J. Anat. (1982), **134**, 1, pp. 103–109
With 6 figures
Printed in Great Britain

Effects of surgically induced instability on rat knee articular cartilage

JAMES M. WILLIAMS*, DAVID L. FELTEN, RICHARD G. PETERSON AND BRIAN L. O'CONNOR

Department of Anatomy, Indiana University School of Medicine, 1100 W. Michigan Street, Indianapolis, Indiana 46223

(*Accepted* 22 *January* 1981)

INTRODUCTION

Degenerative changes in human joints frequently follow mechanical derangements such as meniscal tears, surgical removal of menisci, torn ligaments, recurrent subluxation of the patella and the presence of loose bodies in the joint cavity (Johnson & Brewer, 1979; Smillie, 1951; Fairbank, 1948; Jackson, 1968). The articular cartilage responds to such derangements in characteristics ways. Grossly, the cartilage loses its glistening blue-white appearance and turns yellow. Areas of surface disruption appear which ultimately may develop into pits and ulcerations. Histological changes associated with mechanical derangement may be seen in both the cartilage and the subchondral bone. Cartilaginous changes include focal areas of softness, surface disruptions, cloning and degeneration of chondrocytes and a decrease in matrix proteoglycans. Bony changes include thickening of the subchondral bone, the formation of pseudocysts, eburnation and the development of osteophytes.

Such changes have also been observed in experimental animal models. Ulcerations, fibrillation, eburnation and a fibrous stratum covering the cartilage surface were seen following partial and total patellectomy in rabbit and dog (Bruce & Walmsey, 1942; Cohn, 1944; DePalma & Flynn, 1958). Partial meniscectomy led to cartilage erosions and formation of osteophytes in rabbits (Moskowitz, 1972; Moskowitz *et al.* 1973). Hulth, Lindberg & Telhag (1970) reported cloning of chondrocytes, ulceration of cartilage and development of osteophytes in rabbits following medial meniscectomy and transection of the medial collateral and cruciate ligaments.

Erosion and irregular thickening of cartilage, and osteophytes have been demonstrated in dogs following transection of the anterior cruciate ligament (Marshall, 1969; Nilsson, 1949; Paatsama, 1952).

The present investigation was undertaken to test the rat knee joint as a small animal model for studying the effects of anterior cruciate ligament transection on articular cartilage. Lesions resembling osteoarthritis have been reported in the dog following anterior cruciate section (Marshall, 1969), and are believed to have resulted from the consequent mechanical instability of the joint. On the basis of this one would expect that transection of the anterior cruciate ligament in the rat knee joint would result in degenerative lesions in the articular cartilage. The present study was designed to test this hypothesis under conditions of exercise and no exercise.

* Present address: Department of Medicine, Rheumatology Section, Indiana University School of Medicine, 1100 West Michigan St. - Long 492, Indianapolis, Indiana 46223, U.S.A.

MATERIALS AND METHODS

Thirty adult male Wistar rats (initial weight 325 gm) were used for this study. All animals were individually housed in stainless steel cages with wire mesh floors. Food (Purina Chow) and water were given *ad libitum.*

Operated animals were anaesthetised with sodium pentobarbital (50 mg/kg). The skin overlying the anteromedial aspect of the right knee was incised to expose the patellar ligament. The joint cavity was then opened with an incision medial and parallel to the patellar ligament. After the patella had been dislocated laterally, the anterior cruciate ligament was exposed and transected. The patella was then relocated and the wound was closed. Sham-operated animals were treated in an identical manner without transection of the ligament. Six groups of 5 animals each were prepared in the following manner:

Group	Procedure
1	Ligament section – Exercise
2	Ligament section – No exercise
3	Sham section – Exercise
4	Sham section – No exercise
5	Non-operated – Exercise
6	Non-operated – No exercise

The exercised animals were conditioned to run on a motorized treadmill and were exercised 10 times during the month following surgery. Each exercise session lasted 30 minutes and covered a treadmill running distance of 1500 feet. These exercise sessions required the rats to run at a moderate pace.

