SULPHATE ($^{35}\text{SO}_4$) FIXATION BY HUMAN ARTICULAR CARTILAGE COMPARED IN THE KNEE AND SHOULDER JOINTS

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The fixation of labelled sulphate ion, $^{35}\text{SO}_4$, is a measure of chondroitin-sulphate synthesis by cartilage cells, and a most satisfactory way of testing the viability of these cells (Curran and Gibson, 1956). In the case of human tissues, the test must be made *in vitro*, by incubating suitable slices of cartilage, obtained at biopsy or necropsy, in a medium containing a known amount of sulphur isotope, the uptake of which by the cells included in the cartilage sample can then be measured radiochemically and observed in autoradiographs.

McElligott and Collins (1960) used this technique to study human costal cartilage and the articular cartilage of the patella. Rib cartilage showed a high degree of sulphate utilization in infants, less in older children, and very little in the adult and elderly. In the articular cartilage, by contrast, sulphate utilization was found to increase as age advanced. It was concluded from the assays and from subsequent histochemical and autoradiographic studies (Collins and McElligott, 1960) that the increased avidity for sulphate in the older articular cartilages was related to the onset of osteo-arthritic cartilage fibrillation.

The influence of ageing, however, was not excluded as one of the causes of this rise in chondrocyte metabolism in the joint cartilages; most patellae of persons over 35 years of age show osteo-arthritic changes of some kind and aged non-arthritic cartilages are rare. In order to examine the effect of ageing alone on cellular function in articular cartilage, a study has now been made on paired specimens of cartilage from the patella and from the upper end of the humerus, since the shoulder joint, in contrast to the knee joint, seldom suffers from severe osteo-arthritis and is often free from pathological changes even at an advanced age (Heine, 1926).

Material

A shoulder joint and a knee joint on the same side, usually the left, were opened in the *post-mortem* room in the course of a number of randomly chosen necropsies on persons of either sex dying in the wards of the Royal Infirmary, Sheffield. The age at death ranged from 25 to 82 years. The upper end of the humerus and the whole of the patella were removed, and suitable preparations were made from them at once. If inspection of either joint showed any form of articular disease other than osteo-arthritis, the case was excluded from the study. Analyses were completed in 38 cases (Table I).

Methods

The articular surfaces were carefully inspected and the degree of osteo-arthritic change recorded. A central transverse block of each cartilage with underlying bone was taken; this after incubation in the sulphate medium provided material for histology and autoradiography. Adjoining blocks of cartilage stripped from the bone were taken and sliced finely before incubation for the purposes of quantitative assay of sulphate fixation.

The incubation medium was a salt solution containing buffered glucose and 4.8 mg. per litre of sulphate ions, as sodium sulphate (Kodicek and Loewi, 1955). To this was added approximately 5 microcuries (μC) of ^{35}S per ml.

The radiochemical technique was that described in detail by McElligott and Collins (1960). Quantitative results are expressed as micrograms (μg) of sulphate fixed per gram (g.) dried weight of cartilage. Control tests showed no significant uptake by boiled cartilage specimens. Autoradiographs were prepared as described by Collins and McElligott (1960), but were generally examined unstained or after light contrast staining with alcian green.

Grading of Osteo-Arthritis.—This followed that proposed by Collins (1949) and was based on naked-eye inspection of the joint surfaces supplemented by microscopical examination of the cartilage section.

- Grade 0* Smooth cartilage surface.
- Grade 1* Fibrillation of cartilage limited to the superficial zones of tangential and oblique fibres.
- Grade II* Fibrillation extending into the deep zone of vertical fibres, but no denudation of bone.
- Grades III and IV* Deep fibrillation with loss of cartilage exposing bare bone at one or more areas.

Results

Quantitative Results.—The results of the radiochemical assay in 38 paired specimens of cartilage from humerus and patella are all shown in Table I. Here the cases are grouped primarily according to the presence or absence of osteo-arthritis at the upper end of the humerus and secondarily according to the degree of osteo-arthritis at the patella. There is a considerable range of the numerical results from case to case; this is in line with our previous experience and is due, we believe, to biological

variability, since the influence of technical factors has been controlled (McElligott and Collins, 1960). In any case, the important results in column 9 are expressed as a ratio of sulphate-fixing activity between paired specimens from the same individual processed in parallel.

