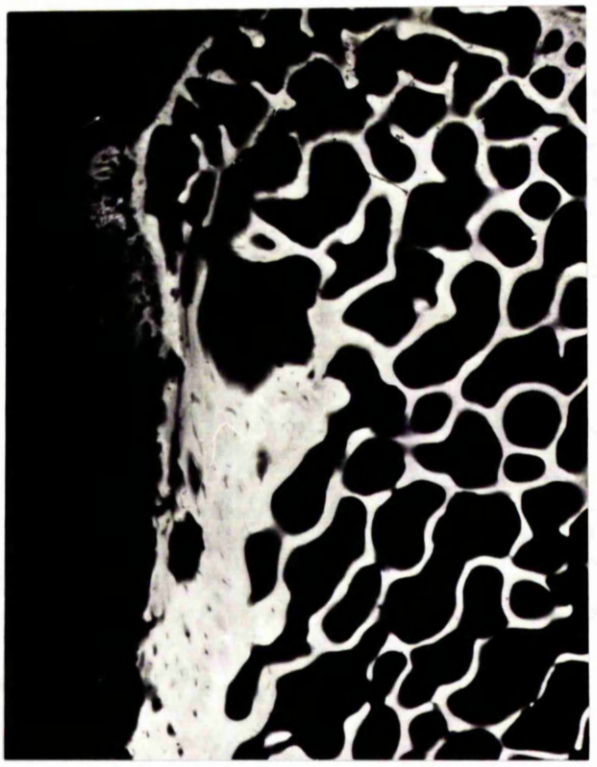

*
*
SM

Fig. 89a: Medial trochlear ridge from dog 4/1. Note three small perfused blood vessels in synovial membrane overlying osteophyte.

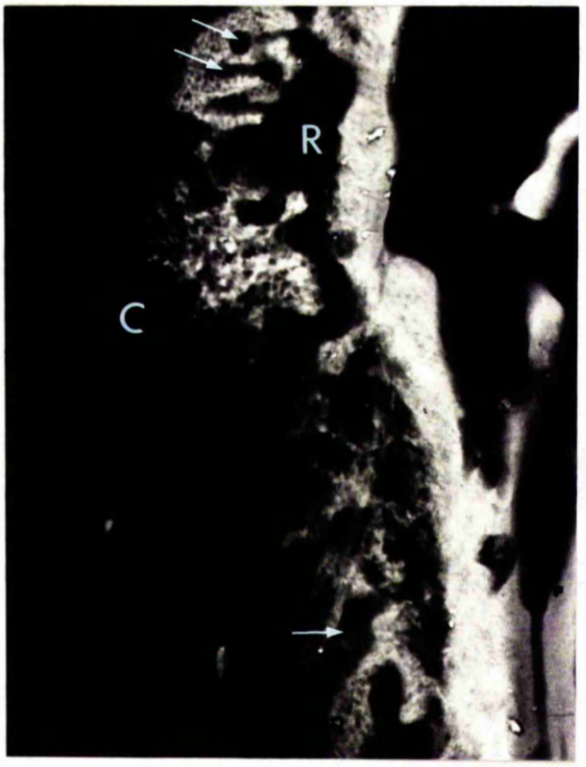
(X 12)

Fig. 89b: Enlargement of (a). The following features may be identified:
Calcified cartilage (C), vascular channels (arrowed) and resorption cavities (R).
The femoral cortex is intact.

(X 40)



a



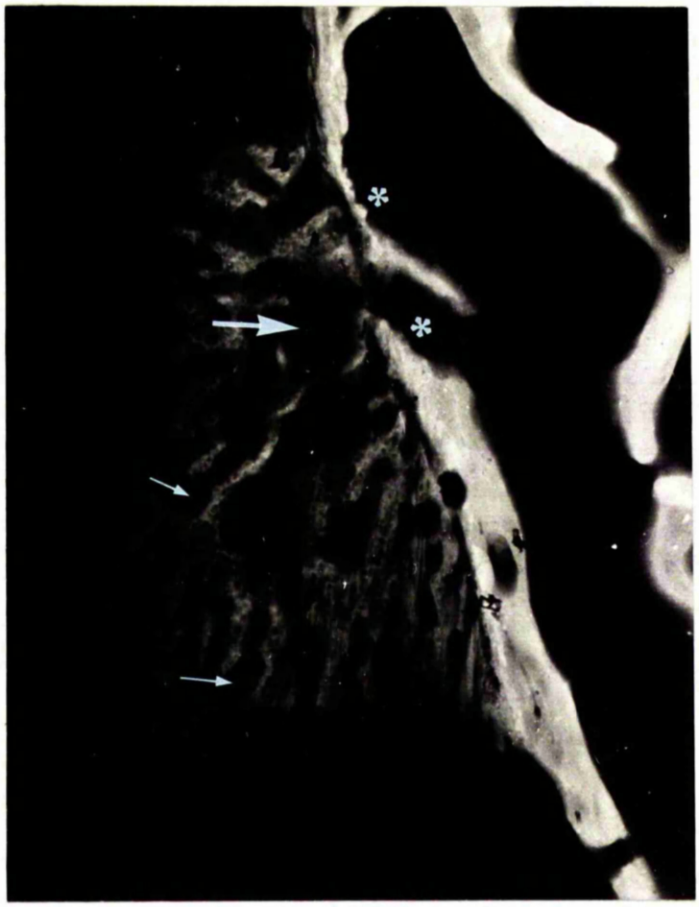
b

Fig. 90: Medial osteophyte from dog 5/1. Small regular vascular channels have been arrowed (small arrows). Larger irregular resorption cavities (large arrow) and resorption of the femoral cortex (*) may be identified. The stippled appearance of the bone is due to the presence of many osteocyte lacunae. Compare fig. 70.

(X 40)

Fig. 91: Lateral osteophyte from dog 5/2 showing large area of calcified cartilage (C) at outer edge. Resorption of the femoral cortex has occurred at *. Compare fig. 69.

(X 40)




C

Fig. 92: Large osteophyte on lateral trochlear ridge at 8 weeks (dog 8/1). A few small Micropaque-filled vessels may be seen (arrowed). Communication between bone marrow spaces of femur and osteophyte is obvious. (X 12)

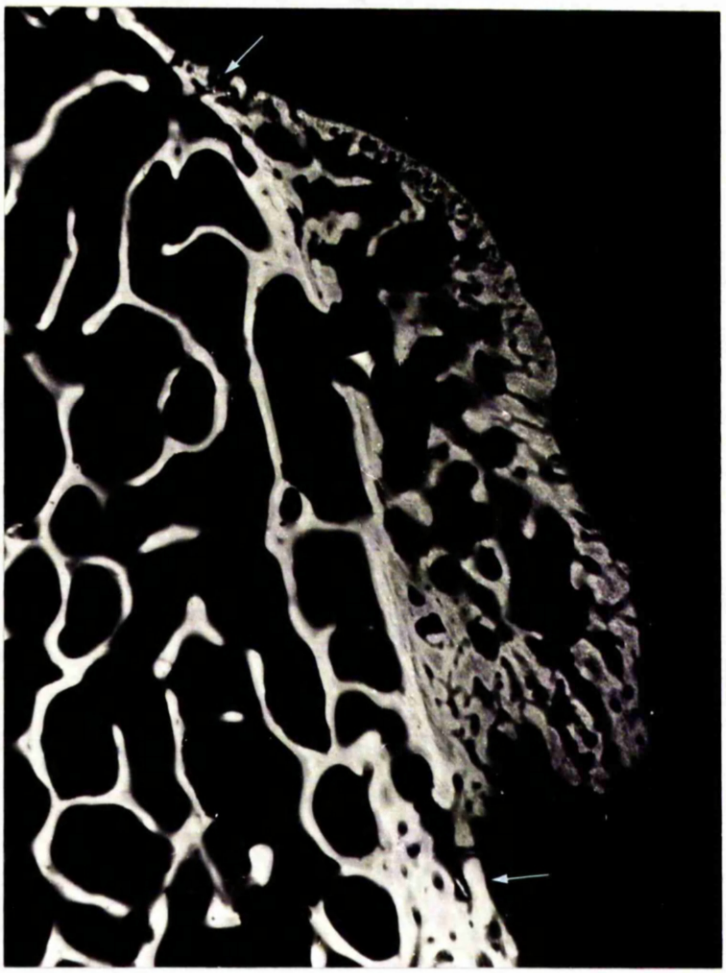


Fig. 93: Higher magnification of lower edge of osteophyte of fig. 92 (lateral osteophyte from dog 8/1), showing the cellular woven bone perforated by smooth regular vascular channels in which small amounts of Micropaque are visible (arrowed).

(X 40)

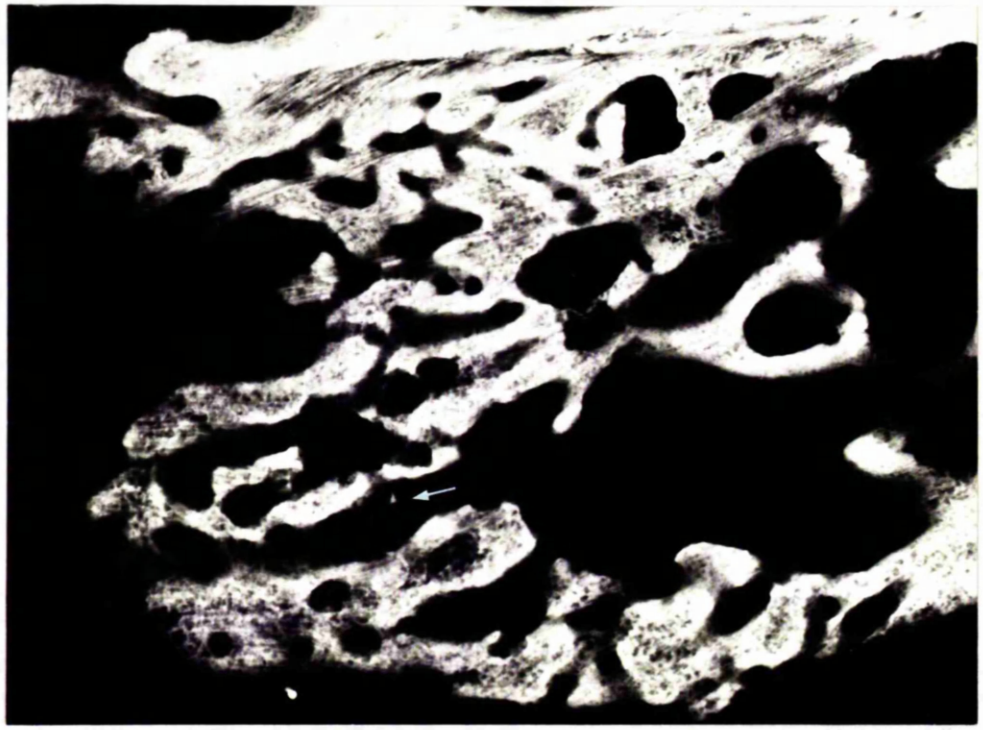


Fig. 94: Medial osteophyte from dog 12/3 showing the irregular and somewhat fragmented arrangement of the trabeculae with wide inter-trabecular spaces, and the outer edge of the osteophyte (beneath the very faintly radiopaque cartilage) perforated by many vascular channels.

(X 35)

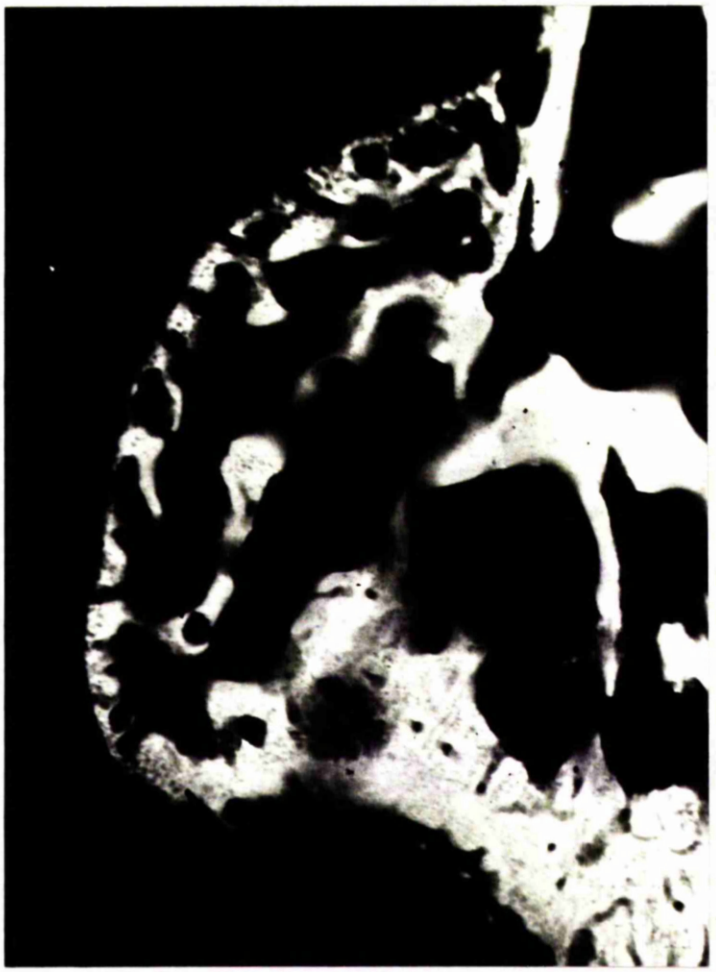


Fig. 95: Microradiograph of lateral trochlear ridge from dog 16/3, the prominent osteophyte consisting of well mineralised cancellous bone having a slightly fragmented trabecular pattern, and a broad fringe of cellular woven bone containing many vascular channels and resorption cavities. A zone of calcified cartilage may be seen on the outer edge (arrowed). Compare with fig. 75 .

