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Ultrastructural evidence for fibril-to-fibril associations in articular cartilage and their functional implication

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INTRODUCTION

Examination of published ideas relating the fine structure of articular cartilage to its role of load-bearing presents a rather confused picture. For example, Serafini-Fracassini & Smith (1974), on the basis of transmission electron microscope studies, have interpreted the apparently random arrangement of collagen fibrils in what they term the wide central zone as supporting the idea that the primary role of the fibrils is to ensure the fixation of the elastic domains of hydrated proteoglycan molecules as they deform under stress, and that the fulfilment of this function does not require any specific fibril orientation. As supporting evidence, they cite the much earlier experiments carried out by Fessler (1960) in which he demonstrated that a very small amount of hyaluronate contained within a given entanglement of collagen fibrils can trap four times the weight of water trapped by the same hyaluronate-free sample. Serafini-Fracassini & Smith contrasted the apparently random fibrillar architecture in articular cartilage with the more orderly arrangement in fibrous cartilage where, they contend, the collagen fibrils seem to be orientated in the planes of tensile stress. Kempson (1979) has examined the relationship between the compressive creep modulus of articular cartilage and its primary chemical constituents – namely, the total glycosaminoglycan content (chondroitin and keratan sulphate) and collagen – by sampling various regions of the human femoral head *in vitro*. He concluded that the compressive stiffness of articular cartilage is determined more by the glycosaminoglycan content than the collagen.

The above conclusions might be interpreted as implying that the presence of collagen and its particular architecture in articular cartilage is less important than that of the proteoglycans. This of course is known to be incorrect. Without the fibrillar meshwork, there would be no fixation of the proteoglycan aggregates thus permitting them to expand to the largest volume or domain of solution available (Mow, Mak & Lai, 1984) with virtually no functional load-bearing properties. The collagen meshwork must therefore resist shape changes in the confined proteoglycans as load is applied (or as swelling occurs) and will therefore be mechanically stressed. It can then be argued that if the fibrils are stressed their spatial pattern or arrangement will be important.

Kempson's (1979) conclusions must be considered within carefully defined functional limits. He was analysing a working tissue system and in that sense there would reasonably be a range of collagen levels over which a fully competent architecture of fibrils could be achieved without creating too large a variation in trapping efficiency of the proteoglycan component; this in turn being reflected in the tissue's

compliance. If one imagines now a progressive reduction in the collagen level, a threshold will be reached when the remaining fibrillar arrangement cannot effectively restrain the proteoglycan component (e.g. through an increased permeability of the meshwork and an insufficient number of fixation sites being available on the remaining fibrils) thus resulting in a composite structure with functionally inadequate properties.

Although collagen fibril segments in all orientations may be found in regions of the cartilage thickness below the articular surface (Boyde & Jones, 1983) it is now generally accepted that articular cartilage can be divided into three relatively distinct zones: a surface layer in which fibrils lie principally in the plane of the articular surface, a transition or intermediate zone where they are more or less randomly arranged, and a deep zone with a predominantly radial orientation (Meachim & Stockwell, 1979; Yarker, Aspden & Hukins, 1983; Myers & Mow, 1983). However even this description, although a refinement of the earlier idea of randomness is, in the view of the present authors, incomplete. For example it says nothing about whether or not fibrils have large scale interzonal continuity within the matrix. This is important to resolve if we are to understand how overall structural cohesion is achieved. If fibrils do in fact traverse large distances within the matrix, both radially and obliquely, how are the strongly radial fibrillar arrays that are so prominent in various forms of degenerate cartilage (Weiss, 1973; Meachim, 1976) actually developed? If they are a derivative of the original healthy fibrillar architecture, it is difficult to conceive of a mechanism whereby long spans of oblique fibrils could be reorientated into a radial aspect.

Recent studies, using a new experimental technique involving the propagation of a microscopic notch through the cartilage general matrix* both perpendicular and parallel to the articular surfaces, have established that the matrix is mechanically highly anisotropic and reflects a fundamental difference in structural configuration in these two primary directions (Broom, 1984*a*, *b*). Rather than the fibrillar architecture being developed from an array of fibrils variously orientated over relatively large distances about a radial mean, there is now substantial evidence that a pseudo-random meshwork is developed from a principally radial array in which each fibril is repeatedly deflected sideways into short-range oblique orientations along its length (Broom, 1982; 1985*a*). Although giving the appearance of randomness particularly when viewed in thin section by transmission electron microscopy, this structure is in fact highly directional in its overall configuration. Further, such an array of fibrils when packed out with hydrated proteoglycan molecules effectively functions as a braced structural system capable of resisting shear forces associated with direct compression. This concept has been convincingly demonstrated very recently with a working physical model (Broom & Marra, 1985).

Other recent studies have shown that dramatically reduced compressive compliance in cartilage resulting from both non-progressive and osteoarthritic degeneration is directly associated with what appears to be a reversion of these 'sideways' deflected fibril segments to a more radial configuration. The fibrillar array then loses its pseudo-random appearance, instead forming radially aligned groups of fibrils frequently displaying an in-phase waveform or crimp (Broom, 1984*b*).

In another recent study (Broom, 1985*b*) it has been shown that a pseudo-random

* The term 'general matrix' in this study refers collectively to the middle and deep zones which comprise the bulk of the total cartilage thickness. In these zones there is a relatively common structural configuration distinct from that found in the superficial zones.

fibrillar structure characteristic of normal cartilage is also susceptible to a stress-induced structural transformation. Repeated impact loading of healthy articular cartilage attached to its subchondral bone, if intense enough to disrupt the articular surface, will create radially aligned parallel arrays of crimped fibrils virtually identical to those described above.

It is concluded, therefore, that there must be some form of fibril-to-fibril interconnection in cartilage in order to tie the radial arrays of repeatedly deflected fibril segments into a coherent structure in the transverse direction. In the authors' view, any breakdown in these interconnections, whether through pathological processes or through overloading, will result in the fibrils reverting to a low energy aligned radial configuration lacking the bracing features characteristic of the healthy architecture and therefore deficient in compressive strength.

It is recognised that the functional properties of articular cartilage must depend on a specific interaction between the collagen and the proteoglycans (Mow, Holmes & Lai, 1984) but its nature is not well understood (Muir, 1979). A minority of the proteoglycans in cartilage appears to be more tightly bound to the collagen than the remainder and Muir (1979) has suggested that these may in fact act as a bonding agent between the collagen fibrils, spanning distances too great for crosslinks to develop. Serafini-Fracassini & Smith (1966), using transmission electron microscopy, examined the interaction between the ground substance and collagen and concluded that some proteoglycans in cartilage are coiled transversely around the fibril repeatedly at specific bonding sites. Another possibility discussed by Meachim & Stockwell (1979) is that proteoglycan is bound to collagen via a glycoprotein. However to date none of these ideas has been given firm substance by empirical testing on functioning cartilage.

