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The interface between bone and tendon at an insertion site: a study of the quadriceps tendon insertion

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ABSTRACT

Traumatic avulsions of ligament or tendon insertions rarely occur at the actual interface with bone, which suggests that this attachment is strong or otherwise protected from injury by the structure of the insertion complex. In this study we describe the terminal extent of quadriceps tendon fibres where they insert into the patellae of adult rabbits, humans, dogs and sheep. Specimens were examined by scanning electron microscopy (SEM) and light microscopy (LM). To facilitate tracing of tendon fibres the specimens were decalcified for SEM, and polarised light microscopy (PLM) was used in the LM segment of the study. By SEM it was possible to identify mature bone by the presence of osteocytes and a lamellar organisation. PLM and SEM showed that, unlike tendon fibres elsewhere, those in the calcified fibrocartilage were not crimped. No specific cement line was identified by SEM. Tendon fibres interdigitated among separate bone lamellar systems, (osteons or marrow spaces), but did not merge with the collagen systems of individual lamellae. The interdigitation was more extensive and the margin between tendon and bone was less distinct in the anterior third of the insertion. The segment of calcified tendon which interdigitated with bone stained less intensely blue and was less cellular than the more proximal calcified fibrocartilage zone adjacent to the tidemark. Lamellar collagen fibres of the bony trabeculae in the anterior patella were unusually parallel and longitudinal in orientation, making distinction of interposed tendon fibres difficult on LM and PLM sections. LM, SEM and transmission electron microscopy of rabbit patellae at birth revealed that anterior quadriceps tendon fibres extended over the patella in a fibrous cellular layer. By 2 wk of age, this layer had acquired chondroid features (i.e. cell lacunae and metachromasia) and contained vessels extending from patellar marrow. At 6 wk of age, part of this fibrocartilaginous layer was replaced by mature bone and osteoid. In the young adult animal, the quadriceps tension interdigitates extensively with the patellar bone. This segment of the insertion is perhaps the remnant of calcified fibrocartilage which has been remodelled by bone formation.

Key words: Entheses; collagen; fibrocartilage; patella.

INTRODUCTION

Dolgo-Saburoff (1929) published a description of the transformation which occurs in the structure of the patellar ligament where it inserts into bone. In this schema, which has been generalised to tendon attachments into epiphyses/apophyses, the insertion complex includes 4 zones: tendon, fibrocartilage, calcified fibrocartilage and bone itself. These attachments which contain cartilage, are separately classified as 'chondral' insertions. To date, the structure of the actual transition occurring between tendon fibre and lamellar bone in chondral insertions has remained somewhat unclear. By light microscopy (LM) the ligament usually appears to terminate at the bone along a distinct border sometimes called a 'cement line' (Gao & Messner, 1996). In a transmission electron microscopy (TEM) study of nondecalcified specimens, Cooper & Misol (1970) observed that the collagen fibres of ligaments 'blend imperceptibly' with the bone matrix. Others have described the existence of specialised 'chondroid bone' at insertion

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sites, raising the possibility of a transitional tissue within the insertion complex (Haines & Mohuiddin, 1968; Benjamin et al. 1986).

The morphology of the interface between tendons or ligaments and bone is of particular interest to those who study the mechanical properties of insertions and the response of bone to stress. Theoretically, tensile forces borne by a ligament or tendon are transmitted to the bone at the insertion, yet bone is thought to be relatively weak in tension. Traumatic failures of ligaments occur within the bone and within the ligament substance proximal to the insertion, but rarely at the ligament-bone junction itself (Noyes et al. 1974; Gao et al. 1996). The location of the failure is influenced by the age of the subject and the rate and direction of loading (Noyes & Grood, 1976). Cast immobilisation, ageing and exercise are accompanied by remodelling of bone at the insertion of the medial collateral ligament, but it is not clear if or how this process alters the strength of the complex, especially at chondral insertions (Laros et al. 1971). Generally, the insertion complex appears to provide a durable and functional bond throughout life.

Several investigations have provided structural information supporting the concept that insertion sites function to minimise stress concentrations and that variations in morphology reflect differences in load direction or magnitude. Specifically, Benjamin et al. (1986, 1991) have postulated that both the curvature of fibres and the total amount of calcified tissue vary in response to loading conditions (Benjamin et al. 1986; Evans et al. 1991). Gao & Messner (1996) have shown that the width and contour of the calcified zone vary between types of insertions and within individual insertions and suggest that the greater interdigitation of calcified fibrocartilage with bone could increase the strength of the bond.

In this study, we describe the most terminal insertion of quadriceps tendon collagen fibres into patellar bone using scanning electron microscopy (SEM) and polarised light microscopy (PLM) of decalcified specimens in several species. When the calcium salts are removed, collagen fibres in calcified tissues are exposed and can be traced using the unique 3-dimensional capabilities of the SEM (Frasca et al. 1978). PLM is capable of tracing groups of parallel collagen fibres at lower magnifications and provides a way to correlate conventional light histology with SEM (Speer & Dahners, 1979), particularly when used with collagen stains such as alizarin red (Zambrano et al. 1982). Several investigators have shown that the mature form of insertions can be better understood through developmental studies. (Ogden et al. 1977; Matyas et al. 1990; Ralphs et al. 1992; Wei & Messner, 1996). For this reason, we examined the interface in rabbits at different ages.

MATERIALS AND METHODS

New Zealand white rabbits were the primary source of material in this study. The rabbits were newborn or 2, 6 or 52 wk old. Six animals at each age were killed using intravenous barbiturate under conditions approved by our institutional Animal Care Committee. The patella with the tendinous segment of the quadriceps insertion was removed from each knee and prepared for microscopy. The tendon-patella segment was also harvested from 2 adult mongrel dogs and 3 adult female sheep which had been killed as part of an unrelated experiment and from 3 fresh adult male human cadavers aged 73, 74 and 81 y.

Preparation for microscopy

All specimens were fixed at room temperature by immersion in 2% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.3. Immersion time ranged from 2 to 24 h depending on specimen size. Rabbit specimens studied by light microscopy and transmission electron microscopy (TEM) were postfixed in buffered 1% osmium tetraoxide and were not decalcified. Three 2-wk, 3 6-wk, and 6 52-wk-old rabbit patellae were decalcified using EDTA and prepared for SEM. All the human, sheep and dog patellae were decalcified using formic acid. Following fixation and decalcification, each specimen was washed in buffer and dehydrated using graded ethanol solutions.

