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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

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In order to observe phenomena accompanying the development of osteoarthritis of the rabbit knee, consistently appearing within 4–6 weeks as a consequence of immobilization of this joint, studies on ^{35}S-sulphate ($Na_2\,^{35}SO_4$) uptake were carried out. The investigation suggests that immobilization of the knee leads within 2–4 weeks to increased sulphate uptake in bone, cartilage and ligaments in the knee region and in the hip (not immobilized) of the same leg. The first marked changes in ^{35}S-sulphate uptake were found in the ligaments and the articular cartilage as early as after 4 days of immobilization.

Key words: osteoarthritis; radioactive sulphate; immobilization; knee; hip; articular cartilage

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In order to find an experimental model of osteoarthritis on which studies concerning pathogenesis and therapy could be based, a series of experiments were carried out with 260 adult rabbits, using immobilization of the knee as the factor provoking degenerative changes in this joint (Langenskiöld et al. 1975).

It has been shown by other authors that long-term immobilization may cause degenerative changes in joints (Evans et al. 1960, Salter & Field 1960, Trias 1961, Hall 1963 a, b, Thaxter et al. 1965, Salter 1967, Thompson & Bassett 1970, Moskowitz 1972). In our own series of experiments it was demonstrated that immobilization of the knee consistently resulted in osteoarthritis of this joint when the immobilization period exceeded 3 weeks. The degenerative changes were demonstrated by radiography and histological examination (Figure 6 c and d). However, it was desirable to detect, by more sensitive methods, the reactions of the joint and the surrounding tissues to immobilization and subsequent remobilization. Determination of the ^{35}S-sulphate uptake of different tissues of the knee region in the immobilized limb and a comparison with the sulphate incorporation of the corresponding tissues in the contralateral leg was considered a means of demonstrating early reactions to immobilization. It could be expected that

changes in ^{35}S-sulphate uptake would appear before changes demonstrable in radiographs and histological sections.

METHODS

With the aid of ^{35}S-sulphate an idea can be obtained of the formation of connective tissue (Amprino 1955). In particular the glycosaminoglycan synthesis of chondrocytes and fibroblasts has been studied by means of ^{35}S-sulphate (Dziewiatkowski 1951, Belanger 1954, Mancini et al. 1961, Crane 1962, Maroudas 1975).

Within 1–2 hours of the administration of ^{35}S-sulphate to test animals the label can be demonstrated within the cell, as was shown by Picard et al. (1964). Furthermore, it has been shown that the maximum specific activity of labelled chondroitin sulphate is reached within 24 hours after isotope administration. One to two days after injection of ^{35}S-sulphate, radioactivity can be traced in the cells and the extracellular matrix. Six to seven days after the injection of the label the radioactivity is located almost exclusively in the organic matrix (Belanger 1954, Dziewiatkowski 1949).

In the series of experiments described in this article the knees of adult rabbits aged more than 9 months were immobilized in extension by means of a plastic splint or by bandaging the knee region with Tensoplast®. The hip remained free and movable. This method of immobilization has previously been described (Michelsson & Langenskiöld 1972 b, Michelsson & Langenskiöld 1974).

The ^{35}S-sulphate uptake in ligaments, cartilage and bone in the knee and hip regions was studied by two methods, by scintillation counting and by autoradiography.

In the series studied by scintillation counting every animal was given 1 mCi ^{35}S-sulphate (carrier free, The Radiochemical Centre, Amersham) intramuscularly in the scapular region 24 hours before being killed. Samples were taken from both hind limbs from the cartilage of the head of the femur, the joint capsule of the hip, the cortical and the spongy bone of the femoral diaphysis, the proximal articular cartilage of the tibia, the medial collateral ligament of the knee, the medial meniscus and the spongy bone of the proximal metaphysis of the tibia. The samples of soft tissues were taken with a knife and the bone samples with a revolving saw (Figure 1).

The samples were dried, weighed, wet-combusted and precipitated in the form of barium sulphate *ad modum* Moltke (1957). The precipitate was then mixed in scintillation gel and the radioactivity was determined with a liquid scintillation counter. The measured radioactivities (cpm/mg dry tissue) were tested using analysis of variance (two-factor design). In order to scale the raw data to a common arbitrary level the radioactivities measured for each tissue in the control limb not immobilized were given the value 100.