Clearly there is still a need to elucidate what appears to be an important structural feature in articular cartilage. Therefore, this paper attempts to bring together various strands of ultrastructural evidence supporting the concept of a generalised fibril-to-fibril structural association. It is hoped that the presentation of this structural evidence will stimulate a more focussed enquiry at the biochemical level.

METHODS AND MATERIALS

Articular cartilage from a variety of sources was examined by conventional transmission electron microscopy and scanning electron microscopy using both cryopreservation and conventional dehydration procedures.

Transmission electron microscopy (TEM)

Full depth blocks of cartilage were taken from 5 normal human femoral heads at postmortem, and from 2 osteoarthritic human femoral heads removed from patients undergoing replacement arthroplasty. Blocks of normal cartilage were also taken from the patellar groove region of 10 mature bovine animals. In addition, blocks of cartilage exhibiting non-progressive degenerative softening were taken from the central region of the tibial plateaux of 3 mature bovine animals.

Blocks were fixed for 2 hours in 2 % glutaraldehyde in 0·10 M sodium cacodylate buffer at pH 7·3. After postfixation in 1 % osmium tetroxide in 0·1 M sodium cacodylate buffer at 7·3 for 2 hours, the tissue was stained *en bloc* overnight at 60 °C with saturated aqueous uranyl acetate. The samples were then dehydrated with ethanol, embedded in Epon and polymerised at 60 °C for 3 days. Silver–gold sections were

cut and stained with uranyl acetate and lead citrate and then viewed in a Philips EM300 electron microscope at 80 kV.

Scanning electron microscopy (SEM) with cryopreservation

Samples of articular cartilage were examined from 5 normal human femoral heads and from the femoral condyles and patellar grooves of 15 mature bovine animals. In addition, cartilage samples exhibiting non-progressive degenerative softening were taken from the central region of the tibial plateaux of 11 mature bovine animals.

An Em-scope SP 2000 sputter cryosystem attached to a Philips SEM 505 was used for this aspect of the investigation. The fresh cartilage samples were mounted on the specimen transfer device, plunged into slushing liquid nitrogen, fractured while frozen in planes incorporating the full zonal thickness, and etched for between 10 and 20 minutes at a nominal indicated temperature of −70 °C. It was found that with the particular system used indicated temperatures below −70 °C yielded virtually no fine detail in the fractured cartilage matrix surface indicating that little ice had sublimed from the tissue (Echlin, 1978). The frozen-etched sample was then sputter coated with gold and transferred under vacuum to the microscope cold stage for examination at 30 kV.

Scanning electron microscopy (conventional)

Full thickness blocks of articular cartilage from 5 normal human femoral heads were fixed in 2 % glutaraldehyde in 0·01 M sodium cacodylate at 4 °C for 2 hours followed by postfixation for 1 hour in 1 % osmium tetroxide in 0·10 M sodium cacodylate. This was followed by dehydration through ethanol, freeze-fracturing in liquid nitrogen, immersion in 100 % acetone and critical point drying. The fracture surfaces were finally sputter coated with gold and examined in the scanning electron microscope at 30 kV.

RESULTS

Transmission electron microscopy

Examination of thin sections by TEM provides a strictly two dimensional view of the fibrillar architecture of articular cartilage. Caution is therefore required when attempting to construct a three dimensional picture from such data. Any given fibril needs only to deflect slightly out of the plane of the thin section to disappear entirely from view. Individual fibril continuity is difficult to follow over any appreciable distance through the cartilage matrix.

The present TEM studies confirmed the generally accepted view that the general matrix ultrastructure contains fibrils variously orientated about a mean radial direction. However, on closer examination this spectrum of fibril orientations was not seen unformly throughout the matrix at a given zonal depth. Rather, there frequently appeared to be a distinct partitioning of the fibrillar architecture into local regions where fibril segments were grouped into parallel or near parallel aggregates or 'nodes' with a generally pronounced radial orientation, and between these the matrix exhibited a more open and irregular fibril mesh (Figs. 1–4).

