SULPHATE ($^{35}\text{SO}_4$) UPTAKE BY CHONDROCYTES IN RELATION TO HISTOLOGICAL CHANGES IN OSTEO-ARTHRITIC HUMAN ARTICULAR CARTILAGE

BY

D. H. COLLINS AND T. F. McELLIGOTT

Department of Pathology, University of Sheffield

Quantitative studies of sulphate uptake by the cells of human cartilage (McElligott and Collins, 1960) have shown that chondrocyte activity increases in articular cartilage as age advances, while it decreases in costal cartilage. A considerably enhanced activity of articular over costal cartilage cells was recorded in cases where there was naked-eye evidence of destruction and fibrillation of the articular cartilage. We postulated a possible relationship between the increased sulphate utilization of ageing articular cartilage, the degree of osteo-arthritis, and the peculiar rearrangement and grouping of chondrocytes that are known to occur in these conditions. In order to investigate this, a large number of autoradiographs of cartilage incubated with labelled sulphate have been prepared and studied side by side with parallel sections stained by various methods including those that demonstrate mucopolysaccharides. It is the results of these studies that we now report.

Material and Methods

A patella was removed at each of 34 necropsies from persons ranging in age from 4 to 83 years. Specimens were processed as soon as possible after removal and in all cases within 2 hours. During this delay the specimens were stored at 4° C. in the incubation medium, a procedure that reduces the rate of loss of chondrocyte activity. The intervals between death and autopsy and between the collection of the specimen and its processing were carefully noted.

Preparation of Specimens.—The patellae were inspected and changes of osteo-arthritis, if present, were graded 0-4 according to the classification proposed by Collins (1949, p. 76). Blocks of cartilage down to the bone were removed from the complete transverse diameter of the patellae and processed for quantitative measurement of sulphate fixation in 31 cases, as previously described (McElligott and Collins, 1960).

Another block of cartilage with its subjacent bone

was then cut from the specimen; this second block of cartilage adjoining that removed for radiochemical assay was used for the histological procedures to be described. The cut surfaces of the cartilage were then shaved with a razor so as to produce a block of the complete depth of the articular cartilage 1 mm. or less in thickness resting on a broader supporting block of the underlying bone, since thicknesses of cartilage greater than 1 mm. impair the uptake of $^{35}\text{SO}_4$ (Boström and Månsson, 1953).

Incubation Medium.—The medium used for incubating the specimens and in which they were transported was that described by Kodicek and Loewi (1955). It is a salt solution containing glucose buffered to pH 7.5. For quantitative estimations of sulphate fixation, sulphate as sodium sulphate 4.8 mg. per litre was added. In the preparation of autoradiographs no sulphate was added as carrier, as Layton (1950) reports that higher uptake of radioactive sulphate occurs where carrier is omitted.

Radiochemical Technique.—The quantitative procedure, based on the methods fully set out by Layton (1949, 1950) and by Kodicek and Loewi (1955), has been previously described (McElligott and Collins, 1960). In the preparation of autoradiographs the blocks of cartilage and bone were incubated in 10 ml. medium at 37° C. in a water bath, in flasks mounted in a mechanical rocker. Approximately 5 microcuries ($\mu\text{C.}$) of ^{35}S per ml. (carrier-free sulphate supplied by Radiochemical Centre, Amersham) was added to the incubation medium. After 4 hours incubation, the blocks were rinsed with ice-cold isotonic (2.5 per cent.) sodium sulphate and fixed in 10 per cent. neutral formalin. After decalcification, dehydration, and double embedding by Peterfi's method, sections were cut at 7 μ .

Autoradiographs were prepared by the stripping film technique of Doniach and Pelc (1950) using Kodak A.R.10 film. The preparations were exposed in the dark in a refrigerator, generally for 60 days. Some sections were stained before mounting by the periodic acid-Schiff (P.A.S.) technique; others were stained with 1 per cent. safranin through the overlying film after exposure and development.

Histological Methods.—Serial sections were stained in the following order:

- (1) Ehrlich's acid haematoxylin and eosin.
- (2) Periodic acid-Schiff (P.A.S.) omitting the bisulphite rinse (Gomori, 1952).
- (3) Alcian blue (Zugibe, Brown, and Last, 1959).
- (4) Phosphotungstic acid haematoxylin (P.T.A.H.).
- (5) Van Gieson's stain with Weigert's iron haematoxylin.
- (6) Laidlaw's silver carbonate method for reticulin (Laidlaw, 1929).
- (7) Toluidine blue for metachromasia, the stained and rinsed sections being fan-dried before clearing and mounting.