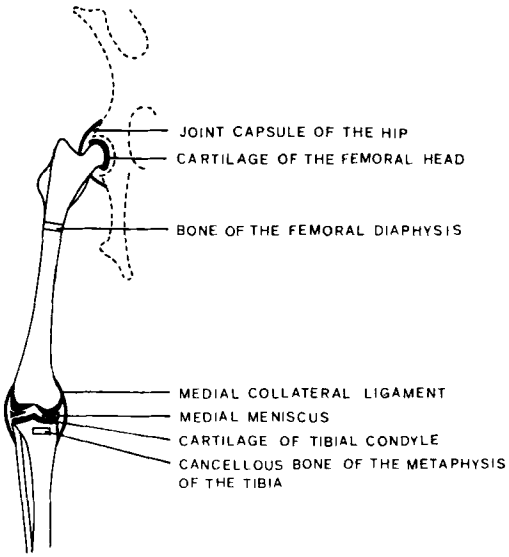

Figure 1. The origin of the samples for investigation.

In the series studied by autoradiography each rabbit was given 2.5 mCi ^{35}S-sulphate intra-abdominally, and one day after the injection the animals were killed. After fixation in formalin and decalcification, histological sections were made. The autoradiograms were made using dental X-ray film (the technique has been published earlier by Langenskiöld et al. 1967). The film was exposed for 6–8 weeks and developed in the usual way. The histological sections were dyed using Alcian blue-PAS.

MATERIAL

The material consisted of three groups of animals. In *the first group* consisting of 16 animals the knees were immobilized for periods varying from 1 to 130 days. Eight of the rabbits were killed 1 day after injection of sulphate and removal of the immobilizing bandage and eight were killed before the bandage was removed. Samples were taken for scintillation counting studies and prepared in the way described above.

The second group consisted of six rabbits whose right knee was immobilized in the way described for 40 days, after which the splint was removed and the limb remobilized. The animals

were given injections of sulphate 1 day before killing and killed 4 to 30 days after removal of the immobilizing bandage.

From the animals of the first and the second group a total of 337 tissue samples were studied for their ^{35}S-sulphate content.

The third group consisted of six rabbits; three of them had one knee immobilized for 2 weeks and three others for 4 to 6 weeks. From these rabbits histological sections and autoradiograms were obtained in the manner described.

RESULTS

The radioactivities in the various tissues of the immobilized and non-immobilized (control) limbs were compared, and the results are shown in Figures 2–7.

Medial collateral ligament of the knee (Figures 2 and 3). In the immobilized limb the ^{35}S-sulphate uptake of the ligament had increased twofold within 4 days of immobilization. This increase reached its peak value, more than four times the control value, after 1 month. Then the uptake began to decrease gradually until, after an immobilization of four and a half months, it was only slightly above the control value.

When the knee was remobilized after 40 days of immobilization the uptake was found to remain at the high level of more than three times the control value for at least 10 days.

The autoradiograms showed a very powerful radioactivity in the right knee compared with the control limb in all ligaments adherent to the joint capsule (Figure 6 a and b).

Medial meniscus of the knee. During immobilization and remobilization the ^{35}S-sulphate uptake of the meniscus of the immobilized knee increased in a similar way to that of the ligaments but to a lesser degree (Figures 1, 2 and 3).

The proximal articular cartilage of the tibia. The sulphate uptake of the articular cartilage of the tibia increased as early as 4 days after immobilization. After 4 weeks it was increased about 50 per cent and remained at this level when immobilization was prolonged (Figures 2 and 3).

After 2 weeks of immobilization the articular cartilage usually disappeared in the weightbearing areas. Therefore the results obtained after 60 and 135 days of immobilization are based on the cartilage which could be taken from the peripheral areas.

In the autoradiograms the radioactivity was low in the central areas of the condyles but high in the peripheral osteophytes and in the cartilaginous islets in the bone tissue (Figure 6 a and b).

Tibial bone. In the metaphyseal bone of the tibia of the immobilized limb the sulphate uptake increased but did so later and to a less marked degree than that of the ligaments. After 3 months of immobilization the sulphate level decreased to below control values but because local structural changes develop in the metaphysis due to a prolonged period of immobilization, no absolute conclusions should be drawn from these results (Figures 2 and 3).

