Acta orthop. scand. 47, 480–485, 1976

INTRA-ARTICULAR INJECTION OF HIGH-MOLECULAR HYALURONIC ACID

An Experimental Study on Normal Adult Rabbit Knee Joints

ANDERS WIGREN, OWE WIK & JOHN FALK

Department of Orthopaedic Surgery, University Hospital, Uppsala, and Departments of Experimental Medicine and Toxicology, Pharmacia, Uppsala, Sweden.

High-molecular hyaluronic acid was injected repeatedly into normal knee joints of adult rabbits. Histologically the articular cartilage was not affected by this treatment. The content of chondroitin sulphate, assessed by a histochemical method, was not altered and neither was the water content of the hyaline articular cartilage. On the other hand, in the synovial membrane of joints treated with hyaluronic acid a diffuse infiltration of plasma cells and lymphocytes was observed, indicating that the hyaluronic acid administered exerts its action via the synovial membrane.

Key words: articular cartilage; normal joints; hyaluronic acid; implantation; synovial membrane

Accepted 29.vi.76

Hyaluronic acid is an essential component of synovial fluid and also of articular cartilage even if present in small amounts (Balazs et al. 1966, 1974, Hardingham & Muir 1972, Laurent 1972, Sundblad 1970). Its physical properties have been studied *in vitro* by Balazs (1974) who has shown, among other things, that high-molecular hyaluronic acid inhibits lymphocyte migration. The phagocyting and chemotactic capacities of the leucocytes are also inhibited (Brandt 1974). It has also been demonstrated that the permeability of the synovial membrane is reduced by administration of hyaluronic acid (Sundblad 1953, 1970). The large hyaluronic acid molecule has a very strong water-binding capacity (Ogston 1966, Laurent 1972) and variations in its concentration in hyaline cartilage should influence the water content of this cartilage.

Hyaluronic acid has been prepared in a high-molecular form and is available as sodium hyaluronate with a molecular weight of about 1.5×10^6. However, its mechanism of action is still unknown. The aim of the present investigation was to study the effect of repeated injections of high-molecular hyaluronic acid into normal adult rabbit joints over a period of 8 weeks.

MATERIAL AND METHODS

Twelve adult Dutch rabbits, 24 months old and weighing about 1.5 kg, were used for the experiment. They were kept in cages with a floor area of 45 × 55 cm. An injection of 0.3 ml of high-molecular hyaluronic acid (Sodium hyaluronate, concentration 10 mg per ml, produced by Phar-

macia AB, Uppsala, Sweden) was given into the right knee joint under sterile conditions twice weekly over a period of 8 weeks. The other knee was left untreated as a control. The rabbits were allowed to move freely in their cages. Four to five days after the last injection they were anaesthetized with urethane. Both the treated and the control joints were dissected free and inspected macroscopically with special regard to any damage caused by the injection or the presence of infection. Thereafter osteochondral specimens for histological and histochemical examination were taken from the femoral condyles with an oscillating saw. Using a scalpel, the articular cartilage was removed from the surface of the tibial condyles for determination of its water content. Specimens intended for histological and histochemical examination were fixed in 10 per cent neutral formalin and specimens for water-content determination were immediately placed in weighed plastic tubes. For histological evaluation of the synovial membrane, specimens were taken from the anteromedial part of the joint capsule and fixed in formalin. When all specimens had been removed, the animal was killed with an overdose of urethane.

After fixation, the osteochondral preparations were decalcified in a mixture of formic acid and sodium citrate, embedded in paraffin, serial-sectioned and stained with haematoxylin and eosin, van Gieson's stain and Alcian blue at a magnesium chloride concentration of 0.4 M (Stockwell & Scott 1965). The synovial membrane was embedded in paraffin, sectioned and stained with haematoxylin and eosin and van Gieson's stain.

The cartilage specimens from the tibial condyles were weighed on a rapid-weighing balance (Mettler H 20 T) with a precision of 0.1 mg. They were then freeze-dried to a stable weight and the percentage water content determined.

