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S. Milz · M. Benjamin · R. Putz

Molecular Parameters Indicating Adaptation to Mechanical Stress in Fibrous Connective Tissue

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Molecular Parameters Indicating Adaptation to Mechanical Stress in Fibrous Connective Tissue

With 15 Figures and 13 Tables

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1 Introduction

1.1 General Remarks

The connective and supportive tissues constitute a considerable amount of the biomass in human and animal organisms. Characteristically, the osseous, cartilaginous, and fibrous tissues each connect a vital part (cells) with a non-vital part, i.e., extracellular matrix (ECM). The composition of the ECM constitutes the mechanical qualities of the respective tissue.

The functional role of the bone and cartilage tissues is exhaustively discussed in the relevant literature. Whereas bone tissue provides the static and dynamic stability of the system as a whole, cartilage tissue accounts for the power transfer between bones. The articular cartilage insures a fairly friction free mobility of skeletal elements; likewise, cartilage interposed between skeletal elements allows mobility due to its reversible deformability. Under both static and dynamic conditions, the powers transferred are remarkably large, while the ensuing mechanical force on various tissue zones varies.

Tight connective tissue, especially muscle tendons and ligaments, are also part of the skeletal power transfer system. facilitating the transfer of tensile forces. The tendons of the locomotor system serve the purpose of transferring muscular energy to those skeletal elements to which they are attached. In this function, they are referred to as tensile tendons (“*Zugsehnen*”). In several body parts, however, tendons appear in a peculiar mechanical situation: they pass around so-called hypomochlia, i.e., bony pulleys. Hence, these tendons are referred to as wrap-around tendons (“*Gleitsehnen*”). On account of this forced change of direction, they are exposed to compressive as well as to tensile powers. The tendon’s metamorphosis to a fibrocartilaginous phenotype, which occurs at such positions, was described relatively early (Ploetz 1938). This fibrocartilage can be distinguished from that which typically occurs at the tendons’ bony attachments (Vogel and Koob 1989; Benjamin and Ralphs 1995; Rufai et al. 1996).

Numerous long tendons of the extremities are diverted by hypomochlia. Such an alteration of the direction of pull may occur either irrespective of the position of the limb—e.g., in the case of the m. fibulares or the tendons of the foot flexors that run behind the malleoli—or it may occur only if the joint is in a certain position, which is the case with certain extensor tendons of the hand.

The cells of the tight (collagenous) connective tissue possess spindle-shaped nuclei. Generally, their processes are long and have thin ends. Hereafter, they shall be referred to as fibroblasts, rather than as the more exact “tenocytes” or “fibrocytes.”

Cell morphology in these areas of diversion differs from that in tensile tendons, and the distribution of collagens and glycosaminoglycans differ in various ways (Berenson et al. 1996).

The occurrence of fibrocartilage in tendons and ligaments has been described both as sesamoid fibrocartilage, in the area of bony pulleys, and as so-called enthesis fibrocartilage at the bony attachments (Benjamin and Ralphs 1995, 1997, 1998). Distinct sesamoid fibrocartilage occurs more frequently in tendons than in ligaments. A remarkable instance of fibrocartilage in a ligament is the case of the annular ligament of the radius (Benjamin et al. 1986). This differentiation toward fibrocartilage allows the tendon or ligament to permanently resist the local compressive force at the bony pulley.

Fibrocartilaginous tissue is avascular. Its water content is high, which indicates a special composition of the ECM (Benjamin and Ralphs 1995 1997; Vogel 1995; Berenson et al. 1996). The ECM contains a number of molecules that are typical of hyaline articular cartilage, especially sulfated glycosaminoglycans (GAGs), aggrecan, and type II collagen (Vogel 1995).

The aim of the present study is to contribute to the understanding of the mutual relationship between the mechanical situation of tendons and ligaments and their inner structure. The groundwork is laid in several of the authors' publications and this study attempts to bring these to coherence in order to take a further step toward a relative quantification of the ECM's restructuring processes.

1.2 Adaptation of Connective and Supportive Tissues to Their Respective Functions

Due to the evident mechanical significance of connective and supportive tissues, the functional mechanisms that insure the efficiency of the tissues, in particular of the cartilaginous and osseous tissues, have been the object of much research in the last 150 years. Numerous studies have described the biological parameters that are subject to change in the course of adapting to the mechanical function, and which thereby allow statements about what has been called the tissue's "loading history" (Kummer 1959, Pauwels 1960, 1965, 1976; Carter 1984, 1987; Carter et al. 1989, 1991).

The ability of tendons to adapt to tensile and local compressive force was described surprisingly early. The term wrap-around tendon ("*Gleitsehne*"), introduced at an early stage (Hyrtl 1863, Testut and Jacob 1905), refers to that section of a muscular tendon, which is distracted by a bony pulley, i.e., a protruding bone or a retinacle. The remaining part of the tendon—subject exclusively to tensile force—is referred to as tensile tendon. Ploetz (1938) was able to demonstrate by animal experiments that the tendon changes its micromorphological appearance in regions of compressive force. Corresponding to functional adaptation, zones of restructuring appear which are, however, regionally restricted and vanish again when the original situation, exclusively tensile force, is reestablished.

In those areas where their direction of pull is altered by bony pulleys, tendons (i.e., wrap-around tendons) are frequently avascular and show fibrocartilaginous metamorphoses (Ploetz 1938; Tillmann 1978; Benjamin and Ralphs 1995, 1997;

Vogel 1995; Berenson et al. 1996). However, this statement is somewhat simplistic. One has to take into account the continuous spectrum of different phenotypes of tissue between the areas of tensile tendon and wrap-around tendon, and even more so the area of attachment (Benjamin et al. 1995). In the respective extremes mechanical situation, the histomorphological conditions are to a great extent clear. Fibroblasts from tensile tendons are longer and exhibit a characteristic stellate shape. They are connected with adjacent cells by distinct extensive processes (McNeilly et al. 1996). In contrast, fibrocartilage cells are relatively large, shaped rotund or oval, with few processes.

Beyond the cell level, differences may also be found in the ECM. The most important components here are the heteropolysaccharides, such as the GAGs and the proteoglycans (PGs), as well as the fibrous proteins that occur as collagens of various types. These groups of molecules form the most important components of the basic substance of all animal and human connective and supportive tissues (Lehninger et al. 1994). The ECM typical of fibrocartilage frequently contains molecules that are typical of articular cartilage. Among those in particular are the sulfated GAGs, aggrecan—a proteoglycan—and type II collagen (Vogel 1995). By their functional interplay, these molecules insure the tissue's resistance against local pressure.

The notion of a functional adaptation to a mechanical force is the basis for a number of studies hitherto carried out on the osseous and cartilaginous parts of the passive locomotor system. These have made possible basic statements referring to the regular adaptation of tissue to the predominant force, which happens according to the "form follows function" principle. The statements were consequently placed under a theory known today as "*Wolff's Law*" (grounded primarily on the research carried out by Meyer 1867, Wolff 1870, 1884, 1892; Roux 1885, 1896) and "causal histogenesis" (grounded primarily on research carried out by Pauwels 1949, 1960, 1963, 1965, 1974, 1976 and Kummer 1959, 1962, 1968, 1978, 1980, followed among others by Peters 1975, Milz and Putz 1994, Milz et al. 1995). Tillmann (1978) worked on the problem with regard to the articular cartilage. In comparison to that, relatively few studies, however, are concerned with the adaptation processes in tendons and ligaments (Ploetz 1938; Tillmann 1978; Benjamin and Evans 1990; Milz et al. 1998, 1999). From the studies of Kummer (1959, 1962, 1978) and Pauwels (1965, 1976) it has already been pointed out that a certain mechanical surrounding proves favorable to the shaping of tendons and ligaments; yet it is mostly from the results of the investigations of Ploetz (1938) that their argument rests on.

It was left to Benjamin et al. (1986, 1990) to return to these questions and to study the morphological changes observable in various tendons and ligaments regarding their mechanical background. Despite the mentioned approaches, however, a summarizing description of the regularity of the functional adaptation process of the tendinous and ligamentous tissues on a molecular level is still absent. What seems to be established is that both the histomorphological tissue structure and the molecular composition of ECM are directly correlated with the local mechanical

force. Remaining unclear are, first, the sequence in which various characteristic molecules of the ECM occur and, second, the way in which these molecules are regionally distributed in relation to mechanical load.

1.3 Parameters of Functional Adaptation

Various morphological parameters have long been identified as indicators of functional adaptation. They are either quantifying parameters—such as volume and distribution of certain tissues—or qualifying characterizations of tissue components.

Among the first group rank, e.g., the thickness distributions of the subchondral mineralization zone and the hyaline articular cartilage, which are regarded as different morphological parameters of compressive force (Pauwels 1965; Kummer 1968; Tillmann 1978; Müller-Gerbl et al. 1989, 1990, 1992; Milz and Putz 1994, Milz et al. 1995, Eckstein 1996): The distribution of the local (X-ray) density of the subchondral mineralization zone reflects the "prevailing" mechanical force (Pauwels 1965) exerted on a joint. Other force-dependent parameters are the local thickness and density of trabeculae, the shape of trabeculae, the degree of interconnection and porosity, the layout of osteons, and the local thickness and density of the corticalis of the tubular bones.

Analogously, various dimensions of a tendon or ligament, such as the cross-section, diameter, arrangement of fibrous fascicles, or texture, may function as parameters of force. This confronts us with the question of the molecular parameters of mechanical load and of the indicators of a molecular "loading history." This is of particular interest considering the appearance of single components of ECM in relation to a certain amount of mechanical load.

Understandably, a knowledge of the physiological adaptation reactions leads toward an appreciation of pathological processes insofar as these may be attributed to changes of the molecular composition. It is useful here to take into consideration the various molecular compounds' topography within the tissue, since different reactions of one and the same tissue in the course of a disease may be explained by it.

