J. Anat. (1991), **179**, pp. 127–136
With 4 figures
Printed in Great Britain

Age-related changes in tendon fibrocartilage

M. BENJAMIN*, R. N. S. TYERS AND J. R. RALPHS

Department of Anatomy, University of Wales College of Cardiff, PO Box 900, *Cardiff CF*1 3*YF, UK*

(*Accepted* 5 *July* 1991)

INTRODUCTION

Fibrocartilage is found in tendons that wrap around bony pulleys (compressive fibrocartilage) and at the attachments of epiphyseal tendons and ligaments to bones (attachment-zone fibrocartilage) (Woo *et al.* 1988; Benjamin & Evans, 1990). Attachment-zone fibrocartilage helps to dissipate stresses by providing a gradual transition in mechanical properties between the tendon or ligament and bone (Benjamin, Evans & Copp, 1986; Woo *et al.* 1988). Compressive fibrocartilage resists the pressure of the tendon against the bone (Merrilees & Flint, 1980) and provides articulating surfaces. We have recently studied compressive fibrocartilage in the quadriceps tendon of 3-month-old rats (Ralphs, Benjamin & Thornett, 1991). The fibrocartilage forms the suprapatella, which is visible as a white plaque on the deep surface of the tendon, immediately above the patella proper. The suprapatella articulates with the femur between the condyles when the knee is flexed. At 3 months its cells are large, pale-staining and packed with intermediate filaments. They are embedded in extracellular matrix that contains chondroitin sulphate but lacks keratan sulphate and type II collagen. Studies by other workers on compressive fibrocartilage in tendons have been restricted to the tendon of flexor digitorum profundus (FDP) (Gillard, Reilly, Bell-Booth & Flint, 1979; Merrilees & Flint, 1980; Okuda *et al.* 1987*a*, *b*; Vogel & Koob, 1989). This fibrocartilage contains a greater variety of matrix components than the suprapatella, including keratan sulphate and type II collagen.

The fibrocartilaginous regions of wrap-around tendons are subject to tendonitis and many attachment zones are affected by rheumatic disease and overuse injury, typically with the formation of bony spurs (Peterson & Renstrom, 1986; Littlejohn, 1989). However, little is known about how the composition of tendon fibrocartilage varies with age or is related to its function and pathology. We report significant age-related changes in the cells and matrix of the rat suprapatella and comment briefly on changes in the adjacent attachment-zone fibrocartilage of the quadriceps tendon.

MATERIALS AND METHODS

Animals

For light microscopy, 2 white Wistar rats were used at each of the following ages: 11–14 weeks; 12, 18 and 24 months. Animals were killed by cervical dislocation after anaesthetising with ether. One hind limb was used for histology and the other for immunohistochemistry. For electron microscopy, suprapatellae from the hind limbs of three 15-month-old rats were used.

* Corresponding author.

Histology

The patella and quadriceps tendon were fixed for 1 week in 10% neutral buffered formol saline. Material was decalcified in 2% nitric acid, dehydrated in graded alcohols, cleared in Inhibisol and embedded in paraffin wax. Sagittal sections were cut at 10 μm and stained with haematoxylin and eosin, Masson's trichrome or toluidine blue.

Electron microscopy

Material was fixed in 3% glutaraldehyde in 0·1 M cacodylate buffer at pH 7·3 for 4 h, rinsed overnight in buffer, postfixed for 20 min in 1% osmium tetroxide in cacodylate buffer, dehydrated in graded alcohols and embedded in Araldite. Silver-gold sections were cut with a diamond knife, mounted on 200 mesh, formvar-coated copper grids and stained with lead citrate and uranyl acetate. Photographs were taken on a Philips EM 401 electron microscope.

Immunohistochemistry

The suprapatellae were dissected out and processed as described previously (Ralphs *et al.* 1991). Briefly, material was fixed in cold 90% alcohol, decalcified in 5% EDTA, infiltrated overnight with phosphate-buffered saline containing 5% sucrose, frozen using dry ice and 10 μm cryostat sections mounted on poly-l-lysine coated slides.

The sections were labelled by indirect immunofluorescence using monoclonal antibodies to extracellular matrix components and to the intermediate filament vimentin, as previously described (Ralphs *et al.* 1991).

Primary antibodies

Type II collagen. Monoclonal antibody CIIC1 (Holmdahl *et al.* 1986) was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, and the Department of Biology, University of Iowa, Iowa City, IA, under contract NO1-HD-6-2915 from the NICHD. It was raised to rat type II collagen and binds to an epitope in the fibrous part of the molecule. Sections were pretreated with testicular hyaluronidase (Sigma type I-S, 1·45 IU/ml) and chondroitinase ABC (Sigma, 0·25 IU/ml) for 30 min at 37 °C prior to incubation with a 1:2 dilution of CIIC1 culture supernatant.

