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## The Thickness of the Calcified Layer in Different Joints of a Single Individual

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### ABSTRACT

The thickness of the articular cartilage and its calcified zone were both measured at specially chosen places in several limb joints from five subjects. The volume of the calcified zone expressed as a percentage of the total cartilage was not only constant for one joint, but also in all the joints of a single individual. Nevertheless, the variation between subjects ranges from 6.9 to 8.6%. In two cases both sides of the body were investigated. As was the case in an earlier investigation on the femoral head, the bilateral distribution of the thickness was the same. Since the thickness of the total cartilage varies with the local distribution of loading in the joint, it follows that the thickness of the calcified layer also depends upon mechanical factors. Five subjects is too few to allow correlation with age or sex to be either refuted or confirmed. There is some evidence in the existing literature that the thickness of the calcified zone may be altered by disease.

**KEYWORDS:** articular cartilage – calcified cartilage – load – distribution of thickness.

### INTRODUCTION

Examination of articular cartilage with the light microscope reveals the existence of a superficial layer with tangentially ordered fibres, an intermediate zone, a radial zone and, close to the bone, a layer of calcification. The 'tidemark' is an undulating line – basophilic with H and E staining – which marks the boundary between the calcified and uncalcified layers.

Although the literature on cartilage itself is almost too large to be taken in, there is surprisingly little work reported on the calcified region and therefore relatively little is known about this part of the articular cartilage (Fick, 1912; Ishido, 1923; Holmdahl & Ingelmark, 1948; Ekholm & Ingelmark, 1952; Fawns & Landells, 1953; Green et al., 1970; Stougaard, 1974; Redler et al., 1975; Lane et al., 1977; Pedley & Meachim, 1979; Dmitovsky et al., 1978; Lane & Bullough, 1980; Bullough & Jagannath, 1983; Donohue et al., 1983).

In a previous investigation carried out on several femoral heads we measured the thickness of both the whole cartilage and the calcified layer in regularly distributed regions of the joint surface (Müller-Gerbl et al., 1987), and we were able to establish that, for each femoral head, the thickness of the calcified layer corresponds closely to that of the total cartilage. In addition we found that although the volume of the calcified zone expressed as a percentage of the total cartilage was the same for all parts of the same head, the values for the calcified

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layer varied from specimen to specimen by as much as 5.08% (range: 3.72%-8.80%).

Our interest then turned to the question: to what extent is the ratio between the total cartilage and the calcified layer constant within a single individual?

## MATERIAL AND METHOD

Five subjects showing no (or only very slight) degenerative changes to the naked eye were available to us (age range: 62-82 years, sex: 3 female, 2 male – see Table 1). Details of the joints used and the number of samples taken are also shown in Table 1.

Table 1. List of the joints investigated and number of samples taken of each articular surface.

Joint surface	No. 1	No. 2 (r/l)	No. 3	No. 4 (r/l)	No. 5
Sup surf talus	11	10/ 6	5	12/12	11
Inf art surf tib/fib	5	5/ 5	5	9/12	7
Patella	7	9/10	6	12/ 9	7
Tib condyles	3	18/17	10	18/21	5
Fem condyles	20	21/21	16	21/23	14
Femoral head	14	16/22	5	17/22	12
Acetabular cart	7	3/ 3	4	11/12	4
Humeral head	13	13/10	8	14/16	11
Glenoid cavity	4	8/ 7	8	6/ 4	3
All joints	84	103/101	67	120/131	84
Total			690		

From each joint, samples were collected at carefully selected points with an 8 mm punch, decalcified, embedded in paraffin and stained with light green (Fig. 1). We chose this stain because it is easy to use and clearly shows up both the outside limit of the calcified layer (the “tidemark”) and the inside transition from cartilage to subchondral bone.

Metrical assessment of the histological specimens was carried out online with an IBAS 2000 image analyser (Zeiss, Oberkochen) connected to a TV system. The surfaces to be measured could be traced directly on the computer monitor with the cursor, and the values obtained for the surface area used to derive the average width of the field measured. Five measurements were carried out on each section and the mean values and standard deviations obtained (Fig. 2).

We used this method of approach because the contours of both the tidemark and the cartilage-bone boundary are extremely irregular, and linear measurements of the distance between them would have resulted in too great a discrepancy between values. Calculating the width of the zone from the area by integration eliminates these differences (see also Müller-Gerbl et al., 1987).

