J. Anat. (1983), **137**, 4, pp. 653–663
With 18 *figures*
Printed in Great Britain

Ultrastructural changes in dog femoral condylar cartilage following anterior cruciate ligament section

C. R. ORFORD, D. L. GARDNER AND P. O'CONNOR

Department of Histopathology, University of Manchester, Oxford Road, Manchester M13 9PT and The University Hospital of South Manchester, Withington, Manchester M20 8LR

(*Accepted* 17 *February* 1983)

INTRODUCTION

Surgical division of an anterior cruciate ligament in beagle dogs causes a brisk sterile synovitis, slight mechanical instability of the stifle joint and a sequence of biochemical and histological disorders in the articular cartilage of the femoral condyles. Among the phenomena that have been described are an increase in water content, in dry weight, in DNA and in collagen synthesis and a decrease in proteoglycan aggregation and molecular size (McDevitt, Gilbertson & Muir, 1977; Gardner, Carney, O'Connor, Bradley & Orford, 1982; Schwartz & Greenwald, 1979–1980). Histological abnormalities include depletion of metachromatic material, increased cellularity (cell density) and a late and conspicuous increase in cartilage thickness with a reversion of cell density towards normal. Early in this sequence a remarkable series of alterations in the morphology of zone I (superficial) and II (middle) chondrocytes becomes evident: there is a move towards a less mature structure so that, 14–28 days after operation, the cell morphology appears 'inappropriate' for the superficial zones (Gardner *et al.* 1982).

Many of the cell and matrix disturbances could be detected but not analysed by light microscopy. It became clear that transmission and scanning electron microscopy would be essential for this purpose. The present paper describes the ultrastructural changes observed by transmission electron microscopy.

MATERIAL AND METHODS

Fifteen normal inbred beagle dogs (14) and bitches (1), aged 9–20 months, weighing 8·1–18·3 kg, underwent closed surgical division of the left anterior cruciate ligament. The right stifle joint served as a sham-operated control: it was opened but the ligament was not severed. Three unoperated animals, aged 10–17 months, weighing 10·9–14·0 kg, were used as normal controls.

The operation was carried out under intravenous thiopentone sodium and halothane/oxygen anaesthesia, after pre-medication with atropine and acetyl promazine. Through an incision medial to the patellar ligament, the periarticular tissue was divided and the stifle joint opened. The anterior cruciate ligament was cut by passing a curved scalpel blade parallel to the medial meniscus, around the ligament. The adequacy of section was confirmed by manual manipulation of the joint.

Animals were housed untethered in individual pens for 14 days after operation. Thereafter they were housed in larger pens with other dogs. Exercise was, therefore, not significantly restricted at any time.

Experimental dogs were selected at random at 3, 7, 14/15, 28, 42, 56, 84, 112 and 168 days after operation. All dogs were killed by intravenous pentobarbitone sodium. The joints were opened and blocks of cartilage, 1·0 × 1·0 × 0·5 mm, were taken from the central load-bearing areas of the lateral femoral condyles of operated and sham-operated joints of each dog and from the right joints of the normal control dogs. Blocks were fixed for 24 hours at 4 °C in sodium cacodylate-buffered 2·5 % glutaraldehyde pH 7·4. After washing in buffer, they were post-fixed for one hour in cacodylate-buffered 1 % osmium tetroxide, washed, dehydrated and embedded in Agar 100 resin. Blocks were sectioned perpendicularly through the articular surface at 70 nm using an LKB Ultrotome IV with glass or diamond knives. Sections were stained with uranyl acetate and lead citrate or with phosphotungstic acid followed by uranyl and lead. They were examined in a Philips 301 transmission electron microscope at an accelerating voltage of 60 kV.

RESULTS

Normal cartilage

A continuous 100 nm lamina of amorphous material covered the cartilage surface (Figs. 1, 2). Chondrocytes close to the surface were flattened in the tangential plane (Fig. 3). Cell membranes proximal to the articular margin were smooth but their deep surfaces bore numerous short cell processes. These chondrocytes had large nuclei, often almost filling the cell. The cytoplasm contained varying amounts of endoplasmic reticulum, Golgi apparatus, mitochondria, secretory vacuoles and vesicles. Micropinocytotic vesicles lined the cytoplasmic aspect of the cell membrane. A single centriole was occasionally observed. Intracytoplasmic fine filaments were present in small amounts throughout the cytoplasm. Chondrocytes in zone II were rounded and exhibited short cell processes on all aspects of the cell. Frequently, a distinct pericellular matrix lacking mature collagen fibrils was evident. Zone II chondrocytes often contained lipid droplets and pools of glycogen in addition to the usual cytoplasmic organelles (Fig. 4). Large whorls of cytoplasmic fine filaments were common (Fig. 5).

Collagen fibrils in the superficial zone matrix were ~ 25 nm in diameter. They were closely packed and orientated parallel to the surface (Fig. 1). The interfibrillar ground substance remained largely unstained although there was evidence of fine filaments between the collagen fibrils (Fig. 2). In zone II, collagen fibrils of varying

---

Fig. 1. Normal cartilage. Articular surface (*s*) and zone I matrix. Collagen fibrils are closely packed and lie parallel to the articular surface. × 24700.

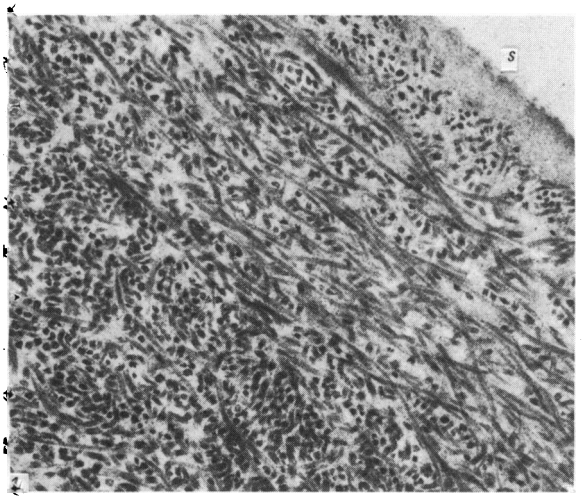
Fig. 2. Normal cartilage. Articular surface (*s*) and zone I matrix. × 58500.

