

## THE HEALING OF CARTILAGE INJURIES UNDER THE INFLUENCE OF JOINT IMMOBILIZATION AND REPEATED HYALURONIC ACID INJECTIONS

### *An Experimental Study*

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In an experimental animal model the influence of intraarticularly injected high-molecular hyaluronic acid on the healing of superficial and deep lesions of the articular cartilage in freely mobile and immobilized joints was investigated. In the right knee joint in 42 adult rabbits two intracartilaginous lesions were produced in one of the femoral condyles and an osteochondral lesion in the other. In half of the animals the injured knee joint was immobilized in plaster of Paris, while the other half were allowed to move the joint freely. Half of the injured joints in each group were then injected with high-molecular hyaluronic acid once a week for 8 weeks. After 2 months the knee joints were examined histologically, histochemically and microangiographically and the water content of the articular cartilage was determined. The intracartilaginous lesions remained unchanged in all knee joints. The wound margins of the osteochondral lesions showed better closure in the immobilized animals. As a rule the osteochondral lesion healed with fibrous connective tissue. In a few joints, however, hyaline cartilage, with production of chondroitin sulphate, developed during healing of the osteochondral lesion. In the vicinity of the joint surface this tissue was transformed into fibrocartilage. Immobilization of the joint invariably led to pannus formation. The hyaluronic acid injections appeared to have no effect, either positive or negative, on the healing of intracartilaginous and osteochondral joint lesions. In mobile joints, however, these injections prevented a reduction of the water content of the articular cartilage, which was regarded as favourable.

*Key words:* articular cartilage; experimental injury; immobilization; hyaluronic acid

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It is usually considered that osteochondral joint lesions heal by invasion of connective tissue into the cartilage defect from the subchondral bone, with subsequent development into fibrocartilage (Bennet et al. 1932, Bennet & Bauer 1935, Campbell 1969, Lemperg & Hjertquist 1971, Puhl et al. 1973).

Sometimes metaplasia to hyaline cartilage has been observed (De Palma et al. 1966, Mitchell & Shepard 1976), although this cartilage gradually shows a greater resemblance to fibrocartilage (Lemperg et al. 1971, Mitchell & Shepard 1976). It has also been found that immobilization of a joint in which an

experimental osteochondral lesion has been produced results in growth of richly vascular connective tissue—pannus—over the articular cartilage from the subchondral bone, causing cartilage destruction (Wigren & Olerud 1971, 1973, Wigren 1975). Intra-articular lesions produced experimentally have not given rise to osteoarthritis and have not healed even after long periods of observation (Thompson 1974, 1975). It has been claimed that intra-articular injections of high-molecular hyaluronic acid promote the healing of cartilage lesions (Balazs 1974), and very good results in treatment of traumatic arthritis in race-horses has recently been reported (Åsheim & Lindblad 1976). In a previous study this substance, when injected in high doses, to some extent prevented articular cartilage degeneration caused by immobilization (Wigren et al. 1975). The aim of the present investigation was to examine, in a larger material of adult rabbit knee joints, the effect of high-molecular hyaluronic acid on the healing of superficial and deep experimental cartilage lesions treated with and without immobilization of the joint.

## MATERIAL AND METHODS

The study was performed on 42 adult rabbits of the Belgian Giant strain, 2–3 years old and weighing 3.5–6 kg. All rabbits were operated on under general barbiturate anaesthesia. The right knee joint was exposed through a lateral parapatellar incision. Using an oscillating saw, a 200 µm wide osteochondral lesion was produced longitudinally

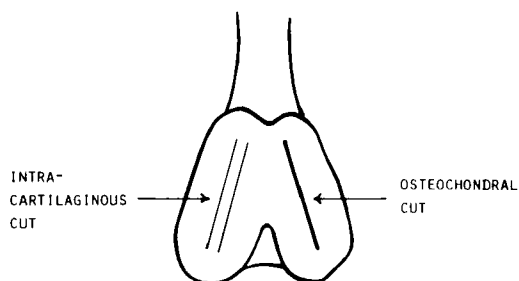


Figure 1. Schematic drawing of the location of the experimental injuries on the femoral condyles.

on the medial femoral condyle, and with a scalpel two parallel intracartilaginous incisions were made longitudinally on the lateral femoral condyle (Figure 1). The joint capsule was then closed with silk sutures applied without tension. No drainage was used. Twenty-one of the rabbits were immobilized with the knee joint in a neutral position in a plaster cast, applied from the hip to the tip of the paw, while the other 21 rabbits were allowed to move the knee joint freely. A window was left in the plaster over the medial side of the knee joint for intra-articular injections. In all animals the left knee joint was left intact as a control. Once a week, starting 3–6 days after the operation, 10 freely mobile and 10 immobilized knee joints were injected with 0.3 ml high-molecular hyaluronic acid (Sodium hyaluronate, concentration 10 mg/ml, molecular weight  $2 \times 10^6$ , Pharmacia AB), while the other injured knee joints were left untreated (Table 1). The treatment was continued for a total of 8 weeks, during which time the animals were allowed to move about freely in their cages. Within 1–2 weeks after the last injection, i.e., 2–2.5 months after operation, Indian Ink (Günter-Wagner Pelikan ink in isotonic saline solution) was infused under general anaesthesia, through a cannula inserted in the abdominal aorta,

Table 1. Macroscopic state of joint 2–2.5 months after osteochondral injury. (The number of hyaluronic acid injected joints is shown in the table.)