Joint capsule of the hip. The sulphate uptake of the capsule of the hip joint had definitely increased in the immobilized limb after 4 days of immobilization. Two months after application of the immobilizing bandage it was more than four times as high as the control level. After four and a half months of immobilization the uptake was still twice as high as the control level. During remobilization, after 40 days of immobilization, the uptake was found to have increased to more than five times the control level. After this there was an extremely sharp decrease for some days until the uptake stabilized at about twice the control level (Figures 4 and 5).

The autoradiograms showed the capsule of the right hip to be definitely more radioactive and thicker than the capsule on the other side (Figure 7).

Cartilage of the femoral head. In the immobilized limb the sulphate uptake of

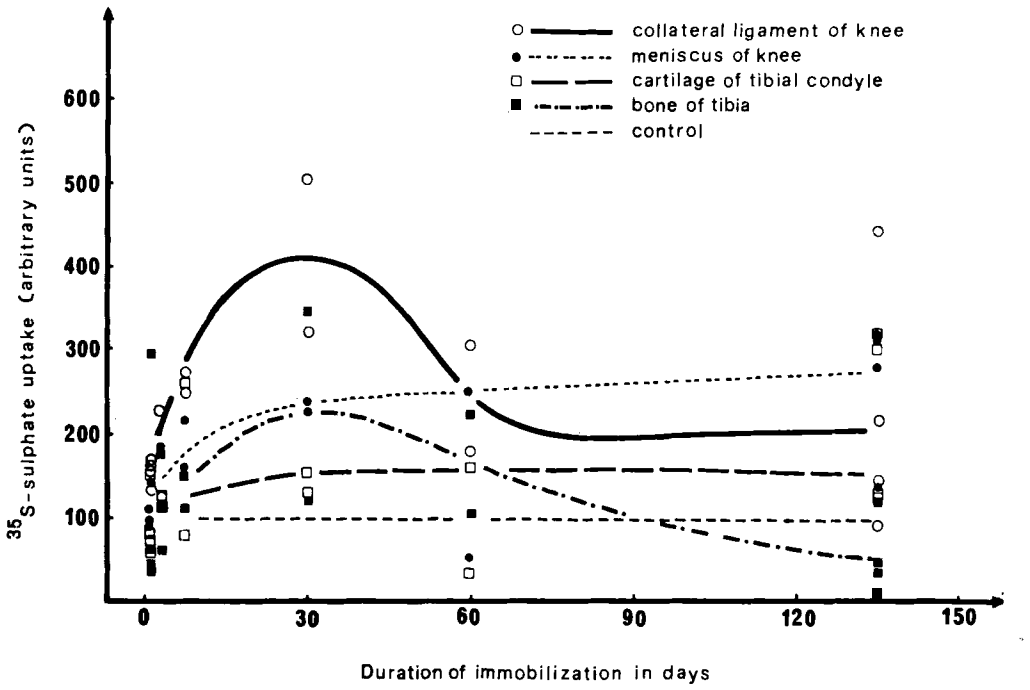

Figure 2. ^{35}S-sulphate uptake in different tissues of the knee after various periods of immobilization.