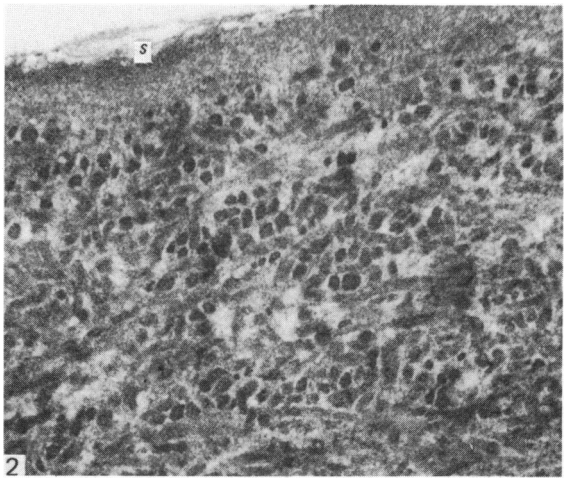
Fig. 3. Normal cartilage. Zone I chondrocyte flattened in a plane parallel to the articular surface (*s*). × 5700.

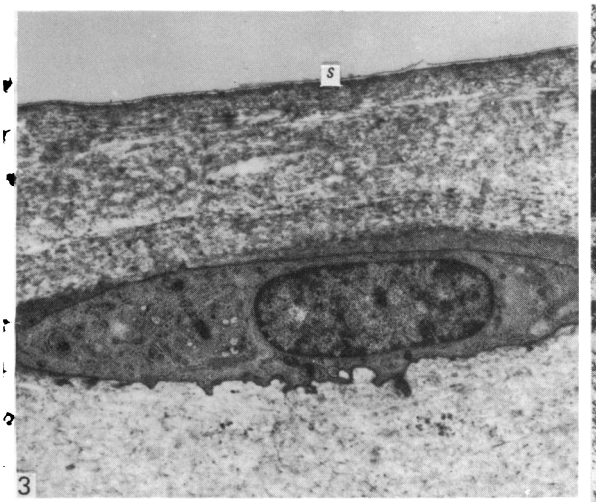
Fig. 4. Normal cartilage. Zone II chondrocyte with cytoplasmic glycogen (*g*) deposits and lying in a narrow pericellular lacuna (*p*). × 5700.

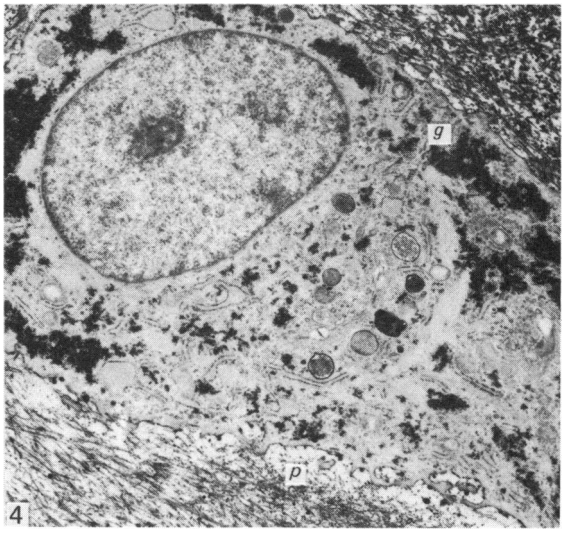
Fig. 5. Normal cartilage. Zone II chondrocyte with a whorl of intracytoplasmic fine filaments (*w*). × 5700.

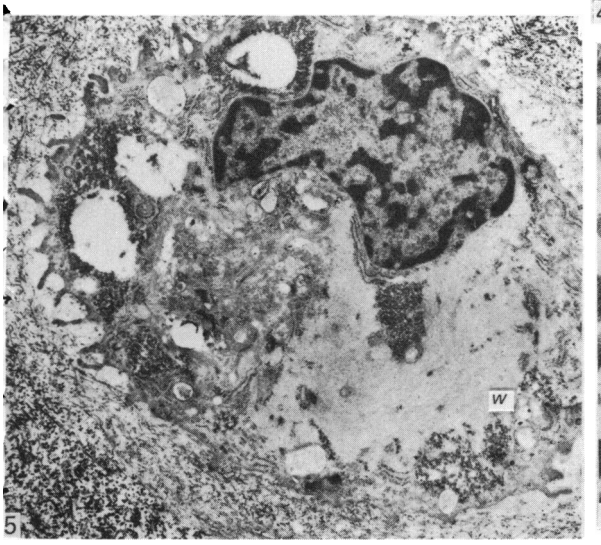
Fig. 6. Normal cartilage. Zone II matrix. Collagen fibrils are randomly arrayed. × 58500.

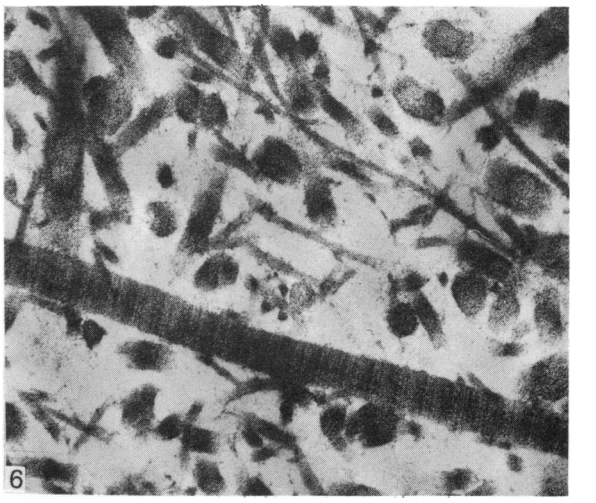

s


s


s


g
p


w



diameters (25–80 nm) formed a random network contrasting with the ordered array in zone I (Fig. 6).