Explanation of Terms.—Reference will repeatedly be made to Articular Cartilage Zones 1-4 and Osteo-Arthritis Grades 0-4. These terms must therefore be clearly defined.

CARTILAGE ZONES.—Basing his observations on those of Benninghoff (1925), Collins (1949, p. 22) recognized four strata in normal human articular cartilage, not only by the position of the collagen fibres but also by the character of the cells, *viz.*:

- Zone 1** Superficial; numerous small cells, fibres arranged tangentially.
- Zone 2** Transitional; cells moderately large and equally spaced, fibres pursuing an oblique course and interlacing.
- Zone 3** Deep; larger cells, often in groups of four to eight, in rows between radially arranged parallel fibres.
- Zone 4** Calcified zone adjacent to bone; lying beneath the wavy basophilic line identifiable in decalcified sections of every adult bone end and appropriately termed the "tide-mark" by Fawns and Landells (1953).

GRADES OF OSTEO-ARTHRITIS.—This pathological grading of the cartilage and bone changes of age and osteo-arthritis was fully described by Collins (1949, p. 76). In summary, the principal distinguishing features of each grade are as follows:

- Grade 0** Normal healthy joint with smooth cartilage.
- Grade I** Superficial flaking of cartilage in areas of pressure and movement.
- Grade II** More extensive destruction of cartilage not denuding bone.
- Grade III** Total loss of cartilage in one or more pressure areas and obvious marginal osteophytes.
- Grade IV** Complete loss of cartilage from large areas with eburnation of bone; prominent osteophytes.

This scheme of grading depends on naked-eye assessment. The extent of cartilage destruction may vary from area to area, but changes that fairly represent each grade are described and illustrated in what follows.

Results

The results fall into two sections:

- (a) The quantitative results of radio-assay, which indicate the over-all avidity for sulphate displayed by all the chondrocytes in the sample of cartilage;
- (b) The results of our study of histological sections and their autoradiographs, from which we can deduce the behaviour of individual chondrocytes or clusters of chondrocytes in relation to the different zones of cartilage and to the general matrix under varying conditions of cartilage injury and disintegration.

A total of 34 patellae from 34 different persons provided histological material (Table I). Quantitative assays of sulphate utilization *in vitro* were made in 31 cases. Sex made no difference to the results; increasing grades of osteo-arthritis tended to occur with increasing age, but individual variation in this respect was considerable.

TABLE I
SUMMARY OF MATERIAL EXAMINED QUANTITATIVELY AND HISTOLOGICALLY

Grade of Osteo-Arthritis	Total No. of Cases (each fully studied histologically)	Age Range (yrs)	No. quantitatively Assayed
0	4	4-35	4
I	12	20-75	11
II	10	44-75	10
III and IV	8	68-83	6
Totals	34	4-83	31

Quantitative Results.—These show increased sulphate utilization by the chondrocytes in the more severe grades of osteo-arthritis (Table II).

TABLE II
UPTAKE OF SULPHATE BY PATELLAR ARTICULAR CARTILAGE *IN VITRO* ($\mu\text{g. SO}_4/\text{g. DRIED CARTILAGE}$)
31 NECROPSIES IN WHICH VARYING GRADES OF OSTEO-ARTHRITIS WERE ENCOUNTERED

Grade of Osteo-Arthritis	No. of Specimens	SO ₄ Uptake ($\mu\text{g./g.}$)	
		Mean	Range
0	4	0.18	0.12 to 0.29
I	11	0.56	0.14 to 1.0
II	10	0.80	0.35 to 1.89
III and IV	6	1.04	0.49 to 1.72

Quantitative results in individual cases are not closely related to the recorded grade of more advanced osteo-arthritis. Sulphate uptake clearly depends to some measure on the particular area sampled; a slice that includes much totally exposed bone, for example, would obviously contain few functioning chondrocytes.

The ages of the patients have not been included in Table II. Sulphate utilization in articular cartilage, but not in costal cartilage, tends to increase with age, but this is probably due to the structural changes of osteo-arthritic pattern that commonly develop in the elderly knee joint, since age causes a diminution of chondrocyte activity in costal cartilage. Figures previously published (McElligott and Collins, 1960) show that the degree of osteo-arthritis is more important than age alone in increasing the ratio of activity between articular and costal cartilage. Furthermore, investigations now in hand (Meachim and Collins, to be published) indicate that mere ageing of the articular cartilage of a joint that is not subject to osteo-arthritis is not associated with increased sulphate utilization.

