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GLYCOSAMINOGLYCAN METABOLISM IN EXPERIMENTAL OSTEOARTHRITIS CAUSED BY IMMOBILIZATION

The Effects of Different Periods of Immobilization and Follow-up

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Glycosaminoglycan metabolism at different developmental stages of osteoarthritis provoked by immobilization was studied in rabbits. Tissue concentrations and the specific radioactivity of glycosaminoglycans (GAG) after a long immobilization (IZ) period (12 weeks) without follow-up, and a moderate IZ time (4–7 weeks) with a long follow-up period (average 22 weeks) were compared with the results obtained after a short IZ time (17 days). In tibial weight-bearing cartilage the specific ^{35}S-activity had increased and the galactosamine, glucosamine and uronic acid concentrations had decreased in all the IZ groups examined. In the marginal cartilage, the menisci and the collateral ligament, the specific radioactivity and GAG concentrations had decreased or were normal after a long IZ with or without follow-up, although they were markedly elevated after a short IZ time. The depletion of GAG correlated roughly with the IZ and follow-up times, except for periodically immobilized rabbits. In long-term "chronic" disease the specific radioactivities and GAG concentrations were depressed on the contralateral, non-IZ sides. The changes in the non-IZ knee are discussed. In studies of experimental osteoarthritis it is important to determine the developmental stage of the disease to be studied.

Key words: animal experiments; articular cartilage; glycosaminoglycans; immobilization; joint capsule; osteoarthritis; ^{35}S-sulphate

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Several experimental models are used for studying degenerative joint diseases. Information about the changes occurring at different developmental stages of the experimental disease is important for studies of pathogenesis and therapy.

Immobilization (IZ) of the rabbit knee in extension produces progressive osteoarthritis (OA) with typical radiographic, histological and macroscopic findings (Langenskiöld et al. 1979). The experimental disease corresponds biochemically to human OA, i.e., increased synthesis of sulphated glycosaminoglycans (GAG) in the articular connective tissues and GAG depletion in the weight-bearing cartilage are observed (Videman et al. 1976, Eronen et al. 1978).

Changes in the articular cartilage of human and experimental OA have been extensively studied (for reviews, see Mankin 1976 and Muir 1977), but less is known about possible alterations in other articular and periarticular tissues. IZ of rabbit knee *in flexion* for 9 weeks causes joint contracture and a depletion of water and GAG, but not of collagen, in periarticular connective tissues (Akeson et al. 1973). IZ of rabbit knee *in extension* for 2 to 12 weeks, on the other hand, produces an increase in the synthesis and con-

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centration of GAG in all the non-cartilaginous connective tissues examined in the knee region (Videman et al. 1979).

The purpose of this study was to investigate the GAG metabolism of articular tissues after a long IZ with or without follow-up in comparison with that after a short IZ. The discrepancy between our earlier results (Videman et al. 1979) and the results of other workers (Akeson et al. 1973) concerning the GAG metabolism of soft connective tissues in experimental OA also required elucidation.

MATERIAL AND METHODS

A total of 29 rabbits older than 9 months were used. Nine rabbits maintained under normal conditions served as controls (group 0). The right knees of 20 rabbits (see Table 1) were immobilized according to the method of Langenskiöld et al. (1979). IZ was applied to 8 rabbits (group 1) for 17 days in order to produce developing OA and to 4 rabbits for 12 weeks in order to induce established OA (group 2).

Two methods for producing long-term "chronic" disease were employed. With the first method (4 rabbits) the knee was immobilized for four 1-week periods, the right knee being kept free for 1 week between each period (group 3). This kind of IZ produces a progressive, degenerative joint disease. After the last IZ period the animals were followed from 16 to 22 weeks before being killed. The other method (4 rabbits) consisted of immobilizing rabbits for 5 to 7 weeks and following it with a period of 16 to 22 weeks free of the splint (group 4).

The mobility of the knee joint was measured with a goniometer without the application of force (0° = full extension; 170° = maximal normal flexion). The degree of OA was estimated from radiographs with the scale: normal, slight OA, moderate OA and severe OA (Videman et al. 1977). Samples were taken from the articular cartilage, medial meniscus and medial collateral ligament of both hind limbs. Articular cartilage was sampled from the tibial weight-bearing region and the tibial margin (not pure cartilage).

The administration of $^{35}SO_4$, the processing of the samples and the determinations of hexosamines, uronic acid (UA) (reported as μg/mg dry, defatted tissue) and specific ^{35}S-activity (DPM/μg galactosamine (GalA)) of the isolated GAGs were performed as previously described (Eronen et al. 1978).

Statistical significances were evaluated with Student's t-test for non-correlating means. Differences were considered significant when the P value was <0.05.

