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A histological study of human femoral condylar articular cartilage

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INTRODUCTION

The structure of articular cartilage has been studied extensively by many authors using sections cut perpendicular to the free surface (perpendicular sections). Superficial, middle and deep zones have been distinguished at different levels from the surface and their histological characteristics described (see Stockwell, 1979, for review).

The purpose of this study is to report some histological features of human femoral condylar cartilage using sections cut parallel to the surface (parallel sections) as well as perpendicular sections. The chondrocytes are flattened in the plane parallel to the surface so that parallel sections reveal details of cell shape and distribution which may remain hidden in perpendicular sections. Specimens were obtained from infants, children and adults. A preliminary report has been published (Gilmore & Palfrey, 1984).

MATERIALS AND METHODS

The distal end of the femur was obtained at autopsy from 9 cadavers aged 26 and 30 weeks gestation, 1 day, 6 days, 2, 5, 6, 31 and 35 years. Individuals whose cause of death involved connective tissue disease, local trauma or prolonged immobilisation were excluded from the study.

Using a small hand saw, the central area of each lateral condyle was cut into an approximately square block including the underlying bone, where ossification had occurred. Fixation for up to one week in 10% neutral formal saline was followed by decalcification when necessary; paraffin blocks were prepared by routine processing. Representative sections from each block were cut perpendicular to the articular surface. Serial sections of 10 μm thickness were cut parallel to the articular surface until subchondral bone appeared in the centre of the section. The thickness in micrometres of the articular cartilage was calculated by counting the number of sections up to this level and multiplying by 10. In the infant specimens, where the entire condyle was cartilaginous, serial parallel sections were cut to a depth of 2 mm from the surface.

Sections were stained with haematoxylin and eosin and were examined using a Leitz Orthoplan microscope with camera attachment. In the infant specimens only those sections cut between the surface and the appearance of the first cartilage canals were included in the study.

RESULTS

The thickness of the articular cartilage in each specimen is given in Table 1. The form and distribution of the chondrocytes within the matrix was examined in each

Table 1. *Thickness of articular cartilage of specimens, estimated from parallel sections*

Age	Thickness (μm)
26 weeks gestation	290
30 weeks gestation	1000
1 day	1200
6 days	1100
2 years	1320
5 years	820
6 years	1000
31 years	1700
35 years	1100

specimen. Superficial, upper middle, lower middle and deep zones were identified, first in perpendicular sections and then in parallel sections, using the criteria of Davies, Barnett, Cochrane & Palfrey (1962). Two main patterns of chondrocyte distribution were recognised. These were termed *infantile* and *mature*. The infantile pattern was characteristic of specimens up to and including two years of age and the mature pattern became increasingly predominant thereafter, until in the adult only traces of the infantile pattern could be identified.