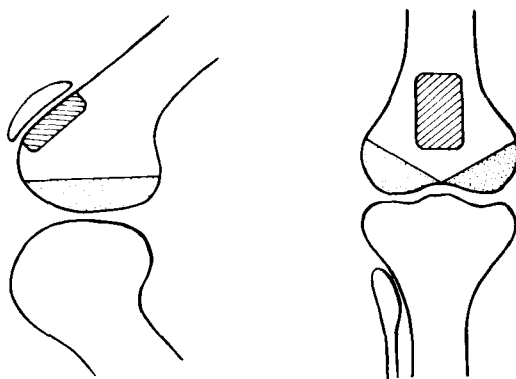
	Mobile joint n = 21		Immobilized joint n = 21	
	Inj. with hyaluronic acid n = 10	Not inj. n = 11	Inj. with hyaluronic acid n = 10	Not inj. n = 11
Vascular pannus over articular surface	1	3	8	5
Intra-articular adhesions	0	0	2	1

according to the method described by Olerud & Hult (1963). The animal died during this infusion, which was continued under positive pressure until 500–700 ml of the solution had been given. The knee joint was then opened and carefully examined with respect to any signs of infection or injection injuries. Specimens were taken from the medial and lateral femoral condyles for histological, histochemical and microangiographic examination. Using a scalpel, 20–40 mg of cartilage was taken from the femoral part of the femoro-patellar joint for analysis of its water content (Figure 2). Specimens for light microscopy and histochemical examination were fixed in 10 per cent neutral formalin, decalcified in a mixture of sodium citrate and formic acid, and embedded in paraffin. After serial sectioning, the sections were stained with haematoxylin-eosin, van Gieson stain for collagen, and Alcian Blue in the presence of 0.4 M and of 0.9 M  $MgCl_2$  (Stockwell & Scott 1965) for evaluation of the occurrence of chondroitin sulphate. Parts of the condyles were sawn into 0.5 mm thick slices and treated by the Spalteholz method (1914) for microangiography. For determination of the water content of cartilage specimens, these were placed in weighed plastic tubes which were then reweighed, and freeze-dried to a stable weight in an automatic freeze-dryer (Leybold-Heraeus GT 4). They were then weighed on a balance (Mettler H 20 T) with a precision of 0.1 mg.

## RESULTS

### *Macroscopic observations*

At the arthrotomy which completed the experiment 2–2.5 months after the operation, both the superficial and the deep cartilage lesions were visible in all injured joints. No signs of intraarticular injection injuries or infection were seen. In both immobilized and mobile hyaluronic-acid-treated knee joints the synovial fluid was normal in appearance and amount. In some of the mobile joints the tissue that had formed in the osteochondral lesion was strikingly light, i.e. poorly vascularized. Vascular pannus over the surface, arising from the osteochondral lesion or the margin of the articular surface, was seen in 4 of 21 mobile knee joints and in 13 of 21 immobilized joints. This difference is statistically significant ( $P < 0.01$ ). Of the four mobile joints with growth of pannus, one had been treated with



*Figure 2. Schematic drawing of the sites on the femoral condyles from which the specimens were taken. Dotted areas: Specimens for histology, histochemistry and microangiography. Hatched area: Specimens for determination of water content.*

hyaluronic acid, while in the 13 immobilized joints with pannus 8 had received this treatment (Table 1). Intraarticular adhesions were observed in a further three immobilized injured knee joints. Two of these had been treated with hyaluronic acid. Vascular pannus and/or intraarticular adhesions were thus found macroscopically in 4 out of 21 mobile joints and in 16 out of 21 immobilized joints. This difference is statistically significant ( $P < 0.001$ ).

### *Histology and histochemistry*

*The intracartilaginous lesion.* At examination of serial-sectioned specimens stained with haematoxylin-eosin and van Gieson's stain, all intracartilaginous lesions were found to be still open, with no healing reaction (Figure 3). No difference between the groups was seen in this respect. In no case did the scalpel incisions penetrate into the subchondral bone. The hyaline cartilage immediately bordering on the incision had a normal histological appearance and showed no reaction to the injury (Figure 3).

*The osteochondral lesion in mobile knee joints.* The wound margins had closed well in six

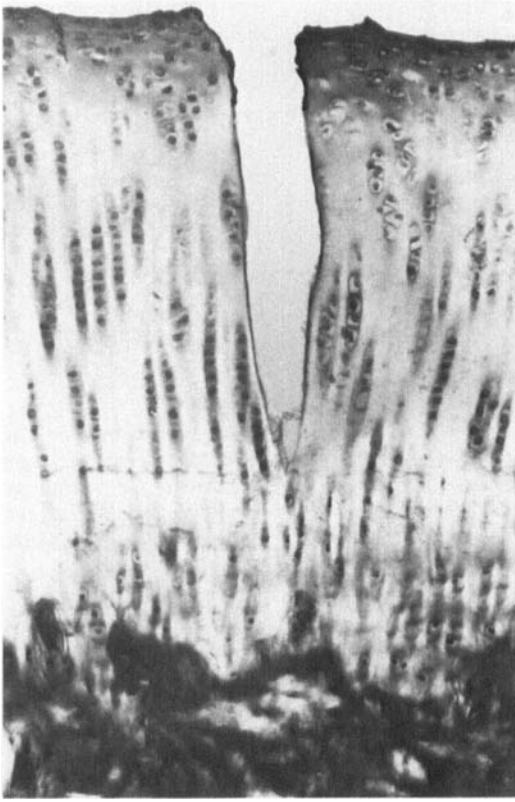


Figure 3. Open intracartilaginous lesion after 2½ months. The absence of a healing reaction is illustrated. Haematoxylin-eosin  $\times 100$ .