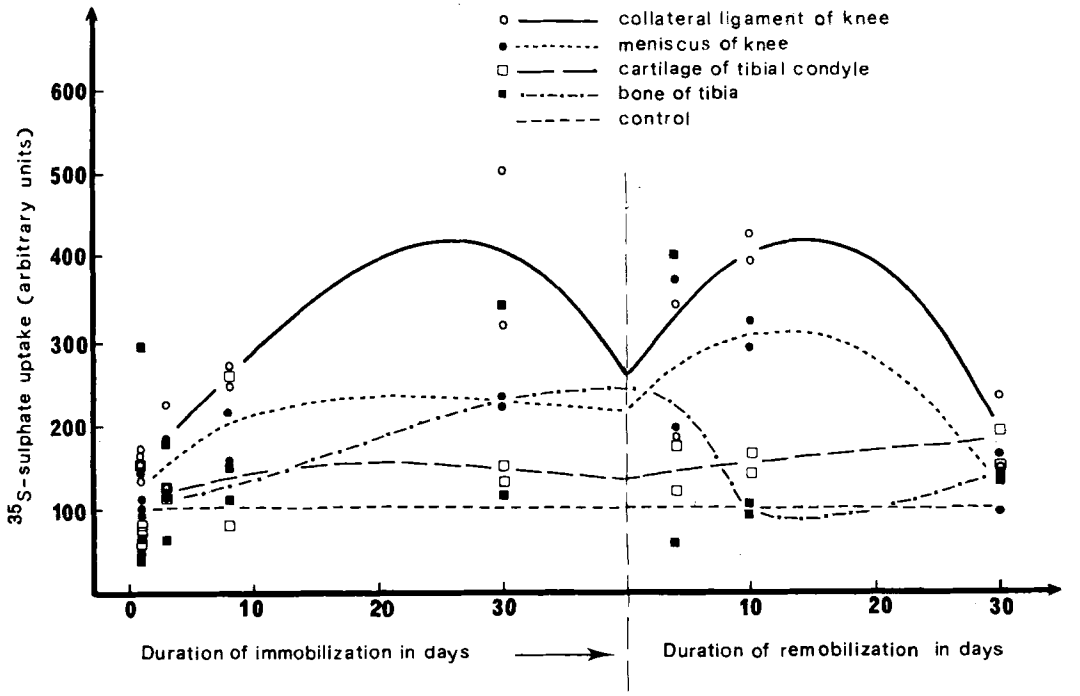

Figure 3. ^{35}S-sulphate uptake in different tissues of the knee after immobilization and subsequent remobilization.

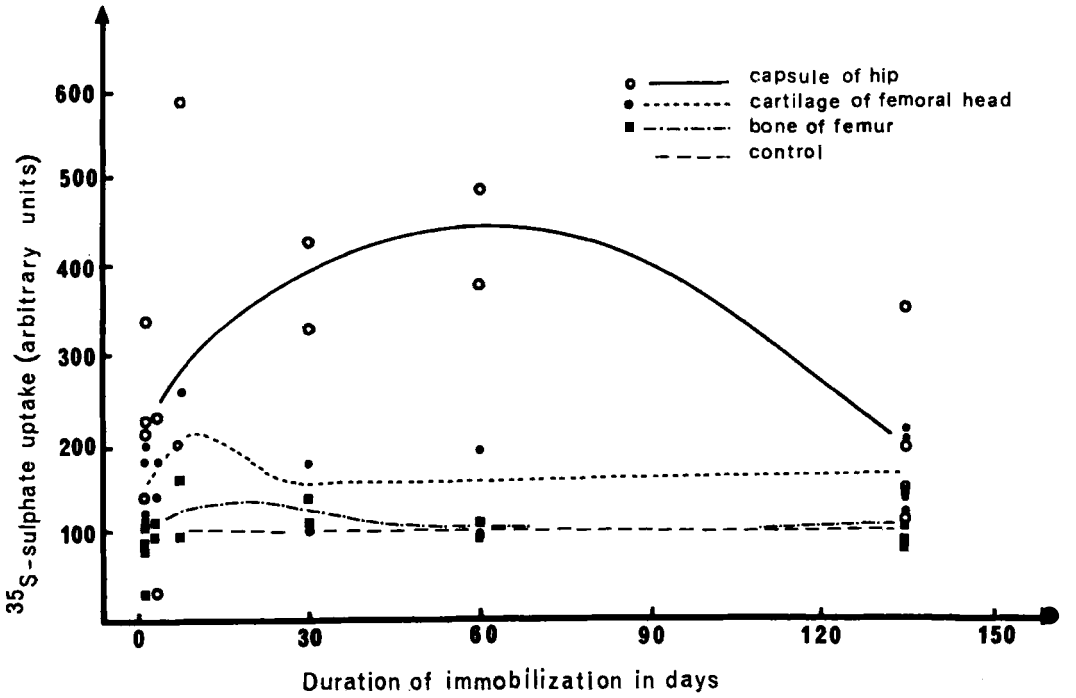

Figure 4. ^{35}S-sulphate uptake in different tissues of the hip and in the femoral bone after various periods of immobilization.

