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OSTEOARTHRITIS and CARTILAGE

Cyclic loading is harmful to articular cartilage from which proteoglycans have been partially depleted by retinoic acid

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Summary

We studied whether cyclic loading is harmful to degraded cartilage. Sets of four cartilage-bearing sesamoid bones were dissected from 5-year-old cows. One bone from each set was cultured for 17 h in control medium to serve as an *ex vivo* control. The three others were cultured for 1 week in control medium to which 0, 10 or 300 ng/ml retinoic acid (RAc), which depletes the cartilage matrix of proteoglycans, had been added. Two were then cultured for another week in control medium. During the last week, one of the two was subjected to a cyclic load (1 MPa, 0.2 Hz). Following treatment with RAc, glycosaminoglycan content and synthesis were significantly decreased, as confirmed by safranin O staining and autoradiography. They were further diminished by loading during the second week of culture. Increased amounts of 3-B-3(–) epitope were found in cartilage that had been treated with 300 ng/ml RAc and then loaded. While loading cartilage matrix that was only slightly degraded proved to be damaging, loading severely degraded cartilage matrix apparently induced osteoarthritic-like changes.

Key words: Articular cartilage, Glycosaminoglycan, Osteoarthritis, Retinoic acid.

Introduction

The ability of articular cartilage to withstand compressive loads and distribute them over the subchondral bone depends on the composition and structural integrity of its extracellular matrix [1, 2]. The large aggregating proteoglycan, aggrecan, contains up to 100 highly polyanionic glycosaminoglycan (GAG) chains with many sulfate and carboxylate groups. Aggrecan is immobilized in high concentrations within the collagen network. This creates a high osmotic swelling pressure, which draws water into the tissue [3, 4]. The swelling pressure is balanced by tension in the collagen fibers [3, 4]. Other components of cartilage extracellular matrix are small proteoglycans and noncollagenous proteins [5].

Chondrocytes, which are sparsely distributed in the matrix, are responsible for the synthesis and maintenance of the matrix components [4, 6]. In degenerative joint diseases, such as osteoarthritis (OA), the balance between the anabolism and catabolism of the matrix components is disturbed [7]: there is a net loss of the components, which alters the biomechanical properties of the matrix.

There is evidence that the chondrocytes also contribute to the breakdown of the matrix [8–10]. The *in vitro* addition of exogenous agents, such as interleukin-1, bacterial lipopolysaccharides, and analogs of retinol, can induce chondrocytic production of metalloproteinases (for references see [11]). The latter are known to degrade cartilage matrix macromolecules [12, 13].

In OA, the loss of matrix components is probably responsible for impaired joint function and pain. The overuse of already damaged joints, which could be facilitated by the use of analgesics, may accelerate the process of joint destruction [14]. To determine whether loading is indeed harmful to degraded cartilage, we studied the effect of cyclic loading on partially degraded cartilage matrix *in vitro*. Cartilage matrix degradation was induced by culturing anatomically intact articular cartilage in the presence of retinoic acid (RAc), which depletes it of proteoglycans [15, 16]. Cyclic loading then took place in the loading apparatus designed in our laboratory [17, 18].

This is, to our knowledge, the first report to describe the effects of loading on a proteoglycan-depleted cartilage matrix *in vitro*.

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Materials and methods

CULTURE OF SESAMOID BONES

The two middle sesamoid bones were dissected from both metacarpophalangeal joints of 5-year-old cows, as described by Korver *et al.* [19], and washed in phosphate buffered saline (PBS). The cartilage from the sesamoid bones of any given animal is uniform with respect to GAG synthesis, GAG content, and proteoglycan composition [16]. Each set of four sesamoid bones from the same cow made up an experimental group. Three sets of experiments were performed, with $N=4$ in the first, $N=6$ in the second and $N=8$ in the third.

In each experiment, one sesamoid bone from the experimental group was incubated *ex vivo* for 17 h in 10 ml control medium in the presence of 10 μCi/ml [^{35}S]sulfate (New England Nuclear, Boston, MA, U.S.A.) and used as a control. The control medium consisted of Ham's F-12 culture medium (Gibco, Paisley, Strathclyde, U.K.), supplemented with 20 ng/ml human recombinant insulin-like growth factor 1 (IGF-1; Boehringer Mannheim GmbH, Germany), 0.1% bovine serum albumin (BSA), globulin-free fraction V (Sigma, St Louis, MO, U.S.A.), 0.1% penicillin/streptomycin, 0.1% fungizone, and sodium sulfate in a concentration of 500 μM [20]. One of the three remaining sesamoid bones was cultured for 1 week, the other two for 2 weeks. The three sets of experiments were distinguished by the culture medium in which these three sesamoid bones were incubated during the first week. This consisted of control medium in the first set, control medium to which 10 ng/ml all-*trans* RAc (Sigma) had been added in the second, and control medium to which 300 ng/ml all-*trans* RAc had been added in the third. The RAc was dissolved in dimethylsulfoxide, which had been diluted in culture medium to 0.1% (v/v). In all experiments, the two sesamoid bones of each group that remained in culture after the first week were incubated further in control medium. During the second week of culture, one of the two was subjected to cyclic loading with a stress of 1 MPa at a frequency of 0.2 Hz, as described below. Media were changed every other day. [^{35}S]sulfate (10 μCi/ml) was added to the culture medium of each sesamoid bone during the last 17 h of its culture period.

