J. Anat. (1984), **139**, 2, pp. 341–352
With 10 *figures*
Printed in Great Britain

Topographical variation in the calcified zone of upper femoral articular cartilage

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(*Accepted* 26 *January* 1984)

INTRODUCTION

In the general population, degeneration of articular cartilage is more common on the inferomedial aspect than on the zenith of the femoral head (Meachim & Emery, 1973), but bone exposure in patients with osteoarthritis usually develops on or near the zenith (Meachim *et al.* 1980). This paradox has led Byers to conclude that cartilage lesions on the inferomedial aspect have only a limited potential to progress to osteoarthritic bone exposure, whereas those on the zenith, although less common, have a greater osteoarthritic potential (Byers, Contepomi & Farkas, 1970). Possible factors which might account for the topographical difference in the natural history of the lesions would be site-to-site variation in:

the amount and nature of mechanical stresses acting on the cartilage;

the biochemical profile, mechanical properties and nutrition of the uncalcified cartilage;

the features of the calcified cartilage base and subarticular bone.

The present investigation has developed quantitative methods for studying 'remodelling' and other histological features in the calcified zone of articular cartilage, and compares the findings in inferomedial samples with those in zenith samples from a series of adult human femoral heads. Its aim is to look for local differences between the two sites and is not concerned with the possibility that the total amount of histological remodelling over the whole femoral head might be sufficient to alter the macroscopic contour of the hip joint (Johnson, 1959).

MATERIAL AND METHODS

The left femoral head was obtained at necropsy from 27 subjects (19 men; 8 women) whose ages ranged from 19 to 86 years. Femoral heads with osteoarthritic bone exposure, leukaemic infiltration, bone necrosis or full-thickness loss of the original cartilage on the inferomedial aspect were excluded. The articular surface was painted with India ink and the state of the articular surface at the two sites sampled was assessed (Meachim & Emery, 1973). The specimen was then fixed in formalin. A mid-coronal slab was sawn out, radiographed on fine grain film, and used to provide a tissue block from each of two sites (Fig. 1). The sample from the inferomedial aspect (site A) was centred midway between the fovea and the inferomedial margin of the articular surface, and that from the zenith (site Z) was centred one third of the distance from the fovea to the lateral margin.

Histological sections in a plane normal to the articular surface were cut from the blocks after decalcification with sulphosalicylic acid and embedding in paraffin. For each block, a set of four photomicrographs was taken from one of the sections at a

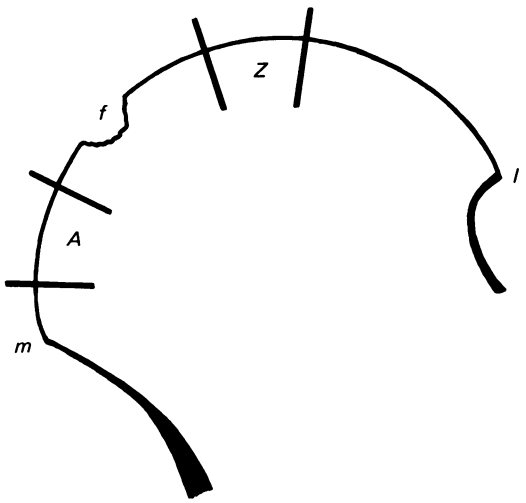

Fig. 1. Diagram of mid-coronal slab of a femoral head, showing the position of the inferomedial (*A*) and of the zenith (*Z*) sample in relation to the foveal (*f*), inferomedial (*m*) and lateral (*l*) margins of the articular surface.

magnification of ×60, and assembled into a montage on which a length of 42 cm was measured out tangentially along the zone of calcified cartilage. The length was curved so as to follow the articular contour and represented a tangential length of 7 mm on the histological section. This standard length was used in all the quantitative methods.

Tidemark identification and mapping

Quantitation of 'remodelling' in the calcified zone of the articular cartilage necessitated identification and mapping of its 'tidemarks' (Fawns & Landells, 1953). Their staining intensity and appearance in the plane of section varied from mark to mark and from segment to segment along the same mark. Those at the interface between calcified and uncalcified cartilage stained strongly with Ehrlich's haematoxylin and eosin, and were easily identified. Others could be fainter and less readily apparent, this sometimes being so in the case of tidemarks embedded within the calcified zone.