Sex (Table I, col. 3) did not influence the results, a fact clearly shown in Table II.

TABLE II

SULPHATE FIXATION BY CHONDROCYTES OF ARTICULAR CARTILAGE OF HUMERUS AND PATELLA, BY SEX

Sex	Humerus		Patella	
	No. of Cases	Mean SO ₄ ²⁻ Uptake (μg./g.)	No. of Cases	Mean SO ₄ ²⁻ Uptake (μg./g.)
Male	22	1.055	22	0.973
Female	16	1.052	16	1.008

TABLE I

CHONDROCYTE ACTIVITY (EXPRESSED AS μg. SO₄²⁻ TAKEN UP BY 1 g. DRIED CARTILAGE) OF ARTICULAR CARTILAGE OF UPPER END OF HUMERUS AND OF PATELLA IN 38 CASES AND THE RATIO OF ACTIVITY IN THE TWO CARTILAGES (EXPRESSED AS PATELLA/HUMERUS)

Specimen No.	Age of Patient (yrs)	Sex	Time after Death (hrs)	Humerus		Patella		P/H Uptake Ratio
				Grade of Osteo-arthritis	SO ₄ ²⁻ Uptake (μg./g.)	Grade of Osteo-arthritis	SO ₄ ²⁻ Uptake (μg./g.)	
R20	25	M	19	0	0.278	0	0.126	0.45
R28	30	F	13	0	1.568	0	1.039	0.66
R31	45	M	22	0	0.839	0	0.931	1.11
R34	37	F	23	0	1.603	0	0.995	0.62
R38	37	F	23	0	0.710	0	0.434	0.61
R45	33	F	21	0	1.364	0	1.004	0.74
R10	63	M	15	0	1.979	I	1.115	0.56
R15	35	M	22	0	0.425	I	0.353	0.83
R17	42	F	21	0	1.707	I	1.811	1.06
R23	63	M	24	0	0.493	I	0.453	0.92
R24	43	M	24	0	0.605	I	0.225	0.37
R30	50	M	6	0	0.165	I	0.247	1.50
R39	39	F	15	0	0.708	I	0.725	1.02
R41	60	M	7	0	2.132	I	1.223	0.57
R43	53	F	17	0	1.020	I	1.201	1.18
R 2	65	F	12	0	0.557	II	0.704	1.26
R 3	62	M	14	0	0.394	II	0.505	1.28
R 5	59	F	6	0	1.274	II	1.658	1.30
R 7	49	M	14	0	1.487	II	1.235	0.83
R11	66	F	10	0	1.342	II	0.648	0.48
R13	59	M	22	0	1.607	II	1.449	0.90
R18	74	F	11	0	0.436	II	0.366	0.84
R19	72	M	25	0	1.780	II	1.644	0.92
R25	60	M	8	0	1.627	II	0.528	0.32
R27	73	M	8	0	0.462	II	1.131	2.45
R35	53	M	19	0	0.534	II	1.925	3.60
R 4	75	M	21	0	2.117	III-IV	2.278	1.08
R21	70	F	12	0	0.467	III-IV	1.036	2.22
R29	68	F	24	0	0.580	III-IV	1.620	2.80
R14	52	M	22	I	0.286	I	0.212	0.74
R16	39	M	25	I	1.287	I	1.298	1.01
R33	72	M	16	I	0.370	II	0.983	2.66
R36	80	M	9	I	1.188	II	0.817	0.69
R37	74	M	17	I	1.762	II	1.537	0.87
R 6	67	F	21	I	0.435	III-IV	0.569	1.31
R12	80	F	10	I	1.946	III-IV	1.531	0.79
R40	71	F	5	I	1.116	III-IV	0.793	0.71
R44	82	M	15	I	1.382	III-IV	1.200	0.87

The time after the patient's death at which incubation of the cartilage was started (Table I, col. 4) ranged from 5 to 25 hours. The activity of cartilage does deteriorate when retained in the body after death, partly because of the slow rate of cooling and partly because it remains in contact with perichondrial tissues. McElligott and Collins (1960) have considered these influences and found that measurable amounts of sulphate can still be fixed by cartilage not excised until 36 hours after death. No statistical relationship exists in Table I between the length of time after death that the cartilage was collected and the result of the individual radiochemical assay. We assume that external influences would have affected the patella and humerus cartilages equally and that the P/H ratio in Table I, col. 9, is therefore valid.

