AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS;
EARLY MORPHOLOGICAL AND BIOCHEMICAL CHANGES

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An experimental model of osteoarthritis resulting from laxity of the joint was induced in eighteen mature
dogs (at least two years old) by sectioning the anterior cruciate ligament of the right knee (stifle) with a stab
incision, the left knee providing a control. A sham operation was also performed in three other dogs, in which
a stab incision was made but the ligament left intact. The dogs were killed at various intervals from one to
forty-eight weeks later. Morphological changes in bone, cartilage, synovial membrane and joint capsule were
examined in all the joints and biochemical changes in the cartilage of three dogs killed after two, eight, and
sixteen weeks. All the changes resulting from the operation progressed with time and became indistinguishable
from those found in three dogs with natural osteoarthritis of the knee. There were no changes in the joints
which had sham operations. As the time of onset is known, this experimental model in a larger species enables
a study to be made of the biochemical as well as the morphological changes in the early stages of osteoarthritis.

Osteoarthritis is characterised by progressive deterioration and localised erosion of articular cartilage,
accompanied by remodelling of bone at the joint margins (Gardner 1965; Sokoloff 1969; Lee, Rooney, Sturrock,
Kennedy and Dick 1974). The two principal constituents of articular cartilage, whose main function is to bear
intermittent compressive loads, are collagen and proteoglycans. Proteoglycans are hydrophilic polyanions of
very large hydrodynamic size that occupy a large solvent volume, and as they are entrapped in the collagen
network they function in arresting the flow of interstitial water when an external force is applied, so that the
compressive stiffness of healthy cartilage depends upon the proteoglycan content (Kempson, Muir, Swanson and
Freeman 1970). The complex molecular organisation of the proteoglycans in cartilage is illustrated by the fact
that different populations may be extracted selectively by solutions of increasing ionic strength (Hardingham and
Muir 1974), and that a minority appear to be firmly associated with collagen and cannot be extracted.

Early biochemical and cellular changes of osteoarthritis are almost impossible to study in the natural
disease because the time of onset is not usually known. In experimentally induced osteoarthritis, however, the
initial phases of the disease can be studied with the added advantage that control tissue is available from the same
animal, thus eliminating variations between individuals.

Cutting the anterior cruciate ligament of the knee (Pond and Nuki 1973) produces laxity of the joint which
leads to biochemical changes resembling those of natural osteoarthritis (McDevitt, Muir and Pond 1974; McDevitt
and Muir 1976). In this study the progressive histological and biochemical changes and the early development of
osteophytes, which were indistinguishable from those of natural osteoarthritis, are described.

MATERIALS AND METHODS
Experimental animals
A total of twenty-four adult dogs of both sexes was used in this study; eighteen were selected for experimental osteo-
arthritis, three for sham operations, and three had natural osteoarthritis. All the dogs selected for operation were at
least two years old and weighed 12 to 30 kilograms. They were clinically normal and were mainly foxhounds, collies
and alsatian crosses. Skeletal maturity and absence of deformities or osteoarthritis of the knee were established by radiography.

Induction of the experimental osteoarthritis
Section of anterior cruciate ligament—The experimental osteo-
arthritis was induced in dogs by section of the anterior cruciate
ligament of the right knee (stifle) through a stab incision as
described by Pond and Nuki (1973), the left knee serving as the
control. The dogs were premedicated with 1 milligram of
acepromazine and then given sodium thiopentone to induce
anaesthesia which was maintained with a mixture of oxygen,
nitrous oxide and halothane administered by closed circuit. The animals were placed in left lateral recumbency and the right hind leg was shaved and the region of the stifle washed with antiseptic solution. The joint was held in partial flexion, a scalp blade (Gillette No. E 11) 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 cut by turning the blade and withdrawing it in a lateral direction. In most dogs, sectioning of the ligament was achieved with very little joint injury; in a few dogs, however, joint cartilage was incised. Penicillin was given routinely after operation.

Procedure after operation—The dogs were housed in individual kennels and allowed exercise in concrete runs twice daily for half to one hour. The dogs, including those having sham operations, were examined every three to four days for four weeks after operation, and at weekly, two-weekly or longer intervals thereafter. On each occasion the degree of lameness and any other clinical features were noted. In addition, each dog was injected with fluorochrome bone labels after various times (Gilbertson 1975).