Histological and Autoradiographic Results

Grade 0.—In normal articular cartilage the chondrocytes are fairly evenly distributed though varying somewhat in size and tending to lie in small clusters in Zone 3. In children, all staining reactions, basophilia with acid haematoxylin, metachromasia,

P.A.S., and alcian blue, are evenly intense throughout the thickness of cartilage. In adults the staining of the superficial layer (Zone 1) becomes weaker. P.A.S. staining remains in the deeper part of Zone 3 and as collars around the cells in Zone 4 (Fig. 1). Metachromasia, basophilia, and alcian-blue staining tend to fade from the deep Zone 3 though remaining strong in upper Zone 3, forming pools of colour around the chondrocytes (Fig. 2) and in Zone 2 where the staining is often quite diffuse. These observations agree in the main with those of Fawns and Landells (1953), who rightly point out that in older people the matrix towards the basal zone becomes eosinophilic in the ordinary haematoxylin and eosin stain, though the included cells remain strongly basophil and metachromatic.

The autoradiographs reveal an intense image over the cells in Zones 2 and 3. The smaller cells of Zone 1 are feebly active or inactive (Fig. 3, opposite) and this confirms what the ordinary section (Fig. 4, opposite) suggests, that these superficial cells are effete. The cells in Zone 4 generally show no uptake of sulphate, although surrounded by a P.A.S.-positive collar (Fig. 5, opposite).

The autoradiographic picture of human articular cartilage after sulphate ($^{35}\text{SO}_4$) incubation has not hitherto been described, so far as we are aware. Our results show that in undamaged cartilage all the cells in Zones 2 and 3, that is, in at least four-fifths of the cartilage depth, are actively synthesizing sulphated mucopolysaccharides (chondroitin),

Fig. 1.—Normal patellar cartilage (Grade 0) from a man aged 35. Strong P.A.S. reaction in Zone 3 and P.A.S.-positive collars around cells in the calcified Zone 4 adjoining the bone. (J.24) P.A.S. $\times 125$.

Fig. 2.—Normal patellar cartilage (Grade 0). Strong staining around chondrocytes in upper Zone 3 and more diffusely in Zone 2. (J.24) Alcian blue $\times 50$.

Fig. 3.—Normal patellar cartilage (Grade 0). Autoradiograph showing sulphate uptake by chondrocytes in Zone 2, but little in the superficial Zone 1. (J.24) $\times$ 125.

Fig. 5.—Normal adult patellar cartilage. Autoradiograph with P.A.S. stain shows diffuse P.A.S. reaction in Zone 3, except around the very active chondrocytes, and weak P.A.S. reaction in Zone 4, except for collars around the chondrocytes which here show no sulphate fixation. (J.24) P.A.S. $\times$ 125.

Fig. 4.—Normal patellar cartilage (Grade 0) from a boy aged 14. Diffusely positive P.A.S. staining. Small effete cells in superficial Zone 1 which shows an insignificant amount of flaking. (J.9) P.A.S. $\times$ 50.

whereas this function tends to be lost by the effete cells of the superficial Zone 1 and by those cells that have become interred in the calcified Zone 4.

Grade I.—Histologically, the cartilage here shows tangential flaking of the superficial Zone 1 (Fig. 6).

In some of the twelve specimens included in this group, destruction of cartilage had advanced to the extent of vertical or oblique fibrillation of Zone 2

Fig. 6.—Patellar cartilage showing Grade I changes. Tangential flaking of surface (Zone 1). Loss of P.A.S. reaction in the matrix and of chondrocytes in this zone. (J.6) P.A.S. $\times$ 125.

(Fig. 7), but never more deeply. The normally effete cells of Zone 1 are not stimulated to activity when tangential flaking occurs. They tend to disappear even before the flakes of matrix separate (Fig. 6), and the autoradiograph shows no increased fixation of sulphate by any of the cells of the cartilage, provided that fibrillation is restricted to Zone 1 (Fig. 8). When, however, the cracks in the fibrillated cartilage reach obliquely into Zone 2 and begin to fibrillate vertically, reactive cell clumps appear (Fig. 7), and these demonstrate enhanced radioactivity through sulphate fixation. This is not a general feature in the specimens that fall within this grade, since most cartilages showing deep vertical fibrillation come to be graded as II

Fig. 7.—Patellar cartilage showing Grade I changes, here including shallow fibrillation of Zone 2; the tangentially flaked fragments of Zone 1 have mostly become detached. Small clumps of chondrocytes appear. (J.3) P.A.S. $\times 50$.

Fig. 8.—Grade I. Autoradiograph showing poor uptake of sulphate by the cells of the broken up superficial Zone 1 as compared with still normal chondrocytes in deeper Zones 2 and 3. (J.21) $\times 125$.

or III. The section of one patella (J.16) that to the naked-eye revealed no more than Grade I changes

Fig. 9.—Autoradiograph showing a single deep fissure, with fracture of the subchondral bony plate, in a cartilage showing elsewhere only Grade I changes. The chondrocytes of Zones 2 and 3 have here formed large clumps and show active sulphate fixation. (J.16) $\times 125$.