A histological section from each site was studied along a succession of microscopic fields, and the position of tidemarks inked in on the photomicrographic montage. Reference was made to a set of drawings showing the appearance at high magnification of tidemarks which had already been firmly identified (Fig. 2). Staining with Ehrlich's haematoxylin and eosin was used throughout the present study. As seen by light microscopy of sections stained by this method, a typical segment of tidemark showed basophilic stippling which had an abrupt lower border but decreased more gradually on the more irregular upper aspect toward the uncalcified cartilage (Figs. 2, 3). In some segments there was an 'undulating tramline' appearance (Fig. 2, right; Fig. 4), and care was taken to distinguish this from genuine duplication of the tidemark. In some segments, a homogeneous narrow band was seen below the stippling (Fig. 2, lower).

Study of the photographic montage was helpful during and after inking in: identification of fainter parts of a tidemark could be confirmed by tracing their

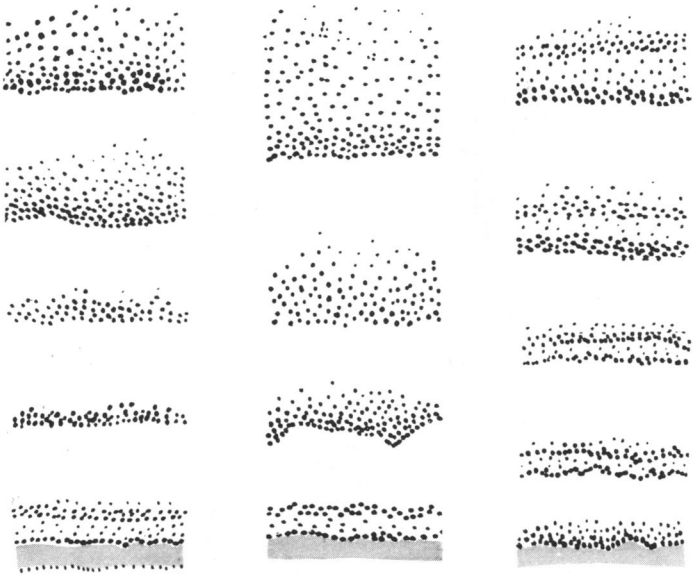

Fig. 2. Drawings of segments from tidemarks, as observed during light microscopy at high magnification of sections stained with Ehrlich's haematoxylin and eosin

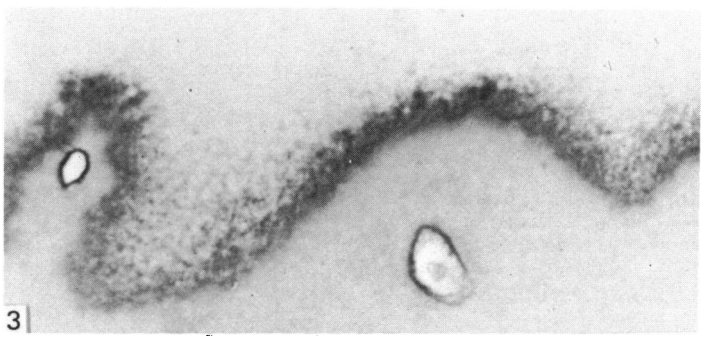

Fig. 3. Photomicrograph of a tidemark. In the segment shown the stippling has a characteristically abrupt lower border and decreases more gradually on the more irregular upper aspect. This and all subsequent photomicrographs are from paraffin sections stained with Ehrlich's haematoxylin and eosin. × 600.

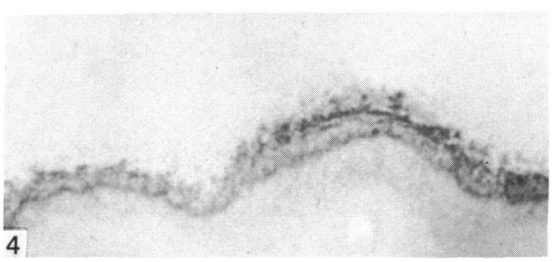

Fig. 4. Photomicrograph of a tidemark segment with an 'undulating tramline' appearance. × 600.