Light and electron microscopy: nondecalcified rabbit patellae. Six nondecalcified patellae from rabbits of each age were embedded in epoxy resin (Eponate 12, Pella, Redding CA). The patellae of the 6-wk and 52 wk-old rabbits were first divided into quarters by cutting them in their transverse and sagittal planes. The embedded patellae were then sectioned along the sagittal plane using an ultramicrotome (Reichert) with glass or diamond knives. For light microscopy, sections were cut approximately 4 μm thick and stained with toluidine blue. Thin sections for TEM were cut from selected areas of the same blocks and stained with lead citrate and uranyl acetate. The LM sections were photographed using an Olympus BHT photomicroscope (Olympus, Japan) equipped with polarising filters and a quarter lambda filter. The thin sections were viewed in a JEOL JEM 1200EXII TEM.

Polarised light microscopy-decalcified rabbit, dog, sheep and human patellae. Following decalcification,

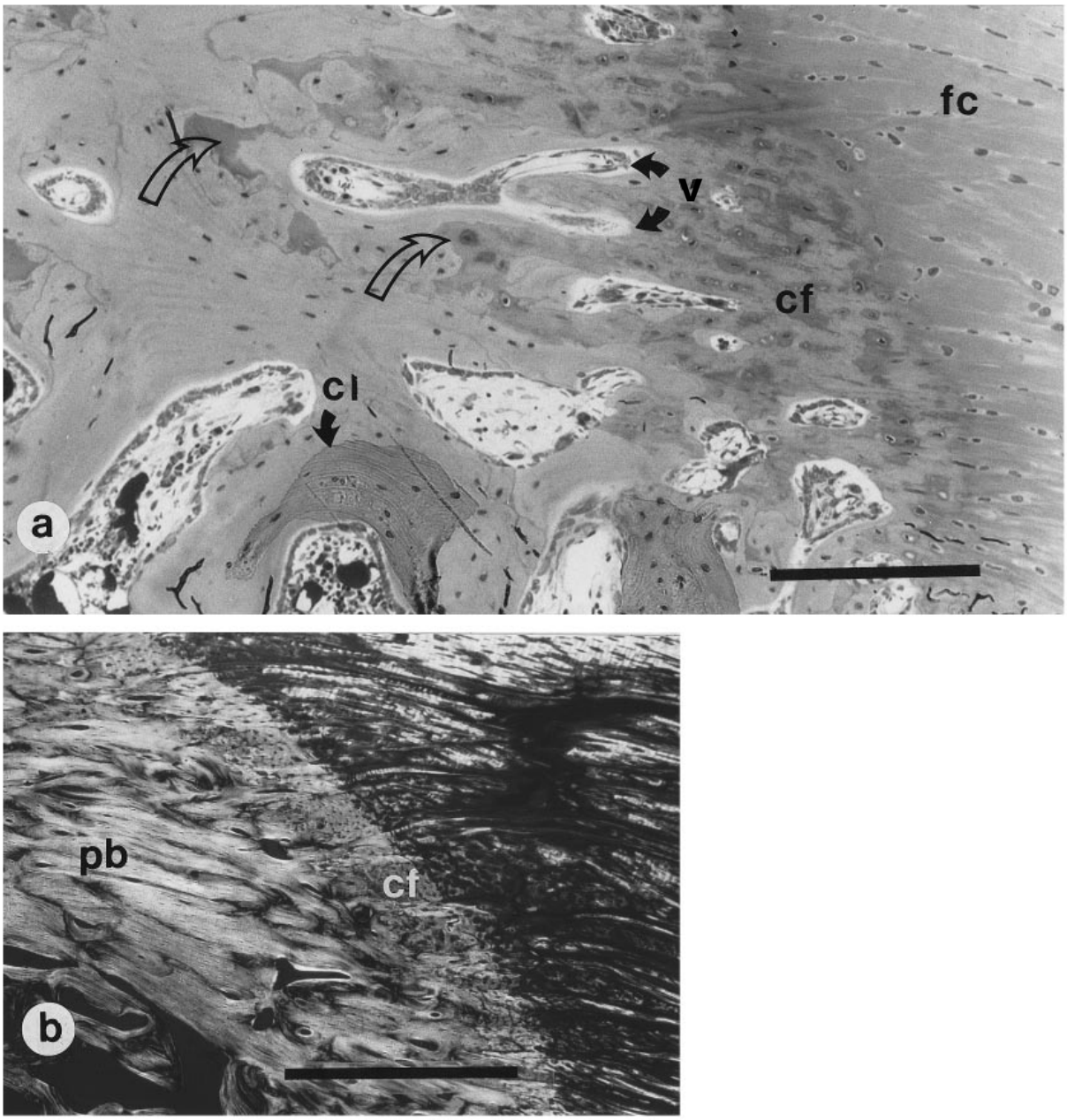

Fig. 1. Light micrographs of quadriceps tendon insertion into the patella. (*a*) Nondecalcified rabbit specimen in epoxy resin stained with toluidine blue. The fibrocartilaginous zone (fc) contains strings of cuboidal cells. The calcified fibrocartilage (cf) around the chondrocytes stains darker blue than other areas in these preparations. Patellar bone stains lightly and contains almond-shaped osteocytes. Within this bone, islands of metachromatic (pink-blue) tissue containing few cells are visible (open arrows). Marrow spaces containing small blood vessels (v) approach the calcified zone of the insertion and flank the hypocellular metachromatic formations. With this stain, cement lines (cl) delineating areas of new bone formation can be seen. Bar, 200 μm. (*b*) Canine specimen stained with haematoxylin and eosin and viewed with polarised light. The region of calcified fibrocartilage (cf) can be identified using polarised light because of its uniform birefringence pattern, but the terminal extent of the tendon cannot be differentiated from bone. Bone (pb) in the anterior patella displays a uniform pattern of birefringence which is similar to that of the calcified tendon. Bar, 100 μm.

a 5 mm thick, sagittally-oriented slab including the tendon insertion was removed from the centre of each patella. The slabs were embedded in paraffin, and sagittal sections were cut and mounted on glass slides. The sections were stained with haematoxylin and eosin or Masson's trichrome and viewed/photographed with the light microscope.

Scanning electron microscopy. For ages 2, 6 and 52 wk, 3 nondecalcified and the EDTA-decalcified rabbit patellae were prepared for SEM by cryofracture (Humphreys et al. 1974). Following dehydration into alcohol the specimen was flash-frozen by immersion in liquid nitrogen and then broken into halves, either transversely or longitudinally in the sagittal plane.