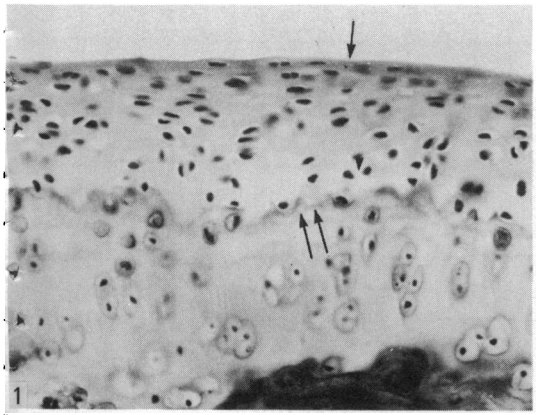
At the end of the one month post-surgical period, all animals were killed by decapitation. The knee joints were opened by cutting through the quadriceps tendon and reflecting the patella distally for viewing the fresh, unfixed femoral condyles. The joint surfaces were kept moist with normal saline to prevent drying during the examination. The distal femurs were then removed and fixed in 10% buffered formalin. After fixation, the joints were decalcified in Decalcifier I (Surgipath Medical Industries, Inc., Glenview, Illinois, U.S.A.) and processed for paraffin sections. Parasagittal serial sections of 8 μm thickness were taken from the central weight-bearing area of the medial femoral condyle. In all, 40 slides were taken from each animal. Every fifth slide was stained with haematoxylin and eosin and all other slides were stained with safranin 0, fast green, FCF to demonstrate matrix proteoglycans (Lillie, 1965; Rosenburg, 1971). Samples from all six groups were stained and examined together to control for variations in the staining procedure.

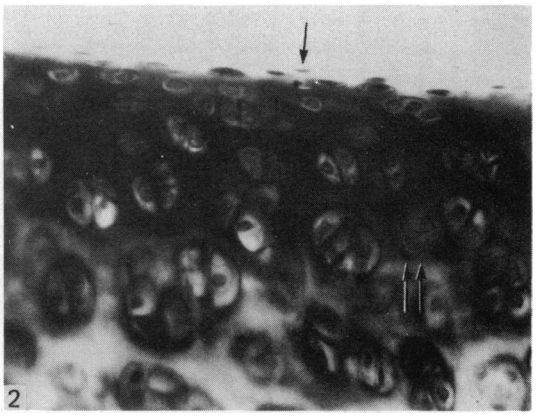
RESULTS

Gross examination

Femoral condyles from Groups 2–6 were normal in gross appearance. The cartilage was white with a glistening surface. Occasionally, very narrow areas were observed where the cartilage appeared translucent and revealed the underlying subchondral bone. These narrow, translucent areas were consistent with observations made in this laboratory of normal rat knee articular cartilage. No osteophytes or synovial effusions were seen in any of the six groups.

The medial condyles from Group 1 were somewhat flattened. The cartilage





Figs. 1–2. Normal rat knee articular cartilage. Articular surface (single arrow). Tidemark (double arrows). (1) haematoxylin and eosin. (2) safranin O, fast green, FCF. ×200.

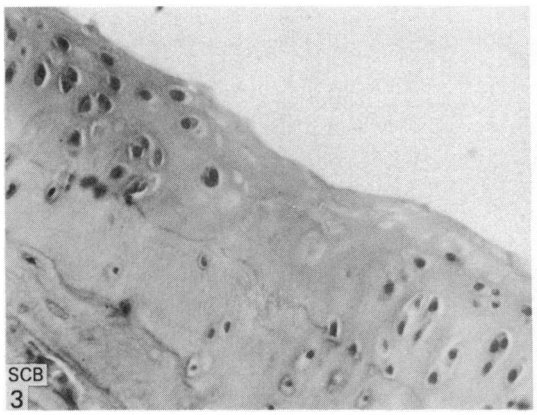
appeared to be swollen and the cartilage surface was a lacklustre grey colour. When viewed with a dissecting microscope, these surfaces appeared to be slightly roughened as compared to those of Groups 2–6.