LOADING OF SESAMOID BONES

The method of loading sesamoid bones has been described previously [17, 18]. In short, each sesamoid bone was fixed in a stainless steel holder, which was then placed in a glass incubation vessel. The holder can be driven both up and down, pulling the cartilage away from or pushing it against a surface at the bottom of the vessel.

That surface was an exact replica of the metacarpal surface with which the sesamoid bone had been in contact in the metacarpal joint. An imprint of the metacarpal surface was made in a two component silicone elastomer. After vulcanization of this elastomer, the metacarpal surface was removed and the imprint filled with polymethylmethacrylate. After polymerization of the polymethylmethacrylate, the nonporous replica of the metacarpal surface was ready for use. The contact area between sesamoid bone and the replica of the metacarpal surface, determined after the experiment on pressure sensitive film as described by Afoke *et al.* [21], was 30–40%.

EXTRACTION OF PROTEOGLYCANS

After they had been labeled, the sesamoid bones were rinsed once in cold PBS, washed in cold PBS at 4°C for 30 min, and wiped dry. Cartilage was removed from the bones in 1- to 2-mm^2 explants, leaving a full-depth central slice (2 mm) for histological analysis. The explants were weighed (wet weight) and washed three times in cold PBS at 4°C. They were then rinsed in water, lyophilized for 48 h, and weighed again [dry weight (dw)]. Finally, they were cut into 20-μm cryostat sections and lyophilized for another 24 h.

The proteoglycans were extracted from the sections with 4 M guanidine hydrochloride (GuHCl; Sigma, St Louis, MO, U.S.A.) in 0.15 M potassium acetate (60 μl/mg dw), pH 5.8, at 4°C for 60 h in the presence of proteinase inhibitors [22]. Extracts and their residues were separated. The residue was washed with extraction buffer, which was then added to the extract. The residue was then dissolved in 0.5 M NaOH at 60°C for 17 h. Samples of extracts and residues were analyzed for total GAG content by the dimethylmethylene blue assay [23] and for ^{35}S-labeled GAGs by liquid scintillation counting.

ANALYSIS OF GAG CHAINS

Aggrecan was isolated from the extracts by CsCl equilibrium density gradient centrifugation in 4 M GuHCl plus inhibitors, initial density 1.50 g/ml. The bottom third (D1-2) of the gradient was dialyzed, lyophilized, and dissolved in 700 μl 0.1 M Tris-HCl buffer, pH 7.5, with 1 mM $CaCl_2$. Sepharose CL-2B (Pharmacia, Uppsala, Sweden) chromatography of the D1-2 and D3-6 fractions of a test sample showed that 79% of all GAGs were contained in the D1-2 fraction and that 94% of these were aggrecans and 6% small proteoglycans. After the D1-2 samples

had been digested with proteinase K (*Tritirachium album*) at 63°C for 3 h and the proteinase had been denatured at 100°C for 5 min, GuHCl was added to a part of the digest, yielding a final concentration of 4 M. These samples were then applied to a Sepharose CL-6B (Pharmacia, Uppsala, Sweden) column (145 × 0.7 cm) to determine GAG chain length. The column was eluted at 2.7 ml/h with 4 M GuHCl in 0.1 M sodium sulfate, 0.05 M sodium acetate, and 0.1% Triton-X100, pH 6.1. Fractions of 700 μl were analyzed for radiolabeled GAGs.