Keratan sulphate. Monoclonal antibody MZ15 was a gift from Dr F. Watt (ICRF, London). It was raised to pig chondrocytes, and is specific for keratan sulphate (Zanetti, Ratcliffe & Watt, 1985; Mehmet *et al.* 1986). Sections were pretreated as above and incubated in a 1:1000 dilution of ascitic fluid.

Chondroitin sulphate. Monoclonal antibody CS56 was obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK. It was raised to a preparation of chicken gizzard membranes and binds to the glycosaminoglycan moieties of chondroitin 4 and 6 sulphate-containing proteoglycans. It is not cell or species specific (Avnur & Geiger, 1984) and was used at a dilution of 1:200 of ascitic fluid.

Vimentin. Monoclonal antibody VIM 13·2 was obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK. This was produced using extracts of human foreskin fibroblasts as the immunogen, and used at a dilution of 1:200 of ascitic fluid. The antibody labels vimentin in immunoblots, and binds to it in fibroblasts, endothelial cells, lymphoid tissue, and melanocytes and tumours derived from them. In cultures of rat fibroblasts the antibody labelled a fibre population extending from

a bright perinuclear region to the cell periphery, as expected for intermediate filaments (Robson, 1989; Ralphs *et al.* 1991).

RESULTS

Structure and molecular composition

The histology of the suprapatella in aged rats was similar to that described in younger animals (Fig. 1*a*) (Ralphs *et al.* 1991). There were numerous large, pale-staining cells between which was a basket-weave arrangement of collagen fibres. This contrasts with the smaller number of cells and the parallel collagen fibres in the overlying tendon.

The extracellular matrix of the suprapatella of young rats labelled strongly with antibody CS56 to chondroitin sulphate. The labelling intensity decreased with age. In young animals label was uniformly distributed throughout the matrix (Fig. 2*a*), whereas in older animals it was restricted to the pericellular region of some cells (Fig. 2*b*). Small amounts of chondroitin sulphate were found in the quadriceps tendon superficial to the suprapatella. Immunolabelling with antibody CIIC1 to type II collagen was absent from young rats but present at 12 months in the deep part of the suprapatella near the articular surface. Immunolabel became stronger and more widespread in 18 and 24-month animals (Fig. 1*b–d*). Labelling with antibody MZ15 to keratan sulphate was only detected in 12-month rats (Fig. 2*c*). The cells of the suprapatella were strongly labelled with antibody VIM 13·2 to vimentin at all ages. Immunolabel was often concentrated in bright foci (Fig. 2*d*).

The fibrocartilage of the adjacent attachment zone of the quadriceps tendon to the patella labelled strongly for type II collagen in both young and old animals. In 12, 18 and 24-month animals, label extended further into the tendon (Fig. 2*e*, *f*). Chondroitin sulphate was present in the attachment zone at all ages, but there was no label for keratan sulphate at any age. Vimentin was detected in fibrocartilage cells of the attachment zone and in fibroblasts of the quadriceps tendon, although the labelling intensity was much less than that in the suprapatella.

Ultrastructure of aged suprapatella

Most cells had large numbers of lipid droplets and glycogen granules but few organelles. Large masses of intermediate filaments were present in all cells. In some cases they formed prominent whorls easily recognised at low magnification (Figs 3, 4*a*). In many cells there were large lakes of glycogen and numerous lipid droplets. Indeed, these cells contained little else but filaments, glycogen and lipid with other cytoplasmic components restricted to a thin peripheral rim and the perinuclear region (Fig. 3). The cells had a small Golgi apparatus, a few cisternae of rough endoplasmic reticulum (RER) and a few small mitochondria (Fig. 4*c*). Polyribosomes were present in perinuclear cytoplasm. The form of the nucleus varied greatly. In some cells it was rounded, but in others it was frequently deeply indented or multilobed (Fig. 4*c*). In extreme cases, the nucleus was highly irregular (Fig. 3). Despite the great variation in shape, most nuclei had conspicuous nucleoli (Fig. 4*c*).

The extracellular matrix of the suprapatella often had distinct pericellular and interterritorial regions (Fig. 3), although the former region was sometimes absent. This corresponded with the pattern of chondroitin sulphate labelling described above. The pericellular matrix was often rich in electron dense granules and strands that are characteristic of proteoglycans, and contained a few banded collagen fibres (Fig. 4*d*). Membrane-bound vesicles were often conspicuous in and around this region. The

