

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

NUCLEIC ACIDS IN ARTICULAR CARTILAGE FROM RABBITS OF DIFFERENT AGES

H. TELHAG & T. HAVDRUP

Accepted 16.xi.74

The joint cartilage varies considerably with age (Rosenthal et al. 1941, Barnett et al. 1963, Mankin 1963, Mankin & Baron 1965, Davies 1969 and Mankin 1970). Histologically, cartilage is a special type of tissue and has one of the lowest ratios of cell mass to matrix (Hamerman & Schubert 1962). The chondrocytes are sparse even in very young cartilage. A decrease in the number of chondrocytes with advancing age in the cartilage of rabbits has been found in some investigations (Barnett et al. 1963, Mankin & Baron 1965). Mankin (1968) counted the chondrocytes in cartilage from rabbits of various ages and found that in immature animals (2 months) the counts are in the range of 2.55×10^5 cells/mm³, at 6 months 2.26×10^5 and in fullgrown animals (18 months) 1.92×10^5 cells/mm³. Meachim & Collins (1962) found no such decrease in man. However, Stockwell (1967) found that the cell density in articular cartilage diminished during maturation (0-30 years) by about sevenfold but during ageing (31-89 years) there was no significant change in the cell density of the whole thickness of articular cartilage.

This paper concerns quantitative determination of nucleic acids in the articular cartilage from rabbits of varying age and the new formation of DNA.

MATERIAL AND METHODS

The material consisted of three groups of animals (grey silver), five immature animals (6 weeks old) weighing 470 to 640 g (mean 535 g), six fullgrown animals (1-1½ years old) weighing 2,110 to 2,530 g (mean 2,260 g) and five old rabbits

Supported by Herman Järnhardts stiftelse, Ulla och Gustaf af Ugglas fond and Svenska Läkaresällskapets fonder.

(more than 4 years old), weighing between 3,280 and 5,150 g (mean 3,840 g). Forty $\mu\text{Ci } ^3\text{H-thymidine}^*$ was injected into both knees of all animals. Four hours later the animals were killed by an overdose of nembutal. Immediately after the animals had died the cartilage from the articular surfaces of the tibia and the femur were dissected with a knife, care being taken to avoid inclusion of subchondral bone and other tissue. The cartilage of each knee was treated separately. The material was weighed (wet weight).

The excised cartilage was placed in a mortar and covered with liquid nitrogen and pulverized. It was then homogenized in 5 ml of 10 per cent TCA (trichloroacetic acid), and the acid-soluble nucleotides were separated by centrifugation and washed twice, each time with 5 ml of the same solvent. The insoluble residue was treated with 10 ml of 0.3 M potassium hydroxide for 18 hours at 37° C to hydrolyze RNA, which thereby was brought into solution. The solution was adjusted to pH 7 by addition of 70 per cent perchloric acid. An equal volume of 5 per cent TCA was added to the neutral solution. This resulted in precipitation of protein and DNA, which were separated from the solution by centrifugation and washing of the precipitate twice, each time with 5 ml of 5 per cent TCA.

RNA and DNA in the separated fractions were determined with the orcinol reaction (Mejbaum 1939) and the Ceriotti procedure (Ceriotti 1952), respectively. The separation of RNA and DNA was checked in all experiments by applying both reactions to the RNA as well as the DNA fraction.

Each sample was assayed for radioactivity ($^3\text{H-thymidine}$) after suspension in 10 ml of Instagel (Packard), using a two-channel Packard Tri-Carb liquid scintillation spectrometer. The results were recorded as counts per milligram of wet weight per minute.

RESULTS

The results are summarized in Table 1. The concentration of DNA and RNA relative to the amount of tissue was significantly lower in cartilage from fullgrown and old rabbits when compared with that in young rabbits.

The synthesis of DNA was significantly less in joint cartilage from fullgrown and old animals than in cartilage from young animals, both in relation to the amount of tissue and to the amount of DNA.

The concentrations of DNA and of RNA were the same in fullgrown rabbits as in the old animals. The synthesis of DNA relative to the amount of tissue and the amount of DNA was less in the old animals than in the fullgrown ones.

* From the Radiochemical Center, Amersham. Aqueous solution containing 5.000 mCi/mM.

Table 1. Concentration of nucleic acids and synthesis of DNA in articular cartilage from immature, fullgrown and old rabbits.