The values for each joint were plotted as rectangular coordinates, so that any regression lines which appeared could be easily identified. The percentage of

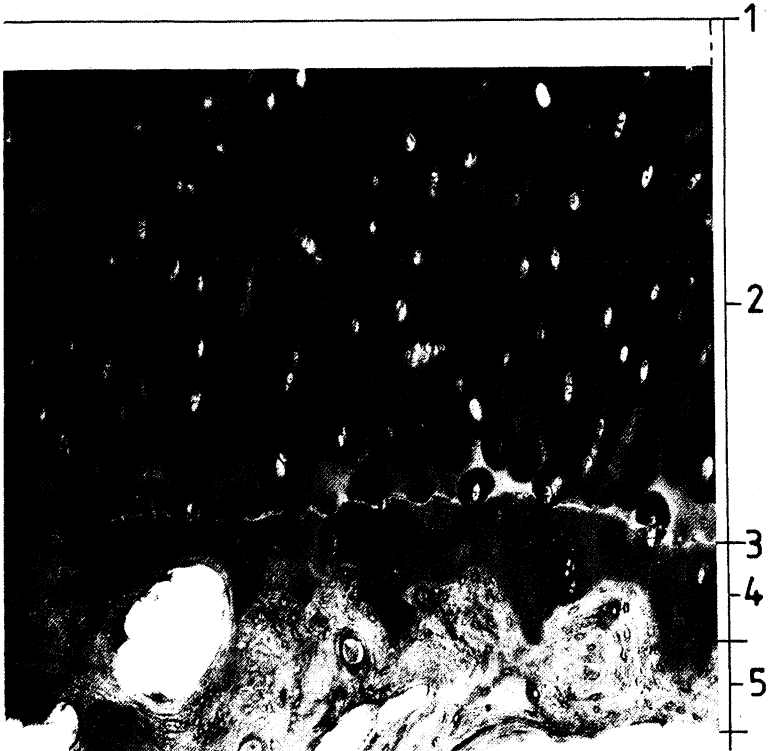


Fig. 1. Histological section through the articular cartilage ca. x20 1 surface of the cartilage, 2 uncalcified cartilage, 3 "tidemark", 4 calcified zone, 5 osteocartilaginous boundary.

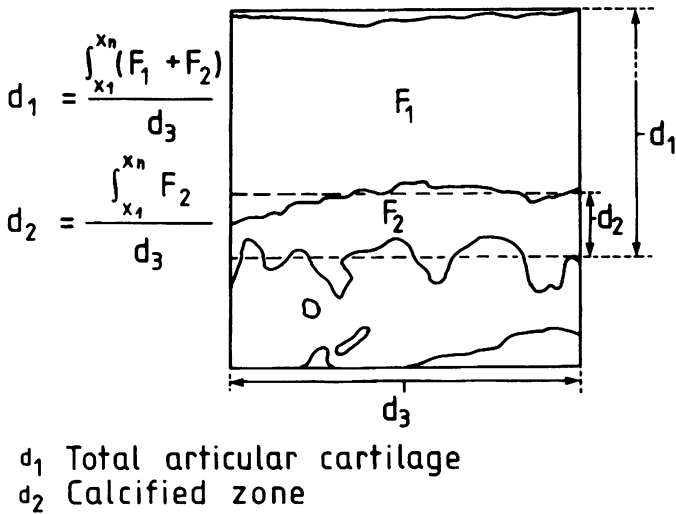


Fig. 2. Method of deriving the thickness of the two zones from their areas.

cartilage occupied by the calcified zone was also calculated for every point from which a sample was taken.

