Cartilage of the hip joint

Topographical variation of glycosaminoglycan content in normal and fibrillated tissue

A. MAROUDAS, H. EVANS, AND L. ALMEIDA*
Biomechanics Unit, Imperial College, University of London

In recent years several authors have studied patterns of degradation of articular cartilage with special reference to different areas on a given joint (*e.g.* Goodfellow and Bullough, 1967; Byers, Contepomi, and Farkas, 1970). The hip joint, because of its relatively simple geometry, has received most attention. It has been suggested that the different patterns of degradation observed might be related to differences in the load-bearing characteristics of the individual areas. Accordingly, Greenwald (1970) studied qualitatively the contact areas in the hip under physiological loads and Kempson, Spivey, Freeman, and Swanson (1969) examined topographical variations of stiffness over the area of the femoral head. It has also been shown (Hirsch, 1944; Kempson, Muir, Swanson, and Freeman, 1970) that cartilage stiffness is related to the glycosaminoglycan concentration in the tissue.

Whilst several authors (*e.g.* Miles and Eichelberger, 1964; Anderson, Ludowieg, Harper, and Engleman, 1964; Bollet and Nance, 1966; Stockwell and Scott, 1965, 1967) have studied the glycosaminoglycan content of articular cartilage in relation to age and osteoarthritic changes, these investigations were usually carried out on pooled samples of cartilage without reference to specific sites. Thus no data were available in the literature relating to topographical variations in the glycosaminoglycan content over the area of a joint. The purpose of the present work was to investigate in detail the concentration of glycosaminoglycans at different sites of the femoral head and the acetabulum in immature as well as mature subjects, covering, as far as possible, the complete age range. The results were related to the visual appearance of each area. In some specimens, apart from studying the mean glycosaminoglycan content at each site, the variation with depth below the articular surface was also investigated.

It has been shown that the glycosaminoglycan content in cartilage is quantitatively related to the concentration of negatively charged fixed groups as determined by physico-chemical methods (Maroudas,

Muir, and Wingham, 1969; Maroudas and Thomas, 1970). In the present study the concentration of negatively charged fixed groups has been chosen as a parameter representing the glycosaminoglycan content because its experimental determination is rapid and can be carried out on small quantities of tissue (Maroudas, 1970). It is thus well suited for an investigation involving large numbers of small specimens. Apart from experimental convenience, the advantage of measuring directly fixed charge density is that it is in itself an important parameter in determining the physiological behaviour of the tissue. Thus, for instance, the negatively charged groups of the glycosaminoglycans are partly responsible for the osmotic pressure in the cartilage matrix (Snowden, Kempson, and Maroudas, in preparation). This pressure, in turn, is thought to play a considerable part in the load-bearing process.

Material and methods

Human *post mortem* material was used throughout the present investigation. The subjects ranged in age from new born to 88 years.

The femoral head and acetabulum were carefully examined visually and the features characterizing the different sites were recorded. The surface of the joint was often gently rubbed with Indian ink in order to show clearly the transition from intact to fibrillated cartilage.

Four sites on the femoral head and three sites on the acetabulum were studied. The sites from which the specimens were usually obtained are shown in Figs 1 and 2 (overleaf).

Site 1 was on the superior surface of the femoral head, which is generally considered to be the weight-bearing area, while Sites 2, 3, and 4 were all close to the fovea: Site 3 was below the fovea (feature 9 in Byers' classification), Site 2 on the posterior facet, and Site 4 on the anterior facet respectively (Byers' features 10 and 11).

Site A was in the triangular area near the roof of the acetabulum. This area usually appears very soft. Specimens AA and AP were taken approximately in the middle of the anterior and the posterior branches of the acetabulum respectively.

FIG. 1 Diagram of femoral head, showing sites from which specimens were removed for testing

Determination of FCD

The tracer cation method (Maroudas and Thomas, 1970) was used for the determination of fixed charge density. The principle of the method is as follows:

If cartilage is equilibrated in a dilute electrolyte solution, free electrolyte is virtually excluded from the tissue because of the Donnan equilibrium (Maroudas, 1970). The only ions left in cartilage under these conditions should be the cations balancing the negatively charged fixed groups. Thus, by measuring the concentration of these cations, one can obtain a value from the total concentration of negatively charged fixed groups.