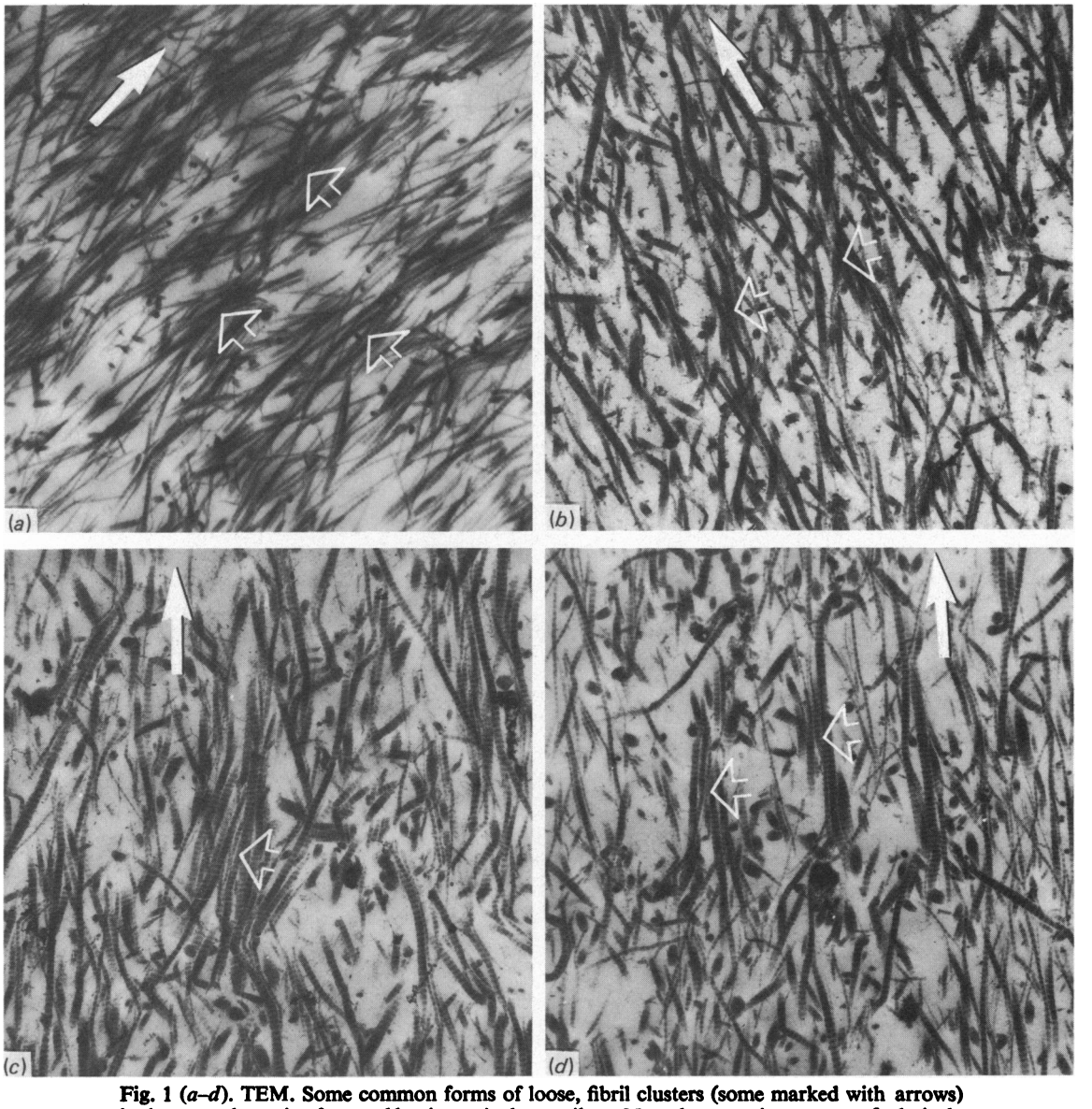


Fig. 1 (*a–d*). TEM. Some common forms of loose, fibril clusters (some marked with arrows) in the general matrix of normal bovine articular cartilage. Note the repeating pattern of relatively dense fibril clusters in (*a*) femoral condyle, × 13800; (*b*) patellar grove, × 18000; (*c–d*) patellar groove, × 13800. The radial direction in each micrograph is indicated by a solid arrow.

The precise form of these aggregates or nodes varied considerably within the range of tissues examined. Some common forms are shown in Figure 1 *a–d*. Here the nodes lack exact definition, consisting of varying numbers of fibril segments loosely aggregated about a radial direction over distances of the order of 0·5–5 μm.

Other examples of the matrix, as well as containing loose clusters like those shown in Figure 1, incorporated a type of aggregation which reflected a more intimate, aligned relationship between the constituent fibril segments (Figs. 2, 3). Mature articular cartilage is known to exhibit a typically bi-modal distribution of collagen

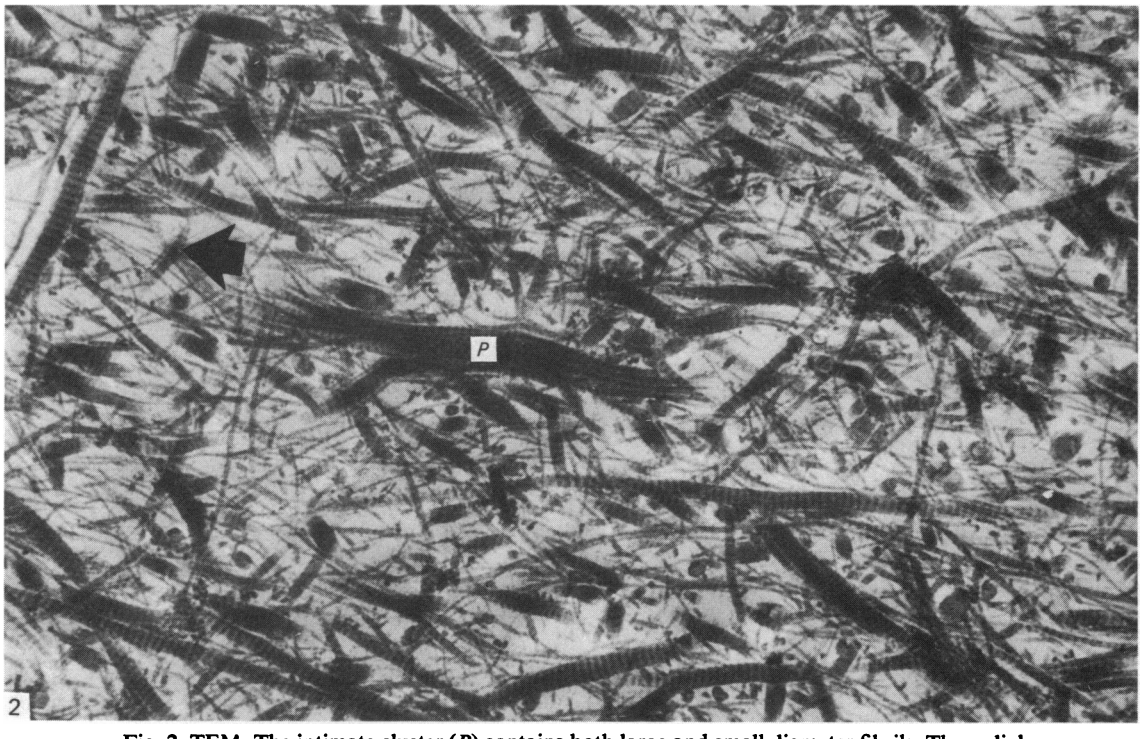


Fig. 2. TEM. The intimate cluster (*P*) contains both large and small diameter fibrils. The radial direction is indicated by an arrow. Normal human head of femur; male, 59 years. ×20000.

Fig. 3. TEM. Region of general matrix containing extended associations of several fibrils (open arrows). The large arrow indicates the radial direction. Normal human head of femur; male, 23 years. ×10000.