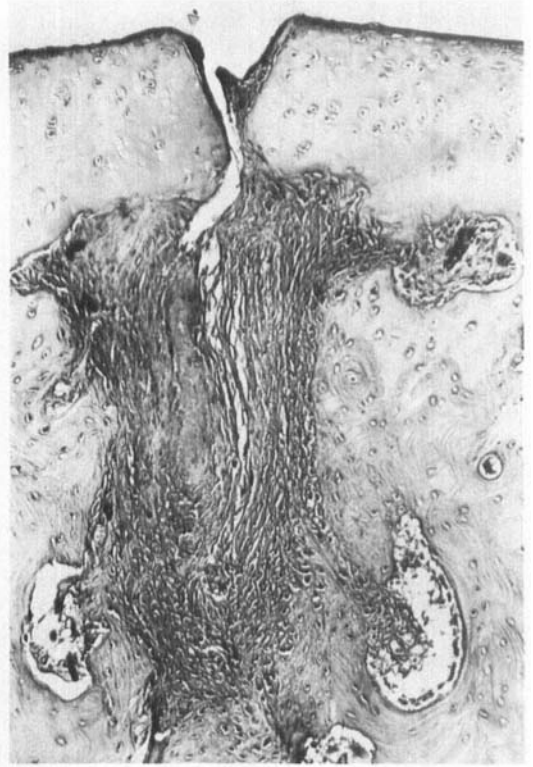


Figure 4. Fibrous connective tissue fills out the deeper parts of this osteochondral lesion. The superficial part remains open. The joint was mobile and treated with hyaluronic acid. Haematoxylin-eosin  $\times 63$ .

untreated joints and in four joints treated with hyaluronic acid (Table 2). This difference is not statistically significant. In the remaining injured joints the osteochondral wound was still more or less open to the cartilage surface.

In most cases a vascularized fibrous

connective tissue had formed in the osteochondral wound cavity during the healing process (Figure 4). In two joints each from the hyaluronic-acid-treated and the untreated group the newly formed tissue resembled hyaline cartilage, being avascular and having

Table 2. Status of the osteochondral wound 2–2.5 months after the lesion

	Mobile knee joints		Immobilized knee joints	
	Open wound	Closed wound	Open wound	Closed wound
Injected with hyaluronic acid	6	4	3	7
Not injected with hyaluronic acid	5	6	3	8

sparse fibrous components. In occasional joints the basal part of the cartilage defect was filled with bone tissue.

The blood vessels of the newly formed fibrous tissue communicated with vessels in the subchondral bone. In the deeper part of the lesion the collagen fibres ran upwards from the subchondral bone towards the surface of the cartilage, while in the superficial regions the fibres were mostly parallel with the articular surface. A moderate number of spindle-shaped fibrocytes were seen in the fibrous tissue. The intercellular substance did not stain with Alcian Blue.

In three lesions from either group, large oval or round chondrocytes were observed, surrounded by a territorial zone which stained strongly with Alcian Blue in the presence of 0.4 M but not 0.9 M  $MgCl_2$ , indicating production of chondroitin sulphate. The chondrocytes formed hyaline cartilage, mainly in the basal part of the lesion. In one case this

hyaline cartilage contained cluster formations, and a proliferation of the tissue, which was undergoing transformation to fibrocartilage, formed a mushroom-like structure over the articular surface (Figure 5).

The amount of newly formed tissue varied considerably from knee joint to knee joint. In this respect there appeared to be no difference between the untreated and the hyaluronic-acid-treated groups.

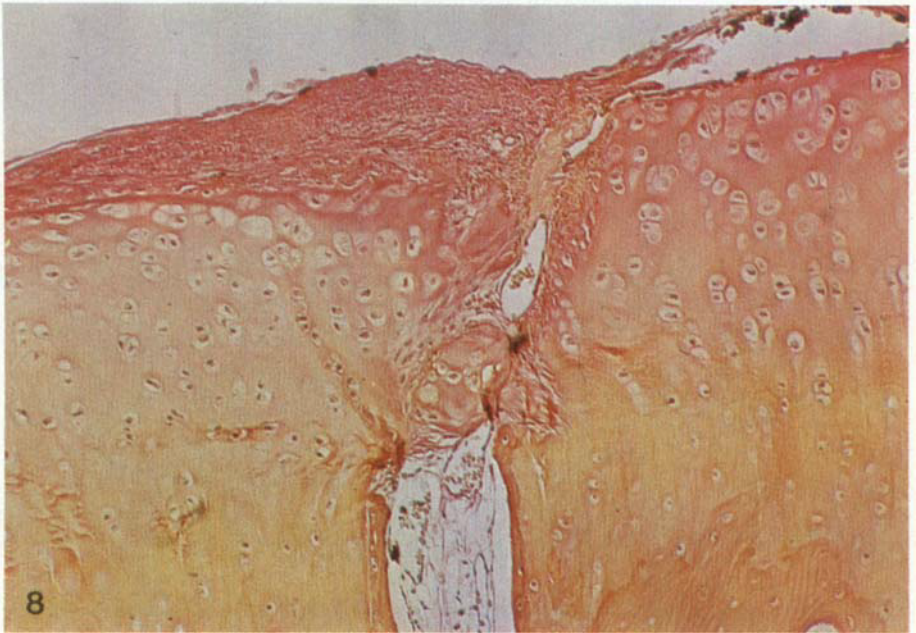
In five joints the newly formed fibrous tissue was most extensive at the level of the calcification zone of the cartilage. In most joints the fibrous tissue decreased in amount in the superficial parts of the lesion and its surface lay at a lower level than the surrounding articular surface (Figure 4). In two cases from either group the newly formed tissue had grown beyond the level of the joint surface.

