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GLYCOSAMINOGLYCAN METABOLISM IN EXPERIMENTAL OSTEOARTHROSIS CAUSED BY IMMOBILIZATION

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A study was made of glycosaminoglycan metabolism of articular cartilage in developing experimental osteoarthrosis caused by immobilization of the rabbit knee. Cartilage samples from various sites in the knee and hip joints were analysed, samples from the mobile limb serving as controls. The concentration of glycosaminoglycans in the tissue was measured by determinations of hexosamine and uronic acid after prior papain proteolysis and subsequent purification. The uptake of ^{35}S-sulphate was used as an indicator of the synthesis rate of sulphated glycosaminoglycans. Metabolic changes characteristic of osteoarthrosis, i.e., glycosaminoglycan depletion and increased uptake of ^{35}S-sulphate, were found in tibial weight-bearing and femoral condylar cartilages. Net synthesis of glycosaminoglycans occurred in the tibial marginal cartilage.

Key words: osteoarthrosis; articular cartilage; immobilization; glycosaminoglycans; ^{35}S-sulphate

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The availability of a progressive and irreversible model arthrosis consistently reproducible in animals and recognizable radiographically is of great importance for the study of the pathogenesis of osteoarthrosis (OA) and for the evaluation of the various forms of treatment. The immobilization of the rabbit knee in extension as described earlier (Langenskiöld et al. 1975) produces a model OA satisfying these demands. In this model, 4 days of immobilization caused a marked increase in ^{35}S-sulphate uptake, indicating an accelerated synthesis of sulphated glycosaminoglycans (GAG) in various articular and periarticular tissues of the knee and hip (Videman et al. 1976). After 10 days histological and macroscopic changes of a degenerative nature could be seen in the knees. Radiologically demonstrable OA was induced by 4 weeks of immobilization, which, if continued for over 7 weeks, produced irreversible restriction of knee joint motion (Videman et al. 1977, Michelsson et al. 1977).

Characteristic biochemical changes in articular cartilage in OA are an increased water content (Lindahl 1948), an unchanged content of collagen and a decreased content of GAG (Bollet et al. 1963, Mankin & Lippiello 1970, 1971). Biochemical information concerning the initial metabolic changes in developing OA is incomplete. This may be due to the fact that most experimental OA models are more suitable for the study of advanced than of incipient disease. The rabbit model used here produces an OA of a progressive, non-traumatic nature. This is why it is also well suited for the study of the initial metabolic changes associated with the disease. A loss of proteoglycans and thus of GAG from the cartilage matrix is generally considered an initial change in OA. Hence we decided to study the GAG metabolism of articular cartilage in our experimental OA model with the object of answering the following questions: 1. Is the development of OA in this model associated with changes in GAG metabolism similar to those reported to occur in human OA? 2. How early in developing OA are changes in GAG metabolism detectable and what are these changes? 3. Are differences in GAG metabolism discernible in articular cartilage taken from different sites during developing OA?

MATERIALS AND METHODS

The right knees of 16 rabbits older than 9 months were immobilized in extension by means of a plastic splint (PVC) and by bandaging the knee region with Tensoplast®, while the hips remained partly movable (Michelsson et al. 1977). Six rabbits were used for pilot experiments. Of the remaining ten rabbits, two were killed after 2, 6, 10, 17 and 30 days of immobilization. Twenty-four hours before they were killed, the animals were given 0.26 mCi/kg ^{35}S-sulphate (carrier-free, the Radiochemical Centre, Amersham, U.K.) intramuscularly. Samples were taken with a knife from both hind legs, tissues from the left leg serving as control material. Samples consisted of articular cartilage from the following sites: tibial weight-bearing region, tibial margin, femoral condyle and femoral head.

The samples were defatted by multiple extractions with acetone, dried to a constant weight at 40°C in a vacuum oven, weighed, and digested with papain (Merck 7144) (Wasteson et al. 1972). Proteins were precipitated with 1/3 volume of 40 per cent trichloroacetic acid (TCA). After standing at +4°C overnight the precipitates were centrifuged off and washed once with 10 per cent TCA. The combined supernatants and washings were dialysed against three changes of distilled water at +4°C for 48 hours with stirring. The retentates were lyophilized, dissolved in water and stored at −20°C if not immediately analysed.

