Chemical composition of human femoral head cartilage: influence of topographical position and fibrillation

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SUMMARY Topographical variations in the composition of cartilage have been described in post-mortem femoral head cartilage. Weight bearing cartilage of the superior region was considerably thicker and had a higher glycosaminoglycan content and lower water and collagen content than cartilage at the periphery and below the fovea. These topographical variations in composition may result both from variations in thickness of the cartilage and from regional areas of degeneration. The composition of cartilage at different depths and with different surface characteristics from different areas of the femoral head was measured. Fibrillated cartilage both from the inferior and superior perifoveal areas had a reduced glycosaminoglycan content and higher water content than intact post-mortem specimens. Cartilage adjacent to fibrillated areas from the superior region did not differ in composition from intact areas of cartilage from the zenith of the femoral head.

The chemical composition of articular cartilage varies with age (Hjertquist and Lemperg, 1972; Ruttner et al., 1974; Venn, 1978) and with degenerative change (Ficat and Maroudas, 1975; Venn and Maroudas, 1977). Degenerative changes themselves are age-related (Collins and Meachim, 1961; Bullough et al., 1973). In addition cartilage composition varies between different joints (Maroudas, 1975) and with topography on a single joint (Bjelle, 1974). Cartilage composition is known to influence the mechanical functioning of the tissue. For instance, the weight bearing areas of the femoral head are associated with both high compressive stiffness and high glycosaminoglycan content (Kempson et al., 1970). Thus a detailed study of topographical variations in the chemical composition of normal cartilage is important both for an understanding of the mechanism of joint function and as a basis for the study of the changes in composition which occur as a result of age and degeneration.

A certain amount of confusion exists in the literature regarding the degenerative changes of clinical osteoarthrosis and normal post-mortem fibrillation, and in some cases the terms have been used synonymously. However, in the post-mortem femoral head fibrillation generally occurs in the inferior region in the perifoveal area, and at the periphery, in contrast to osteoarthritic specimens, where bone exposure at the zenith of the femoral head is frequently surrounded by areas of thinned or fibrillated cartilage (Byers et al., 1970). Thus the 'progressive' fibrillation in weight bearing areas leading to development of osteoarthrosis is probably caused by different factors from those which cause degeneration of the non-weight bearing areas, and osteoarthrosis of the hip may be the end result of several different pathological processes (Kellgren, 1961). If this is the case, the sequence of biochemical events may well differ between fibrillation and osteoarthrosis, and the assumption that the degenerative changes noted in fibrillated post-mortem cartilage merely differ in extent from those which occur in osteoarthrosis may not be valid.

The present study is an extension of work previously reported (Venn and Maroudas, 1977) and has been undertaken to establish the effect of topographical situation on the chemical composition of femoral head cartilage and to evaluate the effect of fibrillation in cartilage from different areas of the femoral head.
Materials and methods

Materials
Human femoral heads were obtained at necropsy and stored at −20°C until use. Surface characteristics were visualised by Indian ink staining (Meachim and Stockwell, 1973); all specimens which showed uptake of stain after gentle wiping were classed as fibrillated. Full-depth cartilage plugs were cut with a cork borer (1 cm diameter) and were excised from the bone with a sharp scalpel. In order to determine biochemical variations with depth of 200 μm slices were cut parallel to the cartilage surface with a freezing microtome.

Methods
Cartilage thickness was measured with a millimeter micrometer. The wet weight of cartilage specimens was determined after soaking for 30 minutes in 0.15 M NaCl in order to ensure rehydration after dissection. Negligible amounts of glycosaminoglycans were lost in this period. The dry weight of each sample was determined by weighing to constant weight after drying to 67°C. Fixed charge density (FCD) was determined by the tracer cation method as previously described (Maroudas and Thomas, 1970). Cartilage was digested in papain solution (1 mg per 20 mg dry weight) overnight at 67°C (Hjertquist and Lemperg, 1967) prior to chemical analysis. Uronic acid was determined by the Bitter and Muir (1962) procedure as modified for automated analysis (von Berlepsch, 1969). Hexosamine was determined by the Elson and Morgan reaction (1933) after hydrolysis for 4 hours at 100°C and drying in vacuo overnight. Hydroxyproline content was determined using the Stegemann (1958) method modified for automated analysis by Grant (1964).