RESULTS

Joint mobility and radiographic findings

The radiographic grade of OA and the ranges of motion of the IZ knees are shown in Table 1. For rabbits immobilized for 17 days (group 1), no clear degenerative changes appeared in the radiographs, and the mean mobility to flexion was 63°. In the rabbits immobilized for 12 weeks (group 2) the joint mobility was markedly re-

Table 1. Radiographic changes and mobility of knees in the immobilization (IZ) and follow-up groups

Rabbit no.	Group	Duration of IZ	Follow-up period after IZ	Grade of osteoarthritis in radiographs	Mobility of knees	
					Extension	Flexion
C 1–8	1	17 days	0	normal-slight	0 –	63
B 66		12 weeks	0	severe	0 –	(+) 15
B 68		12 weeks	0	severe	(−) 40 –	(+) 25
B 69	2	12 weeks	0	slight-moderate	0 –	(+) 60
B 71		12 weeks	0	moderate	(−) 45 –	(+) 10
B 29		4×1 = 4 weeks	22 weeks	moderate	(−) 15 –	(+) 165
B 38	3	4×1 = 4 weeks	16 weeks	moderate-severe	(+) 30 –	(+) 150
B 40		4×1 = 4 weeks	16 weeks	moderate	(+) 5 –	(+) 130
A 146		5×1 = 5 weeks	16 weeks	moderate	(−) 5 –	(+) 85
A 62		6 weeks	24 weeks	slight	0 –	(+) 165
A 101		7 weeks	14 weeks	severe	(−) 35 –	(+) 5
A 141	4	5 weeks	44 weeks	moderate	0 –	(+) 170
B 16		7 weeks	28 weeks	moderate-severe	(−) 70 –	0

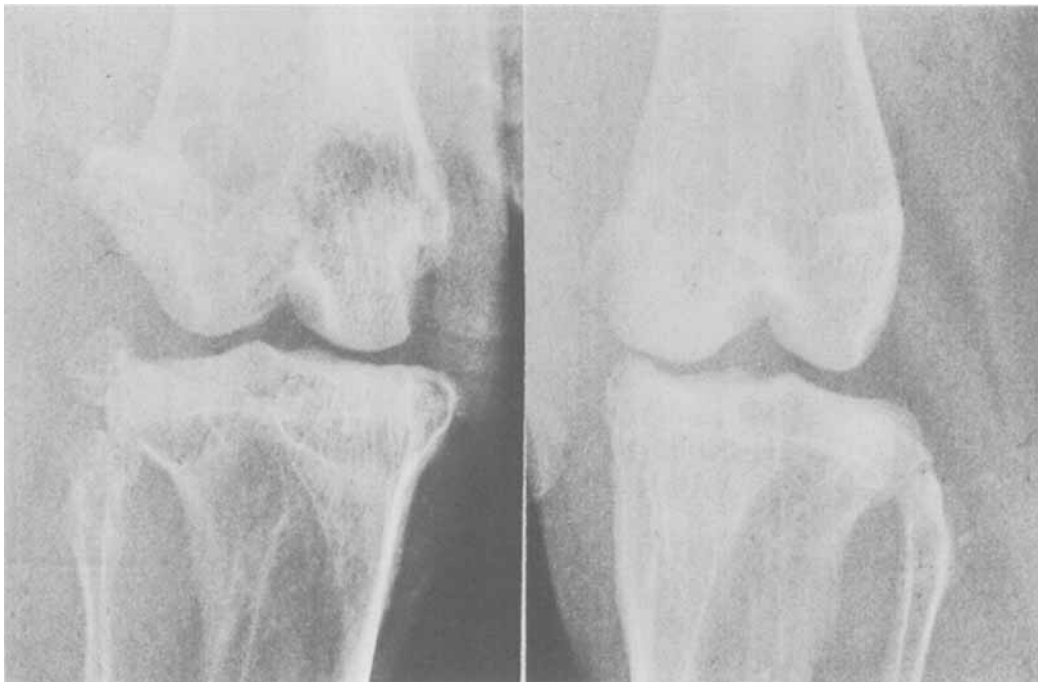

Figure 1. X-rays of rabbit knees (group 3). The knee on the left was immobilized for four 1-week periods with intervals of 1 week and was thereafter followed for 16 weeks. There are marked osteophytes, sclerosis and erosions in the subchondral bone, but the joint space is not narrowed. (The total mobility of this joint was 120°). There are also slight OA changes in the non-IZ knee on the right.

duced, and the radiographic degree of OA was from moderate to severe. The radiographic grade of OA in rabbits with "chronic" OA (groups 3 and 4) was moderate or severe at the end of the follow-up (Figures 1, 2). The range of the mobility of flexion of the IZ knees at the time of killing was from 0 to 165°. In the contralateral non-IZ knees there were also slight radiographic OA changes, but normal joint mobility.

Biochemical results

(The findings are shown in Figures 3–6).

Group 1. The IZ knees of group 1 displayed a depletion of GAG in the weight-bearing cartilage and an accumulation of GAG in the other tissues (except glucosamine (GluA) in meniscus). Increased $^{35}SO_4$-uptake was found in all tissues. As compared with the knees of normal rabbits (group 0), the non-IZ knees showed an elevated GalA level in the meniscus and a decreased concentration of UA in the collateral ligament.

