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EXPERIMENTAL OSTEOARTHRITIS IN THE RABBIT

I. HISTOLOGICAL CHANGES OF THE SYNOVIAL MEMBRANE

By

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SVALASTOGA, EILIV and INGE REIMANN: *Experimental osteoarthritis in the rabbit. I. Histological changes of the synovial membrane.* Acta vet. scand. 1985, *26*, 313—325. — Based on an experimental model of osteoarthritis in 42 full-grown rabbits the histological changes were studied during the development of osteoarthritis after operative induction of instability of the knee joint. The changes were followed from 2 week to 1½ years after the induction.

The first changes were observed at 1 week stage as a proliferation of the lining cells. During the time of observation additional changes were seen such as hypertrophy of villi, infiltrations with plasma cells and lymphocytes, increased vascularity and interstitial edema and fibrosis. Edema was only seen during the first months, later increasing fibrosis was predominant.

The first cartilage change was reduction of the staining ability, expressing depletion of GAG, this was seen already at the one week stage whereas morphological changes were present after 4—6 weeks.

It was concluded that the synovial membrane in this model shows changes that may contribute to the development of osteoarthritis.

joint model; joint pathology.

Changes of the synovium are an integral part of osteoarthritis. However, the significance of the synovial changes in the pathogenesis of osteoarthritis is still discussed (*Arnoldi & Reimann* 1979). Many authors seem to agree with *Lloyd-Roberts* (1953) that the changes in the synovium are secondary to the cartilage destruction and result from displacement of detritus into the synovium. However, synovitis is a constant very early clinical feature. To elucidate some of these problems it is necessary to investigate the synovial membrane in the very early stage of osteoarthritis. This is only possible in an experimental model.

Although the presence of synovial reaction has been mentioned in some animal models (*Langenskiöld et al.* 1979) a description in detail during the development of osteoarthritis has not been reported previously.

The purpose of the present study is to explore the histological features of the synovial membrane in a well documented experimental model of osteoarthritis in the knee joint in rabbits and to study the changes at the different stages during the development of osteoarthritis. Furthermore, an attempt is made to relate the synovial changes to the cartilage destruction.

MATERIAL AND METHODS

The knee joints from 42 full-grown, New Zealand White rabbits were used for this study. One of the knee joints was operated upon according to the method of *Hulth et al.* (1970). Following intravenous anaesthesia (Mebumal 5 %) the medial collateral ligament was excised, the medial meniscus was extirpated and the anterior and posterior cruciate ligaments were divided. The other knee joint served as control (an arthrotomy was performed as a sham operation). The rabbits were killed by an overdose of Mebumal, in groups of 6, and at varying times after operation (1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, 26 weeks and 1½ years, respectively). The knee joints were opened and 2 swabs from each joint were cultivated for aerobic and anaerobic bacterial growth. The knee joints were removed and divided with a saw in the corona plane and the half which was used in this study was fixed in 4 % phosphate buffered formaldehyde, decalcified in 22 % formic acid with 10 % sodium citrate and double-embedded in celloidin-paraffin. Sections 6 µm thick were stained with hematoxylin-eosin and Safranin 0.

Examination by light microscopy

From each knee joint serial sections were prepared. Most pronounced changes were present at the medial joint chamber and this place was chosen for evaluation of the synovial changes in all the joints. The grading method of *Salvati et al.* (1977) was used as basis, thus the following parameters were taken into consideration: proliferation of lining cells, infiltration of plasma cells and lymphocytes, hypertrophy of villi, edema, fibrosis and dilation of venules.

From the Safranin 0 stained sections the articular cartilage of tibial and femoral condyles were evaluated. The grading system of *Mankin et al.* (1977) was used as basis and the following parameters were taken into consideration: Reduction in the Safranin 0 staining (an expression of glycosaminoglycan (GAG) depletion) as well as changes in structure and cells.

RESULTS

Joint effusion was present in all the knee joints where osteoarthritis was induced from first to 12th week. The amount varied from 0.5 to 3 ml. No case of infection was found.

The control side showed no or insignificant joint effusion during the first 2 weeks and there was no case of infection.

Gross inspection

Synovial membrane

Osteoarthritic side: in all cases there were changes of different degrees with edema, hyperaemia, hypertrophy of villi and fibrosis.