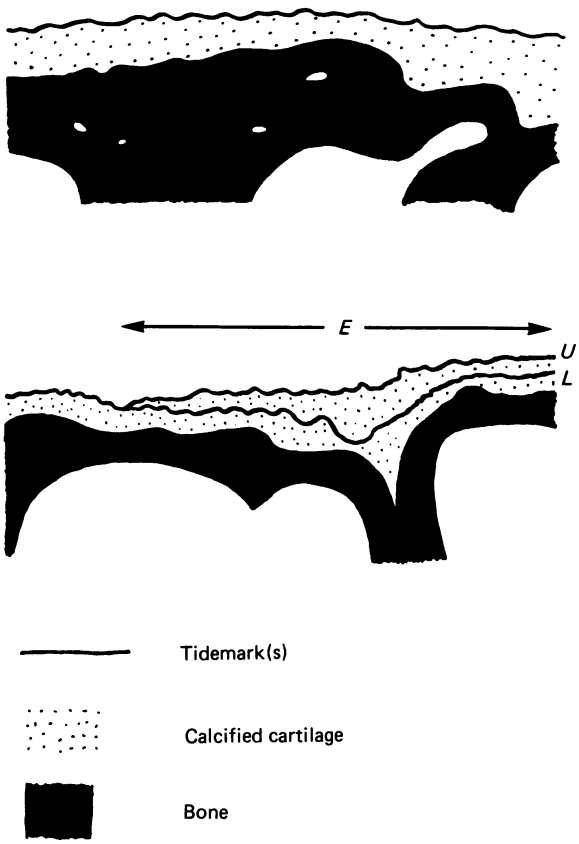

Fig. 5. Diagrams of calcified cartilage and subarticular bone, based on tracings from ×60 photomicrographs. In the upper example there is only one tidemark, and thus no evidence of an extra phase of cartilage calcification. In the lower example, part of the cartilage has two tidemarks (*U*, *L*), thus indicating that there has been extra-phase calcification (*E*) of cartilage along this part of the tangential length shown.

continuity with places where that tidemark was more distinct; gaps in a tidemark due to breaches by ossification could be recognised; the topography of junctions where two tidemarks merged into one could be clarified.

Quantification of cartilage calcification

A tidemark, when single, showed where calcification advance into articular cartilage had halted during skeletal maturation (Fig. 5, top). Thus the presence of two or more tidemarks indicated that calcification advance had subsequently been reactivated (Fig. 5, lower; Fig. 6), with one or more extra phases of cartilage calcification. To compare the two femoral sites, the spatial extent of extra calcification was measured in preference to counting the total number of tidemarks: tidemark counts simply indicate whether or not extra calcification has occurred (Green, Martin, Eanes & Sokoloff, 1970).

The tangential extent of extra calcification at the site sampled was measured on the photomicrographic montage, as depicted in Figures 5 and 6, and expressed as a percentage of the standard tangential length. It was noted that this measurement could be greater than the total length of the lowest tidemark, because the lower mark

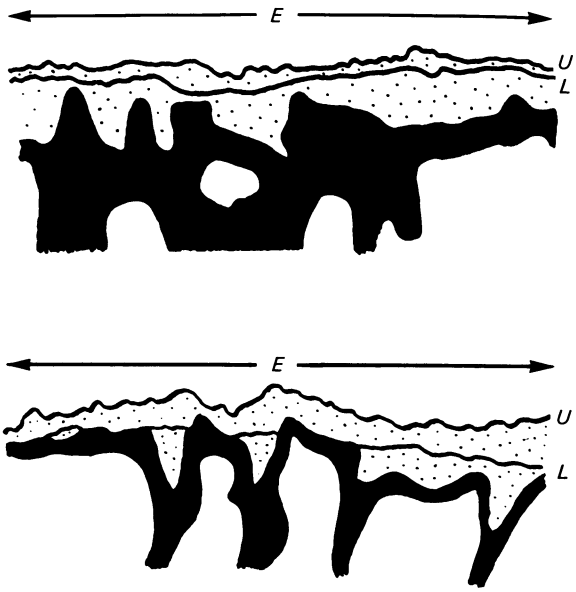

Fig. 6. Further diagrams of calcified cartilage and subarticular bone, based on tracings from × 60 photomicrographs. Key as in Figure 5. In some instances three or more tidemarks were apparent in the sections: only the uppermost (*U*) and the lowest (*L*) tidemarks were used when quantifying extra-phase calcification. In the upper diagram the extent of line *L* is readily determined. The lower diagram shows tidemark *L* interrupted by an advance of ossification into the cartilage: however, its original extent can still be deduced by extrapolating between its remnants, thus indicating that, as in the upper diagram, there has been extra-phase calcification (*E*) over the whole extent of the tangential length shown.

sometimes had gaps where it had been breached by ossification (Fig. 6, lower). Indeed, it was theoretically possible that an original lowest mark might have been completely obliterated by subsequent ossification, thus removing the evidence of extra cartilage calcification.