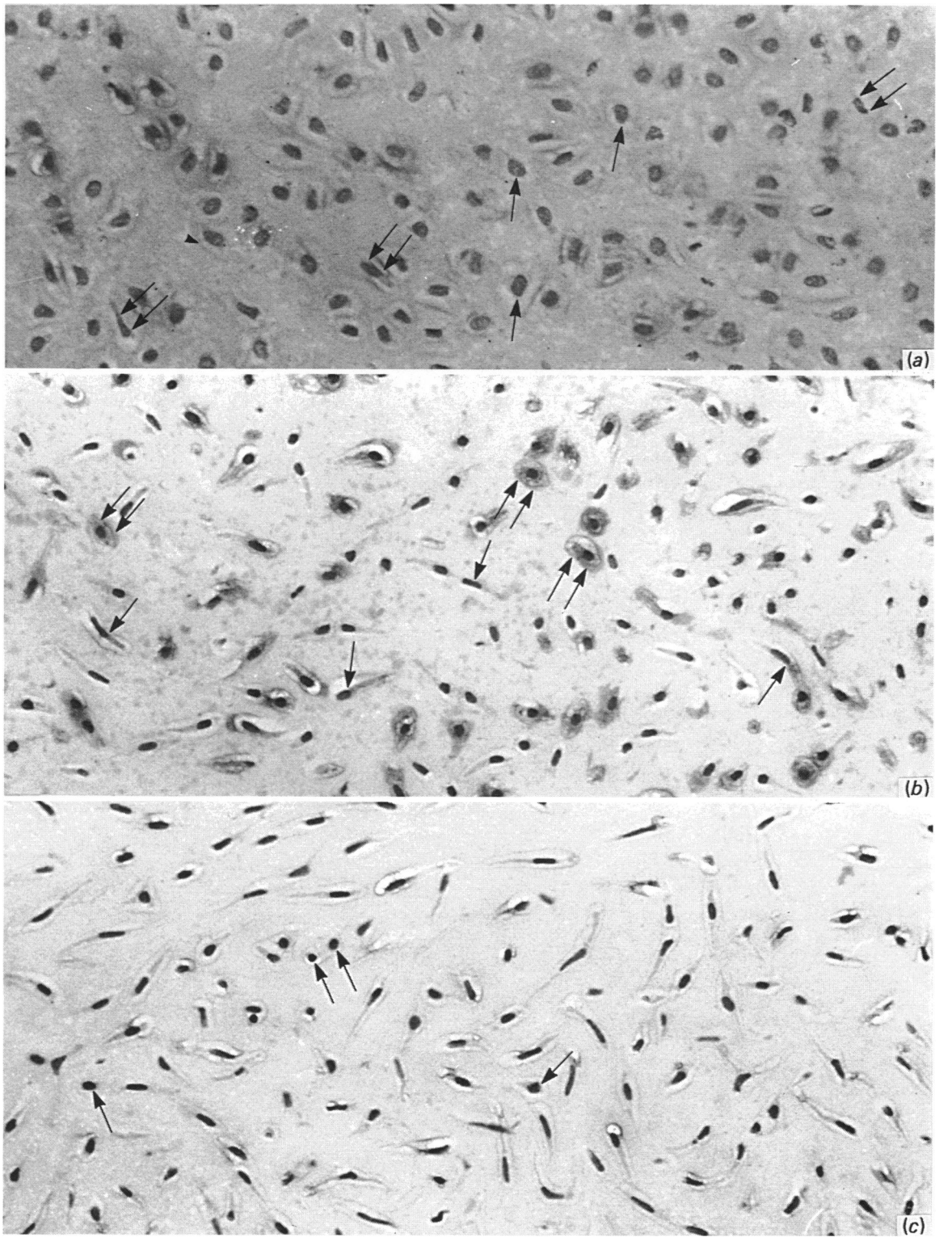
In all specimens, two discrete groups of chondrocytes were present. These were distinguished by their shape. One group comprised fusiform cells with long thin nuclei while the other consisted of cells of the more common rounded shape. The fusiform cells were more numerous in infantile cartilage while the rounded cells predominated in mature specimens.

Infantile pattern

The superficial zone (Fig. 1 *a*) contained a random arrangement of closely packed, rounded cells with no obvious lacunae. The great majority of these cells were of one type, being slightly oval in shape with a well-rounded nucleus about half the diameter of the cell. In some of the cells the nucleus was lightly stained, in others moderately, while a very few were densely stained. Occasionally a second cell type was distinguished – the appearance of the cytoplasm was similar to that of the predominant cell type, but the nucleus was thinner and darker.

