Acta orthop. scand. 44, 604–610, 1973

The Department of Orthopaedic Surgery, Malmö General Hospital,
University of Lund, Malmö, Sweden.

DNA-SYNTHESIS IN DEGENERATED AND NORMAL JOINT CARTILAGE IN FULL-GROWN RABBITS

HANS TELHAG

Accepted 27.ii.73

It is known that joint cartilage in young growing animals grows by mitosis (Elliott 1936, Mankin 1962 a, 1963 a, 1963 b, 1964 and 1968). However, once the animal is full-grown, such mitotic growth appears to cease, for mitoses have never been demonstrated in normal joint cartilage in adult human beings or animals (Elliott 1936, Clark & Clark 1942, Crelin 1957, Crelin & Southwick 1960, Mankin 1963 a, 1963 b, 1964, 1968, Hulth et al. 1972, Telhag 1972). It has been shown that joint cartilage can react to trauma with cellular proliferation (Crelin 1957, Crelin & Southwick 1960, Mankin 1962 b, Telhag 1972) and increased synthesis of glycosaminoglycans in the matrix (Collins & McElligott 1960, Bollet & Nance 1966, Bollet 1969, Mankin & Lippiello 1970, Telhag 1973). Crelin (1957) and Crelin & Southwick (1960) demonstrated that experimental compression of the knee joints in fullgrown rabbits is followed by rapid, progressive degeneration of the joint cartilage. With the aid of intraperitoneal injection of colchicine single mitosis of chondrocytes was demonstrated in the degenerated cartilage but no mitoses were found in the normal joint cartilage.

The present investigation was undertaken to find out:

1. Whether cartilage cells in degenerative joint disease can be labelled with ^{3}H-thymidine,
2. whether ^{3}H-thymidine can be taken up by normal cartilage cells in full-grown rabbits,
3. whether ^{3}H-thymidine can be taken up by cartilage cells after sham-operations, and

Financial support was obtained from Riksförbundet mot Reumatism, Ulla och Gustaf af Ugglas fond and the Swedish Medical Research Council, No. B 72-17X-2436-05.

4. whether ^{3}H-thymidine can be taken up by cartilage cells in "old" rabbits.

MATERIAL AND METHODS

Forty-two full-grown rabbits were used.

Experiment 1

Twenty-two rabbits were operated upon in one of the knee-joints according to a method described by Hulth et al. (1970) to produce degenerative changes in the joint. A piece of the medial collateral ligament was excised, the medial meniscus was extirpated and the anterior and posterior cruciate ligaments were divided. The other knee served as a control.

The animals were killed 2 at a time by intravenous injection of Nembutal in a lethal dose, 1, 3, 5, 7, 10, 15 days and 1, 2, 3, 4, 5 months, respectively, after the operation. Four hours before the animals were killed 20 μCi ^{3}H-thymidine (from the Radiochemical Centre, Amersham, aqueous solution containing 5000 μCi/mM) in 0.2 ml physiological saline was injected into each knee joint.

Both knees were removed and fixed in 10 per cent formalin. The tibial and the femoral ends of the joints were dissected free and treated separately. Both the tibial and the femoral parts were afterwards divided in the frontal plane into two halves with a saw. The 4 halves were decalcified in formic acid and afterwards embedded in paraffin. The preparations were sectioned (7 μ). Autoradiograms of routine histological preparations were made with Ilford K2 liquid emulsion. After exposure for 2 and 3 weeks, respectively, the autoradiograms were developed in Gevaert X-ray developer G 230 and fixed in Gevaert X-ray fixer G 305. The sections were stained through the emulsion with Mayer's haematoxylin. Two to 5 autoradiograms of the tibia and the femur, respectively, of each knee joint of each animal were examined.