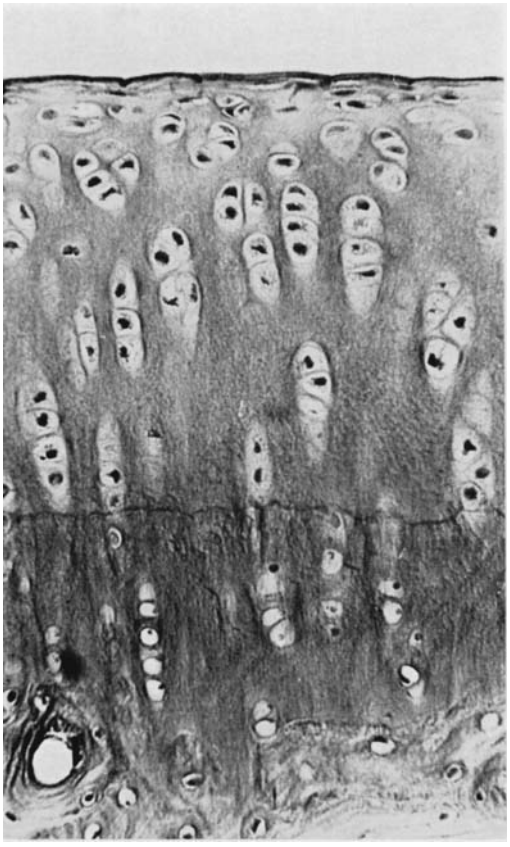

Figure 1. The articular cartilage, after repeated intra-articular injections of hyaluronic acid, is histologically normal.

RESULTS

The rabbits showed no negative reactions to the repeated intra-articular injections. None of them died during the experimental period and no infections occurred in the injected knee joints. When the joints were opened no signs of inflammation were seen macroscopically, but at the time of sacrifice several of the injected joints contained an increased amount of clear, very viscous fluid.

Histological and histochemical examination

The hyaline articular cartilage and subchondral bone of both knee joints were histologically normal, as evaluated from serial sections stained with haematoxylin and eosin and van Gieson's stain (Figure 1). No growth of pannus over the cartilage surface or vascular invasion of the cartilage from the subchondral bone was observed. Cartilage from the treated knee joint showed no deviations in territorial or inter-territorial staining with Alcian blue at 0.4 M $MgCl_2$ as compared with cartilage from the untreated joint in the same animal.