Fig. 10.—Grade I. Fibrillation extending into the transitional oblique-fibred Zone 2. The general matrix in the fibrillated area fails to stain metachromatically and appears colourless when photographed through an orange filter. (J.10) Toluidine blue $\times 50$.

happened to include a fracture through the cartilage and the subchondral plate, beneath which was a focus of reparative fibrosis. This single deep fissure, almost certainly originating from a specific, though unspecified, injury, was bordered by large clumps of very actively functioning chondrocytes (Fig. 9). P.A.S. staining reactions accord with their distribution in normal cartilage. The most strikingly abnormal histochemical reaction seen was the loss of metachromasia in the normally positive Zone 2 when this becomes fibrillated (Fig. 10, opposite). It is the general matrix of this zone that now stains orthochromatically; metachromasia is still seen around the proliferated and sulphate-fixing chondrocyte groups.

Grade II.—In this group the usual cartilage changes are the total loss of Zone 1, a fringe of tags surviving from Zone 2, and vertical fibrillation causing clefts between the radial fibre lines of Zone 3. The clefts do not generally extend to the base of Zone 3; when this happens blocks of cartilage are liable to break off from their calcified or bony base and this constitutes a Grade III event.

The chondrocytes of Zones 2 and 3 are viable cells that respond to the breaking up and apparent leaching away of matrix by multiplication and the formation of cell clusters in which twenty or more nuclei may be counted (Fig. 11). These cell groups are very active in fixing sulphate and an intense autoradiographic image forms over them (Fig. 12).

Fig. 11.—Grade II. Clumps of proliferated and viable chondrocytes in fibrillated area of Zone 2. The outlying matrix has lost its basophilia. (J.26) Haematoxylin and eosin $\times 125$.

Fig. 12.—Grade II. Autoradiograph showing fibrillation of cartilage Zones 2 and 3, and intense image over the large cell clusters shown in Fig. 11. (J.26) $\times 125$.

The same cells show strong metachromatic reaction with toluidine blue, which is most strong over the cell clusters but also lightly diffused around them (Fig. 13).

Fig. 13.—Grade II. Strong metachromasia of cell clumps in fibrillated cartilage of Zones 2 and 3. (J.28) Toluidine blue with orange filter $\times 125$.

A strong metachromasia is also seen around the cell groups in the more deeply fibrillated zone (Fig. 14).

Fig. 14.—Grade II. Metachromasia over and in the vicinity of chondrocytes in the deeper fibrillation of Zone 3 cartilage. (J.32) Toluidine blue with orange filter $\times 50$.

Alcian-blue staining follows exactly the same pattern of distribution as metachromasia, and metachromasia and intense alcian-blue staining are localized within and around cell groups that always show intense autoradiographic images. It is to be deduced therefore that these two histochemical reactions occur where sulphated mucopolysaccharides are being synthesized.

The P.A.S. reaction, however, is distributed in an inverse manner. When the autoradiographic section is stained P.A.S., the sulphate-fixing chondrocytes are seen to lie within a halo of unstaining matrix, although the intervening matrix, further removed from the cell groups, is P.A.S. positive (Fig. 15). This finding is the corollary of the observation already illustrated in Figs 1 and 5, that the chondrocytes of Zone 4 are generally surrounded by a collar of P.A.S. material but are inactive in sulphate utilization.

Fig. 15.—Grade II. Autoradiograph showing fibrillation extending through Zone 2 to Zone 3. All chondrocytes show sulphate fixation, well marked in the fibrillated areas. Each group of these actively sulphating cells is surrounded by a halo of P.A.S.-negative material. Note also that P.A.S.-positive material tends to remain in the superficial tags of Zone 2. (J.32) P.A.S. $\times 50$.

Grades III and IV.—The division between Grades III and IV of osteo-arthritis is based on the naked-

eye appearances of the joint. Microscopy of small areas of cartilage does not distinguish between them. In both grades, cartilage destruction somewhere has proceeded to total loss of the uncalcified cartilage of Zones 1 to 3 and denudation of bone, perhaps, incorporating some residues of the calcified cartilage of Zone 4 (Fig. 16).