Control side: only very slight edema and hyperaemia were seen in 3 cases during the first 2 weeks.

Articular cartilage

Osteoarthritic side: the first changes were seen after 4 to 6 weeks as moderate changes of the articular cartilage in the medial joint chamber with lustreless surface and marginal osteophytes. Later the destruction progressed with destruction of the cartilage until complete loss of tissue in spots.

Control side: macroscopic changes were not observed.

Light microscopy (Table 1)

Synovial membrane

Figs. 1—7 illustrate the different changes at the different stages.

Osteoarthritic side: proliferation of the lining cells were always present. The typical observation in the osteoarthritis joint was 3—5 layers of synoviocytes although a large variation in the numbers of layers of cells were observed between animals in each group especially in the early stages. During the first weeks there

Table 1. Histological evaluation of the synovial membrane in experimental osteoarthritis.

Stage*	1 week		2 weeks		4 weeks		6 weeks		3 months		6 months		1½ years	
	OA	C	OA	C	OA	C	OA	C	OA	C	OA	C	OA	C
Lining cells $\bar{x} \pm$ SEM	3.9±0.6	1.3±0.2	4.6±0.9	1.4±0.1	4.0±0.5	—	4.1±0.5	—	4.1±0.4	—	3.1±0.4	—	2.4±0.4	—
Inflammation	+	—	+	—	++	—	+	—	+	—	+	—	+	—
Hypertrophy of villi	+	—	+	—	++	—	++	—	++	—	++	—	+	—
Edema	++	+	++	—	+	—	+	—	+	—	—	—	—	—
Fibrosis	—	—	(+)	—	+	—	+	—	++	—	+++	—	+++	—
Vascularity	—	—	(+)	—	+	—	+	—	+	—	++	—	++	—

* Six rabbits were examined at each stage
OA: Osteoarthritic joint
C: Control joint

— Normal
(+) Present in 1—2 cases
\+ Slight
++ Moderate
+++ Severe

Figures 1—7. Light microscopic view of histological sections of the synovial membrane prepared at various postoperative intervals. Hematoxylin-Eosin, magnification × 25.

A: Histological sections from instable (osteoarthritic) joints.
B: Histological sections from controlateral joints.