Experiment 2

Twenty μCi ^{3}H-thymidine in 0.2 ml physiological saline was injected intraarticularly into each knee of 3 full-grown rabbits. The injection was repeated every sixth hour for 2 days, i.e. each knee received altogether 8 injections. Six hours after the last injection the animals were killed. Both knee joints were removed and treated in the same way as in Experiment 1. Sections were cut at various levels of each preparation for histological examination and for autoradiography. A few preparations of unsatisfactory quality were discarded.

Altogether more than 500 sections of the tibia, femur and patella were obtained for autoradiographic examination.

Experiment 3

Ten full-grown rabbits were operated upon with arthrotomy via a medial parapatellar incision. The unoperated knee joint served as a control. When the joint was opened, care was taken that the capsule bled into the joint, if necessary by a small incision in the capsule. The joint was then closed. The animals were killed

2 at a time 3, 5, 10, 15 and 30 days after the operation. Four hours before the animals were killed by a lethal dose of Nembutal i.v., 20 μCi ^{3}H-thymidine in 0.2 ml physiological saline was injected into each knee joint. Both knee joints were removed and treated in the way described in Experiment 1. Sections for histological and for autoradiographic examinations were cut at various levels of each part of the tibia and femur. More than 450 autoradiograms were examined. Some preparations of unsatisfactory quality were discarded.

Experiment 4

Seven rabbits more than 4 years old, including 2 more than 5 years, received an intraarticular injection of 20 μCi ^{3}H-thymidine in 0.2 ml physiological saline in both knee joints. Four hours later the animals were killed with an overdose of Nembutal i.v. The tibial and femoral ends of the joint were removed and treated in the way described above, after which sections were cut for histological as well as for autoradiographic evaluation. At least 5 autoradiograms from each part of the tibia and 5 from each part of the femur, i.e. in all, about 320, were examined.

RESULTS

Experiment 1

The cartilage of one of the *unoperated knee* joints was found to contain several thymidine labelled chondrocytes in the superficial and transitional layers in the medial tibial condyle. Cellular death was suspected in the superficial layer and possibly also in the transitional layer and, in some of the preparations, also flaking of the superficial layer. No thymidine labelled chondrocytes were found in preparations from the remaining 21 unoperated knee joints.

In the *operated knees* thymidine labelled chondrocytes were found in animals examined 5 days to 5 months after the operation. Such cells were found in all the animals except 3, the latter examined 5, 7 and 150 days respectively, after the operation. In the preparations studied 5, 7 and 10 days after the operation the labelled cartilage cells were situated mainly in the superficial and transitional layers near the medial or lateral margins and near the attachments of the anterior and posterior cruciate ligaments. In preparations removed 15 days and 1 month after the operation, the labelled chondrocytes were scattered more and more evenly over the entire surface of the joint cartilage and more and more often in the columnar layer. These preparations also showed clones containing labelled cartilage cells, usually in the superficial layer but also in the transitional and the columnar layers. As a rule, these clones contained only one labelled nucleus, occasionally 2 or more. In the preparations obtained 2, 3, 4 and 5 months after the

operations labelled cartilage cells were more common in the transitional and columnar layers and occasionally such cells were found near the border of the calcified layer. In the 3-month sections occasional clones with labelled cartilage cells were seen near the calcification line.

Experiment 2

The histological sections showed no signs of degenerative changes of the cartilage. Only one knee joint showed some labelled cartilage cells.

Experiment 3

The histological preparations showed no degenerative changes in the cartilage. In 3 knee joints examined 5, 10 and 30 days after arthrotomy, cellular proliferation was seen in the margins near the periosteum. The above-mentioned 10 and 30 day preparations showed also a moderate osteoblast activity subperiostally in the margins. No labelled cartilage cells were found in the autoradiograms.

Experiment 4

In one animal both knees showed signs of degenerative changes of the cartilage with clones, flaking and fibrillation. Cartilage cells were occasionally seen in the superficial and transitional layers. The remaining preparations showed no degenerative changes with certainty and no labelled chondrocytes.

