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EXPERIMENTAL OSTEOARTHRITIS IN THE RABBIT

A Study of ^{133}Xenon Washout Rates from the Synovial Cavity

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Synovial perfusion in 6 rabbit knees, with experimentally induced osteoarthritis (joint instability), was studied by recording the initial ^{133}Xe washout rates from the joint space. The unstable, osteoarthritic knee was compared with the contralateral sham operated control knee at intervals of 6 to 96 weeks postoperatively. Within the first half year the ratio between the ^{133}Xe washout rates in the osteoarthritic and control joints was significantly increased. These findings were supported by the increased blood flow to the joint region, visualized by scintigraphy of osteoarthritic rabbits given ^{99m}Tc-microspheres intracardially. However, methodological sources of error do not allow any conclusions regarding the much less increased ^{133}Xe washout rates found in advanced osteoarthritis.

The initially increased synovial blood flow coincided with the existence of joint effusion and the early development of osteophytes, all conditions supposed to be a consequence of posttraumatic synovitis. Attention is drawn to these pathogenic phenomena in studies dealing with the initial changes in experimental models of osteoarthritis and to a possible etiological significance.

Key words: osteoarthritis; synovitis; ^{133}Xenon

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The pathophysiology of joints has been investigated by studying the removal of various agents given intraarticularly. Substances such as phenolsulphonphthalein (Nakamura et al. 1967), radiosodium (Harris & Hillard 1956, Jacox et al. 1952, Scholer et al. 1968), deuterium (Scholer et al. 1968), triated water (Simkin & Pizzorno 1979) and technetium-pertechnetate (Tanaka et al. 1973) have been used, but none of these are ideal as they are biologically active and recirculate in the blood.

^{133}Xe is an inert lipophilic gas that can easily cross cellular membranes. Its recirculation can be considered negligible as almost all Xenon is expired in one circulation through the lungs (Lassen 1946, Lassen et al. 1964).

The disappearance rate of Xenon from synovial cavities is determined by the regional blood flow (Dick et al. 1970a, Phelps et al. 1972) and has been used to measure indirectly the degree of inflammation in arthritic joints and the effect of anti-rheumatic compounds on synovial perfusion (Dick et al. 1970b, Onge et al. 1968).

In osteoarthritis, increased attention has been focused on synovial changes as a pathogenetic factor (Arnoldi & Reimann 1979). It is the purpose of this study to elucidate synovial involvement or response as evaluated by synovial perfusion in osteoarthritis induced experimentally by joint instability and to elucidate any flow changes during the successive stages of osteoarthritic development.

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MATERIAL AND METHODS

Six adult rabbits were operated in one knee joint according to the method described by Hulth et al. (1970) in order to induce slowly progressive osteoarthritic degeneration. The knee was made unstable by excision of the medial collateral ligament, extirpation of the medial collateral meniscus and division of both cruciate ligaments. The other knee served as a control after a sham arthrotomy.

^{133}Xe clearance measurements were made postoperatively at various intervals beginning at 6 weeks and terminating at 1 year for 4 animals and at 2 years for 2 animals. The rabbits were anesthetized with Mebumal (5 per cent) (DAK) given intravenously and the ^{133}Xe clearance was obtained after injecting 0.2 ml of a sterile solution of ^{133}Xe isotonic saline (Radiochemical Centre, Amersham, England, 3.3 mCi/ml Code XAS 120 P) using a fully aseptic technique. Injections were made simultaneously in both knees by a lateral infrapatella approach and the isotope washout was measured at the end of the injection using a widely collimated NaI crystal detector connected to a pulse scaler with a time constant of 10 seconds. The initial washout rate constant K_{init} was computed from the initial monoexponential part of the washout curve, using the logarithmic transformed count rates corrected for background activity and the formula:

$$K = \frac{\ln 2}{T_{1/2}}$$

To determine whether the two clearance phases depended on intact circulation, curves were obtained with tourniquet obstruction of the femur applied during both the initial phase and the slope.