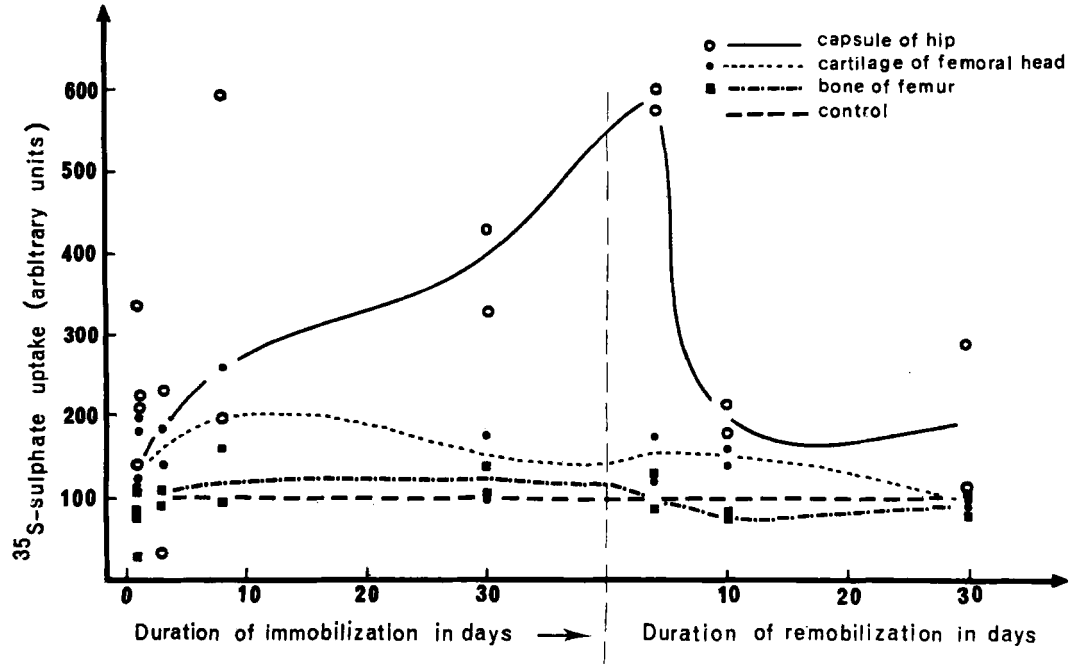

Figure 5. ^{35}S-sulphate uptake in different tissues of the hip and in the femoral bone after immobilization and subsequent remobilization.