Calculations
Collagen content was calculated by multiplying the hydroxyproline content by 7.6 (Eyre, private communication). Chondroitin sulphate content was estimated from the uronic acid content (mM/g) and keratan sulphate was estimated by subtracting the uronic from the hexosamine content (mM/g) as previously described (Venn and Maroudas, 1977).

Results

Chemical composition with position on the femoral head
Full-depth plugs of cartilage 1 cm diameter were excised adjacent to each other at a distance 1 cm from the fovea. Cartilage plugs were also cut across the superior surface of the femoral head to give a profile from the fovea to the outer peripheral region of the femoral head. Measurements were made on 8 femoral heads (mean age 48, range 36 to 58) selected to include only specimens with cartilage in good condition. Surface fibrillation as shown by Indian ink staining occurred at position points 1, 2, 12, 13, and 16 (Fig. 1), that is, surrounding the fovea, in the inferior region, and at the periphery. Mean values for thickness, collagen, water, fixed charge density, chondroitin, and keratan sulphate contents are shown in Table 1 (position points as in Fig. 1). A similar pattern was found in all specimens. In general, cartilage was thickest at the zenith of the femoral head and thinnest below the fovea and at the periphery.

Water content was lowest in the superior region and greatest in the fibrillated regions below the fovea and at the periphery. Total glycosaminoglycan content as measured by fixed charge density was maximal in the superior region and at a point midway between the fovea and the periphery (Table 1 and Fig. 1). Both the chondroitin and keratan sulphate contents showed a similar variation with...
Table 1  Variation of thickness, water content, collagen content, fixed charge density, chondroitin, and keratan sulphate content with position over the femoral head. Same specimens as Fig. 1. Position points as in Fig. 1.

<table>
<thead>
<tr>
<th>Position point</th>
<th>Thickness (mm)</th>
<th>Water content (% wet weight)</th>
<th>Collagen content (% dry weight)</th>
<th>FCD meq/g wet weight</th>
<th>Chondroitin sulphate mM/g wet weight</th>
<th>Keratan sulphate mM/g wet weight</th>
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position (Table 1 and Fig. 1), though keratan sulphate was more variable than the chondroitin sulphate content. Collagen content (percentage dry weight) was greatest in the fibrillated regions below the fovea and at the periphery, as would be expected in regions of low glycosaminoglycan content.

The gradual increase of glycosaminoglycan content from a minimum below the fovea to a maximum in the superior region could not be entirely accounted for by surface fibrillation, since in the specimens selected Indian ink uptake was confined to limited areas below the fovea, surrounding the fovea, and at the periphery (Fig. 1). In addition similar topographical variations have been observed in young specimens (under 20 years of age), though to a less marked extent owing to the small amount of keratan sulphate present in this age group. However, the topographical variation of glycosaminoglycan content in intact cartilage correlated well with cartilage thickness (Fig. 2), while fibrillated cartilage always had a lower glycosaminoglycan content than intact cartilage of the same thickness.

**EFFECT OF FIBRILLATION WITH DEPTH IN CARTILAGE FROM DIFFERENT REGIONS**

Fibrillated cartilage was removed from post-mortem femoral heads at a position either above or below the fovea as illustrated (Fig. 3). Adjacent areas with an intact surface were also analysed together with a control plug from the superior region. Fibrillated cartilage from both inferior and superior regions had a lower glycosaminoglycan content than the control cartilage (Fig. 4). Cartilage adjacent to fibrillated areas from the superior surface had a similar glycosaminoglycan content to the control cartilage. Areas adjacent to fibrillation in the inferior region showed some slight surface degeneration. They had a lower glycosaminoglycan content than adjacent areas from the superior surface, consistent with the findings of full depth cartilage from this area.

Similar results were obtained for chondroitin (Fig. 5) and keratan sulphate (Fig. 6) contents, though keratan sulphate was more variable than chondroitin sulphate and proportionally lower in fibrillated than intact cartilage.

There was a marked increase in water content in fibrillated cartilage throughout the depth of the
cartilage (Fig. 7). This was especially true in fibrillated areas below the fovea. Adjacent areas from the inferior region had a slightly higher water content than the intact cartilage, but there was no significant difference between intact and adjacent samples in the superior region. In all groups of cartilage, fibrillated or not, the slope of the water content profile with depth was similar, being greatest at the surface and decreasing gradually with depth.