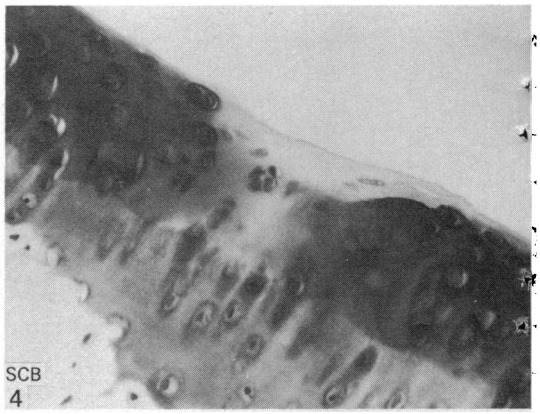
Histological examination

Articular cartilage of the normal rat stained similarly to articular cartilage from other commonly used laboratory animals. The chondrocytes and tidemark were basophilic while the matrix was eosinophilic when stained with haematoxylin and eosin. The cells were arranged into four layers according to the cell shape and orientation to the surface. On the surface, a very thin lamina splendens covered the tangential layer of cells. Beneath the tangential layer was a thicker transitional layer which blended into the radial layer. These three layers comprised the uncalcified zone of the articular cartilage which was separated from the fourth layer, of calcified cartilage, by the tidemark (Fig. 1). The safranin 0 stain, used to demonstrate matrix proteoglycans, revealed differential staining properties of the matrix. Thus, the calcified layer stained less intensely or not at all compared to the overlying uncalcified layer. The overlying lamina splendens as well as the underlying subchondral bone stained green when fast green was used as a counterstain (Fig. 2).

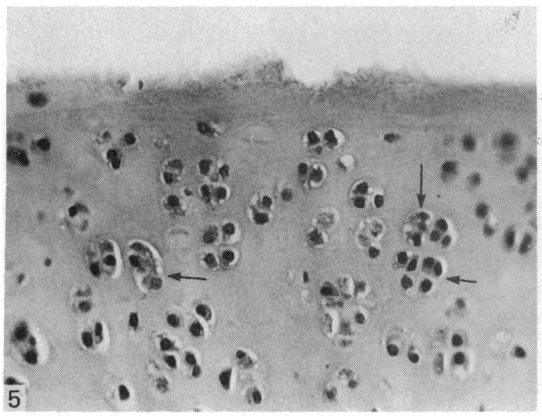
No lesions were present in the articular cartilage and subchondral bone from the non-operated control groups. Surface disruptions and matrix changes of varying severity were seen in the remaining four groups.

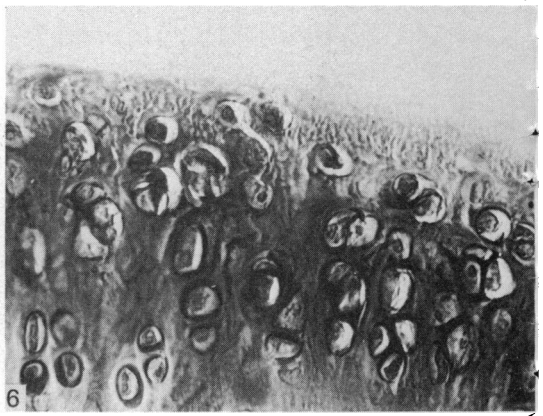
In contrast to the non-operated controls, lesions were consistently present in all the experimentally operated plus exercise animals (Group 1) and were present in three of the five experimental operated minus exercise animals (Group 2). Focal lesions were identified which included surface disruptions, at times extending to the radial layer of cells, a loss of cells and the reduction of safranin 0 staining (Figs. 3, 4). Larger areas of surface disruptions that sometimes extended into the radial layer of cells were occasionally noted. Associated with these larger areas of surface disruption was a loss of cells in the tangential and transitional layers and a corresponding reduction in safranin 0 staining (Figs. 5, 6). Enlarged lacunae, some of which were devoid of cells, were occasionally present deep in the calcified layer, and were suggestive of hypertrophic cartilage. Cloning was occasionally observed in the uncalcified zone where numerous cells occupied a single lacuna.