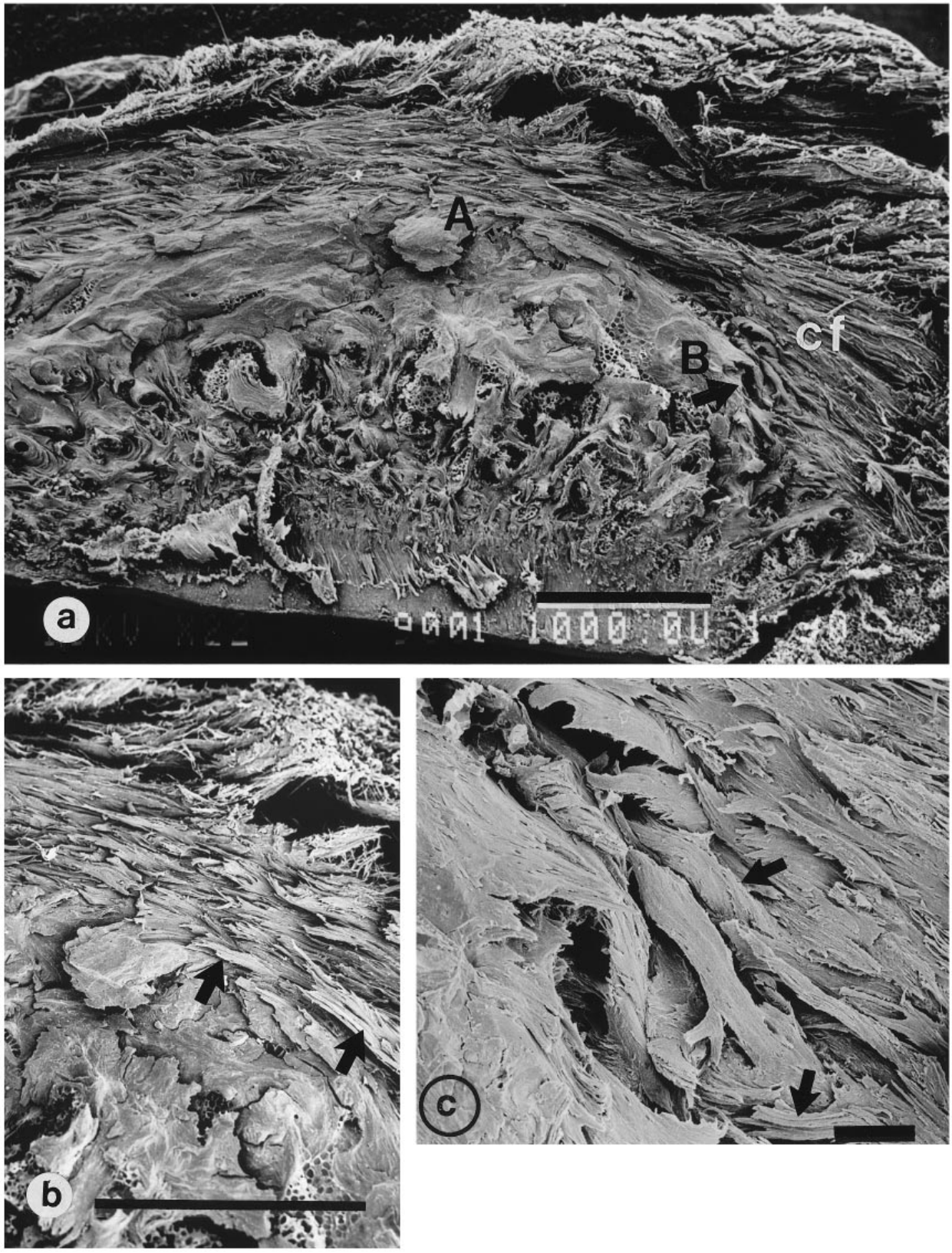

Fig. 2. Scanning electron microscope image of sagittal cryofracture through patella of a 52-wk-old rabbit. (*a*) Low magnification overview of decalcified patella body is equivalent to conventional images, showing the anterior cortex covered by a fibrous layer, (top), trabecular bone in the body and an articular surface (bottom). The quadriceps tendon insertion (right) is apparent, and the region of calcified fibrocartilage (cf) is visible because the fibres are straight and parallel in that region. In these SEM preparations, 2 insertion morphologies are visible, one anteriorly where the fibres remained parallel (A) and another where the fibres appear to deform where they insert into the body (B). Bar, 1 mm. (*b*) Detail of region A. The tendon fibres (arrows) remain parallel and terminate along an irregular interface with bone. The collagen

The patellae from newborn animals were too small to fracture.

Dog, sheep and human patellae (2 each) were prepared in a similar fashion. Decalcified segments adjacent to the pieces used for light microscopy were dehydrated, frozen and fractured in the sagittal plane. The large human patellar fragments were trimmed to 1 cm cubes before fracture. All other specimens were fractured whole.

The fractured specimens were dried by the critical-point method and coated with gold-palladium in a sputter coater. Each was mounted fractured surface up, and viewed in a JEOL JSM35C SEM.

RESULTS

Mature animals

Light microscopy: general. By light microscopy, the structural features of the human, ovine, canine and 52-wk-old rabbit patellae were similar. The body of the patella was composed of lamellar bone, with discrete trabeculae and marrow spaces. Bone in the anterior part of the body—sometimes called the cortex—contained less marrow space than that closer to the articular surface. The entire quadriceps tendon inserted into patellar bone; tendon fibres continued along the entire dorsal surface, but eventually curved in a ventral direction and terminated at a bone interface.

Light microscopy: nondecalcified rabbit patellae. In light microscope preparations of the nondecalcified rabbit patellae stained with toluidine blue, the calcified fibrocartilaginous region of the tendon insertion could be identified, primarily because the calcified tissue surrounding the cell groups stained a dark blue (Fig. 1). A distinct 'cement line' between this part of the calcified tendon and bone was visible but not continuous. The patellar bone immediately adjacent to the tendon insertion was composed of lamellar bone around marrow spaces, but hypocellular regions of homogeneous purple tissue were visible within the bone and between the bone and the more heavily calcified zone of the tendon insertion. Cement lines were clearly visible between some areas of the lamellar bone.

Capillaries were frequently observed at the interface between the tendon and bone (Fig. 1*a*). The vessels extended among the more lightly stained metachromatic areas adjacent to the tendon insertion. Vessels did not penetrate into the more darkly blue-stained region of the tendon fibrocartilage.