Cartilage from operated joints

A number of disorders, some widespread but others localised, followed division of the anterior cruciate ligament. The focal changes created variability between blocks; areas exhibiting normal structure persisted for 24 weeks after surgery. Tissue taken from the operated joints at 3, 7, 14/15 and 28 days after surgery showed no abnormalities. Alterations in the characteristics of the surface layers of cartilage were noticeable 42 days after surgery and were striking after 168 days.

The surface lamina of amorphous material varied in thickness between animals, between joints and even between different sites on the same joint surface. It was unusually thickened, however, in operated joints and exceeded 1 μm in depth 168 days after surgery (Fig. 7). At some sites, the thickening appeared to be due to increased deposition of amorphous material on the surface, but at other sites it might have been due to the laminar material infiltrating zone I and forming an additional amorphous layer beneath a narrow band of collagen fibrils (Fig. 8).

Alterations in zone I chondrocytes were first noted 42 days after surgery. Although many cells appeared normal, a few of them were rounded and resembled chondrocytes from deeper zones. These zone II-type cells, positioned inappropriately near the articular surface (Fig. 9), occurred more frequently at longer time intervals after operation. After 168 days, they had entirely replaced the population of flattened superficial cells. They often occurred in pairs, were well endowed with granular endoplasmic reticulum (Figs. 9, 10) and were situated in unusually large electron-lucent lacunae which lacked mature collagen fibrils (Fig. 10). In zone II, the frequency of paired chondrocytes (Fig. 11) was higher than in unoperated or sham-operated joints. Paired cells were positioned in a single, large lacuna containing fine filaments and sparse collagen fibrils (Fig. 12). Most cells contained extensive endoplasmic reticulum and many exhibited a prominent Golgi apparatus. Glycogen deposits and large whorls of intracytoplasmic filaments were rare. There was no evidence of chondrocyte pyknosis or death.

Loss of the ordered array of zone I collagen fibrils was evident after 56 days. Although the fibrils lay predominantly tangentially to the surface, they exhibited a 'wavy' appearance and occasional fibrils ran obliquely (Fig. 13). At 168 days, the breakdown of the collagen fibril network had reached an advanced stage. The fibrils had lost their tangential orientation and were widely spaced (Fig. 14), separated by electron-lucent areas containing only sparse filamentous threads or clumps of fine fibrils (Fig. 15). Separation of the fibrils was observed in the matrix of zone

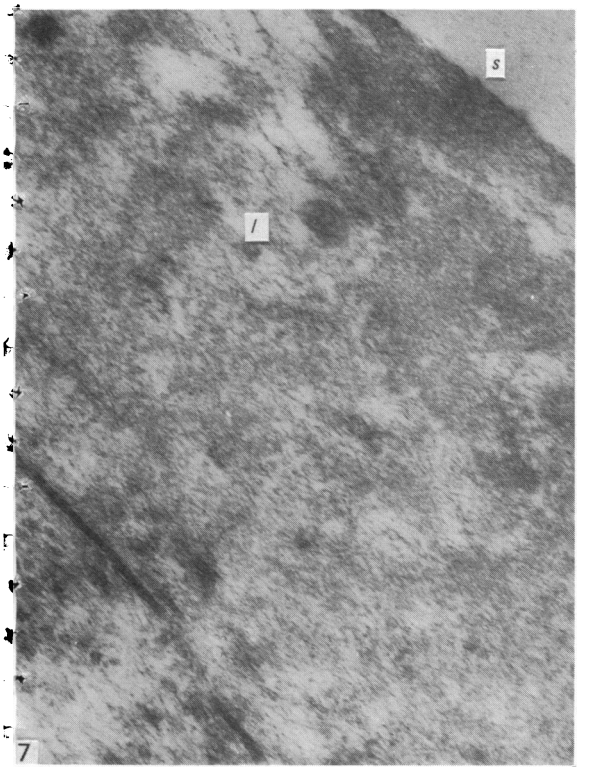
---

Fig. 7. Operated joint, 168 days. Articular surface (*s*). Thickened surface lamina of amorphous material (*l*). (Compare with Fig. 2). ×58500.

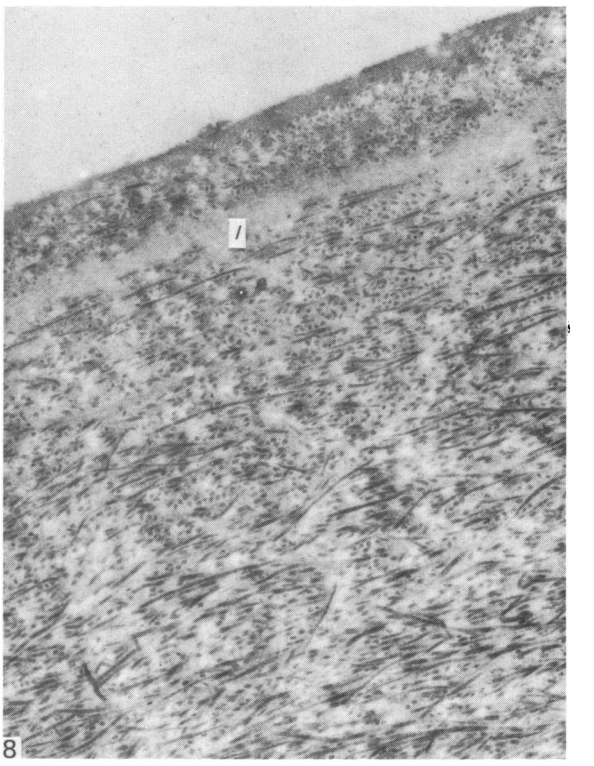
Fig. 8. Operated joint, 112 days. Surface laminar material (*l*) infiltrating zone I matrix. ×16500.

Fig. 9. Operated joint, 168 days. Zone I chondrocytes close to articular surface (*s*) exhibiting 'inappropriate morphology'. Note the rounded shape and extensive rough endoplasmic reticulum (*e*). (Compare with Fig. 3). ×7100.