Group 2. In the weight-bearing cartilage of group 2 the concentration of GalA, GluA and UA was lower in the IZ legs than in the corresponding cartilage of groups 0 and 1. Similar GAG depletion of a lesser magnitude was also seen in the non-IZ knees. The ^{35}S-uptake in the IZ knees was significantly higher, and in the non-IZ knees significantly lower, than in the controls.

In marginal cartilage (which also showed an accumulation of GluA) and in the meniscus of the IZ knees of group 2 the concentrations of GalA and UA were above the control levels and similar to those observed in group 1. Non-IZ knees differed from controls by showing decreased concentrations of GalA (marginal cartilage) and UA (meniscus) and an increased concentration of GluA (marginal cartilage). ^{35}S-uptake was lower in both knees than in group 1 rabbits, but it did

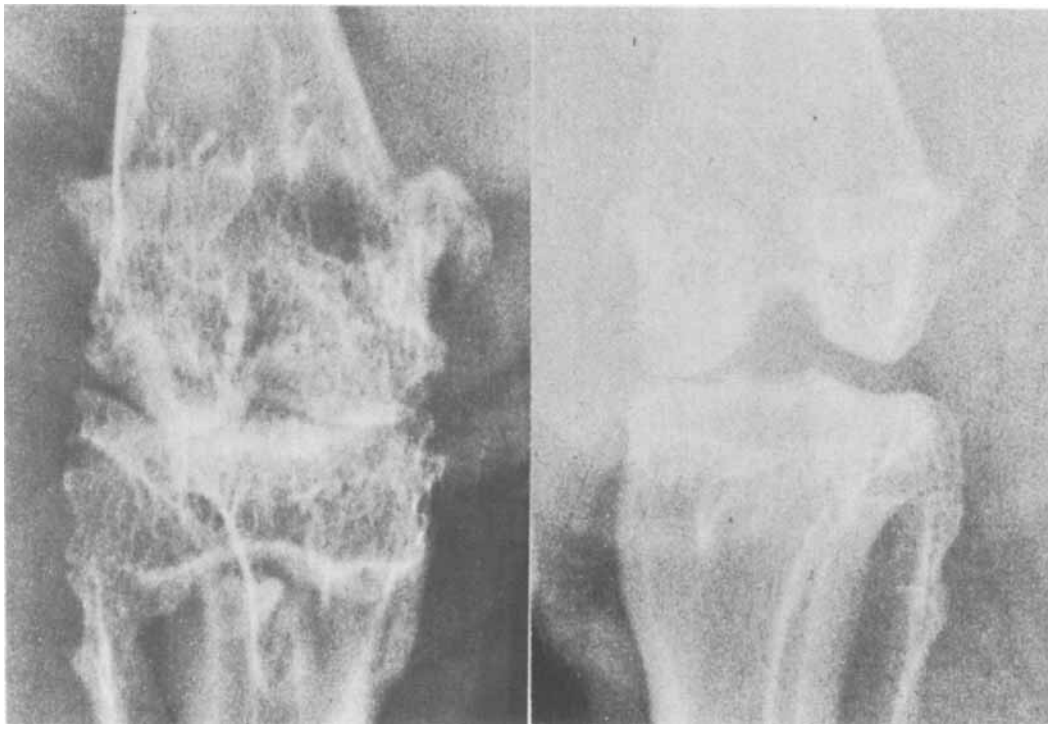

Figure 2. X-rays of rabbit knees (group 4). The knee on the left was immobilized for 7 weeks and was thereafter followed for 14 weeks. The knee is in recurvatum, the degenerative changes in the region of the joint are severe, and the joint space has disappeared. (The mobility of this joint was 40°). There are also slight OA changes in the non-IZ knee on the right.

not differ significantly from ^{35}S-uptake in controls.

In the collateral ligament of group 2 rabbits the concentrations of GalA (both knees) and of UA (IZ knees) were above the control levels. ^{35}S-uptake was similar in both legs and below that of group 1, but not significantly below the control level.

Group 3. Findings in the tibial weight-bearing cartilage of group 3 were similar to those of group 1. In comparison with controls a slight depletion was discernible, as evidenced by reduced concentrations of UA and GluA in the IZ knees. ^{35}S-incorporation was below the control level in the non-IZ knees.

In the marginal cartilage and menisci of the IZ knees of group 3 the GalA and UA concentrations were above the control level. Collateral ligament showed an increase of GluA. ^{35}S-incorporation was lower than in group 0 in the collateral ligaments from both knees, and in the menisci from the non-IZ knee.

Group 4. In the weight-bearing cartilage the concentration of GalA and UA was lower in both knees of rabbits in group 4 than in those of any other group. GluA was also below the control level in IZ knees, which still showed increased ^{35}S-uptake.

In the marginal cartilage of the IZ knees GAG depletion was evident in group 4 when it was compared with groups 1–3, but not significant in comparison with the controls. In the IZ knees ^{35}S-uptake was below the level of group 0.