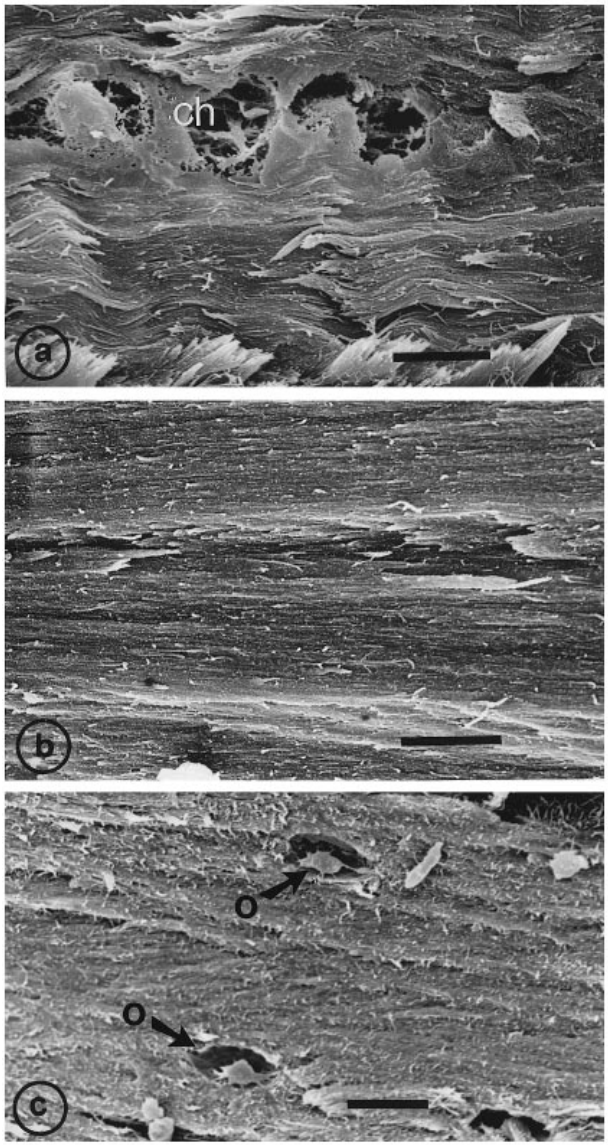

Fig. 3. SEM appearance of regions of the rabbit quadriceps tendon insertion in decalcified specimens. (*a*) Fibrocartilage. This region could be identified by the presence of chondrocytes (ch) in longitudinal clusters surrounded by a fibrous capsule. The tendon collagen fibres are parallel and display a uniform crimp. Bar, 100 μm. (*b*) Calcified fibrocartilage, decalcified specimen. With the calcium salts removed, the collagen fibrils are easily seen and traced. In this zone they are closely packed and display no crimp. Bar, 100 μm. (*c*) Lamellar bone. Mature bone was identified by the presence of lamellae and interposed osteocytes (o). Bar, 50 μm.

Light microscopy: decalcified patellae. In decalcified specimens of rabbit, dog and human patellae stained with Masson's trichrome, the zone of calcified fibrocartilage was difficult to identify unless polarised light was used (Fig. 1*b*). With PLM, the entire

fibres of the adjacent cortical bone also are parallel and run in a longitudinal direction. Bar, 1 μm. (*c*) Detail of region B. Fingers of calcified fibrocartilaginous tendon (arrows) splay as they enter the bone. The bone at this site is lamellar trabecular bone with rounded marrow spaces. Bar, 100 μm.

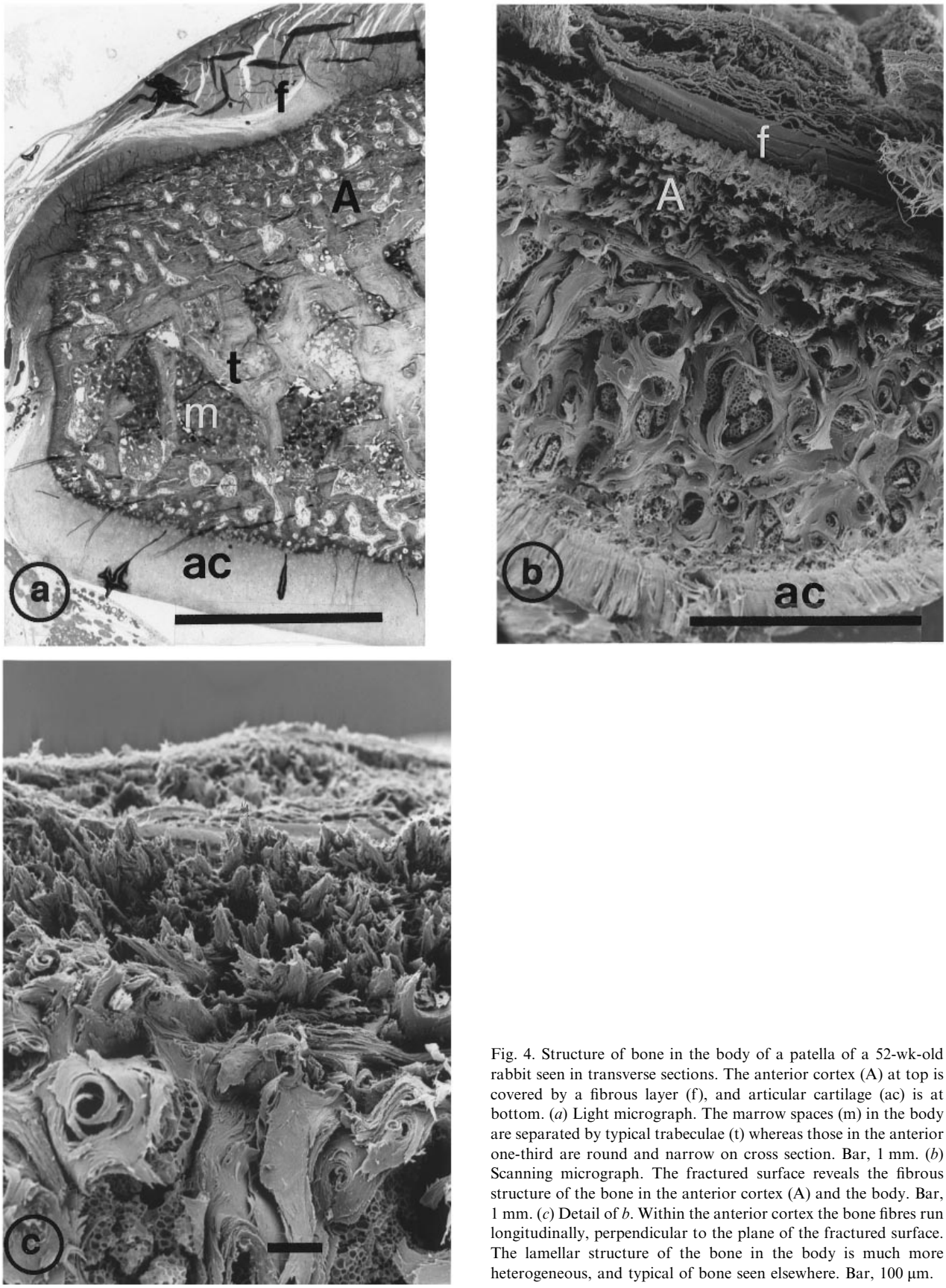

Fig. 4. Structure of bone in the body of a patella of a 52-wk-old rabbit seen in transverse sections. The anterior cortex (A) at top is covered by a fibrous layer (f), and articular cartilage (ac) is at bottom. (*a*) Light micrograph. The marrow spaces (m) in the body are separated by typical trabeculae (t) whereas those in the anterior one-third are round and narrow on cross section. Bar, 1 mm. (*b*) Scanning micrograph. The fractured surface reveals the fibrous structure of the bone in the anterior cortex (A) and the body. Bar, 1 mm. (*c*) Detail of *b*. Within the anterior cortex the bone fibres run longitudinally, perpendicular to the plane of the fractured surface. The lamellar structure of the bone in the body is much more heterogeneous, and typical of bone seen elsewhere. Bar, 100 μm.

