

OSTEOARTHRITIS OF THE KNEE IN THE RABBIT PRODUCED BY IMMOBILIZATION

Attempts to Achieve a Reproducible Model for Studies on Pathogenesis and Therapy

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In order to obtain a reproducible experimental model of osteoarthritis a method of immobilizing the rabbit's knee in extension by means of a plastic splint was developed. The right knees of the rabbits were immobilized for periods varying from 4 days to 24 weeks. With the left knees as controls the knees were studied in a variety of ways among these being radiography (126 rabbits), histological sections stained with Alcian Blue (88 rabbits), analysis of ³⁵S-sulphate uptake (22 rabbits) and ³⁵S-autoradiography (6 rabbits). In 27 rabbits the regaining of mobility after immobilization was studied. After 5-6 weeks of immobilization most of the knees showed moderate or severe changes including loss of articular cartilage and osteophyte formation. Immobilization of the rabbit's knee by this method provokes a fairly easily reproducible type of degenerative joint disease showing similarities to advanced osteoarthritis as seen in humans.

Key words: animal experiments; immobilization; osteoarthritis; knee

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Studies in man have not been able to clarify the pathogenetic chain of factors giving rise to progressive osteoarthritis. Bentley wrote in 1974: "... a model arthrosis which is consistently reproducible in animals, capable of recognition radiographically without sacrifice of the animal and irreversible, would be valuable in facilitating further study of the pathogenesis of the disease and the effect of different methods of treatment on it".

Reviews of the methods for the production of experimental models of osteoarthritis have been given by several authors (Moskowitz 1972, Bentley 1974). That degenerative changes are caused by long-term immobilization of joints with or without compression of the articular cartilage has been demonstrated

in many studies (Ely & Mensor 1933, Evans et al. 1960, Salter & Field 1960, Trias 1961, Hall 1963, 1964, Thaxter et al. 1965, Field & Hueston 1970, Roy 1970, Thompson & Basset 1970, Sood 1971, Finsterbush & Friedman 1973, 1975, Videman et al. 1976). However, an attempt at a systematic mapping of the changes appearing after different periods of immobilization of joints in experimental animals has not been made. The purpose of this investigation has been to develop a method of producing a "standard osteoarthritis" of the knee of the rabbit without operative trauma, to define the immobilization time producing the most suitable model and to map the changes appearing in different parts of the joint.

MATERIALS AND METHODS

In pilot studies it was soon found that immobilization of the rabbit's knee in extension for 6 weeks or more gave rise to degenerative joint disease. However, the standardization and reproducibility of the condition necessarily required standardization of the method of immobilization. The animal should be able to keep the immobilizing device on the limb for months without direct damage to skin and muscles. Immobilization by plaster of Paris or by plastic tubes was tried in various ways but these methods did not fulfil the requirements. For the development of a standard method of immobilization, 152 rabbits were used. These experiments were considered preliminary and although most of the animals developed osteoarthritic changes they are not included in the series reported in this article. The rabbits were considered adult when older than 9 months (Heikel 1959). In addition to the reported series of adult rabbits, the right knees of 65 immature rabbits were immobilized.

The methods used for studying the changes in the knee joints following immobilization are shown in Table 1. In the same table the number of rabbits studied after different periods of immobilization is seen.

Method of immobilization. The method of immobilization finally adopted and used in the reported series of 154 adult and 65 growing rabbits is shown in Figure 1. A straight and somewhat elastic splint of PVC-plastic measuring about $300 \times 30 \times 1$ mm was applied on the dorsal aspect of the right leg from the proximal end of the thigh to the distal end of the limb. The splint was tied to the limb by 3–6 turns of a

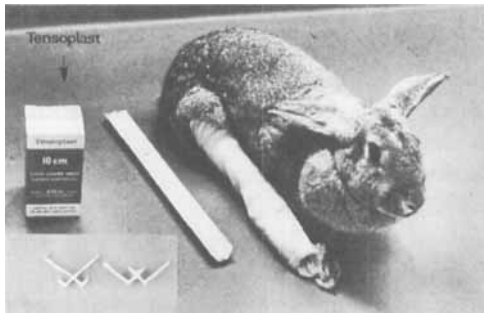


Figure 1. The immobilization method: A rabbit with right hind limb immobilized with the knee in extension showing the splint and bandage used. In the left lower corner of the picture the cross sections of two different splints used (PVC—plastic, $300 \times 30 \times 1$ mm).

Tensoplast® bandage. The splint and the bandage kept the knee straight or somewhat flexed. Flexion up to 20 degrees was possible with the splint applied. A too-tight bandage causing hyperextension and rapid deterioration of the joint was avoided.

When the splint was applied, the hamstring muscles and the muscles of the calf were tight, the flexion of the hip joint was restricted and the foot was drawn into an equinus position.

The non-immobilized left knees were used as controls. These were compared with 15 knees of non-immobilized rabbits.

Radiography. In 126 rabbits both knees were radiographed in the antero-posterior projection at intervals varying from 1 week, as in most experiments, to 4 months when the follow-up period was over a year. These radiographs were taken with the animal lying prone with the hips and knees extended. Side views were taken of both knees at least at the beginning and at the end of the immobilization period. The film used was Kodak® PE 4006 and the focus distance was 100 cm. The results were analyzed statistically using the *t*-test.

Histological methods. Histological sections were made of both knees of 88 animals (61 adult). The whole limbs with the skin stripped off or the separated ends of the femur and the tibia were fixed in 10 per cent neutral formalin and were decalcified in New Decalc® solution (Hist-Lab, Betlehem Trading Ltd, Göteborg, Sweden) on an average for 1 week at room temperature. Then the knees, mostly including periarticular soft tissues, were embedded in hard paraffin in the usual way. Sections were made in different planes and most of them were stained with the Alcian Blue—PAS method.

With Alcian Blue—PAS staining the acidic glycosaminoglycans of connective tissue and sialomucins give a blue colour while neutral mucosubstances, all polysaccharides and mucosubstances containing hexoses or desoxyhexoses with vicinal glycol groups, stain red (McManus & Mowry 1964, Luna 1968). The appearance of the blue colour in the areas of cartilage with pathological location in connection with immobilization chemically indicates enrichment of acidic glycosaminoglycans. Histologically it indicates newly formed cartilaginous tissue (Eronen et al. 1978).