In the upper middle zone (Fig. 1 *b*) most of the cells were fusiform in shape, but a population of larger, rounded cells was present. The fusiform cells were arranged either in rows, with three to five cells lying end to end, or in swirling patterns. Each cell was approximately two or three times longer than its nucleus. The nucleus was moderately, but not darkly stained and when cut transversely, was surrounded by only a thin rim of cytoplasm. The larger cell type was rounded in shape with a round nucleus only slightly larger than the nucleus of the fusiform cell, but more darkly staining. Many of the rounded cells contained cytoplasmic vacuoles often arranged to one side

Fig. 1 (*a–c*). (*a*) One day old infant. Superficial zone. Parallel section, 50 μm from surface. Predominant cell type with rounded nucleus indicated by single arrows. Second cell type with thinner nucleus indicated by double arrows. The matrix staining is more basophilic than at deeper levels. $\times$ 360. (*b*). Twenty six weeks gestation fetus. Upper middle zone. Parallel section, 100 μm from surface. Fusiform cell type indicated by single arrows. Larger, rounded cell indicated by double arrows. $\times$ 360. (*c*). Six days old infant. Lower middle zone. Perpendicular section. The swirling pattern of cell distribution is reminiscent of mesenchyme. Rounded cells are arrowed. $\times$ 360.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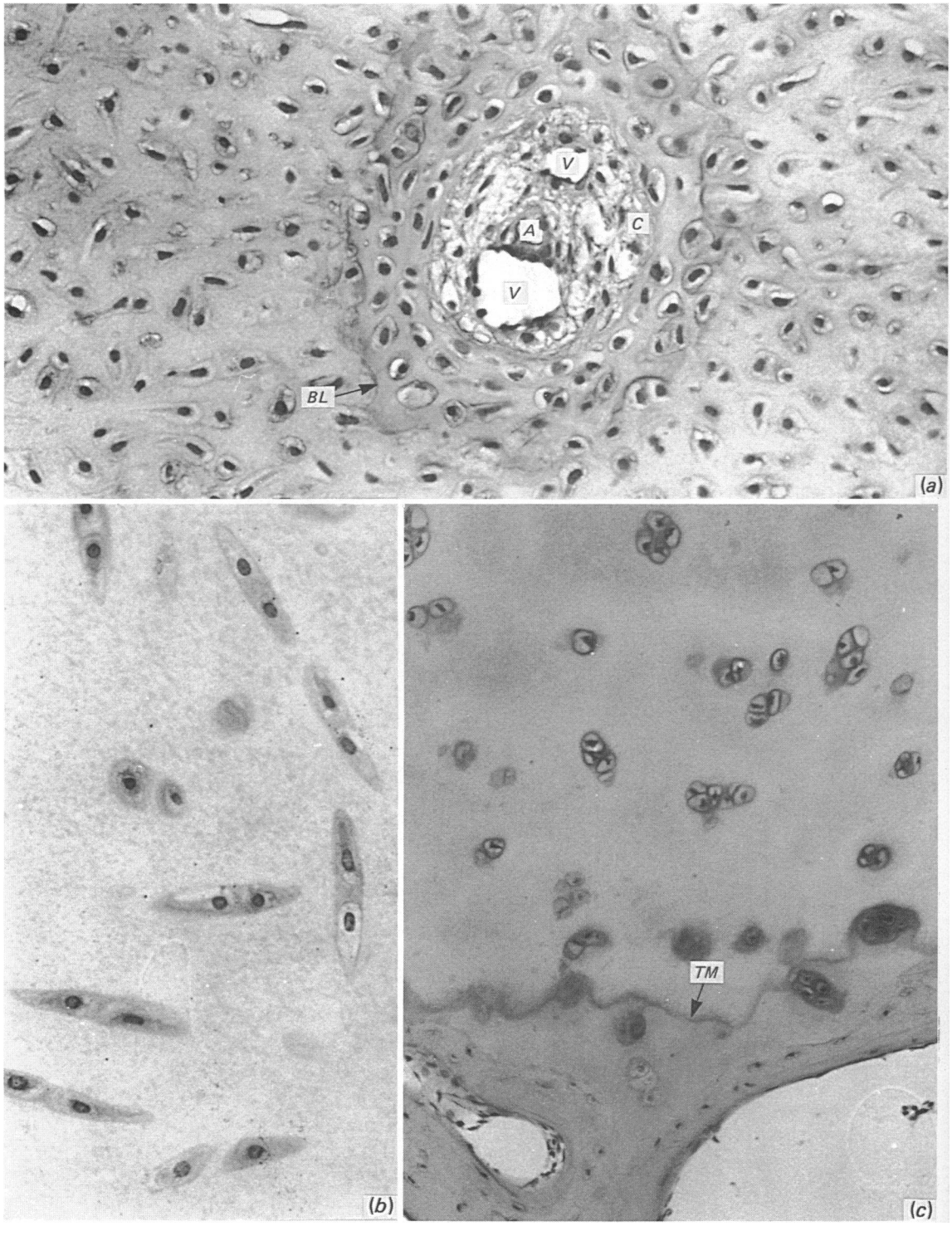
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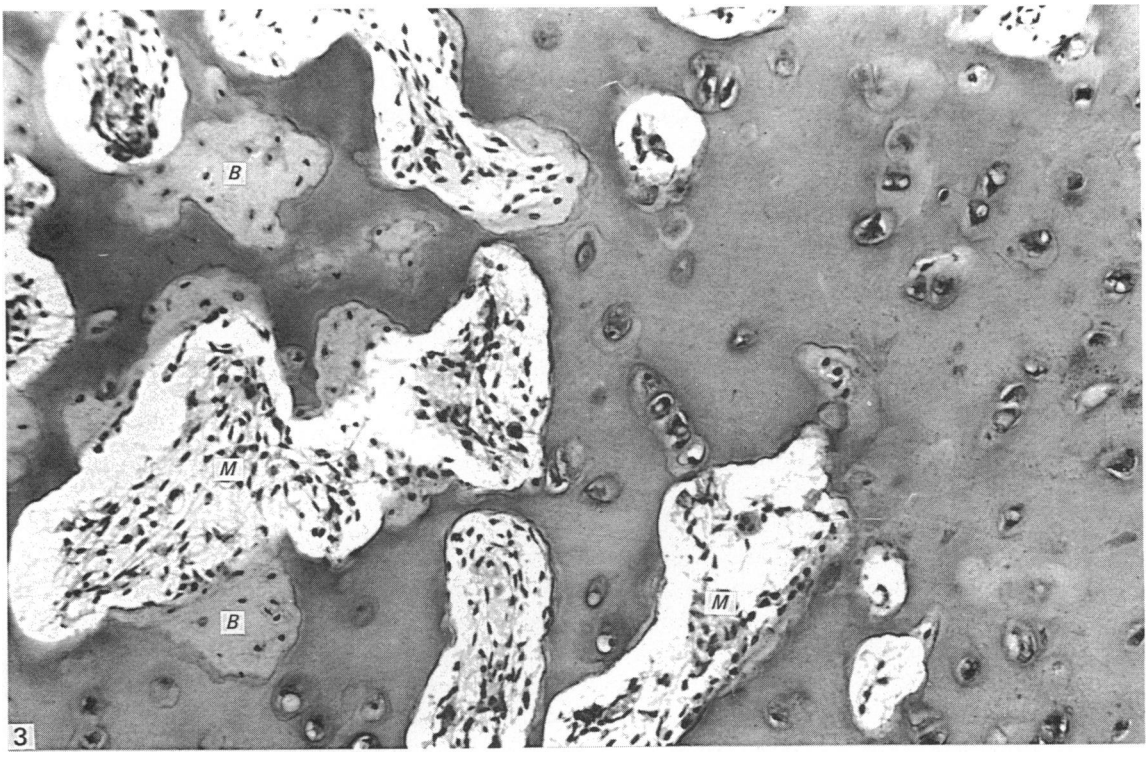

Fig. 3 Six years old child. Region of the cartilage–bone junction. Parallel section 1400 μm from the surface. The irregularity of the junction can be appreciated. Islands of bone (*B*) and mesenchymal tissue (*M*) are in close association with cartilage. × 110.

of the cell. Around some of these cells the matrix was stained more densely, though not evenly, around the cell perimeter. Some cells of both types had a clear space partly surrounding the cell. These were taken to be early lacunae.

