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The hip joint: the fibrillar collagens associated with development and ageing in the rabbit

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ABSTRACT

The fibrillar collagens associated with the articular cartilages, joint capsule and ligamentum teres of the rabbit hip joint were characterised from the 17 d fetus to the 2-y-old adult by immunohistochemical methods. Initially the putative articular cartilage contains types I, III and V collagens, but when cavitation is complete in the 25 d fetus, type II collagen appears. In the 17 d fetus, the cells of the chondrogenous layers express type I collagen mRNA, but not that of type II collagen. Types III and V collagens are present throughout life, particularly pericellularly. Type I collagen is lost. In all respects, the articular cartilage of the hip joint is similar to that of the knee. The joint capsule contains types I, III and V collagens. In the fetus the ligamentum teres contains types I and V collagens and the cells express type I collagen mRNA; type III collagen is confined mainly to its surface and insertions. After birth, the same distribution remains, but there is more type III collagen in the ligament, proper. The attachment to the cartilage of the head of the femur is marked only by fibres of type I collagen traversing the cartilage; the attachment cannot be distinguished in preparations localising types III and V collagens. The attachment to the bone at the lip of the acetabulum is via fibres of types I and V collagens and little type III is present. The ligament is covered by a sheath of types III and V collagens. Type II collagen was not located in any part of the ligamentum teres. The distribution of collagens in the ligamentum teres is similar to that in the collateral ligaments of the knee. Its insertions are unusual because no fibrocartilage was detected.

Key words: Joints; cartilage; ligamentum teres.

INTRODUCTION

A synovial joint is a complex structure comprising both hard and soft tissues. During development the growth of the bone, articular cartilage, ligaments and tendons is coordinated. Apart from gross changes in shape and size, more subtle changes occur in the content and arrangement of the macromolecules in the matrices during fetal and postnatal development. The precise composition and organisation of these matrices is crucial for their mechanical function and the proper development of the joint surfaces.

The hip joint is particularly susceptible to developmental abnormalities, the most common of which is developmental dysplasia of the hip (DDH) (formerly known as congenital dysplasia of the hip). The causes of DDH are unknown. There is some evidence that the strength of the joint capsule is important in holding the head of the femur and acetabulum close together so that they develop appropriate congruent curvatures with a deep acetabulum (Engesaeter et al. 1990). The collagens in the capsule determine its strength and in some cases of DDH the ratio of types I to III collagens is abnormal; this could result in a decrease in capsular strength (Skirving et al. 1984). Others consider that the soft tissues have little influence on hip joint formation (Weinstein, 1998). It is probable that several factors, including genetic ones, are involved.

Little is known about the distribution of the collagens in developing hip joints, although previous studies of the rabbit knee joint have shown that there is a precise sequence in the appearance of the fibrillar collagens during development and that further changes occur with ageing. These changes are found in the articular cartilage and in the ligaments and tendons

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associated with the joint (Bland & Ashhurst, 1996*a*, *b*). Nothing is known of the collagens in the capsules. Changes in the distributions of the proteoglycans and other matrix macromolecules also occur during development and ageing (Kavanagh & Ashhurst, 1999).

Because the collagens are crucial for the strength of the joint capsule and ligaments, the fibrillar collagens that are synthesised during normal development of the rabbit hip joint have been determined. A knowledge of the collagens of normal joints is essential for an analysis of the changes that occur in developmental abnormalities such as DDH.

MATERIALS AND METHODS

Preparation of hip joints

Hindlimbs and pelvic bones were removed from New Zealand White rabbit fetuses aged 15, 17, 20 and 25 d. Hip joints were dissected from newborn and 1, 3, 6 and 12 to 14-wk-old, 8-mo-old and 2-y-old rabbits. At least 2 animals were used at each stage for the immunohistochemical and in situ hybridisation procedures. The tissues were fixed in 4% paraformaldehyde in 0.05 M Tris-HCl buffer, pH 7.3, for 18 h and then washed in buffer. With the exception of those from the 15 and 17 d fetuses, the tissues were decalcified in 14.3% EDTA, pH 7.0, until radiographically free from calcium. After washing, the tissue was dehydrated in graded ethanol solutions, cleared in methyl salicylate and embedded in paraffin wax. Sections were cut at 7 μm. To follow the development of the joint, serial sections through the whole joint were stained with haematoxylin and eosin.

Immunohistochemistry of the collagens

Polyclonal antibodies to purified rabbit types I, II, III and V collagens were raised in goats and affinity purified (Page et al. 1986).

