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CONTENT AND SYNTHESIS OF NUCLEIC ACIDS IN THE CARTILAGE IN CHONDROMALACIA PATELLAE

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The content and the synthesis of nucleic acids in chondromalacian, osteoarthritis and normal cartilage was compared. The chondromalacian cartilage differed from osteoarthritis in that the content of nucleic acids was less. Also, the cell density was less in chondromalacian than in normal cartilage as opposed to previous findings in osteoarthritis. The synthesis of DNA was greater in chondromalacian than in normal cartilage but less than in osteoarthritis. With regard to the RNA synthesis, however, the chondromalacian cartilage showed a higher rate than both normal and osteoarthritic cartilage.

Key words: chondromalacia patellae; articular cartilage; autoradiography; nucleic acids

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Chondromalacia patellae is defined as a degenerative condition of the articular cartilage of the patella. Usually, it is seen in young adults but it may also occur in later life. The etiology is debatable. Trauma, incongruity and nutritional causes have been discussed. There are numerous biochemical studies of osteoarthritis, whereas studies of chondromalacia patellae have been noticeably few. In an earlier investigation (Shoji et al. 1974) no differences in the DNA content compared with normal articular cartilage were found, nor any differences in the content of hexosamine, non-collagenous protein, acid phosphatase or beta-glucuronidase. However, the content of Cathepsin D was found to be significantly elevated in both chondromalacia and osteoarthritis. Owing to these results the authors suggested that chondromalacia seemed to be a disease developing into osteoarthritis.

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The purpose of the present study was to compare parameters of nucleic acid content and synthesis in normal joint cartilage, chondromalacian cartilage and the cartilage of joints with osteoarthritis.

MATERIAL AND METHODS

Samples of the patellar lesions in 28 patients with chondromalacia patellae of grade I–III (Outerbridge 1961) were obtained in conjunction with surgery. The age range of the patients was 15–45 (average 28). In nine patients, cartilage was also taken from macroscopically normal parts of the patellar joint surface and in eight from a non-weight-bearing part of the femoral condyles also of normal appearance. In addition, cartilage biopsies were obtained from 19 individuals with the same range of age in conjunction with arthrotomies for removal of ruptured semilunar cartilages. In none of the latter patients were there any macroscopic signs of degenerative joint disease including chondromalacia patellae. Finally, cartilage samples were obtained from the femur condyles in eight patients with osteoarthritis of the knee in conjunction with surgical reconstruc-

tion of the joint. In these cases the cartilage was taken from the weight-bearing part of the femur condyle joint surface. The age of this latter group ranged from 29 to 69 with an average of 53. The 29-year-old was a man with post-traumatic osteoarthritis of the knee, otherwise the osteoarthritis was classified as primary.

The specimens were divided into two pieces, one for biochemical investigation, and one for histologic and autoradiographic studies. They were immediately placed in 10 ml of Eagle's solution at 37°C containing 50 μ Ci ^{3}H-thymidine or ^{3}H-uridine and shaken for 4 hours.

The specimens for the histologic-autoradiographic study were washed in physiologic saline solution and fixed in 10 per cent neutral, buffered formalin, decalcified in EDTA and embedded in paraffin. The histologic specimens were stained with hematoxylin-eosin, with Safranin-O and toluidine blue.

In seven of the patients with chondromalacia patellae, the abundance of chondrocytes was estimated in a) areas of chondromalacia; b) patellar articular cartilage of normal appearance; and c) condylar cartilage also of normal appearance. In each sample, five fields (0.5 × 0.5 mm) were selected at random and counted. The average variation within each sample was 11 per cent (c/v).

In all but the samples from osteoarthritis cases, autoradiographs of routine histologic sections, 5 μ thick, were prepared according to the dipping method with Ilford K2 liquid emulsion. After 2 and 4 weeks exposure, the autoradiographs were developed in Geveart X-ray developer G 230 and fixed in Geveart X-ray fixer G 305. The sections were stained through the emulsion with Mayer's hematoxylin. The specimens for the biochemical study were placed in acetone after shaking. The material was weighed (dry weight). The cartilage was placed in a mortar, covered with liquid nitrogen, and pulverized. It was then homogenized in 6 ml 10 per cent TCA (trichloroacetic acid) and the acid-soluble nucleotides were separated by centrifugation. The precipitate was washed twice, each time with 6 ml TCA. 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 the addition of 70 per cent perchloric acid. Five ml 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, 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 to the DNA fraction. The determinations were carried out in duplicate and the average used for further calculation.