The procedure consisted of the following steps:

The specimens were cored out of the joint with a cork borer, 0.5 cm. in diameter. The bone was then carefully removed, leaving full-depth specimens of cartilage. The latter were soaked for a few minutes in Ringer's solution and weighed. Each specimen was then transferred into 5 ml. of a 0.015 M NaCl solution where it was left for a few hours. Under these conditions, free electrolyte diffuses out of the tissue so that the remaining sodium ions present are equal to the total concentration of negatively charged fixed groups. The specimen was finally transferred for 12 to 18 hrs into a 0.015 M NaCl solution, labelled this time with the radioactive isotope Na^{22} . All solutions in which cartilage specimens were being equilibrated were kept stirred at 4°C. At 4°C. autolysis of the cartilage during a period of 24 hr is insignificant.

After equilibration the specimens were removed from the radioactive solution, mopped dry, and transferred into a γ -radiation scintillation counter for determining their count-rates. Fixed charge density was calculated from the following formula:

$$\text{fixed charge density} = \frac{(\text{count-rate due to cartilage specimen}) \times (\text{molarity of solution})}{(\text{weight of cartilage specimen}) \times (\text{count-rate due to } 1 \text{ cm}^3 \text{ of equilibrating radioactive solution})}$$

Where the variation of fixed charge density with depth was also investigated, full-depth specimens were cut, after counting, into 200 μ sections using a Leitz Thermal Module freezing microtome. The sections were then 'counted' individually. They were subsequently resoaked in Ringer's solution and weighed on an electromagnetic microbalance.

As a final step, all specimens (both full-depth and slices) were dried to constant weight in a desiccator over phosphorus pentoxide and were weighed on the microbalance.

FIG. 2 Diagram of acetabulum, showing sites from which specimens were removed for testing

Results and discussion

A summary of results obtained on the femoral head and the acetabulum are given in Tables IA and IB respectively. The complete results for the femoral head are shown in Figs 3 to 5. Fig. 3 shows a graph of fixed charge density *v.* age for all four sites on the femoral head, fixed charge density being calculated per weight of wet tissue; Fig. 4 shows the same results, but this time expressed on a dry basis. Fig. 5 shows a graph of water content *v.* age, again for all four sites on the femoral head. The results of fixed charge density per wet weight *v.* age for the acetabulum are shown in Fig. 6.

Variation of fixed charge density and water content with age

The following points emerge from an examination of Figures 3 to 6 and Tables IA and B.

It can be seen that in the new born and in early childhood fixed charge density is low when expressed per weight of whole tissue (Fig. 3) but high when calculated on a dry weight basis (Fig. 4). This is consistent with the high water content of immature cartilage. From the physiological point of view, it is the fixed charge density on a whole tissue basis which is the significant parameter, since it determines both the hydraulic permeability and the osmotic pressure of cartilage. Since low fixed charge density of the tissue is associated with a high rate of fluid movement

and a low osmotic pressure, immature cartilage would be expected to be more readily deformable than the mature tissue. It should be borne in mind, however, that cartilage is thicker and stresses probably lower in children than in adults.

In mature normal specimens no statistically significant correlation was obtained between fixed charge density, whether expressed on a dry or wet basis, and age. This is consistent with the findings of

FIG. 3 Graph of fixed charge density calculated on a whole tissue basis, versus age, for four sites on the femoral head

FIG. 4 Graph of fixed charge density, calculated on dry weight basis, versus age, for four sites on the femoral head

FIG. 5 Graph of water content, versus age, for four sites in the femoral head

several authors (e.g. Miles and Eichelberger, 1964; Anderson and others, 1964; Maroudas and others, 1969; Bollet and Nance, 1966). There is also no correlation between water content and age in mature normal cartilage and this again is consistent with the conclusions of other workers.