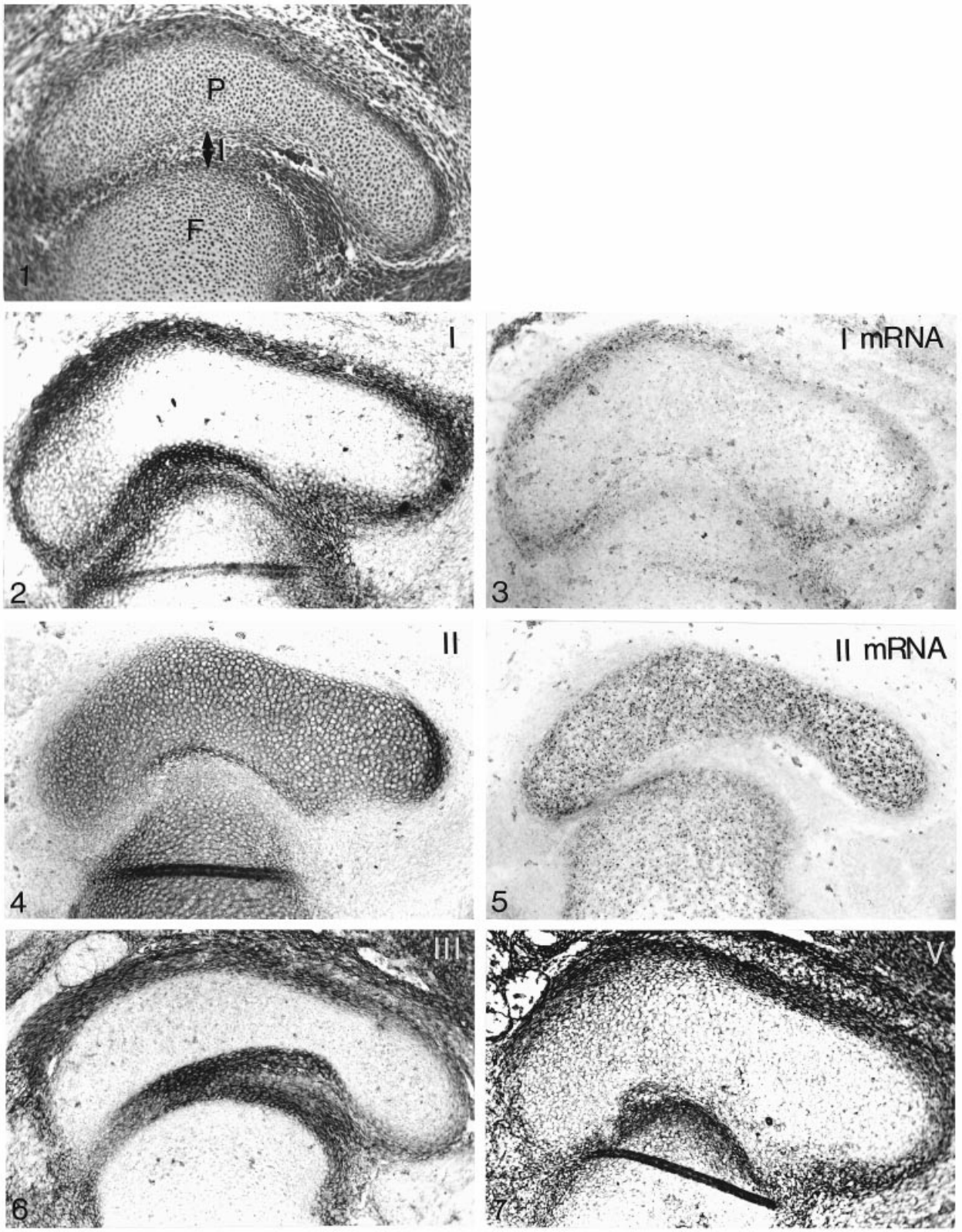
The sections were rehydrated and the following pretreatments performed: (1) 0.1% trypsin (EC 3.4.21.4) in Tris-saline, pH 7.8, with the addition of 0.1% calcium chloride, for 1 h at 37 °C; (2) 2% hyaluronidase (EC 3.2.1.35), bovine, testicular (Sigma, Poole, UK) in phosphate-buffered saline (PBS) for 1 h at 37 °C; (3) 2% L-lysine in PBS for 15 min at room temperature; the sections were washed with PBS between the treatments. To eliminate nonspecific antibody binding, the sections were incubated with undiluted heat-inactivated normal rabbit serum, to which 4% BSA and 0.3% Triton X-100 were added, for 30 min. This was drained from the slides and replaced by the appropriate anticollagen antibody at optimal dilution in 1% BSA in PBS and left overnight at 4 °C. After washing, the sections were exposed to a rabbit antigoat IgG antibody conjugated to alkaline phosphatase in Boehringer blocking buffer (Roche, Lewes, UK) containing 0.3% Triton X-100. The bound antibodies were located using the following substrate; 5 mg naphthol AS-BI phosphate was dissolved in 1 drop dimethyl formamide and added to 5 mg Fast red TR in 10 ml veronal acetate buffer, pH 9.2. Levamisole (1 mg/ml) was added to this substrate to inhibit endogenous alkaline phosphatase activity. The sections were incubated for 20 to 40 min in substrate, washed and mounted in glycerine jelly. For controls, some sections were not exposed to specific antibody; none showed a positive reaction.

In situ hybridisation for collagen mRNAs

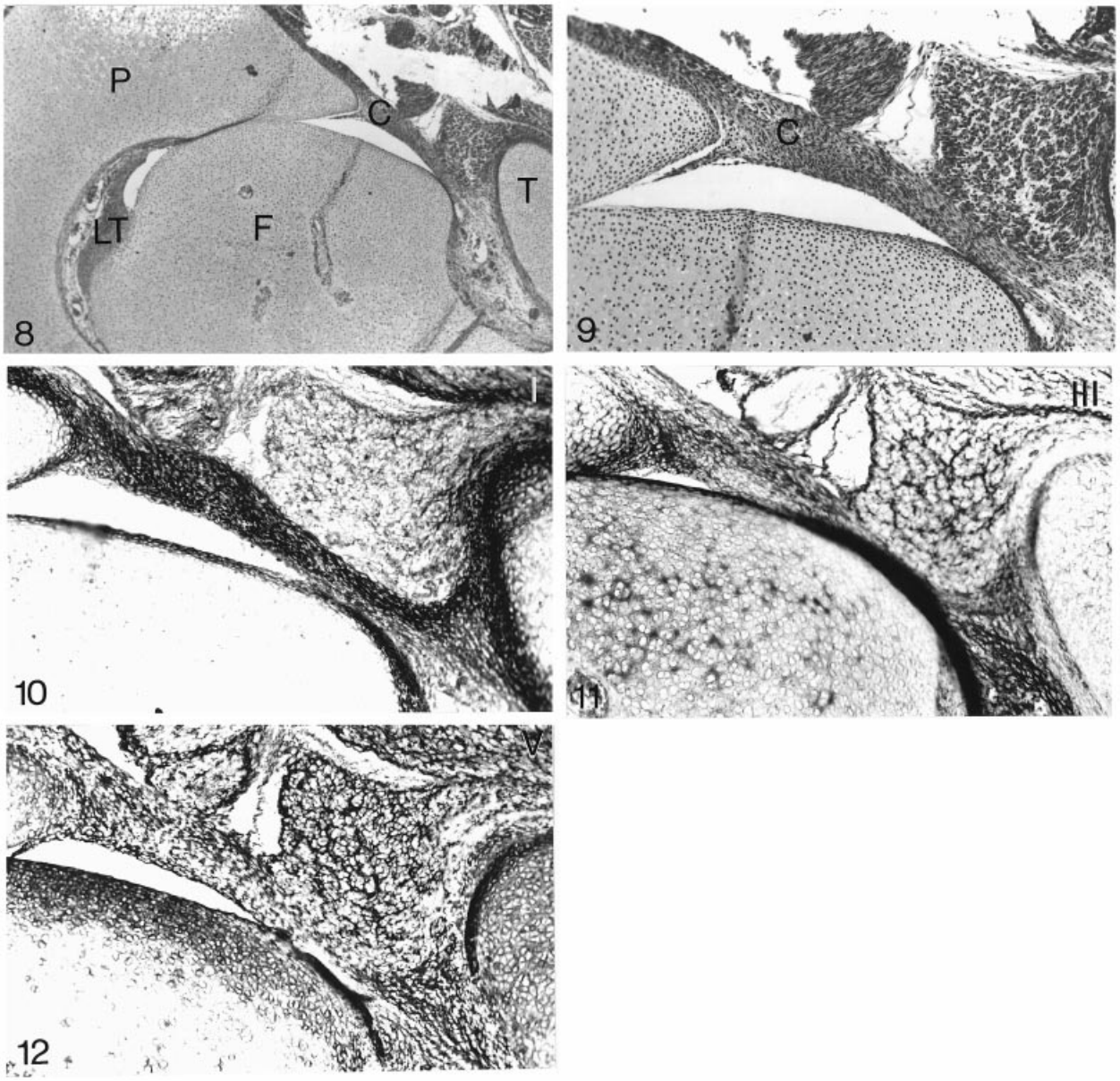
Preparation of riboprobes for types I and II collagens. The plasmid pGM3C1a1 contains a 372 bp fragment of the C-terminal telopeptide and part of the C-propeptide of human proα1 (I) collagen (Critchlow et al. 1995). To generate the antisense probe, pGM3C1a1 was linearised with HindIII and incubated with T7 polymerase; for the sense probe, it was linearised with EcoRI and incubated with SP6 polymerase.