DISCUSSION

Joint cartilage was formerly regarded as an inert tissue and mitosis in cartilage in full-grown animals was considered rare. Research during the last 15–20 years has, however, shown that adult joint cartilage can react to trauma with cellular proliferation (Crelin 1957, Crelin & Southwick 1960, Mankin 1962 b, Telhag 1972) and an increased synthesis of glycosaminoglycans (Collins & McElligott 1960, Bollet & Nance 1966, Bollet 1969, Mankin & Lippiello 1970, Telhag 1973) in the matrix. In degenerative joint disease also the synthesis of collagen is increased (Repo & Mitchell 1971). Although Pelc (1964, 1968) has shown that some cells in non-dividing tissues, for example, can form so-called metabolic DNA, which has nothing to do with cell division, it may

be assumed (Hughes 1959) that DNA synthesis occurs mainly in association with cellular proliferation. The purpose of the present investigation was to find out whether the production of degenerative changes of the cartilage in the knee joint in full-grown rabbits by surgically induced instability of the joint results in an increased number of labelled cells in the joint cartilage. This possibility was confirmed in the present investigation, which showed labelled cartilage cells in the operated knee of 19 of 22 animals. As early as 5 days after the operation thymidine labelled cartilage cells were demonstrated, while histological degenerative changes of the cartilage did not appear until 15–30 days after the operation. Labelled cartilage cells were also demonstrated in those groups of cells (clones) which are typical of osteoarthritis, which suggests that such clones may be the result of mitosis. These findings agree with those found *in vitro* in human joint cartilage (Hulth et al. 1972).

Control examinations confirm that in full-grown rabbits ^{3}H-thymidine labels a larger number of cells in cartilage degenerated by instability of the knee joint than in normal cartilage. Such labelling was found in the unoperated side in only one of 22 knees. It cannot be excluded *a priori* that mitosis may occur in normal, adult cartilage, but if so, the G_1-phase is so long that it cannot be demonstrated by ordinary flash labelling. ^{3}H-thymidine was therefore given 8 times within 2 days in both knees of 3 full-grown rabbits. In only one of the 6 knee joints could any labelled chondrocytes be demonstrated. In addition, knee joints of old rabbits (more than 4 years) were examined for "spontaneous" mitosis. In only one of the 7 knee joints studied could such "spontaneous" labelling be demonstrated and in that knee the joint cartilage was degenerated.

Another question is whether the operative trauma *per se* can cause a reaction of the joint cartilage. Sham-operations consisting of arthrotomy and induced bleeding into the joint were performed on 10 rabbits. In none of them could labelling with ^{3}H-thymidine be demonstrated. The degenerative disease of cartilage produced by instability of the knee joint resembles spontaneous osteoarthritis in some respects. The histological changes show the same pattern (Collins 1949). Earlier investigations (Mankin & Lippiello 1970, Telhag & Gudmundson 1972) have shown that the DNA-synthesis is increased in degenerative joint disease in man and in animals. This has been confirmed in the present investigation. The experimental model used produces degenerative osteoarthritic-like joint disease. Attempts to repair degenerative

changes in the matrix may be considered typical of osteoarthritis. As shown in the present investigation, this attempt to repair is associated with an increased number of cells labelled with ^{3}H-thymidine, which may suggest mitoses, a phenomenon hardly ever seen in normal full-grown rabbits.

SUMMARY

Chondrocytes in the joint cartilage in full-grown rabbits with degenerative changes of the cartilage can be labelled with ^{3}H-thymidine. Sham-operations do not produce degeneration of the cartilage or result in the labelling of cartilage cells. In old animals primary osteoarthritis with cartilage cells capable of taking up the label may perhaps develop. Division of cartilage cells occurs only rarely, if ever, in adults.

REFERENCES

Bollet, A. J. & Nance, J. L. (1966) Biochemical findings in normal and osteoarthritic articular cartilage. II. Chondroitin sulphate concentration and chain length, water and ash content. *J. clin. Invest.* **45**, 1170.