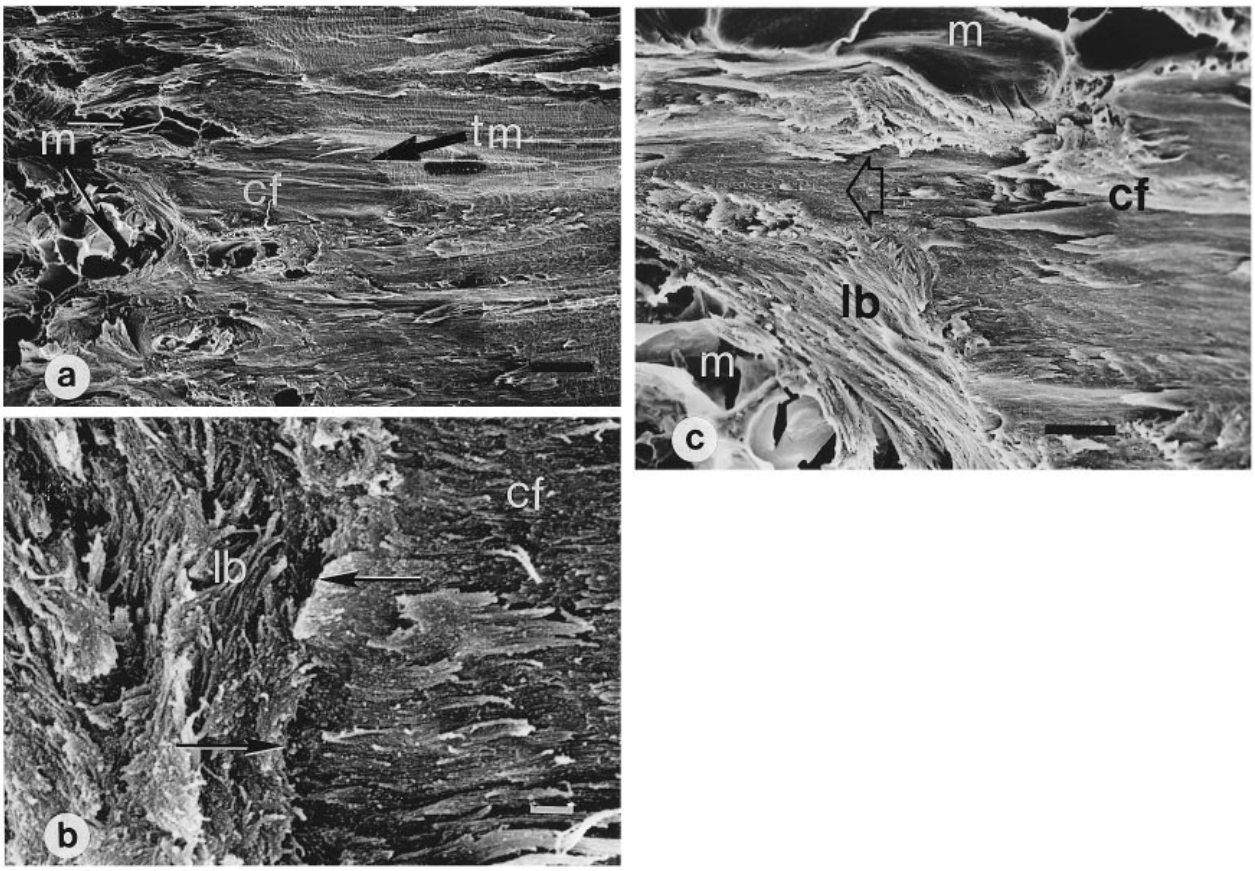

Fig. 5. Morphology of the interface between quadriceps tendon and lamellar bone in decalcified human patella. (*a*) Tendon fibres in the fibrocartilage (right) can be traced across the tidemark (tm). The calcified fibrocartilage (cf) ends along an interface with lamellar bone containing marrow spaces (m), filled with hollow fat cells. Bar, 100 μm. (*b*) Here, the calcified fibres (cf) end abruptly at a distinct interface (arrows) with lamellar bone (lb). Bar, 50 μm. (*c*) At this site, the fibres of the calcified fibrocartilage (cf) form a narrow slip (open arrow) which extends between 2 marrow spaces (m) which are surrounded by lamellar bone (lb). Bar, 1 μm.

insertion complex was uniformly birefringent and the calcified tendon could be illuminated separately with a few degrees of stage rotation. Even in this circumstance the border between the bone and tendon often was indistinct in rabbits.

The calcified fibrocartilage stained more uniformly in the decalcified specimens than in nondecalcified ones. A 'cement line' between bone and tendon insertion was visible as a light blue line in the decalcified patellae stained with haematoxylin and eosin. The cement line separating tendon was most distinct in the human patellae.

The adult lamellar bone was birefringent by PLM in decalcified material. In the anterior aspect of the patella, all the bone transmitted light at approximately the same angle (Fig. 1*b*). At the insertion, bone and calcified tendon transmitted light at almost the same angle, rendering the interface there indistinct by PLM. Trabeculae in the ventral body of the patella displayed a heterogeneous lamellar pattern.

In general, the apparent width of the calcified zone as seen under PLM was uniform over the entire insertion. The total amount of bone adjacent to the insertion was greater in the anterior half, because larger marrow spaces were present in the body of the patellae while those in the anterior bone were small, narrow and longitudinal in orientation. This difference was most prominent in the sheep and dog, which had relatively smaller marrow spaces in the anterior cortex than did the human and rabbit. The apparent border between bone and calcified tendon was more irregular in the rabbit than in the other species.

Scanning electron microscopy. Both bone and tendon collagen fibres were visible by SEM in the cryofractured, decalcified specimens from all species studied (Figs 2–6). In these specimens, the tendon collagen fibres could be traced to their termination within the bone.

