

CHANGES IN BONE FORMATION DURING IMMOBILIZATION AND DEVELOPMENT OF EXPERIMENTAL OSTEOARTHRITIS

A Study using Oxytetracycline in Rabbits

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The effect of immobilization on bone formation in the knee and hip was studied by means of oxytetracycline. One leg of 11 growing and 13 adult rabbits was immobilized so that the knee was held in extension but the hip was free. In the knee, degenerative changes developed consistently with a severity proportional to the length of the immobilization time. In most of the rabbits given oxytetracycline 1-13 days before they were killed considerably more oxytetracycline was incorporated in the bone in the immobilized knee region and slightly more in the hip region of the same leg than in the corresponding areas of the other leg. This indicates an increased turnover of bone tissue in the immobilized leg compared with the non-immobilized leg.

Key words: bone formation; experimental; immobilization; osteoarthritis; tetracycline

Accepted 9.ii.76

Degenerative joint changes have been produced experimentally in animals by various methods, for instance, prolonged compression (Salter & Field 1960, Trias 1961), induced instability (Telhag & Lindberg 1972), or immobilization of the joint in flexion or extension (Evans et al. 1960, Hall 1963 a, b). Immobilization of the knee in extension in young rabbits consistently causes deformities of the hip (Michelsson & Langenskiöld 1972 a, b, Michelsson & Langenskiöld 1974). In adult rabbits immobilization of the knee in extension for 6 weeks or more results in every case in degenerative changes in the knee and in some cases also in the hip (Langenskiöld et al. 1975, Videman

et al. 1975, Michelsson et al. 1977, Videman et al. 1977 a, b).

When oxytetracycline is administered to a rabbit, a part of the antibiotic becomes bound to the bone tissue. Deposition of oxytetracycline in bone is associated with the ossification process (Milch et al. 1957). "Adventitious" tetracycline disappears from the bone 2 days after the administration of the antibiotic and the residual antibiotic in newly-formed bone disappears only through resorption of bone (Frost et al. 1960). As tetracyclines fluoresce in ultraviolet light (Saltzman 1950), they can be used as bone labels. The tetracycline technique is a valuable supplementary aid in the

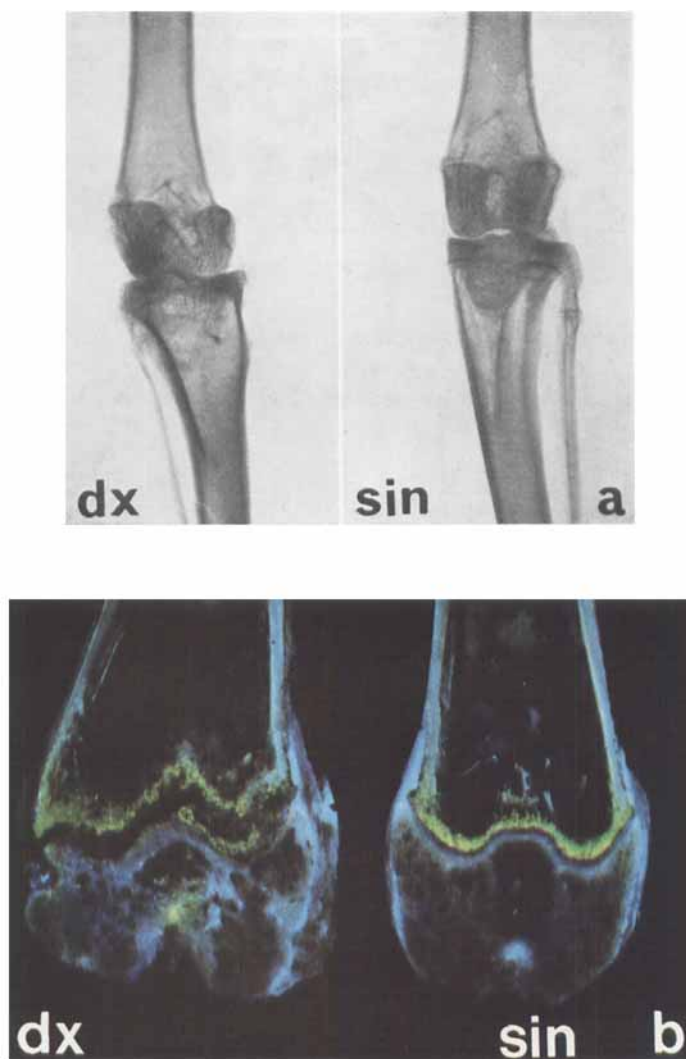


Figure 1 a, b. A 3-month-old rabbit (295) was immobilized with the right knee in extension for 5 weeks. Oxytetracycline was given before it was killed at the age of 6 months. Deformation of the right knee had developed. a) Radiograph taken of specimens. b) In the specimen of the distal end of the right femur a deformed, thickened epiphyseal plate with oxytetracycline labelled bone on both the metaphyseal and epiphyseal sides is visible.

study of the bone formation occurring during immobilization.