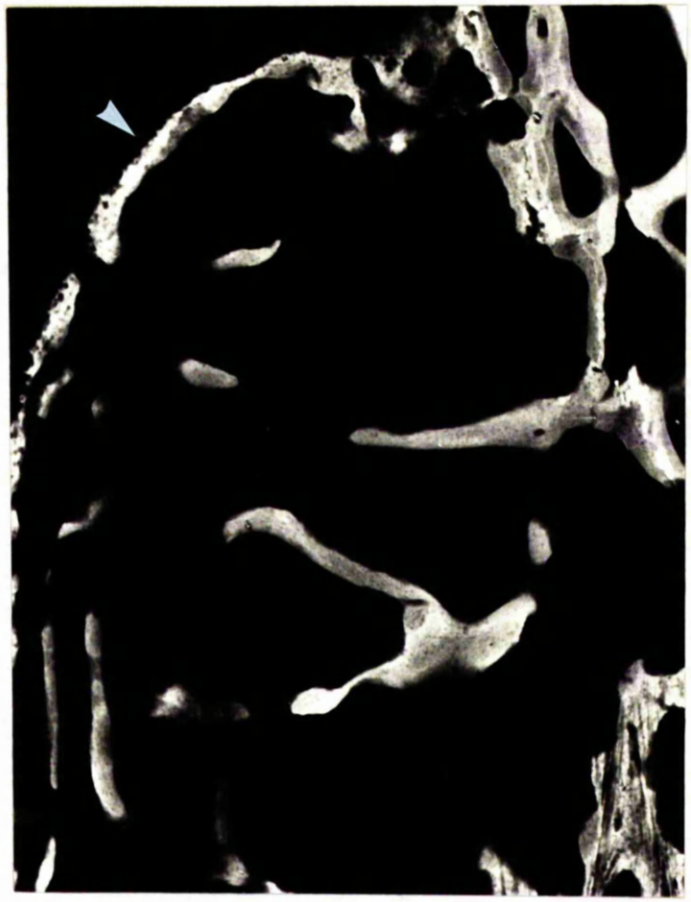
(X 12)

Fig. 96: Part of a 16 week osteophyte (dog 16/4) showing the smooth trabeculae and continuity between epiphyseal trabeculae (on the right) and those of the osteophyte. The outer border of the osteophyte (arrowed) is formed by a thin shell of bone beneath fibrocartilage.

(X 40)

Fig. 97: The edge of a 16 week osteophyte from a perfused specimen showing numerous Micropaque-filled blood vessels visible as densely-opaque markings within the new bone.

(X 40)



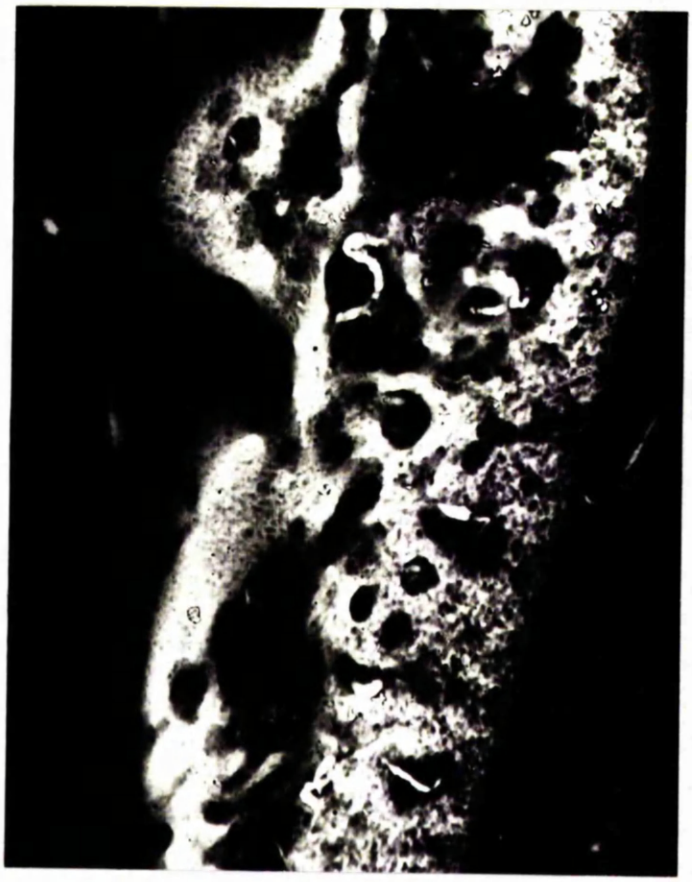


Fig. 98: Microradiograph of the lateral trochlear ridge from dog 24/1, showing a well developed ridge of new bone at the marginal zone comprised largely of trabecular bone. A fringe of woven bone may be identified at the proximal part of the outer edge. Distally the osteophyte is continuous with a broad band of periosteal new bone.

(X 12)

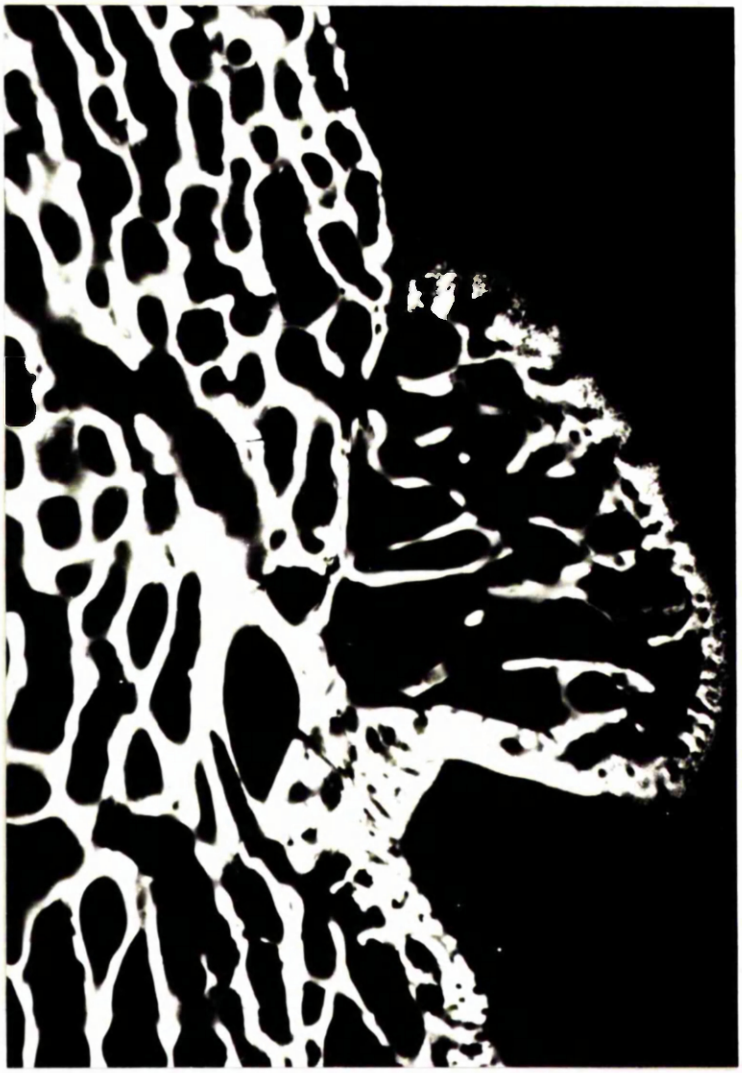


Fig. 99: Microradiograph of osteophyte on medial trochlear ridge from dog 24/2. The osteophyte consists of well-mineralised trabeculae of a slightly irregular shape and distribution. The outer plate of cellular bone is perforated by many vascular channels. A few small Micropaque-filled vessels (arrowed) may be seen within the osteophyte.

(X 35)

Fig. 100: Microradiograph of lateral trochlear ridge 48 weeks after cruciate section (dog 48/2). The areas of subchondral remodelling, marginal osteophyte formation and periosteal new bone deposition are confluent, resulting in recontouring of the outer face of the trochlear ridge.

(X 12)

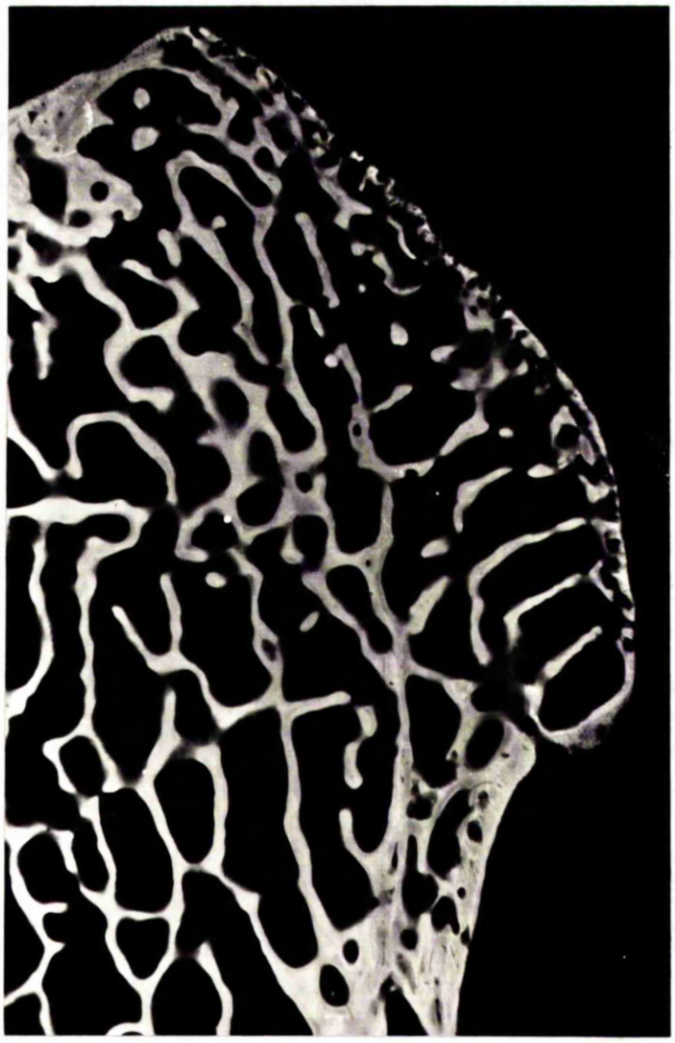


Fig. 101: Higher magnification of part of fig. 100. A few small micropaque-filled blood vessels have been arrowed. Compare with fig. 79a.