The plasmid, pKCol2a1-1, carries a 400 bp cDNA insert which covers the 3′-untranslated region and a small part of the C-propeptide of rabbit proα1 (II) collagen mRNA (Metsäranta et al. 1996). To generate the probes, pKCol2a1-1 was linearised with SmaI and incubated with T3 polymerase to give the antisense probe, or with HindIII and T7 for the sense probe. The riboprobes were labelled with digoxigenin according to the protocols provided with the kit (Roche, Lewes, UK).

In situ hybridisation. A standard procedure was used. The sections were pretreated sequentially with (1) 0.2 N hydrochloric acid, (2) 6% hydrogen peroxide, (3) 20 μg/ml proteinase K (Sigma, EC 3.4.21.14) in Tris-EDTA buffer, pH 8.0, for 10 min at 37 °C, (4) 4% paraformaldehyde, pH 7.4 for 20 min, (5) 0.1 M glycine (2 × 15 min) and (6) 0.25% acetic anhydride for 10 min. They were dehydrated in graded ethanol solutions and 15 μl hybridisation solution was applied. The hybridisation solution (high stringency) contained 50% formamide, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, Denhardt's solution, 0.5% SDS, 150 mM NaCl, 10% dextran sulphate, 0.5 mg/ml yeast or E. coli tRNA and the labelled antisense, or sense, probe, as appropriate. Hybridisation was for 18 h at 55 °C in



Figs 1–7. Photomicrographs of serial sections through the developing hip joint of a 17 d fetus before cavitation. Fig. 1. Head of the femur (F) and pelvic bone (P) with the interzone (I) (H & E). Fig. 2. Immunohistochemical localisation of type I collagen. Fig. 3. In situ hybridisation using riboprobe to type I collagen mRNA. Fig. 4. Immunohistochemical localisation of type II collagen. Fig. 5. In situ hybridisation using riboprobe to type II collagen mRNA. Fig. 6. Immunohistochemical localisation of type III collagen. Fig. 7. Immunohistochemical localisation of type V collagen. All × 84.



Figs 8–12. Photomicrographs of part of the hip joint of a 25 d fetus after cavitation. Figs 8 and 9 show the head of the femur (F) in the acetabulum of the pelvic bone (P); the ligamentum teres (LT) is present in this section. The joint capsule (C) links the pelvic bone to the greater trochanter (T). (H & E). Fig. 10. Immunohistochemical localisation of type I collagen. Fig. 11. Immunohistochemical localisation of type III collagen. Fig. 12. Immunohistochemical localisation of type V collagen. Fig. 8, × 33, Figs 9–12, × 84.

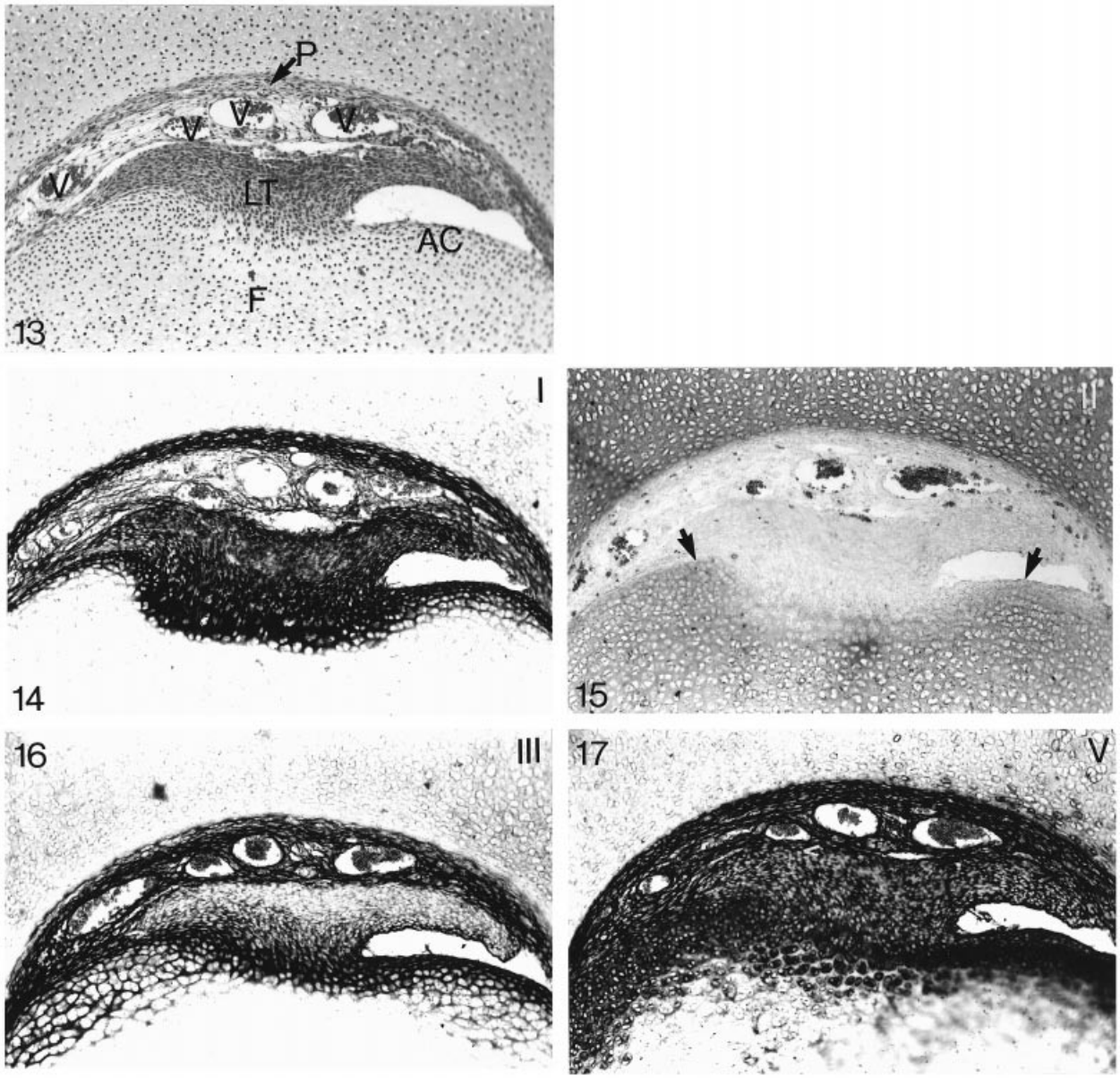
a humid chamber. After hybridisation, the sections were treated with 20 μg/ml RNAse in Tris-EDTA-NaCl buffer, pH 8.0. The sections were washed initially with standard saline citrate (SSC), followed by 0.5 times, then 0.1 times SSC; all washes, except the final wash, were at 55 °C. The digoxigenin label was detected using the Roche kit, except that 0.3 % Triton X-100 was added to the antibody solution. This blocks the nonspecific antibody-binding in the cartilage matrices that arises after treatment with proteolytic enzymes (Bland et al. 1991).

