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EXPERIMENTAL OSTEOARTHRITIS IN THE RABBIT KNEE JOINT

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The development of arthrotic-like changes following resection of the cruciate ligaments in the knee joint of rabbits has been studied at intervals from 2 weeks to 10 months in 35 animals. Signs of cartilage degeneration were followed by changes in the subchondral bone, where formation of osteophytes and condensation took place. An increased vascular supply was demonstrated by microangiographic and scintigraphic investigations. The uptake of ^{18}F and ^{99m}Tc-polyphosphate reached a maximal value about 2 months after the operation and then diminished despite further development of arthrotic changes.

Key words: osteoarthritis; cartilage; subchondral bone; osteophytes; scintigraphy; blood flow

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In osteoarthritis, changes in blood circulation take place which may help to understand the processes involved. Thus the important part played by hypervascularization of the subchondral bone has been demonstrated by microangiographic investigations (Harrison et al. 1953) and scintigraphic results support the contention that the blood supply to the diseased joint is increased (Danielsson et al. 1963, Jeremy et al. 1969, Muheim & Crutchlow 1971). The presence of delayed venous outflow in osteoarthritis has been demonstrated by various methods (Ficat & Arlet 1975, Phillips 1966, Hernborg 1969, Arnoldi et al. 1972). Animal experiments have shown that venous obstruction results in an increased density of the subchondral bone (Brookes & Helal 1968, Lilly & Kelly 1970), and thus may initiate arthrotic changes. On the other hand, it is the general opinion that changes in the subchondral bone are secondary to degenerative processes in the joint cartilage which seem to give rise to an increased blood supply (Collins 1949, Sokoloff 1969, Freeman 1972).

In the present investigation the development of arthrotic-like changes, induced experimentally in the knee joint of rabbits, has been followed, with special attention being paid to the vascular reaction.

MATERIALS AND METHODS

Thirty-six adult male white rabbits, weighing about 4 kg, were submitted to an operation on the right knee using a modification of the method of Hulth et al. (1970). Under strictly sterile conditions the knee joint was opened by transection of the medial collateral ligament. The medial meniscus was removed *in toto* and both lig. cruciata resected. The joint was then unstable and in order not to provoke sub-

luxation the medial collateral ligament was resutured with plain catcut No. 4–0. Stability was further ensured by suturing of the fascia, and the skin was closed with atraumatic Nylon. The operation was performed under Nembutal anesthesia. In five animals, serving as controls, the same operation was performed, including removal of the medial meniscus, but the lig. cruciata were left intact. The animals recovered soon after the operation and in the course of a few days were able to move freely about the cage. One had to be excluded from the study because of destructive inflammation of the joint, but otherwise no infection occurred. Two animals died of intercurrent diseases.

The rabbits were sacrificed at intervals beginning at 2 weeks and extending to 10 months. From both knee joints the medial and lateral condyles of the femur and tibia were isolated and the patella removed, thus obtaining five different samples from each joint. After fixation in formalin and dehydration in alcohol most specimens were embedded in methylmethacrylate, but some were decalcified in formic acid followed by embedding in paraffin. Microradiographic investigation was performed on 100 μ thick specimens obtained by cutting with a saw and grinding. A Machlett O. E. G. 50. X-ray tube with Wolfram anode was used for contact exposures on Kodak Spectroscopic film 649–0. Sections of 8 μ thickness were obtained from both decalcified and undecalcified specimens with a Jung microtome and stained with toluidine blue, haematoxylin and eosin and soluchrome. In seven instances a microangiographic investigation was made according to the method of Trueta & Harrison (1953) with perfusion of 25 per cent micropaque through the abdominal aorta in the anaesthetized animal just prior to sacrifice. Tetracycline labelling was performed in a few animals using two i.m. injections (with an interval of 1 week) of 150 mg Reverin (Pyrrolidinomethyl-tetracyclin) before sacrificing.

Scanning with radioactive fluoride (^{18}F) or technicium labelled polyphosphate (^{99m}Tc-PP) was done in all animals one or several times postoperatively using a 5 inch Elscint whole body rectilinear scanner with videodisplay and processing for quantitative measurements. Contact autoradiographies were obtained by placing ½ mm thick sections on Kodak film P.E. 4006 using an ultrathin P.W. membrane between section and film.