(X 40)

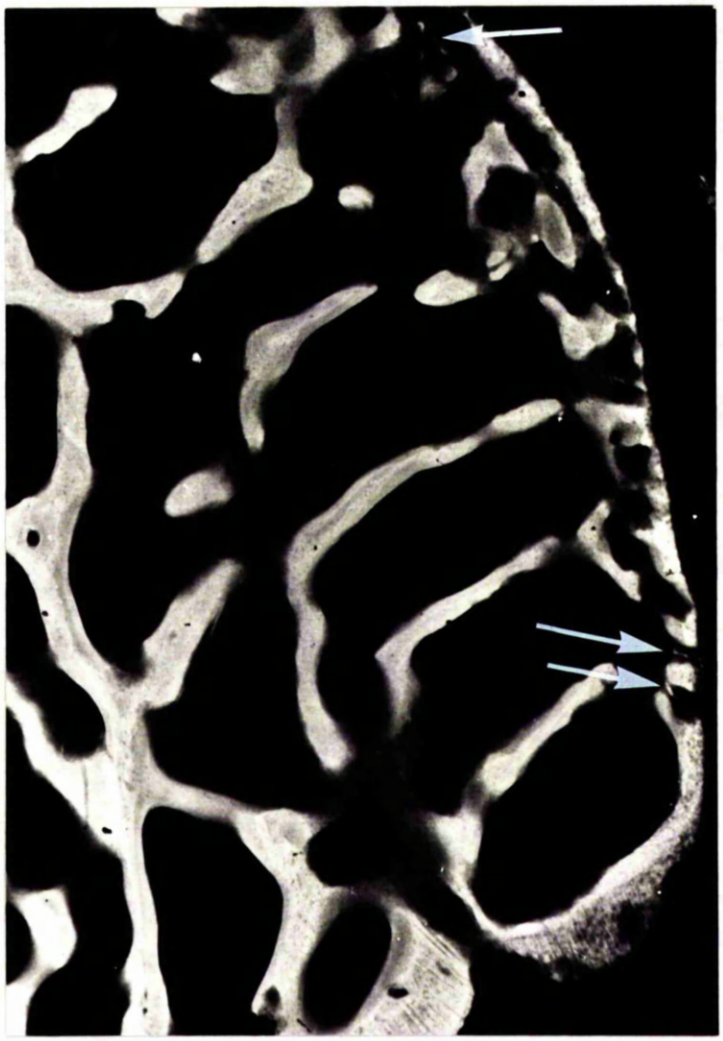
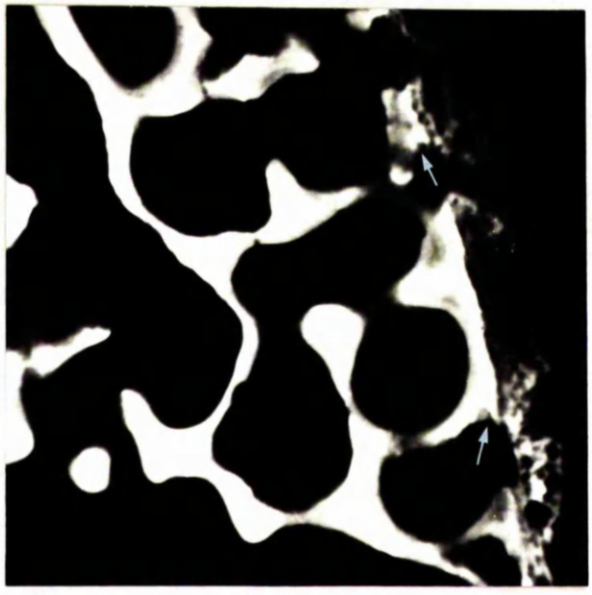


Fig. 102: Subchondral zone remodelling of lateral trochlear ridge from dog 8/2. Small foci of bone resorption in subjacent bone are arrowed.
(X 35)

Fig. 103: Subchondral remodelling of lateral trochlear ridge from dog 7/3. Note perfused blood vessels and trabecular remodelling; new bone deposition (short arrows) and bone resorption (long arrows)
(X 35)

Fig. 104: Higher magnification of subchondral zone from dog 7/2, showing spongey new bone deposition and associated blood vessels (perfused).
(X 100)



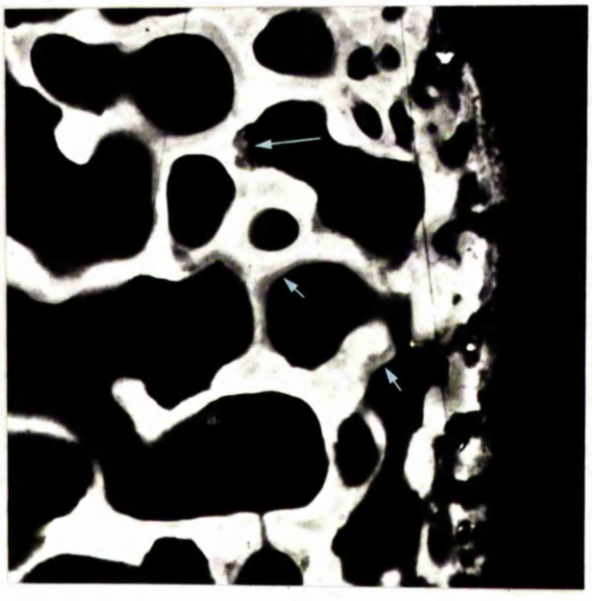


Fig. 105: Part of the lateral trochlear ridge at 12 weeks (dog 12/3) showing trabecular remodelling in the subchondral zone. The remodelled trabeculae are of irregular width and shape.

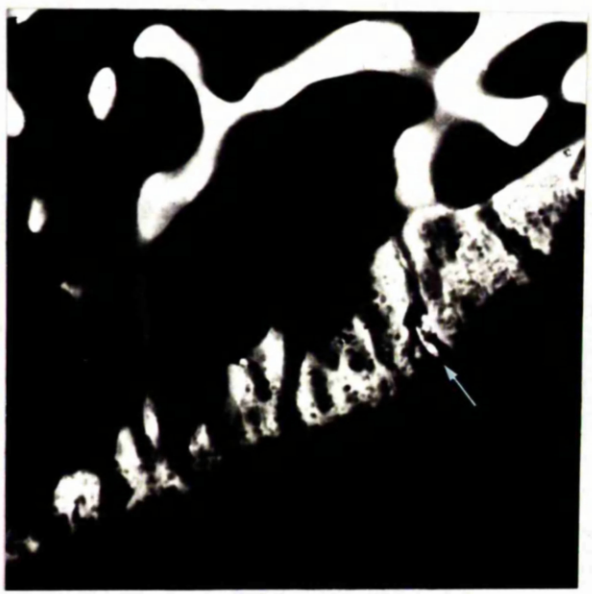
(X 40)

Fig. 106: Subchondral zone remodelling of medial trochlear ridge from dog 24/1.

(X 35)

Fig. 107: Subchondral zone remodelling at tip of lateral trochlear ridge from dog 24/2. Marked increase in width of the trochlea has resulted. A small Micropaque-filled blood vessel is arrowed.

(X 35)



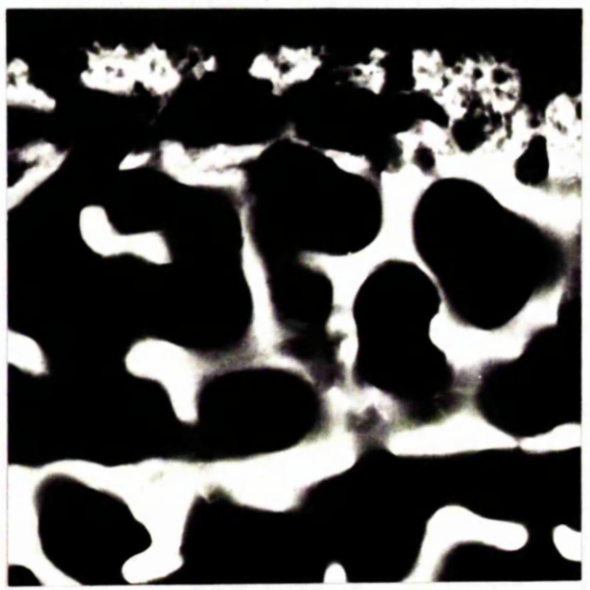
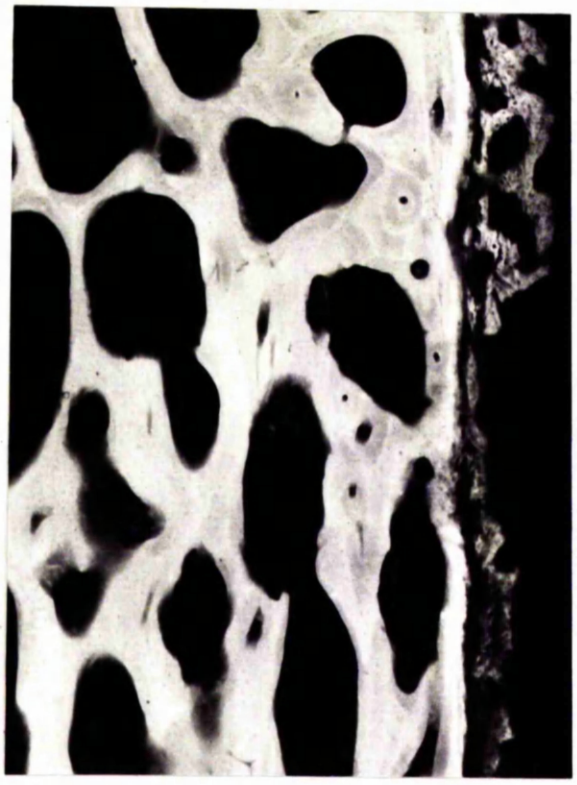


Fig. 108: Lateral femoral cortex from dog 4/2 showing the deposition of very cellular, spongey new bone on the periosteal surface.

(X 35)

Fig. 109: Periosteal new bone deposition on lateral femoral cortex from dog 8/2, showing the distinctive sponge-like appearance.

(X 35)



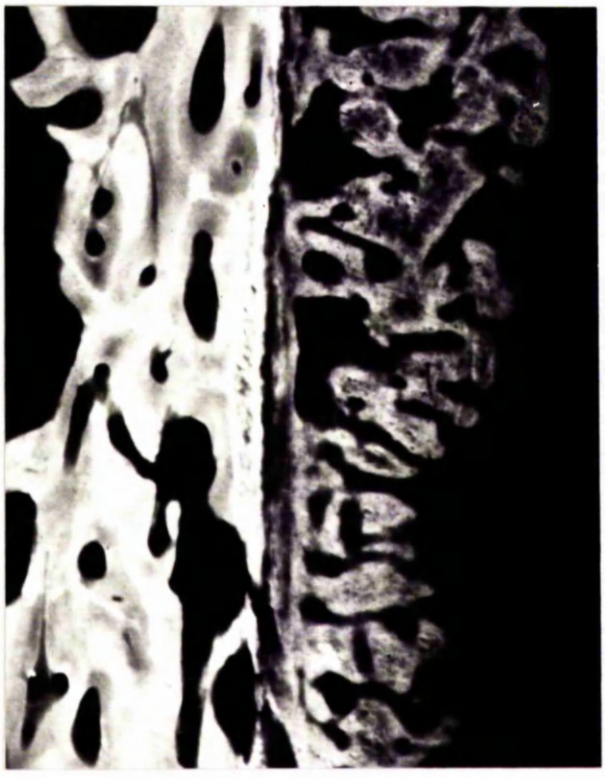
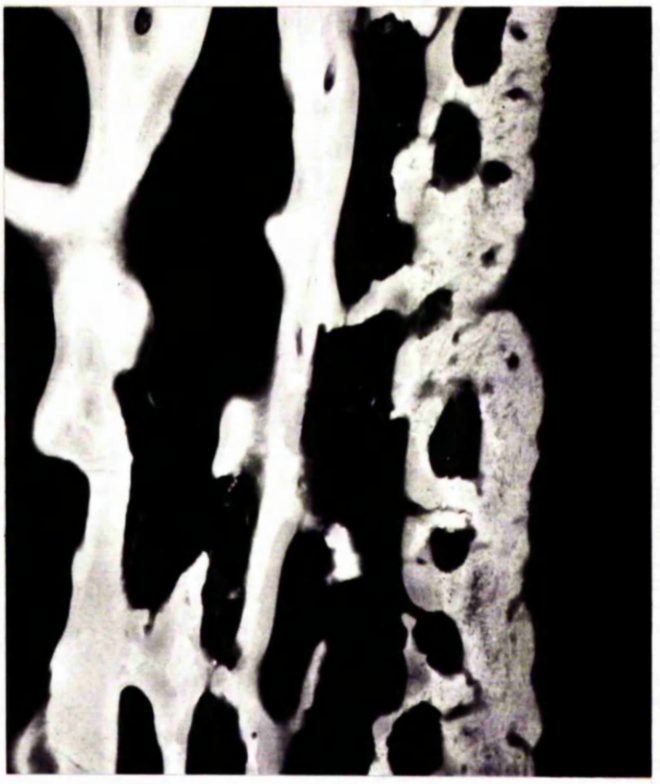


Fig. 110: Periosteal new bone deposition on the lateral femoral cortex of dog 12/3. The outer edge is composed of woven bone but resorption and remodelling has occurred adjacent to the original cortex.
(X 35)

Fig. 111: Periosteal remodelling of the lateral femoral cortex from dog 24/1. On the outer edge of the broad band of irregular trabeculae is a fringe of woven bone.
(X 40)