The lower middle zone (Fig. 1 *c*) showed an increased proportion of fusiform cells when compared to the upper middle zone, these cells being more elongated and the nuclei more darkly staining. Like those in the upper middle zone the elongated cells were arranged in swirling patterns, reminiscent of mesenchyme. The rounded cells contained somewhat flattened nuclei also darkly staining and with numerous cytoplasmic vacuoles.

In the deep zone, that is the layer of cartilage just superficial to that containing cartilage canals, most of the cells were of the larger, rounded type. These cells were often arranged in rows, pairs or triplets. The nuclei were mostly darkly staining, rounded and smaller than in more superficial sections. The cytoplasm was vacuolated in all but a few cells. The matrix was lightly stained, except around some cells where the staining reaction was more intense.

Fig. 2 (*a–c*). (*a*) Twenty six weeks gestation fetus. Deep zone. Perpendicular section showing a cartilage canal (*C*). The canal contains an arteriole (*A*) and two venules (*V*). There is a basophilic line (*BL*) surrounding the canal. × 360. (*b*). Six years old child. Upper middle zone. Parallel section 600 μm from the surface. Pairs of elongated and rounded chondrocytes are shown. × 360. (*c*). Thirty one years old adult. Deep zone. Perpendicular section from the region of the tidemark (*TM*). Cell clusters are seen, some lying deep to the tidemark. The undulating cartilage–bone junction also lies deep to the tidemark. × 110.

Cartilage canals were found in the specimens up to and including 2 years of age (Fig. 2*a*). Serial sections showed the canals to be blind-ended with long axes at right angles to the surface. A typical canal contained two or three vessels, at least one of which had the histological features of a small arteriole, the others being venules and capillaries. The vessels were ensheathed in loose connective tissue which merged imperceptibly with the cartilage matrix. The canals were surrounded by a cuff of chondrocytes with rounded or elongated profiles. Within this cuff a basophilic line was seen, generally not complete but in some parts very obvious.

Mature pattern

The superficial zone was very similar in appearance to that of the infantile cartilage except that the cells were larger and often arranged in pairs, each cell being surrounded by a distinct lacuna.

In the upper middle zone cells were distributed in an irregular manner (Fig. 2*b*). Most were paired, and some lay in rows or groups which shared a single lacuna. Some cells contained intracytoplasmic vacuoles. Remnants of the swirling patterns of infantile cartilage were recognisable.

In the lower middle and deep zones, pyknotic nuclei were present and intracytoplasmic vacuoles were more common. In the deep zone the cells were large and of less ordered appearance (Fig. 2*c*). The narrow junction between calcified and uncalcified zones of cartilage known as the tidemark (Fawns & Landells, 1953) could be seen as a reddish-purple line in perpendicular sections but could not be identified in parallel sections. Superficial to the tidemark, cell clusters of two to six cells were seen. Some clusters lay across the tidemark, and in a few examples it seemed as if the staining reaction of the tidemark ran through individual cells. The cytoplasm of the cells was basophilic, but any territorial basophilia present at more superficial levels faded out gradually to a more uniform pale matrix basophilia as the tidemark was approached. This staining reaction was present deep to the tidemark and chondrocytes, either in clusters or occurring singly, were seen at this level.

The junction between the cartilage and the subchondral bone lay deep to the tidemark (Fig. 2*c*); perpendicular sections displayed the sudden change in appearance between the cartilage and bone matrix, the latter being strongly eosinophilic. However, the undulating nature of the junction was appreciated best in parallel sections where finger-like processes of vascular bone appeared as isolated islands within a region of calcified cartilage (Fig. 3). Blood vessels from the underlying bone passed up to the tidemark and, in some instances, were in contact with cell clusters which passed through it, but vessels were not seen to penetrate into the deep zone.