In order to vizualize blood flow differences in the osteoarthritic and the control knee region, scintigrams were taken after ^{99m}Tc-labeled albumin microspheres were injected into the left ventricle of the heart. Under Mebumal anesthesia a catheter (Intracath No. 3124, Deseret Pharmaceutical Co., Inc. Sandy, Utah) was inserted into the right carotid artery and passed into the heart. Human albumin microspheres (Instant microspheres 3M, St. Paul, Minnesota) with a mean diameter of about 20 micrometers were labeled with ^{99m}Tc (Tecegen generator, Beringwerke AG, Frankfurt, Germany) according to the manufacturers' instructions, vigorously shaken and ultrasonically vibrated for 5 minutes. 10–12 mCi with approximately 1 million labeled particles were injected into the heart and dynamic scintigraphic measurements made from the beginning of the injection with a gamma-camera (Portacamera, General Electric Company, Houston, Texas) connected to a digital computer system (PDP 11, Digital Equipment Corporation, Maynard, Mass.).

Three rabbits were examined: one normal rabbit was studied to ensure that the method resulted in uniform distribution of the microspheres to the hind legs, and 2 osteoarthritic rabbits were studied 3½ months and 16 months postoperatively.

In addition, the last rabbit was bone scanned 4 days later with 5 mCi ^{99m}Tc-MDP (IFA, Kjeller, Norway) given intravenously 3 hours prior to the scintigraphic procedures.

The rabbits were sacrificed with an overdose of Mebumal. Double cultivation for aerobic and anaerobic bacteria from each synovial cavity was performed to ensure that no bacterial arthritis had occurred. The development of osteoarthritis was confirmed by radiographic and macroscopic examination of the joints.

RESULTS

The washout of ^{133}Xe from the joints was found to be biexponential.

Typical curves are shown in Figure 1.

The results of the calculations of the initial washout rates k_{init}, expressed as the ratio between k_{init} obtained on the osteoarthritic side and k_{init} obtained on the control side are given in Figure 2.

The ratios were significantly higher in the early stages of the disease, i.e. less than 6 months postoperatively, than later on ($P < 0.001$, Mann-Whitney test). In both the early and the late stages, the clearance was found to be faster (ratios above 1) in the osteoarthritic knee compared with the control knee ($P < 0.01$, the sign test).

These findings were supported by the scintigram taken during and after administration of ^{99m}Tc-microspheres intracardially. This method resulted in uniform distribution in the hind legs of the normal rabbit with maximal accumulation of radioactivity in the knee region being achieved within the first 10 seconds after the start of the injection and maintained. This uptake remained constant for at least the first 9 minutes measured (Figure 3). The blood flow of the osteoarthritic knee region was found to be particularly high 3 months postoperatively (Ratio 1.8) (Figure 3), but increased flow was still found 16 months postoperatively (Ratio: 1.3) (Figure 4).

Infections were not found in the joints, and osteoarthritic changes were confirmed by radiography and macroscopic examination after sacrifice. In the one rabbit sacrificed 3½ months postoperatively, osteophytes were prominent, but