In no instance was there a positive result in the control sections hybridised with sense probes.

RESULTS

Development of the articular surfaces

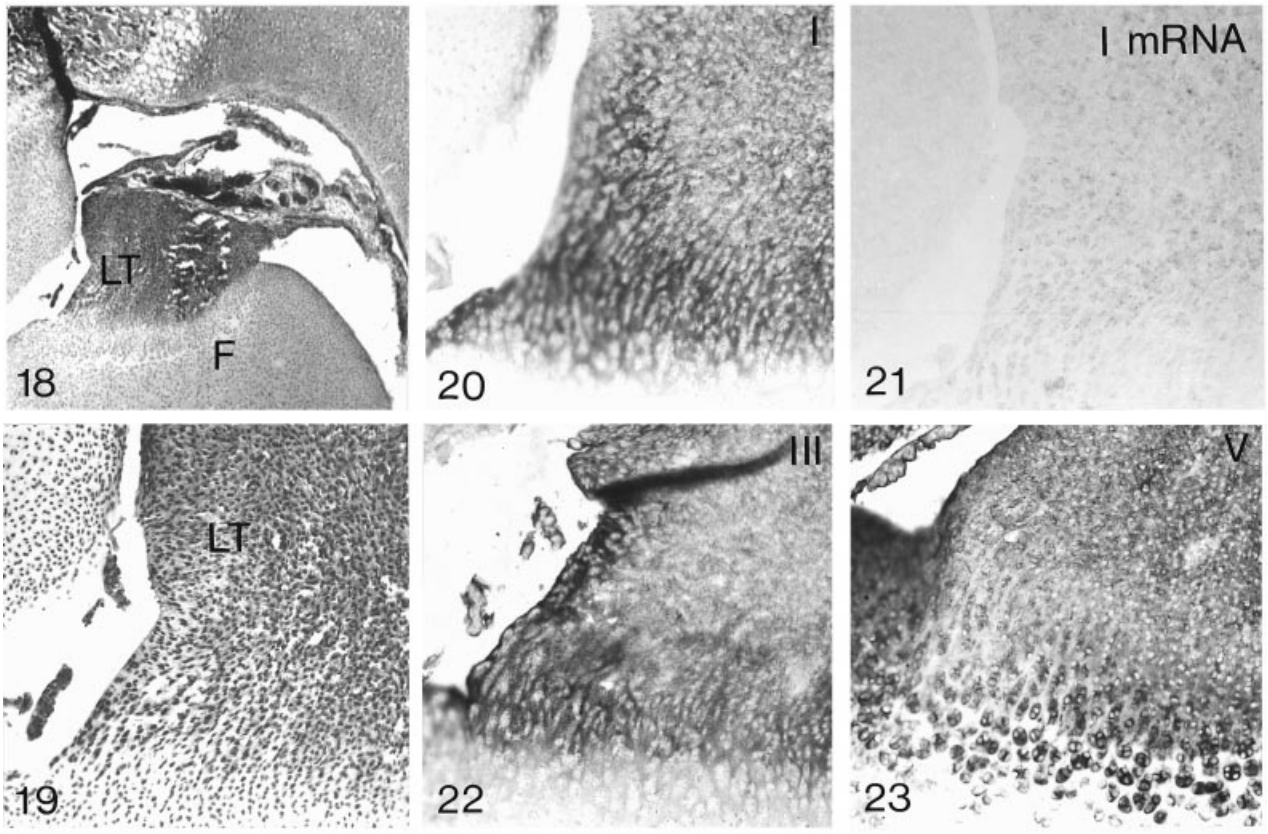
The developing femur and pelvis can be distinguished as 2 cellular condensations in the 15 d rabbit fetus, but in the 17 d rabbit fetus the cartilaginous anlagen of the femur and pelvis can be clearly seen (Fig. 1). Where the joint will develop, the 2 anlagen are separated by the interzone which consists of 2 chondrogenous layers of closely packed cells and the intermediate layer of flattened cells between (Andersen, 1962). The matrix of the interzone binds antibodies to types I, III and V collagens, but not



Figs 13–17. Photomicrographs of the ligamentum teres of a 25-d fetus. Fig. 13. Ligamentum teres (LT) and its insertion into the head of the femur (F). A region of fibrous tissue containing several blood vessels (V) separates the ligamentum teres from the periosteum (P) that lines this part of the acetabulum. A small region of articular cartilage (AC) can be seen on the femur (H & E). Fig. 14. Immunohistochemical localisation of type I collagen. Fig. 15. Immunohistochemical localisation of type II collagen. The developing articular cartilage (arrows) now binds the antibody. Fig. 16. Immunohistochemical localisation of type III collagen. Fig. 17. Immunohistochemical localisation of type V collagen. Comparison of Figs 14 and 16 shows that the main part of the ligamentum teres binds antibodies to type I collagen strongly, but those to type III collagen weakly. In contrast, the fibrous tissue around the blood vessels binds antibodies to type III collagen more strongly than those to type I. All × 84.

those to type II collagen (Figs 2, 4, 6, 7). The matrix of the epiphyseal cartilage of the femur and pelvis binds antibodies to type II collagen (Fig. 4). The cells in the interzone and in the perichondrium give a positive reaction for type I collagen mRNA (Fig. 3). The chondrocytes in the cartilage anlagen are expressing type II collagen mRNA (Fig. 5). There is no overlap in the distribution of these cells.