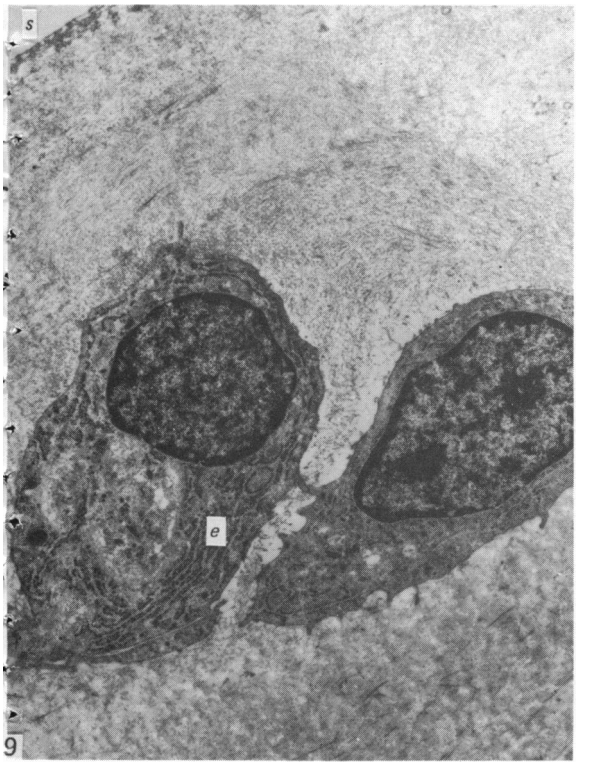
Fig. 10. Operated joint, 168 days. Zone I chondrocytes close to articular surface (*s*) in large electron-lucent pericellular lacuna (*p*). ×4100.