GAG depletion was also seen in the menisci of group 4 as compared with groups 1–3. GalA concentrations from both knees were lower than in group 0, and ^{35}S-incorporation was reduced in the non-IZ knees.

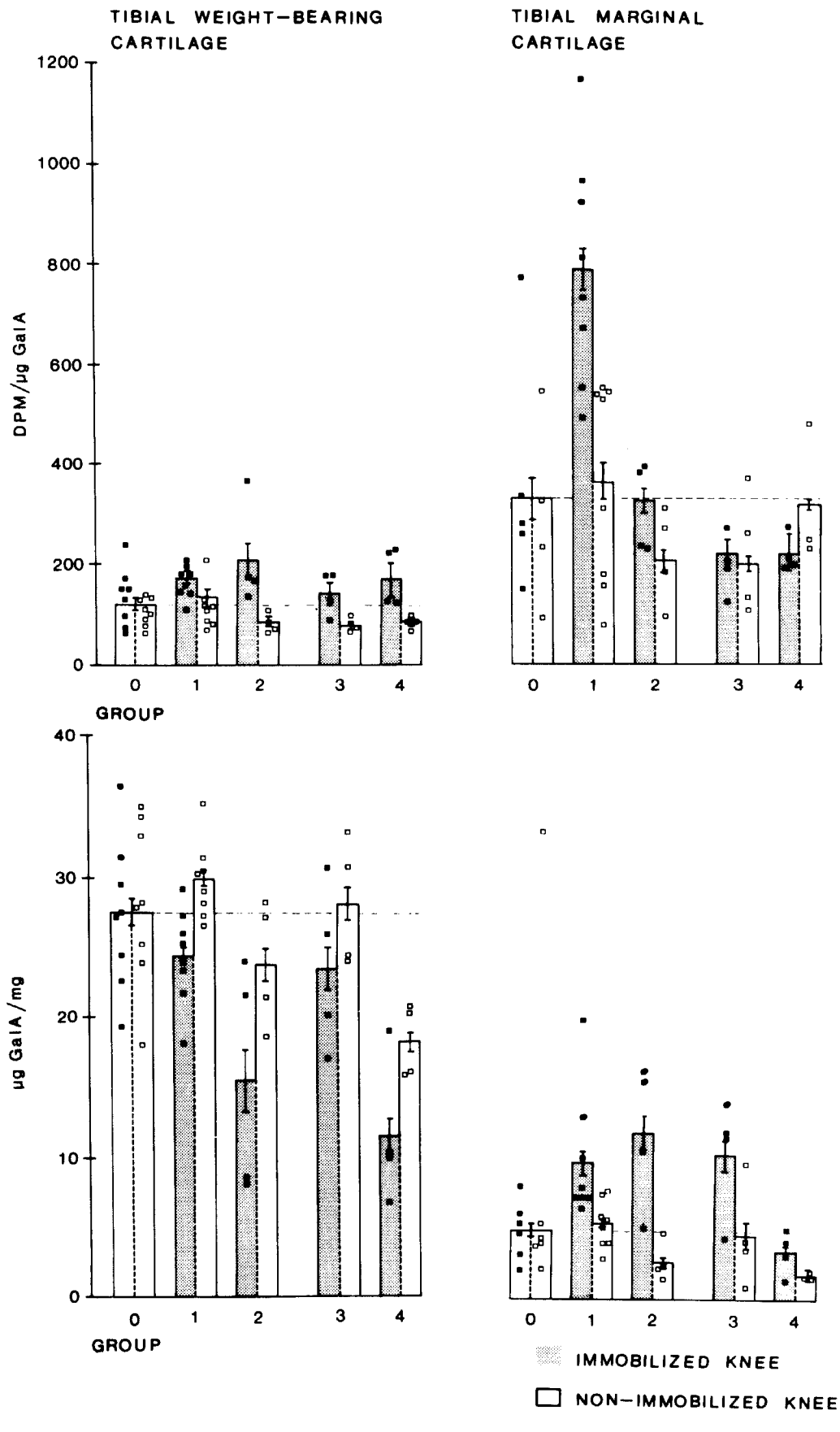

Figure 3. Radioactivity and concentration (mean ± SEM) of galactosamine (GalA) in tibial weight-bearing cartilage and tibial marginal cartilage after various periods of immobilization and follow-up (for groups 1–4, see Table 1).