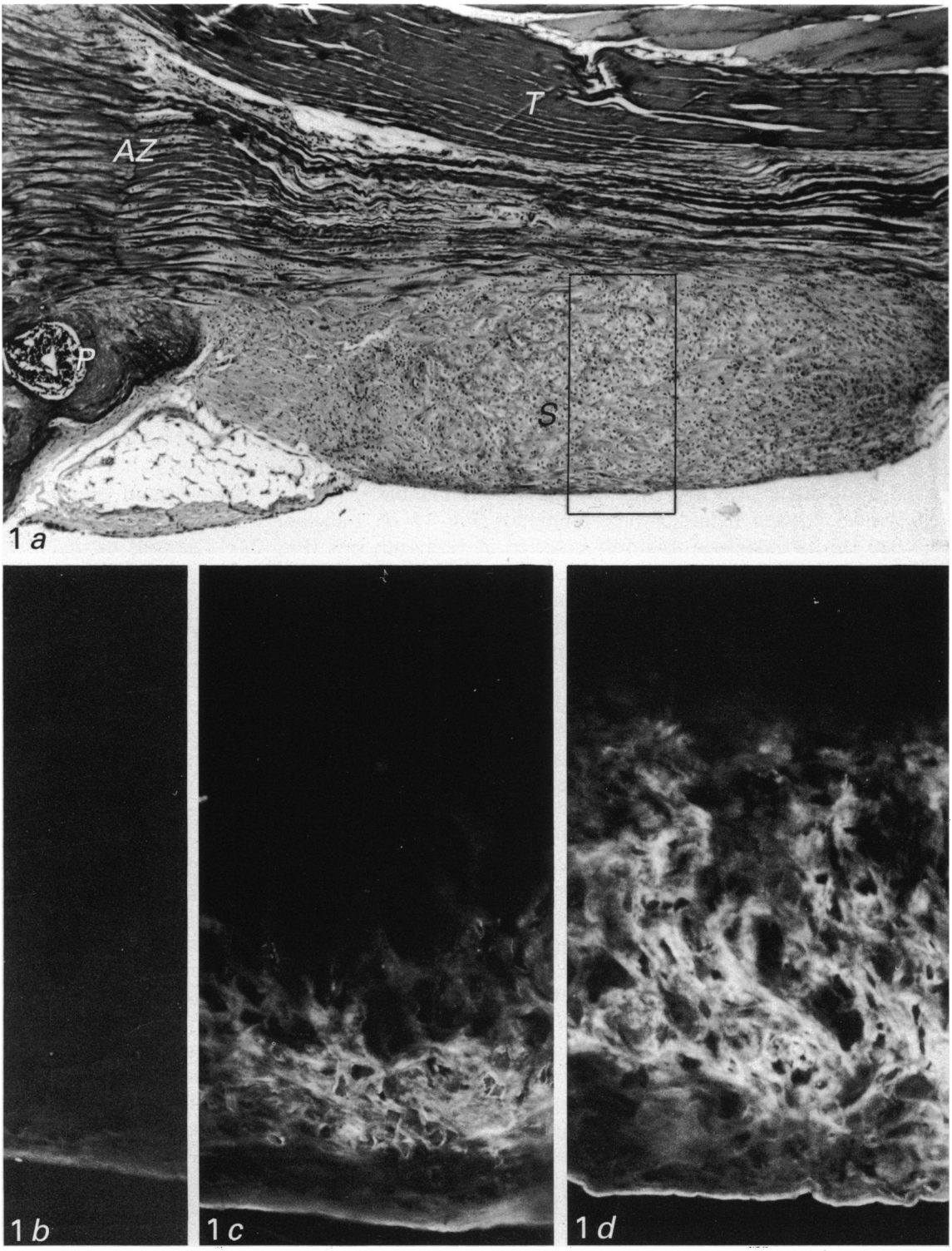

Fig. 1 (*a–d*). (*a*) Sagittal section of the suprapatella (*S*) and the overlying tendon (*T*) from 24-month-old rat. Masson's trichrome; *AZ*, attachment zone; *P*, patella. × 50. (*b–d*). Immunolabel with antibody CIIC1 to type II collagen in suprapatellae of rats aged (*b*) 3 months (*c*) 12 months and (*d*) 24 months. The region shown corresponds to the boxed region in (*a*). × 144.

