The thickness of the calcified layer of articular cartilage: a function of the load supported?

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INTRODUCTION

The hyaline cartilage of a synovial joint varies in thickness from region to region of the articular surface. A correlation between the thickness of the cartilage and the demands made upon it was reported by Holmdahl & Ingelmark (1948) and by Ekholm & Ingelmark (1952), the subsequent work of Pauwels (1965) being carried further, particularly by Kummer and his school (1968, 1969). In places where there is a greater load on the articular cartilage, that cartilage is thicker.

Practically all synovial joints possess a region of calcified cartilage lying immediately over the underlying bone and separated from it by a curved plane, which appears in sections stained with haematoxylin and eosin as a basophilic wavy line. This dividing line was described as the *Trennungsstreifen* by Fick in 1904, the name 'tidemark', by which it is now known, being introduced by Fawns & Landells in 1953.

Although quite a number of works have been published on the nature and distribution of the total cartilaginous layer – particularly in the hip joint (see, for instance, Kurrat, 1977; Oberländer, 1977; Kurrat & Oberländer, 1978) – few authors have so far concerned themselves with the relative thickness of the calcified zone.

In 1970 Green, Martin, Eanes & Sokoloff determined the thickness of the calcified zone of 24 human patellae by measuring samples taken from the centre of the bone and arrived at a mean value of $134 \pm 7 \mu$m. Neither the age of the patient nor the presence of osteoarthritis appeared to have any effect on the thickness of the layer.

Stougard (1974) examined samples from the femoral condyles and patellae from normal and abnormal subjects (for instance, from cases of patellar chondromalacia), and found little difference between the weight bearing and non-weight bearing regions of the joint.

Lane & Bullough (1980) observed a decrease in the thickness of the calcified zone with increasing age in the head of the humerus and femur in man, and also an average thickness of this layer that was some 10–15 % higher in the less heavily loaded regions.

All previous workers had measured the calcified zone at only a few points and not, as in the case of the entire cartilage, in all regions of the joint.

The purpose of our investigation was to measure the thickness of both the entire articular cartilage *and* the calcified zone at a number of carefully selected points in all regions of the femoral head and, by comparing the two, to decide whether a true correlation actually exists.

MATERIALS AND METHODS

We examined 8 femoral heads from subjects undergoing autopsy at the Pathologisches Institut, University of Freiburg. Specimens showing obvious signs of osteoarthritis were discarded, and care was taken to select only subjects in whose
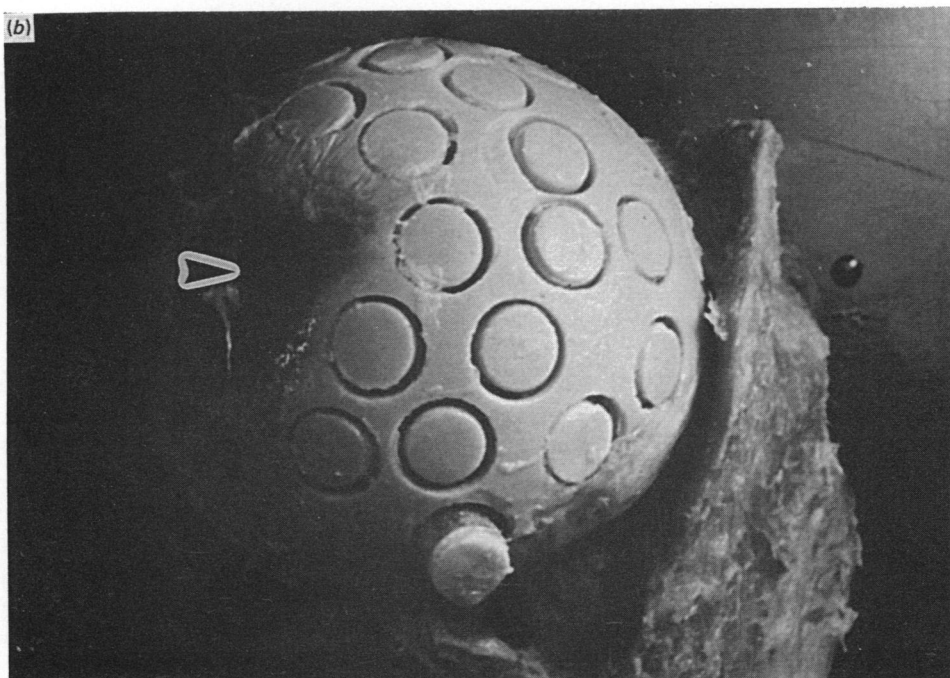
(a)
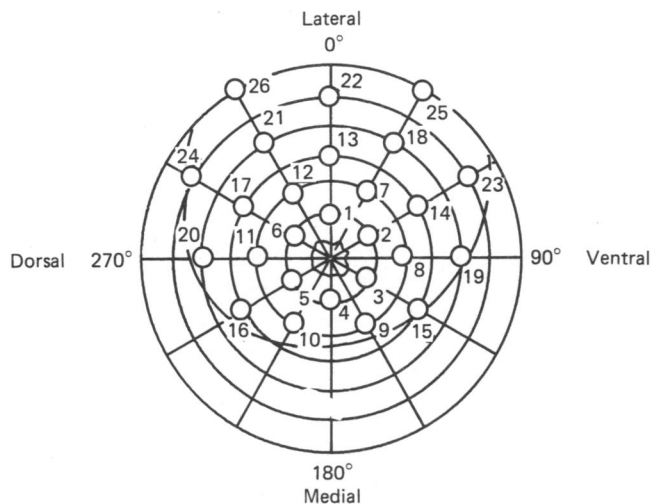
(b)