DISCUSSION

In this investigation, we have used parallel sections in the study of articular cartilage. It is important to recognise that this articular surface is curved, so that the central region of a parallel section lies at a deeper level in the cartilage than the more peripheral parts. It is possible thus to examine a section from the edge to the centre and observe the cell arrangement at progressively deeper levels. Where serial comparisons have been made in these sections, similar, and usually central, parts of the sections have been used. Sections cut perpendicular to the surface were studied in order to orientate the parallel sections, and were used also to examine the tidemark and junctional zones.

The thickness of the articular cartilage was measured in all specimens. The results for the fetal and neonatal specimens compare favourably with published values

(Haines, 1937; Wilsman & van Sickle, 1972). The marked difference in thickness estimates between 26 weeks and 30 weeks fetal specimens may be explained by the fact that the younger fetus was 'small for dates' due to placental insufficency; little is known however about when articular cartilage attains its definitive thickness during development. The values for the older condyles (2–35 years) are lower than those reported by Stockwell (1971 *a*, *b*). We have found that the routine preparation method used in this study reduces values of cartilage thickness by up to 50% in the adult compared to values estimated in the fresh specimen at autopsy (Gilmore & Palfrey, unpublished observations). The problem of cartilage shrinkage is considerable, especially when compounded by the compressive effects of parallel sectioning.

Adult articular cartilage contains several histologically distinct layers or zones – superficial, upper middle, lower middle and deep (Davies, *et al.* 1962). In our specimens the approximate proportion of the total thickness occupied by each zone could be calculated as: superficial 2–3%, upper middle 25%, lower middle 50% and deep 25%. It is not known whether the proportionate thickness of each zone remains constant throughout development. In the present study we assumed that this was the case, and found that the histological differences at the levels sampled correlated well with this presupposition, although further work remains to be done in this area.

Hurrell (1934) showed that cartilage canals are present within a chondro-epiphysis only in the region which eventually will ossify and that the definitive articular cartilage remains avascular. Thus the appearance of canals in parallel sections denotes the transitional zone between the cartilage that will be replaced by bone and the permanent articular cartilage. We have used this observation in the estimation of articular cartilage thickness.

This paper describes two patterns of chondrocyte appearance and arrangement in human lower femoral articular cartilage, *infantile* and *mature*. The infantile pattern was seen in cartilage aged from 26 weeks gestation up to and including 2 years of age. The specimens from older children and adult cartilage showed an essentially similar pattern of cell appearance and distribution, although the cell density was much higher in the cartilage of children than in the adults. The characteristic pattern of the infantile group was the presence of two differently shaped types of chondrocyte – a fusiform cell with a long thin nucleus, and a rounded cell, more like the conventional chondrocyte. It is necessary to consider whether these two forms represent one or two types of cell.

It is possible that all the cells are in fact of the fusiform type and that the rounded cells are merely fusiform cells cut in transverse section. Alternatively, the rounded cells may be a separate population of more mature chondrocytes which have developed from the fusiform type. In some zones there was a greater preponderance of rounded cells than could be explained on the basis of their being transversely sectioned fusiform cells. The fusiform cells have a similar appearance to the mesenchymal cells from which the cartilage developed. Thus it is more likely that there are two morphologically distinct populations of chondrocytes, and that the rounded cell type represents a more mature form.

The swirling patterns demonstrated by the fusiform cells were a striking feature of their arrangement in the infantile cartilage. In this cell population there were two features of note – the pattern of the cells and their orientation. The pattern seemed to be mainly random but rows were a conspicuous feature. It was very striking that many of the cells were orientated along parallel axes which were straight or curved. This may reflect patterns of movement within the matrix, or, if the orientation of the cells is related to that of the collagen fibres, it may indicate the pattern of tension within the

matrix as occurs in fibrocartilage, or perhaps in bone trabeculae (Wolff, 1870, 1899).