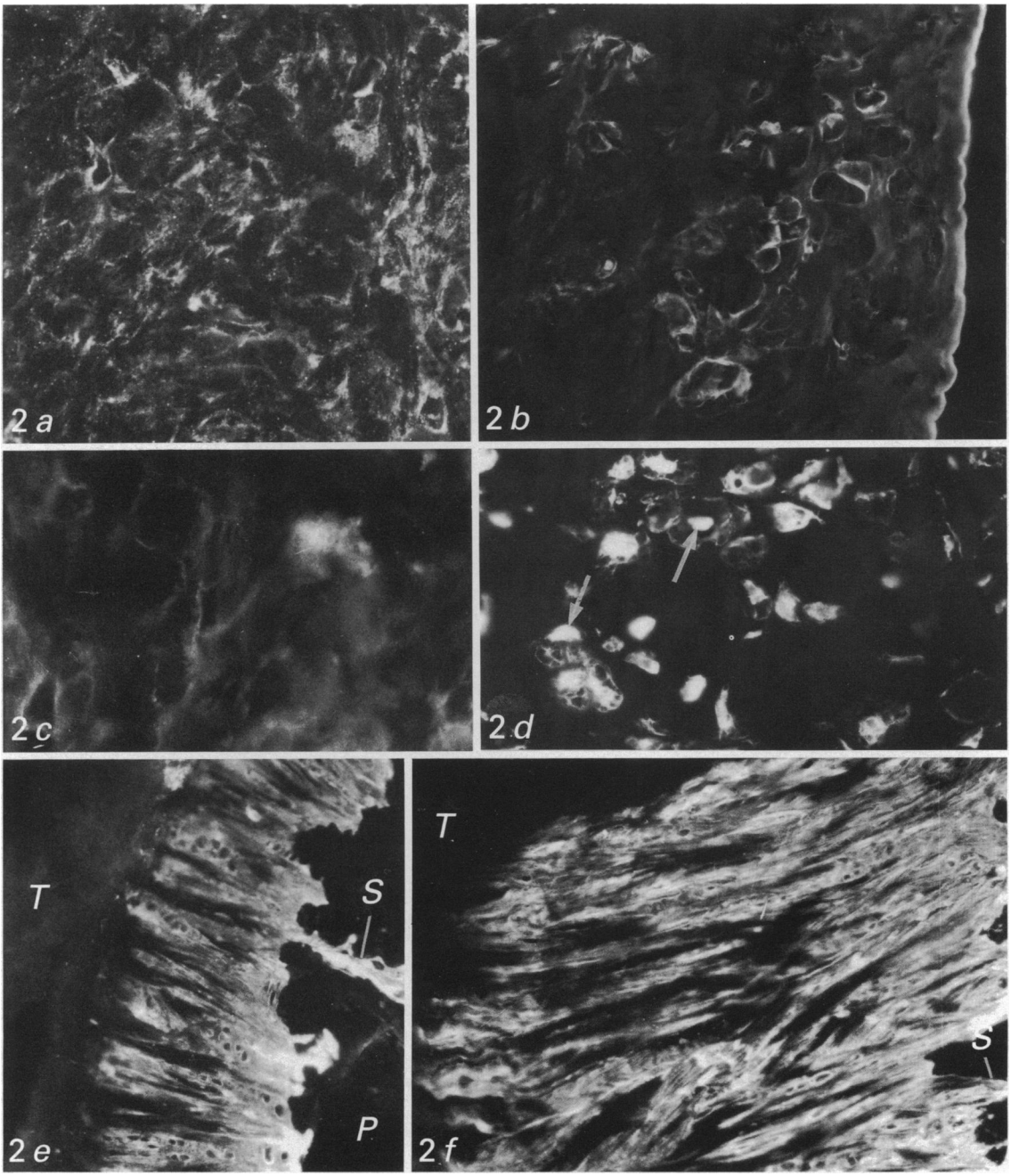

Fig. 2(*a–f*). (*a*, *b*) Age-related changes in immunolabelling of the suprapatella with antibody CS56 to chondroitin sulphate. The labelling is widespread at 3 months (*a*) but restricted to pericellular regions by 24 months (*b*). (*c*) Weak labelling with antibody MZ15 for keratan sulphate only occurs in the suprapatellar matrix at 12 months. (*d*) Strong labelling with antibody Vim 13·2 for vimentin in suprapatellar cells at 24 months. Note the local concentrations in the cytoplasm (arrows). (*e*, *f*) Age-related changes in labelling with antibody CIIC1 for type II collagen in sagittal sections of the attachment zone of the quadriceps tendon. (*e*) 3 months (*f*) 24 months. In both, the type II collagen of the attachment zone is continuous with that in spicules (*S*) of calcified cartilage in the patella (*P*). *T*, Tendon. In (*f*) the bone of the patella starts at the extreme right of the figure. × 360.