Fig. 1 (a–b). (a) Cartesian grid employed. (b) Femoral head, showing method of selecting samples. (The arrow indicates the fovea of the head.)

history no trace of local or general disease of the joints could be found. Seven of the subjects (5 male and 2 female) were between 27 and 86 years old at the time of death. Another, for whom no details were available, brought the total up to 8.

The precise localisation of the samples from each head was determined with a Cartesian grid. This grid was copied from a diagram published by Kurrat & Oberländer (1978), made into a slide and projected on to the articular surface so that the origin coincided with the middle of the fovea capitis femoris (Fig. 1 a). Approximately
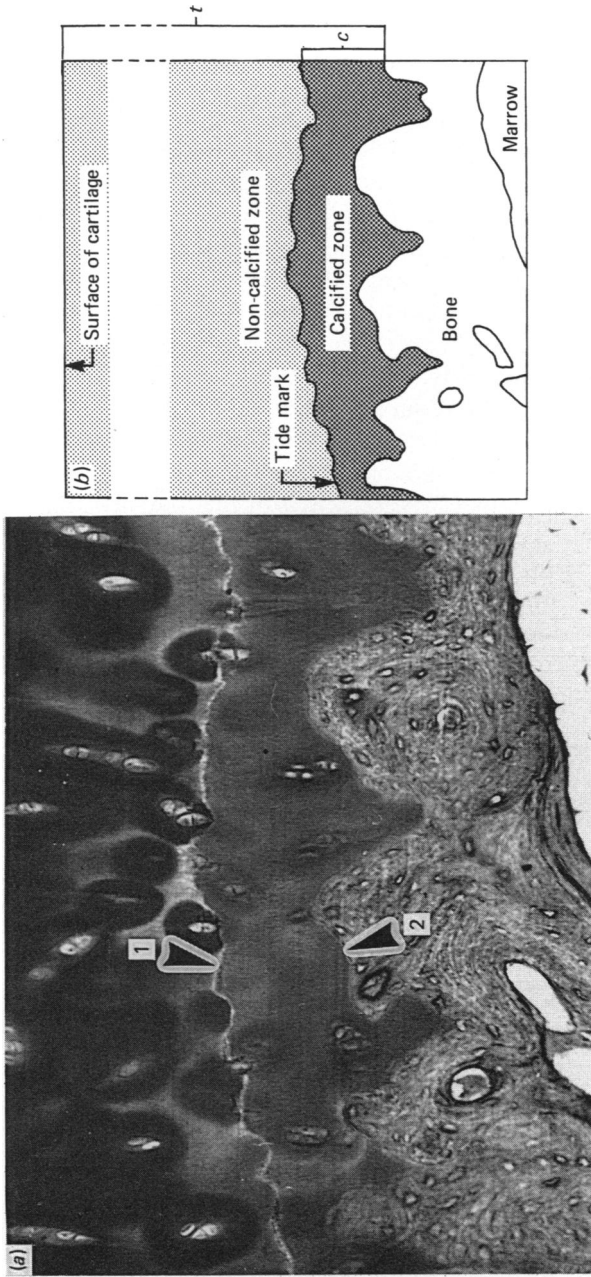
Fig. 2(*a*–*b*). (*a*) Stained section. The area between the 'tidemark' (arrowhead 1) and the contour of the subchondral bone (arrowhead 2) is indicated. × 40. (*b*) Diagram defining the areas of total (*t*) and calcified (*c*) cartilage in a single section.