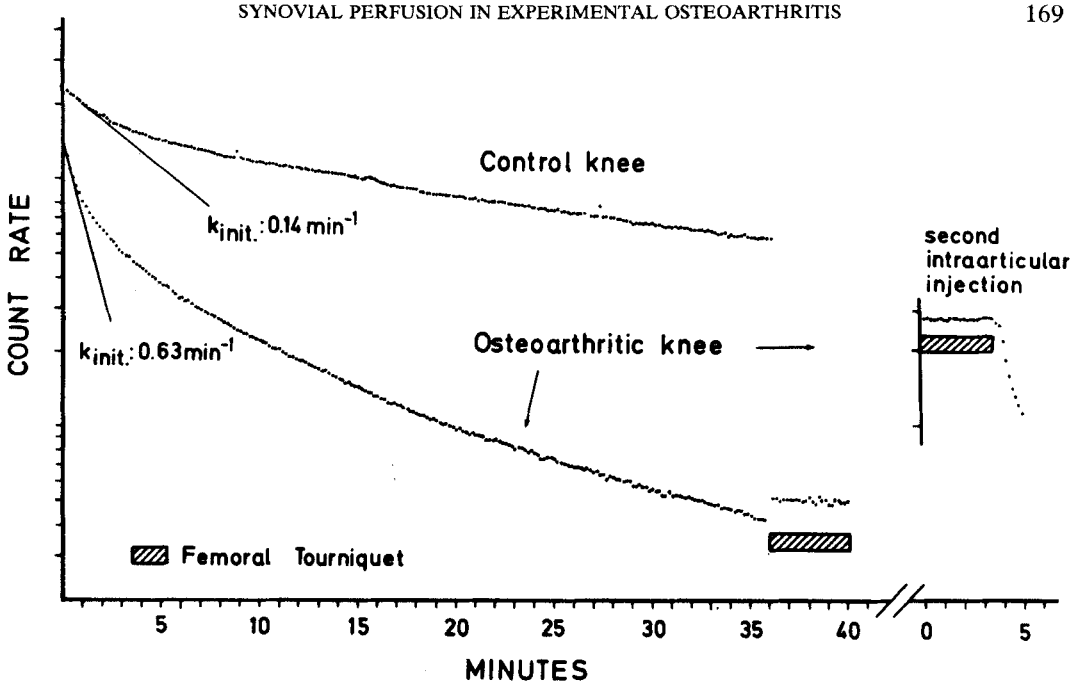


Figure 1. ^{133}Xe *washout curves from the knee joints 23 weeks postoperatively. The biexponential curve and the constant count rate after circulatory arrest is seen both in the late slow phase and the initial fast phase of the washout curve.*

