J. Anat. (1984), **138**, 3, pp. 435–446
With 6 figures
Printed in Great Britain

Temporary immobilisation facilitates repair of chemically induced articular cartilage injury

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(*Accepted* 30 *August* 1983)

INTRODUCTION

Immobilisation of the guinea-pig knee leads to a reduction in chondrocyte death and prevents the formation of bony outgrowths or osteophytes and of fibrillation (splitting) of articular cartilage following intra-articular injection of sodium iodoacetate (Williams & Brandt, 1982). On the other hand, immobilisation itself may lead to atrophy of articular cartilage in the ipsilateral limb (Palmoski, Perricone & Brandt, 1979), with marked losses of Safranin-O staining and uronic acid content, and reduction in proteoglycan synthesis. However, if the period of immobilisation is not excessive, these changes may be promptly and completely reversed upon remobilisation. The present study, therefore, attempts to determine whether temporary immobilisation of the knee not only ameliorates cartilage injury after intra-articular injection of iodoacetate, but also facilitates subsequent repair of established cartilage damage.

MATERIALS AND METHODS

Animals

Adult male albino guinea-pigs (32–40 weeks old) were housed individually in $18 \times 18 \times 18$ in stainless steel wire bottom cages and fed Guinea-pig Chow 5025 (Ralston Purina Company, Richmond, IN) (ascorbic acid content, 1·0 mg/g) *ad libitum*. Food intake was monitored daily. The animals were randomly divided into six experimental groups, as described below. The weight of each animal was recorded at the beginning of the experiment and weekly thereafter. Animals were killed by intraperitoneal injection of T-61 euthanasia solution (Taylor Pharmacal Co., Decatur, IL).

Group 1 (Table 1) consisted of six animals whose left knee was injected with iodoacetate but was not immobilised. These animals were killed six weeks after injection.

Group 2 consisted of six guinea-pigs whose left knee was injected with iodoacetate and was then immobilised for one week, after which the constraint was removed and the animals were permitted five more weeks of ambulation in their cages before they were killed, six weeks after the injection.

Group 3 consisted of four guinea-pigs whose left knee was injected with iodoacetate and was then immediately immobilised and maintained in a constraint until death three weeks after injection.

Group 4 consisted of five animals whose left knee was injected with iodoacetate and was then immediately immobilised for three weeks. The constraints were then removed and the animals permitted to ambulate 'on all fours' in their cages for an additional three weeks when they were killed, six weeks after the injection.

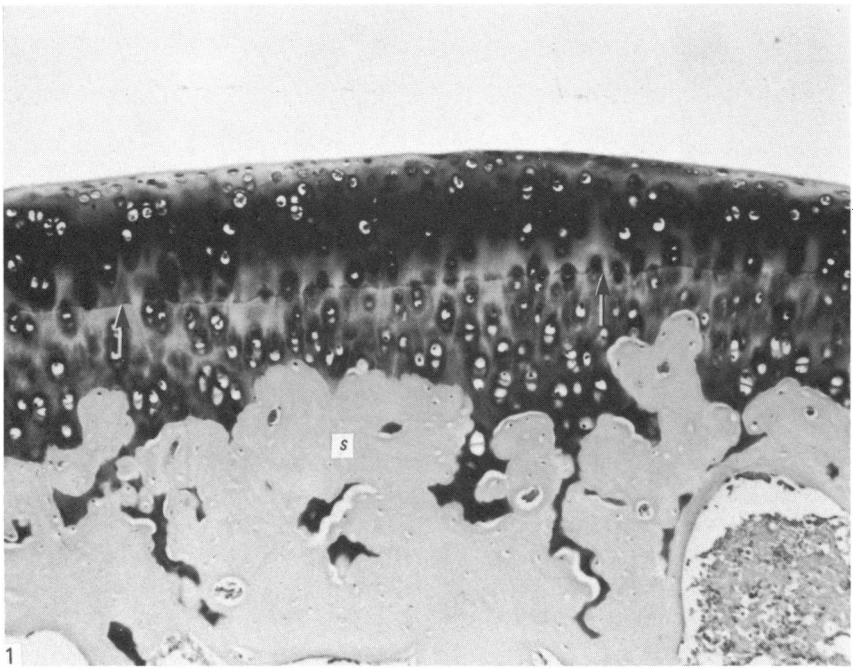

Fig. 1. Normal medial femoral condyle of a control guinea-pig (Group 6), stained with Safranin-O, fast green. *s*, subchondral bone. Arrows indicate tidemark. × 63.