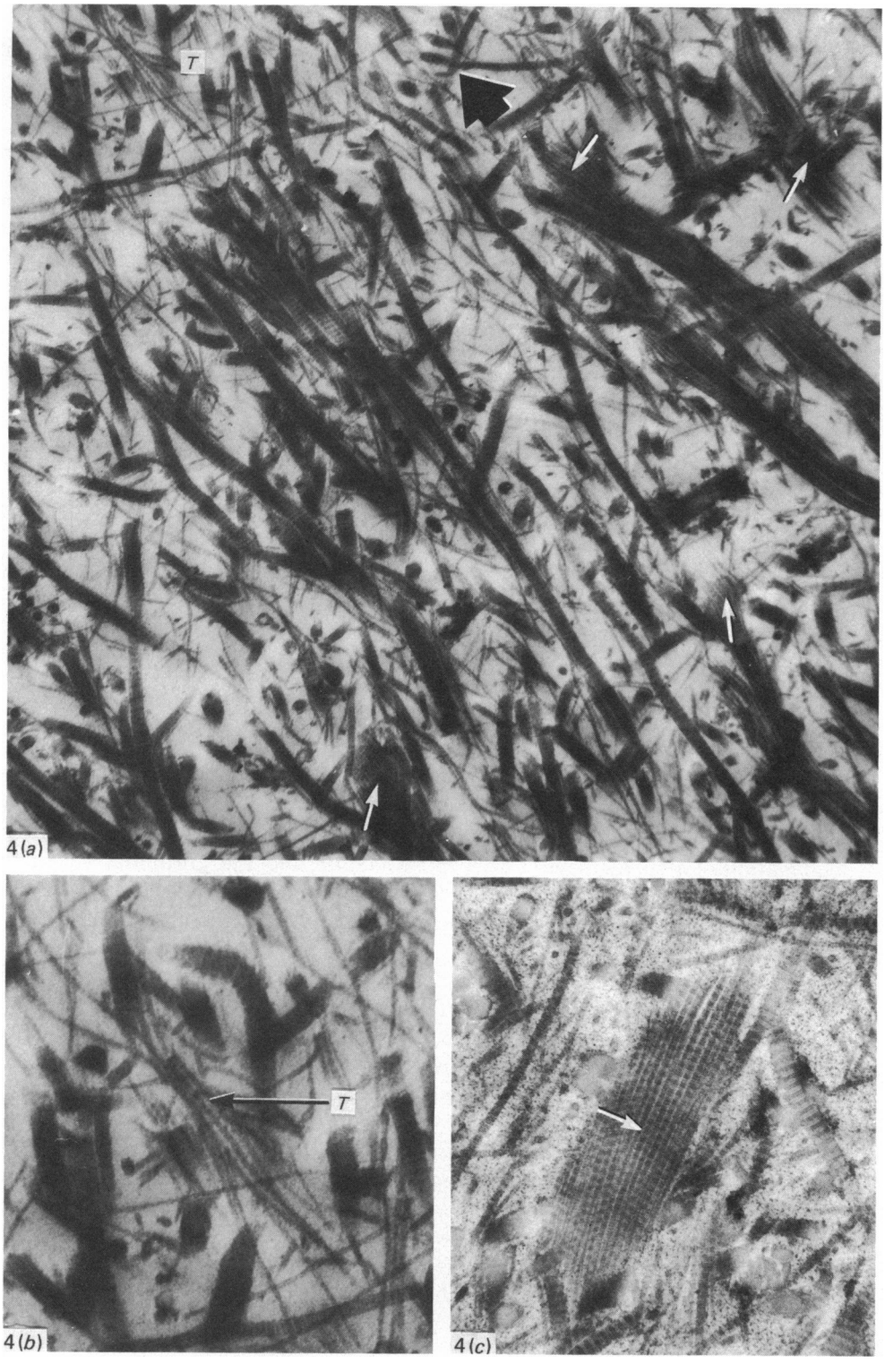


Fig. 4 (*a–c*). TEM. General matrix exhibiting a variety of fibril-to-fibril associations. Small white arrows in (*a*) indicate multistranded parallel arrangement of small diameter fibrils. A similar structure is shown enlarged in (*c*). A transient node of small fibrils is visible at *T* in (*a*), and enlarged in (*b*). Large arrow indicates the radial direction in (*a*). Normal human head of femur; male, 21 years. (*a*) ×17300; (*b*) ×34600; (*c*) ×22500.

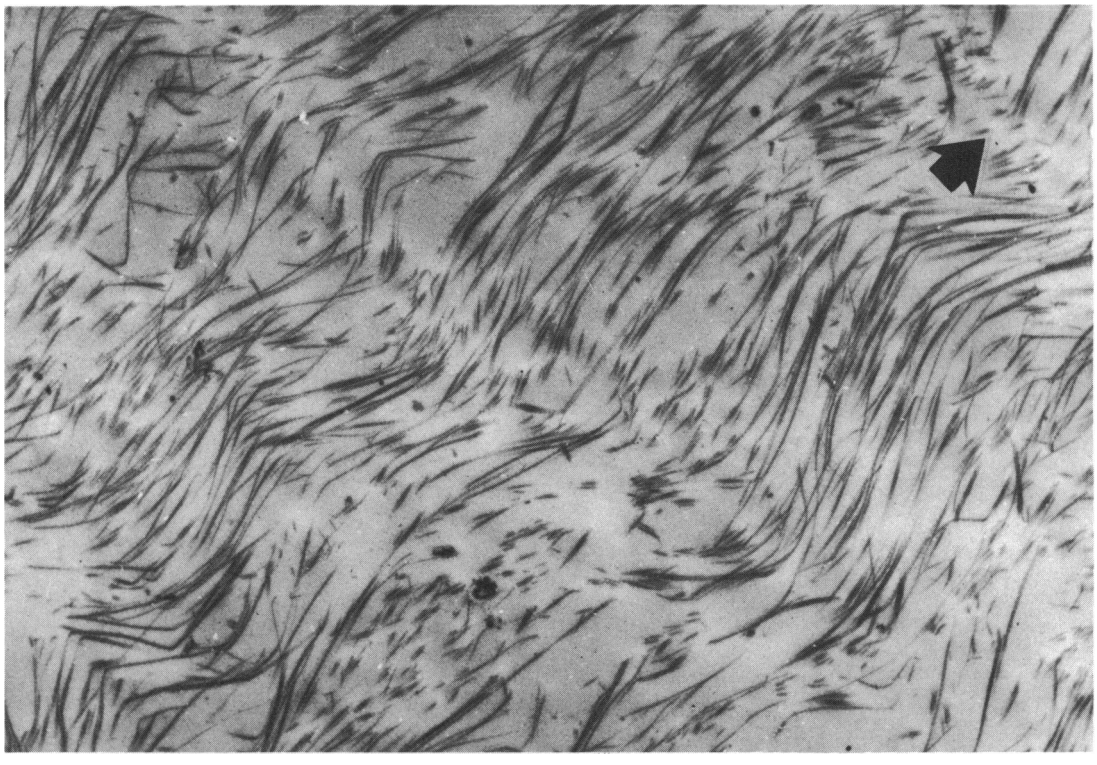


Fig. 5. TEM. Parallel aggregates of fibrils orientated in radial direction and exhibiting a pronounced waveform or crimp. The arrow indicates the radial direction. Softened tibial plateau from bovine knee joint. × 10300.

fibril diameters (Parry, Barnes & Craig, 1978) and this was verified in general in the present study (Figs. 2–4). Fibrils of both size groups were implicated in this process of aggregation. Large diameter fibrils mostly comprised the groupings shown in Figures 1 and 3: in Figure 2, the prominent node *P* contains both large and small diameter fibrils. A transient node of exclusively small fibrils is visible at *T* in Figure 4*a* (enlarged in Figure 4*b*).

Peculiar to the human femoral head cartilage was a highly ordered, multistranded, parallel arrangement of small diameter fibrils sometimes seen in close association with several large diameter fibrils (Fig. 4*a*, *c*). In some sections, this form of aggregation could be traced weaving in and out of the section for distances greater than 30 μm before either passing completely out of the plane of section or dispersing into a 'fan' of individual fibrillar elements.