The articular cartilage around the osteochondral lesion was apparently unaffected, apart from occasional cluster formations in



*Figure 5. From this osteochondral lesion an unusually large amount of fibrocartilaginous tissue has grown over the joint surface. Some clusters are seen at the right-hand margin of the proliferation. The joint was mobile and treated with hyaluronic acid. van Gieson  $\times 40$ .*

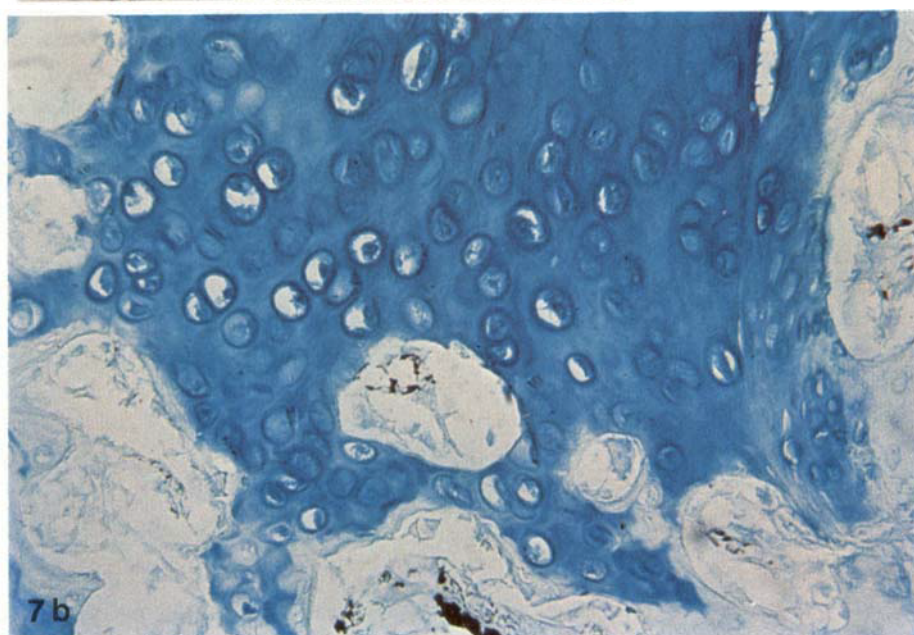
*Figure 6. Most of the wound cavity is occupied by red bone marrow. A thin bone bridge has formed between the bone marrow and the fibrous pannus on the joint surface of this immobilized joint. van Gieson  $\times 63$ .*



*Figure 8. Only a small amount of reparative tissue is seen in this osteochondral wound cavity but a prominent fibrous pannus covers the joint surface of the immobilized knee joint. van Gieson  $\times 40$ .*



*Figure 7a. Microphotograph of the healing of an osteochondral lesion with hyaline-like cartilage in the bottom of the lesion. Towards the surface the tissue has a more fibrous appearance. Alcian Blue (0.4M MgCl<sub>2</sub>) × 40.*



*Figure 7b. Detail from the base of the wound in Figure 7a. Large chondrocytes are seen. Their matrix is strongly stained with Alcian Blue at 0.4 M MgCl<sub>2</sub> but not at 0.9 M, indicating synthesis of chondroitin sulphate. The joint was immobilized but not treated with hyaluronic acid. Alcian Blue (0.4M MgCl<sub>2</sub>) × 160.*

one case. On the whole, the articular cartilage was histologically unchanged. Pannus growing over the articular surface was seen in only one of the joints.

*The osteochondral lesion in immobilized knee joints.* No difference was found between hyaluronic-acid-treated and untreated knee joints with respect to closure of the wound margins (Table 2). The total number of closed lesions (15), on the other hand, was significantly greater than the number of open lesions (6) ( $P < 0.05$ ). As in the mobile joints, the osteochondral lesion healed by formation of a vascularized fibrous tissue. In one non-injected joint, however, the greater part of the newly formed tissue was avascular and its basal portion resembled hyaline tissue, while the superficial portion showed characteristics of fibrocartilage. In seven joints the fibrous tissue was sparse and red subchondral bone marrow penetrated high up into the wound cavity (Figure 6). In 14 joints the fibrous tissue was separated from the red bone marrow by a bone bridge at the level of the calcification zone or surface layer of the cartilage (Figure 6). In these respects there was no difference between hyaluronic-acid-treated and untreated joints.