Cavitation is complete by 25 d. The intermediate layer divides and the cells form the surface layers of the opposing articular cartilages (Figs 8, 9). The cells in the chondrogenous layers are now pushed apart by the developing matrix to form the articular cartilage. The surface of the articular cartilage binds antibodies to types I and III collagens (Figs 10, 11, 14, 16). Type V collagen antibodies are bound throughout the chondrogenous layers, particularly pericellularly (Figs 12, 17). Type II collagen antibodies are bound by the developing articular cartilage immediately after cavitation (Fig. 15). The further development of the



Figs 18–23. Photomicrographs of the ligamentum teres of a 1 wk neonatal rabbit. Figs 18 and 19 show the insertion of the ligamentum teres (LT) into the head of the femur (F). (H & E). Fig. 20. Immunohistochemical localisation of type I collagen. Fig. 21. In situ hybridisation using riboprobe to type I collagen mRNA. Fig. 22. Immunohistochemical localisation of type III collagen. Fig. 23. Immunohistochemical localisation of type V collagen. Fig. 18, × 33; Figs 19–23, × 103.

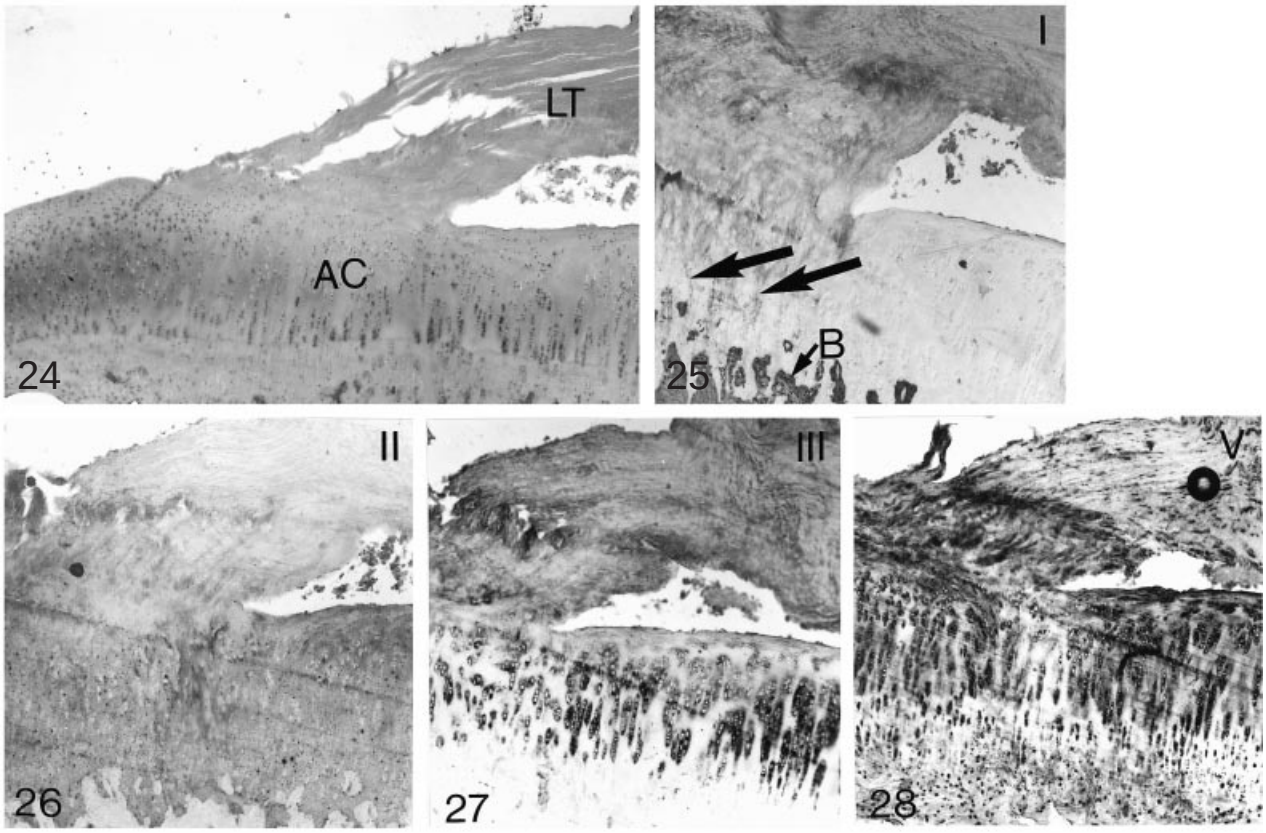
articular cartilage in the hip joint is similar to that of the knee joint which was described in detail by Bland & Ashhurst (1996*a*). The essential features with respect to the collagens are that type I collagen antibody binding at the surface is lost (Fig. 25). Type V collagen antibody binding remains primarily pericellular, although there is some in the interterritorial matrix. Type III collagen antibody binding becomes colocalised pericellularly with that of type V (Figs 27, 28). These properties persist in 2-y-old rabbit cartilage.

The joint capsule

The joint capsule can be seen clearly in the 25 d joint. In Figure 8 it extends from the perichondrium covering the pelvic bones at the edge of the acetabulum and bridges the gap to merge with the dense connective tissue around the neck of the femur (Fig. 9). At this stage it is highly cellular. The matrix binds antibodies to type I collagen very strongly. Those to types III and V collagens are bound less strongly and the distribution of the binding is less uniform (Figs 10–12).

Ligamentum teres

The ligamentum teres develops from a condensation of cells in the interzone that runs between the central part of the head of the femur and the lip of the acetabulum at 17 d (Fig. 1). At 25 d its origin is in a depression in the articular cartilage of the femoral head (Figs 8, 13). Fibres are forming between the cells and these merge later into the developing articular cartilage. Between the ligament and acetabulum there is a layer of loose fibrous tissue containing blood vessels (Fig. 13). This is separated from the cartilage of the pelvis by the developing periosteum; in this region of the acetabulum there will be no articular cartilage. The ligamentum teres binds antibodies to types I and V collagens very strongly and this binding extends into the junctional region of the femur (Figs 14, 17). Antibodies to type III collagen are bound weakly by the ligament proper, but more strongly along its surface and at the insertion (Fig. 16). Type II collagen antibodies are not bound by the ligament (Fig. 15), but they are bound by the cartilage below the developing insertion. The connective tissue around



Figs 24–28. Photomicrographs of the ligamentum teres (LT) and its insertion into the articular cartilage (AC) on the head of the femur of an 8-mo-old rabbit. Fig. 24. H & E. Fig. 25. Immunohistochemical localisation of type I collagen. Bundles of fibres that bind the antibody to type I collagen can be seen in the articular cartilage (arrows). The subchondral bone (B) binds this antibody very strongly. Fig. 26. Immunohistochemical localisation of type II collagen. Fig. 27. Immunohistochemical localisation of type III collagen. Fig. 28. Immunohistochemical localisation of type V collagen. Note the pericellular localisation of types III and V collagens around the chondrocytes. Fig. 24, × 33; Figs 25–28, × 40.