MEASUREMENT OF 3-B-3(–) EPITOPE

To determine the amounts of 3-B-3(–) epitope in the extracts, we applied a modified version of a competitive inhibition enzyme-linked immunosorbent assay (ELISA) for measuring amounts of 3-B-3(–) epitope in synovial fluid lavages and articular cartilage extracts [24]. Bovine proteoglycan monomer (D1D1) was digested with chondroitin ABC lyase (ICN Biomedicals, Costa Mesa, CA, U.S.A.), and dialyzed, and 25 ng protein/ml in 20 mM sodium carbonate buffer was used to coat the wells of microtiter plates (Immulon, Dynatech Laboratories Ltd, West Sussex, U.K.) The plates were washed in Tris incubation buffer (Tris-IB: 0.01 M Tris-HCl, 0.15 M NaCl, 0.1% BSA, and 0.1% nonidet NP40, pH 7.4) and blocked with 1% BSA/Tris-IB. Standard samples, consisting of the same bovine D1D1 preparation that had been used to coat the plates, of known concentrations and experimental samples were prepared in 1% BSA/Tris-IB. The samples were distributed over the wells of the plates: 100 μl per well. One hundred microliters of monoclonal antibody 3-B-3 (Seikagaku Co., Tokyo, Japan), diluted 1:20 000 in 1% BSA/Tris-IB, was also added to each well. The samples were then incubated at 37°C for 90 min. The plates were washed in Tris-IB, incubated with a goat anti-mouse IgG (H + L) alkaline phosphatase conjugated antibody (Promega, Madison, WI, U.S.A.) at 37°C for 1 h, and washed again. Finally, 1 mg/ml p-nitrophenylphosphate, disodium (Sigma) in diethanolamine buffer (1 M diethanolamine and 0.126 mM $MgCl_2$, pH 9.8) was added to each well, and the plates were incubated in the dark at 37°C for 45 min. The absorbance at 405 nm was measured. The concentrations of 3-B-3(–) epitope are expressed as nanograms core protein per milligrams chondroitin sulfate (CS).

SAFRANIN O STAINING AND AUTORADIOGRAPHY

The full depth central slice of cartilage and directly underlying bone was sawn out perpendicular to the articular surface and processed for paraffin embedding as described recently [18]. Five-micrometer sections were cut from the slices with a Leica 1512 microtome. These were destined alternately for safranin O staining or autoradiography. Sections for safranin O staining were hydrated and stained with 0.1% safranin O (Merck, Darmstadt, Germany) in 0.1 M acetic acid buffer, pH 4.6, for 10 min. Sections for autoradiography were hydrated and then coated with Ilford K.5 emulsion, which had been diluted with an equal volume of 1% (w/v) gelatine. Exposure took place at 4°C for 3 weeks. The sections were developed with Kodak D-19 developer (hydroquinone-sodium carbonate) and counterstained with Mayer's hematoxylin. Finally, all sections were mounted in DePeX.

STATISTICAL ANALYSIS

All results, with the exception of those obtained from measurement of 3-B-3(–) epitope, are expressed as mean ± S.E.M. The significance of differences between group means was tested by one-way analysis of variance (ANOVA). Individual comparisons were made with the Newman–Keuls procedure. For statistical analysis of 3-B-3(–) epitope expression the two-way ANOVA model with repeated measurements on one factor was applied. Cells were filled with the triplicate measurements and the individual comparisons between group means were made with the Newman–Keuls procedure.

Results

GAG CONTENT

The GAG content of the extracts derived from the control specimens was 98.0 ± 1.8 μg/mg dw. After a week's culture in control medium in the first set of experiments, GAG content was slightly decreased: 94.6 ± 2.1% of control content [Fig. 1(a)]. During the second week of this culture, GAG content was maintained at the same level: 91.4 ± 1.3% of the control value in the absence of loading and 88.8 ± 2.84% with loading. GAG content after 1 week of culture in the presence of 10 ng/ml RAc was decreased to 90.6 ± 2.8% of the control value. When, in this second set of experiments, the sesamoid bones had been cultured but not subjected to loading for another week in control medium, GAG content further decreased to 82.3 ± 2.1% of that of the controls ($P < 0.01$). Subjection to cyclic loading reduced GAG content to 77.7 ± 2.9% of control content ($P < 0.01$). In the third set of experiments, culture in the presence of RAc reduced GAG content to 64.8 ± 5.2% of the control value