In the newly formed fibrous tissue a moderate number of spindle-shaped fibrocytes were seen, and superficially the collagen fibres ran parallel to the articular surface. The intercellular substance did not stain with Alcian Blue.

In one joint from either group a moderate number of large oval or round chondrocytes were observed in the basal part of the lesion. Their matrices stained strongly with Alcian Blue at the lower of the two electrolyte concentrations used, indicating production of chondroitin sulphate (Figure 7a, b).

As in the mobile group, the amount of newly formed tissue showed great individual variation and the two sub-groups did not differ in this respect.

It was difficult to evaluate the level to which the newly formed tissue had reached, as an outgrowth of connective tissue from the

osteotomy gap, spreading over the articular surface in the form of pannus (Figure 8), was prominent in 10 of 11 untreated knee joints and in all of those injected with hyaluronic acid.

The articular cartilage close to the lesion exhibited no histological signs of osteoarthritis. However, the articular cartilage as a whole showed areas of dead chondrocytes under the fibrous pannus and invasion of blood vessels into the cartilage from the subchondral bone as a reaction to the immobilization. The difference between immobilized injured and non-immobilized injured knee joints with respect to the development of pannus is statistically significant ( $P < 0.001$ ).

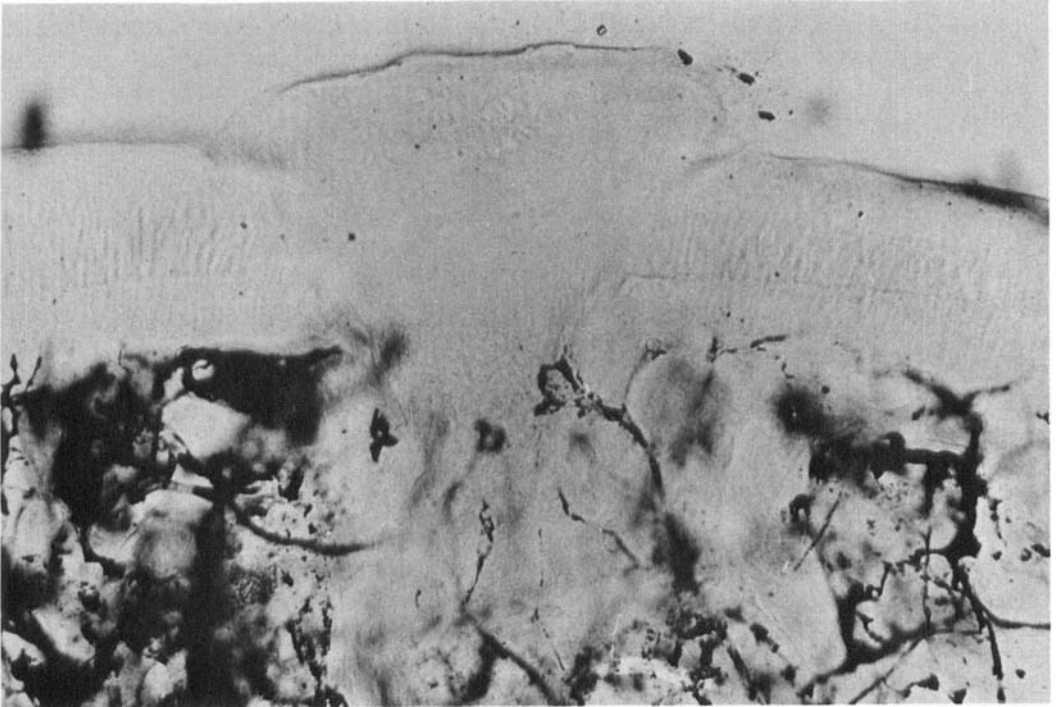
#### *Microangiography*

*Mobile knee joints.* The subchondral area of the osteochondral lesion was richly vascularized, with a growth of occasional blood vessels into the cartilage up to the tide-mark but not beyond it. The newly formed tissue in the gap between the articular surface and the tide-mark was very poorly vascularized. In specimens from the three joints which showed a protrusion of tissue with a hyaline appearance above the level of the articular surface there was no vascular invasion into this tissue (Figure 9). No growth of vascular pannus over the surface was observed.