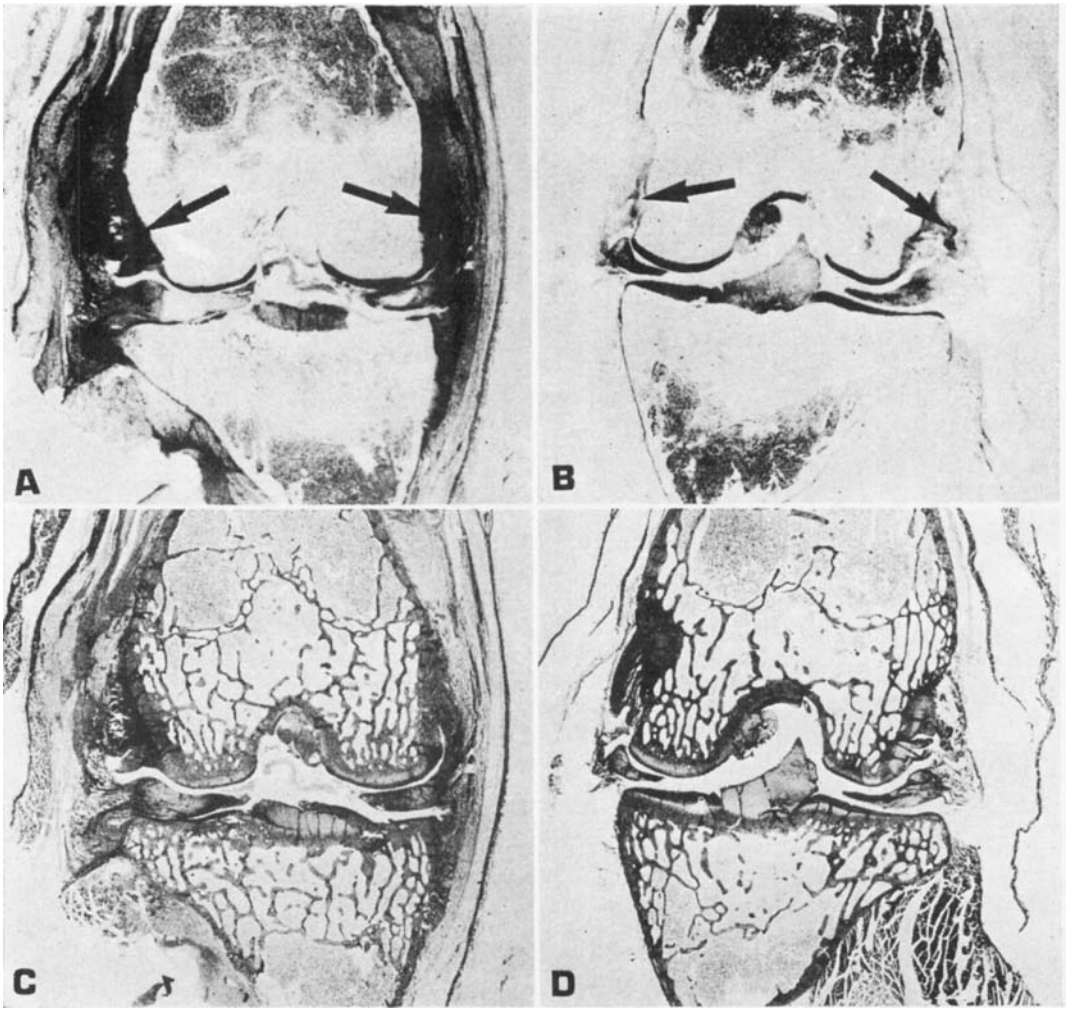

Figure 6. Autoradiograms of the right A) and the left B) knee of an adult rabbit, whose right knee was immobilized for 2 weeks in extension. Histological sections of the right C) and the left D) knee. Thickening and increased radioactivity of the capsule are visible in the right knee. In the articular cartilage of the right knee the radioactivity varies considerably in the different regions.

the cartilage had increased from the first day of immobilization and was twice the control level on the tenth day. The uptake remained relatively stable being about 50 per cent higher than the control level throughout the longer periods of immobilization (Figures 4 and 5).

From the autoradiograms the difference could not be seen equally clearly (Figure 7).

Femoral bone. The changes were very slight, similar to those in the tibial bone (Figures 4 and 5). These samples contained periosteum, cortical and spongy bone and bone marrow.

Summarizing, it can be said that in the tissues of the immobilized limb, with the exception of the bone, the uptake of ^{35}S-sulphate throughout the 130-day period of immobilization was higher than the control level. In principle, the same phenomena were visible in the autoradio-