In the synovial membrane and sub-

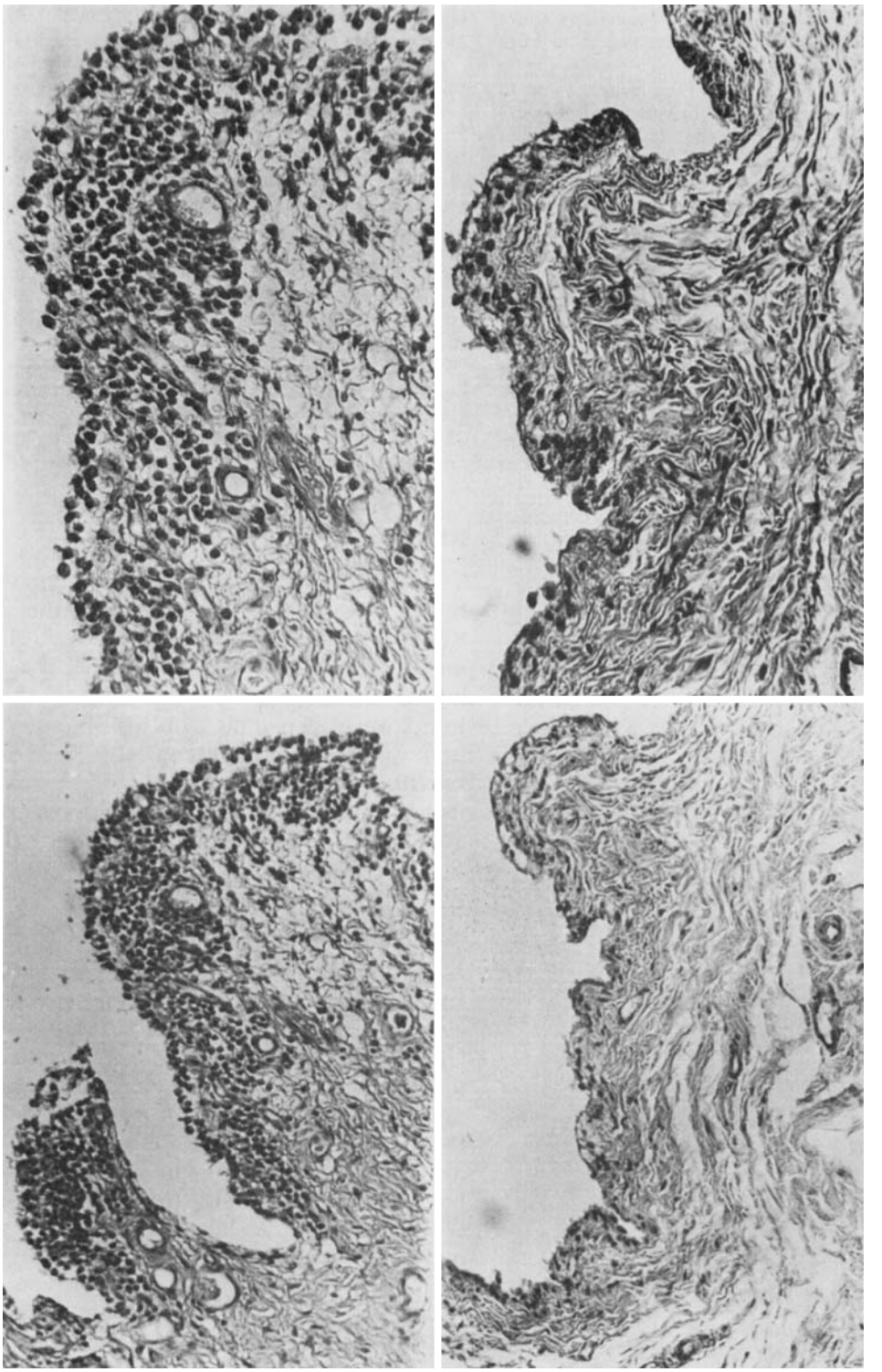

Figure 2. Upper row: Diffuse invasion of basophilic mononuclear cells in the synovial membrane from a knee joint injected with hyaluronic acid. Lower row: For comparison, synovial membrane from the untreated control knee joint of the same rabbit.

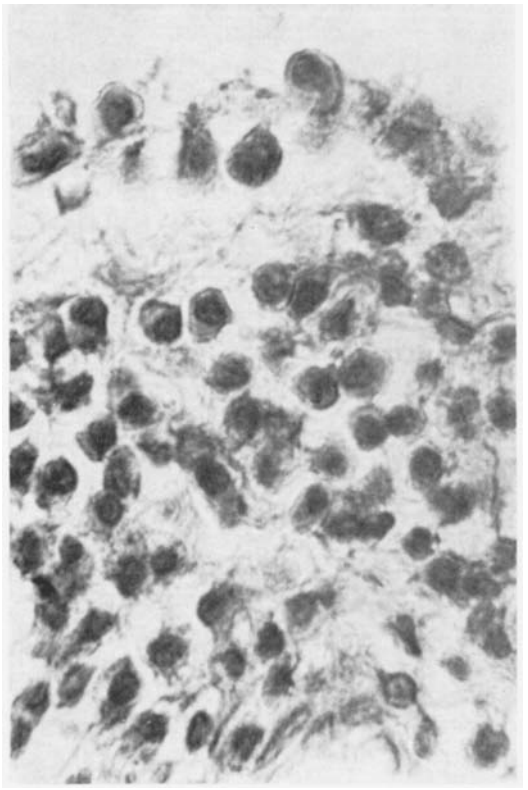

Figure 3. The basophilic mononuclear cells were identified as plasma cells and lymphocytes.

synovial tissue of all treated joints there was diffuse infiltration of basophilic mononuclear cells (plasma cells and lymphocytes; Figures 2, 3). In addition, occasional polymorphonuclear cells, so called pseudoeosinophilic cells, were seen. No corresponding cellular infiltration was observed in the synovial membrane and subsynovial tissue from the contralateral, untreated knee joints.

Determination of water content

No difference in consistency between articular cartilage from hyaluronic acid treated knee joints and cartilage from untreated knee joints was noted on cutting the cartilage. In Table 1 the water content of the cartilage from the hyaluronic acid treated joint is given as a percentage of that from the untreated joint. There was no significant difference in this respect.