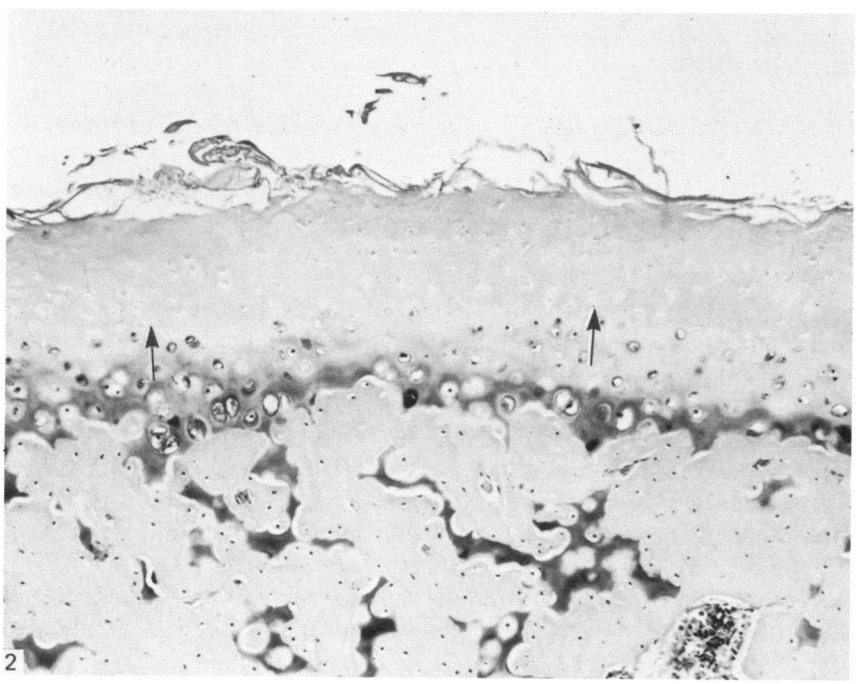

Fig. 2. Medial femoral condyle of a guinea-pig killed 6 weeks following a single intra-articular injection of iodoacetate (Group 1). Safranin-O, fast green stain. Arrows indicate tidemark. Note surface fibrillation, depletion of chondrocytes, absence of interterritorial and pericellular staining throughout the uncalcified cartilage, and partial loss of staining beneath the tidemark. × 63

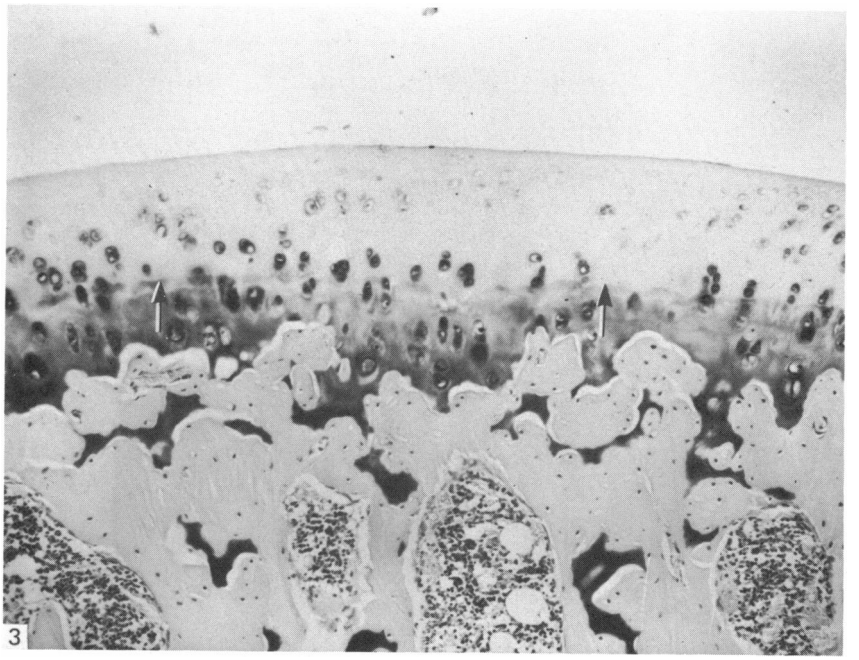

Fig. 3. Medial femoral condyle of guinea-pig in Group 3 stained with Safranin-O, fast green. Arrows indicate tidemark. Note the intact surface and loss of staining in the uncalcified cartilage. × 63.