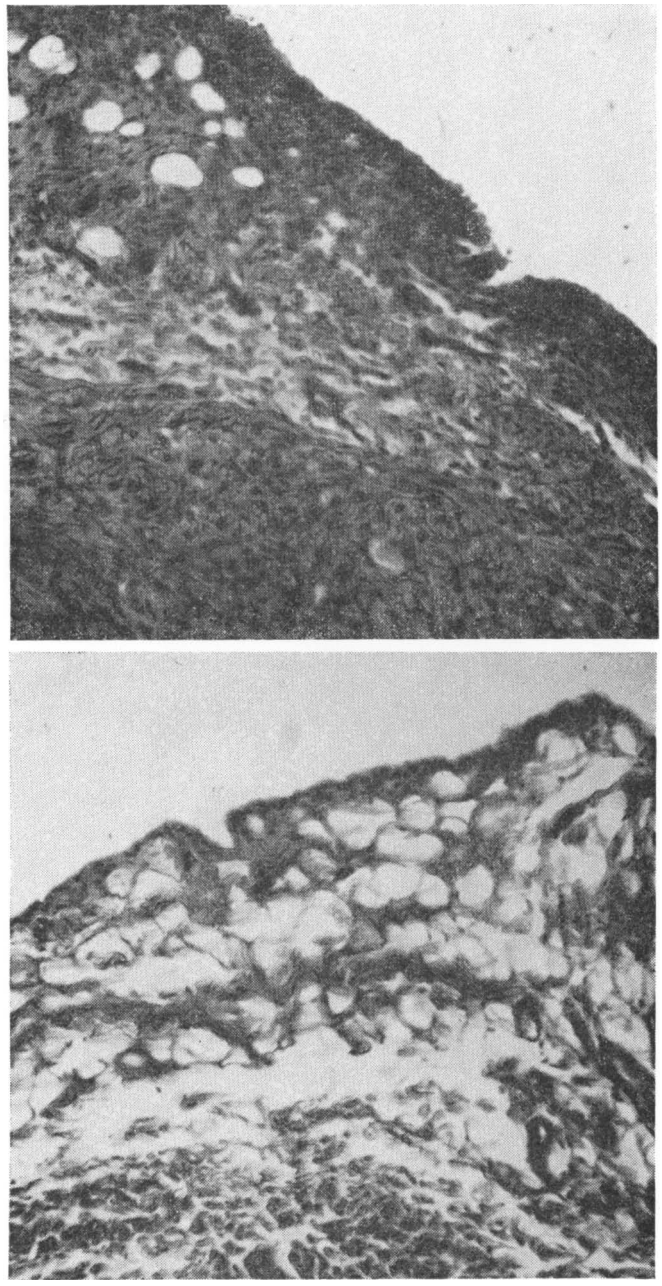

Figure 1. A: One week: A distinct proliferation of lining cells edema are present.

B: Slight proliferation of lining cells.

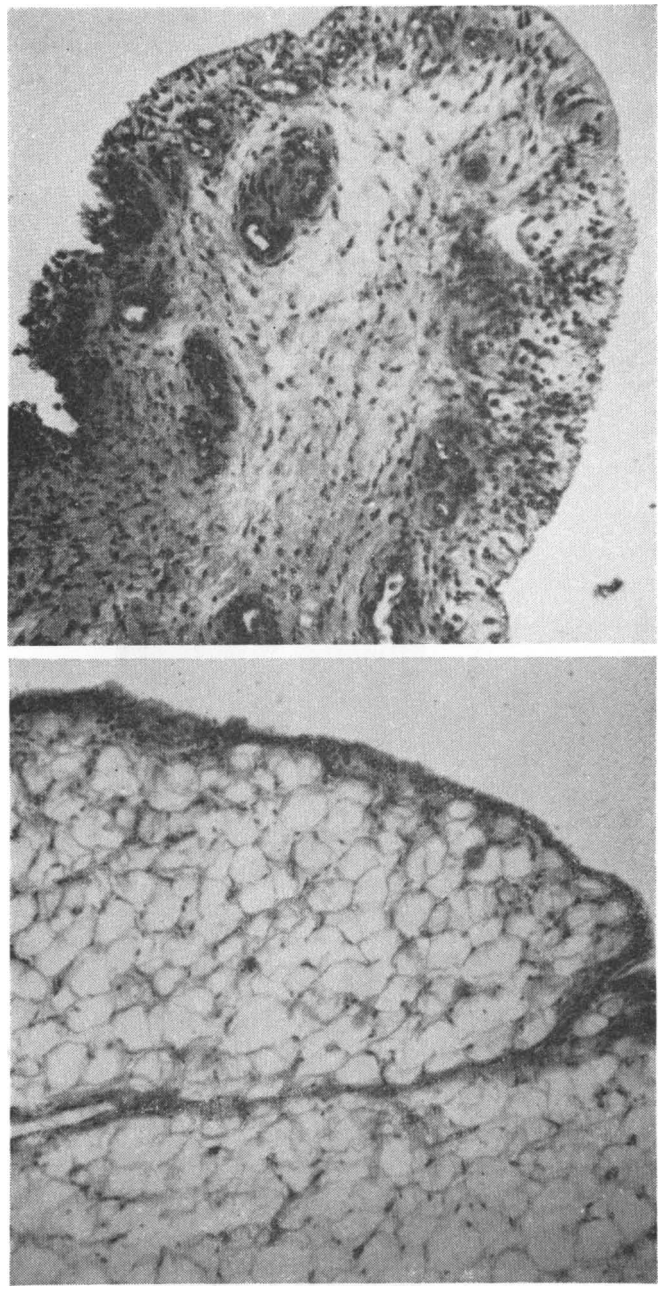

Figure 2. A: Two weeks: Increased proliferation of lining cells, hypertrophy of villi, obvious edema of interstilial tissue and increasing vascularity.

B: Moderate proliferation of lining cells.

