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Exercise increases osteophyte formation and diminishes fibrillation following chemically induced articular cartilage injury

JAMES M. WILLIAMS AND KENNETH D. BRANDT

Rheumatology Division, Indiana University School of Medicine, 541 Clinical Drive – Room 492, Indianapolis, Indiana, U.S.A. 46223

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INTRODUCTION

Studies in the authors' laboratory have shown that a single intra-articular injection of sodium iodoacetate (IA) results in progressive degeneration of guinea-pig knee joint cartilage, with fibrillation, chondrocyte depletion, diminished staining with Safranin-O, and prominent osteophytes (Williams & Brandt, 1982). Recently, it was reported that immobilisation of the injected knee prevents osteophytes and fibrillation and reduces cell loss, but does not prevent loss of proteoglycans (Williams & Brandt, 1984*a*). If, however, a period of constraint is imposed and the animals are then permitted to walk on the injected knee, the articular surface remains intact and small osteocartilaginous proliferations occur at sites where osteophytes develop after IA injection in non-immobilised joints (Williams & Brandt, 1984*b*). These data suggest that the mechanical forces of joint motion acting on damaged articular cartilage play an essential role in osteophyte formation. To examine further this possibility, the present study investigates the effect of treadmill exercise on osteophyte development and on the integrity of articular cartilage following intra-articular injection of IA.

MATERIALS AND METHODS

Animals

Adult male albino guinea-pigs (approximately 32–40 weeks old) were housed individually in 18 × 18 × 18 inches 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 four experimental groups, as described below. The weight of each guinea-pig 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 consisted of 10 animals whose left knee joint was injected with IA but which were not exercised. These animals were killed 1 week ($n = 6$) or 3 weeks ($n = 4$) after the injection.

Group 2 consisted of 12 guinea-pigs whose left knee joint was injected with IA, and which were exercised daily on a treadmill until they were killed 1 week ($n = 5$) or 3 weeks ($n = 7$) after injection.

Group 3 consisted of 11 animals which did not receive intra-articular IA, but which were exercised daily on a treadmill until killed 1 week ($n = 5$) or 3 weeks ($n = 6$) later.

Group 4 consisted of 10 guinea-pigs which served as untreated controls and which were killed at the outset of the experiment. Both knees of each animal in Groups 1–3 were examined daily to assess mobility and swelling.

Intra-articular injection and exercise procedures

Prior to intra-articular injection of IA, animals in Groups 1 and 2 were anaesthetised by an intramuscular injection of ketamine hydrochloride (50 mg/kg). After the skin over the left knee had been shaved and washed with 70% isopropyl alcohol, the injection was performed under aseptic conditions by passing a 26 gauge needle attached to a tuberculin syringe through the joint capsule lateral to the patellar ligament. A sterile solution of 0·3 mg IA in 0·1 ml 0·9 N sodium chloride was injected.

For the exercise procedure (Groups 2 and 3) the animals were placed on a treadmill subdivided into five individual runways which measured 5 inches by 5 feet. Exercise sessions were conducted daily lasting from 15 to 20 minutes and covered a treadmill running distance of 750–1000 t, thus requiring the guinea-pigs to run at a moderate pace.

Tissue analysis

At death, both knees were immediately opened and examined macroscopically. The distal femur was then removed and assessed for articular surface defects according to the method of Meachim (1972). Briefly, the femoral condyles were rinsed with distilled water, dipped in a 50% solution of India ink, rinsed again and examined with reflected light using a $\times 10$ lens.

The femoral condyles and the parapatellar synovial membrane were fixed for 1–3 weeks in 10% buffered formalin and embedded in paraffin. Prior to embedding, the condyles were decalcified in Decalcifier I (Surgipath Medical Industries, Chicago, IL) for 7–10 days. Microscopic sections, 8 μm thick, of the femoral condyles were stained with Safranin-O and fast green to demonstrate matrix proteoglycans (Rosenberg, 1971), or with haematoxylin and eosin. Synovium was stained with haemotoxylin and eosin only. Sections from knees of experimental animals and from untreated controls (Group 4) were stained concurrently to control for variations in uptake of the stain. Measurements of cell density in the uncalcified cartilage were made from analyses of four to six sections from the central region of each femoral condyle, stained with haematoxylin and eosin (Stockwell, 1971).

RESULTS

The daily food intake of each animal ranged from 35 to 70 g throughout the experiment and all animals in Group 1–3 maintained their body weight until death. Thus, there was no evidence that the intra-articular injection of IA produced adverse systemic effects.