The influence of the presence of varying degrees of osteo-arthritis on the results is shown in Table III. Osteo-arthritis of Grades II, III, or IV, which was encountered only in the patella, enhances the activity of the chondrocytes in utilizing sulphate. This is in accord with the results of Collins and McElligott (1960), who concluded that the increasing avidity for sulphate in increasing grades of osteo-arthritis was a cellular function representing a stimulation of cells to manufacture chondroitin sulphate in response to the loss of the surrounding matrix. No greater severity of osteo-arthritis than Grade I was seen in the shoulder joint in this series; in every case

the main depth of the cartilage was intact and, indeed, the destruction of cartilage was in all but one instance no more extensive than a little superficial flaking along the tangential fibre lines.

The absence in the cartilage of the humerus of osteo-arthritis changes likely to stimulate chondrocytes to greater activity enables us to investigate the influence of age alone on the viability of articular cartilage cells. In Table IV the patients are collected into 20-year age groups. Age had no significant effect in the case of the humerus. In the case of the patella, the increase in the mean results with increasing age can be attributed to the frequent incidence of osteo-arthritis of severe grade in this cartilage (Table V).

TABLE V

INCIDENCE OF OSTEO-ARTHRITIC CHANGES OF VARYING SEVERITY IN 38 PAIRED SPECIMENS OF CARTILAGE FROM UPPER END OF HUMERUS AND PATELLA, BY 20-YEAR AGE GROUPS

Age (yrs)	Cartilage	Grades of Osteo-arthritis			
		0	I	II	III-IV
25-44	Humerus	9	1	0	0
	Patella	5	5	0	0
45-64	Humerus	12	1	0	0
	Patella	1	6	6	0
65-84	Humerus	8	7	0	0
	Patella	0	0	8	7

TABLE III

UPTAKE OF SULPHATE BY ARTICULAR CARTILAGE OF HUMERUS AND PATELLA *IN VITRO*, BY GRADE OF OSTEO-ARTHRITIS

Grade of Osteo-arthritis	Humerus			Patella		
	No. of Specimens	SO ₄ Uptake (μg./g.)		No. of Specimens	SO ₄ Uptake (μg./g.)	
		Mean	Range		Mean	Range
0	29	1.043	0.165-2.132	6	0.755	0.126-1.039
I	9	1.086	0.286-1.946	11	0.806	0.212-1.811
II	—	—	—	14	1.081	0.366-1.925
III-IV	—	—	—	7	1.290	0.569-2.278

TABLE IV

MEAN SULPHATE UPTAKE (μg./g.) OF ARTICULAR CARTILAGE FROM HUMERUS AND PATELLA, BY 20-YEAR AGE GROUPS IRRESPECTIVE OF OSTEO-ARTHRITIS GRADES

Age Range (yrs)	No. of Cases	Mean Age of Cases (yrs)	Humerus SO ₄ Uptake (μg./g.)		Patella SO ₄ Uptake (μg./g.)	
			Mean	Range	Mean	Range
25-44	10	36	1.026	0.278-1.707	0.801	0.126-1.811
45-64	13	56	1.064	0.165-2.132	0.976	0.212-1.925
65-84	15	73	1.063	0.370-2.117	1.124	0.366-2.278

Table I shows the P/H (patella/humerus) ratio of chondrocyte activity in the individual cases, but in Table VI the group P/H ratios are calculated from the mean uptake of each cartilage in the cases falling within the appropriate group.