Finally, one marked difference between samples from the different regions of the femoral head is the variation in thickness, cartilage being significantly thinner in the inferior regions.

Discussion

It could be argued that the variations of glycosaminoglycan content with topography noted in this study are due to loss of glycosaminoglycans through
minimal surface defects not visualised on Indian ink staining. Indeed areas of low glycosaminoglycan content (below the fovea and at the periphery of the femoral head) are normal foci of fibrillation in post-mortem specimens (Meachim and Emery, 1973). However, the specimens used in this study were selected for the integrity of their cartilage surface, and the only regions showing surface fibrillation were in the inferior region, around the fovea, and at the periphery. In addition the other characteristics of fibrillated cartilage (high water and collagen contents) were noted only in areas of Indian ink staining. Thus it is likely that the gradual reduction of glycosaminoglycan content from the superior to the inferior region is due to factors other than surface damage.

The most likely explanation for the topographical variation of glycosaminoglycan content is that it is related to cartilage thickness. The observed variation in thickness (cartilage is thickest at the zenith of the femoral head and thinnest at the periphery and below the fovea) correlates well with the variation in glycosaminoglycan content. In thin cartilage from the inferior region the profile of glycosaminoglycan content with depth is similar to that for thicker cartilage, but a higher proportion of the cartilage is made up of the low glycosaminoglycan-containing surface regions and proportionately less consists of the glycosaminoglycan-rich deeper zones.

The results of the present study are in good general agreement with those of Bjelle (1974), who found a reduction of glycosaminoglycan content in the non-weight bearing areas of the femoral condyle and a greater variation in keratan than in chondroitin sulphate with position. However, in that study the effect of cartilage thickness was not taken into account. Cartilage stiffness also varies with position over the femoral head (Kempson et al., 1971); cartilage is stiffest at the zenith of the femoral head, where both thickness and glycosaminoglycan content are greatest. This area of thick cartilage with a high glycosaminoglycan content coincides with the loaded area between the femoral head and the acetabulum (Greenwald, 1970).

Degenerate cartilage is characterised by a low glycosaminoglycan content (Matthews 1953; Bollet et al., 1963; Bollet and Nance, 1966) and a high water content (Bollet and Nance, 1966; Venn and Maroudas, 1977). Glycosaminoglycan content is reduced throughout the cartilage thickness in specimens showing overt fibrillation (Maroudas et al., 1973), and, as has been confirmed in this study, adjacent areas with an intact surface show no reduction in glycosaminoglycan content. In the patella the glycosaminoglycan content does not vary with position in cartilage of the same thickness but is reduced in thin and/or fibrillated cartilage (Ficat and Maroudas, 1975).

One of the most noticeable differences between intact and fibrillated post-mortem femoral head cartilage is the increase in water content in fibrillated specimens. However, the profile of water content with depth remains similar in the 2 groups, unlike that for osteoarthritic femoral head cartilage, where water content is maximal in the mid zones (Maroudas, 1976; Maroudas and Venn, 1977). The swelling of the mid zones of osteoarthritic cartilage is thought to be due to damage of the collagen framework which is no longer capable of restraining the swelling pressure of the proteoglycans. The difference in the pattern observed in osteoarthritis as compared to post-mortem
fibrillated cartilage may result from a difference in the nature of the initial damage to the cartilage, or it may be a result of topographical differences. In osteoarthrosis cartilage is frequently eroded at the zenith of the femoral head (Byers et al., 1970), while fibrillated cartilage occurs in the peripheral, inferior, and perifoveal regions.

It is unlikely that the pattern of water content with depth generally observed in fibrillated post-mortem cartilage could lead to the type of profile seen in osteoarthritic specimens. However, it must be emphasised that the 2 patterns do overlap to some extent, and that occasionally osteoarthritic cartilage shows a profile for water content similar to that for post-mortem cartilage and vice versa. In addition there may be a variation in cartilage from other joints. For example, in fibrillated post-mortem patella cartilage the profile of water content with depth is similar to that for osteoarthritic femoral head cartilage (Maroudas, personal communication).

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References


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