Procedure at euthanasia—The dogs were killed by intravenous injection of sodium pentobarbitone at various times between one to forty-eight weeks after operation. The femoral arteries of both hind limbs of fifteen dogs were cannulated immediately after death and a suspension of barium sulphate in Prussian blue dye was injected (Gilbertson 1975). Each joint was then sectioned and the femoral trochlear region from both left and right knees was set aside for investigation of bone changes. The femoral condyles, tibial plateau and patella of both right and left knees were immediately frozen in small sealed plastic bags at -20 degrees Celsius and reserved for biochemical analysis (three dogs).

Sham operation

Three adult dogs were used. A scalp blade was inserted into the right knee through a lateral, parapatellar stab incision and was then rotated to inflict minor trauma on the synovial membrane while the anterior cruciate ligament was left intact. The dogs were killed two, six, and eight weeks later. Vascular perfusion of dye and administration of fluorochrome bone labels were performed, as described above, on the dogs killed two and eight weeks after the sham operation.

Dogs with natural osteoarthritis

Three dogs, in which naturally acquired osteoarthritis was diagnosed by radiography and clinical examination, were also investigated.

One had no apparent damage to the ligaments and the disease was diagnosed as primary osteoarthritis. The other two had secondary osteoarthritis due to natural rupture of the anterior cruciate ligament, which in the case of a ten and a half-year-old corgi × spaniel cross was diagnosed six and a half years before death.

Grading of cartilage surface

The joints were thawed at 4 degrees Celsius and the quality of the articular surface graded by the Indian ink method of Meachim (1972), as described in detail by McDevitt and Muir (1976). Grade 1 ("intact surface"): These surfaces were normal in appearance and did not retain any ink. Grade 2 ("minimal fibrillation"): These sites appeared normal before staining, but retained the Indian ink as elongated specks or as light grey patches. Grade 3 ("overt fibrillation"): These areas were velvety in appearance and retained the ink as intense black patches.

Erosion of the cartilage with exposure of the underlying bone (corresponding to Grade 4 in Meachim's classification) was not seen in any of the experimental or natural osteoarthritic joints.

Dissection of cartilage

Cartilage from the tibia, femur and patella of each joint was kept separate in every experiment. In most cases the cartilage on the tibia was divided into three areas designated A, B and C (Fig. 1). A small sagittal section of cartilage from each site of the dogs killed one, two, seven and sixteen weeks after surgery was reserved for histology. The remaining cartilage from each region was shaved off the bone with a scalpel blade, and diced into small pieces, care being taken to preclude...
subchondral bone and cartilage from the articular margins. Approximately a quarter of each lot of cartilage was used for determination of molar ratios of galactosamine: glucosamine and the remainder for extraction of proteoglycans.

Histology

Sagittal sections of cartilage of the femur and patella as well as the three sites on the tibia of the operated and of the control joints were fixed in 10 per cent unbuffered formalin for twenty-four hours, embedded in paraffin and sectioned at a thickness of 6μ. Cartilage sections were double stained; proteoglycans by the orthochromatic Safranin O procedure of Rosenberg (1971) and cell nuclei with haematoxylin-eosin.

Extraction of proteoglycans

The diced cartilage (50–200 mg wet weight) was extracted with 2 millilitres of 2M CaCl₂ pH 6–8 for forty-eight hours at 4 degrees Celsius (McDevitt and Muir 1976). The extracts were then filtered through glass wool plugs and dialysed against 0.15M sodium acetate pH 6–8. The cartilage residues were digested with papain and the resulting solution dialysed against 0.15M sodium acetate pH 5–8 as described by McDevitt and Muir in 1976.

The cartilage specimens of the dog killed sixteen weeks after operation were divided into three lots. The first lot was extracted with 2 millilitres of 2M CaCl₂ pH 6–8 as described above; the second with 2M CaCl₂ pH 6–8 containing 0.1M 6-amino-caproic acid, 0.005M benzamidine HCl (Oegema, Hascall and Dziewiatkowski 1975) and 10 micrograms per millilitre of soya bean trypsin inhibitor (Kettenheimer 1974) and the third with 2 millilitres of 2M CaCl₂ pH 6–8 containing 30 μg per millilitre of phenylmethylsulfonyl fluoride (Fahrney and Gold 1963). The three extractions were carried out at 4 degrees Celsius for forty-eight hours and the extract dialysed against 0.15M sodium acetate pH 5–8.