The histological description includes the joint cartilage, the layer of calcified cartilage and the subchondral bone. Metachromasia was evaluated on toluidine blue stained sections in degrees from 3–0, where 3 indicates normal, 2 and 1 loss of metachromatic staining in the superficial and deeper parts of the cartilage, respectively, and

Table 1. Macroscopic and histologic findings on the medial femoral condyle.

Animal no.	Time after op.	Joint cartilage					Calcified cartilage		Subchondral bone			Collins* (degree)
		m. chr.	thickness (μ)	cls. frm.	fbr. lat.	erosion	thickness (μ)	perfor.	endplate (μ)	osteoid	osteophyte	
259	2 w	3	200	0	0	0	120	10	400	0	0	0
240	3 w	2	350	0	0	0	80	8	400	0	0	1
260	1½ m	1	200	++	++	0	50	8	150	+	+	2
261	2 m	1	200	+	+	+	100	10	300	++	0	2
258	3 m	2	300	+	+	+	120	11	500	+	+	3
242	4 m	2	500	+	+	+	100	10	450	+	+	3
255	5 m	2	300	+	+	++	100	10	1000	++	++	3
251	6 m	2	400	+	+	++	100	8	300	+	+	3
267	7 m	1	300	+	+	++	200	10	1500	++	+	3
236	8 m	1	600	+	+	++	300	11	600	+	+	3
266	9 m	0	400	++	++	+++			600	+	+	4
244	10 m	2	400	+	+	+	125	6	700	+	+	3

* Collins (1949).
m. chr. = metachromatic staining ability (see text).
cls. frm. = cluster formation of cartilage cells.
fbr. lat. = fibrillation.
perfor. = vessels perforation into the calcified cartilage from the subchondral bone (number per mm).

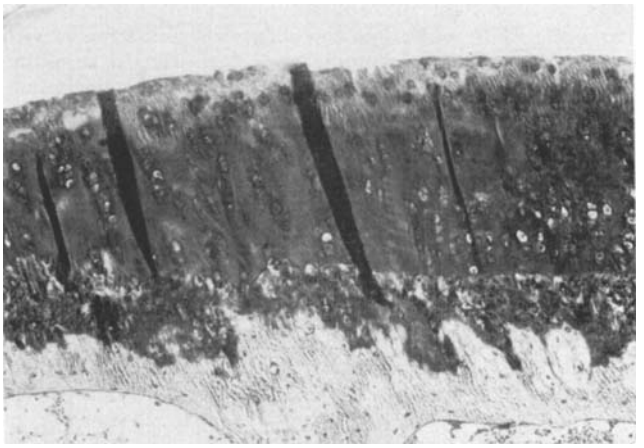

Figure 1. Cartilage from femoral condyle showing loss of metachromasia in the superficial layer, slight fibrillation and formation of cell clusters. (toluidine blue, × 100).

0 total absence of metachromasia. Measurements of the thickness of the cartilage and subchondral bone were made with a micrometric eye-piece. The number of vessels (per mm) perforating the calcified cartilage from the subchondral bone was evaluated from the microradiographs. From the macroscopic and histological findings the degree of arthrotic changes was allotted according to the grading of Collins (1949), where 0 signifies a smooth joint surface and 1–4 increasing degrees of cartilage degeneration and denudation of bone.

RESULTS

At the dissection the operated knee joint appeared swollen but only in a few cases was the amount of synovial fluid increased. The joint capsule was hypertrophic and along its attachment the bone was eroded. The medial meniscus had been partly replaced, whereas recovery of the cruciate ligaments was not seen. To the naked eye the cartilage surface appeared mostly dull, yellowish and often rough with signs of fibrillation. At a later stage the cartilage was eroded leaving the denudated subchondral bone shiny and smooth. This was especially seen on the top of the medial femoral condyle and in the centre of the medial tibial condyle. The macroscopic and histologic changes in the knee joint following the operation varied for the different joint surfaces. In Table 1 figures from the medial femoral condyle are given for a representative selection of animals arranged according to the time after operation. It is seen that as early as 3 weeks after the operation there was some decrease in the metachromatic staining of the cartilage. This was followed by further signs of degeneration including formation of cell clusters and fibrillation (Figure 1). At this stage an increased thickness of the calcified layer was sometimes observed with prolonged extensions into the subchondral bone. About 1½ months after the operation changes appeared also in the subchondral bone with