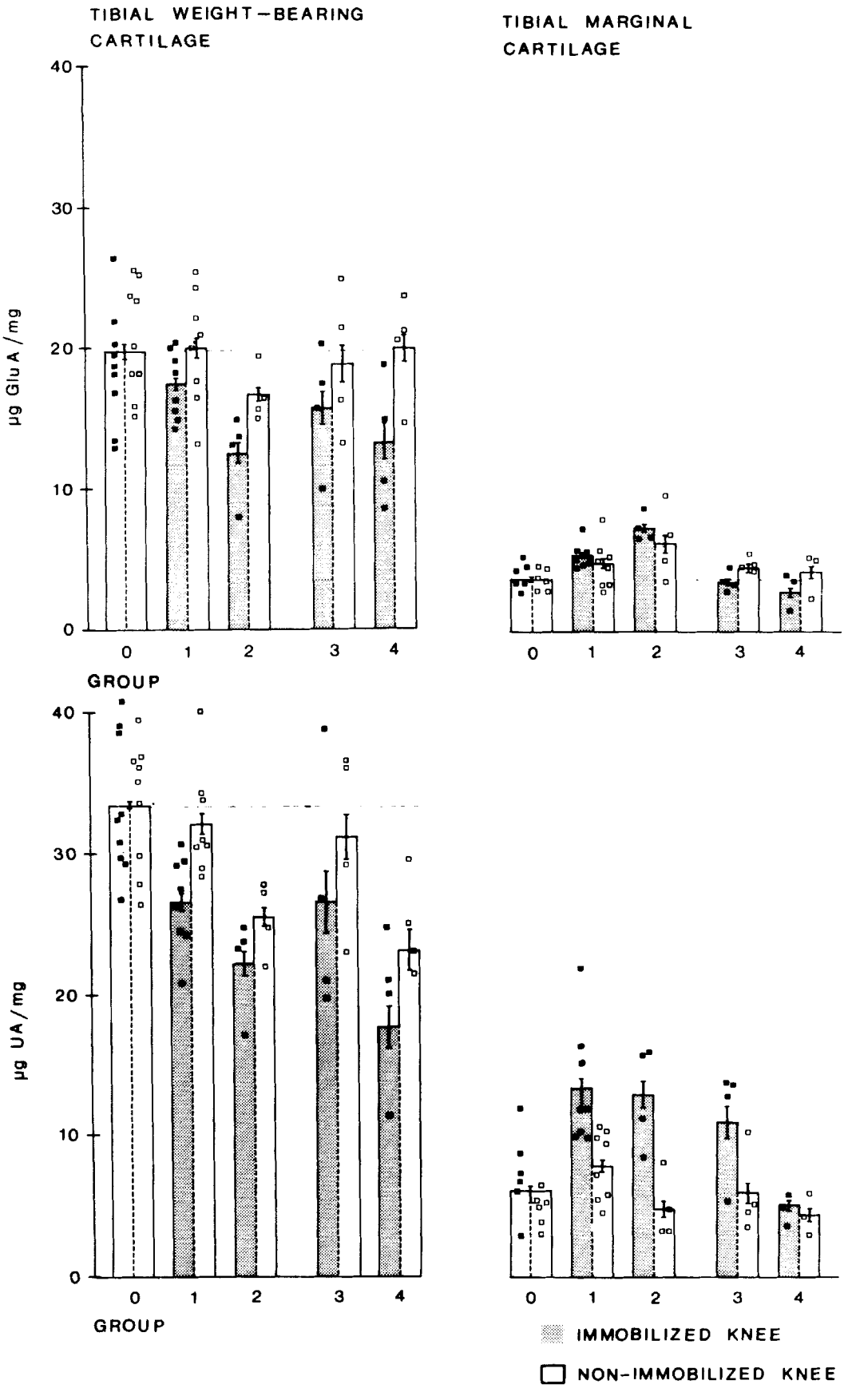

Figure 4. Concentration (mean ± SEM) of glucosamine (GluA) and uronic acid (UA) in tibial weight-bearing cartilage and tibial marginal cartilage.