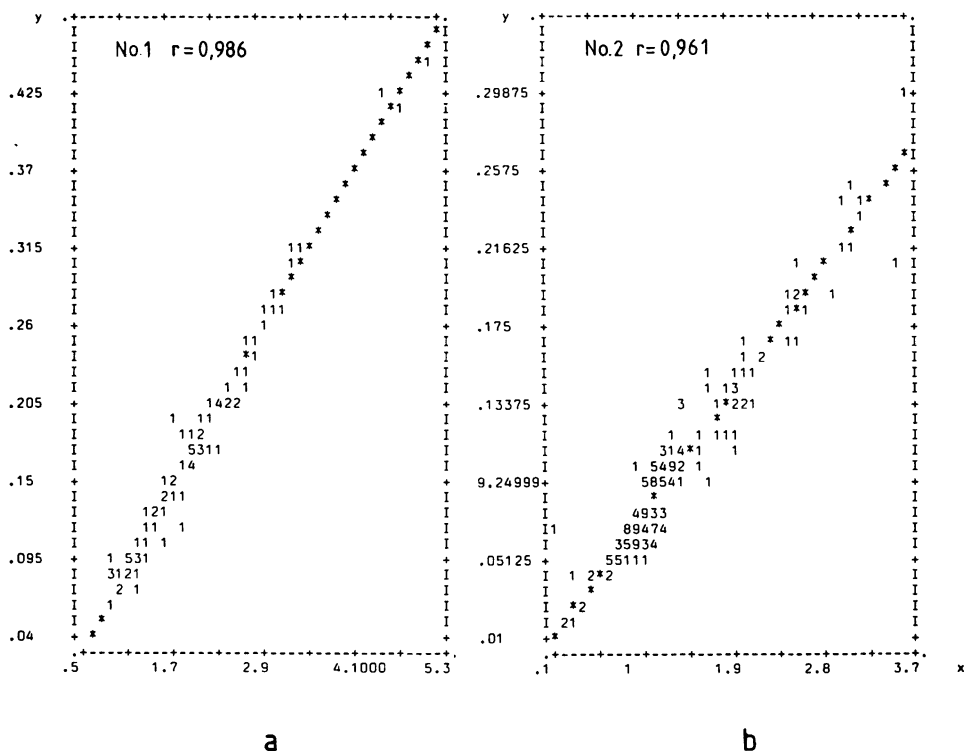
Comparative measurements show that shrinkage due to the histological technique or following fixation amounted to 10-15% of the total thickness of the specimens and need not, so far as the relationship of the zones to one another is concerned, be taken into account.

In order to eliminate effects due to the compression produced by the microtome knife, all blocks were cut in the same plane; that is to say, parallel to the surface of the cartilage.

## RESULTS

Each *joint surface* examined showed a nearly lineal dependence between the thickness of the total cartilage and that of its calcified zone, expressed as the value of the correlation coefficient ( $r$ ). Of the 65 joint surfaces investigated, the value of  $r$  lay between 0.9 and 0.99 in 37 cases, between 0.8 and 0.89 in 12 and between 0.7 and 0.79 in 4. On eight occasions values between 0.5 and 0.69 were found, but in only one case was  $r$  less than 0.5.

The  $x$  and  $y$  values taken from each *individual* were also plotted as rectangular coordinates, and the corresponding regression lines are shown in Figs. 3a-e. The