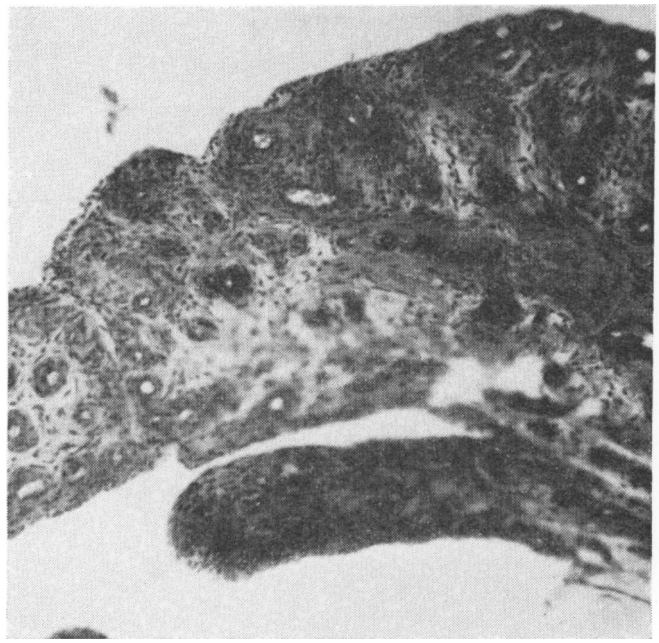

Figure 3. Four weeks: Changes as at 2 weeks (Fig. 2) with increased hypertrophy of villi and vascularity. Slight fibrosis of the interstitial tissue.

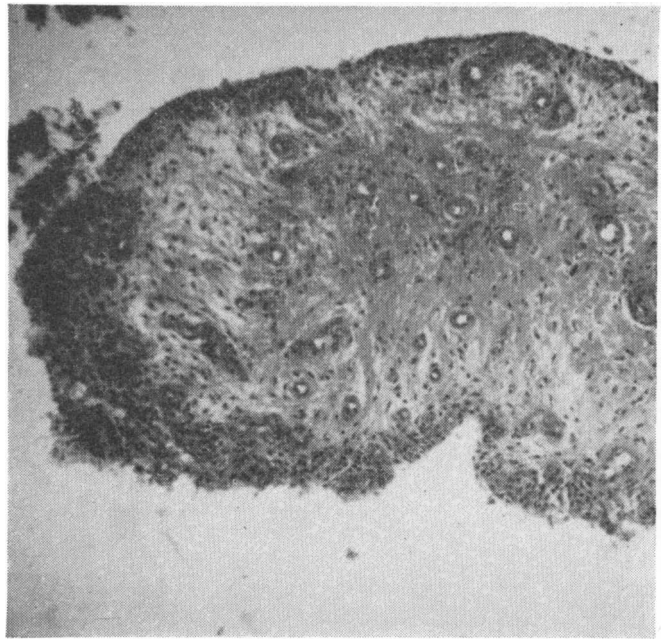

Figure 4. Six weeks: Changes as at 4 weeks (Fig. 3) further increased interstitial tissue edema and fibrosis.

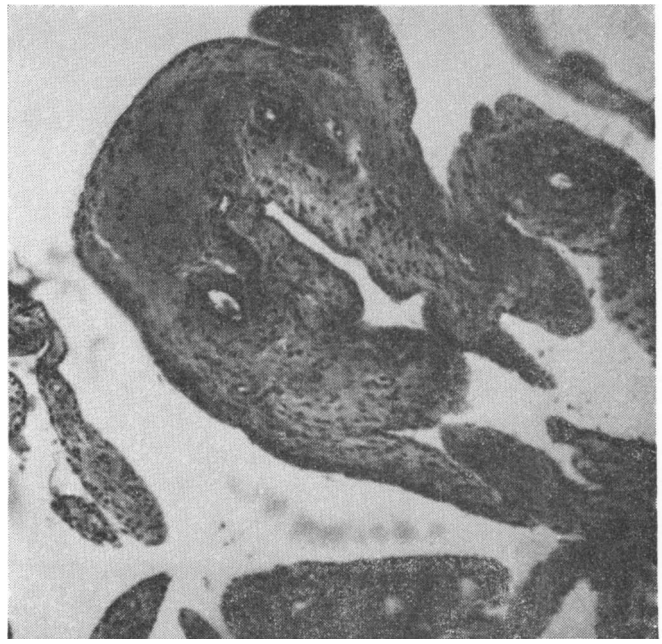

Figure 5. Three months: Marked increased fibrosis compared with the changes at 6 weeks.

