Sulphated glycosaminoglycan synthesis in normal and osteoarthrotic hip cartilage

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SUMMARY The *in vitro* sulphated glycosaminoglycan metabolism and uronate content of human aged and osteoarthrotic hip articular cartilages have been examined. Cartilages were sampled from nonfibrillated weight-bearing areas and cultured in Dulbecco Modified Eagle's medium with $^{35}\text{SO}_4$. Uptake of $^{35}\text{SO}_4$ was corrected for total sulphate concentration and related to uronate levels after papain digestion and dialysis. The levels of uronate in these tissues varied between 0.96% and 2.26% dry weight and did not correlate with either osteoarthrotic grading or sulphated glycosaminoglycan metabolism, irrespective of the source. Glycosaminoglycan metabolism of intact hip articular cartilage from clinically defined osteoarthrotics appears, from these results, to be comparable to that derived from nonsymptomatic hip joints from individuals of similar age. Sodium salicylate was found to depress sulphated glycosaminoglycan metabolism in osteoarthrotic cartilage.

The pathophysiology of osteoarthritis is now well defined. In the early stages it is characterized by softening of articular cartilage followed by fibrillation and loss of the extracellular proteoglycans (McDevitt, 1973; Mankin, 1974). The hydrated proteoglycans of articular cartilage are responsible for its resilience and self lubricating ability (Maroudas, 1973), and their depletion leads to impaired mechanical function (Kempson *et al.*, 1970, 1973).

The chondrocyte response to depletion of its surrounding matrix has been the subject of investigation for many years. Early autoradiographic studies by Collins and McElligott (1960) showed that chondrocytes in osteoarthrotic cartilage exhibited increased $^{35}\text{SO}_4$ uptake. Studies of osteoarthrotic articular cartilage by Mankin and co-workers (Mankin and Lippiello, 1970; Mankin *et al.*, 1971) led these authors to conclude that the synthetic activity of the cartilage cell is increased and it makes some attempt to replace the proteoglycan depleted from the surrounding matrix. Recently, Maroudas (1975) has, however, shown that where articular cartilage is severely fibrillated, $^{35}\text{SO}_4$ uptake is less than normal and uptake in mildly fibrillated cartilage appears to be the same as normal.

These findings are in agreement with those of Bollet and Nance (1966) and Lust *et al.* (1972).

As part of our studies on the effects of anti-inflammatory drugs on human cartilage metabolism (McKenzie *et al.*, 1976a), we compared the sulphated glycosaminoglycan metabolism in human aged normal and osteoarthrotic hip cartilages. The results suggest that comparable rates of sulphated glycosaminoglycan synthesis exist in osteoarthrotic and 'normal' cartilage from subjects in the same age range.

Materials and methods

SOURCE OF ARTICULAR CARTILAGE

Human articular cartilage was obtained from femoral heads removed surgically for osteoarthritis or after subcapital fracture of the femur. 10 normal and 6 osteoarthrotic femoral heads from donors of known age were studied and classified on the grading system of Collins (1949) according to the severity of osteoarthrotic changes. As far as could be ascertained, patients admitted for fracture of the femur had not taken anti-inflammatory drugs one week before surgery. Osteoarthrotics, however, were known to use anti-inflammatory drugs on a regular basis.

For 'normal' femoral heads, cartilage was sampled from the superior surface. Osteoarthrotic femoral heads frequently showed complete erosion of cartilage in this area, and cartilage samples were taken from

adjacent areas which were free of osteophytes or severe visible fibrillation. Maroudas *et al.* (1973) found no significant differences in glycosaminoglycan content over the area of the femoral head, suggesting that the area of cartilage sampled is not critical.

CULTURE METHOD

This procedure has been described in detail elsewhere (McKenzie *et al.*, 1976b). Full depth slices of cartilage 1 mm thick were maintained in organ culture, and experiments on $^{35}\text{SO}_4$ uptake were carried out on the first or second day after cultures were set up when viability is maximal (McKenzie *et al.*, 1976b). The culture medium used was Dulbecco Modified Eagle's minimum essential medium supplemented with 10% fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Victoria).

