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GLYCOSAMINOGLYCAN METABOLISM OF THE MEDIAL MENISCUS, THE MEDIAL COLLATERAL LIGAMENT AND THE HIP JOINT CAPSULE IN EXPERIMENTAL OSTEOARTHRITIS CAUSED BY IMMOBILIZATION OF THE RABBIT KNEE

TAPIO VIDEMAN[1], ILKKA ERONEN[2], CLAES FRIMAN[2] & ANDERS LANGENSKIÖLD[3]

[1]Institute of Occupational Health and the Research Laboratory of the Orthopaedic Hospital of the Invalid Foundation; [2]Fourth Department of Medicine, University Central Hospital; [3]Department of Orthopaedics and Traumatology, University Central Hospital, Helsinki, Finland

A study was made of glycosaminoglycan metabolism in experimental osteoarthritis caused by immobilization of the rabbit knee in extension. Samples from the medial meniscus, the medial collateral ligament of the knee and the hip joint capsule were obtained and analysed after 2, 6, 10, 17, 30 and 87 days of immobilization, samples from the mobile limb serving as controls.

The tissue concentrations of glycosaminoglycans were determined from measurements of hexosamine and uronic acid after prior papain proteolysis and subsequent purification. The uptake of ^{35}S-sulphate (DPM/μg hexosamine) was used as an indicator of the synthesis rate of sulphated glycosaminoglycans. In both early and advanced immobilization osteoarthritis, the synthesis rate and the content of glycosaminoglycans were increased in all tissues.

Key words: glycosaminoglycans; immobilization; joint capsule; osteoarthritis

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The aetiopathogenesis of osteoarthritis (OA) is unclear. Immobilization with or without compression has been successfully employed in the experimental production of degenerative joint changes similar to those of human OA (for review, see Moskowitz 1972). Muscle contraction causing a static compression of the articular cartilage probably contributes to the degenerative changes occurring in immobilized joints (Thaxter et al. 1965, Videman & Michelsson 1977). Changes in the articular cartilage in human OA and in various experimental OA models have been extensively studied (for reviews, see Mankin 1976 and Muir 1977), but comparatively little is known about possible alterations of other articular or periarticular structures. Thickening of the joint capsule is a regular feature of human OA of the hip (Lloyd-Roberts 1953). OA induced by the immobilization of the rabbit knee in extension causes thickening of the knee and ipsilateral hip joint capsules, as well as increased uptake of ^{35}S-sulphate in these structures, in the menisci and in the medial collateral ligament (Videman et al. 1976). Immobilization of the rabbit knee in flexion for 9 weeks, on the other hand, causes joint contracture and a diminished content of water, hyaluronate and chondroitin −4/−6−sulphate of the periarticular connective tissues,

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but no change in total collagen content (Akeson et al. 1973). The concentration of soluble collagen is reduced, however, and the degree of joint stiffness has been found to correlate with the loss of hexosamine from the periarticular connective tissues (Woo et al. 1975), which also display an increase in $NaBH_4$ reducible cross-links in collagen (Akeson et al. 1977).

The purpose of the present study was to investigate further possible changes of glycosaminoglycan (GAG) metabolism in the medial meniscus, the medial collateral ligament and the hip joint capsule after various periods of immobilization of rabbit knees in extension.

MATERIALS AND METHODS

The right knees of 18 rabbits older than 9 months were immobilized according to the method of Langenskiöld et al. (1975). Two rabbits were killed after 2, 6, 10 and 30 days, six after 17 days and four after 87 days of immobilization. Samples were taken from the hind legs and consisted of the medial meniscus, the medial collateral ligament of the knee and the joint capsule (full thickness) of the hip. Tissues from the mobile left leg served as control material. The administration of ^{35}S-sulphate, the processing of the samples and the determinations of hexosamine, uronic acid and ^{35}S-activity (reported as DPM/μg hexosamine) were performed as described in another report (Eronen et al. 1978). Statistical significances were evaluated by Student's *t*-test. Differences were considered significant when the *P* value was < 0.05.