2
Aim of the Study

2.1
Fundamental Considerations

The declared aim of the present study is to offer an approach to understanding regular changes of the ECM of tendons and ligaments that occur in human connective tissue as a result of the molecular adaptation—which is also discernible by other morphological features—to the mechanical function. The anatomical structures selected for this particular purpose are tendons and ligaments whose mechanical situation is so unambiguous that the patterns of molecular distribution may be directly equated with them. Since each of the cases presented is based on a hypothesis, the analysis of the adaptation process follows the principles of an experimental approach in the sense of an '*experimentum naturae*.'

One particular aim is to clarify whether the occurrence of single types of molecules is dependent on a certain amount of mechanical load. Moreover, we want to use the findings of the various tissues' molecular anatomy to draw conclusions as to the clinical relevance of the restructuring and adapting processes. This appears to be of especial interest since certain diseases, particularly rheumatic ones, are related to specific "target" molecules that occur in tendons under certain conditions.

In order to be able to make a statement about the influence of the degree of compressive force, we have deliberately investigated such areas of connective tissue in which only weak forces are at work together with such areas where the compressive and tensile forces are very strong. Thus, we have been able to rank tissue according to the maximal compressive and tensional load exerted on it, and thus to draw conclusions concerning the general reaction mechanisms of the functional adaptation reactions of the extracellular matrix on a molecular level. The result is a "functional" ranking of ECM molecules, which allows a regular insight into the process that remodels a purely tensile tendon into a highly specialized fibrocartilaginous tissue capable of sustaining compressive as well as tensional forces.

Since there exists a series of different animal experiments concerning this topic, the declared aim of the present study is to investigate the situation in the human body. The tissues have been selected in order to ensure that among the selected samples are regions subjected to traction forces alone, as well as regions subjected to intermittent traction and compression forces.

2.2
Structure of the Present Study

The present study falls into two parts. First, the mechanical situation of the area of alteration of the direction of pull and of attachment will be compared to the structural composition in the case of five select tendons and respective ligaments.

Then, the reactions of the investigated structures will be ranked according to the degree of mechanical load.

It has been known for some time that there exists a small joint between the dens axis and the transverse ligament of the atlas. However, it is disputed whether under regular physiological circumstances a transmission of force takes place there, or whether the ligament only serves the purpose of occasionally preventing extreme positions of the upper cervical spine with respect to the joints of the head. In the first case, it might be expected that the ECM's composition is typically cartilaginous, with the extension of the areas in which such a metamorphosis is discernible allowing conclusions to the type, degree, and duration of the strain. However, if one assumes that the ligament only serves as the transverse connection of the atlas' massae laterales, the ECM ought to consist only of material typical of traction tendons.

The question of a possible extension of a fibrocartilaginous area may be answered with the help of an immunohistochemical examination, by which the topographical distribution of the various molecules within the tendon is determined. The pattern thus established should be expected to provide insight as to the quality of the regional adaptation and, moreover, to allow a quantifying interpretation. As in many biological systems, however, a perfectly clean expression of the one or the other type should not be expected.

We shall start with an analysis of the extensor tendons of the toes (PIP area): Their redirection is not highly marked; moreover, they are in direct contact with the articular space in the region of redirection. We shall continue with an analysis of the extensor tendons of the fingers (MCP area), which are redirected to a far greater degree and thus subjected to higher compression tension.

The transverse ligament of the atlas serves as an example of high intermittent compressions exerted on a ligament by a small object.

The transverse ligament of the acetabulum connects the two horns of the lunate articular surface. Due to the incongruence of the hip joint, the femoral head can be expected to exert some compressive load, distributed over a wide area of the midpart of the ligament.

The tendon of the superior oblique muscle is especially strongly redirected in the area of the trochlea and receives the ensuing compressive strain only on a relatively small part of its surface. However, the active muscle force is relatively small.

2.3 Questions

From discussions presented in the preceding sections, the following questions have resulted, to be answered in the course of various subprojects carried out on select anatomical structures:

- Does the molecular composition of the various fibrous connective tissues, such as tendons and ligaments, follow rules that may be adduced in connection with the respective tissue's mechanical function?

- Is there a hierarchy of certain molecules in the ECM that correlates with the degree and duration of the mechanical load?
- Is it possible to predict the occurrence of certain ECM components depending on the intensity of mechanical load?

Further detailed questions result from the particular functional situation of the analyzed tissues and their ensuing clinical significance.

3 Anatomical Structures Investigated

The tendons and ligaments investigated will first be presented in their topographical and functional context. Biomechanical data relevant in connection with the detailed questions will also be mentioned.

3.1 Extensor Tendons of the Toes in the Region of the Proximal Interphalangeal Joint

The tendons of the long extensor of the toes come from the dorsum of the foot, cross the metatarsophalangeal joints and enter the toes' dorsal aponeuroses. During the walking cycle these tendons participate in the transmission of load to the forefoot. Therefore, it may be assumed that in the human body they are flexed more frequently and with more force than the tendons of the fingers (Fig. 1).

On account of the course of the extensor tendons, a pronounced development of fibrocartilage should be expected at those sites where the tendons cross the toe joints, which serve as bony pulleys. But it is not yet known if this is really the case in the area of the PIP joint of the extensor tendons of the toes. The question is of particular interest since the extensor tendon here forms part of the dorsal articular capsule and therefore has direct contact with the articular cavity—apart from the fact that in case of an extension of the flexed toes this tendon is pressed against the upper edge of the distal end of the proximal phalanx.

The situation in the case of the proximal interphalangeal joint (PIP) joint of the 1st toe slightly differs from that of the other toe joints. Here, the extensor tendon is only pressed against the basic phalanx in case of flexion.

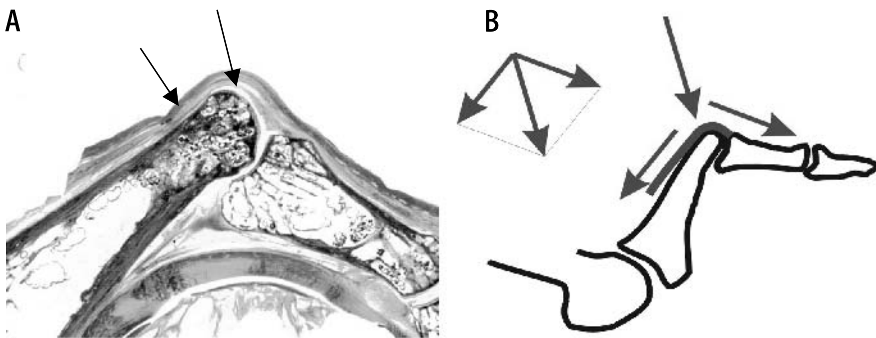


Fig. 1A, B Situation of the extensor tendon at the pulley created by the PIP-joint of a second toe. **A** Anatomical section. The extensor tendon and its intimate contact with the intermediate phalanx are clearly visible (*arrows*). **B** Schematic drawing of the mechanical situation at the pulley, explaining the resulting compressive force at the deep aspect of the tendon

The aim of this subproject is to answer the question whether, besides in the enthesis, i.e., the bony attachment, a second "sesamoid" fibrocartilage may be demonstrated in the deep surface of the extensor tendons in the toes. (In a similar form, such a "sesamoid" fibrocartilage can be found in the extensor tendons of the fingers.) We shall also investigate whether this fibrocartilage exists in all toes. Moreover, we hope to determine the extent to which the immunohistochemical labeling of the enthesis and of the "sesamoid" fibrocartilage—if present—may be compared to the labeling patterns of the finger extensor tendons.

3.2
Extensor Tendons of the Hand in the Region of the MCP Joint

The dorsal tendons of the fingers are nearly straight if the fingers are extended, whereas they are redirected at the heads of the phalanges and of the metacarpalia when the fingers are flexed (Fig. 2).

Previous studies have shown that there exists a sesamoid fibrocartilage on the undersurface of the extensor tendon in the finger where the central pull of the tendon crosses the PIP joint (Benjamin et al. 1993; Lewis et al 1998). At this site, the tendon is only subject to compressive force when the fingers are flexed. The quality of the fibrocartilage differs between various fingers and various individuals. The fibrocartilage of the index tendon shows particularly marked characteristics of cartilage; so does the tendon in cases of a flexion contracture of any finger. In the proximal direction, the torque of the tendon is considerably higher than in PIP joints due to the greater length of the proximal phalanx (Littler and Thompson 1987).

Consequently, it may be expected that at this site the tendon is subjected to a greater compressive strain as well. Moreover, the interphalangeal joints are kept at a relatively constant degree of flexion in the course of numerous finely tuned

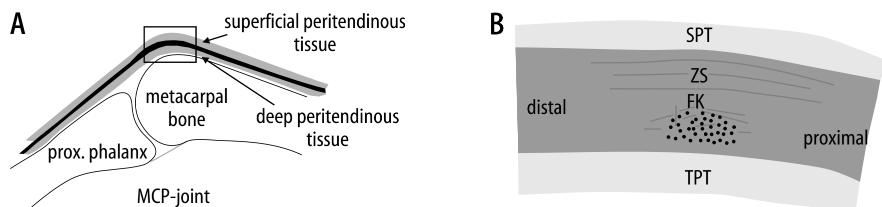


Fig. 2A, B Topographical situation of the index extensor tendon at its pulley. **A** The extensor tendon is surrounded by superficial and deep peritendinous tissue and passes over the MCP-joint without any direct contact with the joint cavity. During flexion the tendon is pressed against the proximal phalanx. In the region of contact (*rectangular marking*) there is a sesamoid fibrocartilage present within the extensor tendon. **B** Detail of the region of contact in **A**. The sesamoid fibrocartilage (*FK*) is found underneath the part of the extensor tendon (*TT*) with most tensile load. The tendon is surrounded by superficial (*SPT*) and deep (*TPT*) peritendinous tissue

movements of the hand—e.g., when the fingertips are raised only a few centimeters, such as in typing. Movement occurs mainly in the MCP joints.