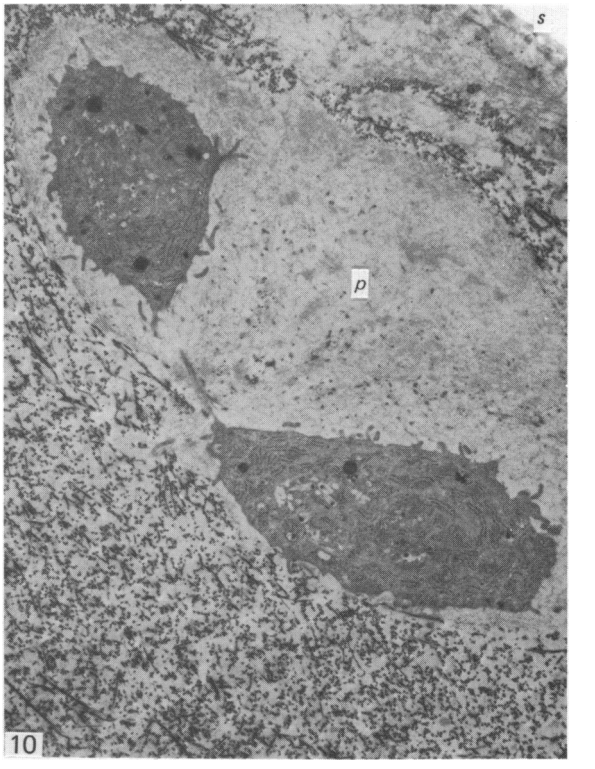
7



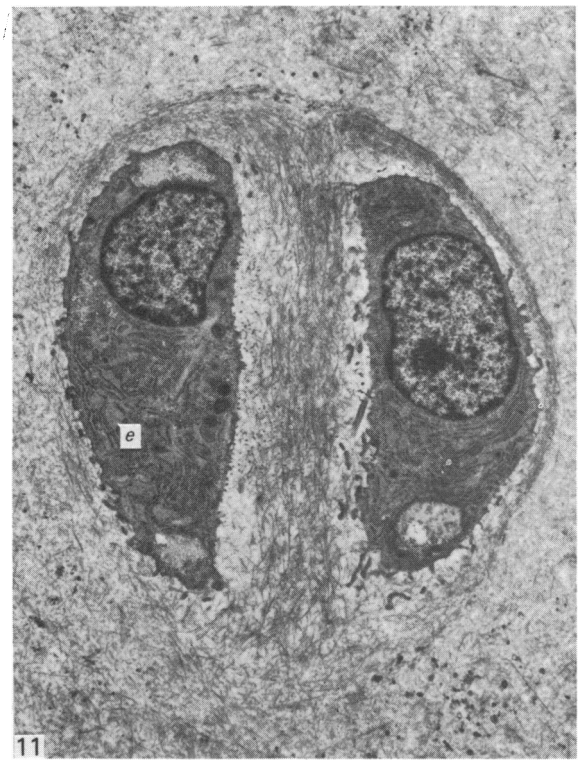
8



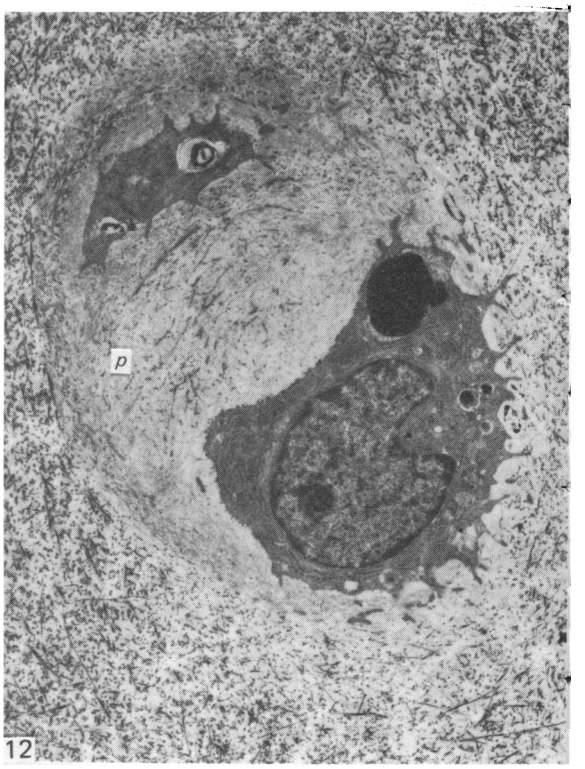
9



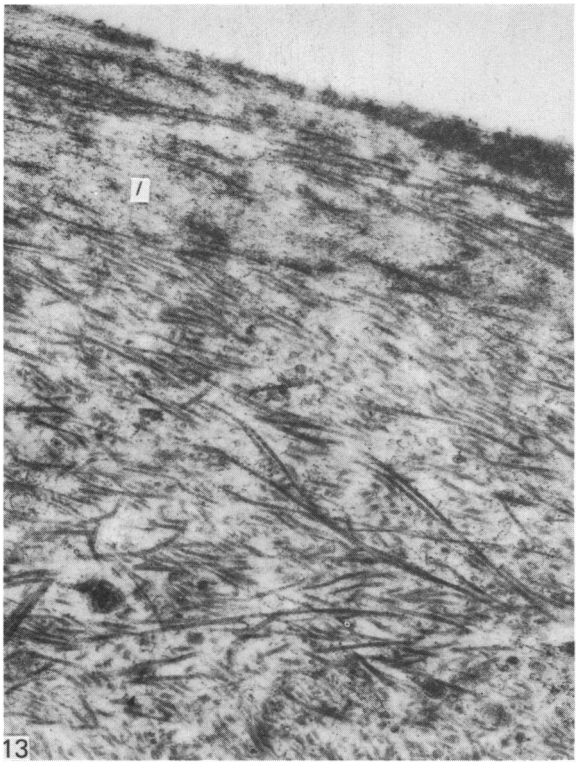
10



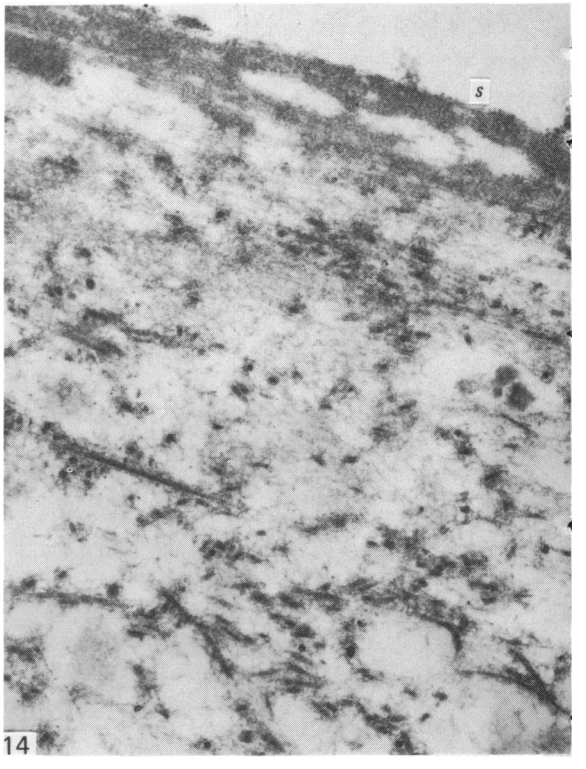
11



12



13



14

II at 168 days only (Fig. 16). Zone II fibrils were of smaller diameter (25–50 nm) than those of normal cartilage.

Cartilage from sham-operated joints

Chondrocyte and matrix changes were not observed in tissue from sham-operated joints (Fig. 17). The surface lamina of amorphous material, however, was considerably thickened, appearing up to 0·5 μm deep in some specimens taken 168 days after surgery (Fig. 18).

DISCUSSION

This experiment demonstrates that conspicuous alterations in the fine structure of chondrocytes and intercellular matrix are precipitated by division of the anterior cruciate ligament. Following surgery there is: (1) a loss of the flattened appearance of superficial chondrocytes: the cells become rounded and resemble cells from deeper zones; (2) a change in the distribution of chondrocytes: many cells from both zones I and II occur in pairs; (3) a striking increase in the size of electron-lucent pericellular lacunae; (4) a separation and disorientation of collagen fibrils in zone I; (5) an increase in the thickness of the surface lamina of amorphous material.

The rounded and paired appearance of zone I chondrocytes confirms histological observations (Gardner *et al.* 1982) and, although mitotic figures were not detected, implies that cell division had occurred. Bizarre nuclear shapes suggesting amitotic division have not been observed; furthermore the theory of amitosis has been largely discredited (Mankin, 1963). It is probable that the rate of division, although elevated after cruciate ligament section, remains too low for mitotic figures to be observed.

Evidence of cellular degeneration, death and shedding from the surface to account for the loss of flattened zone I cells is lacking. This implies *in situ* alterations in chondrocytes rather than migration of cells from deeper zones. The normal chondrocytes of zone I are not effete but their cytoplasmic volume is proportionally less than that of zone II chondrocytes. Thus the change to a more rounded cell type characteristic of deeper zones, together with the presence of extensive endoplasmic reticulum in both zone I and zone II chondrocytes, may represent an increased ability for synthesis of extracellular materials. Chondrocyte proliferation and elaboration of endoplasmic reticulum were noted after immobilisation of rabbit joints (Gritzka, Fry, Cheesman & Lavigne, 1973) and injection of papain into dog hip joints (Scheck, Parker & Sakovich, 1975). The electron-lucent pericellular zones, lacking mature collagen fibrils, suggest increased secretion of non-collagenous material. These zones contain filamentous threads, resemble the interfibrillar ground