RESULTS

In the *meniscus* the tissue concentrations of hexosamine ($P < 0.01$) and uronic acid

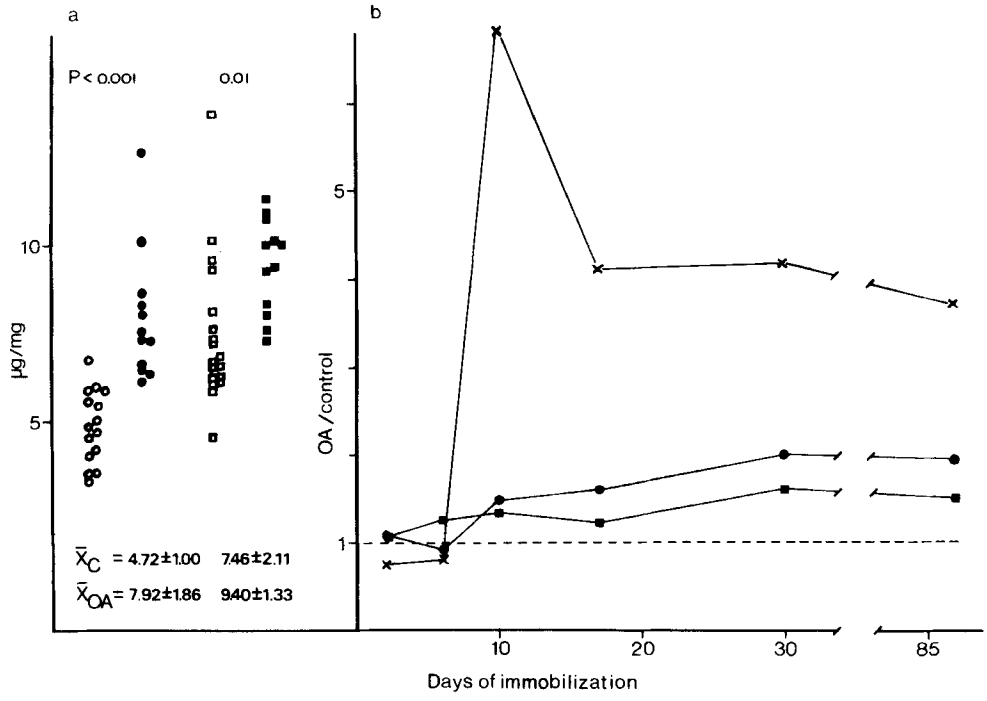

Figure 1. Medial meniscus: (a) Tissue concentrations (μg/mg dry, defatted tissue) of uronic acid (UA) (○ = control values, ● = osteoarthritis values) and of hexosamine (HA) (□ = control values, ■ = osteoarthritis values). The mean values for the immobilized limbs have been calculated with the 2-, 6- and 10-day results excluded, since the disease is not manifest after these short periods of immobilization. (b) Mean ratios of osteoarthritis (OA) control values of parameters measuring glycosaminoglycan metabolism as a function of immobilization time (● = uronic acid, ■ = hexosamine, × = specific ^{35}S-activity).

($P < 0.001$) were significantly higher in the samples from immobilized joints than in those from controls (Figure 1a). The mean concentrations of hexosamine and uronic acid increased during the immobilization until they reached their maximum levels at 30 days. The remained at this level until the 87th day. The average hexosamine and uronic acid concentrations were approximately 150 and 200 per cent of the corresponding control values, respectively. After a small initial decline, specific ^{35}S-activity reached a maximum at 10 days of immobilization, when a sevenfold elevation above the control level was seen, indicating greatly increased synthesis of sulphated GAG. A fourfold elevation of ^{35}S-activity was discerned at 30 days and this elevation persisted until the end of immobilization (87 days) (Figure 1b).

In the *medial collateral ligament* of the knee the tissue concentrations of both hexosamine ($P < 0.001$) and uronic acid ($P < 0.001$) were markedly elevated as compared with the corresponding concentrations in controls (Figure 2a). The mean hexosamine and uronic acid concentrations increased with the immobilization time, were maximal at 17 days, and were still markedly above the control levels at 30 and 87 days of immobilization (Figure 2b). The mean ^{35}S-activity in this tissue also reached a maximum at 10 days (a 17-fold increase above the control level), but it was reduced to a sixfold increase at 30 days and to a twofold increase at 87 days (Figure 2b).