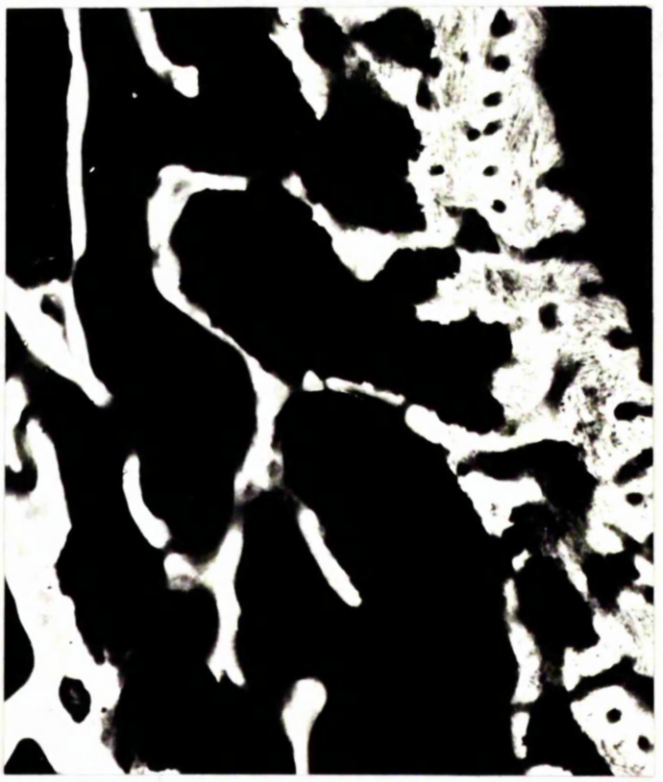
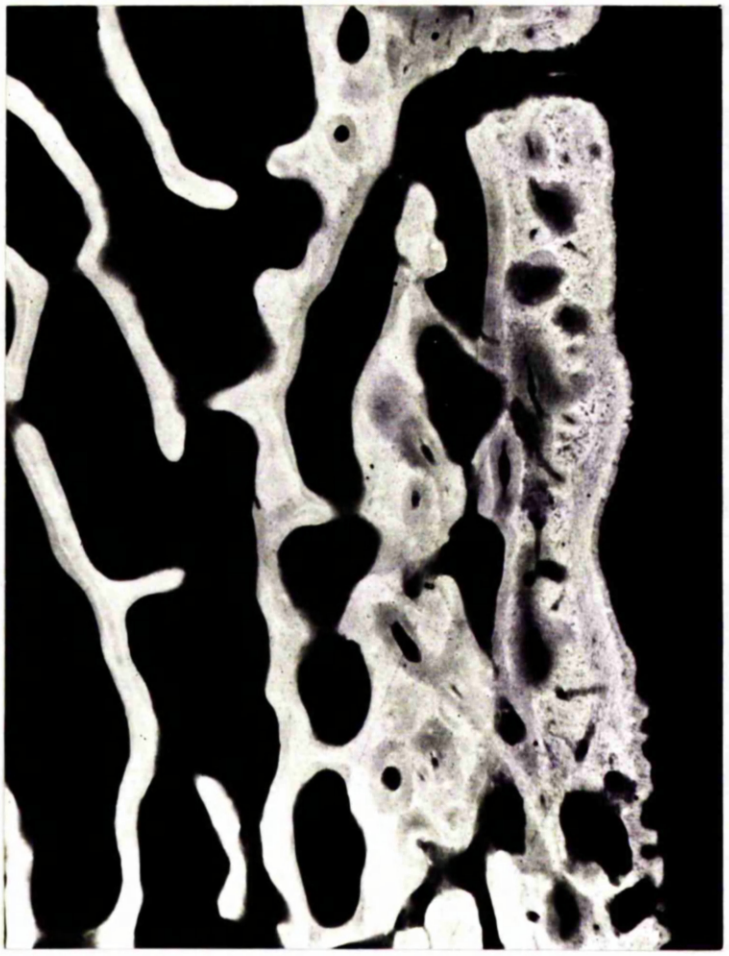


Fig. 112: Lateral femoral cortex from dog 48/2. At this stage new bone deposition and remodelling has resulted in a thickened cortex composed largely of compact bone. Many osteons may be identified at different stages of development.

(X 35)



DISCUSSION

The application of microradiography to undecalcified femoral trochlear sections proved a useful means of assessing the bony remodelling occurring in the stifle joint following section of the anterior cruciate ligament.

Sites of new bone formation were readily identified on the microradiographs, and it was possible from the appearance to draw some conclusions about the nature of the tissue as well as the degree of mineralisation. The association between new bone development and vascular proliferation was clearly demonstrated in those specimens where vascular perfusion with Micropaque-dye mixture had been carried out.

Microradiography has been applied to the study of experimental O.A. by other workers, such as Marshall (1969), Marshall and Olsson (1971) and Paatsama and Sittnikow (1972), but each report gives only very brief descriptions of the results obtained. In Marshall's first series in which the dogs survived from 13 to 58 days after cruciate section, the osteophyte if "recent" did not show communication between the marrow of the osteophyte and that of the femur, but with increasing maturity, "the marrow cavity of the osteophyte was in direct contact with the marrow cavity of the femur", (Marshall 1969). In the second series, in which the duration of survival was longer (minimum of 30 weeks after surgery), the microradiographic appearance of the osteophyte was described as "mature", the marrow spaces apparently blended with those of the preformed bone and the surface of the osteophyte was smooth and regular, (Marshall and Olsson 1971).

Neither of these reports describes the appearance of other areas of bone remodelling apart from the osteophyte. In another experimental series,/

series, Paatsama and Sittnikow (1972) employed 13 dogs, the duration of survival ranging from 3 to 29 weeks after ligament section. However, the microradiographic appearance of the osteophyte was described only from 3 to 6 weeks after surgery. These authors also carried out vascular perfusion with barium sulphate, and a microradiograph from one dog killed 3 weeks after surgery clearly shows contrast-filled blood vessels in association with new bone deposition.

As already indicated, the descriptions of the microradiographic appearance given in each of these 3 reports are not detailed enough for any useful comparison to be made with the results of the present study. Nevertheless, as far as can be ascertained, there appears to be general agreement between the results of the earlier investigations and those recorded in this thesis.

Comparison of microradiographs with corresponding fluorescent photomicrographs proved especially useful in interpretation of the appearance shown at different stages. Discrete deposits of new bone at the marginal zone or on the periosteal surface of the femoral cortex were easy to identify on the microradiograph but sites of appositional bone deposition on trabeculae or early subchondral remodelling were not so readily detected on the microradiograph as on the fluorescent photomicrograph in which small traces of label could be seen very easily. On the other hand, details such as cellular structure, areas of bone resorption, distribution of vascular channels were more readily identified on microradiographs. Even more information about the development of bone remodelling was obtained by correlating microradiograph and fluorescent photomicrograph with the histology of an adjacent decalcified trochlear section..

PART VI

GENERAL DISCUSSION

In spite of pronounced joint instability, most dogs began to use the operated limb quite well within 4 weeks of surgery. Approximately one-third of the dogs were bearing weight as early as 1 week post-operatively when forward drawer movement in the stifle joint in most cases was either "obvious" or "marked".

Indeed, of the 5 dogs found to have both anterior and posterior cruciate ligaments sectioned (and therefore with relatively more unstable joints), only 1 dog (2/3) showed persistent marked lameness, and the others were weight bearing by 1, 2, or 3 weeks after surgery. In general both the degree of lameness and the joint instability diminished with time following surgery, however no direct relationship was established between improvement in limb function and increasing joint stability in the majority of dogs. Some individuals (e.g. 9/2, 10/2) did show less lameness at the same time as a reduction in forward drawer movement was recorded, but more often an obvious or marked drawer forward could be elicited in dogs with relatively mild lameness (e.g. 5/4, 9/1, 10/1, 10/4, 16/1). This lack of correlation between lameness and joint instability is contrary to the observation by Marshall and Olsson (1971) that "lameness decreased or disappeared with decreasing instability".

Marshall and Olsson (1971) also commented that "there was persistent pain as long as marked instability remained", inferring that pain and joint instability are directly related. Again, the results recorded in the present experimental series do not confirm this: no dogs showed pain on manipulation of the joint 1-3 days after surgery and only 7 out of 31 dogs showed definite pain at 1 week although marked instability was present.

The histogram of fig. 4 shows 3 "peaks" of relatively high incidence of pain in the stifle joint: at 3 weeks, corresponding to the onset of significant/

significant synovial effusion in the joint, and at 8 weeks and 12-16 weeks when a pronounced synovial effusion was recorded (figs. 6 and 7). Perhaps manipulation or distension of an inflamed synovial membrane and joint capsule produces pain in the joint. However, some dogs evinced pain in the absence of synovial fluid swelling. Thus although there is an apparent correlation between the presence of pain and the amount of synovial effusion present in the joint, no conclusions can be drawn about a direct "cause and effect" relationship.

The unstable stifle joint may well be more prone to injury than a normal joint, relatively minor trauma such as slipping on a concrete floor having been observed to cause a marked exacerbation of clinical signs. The early use of an unstable joint could be responsible for repeated minor trauma to the joint structures, causing persistent synovial inflammation, meniscal splitting and cartilage degeneration. However if such a relationship exists, it is certainly not obvious from the results of this experimental study. Certainly no correlation was observed between the presence of meniscal damage and the degree of lameness. For example, between 2 and 10 weeks after cruciate section, a total of 23 dogs showed medial meniscal damage varying from mild to severe splitting, of these, 18 dogs were bearing weight and 5 dogs were showing moderate to marked lameness of the operated limb; a further 10 dogs showed either no meniscal damage or very early surface fibrillation and yet were weight-bearing on the operated leg. Similarly, it was not possible to establish any correlation between the degree of cartilage degeneration observed and the presence or absence of lameness.

Rapid onset of atrophy of the quadriceps muscle is regarded as a prominent feature in naturally occurring cases of cruciate rupture (Hickman/

(Hickman 1964), and is also recorded in the experimental series of Paatsama (1952) and Marshall and Olsson (1971). Similarly, in the present experimental series, rapid and pronounced muscle atrophy was recorded in the majority of dogs. Of the 28 dogs in which muscle atrophy was detected 1 to 3 weeks after ligament section, 18 were using the leg well, while 10 showed moderate to marked lameness, suggesting there is little correlation between the presence of atrophy and the degree of lameness. However, in the longest surviving animals, no muscle atrophy was detected in the later stages of the experiment when these dogs had become sound, and it seems logical to assume that the return to 'normal' limb function was responsible for building up the muscle volume. Thus the relationship between muscle atrophy and lameness following cruciate section remains equivocal.

The presence of a "click" on flexion-extension manipulation of the unstable stifle joint was presumed to be due to the femoral condyles slipping over the edges of the menisci (Hickman 1964), and was presumably related to the degree of instability in the joint since it commonly occurred in the first few weeks following cruciate section. There was no evidence of any relationship between the degree of lameness and the presence of a "meniscal click" on manipulation.

There was clearly good correlation between the increasing peri-articular fibrosis and reduction of joint instability with time following surgery. It is however interesting to note that the very marked capsular fibrosis appeared to subside as stabilisation was achieved so that one 48-week dog showed only minimal capsular thickening but the joint capsule was extremely tough on sectioning, indicating a mature, organised fibrous tissue had formed.

* * *

The/

The onset of osteophyte development was first detected radiographically at 3 weeks, and was visible as a slight roughening of the cortex proximal to the trochlear ridge on lateral projection of the joint. In many cases the presence of this early change was noted only because there was a 'normal' lateral radiograph of the same joint for comparison. This may explain the discrepancy between the onset of radiograpically visible osteophytes in experimental cases of cruciate section, and their apparently later appearance in clinical cases of anterior cruciate rupture.

The distribution of osteophytes on lateral radiographs from experimental dogs resembled that described for naturally-occurring cases of cruciate rupture (Tirgari 1972, Lee 1975). The lateral projection of the joint is however of limited value in demonstrating either the pronounced osteophyte formation on lateral and medial trochlear ridge (which in some dogs shows as a mottled appearance in this region) or the osteophytes within the intercondyloid fossa. Tirgari (1972) described the "horse-shoe-shaped" intercondyloid osteophytes on antero-posterior projection of the stifle, commenting that this appearance was characteristic of O.A. following cruciate rupture.

In general, there was good correlation between the macroscopic appearance and distribution. Thus radiographically prominent osteophytes correspond to large protuberant nodules at post mortem. One long-term (48 week) dog, however, showed relatively minor radiographic evidence of new bone deposition, in spite of remodelling of the contours of the joint. This may have been due to the limitations of a lateral projection of the stifle joint.

In over half the dogs, no forward displacement of tibia relative to femur was observed on x-ray. The radiographs were taken without any attempt to produce a drawer forward, so that the situation mimics that of radiography/

radiography of the clinical case and emphasises the fact that absence of a drawer forward on x-ray does not eliminate the possibility of anterior cruciate rupture in the stifle joint (Lee 1975). In both the dogs surviving to 48 weeks the relative positions of tibia and femur appeared normal, indicating that stabilisation of the joint in the normal position had occurred. In clinical cases the joint may stabilise in a forward displaced position (Lee 1975).