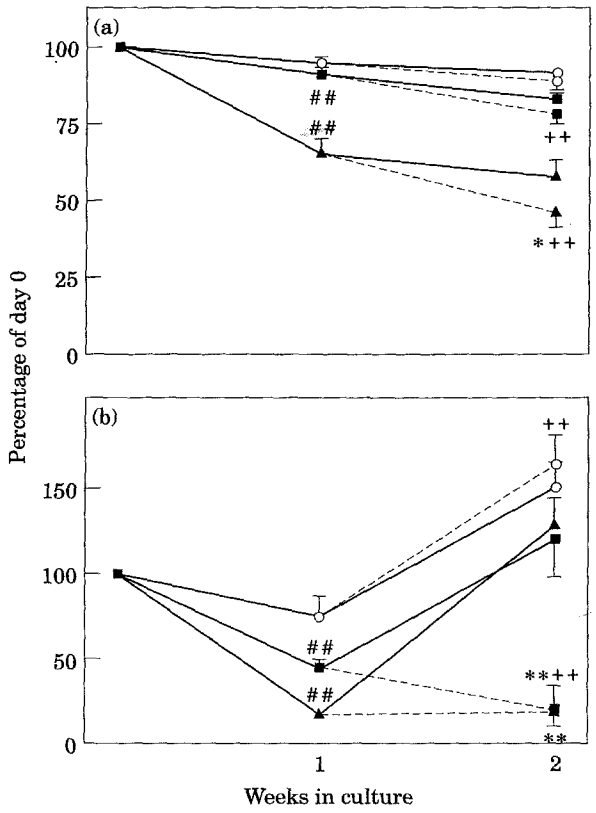

FIG. 1. (a) GAG content and (b) glycosaminoglycan synthesis expressed as percentage of that in control cartilage. Each experimental group consisted of four sesamoid bones of one cow. The control cartilage was derived from one of these and was cultured for 17 h in the presence of [^{35}S]sulfate. The sets of experiments are distinguished by the control medium in which the other three sesamoid bones were cultured during the first week: control medium to which 0 mg/ml (○), 10 ng/ml (■), or 300 ng/ml RAc (▲) had been added. Two sesamoid bones from each group were then cultured for a second week in control medium, during which time one was subjected to a cyclic load of 1 MPa at a frequency of 0.2 Hz (- - -). All significant differences between the 1-week cultures and the controls (#), between 1-week and loaded 2-week cultures (+), and between loaded and non-loaded 2-week cultures (*) are indicated (*$P < 0.05$; ++, **, # # $P < 0.01$). GAG synthesis increased significantly, regardless of culture during the first week, with $P < 0.01$ after the second week.

($P < 0.01$). Subsequent incubation in control medium reduced this further to 57.7 ± 5.2% without loading and to 45.9 ± 4.7% with ($P < 0.01$).

GAG SYNTHESIS

GAG synthesis in the controls was 1.02 ± 0.04 nmol SO_4/mg dw/day. In the first set of experiments, GAG synthesis decreased to 74.7 ± 11.5% of that in the controls, after 1 week [Fig. 1(b)], but, recovered to 151.6 ± 14.4% ($P < 0.01$) after 2 weeks. Under the loading regime, GAG synthesis was 165.0 ± 17.8% of that in the controls. A week's culture in the presence of RAc in the second and third sets of experiments reduced the GAG synthesis to 44.8 ± 5.6% and 17.9 ± 2.7% of the control synthesis ($P < 0.01$). One week after the switch to control medium, the GAG synthesis was, in the absence of loading, 120.7 ± 22.1% of the control synthesis for the second set of experiments and 129.5 ± 15.8% for the third ($P < 0.01$), and, with loading 20.9 ± 14.3% for the second and 19.0 ± 8.5%, the same rate found immediately after depletion, for the third.

SAFRANIN O STAINING

Normal safranin O staining was found in the control slices. All layers except the superficial layer were stained [Fig. 2(A)]. The intensity of staining decreased from the pericellular to the interterritorial matrix. The intensity was unaffected after 1 week of culture in control medium [Fig. 2(B)]. Neither was it affected by subsequent culture, whether with or without loading [Fig. 2(C) & (D), respectively].

While treatment with 10 ng/ml RAc had no effect on the intensity of safranin O staining [Fig. 2(E)], subsequent culture in control medium reduced it in all layers, in particular the lower middle and deep layers [Fig. 2(F)]. In cartilage that had been subjected to loading during the second week, the safranin O staining was reduced in all layers, mainly in the territorial and interterritorial matrix [Fig. 2(G)].

In cartilage cultured for 1 week in medium containing 300 ng/ml RAc, staining was also reduced in all layers [Fig. 2(H)]. The reduction was most pronounced in the territorial and interterritorial matrix of the middle and deep layers. The staining was less affected in the calcified layer. It was further reduced in the deep and lower middle layers, when cartilage was cultured in control medium for another week in the absence of loading [Fig. 2(I)]. When the sesamoid bones were subjected to cyclic loading, the safranin O staining had almost disappeared from all layers except the calcified layer: a slight positive staining was found only in the pericellular matrices of some of the chondrocytes [Fig. 2(J)].

AUTORADIOGRAPHY

In *ex vivo* controls and in sesamoid bones that had been cultured for 1 week in control medium, all chondrocytes, except those in the calcified layer, were active in the uptake of [^{35}S]sulfate [Fig. 3(A) & (B)]. A week's culture in the presence of RAc reduced this activity in all chondrocytes [Fig. 3(E) & (H)]. In the absence of loading, [^{35}S]sulfate uptake increased during the second week of culture,