25 samples were obtained from each bone, an 8 mm punch being applied at the predetermined points (Fig. 1*b*). They were then decalcified, embedded in paraffin, sectioned perpendicular to the surface and stained with light green. We chose this stain because it emphasises both the 'tidemark' and the transitional zone between bone and cartilage equally well (Fig. 2*a*).

Comparison of the width of the unsectioned specimens embedded in the block with the cut sections by means of sliding calipers revealed a shrinkage of 10–15%. To ensure that the distortion remained as far as possible the same throughout the survey all blocks were sectioned in the same direction.

All measurements of the histological sections were made semi-automatically with the image analyser IBAS 2000 (Zeiss/Kontron, Oberkochen, Eching, FRG).

In each section we chose a particular region and measured (1) the area of the entire cartilage and (2) the area of the calcified part (Fig. 2*b*). From these measurements the linear values were derived mathematically.

The images of the sections were input from a Zeiss Standard Universal microscope (objective 10 ×, optovar 1·25) with a Siemens K 30 Vidikon TV camera directly from the stained slide. The microscope was focused until the image of the section was clearly visible on the TV screen of the computer. The thickness of the total articular cartilage as well as the thickness of the calcified zone were measured interactively on the TV screen by following the outlines of the regions of interest with a cursor. The length of each region on the screen corresponded to an object area of 0·5 mm in length.

The mean values and standard deviations were calculated from five randomly selected areas per section. Up to six sections per slide were investigated.

The relative error of measurement was less than 1%. Data were investigated for statistical significance with the Mann–Whitney U test (two-sided). Differences were considered to be significant if $P < 0.05$.

We employed this method of measurement because both the 'tidemark' and the contour of the subchondral bone undulate considerably, so that the expected scatter from a simple linear measurement of the distance would be too great.

Calculating the thickness of the zone over a predetermined area yielded results with remarkably little scatter and good reproducibility.

RESULTS

The thickness of the complete cartilage was found to lie between 0·4 and 3·5 mm. The greatest thickness was always found lateral to the fovea, while over the dorso-medial surface of the head as far as the medial margin of the fovea the layer of cartilage was thinner. Its decrease in thickness took place more slowly on the lateral than on the medial side of the joint (Fig. 3*a*).

The thickness of the calcified zone lay between 20 μm and 230 μm and showed the same variation as the complete layer of cartilage (Fig. 3*b*).

Since the values varied considerably within a single joint, we used the XY coordinates for the selection of corresponding regions of the complete and calcified layers. The regression lines obtained revealed a linear correlation between the two sets of values (Fig. 4).

The calculated correlation coefficients for all eight femoral heads lay between $r = 0.60$ and $r = 0.96$ (Table 1).