Hexosamine was determined (Ludowieg & Benmaman 1967) after hydrolysis for 14 hours in 4 M hydrochloric acid at 100°C. Uronic acid was assayed with m-hydroxydiphenyl using D-glucuronic acid as a standard (Blumenkrantz & Asboe-Hansen 1973). ^{35}S radioactivity was measured with a liquid scintillation counter (LKB-Wallac 81000) using automated external standardization for counting efficiency. Radioactivities were reported as dpm/µg hexosamine. Statistical significances were evaluated by the Mann-Whitney nonparametric test. Differences were considered significant when $P < 0.05$.

RESULTS

In the *tibial weight-bearing cartilage* the tissue concentrations of hexosamine ($P < 0.005$) and uronic acid ($P < 0.025$) are significantly lower in samples from immobilized joints as compared with the controls (Figure 1a). Tissue concentrations of hexosamine and uronic acid decrease with the period of immobilization, the average hexosamine concentration being 50 per cent and the uronic acid concentration 65 per cent of the corresponding control values after 30 days (Figure 1b). On the other hand, specific ^{35}S activity rises, after a small initial decline, indicating an increased synthesis rate of sulphate GAG. Specific radioactivities reach mean values approximately 100, 150 and 125 per cent higher in immobilized as compared with control cartilages after 10, 17 and 30 days of immobilization, respectively (Figure 1b).

In the *femoral condylar cartilage* the metabolic changes are similar to those found in tibial weight-bearing cartilage. Concentrations of hexosamine ($P < 0.005$) and of uronic acid ($P < 0.025$) are significantly lower in cartilage from the immobilized joints (Figure 3a). After only 6 days of immobilization the specific ^{35}S activity of immobilized cartilage is approximately 200 per cent higher than in control cartilage and remains markedly elevated throughout the immobilization period (Figure 3b).

In the *tibial marginal cartilage* the changes in GAG metabolism induced by im-

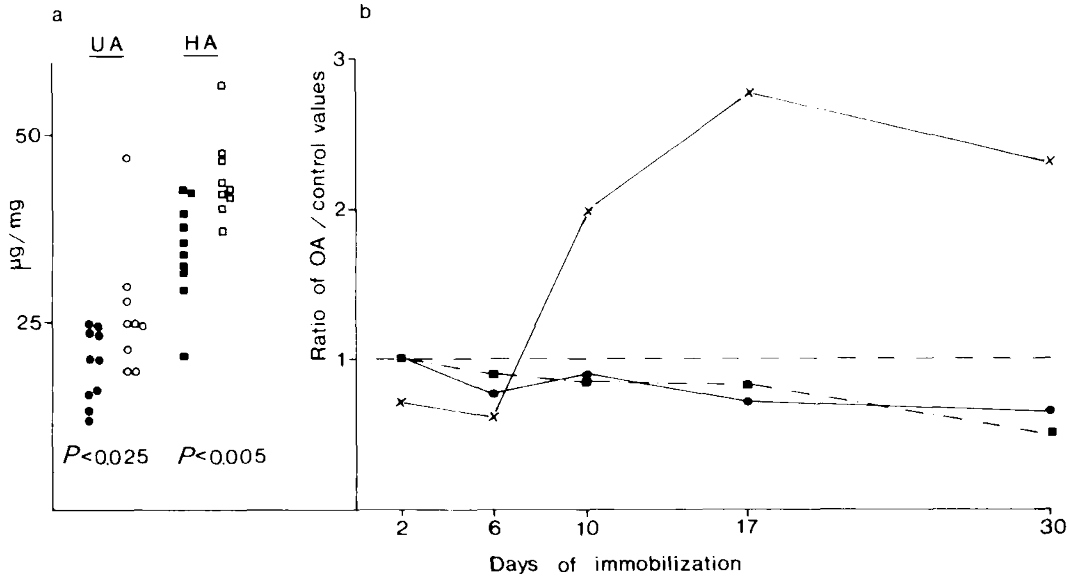

Figure 1. Tibial weight-bearing cartilage.
(a) Tissue concentrations (μg/mg dry, defatted tissue) of uronic acid (UA)
○ = *control values*
● = *osteoarthrosis values*
and hexosamine (HA)
□ = *control values*
■ = *osteoarthrosis values*
(b) Ratios of osteoarthrosis (OA)/control values of parameters measuring glycosaminoglycan metabolism as a function of immobilization time:
● = *uronic acid*
■ = *hexosamine*
× = ^{35}*S activity*

mobilization differ strikingly from those occurring in the cartilages described above. Concentrations of hexosamine ($P < 0.01$) and of uronic acid ($P < 0.05$) are significantly *higher* in immobilized than in control cartilage (Figure 2a). This increase is distinct after 17 days of immobilization and remains so after 30 days. Specific ^{35}S activity of immobilized cartilage increases progressively with the duration of immobilization, being approximately 7-fold, 9-fold and 12-fold higher than in control cartilage after 10, 17 and 30 days, respectively (Figure 2b).