The extensor tendons of the fingers—as opposed to those in the toes—have no direct contact with the articular cavity. Instead, they are separated from the synovial space of the joint by the so-called peritendinous tissue, which, from a phylogenetic point of view, represents a part of a so-called intertendinous fascia (Landsmeer 1955). This relatively undifferentiated connective tissue supposedly represents a rudiment of the original tendon blastema (Lucien 1947).

The purpose of this subproject is to compare the immunohistochemical profile of the extensor tendons at the level of the MCP joints to that of the PIP joints (Lewis et al. 1998). In this context, it is of interest to find whether there exists a fibrocartilaginous region in the tendon comparable to that found associated with the PIP joints, and whether it is located at the spectrum's fibrous or cartilaginous end.

3.3 Transverse Ligament of the Atlas

It is well known that there is a small synovial joint between the transverse ligament of the atlas and the dens of the axis (Fig. 3). In recent times, especially Stofft (1968) and Saldinger et al. (1990) have investigated the morphology and internal structure of the ligament. Where it presses against the bone, its articular surface is described as fibrocartilaginous, whereas adjacent portions of the ligament are purely fibrous (Williams et al. 1995).

Hitherto, the question has remained open whether this joint, whose dorsal portion the transverse ligament represents, is subject to a perpetual compressive strain or whether the ligament is only strained in the course of preventing an extreme

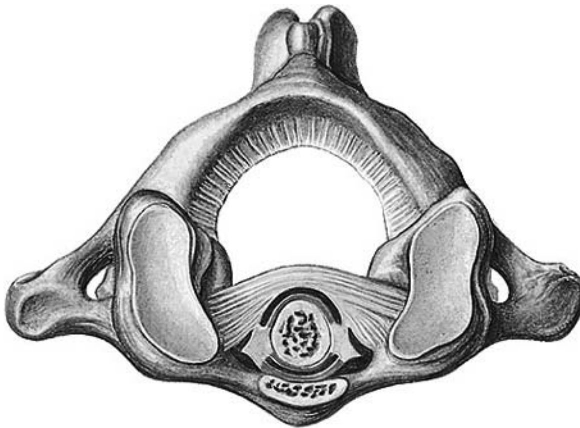


Fig. 3 Topographical situation of the transverse ligament of the atlas (according to Sobotta 1993). The ligament is attached on both sides to the massa lateralis of the atlas (tuberculum lig. transversi atlantis) and it is separated from the dens of axis by a synovial joint

position of the joint. This question is of importance, since alterations or ruptures of this ligament may result in serious neurological problems. A pathological alteration of the ligament as a consequence of rheumatoid arthritis is frequently reported (Lipson 1977; Dastur 1979; Krantz 1980; Dvorak et al. 1988; Zeidman and Ducker 1994: Constantin and Bouteiller 1998; Fujiwara et al. 1998; Rawlins et al. 1998).

If, however, one assumes that the transverse ligament of the atlas represents a girder of the atlas, one should expect it to be composed of mostly tension-proof material. Of course, one will have to take into account the alignment of the fiber fascicles and the geometrical layout of the ligament as a whole with regard to the framework of the atlas.

An examination of the molecular composition of the ECM can contribute to a clarification of this question, since a typically cartilaginous composition, as opposed to a rather fibrous one, would signify that the ligament is under stronger and more frequent strain than would be expected in the case of a pure supportive unit for extreme positions of the joint.

Therefore, the aim of this subproject is to establish the immunohistochemical profile of the various sectors of the transverse ligament of the atlas continually from one bony attachment (enthesis) to the opposing one in order to be able to answer the question as to the degree of local compressive stress exerted by the dens axis.

3.4 The Transverse Ligament of the Acetabulum

The transverse ligament of the acetabulum is a fibrous ligament of about 3–4 cm length, which crosses the acetabular notch and whose upper edge represents a prolongation of the fibrocartilaginous labrum acetabulare (Fig. 4). Thus, it is part of the supportive unit of the hip joint that deepens the socket and widens the articular surface. (Williams et al. 1995).

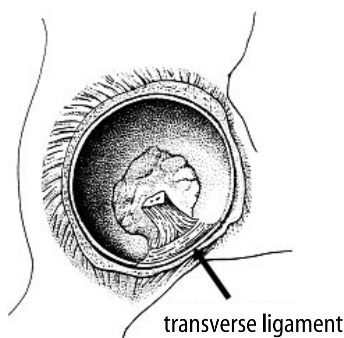


Fig. 4 Topographical situation of the transverse ligament of the acetabulum. The ligament connects both horns of the joint surface (facies lunata; *left side*: dorsal, *right side*: ventral)

Presupposing that the horns of the lunate articular surface of the acetabulum are forced apart if the joint bears load, we assume that the ligament should tighten and possibly stretch, which would inevitably force it against the femoral head. If this mechanical situation is indeed the case, the transverse ligament of the acetabulum ought to show certain features similar to tendons and ligaments whose direction of pull is altered by a bony pulley. This has not yet been demonstrated.

Biomechanical experiments carried out on isolated hip joints have led to the assumption that in case of load bearing of the hip joint, the transverse ligament of the acetabulum can serve as a bridle for the dorsal and ventral portions of the lunate articular surface of the acetabulum (Löhe et al. 1994, Lazennec et al. 1997, Vandenbussche et al. 1999). An inherent limiting factor of such biomechanical studies, however, is the fact that the samples to be investigated have to be fixed artificially, that is non-physiologically, thus making it impossible to simulate all effective muscular pulls.

The ligament is supposed to exert force on both horns of the lunate articular surface of the acetabulum and to limit their drifting apart in the course of load transmission in the acetabulum (Lazennec et al. 1997, Vandenbussche et al. 1999). If isolated human hip joints are subjected to a compressive force of 2,800 N in a material testing device, the acetabular notch will widen about 3.2% (Löhe et al. 1994) or 43 µm (average value of different individuals; Vandenbussche et al. 1999) even where the transverse ligament of the acetabulum is intact. If the transverse ligament of the acetabulum is severed, a compressive force of 1,000 N in the hip joint is sufficient to ensure that the horns of the lunate articular surface of the acetabulum widen more than when the ligament is intact. Any widening of the acetabular notch must necessarily lead to a tightening, i.e., stretching, of the ligament, by which the compressive force with which the ligament is pressed against the femoral head, is increased. A further increase of compressive strain may also be a consequence of the non-congruence of the femoral head and the acetabulum, which manifests itself as load-depending variations in the local width of the articular space (von Eisenhart-Rothe et al. 1997; Eckstein et al. 1997). This means that in the case of higher load bearing, and a corresponding position of the hip joint, the two horns of the lunate articular surface of the acetabulum should be forced apart even farther.

One problem with the studies hitherto mentioned, is the fact that these experiments were carried out on isolated hip joints. In such an experimental setup, the biomechanical situation significantly differs from that encountered in living individuals. Differences exist with regard to the process of walking and standing and with regard to duration and dynamics of the effective forces. In the experiment, the biomechanical situation therefore differs from the natural circumstances not only with regard to the way the joint parts were fixed in the material testing device, but also because of the complete absence of any muscular tensile forces upon the single skeletal elements. Due to such inevitable problems in the course of the simulation of a normal biomechanical situation with isolated skeletal elements, we cannot yet say with certitude whether the transverse ligament of the acetabulum is subject to a local compressive force in the living individual or not.

Also in this subproject the question arises whether the transverse ligament of the acetabulum is subject only to an intermittent tensile force due to a separation of the horns of the lunate articular surface of the acetabulum when the hip joint is loaded due to the incongruence of the hip joint's articulating surfaces, or whether it is not also subject to a local compressive force in the area of its obtuse redirection by the femoral head. In this case, it would have to be expected that its molecular composition correspond to that of other tendons and ligaments whose direction of pull is also altered by a hypomochlion. There should be fibrocartilaginous restructuring zones and characteristic marker molecules, such as aggrecan and type II collagen, demonstrable.

3.5
Tendon and Trochlea of the Superior Oblique Muscle

On its way to the eyeball, the tendon of the superior oblique muscle is redirected in an acute angle by the trochlea, which is attached to the medial wall of the orbit (Sieglbauer 1940, Sevel 1986). The anatomical and biomechanical findings reported allow the conclusion that in the area of its redirection the tendon is subject to a force, which is absolutely low, yet constantly affecting it.

The oblique tendon is in a very interesting biomechanical situation. By its redirection at an acute angle, it is subject to a relatively high compressive force in the contact area even if the muscular force is low. This leads to the conclusion that the tendon as well as the trochlea is subject to a load that is significant in relation to the smallness of the involved structures (Fig. 5).