Bollet, A. J. (1969) An essay on the biology of osteoarthritis. *Arthr. and Rheum.* **12**, 152.

Clark, E. R. & Clark, E. L. (1942) Microscopic observations on new formation of cartilage and bone in the living mammal. *Amer. J. Anat.* **70**, 167.

Collins, D. H. (1949) *Pathology of articular and spinal diseases.* Williams and Wilkins Co., Baltimore.

Collins, D. H. & McElligott, T. F. (1960) Sulphate ($^{35}SO_4$) uptake by chondrocytes in relation to histological changes in osteoarthritic human articular cartilage. *Ann. rheum. Dis.* **19**, 318.

Crelin, E. S. (1957) Mitosis in adult cartilage. *Science* **1925**, 650.

Crelin, E. S. & Southwick, W. O. (1960) Mitosis of chondrocytes induced in the knee joint articular cartilage of adult rabbits. *Yale J. Biol. Med.* **33**, 243.

Elliott, H. C. (1936) Studies on articular cartilage. I. Growth mechanisms. *Amer. J. Anat.* **58**, 127.

Hughes, W. L. (1959) The metabolic stability of DNA. In: *The kinetics of cellular proliferation.* ed. Stohlman Jr. F.R. p. 83. Grune and Stratton, New York.

Hulth, A., Lindberg, L. & Telhag, H. (1970) Experimental osteoarthritis in rabbits. *Acta orthop. scand.* **41**, 522.

Hulth, A., Lindberg, L. & Telhag, H. (1972) Mitosis in human osteoarthritic cartilage. *Clin. Orthop.* **84**, 197.

Mankin, H. J. (1962 a) Localization of tritiated thymidine in articular cartilage of rabbits. *J. Bone Jt Surg.* **44-A**, 682.

Mankin, H. J. (1962 b) Localization of tritiated thymidine in articular cartilage of rabbits. II. Repair in immature cartilage. *J. Bone Jt Surg.* **44-A**, 688.

Mankin, H. J. (1963 a) Localization of tritiated thymidine in articular cartilage of rabbits. III. Mature articular cartilage. *J. Bone Jt Surg.* **45**-A, 529.

Mankin, H. J. (1963 b) The calcified zone (basal layer) of articular cartilage of rabbits. *Anat. Rec.* **145**, 73.

Mankin, H. J. (1964) Mitosis in articular cartilage of immature rabbits—a histologic, stathmokinetic (colchicinic) and autoradiographic study. *Clin. Orthop.* **34**, 170.

Mankin, H. J. (1968) The effect of aging on articular cartilage. *Bull. N.Y. Acad. Med.* **44**, 545.

Mankin, H. J. & Lippiello, L. (1970) Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. *J. Bone Jt Surg.* **52**-A, 424.

Pelc, S. R. (1964) Labelling of DNA and cell division in so called non-dividing tissues. *J. Cell. Biol.* **22**, 21.

Pelc, S. R. (1968) Turnover of DNA and function. *Nature (Lond.)* **219**, 162.

Repo, R. U. & Mitchell, N. (1971) Collagen synthesis in mature articular cartilage of the rabbit. *J. Bone Jt Surg.* **55**-B, 541.

Telhag, H. (1972) Mitosis of chondrocytes in experimental "osteoarthritis" in rabbits. *Clin. Orthop.* **86**, 224.

Telhag, H. & Gudmundson, C. (1972) Nucleic acids in degenerative joint disease – an experimental study in rabbits. *Clin. Orthop.* **88**, 247.

Telhag, H. (1973) Effect of tranexamic acid (Cyklokapron®) on the synthesis of chondroitin sulphate and the content of hexosamine in the same fraction on normal and degenerated joint cartilage in the rabbit. *Acta orthop. scand.* **44**, 249.

Correspondence to:

Hans Telhag
Dept. of Orthopaedic Surgery
Malmö General Hospital
University of Lund
Malmö, Sweden