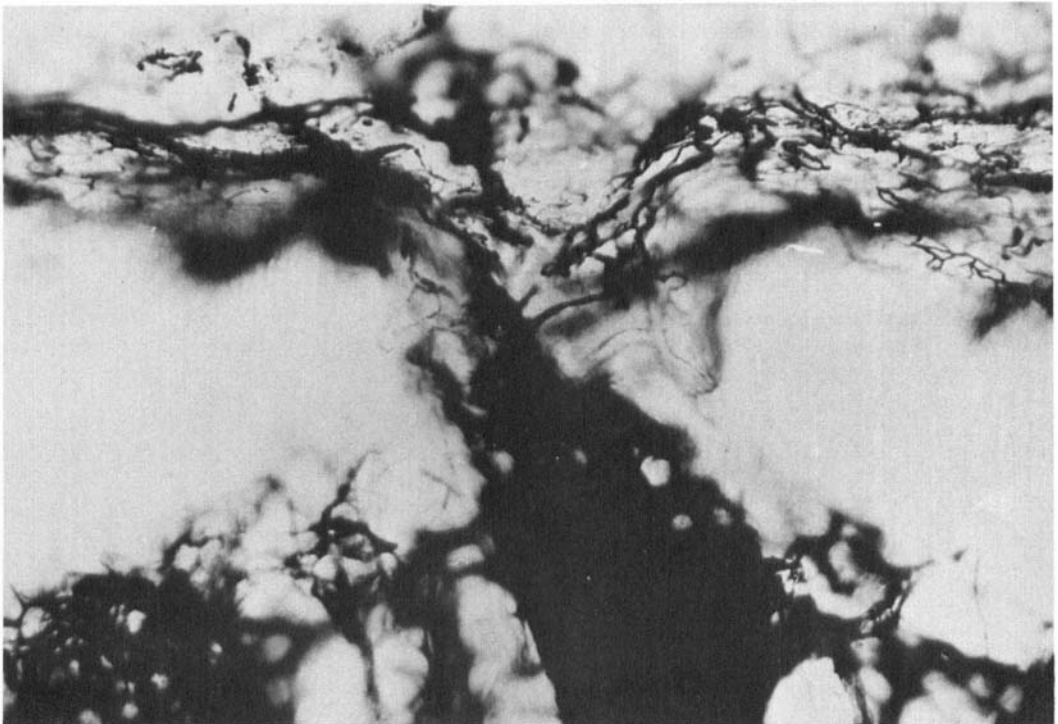
*Immobilized knee joints.* From a richly vascularized tissue in the subchondral region an abundance of blood vessels grew through the lesion to the articular surface and outwards over the surface of the cartilage in all joints (Figure 10). No difference was observed between hyaluronic-acid-treated and untreated joints. Invasion of blood vessels into the cartilage through the tide-mark was seen in specimens from several joints.

#### *Determination of the water content*

*Mobile knee joints.* Owing to technical mishaps, specimens for water content determination were only obtained from 17 of



*Figure 9. Microangiogram showing the absence of blood vessels in the articular cartilage and in the reparative tissue, which is also seen in Figure 5. Spalteholz  $\times 63$ .*



*Figure 10. Microangiogram showing an abundant growth of blood vessels from the lesion and out over the joint surface. Immobilized joint. Spalteholz  $\times 63$ .*

21 joints. The results are given in Table 3. In the group not treated with hyaluronic acid the water content of the articular cartilage was significantly lower in the injured joints ( $P < 0.01$ ) than in the intact joints of the same animals. In the hyaluronic-acid-treated group there was no significant decrease in this water content in the injured joint. Thus there was a significant difference between the water content of the articular cartilage in injured joints treated with repeated injections of hyaluronic acid and injured joints not injected with this substance ( $P < 0.05$ ).

*Immobilized knee joints.* Specimens were obtained from all joints. The results are given in Table 4. Neither untreated nor hyaluronic-acid-treated knee joints showed any significant change in the water content of the articular cartilage.

## DISCUSSION

The treatment of intraarticular injuries is a constantly recurrent problem in orthopaedics.

The question of whether or not osteochondral and intracartilaginous lesions heal with restoration of hyaline cartilage, and of whether degenerative changes of the articular cartilage develop, are of great importance. Immobilization of an injured joint, which is often necessary, may give rise to irreversible damage to the articular cartilage (Evans et al. 1960, Salter & Field 1960, Wigren & Olerud 1971, 1973, Finsterbush & Friedman 1973, Wigren & Wik 1974). High-molecular hyaluronic acid injected into an experimentally injured joint has been reported to promote healing (Rydell & Balazs 1971). It has been found that injections of this substance at short intervals counteract degenerative changes resulting from experimental immobilization (Wigren et al. 1975). The osteochondral lesion produced in the present experiments healed with development of fibrous connective tissue and in some cases fibrocartilage, in the same way as has been reported by Campbell (1969), among others. The white scar tissue observed macroscopically in the superficial cartilage has also been observed by Mitchell & Shepard (1976). They found that after 8 weeks this

*Table 3. The water content of the articular cartilage of adult rabbit femoral condyles 2–2.5 months after an experimental injury to the cartilage. Freely mobile knee joint. The influence of repeated intraarticular implantations of hyaluronic acid. The articular cartilage from the intact knee joint of each rabbit served as an individual control.*

Animal no.	% Water			Animal no.	% Water		
	Operation + hyal. acid	Control knees	Water content in % of normal		Only operation	Control knees	Water content in % of normal
13	70.0	70.1	99.9	2	63.5	68.1	93.2
17	67.9	68.4	99.3	3	67.3	67.1	100.3
18	68.2	67.5	101.0	4	58.8	67.6	87.0
19	69.2	69.9	99.0	7	69.5	69.3	100.3
23	62.7	68.0	92.2	8	56.3	68.8	81.8
25	69.4	67.7	102.5	9	60.8	64.6	94.1
30	64.0	58.6	109.2	11	68.4	68.5	99.9
				12	57.4	67.1	85.5
				28	63.6	64.6	98.5
				29	61.6	66.3	92.9
			$n = 7$				$n = 10$
			mean = 100.4				mean = 93.4
			S.D. = $\pm 5.1$				S.D. = $\pm 6.7$
			95 per cent confidence interval = 95.8–105.1				95 per cent confidence interval = 88.6–98.1

Table 4. The water content of the articular cartilage of adult rabbit femoral condyles 2–2.5 months after an experimental injury to the cartilage. Immobilized knee joint. The influence of repeated intraarticular implantations of hyaluronic acid. The articular cartilage from the intact knee joint of each rabbit served as an individual control.