Biomechanical data (Robinson 1975; Collins et al. 1981) show that the external eye muscles are capable of producing peak forces of 40–100 g. In healthy probands, however, the physiological maximal values are usually 16–20 g (Rosenbaum and

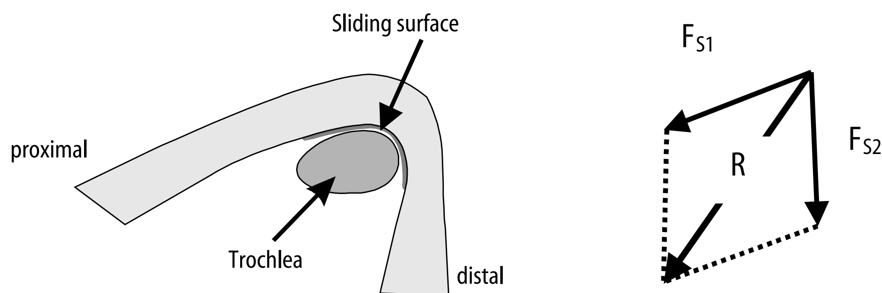


Fig. 5 Schematic drawing showing the topographical situation of the tendon of m. obliquus bulbi superior. The tendon (*proximal* part) follows the medial wall of the orbit until an abrupt change of the direction of pull occurs at the trochlea, which acts as a pulley. The average angle between the proximal and distal tendon is 55 degrees. *FS1* is force transmitted by the proximal part of the tendon. *FS2* is force transmitted by the distal part of the tendon. *R* is the resulting compressive force on the trochlea

Myer 1980), 16–28 g (Tian and Lennerstrand 1994), and 17–40 g (Robinson 1975). It is remarkable to notice that Collins et al (1981) classify as paretic those muscles whose maximum possible (i.e., tetanic) contraction force is less than 45 g.

The varying statements about the height of the physiologically occurring peak forces may be related to the fact that the measuring took place either at the end of the respective movement of the eye (Rosenbaum and Myer 1980) or while the eyeball was in a fixed position (Collins et al. 1981). The external eye muscles must produce forces of such degrees since the tissue surrounding the eyeball shows a certain stiffness that has to be overcome during movement while the tissue is deformed. Furthermore, several external eye muscles are activated at one time and thus operate antagonistically. Collins et al. (1981) have determined that due to the stiffness of the tissue, depending on the direction of movement, the necessary force to turn the eyeball for one degree is 0.8–1.7 g. Since all of these data have been obtained under almost static conditions, one has to presume that far higher maximal forces are to be expected with rapid movements of the eyeball, even if these are only effective for a very brief period.

For adducting and elevating the eye, the superior oblique muscle has to produce force of 17.4 g. The tendon is pulled through the loop of the trochlea for about 5.6 mm. These figures are but insignificantly lower in isolated elevation of the eye (12.7 g, 4.1 mm; Robinson 1975): Forces up to 38 g and an excursion of the tendon of 6 mm have been reported in patients suffering from strabismus (Simonsz et al 1992). Taking into account that these data have been obtained for a tendon that is redirected at a very acute angle (average 55°), it is understandable that a pronounced compressive strain is exerted on the tendon by the hypomochlion. The increased compressive strain on the gliding/articulating lower surface of the tendon ought to cause a functional adaptation of the tissue in the form of a thin fibrocartilaginous zone.

In the relevant literature, the trochlea is described as partly cartilaginous (Hyrtl 1863, Sevel 1986, Schiebler and Schmidt 1987) or as fibrocartilaginous (Testut and Jacob 1905, Sieglbauer 1940, Lang and Wachsmuth 1979, Williams et al. 1995). However, up to this day no data are obtainable concerning the molecular composition of the trochlea or of the tendon that crosses it. This is all the more surprising since a loss of function in this region impairs, among other things, the patient's ability to elevate and adduct the eye, a condition that has long been known as Brown's syndrome (Brown 1950). It is remarkable in this context that a connection between the occurrence of Brown's syndrome and rheumatoid arthritis is reported; further investigation of this apparent coincidence has not yet been undertaken.

The aim of this subproject is to test a hypothesis that allows a new approach to a problem not yet described in the literature. We assume that the tendon of the superior oblique muscle is subjected to a continuous intermittent compressive force in the region of its redirection. Even though the absolute active force is relatively low, we expect that the tendon, as a result of the specific mechanical stress to which it is subjected, will show at least some of the characteristics known from other tendons and ligaments whose direction of pull is altered by a bony pulley.

Here, too, traces of sesamoid fibrocartilage should be discernible, whose cellular morphology should at least be cartilage-like; aggrecan and type II collagen should be demonstrable in the ECM as part of the characteristic molecular composition (Vogel 1995; Berenson et al. 1996; Waggett et al. 1998). These molecules should not only be expected to occur in that region where the tendon is redirected, but also in the trochlea itself, since it is subjected to the same compressive forces as the tendon. The molecular profile of the tendon of the superior oblique muscle in the region where it is redirected and the profile of the trochlea itself shall be established in more detail.

4 Materials and Methods

4.1 Materials

4.1.1 Extensor Tendons of the Toes

The extensor tendons in the region of the PIP joint of all five toes of six forefeet were investigated. The feet were usually removed from unfixed cadavers within 48 h postmortem. In the case of the 1st toe, the examination was restricted to the IP joint. The donors were of both sexes and ranged from 42 to 80 years (Table 1).

The removed IP joints were fixed in 90% methanol at 4°C and were stored at −20°C until use. The extensor tendon, together with its bony attachment (enthesis) was dissected from the articular capsule of the IP joint of the 1st toe and from the PIP joint of the 2nd through 5th toes. None of the specimens showed any macroscopically discernible pathological alterations of the tendon or of the toe as a whole. It was ensured with particular attention that no pressure sores of the skin, such as clavi (i.e., horny skin), etc., were present above the IP joint. Besides, no toes were removed from feet with hallux valgus and/or metatarsus and/or digitus (secundus through quintus) superductus. The decalcified specimens were infiltrated with 5% sucrose solution in PBS for 12 h, they were cryosectioned at 12 μm with the aid of a cryomicrotome.

Table 1 Details of the IP joints investigated

Specimen	Sex	Age (years)	Removal time postmortem	Medical history
Donor 1	Male	42	60 h	Unknown
Donor 2	Female	71	27 h	Unknown
Donor 3	Male	68	24 h	Unknown
Donor 4	Male	68	24 h	Unknown
Donor 5	Female	73	13 h	Unknown
Donor 6	Female	80	5 h	Unknown

4.1.2 Extensor Tendons of the Human Hand in the Region of the MCP Joint

Extensor tendons were taken from all fingers of six donors (ages 39–85 years) in the region overlying the MCP joint (Table 2). In the thumb, the tendon of m. extensor pollicis longus was removed, whereas in the fingers (index, middle, ring, and little), the tendon of m. extensor digitorum was removed. In the case of each index and little finger, care was taken to include parts of the tendons of m. extensor indicis and of m. extensor digiti minimi in the sampled tissue.

Table 2 Details of the extensor tendons investigated at MCP-joint level

Specimen	Sex	Age (years)	Removal time postmortem	Medical history
Donor 1	Male	83	<48 h	Unknown
Donor 2	Male	49	<48 h	Cirrhosis of the liver
Donor 3	Female	85	<48 h	Unknown
Donor 4	Female	85	<48 h	Paralysis with spastic flexion of the MCP joint
Donor 5	Female	81	<48 h	Ovarian carcinoma
Donor 6	Female	39	<48 h	Suicide

None of the removed tendons showed any macroscopically discernible degenerative alterations; all specimens were removed within 48 h postmortem. As with the toes, care was taken that no macroscopically discernible alterations of the fingers and/or of the carpi were present. If the necropsy suggested any suspicion of a rheumatic disease, no specimens were removed. The only pathoanatomical exception was a case of paralysis with spastic flexion of MCP joint; material of this cadaver was taken into the examination on account of the biomechanically relevant situation. The tissue samples were fixed in 90% methanol at 4°C for 24 h minimum and then stored in an identical solution at −20°C.

4.1.3 The Transverse Ligament of the Atlas

Transverse ligaments of the atlas were removed from 13 unfixed human cadavers (both sexes, ages 61–93 years) within 48 h postmortem (Table 3) and fixed in 90% methanol at 4°C for 24 h. The specimens thus obtained were stored in methanol at −20°C until final use.

In seven cadavers, the entire transverse ligament of the atlas was removed including its bony attachment (enthesis). For this purpose, a block of 8 cm × 8 cm × 12 cm, consisting of portions of the occipital bone and the first three cervical vertebrae, was removed immediately after the opening of the skull and the removal of the brain and brain stem. In this block, the cervical medulla was removed from the spinal canal, which made it easier to fix the dura mater, and the ligamentous structures underlying it, in methanol. The fine preparation of the transverse ligament of the atlas was carried out with the aid of a dissecting magnifier after the tissues had been fixed in methanol (90% at 4°C for 24 h; further storage at −20°C). In the remaining six bodies, only the central portion of the ligament, which is articulated immediately with the dens of axis, was removed. This was accomplished through the foramen magnum, after the skull had been opened and the brain removed.

Table 3 Details of the transverse ligaments of the atlas

Specimen	Sex	Age (years)	Removal time postmortem	Medical history	Part of ligament investigated
Donor 1	Female	90	<48 h	Unknown	Central part
Donor 2	Male	91	<48 h	Unknown	Central part
Donor 3	Female	85	<48 h	Unknown	Central part
Donor 4	Female	81	<48 h	Unknown	Central part
Donor 5	Male	82	<48 h	Cancer	Central part
Donor 6	Female	93	<48 h	Unknown	Central part
Donor 7	Female	68	<48 h	Unknown	Entire ligament
Donor 8	Female	87	<48 h	Unknown	Entire ligament
Donor 9	Male	75	<48 h	Unknown	Entire ligament
Donor 10	Female	76	<48 h	Breast cancer	Entire ligament
Donor 11	Male	65	<48 h	Unknown	Entire ligament
Donor 12	Male	75	<48 h	Prostate cancer	Entire ligament
Donor 13	Male	61	<48 h	Carcinoma of the kidney, suicide (hanging)	Entire ligament

4.1.4 The Transverse Ligament of the Acetabulum

In eight human cadavers (both sexes, age 17–36 years), the transverse ligament of the acetabulum was removed within 36 h postmortem (Table 4) and fixed in 90% methanol at 4°C for 24 h. They were stored until final use in 90% methanol at −20°C.