Fig. 16.—Grades III and IV. Total loss of uncalcified articular cartilage giving the gross appearance of a bared bone end, though islands of Zone 4 calcified cartilage often remain, shown here to give a certain metachromatic reaction. (J.30) Toluidine blue $\times 50$

The main strata of cartilage seem at this stage of disintegration to have been shorn off along the “tide-mark” line that normally separates Zones 3 and 4. Later this denuded bone may become still further worn down and compacted like ivory

Fig. 17.—Grades III and IV. Vertical fibrillation of Zone 3 cartilage extending as deeply as Zone 4 and the underlying bone. (J.30) Reticulin stain $\times 50$.

(eburnation) as a result of apposition of new lamellar bone upon the subchondral trabeculae. Eventually most of the Zone 4 cartilage is worn away.

Apart from denudation of bone, the principal cartilage change in Grades III and IV is vertical fibrillation through the deepest part of Zone 3 (Fig. 17). At this stage all of Zone 1 cartilage and most of Zone 2 has already been lost.

Chondrocytes proliferate to form huge clusters of cells in the fibrillated Zone 3 (Fig. 18).

Fig. 18.—Grades III and IV. Large clusters of chondrocytes in fibrillated cartilage of Zone 3. P.A.S.-negative reaction around these cell groups. (J.31) P.A.S. $\times 50$.

P.A.S.-positive material fades from the matrix immediately surrounding these cell groups. Metachromatic material is, however, concentrated around the cartilage cells and in the matrix immediately surrounding the cell clusters (Fig. 19, overleaf), and alcian-blue staining is similarly distributed. The same cells are shown by autoradiography to be very active users of sulphate (Fig. 20, overleaf). Under higher magnification the autoradiographs show very clearly that the uptake of sulphate is localized to the cell groups and often to individual cells within the group (Fig. 21, overleaf).

Some sections gave us the opportunity to study the junction between surviving cartilage and denuded bone. The deeply fibrillated Zone 3 loses more and more of its thickness and tapers down

Fig. 19.—Grades III and IV. Strong metachromasia over and around chondrocyte clusters in fibrillated cartilage of Zone 3. (J.31) Toluidine blue $\times 50$.

Fig. 20.—Grades III and IV. Autoradiograph of fibrillated cartilage in Zone 3 showing great activity of chondrocytes in fixing sulphate. (J.31) $\times 50$.

towards the denuded region, but the chondrocytes are still active and viable, forming the usual large clumps that seem to be their response to fibrillation and loss of matrix, and they still show active sulphate utilization in the autoradiographs and the elaboration of material that stains well with alcian blue and is strongly metachromatic (Fig. 22, opposite).

The final disintegration of the cartilage seems to result from the development of horizontal clefts that meet with the vertical clefts in the fibrillated Zone 3 and cause small masses of cartilage to

Fig. 21.—Grades III and IV. Autoradiograph magnified to show localization of image over cell groups and over individual cells within the group, clearly indicating that sulphate fixation during *in vivo* incubation of cartilage is a vital function of chondrocytes. (J.31) $\times 125$.

crumble away. The horizontal cleavage generally takes place along or close to the tidemark line that is the upper boundary of the calcified cartilage of Zone 4.

A noteworthy feature, well seen in sections stained for reticulin (Fig. 23, opposite), is the withering up of the tags of cartilage when they are nearly isolated from the main mass of cartilage. It seems that the mucopolysaccharide matrix dissolves away, leaving contracted spirals of collagen fibre bundles. In many such situations the chondrocytes within the shrinking tags still show intact nuclei, metachromasia, and sulphate fixation; but sometimes before the fragment of decadent cartilage finally separates the cells seem to die.

Discussion

It has been our aim to illustrate in some detail the cellular changes that develop in articular cartilage as it breaks up and wears away on a joint surface, and to relate these changes in the cells with their capacity for synthesizing sulphated mucopolysaccharides and with the histochemical reactions of the surrounding cartilage matrix.

The disintegration of cartilage so commonly seen in ageing joints that are exposed to trauma,

Fig. 22.—Grades III and IV. Progressive loss of depth of fibrillated Zone 3 at edge of denuded area (to right). Chondrocyte clumps are very large and the cells continue to elaborate metachromatic (dark) material. (J.35) Toluidine blue $\times 125$.