* * *

The application of three different techniques - routine histological examination, fluorescent photomicrography following fluorochrome labelling of bone, and microradiography - proved particularly useful in the study of bone remodelling in this experimental model. Direct comparison was possible between microradiographs and fluorescent photomicrographs since these were obtained from the same bone sections. In this way the information obtained by one method was often complemented and expanded by reference to the other. For example on the microradiographs it was possible to distinguish between areas of calcified cartilage and woven bone, to identify vascular channels and bone resorption cavities, whereas on fluorescent photomicrographs the precise sequence and sites of new bone deposition could be assessed. Not only did fluorochrome labelling establish the early formation of a marginal osteophyte, but it was useful in showing areas of subchondral bone remodelling and appositional bone deposition on epiphyseal trabeculae. In the early stages both these features were difficult to identify either on microradiographs or on histological sections.

There was good correlation between the microradiographic appearance, the distribution of fluorochromes, and the pattern of bone remodelling shown by routine histological examination. Thus the initial stages

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of osteophyte formation, which on histological examination were graded 1 to 3 and consisted of deposits of highly cellular woven bone, corresponded to a pattern of broad, irregular and "hazy" bands of fluorochrome distribution, while microradiographs showed an osteophyte of poorly mineralised, stippled (cellular) bone. As the process of bone remodelling continued, the development of a trabecular structure within the osteophyte was discerned. Histologically, the inter-trabecular spaces were highly cellular and vascular, with the presence of both osteoblasts and osteoclasts indicating that bone deposition and bone resorption were proceeding. This was confirmed by the pattern of fluorochrome distribution and the microradiographic appearance. In the mature osteophyte of the longer surviving dogs, the trabeculae on histological examination consisted largely of lamellar bone with a regular pattern, this was confirmed by fluorescent microscopy which revealed the presence of only small amounts of label in a thin linear arrangement indicating appositional bone deposition, and by the micro-radiographic appearance of regular trabeculae of well mineralised bone.

In a few dogs discrete areas of calcified cartilage within the osteophyte failed to show uptake of fluorochrome label, in spite of the fact that adjacent woven bone was clearly labelled. The reason for this lack of fluorescence is not known. It is possible calcifying cartilage did not have suitable sites for binding xylenol orange, or perhaps a difference in vascularity of the cartilage compared to bone resulted in a lower concentration of label and hence reduced availability for binding. The question of whether or not calcifying cartilage is labelled by fluorochrome uptake has been disputed in the past: several workers have reported differing results in a variety of situations (Milch et al 1958, 1961; Holmes 1963, Urist and Ibsen 1963).

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The similarity between the changes which follow experimental transection of the cruciate ligament and those of naturally occurring O.A., both macroscopically and microscopically, has already been discussed (p.81 and 108). It has also been pointed out that these changes are progressive, becoming increasingly severe with time following ligament section. Thus, the similarity to natural O.A. and the progressive nature of the lesions indicate that the canine stifle joint in which the anterior cruciate ligament has been sectioned provides a useful and valid model for the study of the pathogenesis of O.A. A contrary view is, however, held by Olsson (1971) who considers that the changes which follow cruciate section are not those of true O.A. This contention is based on the absence of, or presence of "minimal", cartilage changes in canine stifle joints with experimentally induced cruciate rupture (Marshall and Olsson 1971). In the present experimental series, however, additional support for the validity of the model was provided by biochemical analysis of articular cartilage. This work was not reported in this thesis, having been carried out separately at the Kennedy Institute of Rheumatology in London. The early results were recorded in a number of publications (McDevitt, Muir and Pond 1973, 1974, McDevitt and Muir 1975). The findings may be summarised as an increased hydration of articular cartilage and a change in quality of the proteoglycan, shown by an increased galactosamine - glucosamine molar ratio. There was also a higher extractability of the proteoglycan aggregates with high molarity $CaCl_2$ solution. The biochemical changes were similar to those found in naturally occurring O.A. It would appear that the cartilage responds to the insult to the joint by assuming an immature behaviour; chondrocytes dividing and synthesising proteoglycans at/

at a faster rate and making a chondroitin sulphate-rich "immature" proteoglycan (McDevitt 1973).

An important feature of early O.A. is that these biochemical changes were not confined to focal areas but were diffused throughout all the cartilage of the operated joints of dogs killed 6 or more weeks after surgery; the changes preceded the appearance of fibrillation (McDevitt et al 1974). It is interesting that the earliest biochemical change of hyaline cartilage was recorded 3 weeks after surgery (McDevitt 1975), whereas the process of bone remodelling began as early as 3 days after ligament section with the onset of osteophyte formation, and subchondral bone deposition was recorded at 13 days.

The early appearance of bone remodelling lends some support to the theory that the initial abnormality of O.A. may occur in the bone. Rádin and colleagues (1972) put forward the hypothesis that subchondral bone remodelling follows repetitive impulse loading of the joint, thereby increasing the stiffness of the bone and subjecting the articular cartilage to increased stress. Foss and Byers (1972) demonstrated above average bone density in associated with O.A. In the experimental model reported here, however, while subchondral and trabecular remodelling certainly occurred, subchondral sclerosis was not a feature of the disease process. Instead, bone resorption appeared at least to equal new bone deposition, and in a number of dogs the trabeculae were reduced in thickness in the operated joint. Unfortunately, it was not possible to establish for each case the extent of subchondral bone remodelling of weight-bearing areas such as femoral condyles and tibial plateau, because these parts of the joint were used for biochemical analysis of cartilage. Nevertheless, it has been clearly demonstrated that increased bone remodelling is an integral part/

part of the development of O.A., in this experimental model.

The development of peri-articular osteophytes was a prominent feature of this experimental O.A. model. It has already been pointed out that osteophyte formation is generally accepted to be due to endochondral ossification of degenerate marginal articular cartilage (Trueta 1968, Freeman 1972). However, this was not found to apply in this situation. Rapid metaplasia of mesenchymal tissue in the marginal zone occurred, and focal deposits of woven bone were laid down, sometimes (but not invariably) containing fibrocartilage. Only in later stages of development was endochondral ossification a prominent feature of osteophyte growth.

The notably early onset of osteophyte formation, prior to any articular cartilage degeneration, does not support the widely accepted view that osteophytes are relatively late manifestations of O.A. and always occur secondary to advanced cartilage destruction (Collins 1949, Gardner 1965, Trueta 1968). It should be remembered however that a marked instability of the joint following cruciate section is a feature of this experimental model, and the instability may well bear a direct relationship to the development of osteophytes as suggested by Morgan (1967), Marshall and Olsson (1971) and Olsson et al (1972). No explanation for the relationship between instability and osteophyte formation was put forward by these authors. However, Bennett and colleagues (1942) suggested that the "transitional structure" of the marginal zone rendered it susceptible to sustained traction, "a functional stress well known to induce hyperplasia and hypertrophy". If this is indeed the case, and if weight bearing on an unstable joint does produce traction at the reflection of the synovial membrane, then the connection between instability and osteophyte development would be at least

partly/

partly explained. Demonstration of a relationship between the degree of instability and weight-bearing and the rate of osteophyte development would help to substantiate this theory. It is possible that, in the early stages of development, there was some correlation between size of osteophyte and degree of instability: dogs 2/1, 2/4 and 3/3 showing slightly larger osteophytes than their contemporaries and evidence of more pronounced joint instability. However, dog 3/3 was also markedly lame, and presumably less likely to subject its synovial membrane to "sustained traction". In the longer surviving dogs, it was not possible to show convincingly that there was any relationship between instability, weight-bearing and osteophyte development.

It is likely that a number of other factors are involved in the development of peri-articular osteophytes, although these factors may themselves be partly due to the instability in the joint. Thus, the connection between osteophyte formation and vascular proliferation was obvious in this experimental series, a close relationship between proliferating blood vessels and developing osteophyte being demonstrated from the earliest stage up to the longest surviving animal. Although it is not surprising that such an association exists, little emphasis has been placed on the role of vascularity in the pathogenesis of bone remodelling. Trueta (1968) commented that "during the productive phase of osteoarthritis" a state of hypervascularity, i.e. profusion and dilation of blood vessels, existed. Paatsama and Sittnikow (1972) provided an illustration of many contrast-filled blood vessels within a developing osteophyte in their paper, but made no comment on the significance of this finding.

A number of other workers have commented on vascular proliferation observed/

observed within bone in osteoarthritic joints (Harrison et al 1953, Sell 1960, Sokoloff 1969), and in recent years the technique of intraosseous phlebography in clinical cases of O.A. has demonstrated impaired venous drainage, mainly in advanced cases of O.A. Lynch (1974) commented that intraosseous hypertension in O.A. could be the result either of increased blood inflow or impaired outflow or both. Phillips (1968) suggested that hyperaemia occurs first in O.A. of the hip and that venous congestion follows, but Brookes (1966) and Brookes and Helal (1968) favoured the concept of venous congestion as a causal factor.

Extensive investigations into bone blood flow have been carried out by Brookes (1971). In experiments on fracture healing he recorded a great increase in the number and calibre of small arterial vessels during the first four weeks after osteotomy and associated with the hypervascularity was a florid deposition of spongey bone. During this time, the pH of blood in the healing area was significantly more alkaline than the pH of blood from a comparable contralateral zone. Later, as the pH fell, compact bone was formed. This occurred approximately 8 weeks post-operatively. At 24 weeks, the pH of the operated side was considerably lower than the contralateral side. This sequence of events in fracture healing correlates remarkably well with the pattern of new bone formation in experimental O.A. For example, florid deposition of spongey bone occurred in the first 4 to 6 weeks of osteophyte development, and by 8 to 10 weeks more mature cancellous bone was being laid down. The associated hypervascularity may be responsible for this bone remodelling by causing alterations in the local tissue conditions (pH, pCO_2 and pO_2) similar to those described by Brookes for fracture healing. In connection with this, it is interesting that, although endochondral ossification was first noted from 4 weeks after cruciate/

cruciate section, it became much more pronounced in later stages of the disease process when a low pH would be operative: Brookes (1971) has stated that a depressed pH and increased pCO_2 are necessary for endochondral ossification to occur.

Because bone deposition was readily labelled by fluorochromes given before death, new bone formation was recorded as early as 3 days after cruciate section whereas vascular proliferation was observed at 7 days. However, there was no possibility of detecting vascularity in the joint other than by perfusion with dye at post mortem examination, and no dog was killed earlier than 1 week. It is likely that vascular changes occurred at least as early as the onset of osteophyte formation, and may have been responsible for the change in environment necessary for ossification to occur.

Although no conclusions can be drawn from the small numbers of long-term survival dogs reported in this thesis, the apparent increase in bone remodelling in the control stifle joints of these animals is of particular interest. Not only was subchondral sclerosis observed but there was evidence of mild histological abnormalities of the articular cartilage, and in one dog biochemical changes as well. Perhaps these changes represent early "spontaneous" O.A. of this joint, since in the dog a relatively high incidence of bilateral stifle O.A. is reported (Tirgari and Vaughan 1975). Further investigation of such "control" or contralateral stifle joints in cases of experimentally induced or natural O.A. is indicated. If it were established that an increase in bone remodelling is followed by degenerative changes in the articular cartilage, this would support Radin's theory that subtle bone changes underlie the pathogenesis of O.A. It is conceivable that, following cruciate/

cruciate section in one stifle joint, repetitive impulse loading of the opposite limb occurs, and a chain of events similar to that proposed by Radin and colleagues (1972) ensues.

There is however another possible explanation of the increased remodelling of the control joint. Work by Rhinelander and Baragry (1962) and Rhinelander (1968) on experimental fracture healing in dogs has shown that there is a hyperaemia of the control bone of the contralateral limb. This increase in blood flow is presumed to be due to vascular reflexes under the control of the sympathetic nervous system (Brookes 1971). Perhaps a similar reflex mechanism is in operation in this O.A. model, producing a change in vascularity, altered local tissue conditions, (e.g. PO_2, pCO_2 and pH) and hence an effect on bone turnover in the contralateral joint and possibly other parts of the skeleton as well.

Thus, in spite of the currently accepted view that vascular abnormalities are secondary to other features of O.A., and not the cause of them (Freeman 1972), the findings of this study of experimental O.A. indicate not only the importance of the vascular component in the development of the disease, but also the possibility that, in some situations, vascular factors may initiate rather than result from O.A.

Various authors have speculated on the reason for osteophyte production in an osteoarthritic joint. Gardner (1965) suggested that, by offering an extended and altered articular surface, osteophyte production is a mechanical correction for the disturbance of the central articular surface (i.e. the central loss of articular cartilage). Similar explanations have been advanced by a number of earlier workers, including Beneke (1897, quoted by Bennett et al 1942) Fisher (1922) and more recently Collins (1949) who commented "adult cartilage can only respond/

respond to changing mechanical conditons by degeneration and destruction, but bone responds by regeneration and reconstruction ..... The process of osteoarthritis is a vicious circle of changing mechanical conditions and attempts at structural adaptations".

Bennett, Waine and Bauer (1942) proposed that weight-bearing in a joint with degenerating cartilage caused sustained traction on the peripheral joint margins and, along with other unknown factors, resulted in marginal proliferations.