Group 5 consisted of four animals which did not receive iodoacetate but whose left knee was immobilised for three weeks. Following removal of the constraint these animals were permitted an additional three weeks of ambulation in their cages before death.

Group 6 consisted of five guinea-pigs which served as untreated controls and were killed at the outset of the experiment.

Both knees of each animal were examined daily (Group 1) or weekly (Groups 2–5) to assess mobility and swelling.

Injection and immobilisation procedures

For intra-articular injection of iodoacetate animals were anaesthetised by an intramuscular injection of ketamine hydrochloride (50 mg/kg). After the left knee was shaved and washed with 70 % isopropyl alcohol, a 26 gauge needle attached to a tuberculin syringe was passed through the joint capsule lateral to the patellar ligament and a sterile solution of 0·3 mg of sodium iodoacetate in 0·1 ml of 0·15 M sodium chloride was injected. Care was taken to avoid contacting the articular surface with the needle.

For the immobilisation procedure, the animals were anaesthetised as described above and the left knee was secured against the trunk in approximately 120 ° of flexion with cloth tape, which was reapplied weekly. Forced compression of the articular surfaces was avoided. Daily inspection confirmed that all taped limbs remained immobilised during the period of constraint.

Analysis of tissue

After killing the animals, both knees were immediately opened and examined macroscopically. The femoral condyles and parapatellar synovial membrane were

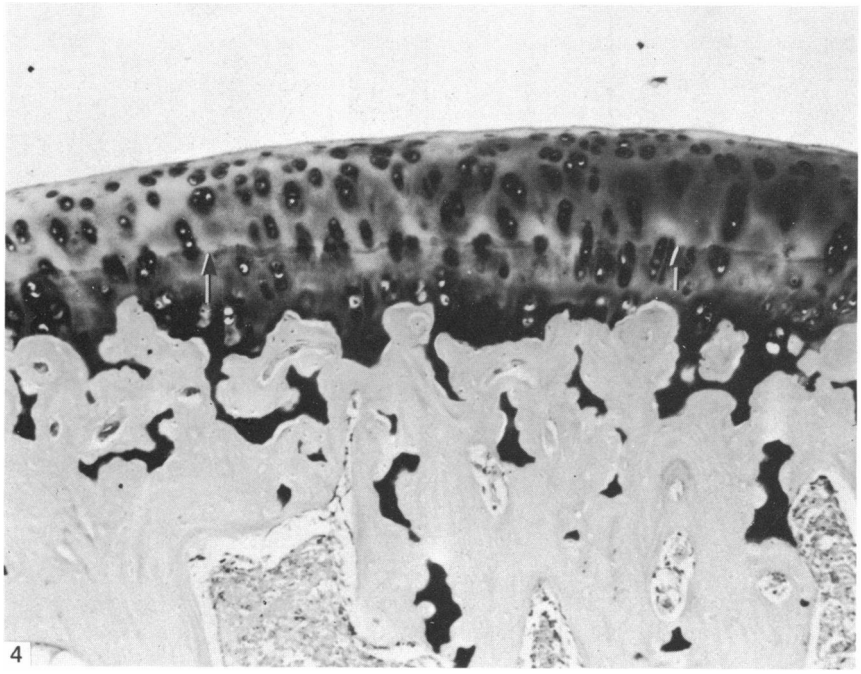

Fig. 4. Medial femoral condyle of guinea-pig in Group 4 stained with Safranin-O, fast green. Arrows indicate tidemark. Note the intact surface, normal pericellular staining throughout and areas of prominent interterritorial staining of the uncalcified cartilage. × 63. (Compare with Figs. 2 and 3).