In all cases, the entire acetabulum including the band and both its bony entheses was removed. For further use, the course of the ligament within the specimens was exactly documented; the position of the anterior horn of the lunate articular surface

Table 4 Details of the transverse ligaments of the acetabulum

Specimen	Sex	Age (years)	Removal time postmortem	Medical history
Donor 1	Male	21	30 h	Unknown
Donor 2	Male	33	25 h	Acute hepatitis B
Donor 3	Male	26	21 h	Unknown
Donor 4	Male	18	24 h	Unknown
Donor 5	Female	17	16 h	Unknown
Donor 6	Female	39	36 h	Unknown
Donor 7	Male	29	36 h	Unknown
Donor 8	Male	22	18 h	Unknown

of the acetabulum was marked with a needle, and the transverse ligament of the acetabulum with both of its bony entheses was removed in toto.

4.1.5
Tendon and Trochlea of the Superior Oblique Muscle

From 11 unfixed human cadavers (both sexes, age 59–94) a portion of tissue consisting of the trochlea itself and the tendon of the superior oblique muscle, which crosses it, was removed within 36 h postmortem (Table 5). The samples were fixed in 90% methanol at 4°C for 24 h and stored in methanol at −20°C until used.

Table 5 Details of the tendon and trochlea of m. obliquus bulbi superior

Specimen	Sex	Age (years)	Removal time postmortem	Medical history
Donor 1	Male	63	24 h	Unknown
Donor 2	Female	92	12 h	Unknown
Donor 3	Female	94	12 h	Unknown
Donor 4	Female	75	12 h	Unknown
Donor 5	Male	87	42 h	Unknown
Donor 6	Female	94	18 h	Unknown
Donor 7	Male	89	18 h	Unknown
Donor 8	Male	76	27 h	Unknown
Donor 9	Male	70	48 h	Unknown
Donor 10	Male	63	48 h	Unknown
Donor 11	Male	59	48 h	Unknown
Donor 12	Male	80	24 h	Unknown

4.2
Methodology of the Immunohistochemical Investigation

Since most specimens also contained the bony entheses, they were generally decalcified prior to the investigation. This was carried out in a 5% solution of EDTA in distilled water, changed daily. Then the decalcified samples were infiltrated with 5% sucrose solution in PBS for 12 h and cryosectioned at 12 µm with the aid of a cryomicrotome.

First, a number of sections were stained with toluidine blue in order to detect metachromasia. The next step was an immunolabeling of the samples with polyclonal antibodies directed against type I and III collagens and monoclonal antibodies directed against type II and VI collagens and with monoclonal antibodies directed against the glycosaminoglycans chondroitin 4 and 6 sulfates, dermatan sulfate, and keratan sulfate (Table 6).

Table 6 List of primary antibodies

Antigen	Antibody	Type	Host	Source
Collagen I	AB-745 (P1)	Polyclonal	Rabbit	Chemicon International, Temecula, CA, USA
Collagen II	CIICI	Monoclonal	Mouse	DSHB
Collagen III	AB-747 (P3)	Polyclonal	Rabbit	Chemicon
Collagen VI	5C6	Monoclonal	Mouse	DSHB
Chondroitin 4 sulfate Dermatan sulfate	2B6	Monoclonal	Mouse	B. Caterson (Caterson et al. 1985)
Chondroitin 6 sulfate	3B3/7D4	Monoclonal	Mouse	B. Caterson
Keratan sulfate	5D4	Monoclonal	mouse	B. Caterson

DSHB, Developmental Studies Hybridoma Bank, University of Iowa.

Together with the antibodies described above, a monoclonal antibody (1C6) was used in the samples of the extensor tendons of the MCP joint. It is primarily directed against rat aggrecan, but is also able to recognize human aggrecan (Calabro et al. 1992). Sections immunolabeled with this antibody have to be reduced with 10 mM dithiothreitol in 50 mM Tris HCl, 200 mM sodium chloride, pH 7.4, for 2 h at 37°C and then alkylated with 40 mM iodoacetamide in PBS for 1 h at 37°C. A possible non-specific binding of the secondary antibody was reduced by blocking with an appropriate serum—here, a mixture of horse and goat serum in excess—for 40 min.

As a control for non-specific antibody binding, either the primary antibody was omitted, or the samples were incubated with normal mouse immunoglobulin (10 μg/ml for all monoclonal antibodies) or normal rabbit serum (1:200 for polyclonal antibodies). In order to unmask the individual epitopes, the sections were exposed to different enzymes (hyaluronidase, chondroitinase AC and ABC; Sigma-Aldrich, Munich). Endogenous peroxidase activity was blocked in all sections by pretreatment with 0.3% hydrogen peroxide in 100% methanol for 30 min. Antibody binding was detected with the Vectastain ABC 'Elite' avidin/biotin/peroxidase kit (Vector Labs., Burlingame, CA, USA). After the immunolabeling was completed, the cell nuclei were counterstained with Mayer's hematoxylin.

The sections of the transverse ligament of the atlas and the neighboring tissues were immunolabeled with a wider panel of monoclonal antibodies (Table 7), such as antibodies directed against various collagens (type I, II, III, IV), glycosaminoglycans (chondroitin 4 and 6 sulfate, dermatan sulfate, and keratan sulfate), and proteoglycans (aggrecan, link protein, and versican). Sections immunolabeled with antibodies directed against aggrecan and link protein were treated with 10 mM dithiothreitol in 50 mM Tris HCl, 200 mM sodium chloride, pH 7.4, for 2 h at 37°C and then alkylated with 40 mM iodoacetamide in PBS for 1 h at 37°C. In these investigations, non-specific binding of the secondary antibody was reduced by an appropriate serum block [highly concentrated horse serum 1:20 (Vector Labs., Burlingame, CA, USA) for 40 min].

Table 7 List of primary antibodies

Antigen	Antibody	Dilution	Enzyme	Source	Reference
Collagen I	Col 1	1:2000	Hyal (1.5 IU/ml) and ChABC (0.25 IU/ml)	Sigma	
Collagen II	CIICI	1:6	Hyal (1.5 IU/ml) and ChABC (0.25 IU/ml)	DSHB	Holmdahl et al. 1986
Collagen III	4H12	1:500	Hyal (1.5 IU/ml) and ChABC (0.25 IU/ml)	ICN	
Collagen V	3C9	1:500	Hyal (1.5 IU/ml) and ChABC (0.25 IU/ml)	ICN Biomedicals, Irvine, CA, USA	
Collagen VI	5C6	1:6	Hyal (1.5 IU/ml) and ChABC (0.25 IU/ml)	DSHB	Hessle and Engvall 1984
Chondroitin 4 sulfate	2B6	1:1500	ChAC (0.25 IU/ml)	B. Caterson	Caterson et al. 1985
Chondroitin 4 and dermatan sulfate	2B6	1:1500	ChABC (0.25 IU/ml)	B. Caterson	Caterson et al. 1985
Chondroitin 6 sulfate	3B3	1:150	ChABC (0.25 IU/ml)	B. Caterson	Caterson et al. 1985
Keratan sulfate	5D4	1:1500	None	B. Caterson	Caterson et al. 1983
Chondroitin 6 sulfate *oversulfated*	7D4	1:350	None	B. Caterson	
Aggrecan	1C6	1: 10	ChAC (0.25 IU/ml) after reduction and alkylation	B. Caterson	Calabro et al. 1992
Link protein	8A4	1:10	ChAC (0.25 IU/ml) after reduction and alkylation	B. Caterson	Calabro et al. 1992
Versican	12C5	1:10	ChAC (0.25 IU/ml)	DSHB	Asher et al. 1991, 1995
Tenascin	T2H5	1:100	ChAC (0.25 IU/ml)	Serotec, Ltd., Kidlington, UK	Verstraeten et al. 1992

ChAC, chondroitinase AC; ChABC, chondroitinase ABC; Hyal, hyaluronidase; DSHB, Developmental Studies Hybridoma Bank, University of Iowa (USA).

5 Results

5.1 Extensor Tendons of the Toes

5.1.1 Histological Findings

In all extensor tendons, the staining with toluidine blue detected a metachromatic zone in the region of the bony enthesis and in the deep surface in the vicinity of the articular surface (Fig. 6). The metachromasia observed here is most pronounced in a state of hydration; however, it is demonstrable to a lesser extent also in a state of dehydration. After a treatment with alcohol it disappears almost entirely.

In these zones, the cell nuclei do not show the longitudinal shape typical of tendon cells (tenozytes—fibroblasts), but are rather rotund, such as is typical of cartilaginous cell nuclei. These nuclei are less intensely stained as those of the neighboring tenozytes, which is a token of a higher content of euchromatin, i.e., active genetic material. Occasionally, a mostly unstained layer of cytoplasm is discernible, which reaches into the tendon a variable distance.