In the *joint capsule of the hip* the changes in GAG metabolism resembled those found in the meniscus and the collateral ligament. The tissue concentrations of hexosamine ($P < 0.001$) and uronic acid ($P < 0.001$) were significantly higher in samples from immobilized joints when compared with those of controls (Figure 3a). This increase was marked at 17 days of immobilization, was

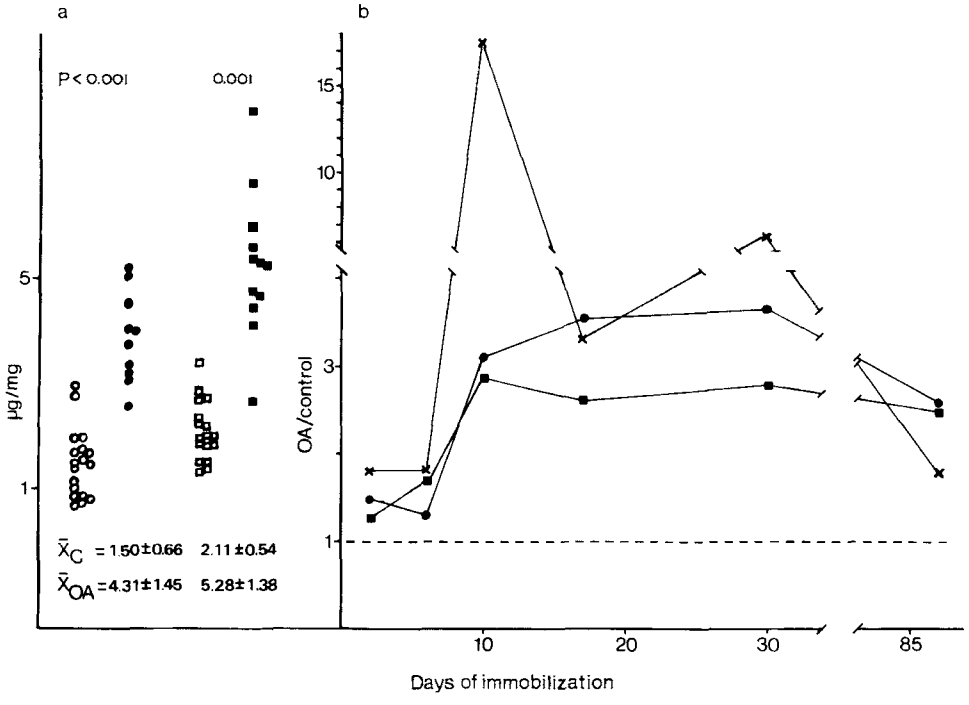

Figure 2. Medial collateral ligament of the knee. (Explanation of symbols can be found in text to Figure 1.)