fixed for one to three weeks in 10 % buffered formalin and were then embedded in paraffin. The condyles were decalcified in Decalcifier I (Surgipath Medical Industries, Chicago, IL) for seven to ten days prior to embedding. Microscopic sections, 5 μm thick, of the distal end of the femur were stained with Safranin-O and fast green to demonstrate matrix proteoglycans (Rosenberg, 1971), or with haematoxylin and eosin. Sections of the parapatellar synovium were stained with haematoxylin and eosin. To control for variations in uptake of stain, samples from both knees of experimental animals and from untreated controls (Group 6) were stained concurrently. Measurements of cell density of the uncalcified cartilage of the central region of each femoral condyle were made on four to six sections stained with haematoxylin and eosin, according to the method of Stockwell (1971).

RESULTS

None of the animals developed joint swelling and no gross instability or limitation of motion occurred as a result of the intra-articular injection or the immobilisation procedure. After three weeks of immobilisation, atrophy of the ipsilateral limb muscles was obvious in all animals in Group 3. However, atrophy was less apparent at the time of death if the animals had been permitted to move freely for three weeks after removal of the constraint, regardless of whether they had received an intra-articular injection of iodoacetate (Groups 4 and 5)

The daily food intake of each animal ranged from 35 to 70 g and thus supplied a non-scorbutic, dietary level of ascorbic acid (Mannering, 1949). All animals in Group 1 maintained normal body weight. In contrast, animals which were im-

Table 1. *Effects of intra-articular iodoacetate injection and immobilisation on articular cartilage of the medial femoral condyle*

Experimental groups	No. of animals	Intra-articular iodoacetate injection	Immobilisation period (weeks)	Duration of experiment (weeks)	Decrease in Safranin-O staining		Fibrillation	Osteophytes
					Interterritorial	Pericellular		
Group 2	6	Yes	0	6	+++	+++	+++	+++
Group 2	6	Yes	1	6	+++	+++	+++	+++
Group 3	4	Yes	3	3	+++	+++	0	0
Group 4	5	Yes	3	6	+−+++	0−+++	0	0−++
Group 5	4	No	3	6	0	0	0	0
Group 6	5	No	0	—	0	0	0	0

0, none; +, slight; ++, moderate; +++, marked.

Table 2. *Effects of intra-articular iodoacetate injection and of immobilisation on chondrocyte density*

Groups	No. of animals	Intra-articular iodoacetate injection	Immobilisation period (weeks)	Duration of experiment (weeks)	Cell density			
					Medial condyle		Lateral condyle	
					Mean no. of cells/mm³ × 10⁻³ (range)	% of contra-lateral knee	Mean no. of cells/mm³ × 10⁻³ (range)	% of contra-lateral knee
Group 1	6	Yes	None	6	22 (11–38)	8 ($P<0.005$)*	31 (15–69)	21* ($P<0.005$)*
Group 2	6	Yes	1	6	65 (0–111)	41 ($P<0.005$)*	177 (102–244)	74 ($P<0.01$)*
Group 3	4	Yes	3	3	114 (105–124)	70 ($P<0.005$)*	146 (120–153)	73 ($P<0.05$)*
Group 4	5	Yes	3	6	158 (78–200)	77	181 (178–185)	86
Group 5	4	No	3	6	160 (140–174)	99	199 (149–232)	101
Group 6	5	No	None	—	185 (137–244)		178 (144–237)	

*Determined by Student's *t* test for paired observations.

mobilised, whether or not they received intra-articular iodoacetate, lost a mean proportion of 12% of their initial body weight during the period of constraint. However, all animals gained weight during the subsequent period of remobilisation, so that at death the weights of animals in Groups 2, 4 and 5 were no different from those of animals in Group 1.

Macroscopic examination

No knee effusions were noted and the synovial membrane appeared normal to the naked eye in all instances. Articular cartilage of animals in Group 6, and from the right (untreated) knees of all animals in Groups 1–5 was translucent and pearly pink, with a smooth, macroscopically intact surface. Cartilage from guinea-pigs whose left knee was immobilised but which did not receive iodoacetate (Group 5) was also normal to the naked eye.

In marked contrast, six weeks after injection of iodoacetate the articular cartilage of every animal in Group 1 had lost its normal pink translucency and was diffusely white and opaque. Immobilisation for one or three weeks following intra-articular iodoacetate injection (Groups 2–4) did not alter the opacity of the cartilage.