In contrast to the general matrix of normal articular cartilage which exhibited this recurring pattern of frequent fibril aggregation with accompanying regions of greater randomness or obliquity, the general matrix of softened cartilage (from the central tibial plateau) exhibited extensive regions containing predominantly parallel radial fibrils; these frequently incorporated a variable in-phase waveform or crimp (Fig. 5). A similar arrangement of fibrils has been noted in regions of softened matrix characteristics of osteoarthritic articular cartilage (Broom, 1984*b*, 1985*a*).

Scanning electron microscopy (low temperature or cryopreservation)

Using this technique, structural distortion resulting from the removal of water bound within articular cartilage can be eliminated. This is significant because water comprises 60–80 % of the wet weight of the tissue (Maroudas, 1979). Rapid freezing is assumed to cause minimal distortion of the tissue structure by reducing or eliminating effects due to ice-crystal formation (Echlin, 1978; Gardner, O'Connor & Oates, 1981).

Whereas TEM provided an unequivocal view of the collagen fibrillar arrangement in a two dimensional sense, with low temperature SEM there was generally some obscuration of the individual fibrils comprising the general architecture. It was assumed that this resulted from residues left in the tissue as the end product of the sublimation of water from the hydrated ground substance. The sublimation process did, however, remove water to some depth below the original fracture plane thus providing a more three dimensional view of the undisturbed fibrillar arrangement.

The general matrix of each of the various samples of normal articular cartilage (i.e. bovine patellar surface, bovine femoral condyle and human head of femur) all exhibited a well developed interconnecting arrangement of fibrillar elements (Fig. 6*a–d*). Individual fibrils extended only short distances before anastomosing and aggregating with adjacent fibrils to create a coherent structure which displayed a pronounced radial texture. Only occasionally were individual fibrils seen to extend for large distances in orientations oblique to the primary radial direction (Fig. 6*d*). Although there was some variation in the degree of radial texture between different tissue specimens, for a given specimen the structural appearance at a particular zonal depth within the general matrix was relatively uniform.

The general matrix of cartilage exhibiting non-progressive degenerative softening was characterised by a number of structural features which contrasted markedly with the 'normal' architecture described above. There was, for example, a considerable variation in texture of the fibrillar structure within a given specimen (Fig. 7*a*). In local areas, fibrils coalesced on a relatively large scale to form prominent, radially extended, parallel bundles: the intervening spaces were either depleted of fibrils or contained an irregular arrangement of individual fibrils (Fig. 7*b*). The coarse radial structure varied in its extent within a given sample, occupying at times almost the entire general matrix or else blending gradually into a matrix whose texture more closely resembled that of normal cartilage (Fig. 7*c*, *d*). Other areas of matrix exhibited simply a grossly coarsened meshwork with shorter lengths of aggregated fibrils (Fig. 8). Figure 8 also demonstrates the familiar crimped appearance characteristic of much of the softened cartilage matrix (Broom, 1982).

Scanning electron microscopy (conventional)

Articular cartilage examined by SEM using the conventional procedures of fixation, dehydration, freeze-fracture and critical point drying is likely to reflect significant artifactual alterations. With the proteoglycan complexes collapsed and the entire water content removed, the remaining structures consist largely of a porous matt of collagen fibrils. This structure, following freeze-fracture, is unsupported and therefore susceptible to rearrangement especially in the fracture surfaces themselves. Further, it is not known whether such preparative techniques induce changes in proteoglycan/fibril relationships (Gardner, O'Connor & Oates, 1981) and therefore in the fibril/fibril interaction occurring in the original hydrated

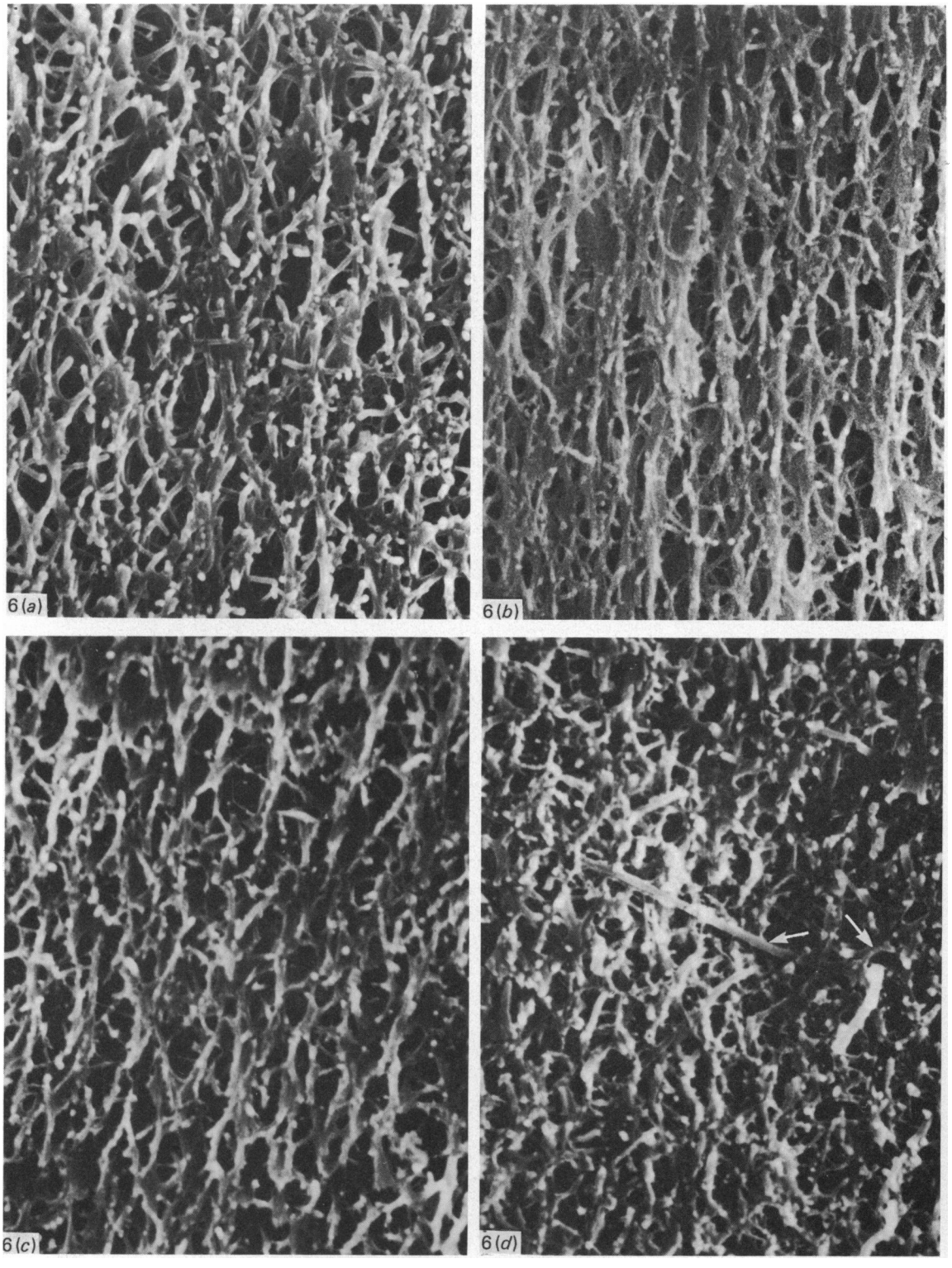
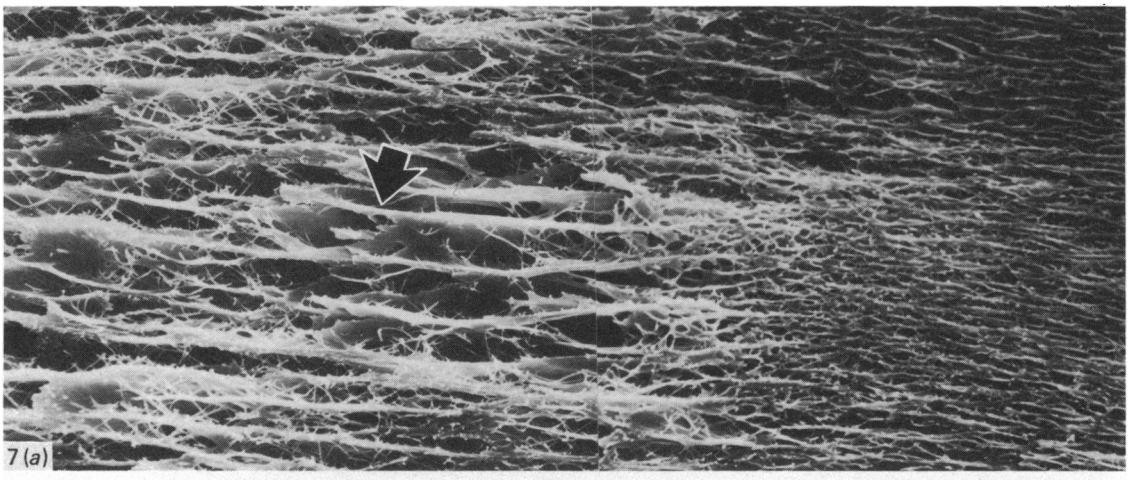