Table 1. The water content of the articular cartilage after treatment with hyaluronic acid, expressed as a percentage of that in the untreated control knee joint. Implantation of high-molecular hyaluronic acid into the joint did not affect the water content of the normal articular cartilage.

Rabbit	Per cent
1	100.4
2	96.8
3	97.6
4	105.9
5	99.7
6	95.7
7	90.2
8	100.3
9	101.0
10	108.9
11	109.2
12	99.7

n = 12
mean = 100.5
S.D. = ± 5.45
95 per cent confidence interval = 97.0–103.9.

DISCUSSION

It seems reasonable to assume that the clear and very viscous fluid observed in several knee joints treated with hyaluronic acid also contained hyaluronic acid. This would indicate that the elimination time of hyaluronic acid, when injected repeatedly into the knee joints of rabbits, may be more than 4–5 days, and also that the absence of findings in this study was not due to under-dosage.

High-molecular hyaluronic acid has been found to have a favourable effect in various experimental articular cartilage injuries (Rydell & Balazs 1971, Wigren et al. 1975). Good results in the treatment of traumatic arthritis in race-horses (Rydell et al. 1970, Lindblad & Åsheim 1974, personal communication) and of degenerative joint disease in man (Helfet

1974, Peyron & Balazs 1975, personal communication) have also been reported. The effect has been ascribed to the surface-protecting properties of the hyaluronic acid (Balazs et al. 1966, Walker et al. 1970, Sundblad 1970). Antonas et al. (1973) demonstrated that intra-articularly injected hyaluronic acid was able to penetrate the articular cartilage of rabbits. The volumes injected (1–2 ml) have been shown, however, to cause a considerable increase in the intra-articular pressure (Wigren et al. 1975), whereas the volume used in the present experiment does not increase the pressure in the rabbit knee joint (Wigren et al. 1975). An intra-articular pressure increase can damage the joint (Smillie 1962) by causing distension injuries to the synovial membrane.

The normal histological appearance of the hyaline articular cartilage after repeated intra-articular injections of hyaluronic acid, over a period of 8 weeks, is consistent with the fact that no injurious effects of implantation of high-molecular hyaluronic acid on articular cartilage have been observed previously. Alcian blue in the presence of 0.4 M $MgCl_2$—the histochemical method used in this study —mainly stains the chondroitin sulphate in the cartilage (Stockwell & Scott 1965). With this method no changes in the content of chondroitin sulphate in the cartilage of the joints treated with hyaluronic acid were seen. Had the hyaluronic acid been able to penetrate into the articular cartilage, which is improbable in view of its large molecular size, a reduced content of chondroitin sulphate might have been expected, as Muir & Wiebkin (1973) have demonstrated *in vitro* that hyaluronic acid has an inhibitory effect on the synthesis of chondroitin sulphate in the chondrocyte.

The water content of articular cartilage, which normally is relatively constant in adult animals (Freeman 1972) is increased in osteoarthrosis (Mankin & Thrasher 1975). After immobilization of a joint in an adult animal this water content is reduced (Akeson et al. 1974, Wigren 1975). The water content of the normal articular cartilage in the present experiment was not altered by repeated injections of hyaluronic acid, possibly due to the inability of the hydrophilic hyaluronic acid molecule to penetrate into the healthy articular cartilage.

The diffuse infiltration of basophilic mononuclear cells into the synovial membrane did not give rise to any macroscopic signs of inflammation. The reaction observed histologically could only partly have represented unspecific irritation, as a corresponding reaction occurred to only a minor extent after repeated injections of physiological saline (Falk 1974, personal communication).

In the latter study two equal-sized groups of adult rabbits were injected intra-articularly with hyaluronic acid or saline. One hundred per cent of the hyaluronic acid injected joints showed moderate plasma cell infiltration of the synovial membrane while a similar though slight reaction was found in only 43 per cent of the saline injected joints.