Changes in mobility of the knee joint. In 27 rabbits the mobility of the knee was estimated after periods of immobilization varying between 2 and 14 weeks (regaining of mobility =

Table 1. The number (n) of rabbits (the right knee immobilized in extension) used for the different methods of examination. In parentheses the number of growing rabbits

Examination method	Immobilization time in days					Total
	4-14 n	15-28 n	29-42 n	43-80 n	> 80 n	
Radiographic follow-up	6 (2)	12 (2)	39 (23)	15 (6)	18 (3)	90 (36)
Histological examination	15 (2)	13 (2)	10 (15)	5 (6)	18 (2)	61 (27)
Regaining of mobility study	— (3)	3 (2)	1 (7)	2 (3)	6 (—)	12 (15)
³⁵ S-sulphate uptake analysis	9	—	6 (2)	3	2	20 (2)
Autoradiography with ³⁵ S-sulphate	2	3	1	—	—	6 (—)
Glycosaminoglycan analysis	10	4	2	—	—	16 (—)
Photography	1	3	3	— (1)	1	8 (1)
Scanning electron microscopy	— (1)	3	3 (1)	2	—	8 (2)
Oxytetracycline fluorescence	1	—	1 (6)	3 (4)	5 (1)	10 (11)
Total	44 (8)	41 (6)	66 (54)	30 (20)	50 (6)	231 (94)

remobilization). The measurements were made with a goniometer regularly once a week for at least 10 weeks after removal of the splint. All measurements were made by only two persons in order to minimize variations in technique. The measurements were made without anaesthesia and the knees were extended and flexed with caution until abnormal resistance was felt. The generally adopted expression of mobility of the knee was used, the fully extended position expressed as 0° and full flexion as 180°. Hyperextension was expressed with negative numbers. The range of movement from maximal extension was expressed in degrees.

Gross appearance. In specimens where the distal end of the femur and the proximal end of the tibia were separated, the gross appearance of the joint surfaces and the periarticular soft tissues was noted. In nine rabbits the soft tissues from the distal ends of the femora and the proximal ends of the tibiae were carefully removed and the gross appearance of the joint surfaces was recorded by photography.

Methods and results described earlier: ³⁵S-sulphate uptake (Videman et al. 1976), ³⁵S-autoradiography (Videman et al. 1976), Labelling with oxytetracycline (Michelsson et al. 1977a), Scanning electron microscopy (Videman et al. 1977), Glycosaminoglycan analysis (Eronen et al. 1978).

RESULTS

Table 1 shows the number of immobilized knees studied by the various methods after different periods of immobilization. The methods used disclosed the changes in the joint from different aspects.

Progression of changes caused by immobilization

Changes appearing after 4-14 days of immobilization. On the fourth day of immobilization a marked increase of uptake of ³⁵S-sulphate in the ligaments of the knee and in the joint capsule could already be demonstrated (Videman et al. 1976). After 6 days thickening of the joint capsule, the collateral ligaments and the patellar tendon could be seen histologically (Figures 2A and 2B). This thickening of the periarticular soft tissue was regularly seen at all stages of immobilization (Figures 2B-D). After 10 days, abnormally proliferating cartilage could be seen peripheral to the edges of the joint cartilage both on the femur and the tibia

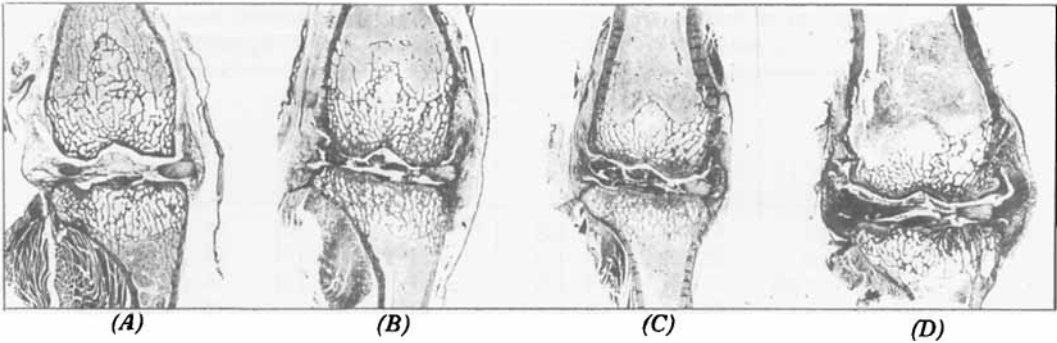


Figure 2A-D. Histological sections of adult rabbit knees in the frontal plane. A. Normal rabbit knee (non-immobilized animal). B. Section of rabbit knee immobilized for 2 weeks. The most marked difference compared with normal is the thickening of the capsule. C. Section of rabbit knee immobilized for 3 weeks. D. Section of rabbit knee immobilized for 10 weeks. Marked thickening and increased density of capsular tissue. Note diminished amount of cancellous bone in the distal end of the femur with increasing duration of immobilization. (Stained with Alcian Blue—PAS and $2.5\times$ magnification).