Fixed charge density and water content of fibrillated cartilage

It is evident from Figs 3 and 4 and Table IA that fibrillated specimens have a much lower fixed

FIG. 6 Graph of fixed charge density, calculated on a whole tissue basis, versus age, for three sites on the acetabulum

charge density than normal whilst their water content is higher. The differences are statistically highly significant ($P < 0.005$). These findings are consistent with those of a number of authors who compared the glycosaminoglycan content of normal and degenerate specimens of cartilage (e.g. Hirsch, 1944; Mathews, 1953; Bollet, Handy, and Sturgill, 1953; Anderson and others, 1964; Bollet and Nance, 1966).

It is notable that differences in fixed charge density between normal and fibrillated specimens are more pronounced when fixed charge density is expressed on a whole tissue basis rather than by dry weight. Thus the mean fixed charge density per wet weight of tissue of normal specimens is 55 per cent. higher than that of the fibrillated material while the difference in the mean fixed charge density between the two sets of specimens when based on dry weight is only 30 per cent. This is due to an increase in the water content in fibrillated cartilage, superimposed on a decrease in the concentration of glycosaminoglycans. It must be borne in mind that it is the fixed charge density based on the weight of the whole tissue which is important from the physiological point of view and hence it is not surprising to find considerable differences, as regards both the permeability and the mechanical characteristics, between normal and fibrillated cartilage.

It may be surprising at first sight to find a lower water content in normal than in fibrillated specimens, since the former have a greater concentration of glycosaminoglycans which are known to be highly hydrophilic. However, it is the elastic force in the collagen network which prevents the glycosaminoglycan molecules within cartilage from swelling. In fibrillated specimens, in which the collagen network is damaged, this force is likely to be less and the glycosaminoglycans, though fewer in quantity, will be able to achieve a higher degree of hydration.

Fixed charge density and water content at different sites of the femoral head

Although, as can be seen from Table IA(2), the mean value of fixed charge density for normal cartilage is very slightly higher at the superior surface (Site 1)

Table IA *Mean values of fixed charge density at different sites of femoral head*

Series	(1) Total results				(2) Normal specimens					(3) Fibrillated specimens				
Site	1	2	3	4	1	2	3	4	Pooled specimens	1	2	3	4	Pooled specimens
No of samples	33	35	38	38	32	22	13	29	96	1	13	25	9	48
Mean fixed charge density (wet tissue basis)	0.1374	0.1174	0.0989	0.1220	0.1405	0.1310	0.1320	0.1320	0.1347	0.050	0.095	0.084	0.0895	0.088
Standard deviation	0.1780	0.0231	0.0282	0.0253	0.0178	0.0176	0.0178	0.0175	0.021	—	0.0155	0.0234	0.0225	0.021
Mean fixed charge density (dry tissue basis)	0.4449	0.3989	0.3457	0.4187	0.444	0.437	0.423	0.439	0.1347	0.4500	0.3401	0.3121	0.3381	0.329
Standard deviation	0.0487	0.0720	0.0896	0.0813	0.0579	0.0579	0.0379	0.0725	0.06003	—	0.0424	0.0864	0.1306	0.0825
Mean water content (percentage of weight of wet tissue)	69.09	70.57	71.38	70.75	68.5	70.3	69.2	69.4	69.24	89	72.68	72.63	75.08	73.2
Standard deviation	3.000	3.026	3.815	3.406	2.98	2.87	1.73	2.83	2.77	—	2.882	4.234	1.976	4.17

Table IB *Mean values of fixed charge density at different sites of acetabulum*

Site	AA*	AP*	Pooled AA and AP	A*
No. of samples	27	27	54	29
Mean fixed charge density (wet tissue basis)	0.117	0.124	0.1205	0.067
Standard deviation	0.026	0.020	0.023	0.022
Mean fixed charge density (dry tissue basis)	0.381	0.417	0.399	0.303
Standard deviation	0.091	0.083	0.087	0.084
Mean water content (percentage of weight of wet tissue)	69.9	69.8	69.85	78
Standard deviation	2.91	2.80	2.855	2.78

* All samples AA and AP were normal and all samples A were soft but not fibrillated

than in the perifoveal region (Sites 2, 3, and 4), the difference is not statistically significant ($P > 0.1$); it must therefore be concluded that *normal* cartilage shows no significant variations with regard to fixed charge density over the area of the femoral head.