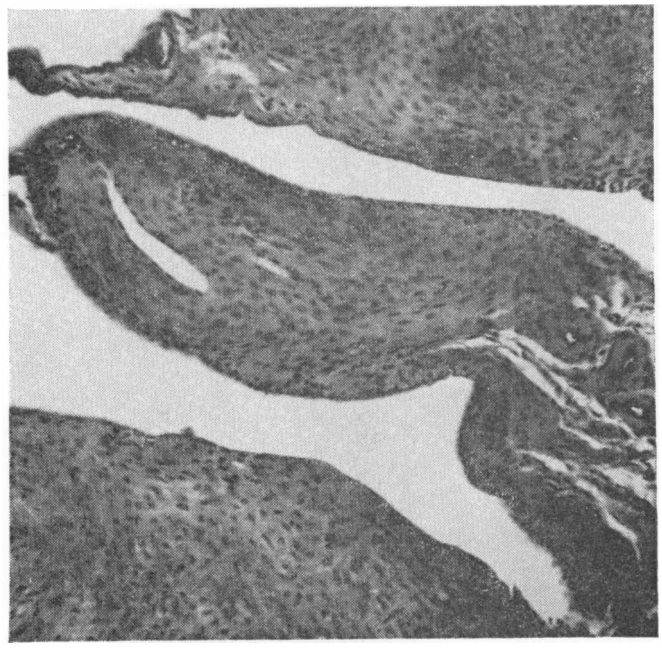

Figure 6. Six months: Interstitial tissue with severe fibrosis.

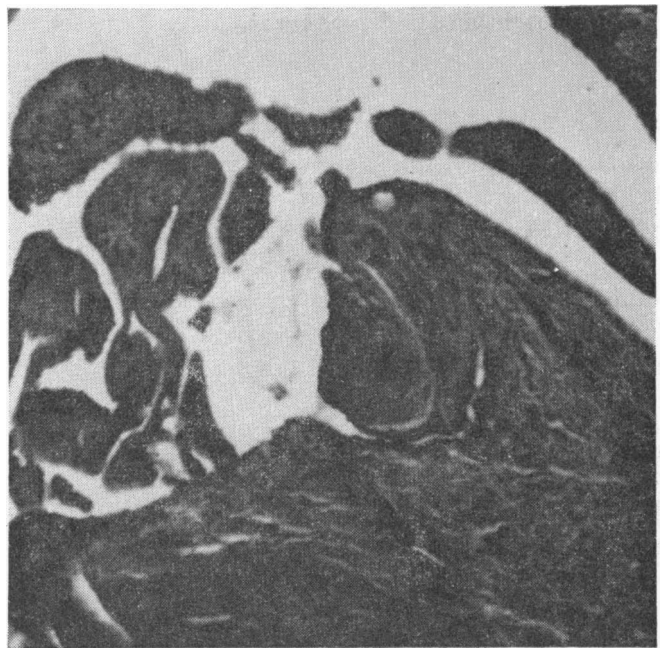

Figure 7. One and a half year: Only moderate proliferation of lining cells. Interstitial tissue totally fibrosized.

was an increase in the proliferation of the lining cells from 3—5 layers until 5—10 layers in some cases. From the third month there was a general tendency of a slight decrease in the proliferation. In the individual animal the proliferation in the osteoarthritic knee joint always exceeded the proliferation on the contralateral sham operated side. Infiltration of plasma cells and lymphocytes was seen only as scattered infiltration in slight to moderate degree.

Hypertrophy of villi was seen in moderate degree in most cases after 4 weeks.

A moderate degree of edema was present during the first weeks after which it subsided. From the third month stage edema was not found in any significant degree.

Interstitial fibrosis was present already after 2 weeks and was rather common after 4 weeks and increased during the development of the disease. Dilation of the venules were seen in a few cases during the first week, later it was noted in most cases.

Control side: only during the first 4 weeks slight changes with proliferation of the lining cells (2—3 layers of synoviocytes)

and edema were observed in few cases. After 4 weeks the synovial membrane was judged as normal.

Articular cartilage

Osteoarthritic side: during the first 2 weeks a decreased Safranin 0 staining at the surface was present as an expression of reduced GAG content. Morphological changes were first seen after 4 to 6 weeks. The first sign was changes at the surface of the medial tibia and/or femoral condyle with flaking and clefts, later desorganization and clooning was found. The cartilage destruction was progressing with time.