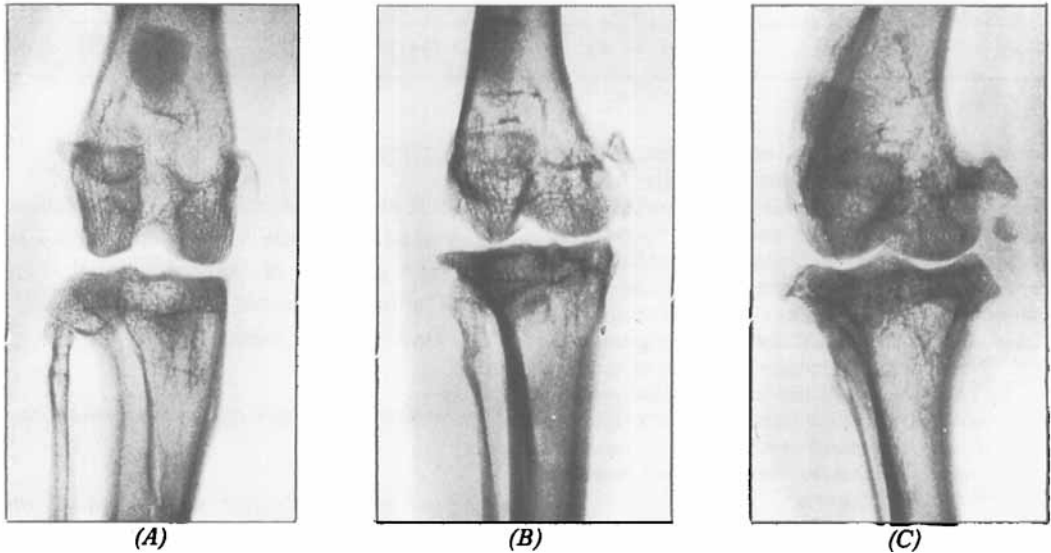


Figure 3A-C. Antero-posterior radiographs of rabbit knees. A. Normal knee. B. Knee immobilized for 7 weeks showing moderate osteoarthritic changes (followed for 6 weeks after immobilization). C. Knee immobilized for 12 weeks, showing severe osteoarthritic changes (followed for 2 years and 2 months after immobilization).

(Figure 5A and B). After 14 days, the definite beginnings of osteophyte formation on the medial side of the tibia could be seen in histological sections. Within 14 days of immobilization only slight narrowing of the joint space was noted as the single sign of joint degeneration in the radiographs.

Changes appearing after 15–28 days of immobilization. After 21 days, fibrillation and loss of cartilage were repeatedly seen at the joint surfaces both on the femoral and the tibial condyles and on the facies patellaris of the femur (Figure 6). All signs of erosion and necrosis of articular cartilage seen by other

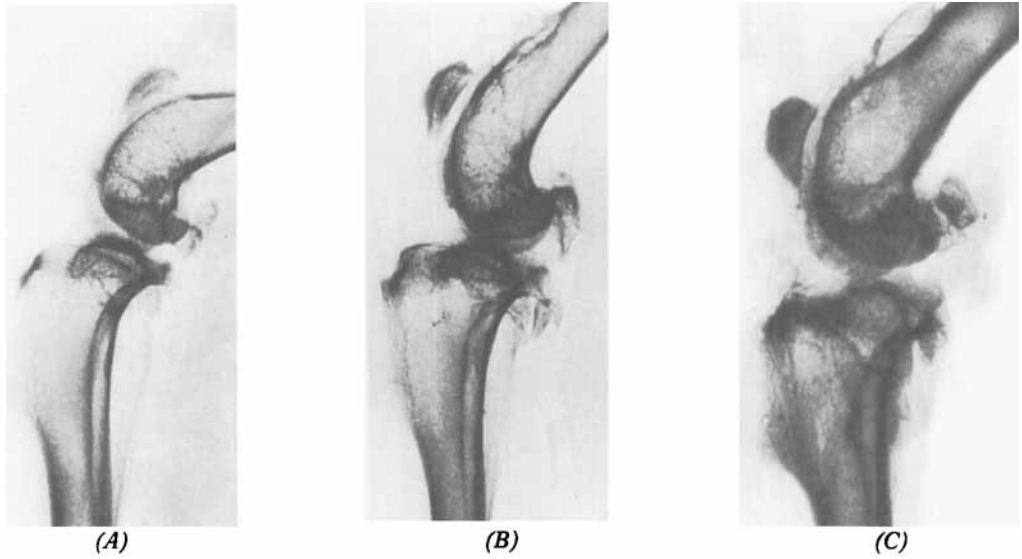


Figure 4A-C. Side-view radiographs of rabbit knees. A. Normal knee. B. Knee immobilized for 8 weeks showing moderate osteoarthritic changes (followed for 2 months after immobilization). C. Knee showing severe osteoarthritic changes after 6 weeks of immobilization (followed for 10 months after immobilization).

authors (Hall 1963, Finsterbush & Friedman 1973, 1975), including fragmentation and formation of fissures, were observed. As histologic artefacts make interpretation of these regressive changes uncertain, less

emphasis is here put on the changes significant for erosion of cartilage and preceding the stage at which subchondral bone is denuded. Abundant abnormal cartilage proliferation at the edges of the joint surfaces was common (Figure 7A and B). Osteophyte formation caused thickening of the distal end of the femur. On the medial aspect of the tibia osteophyte formation was a constant finding (Figures 7A and B, 8A and B). Radiographs often showed osteophytes both on the femur and the tibia.

Changes seen after 29–80 days of immobilization. Loss of articular cartilage at the joint surfaces was more marked than after a shorter period, especially at the contact

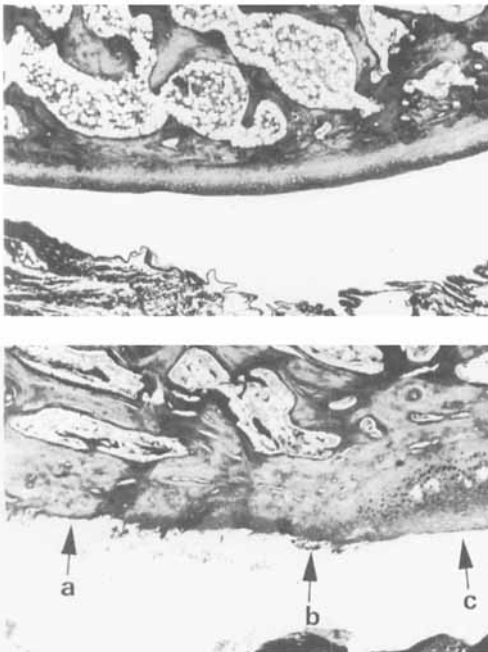


Figure 6. Histological sections of weight-bearing areas of femoral condyles. The upper picture shows a normal articular cartilage. The lower picture shows the corresponding area of a knee after immobilization for 15 days. In one region the articular cartilage has disappeared (arrow a). Other parts show fibrillation (arrow b) or hypertrophy of articular cartilage (arrow c). (Stained with Alcian Blue—PAS and 30× magnification).