In this study oxytetracycline was used to investigate new-bone formation in the knee and hip regions in rabbits in connection with immobilization of the knee and the development of osteoarthritis.

MATERIAL AND METHODS

Eleven growing rabbits 2–5 months old, and 13 adult rabbits aged 9–33 months, made up the experimental series. The right hind limb of the rabbits was immobilized with a plastic splint and Tensoplast® so that the knee remained in extension but the hip was free and movable. A corresponding technique for immobilization has previously been described in detail (Michelsson

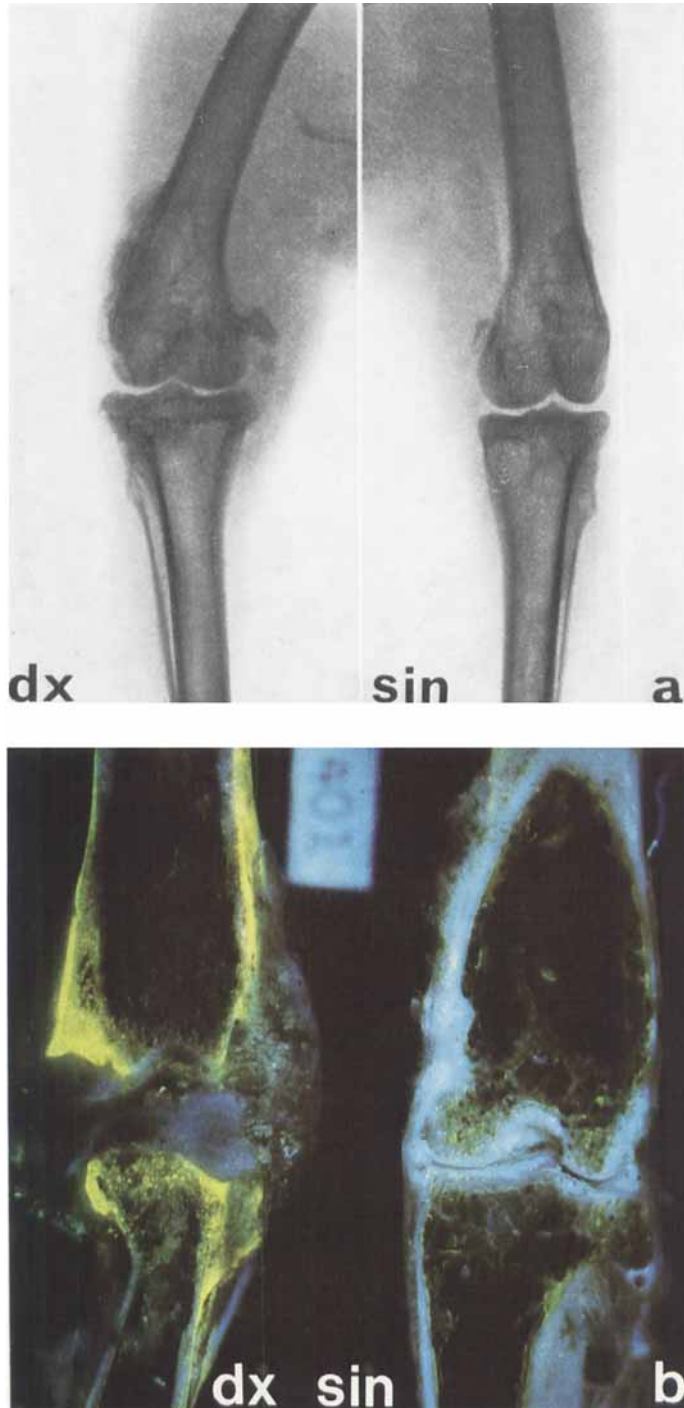


Figure 2 a, b. An adult rabbit (401) was immobilized with the right knee in extension for 7 weeks before it was given oxytetracycline and killed. a) Marked degenerative changes of the right knee had developed and are visible on the radiograph. b) In the specimen from the right knee the amount of oxytetracycline labelled bone was considerably greater than in the left knee (b).

Table 1. Content of oxytetracycline in knee and hip of the immobilized (right) leg compared with the left non-immobilized knee and hip in 20 rabbits. The oxytetracycline was given some days before the animals were killed.

