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NUCLEIC ACIDS IN HUMAN NORMAL AND OSTEOARTHRITIC ARTICULAR CARTILAGE

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During operations on joints of patients with osteoarthritis, specimens of severely degenerated cartilage of the femoral head were removed and cartilage from knee joints taken at meniscectomy was used as control material. DNA and RNA concentrations were reduced in advanced osteoarthritis, while the synthesis of DNA and RNA was increased in relation to the number of cells in the tissue. The result showed that in advanced osteoarthritis the remaining chondrocytes are metabolically very active.

Key words: nucleic acids; articular cartilage; human osteoarthritis; chondrocytes

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Joint cartilage is a unique tissue whose structural, biochemical and metabolic characteristics were unknown until a few decades ago. During the last three decades the biochemistry and metabolism in normal and in osteoarthritic cartilage have received wide attention.

It has been shown that experimental degenerative cartilaginous changes in rabbit cartilage are not accompanied by any change in DNA and RNA concentrations (Telhag & Gudmundson 1972). The concentration of DNA in man has also been shown to be the same in normal and in osteoarthritic cartilage (Mankin & Lippiello 1970, Mankin et al. 1971). A search through the literature failed to reveal any determinations of the RNA-concentration in normal and in osteoarthritic cartilage in man. The purpose of the present investigations was to elucidate the synthesis and concentration of nucleic acids in normal and osteoarthritic cartilage in man.

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MATERIAL AND METHODS

Articular cartilage was obtained during operations on human joints. Specimens were taken from the femoral head in 29 patients, aged 65 ± 8 (range 46–79), with osteoarthritic degeneration of the hip. The specimens were taken from macroscopically thin, fibrillated and partly eroded cartilage with grade 3 and 4 degeneration of the femoral head (Collins 1949). Control biopsy specimens were taken from a non-weight-bearing area of the medial femoral condyle during operations because of injuries to the meniscus in 17 knees from patients, aged 36 ± 10 (range 21–46), without osteoarthritis. The cartilage was cut out with a knife, care being taken not to include underlying subchondral bone. No joint changes were demonstrable at roentgenography or operation. The samples were divided

into two equal pieces and rinsed free of blood. The material was weighed (wet weight). The specimens were immediately placed in 10 ml Eagle's solution (37° C) containing 50 μCi tritiated thymidine or 50 μCi tritiated uridine and shaken for an hour and a half. After washing in physiologic saline solution, the pieces were placed in a mortar and covered with liquid nitrogen and pulverized. It was then homogenized in 5 ml of 10 per cent TCA (trichloro-acetic 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 5 ml of 0.3 M potassium hydroxide for 18 hours at 37° C to hydrolyze RNA, which was thereby 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 three times with 5 ml of 5 per cent TCA.

RNA and DNA in the separated fractions were determined with the orcinol (Mejbaum 1939) and the Ceriotti procedures (Ceriotti 1952), respectively. The separation of RNA and DNA was checked in all experiments by applying both reactions to the RNA as well as to the DNA fraction. Each sample was assayed separately for radioactivity (^{3}H-thymidine or ^{3}H-uridine) 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 DNA concentration was significantly reduced in the degenerated cartilage. The RNA concentration was also significantly reduced in the degenerated cartilage in relation to the tissue content. Relative to the content of DNA, the RNA concentration was increased in degenerated cartilage, but not significantly (Table 1).

The DNA synthesis (incorporation of ^{3}H-thymidine) was significantly reduced in the degenerated cartilage in relation to the tissue content, but significantly increased relative to the content of DNA. The synthesis of RNA (incorporation of ^{3}H-uridine) was significantly increased in degenerated cartilage in relation to the content of DNA and unchanged in relation to the content of tissue and RNA (Table 2).

DISCUSSION

Adult joint cartilage was formerly regarded as a tissue with relatively little metabolic activity. Research during the last few decades has, however, shown that degenerative changes of the cartilage are accompanied by an inreased synthesis of DNA (Crelin & Southwick 1960, 1964, Mankin & Lippiello 1970, Telhag

Table 1. Comparison of quantities of DNA and RNA in osteoarthritic and control articular cartilage.

Cartilage	γDNA/mg (M ± SD)	γRNA/mg	γRNA/γDNA
Control	2.2 ± 0.8	14.8 ± 7.4	7.8 ± 5.8
Osteoarthritic	1.0 ± 0.3	8.9 ± 3.9	10.1 ± 4.2
P	> 0.001	0.01 > p > 0.001	0.2 > p > 0.1

Table 2. Comparison of synthesis of DNA and RNA in osteoarthritic and control articular cartilage.

Cartilage	cpm DNA/mg	cpm DNA/γDNA	cpm RNA/mg	cpm RNA/γDNA	cpm RNA/γRNA
Control	49.7 ± 14.0	24.1 ± 13.2	211 ± 166	102 ± 89	8.2 ± 7.9
Osteoarthritic	33.8 ± 13.6	44.0 ± 29.5	203 ± 98	210 ± 96	13.9 ± 8.4
P	0.05 > p > 0.01	0.05 > p > 0.01	–	0.05 > p > 0.01	0.2 > p > 0.1

1972, Telhag & Gudmundson 1972), GAG (Collins & McElligott 1960, Mankin & Lippiello 1970) and collagen (Repo & Mitchell 1971), changes which are regarded as signs of repair.

The investigation showed that the concentration of DNA in joint cartilage is significantly reduced in advanced osteoarthritis. Mankin et al. (1971) have, however, shown that the concentration of DNA does not change significantly with advancing degeneration. The reason why the results were different in our investigation might be due to the fact that we studied only cartilage with advanced osteoarthritis. The synthesis of DNA per amount of tissue was significantly decreased, but significantly increased per amount of DNA. This result is in agreement with earlier observations in human hips with osteoarthritis (Mankin & Lippiello 1970) in rabbit knee joints (Telhag & Gudmundson 1972) and autoradiographically demonstrated increased uptake of ^{3}H-thymidine in degenerated joint cartilage from man (Mankin & Lippiello 1970, Hulth et al. 1970) and animals (Telhag 1972). The concentration of RNA in advanced osteoarthritis was significantly reduced in the investigation. Telhag & Gudmundson (1972) have shown that the concentration of RNA is unchanged in degenerated cartilage from the rabbit. In the latter investigation, however, cartilage was taken from the entire rabbit knee joint, i.e., the specimens examined consisted of a cartilage in various stages of degenerative joint disease. The synthesis of RNA was significantly increased per amount of DNA. In earlier investigations RNA-synthesis has been described as reduced in the dog (Mankin & Laing 1967) and rabbit (Telhag & Gudmundson 1972) and unchanged in man (Mankin & Lippiello 1970). Since there is a constant relationship between the DNA concentration and the number of cells in the tissue, the investigation shows that the remaining chondrocytes in the markedly degenerated cartilage are metabolically more active than those in normal cartilage.

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