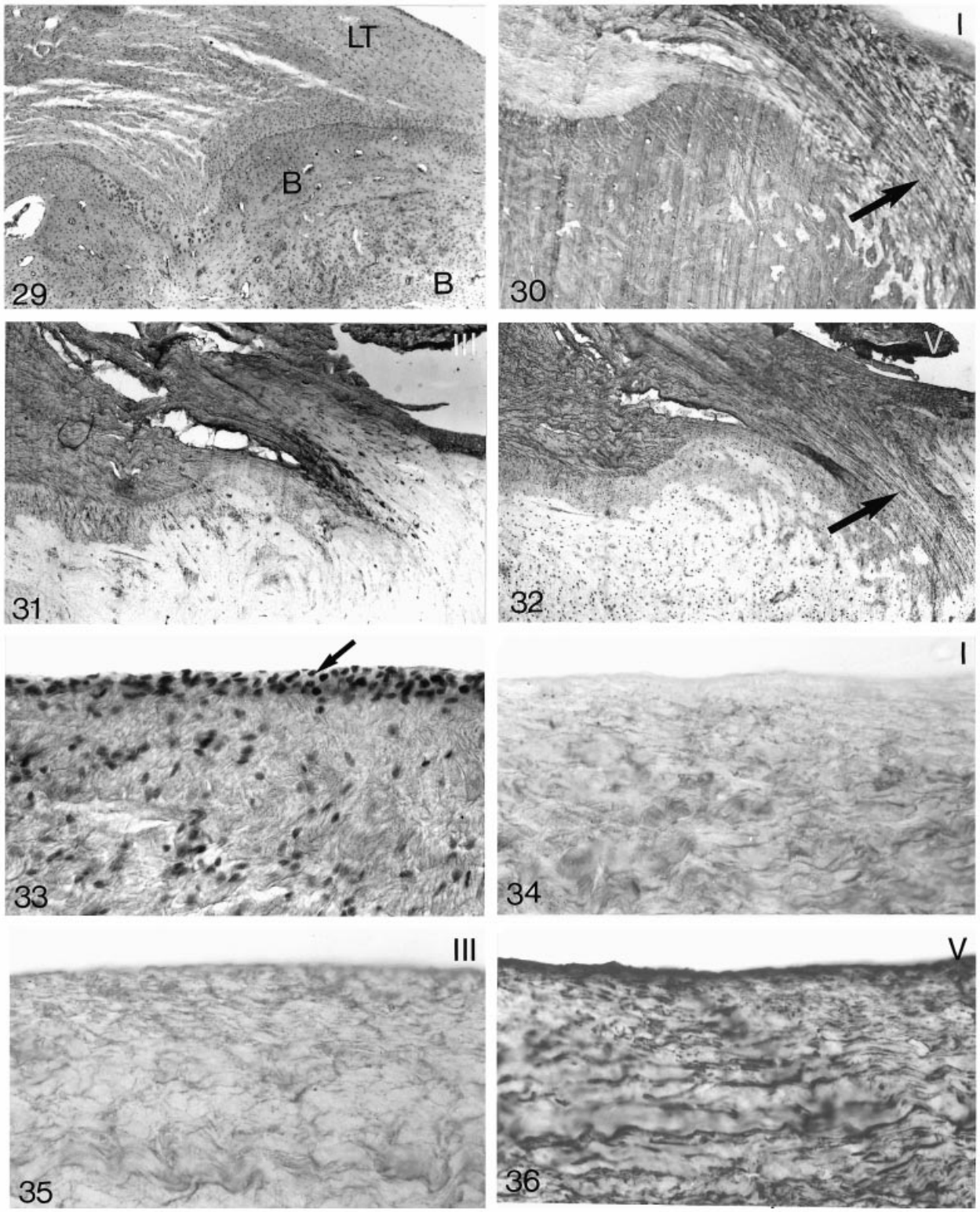
the blood vessels binds antibodies to types III and V collagens strongly, but those to type I collagen less strongly (Figs 14, 16, 17). Immediately postnatal, the ligament is highly cellular (Figs 18, 19) and as it merges into the articular cartilage, the cells are arranged in rows (Fig. 19). Antibodies to types I and V collagens are bound strongly, while those to type III collagen are bound less strongly, except at the insertion and along the surface (Figs 20, 22, 23). The cells in the ligament are expressing the mRNA for type I collagen (Fig. 21).

At 8 mo the rabbit is skeletally mature. The ligamentum teres is attached to the articular cartilage of the head of the femur (Fig. 24). The fibres of the ligament penetrate the cartilage where they bind the type I collagen antibody (Fig. 25). They cannot be distinguished in the preparations in which types II, III and V collagens are localised (Figs 26, 27, 28). The other attachment of the ligament is to the bone on the lip of the acetabulum (Fig. 29). The fibres of the insertion bind types I and V collagen antibodies and penetrate into the bone (Figs 30, 32), but few fibres that contain type III collagen are present at the insertion (Fig. 31). Antibodies to type II collagen are not bound in this region. Fibrocartilage was not seen within the ligamentum teres at any age. The ligament at 2 y resembles the 8 mo ligament in all respects.

The mature ligamentum teres possesses many round cells and is covered by a highly cellular sheath (Fig. 33). The fibres in the main part of the ligament bind antibodies to types I, III and V collagens (Figs 34, 35, 36). The sheath binds antibodies to types III and V collagens only.

DISCUSSION

The stability of the hip joint depends upon the shape of the articular surfaces and also on the soft tissue of the capsule and the ligamentum teres. In this paper we describe the development of the joint and the distribution of the fibrillar collagens from the 17 d fetus to the adult rabbit.



Figs 29–32. Photomicrographs of the insertion of the ligamentum teres (LT) into the bone (B) on the lip of the acetabulum of an 8-mo-old rabbit. Fig. 29. H & E. Fig. 30. Immunohistochemical localisation of type I collagen. Fig. 31. Immunohistochemical localisation of type III collagen. Fig. 32. Immunohistochemical localisation of type V collagen. Note the fibres that bind antibodies to types I and V collagen penetrating the bone (arrows). All × 33.