Gross examination

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 exercise procedure. Synovial effusion was not present in knees of any animal in Groups 1–4 and the synovial membrane in all instances was macroscopically normal. The articular cartilage from knees of all untreated animals (Group 4), from both knees of all

animals which were exercised but did not receive intra-articular IA (Group 3), and from the right (untreated) knees of animals in Groups 1 and 2 was translucent and pink, with a smooth, macroscopically intact particular surface.

One week after injection of IA however, regardless of whether the animal was subsequently submitted to the exercise regimen, the articular cartilage from the left (injected) knees of all animals in Groups 1 and 2 had lost its normal pink translucency and was diffusely white and opaque. Similar opacity was noted in all animals killed three weeks after IA injection.

None of the cartilage specimens retained India ink, with the exception of one sample from an animal in Group 1, which showed moderate uptake of ink on the central portion of the medial femoral condyle three weeks after IA injection.

Microscopic examination

Synovial membrane

The synovial membrane from both knees of every animal in Groups 3 and 4, and from the right uninjected knees of every animal in Groups 1 and 2, was histologically normal (Ghadially & Roy, 1969). It consisted of an intima one to six lining cells in thickness and a subintima of loosely arranged collagen fibres and adipose tissue, richly vascularised with capillary loops beneath the lining cell layer. Occasional villi protruded into the joint cavity.

One week after injection of IA, focal hypercellularity of the lining cell layer was noted in four of the six samples in Group 1 and in one of the six injected joints in Group 2. In all cases, the synovial lining exhibited normal cellularity three weeks after the injection. Neither inflammation nor fibrosis were noted in any sample.

Articular cartilage

Histochemistry. Femoral cartilage from control animals (Group 4) and from the right uninjected knees of animals in Group 1 appeared histologically and histochemically normal. Safranin-O staining revealed differential staining properties of the matrix: orange-red staining of the calcified zone was less intense than that of the overlying uncalcified cartilage (Fig. 1*a*). Infrequently, focal areas of diminished staining of interterritorial matrix were observed in control samples. With the fast green counterstain, the overlying lamina splendens, as well as the subchondral bone stained pale green.

One week after IA injection (Group 1), interterritorial matrix staining with Safranin-O was markedly reduced throughout the uncalcified cartilage on the medial condyles (Table 1). A marked reduction of pericellular staining was noted as well, but was confined to the superficial zone. Cartilage from the ipsilateral knees of all animals which were submitted to treadmill exercise for one week after IA injection (Group 2) showed similar changes. Safranin-O staining of the calcified cartilage was normal in Groups 1 and 2 one week after IA injection.

Three weeks after IA injection the uncalcified cartilage from every injected joint showed a marked loss of pericellular and interterritorial staining (Group 1; Fig. 1*b*). Cartilage below the tidemark, in contrast, continued to appear normal. This marked loss of interterritorial staining was seen also in every injected knee of Group 2 animals after three weeks of exercise (Table 2). However, the depletion of pericellular staining was much less marked in Group 2 than in Group 1 three weeks after IA injection (Fig. 1*c*). Thus, in samples from five animals, pericellular staining was normal, or was diminished only in the superficial zone (Table 2), and was thus