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 Tricarb liquid scintillation spectrometer. The results were recorded as counts per milligram of dry weight per minute.

RESULTS

No labeled chondrocytes or microscopic changes were found in the control specimens. In the chondromalacian cartilage degenerative changes were seen with flaking of the superficial layer and sometimes fibrillation down to the columnar layer (Figure 1). Sometimes the superficial and also the transitional layers were loose, with sparse staining of the matrix. In some slides, fibrillation of the columnar layer, as well as chondrocyte death and clustering were found, similar to the findings in osteoarthritis, and in some blister formation in the superficial and transitional layers (Figure 2). After staining with Safranin-O and toluidine blue, the matrix was less stained in the slides with degenerative changes. The chondrocytes were significantly less abundant in the chondromalacian cartilage as compared with normal patellar and condylar cartilage (Table 1). No labeled chondrocytes were found in the cartilage from patients with chondromalacia.

The content of DNA was significantly decreased in the chondromalacian cartilage as compared with normal cartilage (Table 2). Similar tendencies were found when the chondromalacian cartilage was compared with normal cartilage from the same patella or with femoral condyle cartilage from the same joint (Tables 4 and 6). The DNA content was significantly greater in osteoarthritis cartilage than in chondromalacian cartilage (Table 8) but not quite significantly

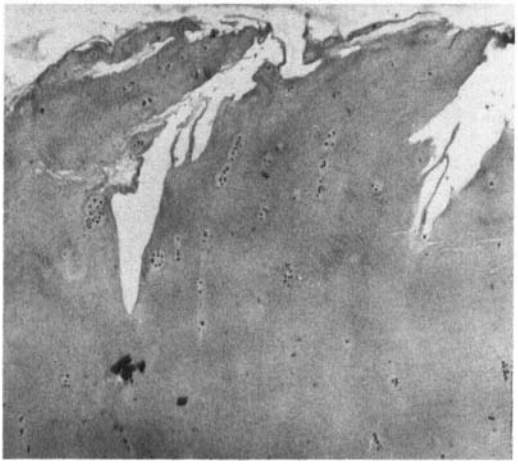

Figure 1. Section of chondromalacian cartilage with fibrillation. Note the scarcity of chondrocytes (Objective × 6.3, hematoxylin-eosin).

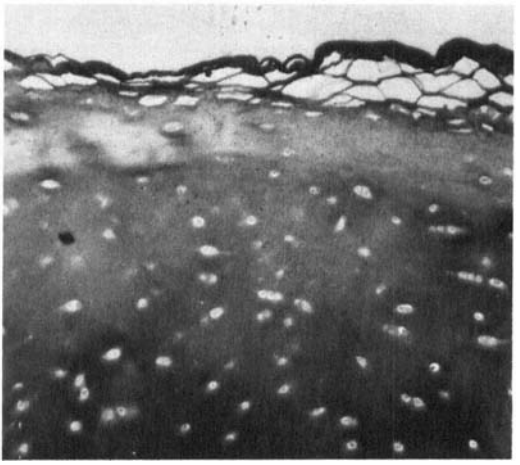

Figure 2. Section of chondromalacian cartilage with blister formation in the superficial and transitional layers (Objective × 6.3, hematoxylin-eosin).

Table 1. Number of chondrocytes per mm² in chondromalacian cartilage as compared with normal patellar and condylar cartilage

Chondromalacian cartilage		Normal patellar cartilage		Normal condyle cartilage	
n	av. s.d.	*n*	av. s.d.	*n*	av. s.d.
7	164 ± 35	7	296 ± 84	7	306 ± 66
	$0.01 > P > 0.001$			$P > 0.1$	
			$P > 0.001$		

Table 2. Nucleic acid content *in patella cartilage of patients with chondromalacia patellae (chondromalacia) and in non-weight-bearing condylar cartilage obtained in conjunction with meniscectomy (control)*

		γDNA/mg	γRNA/mg	γRNA/γDNA
Chondromalacia	($n = 28$)	2.5 ± 1.3	4.5 ± 2.4	2.6 ± 2.9
Control	($n = 19$)	4.0 ± 2.0	7.3 ± 3.2	2.2 ± 0.8
		$0.01 > P > 0.001$	$0.01 > P > 0.001$	—

Table 3. Nucleic acid synthesis *in patella cartilage of patients with chondromalacia patellae (chondromalacia) and in non-weight-bearing condylar cartilage obtained in conjunction with meniscectomy (control)*