Rabbit no.	Age at immobilization in months	Duration of immobilization in weeks	Interval between immobilization and sacrifice in weeks	Oxytetracycline content	
				in right knee	in right hip
Growing rabbits					
295	3	5	8	++	+
411	3	6	1	++	+
412	3	6	1	+	++
220	4	6	28	+	+
223	4	6	30	++	+++
398	5	6½	0	+	+
387	5	7	0	+++	+
389	5	7	2	++	+
388	5	8½	½	++	+
392	5	8½	2	++	++
225	2	13	1	++	+
Adult rabbits					
395	> 12	2	1	+	+
66	12	4	52	+	+
80	9	5	78	—	—
401	> 12	7	0	+++	+
10	> 12	8½	52	+	—
375	13	13	34½	+	—
230	33	13	26	++	—
89	11	17½	1	—	—
303	16	17½	2	++	+

— not more oxytetracycline in the right (immobilized) joint than in the left.
 + slightly more oxytetracycline in the right (immobilized) joint than in the left.
 ++ moderately more oxytetracycline in the right (immobilized) joint than in the left.
 +++ markedly more oxytetracycline in the right (immobilized) joint than in the left.

& Langenskiöld 1972 b, Michelsson & Langenskiöld 1974).

The splint was removed after 2–17½ weeks and the animals were allowed to move freely.

The animals were killed 0–78 weeks after the immobilization period having been given 50 mg oxytetracycline per kg of body weight intramuscularly on three consecutive days. Nine adult and all the 11 growing rabbits were given the oxytetracycline injections 1–13 days before they were killed (Table 1). Four other adult rabbits were given oxytetracycline at the beginning of immobilization and were killed 49–90 days thereafter.

The hips and knees of the animals were dissected, split in the frontal plane and examined in UV-light. The knees and hips of the immobilized and non-immobilized legs were com-

pared principally with regard to their oxytetracycline content.

RESULTS

In 18 of the 20 rabbits that received the oxytetracycline just before they were killed, the oxytetracycline content was higher in the bone tissue of the knee, and in 15 of them also in the hip, of the immobilized (right) leg than in the corresponding joints of the other leg (Table 1).

In the growing rabbits the condyles of

the immobilized *knee* were wider than normal; their structure was more heterogeneous and they were deformed. Higher concentrations of oxytetracycline were found on the immobilized side compared with the normal knee and it was always found to be spread over larger areas. In the distal end of the femur of growing rabbits there is normally a tetracycline marked zone only on the metaphyseal side of the epiphyseal plate, but in the immobilized leg in three rabbits there was bone marked by tetracycline on the epiphyseal side as well (Figure 1 a-b).

In the *adult rabbits* marked degenerative changes were seen in the immobilized *knees*. The condyles were thicker than normal, slightly deformed and their structure was more heterogeneous than normal. Oxytetracycline was found over larger areas, especially at the periphery, and in general in higher concentrations than in the left knee (Figure 2 a, b). These changes occurred in seven of the nine rabbits given oxytetracycline just before they were killed. In two of the four adult rabbits given oxytetracycline at the beginning of the immobilization period only, the oxytetracycline content was higher in the left knee than in the immobilized right knee.

Marked changes were also seen in the *hip joints* of the immobilized hind legs of the rabbits; the capsule was considerably thickened and the cartilage had degenerated. The cartilage and bone alterations were generally less severe than in the knee joints. In some hips, however, noticeable alterations were seen in the head and neck of the femur, where the oxytetracycline content was also higher and covered larger areas than in the hip joint of the non-immobilized leg (Figure 3 a, b). These changes occurred in 15 of the 20 rabbits given oxytetracycline 1-13 days before they were killed. In two of the three rabbits examined and given oxytetracycline at the beginning of the immobilization period, the oxytetracy-

cline content was higher in the left hip than in the right.

DISCUSSION

In which tissues and at what time the various alterations take place when osteoarthritis develops has not been satisfactorily explained. Since it is difficult to study the initial stages of osteoarthritis in humans, studies of experimentally induced arthritis in animals have been commenced. In the studies of other investigators, experimental osteoarthritis has mostly been produced by trauma and has therefore been accompanied by tissue damage. In this series immobilization was used in order to produce osteoarthritis without immediate tissue damage. In our experiments the knee only was immobilized in extension while the hip was left free. Degenerative alterations were consistently produced in the knee as a direct consequence of immobilization, but also in the hip of the same leg marked degenerative alterations were sometimes seen even though it was not immobilized.