Microscopic examination

Synovial membrane

The synovial membrane from both knees of animals in Groups 5 and 6, and from untreated knees of Groups 1–4, was histologically normal. It consisted of an intima 1–6 cells thick and a subintima of loosely arranged collagen fibres and adipose tissue, richly supplied with capillaries. Three weeks and six weeks after iodoacetate injection, regardless of whether the knee was immobilised (Groups 1–4), the synovium exhibited normal cellularity. Cellular infiltration or fibrosis were not observed in any sample.

Articular cartilage

Articular surface

The articular surface was smooth and intact (Fig. 1) in all control samples (Group 6 and untreated knees of Groups 1–5). Immobilisation for three weeks, followed by three weeks of activity (Group 5), did not affect the integrity of the joint surface and no focal areas of cell death or loss of staining suggesting pressure necrosis were seen.

In Groups 1 and 2, horizontal or vertical fibrillation, extending from the surface through the transitional zone was present uniformly on the medial condyle (Fig. 2; Table 1), while the surface of the lateral condyle remained intact. At no time did fibrillation extend through the radial zone of uncalcified cartilage to its junction with the calcified layer at the tidemark. In marked contrast, immobilisation for three weeks following iodoacetate injection (Group 3) prevented fibrillation (Fig. 3), even if, as in Group 4, the animal was permitted three weeks of remobilisation following removal of the constraint (Fig. 4). Furthermore, no focal areas of cell death or loss of staining suggesting pressure necrosis were noted in any of the joints from Groups 3 and 4.

Histochemistry

Articular cartilage from the femoral condyles of untreated controls (Group 6), of uninjected animals whose legs had been immobilised (Group 5), and from the untreated (right) knees of animals in Groups 1–5, appeared histologically and histochemically normal. Furthermore, examination of untreated control samples

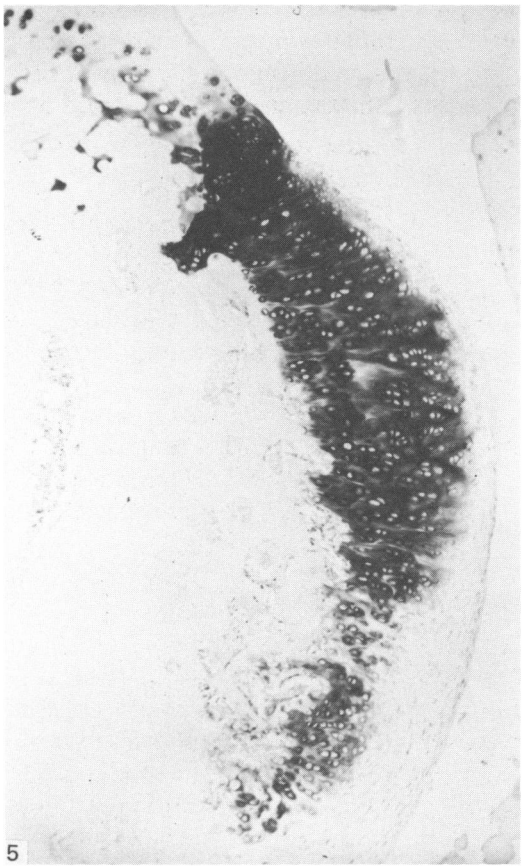

Fig. 5. Cartilaginous osteophyte developing at the medial joint margin in a guinea-pig 6 weeks following intra-articular injection of iodoacetate (Group 1). Safranin-O, fast green stain. × 63.

(Group 6) from each staining rack showed no variation in uptake of stain, thus ensuring valid comparison between each of the experimental groups. Safranin-O staining of the calcified zone was less intense than that of the overlying uncalcified cartilage and the subchondral bone stained pale green with the counterstain (Fig. 1).

The uncalcified cartilage of every injected joint of Group 1 and Group 2 showed a striking, complete loss of pericellular and interterritorial staining (Fig. 2; Table 1). Three specimens each from Groups 1 and 2 also showed some reduction of interterritorial staining beneath the tidemark. Uncalcified cartilage of animals which had been immobilised for three weeks immediately following iodoacetate injection and then killed (Group 3) showed complete loss of Safranin-O staining identical to that seen in Groups 1 and 2 (Fig. 3; Table 1). The tidemark was intact in every specimen from Groups 1–3.