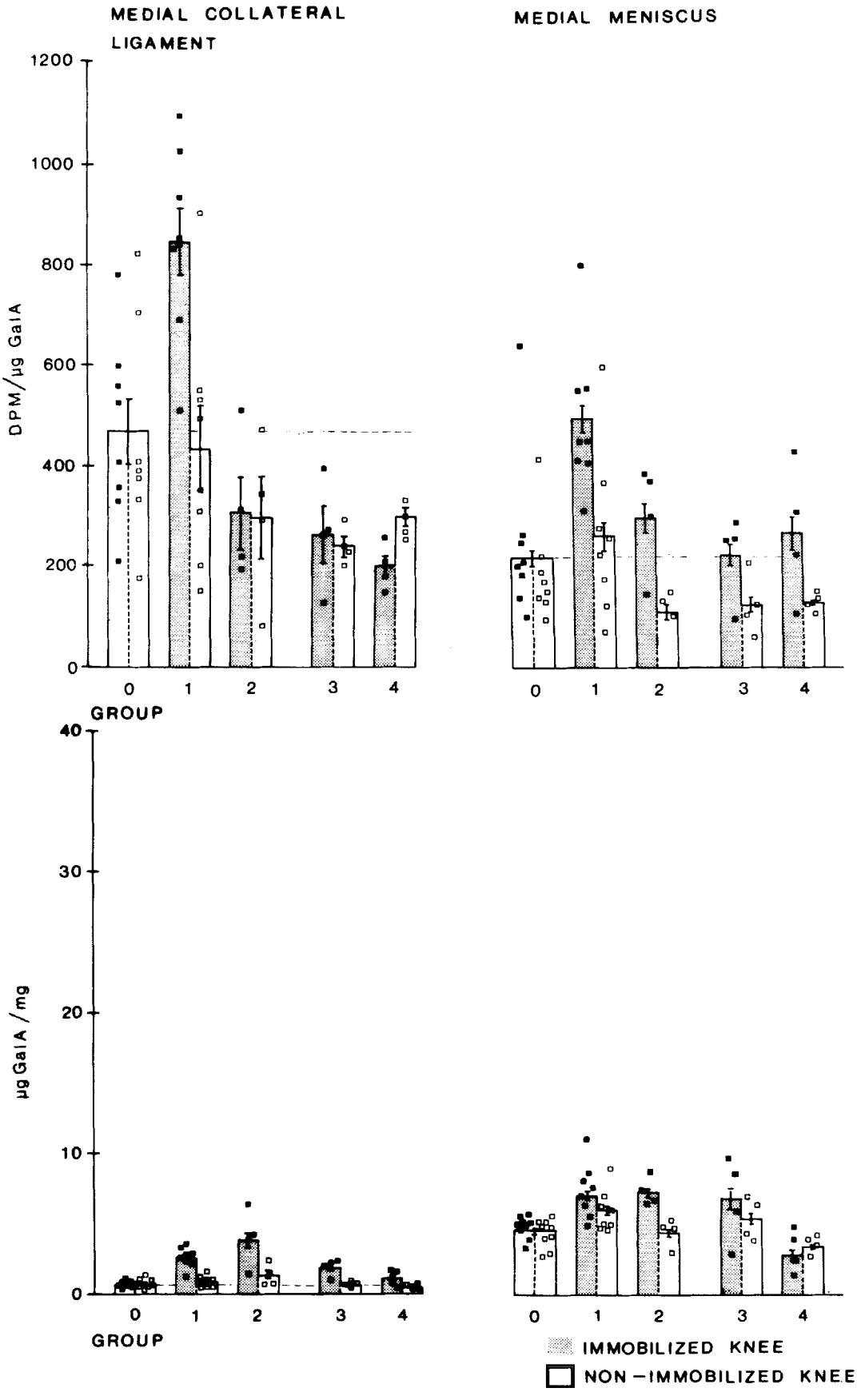

Figure 5. Radioactivity and concentration (mean ± SEM) of galactosamine (GalA) in medial collateral ligament and medial meniscus.