Fig. 23.—Grades III and IV. Disintegration of articular cartilage at the edge of an area of denuded bone (right). Cell clumps still survive in many of the tags, but the amorphous mucopolysaccharide matrix is being lost to expose contracted spirals and wavy outlines of collagen fibre bundles. (J.30) Reticulin stain $\times 50$.

such as the articulation of the patella which we chose for our material, is often referred to as a degeneration and it is known to lead to osteo-arthritis, or, as it is often termed, degenerative arthritis. But we have already shown, by quantitatively comparing the trend of sulphate utilization with age (McElligott and Collins, 1960), that the cells of ageing patellar cartilage show an enhanced capacity to synthesize chondroitin sulphate and, therefore, that osteo-arthritis is not due to a loss of function of the chondrocytes. The anomaly this presented was the revelation that, though we know that there is a loss of substance and a disintegration of articular cartilage in osteo-arthritis, the cells that remain are much more than normally capable of utilizing sulphate, which, for reasons fully explained in our earlier paper, is a measure of the rate of synthesis of sulphated mucopolysaccharide. We thought at that time that there might be a relationship between the enhanced sulphate utilization in ageing articular cartilage, the degree of osteo-arthritis, and the peculiar rearrangement and grouping of chondrocytes that are seen in the conventional histological section of the osteo-arthritic cartilage. Our present studies show that this is indeed the case.

Table II, which is based on quantitative results obtained mostly before our earlier paper and used there for a comparative study with costal cartilage,

shows that increasing degrees of osteo-arthritis lead to increasing uptake of labelled sulphate *in vitro*.

The grades of osteo-arthritis of which we speak are pathological grades. They do not correspond to any clinical grade. They relate only to a single articular surface, that of one patella, and what we have called Grades I and II of osteo-arthritis are most likely to be sub-clinical lesions. No objection can be taken to this, because sub-clinical grades of other disease processes, discoverable only by the pathologist, are very generally recognized. Some may quibble that these are grades of "age change"

and not of osteo-arthritis, but from our parallel studies on costal cartilage, already published, and from our studies of weight-bearing and non-weight-bearing joint surfaces which are still being pursued, we are convinced that the changes outlined in an earlier part of this paper are to be classified only as those of osteo-arthritis.

Autoradiographs clearly show that the increased utilization of sulphate by osteo-arthritic cartilage is a function of the chondrocytes, and especially of those chondrocytes that proliferate to form multicellular clusters in the fibrillated areas of Zone 3 and, to a somewhat less extent of Zone 2. The chondrocytes of superficial Zone 1 in normal cartilage have the appearance of biologically effete cells and fix little or no sulphate; they do not react to the destruction of cartilage by tangential flaking that is seen in the earliest (Grade I) lesion of osteo-arthritis.

Curran and Gibson (1956) state that the isotope technique for estimating sulphate fixation in cartilage is a test of the viability of cartilage cells, and the application of the method to osteo-arthritic articular cartilage has proved that in such cartilage the cells are not dead or functionally inactive. On the contrary, their capacity for synthesizing chondroitin sulphate seems to be stimulated. It seems reasonable to suppose that the cells react in this way to the breaking up and loss of the surrounding cartilage matrix, but whether this is caused by mechanical trauma, by enzymatic action, or by other means is a point on which our present studies offer no evidence.

Experiments involving the depletion of living cartilage matrix by papain are relevant to this matter. Thomas (1956) originally observed that the ears of rabbits injected intravenously with crude papain flopped, and he discovered that this was due to a loss of the mucopolysaccharide matrix. Subsequent work showed that papain affected many other cartilages in the rabbit and that the breaking down of chondro-mucoprotein releases large amounts of chondroitin sulphate into the circulation (Bryant, Leder, and Stetten, 1958; Tsaltas, 1958; Weissmann, Potter, McCluskey, and Schubert, 1959). Quantitative studies with ^{35}S on the costal cartilages of papain-treated rabbits have very recently shown that the depletion of matrix brought about by this enzyme stimulates the chondrocytes to enhanced activity in synthesizing chondroitin sulphate (McElligott and Potter, 1960). This finding strongly supports our view that it is loss of matrix that primarily stimulates the cells in osteo-arthritic cartilage.

The large cell clusters of osteo-arthritic cartilage

almost certainly form by the proliferation, and not by the mere aggregation, of existing chondrocytes. That we have never, in many scores of sections, seen the mitosis of these cells is unimportant; the whole process of osteo-arthritis evolves exceedingly slowly; it is spread over years of time and is therefore not to be compared with such processes as the growth of granulation tissue or neoplasia, in which the pathologist is accustomed to see mitoses; adult cartilage, unlike epidermis or intestinal mucosa, is a highly stable tissue, virtually incapable even of self-repair. Indeed, our present histological studies give no indication that the active chondrocytes of disintegrating osteo-arthritic cartilage are capable of any other function than the elaboration of sulphated mucopolysaccharides; they do not seem to regenerate the collagen fibres of the cartilage, nor do they revert to a fibroblastic or undifferentiated form of mesenchymal cell.