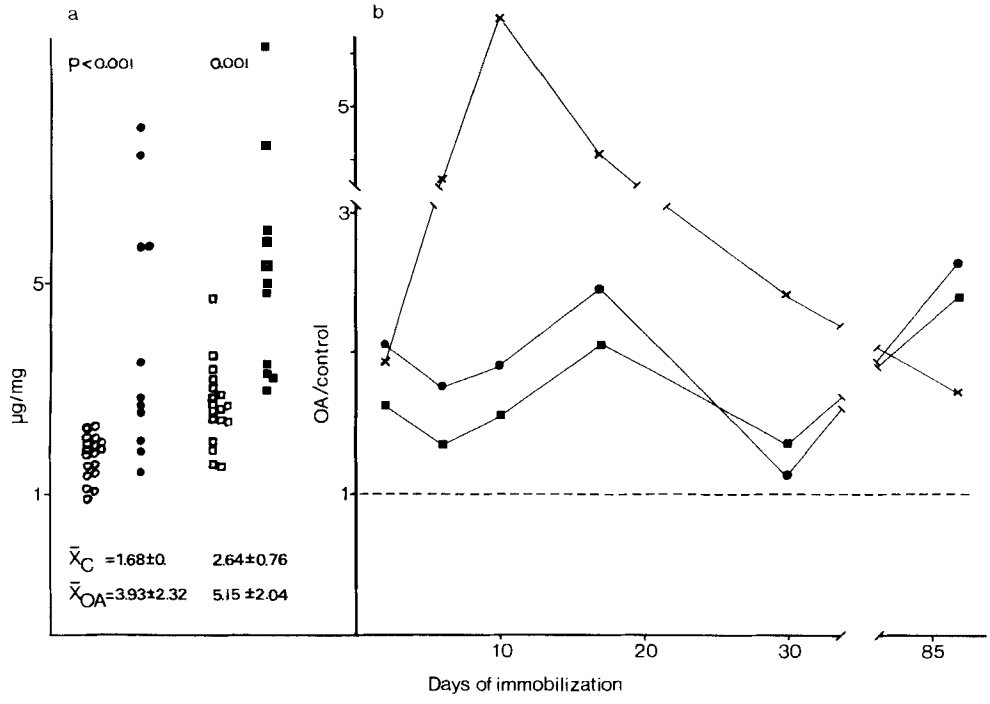

Figure 3. The joint capsule of the hip. (Explanation of symbols can be found in text to Figure 1.)

only slight after 30 days and was again marked at 87 days (Figure 3b). The increase in mean ^{35}S-activity was again maximal at 10 days (almost a sevenfold increase above the control level), and it remained distinctly above the control level at 30 and 87 days.

The results can be summarized as follows: In all the tissues studied immobilization caused an increased uptake of ^{35}S-sulphate, i.e. an increased synthesis rate of sulphated GAG, which led to elevated tissue concentrations of GAG that were discernible even after 87 days of immobilization.

DISCUSSION

The present study and an earlier one (Eronen et al. 1978) showed that immobilization of rabbit knees in extension causes an increased synthesis of sulphated GAG in articular cartilage, meniscus, collateral ligament and joint capsule (also hip). In hyaline cartilage and meniscus increased GAG synthesis was observed after 10 days whereas only 2 days of immobilization sufficed to stimulate GAG synthesis in the joint capsule and collateral ligament. In non-weight-bearing cartilage, meniscus, collateral ligament and joint capsule the GAG concentration rose, i.e. net synthesis occurred, but in weight-bearing cartilage GAG concentration fell. These results are in accordance with our earlier studies using histological, roentgenological and autoradiographic methods (Videman et al. 1977).

It has been shown that immobilization of rabbit knees in flexion leads to a diminished concentration of GAG in menisci and periarticular connective tissues (Akeson et al. 1973). The difference between these and our present results may be due to the fact that immobilization in flexion causes differently oriented forces and stress in the joint

structures than immobilization in extension. In our development work on the OA model, we immobilized rabbit knees in flexion for 4–10 weeks, but only in a few cases was radiographic and macroscopic evidence of joint degeneration obtained, and immobilization in extension was thereafter employed. In human OA the total hexosamine content of degenerative areas of menisci has been found to increase (Ghosh et al. 1975, Peters & Smillie 1972).

In human OA, fibrous thickening, fibroblastic proliferation and, occasionally, local cartilage metaplasia are characteristic histological changes of the hip joint capsule (Sokoloff 1969, Lloyd-Roberts 1953). Similar changes are, to various degrees, observable in the present immobilization OA model (Videman et al. 1976). It is obvious that immobilization causes thickening and shortening of the joint capsule, and these changes increase pressure on the cartilage and thus cartilage damage and other OA changes. Via this mechanism capsular changes may have an important role in the production of OA, and the generally held view that capsular changes are secondary could in many instances be inaccurate.

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Correspondence to: Dr. Tapio Videman, M.D., Institute of Occupational Health, Dept. of Physiology, Laajaniityntie 1, SF-01620 Vantaa 62, Finland.