Figs 33–36. Photomicrographs of a small part of the ligamentum teres of an 8-mo-old rabbit. Fig. 33. The highly cellular sheath (arrow) overlying the dense fibrous tissue of the ligament. (H & E). Fig. 34. Immunohistochemical localisation of type I collagen. Fig. 35. Immunohistochemical localisation of type III collagen. Fig. 36. Immunohistochemical localisation of type V collagen. Note the absence of type I collagen antibody binding in the sheath. All × 260.

Articular cartilage

Cavitation and the development of the joint surfaces is similar to that described previously in the rabbit knee and other mammalian joints including those of man (Gardner & Gray, 1950; Andersen, 1961, 1962; Andersen & Bro-Rasmussen 1961; Mitrovic, 1978; Gardner & O'Rahilly, 1980; Bland & Ashhurst, 1996*a*). The articular cartilage develops from the interzone, that is the chondrogenous and intermediate layers. During cavitation, the cells of the intermediate layer split to form the surface of the articular cartilage of the femoral head and acetabulum. There is no evidence for the death of any cells during cavitation (unpublished observations). In the NZW rabbit cavitation is complete in the hip joint of the 25 d fetus, which is slightly in advance of the knee joint (Bland & Ashhurst, 1996*a*).

Before cavitation, there is no type II collagen in the interzone, but types I, III and V collagens are present. The type V collagen in the chondrogenous layers is pericellular and this feature indicates the region that will become the articular cartilage. In these respects the articular cartilage of the hip joint is identical to that of the knee joint (Bland & Ashhurst, 1996*a*). The further development of the articular surfaces of the femoral head and the acetabulum is similar to that in the knee joint (Bland & Ashhurst, 1996*a*). Throughout life, the chondrocytes are surrounded by types III and V collagens, while type I collagen becomes undetectable in the rabbit. More recently, a similar distribution of types III and V collagens has been found in human and equine articular cartilages (Y. S. Bland, M. A. Bayliss, B. von Rechenberg & D. E. Ashhurst, unpublished observations). Biochemical studies have revealed the heterogeneity of the collagens in articular cartilage (Eyre et al. 1987; Thomas et al. 1994; Wotton & Duance, 1994). The pericellular types III and V (collagens) are probably located in the chondrons (Poole, 1997)

Joint capsule

The joint capsule is very thin and was observed only in the 25 d fetus. Type I collagen is the major collagenous component and is present throughout the tissue, whereas types III and V collagens form networks among the type I fibrils. It was not possible to distinguish the ligaments within the joint capsule.

Ligamentum teres

The ligamentum teres can be distinguished in the rabbit fetus from 17 d. Its origin in the rabbit is the same as in the human fetus (Gardner & Gray, 1950; Andersen, 1962). At the beginning of its development, the ligamentum teres contains types I and V collagens both in the ligament proper and with type III in its insertion into the femur. In the adult ligament a highly cellular sheath which contains only types III and V collagens covers the ligament.

The distribution of the collagens during the development of the ligamentum teres has some similarities with the developing collateral and cruciate ligaments of the rabbit knee joint (Bland & Ashhurst, 1996*b*). Type III collagen is not present in any of the 25 d fetal ligaments, except at the insertion into the cartilage. In the adult type III collagen is present throughout the ligaments, though it is not as widespread as types I and V collagens. The sheaths of both the ligamentum teres and collateral ligaments contain only types III and V collagens (Bland & Ashhurst, 1996*b*). In contrast, in tendons, the major bundles of collagen fibres contain types I and V collagens. Type III is confined to the endotenons and epitenons (Duance et al. 1977; Bland & Ashhurst, 1997). These differences in the collagens of ligaments and tendons are confirmed by biochemical data (Amiel et al. 1984; Watanabe et al. 1994) and are discussed in detail by Bland & Ashhurst (1997).

The ligamentum teres in the adult inserts through fibres of type I collagen into articular cartilage on the head of the femur, but into bone on the lip of the acetabulum. No fibrocartilage was seen in the ligamentum teres. This lack of fibrocartilage distinguishes these attachments from the insertions of the meniscal and cruciate ligaments into bone in the knee joint (Messner, 1997; Messner & Gao, 1998) and of the patellar and other tendons (Benjamin et al. 1986; Evans et al. 1990; Raspanti et al. 1996).

Concluding comments

The development of the rabbit hip joint has many features in common with that of the knee joint, but while the latter is a hinge joint the former is a ball and socket joint. The stability of the hip joint is determined largely by the shape of the bones. The acetabulum is deep and the head of the femur fits it closely. The bones are held in position by the capsule and ligamentum teres.

Abnormalities in the shape of the acetabulum and femoral head cause instability. At birth in man, the acetabulum is shallow and the head of the femur has a low curvature. The depth of the acetabulum and the curvature of the head of the femur develop during postnatal growth. Immediately postnatal this requires

that the joint is abducted and the developing articular surfaces must be held close together by the capsule and ligaments (Carr et al. 1993). In DDH, the acetabulum is too shallow and the head of the femur is too flat, and in addition, there may be generalised joint laxity (Wilkinson, 1963; Carter & Wilkinson, 1964). It is thought that the laxity of the capsule is a major contributory factor (Carr et al. 1993).

Biochemical studies of DDH capsules of patients aged 1–4 y suggest that the amount of type III compared with type I collagen is reduced and changes in cross-linking occur (Skirving et al. 1984), but increased amounts of type III collagen were found in the umbilical cords of newborn DDH babies (Jensen & Fredensborg 1986). There is a similar unresolved discrepancy in values of collagen fibril diameters; Skirving et al. (1984) reported an $\sim 10\,\%$ increase in diameter, whereas Ippolito et al. (1980) found a slight decrease.

The appropriate complement, arrangement and biochemical properties of the component collagens is essential for the proper functioning of all tissues. With increasing information about the soft tissues of the skeletal system it is becoming obvious that each ligament and tendon has its unique complement and arrangement of collagens within it. Abnormalities of the biochemistry, relative proportions of the collagen types or arrangement of these leads to abnormal function as in DDH. Joint hypermobility is now accepted as a syndrome that encompasses various inherited collagen diseases (Child, 1986). It is only by increasing our knowledge of the normal skeletal tissues that the significance of changes found in abnormal tissues will be understood.

REFERENCES

AMIEL D, FRANK C, HARWOOD F, FRONEK J, AKESON W (1984) Tendons and ligaments: a morphological and biochemical comparison. *Journal of Orthopaedic Research* **1**, 257–265.