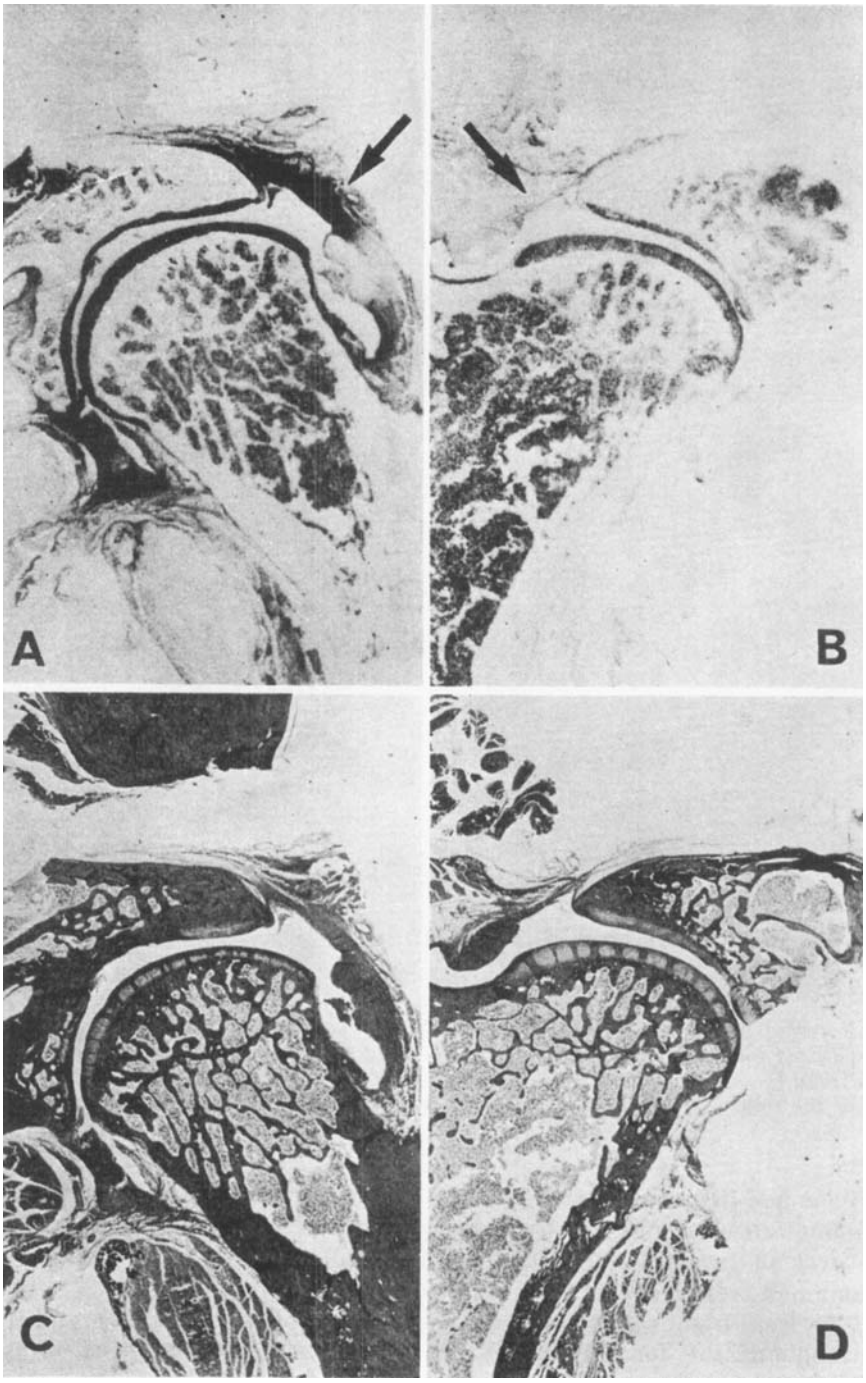

Figure 7. The right knee of an adult rabbit was immobilized in extension for 4 weeks. Autoradiogram of the region of the right A) and the left B) hip and corresponding histological sections of the hip region C) and D). In the articular cartilage and the capsule the radioactivity is much greater in the region of the right hip than in the left. The radioactivity is the same in the bone marrow of both legs.

grams, but especially in the articular cartilage and meniscus the radioactivity varied extensively in the different regions. During the remobilization phase the uptake in all tissues studied was higher than in the control tissues during the first 10 days after removal of the immobilizing bandage and remained that way even after some time, except in the bone and the articular cartilage of the femoral head. The greatest differences in the ^{35}S-sulphate uptake of the various tissues of the immobilized and non-immobilized limb were observed in the joint capsule of the hip, the medial collateral ligament of the knee and the medial meniscus.

According to Wilcoxon's test, removal of the splint or bandage at the time of the sulphate injection 24 hours before killing, or at the time of the killing itself, significantly affected the uptake of ^{35}S-sulphate only in the tibial bone and the articular cartilage of the femoral head.

The effects of both main variables, time of immobilization and type of tissue studied, were statistically significant at a level of $P < 0.01$.

DISCUSSION

On the basis of radiographs and histological and macroscopic studies, degenerative changes were observed in the knee and hip of the immobilized limbs even after only 10 days of immobilization (Langenskiöld et al. 1975). The capsule of both the knee and the hip joint was consistently found to be thickened. A similar thickening of the joint capsule is also a regular feature of human osteoarthritis (Lloyd-Roberts 1953). The severity of the degenerative changes in the joints of the animals was directly correlated with the length of the immobilization period.

The experiments with ^{35}S-sulphate showed, as was expected, that the sulphate uptake in the joint regions of an immobilized limb is modified at a stage when no histological or radiological changes can yet be demonstrated. Immobilization and remobilization both distinctly increased the ^{35}S-sulphate uptake in the tissues of the immobilized limb. The first marked changes in sulphate uptake were found, in the ligaments and the articular cartilage, as early as after 4 days of immobilization. According to the autoradiograms and the histological examination thickening was induced in ligaments, joint capsules and menisci, from which it can be concluded that an increased ^{35}S-sulphate uptake cannot result from, e.g., decreased amount of protein or collagen.

The findings speak in favour of the idea that changes in the soft tissues around osteoarthritic joints play a role in the pathogenesis of osteoarthritis.

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