% Water				% Water			
Animal no.	Operation + hyal. acid	Control knees	Water content in % of normal	Animal no.	Only operation	Control knees	Water content in % of normal
2	72.3	71.5	101.0	1	66.7	67.1	99.3
5	63.7	68.7	92.7	3	63.5	68.4	92.9
7	66.1	66.2	99.8	4	68.3	68.3	99.9
13	69.0	68.3	101.0	6	68.5	68.4	100.2
16	72.4	69.0	105.0	9	62.2	71.5	87.0
17	72.2	71.1	101.5				
19	73.8	73.3	100.7	15	68.5	71.0	96.5
20	70.1	70.1	100.1	18	72.1	69.8	103.2
23	70.6	72.8	97.0	21	75.2	73.6	102.0
26	68.2	68.7	99.2	22	70.1	68.9	101.8
				24	69.0	70.6	97.8
				25	66.0	66.4	99.4
			$n = 10$				$n = 11$
			mean = 99.8				mean = 98.2
			S.D. = $\pm 3.2$				S.D. = $\pm 4.7$
			95 per cent confidence interval = 97.5–102.1				95 per cent confidence interval = 95.0–101.3

tissue consisted of hyaline-like cartilage. Such healing was seen in only a few cases in our study. The relative duration of the observation period may be of significance for the type of tissue found, but Lemperg & Hjertquist (1971) and Mitchell & Shepard (1976) observed that hyaline-like cartilage occurring early in the healing process of a cartilage lesion underwent secondary changes to dense fibrous tissue. In the present study the cartilage defect closed more frequently in the immobilized than in the mobile joints and in all immobilized joints the newly formed scar tissue was more richly vascularized and grew over the articular surface as a pannus. This reaction is a consequence of the immobilization (Evans et al. 1960, Salter 1960, Wigren 1975). In the immobilized joints an invasion into the cartilage by blood vessels was also observed, as an indication of cartilage injury (Mankin 1975). No histological signs of osteoarthritis in the form of cluster formations or fibrillation were observed in the articular cartilage. The experimental lesion did not seem to have initiated osteoarthritis, as has been claimed

by Puhl & Dustmann (1973, 1976). Farkas et al. (1976) have denied such a development, however. It is widely agreed that normal mature articular cartilage does not show chondrocyte mitosis (Mankin 1963, 1974, Otte 1972). Our observation of an accumulation of chondroitin-sulphate-producing chondrocytes in the basal regions of the osteochondral lesion corresponds with the findings of Mitchell & Shepard (1976) and might be explained by a dividing capacity of the chondrocytes which have been freed from the cartilage matrix during the production of the experimental lesion. Differentiation of immature cells to chondroitin-sulphate-producing chondrocytes is another possibility – in this case induced by the trauma.

In a previous investigation (Wigren et al. 1975) immobilization of a joint was found to cause a reduction of the water content of the articular cartilage. This finding is in agreement with the changes observed by Akeson et al. (1974, 1975) in the connective tissue surrounding experimentally immobilized rabbit knee joints. In their study

a reduced water content was associated with a decreased content of hyaluronic acid, a decreased amount of soluble collagen and a decreased content of hexosamine. These changes were also accompanied by impairment of biomechanical properties (Akeson et al. 1975). In the present experiments injection of high-molecular hyaluronic acid counteracted a reduction of the water content of the articular cartilage in the experimentally injured, but mobile joint. How this effect is mediated is not clear (Wigren et al. 1975). The synovial membrane plays some role (Wigren et al. 1976). Adsorption of the greatly hydrophilic hyaluronic acid molecule to the surface of the articular cartilage may be another mechanism. In the immobilized joints the water content of the articular cartilage was not affected. This may have been due to technical difficulties in the sampling (Sokoloff & Hough 1975) or to concealment of a reduction in water content by richly vascular pannus over the cartilage surface, which might have accompanied and influenced the sample.

The intracartilaginous lesion had not healed and showed no reaction whatsoever after 2–2.5 months. This finding is in agreement with reports by Thompson (1974, 1975). No reaction of the subchondral bone beneath the lesion, as was described by Lemperg et al. (1971), was seen. The articular cartilage around the lesion was completely unaffected, and showed no degenerative changes. The need for serial sectioning of specimens in evaluating intracartilaginous lesions must be emphasized, as apparent closure of the wound margins may give the impression that the lesion has healed. Injections of high-molecular hyaluronic acid appeared to have no effect on the intracartilaginous lesion.