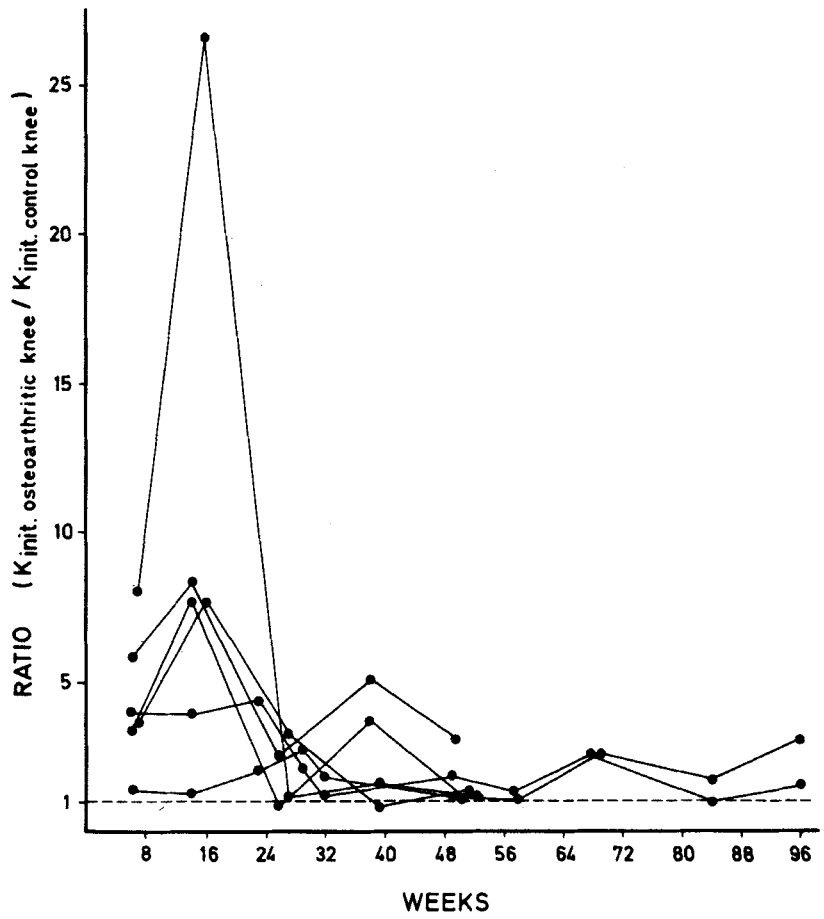
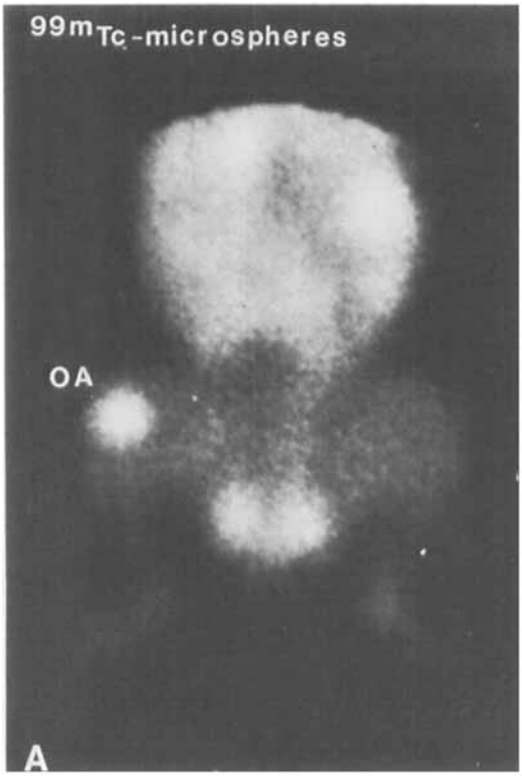
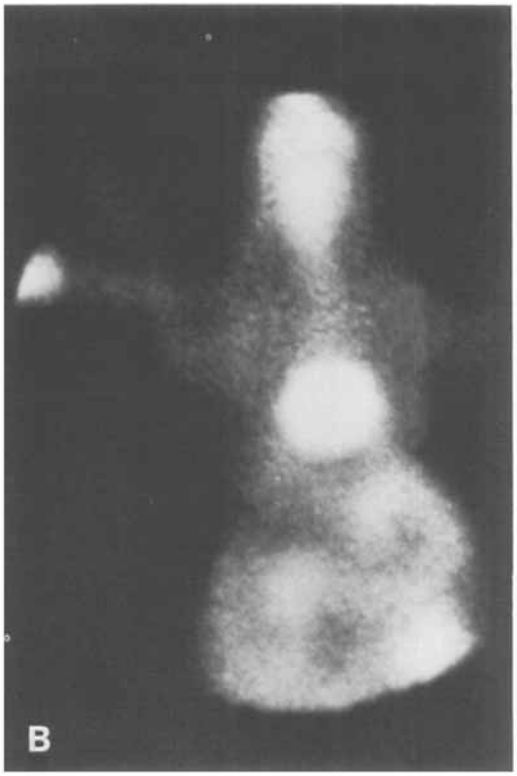