Chemical analyses

Samples of fresh cartilage from each site were dried, weighed, digested with papain and dialysed (McDevitt and Muir 1976). Samples containing 10–15 μg of hexosamine were hydrolysed at 96 degrees Celsius in 2 millilitres of 8N HCl for three hours (Swann and Balazs 1966) and the galactosamine: glucosamine molar ratio determined using a Locarte amino-acid analyser (Tsiganos and Muir 1969).

Uronic acid contents were determined by the method of Bitter and Muir (1962) using an automated procedure (Heingard 1973).

Histology and microradiography of bone

Histology of calcified and decalcified vertical sections of the trochlear region of the femur from the experimental, control and sham operated joints were carried out as described (Gilbertson 1975) as was the microradiography of calcified bone sections.

RESULTS

Cartilage

Gross changes—The cartilage of the control joints was white, shiny and firm with the sole exception of tibial area A of the dog killed twenty-four weeks after operation, which showed minimal (Grade 2) staining with Indian ink. The cartilage of the affected joints of dogs killed six or more weeks after operation was less shiny and softer and was noticeably thicker than the control cartilage, except where severely fibrillated (Grade 3). Invasive synovial pannus was not seen, either by gross or microscopic examination, on the tibial, patellar or femoral condylar cartilage of any of the operated joints.

Indian ink staining showed that in each dog the most severe fibrillation was always located in area A of the tibial cartilage of the operated joint and that the lesions progressed in severity and size with time after surgery (Table I). Complete disruption of the cartilage surface

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>GRADE OF CARTILAGE SURFACE AT DIFFERENT TIMES AFTER SURGERY DETERMINED BY INDIAN INK STAINING (MEACHIM 1972)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks after operation</td>
<td>Number of dogs</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Ligament section</td>
<td></td>
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<td>1</td>
<td>1</td>
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<td>2</td>
<td>1</td>
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<td>24</td>
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<td>48</td>
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<td>2</td>
<td>2</td>
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<tr>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Natural osteoarthritis</td>
<td></td>
</tr>
<tr>
<td>Dog-X</td>
<td>1</td>
</tr>
<tr>
<td>Dog-Y</td>
<td>1</td>
</tr>
<tr>
<td>Dog-Z</td>
<td>1</td>
</tr>
</tbody>
</table>

(Grade 3) of a small area in the centre of area A was evident four weeks after operation, and this lesion developed radially with time after operation until by twenty-four weeks all the surface of area A was severely damaged. The damage to the cartilage surfaces of areas B and C, however, was much less progressive and severe than in area A.

Damage to the femoral condylar cartilage was also less severe. Surface deterioration of the patellar cartilage of operated joints was not demonstrable by Indian ink staining until eight weeks after surgery. Overt fibrillation (Grade 3) was never observed in the patellar cartilage.

The cartilage surfaces of the joints of the dogs killed two and eight weeks after the sham operation was firm, shiny, completely intact (Grade 1) and indistinguishable from that of control joints.
Fibrillation of the tibial cartilage of the two dogs with natural secondary osteoarthritis was also confined to area A where the lesions were somewhat further advanced than Grade 3, particularly in dog Z.

**Microscopical changes**—The histological appearance of the cartilage of the control joints of dogs killed one, two, seven and sixteen weeks after operation was normal in that the surface was intact, no loss of Safranin O staining was evident, the cell density was low and clusters of two or more cells were absent (Fig. 2).

Similarly, the cartilage of the tibia (Fig. 3), femur and patella of joints subjected to sham operations in dogs killed after two and eight weeks was intact, the cell density was similar to that of control tissue, nests of cells were absent and Safranin O staining was not diminished.

The cartilage of area A of the tibia of the experimental joint of the dog killed one week after operation was slightly roughened with occasional small clefts, while the surfaces of B and C were intact. The number and depth of the clefts in the surface of area A was greater two weeks after operation, although the surface of area B was intact while that of area C was slightly roughened. Fibrillation in area A gradually progressed with time after operation until, after seven weeks, deep clefts were evident (Fig. 4) and by sixteen weeks (Fig. 7) erosion of the articular surface layer was complete. Damage to the cartilage surface of the lateral tibial plateau (area C) and the remainder of the surface of the medial condylar cartilage (area B), however, was minimal at seven (Figs. 5 and 6) and sixteen weeks after operation (Table II).