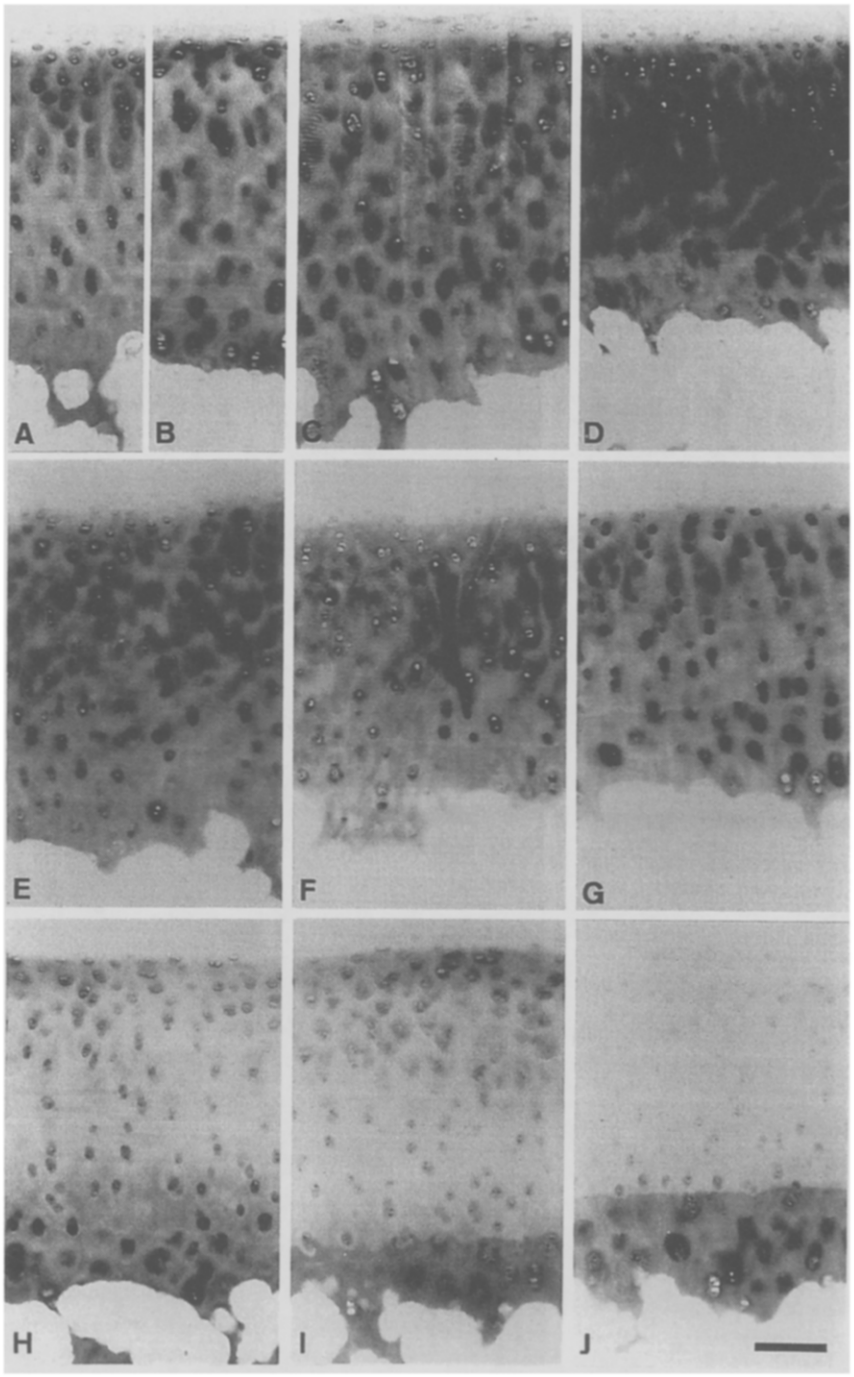

FIG. 2. The distribution of glycosaminoglycans stained by safranin O in cross-sections of sesamoid bone cartilage from 5-year-old cows. Photograph A shows a cross-section from an *ex vivo* control that had been cultured in control medium for 17 h. B, E, and H show cross-sections from sesamoid bones, that had been cultured for 1 week in control medium to which 0, 10 and 300 ng/ml retinoic acid, respectively, had been added. The cross-sections shown in C and D, in F and G, and in I and J were taken from sesamoid bones that had been cultured for 1 week in the same medium as the sesamoid bones from which cross-sections B, E and H, respectively, had been taken and for a second week in control medium. The sesamoid bones from which the cross-sections are shown in D, G and J were subjected to cyclic loading with 1 MPa at a frequency of 0.2 Hz during the second week. Bar = 400 μm.

regardless of the culture medium used during the first week [Fig. 3(C), (F) & (I)]. The most active chondrocytes were those of the superficial and upper middle layers.

Under the loading regime in the first set of experiments [Fig. 3(D)], deep layer chondrocytes were more active in the uptake of [^{35}S] sulfate than those in above layers. In the second and third

set of experiments, cyclic loading restricted the uptake of [^{35}S]sulfate to chondrocytes in the deep and lower middle layers [Fig. 3(G) and (J)].