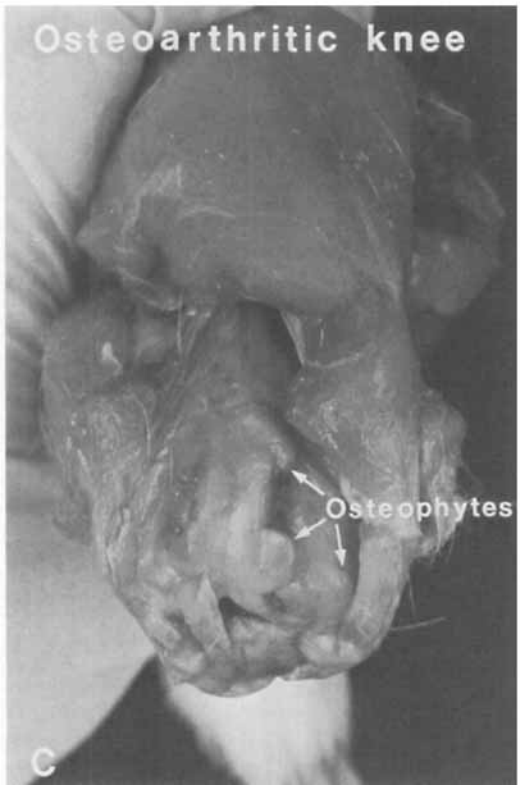
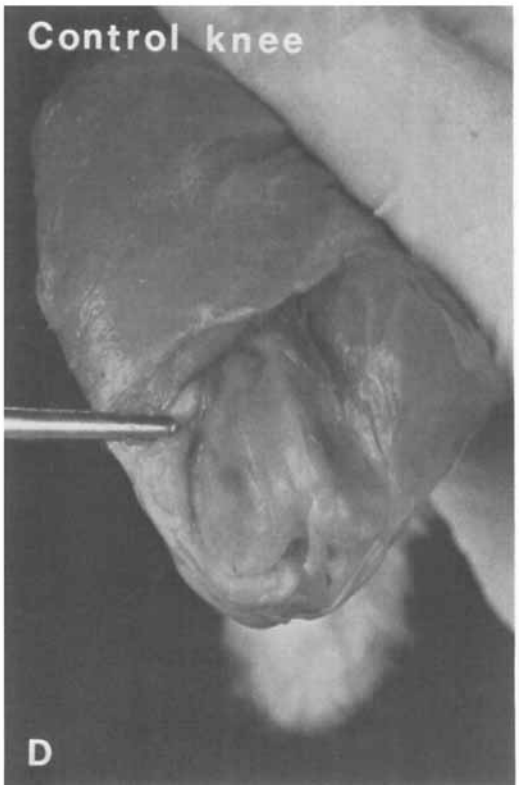