Figs. 3–4. Focal lesion from Group 1, exhibiting minor surface disruptions, loss of cells and a reduction of matrix proteoglycans. Subchondral bone (SCB). (3) haematoxylin and eosin. (4) safranin O, fast green, FCF. ×200.





Figs. 5–6. Large area of surface disruptions accompanied by a reduction in matrix proteoglycans. Chondrocyte cloning is shown (single arrows) in Fig. 5. (5) haematoxylin and eosin. (6) safranin O, fast green, FCF. ×200.

A few small focal areas of superficial surface disruption and superficial matrix proteoglycan reduction were present in two animals from each of the sham-operated groups. Two small focal areas of swollen cartilage, accompanied by a decrease in cells and a reduction in matrix proteoglycans, were noted in two animals from the sham-operated plus exercise group.

DISCUSSION

As expected, lesions were absent from the knee joints from the non-operated controls and were present in experimental operated groups. Lesions in the experimental operated groups, both with and without exercise, resembled published descriptions of histological changes in dogs' knees following anterior cruciate ligament transection (Marshall, 1969) and partial and total patellectomy (Bruce & Walmsey, 1942; Cohn, 1944; DePalma & Flynn, 1958) and were consistent with biochemical alterations in naturally occurring and experimentally induced osteoarthritis (McDevitt, Muir & Pond, 1973; Mankin, Dorfman, Lippiello & Zarins, 1971; Erlich *et al.* 1975). Moreover, experimental operated group lesions were

similar to histological changes in other mechanical derangement models such as partial and total patellectomy in both dogs and rabbits (Bruce & Walmsey, 1942; Cohn, 1944; DePalma & Flynn, 1958), and partial medial meniscectomy (Moskowitz *et al.* 1973) and medial meniscectomy plus transection of the medial and lateral collateral ligaments in rabbits (Hulth *et al.* 1970).

These results suggest that the adult Wistar rat may be a useful small animal model to study the effects of mechanical derangement on articular cartilage. Unlike other animal models, notably dogs and rabbits, rats are inexpensive to purchase and maintain, easily exercised and not often subject to post-surgical infection. Also rat knee joints only infrequently develop degenerative joint lesions spontaneously (Moskowitz, 1972; Sokoloff, 1969).

Lesions were more frequent in the experimental operated plus exercise animals (5 of 5 animals) than experimental operated minus exercise animals (3 of 5 animals). However, lesions in the unexercised group were usually as severe as those of the exercised groups. In a related project, we have noted that following partial denervation of the rat knee joint, lesions were more frequently encountered in treadmill exercised animals. It seems likely from these data that treadmill exercise may be important in contributing to the frequency if not the severity of lesions by introducing a form of microtrauma in addition to that produced by the surgically induced derangement.

The presence of minor changes in joints from the sham-operated controls was unexpected, although similar results have been reported in sham-operated controls to partial meniscectomy in rabbits (Moskowitz *et al.* 1973). When seen, these changes were confined to the surface and were thus qualitatively distinct from the more pronounced focal and widespread changes seen in the experimental operated joints. Several possible explanations may account for the presence of these lesions. One is that dislocating the patella stretched periarticular tissues, altering joint biomechanics and thus creating a mechanically deranged joint.