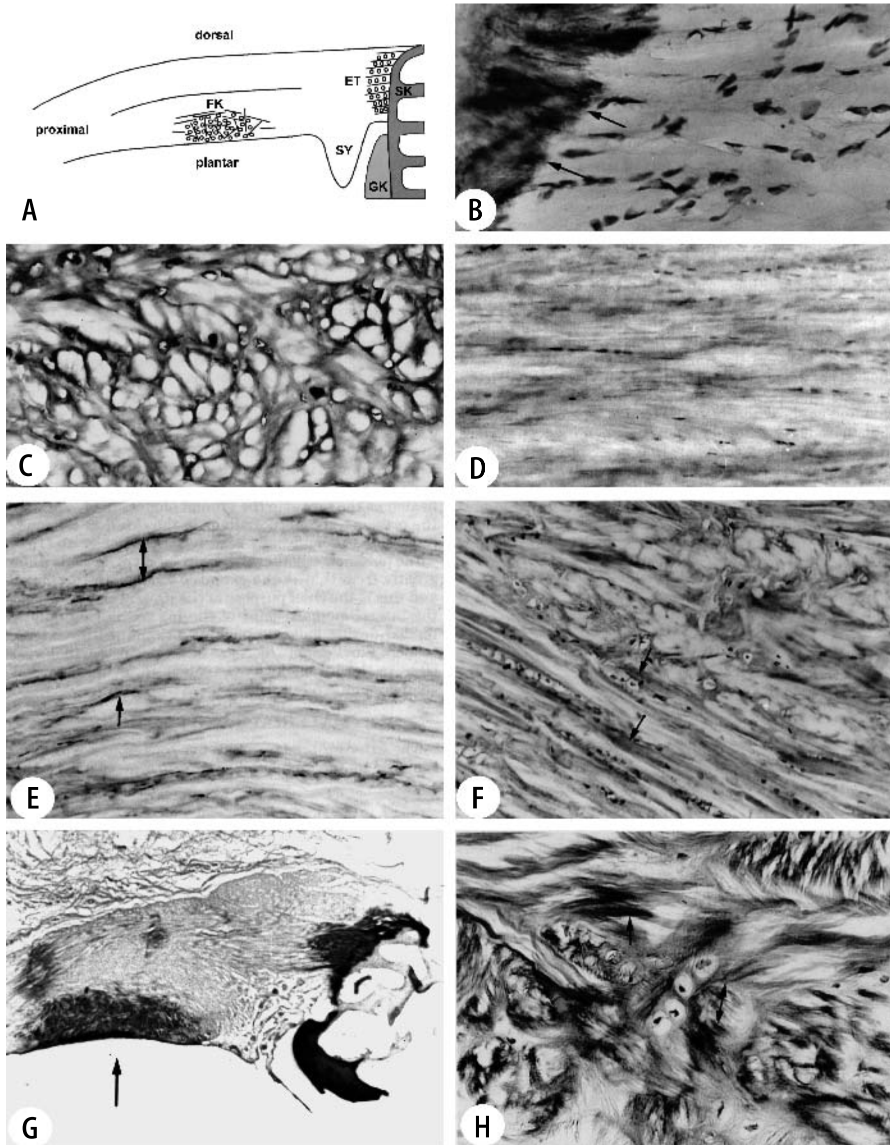
The extension of this metachromatic zone within the tendon varies considerably between the single tendons; however, its existence can always be detected. The same applies to the enthesis, which constantly shows a metachromatic zone reaching variably into the tendon.

Fig. 6 **A** Schematic drawing of the insertion of the extensor tendon to show the position of the enthesis (*ET*) and sesamoid (*FK*) fibrocartilages. *GK*, hyaline cartilage; *SK*, subchondral bone; *SY*, synovial fold. **B** Enthesis of the extensor tendon stained with toluidine blue. The metachromatic region (*arrows*) indicates the extent of the enthesis fibrocartilage (2nd toe, magnification ×3,370). **C** The basket-weave-like network of collagen fibers in the metachromatic, sesamoid fibrocartilage of the extensor tendon (3rd toe, toluidine blue, magnification ×3,370). **D** Labeling of the superficial aspect of the extensor tendon for collagen I (2nd toe, magnification ×3,185). **E** Labeling for collagen III (*arrows*) in the superficial aspect of the extensor tendon (1st toe, magnification ×3,185). **F** Strong pericellular labeling for type VI collagen (*arrows*) around the large cells of the sesamoid fibrocartilage (4th toe, magnification ×3,185). **G** Low-power view of type II collagen labeling in the enthesis and sesamoid fibrocartilages (*arrow*) of an extensor tendon. The adjacent articular cartilage on the base of the proximal phalanx is also strongly labeled (4th toe, magnification ×315). **H** High-power view of the strong pericellular labeling (*arrows*) for collagen II characteristic of the sesamoid fibrocartilage (4th toe, magnification ×3,370). (From Milz et al. 1999, with permission of Wiley-Liss)

5.1.2
Immunohistochemical Findings

5.1.2.1
Collagens

The entheses of all extensor tendons investigated shows a typical distribution of labeling for type II collagen, which reaches variably into the tendons from the sites



of connection to the bone (Fig. 4). In almost all tendons of the 2nd through the 5th toes, a second region with positive labeling for type II collagen can be detected, which varies considerably in extension. This zone is situated in the deep surface adjacent to the articular space in that portion of the extensor tendon proximal to the enthesis. It is, however, not connected with the enthesis. A comparable zone could be detected in only three out of six of the investigated extensor tendons of the 1st toe.

Type I collagen can be detected in the bone and in the tendons. The labeling in the latter is of varying intensity, but positive throughout. Type III collagen is detected in all investigated sections of the tendon. In some cases, the bone in the enthesis region also shows positive labeling.

The antibody that detects type VI collagen, 5C6, demonstrates positive labeling in all investigated sections of the tendon. The labeling does not show any discernible regional differences in the region of the enthesis, where the chromogen precipitate is somewhat stronger. The ECM of the bone at the enthesis shows no labeling; however, some osteocytes showed a pronounced labeling for type VI collagen (Table 8, Fig. 6).

5.1.2.2
Glycosaminoglycans

Keratan sulfate (antibody 5D4) and dermatan sulfate (antibody 2B6 in connection with an enzyme pretreatment with chondroitinase ABC) are detectable in all investigated sections of the toe tendons. The subchondral bone shows no labeling in the intercellular matrix, but mostly pericellular chromogen deposits occur around some osteocytes. In all toe tendons, chondroitin 6 sulfate (antibody 3B3 in connection with an enzyme pretreatment with chondroitinase ABC) is present in the sesamoid fibrocartilage and in the region of the enthesis, where extracellular labeling is found in 25 out of 30 toe tendons.

In 8 of 30 extensor tendons, labeling is detectable in the region of the sesamoid cartilage or of the enthesis fibrocartilage, even without enzyme pretreatment (3B3-). Labeling for 7D4 (antibody against a native epitope of chondroitin 6 sul-

Table 8 Number of positive markings in each region ($n = 6$)

	Insertion (Enthesis)					Sesamoid fibrocartilage					Distal tendon				
Antigen	C6S	C4S	DS, C4S	KS	Col II	C6S	C4S	DS, C4S	KS	Col II	C6S	C4S	DS, C4S	KS	Col II
Toe 1	2	5	6	5	6	2	5	5	5	3	1	3	3	2	0
Toe 2	6	5	6	4	6	6	5	6	4	6	1	2	4	2	0
Toe 3	6	1	5	5	6	6	1	6	6	6	2	1	5	6	0
Toe 4	6	2	6	6	6	6	3	6	6	5	3	3	4	6	0
Toe 5	5	1	6	5	6	6	1	6	5	5	2	0	5	5	0

C6S, chondroitin 6 sulfate; C4S, chondroitin 4 sulfate; DS, dermatan sulfate; KS, keratan sulfate; Col II, collagen II.

fate) was found at the same location in only four out of these eight cases. In five further cases, labeling for 7D4 occurred in the region of the enthesis without a corresponding 3B3 labeling.

Positive labeling for chondroitin 4 sulfate (antibody 2B6 in connection with an enzyme pretreatment of the tissue with chondroitinase AC) is found in 14 out of 30 toes; 11 of these markings were in the 1st or 2nd toes (Table 8, Fig. 7).

5.2 Extensor Tendons of the Hand in the Region of the MCP Joint

5.2.1 Histological Findings

A sesamoid fibrocartilage was constantly present in all of the extensor tendons in regions where each tendon wraps around the metacarpal head of the MCP joint to alter the direction of pull. This fibrocartilage is separated from the articular space by the deep surface of the peritendinous connective tissue and lies between the more typically fibrous portions of the tendon—i.e., those portions that are more like tensile tendons (Fig. 8). Although the fibrocartilage varies in amount, its existence can always be demonstrated. The characteristic picture is of large cartilaginous cells which, scattered in a metachromatic ECM, are surrounded by bundles of collagen fibers interwoven in basket-weave fashion (Fig. 8). The immunohistochemical characteristics of this tissue are summarized in Table 9, where they are compared with patterns of the adjacent parts of the tendon and with the superficial and deep peritendinous tissue.

5.2.2 Immunohistochemical Findings

5.2.2.1 Collagens

Type I, II, and VI collagens are found in all regions of the tendon and, with a somewhat lower intensity of labeling, also on the superficial and deep surface of the peritendinous connective tissue (Fig. 8). Even though no difference could be observed with regard to the intensity of labeling for type IV collagen, the pattern of labeling differs significantly in the fibrocartilaginous region compared with the rest of the extensor tendon. Type VI collagen fibers contribute substantially to the basket-weave network of the fibrocartilage's collagen arrangement. Labeling for type II collagen is characteristic of the fibrocartilage of all extensor tendons of the index finger and can be found in only two or three specimens from the other fingers, including the thumb (Fig. 8). Labeling for type II collagen occasionally extends into the adjacent proximal or distal parts of the tendon, but with no predominance in one finger over another.