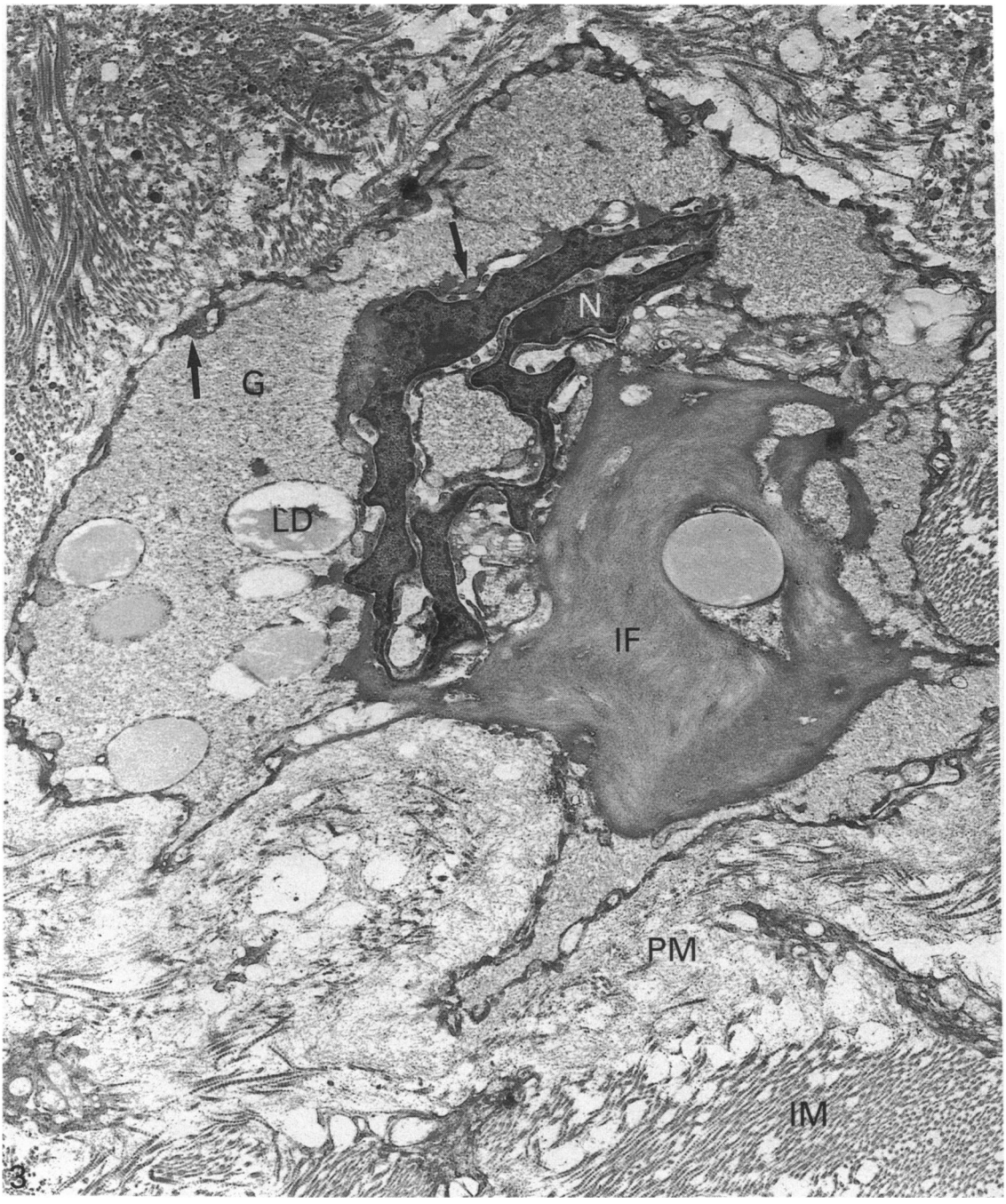

Fig. 3. Electron micrograph of suprapatellar cell from 15-month-old rat. The cell has an irregular nucleus (*N*), large masses of intermediate filaments (*IF*), extensive areas where glycogen (*G*) has been extracted and prominent lipid droplets (*LD*). Other cytoplasmic organelles are restricted to peripheral and perinuclear cytoplasm (arrows). ×9000.