Fig. 6 (*a–d*). SEM (cryopreserved). General matrix structures exposed by fracture and sublimation of frozen tissue. The radial direction is vertical in each micrograph. Note the extended obliquely orientated fibrils (arrows) visible only in (*d*). (*a–b*) Normal bovine patellar groove; (*c*) normal bovine femoral condyle; (*d*) normal human head of femur; male, 59 years. (*a–d*) ×10000.



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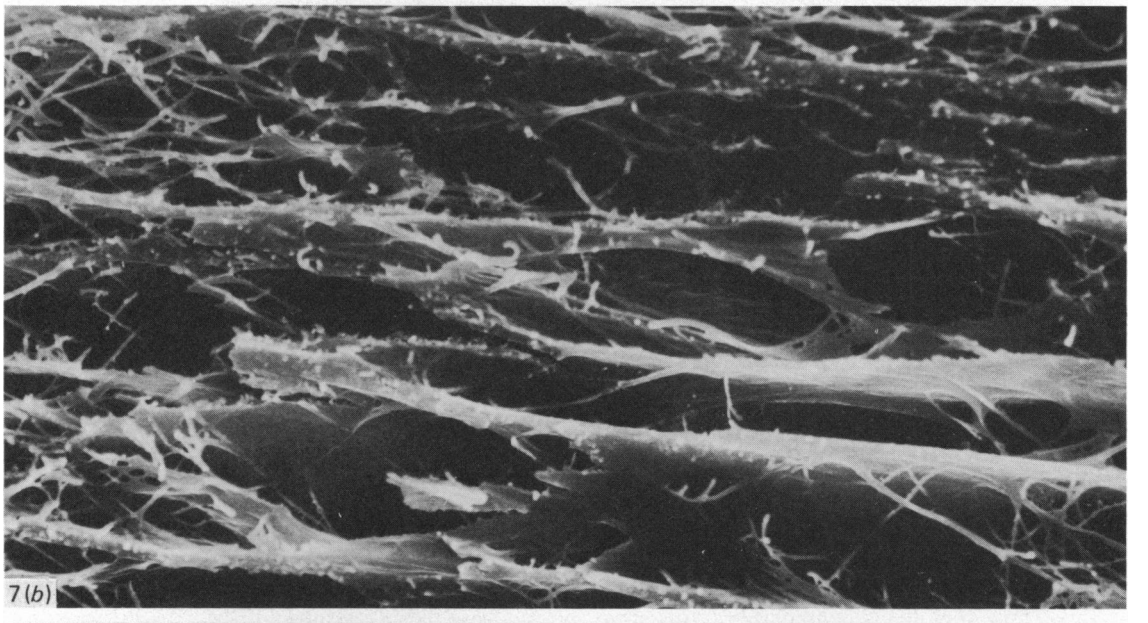

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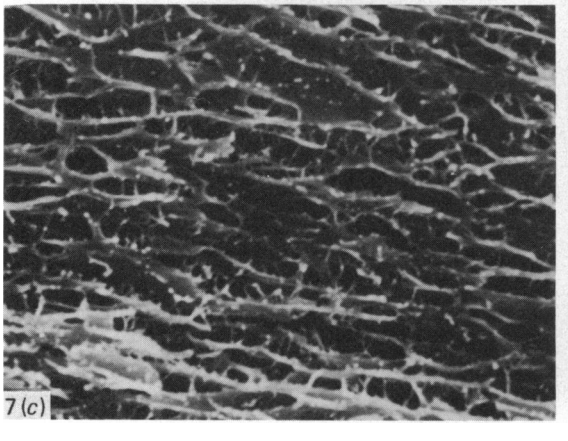
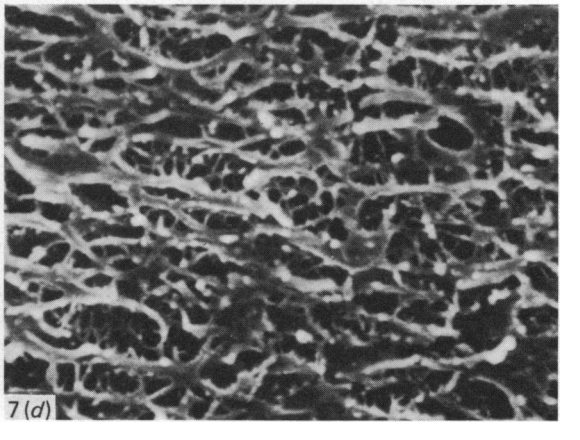


7 (*c*)



7 (*d*)