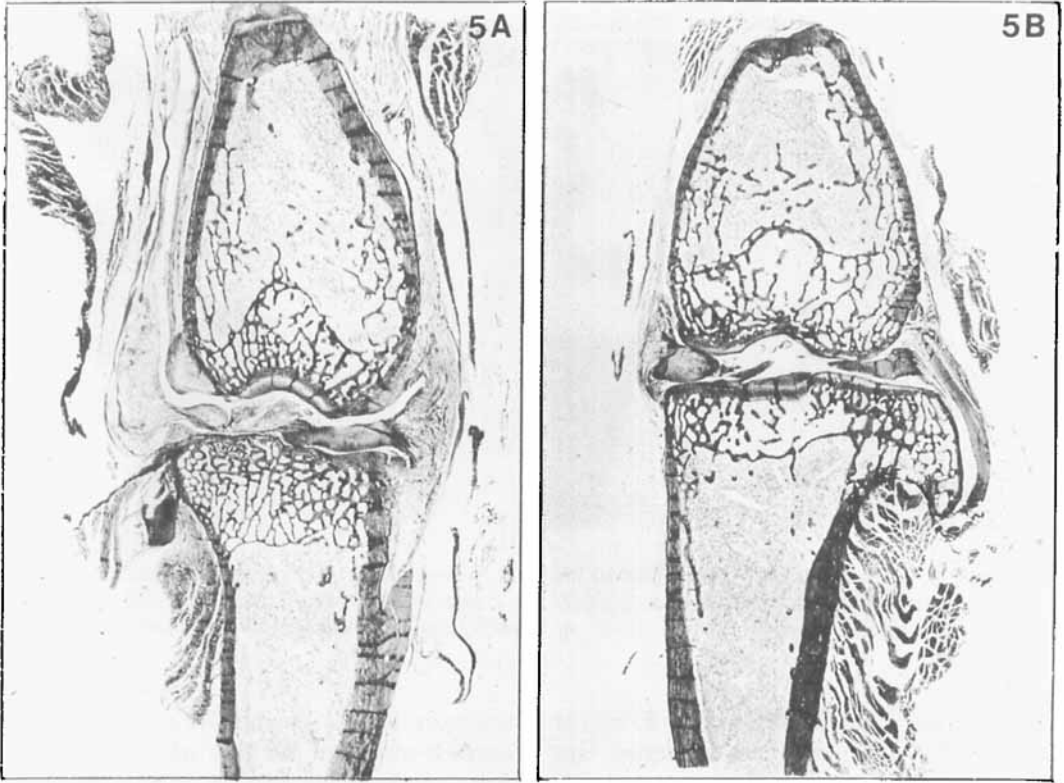


Figure 5A and B. Histological sections of rabbit knees, in the frontal plane, stained by the Alcian Blue—PAS method. B. Non-immobilized knee. A. The other knee of the same animal after immobilization for 2 weeks. Note the thickened capsule and the pathologically situated blue stained portions of cartilage in the immobilized knee. (Stained with Alcian Blue—PAS and 1.5 × magnification).

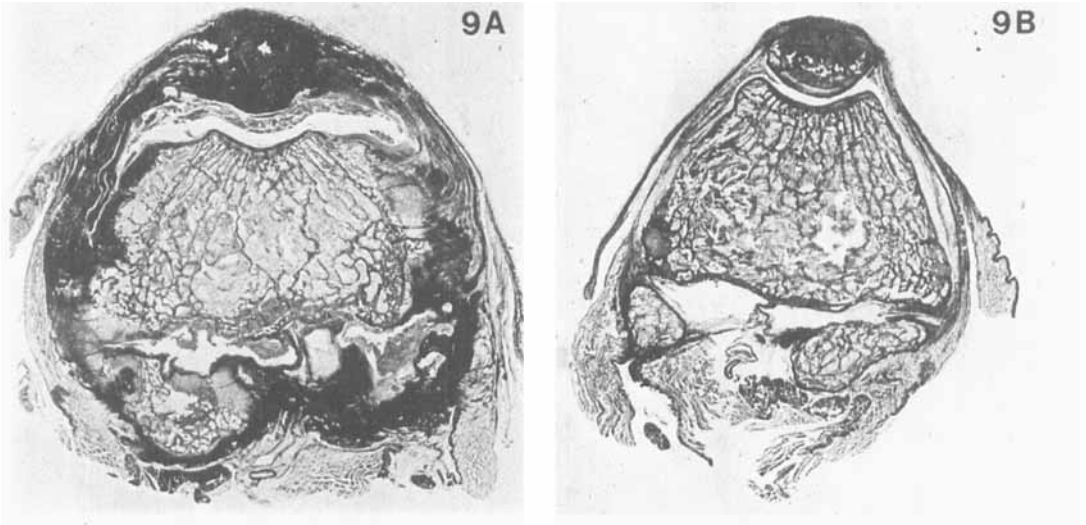


Figure 9A and B. Histological cross sections of the distal parts of the femora of the same rabbit. B. A section of the non-immobilized knee. A. A section of the contralateral knee which was immobilized for 6 weeks (followed for 3½ months after immobilization). Pathological proliferation of cartilage (blue) and enlargement of the condyles and the sesamoid bones are evident. (Alcian Blue—PAS staining and 2× magnification).