Specimens from animals which were injected with iodoacetate, immobilised for three weeks, and then permitted to ambulate for an additional three weeks (Group 4) were strikingly different from those in Groups 1–3. Samples from three joints showed only focal loss of interterritorial staining, with normal pericellular staining (Fig. 4; Table 1). In cartilage from another Group 4 animal, interterritorial staining was absent, but loss of pericellular staining was seen only around the most superficial chondrocytes. Only one animal in Group 4 showed a complete loss of interterritorial

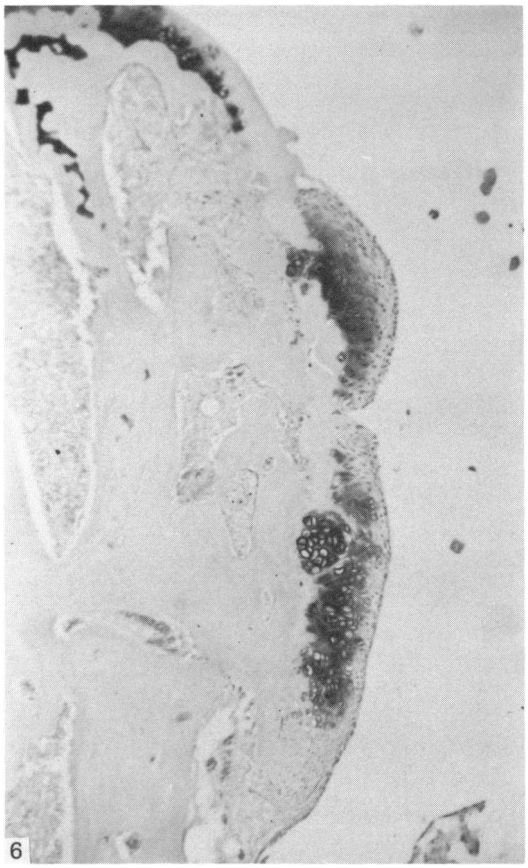

Fig. 6. Osteophyte developing at the medial joint margin in a guinea-pig from Group 4. Safranin-O, fast green stain. × 63.

and pericellular staining such as that seen in Groups 1–3. In all specimens from Group 4 the calcified cartilage was histologically and histochemically normal and the tidemark was intact.

Osteophytes

Osteophytes were not present in Groups 5 or 6 or in the uninjected (right) knees of guinea-pigs in Groups 1–4. In contrast, six weeks after iodoacetate injection (Group 1) cartilaginous osteophytes, which stained intensely with Safranin-O, were present at the medial joint margin and on the medial aspect of the intercondylar groove in every sample (Fig. 5; Table 1). Additionally, they had formed on the lateral aspect of the intercondylar groove in four knees.

One week of immobilisation following iodoacetate injection (Group 2) had no effect on osteophyte formation. However, no osteophyte formation was noted in Group 3, where the ipsilateral leg was immobilised for the entire three weeks interval between iodoacetate injection and death of the animal (Table 1). Group 4, which was also injected with iodoacetate but was permitted three weeks of ambulation after a three weeks period of immobilisation, showed much less tendency to osteophyte formation than Groups 1 and 2; one animal was free of osteophytes, another exhibited a single small osteophyte at the medial joint margin, and three animals developed

small osteophyte buds at the medial joint margin and the medial aspect of the intercondylar groove. These were invariably much less prominent than the osteophytes seen in Groups 1 and 2 (compare Figs. 5, 6).

Cell density

In control animals (Group 6), the cell density averaged 185000 cells/mm^3 in cartilage from the medial condyle and 178000 cells/mm^3 in samples from the lateral condyle (Table 2). The cell density of cartilage from both knees of all animals in Group 5 and from the untreated (right) knees of all animals in Groups 1–4 knees was normal. However, in Group 1 chondrocyte counts of the medial condyle cartilage were only 10 % of control levels ($P < 0{\cdot}005$) while those of the lateral condyle averaged 21 % of controls ($P < 0{\cdot}005$) (Table 2). Immobilisation immediately after intra-articular iodoacetate injection diminished chondrocyte loss. Thus, in animals which were immobilised throughout a three weeks interval between iodoacetate injection and death (Group 3), cell counts of the medial or lateral condylar cartilage were about 70 % of controls. Furthermore, a subsequent three weeks of remobilisation did not result in any apparent depletion of the chondrocyte densities, since cell densities in Group 4 were 77 % (medial condyle) and 86 % (lateral condyle) of control values. However, a comparison of cell densities from Groups 3 and 4 showed no statistically significant difference.