CHAIN LENGTH OF NEWLY SYNTHESIZED GAGS

In the first and second set of experiments, the K_{av} of GAGs that were newly synthesized in cartilage that had been cultured for 1 week, was comparable to the K_{av} of GAGs that were newly synthesized in the *ex vivo* controls (Table I). In the third set of experiments the K_{av} of GAGs that were newly synthesized in cartilage that had been cultured for 1 week was significantly decreased. After continued culture, in all three sets of experiments and regardless of loading regime, the K_{av} of newly

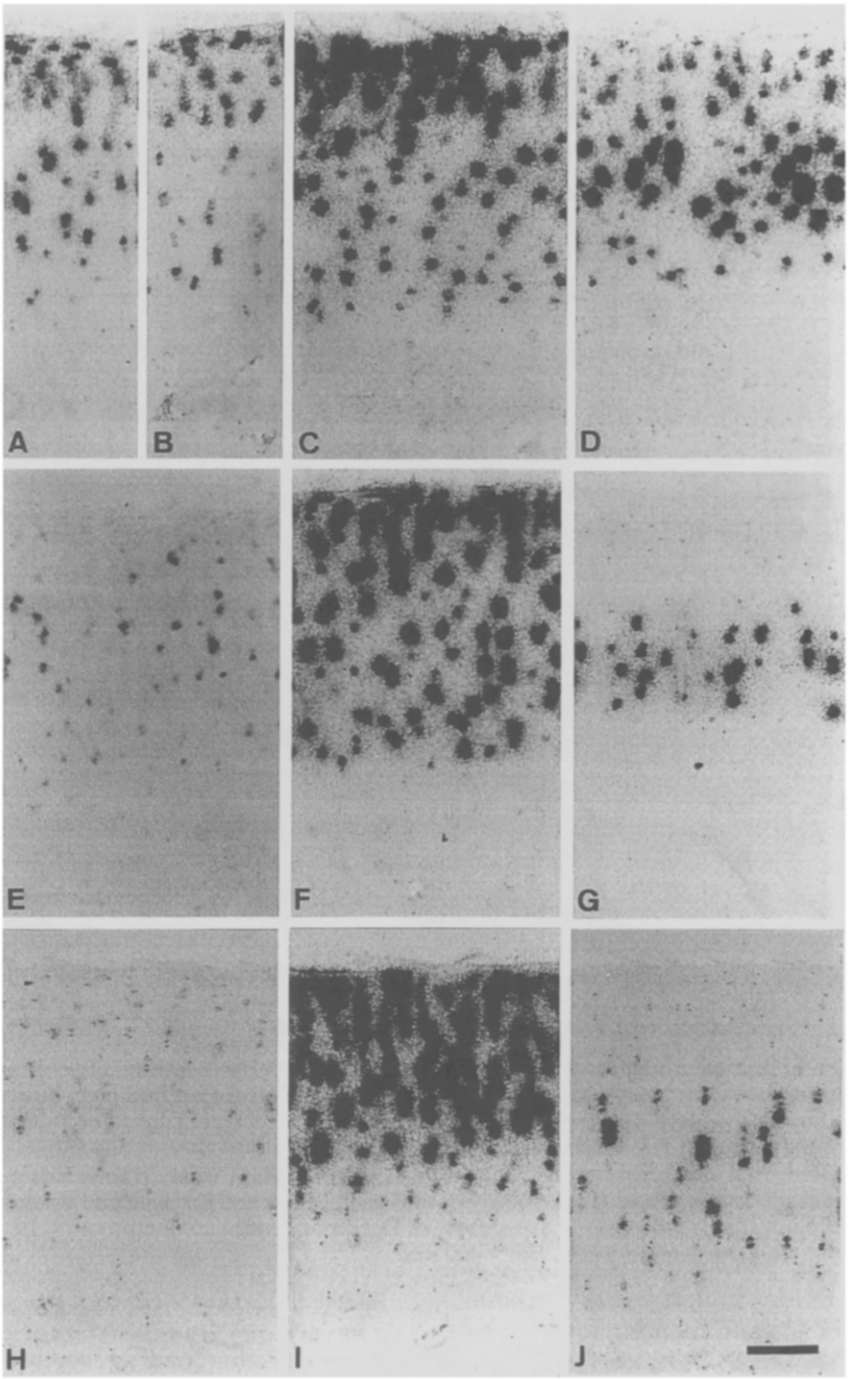

FIG. 3. The distribution of incorporated ^{35}S-label in cross-sections of sesamoid bone cartilage from 5-year-old cows. The culture conditions of the sesamoid bones represented in the autoradiographs are given in Fig. 2. In each case, [^{35}S]sulfate (10 μCi/ml) had been added to the culture medium during the last 17 h of culture. Bar = 400 μm.