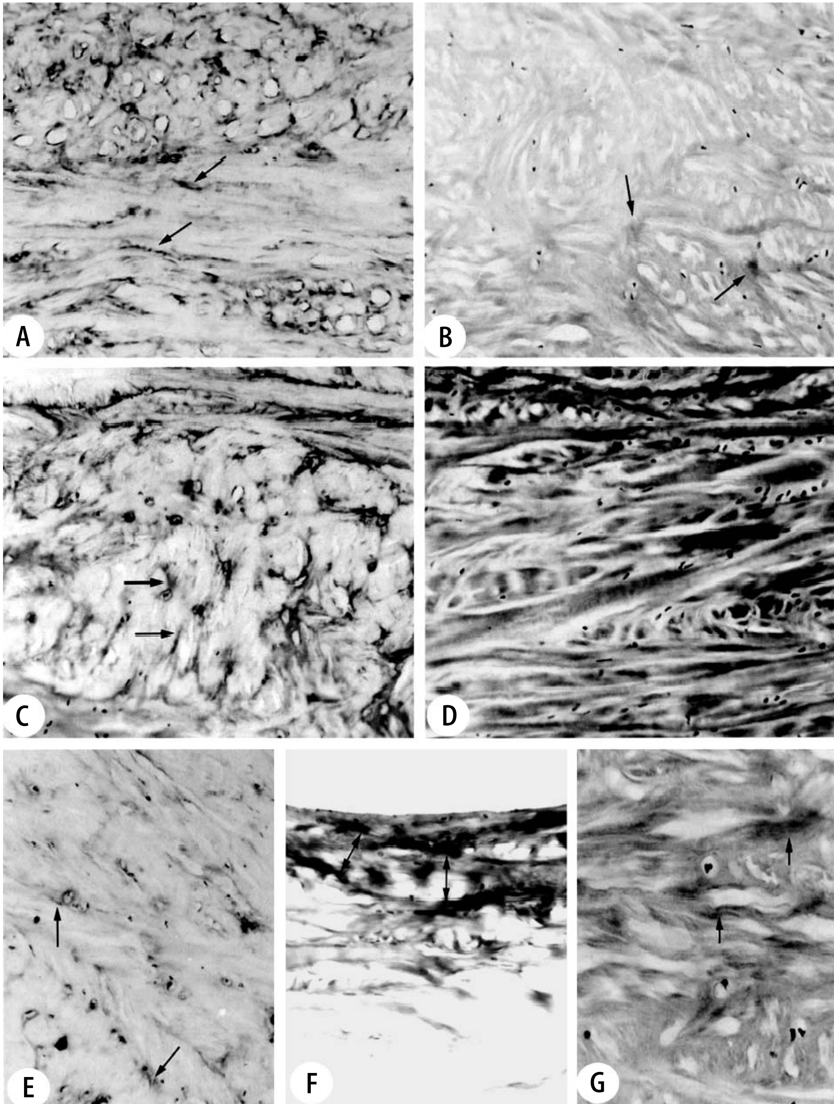


Fig. 7 **A** Sesamoid fibrocartilage of the extensor tendon labeled (*arrows*) for keratan sulfate (antibody 5D4; 3rd toe, magnification ×3,185). **B** Labeling (*arrows*) for chondroitin 4 sulfate (antibody 2B6 + ChAC) in the sesamoid fibrocartilage (4th toe, magnification ×3,185). **C** Labeling (*arrows*) for chondroitin 6 sulfate (antibody 3B3 + ChABC) in the sesamoid fibrocartilage (4th toe, magnification ×3,185). **D** Strong labeling for chondroitin 4 sulfate and dermatan sulfate (2B6 + ChABC) in the sesamoid fibrocartilage (4th toe, magnification ×3,185). **E** Labeling (*arrows*) for the native epitope of chondroitin 6 sulfate in the sesamoid fibrocartilage (antibody 3B3-, i.e., without enzyme treatment; 3rd toe, magnification ×3,185). **F** Labeling for the native epitope of chondroitin 6 sulfate (*arrows*) in the sesamoid fibrocartilage (antibody 7D4; 2nd toe, magnification ×3,185). **G** Labeling (*arrows*) for chondroitin 4 sulfate in the sesamoid fibrocartilage (antibody 2B6 1 ChAC; 4th toe, magnification ×3,370). (From Milz et al. 1999, with permission of Wiley-Liss)

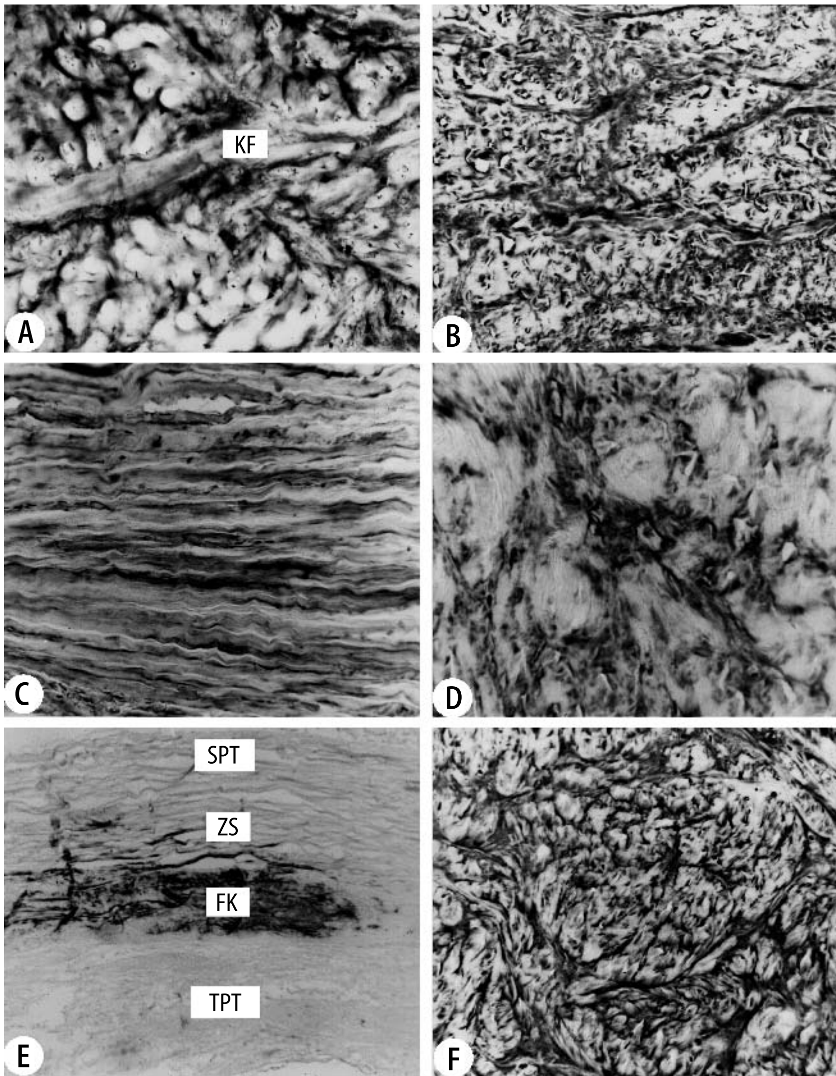


Fig. 8A–F Metachromasia and detection of the various collagens in the extensor tendons of the fingers. **A** Metachromasia in the region of the sesamoid fibrocartilage in the extensor tendon of an index finger. Note the basket weave orientation of the collagen fibers (*KF*) (toluidine blue, ×185). **B** Labeling for collagen I in the region of the sesamoid fibrocartilage (×185). **C** Labeling for collagen III in the superficial part of the extensor tendon of a ring finger (×185). **D** Strong pericellular labeling for collagen VI next to the rounded cells of the sesamoid fibrocartilage of a ring finger (×370). **E** Labeling for collagen II in the sesamoid fibrocartilage (*FK*) of a middle finger at low-power magnification. The neighboring deep (*TPT*) and superficial (*SPT*) peritendinous tissue and the superficial part of the extensor tendon (*ZS*) do not show any marking (×15). **F** Labeling for collagen II in the sesamoid fibrocartilage (*FK*) of a ring finger at higher magnification. Note the pronounced pericellular marking in that region (×185). (From Milz et al. 1998, with permission of Wiley-Liss)

Table 9 Immunohistochemical labeling of the different topographical regions investigated (numbers indicate positive labeling in a certain region; $n = 6$)

Finger	Distal tendon						Sesamoid fibrocartilage						Proximal tendon					
	Agg	C6S	C4S	DS, C4S	KS	CII	Agg	C6S	C4S	DS, C4S	KS	CII	Agg	C6S	C4S	DS, C4S	KS	CII
Thumb	0	1	5	6	6	0	6	6	6	6	6	2	0	0	5	6	6	0
Index	0	0	4	6	3	1	6	6	6	6	3	6	2	0	6	6	3	1
Middle	1	0	6	6	4	0	6	6	6	6	4	3	1	0	6	6	4	0
Ring	0	0	6	6	4	1	6	6	6	6	5	3	0	0	6	6	4	0
Fifth	1	2	5	6	5	0	6	5	6	6	5	3	0	0	5	6	5	1

Finger	Superficial peritendinous tissue						Deep peritendinous tissue					
	Agg	C6S	C4S	DS, C4S	KS	CII	Agg	C6S	C4S	DS, C4S	KS	CII
Thumb	0	0	1	4	6	0	1	0	4	6	6	0
Index	1	0	2	5	5	0	4	3	6	6	4	0
Middle	0	0	3	5	4	0	3	2	4	5	4	0
Ring	0	0	1	5	3	0	3	2	5	6	3	0
Fifth	0	0	1	6	5	0	2	1	5	6	5	0

Agg, aggrecan; C6S, chondroitin 6 sulfate; C4S, chondroitin 4 sulfate; DS, dermatan sulfate; KS, keratan sulfate; CII, collagen II.