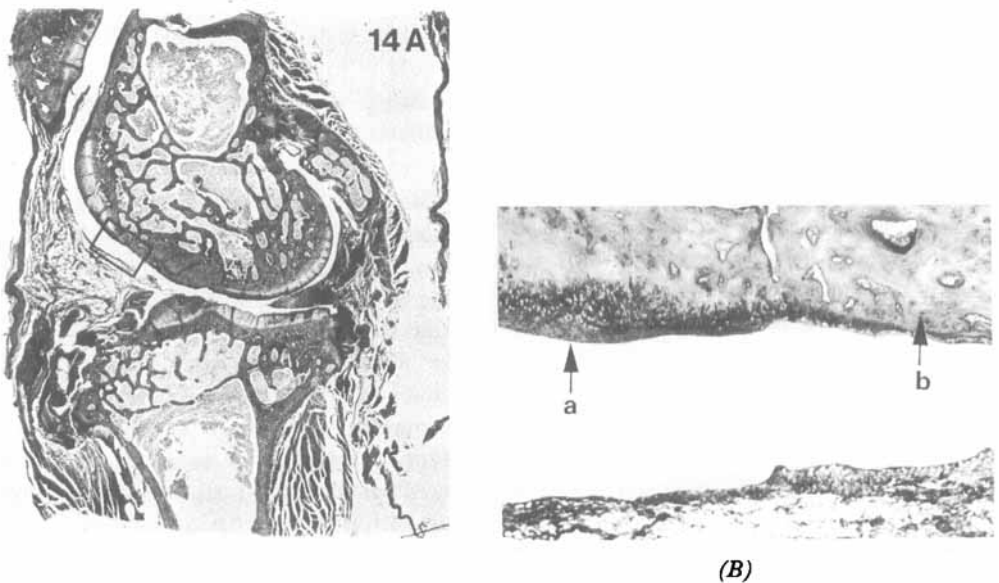


Figure 14A and B. Histological sagittal section of a knee immobilized for 7 weeks and thereafter followed for 3 years before sacrifice of the animal (1.9× magnification). B. A higher magnification (30× magnification) of marked area in A. The phenomena of hypertrophy of articular cartilage (arrow a) and eburnation of bone (arrow b) are seen in the same condyle. (Alcian Blue—PAS staining).

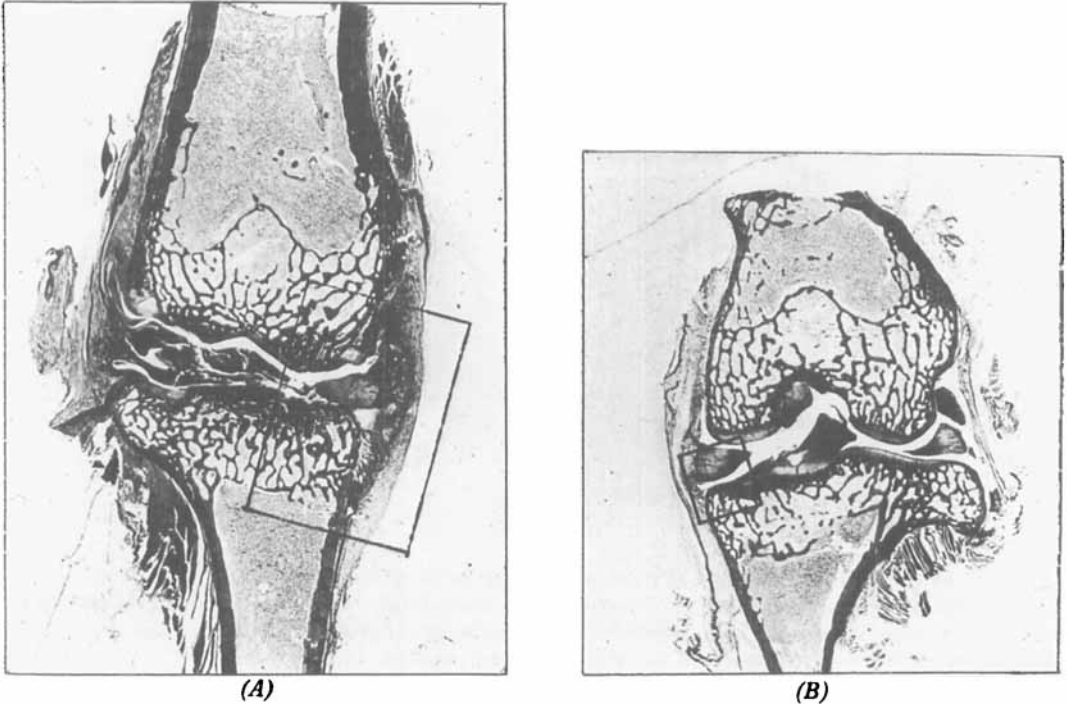


Figure 7A and B. Histological sections in the frontal plane of both knees of the same rabbit. B. The non-immobilized knee. A. The other knee which was immobilized for 3 weeks. Pathological formation of cartilage, marginal osteophytes and fibrous tissue in the capsule are evident in the section. (Stained with Alcian Blue—PAS and $1.8\times$ magnification).

areas. The cortex of the femur appeared thickened and the cancellous bone at the distal end of the femur was markedly diminished. After 6 weeks of immobilization, the sesamoid bones were enlarged and the femoral facies patellaris showed abundant marginal osteophyte formation (Figures 3B and 4B). A regular phenomenon was a marked thickening of the distal end of the femur (Figures 9A and B, 10A and B, 11). A round area of denuded bone at the cranial end of the facies patellaris of the femur was seen in almost every case at this stage (Figures 12A and B, 13).

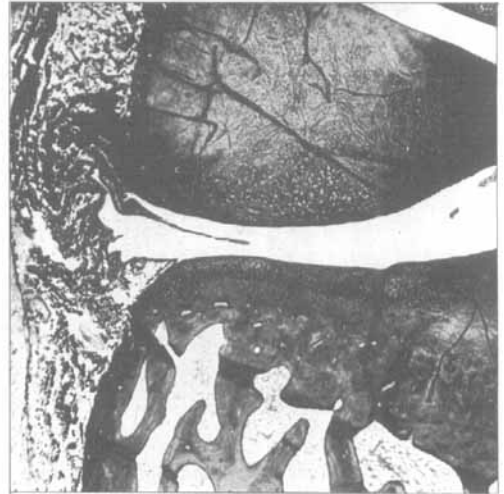
Changes after more than 80 days of immobilization. Eburnation of denuded bone and areas of hypertrophied cartilage were regularly seen at this stage although these phenomena could appear at earlier stages (Figure 14).