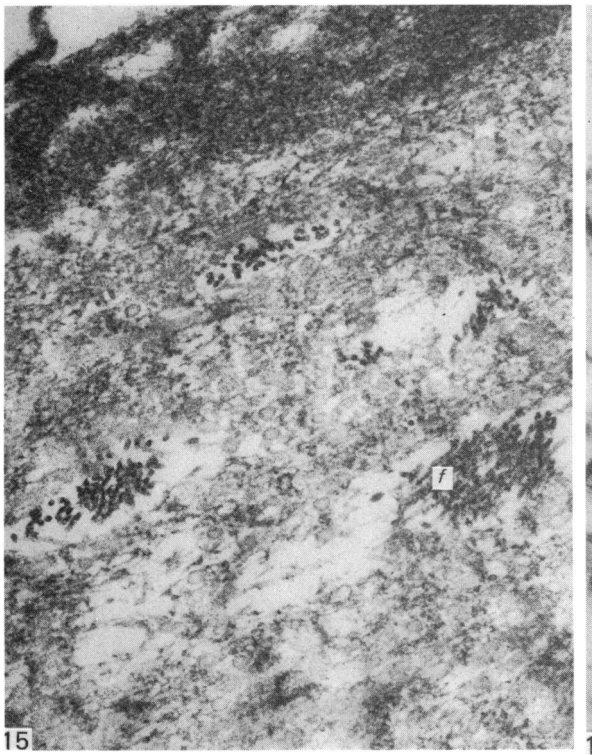
---

Fig. 11. Operated joint, 56 days. Zone II chondrocyte pair. Note extensive rough endoplasmic reticulum (*e*). ×3400.

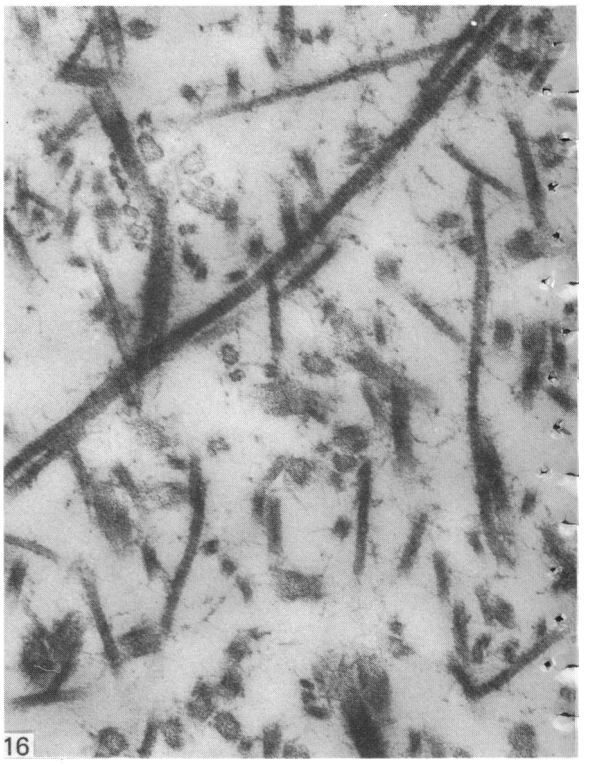
Fig. 12. Operated joint, 112 days. Zone II chondrocyte pair in large, collagen-poor pericellular lacuna (*p*). ×4100.

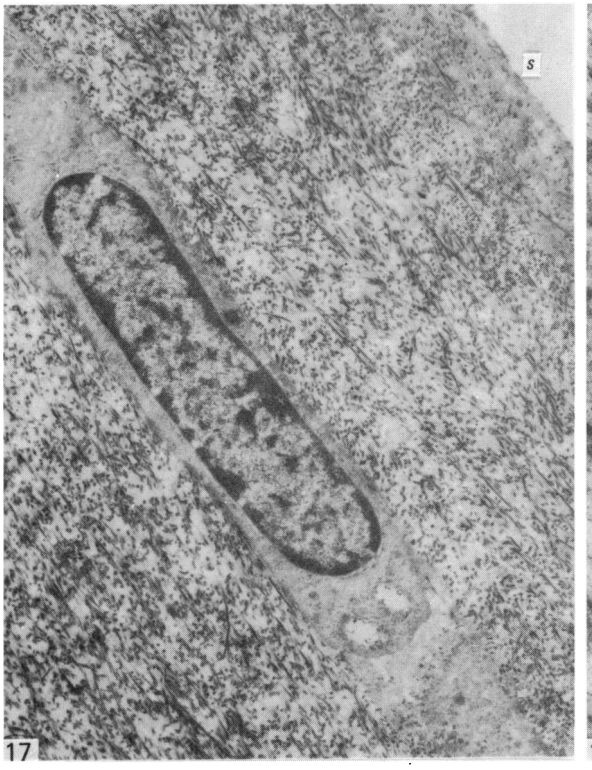
Fig. 13. Operated joint, 84 days. Disrupted zone I matrix. Note disarrayed collagen fibrils and surface laminar material (*l*) infiltrating superficial region. ×11800.

Fig. 14. Operated joint, 168 days. Articular surface (*s*) and disrupted zone I matrix. Note disarrayed and widely separated collagen fibrils. (Compare with Fig. 1). ×24700.

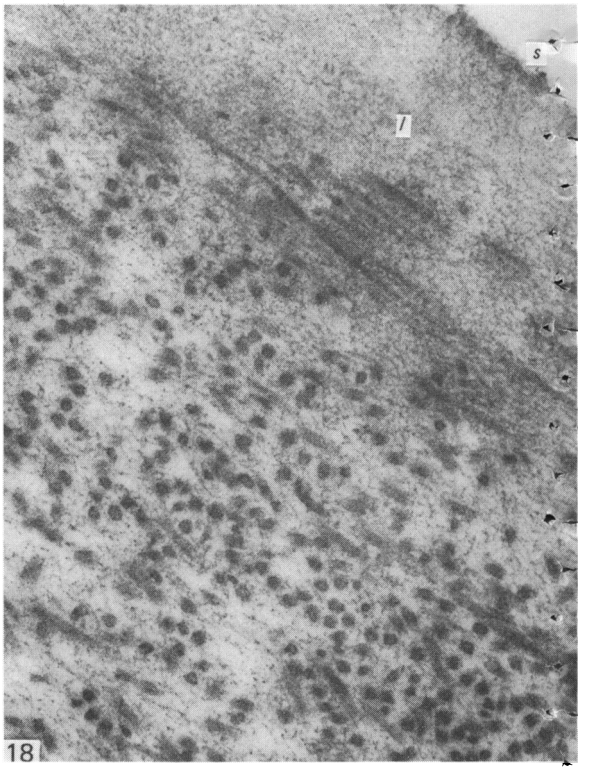


15


16



17



18

substance of normal matrix and may represent proteoglycan; it is hoped to confirm this in future experiments by specific staining methods. Similar pericellular 'halos' appeared after intra-articular injection of papain (Marcelon, 1975; Scheck *et al.* 1975) and surgical production of cartilage defects (Fuller & Ghadially, 1972; Ghadially, Fuller & Kirkaldy-Willis, 1971). In most cases, however, deterioration and death characterise chondrocytes of articular cartilage subjected to experimental insult; the inappropriate chondrocyte structure described here has not been reported for other experimental models of cartilage trauma. Since active synthetic capability is characteristic of immature chondrocytes (Lust & Sherman, 1973), reversion to a juvenile state is postulated.