INCORPORATION OF $^{35}\text{SO}_4$ INTO SULPHATED GLYCOSAMINOGLYCANS

The synthesis of sulphated glycosaminoglycans in 'normal' and osteoarthrotic articular cartilage was estimated by determining the extent of incorporation of $^{35}\text{SO}_4$ into these macromolecules over a 6-hour period. The method, which is described in more detail elsewhere (McKenzie *et al.*, 1976b), consisted of the addition of $^{35}\text{SO}_4$ (Radiochemical Centre, Amersham, UK) to the medium 24 hours after initiation of the cultures, to a final concentration of 40 $\mu\text{Ci/ml}$. Three cartilage slices were removed at hourly intervals over the 6-hour experimental period. Incubations were terminated by placing cartilage slices in 5 ml ice-cold physiological saline containing nonradioactive sulphate (1 mg/ml) as a chaser. The saline was changed three times at 10-minute intervals to remove the majority of unincorporated sulphate from the cartilage. Slices were dehydrated in acetone and alcohol and dried under vacuum at 60°, then dry weight determined. Solubilization of the tissue was achieved by digestion of each slice with 1 ml crystalline papain (Merck, 1.5 mg/ml), in 0.1 M acetate buffer pH 5.6, containing 0.05 M EDTA, and 0.005 M cysteine. The digests were dialysed overnight against water to remove the remaining unincorporated $^{35}\text{SO}_4$. The radioactivity associated with the glycosaminoglycans present in aliquots of the digest was determined by standard liquid scintillation counting techniques using a Triton X-100/toluene scintillation mixture. The uronic acid content of the dialysed digests were determined by the method of Bitter and Muir (1962). The extent of incorporation of $^{35}\text{SO}_4$ into glycosaminoglycans was expressed as cpm/ μg uronic acid for each tissue slice. Results were analysed statistically as described by McKenzie *et al.* (1976a).

EFFECT OF SODIUM SALICYLATE ON SULPHATED GLYCOSAMINOGLYCAN SYNTHESIS IN AGED AND OSTEOARTROTIC ARTICULAR HIP CARTILAGE
The effect of varying concentrations of sodium salicylate on sulphated glycosaminoglycan synthesis was determined using cartilage from an osteoarthrotic femoral head from a patient aged 35 years. This was compared with results published previously (McKenzie *et al.*, 1976a) on the effects of sodium salicylate on articular cartilage from 3 'healthy' femoral heads. The concentrations of sodium salicylate studied were 5.0, 2.5, and 1.0 mmol/l, which encompasses the serum therapeutic levels reached by this substance in man (Domenjoz, 1971).

Results

RATE OF $^{35}\text{SO}_4$ INCORPORATION INTO SULPHATED GLYCOSAMINOGLYCANS OF NORMAL AND OSTEOARTROTIC HIP CARTILAGE IN VITRO

The rate of incorporation of $^{35}\text{SO}_4$ into all samples of aged and osteoarthrotic cartilage was measured over a 6-hour period, 24 hours after initial incubation. The $^{35}\text{SO}_4$ in the incubation media was calculated as parts per million of total inorganic sulphate present for each experiment. As the measured radioactivity only reflects a proportion of total $^{35}\text{SO}_4$ utilized in synthesis, a correction factor was used and the rate of SO_4 uptake determined from the expression

$$\text{Rate} = \frac{\text{Radioactivity/min}}{\text{Uronic Acid } (\mu\text{g})} \times \frac{10^6}{\text{ppm } ^{35}\text{SO}_4} \times \frac{1}{\text{Time (h)}}$$

While no allowance was made of the incorporation of $^{35}\text{SO}_4$ into keratan sulphate, it was considered on the basis of its low metabolic half life (Maroudas, 1975) not to affect our calculations to any great extent.

The results obtained for $^{35}\text{SO}_4$ uptake into cartilage from 16 femoral heads, together with uronic acid content, age, classification, and OA grading are shown in the Table. As the Table shows there is no measurable difference between the rate of $^{35}\text{SO}_4$ uptake into hip articular cartilage classified as normal or osteoarthrotic grades I-III. The total uronic acid content varied between 0.96% and 2.26% on a dry weight basis. There was no observable correlation between uronic acid level and rate of $^{35}\text{SO}_4$ uptake whether 'normal' grade 0, or grades I-III. The effect of sodium salicylate over the concentration range 1.0-5.0 mmol/l on sulphated glycosaminoglycan synthesis in osteoarthrotic cartilage is shown in the Fig. As was previously reported (McKenzie *et al.*, 1976a), sodium salicylate depresses the synthesis of these macromolecules in normal aged articular cartilage, and our results confirm that this is also true for osteoarthrotic cartilage.

Table Results of $^{35}\text{SO}_4$ uptake into cartilage of 16 femoral heads

Classification	OA Grading (Collins, 1949)	Age (years)	Uronic acid (% dry wt)	Rate of $^{35}\text{SO}_4$ uptake/min ($\mu\text{g/h}$) $\times 10^{-1}$ *
F	0	67	2.06	11.3
F	0	78	1.94	8.5
F	I	63	1.60	1.5
F	I	70	1.75	3.6
F	I	70	1.82	1.1
F	I	71	1.33	11.1
OA	I	78	1.99	3.9
F	I	79	1.92	9.8
F	I	83	1.20	2.7
OA	II	70	1.27	1.2
OA	II	80	1.52	2.2
F	II	84	2.26	7.0
OA	III	35	2.17	12.5
OA	III	51	0.96	12.2
OA	III	72	1.40	3.8
F	III	84	1.67	7.0

F=surgically removed for fracture of neck of femur; OA=surgically removed due to osteoarthritis.