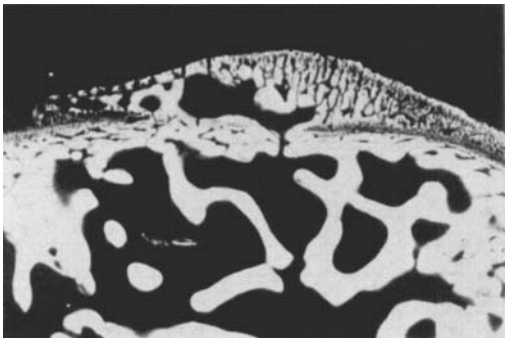

Figure 2. Microradiograph of femoral condyle showing formation of osteophytes and vessels perforating the calcified cartilage (× 25).

Table 2. Quantitative histological evaluation of the specimen.

Specimen		Joint cartilage m. chr.	Joint cartilage thickness	Calcified cartilage thickness	Calcified cartilage perf.	Bone end-plate thickness
Med. fem. condyle	r. (22)	1.7	*365 ± 126	121 ± 55	10	*618 ± 363
	l. (19)	3	249 ± 67	103 ± 30	9	269 ± 67
Lat. fem. condyle	r. (21)	2.3	*317 ± 117	114 ± 42	9	270 ± 134
	l. (16)	3	215 ± 82	101 ± 32	11	210 ± 63
Med. tib. condyle	r. (17)	2.0	664 ± 211	125 ± 65	11	530 ± 301
	l. (18)	3	623 ± 206	86 ± 31	10	404 ± 101
Lat. tib. condyle	r. (19)	2.4	362 ± 126	107 ± 38	13	289 ± 121
	l. (16)	3	305 ± 106	90 ± 44	12	241 ± 122
Patella	r. (15)	2.1	*481 ± 189	112 ± 33	15	318 ± 110
	l. (13)	2.8	257 ± 92	92 ± 17	13	275 ± 107

r. and l. = right and left joint respectively, the number of the specimen examined is given in brackets.
m. chr. = metachromatic staining ability (see text).
Thickness of joint cartilage, calcified cartilage and subchondral end-plate is given in microns.
Perf. signifies vessels perforating into the calcified cartilage from the subchondral bone (number per mm).
The values marked with * differ significantly from the control value ($P < 0.01$).

formation of osteophytes, first as exostoses along the edge of the cartilage, but later also in more central parts of the joint (Figure 2). The formation of osteophytes was associated with a vascular reaction, in which vessels from the subchondral bone penetrated the layer of calcified cartilage and initiated bone formation in the cartilage. A new ossification front was established while the old one gradually disappeared leaving the bone and joint surface elevated (Figure 2). The presence of hyperaemia in the bone tissue was demonstrated on microangiographic pictures (Figure 3) and the amount of osteoid tissue as well as tetracycline labelling (Figure 4) indicated increased bone formation. This ultimately resulted in sclerosis of the subchondral bone especially where the joint cartilage had been eroded with denudation of the bone.

Changes similar to those described take place on the medial tibial condyle and to a lesser extent on the lateral femoral condyle while only slight changes were seen on the lateral tibial condyle. On the patella, exostoses at the edge of the cartilage developed at an early stage in combination with a decrease in metachromatic staining and an increased thickness of the cartilage, which was more pronounced here than in other parts of the knee joint.