		CPM DNA/ mg	CPM DNA/ γDNA	CPM RNA/ mg	CPM RNA/ γDNA	CPM RNA/ γRNA
Chondromalacia	($n = 28$)	88 ± 52	36 ± 26	695 ± 450	410 ± 420	152 ± 80
Control	($n = 19$)	65 ± 33	19 ± 11	1217 ± 689	381 ± 238	184 ± 113
		$0.1 > P > 0.05$	$0.05 > P > 0.01$	$0.01 > P > 0.001$	—	—

Table 4. Nucleic acid content *in the cartilage of chondromalacian lesions (chondromalacia) and in cartilage of normal macroscopic appearance (control) from the same patella*

Cartilage		γDNA/mg	γRNA/mg	γRNA/γDNA
Chondromalacia	($n=9$)	2.9 ± 1.2	6.1 ± 1.8	2.5 ± 0.9
Control	($n=9$)	4.8 ± 2.2	8.5 ± 2.9	2.0 ± 0.9
		$0.05 > P > 0.01$•	$0.05 > P > 0.01$•	—

• *t*-test of pairs.

Table 5. Nucleic acid synthesis *in the cartilage of chondromalacian lesions (chondromalacia) and in cartilage of normal macroscopic appearance (control) from the same patella*

Cartilage		CPM DNA/ mg	CPM DNA/ γDNA	CPM RNA/ mg	CPM RNA/ γDNA	CPM RNA/ γRNA
Chondromalacia	($n=9$)	134 ± 57	49 ± 35	1205 ± 428	537 ± 237	216 ± 53
Control	($n=9$)	131 ± 100	27 ± 15	1089 ± 232	263 ± 72	166 ± 101
		—	$0.1 > P > 0.05$•	—	$0.01 > P > 0.001$•	

• *t*-test of pairs.

Table 6. Nucleic acid content *in the patella cartilage of patients with chondromalacia patella (chondromalacia) and in cartilage of normal macroscopic appearance (control) from the femoral condyle of the same joint*

Cartilage		γDNA/mg	γRNA/mg	γRNA/γDNA
Chondromalacia	($n=8$)	2.6 ± 0.7	6.1 ± 1.9	2.6 ± 0.9
Control	($n=8$)	4.2 ± 2.1	9.0 ± 4.8	2.6 ± 1.7
		$0.1 > P > 0.5$	$0.2 > P > 0.1$	—

Table 7. Nucleic acid synthesis *in the patella cartilage of patients with chondromalacia patellae (chondromalacia) and in cartilage of normal macroscopic appearance (control) from the femoral condyle of the same joint*

Cartilage		CPM DNA/ mg	CPM DNA/ γDNA	CPM RNA/ mg	CPM RNA/ γDNA	CPM RNA/ γRNA
Chondromalacia	($n=8$)	131 ± 60	52 ± 36	1198 ± 457	567 ± 234	210 ± 68
Control	($n=8$)	128 ± 69	32 ± 15	1175 ± 211	327 ± 125	513 ± 1107
		—	$0.2 > P > 0.1$•	—	$0.05 > P > 0.01$•	

• *t*-test of pairs.

Table 8. Nucleic acid content *in the cartilage from the femoral condyle of patients with osteoarthritis of the knee (osteoarthritis) and in chondromalacian lesions of patients with chondromalacia patellae (chondromalacia)*

Cartilage		γDNA/mg	γRNA/mg	γRNA/γDNA
Osteoarthritis	($n=8$)	5.7 ± 2.1	12.3 ± 3.4	2.6 ± 1.3
Chondromalacia	($n=28$)	2.5 ± 1.3	4.5 ± 2.4	2.6 ± 2.9
		$0.001 > P$	$0.001 > P$	—

Table 9. Nucleic acid synthesis *in the cartilage from the femoral condyle of patients with osteoarthritis of the knee (osteoarthritis) and in chondromalacian lesions of patients with chondromalacia patellae (chondromalacia)*

Cartilage	CPM DNA/ mg	CPM DNA/ γDNA	CPM RNA/ mg	CPM RNA/ γDNA	CPM RNA/ γRNA
Osteoarthritis ($n=8$)	222 ± 264	31 ± 14	1355 ± 562	271 ± 144	124 ± 81
Chondromalacia ($n=28$)	88 ± 52	36 ± 26	695 ± 450	410 ± 420	152 ± 80
	$0.05 > P > 0.01$	—	$0.01 > P > 0.001$	—	—

increased in osteoarthritis cases as compared with the 19 controls ($0.1 > P > 0.05$) (Tables 2 and 8).

The RNA content was reduced in chondromalacian cartilage both in comparison with the control patients and with the cartilage of normal appearance in the same joint. When corrected for the DNA content, however, there were no differences in the RNA concentrations (Tables 2, 4 and 6).