Mobility of the joint in relation to immobilization time

Marked degenerative changes usually appeared after 4 weeks of immobilization, but after prolonged immobilization joint mobility was lost and severe destruction of the joint often followed. It was thus desirable to define the length of the immobilization period provoking osteoarthritis without causing permanent contracture of the joint. A preliminary report of a series of 15 immature and 12 adult rabbits studied for this purpose has been presented (Michelsson et al. 1977b). From the curves shown in Figure 15 it appears that an immobilization period exceeding 6 weeks was followed by prolonged restriction of movement. Thus immobilization of the knee in extension for a period of 5–6 weeks is a means of provoking degenerative



(A)



(B)

Figure 8A and B. Detail pictures from Figure 7. B. The medial corner of the upper end of the tibia of the non-immobilized knee. A. The medial corner of the upper end of the tibia of the immobilized knee showing the meniscus and the capsule. The loss of cartilage from the medial condyle of the tibia, the pathological formation of new cartilage and the osteophyte formation on the tibia (arrows) are clearly seen. (Alcian Blue—PAS staining and 7.5 × magnification).



(A)



(B)

Figure 10A and B. Radiographs of the flexed knees of a rabbit taken in a projection tangential to the facies patellaris of the femur. B. The non-immobilized knee. A. The other knee, which was immobilized for 6 weeks (followed for 5½ months after immobilization). The marginal osteophytes of the patella and the thickening of the distal end of the femur are clearly seen.

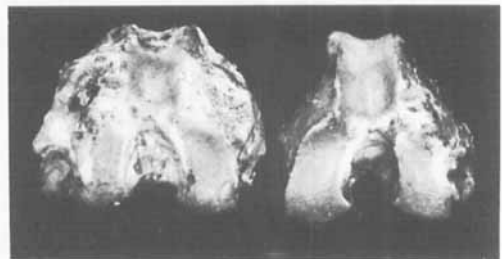


Figure 11. Photographs of the distal ends of both femora of the same rabbit. To the right the non-immobilized knee. To the left the contralateral knee, which was immobilized for 6 weeks (followed for 2 months after immobilization). Compare with Figures 9 and 10.

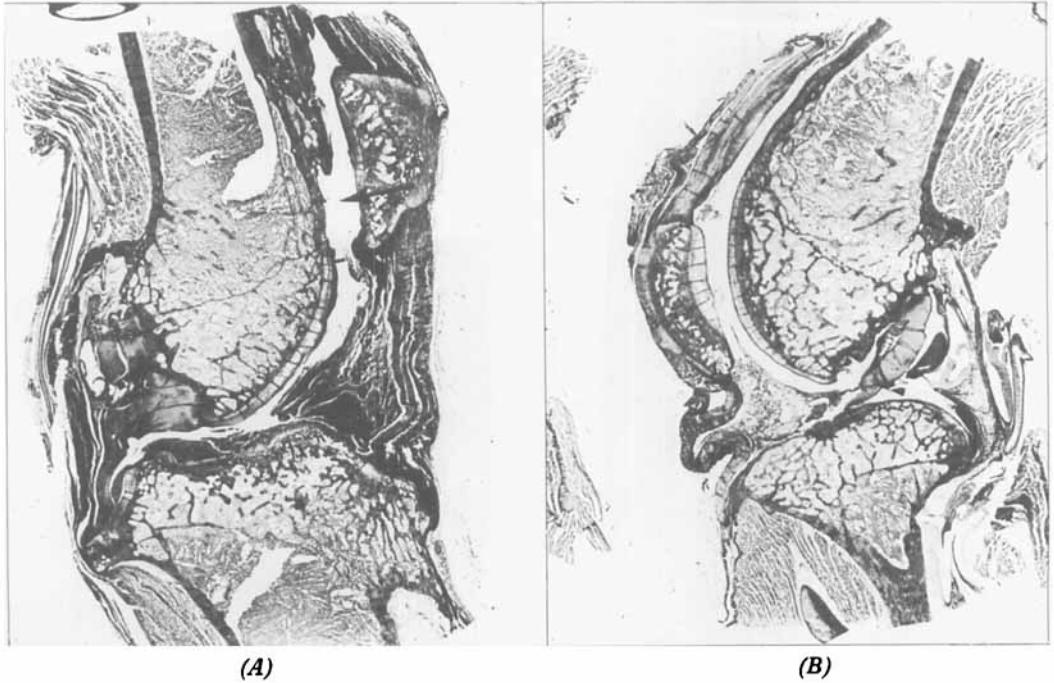


Figure 12A and B. Histological sagittal sections of both knees of the same rabbit. B. The non-immobilized knee. A. The opposite knee, which was immobilized for 3 months (followed for 3 months after immobilization). Note the area of denuded bone in the femoral facies patellaris opposite the patella (arrow). (Alcian Blue—PAS staining and $1.5\times$ magnification).

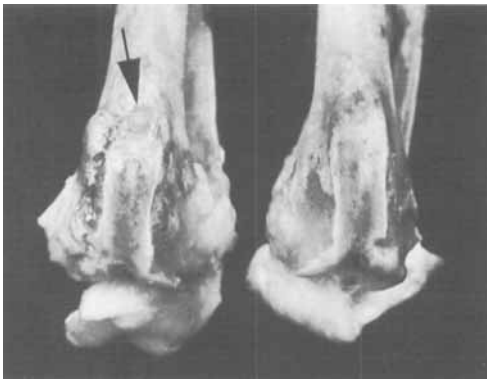


Figure 13. Photographs of the distal ends of both femora of the same rabbit. To the right the femur from the non-immobilized leg. To the left the femur from the opposite leg, which was immobilized for 8 weeks (followed for $1\frac{1}{2}$ years after immobilization). Note the round depression in the proximal part of the facies patellaris (arrow, compare with Figure 12).

disease of this joint without causing permanent restriction of movement.

Radiological assessment of osteoarthritis

The severity of changes suggesting degenerative joint disease in the radiographs was assessed as follows: Grade 0: no change (Figure 3A and 4A). Grade 1: slight; joint space narrowed, bone trabeculae somewhat thickened, lipping at edges of joint surface. Grade 2: moderate; joint space narrowed, marked osteophytes, bone trabeculae markedly thickened, sesamoid bones enlarged, joint surface irregular (Figures 3B and 4B). Grade 3: severe; as grade 2 but more marked changes (Figures 3C and 4C).