However, if no distinction is made between completely normal specimens and those showing varying degrees of fibrillation, then fixed charge density does exhibit variations from area to area, being highest at Site 1 and lowest at Site 3 (see Table IA(1)). This is consistent with the very much greater incidence of degenerative changes in the perifoveal region than on the superior surface of the femoral head. Thus, while out of the 33 specimens examined only one showed fibrillation at Site 1, surface roughening or more serious fibrillation was seen in 25 out of the 38 cases at site 3.

Several authors have commented on the softness of the perifoveal region and Kempson and others (1969) have drawn maps showing a systematic

pattern of variation of stiffness over the area of the femoral head. Since stiffness has been shown to depend on the glycosaminoglycan content (Hirsch, 1944; Kempson and others, 1969), it is not surprising to find that their softest region corresponds to Site 3 of the present investigation. However, it must be stressed that when *normal* cartilage is present in an area, fixed charge density is the same whatever the location of that area (see Table I); one would therefore not expect visually normal cartilage to be any softer in the perifoveal region than on the superior surface. It is therefore suggested that the variations in stiffness reported by Kempson and others (1969) must have been due to the presence of slight surface fibrillation in the perifoveal region in the specimens which they tested.

In order to determine whether localized degeneration at a given site leads to changes in fixed charge density at other sites where cartilage remains visibly normal, the mature femoral heads tested were divided into two groups:

(I) Those which showed somewhere on their surface fairly extensive fibrillation, ulceration, or cartilage loss

(II) Those which were either completely normal throughout or showed small areas of surface roughness only.

The two groups were compared with respect to the mean fixed charge density at Site 1, where the cartilage was normal in 32 out of the 33 heads tested. Table II shows that there was no significant difference between the two groups. It may be concluded that local changes, even if severe, do not affect the concentration of glycosaminoglycans at other sites of the same joint. Degeneration thus appears to be initially

Table II *Fixed charge density of Groups I and II*

Group	No. of samples	Mean fixed charge density at site 1
I	10	0.139
II	22	0.141

a local phenomenon and does not seem to be accompanied by overall changes in the concentration of negatively charged groups in the joint.

This conclusion is in agreement with the previous findings by Anderson and others (1964) relating to the glycosaminoglycan content in the areas of cartilage surrounding arthritic lesions.

Fixed charge density and water content at different sites of the acetabulum

The mean values of fixed charge density and water content of Sites AA and AP lie very close to one another. The fixed charge density is slightly (8 per cent.) lower than the mean value for the normal adult cartilage on the femoral head. In the present series of experiments no fibrillation was observed at Sites AA and AP.

Site A, at the apex of the acetabulum, is almost invariably soft in the adult though it does not appear to be fibrillated.

In immature specimens (see Fig. 6) there is no significant difference in fixed charge density or water content between Site A on the one hand and Sites AA and AP on the other. However, while at Sites AA

and AP, as on the femoral head, fixed charge density per wet weight of tissue was found to increase in the adult and the water content was found to decrease, at Site A the water content remained high and fixed charge density fell. Table IB shows that, in the adult, the mean fixed charge density at Site A was lower than that at any other site either on the acetabulum or on the femoral head, while the water content was higher.

The difference may be a developmental one since Site A overlies the area of bone which in the child formed the tri-radiate junction. It could also be that, since the junction between articular cartilage and the labrum is not always well defined, the cartilage taken from Site A may sometimes not be truly hyaline.

Variations of fixed charge density with depth from the articular surface at different sites of the femoral head

Fig. 7 shows schematic drawings of five typical femoral heads exhibiting varying degrees of degeneration at different sites.

Fig. 8 (overleaf) shows the graphs of fixed charge density per wet weight of tissue *v.* slice number from the articular surface for several sites on each of the five heads.