Loss of Safranin O staining was observed only in severely fibrillated area A of the tibia of the operated joint of the dog killed sixteen weeks after surgery, where it was confined to the superficial zone (Table II). Changes in the appearance and distribution of the chondrocytes preceded damage to articular surfaces and loss of Safranin O staining (Table II). A slight increase in cell density and occasional lacunae with two nuclei were evident in areas A, B and C one week after the operation. The cell density and the number of lacunae with two or more nuclei increased progressively with time after operation in all three areas of the tibial cartilage (Table II) (Figs. 4 to 7) and was most pronounced in area A (Fig. 4). Sixteen weeks after operation the cartilage matrix was highly cellular, clones of two or more cells were abundant particularly around severely fibrillated sites, the lacunae were enlarged compared with controls and many vacant lacunae were evident (Fig. 7). At this time the appearance and distribution of the chondrocytes in area A of the tibia (Fig. 7) closely resembled that of natural secondary osteoarthritis (Fig. 8).

**Biochemical changes**—Chondroitin sulphate chains of proteoglycans contain uronic acid which can be used to measure the proteoglycan content of tissues and extracts. Thus 2M CaCl₂ extracted 35–55 per cent of the total proteoglycan from the cartilage of control joints (Table III). The variation between different joints was mainly due to differences in the ages of the dogs, a lower proportion of the total proteoglycan being extracted from the cartilage of older animals. The proportion of the...
Figure 5—Tibial cartilage from area B (site adjacent to area A of Figure 4) of a dog killed seven weeks after the ligament section operation. Although the articular surface is apparently intact, the cell density is increased and lacunae with two nuclei are evident in the superficial and middle layers. (Safranin O, ×250.) Figure 6—Cartilage from the lateral tibial plateau, area C (medial tibial plateau represented in Figures 4 and 5) of a dog killed seven weeks after the ligament section operations. The articular surface is roughened, cell density is increased and lacunae with two nuclei are evident in the upper and middle zones of the tissue. (Safranin O, haematoxylin and eosin, ×250.)

Figure 7—Tibial cartilage from area A of a dog killed sixteen weeks after the ligament section operation. Severe disruption of the articular surface and a considerable increase in cell density are evident. Clusters of two or more stained nuclei are abundant. The lacunae, some of which are empty, are larger than those of healthy control tissue (Fig. 2). (Safranin O, haematoxylin and eosin, ×250.) Figure 8—Tibial cartilage from area A of the knee of a dog with natural osteoarthritis. The appearance is very similar to the severely fibrillated cartilage (Fig. 7) of the dog sixteen weeks after ligament section. (Safranin O, haematoxylin and eosin, ×250.)
total uronic acid extracted from the tibial, femoral and patellar cartilage of the joints of dogs killed two and eight weeks after the sham operation was very similar to that extracted from the corresponding controls.

In contrast, the total proteoglycan extracted from the cartilage of experimental joints, compared with corresponding controls, was increased even as early as two weeks after operation, although after so short a time the increase was not great and was restricted to the tibial area A and the femoral cartilage (Table III). However, seven weeks after operation, as much as 68–83 per cent of the proteoglycan could be extracted by 2M CaCl₂ from all areas of cartilage of the affected joint (Table III). This change is similar to that previously observed with five dogs killed at various times between three and forty-eight weeks after operation (McDevitt and Muir 1976). Similarly in natural osteoarthritis a much greater proportion of the total proteoglycan was extracted from all areas of cartilage of the joint compared with controls (mean of eight controls). The cartilage from the tibia, femur and patella respectively of each joint of the dog killed sixteen weeks after operation was divided into three lots and extracted with 2M CaCl₂ with or without protease inhibitors, as shown in Table IV. About 50 per cent of the total uronic acid was extracted from the cartilages of the control joint and 60–70 per cent from the cartilages of the operated joint, whether or not inhibitors were present (Table IV).

The relative proportion of chondroitin sulphate to keratan sulphate in proteoglycans is determined by galactosamine:glucosamine molar ratios and in whole cartilage this ratio is an approximate estimate of their relative proportions. In control joints the galactosamine:glucosamine molar ratio of whole cartilage showed variation between different dogs as previously noted (McDevitt and Muir 1976), the ratio being slightly lower in area A than B and C of the same joint (Table V). In contrast, in the dog killed seven weeks after operation the ratio was appreciably higher in all three areas of the tibial cartilage of the operated joint compared with the corresponding controls. A similar difference was consistently found between the cartilages of operated and control joints of four dogs killed between six and forty-eight weeks after the operation (McDevitt and Muir 1976).