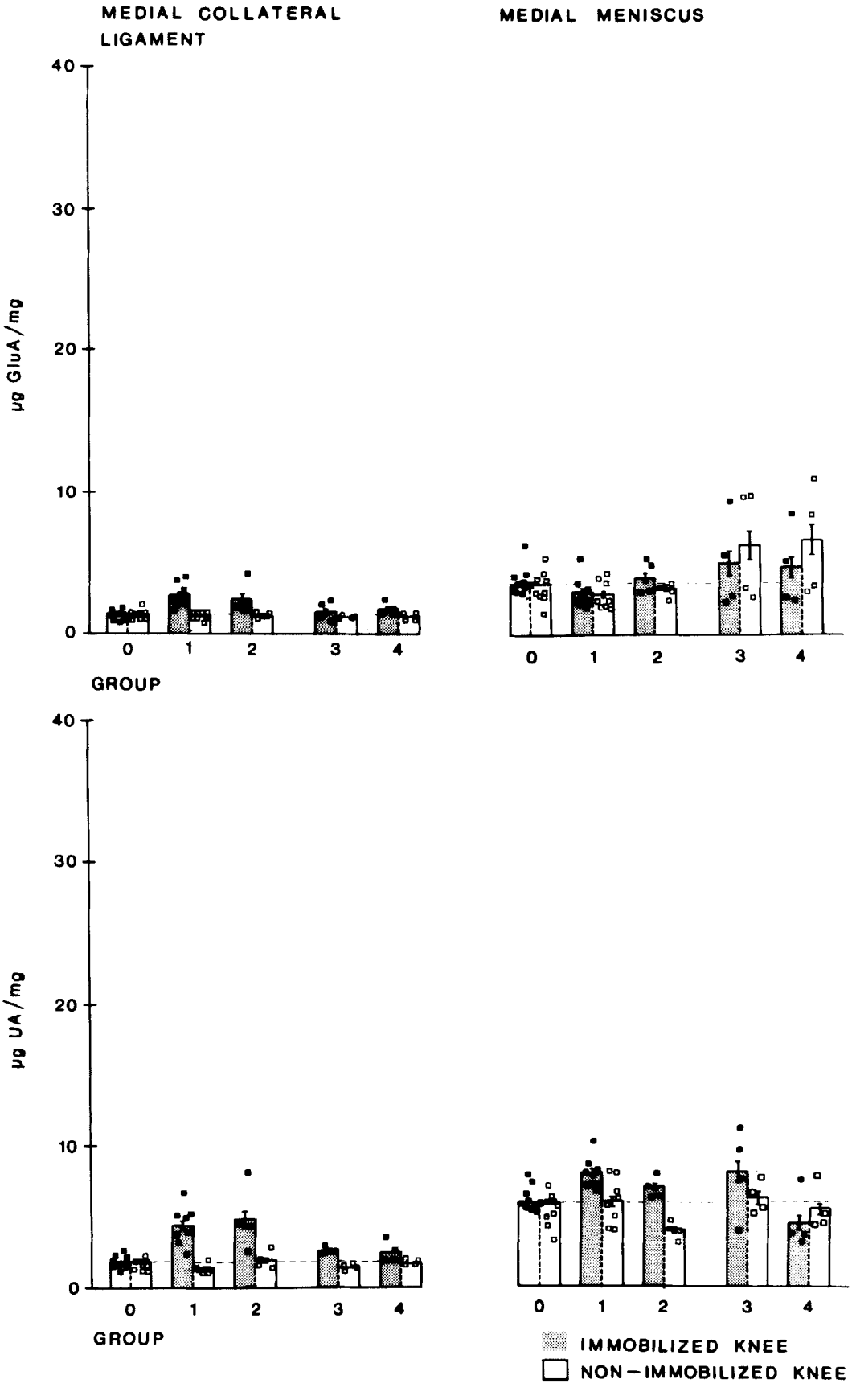

Figure 6. Concentration (mean ± SEM) of glucosamine (GluA) and uronic acid (UA) in medial collateral ligament and medial meniscus.

In the collateral ligament of the IZ knees GalA, GluA and UA concentrations were higher in group 4 than in the controls. In non-IZ knees a clear depletion of GalA was found. Isotope incorporation was lower than in the controls.

The results can be summarized as follows: The specific ^{35}S-activity, i.e., the synthesis of sulphated GAG, was increased in the weight-bearing cartilage of all the groups. In the non-IZ, contralateral knees the opposite effect was observed; after a long IZ with or without follow-up the ^{35}S-activity was below the control level. In the marginal cartilage, the menisci and the collateral ligament, the specific radioactivity was markedly elevated after a short IZ, whereas long IZ periods with or without follow-up were associated with normal or depressed GAG synthesis. Again the non-IZ knees displayed lower levels of ^{35}S-activity than the controls.

GAG depletion was found in weight-bearing cartilage as shown by lowered concentrations of GalA, GluA and UA. The depletion correlated roughly with the length of IZ and follow-up, with the exception of group 3 (= periodic IZ). A depletion of GalA and UA was also seen in the non-IZ knees of group 4, in which both knees yielded increased GluA/GalA ratios. In marginal cartilage, menisci and collateral ligaments from IZ knees the GAG concentration rose after a short IZ, whereas with prolonged IZ it gradually decreased (especially GalA), a phenomenon also occurring in tissues of the contralateral non-IZ knees.

DISCUSSION

The radiographic appearance of IZ joints fulfilled the criteria of OA in both groups with a long follow-up period (groups 3 and 4). Periodic IZ (group 3) produced a condition with a joint mobility closer to that of human OA than did continuous IZ, which led to a greater variation in mobility. The development of mild OA changes in the non-IZ contralateral legs after a long IZ was evident from the radiographs and from the biochemical results. Short-term IZ also causes OA changes which can be observed with a scanning electron microscope also in the contralateral, non-IZ knee after a long IZ (Candolin & Videman 1980). These findings should be kept in mind when comparisons are made between immobilized and contralateral legs.

It is well established that osteoarthritic cartilage shows a depletion of GAG and that the depletion correlates with the severity of the disease. A greater decrease in keratan sulphate (KS) than in chondroitin sulphate (CS) content has been reported (Mankin & Lippiello 1971, McDevitt & Muir 1976), as well as the opposite (Bollett & Nance 1966, Hjertqvist & Lemberg 1972). In our results "established" OA (group 2) and "chronic" OA (group 4) were associated with an increase in the GluA/GalA ratio indicative of an increased KS/CS ratio; concomitantly, however, a decrease in the GalA/UA ratio was observed. Taken together these results may point to an increase in the cartilage hyaluronate content.

Despite the GAG depletion of osteoarthritic cartilage an increased GAG synthesis was preserved even in the animals with the most severe OA (group 4). Other tissues showed reduced ^{35}S-activity after a long IZ, however. These findings are somewhat at variance with the findings in human OA cartilage (Mankin 1976). The discrepancy may be due to the fact that different stages of OA were investigated.

On the basis of isotope incorporation experiments performed on OA cartilage (Mankin & Lippiello 1971), it appears that both cell replication and the synthesis of matrix macromolecules increase in correlation with the severity of OA up to a certain disease stage, beyond which both processes decline rapidly. Articular cartilage obviously responds to injurious stimuli with a reparative process involving increased cell replication and matrix synthesis, but at a critical point reparation fails and irreversible OA ensues (Mankin 1976).