Figure 2. The ratio between the washout rates of the osteoarthritic and the control knee of 6 rabbits at different periods postoperatively. Ratios above 1 indicate increased clearance from the osteoarthritic knee. The highest ratios are seen within the first half year.


99mTc-microspheres
OA
A


B


Osteoarthritic knee
Osteophytes
C


Control knee
D

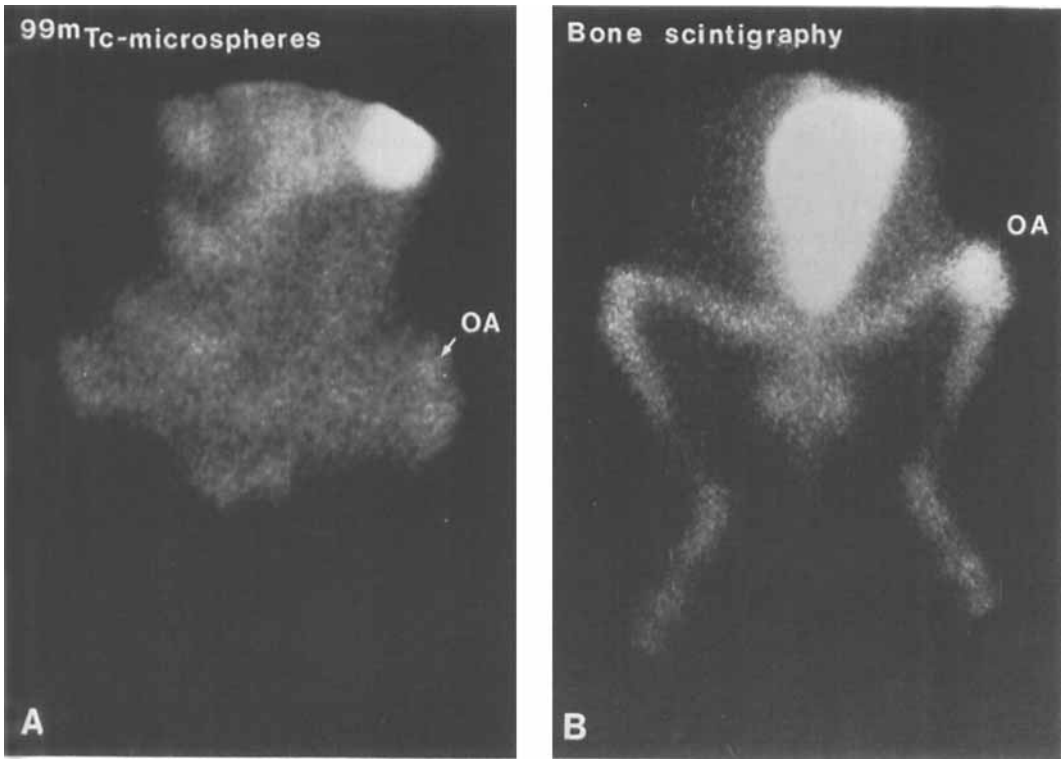


Figure 4. Blood flow distribution of ^{99m}Tc- microspheres 16 months postoperatively and bone scintigraphy of the same animal. Some accumulation of microspheres in the osteoarthritic joint region is seen (frame ratio 1.3), but less than at the early stage of the disease. High accumulation of bone seeking agents is seen in the osteoarthritic joint.

no denuded or eburnated areas of the joint surfaces were seen (Figure 3C).

DISCUSSION

The main finding of the present study was that the initial washout rate in the osteoarthritic knee was greater than in the control. The maximum difference was seen about 16 weeks postoperatively. Thereafter the difference between the two sides diminished with time.

Methodological considerations and sources of error

Phelps et al. (1972) studied the washout of ^{133}Xe from the knee of dogs following intraarticular injection and found that the washout function was typically biexponential. By analyzing the location of ^{133}Xe in the joint during the course of the ^{133}Xe washout, they found that during the initial phase ^{133}Xe was cleared from the synovial membrane but accumulated in the subsynovial intraarticular fat tissue. This indicates that the in-

Figure 3. Blood flow distribution of ^{99m}Tc-microspheres 3 1/2 months postoperatively.
A,B: Scintigraphy illustrating the high accumulation of labeled microspheres in the osteoarthritic knee (frame ratio 1.9).
C,D: Macrophotos of the same knee. Osteophytes can be seen in the osteoarthritic knee.

itial decrease in ^{133}Xe activity was mainly due to washout of ^{133}Xe from the synovial membrane.

Using the data from Phelps et al. (1972), it could be calculated that the fast monoexponential component obtained after curve resolution accounted for about 90 per cent of the initial washout rate. It should be emphasized that the error due to ^{133}Xe accumulation in fat tissue is almost eliminated when the ratio of initial washout rates is used, as long as the effect of the slow component upon the initial washout function is equally weighted on both sides. This is apparently true for normal conditions (Phelps et al. 1972), but may not be quite fulfilled during our experimental conditions, as the content of intraarticular fat in the osteoarthritic knee could change with time. In order to test this possibility the washout of ^{133}Xe was followed for more than 30 minutes after intraarticular injection in seven experiments carried out at intervals of between 7 weeks to 96 weeks after the operation.

Like Phelps et al. (1972) we found that the ^{133}Xe washout curve followed a biexponential course. After curve resolution, it was found that the maximal error due to differences in the contribution of the slow component to the initial washout rate was about 10 per cent; thus the ratio of initial washout rates mainly reflects the relative washout rate of ^{133}Xe from the synovial membrane.