It is notable that there was no change in the galactosamine:glucosamine molar ratio in any specimen taken from joints which had had sham operations compared with corresponding controls.

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**TABLE II**

**HISTOLOGY OF SAGITTAL SECTIONS OF Tibial, Femoral AND PATELLAR CARTILAGE AT DIFFERENT TIMES AFTER LIGAMENT SECTION (Tibial Areas A, B AND C AS DEPICTED IN FIGURE 1)**

<table>
<thead>
<tr>
<th></th>
<th>Experimental osteoarthritis</th>
<th>Femoral condyle</th>
<th>Patella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular surface</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Loss of Safranin O</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cell clones</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Articular surface</td>
<td></td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Loss of Safranin O</td>
<td></td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Cell clones</td>
<td></td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Articular surface</td>
<td></td>
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<td>+ + +</td>
</tr>
<tr>
<td>Loss of Safranin O</td>
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<td>+ + +</td>
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<tr>
<td>Cell clones</td>
<td></td>
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<td>Articular surface</td>
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<tr>
<td>Loss of Safranin O</td>
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<td>Cell clones</td>
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<tr>
<td>Articular surface</td>
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<tr>
<td>Loss of Safranin O</td>
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<td>+</td>
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<tr>
<td>Cell clones</td>
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<td>+ + + + +</td>
<td>+ + + +</td>
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</tbody>
</table>

**KEY TO SCORES**

Quality of articular surface: — intact surface; + slight roughening; ++ roughening and small clefts; +++ roughening and fissures to middle zone; ++++ total disruption of surface layer.

Loss of Safranin O: — no loss of Safranin O staining; + decreased Safranin O staining in superficial zone.

Cell clones: + increased cell density; ++ occasional cell "doublets"; +++ clones of two or more cells evident.
TABLE III

Proportion of total proteoglycan (measured as uronic acid) extracted by 2M CaCl₂ from the cartilage of the tibia, femur and patella of (a) experimental, (b) control, (c) sham operation joints and (d) joints with naturally acquired osteoarthritis (tibial areas A, B and C depicted in Figure 1)

<table>
<thead>
<tr>
<th></th>
<th>Weeks after operation</th>
<th>Uronic Acid Extracted Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tibia</td>
</tr>
<tr>
<td></td>
<td>Operation</td>
<td>Control</td>
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<tr>
<td>Ligament section</td>
<td>2</td>
<td>48.4</td>
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<td>Ligament section</td>
<td>7</td>
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<td>8</td>
<td>45.1</td>
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<td>Natural osteoarthritis</td>
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<tr>
<td>Dog-X</td>
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<td>—</td>
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<tr>
<td>Dog-Y</td>
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</tr>
</tbody>
</table>

† Areas A, B and C of the tibia were pooled.
* The mean of eight control joints was used as a control value for the natural osteoarthritic cartilage.

TABLE IV

Proportion of total proteoglycan (measured as uronic acid) extracted by 2M CaCl₂ with or without protease inhibitors, from cartilage of tibia, femur and patella of experimental operation and control joints of a dog killed sixteen weeks after operation

<table>
<thead>
<tr>
<th></th>
<th>Tibia</th>
<th>Femur</th>
<th>Patella</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Operation</td>
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<td></td>
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<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Solution 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.9</td>
<td>52.3</td>
<td>72.1</td>
<td>49.6</td>
</tr>
<tr>
<td>Solution 2</td>
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<tr>
<td>71.1</td>
<td>47.8</td>
<td>69.6</td>
<td>50.1</td>
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<tr>
<td>Solution 3</td>
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<td></td>
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<tr>
<td>—</td>
<td>—</td>
<td>66.8</td>
<td>47.3</td>
</tr>
</tbody>
</table>

Solution 1: 2M CaCl₂ pH 6.8.
Solution 2: 2M CaCl₂, 0.1M 6-amino caproic acid, 0.005M benzamidine HCl, 1 μg/ml soya bean trypsin inhibitor, pH 6-8.
Solution 3: 2M CaCl₂, 30 μg/ml phenylmethylsulphonyl fluoride pH 6-8.