The quantitative results of the histologic investigations are summarized in Table 2, where mean values (with standard deviations) of the measurements on each of the different joint surfaces are given and compared with the corresponding measurements in the normal knee joint. It is seen that the thickness of the joint cartilage, on the average, increases somewhat on all joint surfaces, but the difference from the normal values is only statistically significant for the femoral condyles and the patella. As regards the calcified cartilage an increased thickness is generally observed, but the variations are too great for any conclusions to be

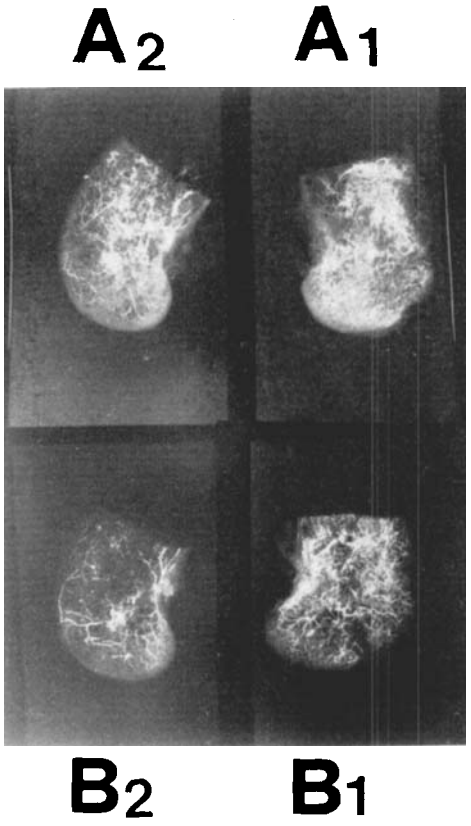

Figure 3. Microangiograph from the medial (A) and lateral (B) femoral condyles. Comparison between right (1) and left (2) sides.

drawn. The number of vessels penetrating into the calcified cartilage from the subchondral bone did not differ from normal values, but the individual perforations were enlarged. In the subchondral bone the thickness of the endplate increased on the average on all joint surfaces. This was most distinct on the medial femoral condyle, where the difference from normal values was statistically significant, especially for specimens obtained more than 3 months after the operation.

The results of the scintigraphic investigations with radioactive fluoride (F-18) and technicium polyphosphate (Tc-PP) are shown in Figure 5, where the ratio between the uptake in the operated and the normal knee joint is given as mean values with standard deviations. It is seen that the uptake of both F-18 and Tc-PP increased on the operated side during the first 2 months after operation to reach a maximal value for the ratio of about 2.7. During the following months the ratio slowly decreased to about 1.6 at 9–10 months after the operation. The uptake of F-18 differed only slightly from that of Tc-PP. Autoradiographic investigations showed that the uptake in the early stages was primarily localized to the periphery of the joint. At a later stage accumulations also took place in more central parts of the subchondral bone (Figure 6).

In the control animals, operated without resection of the cruciate ligaments,

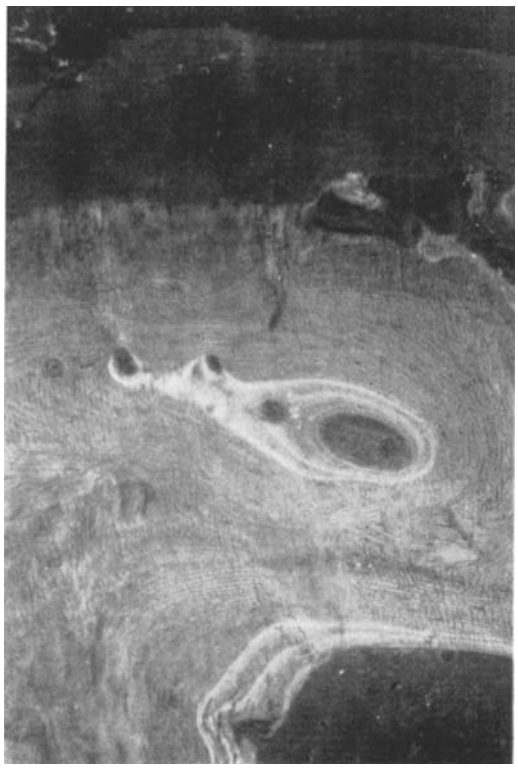

Figure 4. Subchondral bone from femoral condyle. Tetracycline labelling shows increased bone formation. (unstained, U-V light, × 100).