Brookes (1971) suggested that osteophyte production may be an "attempt to improve subchondral circulation by the reactive development of hypervascularity at the free border of Hunter's vascular circle".

Radin, Paul and Rose (1972) put Wolff's Law forward as an explanation of subchondral remodelling in O.A.; this law states that the internal architecture of the bone is directly related to the stress distributions to which it is subjected. This principle may well be operative in effecting the marked remodelling of the joint which occurs after cruciate section, especially since "the magnitude of the forces acting on joints, their site of application, and the duration and rate of their actions must all be altered when the structure becomes unstable", Sokoloff (1969). Nevertheless, this must remain a matter of conjecture at the present time since little is known about the relationship between bone structure and forces acting on it.

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It is widely accepted that the term "osteoarthrosis" is preferable to "osteoarthritis" since the latter name tends to imply an inflammatory aetiology. Most workers agree that the condition is basically a degenerative disease or "joint failure", the primary lesion occurring in the articular cartilage, followed by secondary "inflammatory" responses

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in the synovial membrane, joint capsule and bone (if osteophyte formation, sclerosis and joint remodelling can be regarded as a chronic inflammatory response by bone tissue). However, in this experimental model of O.A. it has been shown that an "inflammatory component" (namely, synovitis and vascular proliferation associated with bone remodelling) is not only an integral part of the disease process but develops very early after the initial joint insult, and before articular cartilage degeneration can be detected. In fact, the avascular articular cartilage is the only tissue showing no evidence of inflammation during the development of the disease. For this reason, "osteoarthritis" rather than "osteoarthrosis" has been used to describe the condition occurring in the canine stifle joint following transection of the anterior cruciate ligament. It has already been pointed out that the naturally occurring condition bears a very close resemblance to the experimental model. It would therefore seem logical also to use the term "osteoarthritis" for spontaneous O.A. in the canine stifle joint.

No conclusions can be reached concerning the pathogenesis of O.A. on the basis of a single experimental study. The results reported in this thesis have shown the remarkably early development of bone remodelling in the canine stifle joint following cruciate rupture. They have also indicated the probable importance of vascular factors in the pathogenesis of O.A. in this experimental model. It remains to be seen whether these factors are important only in this particular situation or whether they apply also to O.A. affecting other joints of the dog, and O.A. in other species.

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APPENDIX 1

Results of Clinical Examinations
of Group A Dogs.

Duration of Survival	Dog No.	Time Interval (weeks) after ligament section																
		1 - 3 days	1	2	3	4	5	6	7	8	9	10	12	14	16	20	24	48
1 WEEK	1	++	+															
	2	++	+															
	3	++++	+++															
	4	+	+															
2 WEEKS	1	++++	+++	+														
	2	++++	++	+														
	3	++	++++	+++														
	4	+	+	+														
3 WEEKS	1		++	++														
	2		++	++														
	3	++	++	+++														
	4	+	+	+														
4 WEEKS	1	++++		++++	++++	++												
	2	+++		+++	+	++												
	3	+++	+	++	+	+												
	4	+++	+++	++	+	(+)												
5 WEEKS	1	+	+	+	+	+++	+											
	2	+++	++		+	+	+											
	3	+++		++	+++	++	+											
	4	+++		++	++	+	+											
6 WEEKS	1		+	+		+		(+)										
	2		+	+		+		+										
	3		++		+		+	(+)										
	4		++		+		++	++										
7 WEEKS	1	+++	+++		+++		+++	+	+									
	2		+++		+++		+	+	+									
	3	+++		+++		++		+++	+++									
	4	++		++		+		+	(+)									
8 WEEKS	1			+		++		+		+								
	2			+		+		+	++	+								
	3	++	++		+		+		+	+								
	4	+++	+++		++		++		++	+								
9 WEEKS	1		+		+		+		+		+							
	2		++++		+++		+		+		(+)							
	3					++	++	++	++		+							
	4					+		++		+++	+							
10 WEEKS	1		+	+				+		+	+	(+)						
	2	++	+++	++				+		+	+	+						
	3				+		+		+			+						
	4				+		+		+			+						
12 WEEKS	1		+++						(+)		(+)		(+)					
	2		+		+						+		(+)					
	3			+		+				+			(+)					
	4			+		+				+			+					
16 WEEKS	1					++				+			+	+	(+)			
	2					++++				+++			++		++			
	3							+				+		N	N			
	4							+++				+++		+	(+)			
24 WEEKS	1		++++			++							+			++	(+)	
	2	++++	++	+			+						N			N	N	
48 WEEKS	1	++	+	+		+							+				N	N
	2	++	+	+						(+)					(+)			N

Grading Of Lameness:

- N = no lameness detected
- (+) = slight lameness, only slight abnormality of gait
- + = lameness obvious, but leg bearing weight at each step
- ++ = moderate lameness, leg held up intermittently
- +++ = marked lameness, weight-bearing approximately 1 step in 3
- ++++ = 100% lameness, leg not used at all during walking or running

Table 1. Degree of lameness.

Duration of Survival	Dog No.	Time interval (weeks) after ligament section																
		1 - 3 days	1	2	3	4	5	6	7	8	9	10	12	14	16	20	24	48
1 WEEK	1	+	+															
	2	++	++															
	3	+	++															
	4	+	++															
2 WEEKS	1	+	+++	++														
	2		+	+														
	3	++	++	+														
	4	++	++	++														
3 WEEKS	1		+	++	+													
	2		++	++	+													
	3	++	+++	++	++													
	4	++	++	++	+													
4 WEEKS	1	+		+	++A	++												
	2	++		++	++	+												
	3		+	+	++A	+												
	4		++	++	+	+A												
5 WEEKS	1	++	++			++	+											
	2	++	++			+	+											
	3	+		+	+	+	+											
	4	++		++	++	++	++											
6 WEEKS	1			++		+		+										
	2			+		+		+										
	3		+		+		+											
	4		N		N		N	++										
7 WEEKS	1		+		++		+		++									
	2		+		++		+		+									
	3			+		?		+	+A									
	4			?		N		N	+A									
8 WEEKS	1			++		+		+										
	2			+		+		+										
	3		N		?		?		?	+A								
	4		++		+		?		+	+								
9 WEEKS	1		+		+		++		++		+A							
	2		+		+		N		N		+A							
	3					+	+	+		+	+							
	4					+		+		+	(+)A							
10 WEEKS	1		+++	++				++		++		++						
	2		++	++				+		N	+	(+)A						
	3			+			N		N			(+)A						
	4			+			++		++			++						
12 WEEKS	1		++						++		+	++	+					
	2		++								+		+A					
	3			+		+				N			(+)A					
	4			+		?				N			NA					
16 WEEKS	1					+				+			+		(+)			
	2					(+)				(+)			(+)		+			
	3							+				+		N	+A			
	4							+				+		N	++A			
24 WEEKS	1		++			++							++			(+)	+A	
	2	++	+	+			+						(+)				(+)A	
48 WEEKS	1	++	++	+		+							(+)				(+)	(+)A
	2	++	++	+						+					+			NA

KEY:

N = no forward drawer
(+) = slight forward drawer
\+ = obvious forward drawer
++ = marked forward drawer
+++ = gross forward drawer
? = uncertain interpretation
A = examined under general anaesthesia

Table 2. Degree of instability.

Duration of Survival	Dog No.	Time intervals (weeks) after ligament section: 1-3 days	1	2	3	4	5	6	7	8	9	10	12	14	16	20	24	48
1 WEEK	1	N	N															
	2	+	N															
	3	(+)	N															
	4	N	+															
2 WEEKS	1	+	+	+														
	2	N	N	(+)														
	3	N	N	(+)														
	4	?	?	(+)														
3 WEEKS	1		+	+	+													
	2		+	+	+													
	3	N	N	N	++													
	4	(+)	N	N	+													
4 WEEKS	1	+		+	++	++												
	2	++		+	++	++												
	3		N	?	+	+												
	4		(+)	++	++	+												
5 WEEKS	1	+	+			N	?											
	2	+	+			++	++											
	3	+		++	++	+	+											
	4	N		+	?	+	+											
6 WEEKS	1			++		++		++										
	2			++		+		++										
	3		+		+		+	+										
	4		+		?		+	+										
7 WEEKS	1		+				N	+	+									
	2		+				N	N	?									
	3			++		N		+	++									
	4			++		+		N	?									
8 WEEKS	1			++		++		++		++								
	2			++		++		++	+++	++								
	3		(+)		++		+		N	?								
	4		N		+		(+)		+	+								
9 WEEKS	1		++		+		N		N		N							
	2		+++		++		+		+		+							
	3					+	+	+		?	?							
	4					+		+		N	N							
10 WEEKS	1		N	N				N		++	++	+						
	2		N	(+)				+		+	+	+						
	3			++			N		+			+						
	4			+			N		?			+						
12 WEEKS	1		++						N		N		N					
	2		++								N		++					
	3			++		N				N			?					
	4			++		++				++			++					
16 WEEKS	1					+				+			N		N			
	2					+				+			N		N			
	3							?				N		N	+			
	4							+				?		N	N			
24 WEEKS	1		+			++							+			N	N	
	2	++	++	++			++						N				N	
48 WEEKS	1	+	++	++		+							+				N	N
	2	+	+	+						N					?			N

KEY:

N	=	no fluid swelling
(+)	=	slight fluid swelling
+	=	moderate fluid swelling
++	=	marked fluid swelling
+++	=	gross fluid swelling
?	=	uncertain interpretation

Table 3. Degree of synovial effusion.

Duration of Survival	Dog No.	1 - 3 days	Time interval (weeks) after section of ligament: 1	2	3	4	5	6	7	8	9	10	12	14	16	20	24	48
1 WEEK	1	N	N															
	2	N	N															
	3	N	N															
	4	N	N															
2 WEEKS	1	N	?	(+)														
	2	N	N	(+)														
	3	N	N	+														
	4	N	N	+														
3 WEEKS	1		N	N	+													
	2		N	+m	+m													
	3	N		+m	+													
	4	N		+m	+													
4 WEEKS	1	N		N	+	+m												
	2	N		N	(+)	(+)												
	3		N	N	?	(+)												
	4		N	+	+	+												
5 WEEKS	1	N	?m			+	++m											
	2	N	N			+	++											
	3	N		N	N	+	+m											
	4	N		N	N	+	+											
6 WEEKS	1			N		+m		+										
	2			N		(+)		++										
	3		N		+		+	+										
	4		N		+m		+m	+										
7 WEEKS	1		N				++		++									
	2		N				(+)		(+)									
	3			N		++		++	++									
	4			+		+		++	++									
8 WEEKS	1			N		+		+		+								
	2			(+)		+		+		++								
	3		N		+		+		+	+								
	4		N		N		N		+	++m								
9 WEEKS	1		N		+		+		+		+							
	2		+		++		++		++		+++							
	3					+	+	+		+	++m							
	4					+		+		+	+							
10 WEEKS	1		N	?				+		++	++	++						
	2		N	+				+m		+	++m	++						
	3			N			+		+			+						
	4			N			+		+			+m						
12 WEEKS	1		?						+		+m		++m					
	2		N								++		++					
	3			N		N				+m			+m					
	4			+		+				++			++					
16 WEEKS	1					++				++			+		++			
	2					N				N			+		(+)			
	3							(+)				+		+	++			
	4							+				++		++	++			
24 WEEKS	1		N			+							++			++m	++m	
	2	N	N	+			+							++			++	
48 WEEKS	1	N	N	?		+							+	+			+	+
	2	N	N	N						(+)					(+)			(+)

KEY:

N	=	no fibrous thickening
(+)	=	slight fibrous thickening
+	=	easily detected fibrous thickening
++	=	marked fibrous thickening
+++	=	gross fibrous thickening
?	=	possible fibrous thickening
m	=	more pronounced medially

Table 4. Degree of peri-articular fibrosis.

APPENDIX 2

Histological Appearance of Each Section cut from Right Femoral Trochlea of Group A Dogs.