The extent of the area (i.e. the product of vertical and tangential extent) of extra calcification was measured by point counting the area it occupied in the photographic montage, using a counting grid with the points arrayed at the corners of 0·5 cm^2 squares. The average was taken from a series of three counts, and the result expressed as the points occupied over the standard tangential length (Fig. 7).

When three or more tidemarks were present, only the uppermost (Fig. 6) and the lowest (Fig. 6) were used during quantification, the separate extra phases being measured together as total 'extra-phase calcification'. This simplification was intentional, since preliminary analysis showed that treatment of each extra phase separately made study and presentation of the data unnecessarily complex (Fig. 8).

The thickness of the complete zone of calcified cartilage was determined by point counting in triplicate the area it occupied in the photographic montage, and was expressed in terms of the points occupied per standard tangential length (Pedley & Meachim, 1979).

Quantification of 'focal contacts'

The position of the uppermost or only tidemark (Figs. 5, 6) was located, and the interface between calcified and uncalcified cartilage examined for features which are termed 'focal contacts' in this study. At a 'focal contact', the lower margin of the

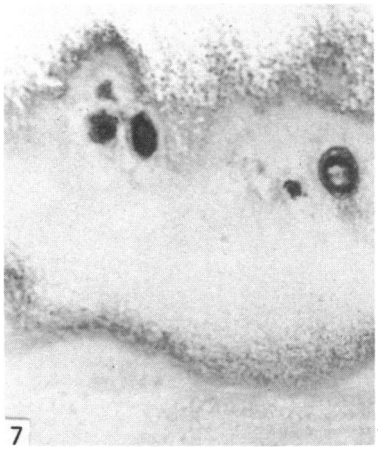

Fig. 7. Photomicrograph of calcified cartilage zone with two tidemarks, the upper being at the interface with the uncalcified articular cartilage and the lower being embedded within the calcified tissue. The area between the upper and lower tidemarks was used for spatial quantification of extra-phase calcification of the cartilage. × 375.

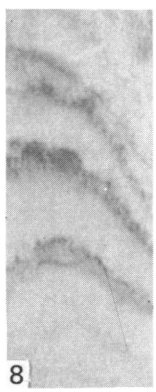

Fig. 8. Photomicrogaph showing part of a 'stratification' of multiple faint tidemarks within the calcified zone. Note the complexity of spatial quantification if the extra phases of calcification were each to be treated separately when there are three or more tidemarks. × 375.

uncalcified articular cartilage (Fig. 9) was in contiguity with the upper margin of tissue other than calcified hyaline cartilage. Using light microscopy, such contacts were counted in two paraffin sections, and the result expressed as the mean count per 7 mm tangential length along the sample. In order to standardise the quantification, instances where a broad segment of subarticular bone had made contact with the uncalcified cartilage were not included in the counts. They were excluded because, as discussed later, the function in adults of 'focal contacts' may perhaps be mainly nutritional, and not solely concerned with the advance of an ossification front of subarticular bone into the cartilage.

Quantification of shearing splits

An attempt was made to quantify shearing damage at the interface of calcified and uncalcified cartilage, by counting tangential splits (Fig. 10) at the uppermost or only tidemark (Meachim & Bentley, 1978). Using light microscopy, such splits

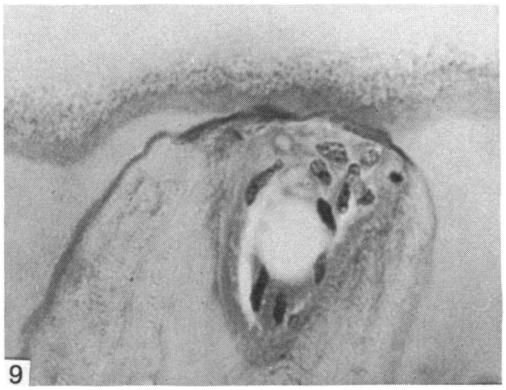

Fig. 9. Photomicrograph of a focal contact. At the tidemark the uncalcified articular cartilage (above) is in contiguity with a 'defect' (below) in the zone of calcified cartilage. In this example the 'defect' contains a blood vessel and bone, and contact is over a tangential length of approximately 40 μm in the plane of section. This Figure coincidently illustrates a tidemark segment of the sort which shows a homogeneous narrow band below the stippling (Fig. 2, lower). ×415.