Another possibility is that the synovial membrane was irritated sufficiently to stimulate enzymatic destruction of the articular cartilage. It has been shown that the synovial membrane can have a destructive effect on articular cartilage, mediated by direct enzymatic action on the cartilage matrix or by an indirect stimulation of destructive enzymes by chondrocytes (Fell & Jubb, 1977; Jubb, & Fell, 1980). Thus it is conceivable that incising the joint capsule, dislocating the patella and suturing the wound with potentially inflammatory silk sutures was sufficient to traumatise the synovial membrane, initiating the destructive process.

A fourth possibility is that small articular nerves were cut, partially denervating periarticular tissues and resulting in altered protective muscular reflexes (= mechanical derangement). In turn, this could lead to an increase in the amount of microtrauma that ultimately leads to articular cartilage damage. In humans, joint lesions resembling those described in the present study, but of considerably greater severity, occur as complications of various deafferenting diseases (Campbell & Doyle, 1954; Seibert-Daiker, 1978; Rodnan, 1972; Bailey & Root, 1947; Delano, 1946). Moreover, neuropathic joints have been produced experimentally in cats (Eloesser, 1917; Corbin & Hinsey, 1939) and rabbits (Finsterbush & Friedman, 1975) as a result of dorsal rhizotomy. More recently, biochemical changes identical to early biochemical alterations in osteoarthritis were present at three weeks and more pronounced at nine weeks following resection of the posterior and medial articular nerves in dogs (Palmoski, O'Connor & Brandt, 1979).

SUMMARY

Degenerative lesions in the articular cartilage were present following transection of the anterior cruciate ligament in the rat. These lesions included surface disruptions, a reduction in matrix proteoglycans, and cellular changes and therefore were similar to lesions seen in dogs following transection of the anterior cruciate ligament as well as lesions seen in other mechanical derangement models. Lesions were more frequently encountered in animals that had been exercised on a treadmill. This suggests that the rat knee joint may be a useful small animal model in studying the effects of mechanical derangement on articular tissues.

The authors wish to acknowledge the work of Paula Lawson-Moore who prepared the photomicrographs for publication. The final copy was typed by Deborah Komlanc. This study was supported principally by a grant from the Association for the Advancement of Mental Health Research and Education, Inc., Indianapolis, Indiana, U.S.A. and in part by the National Institute of Health Grant PHS RO1 AM 26951-01.

REFERENCES

BAILEY, C. C. & ROOT, H. (1947). Neuropathic foot lesions in diabetes mellitus. *New England Journal of Medicine* **236**, 397–401.

BRUCE, J. & WALMSEY, R. (1942). Excision of the patella. *Journal of Bone and Joint Surgery* **24**, 311–325.

CAMPBELL, D. J. & DOYLE, J. O. (1954). Tabetic Charcot's spine. A report of eight cases. *British Medical Journal* **1**, 1018–1020.

COHN, B. (1944). Total and partial patellectomy. *Surgery, Gynecology and Obstetrics* **79**, 526–536.

CORBIN, K. B. & HINSEY, J. C. (1939). Influence of the nervous system on bone and joints. *Anatomical Record*, **75**, 307–317.

DELANO, P. J. (1946). The pathogenesis of Charcot's joint. *American Journal of Roentgenology, Radium Therapy and Nuclear Medicine* **56**, 189–200.

DEPALMA, A. & FLYNN, J. (1958). Joint changes following experimental partial and total patellectomy. *Journal of Bone and Joint Surgery* **40**, 395–413.

ELOESSER, L. (1917). On the nature of neuropathic joints. *Annals of Surgery* **66**, 201–207.

ERLICH, M. G., MANKIN, H. J., JONES, H., GROSSMAN, A., CRISPEN, C. & ANCONA, D. (1975). Biochemical confirmation of an experimental osteoarthritis model. *Journal of Bone and Joint Surgery* **57**, 392–396.

FAIRBANK, T. J. (1948). Knee joint changes after menisectomy. *Journal of Bone and Joint Surgery* (Brit. ed.) **30**, 664–670.