	1 Week	1/1 No Sections						1/2 1	2	3				1/3 1	2	3	4			1/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR																								
	MEDIAL								I	I					?I					?1					
	LATERAL																				I				
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL																								
	LATERAL																								
	B. BONE REMODELLING																								
	MEDIAL																								
	LATERAL																								
	T. GROOVE																								
	III) PERIOSTEAL ZONE																								
	MEDIAL								F																
	LATERAL								F																
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL																								
	LATERAL																								
	T. GROOVE																								
	II) METACHROMASIA																								
	MEDIAL																								
	LATERAL																								
	T.GROOVE																								
	III) DEGENERATION																								
	T.GROOVE																								
(C) SYN. MEMBRANE	THICK									+										(+)	(+)	(+)			
	CELLULAR							(+)	+	+					(+)					+	+	+			
	FIBROUS																								
	VASCULAR							(+)	+					(+)	+	+				+	(+)	+		+	
	VILLOUS																								
	PANNUS																								

		2/1						2/2						2/3						2/4					
	2 Weeks	1	2	3	4			1	2	3	4	5	6	1	2	3	4			1	2	3	4		
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	2c	2c						I					?F						2					
	MEDIAL			2c	I					I	I	I			I	I	I			2c	2	2	?I		
	LATERAL			I						?I					I	?I	I			2c	I				
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL																								
	LATERAL																								
	B. BONE REMODELLING																								
	MEDIAL																								
	LATERAL																								
	T. GROOVE																								
	III) PERIOSTEAL ZONE																								
	MEDIAL			F						F	?F		2		F	?F									
	LATERAL												I												
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL																								
	LATERAL																								
	T. GROOVE																								
	II) METACHROMASIA																								
	MEDIAL																								
	LATERAL																								
	T.GROOVE																(-)								
	III) DEGENERATION																								
	T.GROOVE																								
(C) SYN. MEMBRANE	THICK	+	+							(+)	(+)				(+)	(+)									
	CELLULAR	+	+						(+)	(+)			+		+	+	(+)				(+)				
	FIBROUS																								
	VASCULAR	+	+					+	+	+	+	++				+				+	+	+	+		
	VILLOUS																								
	PANNUS																				+				

	3 weeks	3/1 1	2	3	4	5		3/2 1	2	3	4	5	6	3/3 1	2	3	4			3/4 1	2	3	4		
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	x2						x2	1,2																
	MEDIAL		x2c	2c	2c	2							?1	3	2					1	1	1			
	LATERAL		2c	2	1,2	2			1	?1		?1			2c	1	1			2	1	1			
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL					+																			
	LATERAL					+																+			
	B. BONE REMODELLING																								
	MEDIAL					+			+								+						+		
	LATERAL					+																+			
	T. GROOVE																								
	III) PERIOSTEAL ZONE																								
	MEDIAL			F	B	B									F		B					F	B+		
	LATERAL			B	B	B+											B+						B+		
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL																								
	LATERAL																								
	T. GROOVE																								
	II) METACHROMASIA																								
	MEDIAL																								
	LATERAL																								
	T.GROOVE			(-)	(-)					(-)															
	III) DEGENERATION																								
	T.GROOVE																								
(C) SYN. MEMBRANE	THICK	+	+	+	(+)				+	+		+	(+)			+				+	+	+	+		
	CELLULAR	+	+	+	+			+	+	+	+	+	(+)	+	++	+				+	+	+	+		
	FIBROUS														+	(+)				+	+	+	+		
	VASCULAR	+	+	+	+	+		+	+			+		+	++	+	++			+	+	++	+		
	VILLOUS																				+		+		
	PANNUS																								

	4 Weeks	4/1 1	2	3	4	5	6	4/2 1	2	3	4	5	6	4/3 1	2	3	4	5		4/4 1	2	3	4		
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	x2						x2c	x2c					3	x3						x2				
	MEDIAL		2	2c	2c	2c				2	2c	2c				3c	3c	3				2	2		
	LATERAL		2c	2c	2c	1				2c	2c	2c	2			3c	3c	3				2	1		
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL					+																	+		
	LATERAL			+																					
	B. BONE REMODELLING																								
	MEDIAL			+																					
	LATERAL			+																					
	T. GROOVE																								
	III) PERIOSTEAL ZONE																								
	MEDIAL		F	F			2c						B			F		B+				F	B		
	LATERAL		F	F		B							B									F			
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL																						+		
	LATERAL																								
	T. GROOVE																								
	II) METACHROMASIA																								
	MEDIAL																						(+)		
	LATERAL																								
	T.GROOVE		-		(-)	(-)				-	(-)	(-)	(-)			-	(-)					-	(-)		
	III) DEGENERATION																								
	T.GROOVE					(+)																			
(C) SYN. MEMBRANE	THICK	(+)	+	+	(+)	+		+	++	+	+	+		+	+	++	+	+		+	+	+	+		
	CELLULAR	(+)	+	+	+	+		++	++	+	+	+		+	++	+	+	+		+	++	+	+		
	FIBROUS															+	+	+							
	VASCULAR	(+)	+	++	++	++				+	+	+		+	+	+				+	++	+	+		
	VILLOUS														+										
	PANNUS		+							+						+						+			

	5 Weeks	5/1 1	5/1 2	5/1 3	5/2 1	5/2 2	5/2 3	5/3 1	5/3 2	5/3 3	5/3 4	5/3 5	5/3 6	5/4 1	5/4 2	5/4 3	5/4 4	5/4 5	5/4 6
(A) BONE	I) OSTEOPHYTE																		
	SUPRA-TROCHLEAR				x4			2, 3c	x3					2, 3c	3c				
	MEDIAL	3	3			3	4			3	3	2				2	2	2c	
	LATERAL	3	3			4	4			3	3	2				3c	2c	2	
	II) SUBCHONDRAL ZONE																		
	A. VASCULAR BUDS																		
	MEDIAL										+	+							
	LATERAL										+	+							
	B. BONE REMODELLING																		
	MEDIAL																		
	LATERAL					+	?+												
	T. GROOVE					+				+						+			
	III) PERIOSTEAL ZONE																		
	MEDIAL		F	B++	F	B	B+				F	F	B+		F	F,B	F,B		B+
	LATERAL		F	B++	F	B+	B++		F		F	F,B	B+			F,B		F	B+
	IV) TRABECULAE																		
	THICKNESS																		
	TROCHLEAR WIDTH																		
(B) CARTILAGE	I) SWOLLEN																		
	MEDIAL									(+)									
	LATERAL									(+)		+							
	T. GROOVE																		
	II) METACHROMASIA																		
	MEDIAL																		
	LATERAL									(+)	(+)	(+)							
	T.GROOVE						(-)				(-)	(-)	(-)			-	(-)	(-)	(-)
	III) DEGENERATION																		
	T.GROOVE																		
(C) SYN. MEMBRANE	THICK	++	+	+	+	++	+	+	+	+				++	+	+			+
	CELLULAR	+	+	+	+	+	+	+	+	+	+	+	+	++	++	+	+	+	+
	FIBROUS	++	+	+	+	++	+	+	+			+		+					+
	VASCULAR	+	+	++	+	+	+	+	++	+	+	+	++	+	+			+	++
	VILLOUS																		
	PANNUS	+	+			+				+						+			

	6 Weeks	6/1 1	2	3	4	5		6/2 1	2	3	4	5	6	6/3 1	2	3	4	5	6	6/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	x4												3	3					x3	x3				
	MEDIAL		3	2	3				3c	2c	3c					2	2c	2				2	2	3	
	LATERAL		4	3	3	2c			2c	3c	3c	2c	2c			1	2c	2				3c	2		
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL			+	++	++					+		+												
	LATERAL		+	+	++	+				+	+	+						+							+
	B. BONE REMODELLING																								
	MEDIAL			+	+	+				+	+	+	+												+
	LATERAL		+	+	+						+	+	+					+							+
	T. GROOVE		+	+	+																	+			
	III) PERIOSTEAL ZONE																								
	MEDIAL	F	B	B	B++	3,B++				F		3	3c				F	B++	B+			B+	B+	B++	B++
	LATERAL		F	B+	B++	B+++			F	B			B++				F	B++	B++			B+	B+	B+	B++
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL		+	+							+					(+)	+	+							
	LATERAL		+	+	+	+						+				+	+	+						+	
	T. GROOVE																								
	II) METACHROMASIA																								
	MEDIAL			+	+						(+)														
	LATERAL			+	+	(+)						(+)				+									
	T.GROOVE		-			(-)			-			(-)	(-)			-						(-)	(-)	(-)	
	III) DEGENERATION																								
	T.GROOVE												+						+			(+)			
(C) SYN. MEMBRANE	THICK	++	++	++	+			++	++	++	+		+	+	+	+	+	+			+	+			+
	CELLULAR	+						++	++	+	+		+	++	++	+	+	+	+		++	+	+		
	FIBROUS	++	++	++	+			+	+	++	+	+	+	+	+	+	+	++	+		+	+			+
	VASCULAR	++	++	+	+			+++	++	++	++	++	++	++	++	+	+	++	++		+	+	+	+	++
	VILLOUS							+																	
	PANNUS		+													+						+			

	7 Weeks	7/1 1	2	3	4	7/2 1	2	3	4	7/3 1	2	3	4	5	7/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																			
	SUPRA-TROCHLEAR	B+				2	2,3			x3	x2				x3					
	MEDIAL		2	3		4	4c	3			3	4	4			3c	3	2		
	LATERAL		3	3		4	3	4	3c		3c	3c	4			3c	3	3c		
	II) SUBCHONDRAL ZONE																			
	A. VASCULAR BUDS																			
	MEDIAL							+	+				+						+	+
	LATERAL			+					+			+	+	++					+	+
	B. BONE REMODELLING																			
	MEDIAL								+			+								+
	LATERAL								(+)			+	+	+						
	T. GROOVE						+		+											
	III) PERIOSTEAL ZONE																			
	MEDIAL				F		F,B	B+	B+++		F,B	F	3	B+	F	F	B+	F		B++
	LATERAL				B+		B	B+	B+++		F	B+	B+	B++	B+	F	3	F,B	3c	3c
	IV) TRABECULAE																			
	THICKNESS					-	-	-				-	-							
	TROCHLEAR WIDTH																			
(B) CARTILAGE	I) SWOLLEN																			
	MEDIAL								+				+	++			+			
	LATERAL								(+)				+	+			+	+	+	
	T. GROOVE													(+)						
	II) METACHROMASIA																			
	MEDIAL								+					+			+		(+)	
	LATERAL			(+)										+			+	+	(+)	
	T.GROOVE							-						-		-		(-)	-	-
	III) DEGENERATION																			
	T.GROOVE		(+)			(+)		+					+	+						+
(C) SYN. MEMBRANE	THICK		+	+		++	++	++		+	++	++	++	+	++	++	+	++	+	+
	CELLULAR		+	+		+	++	++	+	+	+	+	+	+	++	+	+	+		
	FIBROUS					++	+	+		+	++	++	++	+		+		+	+	+
	VASCULAR		+		+	+	+	+	+	++	++	++	++	++	++	++	+	++			+
	VILLOUS					+									+					
	PANNUS		+			+	+				+					+				

	8 Weeks	8/1 1	2	3	4	5	6	8/2 1	2	3	4	5	6	8/3 1	2	3	4			8/4 1	2	3	4		
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	3,4	2					x3,4						2,3						3c					
	MEDIAL		4	4	4				4	3	4			4	3	4					4	2			
	LATERAL		4	3,4	4	4			4	4	4		3	2	3	3					4	4			
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL						+					+	+	+		++	++						+		
	LATERAL			+	+	+	+				+		+		+	+							+		
	B. BONE REMODELLING																								
	MEDIAL		+				+				+	(+)	+			+	+						+		
	LATERAL					+	+				+	+			+										
	T. GROOVE		+	+	+	+	+			+	+	+				+									
	III) PERIOSTEAL ZONE																								
	MEDIAL			B+	B++	B+++	B++	F	F	F,B	B+	B+++	B+++		3	B+	B+				B		B+		
	LATERAL		F	B+,3	B++	B++		F	B	B++	B++	B+++	B+		B	B+++	B++					B+	B+		
	IV) TRABECULAE																								
	THICKNESS		-	-	-	-		-	-	-	-	-	-	-	(-)	(-)				-	-	-	-		
	TROCHLEAR WIDTH							+	+	+	+	+	+												
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL			(+)								+	+		+	+									
	LATERAL			(+)	+	(+)				+	+	+	+			+						+	+		
	T. GROOVE						+					++													
	II) METACHROMASIA																								
	MEDIAL														+	+									
	LATERAL			(+)											(+)	+						+			
	T.GROOVE					(-)	-									(-)									
	III) DEGENERATION																								
	T.GROOVE		(+)				+				+	+	+		+	+					(+)				
(C) SYN. MEMBRANE	THICK	++	++	+	++	+	+	+	++	++	++	+		+	+	+	+			+	+	+	+		
	CELLULAR	++	+	+	++	++	+	+	+		+	+		+	+	+	+			+	+	+	+		
	FIBROUS	+	++	+	+	+	+	+	++	++	++	+		+	+	+				+	+				
	VASCULAR	++	++	+	++	+++	++	+	++	++	++	++	++			+	+			+	+	+	+		
	VILLOUS																								
	PANNUS		+						+					+							+				