The finding of infiltration of mononuclear cells is partly in accord with observations on synovial fluid from racehorses treated with hyaluronic acid (Rydell et al. 1970) and indicates that the effect of high-molecular, intra-articularly implanted hyaluronic acid in certain types of osteoarthrosis and traumatic arthritis could be mediated via the synovial membrane. The synovial changes after repeated injections of hyaluronic acid over 8 weeks, together with previously observed effects on cells of the lymphomyeloid system *in vitro*, indicate that intra-articular administration of hyaluronic acid could influence the synovial membrane through a cellular mechanism and might therefore be worth testing in experimental and clinical synovitis. The question could be raised as to

whether the observed infiltration of plasma cells is an expression of an immunological reaction. However, with the aid of the Passive Cutaneous Anaphylaxis test (Richter 1974), no formation of PCA reactive antibodies against this or other hyaluronic acid preparations was observed during or after immunization.

REFERENCES

Akeson, W. H., Woo, S. 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.

Antonas, K. N., Fraser, J. R. E. & Muirden, K. D. (1973) Distribution of biologically labelled radioactive hyaluronic acid injected into joints. *Ann. rheum. Dis.* **32**, 103.

Balazs, E. A., Bloom, G. & Swann, D. (1966) Fine structure and glycosaminoglycan content of the surface layer of articular cartilage. *Fed. Proc.* **25**, 1813.

Balazs, E. A. & Darzynkiewics, Z. (1973) The effect of hyaluronic acid on fibroblasts, mononuclear phagocytes and lymphocytes. In: *The biology of fibroblasts*, p. 237. Academic Press, London.

Balazs, E. A. (1974) The physical properties of synovial fluid and the special role of hyaluronic acid. In: *Disorders of the knee*. Ed. A. Helfet. J. B. Lippincott Co., Philadelphia.

Brandt, K. D. (1974) The effects of synovial hyaluronate on the ingestion of monosodium urate crystals by leucocytes. *Clin. chim. Acta* **55**, 307.

Freeman, M. A. R. (1972) *Adult articular cartilage*, p. 15. Grune & Stratton, New York.

Hardingham, T. E. & Muir, H. (1972) The specific interaction of hyaluronic acid with cartilage proteoglycans. *Biochim. biophys. Acta (Amst.)* **279**, 401.

Helfet, A. (1974) *Disorders of the knee*. J. B. Lippincott Co., Philadelphia.

Laurent, T. C. (1972) The ultrastructure and physical-chemical properties of interstitial connective tissue. *Pflügers Arch. ges. Physiol.* **336**, 21.

Mankin, H. J. & Thrasher, A. Z. (1975) Water content and binding in normal and osteoarthritic human cartilage. *J. Bone Jt Surg.* **57**-A, 76.

Muir, H. & Wiebkin, O. W. (1973) The inhibition of sulphate incorporation in isolated adult chondrocytes by hyaluronic acid. *Febs Letters* **37**, 42.

Ogston, A. G. (1966) On water binding. *Fed. Proc.* **25**, 986.

Richter, W. (1974) Non-immunogenicity of purified hyaluronic acid preparations tested by passive cutaneous anaphylaxis. *Int. Arch. Allergy* **47**, 211.

Rydell, N. W., Balazs, E. A. & Butler, J. (1970) Hyaluronic acid in synovial fluid. *Acta vet. scand.* **2**, 139.

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.

Smillie, I. S. (1962) *Injuries of the knee joint*, 3rd. ed. p. 22. Churchill & Livingstone, Edinburgh and London.

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.

Sundblad, L. (1953) Studies on hyaluronic acid in synovial fluids. *Acta Soc. Med. upsalien.* **58**, 197.

Sundblad, L. (1970) Ledvätskans biokemi. *Opusc. med. (Stockh.)* **15**, 33.

Walker, P. S., Unsworth, A., Dowson, D., Sikorski, J. & Wright, V. (1970) Mode of aggregation of hyaluronic acid protein complex on the surface of articular cartilage. *Ann. rheum. Dis.* **29**, 591.

Wigren, A., Wik, O. & Falk, J. (1975) Repeated intraarticular implantation of hyaluronic acid. *Uppsal. J. med. Sci.*, Suppl. 17.

Wigren, A. (1975) Experimental studies in immobilized knee joints. *Acta Univ. Uppsal.* **231**.

Correspondence to: Anders Wigren, M.D., Department of Orthopaedic Surgery, University Hospital, 750 14 Uppsala, Sweden.