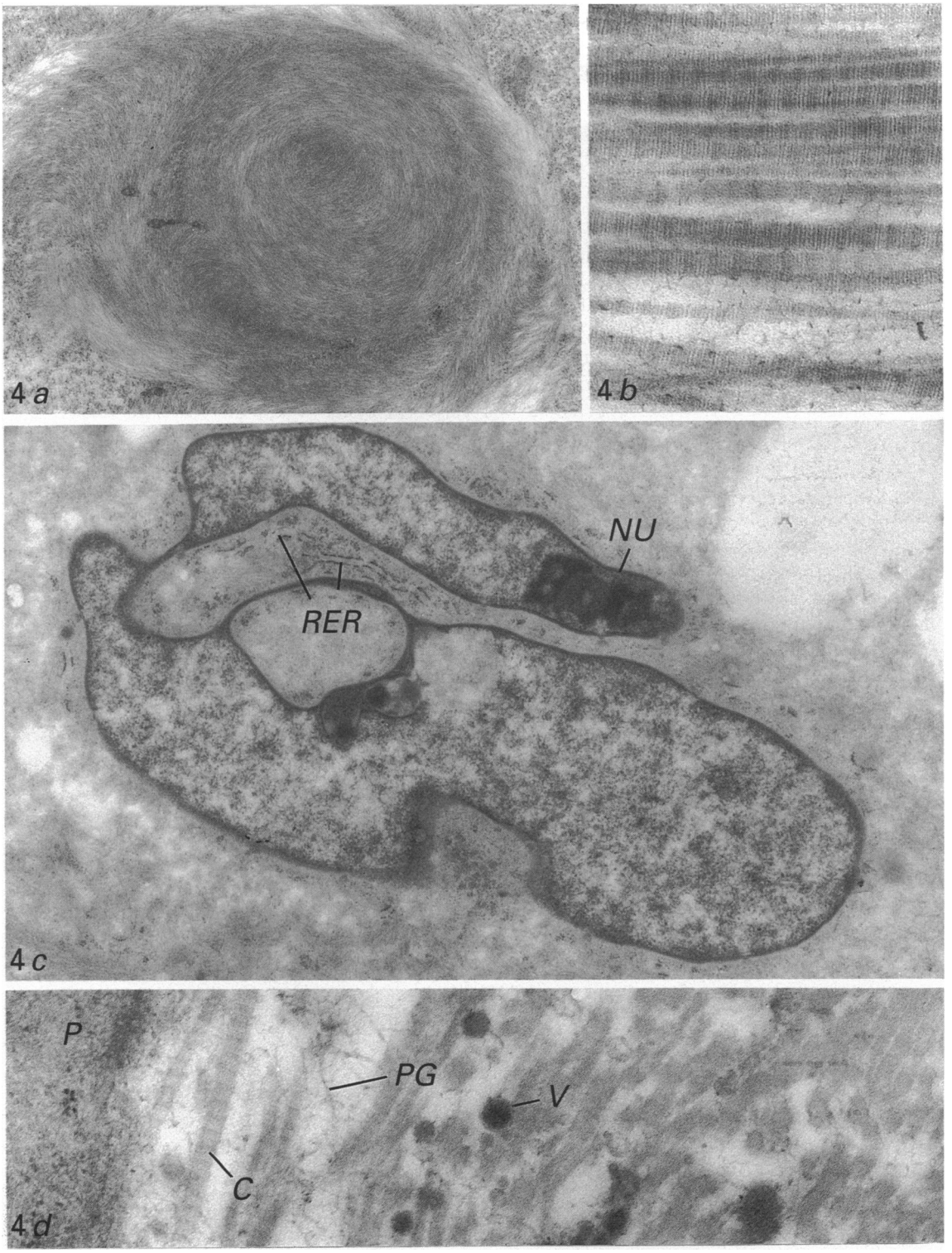

Fig. 4(*a–d*). Electron micrographs of details of the 15-month suprapatella. (*a*) Intracellular whorl of intermediate filaments. × 11 500. (*b*) Banded collagen fibres in the interterritorial matrix. × 70 000. (*c*) Lobed and indented nucleus with a prominent nucleolus (*NU*). A few cisternae of RER are present nearby. × 15 000. (*d*) Pericellular matrix showing membranous vesicles (*V*), strands of proteoglycans (*PG*) and collagen fibres (*C*). (*P*) Periphery of the cell. × 42 500.

interterritorial matrix contained large bundles of banded collagen fibres (Fig. 4*b*). The mean diameter of these was $52{\cdot}3 \pm 2$ nm ($n = 50$), with a banding periodicity of $57 \pm 0{\cdot}5$ nm ($n = 10$). Proteoglycan particles were less prominent than in the pericellular matrix.

DISCUSSION

The present study demonstrates pronounced changes in the collagen composition of compression fibrocartilage with age, with the appearance of type II collagen in animals 12 months and older. This coincides with a significant decrease in the mean diameter of collagen fibres in the interterritorial matrix (52 ± 2 nm at 15 months, $77 \pm 2{\cdot}25$ nm at 3 months; Ralphs *et al.* 1991). Where the molecular composition of fibrocartilages has been studied, type II collagen is often found. It is present in fibrocartilage of menisci, intervertebral discs, tendon attachment zones (Benjamin & Evans, 1990; Ralphs *et al.* 1991) and the developing os penis (Murakami, 1987). Age-related changes have only been reported in intervertebral discs, where there are no major variations in the proportion of collagen types (Eyre, 1988). Although we did not identify other collagens in the suprapatella, it is probably rich in type I collagen (Ralphs *et al.* 1991). As type II collagen appears relatively late and is not distributed throughout the aged suprapatella, it may account for a small proportion of the total collagen as in menisci and intervertebral discs (see Benjamin & Evans, 1990, for review).

The age-related decrease in chondroitin sulphate in the suprapatella parallels that in menisci (Ghosh & Taylor, 1987) and intervertebral discs (McDevitt, 1988) and is similar to changes in articular cartilage (e.g. Dziewiatowski, LaValley & Beaudoin, 1989). Menisci, intervertebral discs and articular cartilage increase in keratan sulphate content with age (McDevitt, 1988). Compressive fibrocartilage of bovine FDP tendon contains keratan sulphate (Vogel & Koob, 1989), which also occurs in developing chick tendon (Craig, Ralphs, Bentley & Archer, 1987). It is unclear why keratan sulphate should only be observed transiently in the rat suprapatella.