On scanning micrographs, the zones of fibrocartilaginous tendon could be identified and differentiated from lamellar bone by the presence of typical chondrocyte lacunae in rows (Fig. 3*a*). Initially, the zone of calcified fibrocartilage was located by com-

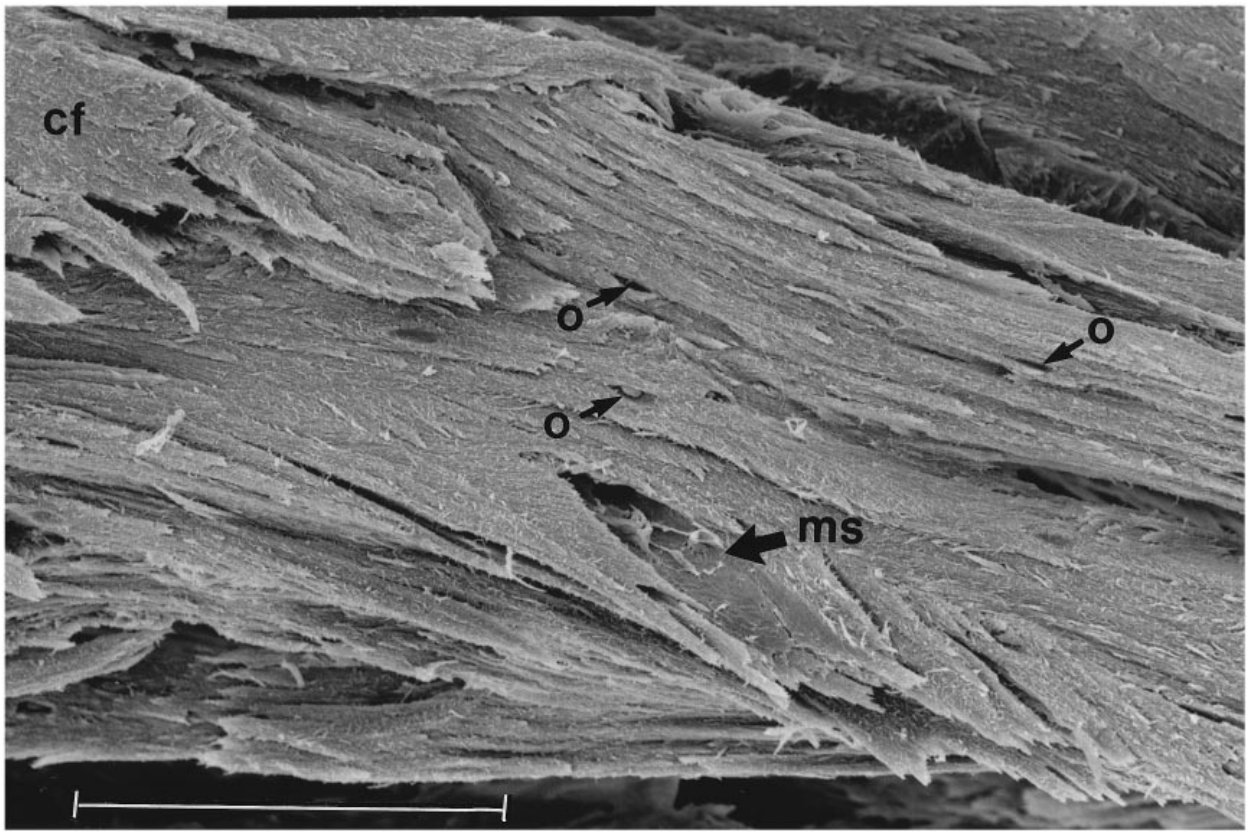

Fig. 6. Structure of bone in the anterior aspect of the patella. Thin lamellae separated by osteocytes (o) surround a cylindrical marrow space (ms). Because of their similar fibre orientation, slips of tendon fibrocartilage (cf) are difficult to trace in this region. Bar, 100 μm.

parison with the LM sections. At all locations and in all ages, the collagen in the calcified zone did not display a crimp pattern (Fig. 3*b*). Thus the calcified zone could reliably be distinguished from the rest of the tendon, which was crimped. Collagen fibres of the tendon could be traced through all zones and were found to be continuous up to the point where they terminated in the patella.

At insertion, the tendon fibres either ended blindly against formations of lamellar bone or interdigitated between lamellar systems (Figs 2, 5). Tendon fibres were never observed merging with those of an individual bony lamella. Where tendon fibres penetrated among lamellar bone formations, usually they were not accompanied by chondrocytes and, in that sense, were not typical of fibrocartilage. In the anterior or dorsal aspect of the patellar insertion, the tendon fibres did not splay and appeared to penetrate more deeply into bone (Fig. 2*a*, *b*). There they were closely interwoven with fingers of trabecular bone which surrounded thin tubular marrow spaces (Fig. 6). In the more ventral aspect of the insertion, the tendon fibres often curved and flattened where they conformed to spherical marrow spaces (Fig. 2*c*).

By SEM the bone in the anterior aspect of the patella was structurally different from that in the body. In all species, the anterior bone was lamellar and contained osteocytes in lacunae, but the collagen fibres within the lamellae were uniformly oriented and parallel to the long axis of the patella (Fig. 6). The longitudinal bias of the anterior bone collagen was confirmed by the transverse sections and fractures, which showed that the collagen fibres ran perpendicular to the plane of section throughout the anterior aspect of the patella (Fig. 4). By contrast, the orientation of fibres in lamellae within trabeculae deeper in the patellar body was heterogeneous (Fig. 4 *c*).

Development

From birth to adulthood the length of the rabbit patella increased form 2.5 mm to 9 mm. During growth, the appearance of the patella, viewed grossly or in midsagittal sections, changed from a rounded, oval shape to one which was more square, but the proportions of each surface occupied by articular cartilage, tendon insertion and ligament origin remained roughly constant.