	9 Weeks	9/1 1	2	3	4	5		9/2 1	2	3	4	5	6	9/3 1	2	3	4	5	6	9/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	B+	B+					B++	3					2,4						F	B+	B+			
	MEDIAL			1	2					4	2				5	5	4	5				1	4	3	4
	LATERAL			2	2					4	4	4			5	5	5	5				4	4	3c	4
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL												++			(+)	+	+	+				+	+	+
	LATERAL											+					+	+	+			+	++	++	++
	B. BONE REMODELLING																								
	MEDIAL											+	+			+	+	+	+				+	+	++
	LATERAL											+	+				+	(+)	(+)			+	+	+	++
	T. GROOVE															(+)	+	(+)				+			
	III) PERIOSTEAL ZONE																								
	MEDIAL			F	B	c		B+	B+	F,B	F,B	B++	B+++		F	B	B+	B++	5			B+	B+	B+	B++
	LATERAL			B+	B+	c					B+	B++	B++		F	B	B+	B++	5		F	B+	B++	B++	B+
	IV) TRABECULAE																								
	THICKNESS								-	?-	-	-		-	-	-	-				-	?-	-	?-	?-
	TROCHLEAR WIDTH									+	+	+		+	?+	+	+	+	+						
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL			(+)	(+)						+	+	+			(+)	+						+	+	+
	LATERAL			(+)	(+)						+	+	+		(+)	(+)	+	+				+	+	+	+
	T. GROOVE												+						+						
	II) METACHROMASIA																								
	MEDIAL			+	(+)						(+)	+	+			+	+						+		
	LATERAL			+	(+)						(+)	+	+			+	+	+				(+)	+		
	T.GROOVE				-	-					(+)	(+)	(+)				(-)	(-)	-			-	(-)	(-)	-
	III) DEGENERATION																								
	T.GROOVE								(+)	+	+	+			(+)			+	+			(+)	+	+	+
(C) SYN. MEMBRANE	THICK	++	++	+	+			++	++	++	++	++	+	++	++	++	++	++	+		++	+	++	++	+
	CELLULAR	++	+	+	+			+	+	+	+	+	+	++	++	++	++	+			++	+	+	+	+
	FIBROUS		+	+				++	++	++	++	+	+	++	++	++	++	++	++		+	+	++	++	+
	VASCULAR	+	++	+	+			++	++	+	+	+	++	++	+	+	++	+	++		+	++	++	++	+
	VILLOUS								+	+															
	PANNUS								+						+							+			

	10 Weeks	10/1 1	2	3				10/2 1	2	3	4			10/3 1	2	3	4	5	6	10/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	B+	x2					3c	x2c					5	x3,4					B++	x2				
	MEDIAL		5	4						4	4					4	5	3			4	4	4	5	4
	LATERAL		5	5					4	3c	3c				4	5	5	5			4	4	4		5
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL									+	++							+				++	+	+	+
	LATERAL			+					+	++	++											++	+	+	+
	B. BONE REMODELLING																								
	MEDIAL									+	+								++				+	+	+
	LATERAL								+	+	+								?				+		+
	T. GROOVE																								
	III) PERIOSTEAL ZONE																								
	MEDIAL		B+	F							B++				?F	F	B+				F		F	4,B++	B++
	LATERAL										B++				F	B	B+	B+			F,B	B+	B+	B++	B+++
	IV) TRABECULAE																								
	THICKNESS																								
	TROCHLEAR WIDTH																								
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL									+	(+)						++	++							+
	LATERAL			+						+						+	++	++					+		+
	T. GROOVE																		++						
	II) METACHROMASIA																								
	MEDIAL										+					+	+	+							
	LATERAL			+												+	+	+							
	T.GROOVE			-												-									
	III) DEGENERATION																								
	T.GROOVE										+						+	+	+						
(C) SYN. MEMBRANE	THICK		+	+				+	+	+	+			+++	++	++	++	++		+	+	+	+	+	+
	CELLULAR		+	+				+	+	+	+			+	+	+	+	+		+	+				+
	FIBROUS		+	++				+	++	+	+			++	++	++	++	++		+	+	+	+	+	+
	VASCULAR		+	+				+		+	+			+++	++	+	++	+++		+	+		+	++	+
	VILLOUS																								
	PANNUS		+													+					?+				

	12 Weeks	12/1 1	2	3	4	5	6	12/2 1	2	3	4	5	6	12/3 1	2	3	4	5	6	12/4 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	B++	2c					B+++	B++	x3				B++	x8++					B+++	4				
	MEDIAL			4	4	4				5	4	5				5	5					3	4	5	
	LATERAL		4	3	4	3c				5	3,4	4				5	5					5	5	5	
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL										+	++	+					+							+
	LATERAL									+	+	+					+	+	+						
	B. BONE REMODELLING																								
	MEDIAL										+	+				+	+	+							+
	LATERAL		+							+		+				+		+							+
	T. GROOVE									+						+									
	III) PERIOSTEAL ZONE																								
	MEDIAL		B+	B+	B+	F,B	B++			B+	B++	B++	5			F,B	B++	5	5				B	B	B++
	LATERAL		F	F	B+	B++				F	B++	B+++	5			B+	B++	5	5		F	B	B+	B	B+
	IV) TRABECULAE																								
	THICKNESS		-	-	-	-				-		±	±			-							-	-	-
	TROCHLEAR WIDTH											(+)	(+)				+	+					+	-	
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL				+	+	+			+		+							+					+	
	LATERAL			+	+	+	+				+						+		+				+	+	
	T. GROOVE																+		+			+			±
	II) METACHROMASIA																								
	MEDIAL			(+)	(+)	(+)	+			+		+												+	
	LATERAL			(+)	(+)		(+)				(+)						(+)						+	(+)	
	T.GROOVE			-	(-)	(-)										-		(-)	-			-			
	III) DEGENERATION																								
	T.GROOVE			(+)		+	+			(+)	+	+	+			+	(+)	+	++			+	+	?+	?+
(C) SYN. MEMBRANE	THICK	++	++	++	++	++	++	++	++	++	++	++	++		++	++	++	++	+		++	+++	++	++	+++
	CELLULAR	++	+	+	+	+	++	+	+	+	++	+	+		++	+	+	+	+		+	+	+	++	++
	FIBROUS	++	++	++	++	++	++	++	++	++	++	+	++		+	++	++	++			++	++	++	++	+++
	VASCULAR	++	++	+	++	+	++	+	+	+	++	+	+		++	++	++	++	++		++	++	++	++	++
	VILLOUS	+					+		+						+										
	PANNUS		+	+						+						+						+			

	16 Weeks	16/1 1	16/1 2	16/1 3	16/1 4	16/1 5		16/2 1	16/2 2	16/2 3	16/2 4	16/2 5	16/2 6	16/3 1	16/3 2	16/3 3	16/3 4	16/3 5		16/4 1	16/4 2	16/4 3	16/4 4	16/4 5	16/4 6
(A) BONE	I) OSTEOPHYTE																								
	SUPRA-TROCHLEAR	B	B÷					2,4					* B+(i/c)	5	5					4	4				
	MEDIAL		5	5	5				4	4	4				5	4	5	5		5	5	5	5		
	LATERAL		5	5	5				4	4	4				5	5	5	5		5	5	5	5	6	5
	II) SUBCHONDRAL ZONE																								
	A. VASCULAR BUDS																								
	MEDIAL			+	+	++				+	++	+	+					+		⊛	⊛	++	++	++	++
	LATERAL		+	++	++	+				+	++	+					+	++			++	+	+	+	+
	B. BONE REMODELLING																								
	MEDIAL			+		+				+	+	+	+		+	+	+	+		++		+	+	+	++
	LATERAL		+	+	+	+					+	+	+		++	+	+	+		++			++	+	+
	T. GROOVE		+										+(i/c)		++	+				++	++				
	III) PERIOSTEAL ZONE																								
	MEDIAL		B	B++	B++	B++			B+	B+	B++	B+++	B+++	B++	B+	F,B	B++	B+					B+	5	5
	LATERAL		B+	B++	B++	5			B+	B+	4,B++	B+++	B+++	B+	B	B++	B++	B++			B+	B+	B++	5	5
	IV) TRABECULAE																								
	THICKNESS				-						-	-									?		-	-	
	TROCHLEAR WIDTH										+	+													
(B) CARTILAGE	I) SWOLLEN																								
	MEDIAL			+	+	++			+		+	+			+	+	++	++		+	+	+	+	+	+
	LATERAL		+	+	+	++			+	+	+	+			+	+	++	++		+	+	+	+	+	+
	T. GROOVE			+	+	++							++		+	+	+	++		+	+		+	+	+
	II) METACHROMASIA																								
	MEDIAL				(+)	+					(+)				+	+	+	+			+				
	LATERAL				(+)	+				(+)	(+)				+	+	+	+			+	+			
	T.GROOVE				-	-			-	(-)			-		-	-	-	-			-				-
	III) DEGENERATION																								
	T.GROOVE		(+)	+	+	+							++		(+)	++	++	++			+	+	+	+	+
(C) SYN. MEMBRANE	THICK	++	++	++	+	++		++	+++	++	+	++	+++	+++	++	++	++	++		+++	++	++	+		++
	CELLULAR	++	+	+	+	+		++	++	+	+	+		++	++	++	+	+		+	+	+	+		
	FIBROUS	+	+	++		++		++	+++	++	+	++	++	++	++	++	++	++		++	++	++	+		++
	VASCULAR	++	++	+	+	+		++	++	+	+	++	++	++	++	++	++	++		++	+	+	+		++
	VILLOUS		+													+									
	PANNUS		+					+	+						+	+				+	+				

* i/c = intercondyloid notch. ⊛ Vascular budding in trochleer groove.

	24 Weeks	24/1 1	2	3	4	5	6	24/2 1	2	3	4	5	6
(A) BONE	I) OSTEOPHYTE												
	SUPRA-TROCHLEAR	x5	x5	5				B++					
	MEDIAL			5	5	5	5		6	6	6	5	
	LATERAL			5	5	6	5		6	6	6	6	
	II) SUBCHONDRAL ZONE												
	A. VASCULAR BUDS												
	MEDIAL					++			++	++	++	++	++
	LATERAL						+		++	++	++	++	+
	B. BONE REMODELLING												
	MEDIAL					+	+		++	++	++	++	+++
	LATERAL					(+)	+		++	++	++	++	+
	T. GROOVE			+			?+	++	+	+	+	+	+
	III) PERIOSTEAL ZONE												
	MEDIAL	B+	B+	B++	B++	5	B+++	R	B+	B+	B+	B++	B+++
	LATERAL	B++	B++	B++	B+++	B+++	5,B+++	R	B+++	B+++	B+++	B++	B++
	IV) TRABECULAE												
	THICKNESS			-	-	?-	?-	-	-	-	-	?	
	TROCHLEAR WIDTH	+	+	(+)		(+)		+	++	++	++	++	
(B) CARTILAGE	I) SWOLLEN												
	MEDIAL				++	++	++		+	+	+	+	+
	LATERAL			+	++	++	++		+	+	+	+	+
	T. GROOVE				+	+	+		+	+	+	+	++
	II) METACHROMASIA												
	MEDIAL												
	LATERAL												
	T.GROOVE								-			(-)	(-)
	III) DEGENERATION												
	T.GROOVE				+	+	++		+	+	+	+	+
(C) SYN. MEMBRANE	THICK	++	++	++	++	++	++	++	+++	+++	+++	++	+++
	CELLULAR	+	+	+	++	+	++	++	++	++	++	+	++
	FIBROUS	++	++	++	++	++	++	+	++	++	++	++	++
	VASCULAR	++	++	++	++	++	++	++	++	++	++	++	++
	VILLOUS	+	+	+	+	+	(+)	+	+	+		+	+
	PANNUS			+					+				

	48 Weeks	48/1 1	2	3	4	5	48/2 1	2	3	4
(A) BONE	I) OSTEOPHYTE									
	SUPRA-TROCHLEAR		B+				R	⊠7		
	MEDIAL			7	7	7			7	7
	LATERAL			7	7	7			7	7
	II) SUBCHONDRAL ZONE									
	A. VASCULAR BUDS			⊛						
	MEDIAL				+	++			+	+
	LATERAL		+	+		++			+	+
	B. BONE REMODELLING									
	MEDIAL			+	++	+			+	+
	LATERAL			++	++	+			+	+
	T. GROOVE			++	+					
	III) PERIOSTEAL ZONE									
	MEDIAL			R	R	R		R	R	
	LATERAL			R	R			R	?R	?R
	IV) TRABECULAE									
	THICKNESS							-	-	-
	TROCHLEAR WIDTH		+	++	++	++			+	
(B) CARTILAGE	I) SWOLLEN									
	MEDIAL				(+)	+				
	LATERAL				+	+				
	T. GROOVE			+	+	++			+	+
	II) METACHROMASIA									
	MEDIAL				+	+				
	LATERAL				+	+				
	T.GROOVE			+					-	-
	III) DEGENERATION									
	T.GROOVE			+	++	+			+	+
(C) SYN. MEMBRANE	THICK		+	+	+	+	++	+	+	+
	CELLULAR		+	+	+	+	+	++	+	++
	FIBROUS			+	+	+	++	+		
	VASCULAR						+	+		+
	VILLOUS		+		(+)			+	+	
	PANNUS							+		

⊛ Vascular budding in trochlear groove.