The most interesting regions of the mature cartilage were the middle zone, where remnants of the swirling patterns of infantile cartilage could be recognised, and the region around and deep to the tidemark, where the undulating nature of the cartilage–bone junction was a marked feature. Chondrocytes coexisted in close proximity to bony islands. Blood vessels extended as far as the tidemark, but none were seen to penetrate it in the present study, although this has been reported elsewhere (see Stockwell, 1979, for review). Parallel sections demonstrated that fingers of vascular bone penetrated deeply into the calcified layer of the deep zone, but the blood vessels were separated from the chondrocytes by a layer of bone matrix.

The tidemark was a conspicuous feature of mature cartilage examined using perpendicular sections but it could not be identified in parallel sections. This intensely basophilic line is narrow (2–5 μm) and undulating (Fawns & Landells, 1953; Redler, Mow, Zimny & Mansell, 1975). In a 10 μm parallel section the tidemark forms only a proportion of the total thickness and probably is represented by an imperceptible increase in matrix basophilia. In contrast, if the tidemark is present in a 10 μm perpendicular section, it will be evident at the same level throughout the thickness. The basophilic line within the cuff of chondrocytes surrounding a canal was seen in both parallel and perpendicular sections. This can be explained by the same rationale if the basophilic line represents a thin cylindrical layer of matrix concentric with the canal.

Cartilage canals are not a feature of adult cartilage so that this tissue is said to be avascular (Ham & Cormack, 1979; Williams & Warwick, 1980). The canals however are an accepted feature of developing cartilage, for example at the ends of long bones. Haines (1933, 1937) showed that where canals are present ossification eventually will occur. It is difficult to suggest an improved method of discriminating histologically between articular cartilage and that destined for ossification, in a homogeneous mass of hyaline cartilage. In this study we saw no histological difference between the two regions of cartilage. However, the questions which remain are whether the definitive articular layer is indeed avascular throughout its development and at what age the final thickness is determined.

Blood vessels were present in the specimens of the mature group, enclosed within fingers of bone, extending in close proximity to chondrocytes in the calcified layer of the definitive articular cartilage. Other workers have described vessels actually penetrating the tidemark (Mital & Millington, 1970; Redler *et al.* 1975; Stockwell, 1979). Thus this region of articular cartilage can hardly be said to be avascular. The pattern of blood vessel distribution within the epiphysis of a long bone is determined by the nutritional requirements of the tissue (Haines, 1933, 1937). The articular cartilage may lie close enough to the synovial cavity for its nutritional needs to be supplied from the synovial fluid (Bywaters, 1937) but diffusion through cartilage can take place only over limited distances (Maroudas, Bullough, Swanson & Freeman, 1968) and it is unclear how this correlates with known differences in thickness of articular cartilage across this articular surface.

SUMMARY

Paraffin sections cut both parallel to and perpendicular to the surface were used to study the histological structure of the articular cartilage of the lateral femoral condyle of infants, children and adults. Two main cell types were present – fusiform

chondrocytes lying in swirling patterns were the predominant cell type of the cartilage up to and including two years, while a more rounded cell, randomly arranged, was commoner in the older specimens. Evidence suggests that these cells represent two distinct populations of chondrocyte, the round cells being derived from the earlier fusiform cells.

Cartilage canals were a feature of the deeper regions of the presumptive articular cartilage in young specimens in which the epiphysis was still cartilaginous.

The basophilic tidemark which marks the junction between the calcified and uncalcified cartilage in perpendicular sections was not seen in parallel sections. The calcified cartilage layer contained numerous processes of vascular bone which extended up from the subchondral bone and were a characteristic feature of the cartilage–bone interface. This layer of the articular cartilage cannot therefore be considered to be truly avascular.

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