At birth, the quadriceps tendon, including the insertion, was highly cellular, and the cells were stellate in shape. With age, the collagen component

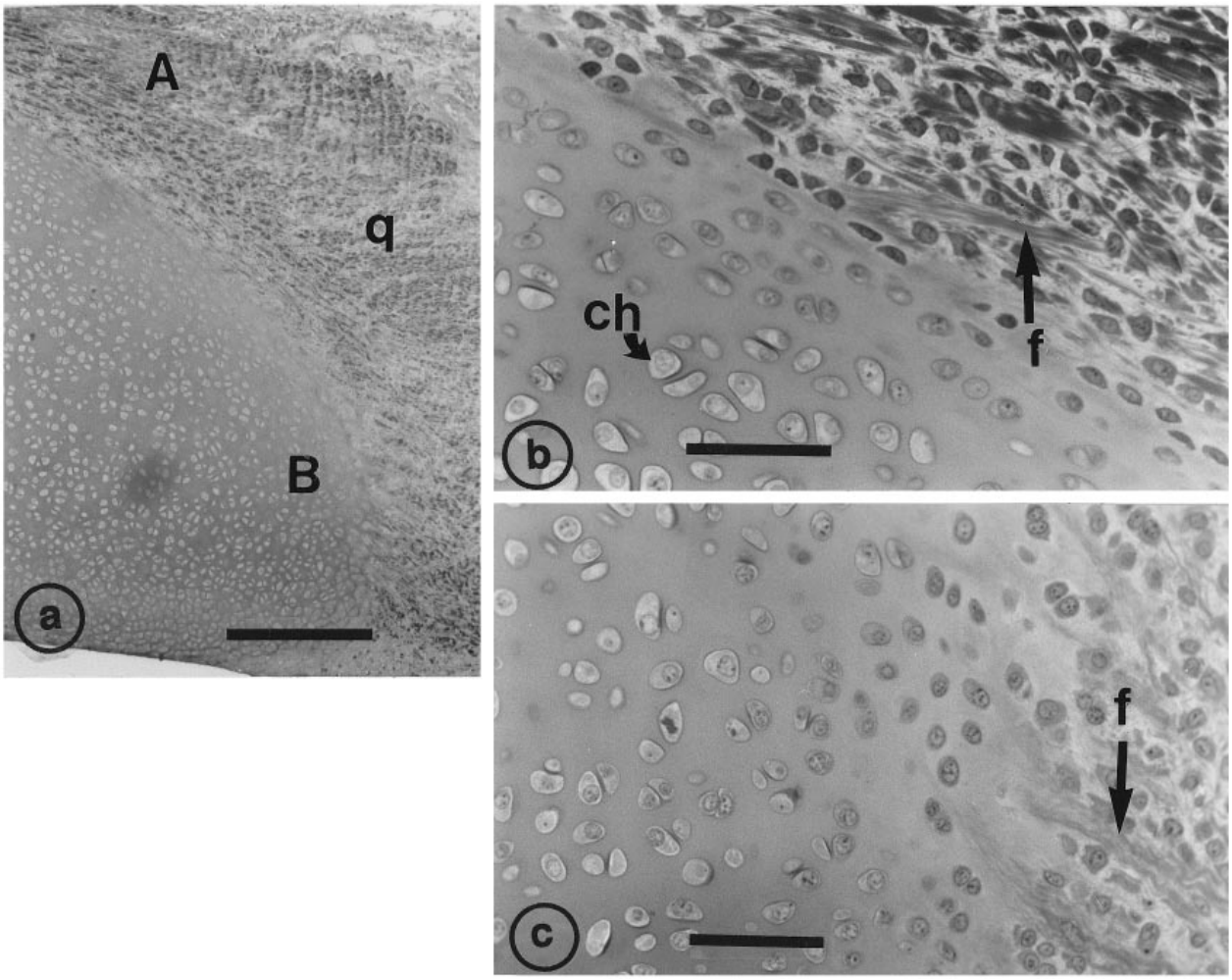

Fig. 7. Newborn rabbit patella, embedded in epoxy resin. (*a*) The quadriceps muscle (q) tendon inserts directly on the body of the patella (B) and also passes over the anterior aspect (A) of the hyaline cartilage anlage. Bar, 0.5 μm. (*b*) The interface between the anterior tendinous region and the patella. Collagen fibres (f) of the tendon approach, but never penetrate hyaline cartilage of the body, which is identified by the presence of chondrocytes (ch) in round lacunae with distinct borders. Bar, 100 μm. (*c*) At the proximal pole of the patellar cartilage, the fibres (f) end blindly without penetrating into the cartilage. Bar, 100 μm.

became predominant and the cell silhouettes changed to fusiform in the tendon proper and cuboidal in the insertion complex.

Newborn. The centre of the patella in newborns was composed of typical hyaline cartilage with clusters of round cell lacunae in a random fibrous matrix. The anterior or dorsal 20% of the patella was more cellular and fibrous than the body (Fig. 7). By LM and TEM, this segment was continuous with, and structurally identical to the rest of the tendon. The tendon fibres appeared to end abruptly at an interface with the hyaline cartilage of the body of the patella. At the interface, the tendon collagen fibre bundles splayed and interdigitated minimally with the random fibrillar network of the hyaline cartilage. The stellate tendon cells were distinct from the more spherical chondrocytes of the adjacent hyaline cartilage. The insertion extended continuously from the proximal pole of the patella to cover the entire anterior surface, with the only structural variation being the angle at which tendon fibres intersected the body (Fig. 7*b*, *c*).

Two weeks. At 2 wk of age, an anterior segment of the quadriceps tendon still appeared to pass over the patellar body. The tendon had changed in several ways. The area occupied by collagen fibres increased and, proximal to the insertion site, the tenocytes were spindle-shaped. The tendon cells in proximity to the patella were cuboidal and surrounded by capsules consisting of small unbanded fibrils, typical of chondrocyte lacunae. These 'fibrocartilaginous' characteristics were apparent throughout the portion of the tendon that passed over the dorsal aspect of the patella and also extended 1 mm into the volar segment inserting directly into the proximal end of the patella. At the interface with the body of the patella, the tendon matrix, including the fibre bundles and the chondrocyte lacunae, was calcified and stained a dark blue with toluidine blue. The chondrocyte lacunae at

this interface were larger and more round than those more proximal in the tendon.

The body of the patella, which at birth was composed of hyaline cartilage, was largely ossified and composed of marrow elements, capillaries and immature bone. Small regions of hyaline cartilage remained in those areas of the proximal and distal poles of the patella where tendon and ligament did not attach. Capillaries budding from the ossific nucleus penetrated into the fibrocartilaginous region on the anterior and proximal aspects of the patella. Usually, these capillaries ran parallel to the tendon fibres.

Six weeks. At 6 wk of age the patella had grown to approximately 80 % of the adult length. The anterior aspect of the patella was no longer fibrocartilage. This region had been replaced by a combination of metachromatic calcified fibrocartilage, lamellar bone and longitudinally-oriented narrow marrow spaces. The fibrocartilage in this region was hypocellular and, in these nondecalcified specimens, was stained pink or purple by toluidine blue. SEM and TEM of this tissue showed that the fibres within this metachromatic tissue were collagen fibres continuous with those of the calcified tendon inserting along the proximal pole. Lamellar bone lined the marrow spaces. This bone was easily distinguished from calcified tendon and osteoid by the presence of osteocytes, a lamellar structure and, by TEM, canaliculi.