According to the Kety formula (Kety 1951) blood flow can be calculated as

$$f = \lambda \cdot k \cdot 100 \quad ml \cdot 100\ g^{-1} \cdot min^{-1}$$

where k is the washout rate constant in min^{-1} and λ is the tissue to blood partition coefficient in $ml \cdot g^{-1}$. Assuming that λ for the synovial tissue is equal on both sides, the relative blood flow can be calculated as

$$f_A/f_C = k_{init\ A}/k_{init\ C}$$

where $k_{init\ A}$ and $k_{init\ C}$ are the washout rates obtained from the initial monoexponential part of the washout curve measured in the osteoarthritic knee and the control knee, respectively. However, λ for the synovial tissue will decrease on the osteoarthritic side with a decrease in the fat content in the synovial tissue. Phelps et al. (1972) found that λ in the synovial tissue was 0.67 $ml \cdot g^{-1}$ with a fat content of 4 per cent and a hematocrit of 50 per cent. Assuming that all fat in the osteoarthritic knee is lost, which is unlikely, the maximal error in the calculation of relative blood flow would be about 40 per cent. Thus a decrease in λ on the osteoarthritic side tends to result in an overestimation of f_A/f_C with a maximal error of 40 per cent. The results can also be influenced by accumulation of fluid in the osteoarthritic knee. This error tends to result in an underestimation of f_A and thereby an underestimation of f_A/f_C. Phelps et al. (1972) found that increasing the intraarticular volume approximately tenfold had only a minimal influence on ^{133}Xe washout.

The initial part of the washout curve might be influenced by changes in counting geometry due to intraarticular re-distribution of ^{133}Xe. This error was tested by injecting ^{133}Xe during circulatory arrest. The count rate remained constant with time until circulation was restored which indicated that this error was insignificant (see Figure 1).

The minor error due to washout of ^{133}Xe via the lymph (Dick et al. 1970a) seems insignificant and was therefore ignored in the calculations of relative blood flow.

Thus the observed increase in $k_{init\ A}$, by about 500 per cent or more, can not be explained by the sources of error mentioned above. Thus, blood flow in the synovial tissue on the osteoarthritic side increases to a maximum level about 16 weeks postoperatively, compared to the control side. The increased accumulation of microspheres on the osteoarthritic side supports this conclusion. However, considering the sources of error conclusions can not be made regarding the slightly increased washout rates found more than 6 months postoperatively as being indicative of increased flow.

The initial hypervascularized period could be explained by posttraumatic synovitis, a condition still poorly defined. By comparing the synovial changes in clinical posttraumatic synovitis and osteoarthritis (Soren et al. 1976), the similarity of the histopathological changes suggested common pathogenic factors. Although small differences

exist, both conditions are characterized by some hypertrophy of the synovial lining cells and only focal perivascular infiltration with lymphocytes, indicating that the term synovitis is justified, but with some limitations.

The fibroblastic synovitis in mature osteoarthritis is further characterized by increased lysosomal activity in the lining cells (Reimann & Christensen 1979). It is still uncertain to what degree these changes are secondary, based on phagocytosis of cartilage fragments of split products (Lloyd-Roberts 1953), or are more primary in nature.

In this experimental model, however, a very early synovial reaction was seen with increased synovial perfusion, which coincided with the existence of joint effusion and the early development of osteophytes. This early reaction is seen not only in this model but also after sectioning of the anterior cruciate ligament (Gilbertson 1975). Joint effusion here was seen until 12 weeks postoperatively, but subsided thereafter. Finally, in the rabbit experimental model for osteoarthritis based on immobilization (Langenskiöld et al. 1979), osteophytes, joint effusion and traumatic synovitis are also seen (Finsterbush & Friedman 1973).

As the animal models are usually used for studies of the early stages of osteoarthritis, it should be stressed that the synovial reaction could contribute to the results obtained. The initial synovial reaction is most likely a consequence of the joint damage induced, but in addition it may be a participant in the pathogenetic mechanisms causing osteoarthritis. It is believed that even primary osteoarthritis may in the early stages be combined with or preceded by a synovial reaction resembling posttraumatic synovitis.

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