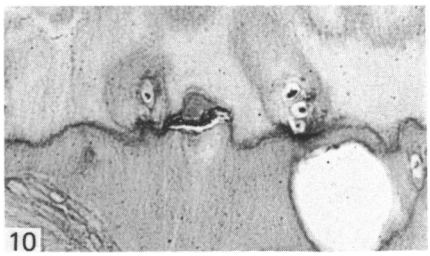

Fig. 10. Photomicrograph of a horizontal split (centre), approximately 50 μm in tangential length in the plane of section, along the interface of the calcified with the uncalcified cartilage. ×150.

were counted in three paraffin sections from the tissue block, and the result expressed as the mean count per 7 mm tangential length along the sample. Blocks were then re-embedded in low-viscosity nitrocellulose and cut in a direction which was at right angles to that used for the paraffin sections, although still in a plane vertical to the articular surface; splits were counted in three nitrocellulose sections and the result again expressed as the mean count per 7 mm tangential length. The final count was taken as the average of the paraffin and the nitrocellulose section results. All counts of tangential splits were made by the same observer.

RESULTS

Articular surface

Assessment of the state of the articular surface and the uncalcified articular cartilage at the two sites sampled using the India ink method supplemented by histology gave the results shown in Table 1.

Cartilage base

At both sites, the base of the uncalcified articular cartilage comprised a zone of calcified cartilage resting on a subarticular plate of compact bone which was supported by osseous arcades and struts in continuity with the trabeculae of the underlying cancellous bone (Figs. 5, 6). Histologically, the compact bone of the subarticular

Table 1. *State of the articular surface of the femoral head assessed by light microscopy using India ink (Meachim & Emery, 1973)*

	Inferomedial	Zenith
Intact articular surface	1	23
Minimal fibrillation	17	3
Overt superficial fibrillation	8	1
Deep fibrillation	1	0
Total number of specimens	27	27

plate was mainly of the parallel lamellar fibre type, although admixed with small foci of woven fibre texture. It usually included some concentric Haversian systems. In many instances, there were microscopic foci of bone hypocellularity or acellularity, with empty osteocyte lacunae. A tidemark (Figs. 3, 4) was always apparent at the interface of the calcified and uncalcified cartilage. In many of the samples, other tidemarks were also identified, embedded within the zone of calcified cartilage (Fig. 7); their number varied from one to many (Fig. 8). Extensive necrosis of chondrocytes was observed in the calcified zone.

Extra-phase calcification of cartilage

The tangential extent of extra-phase calcification of cartilage varied from sample to sample, and individual results ranged from 0 % to 100 % at both sites. Taking the series as a whole, the mean of the results was greater for the inferomedial samples (59 %) than for the zenith samples (27 %). In keeping with this difference, the mean of the results for the area extent (the product of vertical and tangential extent) of extra-phase calcification was also greater for the inferomedial samples (44 points) than for the zenith samples (16 points).

When the results were analysed according to the state of the articular surface, regardless of site, the mean tangential extent of extra-phase calcification was greater for the 20 samples where the surface showed minimal fibrillation (57 %) than for the 24 samples where it was still intact (21 %).

For each of these three comparisons (tangential extent in inferomedial compared with zenith samples, area extent in inferomedial compared with zenith samples and tangential extent in fibrillated compared with intact samples) Student's t test gave a P value of less than 0·01.

Thickness of the calcified zone

The complete zone of calcified cartilage comprised both the original structure and any additional contribution by extra-phase calcification. Taking the series as a whole, the mean of the results for the thickness of the calcified zone was greater for the inferomedial samples (135 points) than for the zenith samples (96 points). However, this difference may have been due simply to the more extensive extra-phase calcification inferomedially, since the inferomedial (81 points) and the zenith (91 points) means were similar if samples showing extra-phase calcification were excluded from the site comparison. In keeping with this interpretation, the mean thickness for inferomedial samples showing extra-phase calcification (145 points) was greater than for inferomedial samples which did not (81 points).

Ossification gaps in the lowest tidemark

Where there was only a single tidemark it formed a continuous line (Fig. 5, top). Where there was more than one tidemark, the lowest mark sometimes had gaps where it had been breached by ossification into the calcified cartilage (Fig. 6, lower). The mean percentage of the standard tangential length showing such gaps was similar at the two sites sampled.