TABLE V

Galactosamine, glucosamine molar ratios of samples of whole cartilage from tibia, femur and patella after experimental, control and sham operations. The tissue was first made soluble by proteolysis and then hydrolysed in 8M HCl (for details, see text) (tibial areas A, B and C depicted in Figure 1)

<table>
<thead>
<tr>
<th></th>
<th>Weeks after operation</th>
<th>Tibia</th>
<th>Femur</th>
<th>Patella</th>
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<tbody>
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<td>Operation</td>
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<tr>
<td>Control</td>
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<td></td>
</tr>
<tr>
<td>Ligament section</td>
<td>7</td>
<td>2.8</td>
<td>2.5</td>
<td>3.5</td>
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<td>8</td>
<td>2.6</td>
<td>2.6</td>
<td>2.9</td>
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Bone

Gross changes—Osteophyte formation was not demonstrable in the control joints. The earliest detectable osteophyte formation was noted at the proximal limit of the femoral trochlea two weeks after cruciate section, and consisted of a tiny ridge of new bone. By four weeks, this had increased to discrete small nodules, and by eight weeks an irregular ridge of semi-confluent nodules was present at the margins of the femoral trochlea (Fig. 10); osteophytes were also obvious in the intercondylar fossa, on the tibial spine, on the edges of the tibial plateau and on the proximal and distal margins of the patella. Further increase in size and extent of osteophyte formation at the margins of the joint was noted with time after operation.
By sixteen weeks, a prominent smooth ridge of large nodules of new bone was present, and in the longest surviving dogs, killed at forty-eight weeks, remodelling of the trochlear ridges, femoral condyles and tibial plateau had occurred.

**Microscopical changes**—Fluorochrome uptake due to new bone deposition in the marginal zone was recorded when given three days after operation. The earliest bone marrow spaces of the osteophyte and femoral epiphysis. At sixteen weeks, the protuberant osteophyte ridge was composed largely of trabecular bone covered by a thick layer of cartilage, and at forty-eight weeks remodelling had resulted in marked widening of the trochlear ridge, the new bone consisting of mature trabeculae which appeared confluent with those of the epiphysis.

![Photomicrograph of control femoral trochlear ridge. (Haematoxylin and eosin, ×8.)](image1)

![Femoral trochlear ridge eight weeks after ligament section operation showing osteophyte development at marginal zone and a thick layer of woven bone on the periosteal surface of the femoral cortex. (Haematoxylin and eosin, ×8.)](image2)

Histological appearance of marginal osteophyte formation recorded one week after cruciate section consisted of a small focal collection of mainly spindle-shaped fibroblast-like cells. By two to four weeks, cellular woven bone had been deposited with a few scattered or focal accumulations of chondrocytes present in some sections. By eight weeks an early trabecular structure within the osteophyte was evident (Fig. 11), and resorption of the femoral cortex had established communication between synovial membrane and menisci.

At each stage, from one week to forty-eight weeks after ligament section, vascular proliferation associated with the new bone development was clearly demonstrated by perfusion with the dye mixture.

**Synovial membrane and menisci**

**Gross changes**—Pathological changes were noted in the synovial membrane, joint capsule and menisci.
Vascular proliferation in the synovial membrane was shown by the injection of dye one week after operation. Generally, the vascularity became more intense with time up to about twelve weeks and thereafter subsided. Increased thickness of the synovial membrane with yellowish discoloration and the presence of villous folds and adhesions were also noted two weeks or more after cruciate section. In some dogs vascular granulation tissue was observed encroaching on to the proximal limit of the femoral trochlea.

Increased thickness of the joint capsule was often most pronounced on the medial aspect of the joint and was seen in every dog killed three or more weeks after sectioning the ligament.

Damage to the meniscus was noted in seventeen dogs, the medial meniscus being most often affected. Initially, there was slight surface splitting, and later, severe splitting or tearing or even complete disintegration of the meniscus.

**Microscopical changes**—Increased cellularity of the superficial layers of the synovial membrane was due to proliferation of lining cells and infiltration by mononuclear cells. The earliest change was detected at one week, and by four weeks the synovial layer was many times thicker than normal (Fig. 12). A slight increase in vascularity of the synovial membrane was also noted one to two weeks after operation, which became more pronounced with time, being moderate to marked by six to eight weeks and persisting up to twenty-four weeks after operation and thereafter subsiding, as fewer blood vessels were observed in the longest surviving dogs. In a number of animals the thickened synovial membrane was thrown up into small folds or villi. Fibrosis of the subintimal layers of the synovial membrane was noted from three to four weeks after operation and was especially pronounced in dogs killed after twelve to twenty-four weeks.