To make the interdependence between the thickness of the two regions easily recognisable the findings are shown in the form of a histogram (Fig. 5). From this diagram it can be clearly seen that the thickness of the calcified zone varies *pari passu*
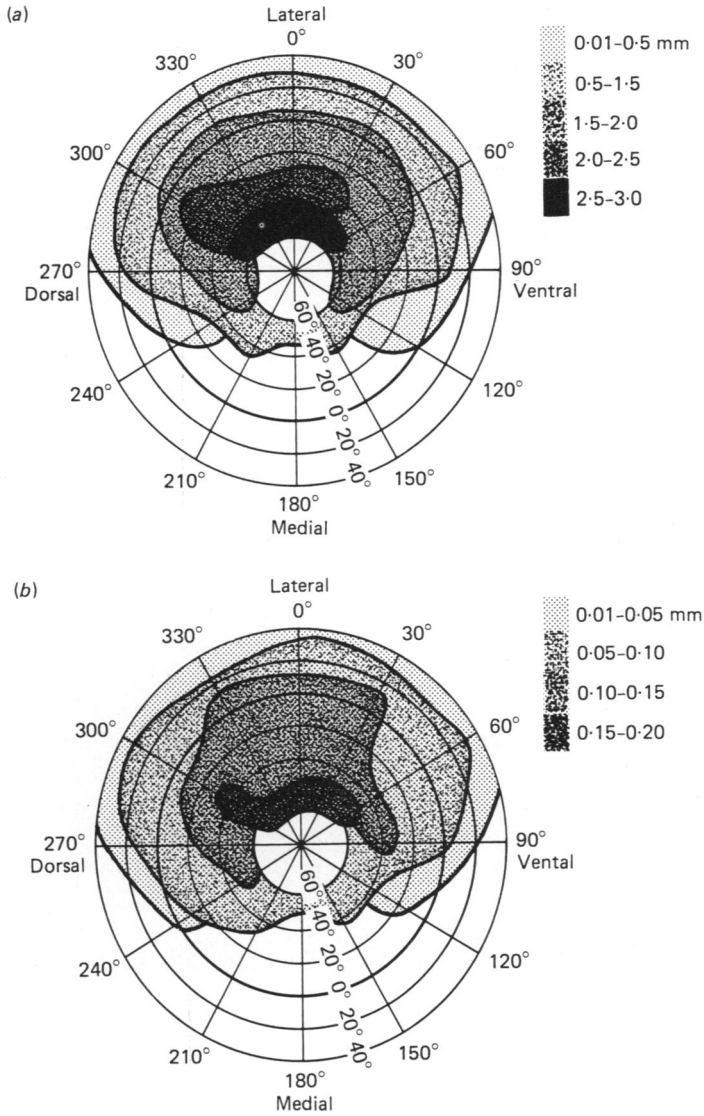
(a)

Lateral
0°

330°   30°

300°   60°

270° Dorsal   90° Ventral

240°   120°

210°   150°

180°
Medial

0·01–0·5 mm
0·5–1·5
1·5–2·0
2·0–2·5
2·5–3·0

(b)

Lateral
0°

330°   30°

300°   60°

270° Dorsal   90° Vental

240°   120°

210°   150°

180°
Medial

0·01–0·05 mm
0·05–0·10
0·10–0·15
0·15–0·20

Fig. 3 (*a–b*). Femoral head no. 8 (34 years old). (*a*) Distribution of total cartilage. (*b*) Distribution of calcified layer.

No. 8

Calcified zone (mm)

0·2

0·1

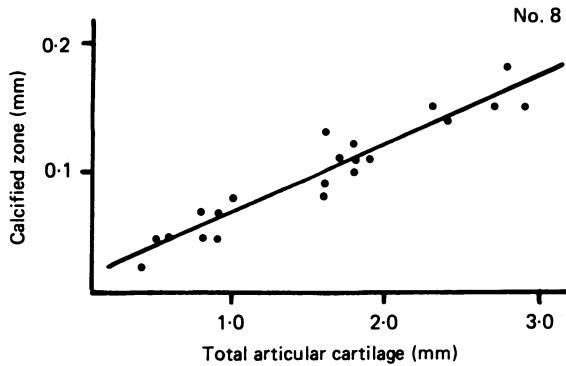Total articular cartilage (mm)

1·0   2·0   3·0

Fig. 4. Regression line between the thickness of the calcified layer and the complete layer of cartilage (femoral head no. 8, 34 years old).