Focal contacts

Both sites showed focal contacts, where the lower margin of the uncalcified articular cartilage was in contiguity with the upper margin of tissue other than calcified hyaline cartilage (Fig. 9). The tissue beneath such contacts contained one or more of the following components: blood vessels, bone of parallel lamellar or woven fibre structure, osteochondroid tissue, new chondroid tissue, fat and unidentified soft tissue or debris. These components mostly corresponded to those found in structures described and illustrated as 'types A, B and C defects' in the zone of calcified cartilage by Woods, Greenwald & Haynes (1970). In the plane of section a 'defect' may or may not appear vascularised, and may or may not show contiguity with the uncalcified articular cartilage. In the present study, only those 'defects' in contact with the uppermost or only tidemark were quantified.

For the individual femoral heads, the number of focal contacts in the zenith sample was consistently higher than that in its paired inferomedial sample. Taking the series as a whole, the mean of the zenith samples (7·7 contacts) was greater than the mean of the inferomedial samples (1·2 contacts). Student's t test gave a P value of less than 0·001, and Wilcoxon's rank sum test for paired samples showed that the site difference in focal contacts was significant at the 1 % level.

Shearing splits

Tangential splits along the uppermost or only tidemark (Fig. 10) were found in 24 of the 27 zenith samples and also in 24 of the 27 inferomedial samples. Although such splits constitute evidence of shearing damage at the interface of the calcified and uncalcified cartilage (Meachim & Bentley, 1978), it must also be acknowledged that genuine splitting may be difficult to distinguish from technical artifact. Subject to this important reservation, the mean number of splits in the zenith samples (2·7 splits) was closely similar to the mean number in the inferomedial samples (2·7 splits). Most splits were 5–100 μm in tangential length; examples where one or more of them exceeded 100 μm were noted in four of the inferomedial and one of the zenith samples.

Horizontal splitting was observed both in cartilage with an intact articular surface and in fibrillated cartilage.

Horizontal splits were sometimes found along tidemarks which had become embedded within the calcified cartilage below the contemporary level of the interface of the calcified and uncalcified cartilage. These were not counted.

DISCUSSION

Extra-phase calcification of cartilage

Demonstration of more than one tidemark does not in itself indicate when the extra phase or phases of cartilage calcification occurred. In theory, some of the additional marks could have formed during transient interruptions to calcification in the closing stages of skeletal maturation (perhaps analogous to radiological growth arrest lines), and not during later adult years. Against this suggestion, tidemark reduplication in patellar cartilage is reported not to be a feature of young joints with intact articular surfaces (Green *et al.* 1970). Sokoloff (1980, p. 5) states that changes in the osteochondral junction and reduplication of the tidemark take place throughout adult life, even in the absence of cartilage fibrillation. In the present study there were insufficient specimens from young adults to warrant the inclusion of an analysis of the effects of age, but an informal examination of the data does show age trends consistent with Sokoloff's view.

The results show that extra-phase calcification of cartilage, whether or not it is termed 'remodelling', is in general more extensive on the inferomedial aspect than on the zenith of the femoral head. Its mean extent is greater in samples where the articular surface shows minimal fibrillation than in samples where it is still intact. Considered together, these findings could either be due to an inherent difference in the cartilage base at the two sites, or could simply reflect the greater susceptibility to fibrillation of the overlying uncalcified cartilage on the inferomedial aspect. In the case of the patella, Green *et al.* (1970) have shown that the mean number of tidemarks is greater in fibrillated than in non-fibrillated specimens. This would be in keeping with the site difference noted here in the femoral head, since surface fibrillation is much more common in the inferomedial than in the zenith samples. Whether extra-phase calcification precedes or follows fibrillation is not known.

Focal contacts

In the present study, focal contacts (Fig. 9), between the uncalcified articular cartilage and 'defects' in the calcified zone, are more common at the zenith than in the area inferomedial to the fovea of the femoral head. This result agrees with observations previously made by others (Lane, Villacin & Bullough, 1977; Woods *et al.* 1970). Its interpretation depends on what function is attributed to the focal contacts.

One possibility is that, when vascularised, they are concerned in remodelling by ossification at the cartilage base (Lane *et al.* 1977). However, if, in adults, their main function is in remodelling, it is then difficult to explain the observation that extra-phase calcification is not likewise more associated with the zenith than with the inferomedial site. It is also difficult to explain the observation that gaps due to ossification breaching the lowest tidemark (Fig. 6, lower) are of similar extent at the two sites, since it might be expected that such gaps would be more numerous in the zenith site, which has more focal contacts. These comments emphasise the difficulties in interpreting remodelling, which implies a change over a period of time, from specimens representing only single points in time.