DISCUSSION

The data from Group 3 confirm the authors' previous observation that immobilisation for three weeks after injection of iodoacetate into the guinea-pig knee joint prevents fibrillation and osteophyte formation *during that period* (Williams & Brandt, 1983). Immobilisation alone does not cause any pathological changes in uninjected animals killed after three weeks of constraint (Williams & Brandt, 1983) or, as shown here, in those permitted to survive for a subsequent three weeks period of remobilisation (Group 5). The major significance of the present paper lies in the results in Group 4, which show that this temporary period of constraint also prevents fibrillation and markedly reduces osteophyte formation *subsequently*, when the animal resumes walking 'on all fours'.

The factors underlying initiation and development of osteophytes, which are proliferations of cartilage and bone projecting into the joint space, ligaments or tendons (Sokoloff, 1979), are uncertain. Chrisman, Fessel & Southwick (1965) suggest that osteophyte formation in osteoarthritis is due to stimulation of cells at the chondrosynovial junction by polysaccharides derived from degradation of articular cartilage, which accumulate in the synovial recesses.

Could cartilage breakdown products have stimulated osteophyte formation in the present study? Fibrillation, widespread in Group 1, is absent in Group 4. Possibly in Group 1, the loss of surface integrity which results from load-bearing on chemically damaged cartilage, facilitates egress of matrix breakdown products, so stimulating osteophyte formation at the chondrosynovial junctions. In Group 4, immobilisation may prevent, or reduce, delivery of such products to these sites by preventing fibrillation. Additional studies are required to determine whether the small osteophytes in Group 4 would ultimately become as large as those seen in animals which were not immobilised.

On the other hand, it should be considered that osteophyte formation may be

wholly independent of the degeneration of the articular cartilage. Consistent with this possibility, Danielsson & Hernborg (1970) observe changes in articular cartilage in only about one third of human knees with osteophytes.

Bennett & Bauer (1937) have noted osteophyte formation after dislocation of the patella in rabbits. They suggest that stretching of the synovial membrane at its insertion is a stimulus. Although the volume of iodoacetate solution injected (0·1 ml) in the present study may have stretched the synovial attachments, it was rapidly absorbed and no joint distension was noted 24 hours after injection. Furthermore, single or weekly intra-articular injections of 0·1 ml of normal saline (the vehicle used in this study) do not produce gross or histological abnormalities after three weeks (Williams & Brandt, 1982).

Mechanical instability has also been suggested as a factor in osteophyte formation by Marshall & Olsson (1971) and by Telhag & Lindberg (1972). Osteophytes do not develop in tendons of osteolathyritic rats if the muscles or their motor neurons are transected (Hamre & Yeager, 1957, 1958). Osteophytes which appear after transection of the anterior cruciate ligament in the dog (Gilbertson, 1975) do not develop if the unstable knee is immobilised (Palmoski & Brandt, 1982). However, in the present study mechanical instability of the knee was not apparent in any of the experimental groups.

Safranin-O staining of the uncalcified cartilage in Group 4 is much more widespread and intense than that in Group 1 (compare Figs. 2 and 4). It is also much more prominent than that seen in animals injected with iodoacetate which were killed after the ipsilateral limb had been immobilised for three weeks (Group 3; Fig. 3). This period of immobilisation following iodoacetate injection appears to protect the surviving chondrocytes sufficiently to permit them to synthesise new matrix during the period of remobilisation. The newly synthesised matrix, furthermore, is sufficiently normal to preserve its gross structural integrity when subjected to load-bearing forces during the period of remobilisation. In contrast, immobilisation for only one week (Group 2) fails to permit recovery of the damaged cartilage and does not protect against fibrillation, loss of Safranin-O staining or osteophyte formation.