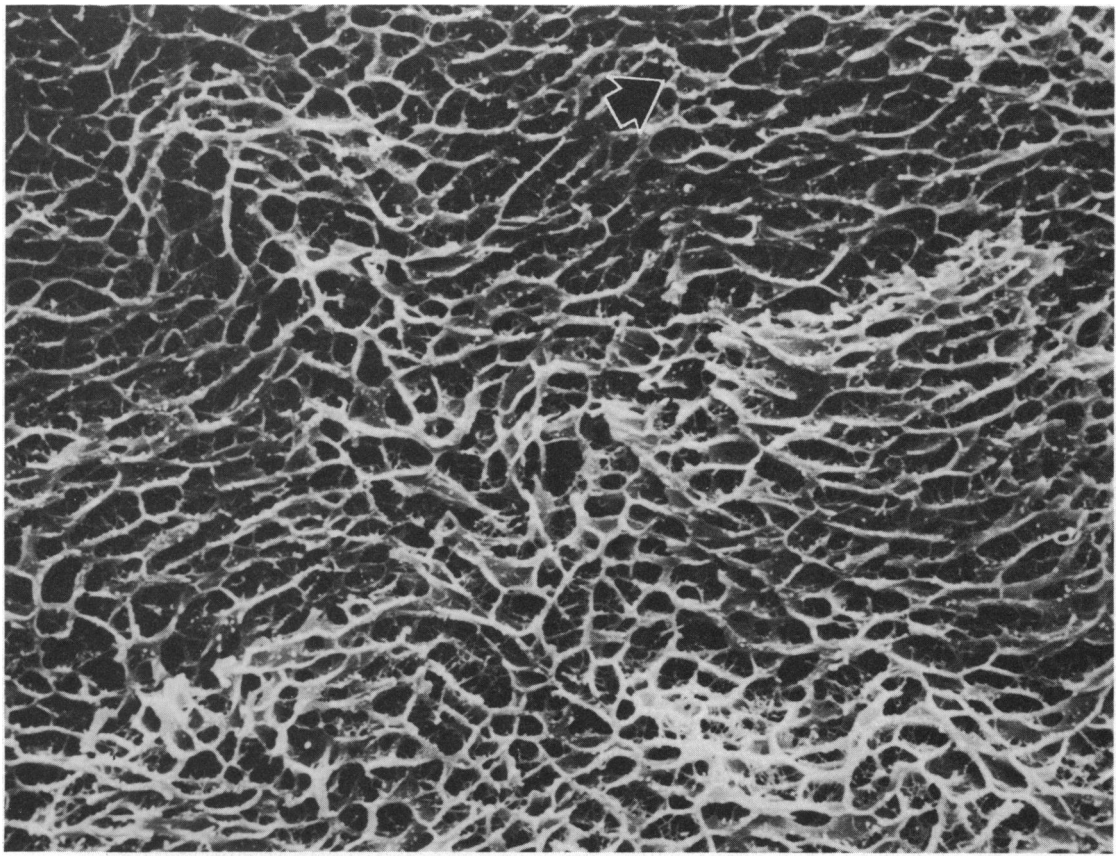


Fig. 8. SEM (cryopreserved). Grossly coarsened fibrillar meshwork in general matrix of softened bovine tibial plateau with superimposed waveform or crimp. Arrow indicates the radial direction. × 5000.

structure. Any morphological conclusions arising from the application of this technique must therefore be viewed with considerable caution.

The conventional SEM studies did in fact reveal a variety of fibril-to-fibril associations that were ostensibly consistent with those observed by TEM. The loose irregular cluster in Figure 9*a* should be compared with those shown in Figure 1*a–d*. In Figure 9*b* and *c* extended aggregates of fibrils of varying diameter and exhibiting different levels of intimacy are clearly visible. These appeared similar to the aligned groupings of fibril seen for example in Figures 2 and 3. The multistranded aggregate of tightly packed, small diameter, parallel fibrils in Figure 9*d* is probably an example of the fine aggregate structure shown in Figure 4*c*.

Fig. 7 (*a–d*). SEM (cryopreserved). (*a*) General matrix of softened bovine tibial plateau articular cartilage exhibiting a pronounced radial structure comprising aggregates of fibrils. (*b*) Enlargement of region near arrow in (*a*). The radial arrays blend into a structure on the right hand side of the Figure which has a finer and more interconnecting texture shown in (*c*). (*d*) matrix still more distant from the prominent arrays than that in (*c*) with a structure more typical of normal matrix. The radial direction is horizontal in each micrograph. (*a*) × 1785; (*b*) × 5400; (*c*) × 5300; (*d*) × 10700.