FIG. 7 Diagrammatic drawings of five femoral heads, showing areas of degeneration and sites from which specimens were removed for testing:

- (i) Head 1, age 58 (ii) Head 2, age 51 (iii) Head 3, age 53 (iv) Head 4, age 68 (v) Head 5, age 72

FIG. 8 Graphs of fixed charge density calculated on a whole tissue basis, versus slice number from articular surface, for the different sites tested on heads 1-5. Ages as in Fig. 7

The graphs will be discussed in detail for each head.

HEAD 1 (age 58) was completely normal over its entire surface and the mean values of fixed charge density for

the full-depth specimens were very close to one another at all four sites. The curves of fixed charge density *v.* depth also lie close to one another. In the middle zone fixed charge density is about twice as high as in the superficial

zone. It decreases again in the deep zone but remains higher than in the superficial zone.

HEAD 2 (age 51) showed surface roughening at an area below the fovea and a narrow band around the fovea; all other areas were completely normal. Specimens 1, 2, and 4 were taken from normal areas, Specimen 3 was taken from the slightly rough area below the fovea. Specimen 3a was on the edge of this area, towards the posterior facet. Fig. 8(ii) shows that the curves for Specimens 1, 2, and 4 practically coincide, the fixed charge density in the middle zone rising again to about twice its value in the superficial zone. Specimen 3 shows a lower fixed charge density throughout, while the curve for Specimen 3a lies a little above that for Specimen 3 but below those for Specimens 1, 2, and 4. In the superficial zone curves 3 and 3a coincide.

HEAD 3 (age 53) showed a similar appearance to Head 2 except for the presence of a somewhat more fibrillated area below the fovea and the curves of fixed charge density *v.* depth followed a similar pattern, curves for specimens 1, 2, 4 being normal.

HEAD 4 (age 68) showed more severe local signs of degeneration than the previous specimens. Thus there was fairly extensive fibrillation in the superior and posterior perifoveal region while the area below the fovea looked rough and lumpy. The superior surface, however, appeared entirely normal.

The curve for Specimen 1 is similar to those obtained for Heads 1, 2, and 3. The curve for Specimen 2 shows very low values of fixed charge density in the superficial zone. This corresponded to a markedly fibrillated appearance of the first slices. Fixed charge density rose considerably with distance from the articular surface, until in the deep zone it almost approached the values for Specimen 1. It should be noted that Specimen 2 was a thick specimen. Specimen 3, on the other hand, was very thin and this may explain why, although its surface appeared less fibrillated and the fixed charge density of the surface slice was higher than that of Specimen 2, the average fixed charge density was lower than that of Specimen 2. Specimens 2a and 4a were taken from sites on the edges of the fibrillated zone and their fixed charge density curves occupy an intermediate position between the curve for the normal Site 1 and those for the fibrillated Sites 2 and 3.

HEAD 5 (age 72) showed an area on the posterior facet close to the fovea which was worn down to the bone. There was also a very fibrillated zone below the fovea, extending to the anterior facet. Specimens 1, 2a, and 4a came from visually normal areas; Specimen 2 came from a roughened area adjacent to the area depleted of cartilage; Specimen 3 came from a badly fibrillated area below the fovea and Specimen 4 came from a similar area just anterior to the fovea.

The three curves obtained for the visually normal specimens lie close to one another and well above those from fibrillated areas. Specimen 3 was the thinnest of the three abnormal specimens and this is probably why it had the lowest overall fixed charge density, though in fact near the surface its fixed charge density was higher than that of Specimen 4.

The following general points arise from the examination of the fixed charge density curves for the various sites:

Curves for specimens showing fibrillation invariably lie well below the curves for visually normal specimens. Curves for specimens on the edge of fibrillated areas usually lie in an intermediate region possibly because of the presence of microscopic surface cracks.