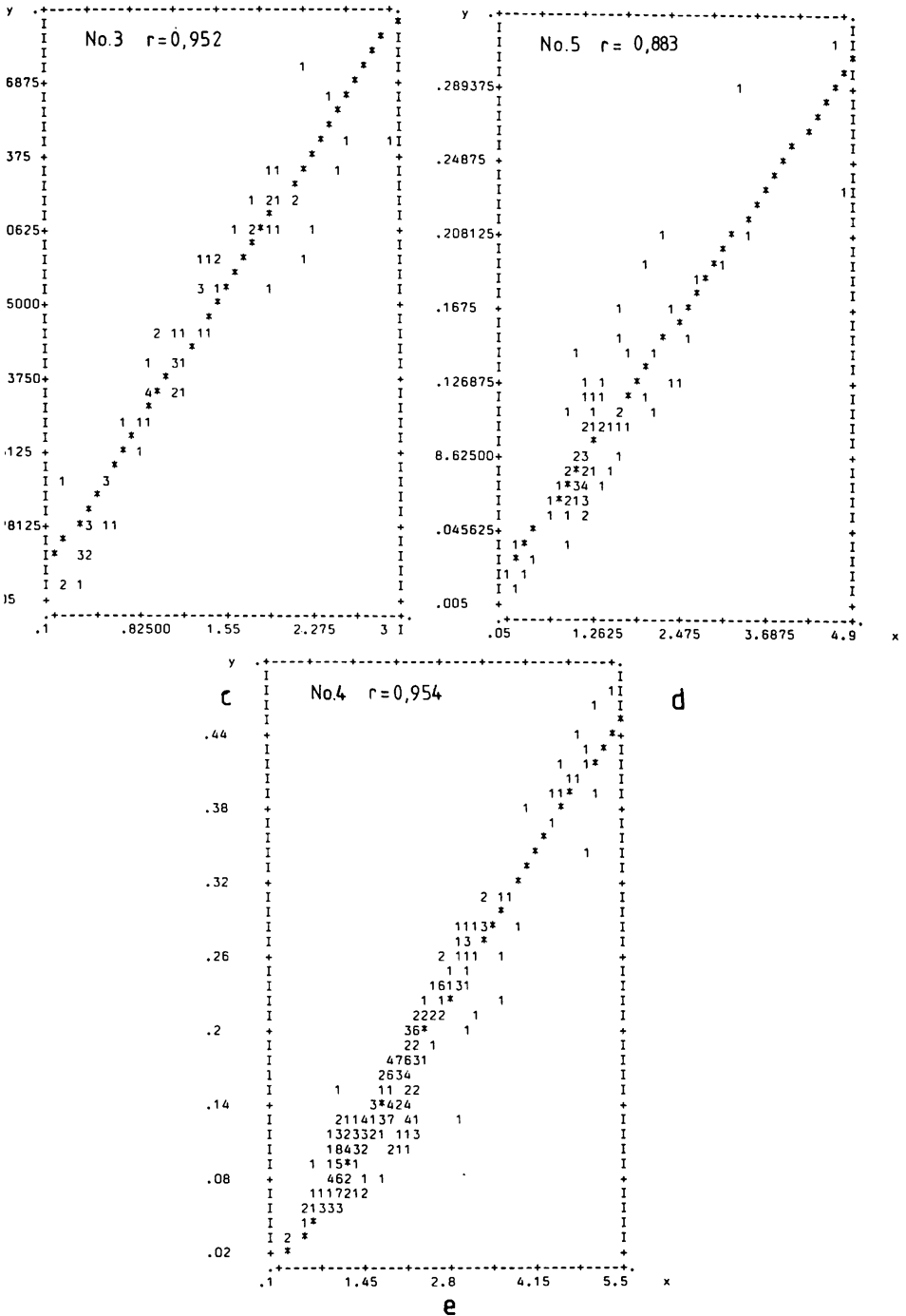


Fig. 3. a-e: Each diagram shows the correlation (r) of the calcified zone (ordinate) and the total cartilage (abscissa) for all samples from the joints of ONE individual (in mm).



thickness of the calcified zone expressed as a percentage of the total thickness of the cartilage was constant for each joint, as is shown by the narrow standard deviations of the means. As an example, the results from body No. 2 are shown in Table 2, from which it can be seen that the means from all joints of this individual are constant. The same result was found every time, so that for each person examined the relationship between the thickness of the calcified zone and that of the whole cartilage is a constant. These values are listed in Table 3, and it can be seen that the differences *between* individuals can vary considerably.

In those cases where the same joint had been investigated for both sides of the body (Subjects 2 & 4) the corresponding values for the joints of the right and left were compared. The "t" test was used to show that in one case (Subject 2) no significant differences existed between the two sides, whereas with Subject 4 the

Table 2. Calcified zone expressed as a percentage of the total articular cartilage for the right and left sides of Subject No. 2: means, standard deviations and correlation coefficients.

Joints of the Right Side		
Joint Surface	Mean (%) + SD	Correlation Coefficient
Sup Surf talus *	7.64 ± 0.7	0.98
Inf art surf tib/fib	7.14 ± 0.6	0.97
Med tib condyle	7.48 ± 1.4	0.89
Lat tib condyle	7.94 ± 0.8	0.97
Fem condyles	8.02 ± 1.6	0.94
Patella	7.62 ± 1.0	0.83
Femoral head	7.68 ± 1.0	0.94
Acetabular cartilage	7.77 ± 1.2	0.5
Humeral head	7.29 ± 1.1	0.89
Glenoid cavity	7.67 ± 0.9	0.99
All joints from ride side	7.67 ± 1.11	
Joints of the Left Side		
Joint Surface	Mean (%) + SD	Correlation Coefficient
Sup Surf talus	8.08 ± 0.3	0.99
Inf art surf tib/fib	7.20 ± 0.9	0.95
Med tib condyle	8.37 ± 0.7	0.91
Lat tib condyle	8.38 ± 1.2	-
Fem condyles	6.75 ± 1.0	0.95
Patella	7.77 ± 0.6	0.98
Femoral head	7.34 ± 0.8	0.96
Acetabular cartilage	8.31 ± 0.7	0.89
Humeral head	7.87 ± 0.6	0.96
Glenoid cavity	7.18 ± 0.9	0.95
All joints from left side	7.55 ± 0.83	

Table 3. Means and standard deviation as in Table 2 for all joints of each subject including age and sex.