Table 1. *Means and standard deviations for all femoral heads, together with correlation coefficients*

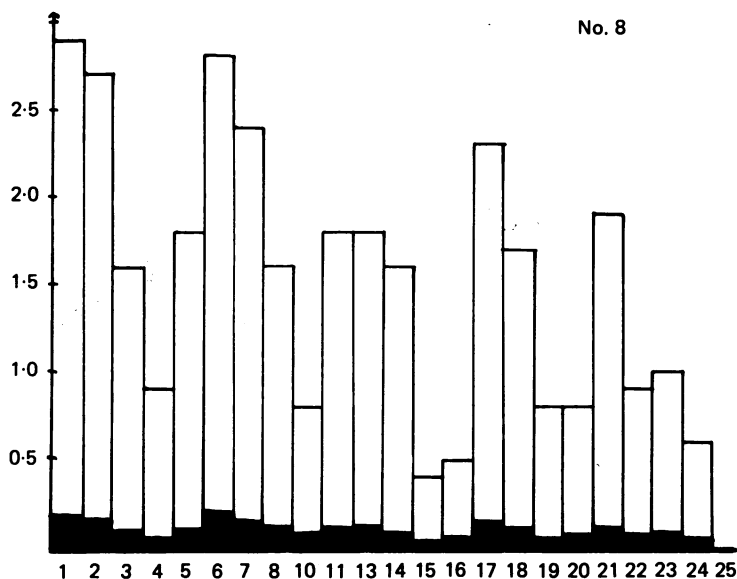| Head no.	Mean (%)	S.D.	Correlation coefficient
1	3·72	1·45	0·716
2	8·28	1·45	0·920
3	7·30	1·66	0·785
4	5·80	1·69	0·596
5	7·93	1·48	0·943
6	8·80	1·42	0·805
7	7·82	1·56	0·802
8	6·90	1·77	0·958

Fig. 5. Histogram showing interdependence between the thickness of the calcified layer (black) and the complete layer of cartilage (white). (Femoral head no. 8, positions of samples indicated in Fig. 1 a).

with that of the entire hyaline layer. The percentage of cartilage taken up by the calcified zone at each point of a femoral head is very nearly constant.

Finally, the means and standard deviations of the percentages for all the femoral heads are also listed in Table 1. Comparing these results with each other confirms that the percentage variation lies between 3·23 and 8·8 %. In other words, the percentage taken up by the calcified zone of the cartilage remains constant within a single femur, but shows considerable variation between bones. Any attempt to relate these differences to age or sex has produced no recognisable pattern, although the number of specimens we examined was naturally too small to allow us to exclude such a relationship entirely.

DISCUSSION

The investigations of Holmdahl & Ingelmark (1948) and Ekholm & Ingelmark (1952) established that short term demands made upon the cartilage of a joint produce a corresponding increase in its thickness. In addition to these reversible signs of

temporary adaptation, which can be seen following short term overloading and which must be attributed to swelling of the tissue, the results of long term work carried out on rabbits by Holmdahl & Ingelmark (1948) reveal an absolute increase in the cellular components and matrix of cartilage subjected to increased functional demands. It therefore appears that the thickness of the layer of cartilage is proportional to the load it has to bear.

The distribution of the loading forces and patterns within the hip joint are well documented (Greenwald & O'Connor, 1971; Kummer, 1968, 1969), and our own findings confirm this relationship between load and response. In places where a severe load is supported the cartilage is thicker. This part of our results is in complete agreement with the findings of Holmdahl & Ingelmark (1948) and those of Kurrat (1977), who produced a map showing the distribution of the thickness of the articular cartilage in the femoral head.