The longest follow-up periods in our experiments were short compared with the duration of human OA. The decline in ^{35}S-activity observed in the tissues of the non-IZ knee after a long IZ is interesting; mild OA should be associated with increased and not depressed GAG synthesis. Whether humoral or local mechanical factors are responsible for this phenomenon remains to be elucidated.

The same kind of phenomenon also occurred in extra-cartilaginous tissue GAG concentrations; after an initial increase the GAG concentration fell. This decrease was also found in the non-IZ knees, in which the GalA concentrations (corresponding to chondroitin sulphates) were markedly reduced, especially in tibial margins and in collateral ligaments. Akeson et al. (1973) also reported a decreased GAG concentration in periarticular connective tissues following IZ of the rabbit knee in flexion for 9 weeks. The present study produced the opposite results after short IZ periods, but this discrepancy may arise from the different IZ technique and/or from the fact that our results may have been derived from investigations of developing OA and those of Akeson from investigations of chronic, irreversible disease. Our results also showed a tendency (group 4) towards a GAG decline after a long IZ and/or remobilization periods.

The tissue of the joint capsule may play an important role in the pathogenic chain leading to OA (Videman et al. 1979, Langenskiöld et al. 1979). Mechanical measurements of the compressive forces in IZ joints (Videman, unpublished results) show a certain parallelism with our GAG results; thickening of the joint capsule itself can cause increased pressure inside the joint, but the role of GAG, and especially of an altered CS/HA ratio, in soft connective tissue biomechanics is unclear. IZ is followed by an increased amount of cross-links (Akeson et al. 1977) in periarticular collagen, possibly causing stiffness. In the formation of these cross-links, an altered composition of GAGs, together with a lack of fibre-orienting motion, may have an important role.

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REFERENCES

Akeson, W. H., Woo, S. L. Y., Amiel, D., Coutts, R. D. & Daniel, D. (1973) The connective tissue response to immobility: Biochemical changes in periarticular connective tissue of the immobilized rabbit knee. *Clin. Orthop.* **93,** 356–362.

Akeson, W. H., Amiel, D., Mechanic, G. L., Woo, S. L. Y., Harwood, F. L. & Hamer, M. L. (1977) Collagen cross-linking alterations in joint contractures: Changes in the reducible cross-linkings in periarticular connective tissue collagen after nine weeks of immobilization. *Connect. Tissue Res.* **5,** 15–19.

Bollet, A. J. & Nance, J. L. (1966) Biochemical findings in normal and osteoarthritic articular cartilage. II. Chondroitin sulphated concentration and chain length, water and ash content. *J. clin. Invest.* **45,** 1170–1177.

Candolin, T. & Videman, T. (1980) Surface changes in the articular cartilage of rabbit knee during immobilization. A scanning electron microscopic study of experimental osteoarthritis. *Acta path. microbiol. scand.* (In press).

Eronen, I., Videman, T., Friman, C. & Michelsson, J.-E. (1978) Glycosaminoglycan metabolism in experimental osteoarthrosis caused by immobilization. *Acta orthop. scand.* **49,** 329–334.

Hjertqvist, S. O. & Lemberg, R. (1972) Identification and concentration of the glycosaminoglycans of human articular cartilage in relation to age and osteoarthrosis. *Calcif. Tiss. Res.* **10,** 223–237.

Langenskiöld, A., Michelsson, J.-E. & Videman, T. (1979) Osteoarthritis of the knee in the rabbit produced by immobilization. *Acta orthop. scand.* **50,** 1–14.

Mankin, H. J. & Lippiello, L. (1971) The glycosaminoglycans of normal and arthritic cartilage. *J. clin. Invest.* **50,** 1712–1719.

Mankin, H. J. (1976) Biochemical changes in articular cartilage in osteoarthritis. *A. A. S. Symposium on Osteoarthritis,* pp. 1–22. Mosby, St. Louis.

McDevitt, C. A. & Muir, H. (1976) Biochemical changes in the cartilage of the knee in experimental and natural osteoarthrosis in the dog. *J. Bone Jt Surg.* **58-B,** 94–101.

Muir, H. (1977) Molecular approach to the understanding of osteoarthrosis (Heberden Oration, 1976). *Ann. rheum. Dis.* **36,** 199–208.

Videman, T., Michelsson, J.-E., Rauhamäki, R. & Langenskiöld, A. (1976) Changes in ^{35}S-sulphate uptake in different tissues in the knee and hip regions of rabbits during immobilization, remobilization and the development of osteoarthritis. *Acta orthop. scand.* **47,** 290–298.

Videman, T., Michelsson, J.-E. & Langenskiöld, A. (1977) The development of radiographic changes in experimental osteoarthritis provoked by immobilization of the knee in rabbits. *IRCS Med. Sci.* **5,** 62.

Videman, T., Eronen, I., Friman, C. & Langenskiöld, A. (1979) 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. *Acta orthop. scand.* **50,** 465–470.

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