Table I
K_{av} *of newly synthesized glycosaminoglycans on a sepharose CL-6B column*

[RAc] (ng/ml)	*ex vivo*	1 week	2 weeks	2 weeks loaded
0	0.53 ± 0.01	0.50 ± 0.00	0.53 ± 0.01	0.54 ± 0.02
10	0.55 ± 0.01	0.53 ± 0.02	0.53 ± 0.02	0.55 ± 0.01
300	0.53 ± 0.01	0.51 ± 0.00*	0.53 ± 0.00	0.53 ± 0.01

Three of the four sesamoid bones from one cow were cultured for 0 (*ex vivo* control), 1 and 2 weeks. The fourth was cultured for 2 weeks and subjected to a cyclic loading during the second. The *ex vivo* control was labeled with [^{35}S]sulfate in control medium for 17 h following excision. For the remaining three, culture during the first week was in control medium to which 0, 10 or 300 ng/ml RAc had been added, according to the set of experiments to which they had been assigned. Culture during the second week (two bones) took place in control medium. In each case, [^{35}S]sulfate was added to the medium during the last 17 h of culture. Values are expressed as mean ± S.E.M. with $N=4$, $N=6$, $N=8$ for treatment with 0, 10 and 300 ng/ml RAc, respectively.
*$P < 0.01$ compared to the other three cultures from the same set of experiments.
RAc, retinoic acid.

synthesized GAGs was comparable to that of the corresponding *ex vivo* controls.

3-B-3(–) EPITOPE EXPRESSION

The amounts of 3-B-3(–) epitope found in the *ex vivo* controls in the first, second and third sets of experiments were 6.3 ± 2.3, 6.8 ± 1.4 and 7.9 ± 1.9 ng core protein/mg chondroitin sulfate (CS) (Fig. 4). Further culture, without loading, in control medium did not affect the amount of 3-B-3(–) epitope, which was 5.5 ± 1.7 ng core protein/mg CS after 1 week and 5.8 ± 1.4 ng core protein/mg CS after 2 weeks. With loading during the second week, the amount of 3-B-3(–) epitope was 8.5 ± 1.5 ng core protein/mg CS. Comparable results were obtained in the second set of experiments: 7.2 ± 2.3 ng core protein/mg CS after 1 week, 7.1 ± 1.3 after 2 weeks without loading, and 8.3 ± 1.2 after 2 weeks with loading. In the third set of experiments, we found 7.6 ± 1.1 ng core protein/mg CS after the first week of culture. After the second, we found 8.0 ± 2.3 ng core protein/mg CS in samples that had not undergone loading. The amount of 3-B-3(–) epitope was increased to 14.3 ± 3.8 ng core protein/mg CS in those that had ($P < 0.05$).

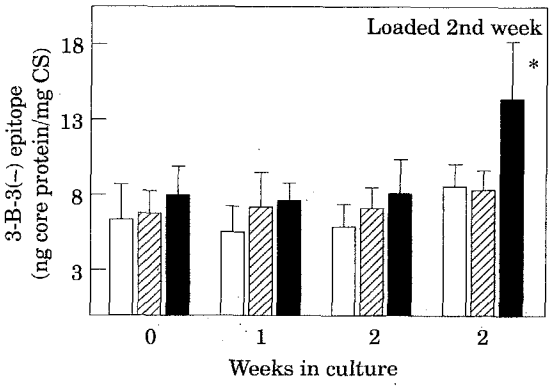

FIG. 4. The amount of 3-B-3(–) epitope in cartilage from bovine sesamoid bones, expressed as nanograms core protein per milligrams chondroitin sulfate (CS). (□), (▨) and (■) bars represent sets of experimental groups in which the culture medium of the first week consisted of control medium to which 0, 10, and 300 ng/ml, respectively, had been added. All continued culture was in control medium only. Cultures that underwent loading during the second week were subjected to a stress of 1 MPa at a frequency of 0.2 Hz. *$P < 0.05$ compared to the amounts of 3-B-3(–) epitope found in the other three cultures from the same set of experiments.

Discussion

RAc acid stimulates chondrocytic production of metalloproteinases, which can degrade cartilage matrix molecules [11–13]. Retinol-induced collagen breakdown has been observed in explants of articular cartilage from adult pigs. The explants were cultured in medium containing 3000 ng/ml retinol, for at least 10 days. Hydroxyproline was found in the culture medium, which was, as shown by histochemistry, to have been released from the explants just above the cut surface [25]. Exposing cultured chondrocytes to RAc decreases type II collagen synthesis [26–28].