ANDERSEN H (1961) Histochemical studies on the histogenesis of the knee joint and superior tibio-fibular joint in human foetuses. *Acta Anatomica* **46**, 279–303.

ANDERSEN H (1962) Histochemical studies of the development of the human hip joint. *Acta Anatomica* **48**, 258–292.

ANDERSEN H, BRO-RASMUSSEN F (1961) Histochemical studies on the histogenesis of the joints in human fetuses with special reference to the development of the joint cavities in the hand and foot. *American Journal of Anatomy* **108**, 111–122.

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

BLAND YS, ASHHURST DE (1996*a*) Development and ageing of the articular cartilage of the rabbit knee joint: distribution of the fibrillar collagens. *Anatomy and Embryology* **194**, 607–619.

BLAND YS, ASHHURST DE (1996*b*) Changes in the distribution of fibrillar collagens in the collateral and cruciate ligaments of the rabbit knee joint during fetal and postnatal development. *Histochemical Journal* **28**, 325–334.

BLAND YS, ASHHURST DE (1997) Fetal and postnatal development of the patella, patellar tendon and suprapatella in the rabbit; changes in the distribution of the fibrillar collagens. *Journal of Anatomy* **190**, 327–342.

BLAND YS, CRITCHLOW MA, ASHHURST DE (1991) Digoxigenin as a probe label for in situ hybridization on skeletal tissues. *Histochemical Journal* **23**, 415–418.

CARR AI, JEFFERSON RJ, BENSON MKD (1993) Joint laxity and hip rotation in normal children and in those with congenital dislocation of the hip. *Journal of Bone and Joint Surgery* **75B**, 76–78.

CARTER C, WILKINSON J (1964) Persistent joint laxity and congenital dislocation of the hip. *Journal of Bone and Joint Surgery* **46B**, 40–45.

CHILD AH (1986) Joint hypermobility syndrome; inherited disorder of collagen synthesis. *Journal of Rheumatology* **13**, 239–243.

CRITCHLOW MA, BLAND YS, ASHHURST DE (1995) The expression of collagen mRNAs in normally developing neonatal rabbit long bones and after treatment of neonatal and adult rabbit tibia with transforming growth factor-β2. *Histochemical Journal* **27**, 505–515.

DUANCE VC, RESTALL DJ, BEARD H, BOURNE FJ, BAILEY AJ (1977) The location of three collagen types in skeletal muscle. *FEBS Letters* **79**, 248–252.

ENGESAETER LB, WILSON DJ, NAG D, BENSON MKD (1990) Ultrasound and congenital dislocation of the hip. *Journal of Bone and Joint Surgery* **72B**, 197–201.

EVANS EJ, BENJAMIN M, PEMBERTON DJ (1990) Fibrocartilage in the attachment zones of the quadriceps tendon and patellar ligament of man. *Journal of Anatomy* **171**, 155–162.

EYRE DR, WU JJ, APONE S (1987) A growing family of collagens in articular cartilage: identification of 5 genetically distinct types. *Journal of Rheumatology* **14**, 25–27.

GARDNER E, GRAY DJ (1950) Prenatal development of the human hip joint. *American Journal of Anatomy* **87**, 163–192.

GARDNER E, O'RAHILLY R (1980) The early development of the knee joint in staged human embryos. *Journal of Anatomy* **102**, 289–299.

IPPOLITO E, ISHII Y, PONSETI IV (1980) Histologic, histochemical, and ultrastructural studies of the hip joint capsule and ligamentum teres in congenital dislocation of the hip. *Clinical Orthopaedics and Related Research* **146**, 246–258.

JENSEN BA, FREDENSBORG N (1986) Collagen type III predominance in newborns with congenital dislocation of the hip. *Acta Orthopaedica Scandinavica* **57**, 362–365.

KAVANAGH E, ASHHURST DE (1999) Development and ageing of the articular cartilage of the rabbit knee joint: distribution of biglycan, decorin and matrilin-1. *Journal of Histochemistry and Cytochemistry* **47**, 1603–1615.

MESSNER K (1997) Postnatal development of the cruciate ligament insertions in the rat knee. Morphological evaluation and immunohistochemical study of collagens types I and II. *Acta Anatomica* **160**, 261–268.

MESSNER K, GAO J (1998) The menisci of the knee joint. Anatomical and functional characteristics, and a rationale for clinical treatment. *Journal of Anatomy* **193**, 161–178.

METSÄRANTA M, KUJALA UM, PELLINIEMI L, ÖSTERMAN H, AHO H, VUORIO E (1996) Evidence for insufficient chondrocytic differentiation during repair of full-thickness defects of articular cartilage. *Matrix Biology* **15**, 39–47.

MITROVIC D (1978) Development of the diarthrodial joints in the rat embryo. *American Journal of Anatomy* **151**, 475–486.

PAGE M, HOGG J, ASHHURST DE (1986) The effects of mechanical stability on the macromolecules of the connective

tissue matrices produced during fracture healing. I. The collagens. *Histochemical Journal* **18**, 251–265.

POOLE CA (1997) Articular cartilage chondrons: form, function and failure. *Journal of Anatomy* **191**, 1–13.

RASPANTI M, STROCCHI R, DE PASQUALE V, MARTINI D, MONTANARI C, RUGGIERI A (1996) Structure and ultrastructure of the bone/ligament junction. *Italian Journal of Anatomy and Embryology* **101**, 97–105.

SKIRVING AP, SIMS TJ, BAILEY AJ (1984) Congenital dislocation of the hip: a possible error of collagen metabolism. *Journal of Inherited and Metabolic Diseases* **7**, 27–31.

THOMAS JT, AYAD S, GRANT ME (1994) Cartilage collagens: strategies for the study of their organisation and expression in the extracellular matrix. *Annals of Rheumatic Diseases* **53**, 488–496.

WATANABE M, NOJIMA M, SHIBATA T, HAMADA M (1994) Maturation-related biochemical changes in swine anterior cruciate ligament and tibialis posterior tendon. *Journal of Orthopaedic Research* **12**, 672–682.

WEINSTEIN SL (1998) Developmental hip dysplasia and dislocation. In *Skeletal Growth and Development: Clinical Issues and Basic Science Advances* (ed. Buckwatter JA, Erhlich MG, Sandell LJ, Trippel SB), pp. 449–470. Rosemont, IL: American Academy of Orthopaedic Surgeons.

WILKINSON JA (1963) Prime factors in the etiology of congenital dislocation of the hip. *Journal of Bone and Joint Surgery* **45B**, 268–283.

WOTTON SF, DUANCE VC (1994) Type III collagen in normal human articular cartilage. *Histochemical Journal* **26**, 412–416.