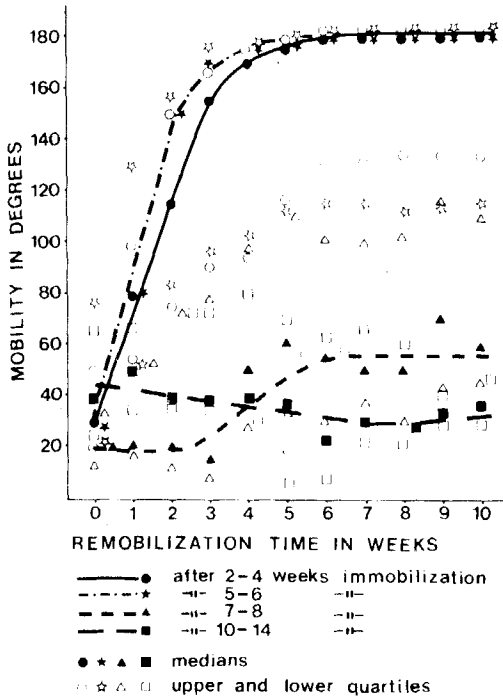


Figure 15. The development of mobility of the knee after different periods of immobilization. The curves are drawn according to the medians and the distribution symbols are the upper and lower quartiles. In both the 2-4 and the 5-6 weeks immobilization groups there were 8 rabbits. The limitation of mobility was irreversible when the immobilization period was increased from 6 to 8 weeks with this immobilization method. During the first month after immobilization the mobility was greater in the 10-14 week immobilization group (6 rabbits) than in the 7-8 week group (5 rabbits). This phenomenon was due to the hyperextension in the 10-14 week group.

The effect of the length of the immobilization period on radiographic changes

The result of assessment of the radiographic changes of 126 knees is seen in Table 2: It shows that slight or moderate changes were already present after 2-4 weeks of immobilization, but also that the period after the end of immobilization affects the final condition. When the duration of immobilization increases, the radiographic grade of degenerative signs also changes statistically

significantly. The shortest statistically significant period was when the immobilization period was increased from 15-28 days to 29-42 days.

The effect of the length of the immobilization period on histological changes

The progression of the proliferative changes in the joint capsule and ligaments around the knee was most marked during the third week of immobilization. During further immobilization for 2 months progression of these changes was slower. The signs of cartilage necrosis and erosion and eburnation of bone progressed continuously with increasing immobilization time.

The effect of the time following the immobilization period

It appears from Table 2 that moderate or severe radiographic changes dominated when the follow-up period after removal of the immobilizing splint exceeded 57 days. The degenerative changes progressed during the remobilization period. The difference in severity between the radiographic changes present at the end of immobilization and those found 8 weeks later was statistically highly significant ($P < 0.001$).

Moderate osteoarthritis predominated in the group immobilized for more than 6 weeks. Histologically, it was noted that the proliferated cartilage close to the osteophytes was less abundant when a long time had elapsed from the immobilization period.

DISCUSSION

Osteoarthritis has earlier been considered an incurable condition because of the inability of the articular cartilage to repair itself after injury. However, it has recently been shown in experiments that regeneration of joint cartilage is possible (Sokoloff 1974). Clinical facts have also provided evidence which

Table 2. The distribution of 126 immobilized knees after radiographic assessment of grade of osteoarthritis. Grouping according to duration of immobilization and the follow-up period after removal of the immobilizing splint*

Grade of osteoarthritis	Duration of immobilization in days										Total	
	0-14	15-28	29-42	43-79	≥80	Follow-up periods after immobilization in days						
	0-14	15-56	≥57	0-14	15-56	≥57	0-14	15-56	≥57	0-14	15-56	≥57
No changes	4		3									7
Slight	2	1	4	2	8	7	9	2	1	3	1	3
Moderate				5	6	7	24	1	1	11	1	12
Severe				1			1			3	1	3
Total	8	14		62	21	21						126

* The difference between the following groups was significant:

Immobilization: 0-14 and 29-42 days ($P < 0.001$); 15-28 and 29-42 days ($P < 0.01$); 28-42 and ≥ 80 days ($P < 0.01$).

Follow-up: 0-14 and 15-56 days ($P < 0.01$); 0-14 and ≥ 57 days ($P < 0.001$).

justifies optimism as far as the curability of osteoarthritis is concerned. The improvement which has been seen in osteoarthritic hips after intertrochanteric osteotomy is still unexplained (Ottolenghi & Frigerio 1962, Nissen 1963, Blount 1964, Ferguson 1971, Nissen 1971, Salenius et al. 1971, Hirsch et al. 1972, Detenbeck et al. 1972, Byers 1974). Our knowledge of the pathogenesis of osteoarthritis and its curability would greatly increase if the curative factors released by osteotomy were discovered and studied by means of experiments in animals.

The purpose of this investigation, the production of a reproducible degenerative joint disease in rabbits, with changes corresponding to those seen in a severe and advanced stage of human osteoarthritis, has been fulfilled. This can be seen in Table 2 and Figure 15. Characteristic of the early changes in the model described is the hypertrophy of the capsule of the joint and of the collateral ligaments. It seems that this phenomenon is an important link in the pathogenetic chain when immobilization of the rabbit's knee leads to degeneration of the joint.

The importance of thickening of the capsule and the periarticular ligaments in the pathology and pathogenesis of advanced osteoarthritis of the hip in the human has been especially stressed by Lloyd-Roberts (1953 and 1955).

The condition seen after 5–6 weeks of immobilization of the rabbit knee is regarded as a model of advanced disease suitable for studies on pathogenesis and treatment of the corresponding stage of human osteoarthritis.