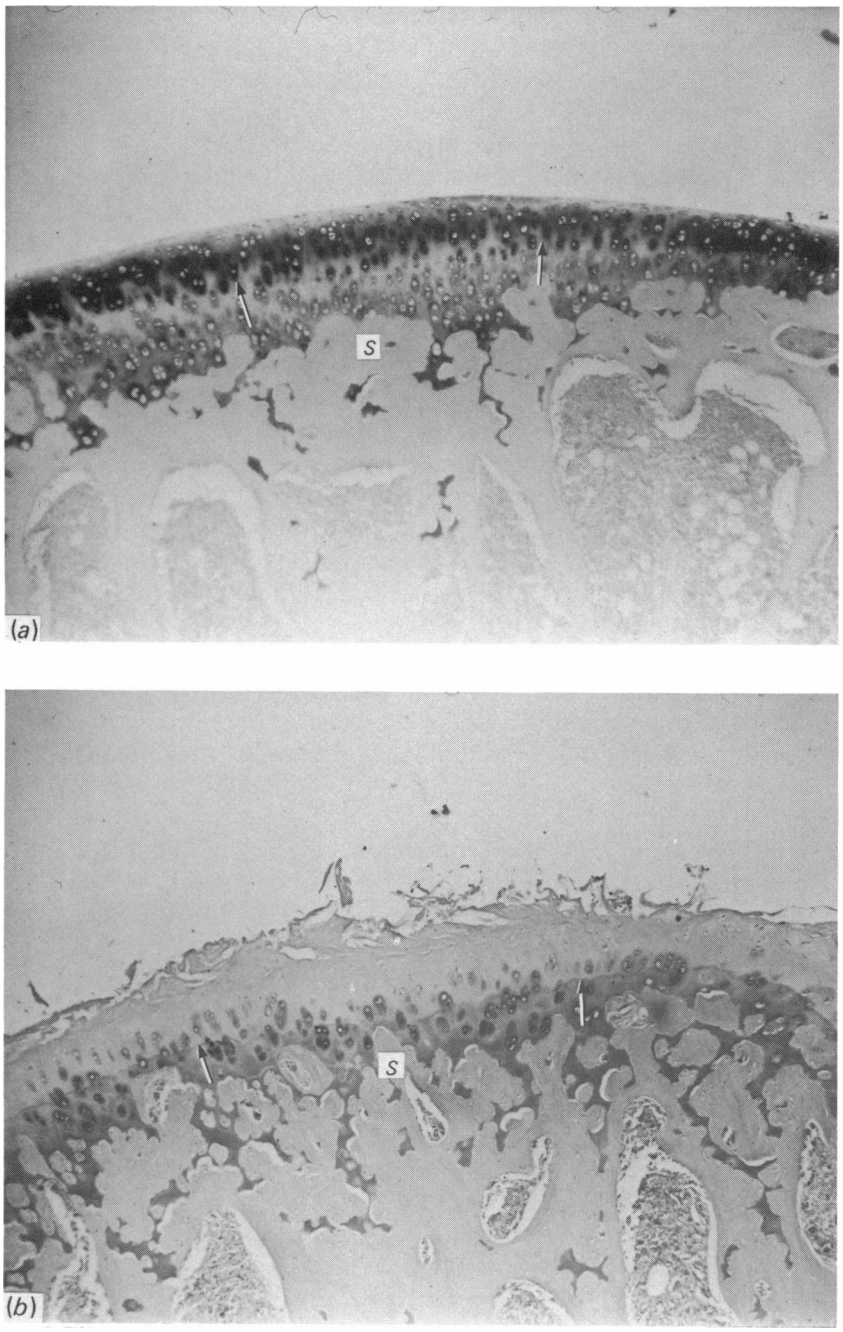

Fig. 1 (*a–c*). Medial femoral condyle from (*a*) control guinea-pig (Group 4); (*b*) guinea-pig three weeks after injection of iodoacetate into the ipsilateral knee joint (Group 1), exhibiting depletion of chondrocytes, loss of interterritorial and pericellular staining with Safranin-O, and extensive fibrillation; (*c*) guinea-pig which was exercised daily for three weeks on a treadmill after intra-articular injection of iodoacetate (Group 2). Note the absence of fibrillation and persistence of pericellular staining. The intense Safranin-O staining of the calcified region (below the tidemark) is commonly observed in samples from normal (32–40 weeks old) guinea-pigs. Safranin-O, fast green. *S*, subchondral bone; arrows indicate tidemark. × 60.