The *DNA synthesis,* related to the DNA concentration, was significantly increased in chondromalacian cartilage as compared with control cartilage (Table 3). This tendency was not contradicted by the comparison with cartilage samples from the same joint (Tables 5 and 7). However, per unit tissue, the DNA synthesis did not significantly differ between control and chondromalacian cartilage and was identical when a comparison was made between normal and chondromalacian cartilage in the same joint (Tables 3, 5 and 7). In comparison with osteoarthritis there was no difference in DNA synthesis except when related to the amount of tissue (Table 9). When corrected for the DNA content there was no difference.

The *RNA synthesis* was about half in chondromalacia as compared with controls when related to the amount of tissue (Table 3). However, when corrected for the DNA concentration, there was no significant difference. When, on the other hand, the data were compared between chondromalacian cartilage and cartilage of normal appearance within the same joint there was a significant increase. To summarize, chondromalacian cartilage demonstrated the highest rate of synthesis of RNA, whereas osteoarthritis in this study represented the lowest value with the control data in an intermediate position.

DISCUSSION

The histological changes in osteoarthritis are well known and have been described by Collins (1949) and others.

In chondromalacia patellae, microscopic degenerative changes of the articular cartilage were found similar to those in osteoarthritis, with flaking of the cartilage surface and fibrillation (Figure 1) sometimes down to subchondral bone. As an expression of the

degeneration, the ground substance was often less stainable with Safranin-O and toluidine blue in chondromalacia, thus indicating a loss of glycosaminoglycans from the cartilage matrix, changes similar to those in osteoarthritis.

Goodfellow et al. (1976) described fasciculation of the cartilage of the patella in young people suffering from intractable patello-femoral pain. They also found an intermediate stage (between fasciculation stages 1 and 2) with blistering of the cartilage. This was confirmed in our investigation where blistering was found especially in the superficial and transitional layers (Figure 2).

In earlier investigations, an increased thickness of the cartilage was found in human osteoarthritis (Meachim & Collins 1962). In comparison with normal articular cartilage from the patella or from the femoral condyle, there were less chondrocytes in chondromalacian cartilage. According to Freeman (1973), there is an approximately inverse relationship between cell density and cartilage thickness. We could not find any difference between normal cartilage from the patellae and cartilage from the femoral condyle either histologically or biochemically. In earlier investigations it has been shown that mitoses of the chondrocytes, indicating a higher DNA synthesis, are found in degenerated articular cartilage from humans and animals (Hulth et al. 1972, Telhag 1972). In chondromalacia patellae no thymidine-labeled chondrocytes were seen, either as single chondrocytes, or in so-called clusters. In osteoarthritis the DNA synthesis is increased (Mankin & Lippiello 1970, Mankin et al. 1971). In the present study the same was found to occur in chondromalacian cartilage but not to a sufficient extent to label the chondrocytes.

Shoji et al. (1974) found that the amount of DNA in chondromalacian cartilage compared with normal cartilage was unchanged. In the present investigation a significantly decreased content of DNA was found in chondromalacian cartilage. This is in agreement with the decreased chondrocyte density. As reported in the past (Mankin & Lippiello 1970, Telhag & Gudmundson 1972), the DNA content in osteoarthritis as compared with normal cartilage is unchanged, but in the present study there was a significant increase in osteoarthritis cartilage compared with chondromalacian.

The RNA content has not previously been determined in chondromalacian cartilage. We found a reduced quantity of RNA per amount of tissue as compared with normal cartilage. In relation to the DNA content, the quantity of RNA was unchanged. This is in agreement with the reduced cell density in chondromalacian cartilage found histologically.

In a recent study, the RNA synthesis in human osteoarthritis cartilage was not found to differ significantly from that of normal articular cartilage (Mankin & Lippiello 1970). Mankin & Laing (1967) demonstrated a decreased synthesis of RNA in osteoarthritis cartilage from dogs, which is in agreement with the findings in rabbits by Telhag & Gudmundson (1972). In the present study no significant difference was found in the RNA synthesis in chondromalacian cartilage as compared with normal cartilage. When comparing cartilage from chondromalacian lesions and cartilage of normal appearance within the same joint, there was an increase in the synthesis.

In the present investigation some factors indicate that chondromalacia patellae is a different disease from osteoarthritis: viz., the reduced chondrocyte density, the absence of thymidine-labeled chondrocytes and the concentration of nucleic acids which is low as compared with normal articular cartilage as well as osteoarthritis cartilage.

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