The repair mechanisms of articular cartilage are poorly understood. They have important implications, however, with respect to the reversibility of cartilage damage. Full thickness defects of articular cartilage which penetrate subchondral bone are filled by repair tissue resembling either fibrocartilage or hyaline cartilage (Ghadially, Ghadially & Ghadially, 1977; Salter *et al.* 1980), In contrast, superficial lacerations which do not extend into subchondral bone, fail to repair (DePalma, McKeever & Subin, 1966; Ghadially, Ailsby & Oryschak, 1974). In the present study, however, repair occurs without invasion of the tidemark by capillaries from subchondral bone.

As noted previously (Williams & Brandt, 1983), three weeks of immobilisation reduces cell depletion following iodoacetate injection (Table 2). Although the difference is not statistically significant, the chondrocyte density in Group 4 is greater than that in Group 3, suggesting that cell replication may occur in the period of remobilisation. Although cell division occurs in immature articular cartilage, it has been noted only rarely in normal adult articular cartilage (Mankin, 1963). On the other hand, cloning of chondrocytes is common in osteoarthritic cartilage (Sokoloff, 1969), in which thymidine incorporation is also increased, suggesting active cell division (Mankin, Dorfman & Lippiello, 1971). Although mitoses have not been observed in the present study, cell division may occur in the three weeks remobilisation period, during which tissues have not been examined.

There are several differences between the medial and lateral femoral condyles after intra-articular injection of iodoacetate. Although decreases in Safranin-O staining are similar, fibrillation occurs only on the medial condyle. Osteophytes develop on the medial joint margin but are never noted on the lateral joint margin. Furthermore, chondrocyte loss is consistently less marked on the lateral than on the medial condyle. These findings may be related to differences in load-bearing between the two regions. The guinea-pig knee is normally maintained in varus, suggesting that proportionately more load is borne by the medial than by the lateral condyle. The earliest changes in rat knees following immobilisation also occur in the medial compartment (Evans, Eggers, Butcher & Blumel, 1960). Osteoarthritic changes in dogs which have undergone transection of the anterior cruciate ligament appear first in the medial compartment of the knee joint (McDevitt, Muir & Eyre, 1980). Moskowitz, Goldberg & Malemud (1981) report pitting, ulceration and osteophytes on the medial femoral condyle, with no macroscopic changes on the lateral condyle, six weeks after partial meniscectomy in rabbits. Incorporation of [^{3}H]thymidine and [^{14}C]glycine is lower on the medial than on the lateral femoral condyle at three weeks after operation.

Iodoacetate is a broad metabolic poison which is known to specifically inhibit glycolysis in bovine cartilage (Rosenthal, Bowie & Wagoner, 1942). Preliminary results in the authors' laboratory show that incubation of proteoglycan with iodoacetate *in vitro* for 18 hours does not affect the hydrodynamic size of either proteoglycan aggregate or monomer. However, the exact mechanism of action of iodoacetate in this model remains uncertain.

SUMMARY

Recent studies have indicated that immobilisation of the lower limb may prevent surface fibrillation and osteophyte formation, and reduce cell depletion, following injection of iodoacetate into the ipsilateral knee of the guinea-pig. The present study shows that temporary immobilisation also facilitates repair of the damaged cartilage during a subsequent period of remobilisation in which the animal is permitted to move 'on all fours'. Thus, in animals killed six weeks after a single intra-articular injection of iodoacetate (0·3 mg in 0·1 ml saline), and in which the injected knee had been immobilised for three weeks, Safranin-O staining of the articular cartilage was more intense, chondrocyte density greater, and osteophytosis much less marked than in animals injected with iodoacetate but killed *immediately* after the three weeks immobilisation period. By contrast, immobilisation for only one week failed to protect against degenerative changes and osteophytes caused by iodoacetate injection. Immobilisation alone produced no apparent pathological changes in animals which did not receive iodoacetate.

This work was supported by grants AM 20582 and AM 27075 from the National Institutes of Health and a grant from the Grace M. Showalter Trust. Dr. Williams is a Postdoctoral Fellow of the Arthritis Foundation. The authors would like to thank Suzanne Schnaitter for technical assistance, Joe Demma for photographic assistance and Roberta Fehrman for secretarial support.

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