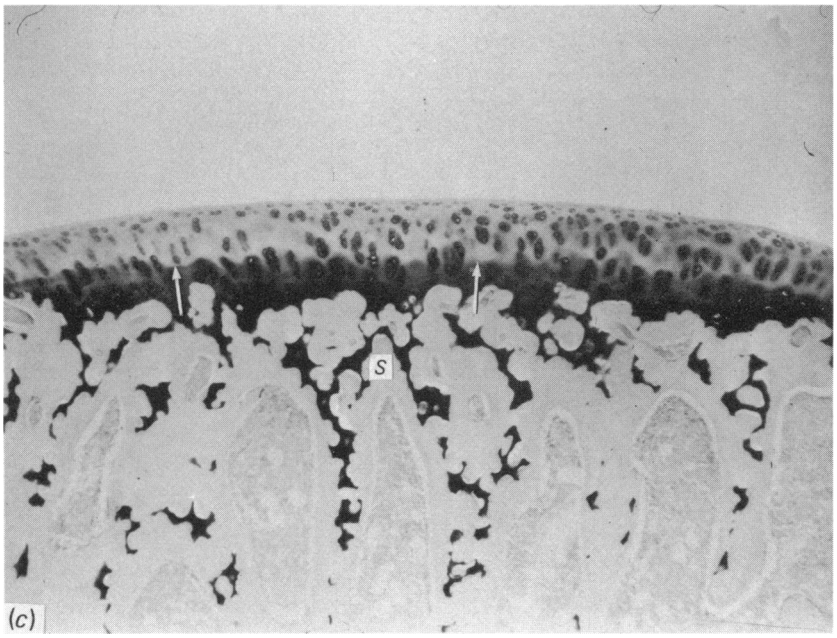

Table 1. *Effects of treadmill exercise on changes in the guinea-pig knee one week after intra-articular injection*

Experimental groups	No. of animals	Intra-articular iodoacetate injection	Treadmill exercise	Decrease in Safranin-O staining*		Defects in surface integrity	Osteophyte formation
				IT	PC		
1	6	Yes	No	+++	+	0–+	0
2	5	Yes	Yes	+++	+	0	+++
3	5	No	Yes	0–+	0	0–+	0
4	10	No	No	0	0	0	0

* IT, interterritorial; PC, pericellular; 0, none; +, slight; ++, moderate; +++, marked.

similar to Group 1 and 2 samples obtained one week after IA injection (Table 1). In samples from the other two Group 2 animals, pericellular staining was extensively reduced.

Femoral cartilage from all five animals which were exercised for one week but not injected with IA (Group 3) was histologically and histochemically normal. Cartilage from both knees of three of the six animals in Group 3 which were submitted to three weeks of exercise was also normal. However, samples from the other three animals in Group 3 showed diffuse loss of interterritorial staining and fibrillation (see below). These changes occurred bilaterally on the medial condyles in one animal and unilaterally, on the medial or lateral condyle, in the other two animals.

The tidemark remained intact and the calcified cartilage was normal in all uninjected joints, and no other changes were seen in these areas as a result of the exercise regimen.

Cell density. Cell densities calculated for cartilage from the medial condyle of normal untreated animals (Group 4) and from untreated knees in Groups 1 and 2

Table 2. *Effects of treadmill exercise on changes in distal femoral articular cartilage three weeks after injection of iodoacetate into the guinea-pig knee*

Experimental groups	No. of animals	Intra-articular iodoacetate injection	Treadmill exercise	Decrease in Safranin-O staining*		Defects in surface integrity
				IT	PC	
1	4	Yes	No	+++	+++	++
2	7	Yes	Yes	+++	0–++	0
3	6	No	Yes	0–+	0	0–+
4	10	No	No	0	0	0

* IT, interterritorial; PC, pericellular; 0, none; +, slight; ++ moderate; +++, marked.

Table 3. *Effects of treadmill exercise on changes in cell density of distal femoral articular cartilage three weeks after injection of iodoacetate into the guinea-pig knee*

Experimental groups	No. of animals	Intra-articular iodoacetate injection	Treadmill exercise	Cell density of an uncalcified cartilage from medial condyle	
				Mean number of cells/mm^3 × 10^{-3} of left knee (range)	% of contralateral right knee
1	4	Yes	No	30 (20–40)	17 ($P < 0{\cdot}05$)*
2	7	Yes	Yes	132 (70–183)	60 ($P < 0{\cdot}01$)*
3	6	No	Yes	195† (102–261)	101
4	10	No	No	185 (144–253)	99

* Determined by Student's *t* test for paired observations.
† These values were not significantly different from those for Group 4.

were on average 185000 cells/mm^3 (Table 3). Values for Group 3 were slightly higher than those of Group 4 (199000 and 195000 cells/mm^3, after one and three weeks of treadmill exercise, respectively; Table 3), although the difference was not significant.

In contrast, one week after IA injection (Group 1) the mean cell density of cartilage from the medial condyle had fallen to 69% of control values ($P < 0{\cdot}01$). The depletion of chondrocytes was progressive, so that three weeks after IA injection, chondrocyte counts on medial condyle cartilage were only 17% of control levels ($P < 0{\cdot}05$; Table 2). Notably, one week of treadmill exercise following intra-articular IA (Group 2) resulted in a smaller reduction in cell density than that seen in animals which received IA but were not exercised (76% of control values; $P < 0{\cdot}01$). Furthermore, in animals submitted to three weeks of exercise following the IA injection, mean cell densities were strikingly greater (60% of control, $P < 0{\cdot}01$) than those of Group 1 animals at the corresponding time interval (17% of controls).