The cell clumps in fibrillated cartilage have been known for 40 years or more; their significance was never clear, because no one knew just what these cells were doing. Now it has been demonstrated that they are attempting to make good the loss of mucopolysaccharide matrix. Our findings seem finally to refute the view that the initial cartilage lesion is a cellular necrosis, and they support the more widely-held view (see Bennett, Waine, and Bauer, 1942, for discussion) that the earliest disturbance is one of the intercellular substance. Silberberg, Frank, Jarrett, and Silberberg (1959) noted certain changes in the histochemical reactions of cartilage matrix in human sternoclavicular joints, indicating biochemical and biophysical changes, which they believed might interfere with the restraining influence usually exerted by the matrix on cell growth and might thereby release the dormant growth potential of the cartilage cells. This, on general principles, is an acceptable idea. The results of the stimulated chondrocyte growth and activity, however, fall short of an effective repair of the cartilage's defects, though, perhaps, the new production of sulphated acid mucopolysaccharides may for long delay its final disintegration.

The staining reactions which we have observed in articular cartilage are in line with those recorded by Fawns and Landells (1953). Particular points on which we agree are the general reciprocity of P.A.S. and metachromatic staining, the feeble staining of any mucopolysaccharides in Zone 1, the metachromatic pools around the cells in Zones 2 and 3, the absence of metachromasia in deep Zone 3 in older subjects, and the great intensity of P.A.S. staining in haloes around the cells of the calcified Zone 4.

Some additional points emerge from our studies: first, confirmation of the fact that alcian blue stains strongly in areas that also show metachromasia; secondly, the association of metachromasia and alcian-blue staining with active synthesis of chondroitin sulphate, as shown in the autoradiographs, and the negative P.A.S. reaction around cells that are fixing sulphate. The cells in the calcified zone of articular cartilage that are surrounded by a P.A.S.-positive halo seem to have little or no power of utilizing sulphate.

It is now generally agreed that alcian-blue staining and metachromasia both indicate the presence of acid mucopolysaccharides, commonly, but not necessarily, sulphated. A positive reaction with P.A.S. is much less specific, since P.A.S.-positive substances are of many kinds and include polysaccharides like glycogen, neutral mucopolysaccharides, mucoproteins and glycoproteins, glycolipids and some other unsaturated lipids, and phospholipids (Pearse, 1960). Some of the P.A.S. staining in cartilage may be more closely associated with its collagen fibrils than with its diffuse matrix. Basophilia with an acid haematoxylin corresponds mainly with metachromasia and likewise indicates the probable presence of acid mucopolysaccharides so far as haematoxylin staining of intercellular material is concerned.

When we consider all that may be observed in the various histological preparations we have used, we are bound to conclude that at every stage of osteo-arthritis it is loss of mucopolysaccharide matrix (chondroitin sulphate) that constitutes the primary lesion in the cartilage.

At the earliest stage, the superficial cartilage matrix changes its staining properties and seems to shrink and to crack along the tangentially disposed fibre lines (Fig. 6). Fibrillation then extends into the oblique-fibred Zone 2 (Figs 10 and 11) and then vertically through Zone 3 (Figs 15, 17, and 18). Metachromasia, basophilia, and alcian-blue staining are lost from this disintegrating matrix, but the fibres survive (Fig. 23) and the P.A.S. reaction is sometimes maintained in the region of these fibres. Finally, all the uncalcified cartilage of Zones 1 to 3 is sheared off at the level of the calcified Zone (Fig. 16), and ultimately such an area may be worn down to a polished eburnated surface of hyperplastic bone with no cartilaginous residues.

The reaction of the chondrocytes to this loss of matrix is vigorous in all areas of uncalcified cartilage except the most superficial. Proliferation of cartilage cells is accompanied by enhancement of chondroitin sulphate synthesis, but, because this seems to be the only capacity they retain, the

cellular reaction is incapable of permanently preventing the ultimate disintegration of the cartilage or of repairing it in any structurally effective way.

Summary

The uptake of labelled sulphate ($^{35}\text{SO}_4$) *in vitro* by normal and osteo-arthritic human articular cartilage has been measured and studied in autoradiographs. The findings have been considered in conjunction with observations on histological sections of the same cartilages stained for cells and for the mucopolysaccharide and fibrillar components of matrix.

Increasing avidity for sulphate is displayed by cartilage that shows increasing grades of osteo-arthritis. This is a cellular function and represents a stimulation of cells to manufacture chondroitin sulphate in response to loss of the matrix that surrounds them.

The cells most active in this respect are those that have proliferated to form large clusters lying between the fissures of fibrillated cartilage in the middle Zones (2 and 3); cells of the superficial zone (Zone 1) are effete, and they and the cells incarcerated in the calcified zone of cartilage (Zone 4) do not respond to any extent either by proliferation or by enhanced sulphate utilization.