Fibrillated specimens show a decrease in the glycosaminoglycan content throughout most of their depth, not only at the surface. However, some thick specimens from fibrillated areas show relatively smaller departures from the normal specimens in the deep zone than in the superficial and the middle zones. It should also be noted in this context that the thin specimens in the fibrillated areas below the fovea usually have a low fixed charge density throughout their thickness. These findings would suggest that glycosaminoglycan loss from fibrillated cartilage might proceed by a gradual 'leakage' of the large molecules, beginning at the articular surface. The thicker the cartilage the slower the overall process of depletion would be. In this connection it is notable that in normal specimens the curves are usually steep near the surface, whilst in fibrillated areas the curves are considerably flatter.

Although, in fibrillated specimens, fixed charge density is considerably lower than in normal cartilage, it is never close to zero, even in the superficial zone.

The lowest level of fixed charge density encountered in any of the specimens in this study was around 0.03 mEq./g. wet tissue. It may be that this represents the same proteoglycan fraction which is so difficult to separate from collagen by extractive biochemical procedures and which is thought to be intimately associated with the collagen fibres.

The curves in Fig. 8 are all for fixed charge density based on wet weight of tissue. For comparison two sets of results for Head 2 are plotted on both a wet and a dry basis (Fig. 9, overleaf). The curves based on dry weight dip more in the deep zone than the curves based on wet weight. This happens because the water content decreases considerably in the deep zone (Fig. 10, overleaf).

It should be noted that a similar tendency for the water content to decrease with the distance from the articular surface has been observed previously in the cartilage from the femoral condyles (Maroudas and others, 1969). However, the average water content in the femoral head appears to be 10 per cent. lower than in the femoral condyles, while the average fixed charge density is some 10 to 20 per cent. higher.

Fig. 9 shows that, when expressed on a dry weight basis, fixed charge density is nearly as low in the deep as in the superficial zone. However, the reason for this low value is different in the two zones. Thus, in the superficial zone, there is a high concentration of

FIG. 9 Comparison between typical curves of fixed charge density, versus slice number from articular surface, based on wet and dry weight of cartilage respectively

FIG. 10 Typical curves of water content, versus slice number from articular surface

collagen and a very low concentration of chondroitin sulphate (Maroudas and others, 1969). In the deep zone there is much less collagen, but there appears to be a, so far unidentified, non-collagenous neutral protein (Muir, Bullough, and Maroudas, 1970). Furthermore, the main glycosaminoglycan present in the deep zone is keratan sulphate which has only one acidic group per molecule (whilst chondroitin sulphate has two). Thus even fairly large amounts of keratan sulphate are associated with a low fixed charge density.

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Summary

Using a fixed charge density micro-method, the concentration of glycosaminoglycans has been measured at different sites over the femoral head and the acetabulum.

Forty hips were studied, from subjects ranging in age from newborn to 88 years.

Topographical variation in fixed charge density over the femoral head was found to correlate closely with local fibrillation. Normal cartilage exhibited negligible variation in glycosaminoglycan content from site to site. Areas which showed signs of fibrillation, however slight, had a lower fixed charge density than normal.

Although all fibrillated specimens had a lower fixed charge density throughout their depth than normal cartilage, the extent of glycosaminoglycan depletion appeared to be relatively more pronounced in very thin specimens and in the slices nearer the articular surface in the case of thick specimens. This observation might be interpreted as due to a diffusive leakage of glycosaminoglycans via the articular surface in fibrillated areas.

Degeneration at a given site of the femoral head appeared to have no effect on the level of fixed charge density at other sites on the joint surface. This points to a focal nature of degenerative change rather than to a generalized degradative process.

In normal cartilage from adult subjects, neither fixed charge density nor water content showed any variation with age.

In the child fixed charge density was lower than in the adult when expressed on a whole tissue basis but higher when expressed per dry weight; this is consistent with the high water content in immature cartilage.

In the acetabulum, the same fixed charge density was observed on the anterior and posterior branches, but the soft area near the apex had a very low fixed charge density. It is concluded that the reason for this low value is anatomical rather than pathological.

The fixed charge density micro-method can give detailed information on the topographical variation of acid glycosaminoglycans, which is more accurate than histological methods. The technique is more applicable to small samples than biochemical procedures.

Our thanks are due to Dr. Paul Byers for his advice and help in grading the specimens. This work was supported by a grant from the Medical Research Council.

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