Articular surface. The articular surface was intact in all control samples both in Group 4 and in the right (untreated) knees of Groups 1 and 2 (Fig. 1*a*; Table 1).

Table 4. *Effects of treadmill exercise on osteophyte formation three weeks after injection of iodoacetate into the guinea-pig knee*

Experimental groups	No. of animals	Intra-articular iodoacetate injection	Treadmill exercise	Size of osteophytes: Medial joint margin	Intercondylar groove: Medial	Intercondylar groove: Lateral	Lateral joint margin
1	4	Yes	No	+++-+++++	++-+++	0	0
2	7	Yes	Yes	+++-+++++	++-+++	++-+++	+
3	6	No	Yes	0	0	0	0
4	10	No	No	0	0	0	0

0, absent; +, early cartilage metaplasia with no outgrowth; ++, small osteophyte; +++, medium sized osteophyte; ++++, large osteophyte.

Changes in the integrity of the articular surface appeared after IA injection, but developed more slowly than loss of chondrocytes. Thus, one week after IA injection (Group 1), except for solitary small focal defects on the medial condyles of two joints, the articular surface remained intact (Table 1). One week of treadmill exercise following IA injection appeared to have no effect on the articular surface, which was smooth and intact in all cases (Group 2).

These changes in Group 1 were progressive, so that three weeks after IA injection surface defects were present on the medial condyle of all injected joints. These ranged from minor surface irregularities (two joints) to widespread vertical fibrillation extending through the transitional zone (two joints) (Fig. 1*b*). Occasionally, particles of India ink were seen trapped in the fibrillated areas, even though staining was not apparent to the naked eye.

Treadmill exercise for one week (Group 3) did not produce any abnormality in the articular surface (Table 1). However, after three weeks, fibrillation covering approximately one third of the weight bearing surface and extending into the transitional zone was seen bilaterally in the medial condyles of one animal in Group 3, and unilaterally on the medial or lateral condyle of two other animals; it was associated with a decrease in Safranin-O staining, as described above (Table 2).

Treadmill exercise for three weeks following the IA injection appeared to have a striking protective effect, since the articular surface in Group 2 was invariably smooth and intact (Fig. 1*c*; Table 2).

Osteophytes. Osteophytes were not observed in the knees of any animals in Groups 3 or 4, or in the uninjected knees of animals in Groups 1 or 2 (Table 4). The normal medial joint margin in the guinea-pig was characterised by a gradual thinning and disappearance of the articular cartilage of the medial condyle, which blended into the periosteum and the reflection of the synovial membrane, creating a deep synovial recess (Fig. 2). The intercondylar groove was similarly marked by a gradual thinning of articular cartilage and by the origin of the cruciate ligaments, which were covered by synovium.

Although no osteophytes were seen one week after intra-articular IA injection in animals which were not exercised (Group 1; Table 1), prominent osteophytes, which were as large as those seen in joints of non-exercised animals *three* weeks after IA injection (Group 1) (see below), were noted in injected joints of all animals which were exercised for one week after IA injection (Group 2). In addition, small to