Metachromasia and alcian-blue staining are found where sulphated acid mucopolysaccharides are being formed. These reactions tend to be lost as cartilage disintegrates.

The periodic acid-Schiff reaction (P.A.S.) of articular cartilage is difficult to interpret: it is usually absent where metachromasia is strong and *vice versa*; it seems often to be associated with the collagen fibril bands in cartilage and, with the fibres, may still be observed in disintegrating cartilage after the metachromatically staining material has been dispersed.

All our observations lead to the conclusion that the original lesion of cartilage in osteo-arthritis is a dissolution of the acid mucopolysaccharide fraction of its matrix.

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Absorption de sulfate ($^{35}\text{SO}_4$) par les chondrocytes en relation aux altérations histologiques dans le cartilage articulaire humain ostéoarthritique

RÉSUMÉ

On a mesuré et étudié à l'autoradiographie *in vitro* l'absorption de sulfate marqué ($^{35}\text{SO}_4$) par le cartilage articulaire humain, normal et ostéoarthritique. On a considéré les résultats en relation aux observations des coupes histologiques des mêmes cartilages, colorées pour mettre en évidence les cellules et les composants mucopolysaccharides et fibrillaires de la matrice.

L'absorption de sulfate par le cartilage augmente au fur et à mesure que l'ostéoarthrite s'empire. C'est une fonction cellulaire et représente une stimulation des cellules à produire du sulfate de chondroïtine en réponse à la perte de la substance de base.

Les cellules les plus actives de ce point de vue sont celles qui ont proliféré de grands amas situés entre les fissures du cartilage fibrillaire dans les zones moyennes (2 et 3); les cellules de la zone superficielle (zone 1) sont épuisées et, comme les cellules incarcérées dans la zone calcifiée du cartilage (zone 4), ne répondent guère par la prolifération ni par l'utilisation augmentée de sulfate.

On trouve de la métachromasie et de la coloration au bleu d'alcan à où se forment des mucopolysaccharides acides sulfatés. Ces réactions tendent à disparaître à mesure que le cartilage se décompose.

La réaction acide-Schiff périodique (P.A.S.) du cartilage articulaire est difficile à interpréter: elle est généralement absente quand la métachromasie est prononcée et vice versa; souvent elle semble être associée

aux bandes fibrillaires de collagène dans le cartilage et on peut encore l'observer, avec les fibres, dans le cartilage en désintégration, après que le matériel coloré métachromatiquement ait été dispersé.

Toutes nos observations mènent à la conclusion que la lésion originale du cartilage dans l'ostéoarthrite est la dissolution de la fraction mucopolysaccharidique acide dans la matrice.

Absorción de sulfato ($^{35}\text{SO}_4$) por los condrocitos en relación con los cambios histológicos en el cartilago articular humano osteoartrítico

SUMARIO

La absorción de sulfato marcado ($^{35}\text{SO}_4$) por cartilago articular humano, normal y osteoartrítico, ha sido estudiado *in vitro* por mediciones y autorradiografía. Los hallazgos se han considerado en relación con observaciones de secciones histológicas de los mismos cartilagos teñidas para visualizar las células así como los componentes mucopolisacárido y fibrilar de la substancia de base.

Progresiva avidez por el sulfato se manifiesta en el cartilago que presenta grados progresivos de osteoartritis. Se trata aquí de una función celular, representando una estimulación de las células a la producción de sulfato de condroitina como respuesta a la pérdida de la substancia básica que la rodea.

Las células más activas a este respecto son aquellas que han proliferado procedentes de grandes grupos situados entre las fisuras del cartilago fibrilado en las zonas medias (2 y 3); las células de la zona superficial (zona 1) están agotadas y ellas, así como las células engrosadas en la zona de cartilago calcificado (zona 4), no responden en absoluto ni por proliferación ni por utilización mayor de sulfato.

Metacromasia y tinción con azul-alcan aparecen donde mucopolisacáridos ácidos sulfatados están en formación. Estas reacciones tienden a desaparecer a medida que el cartilago se desintegra.

La reacción ácido-Schiff periódica (P.A.S.) del cartilago articular es difícil de interpretar: está usualmente ausente cuando la metacromasia es pronunciada y viceversa; a menudo parece estar asociada con fajas fibrilares de colágeno en el cartilago y puede aún ser observada con las fibras en cartilago en desintegración después que el material teñido metacromáticamente ha sido dispersado.

Todas nuestras observaciones conducen a la conclusión de que la lesión original del cartilago en la osteoartritis es una disolución de la fracción mucopolisacárida ácida de su substancia base.