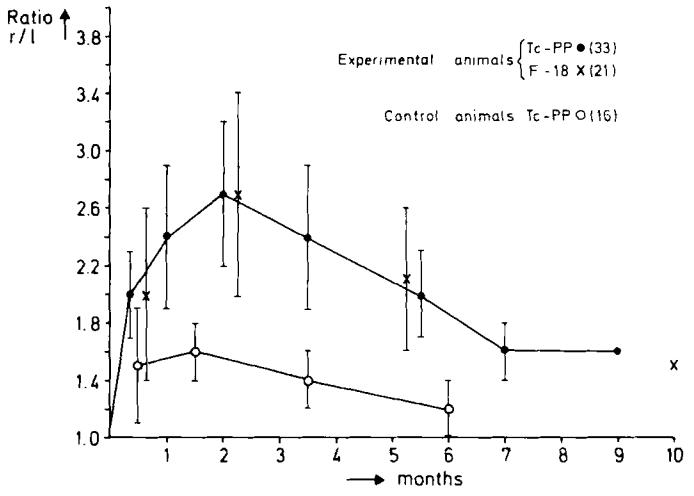

Figure 5. Results of scintigraphic measurements.

only slight histological changes occurred corresponding to Collins' degrees of 0–1. The uptake of F-18 and Tc-PP, although increased, was significantly less than in the experimental animals and the ratio declined to almost normal values during the course of about 6 months after the operation as seen in Figure 5.

DISCUSSION AND CONCLUSIONS

From the present investigation it is seen that changes similar to those observed in human osteoarthritis take place in the knee joint of rabbits following resection of the cruciate ligaments. This has previously been shown by Hulth et al. (1970) and similar results have been obtained by other methods (Salter & Field 1960, Trias 1961, Thompson & Bassett 1970, Meachim 1963, Lemperg et al. 1971 and Moscowitz et al. 1973). In accordance with observations on human osteoarthritis, signs of cartilage degeneration preceded changes in the subchondral bone. The increased ability for metachromatic staining is considered due to enzymatic degradation of mucopolysaccharides (Sokoloff 1969, Mankin & Lipiello 1970). The observed increase in thickness of the cartilage has also been described in human osteoarthritis (Meachim & Collins 1962) and seems due to proliferation of cartilage cells (Mankin et al. 1971, Hulth et al. 1972, Telhag 1972). In the subchondral bone changes are accom-

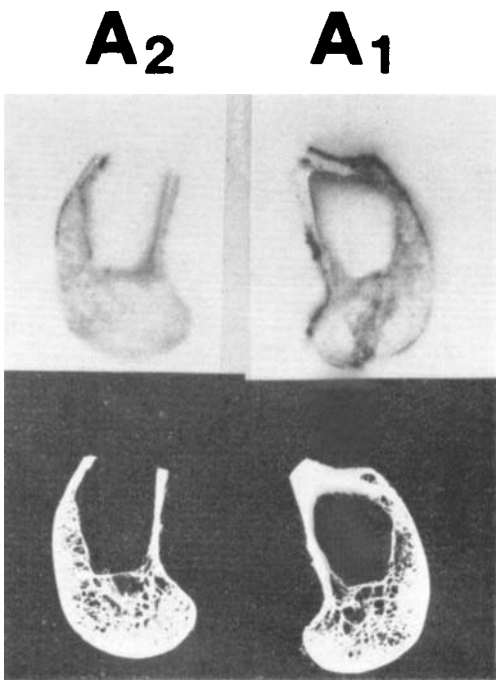

Figure 6. Autoradiograph above and radiograph below of femoral condyle. Right (1) and left (2).

panied by an increased blood supply demonstrated especially as regards the formation of osteophytes (Harrison et al. 1953). The uptake of F-18 and Tc-PP in the bone tissue, whether due to exchange processes or incorporation into apatite crystals, is generally considered to be primarily dependent upon the blood flow (Van Dyke et al. 1965, French & McCready 1967, Wootton 1974 and McGrail et al. 1974). From the scintigraphic results it therefore appears that the hyperaemia is connected with the development of arthrotic changes. The fact that the uptake of radioactive indicators decreases somewhat, despite further progression of the arthrotic changes, indicates that hyperaemia is less involved in these later processes including condensation of the subchondral bone (Danielsson et al. 1963). Although it seems from the present investigation that the hyperaemia is secondary to the degenerative processes in the cartilage, it cannot be excluded, however, that hyperaemia may be a primary cause of osteoarthritis.

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