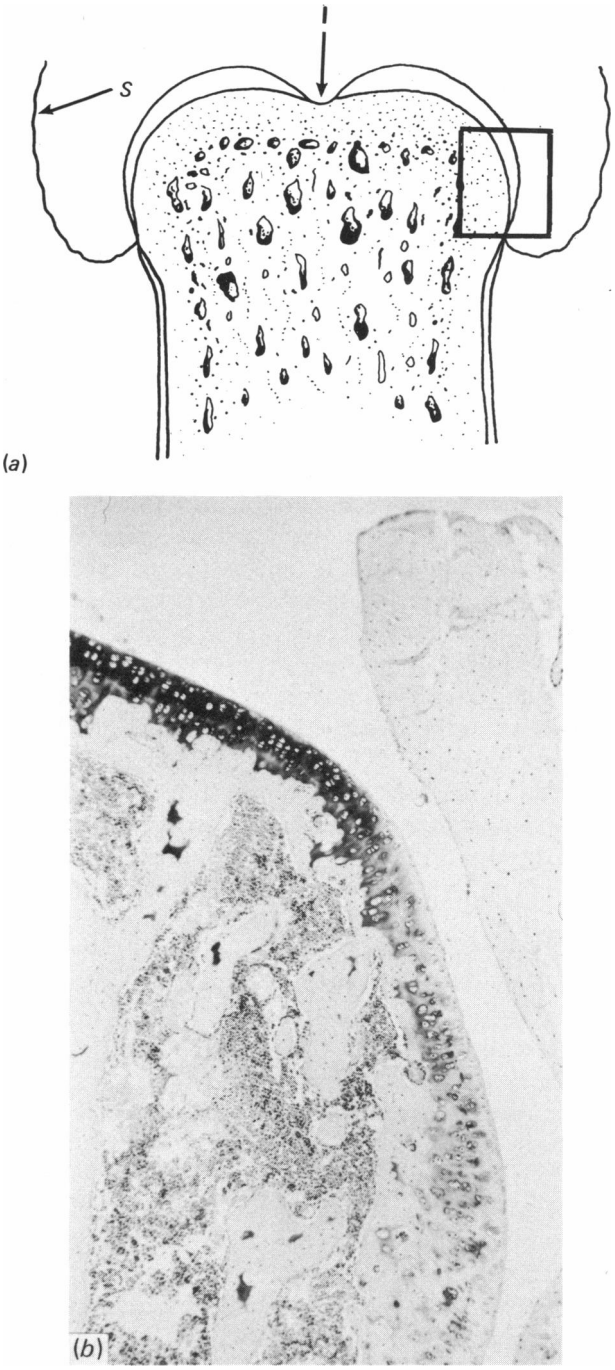

Fig. 2 (*a–b*). (*a*) Schematic drawing of femoral condyles of guinea-pig knee showing synovial reflection. Arrow indicates the intercondylar groove; *S*, synovial reflection. Inset depicts the area from which tissue shown in Figures 2 (*b*), 3 (*a*) and 3 (*b*) was obtained; (*b*) histological appearance of the normal medial joint margin from the uninjected (right) knee of an animal in Group 1. Note the absence of osteophytes. Safranin-O, fast green stain. × 63.