TABLE VI
COMPARISON OF CHONDROCYTE ACTIVITY IN ARTICULAR CARTILAGES OF HUMERUS AND PATELLA BY GRADE OF OSTEO-ARTHRITIS AFFECTING THE PATELLA. RATIO OF MEANS (P/H) CALCULATED FROM THE SULPHATE-FIXING ACTIVITY OF EACH CARTILAGE

Grade of Osteo-arthritis		No. of Cases	P/H Ratio Mean sulphate uptake by patella Mean sulphate uptake by humerus
Humerus	Patella		
0-I	0	6	0.71
	I	11	0.82
	II	14	1.01
	III-IV	7	1.12

It is clear that the patella cartilage tends to become more active than the humerus cartilage in sulphate utilization as soon as osteo-arthritic changes in the patella have advanced beyond Grade I, that is to the point where fibrillation has led to substantial loss of matrix and has stimulated a reactive synthesis of chondroitin sulphate by the cartilage cells. This enhancement of sulphate fixation by the osteo-arthritic cartilage of the patella as compared with the virtually unchanged cartilage of the humerus is the more noteworthy in that patellar cartilage showing only Grade I osteo-arthritis or none is evidently less biologically active than the humerus cartilage.

Autoradiographic Results.—Autoradiographs of sections of cartilage from humerus and patella were prepared in every case. Figs 1 and 2 illustrate the enhancement of chondrocyte activity with the onset of osteo-arthritis in the patella.

The autoradiographs also confirmed the obser-

Figs 1 and 2.—Autoradiographs showing sulphate ($^{35}\text{SO}_4$) uptake by chondrocytes of articular cartilage of humerus (Fig. 1) and patella (Fig. 2). Both specimens came from a woman aged 65 years and were processed in parallel. The shoulder joint was free from osteo-arthritis and the humerus cartilage is intact (Fig. 1). There was Grade II osteo-arthritis in the knee-joint and the patellar cartilage (Fig. 2) shows fibrillation, loss of matrix, and clumping of chondrocytes, with a dense radiographic shadow over each clump. Radiochemical assay gave a sulphate uptake of 0.557 $\mu\text{g./g.}$ for the humerus cartilage and 0.704 $\mu\text{g./g.}$ for the patellar cartilage, and a P/H ratio of 1.26. Autoradiographic exposure 60 days. Magnification of photo-micrographs $\times 100$.

vements recorded by Collins and McElligott (1960), and they showed that the increased utilization of sulphate by osteo-arthritic cartilage is a function of the chondrocytes, particularly of those cells that proliferate to form multicellular clusters in the fibrillated area of the deep vertical-fibre zone. This appearance was, of course, seen only in the patella in our series of cases. In both the patella and the humerus, the cartilage cells of the superficial zones fixed little or no sulphate. The small cells very close to the surface of the intact cartilages seldom had granules over them, and those a little more deeply situated showed no reaction when there was tangential flaking as seen in the earliest (Grade I) phase of osteo-arthritis.

Summary and Conclusions

38 paired specimens of adult articular cartilage from the upper end of the humerus and from the patella were obtained from human autopsy material. Their ability to fix sulphate *in vitro*, using $^{35}\text{SO}_4$, was studied by radiochemical assay and autoradiography.

In the absence of osteo-arthritis, the cartilage from the patella tended to be less active in utilizing sulphate than that from the humerus. Grade I osteo-arthritis did not significantly increase this activity in either cartilage.

Osteo-arthritis of Grades II to IV, encountered only in specimens of patella, led to enhanced uptake of sulphate by the affected cartilage and to the situation in which, in paired specimens, the patellar cartilage became quantitatively more active than that from the humerus.

Autoradiographic studies confirmed the quantitative results and showed a heavy uptake of labelled sulphate by clumps of chondrocytes bordering the fissures of fibrillated osteo-arthritic cartilage.

The sex of the patient had no influence on the results.

In the case of cartilage from the humerus, the age of the patient did not affect the mean results. In the case of the patella, the increase of chondrocyte activity in older patients could not be dissociated from the increasing incidence of osteo-arthritis.

It is concluded, therefore, that the ability of the cells of adult human articular cartilage to synthesize sulphated mucopolysaccharides is uninfluenced directly by ageing. The enhancement of chondrocyte activity, seen for example in many specimens of patella with damaged cartilage, appears to be a reactive phenomenon to local conditions and the loss of mucopolysaccharide from the matrix associated

with the process of osteo-arthritis within the affected joint.