Where the quadriceps tendon inserted, the zones of tendon fibrocartilage and calcified fibrocartilage were more distinct than at 2 wk of age. At the insertion, the chondrocyte lacunae were relatively large and round in comparison with other tendon cells and the matrix about them stained more darkly blue. Narrow (50 μm) extensions of the bone marrow space, containing thin walled vessels, frequently contacted the calcified tendon. These fingers of marrow space usually ran between the hypocellular regions of calcified tendon, and appeared to follow and replace the chondrocyte-laden planes. The hypocellular regions of calcified tendon on either side of these spaces did not stain as darkly as did the fibrocartilage adjacent to the chondrocyte rows in the insertion. Yet, by EM, the collagen fibres of the 2 regions were continuous.

DISCUSSION

This study confirms that adult quadriceps tendon collagen fibres are continuous through the fibrocartilaginous zones and penetrate to some degree into the bone of the patella. These fibres interdigitate between individual lamellar systems but are not integrated into the lamellar structure of the bone. The terminal end of the tendon attachment is an extension of the zone of calcified fibrocartilage. The fibres there are calcified and continuous with the tendon, but the tissue has few or no chondrocytes and the fibres sometimes turn and splay as they interdigitate with bone. On non-decalcified LM sections, the terminal end of the tendon does not stain as darkly blue with toluidine blue as does the intact calcified fibrocartilage. For these reasons, the termination of the tendon is not readily apparent by standard light microscopy.

These observations may explain the reports of metachromatic 'chondroid' or 'metaplastic' tissue at other insertion sites (Haines & Mohuiddin, 1968). In our adult specimens, what appeared to be islands of immature bone or osteoid due to their pink/purple staining character on LM sections were found by SEM to be the acellular or hypocellular terminal ends of inserting tendon fibres. Therefore, in the rabbit, dog, sheep and human quadriceps tendon insertion, no separate tissue layer is interposed between tendon and bone.

This study does not explain why tendon avulsions tend not to occur at the bone-tendon interference. In some areas, slips of tendon fibre penetrated deeply into the patellar bone but, because the fibres do not blend into the bone collagen matrix, the mechanism of adhesion between bone and tendon is not apparent. In a study of insertion failures Gao et al. (1996) described avulsions which follow the bone-tendon interface. Their illustration of such a tear at the rabbit medial collateral insertion seems to show that the deeply-penetrating slips of tendon are pulled free from bone, indicating that the bond is not unbreakable.

Ralphs et al. (1992) observed by light microscopy that the quadriceps tendon fibres appear to penetrate deeply into the patellar anlage in rats up to 4 wk of age. Bland & Ashurst (1997) and Metsaranta et al. (1996), however, showed that the hyaline cartilage forming the patellar body in 28 d fetal rabbits does not contain type I collagen. As in our newborn rabbits, the tendinous structure, containing type I collagen, was restricted to the anterior aspect of the patella. Thus it appears that the ventral body of the rabbit patella, like other bones, develops from a hyaline cartilaginous anlage, and in that sense is a sesamoid bone, not an ossified tendon. However, this is not necessarily true of the dorsal, anterior aspect.

At birth the tendon adjacent to, and passing anteriorly over the patellar anlage had no chondroid structural features and was easily distinguished from the hyaline cartilage of the patella. By 2 wk of age, the same regions of the tendon were 'fibrocartilaginous' in that the cells had acquired structural characteristics

of chondrocytes. This fibrocartilage had been remodelled into a combination of osteoid, bone and marrow spaces by 6 wk of age. If this sequence is correct, the bone adjacent to the quadriceps tendon insertion—including all the anterior part—has replaced tendon fibrocartilage and not patellar hyaline cartilage. Thus the patellar bone may derive from 2 distinct tissues.

The bone in the dorsal aspect of the adult patella is unusual in that the collagen fibre systems within lamellae do not spiral obliquely, but generally are longitudinal and parallel (Figs 4, 6). This uniformity may reflect the origin of the bone, if it has formed along the longitudinal fibres of calcified tendon which covered the anterior aspect of the patella during development. In that case, the bone would be analogous to the primary spongiosa adjacent to a physis. As described by Ogden et al. (1977), the tibial tubercle at the insertion of the patellar ligament, develops from fibrocartilage.

The orientation of fibres in secondary osteons usually varies and reflects the local mechanical properties of the bone (Ascenzi & Bonucci, 1968; Frasca et al. 1977; Pidaparti & Burr, 1992). The fibre orientation is most oblique in bone which has the greatest compressive modulus and more longitudinal in bone with higher tensile strength. Using finite element analysis, Hayes et al. (1977) have modelled the stress patterns in the patella. The bone collagen alignment observed here was principally longitudinal in the areas which they predicted would be subject to tensile stress. Therefore the collagen orientation within the appositional bone surrounding the narrow marrow spaces in the anterior patellar cortex is probably adapted to tensile loads. Although the concept of 'tensile trabeculae' is old, we are aware of no previous description of a unique collagen orientation within bone bearing tensile loads.

The absence of crimp in the calcified zone of the insertion has not been reported before. Crimping in the calcified tendon insertion was not observed at any age studied, but the noncalcified segment of the tendon was clearly crimped in all specimens at all ages. Crimping is thought to be a consistent feature of all tendons and ligaments (Gathercole & Keller, 1991), contributing to the viscoelasticity of the tissue (Stouffer et al. 1985). The calcium salts probably stiffen the calcified tendon and, in that case, crimping would not change the elasticity in that segment.

In bone histology, a cement line marks the point where bone resorption ceased and new bone formation began during remodelling. These lines have unique staining qualities and composition, giving rise to speculation that they affect bone strength (Bain et al. 1990; Burr et al. 1988). Use of the term 'cement line' to designate the terminal extent of a tendon or ligament insertion is not as precisely defined, nor has a mechanical role been elucidated. In our nondecalcified rabbit specimens, such a line was not always visible. This was probably due to the small thickness of our epoxy-embedded sections. Greater knowledge of the composition and structure of the material at this interface is needed.

Microscopy of insertion sites is difficult due to the presence of the calcium salt, which complicates sectioning for transmission microscopy and obscures fibre anatomy in SEM. Decalcification was helpful, particularly with SEM, where it enabled us to trace fibres which otherwise were hidden. Decalcification has undesirable effects, and may denature proteins or remove proteoglycan (Kiviranta et al., 1984). The nondecalcified LM sections cut from epoxy-embedded tissue were particularly interesting because the calcified tissues displayed an unexpected range of staining intensity, especially when compared with the decalcified paraffin sections. While one conclusion of this study is that tendon fibres insert directly into bone, the epoxy sections indicate that the biochemical composition of the most terminal extent of the tendon insertion differs from the adjacent calcified fibrocartilage.

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