The majority of cells differed ultrastructurally from those of young animals (Ralphs *et al.* 1991). They had prominent whorls of intermediate filaments, large lakes of glycogen and unusually shaped nuclei. The aged cells had less RER and Golgi membranes than cells from younger animals. This suggests low secretory activity, although there must be some because of matrix turnover that leads to the appearance of type II collagen. Both lipid and glycogen are present in younger animals but not in such quantity (Ralphs *et al.* 1991). They are normal and constant inclusions in other cartilage cells and accumulate with age (Stockwell, 1967; Gyarmati, Foldes, Kern & Kiss, 1987; Labandeira-Garcia, Guerra-Seijas & Suarez-Nunez, 1987). They are present in the fibrocartilage of the FDP tendon (Okuda *et al.* 1987*a*) and the os penis (Yamamoto, 1989). The significance of such accumulations is unclear. It has been suggested that glycogen could provide a source of reserve materials and energy for synthesising matrix glycosaminoglycans (Townsend & Gibson, 1970; Stockwell, 1979). However, the observation that chondroitin sulphate labelling decreased with age suggests that cells from aged animals are synthesising little glycosaminoglycan. It may be that glycogen accumulates because it is no longer needed for matrix synthesis. Lipids may play a role in the calcification of cartilage (Boskey, 1981). Yamada (1976) suggested that the vesicles derived from degenerate cells in attachment-zone fibrocartilage may be associated with the onset of calcification. Similar structures were observed in the matrix of the suprapatella, but no apatite crystals were seen and there is no evidence that the suprapatella calcifies or ossifies with age.

The distribution of intermediate filaments in the suprapatellar cells changed dramatically with age. Although some cells retained the arrangement of filaments characteristic of young animals (Ralphs *et al.* 1991), others developed large, whorl-like concentrations that corresponded with the pattern of vimentin labelling seen by fluorescence microscopy. Accumulation of intermediate filaments is a normal cellular characteristic of various cartilages (see Ralphs *et al.* 1991). However, the formation of large whorls of filaments may be abnormal in fibrocartilage: large numbers of filaments occur in cells of the intervertebral disc, but large whorls only appear in recurrent herniated discs (Postacchini, Bellocci, Ricciardi-Pollini & Modesti, 1982). It may be that the whorls found here in aged suprapatellar cells are signs of senescence.

The composition of the attachment-zone fibrocartilage was similar to that in young animals (Ralphs *et al.* 1991), although the region was more extensive; type II collagen spreads from the fibrocartilage into the tendon in older animals. This may be of pathological significance, as spreading of fibrocartilage from attachment zones has been observed in human lumbar ligaments with increasing age and is associated with the formation of bony spurs (Scapinelli, 1989).

SUMMARY

Age-related changes are reported in the rat suprapatella: a fibrocartilage that resists compression of the quadriceps tendon against the femur in the flexed knee. The suprapatella was studied by histology, immunohistochemistry and electron microscopy in rats aged 11–14 weeks, and 12, 15, 18 and 24 months. Type II collagen was absent in the matrix of animals 11–14 weeks old, but appeared by 12 months; immunolabelling increased further with age. Chondroitin sulphate was present in all animals, although immunolabelling decreased with age. Keratan sulphate appeared transiently at 12 months. The structure of the suprapatellar cells also changed with age. In some respects the suprapatellar cells of aged rats are similar to those of younger animals; they contain relatively few organelles and their cytoplasm is packed with intermediate filaments which contain vimentin. However, lipid droplets and glycogen are more prominent in older animals, and the nuclei become elaborately infolded and multilobed. Type II collagen was present in rats aged 11–14 weeks in fibrocartilage of the attachment of quadriceps femoris to the patella, but with increasing age it spread proximally, further into the tendon.

REFERENCES

AVNUR, Z. & GEIGER, B. (1984). Immunocytochemical localisation of native chondroitin-sulfate in tissues and cultured cells using specific monoclonal antibody. *Cell* **38**, 811–822.

BENJAMIN, M., EVANS, E. J. & COPP, L. (1986). The histology of tendon attachments to bone in man. *Journal of Anatomy* **149**, 89–100.

BENJAMIN, M. & EVANS, E. J. (1990). Fibrocartilage. *Journal of Anatomy*, **171**, 1–15.

BOSKEY, A. L. (1981). Current concepts of the physiology and biochemistry of calcification. *Clinical Orthopaedics and Related Research* **157**, 225–257.

CRAIG, F. M., RALPHS, J. R., BENTLEY, G. & ARCHER, C. W. (1987). MZ15, a monoclonal antibody recognizing keratan sulphate, stains chick tendon. *Histochemical Journal* **19**, 651–657.

DZIEWIATOWSKI, D. D., LAVALLEY, J. & BEAUDOIN, A. G. (1989). Age-related changes in the composition of proteoglycans in sheep cartilages. *Connective Tissue Research* **19**, 103–120.

EYRE, D. R. (1988). Collagens of the disc. In *The Biology of the Intervertebral Disc*, vol. 1 (ed. P. Ghosh), pp. 171–188. Boca Raton: CRC.

GHOSH, P. & TAYLOR, T. K. F. (1987). The knee joint meniscus. A fibrocartilage of some distinction. *Clinical Orthopaedics and Related Research* **224**, 52–63.

GILLARD, G. C., REILLY, H. C., BELL-BOOTH, P. G. & FLINT, M. H. (1979). The influence of mechanical forces

on the glycosaminoglycan content of the rabbit flexor digitorum profundus tendon. *Connective Tissue Research* **7**, 37–46.