Subject No. 1	female	62 years	8.62 ± 0.6
Subject No. 2	female	75 years	7.61 ± 0.9
Subject No. 3	female	82 years	6.88 ± 1.1
Subject No. 4	male	75 years	8.21 ± 1.4
Subject No. 5	male	77 years	7.03 ± 1.1

differences were found to be significant, although the mean values of 7.9% and 8.5% are not very far apart.

## DISCUSSION

We have been able to confirm that our earlier finding (Müller-Gerbl et al., 1978) – namely, that the thickness of the calcified zone of the femoral articular cartilage is a constant percentage of the thickness of the total cartilage *within* each joint examined – is also true for other limb joints. Or, in other words, that the calcified zone is thick in those places where the articular cartilage is thick.

Since this is so, and since the total thickness of the cartilage is dependent upon the distribution of the load (Oberländer, 1977; Kurrat, 1977; Kurrat & Oberländer, 1978), it seems apparent that the thickness of the calcified zone at any particular point is also determined by mechanical factors.

In spite of reports published by other authors (Holmdahl & Ingelmark, 1948, Green, 1970), the thickness of the calcified zone is not the same in all regions of the same joint, but varies from place to place as much as the cartilage itself.

Authors such as Bennett et al. (1942), Trueta (1968), Stougard (1974) and Lane & Bullough (1980) have already stated that they found variations in the thickness of the calcified zone between the weakly and strongly loaded regions of a joint; although Lane & Bullough (1980) observed – in direct opposition to our own findings – that the calcified zone of the articular cartilage of the femoral and humeral heads showed an average increase of 10-15% in thickness in places where the mechanical load was *less*. It seems probable that these differences are due to variations in accuracy of the methods used.

Stougard examined specimens from three different parts of the femoral condyles and one part of the patella. In addition, he included a patella that already showed the pathological changes of *chondromalacia patellae*. He found that the thickness of the calcified cartilage differed within wide limits. It was least in the non-weight-bearing regions of the femur and greatest in the specimens taken from the diseased surface of the patella. By reason of the large number of samples taken from each articular surface and the regular distribution of the sites from which the samples were taken, we are able from our past and present investigations to extend and confirm Stougard's findings that a strong correlation exists between the thickness of the total cartilage and that of the calcified zone and, finally, that these depend upon the mechanical load on the joint.

The question still remains: what factors are responsible for producing the constant ratio between the thickness of the calcified zone and that of the articular cartilage? Lane and Bullough reported an absolute decrease of the thickness of the calcified zone with increasing age, but five subjects are too few for us to make any pronouncement on a possible correlation with either sex or age.

Even if the literature available does not allow us to offer any conclusive explanation for the phenomena observed, there is nevertheless some suggestion in the work of other authors of factors which may possibly influence the absolute thickness of the calcified zone.

According to Stougaard (1974), pathological changes such as those of *chondromalacia patellae* can lead to thickening of this zone.

In an investigation on the effect of "subfracture loads" on articular cartilage, Donohue and his co-workers (1983) suggested that an activation of the zone of calcified cartilage might be the first change associated with ultrastructural alterations in the superficial and radial zones. Such a change includes increase in the number of cellular clones, vascular invasion and an increase in the proteoglycan content of the matrix.

Townsend and his co-workers (1977) suggested that the distribution of degenerative changes in the uncalcified articular cartilage of the patella may be related to the distribution of the density and texture of the subarticular calcified tissue. Subarticular density must influence the resilience of the articular cartilage base, and so affect the ability of the uncalcified cartilage to withstand damage during compressive loading (Freeman & Meachim, 1973).

All these findings and reports make it clear that the calcified zone of the articular cartilage plays a more important role in the causation of the various diseases that affect the joints than has hitherto been realised.

However, to make it possible to appreciate the significance of pathological changes in the calcified zone, it is first necessary to learn more about the normal properties of that layer.

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