We have previously reported that in adult rabbits knee immobilization simultaneously increases the uptake of ^{35}S -sulphate in the joint capsules of the knee and hip, and in the cartilage and bone tissue (Videman et al. 1975, 1976). The oxytetracycline content was usually higher in the right immobilized knee and hip in the rabbits given oxytetracycline just before they were killed, but in the non-immobilized knee and hip of the rabbits given oxytetracycline at the beginning of immobilization. This indicates an increased turnover of the bone tissue in the immobilized leg compared with the other leg.

This study increases our understanding of how bone tissue reacts during immobilization and the development of osteoarthritis.

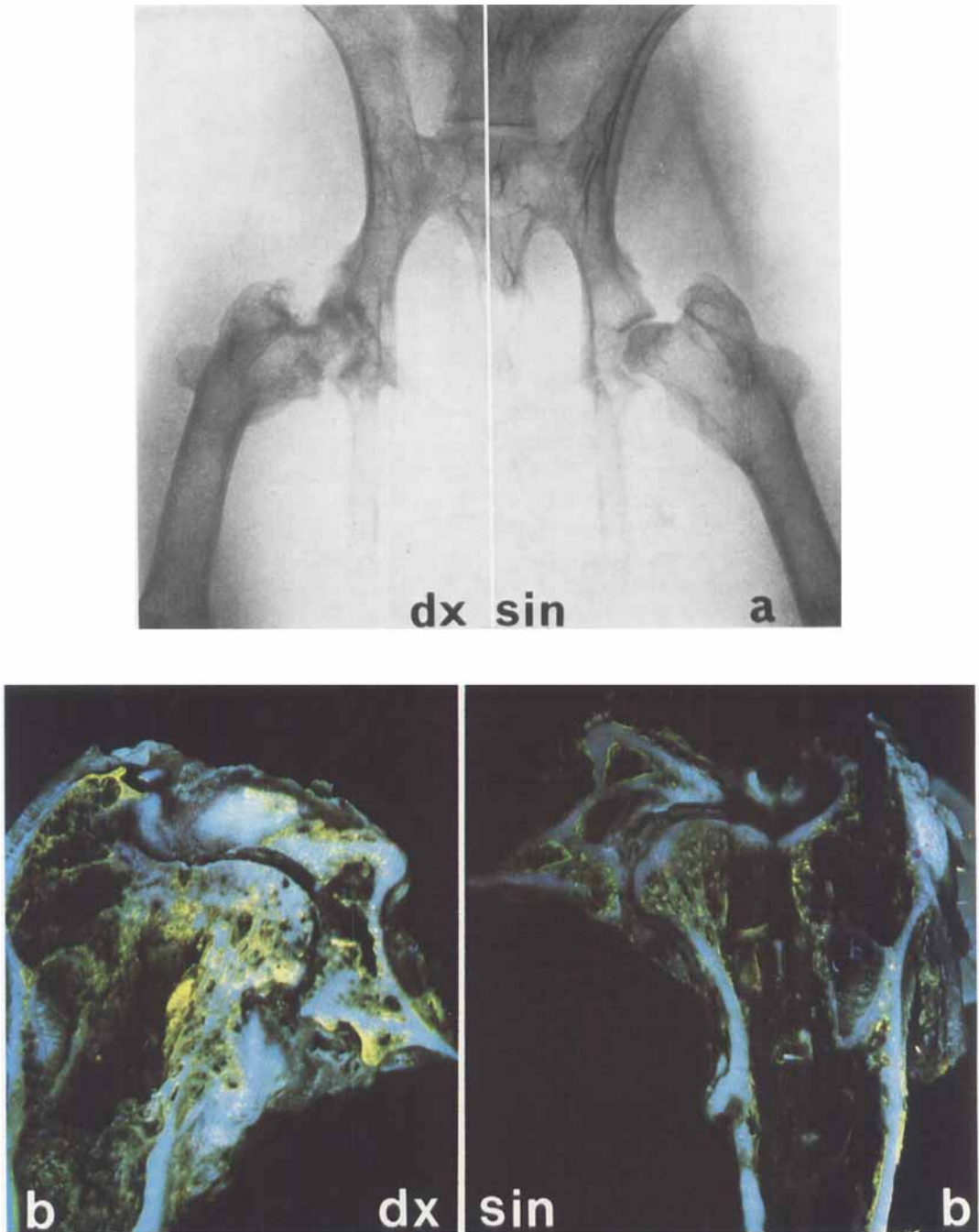


Figure 3 a, b. A 4-month-old rabbit (223) was immobilized with the right knee in extension for 6 weeks. Seven months later the animal was given oxytetracycline and killed, a) Degenerative changes are clearly visible on the radiograph of the right hip. b) Increased content of oxytetracycline as a sign of increased bone formation in the specimen of the right hip.

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