FELL, H. B. & JUBB, R. W. (1977). The effect of synovial tissue on the breakdown of articular cartilage in organ culture. *Arthritis and Rheumatism* **20**, 1359–1371.

FINSTERBUSH, A. & FRIEDMAN, B. (1975). The effect of sensory denervation on rabbits' knee joints – A light and electron microscopic study. *Journal of Bone and Joint Surgery* **57**, 949–955.

HULTH, A., LINDBERG, L. & TELHAG, H. (1970). Experimental osteoarthritis. *Acta orthopaedica scandinavica* **41**, 522–530.

JACKSON, J. P. (1968). Degenerative changes in the knee after meniscectomy. *British Medical Journal* **2**, 525–527.

JOHNSON, R. P. & BREWER, B. J. (1979). Internal derangement of the knee. In *Arthritis* 9th ed., ch. 65. (ed. D. J. McCarty), Philadelphia: Lea & Febiger.

JUBB, R. W. & FELL, H. B. (1980). The effect of synovial tissue on the synthesis of proteoglycans by the articular cartilage of young pigs. *Arthritis and Rheumatism* **23**, 545–555.

LILLIE, R. D. (1965). *Histopathologic Technic and Practical Histochemistry*, 3rd ed. New York: McGraw-Hill.

MANKIN, H. J., DORFMAN, H., LIPPIELLO, L. & ZARINS, A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *Journal of Bone and Joint Surgery* **53**, 523–537.

MARSHALL, J. L. (1969). Periarticular osteophytes. Initiation and formation in the knee of the dog. *Clinical Orthopaedics and Related Research* **62**, 37–47.

MCDEVITT, C. A., MUIR, H. & POND, M. J. (1973). Canine articular cartilage in natural and experimentally induced osteoarthritis. *Biochemical Society Transactions*. **1**, 287–288.

MOSKOWITZ, R. W. (1972). Experimental models of degenerative joint disease. *Seminars in Arthritis and Rheumatism* **1**, 95–114.

MOSKOWITZ, R. W., DAVIS, W., SAMMARCO, J., MARTENS, M., BAKER, J., MAYOR, M., BURNSTEIN, A. H. & FIANKEL, V. H. (1973). Experimentally induced degenerative joint disease following partial menisectomy in the rabbit. *Arthritis and Rheumatism* **16**, 397–405.

NILSSON, F. (1949). Meniscal injuries in dogs. *North American Veterinarian* **30**, 504–516.

PAATSAMA, S. (1952). *Ligament Injuries in the Canine Stifle Joint: A Clinical and Experimental Study.* Thesis, University of Helsinki.

PALMOSKI, M., O'CONNOR, B. L. & BRANDT, K. (1979). Sectioning of articular nerves impairs the integrity of articular cartilage. *Arthritis and Rheumatism* **22**, 644.

RODNAN, G. P. (1972). Neuropathic Joint Disease (Charcot Joints). In *Arthritis and Allied Conditions* (ed. J. L. Hollander & D. J. McCarty, Jr.), 8th ed., ch. 72. Philadelphia: Lea & Febiger.

ROSENBURG, L. (1971). The chemical basis for the histological use of safranin O in the study of articular cartilage. *Journal of Bone and Joint Surgery* **53**, 69–82.

SEIBERT-DAIKER, F. M. VON. (1978). Die Osteoarthrosis, Syringomyelia und ihre Fehldeutungen. *Beitrag zur Entwicklung des Krankheitsbildes an einer Roentgenverlaufsserie* **128**, 79–82.

SMILLIE, I. S. (1951). Treatment, after-treatment and complications of injuries of menisci. In *Injuries of the Knee Joint*, 2nd ed. Baltimore: Williams and Wilkins Co.

SOKOLOFF, L. (1969). *The Biology of Degenerative Joint Disease.* University of Chicago Press.