Cartilage	γ DNA/mg	γ RNA/mg	γ RNA/ γ DNA	cpm DNA/mg	cpm DNA/ γ DNA
1. Immature rabbits	1.41 ± 0.53	14.18 ± 4.32	10.51 ± 2.51	72.94 ± 62.99	46.83 ± 28.85
2. Fullgrown rabbits	0.86 ± 0.28	8.94 ± 2.08	10.85 ± 2.17	7.25 ± 2.34	9.19 ± 4.20
3. Old rabbits	0.83 ± 0.37	7.88 ± 1.05	10.60 ± 3.31	3.41 ± 1.27	4.87 ± 2.92
probability level 1-2	$.01 > P > .001$	$.01 > P > .001$	—	$.01 > P > .001$	$P > .001$
probability level 1-3	$.05 > P > .01$	$P > .001$	—	$.01 > P > .001$	$P > .001$
probability level 2-3	—	$.2 > P > .1$	—	$P > .001$	$.05 > P > .01$

DISCUSSION

Immature cartilage grows by mitotic division of the chondrocytes. In the immature cartilage, cell division occurs in two zones: one just below the articular surface, probably the growth zone of the joint cartilage and the other zone in the basal parts of the cartilage, probably constituting the growth zone of the epiphyseal nucleus. When the rabbit approaches maturity the outer zone disappears and later also the proliferation zone adjacent to the epiphyseal nucleus. When a well-defined zone with calcified cartilage has formed the chondrocytes cease to divide. Mitosis has 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, 1973). The present investigation showed that the amounts of DNA and RNA decreased when the animals reached adult age, but that no further change occurred in the nucleic acids with subsequent increase in age. There is a constant ratio between the concentration of DNA and the number of cells in the tissue. The results agree with those reported by earlier investigators (Rosenthal et al. 1941, Barnett et al. 1963, Mankin 1963 a, Mankin & Baron 1965, Mankin 1968, Davies 1969 and Mankin 1970) regarding the amounts of DNA while the RNA concentration, as far as we know, has never before been studied as regards variation with age.

The chondrocytes of articular cartilage appear to "turn off" the switch for DNA synthesis at maturity rather than "breaking" the switch (Mankin 1970). The same author (Mankin 1968) pointed out in another investigation that considerable changes occur in the articular

surfaces in the period of maturation, but that little variation is noted with senescence. The present investigation showed, firstly, that once the animal reaches maturity the synthesis of DNA decreases markedly and, secondly, that the DNA synthesis decreases further with the advancing age of the animal. This might suggest that the ability of the cartilage to proliferate decreases with age.

SUMMARY

The joint cartilage of immature, adult and old rabbits was examined. The joint cartilage in adult and old rabbits contained less nucleic acids than that in young rabbits. The synthesis of DNA decreases with advancing age, while no difference was found in the concentration of nucleic acids between adult and old rabbits.

REFERENCES

- Barnett, C. H., Cochrane, W. & Palfrey, A. J. (1963) Age changes in articular cartilage. *Ann. rheum. Dis.* **22**, 389.
- Cerioti, G. (1952) A microchemical determination of deoxyribonucleic acid. *J. biol. Chem.* **198**, 297.
- 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.
- Crelin, E. S. (1957) Mitosis in adult cartilage. *Science* **125**, 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.
- Davies, D. V. (1969) The fine structure and ageing of articular cartilage. *Orthop. Gemeinschaftskongress* **4**, 12.
- Elliott, H. C. (1936) Studies on articular cartilage. I. Growth mechanism. *Amer. J. Anat.* **58**, 127.
- Hamerman, D. & Schubert, M. (1962) Diarthrodial joints; an essay. *Amer. J. Med.* **33**, 555.
- 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. (1963 a) The calcified zone (basal layer) of articular cartilage of rabbits. *Anat. Rec.* **145**, 73.
- Mankin, H. J. (1963 b) Localization of tritiated thymidine in articular cartilage of rabbits. III. Mature articular cartilage. *J. Bone Jt Surg.* **45-A**, 529.
- Mankin, H. J. (1964) Mitosis in articular cartilage of immature rabbits—a histologic, stathmokinetic (colchicine) and autoradiographic study. *Clin. Orthop.* **34**, 170.
- Mankin, H. J. & Baron, P. A. (1965) The effect of aging on protein synthesis in articular cartilage of rabbits. *Lab. Invest.* **14**, 658.

- Mankin, H. J. (1968) The effect of aging on articular cartilage. *Bull. N. Y. Acad. Med.* **44**, 545.
- Mankin, H. J. (1970) The articular cartilage: a review. AAOS Instructional Course Lectures **19**, 204.
- Meachim, G. & Collins, D. H. (1962) Cell-counts of normal and osteo-arthritic articular cartilage in relation to the uptake of sulphate ($^{35}\text{SO}_4$) in vitro. *Ann. rheum. Dis.* **21**, 45.
- Mejbaum, W. (1939) Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsäure. *Z. Physiol. Chem.* **258**, 117.
- Rosenthal, O., Bowie, M. A. & Wagoner, G. (1941) Studies in the metabolism of articular cartilage. I. Respiration and glycolysis of cartilage in relation to its age. *J. cell. comp. Physiol.* **17**, 221.
- Stockwell, R. A. (1967) The cell density of human articular and costal cartilage. *J. Anat. (Lond.)* **101**, 753.
- Telhag, H. (1972) Mitosis of chondrocytes in experimental "osteoarthritis" in rabbits. *Clin. Orthop.* **86**, 224.
- Telhag, H. (1973) DNA-synthesis in degenerated and normal joint cartilage in full-grown rabbits. *Acta orthop. scand.* **44**, 604.

Key words: cartilage; nucleic acid; autoradiography; rabbit

Correspondence to:

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