In the *femoral head cartilage* the concentrations of hexosamine and uronic acid are not affected by immobilization of the knee. Specific ^{35}S activity of cartilage from the immobilized limb, however, is increased about 100 per cent above the control activity after 10 days and remains increased by some 50 per cent throughout immobilization.

The results can be summarized as follows: Immobilization caused an increased specific ^{35}S activity of varying degree in cartilage from all sites studied, i.e., an increased synthesis rate of sulphated GAG. This increase was most pronounced by far in tibial marginal cartilage, where there was a rise in GAG concentration as well, indicating that net synthesis occurred. In tibial weight-bearing and in femoral condylar cartilages immobilization caused GAG depletion indicating that the GAG degradation rate was increased more than the synthesis rate. In femoral head cartilage the GAG concentration was not affected by immobilization.

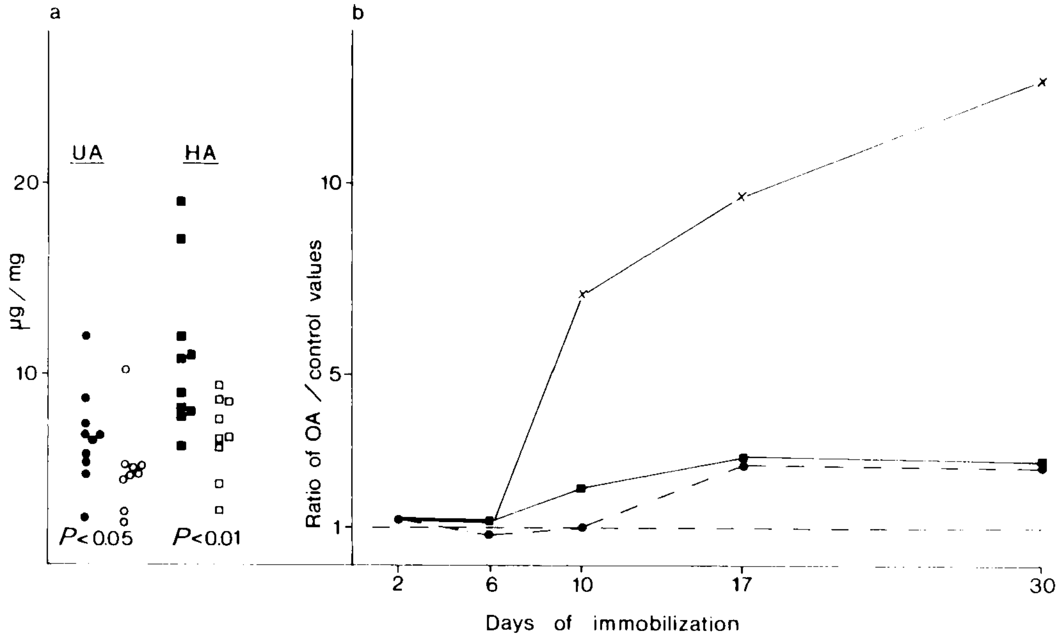

Figure 2. Tibial marginal cartilage.
(a) As Figure 1a.
(b) As Figure 1b.