GYARMATI, J., FOLDES, I., KERN, M. & KISS, I. (1987). Morphological studies on the articular cartilage of old rats. *Acta morphologica hungarica* **35**, 111–124.

HOLMDAHL, R., RUBIN, K., KLARESKOG, L., LARSSON, E. & WIGZELL, H. (1986). Characterisation of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. *Arthritis and Rheumatism* **29**, 400–410.

LABANDIERA-GARCIA, J. L., GUERRA-SEIJAS, M. J. & SUAREZ-NUNEZ, J. M. (1987). Quantitative histochemical study of changes with age in intracellular lipids of xiphisternal cartilage in rat and rabbit. *Anatomischer Anzeiger* **164**, 255–264.

LITTLEJOHN, G. O. (1989). More emphasis on the enthesis. *Journal of Rheumatology* **16**, 1020–1022.

MCDEVITT, C. A. (1988). Proteoglycans of the intervertebral disc. In *The Biology of the Intervertebral Disc*, vol. 1 (ed. P. Ghosh), pp. 151–170. Boca Raton: CRC.

MEHMET, H., SCUDDER, P., TANG, P. W., HOUNSELL, E. F., CATERSON, B. & FEIZI, T. (1986). The antigenic determinants recognized by three monoclonal antibodies to keratan sulphate involve sulphated hepta- or larger oligosaccharides of the poly (N-acetyllactosamine) series. *European Journal of Biochemistry* **157**, 385–391.

MERRILEES, M. J. & FLINT, M. H. (1980). Ultrastructural study of tension and pressure zones in a rabbit flexor tendon. *American Journal of Anatomy* **157**, 87–106.

MURAKAMI, R. (1987). Immunohistochemical and immunoblot analyses of collagens in the developing fibrocartilage in the glans penis of the rat. *Acta morphologica neerlando-scandinavica* **25**, 279–282.

OKUDA, Y., GORSKI, J. P. & AMADIO, P. C. (1987*a*). Effect of postnatal age on the ultrastructure of six anatomical areas of canine flexor digitorum profundus tendon. *Journal of Orthopaedic Research* **5**, 231–241.

OKUDA, Y., GORSKI, J. P., AN, K-N. & AMADIO, P. C. (1987*b*). Biochemical, histological and mechanical analyses of canine tendon. *Journal of Orthopaedic Research* **5**, 60–68.

PETERSON, L. & RENSTROM, P. E. R. (1986). *Sports Injuries. Their prevention and treatment*. London: Dunitz.

POSTACCHINI, F., BELLOCCI, M., RICCIARDI-POLLINI, P. T. & MODESTI, A. (1982). An ultrastructural study of recurrent disc herniation. A preliminary report. *Spine* **7**, 482–497.

RALPHS, J. R., BENJAMIN, M. & THORNETT, A. (1991). Cell and matrix biology of the suprapatella in the rat: a structural and immunocytochemical study of fibrocartilage in a tendon subject to compression. *Anatomical Record*, in press.

ROBSON, R. M. (1989). Intermediate filaments. *Current Opinion in Cell Biology* **1**, 36–43.

SCAPINELLI, R. (1989). Morphological and functional changes of the lumbar spinous processes in the elderly. *Surgical and Radiological Anatomy* **11**, 129–133.

STOCKWELL, R. A. (1967). The lipid and glycogen content of rabbit articular hyaline and non-articular hyaline cartilage. *Journal of Anatomy* **102**, 87–94.

STOCKWELL, R. A. (1979). *Biology of Cartilage Cells*. London, New York: Cambridge University Press.

TOWNSEND, F. J. & GIBSON, M. A. (1970). A histochemical study of glycogen metabolism in developing cartilage and bone. *Canadian Journal of Zoology* **48**, 87–95.

VOGEL, K. G. & KOOB, T. J. (1989). Structural specialization in tendons under compression. *International Review of Cytology* **115**, 267–293.

WOO, S., MAYNARD, J., BUTLER, D., LYON, R., TORZILLI, P., AKESON, W., COOPER, R. & OAKES, B. (1988). Ligament, tendon and joint capsule insertions to bone. In *Injury and Repair of the Musculoskeletal Soft Tissues* (ed. S. L.-Y. Woo & J. A. Buckwalter), pp. 133–166. Illinois: American Academy of Orthopedic Surgeons.

YAMADA, M. (1976). Ultrastructural and cytochemical studies on the calcification of the tendon-bone joint. *Archivum histologicum japonicum* **39**, 347–378.

YAMAMOTO, M. (1989). An electron microscopic study of the distal segment of the os penis of the rat. *Archives of Histology and Cytology* **52**, 529–541.

ZANETTI, M., RATCLIFFE, A. & WATT, F. M. 91985). Two subpopulations of differentiated chondrocytes identified with a monoclonal antibody to keratan sulphate. *Journal of Cell Biology* **101**, 53–59.