Campbell and Handley found that RAc (300 ng/ml) added to bovine articular cartilage explant cultures specifically inhibited proteoglycan synthesis but did not affect total protein synthesis [11]. It has been suggested that chondrocytes that have been treated with RAc cease to produce the precursor protein of cartilage proteoglycan [27]. The addition of GAG chains to these precursor proteins,

or core proteins yields proteoglycans, which are then released into the extracellular matrix [29]. When synthesis of the core protein is down-regulated, so are enzymes and the mechanism of chain synthesis [30, 31]. On the basis of the foregoing, it is reasonable to assume that the concentration of RAc used in our system affected the proteoglycans only.

The cyclic load applied in this study is well within the physiological range and corresponds to that produced by normal walking of the animal. The absence of muscles, ligaments, and posture corrections *in vitro*, however, limits the validity of any comparison of the *in vitro* stress with the physiological range [17].

It has been suggested that decrease in GAG content is directly proportional to severity of OA [32]. In the histochemical grading system of OA of Mankin *et al.*, reduction of safranin O staining is used as a parameter for severity of OA [33]. We found the depletion of proteoglycans induced by RAc treatment to be most pronounced in the lower middle and deep layers of our cartilage specimens. This suggests that the surface and upper middle layers are less sensitive to RAc-induced degradation. A similar phenomenon has been reported for articular cartilage from rats [34].

Autoradiography showed that both the mild and severe RAc treatment reduced GAG synthesis in all chondrocytes. Culture in the presence of 300 ng/ml RAc did not stop GAG synthesis, as was shown by the presence of grains around the chondrocytes.

The restoration of GAG synthesis found after removal of RAc from the culture medium suggests that the binding of RAc to its receptor is reversible. The recovery of GAG synthesis to above control levels corroborates the hypothesis that the rate of synthesis by the chondrocytes is controlled by the extracellular concentrations of cartilage macromolecules [35]. The loss of proteoglycans in both human and experimental OA [36, 37] is also accompanied by increased rates of proteoglycan synthesis, and may indicate a process of cellular repair [35]. In cartilage that is subjected to loading after it has been treated with RAc, only deep layer chondrocytes were found to be engaged in active GAG synthesis. Deep layer and, to a lesser extent, middle layer chondrocytes have also been reported to be the most active in the uptake of [^{35}S]sulfate in human OA cartilage [36].

In the Pond-Nuki model, in which OA is experimentally induced by transection of the anterior cruciate ligament of canine stifle joints [38], newly synthesized proteoglycans are longer than those from control joints [39]. The lower K_{av} of the newly synthesized GAGs at the end of 1 week of culture in medium with 300 ng/ml RAc that we found, similarly shows that these GAGs were longer than those in the controls. However, the proteoglycans that were newly synthesized after subsequent culture in control medium, regardless of loading regime, had GAGs as long as those synthesized in the controls.

Monoclonal antibody 3-B-3 has been raised against the terminal unsaturated residue left after digestion of 6-sulfated chondroitin sulfate proteoglycans with chondroitin ABC lyase [40]. Without digestion, a native epitope, the 3-B-3(–) epitope has been found in developing leg bones of chick embryos [41] and in proteoglycans extracted from OA joints in the Pond-Nuki model [42–45]. For the latter, it has been suggested that the increased expression of the epitope might serve as a marker of early events in the pathogenesis of OA [42–44].

We recently reported that cyclic loading induces the expression of 3-B-3(–) epitope in cartilage from sesamoid bones after the tissue is brought into culture and loaded directly for 1 week [18]. In the present study, no change in the amount of 3-B-3(–) epitope was found after either 1 or 2 weeks, whether or not the cartilage had been depleted of proteoglycans and provided the cartilage was not subjected to loading during the second week. Even with loading, the amount of 3-B-3(–) epitope in cartilage that was cultured in control medium only, or in control medium with 10 ng/ml RAc during the first week, remained unaffected. This may indicate that the chondrocytes were responding adequately to the changes induced in their environment. The adaptive mechanism apparently failed when cartilage was subjected to loading after severe RAc treatment. The increased expression of the 3-B-3(–) epitope that was found might indicate that OA-like changes had occurred.

A mild release of proteoglycans from the cartilage matrix is induced by culturing articular cartilage in the presence of 10 ng/ml RAc. Although GAG synthesis recovers, GAG content decreases further if the agent is then omitted from the culture medium. The further decrease that follows upon loading, shows that loading even slightly degraded cartilage matrix is detrimental to the tissue. The significant decrease in GAG content, the near absence of safranin O staining, the very low GAG synthesis rate, and the increased expression of 3-B-3(–) epitope that were observed after severely degraded cartilage matrix had been subjected to loading indicate that the tissue had been damaged and that OA-like changes had occurred.

Our results suggest that the excessive loading of osteoarthritic joints will exacerbate damage to the cartilage. Care must, therefore, be taken to avoid overusing these joints, particularly when analgesics are being used.

Acknowledgments

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