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REFERENCES

Bentley, G. (1974) Experimental osteoarthrosis. Normal and osteoarthrotic articular cartilage,

- Ed. Ali, S. Y. Elves, M. W. & Leaback, D. H. pp. 259–284. Kingswood Press, England.
- Blount, W. P. (1964) Osteotomy in the treatment of osteoarthritis of the hip. *J. Bone Jt Surg.* **46-A**, 1297–1325.
- Byers, P. D. (1974) The effect of high femoral osteotomy on osteoarthritis of the hip. *J. Bone Jt Surg.* **56-B**, 279–290.
- Detenbeck, L. C., Coventry, M. B. & Kelly, P. J. (1972) Intertrochanteric osteotomy for degenerative arthritis of the hip. *Clin. Orthop.* **86**, 73–78.
- Ely, L. W. & Mensor, M. C. (1933) Studies on the immobilization of normal joints. *Surg. Gynec. Obstet.* **57**, 212–215.
- Eronen, I., Videman, T., Friman, C. & Michelsson J.-E. (1978) Glycosaminoglycan metabolism in experimental osteoarthrosis caused by immobilization. *Acta orthop. scand.* **49**, 329–334.
- Evans, E. B., Eggers, G. W. N., Bulter, J. K. & Blumel, J. (1960) Experimental immobilization and remobilization of rat knee joints. *J. Bone Jt Surg.* **42-A**, 737–758.
- Ferguson, A. B. (1971) The pathology of degenerative arthritis of the hip and the use of osteotomy in its treatment. *Clin. Orthop.* **77**, 84–97.
- Field, P. L. & Hueston, J. T. (1970) Articular cartilage loss in longstanding immobilization of interphalangeal joints. *Brit. J. Plast. Surg.* **23**, 186–191.
- Finsterbush, A. & Friedman, B. (1973) Early changes in immobilized rabbits knee joints: A light and electron microscopic study. *Clin. Orthop.* **92**, 305–319.
- Finsterbush, A. & Friedman, B. (1975) Reversibility of joint changes produced by immobilization in rabbits. *Clin. Orthop.* **111**, 290–298.
- Hall, M. C. (1963) Cartilage changes after experimental immobilization of the knee joint of the young rat. *J. Bone Jt Surg.* **45-A**, 36–44.
- Hall, M. C. (1964) Articular changes in the knee of the adult rat after prolonged immobilization in extension. *Clin. Orthop.* **34**, 184–195.
- Heikel, H. V. A. (1959) On ossification and growth of certain bones of the rabbit; with a comparison of the skeletal age in the rabbit and in man. *Acta orthop. scand.* **39**, 171–184.
- Hirsch, C., Goldie, I. & Ryba, W. (1972) Intertrochanteric osteotomy for osteoarthritis of the hip. A radiological evaluation. *Clin. Orthop.* **86**, 63–67.
- Lloyd-Roberts, G. C. (1953) The role of capsular changes in osteoarthritis of the hip joint. *J. Bone Jt Surg.* **35-B**, 627–642.
- Lloyd-Roberts, G. C. (1955) Osteoarthritis of the hip: A study of the clinical pathology. *J. Bone Jt. Surg.* **37-B**, 8–47.

- Luna, L. G. (Editor) (1968) *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd ed., pp. 163–165. The Blakiston Division McGraw-Hill Book Company, New York, Toronto, London, Sydney.
- McManus, I. F. A. & Mowry, R. W. (1964) *Staining methods histological and histochemical*, pp. 63–64. Harpes & Row, New York, Evanston & London (A Hoeber International Reprint).
- Michelsson, J.-E., Videman, T. & Langenskiöld, A. (1977a) Changes in bone formation during immobilization and development of experimental osteoarthritis. A study using oxytetracycline in rabbits. *Acta orthop. scand.* **48**, 443–449.
- Michelsson, J.-E., Videman, T. & Langenskiöld, A. (1977b) Contractures of the knee in provoking osteoarthritis in rabbits by immobilization. *Int. res. comm. syst. Med. Sci.* **5**, 61.
- Moskowitz, R. W. (1972) Experimental models of degenerative joint disease. *Seminars in Arthritis and Rheumatism*. **1**, 95–116.
- Nissen, K. I. (1963) The arrest of early primary osteoarthritis of the hip by osteotomy. *Proc. roy. Soc. Med.* **56**, 1051–1060.
- Nissen, K. I. (1971) The rationale of early osteotomy for idiopathic coxarthrosis (epichondro-osteoarthrosis of the hip). *Clin. Orthop.* **77**, 98–104.
- Ottolenghi, C. E. & Frigerio, E. (1962) Intertrochanteric osteotomies in osteoarthritis of the hip. *J. Bone Jt Surg.* **44-A**, 855–895.
- Roy, S. (1970) Ultrastructure of articular cartilage in experimental immobilization. *Ann. rheum. Dis.* **29**, 634–642.
- Salenius, P., Langenskiöld, A. & Österman, K. (1971) Intertrochanteric displacement osteotomy in the treatment of osteoarthritis of the hip. Results of follow-up examination. *Acta orthop. scand.* **42**, 63–77.
- Salter, R. B. & Field, P. (1960) The effects of continuous compression on living articular cartilage: An experimental investigation. *J. Bone Jt Surg.* **42-A**, 31–49.
- Sokoloff, L. (1974) The general pathology of osteoarthritis. *Normal and osteoarthrotic articular cartilage*, Ed. Ali, S. Y., Elves, M. W. & Leaback, D. H., pp. 111–124. Kingswood Press, England.
- Sood, S. C. (1971) A study of the effects of experimental immobilization on rabbit articular cartilage. *J. Anat. (Lond.)* **108**, 497–507.
- Thaxter, T. H., Mann, R. A. & Anderson, C. E. (1965) Degeneration of immobilized knee joints in rats: histological and autoradiographic study. *J. Bone Jt Surg.* **47-A**, 567–585.
- Thompson, R. C. & Basset, C. A. L. (1970) Histological observations on experimentally induced degeneration of articular cartilage. *J. Bone Jt Surg.* **52-A**, 435–443.
- Trias, A. (1961) Effect of persistent pressure on the articular cartilage: An experimental study. *J. Bone Jt Surg.* **43-B**, 376–386.
- Videman, T., Langenskiöld, A., Michelsson, J.-E. & Candolin, T. (1977) A macroscopic and scanning electron microscopic study of experimental osteoarthritis of the knee. *Int. res. comm. syst. Med. Sci.* **5**, 232–233.
- Videman, T., Michelsson, J.-E., Rauhamäki, R. & Langenskiöld, A. (1976) Changes in ³⁵S-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.

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