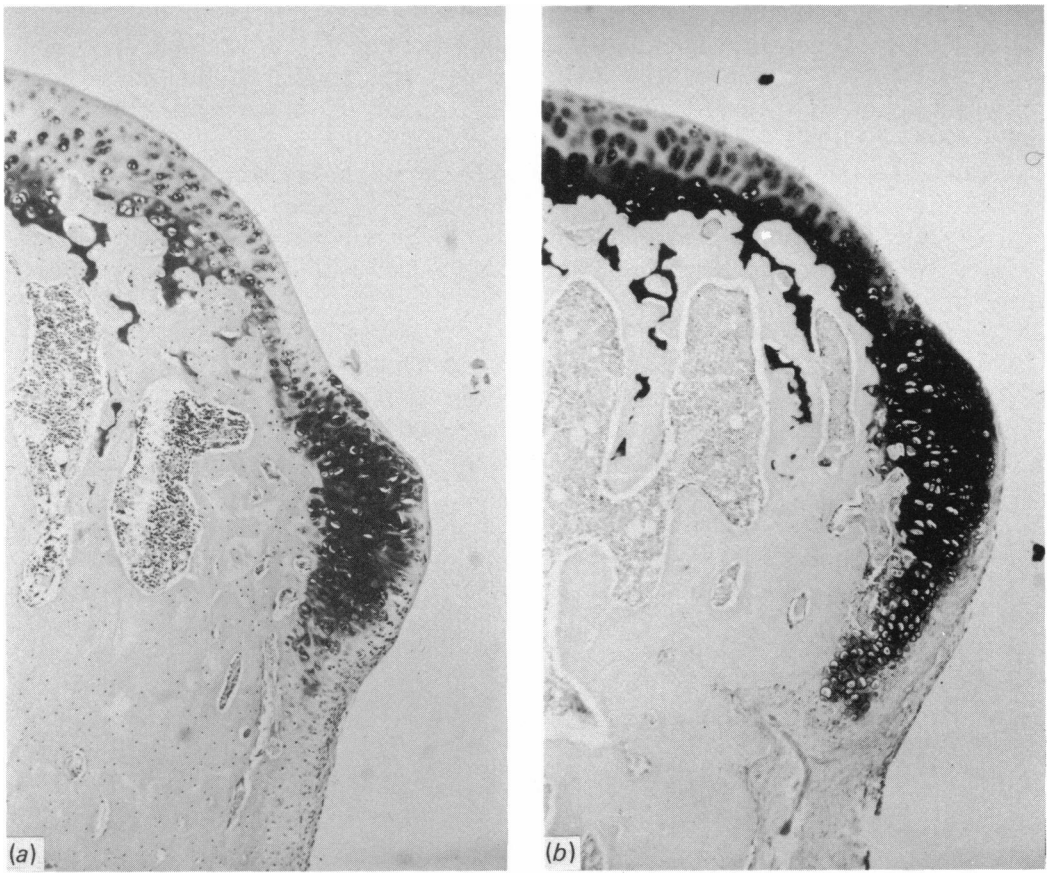

Fig. 3(*a–b*). (*a*) Cartilaginous osteophyte (the hump in the cartilage) developing at the medial joint margin in a guinea-pig three weeks after intra-articular injection of iodoacetate (Group 1); (*b*) osteophyte developing at the medial joint margin in a guinea-pig which was exercised daily for three weeks after IA injection (Group 2). Safranin-O, fast green stain. × 63.

medium sized osteophytes were seen at the medial aspect of the intercondylar groove in all but one of these animals.

Three weeks after the IA injection, osteophytes, which stained intensely with Safranin-O, were invariably present in Group 1 (Table 4). Those at the medial joint margin (Fig. 3*a*) tended to be larger than those at the medial aspect of the intercondylar groove. In animals which were exercised for three weeks after IA injection (Group 2), osteophytes at these sites were similar to those in Group 1 (Fig. 3*b*). However, osteophytosis was clearly more widespread in Group 2, and was present at the lateral aspect of the intercondylar groove in six of seven knees. In addition, focal clusters of chondrocytes surrounded by a matrix which stained with Safranin-O, suggesting early osteophyte formation, were seen at the lateral joint margin in six knees. In contrast, no osteophytes were noted at these sites in any animals in Group 1 (Table 4).

DISCUSSION

The data demonstrate that daily treadmill exercise for three weeks prevents fibrillation, reduces chondrocyte loss, and preserves pericellular matrix proteoglycans following intra-articular injection of IA. On the other hand, osteophyte formation is clearly accelerated so that osteophytes, which do not occur in injected joints of Group 1 animals one week after IA injection, are seen in all Group 2 animals after only one week of exercise. They tend to be as large as those seen three weeks after IA injection in Group 1 animals which are not exercised. Additionally, osteophytes are more widespread in injected joints which are exercised than in those which are not.

Slight hypercellularity of the lining cell layer and mild infiltration of the subsynovial layer with inflammatory cells has not been noted 24 hours after IA injection (Williams & Brandt, unpublished observations). In the present study, focal hypercellularity of the lining cell layer is present one week after injection in four of six samples, from Group 1, but in only one of the five samples from Group 2. In all other cases, the synovial membrane is normal.

The factors underlying the initiation and development of osteophytes are not clear. 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 the articular cartilage. Possibly, the treadmill exercise regimen facilitates egress of matrix breakdown products from the chemically damaged cartilage and these then stimulate osteophyte formation at the chondrosynovial junctions. On the other hand, osteophyte formation may occur without articular cartilage damage. In dogs which have undergone transection of the anterior cruciate ligament, Gilbertson (1975) notes osteophyte development as early as three days following destabilisation of the knee, i.e. prior to any apparent structural changes in the articular cartilage. In a radiological study over periods of up to 16 years, Danielson & Hernborg (1970) note that associated structural changes develop in only one third of human knee joints which initially exhibit osteophytes alone.

Mechanical instability has 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). Similarly, osteophytes do not develop after transection of the anterior cruciate ligament in the dog if the unstable knee is immobilised (Palmoski & Brandt, 1982). Osteophytes which appear in guinea-pigs within three weeks of a single intra-articular injection of IA do not develop if the injected knee is immobilised (Williams & Brandt, 1984*a*). Mechanical instability has not been obviously apparent in any of the experimental groups of the present study, however, so that it must be considered that immobilisation may protect against osteophyte formation via some other mechanism.

It has previously been reported that immobilisation of the ipsilateral knee prevents fibrillation following IA injection, presumably because of a reduction in the mechanical forces acting on chemically damaged cartilage (Williams & Brandt, 1984*a*). Furthermore, temporary immobilisation of the injected knee, when followed by a period of remobilisation, permits partial restoration of matrix proteoglycans and prevents fibrillation during subsequent weight bearing on the damaged cartilage (Williams & Brandt, 1984*b*). The present study shows that treadmill exercise may also prevent fibrillation after IA injection. Presumably, it does so by reducing cartil-

age damage and promoting repair of the cartilage injury. That this indeed occurs is suggested by the relative preservation of chondrocytes and of pericellular staining with Safranin-O, and by the ability of the damaged cartilage in Group 2 to tolerate loading without fibrillating.