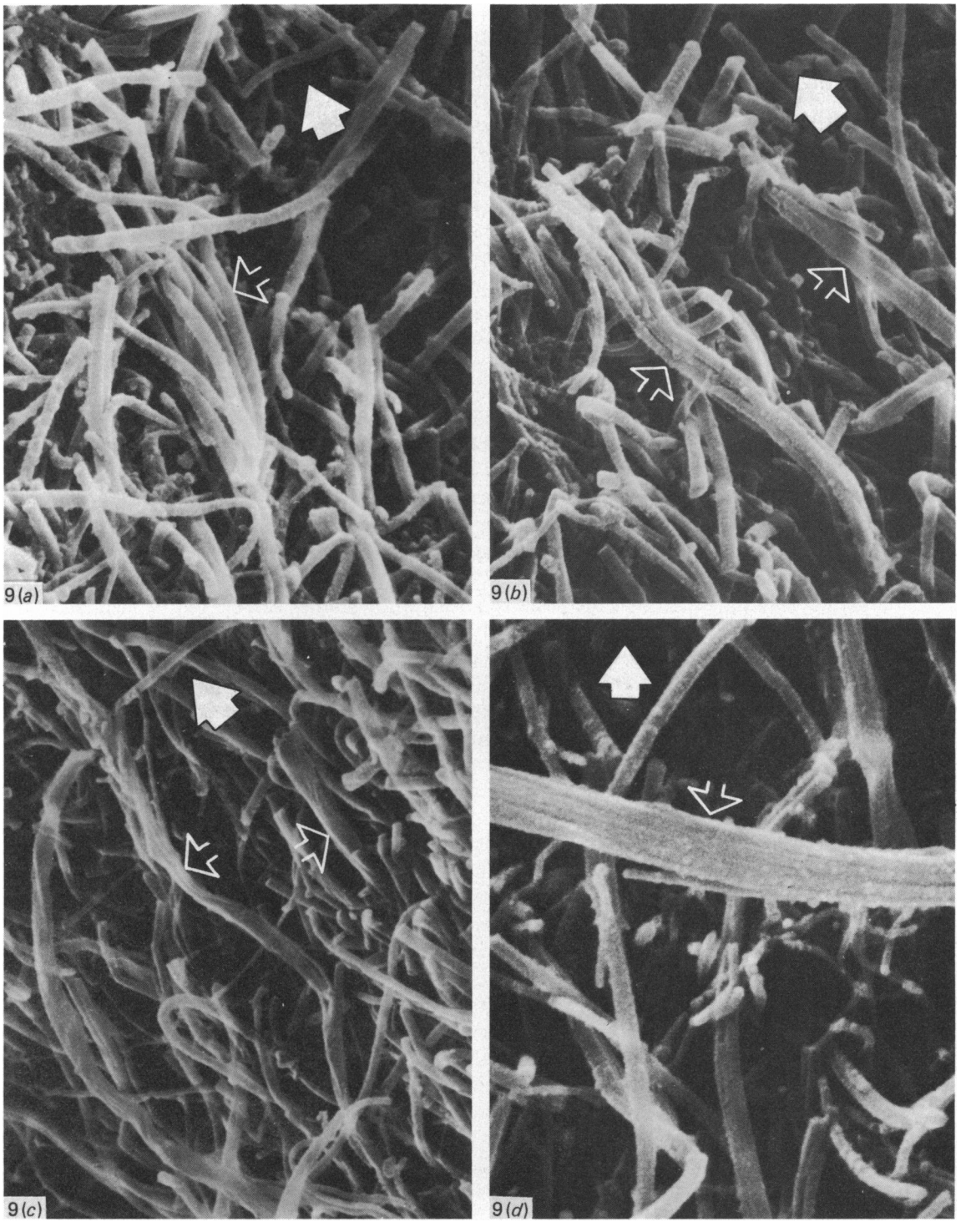


Fig. 9 (*a–d*). SEM (conventional technique). Various fibril associations in the dehydrated general matrix exhibiting characteristics similar to those observed by TEM. Small open arrows indicate fibril groupings in each micrograph. Approximate radial direction is shown by solid arrows. All specimens normal human head of femur. (*a*) Male, 56 years × 28000. (*b*) Male, 59 years × 28000. (*c*) Male, 23 years × 20000. (*d*) Male, 21 years × 18000.

DISCUSSION

The present study provides strong morphological evidence in support of a variety of specific associations between the elements comprising the fibrillar architecture of articular cartilage. These associations, seen most clearly by TEM throughout the range of cartilage types examined, range from a relatively loose, transient clustering of several fibrils through to fibrils so intimately bound as to suggest some sort of highly ordered chemical affinity. Further elucidation of the nature of such interactions lies outside the scope of the present investigation.

Although not immediately apparent from a superficial examination of the TEM thin sections, the low temperature SEM studies, together with subsequent re-examination of the TEM data, reveal that these fibril-to-fibril associations serve a higher structural purpose, i.e. they create from a primary radial arrangement of fibrils in the general matrix of cartilage a three dimensional interconnecting meshwork. It has been argued (Broom, 1982, 1985*a*), that this structural concept not only unifies the wide spectrum of light microscopic and ultrastructural data now available for the fibrillar meshwork of articular cartilage, but also provides an important key to understanding its functional properties.

In all specimens of cryopreserved matrix examined by SEM (i.e. exposed by fracture) there was only scant evidence of any long range oblique fibril orientation (Fig. 6*d*). With a significant depth below the exposed matrix surface revealed by sublimation, any long range fibril obliquity should be readily apparent; this was not the case. It is therefore suggested that the preferred orientation studies carried out by Aspden & Hukins (1981), using angle X-ray diffraction, be interpreted as quantifying the angular spread of the short segments of much longer radial fibrils that have been repeatedly deflected into oblique orientations. Such a system of interconnecting fibrils packed out with highly deformable hydrated proteoglycan molecules forms the braced composite structure as recently described (Broom, 1985*a*; Broom & Marra, 1985).

If 'normal' cartilage is defined structurally as comprising fibril segments that are optimally constrained in a repetitive, obliquely intermeshing arrangement, then any breakdown of the forces or constraints sustaining this high energy configuration will encourage it to revert to a more overtly radial configuration. The authors contend that such a process produced the extensive parallel aggregates of radial fibrils and the depleted intervening spaces seen in Figure 7*b*. Less advanced degrees of interfibrillar constraint breakdown would in turn be represented by both the transition structure depicted in Figure 7*c* and the coarse interconnecting structure in Figure 8.

Studies of the response of fresh hydrated articular cartilage have been made using simultaneous micromechanical compression and differential contrast microscopy. These demonstrate that matrices containing parallel aggregates of radial fibrils deform under extremely low compression stresses via a mechanism involving an in-phase collapse of the fibrillar structure, thus indicating the crucial role of the oblique fibril segments in providing shear bracing in healthy cartilage (Broom, 1982, 1984*b*). It must be emphasised that the fibrillar meshwork can function in this way only in co-operation with the hydrated proteoglycan complexes bound within it. In all probability there is some relationship between the size of the inter-fibril 'mesh' and that of the huge, stiffly extended proteoglycan macromolecules. At some average mesh size there will be optimum containment of the hydrated ground substance and therefore maximum mechanical rigidity of the tissue.

However, increasing the interfibrillar mesh size while maintaining the same level of proteoglycan in the matrix is likely to have functional consequences that reflect more than a simple reduction in the level of structural bracing. It is generally assumed, for example, that some form of electrostatic interaction exists between collagen and the proteoglycans in articular cartilage (Muir, 1979; Lash & Vasan, 1983). Unfortunately, little is known about this relationship in a functional sense. Depending on the magnitude of such interactions, any increase in the interfibrillar mesh size through increased fibril aggregation would tend to reduce the level of collagen/proteoglycan bonding. The proteoglycans, thus less subjected to constraining forces within the enlarged interfibrillar spaces, would in turn contribute less to the overall rigidity of the tissue.

As far as the authors are aware, there are no reports to date of any attempt to correlate variations in the mechanical properties of articular cartilage with the pattern of loading within the joint. That such differences do exist both within a given joint and between joints is well recognised (Kempson, 1979; Broom, 1984*a*). We can therefore envisage a whole range of mechanical properties in articular cartilage suitably matched to the loading requirements of the entire joint surface; this might be achieved by varying the frequency and extent to which the fibrils are interconnected ultrastructurally in this braced configuration.

SUMMARY

This study presents ultrastructural evidence for the presence of a variety of fibril-to-fibril interactions or associations in the architecture of the general matrix of articular cartilage. These interactions are believed to serve a higher purpose of repeatedly constraining an overall radial arrangement of fibrils into an array of oblique interconnecting segments thus creating a three dimensional meshwork within which the hydrated ground substance is constrained.

It is argued that any reduction in these interfibrillar interactions will allow the oblique fibril segments to revert to a low energy radial configuration, thus explaining the presence of such arrays prominent in various degenerate forms of articular cartilage.

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