These observations can be explained by the investigations of Pauwels (1960) into the effect of mechanical irritation on the differentiation of the hard connective tissues. In this case the hydrostatic pressure produces a specific stimulus to growth in cartilaginous tissue, while the varying forces that appear during the transfer of pressure in the joint are responsible for its shape – although it should not be forgotten that *intermittent* pressure stimulates growth, whereas *continuous* pressure inhibits it (Jones, Klämfeldt & Sandström, 1982). Tillmann (1969), in agreement with Kummer (1963), related this stimulus to the degree of deformation occurring, which lies between definite maximum and minimum values. Within this range severe deformation leads to an increase in thickness of the cartilage, and a reduced intracapsular pressure to a decrease. Failing to reach the minimal value results in destruction of cartilage in favour of the underlying bone, whereas exceeding the maximum produces a transformation of hyaline cartilage into fibrocartilage and the appearance of a pathological fibrocartilaginous degeneration (Tillmann, 1973). In other words, a direct relationship exists between thickness of the cartilaginous layer and mechanical stress.

Although our results regarding the thickness of the complete articular cartilage are in agreement with those of other authors, our findings on the thickness of the calcified zone are not. Here we were also able to detect a clear distribution pattern of both the total thickness and that of the calcified zone, with the values closely and positively correlated between the two layers.

Holmdahl & Ingelmark (1948) reported a simple overall thickness for the calcified layer of cartilage within a joint, and emphasised their opinion that function seemed to have no influence upon it. Green *et al.* (1970) also established a simple mean value for the thickness of the calcified zone in the human patella, although their method involved the examination of only a single specimen from the middle of each bone.

Stougard (1974) and Lane & Bullough (1980) certainly claimed to have found a difference between specimens taken from regions of greater or lesser loading, but they reported a thicker calcified region in the non-weight bearing areas. Our own findings, of course, were exactly the opposite to this.

It is possible that these contradictory results are the consequence of differences in the technique used for measuring. The 'tidemark' is certainly wave-like, although its overall course is relatively straight. The border between cartilage and bone, on the other hand, pursues a most irregular course and at times wanders far into the calcified zone. With the linear methods of Stougard (1974), for instance, the distances recorded depend greatly on the points chosen for measurement, and can show considerable variation. Lane & Bullough (1980) determined the thickness of the calcified zone by

using an eyepiece equipped with a grid and dividing the area of this zone by its length. Such a method certainly gives better and more consistent results, although the area can only be crudely estimated by the examiner and it hardly takes into account all the irregularities of its boundaries. On the other hand, we used an electronic image analyser to measure the areas and allowed the computer to arrive at a value for the width. This means that the reproducibility of our results must surely be a great deal more certain. Our measurements were based on a miminum of 5 sections from every single specimen of cartilage examined. While the variance within each sample was very small, we are justified in concluding that these surface measurements must be closely correlated with the volume of the cartilage.

Since, as we have already stated, the thickness of the two layers shows a close correlation – with the total thickness directly dependent upon the distribution of the load – we feel justified in concluding that the pattern of thickness seen in the calcified zone is also influenced by mechanical factors. No explanation can be offered for the large individual variations from bone to bone in the percentage relationship between the two layers – as little as 3·2% in one case, and as much as 8·8% in another.

SUMMARY

The thickness of both the articular cartilage and its calcified zone were measured at 25 carefully selected points in 8 human femoral heads, and the ratio of one to the other was found to be remarkably constant for each bone.

The thickness of the calcified zone therefore shows the same distribution pattern as that of the total cartilage and, since the latter is dependent upon the distribution of the load, the thickness of the calcified region also appears to be related to mechanical stress.

The volume of the calcified zone, however, expressed as a percentage of the total cartilage, varied considerably from one bone to another within the range from 3·23 to 8·8%.

Too few specimens were examined to allow correlation with age or sex to be either refuted or confirmed.

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