Notably, three weeks after IA injection the reduction in pericellular staining is less marked in Group 2 than in Group 1 (Table 2). In Group 2, furthermore, it is less marked at three weeks (Table 2) than at one week (Table 1), especially in the superficial zones. This suggests that some recovery of proteoglycan synthesis by surviving chondrocytes may have occurred during this interval in Group 2. As noted, cell counts in Group 2 are slightly higher one week after injection than at three weeks (76% and 60% of control values, respectively).

A number of studies suggest that exercise is beneficial to joint cartilage. Salter *et al.* (1980) report healing of full thickness defects with hyaline cartilage in only 3% of rabbit knees which had been immobilised and in 5% of knees of rabbits permitted free cage activity, but in 44% of rabbits whose knees had undergone continuous passive motion until killed. Lanier (1946) reported a lower incidence of spontaneous articular cartilage degeneration in male strain D mice which are exercised for one year than in non-exercised littermate controls. Videman (1982) has shown that exercise does not affect development of joint capsule thickening, fibrillation or cartilage thickness in rabbit knees with experimentally induced osteoarthritis.

Palmoski, Colyer & Brandt (1980) have demonstrated a decrease in thickness of canine femoral condylar cartilage with proteoglycan depletion and reduction in proteoglycan synthesis, following transection of the ipsilateral paw, which reduces joint loading but permits essentially normal oscillatory motion of the knee joint. Cyclic compressive stresses of reasonable magnitudes have been shown to increase net glycosaminoglycan synthesis by normal canine femoral cartilage slices *in vitro*, whereas static stresses lead to reductions in glycosaminoglycan and protein synthesis and uronic acid content, and to an increase in water content of the cartilage presumably reflecting damage to the collagen network (Palmoski & Brandt, 1984). In the present study, treadmill running may have provided sufficient cyclic compressive stress to stimulate the surviving chondrocytes to synthesise new matrix notably sufficiently normal to prevent fibrillation under conditions of repetitive impulsive loading.

Simon *et al.* (1976) have demonstrated progressive cartilage degeneration in rabbit knees following the production of focal chondrocyte death by freezing. Within six months, cartilage from the area of injury shows absence of chondrocytes, severe reduction in Safranin-O staining and loss of [^{35}S]sulphate uptake. By twelve months, fibrillation and chondrocyte clones surrounded by Safranin-O staining have developed. Notably, these changes are confined to the area of freezing. Although the cartilage changes which develop after IA injection can be similarly attributed to chondrocyte death, the effects are much more extensive than those seen after local freezing, consistent with the extensive chondrocyte damage which occurs after injection of a metabolic poison into the joint space.

Finally, it is noteworthy that diffuse fibrillation associated with reduction in Safranin-O staining occurs in three of the six animals exercised for three weeks without prior intra-articular IA injection (Group 3). It is possible that the changes in these joints are related to exercise alone. However, cartilage fibrillation has not been noted previously following treadmill exercise similar to the regimen used in the present study in normal guinea-pigs (Saaf, 1950). On the other hand, spontaneous degeneration of knee cartilage may occur in guinea-pigs in the age group employed

here (32–40 weeks). The authors have observed fibrillation, similar to that seen in Group 3, in knee cartilage in 15% of normal guinea-pigs at this age. Cartilage degeneration may be much more common in still older guinea-pigs, however, since Silverstein & Sokoloff (1958) reported cartilage loss with eburnation of the femoral condyles in 24 of 26 knees of animals 123 weeks old.

SUMMARY

The present study shows that a treadmill exercise regimen imposed on guinea-pigs whose articular cartilage has been damaged by intra-articular injection of IA reduces chondrocyte depletion, results in an increase in pericellular Safranin-O staining around surviving chondrocytes, and prevents fibrillation of the articular surface. The data suggest that exercise protected, or facilitated recovery of, chondrocytes subjected to chemical injury, and that the surviving cells then synthesised a matrix which was sufficiently normal to withstand impulsive joint loading. On the other hand, the exercise regimen accelerated osteophyte formation, and led to formation of osteophytes in sites at which they did not develop in animals which received intra-articular IA but which were not exercised.

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