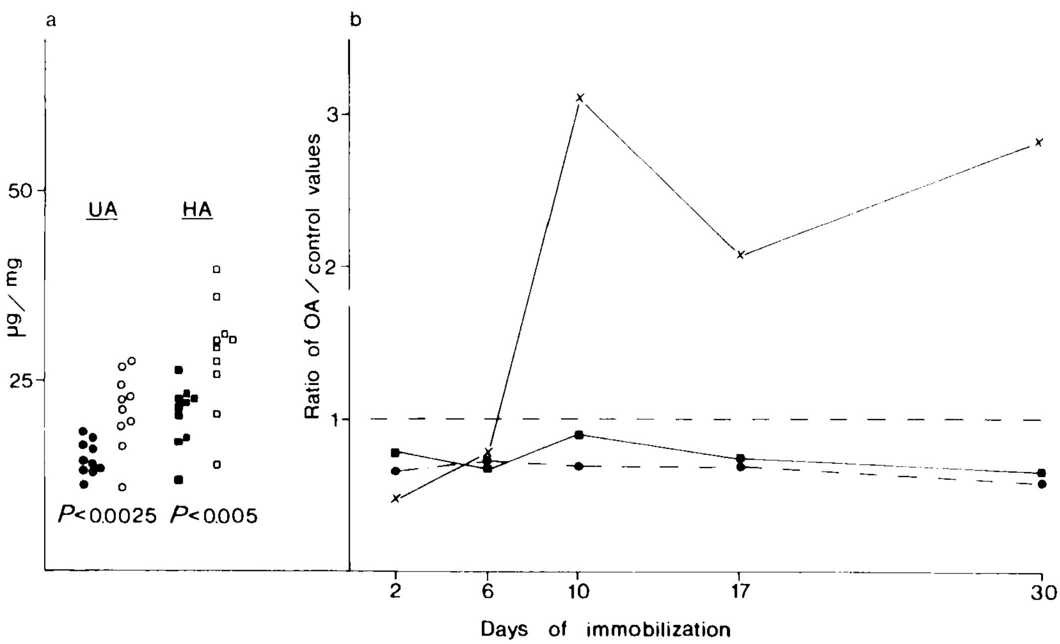

Figure 3. Femoral condylar cartilage.
(a) As Figure 1a.
(b) As Figure 1b.

DISCUSSION

The results show that immobilization of the rabbit knee in extension induces changes in GAG metabolism in weight-bearing cartilage of the knee joint similar to those reported to occur in human OA, i.e., decreased GAG concentration and increased synthesis rate of sulphated GAG. Since these alterations of GAG metabolism are generally considered early events in the development of OA, the model arthrosis described appears appropriate for the study of the pathogenesis and the treatment of OA.

Immobilization of the rabbit knee in extension rapidly produces the changes characteristic of OA in weight-bearing cartilage. After 10 days the GAG synthesis rate increases some 100 per cent and GAG depletion is discernible. Besides the rapidity of OA induction, other advantages of the present model arthrosis are its simplicity and its non-traumatic nature as compared with surgical manoeuvres employed in the production of experimental OA, e.g., the Hulth procedure (Hulth et al. 1970, Ehrlich et al. 1975), partial meniscectomy (Moskowitz et al. 1973) and anterior cruciate ligament section (Pond & Nuki 1973, Telhag & Lindberg 1972).

The real reasons for the OA changes in joints following immobilization are mostly unknown. Immobilization of the knee in extension causes a continuous compression of the weight-bearing cartilages in the joint. This compression may disturb normal chondrocyte function and lead to the changes observed. It has been found that distraction of a joint makes it possible to diminish the OA changes in rabbit knee developing during immobilization (Videman & Michelsson 1977).

An interesting feature is the initial decline in the GAG synthesis rate visible in tibial weight-bearing and femoral condylar cartilages after 2 and 6 days of immobilization, a phenomenon also observed in an earlier study (Videman et al. 1976). The immobilization may disturb chondrocyte function and depress GAG synthesis until degradation of cartilage matrix provides a stronger stimulus leading to increased GAG production by the cells. A normal synthetic rate together with incipient degradation of GAG could also lead to diminished uptake of ^{35}S-sulphate as compared with controls.

Cartilage proliferation peripheral to the edges of the joint cartilage on both the femur and the tibia is found after 10 days of immobilization, and after 14 days definite osteophyte formation occurs on the medial aspects of the tibia (Langenskiöld et al., in press). The finding of net synthesis of sulphated GAG in tibial marginal cartilage reported here agrees well with these morphological observations. The opposite changes in GAG concentrations in weight-bearing and marginal cartilage make it important to determine exactly the sampling regions of cartilage, especially in the fully developed disease, when the weight-bearing cartilage may have almost disappeared. An important future task appears to be the elucidation of the stimulatory mechanisms underlying the hypertrophy of the marginal cartilage of an osteoarthrotic joint.

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