Control side: during the first 2 weeks very slight decreased staining at the surface was observed after which the articular cartilage at the control side was judged as normal.

DISCUSSION

Before discussing the significance of the data obtained by the histological study of the synovial membrane it is important to review the model applied. Numerous animal models of degenerative joint disease have been described (*Adams & Billingham* 1982). The present study was based on a model for producing a slowly progressing osteoarthritis in rabbits ad modum *Hulth et al.* (1970) by development of instability of this model in the study of osteoarthritis (*Telhag & Lindberg* 1972, *Ehrlich et al.* 1975). However, this model may be criticized as the very early synovial reaction may be the result of the operative opening of the joint. To take this into account the other knee joint which served as a control in the present investigation was sham operated with an arthrotomy. The control joints showed only transient changes and the synovial membrane was normalized between 2 and 4 weeks after the arthrotomy. Furthermore, it may be mentioned that early synovitis and joint effusion has also been described in an osteoarthritic model based on immobilization of the joint (*Finsterbush & Friedman* 1973, *Langenskiöld et al.* 1979).

Therefore we find the model used in this study (*Hulth et al.* 1970) usable for registration of the sequential changes in the synovial membrane during development of osteoarthritis in the knee joint.

It was obvious that a very early synovial reaction was present in the knee joints where osteoarthritis was induced, as a marked

proliferation of the lining cells was seen after 1 week. This further progressed after 2 weeks together with the appearence of hypertrophy of villi and increased vascularity. In this model the maximum level of proliferation was observed within the first 3 months after the operation after which the synovium became more and more fibrosized. This is in accordance with human osteoarthritis although a stage of more proliferative synovitis with marked edema and dilated venules and capillaries is often present before the fibrous stage (*Arnoldi & Reimann* 1979).

In this context it is of importance that the model provides additional information not accessible in naturally occurring osteoarthritis, especially concerning the early changes in the synovial membrane.

By comparison with the degenerative changes of the articular cartilage it was of special interest to note that corresponding to the early signs of synovitis a surface depletion of GAG in the hyaline cartilage was seen. Morphological changes of the chondrocytes and surface irregularities, however, were not present in the cartilage until 4 to 6 weeks after the induction of osteoarthritis, the stage which corresponds to the maximum of synovial proliferation.

It is well known that depletion of GAG in articular cartilage is an early sign of osteoarthritis (*Mankin & Lippiello* 1971), although it has been shown that depletion without concomitant morphological alterations of the articular cartilage may be reversible (*Reimann et al.* 1982).

In this model of osteoarthritis the early synovial changes have been demonstrated to coincide with slight depletion of GAG in the articular cartilage and the changes in the synovial membrane as well as cartilage progressed with time. It is difficult to say whether the early changes of synovium are of primary nature or rather a result of traumatic synovitis. However, it may be stressed that the synovial reaction is an early contributing factor in the development of osteoarthritis. Furthermore it seems clear that the early synovial changes are not the result of cartilage alterations and as such based on phagocytosis of cartilage fragments.

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SAMMENDRAG

Eksperimentel osteoarthritis hos kaniner. I. Histologiske forandringer i synovialmembranen.

Der redegøres for de histologiske forandringer i synovialmembranen under udviklingen af eksperimentel osteoarthritis i knæleddet hos 42 udvoksede kaniner efter en instabilitetsoperation. De histologiske forandringer blev fulgt fra 1 uge til 1½ år efter operationen.

De første forandringer, der blev iagttaget en uge efter operationen, var en proliferation af synoviocytterne. I løbet af forsøgsperioden observeredes yderligere forandringer med hypertrofi af villi, infiltra-

tion med plasmaceller og lymfocytter, øget vaskularitet, ødem og fibrose. Ødem blev kun iagttaget i de første måneder, i de senere stadier dominerede en fremadskridende fibrose.

På et uges stadiet blev der iagttaget nedsat farvbarhed af brusken som udtryk for depletering af GAG. Morfologiske forandringer blev først iagttaget efter 4—6 uger.

Det konkluderes, at synovialmembranen i denne model udviser forandringer, som kan være medvirkende årsag ved udviklingen af osteoarthritis.

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