Biochemical analysis of cartilage from operated joints reveals a significant increase in hydration but not in proteoglycan content per unit dry weight (Gardner *et al.* 1982). Thus, the electron-lucent spaces between collagen fibrils may represent increased amounts of water (Adams & Billingham, 1982). 'Loosening' of the cartilage matrix with fragmentation and disorientation of collagen fibrils follows prolonged immobilisation of the rabbit knee joint (Roy, 1970). The diminished size of zone II collagen fibrils contributes to the impression of increased interfibrillar space. These sparsely packed, narrow-diameter, fibrils resemble those of young canine articular cartilage (Lust & Sherman, 1973) and may be the consequence of secretion of an immature type of collagen by chondrocytes. Alternatively, they may be the result of shrinkage of collagen fibrils or their degradation into smaller moieties.

Deposits of amorphous or fibrillar material at the articular surface or in the interfibrillar spaces follow surgical resection of a portion of cartilage (Fuller & Ghadially, 1972), intra-articular injection of papain (Scheck *et al.* 1975) and compression of a moving joint (Gritzka *et al.* 1973). In the present study, the increased depth of the surface lamina of amorphous material may correspond to surface deposits; small clumps of fine fibrils within the matrix may represent the initial stages of more widespread interfibrillar deposits. The minor increase in thickness of the lamina of amorphous material in sham-operated joints suggests that these limbs are also affected by the operation. This could be a consequence of altered gait or of the surgical trauma.

Deterioration of the collagen network occurs under an intact surface and is not a consequence of fibrillation. There is no evidence of injury to chondrocytes, with ensuing degeneration, pyknosis and death, such as that noted by Gritzka *et al.* (1973) after compression of a rabbit joint. Lysosomal proteases such as cathepsin D and cathepsin B_1 may provide a basis for pathological degradation of matrix components since catheptic activity is elevated in fibrillated cartilage (Ali & Bayliss, 1974; Ali & Evans, 1973). It is possible that the enlarged pericellular zones observed

---

Fig. 15. Operated joint, 168 days. Bundles of fine electron-dense fibrils (*f*) in the zone I matrix. × 58500.

Fig. 16. Operated joint, 168 days. Zone II matrix. Note narrow, widely separated collagen fibrils. (Compare with Fig. 6). × 58500.

Fig. 17. Sham-operated joint, 168 days. Apparently normal articular surface (*s*) and zone I matrix with ordered array of collagen fibrils and flattened chondrocyte. (Compare with Figs. 9 and 10). × 7400.

Fig. 18. Sham-operated joint, 168 days. Articular surface (*s*) and zone I matrix. Note normal array of collagen fibrils, but thickened surface lamina of amorphous material (*l*). (Compare with Fig. 2). × 58500.

in the present study are a result of matrix degradation by enzymes released from chondrocytes, but such an interpretation must remain speculative since the nature of these zones is unclear. It is unlikely that lysosomal enzymes account for the more widespread deterioration of the collagen network since this is not confined to areas of matrix around individual chondrocytes. Furthermore, the mode of release of enzymes requires explanation; the frequency of lysosomes is not increased after division of the anterior cruciate ligament and chondrocytes are not damaged in such a way as to allow leakage of cytoplasmic or lysosomal contents. On the contrary, the chondrocyte response is one of hypertrophy and the evidence implies elevated levels of matrix component synthesis rather than catabolism.

If the consequence of cruciate ligament division is primarily one of inappropriate cell function, it is possible that the ensuing matrix disturbance may in turn alter chondrocyte behaviour. The manner in which chondrocytes divide and distribute themselves may be a consequence of the orientation of collagen fibrils (Ali & Wisby, 1975) and chondrocyte secretion may be regulated by proteoglycan structure via a feed-back mechanism (Fitton Jackson, 1970; Wiebkin & Muir, 1973; 1975).

Similarities exist between the electron microscopical characteristics of spontaneous osteoarthrosis and those of the surgically-produced disorder described in this paper. Separation and disorientation of zone I collagen fibrils occur in human osteoarthrosis (Weiss, 1973) and aged cartilage (Meachim, Denham, Emery & Wilkinson, 1974) and may precede fibrillation (Meachim & Roy, 1969). Whereas 'inappropriate' morphology of superficial cells has not been reported in human osteoarthrosis, reactive cell changes producing clusters of active cells do occur in early lesions. In more advanced lesions, degenerate and necrotic changes become the prominent feature of superficial chondrocytes (Weiss, 1973; Weiss & Mirow, 1972). In naturally occurring canine osteoarthrosis (Wiltberger & Lust, 1975), rounded, paired chondrocytes surrounded by a prominent electron-lucent halo and containing extensive endoplasmic reticulum are located close to the articular surface; there are few degenerate cells. The superficial layer of close-packed, tangential collagen fibril bundles is absent, there is an increased percentage of narrower collagen fibrils in the intermediate zone and although the fibrils appear to be more numerous than in normal cartilage, their diminished thickness gives an impression of increased interfibrillar space. Fibrillar or amorphous deposits accumulate at the articular surface and in the intercellular matrix; Wiltberger & Lust (1975) consider them a diagnostic feature of osteoarthrosis. Small clumps of fine filaments observed in the present study may represent the initial stages of such deposits.