**Bone and synovial membrane after sham operation**

Gross and microscopical examination of the femoral sections of the joints subjected to sham operations in dogs killed two and eight weeks later showed no osteophytes at the joint margins. Furthermore, fluorescent microscopy of calcified bone sections from such joints showed no increase in fluorochrome uptake compared with the controls. Both lateral and medial menisci were intact and no change was evident in the joint capsule.

The dog killed two weeks after the sham operation showed mild synovitis in the affected joint; the synovial membrane was somewhat discoloured and there was a slight increase in cellularity and in vascularity of the superficial layers of the synovial membrane, revealed...
after perfusion with the dye mixture. These changes were not seen after the sham operation in the dog killed eight weeks later.

**Bone and synovial membrane in natural osteoarthritis**

Well developed osteophytes were present in the joints of all three dogs with naturally acquired osteoarthritis. The osteophytes were largest on the femoral trochlear groove, the distal pole of the patella and the margins of the medial tibial plateau. Although the volume of the synovial fluid was normal, the synovial membrane of each joint was discoloured with obvious vascular proliferation.

**DISCUSSION**

Natural osteoarthritis of the knee (stifle) joint in dogs is characterised by a progressive erosion of the articular cartilage, osteophyte formation, thickening of the joint capsule and hyperaemia and proliferation of the synovial membrane (Tirgari and Vaughan 1975) and is thus very similar to the human disease (Gardner 1965). Isolated rupture of the anterior cruciate ligament occurs naturally in humans (Kennedy, Weinberg and Wilson 1974; Wang, Rubin and Marshall 1975) and in dogs (Tirgari and Vaughan 1975) and the resulting joint laxity eventually produces changes in the joints of humans (Frankel, Burstein and Brooks 1971) and dogs (O'Donoghue, Frank, Jeter, Johnson, Zeiders and Kenyon 1971; Tirgari and Vaughan 1975) which macroscopically resemble those of osteoarthritis resulting from other causes.

Joint laxity produced by the operation induced changes in the joint capsule, synovial membrane, bone and cartilage. The location of the lesions in bone and cartilage and their macroscopical and microscopical appearance were indistinguishable from those seen in the natural disease. Furthermore, as assessed histologically, the lesions in cartilage and bone progressed in severity with time after operation. It should be pointed out that the Indian ink staining procedure of Meachim (1972) reveals the quality only of the surface of the cartilage, so that the macroscopical appearance of the lesion shown by this means does not progress with time in a linear fashion from Grade 1 to Grade 4. Thus, although Grade 3 (severe fibrillation) was recorded in tibial area A four weeks after surgery, complete erosion of the articular cartilage (Grade 4) was not seen even forty-eight weeks after surgery, nor was a Grade 4 lesion seen in area A of the cartilage of a dog whose anterior cruciate ligament had ruptured naturally six and a half years before death. It is noteworthy that chondrocyte cloning, a feature of advanced human osteoarthritis (Meachim and Collins 1962), was abundant in severely fibrillated lesions in both the natural and experimentally induced disease in dogs. This observation is consistent with the findings of Tirgari and Vaughan (1975) but is difficult to reconcile with the report that cell cloning was uncommon in degenerative lesions in the cartilage of the femoral head of dogs with hip dysplasia (Lust, Pronsly and Sherman 1972).

Osteoarthritis due to natural rupture of the anterior cruciate ligament is usually associated with damage to the medial meniscus in dogs (Tirgari and Vaughan 1975) and in humans (Kennedy et al. 1974) and this effect was also reproduced after section of the cruciate ligament.

Natural rupture of the anterior cruciate ligament elicits a mild inflammatory response in canine joints (Tirgari and Vaughan 1975), and the increased vascularity and proliferation of the synovial membrane which were observed after surgical resection of the anterior cruciate ligament were similar to the changes reported by Tirgari and Vaughan (1975) in the natural disease. Moreover, in surgically induced osteoarthritis, the morphological changes in cartilage and bone were similar to those seen in natural osteoarthritis, and the biochemical changes (McDevitt et al. 1974; McDevitt and Muir 1976) were identical with those found in the articular cartilage of the hip of a dog with hip dysplasia (McDevitt, Muir and Pond 1973). On the other hand, in joints which had the sham operation there were no changes in bone or cartilage so that it is unlikely that, in those joints in which the anterior cruciate ligament had been cut, the changes resulted from the transient inflammation in the synovium due to insertion of the scalpel blade.