## REFERENCES

- Åsheim, Å. & Lindblad, G. (1976) Intraarticular treatment of arthritis in race-horses with sodium hyaluronate. *Acta vet. scand.* **17**, 379–394.
- Akeson, W. H., Woo, L. Y., Amiel, D. & Matthews, J. V. (1974) Biomechanical and biochemical changes in the periarticular connective tissue during contracture development in the immobilized rabbit knee. *Connect. Tiss. Res.* **4**, 315–323.
- Akeson, W. H., Woo, S. L.-Y., Amiel, D., Matthews, J. V. & Rutherford, L. (1975) Lubrication defect of immobilized connective tissue matrices. *Ann. rheum. Dis.* **34**, Suppl. 2, 100–103.
- Balazs, E. A. (1974) The physical properties of synovial fluid and the special role of hyaluronic acid. In: *Disorders of the knee*. Ed. Helfet, A., J. B. Lippincott Co., Philadelphia.
- Bennet, G. A., Bauer, W. & Maddoch, S. (1932) A study in the repair of articular cartilage. *Amer. J. Path.* **8**, 499–525.
- Bennet, G. A. & Bauer, W. (1935) Further studies concerning the repair of articular cartilage in dog joints. *J. Bone Jt Surg.* **17**, 141–150.
- Campbell, C. J. (1969) The healing of cartilage defects. *Clin. Orthop.* **64**, 45–63.
- Evans, E. B., Eggers, G. W., Butler, J. K. & Blumel, J. (1960) Experimental immobilization and remobilization of rat knee joints. *J. Bone Jt Surg.* **42-A**, 737–758.
- Farkas, T. A., Biro, T. & Bihari-Varga, M. (1975) Early changes in the composition of rabbit articular cartilage following experimentally produced intra-articular fractures. *Acta orthop. scand.* **46**, 716–726.
- Finsterbush, A. & Friedman, B. (1973) Early changes in immobilized rabbits knee joints. A light and electron microscopic study. *Clin. Orthop.* **92**, 305–319.
- Lemperg, R. & Hjertquist, S. O. (1971) Histological, autoradiographic and microchemical studies of spontaneously healing osteochondral articular defects in adult rabbits. *Calcif. Tiss. Res.* **8**, 54–72.
- Lemperg, R., Boquist, L. & Rosenquist, J. (1971) Intracartilaginous defects in adult sheep. *Virchows Arch. path. Anat.* **354**, 1–16.
- Mankin, H. J. (1963) Localization of tritiated thymidine in articular cartilage of rabbits III. Mature articular cartilage. *J. Bone Jt Surg.* **45-A**, 529–540.
- Mankin, H. J. (1974) The reaction of articular cartilage to injury and osteoarthritis. *New Engl. J. Med.* **291**, 1284–1292 and 1335–1340.
- Mitchell, N. & Shepard, N. (1976) The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J. Bone Jt Surg.* **58-A**, 230–233.
- Olerud, S. & Hulth, A. (1963) Morfologiska studier av callusbildningen. *Acta Soc. Med. upsalien.* **67**, 307–311.
- Otte, P. (1972) Die Verpflanzung von Gelenkknorpeln: Die Biologie des Gelenkknorpels im Hinblick auf die Transplantation. *Z. Orthop.* **110**, 677–685.

- de Palma, A. F., McKeever, C. D. & Subin, D. K. (1966) Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin. Orthop.* **48**, 229–242.
- Puhl, W., Dustmann, H. O. & Quosdorf, U. (1973) Tierexperimentelle Untersuchungen zur Regeneration des Gelenkknorpels. *Arch. orthop. Unfall-Chir.* **74**, 352–365.
- Puhl, W. & Dustmann, H. O. (1976) Altersabhängige Heilungsmöglichkeiten von Knorpelwunden. *Z. Orthop.* **114**, 749–764.
- Rydell, N. W. & Balazs, E. A. (1971) Effect of intraarticular injection of hyaluronic acid on the clinical symptoms of osteoarthritis and on granulation tissue formation. *Clin. Orthop.* **80**, 25–32.
- 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. & Hough, A. J. (1975) Tissue sampling as a potential source of error in experimental studies of cartilage. *Connect. Tiss. Res.* **3**, 27–31.
- Spalteholz, K. W. (1914) *Über das Durchsichtmachen von menschlichen und tierischen Präparaten*. Zweite Auflage. S. Hirzel, Leipzig.
- Stockwell, R. A. & Scott, J. E. (1965) Observations on the acid glycosaminoglycan (Mucopolysaccharide) content of the matrix of ageing cartilage. *Ann. rheum. Dis.* **24**, 341–350.
- Thompson, R. (1974/1975) Experimental evidence for an injury threshold for articular cartilage. In: *Conference on articular cartilage*, September 2–6, 1974. *Ann. rheum. Dis.* **34**, Suppl. 2, 140–142.
- Wigren, A. & Olerud, S. (1971) Reincorporation of an avascular articular surface bearing fragment. *Acta Soc. Med. upsalien* **76**, 63–76.
- Wigren, A. & Olerud, S. (1973) Reincorporation of an avascular articular surface bearing bone fragment. *Upsal. J. Med. Sci.*, Suppl. 14.
- Wigren, A. & Wik, O. (1974) The influence of hyaluronic acid on immobilized knees. *Upsal. J. Med. Sci.*, Suppl. 16.
- Wigren, A., Wik, O. & Falk, J. (1975) Repeated intraarticular implantation of hyaluronic acid. An experimental study in normal and immobilized adult rabbit knee joints. *Upsal. J. Med. Sci.*, Suppl. 17.
- Wigren, A. (1975) Experimental studies in immobilized knee joints. *Acta Univ. Upsal.* Thesis No. 231.
- Wigren, A., Wik, O. & Falk, J. (1976) Intraarticular injection of high-molecular hyaluronic acid. An experimental study on normal adult rabbit knee joints. *Acta orthop. scand.* **47**, 480–485.

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