The overall impression gained from surgically-induced canine degenerative joint disease is one of increased cellular activity associated with an abnormal intercellular matrix: characteristics which resemble those of natural osteoarthrosis.

SUMMARY

Canine femoral condylar cartilage from stifle joints subjected to surgical division of the anterior cruciate ligament showed conspicuous, widespread alterations in chondrocyte morphology and matrix organisation when compared by transmission electron microscopy with cartilage from sham-operated and non-operated joints. Chondrocyte changes were of a kind associated with increased synthesis of matrix components; zone I cells lost their flattened shape and became rounded, cells in zones I and II developed an extensive endoplasmic reticulum, occurred in pairs

more often than usual and were situated in enlarged electron-lucent lacunae. Associated with altered chondrocyte function and increased hydration, the matrix became increasingly disorganised; collagen fibrils were widely separated, disorientated and of small diameter. It is postulated that the disorder is primarily one of inappropriate cell function.

The expert assistance of W. A. Bradley and P. Skelton-Stroud with the veterinary aspects of this work is gratefully acknowledged. Throughout this investigation, the laboratory was generously supported by the Arthritis and Rheumatism Council for Research, by the Medical Research Council and by Ciba–Geigy (Pharmaceuticals Division).

REFERENCES

ADAMS, M. E. & BILLINGHAM, M. E. J. (1982). Animal models of degenerative joint disease. *Current Topics in Pathology* **71**, 265–297.

ALI, S. Y. & BAYLISS, M. T. (1974). Enzymic changes in human osteoarthrotic cartilage. In *Normal and Osteoarthrotic Articular Cartilage* (ed. S. Y. Ali & P. H. Leaback), pp. 189–205. London: Institute of Orthopaedics.

ALI, S. Y. & EVANS, L. (1973). Enzymatic degradation of cartilage in osteoarthritis. *Federation Proceedings* **32**, 1494–1498.

ALI, S. Y. & WISBY, A. (1975). Ultrastructural aspects of normal and osteoarthrotic cartilage. *Annals of the Rheumatic Diseases* **34**, Suppl. 2, 21–23.

FITTON JACKSON, S. (1970). Environmental control of macromolecular synthesis in cartilage and bone: morphogenetic response to hyaluronidase. *Proceedings of the Royal Society* B **175**, 405–453.

FULLER, J. A. & GHADIALLY, F. N. (1972). Ultrastructural observations on surgically produced partial-thickness defects in articular cartilage. *Clinical Orthopaedics and Related Research* **86**, 193–205.

GARDNER, D. L., CARNEY, S. L., O'CONNOR, P., BRADLEY, W. A. & ORFORD, Ç. R. (1982). Biochemical and histological changes in dog cartilage following anterior cruciate ligament section. (In preparation).

GHADIALLY, F. N., FULLER, J. A. & KIRKALDY-WILLIS, W. H. (1971). Ultrastructure of full-thickness defects in articular cartilage. *Archives of Pathology* **92**, 356–369.

GRITZKA, T. L., FRY, L. R., CHEESMAN, R. L. & LAVIGNE, A. (1973). Deterioration of articular cartilage caused by continuous compression in a moving rabbit joint. *Journal of Bone and Joint Surgery* **55** A, 1698–1720.

LUST, G. & SHERMAN, D. M. (1973). Metabolic and ultrastructural studies on articular cartilage of developing canine hip joints. *Cornell Veterinarian* **63**, 94–105.

MCDEVITT, C. A., GILBERTSON, E. & MUIR, H. (1977). An experimental model of osteoarthritis; early morphological and biochemical changes. *Journal of Bone and Joint Surgery* **59** B, 24–35.

MANKIN, H. J. (1963). Localization of tritiated thymidine in articular cartilage of rabbits. III. Mature articular cartilage. *Journal of Bone and Joint Surgery* **45** A, 529-540.

MARCELON, G. (1975). Ultrastructural and histological studies on articular cartilage in experimental osteoarthrosis. *Annals of the Rheumatic Diseases* **34**, Suppl. 2, p. 136.

MEACHIM, G. & ROY, S. (1969). Surface ultrastructure of mature adult human articular cartilage. *Journal of Bone and Joint Surgery* **51** B, 529–539.

MEACHIM, G., DENHAM, D., EMERY, I. H. & WILKINSON, P. H. (1974). Collagen alignments and artificial splits at the surface of human articular cartilage. *Journal of Anatomy* **118**, 101–118.

ROY, S. (1970). Ultrastructure of articular cartilage in experimental immobilization. *Annals of the Rheumatic Diseases* **29**, 634–642.

SCHECK, M., PARKER, J. & SAKOVICH, L. (1975). The fine structure of proliferating cartilage cells: structural changes in an experimental model. *Journal of Anatomy* **119**, 435–452.

SCHWARTZ, E. R. & GREENWALD, R. A. (1979–1980). Experimental models of osteoarthritis. *Bulletin on the Rheumatic Diseases* **30**, 1030–1033.

WEISS, C. (1973). Ultrastructural characteristics of osteoarthritis. *Federation Proceedings* **32**, 1459–1466.

WEISS, C. & MIROW, S. (1972). An ultrastructural study of osteoarthritic changes in the articular cartilage of human knees. *Journal of Bone and Joint Surgery* **54** A, 954–972.

WIEBKIN, O. W. & MUIR, H. (1973). The inhibition of sulphate incorporation in isolated adult chondrocytes by hyaluronic acid. *FEBS Letters* **37**, 42–46.

WIEBKIN, O. W. & MUIR, H. (1975). Influence of the cells on the pericellular environment. The effect of hyaluronic acid on proteoglycan synthesis and secretion by chondrocytes of adult cartilage. *Philosophical Transactions of the Royal Society* B **271**, 283–291.

WILTBERGER, H. & LUST, G. (1975). Ultrastructure of canine articular cartilage: comparison of normal and degenerative (osteoarthritic) hip joints. *American Journal of Veterinary Research* **36**, 727–740.