Earlier analyses of different regions of cartilage in experimentally induced osteoarthritis showed that the tissue became swollen and more hydrated and the properties of the proteoglycans changed before the articular surface was damaged or there was a loss of glycosaminoglycans (McDevitt et al. 1974; McDevitt and Muir 1975a, 1976). Thus, purified proteoglycans extracted from such pathological cartilage differed from those of normal tissue in that they contained relatively more chondroitin sulphate and less keratan sulphate (McDevitt and Muir 1975b). They were also more easily extracted (McDevitt and Muir 1976) so that either their association with collagen was reduced, or they were less tightly entrapped in the collagen network (McDevitt et al. 1974; McDevitt, Muir and Gilbertson 1975; McDevitt and Muir 1975a, 1976). Initially, that is three weeks after operation, these changes were confined to area A of the tibial condyle, but thereafter the changes were evident throughout all regions of the cartilage of the tibiae, femora and patellae of the operated joints. The greater ease of extraction of proteoglycans from osteoarthritic cartilage was not reduced by the presence of protease inhibitors in the extraction medium and would therefore appear to be the result of some change in vivo in the matrix itself, rather than to the action of degradative enzymes during the extraction procedure.

The increased hydration and swelling in the non-fibrillated regions of the cartilage of the operated joint imply that the swelling pressure of the proteoglycans in the matrix (Maroudas 1973) was less resisted by the
collagen fibres. These findings in the dog are consistent
with the increased distance between collagen fibres shown
by electron microscopy in human osteoarthritis (Weiss
and Mirow 1972).

It is evident from the present study that osteophyte
formation in bone (Gilbertson 1975) and biochemical
changes in the cartilage are concurrent processes in the
early stages of experimental osteoarthritis. In a few
animals killed twenty-four weeks or more after operation,
the laxity of the right knee may have induced some minor
changes in the cartilage and bone of the left control joint.
The conclusions of the present study, however, were
mainly based on results obtained with animals killed
after much shorter intervals when the unoperated knee
appeared to be a valid control as assessed by Indian ink
staining, histology, chemical composition of the tissue
and the proportion of proteoglycans extracted from it.
Moreover, no changes in bone or synovial membrane
were seen in control joints up to sixteen weeks after
operation.

Although a number of experimental models of
osteoarthritis have been developed, biochemical evalua-
tion is largely lacking in most. Recently, however,
chemical and metabolic findings have supported the
validity of a model of osteoarthritis in rabbits. Joint
laxity was induced by the more drastic procedure of
severing the medial collateral and both cruciate ligaments
and resecting the medial meniscus (Ehrlich, Mankin,

There are several features that recommend the
present model of osteoarthritis in the dog developed by
Pond and Nuki (1973). Firstly, rupture of the cruciate
ligament occurs naturally in dogs (Tirgari and Vaughan
1975) and in humans (Kennedy et al. 1974; Wang et al.
1975) when it initiates secondary osteoarthritis (Tirgari
and Vaughan 1975); and hence sectioning this ligament
is analogous to a natural event. Secondly, the osteo-
arthritis that results from this procedure has morpho-
logical and biochemical changes which are indistinguish-
able from those of the natural disease. Thirdly, although
progressive changes occur during development in the
chemical composition of articular cartilage of humans
and dogs, once maturity is reached the composition of
normal cartilage changes little with age in either species
(Maroudas, Muir and Wingham 1969; Kempson, Muir,
Pollard and Tuke 1973; McDevitt 1973). Fourthly, the
proteoglycans of mature canine and human articular
cartilage are analogous in containing comparable
amounts of keratan sulphate relative to chondroitin
sulphate. The relative proportions of these glycosaminogly-
cans tend to vary between different species, and
between different populations of proteoglycans (Tsiganos,
Hardingham and Muir 1971).

This experimental model of osteoarthritis appears to
be closely analogous to the natural human and canine
disease. It has the advantage that the size of the joints is
large enough to enable topographical biochemical
changes as well as morphological changes to be studied.
Since the time of onset is known the disease can be
followed in its early stages before overt lesions develop,
since these first appear at the same site of the tibial
condyle in each animal.

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REFERENCES