Although some of the focal contacts and 'defects' may be concerned in remodelling the cartilage base, it is possible that their main function in adults is to aid nutrition of the overlying uncalcified articular cartilage. Since the uncalcified cartilage is avascular, that at the zenith, being thicker (Vignon, 1973, Fig. 20), may require

more marrow contacts than that inferomedial to the fovea. Woods *et al.* (1970) found that the proportion of vascularised to non-vascularised 'defects' in the calcified cartilage decreases with age at both sites, and have speculated that decreased vascularity might impair the nutrition of the overlying uncalcified cartilage and influence the natural history of its degenerative changes.

Relationship to osteoarthritic cartilage degeneration

No evidence was found that any of the following features are confined to, or more common at, the femoral zenith, where the potential for degeneration of articular cartilage to progress to osteoarthritic bone exposure is greater than on the inferomedial aspect (Byers *et al.* 1970): extra-phase calcification of cartilage; ossification gaps in the lowest tidemark; shearing splits at the uppermost or only tidemark. Speculations about the significance of topographical variation in focal contacts and 'defects' (Lane *et al.* 1977; Woods *et al.* 1970) have already been mentioned.

In the case of the patella, a previous study suggested that the natural history of cartilage degeneration might be influenced by subarticular resilience (Pedley & Meachim, 1979). This mechanical property is dependent on morphological features such as the trabecular pattern of the subarticular bone and the relative amounts of calcified to uncalcified tissue in the subarticular base of bone, marrow and cartilage. In the case of the femoral head, recent studies of these morphological features in the authors' laboratory have yielded data which seem to show no consistent difference between zenith and inferomedial resilience (Meachim, Allibone & Leigh, unpublished observations).

Osteoarthritis of the hip affects only a proportion of the general population, and the bone exposure which develops in the hip joint of such patients is the common end point resulting from a number of initially different pathogenic pathways (Meachim *et al.* 1980). Femoral heads destined to become osteoarthritic might perhaps show topographical variations different from those in the present series.

SUMMARY

A series of 27 adult human femoral heads has been examined for topographical variation in 'remodelling' and other histological features of the calcified zone at the base of the articular cartilage. The specimens were obtained from necropsies; hip joints with osteoarthritic bone exposure were excluded. A tissue sample from the inferomedial aspect was compared with one from the femoral zenith, using a standard length along the articular contour at each site. Histological sections were cut in a plane vertical to that of the articular surface.

A study was made of the various patterns seen within cartilage tidemarks when these were examined at high magnification in paraffin sections stained with Ehrlich's haematoxylin and eosin. Special attention was paid to the identification of tidemark segments which stained faintly and were not readily apparent. The tidemarks were mapped on a photomicrographic montage from each of the tissue samples. When a sample showed evidence of one or more extra phases of cartilage calcification, as indicated by the presence of more than one tidemark, the spatial extent of the extra calcification was quantified by linear measurement and by point counting on the photomicrographic montage.

The mean of the results for the spatial extent of extra-phase calcification of the cartilage was greater for the inferomedial than for the zenith samples. However, it

was also greater for samples where the articular surface showed minimal fibrillation than for samples where the surface was still intact, and it was noted that surface fibrillation was much more common in the inferomedial than in the zenith samples.

Where there was more than one tidemark, the lowest sometimes showed gaps where it had been breached by an advance of ossification into the calcified cartilage. The mean value of the tangential extent of such gaps was similar at the two sites sampled.

Focal contacts, where the uncalcified articular cartilage was in contiguity with calcified zone 'defects' containing tissue other than calcified hyaline cartilage, were more numerous at the femoral zenith than inferomedial to the fovea.

Counts were also made of tangential shearing splits at the interface of the calcified and uncalcified cartilage. Subject to the reservation that genuine splitting may be difficult to distinguish from technical artifact, the mean number was closely similar at the two sites sampled.

The interpretation of the findings is discussed in relation to remodelling changes in the cartilage base and to degenerative changes in the overlying articular cartilage.

Financial support from the Arthritis and Rheumatism Council and help from discussions with Professor E. L. Radin are gratefully acknowledged.

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