The work here reported was performed with the aid of a research grant from the Empire Rheumatism Council for which we are very grateful.

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Fixation comparée du sulfate ($^{35}\text{SO}_4$) par le cartilage articular humain de l'épaule et par celui du genou

RÉSUMÉ

On obtint à l'autopsie 38 prélèvements appariés de cartilage articulaire humain adulte de l'extrémité supérieure de l'humérus et de la rotule. Sa capacité pour fixer le sulfate *in vitro*, usant $^{35}\text{SO}_4$, fut étudiée par dosage radiochimique et par l'autoradiographie.

En l'absence d'ostéoartrite, le cartilage rotulien tendait à absorber moins de sulfate que le cartilage huméral. La présence d'ostéoartrite du grade I n'augmentait pas appréciablement l'activité des deux cartilages.

L'ostéoartrite des grades II à IV, présente seulement dans les cartilages rotuliens, faisait augmenter l'absorption de sulfate par le cartilage affecté, produisant ainsi une situation où, lorsque les prélèvements venaient du même sujet, le cartilage rotulien devenait quantitativement plus actif que le cartilage humoral.

Les études autoradiographiques ont confirmé les résultats quantitatifs et indiqué une absorption considérable de sulfate marqué par les amas des chondrocytes bordant les fissures du cartilage fibrillaire ostéoarthritique.

Le sexe de la personne de laquelle provenait le cartilage n'avait aucune influence sur les résultats.

Pour le cartilage huméral, l'âge de la personne n'affectait pas les résultats moyens. Pour la rotule, l'augmentation de l'activité des chondrocytes avec l'âge ne pouvait pas être dissociée de la fréquence croissante d'ostéoartrite.

On conclut que la capacité du cartilage articulaire humain pour synthétiser des mucopolysaccharides sulfatés n'est pas affectée directement par l'âge. L'augmentation de l'activité chondrocytaire, observée, par exemple, dans les prélèvements de cartilage rotulien lésé, semble être un phénomène de réaction contre des conditions locales et la perte des mucopolysaccharides de la matrice, associée au processus d'ostéoartrite dans l'articulation affectée.

Comparación de la fijación de sulfato radioactivo ($^{35}\text{SO}_4$) por el cartilago articular humano del hombro y por el de la rodilla

SUMARIO

Se obtuvieron en autopsia 38 muestras apareadas de cartilago articular humano adulto procedentes del cabo superior del húmero y de la rótula. Se estudió radioquímicamente y por autorradiografía su capacidad para fijar sulfato *in vitro*, usando $^{35}\text{SO}_4$.

En ausencia de osteoartritis, el cartilago rotuliano tendía a ser menos activo en la utilización del sulfato que aquel procedente del húmero. Presencia de osteoartritis del grado I no aumentaba significativamente la actividad de ninguno de ambos cartilagos.

Osteoartritis de grados II a IV, presente solamente en cartilagos rotulianos, produjo incremento en la absorción de sulfato por el cartilago afecto, creando una situación en que, en muestras procedentes del mismo individuo, el cartilago rotuliano fué cuantitativamente más activo que el humeral.

Estudios autorradiográficos confirmaron los resultados cuantitativos y mostraron una considerable absorción de sulfato marcado en los acúmulos de condrocitos bordeando las fisuras del cartilago fibrilar osteoartítico.

El sexo de la persona a quien pertenecía el cartilago no tuvo influencia alguna.

En el caso del cartilago humeral, la edad de la persona no afectó los resultados medios. En el caso del cartilago rotuliano, el aumento de actividad condrocítica con la edad no pudo separarse de la creciente incidencia de osteoartritis.

Se concluye que la habilidad del cartilago articular humano para sintetizar mucopolisacaridos sulfatados no es directamente afectada por la edad. El aumento de la actividad condrocítica, observada por ejemplo en muchas de las muestras de cartilago rotuliano lesionado, parece ser un fenómeno reactivo a condiciones locales y a pérdida de mucopolisacaridos de la matriz, asociada con procesos osteoartíticos dentro de la articulación afectada.