* Rate of $^{35}\text{SO}_4$ uptake over a 6-hour period was adjusted for total sulphate concentration and its distribution in media and cartilage (Maroudas, 1975) according to the expression—

$$\text{Rate} = \frac{\text{Radioactivity/min}}{\text{Uronic Acid } (\mu\text{g})} \times \frac{10^6}{\text{ppm } ^{35}\text{SO}_4} \times \frac{1}{\text{Time (h)}}$$

Fig. Effects of sodium salicylate at various concentrations on the uptake of $^{35}\text{SO}_4$ into osteoarthrotic cartilage of a femoral head.

Discussion

Osteoarthritis is characterized clinically by pain, stiffness, and certain radiological criteria (Sokoloff, 1969). As articular cartilage is aneural, avascular, and alymphatic, pain must arise from the synovium, joint capsule, and other periarticular tissues but the exact mechanisms of pain production are unknown. From the present studies it is apparent that there is

no clear relationship between the severity of cartilage damage, assessed by the grading method of Collins (1949), and the appearance of symptoms characteristic of osteoarthritis, thus, 8 of 9 hips of graded type I and 2 out of 7 graded II–III were derived from patients who came to surgery for fracture of neck of femur and had no previous joint symptoms (Table). The rates of incorporation of $^{35}\text{SO}_4$ into glycosaminoglycans were found to be independent of the grading. It should be noted that in all instances visually intact cartilage was sampled and fibrillated areas avoided. This, however, may not be necessary criteria for cartilage integrity, as noted by McDevitt and Muir (1976) who reported that alteration in cartilage glycosaminoglycans may occur before macroscopical change is apparent. While these findings are in reasonable agreement with the studies of Maroudas (1975), Lust *et al.* (1972), and Bollet and Nance (1966), they contrast sharply with those of Mankin and co-workers (Mankin and Lippiello, 1970; Mankin *et al.*, 1971; Mankin, 1974) and Mayor and Moskowitz (1974) who consider that $^{35}\text{SO}_4$ incorporation and glycosaminoglycan synthesis is increased in osteoarthrotic cartilage relative to normal. Mankin *et al.* (1971) also reported a direct correlation between the rate of glycosaminoglycan synthesis and the condition of articular cartilage as assessed by a grading method. We were unable to show such a relationship. Several factors emerge as possible explanations for these differences. First, we sampled only 'healthy' looking cartilage, carefully avoiding areas of obvious damage. Chondrocytes within these localized fibrillated areas of osteoarthrotic cartilage may show a greater metabolic activity in response to the lower levels, size, composition, and heterogeneity of surrounding glycosaminoglycans (Bollet and Nance, 1966; Bjelle *et al.*, 1972; Hjertquist and Lemperg, 1972; Hjertquist and Wasteson, 1972; Brandt, 1974; Maroudas and Evans, 1974; Ficat and Maroudas, 1975). Second, the origin of the osteoarthrotic cartilage that was examined. Apart from one specimen from a young male, all of those examined could be considered as aged. It is possible that the metabolism of aged and structurally fatigued osteoarthrotic cartilage (Freeman and Meachim, 1973) varies from cartilages derived from osteoarthritis of other aetiology. Last, we initiated our investigations of $^{35}\text{SO}_4$ uptake 24 hours after the excised cartilages had been in culture. Previous studies (McKenzie *et al.*, 1976b) had established that cell viability was optimum at this time, and cell 'shock' had probably subsided (Biggers, 1965). After this 24-hour preincubation period, the medium was replaced by fresh solution containing the $^{35}\text{SO}_4$, thereby removing foreign substances such as drugs which may have been

present within the cartilage during the initial incubation period. Some of the most commonly used anti-inflammatory drugs are known to inhibit proteoglycan synthesis (Whitehouse and Bostrom, 1962; McKenzie *et al.*, 1976a) and the degradation of cartilage proteoglycans by neutral proteases (Perper and Oronsky, 1974; Leiss and Kalbhen, 1976).

In previous studies we have shown that the majority of commonly prescribed anti-inflammatory drugs, including sodium salicylate, depress the synthesis of glycosaminoglycans in aged human cartilage (McKenzie *et al.*, 1976a). The comparable rates of $^{35}\text{SO}_4$ incorporation into osteoarthrotic and aged human cartilage suggest that these same anti-inflammatory drugs would also depress the synthesis of glycosaminoglycans in osteoarthrotic cartilage. The study on the effects of sodium salicylate on glycosaminoglycan synthesis in osteoarthrotic cartilage reinforces this conviction, and once more questions the place of these substances in the management of osteoarthritis.

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