5.2.2.2 Glycosaminoglycans and Proteoglycans

Keratan sulfate, dermatan sulfate, and chondroitin 4 sulfate are found in all investigated parts of the extensor tendons and in the superficial and deep peritendinous tissue (Fig. 9). In distinct contrast, labeling for chondroitin 6 sulfate (6 out of 28 fingers) is restricted almost entirely to the tendon's fibrocartilaginous regions, with the intensity of labeling varying from relatively weak to considerably strong (Fig. 9). Occasionally, labeling is seen to extend into the deep peritendinous tissue and into the distal parts of the extensor tendon. Labeling is predominantly pericellular and distributed around the basket-weave network of fiber bundles. In one single case a positive labeling with the antibody 3B3 without enzyme pretreatment (3B3-) is discernible, whereas in 21 investigated extensor tendons positive labeling for the antibody 7D4 is discernible (Fig. 9). This antibody recognizes a native epitope of chondroitin 6 sulfate, which is different from that which is recognized by the antibody 3B3-. Generally, the labeling pattern for the glycosaminoglycans seems to be less variable among the different fingers of one hand than among the same fingers of different individuals.

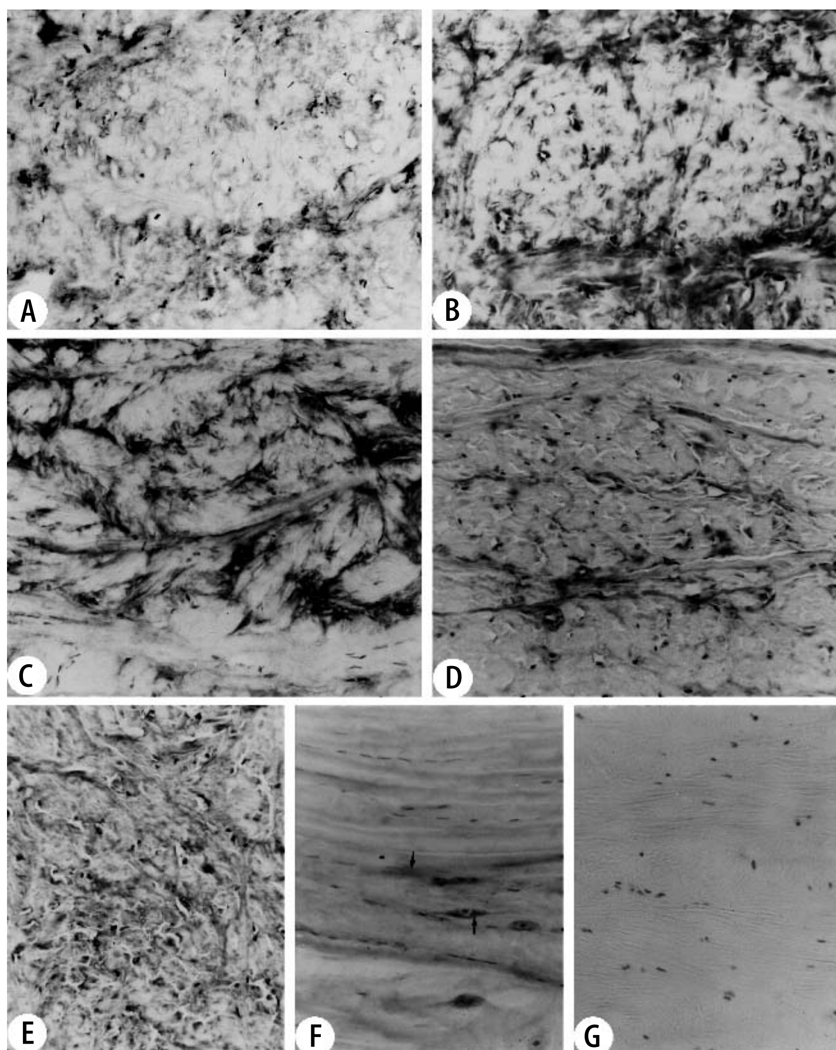


Fig. 9A–G Detection of chondroitin 6 sulfate and dermatan sulfate in the extensor tendons of the fingers. **A, B** Comparison of the labeling patterns for chondroitin 4 sulfate (**A**: antibody 2B6 + ChAC) and chondroitin 4 sulfate together with dermatan sulfate (**B**: 2B6 + ChABC) in the sesamoid fibrocartilage of an index. The difference in labeling shows that both glycosaminoglycans are present but have a different regional distribution within the fibrocartilage (185 x). **C** Pronounced labeling for chondroitin 6 sulfate (Antibody 3B3 + ChABC) in the sesamoid fibrocartilage of a ring finger (×185). **D** Detection of the native epitope of chondroitin 6 sulfate (Antibody 7D4) in the sesamoid fibrocartilage of a middle finger (×185). **E** Labeling for keratan sulfate (Antibody 5D4) in the sesamoid fibrocartilage of a middle finger (×185). **F** Pericellular labeling (*arrows*) for aggrecan (Antibody 1C6 +ChAC) in the sesamoid fibrocartilage of a middle finger (×185). **G** Control without any labeling. The primary antibody was omitted and the section was incubated with normal mouse immunoglobulins (×185). (From Milz et al. 1998, with permission of Wiley-Liss)

Labeling for aggrecan is detectable in the sesamoid fibrocartilages of all extensor tendons (Fig. 9) and sometimes extends into the adjacent proximal and distal portions of the tendon. In a few cases, positive labeling for aggrecan occurred in regions of the tendons that do not label for chondroitin 6 sulfate (in neighboring sections). The intensity of labeling differs considerably between tendons: the strongest and most extensive labeling is seen in the extensor tendon of an index finger, the weakest in that of a thumb. Generally, however, labeling is distributed relatively diffusely throughout the entire ECM. In several fibrocartilages, a strong pericellular labeling pattern is seen, which resembles the distribution of chondroitin 6 sulfate.

5.3 The Transverse Ligament of the Atlas

5.3.1 Histological Findings

In the center of each investigated transverse ligament of the atlas a sesamoid fibrocartilage is invariably found and opposed by a distinct enthesis fibrocartilage at either attachment site to the atlas. The remaining parts of the tendon show the parallel fibers of connective tissue to be typically expected of a ligament. This setup is shown in Figure 10. Even though the extension of the sesamoid fibrocartilage differs from individual to individual, the morphological appearance is regularly characterized by large round fibrocartilage cells, which are scattered in a highly metachromatic ECM (Fig. 11). In the region of the articular surface toward the dens axis, there is a thin yet distinct layer of orthochromatic extracellular matrix

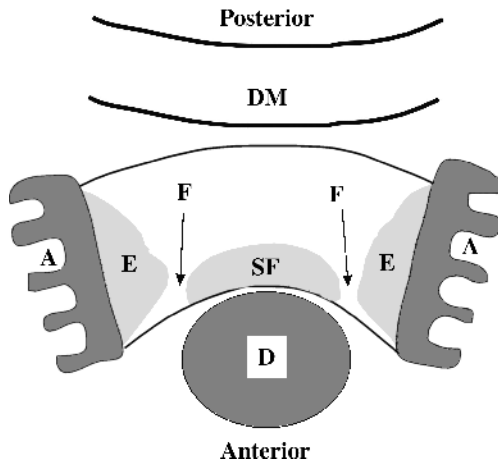


Fig. 10 Topographical situation of the transverse ligament of the atlas (lig. transversum atlantis) with its sesamoid (*SF*) and enthesis fibrocartilages (*E*); fibrous zone (*F*); dens of axis (*D*), dorsal to the ligament membrana tectoria and dura mater (*DM*)

(Fig. 11). The immunohistochemical characteristics of the sesamoid fibrocartilage are summarized in Table 10, where they are compared with the various labeling patterns of the enthesis fibrocartilage and the dura mater lining the vertebral canal.

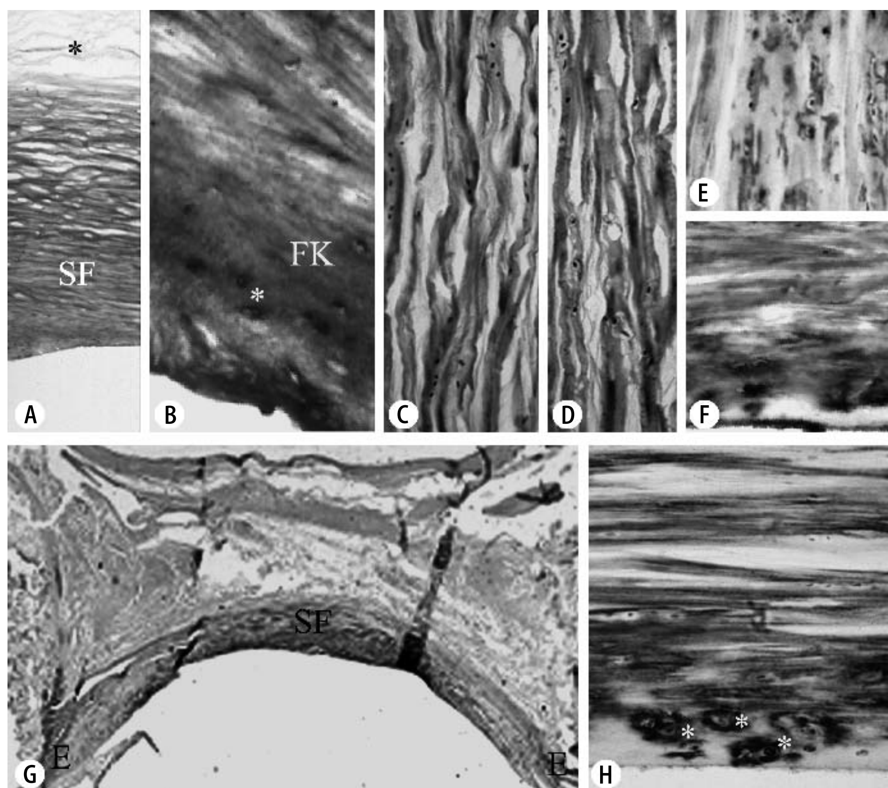


Fig. 11A–H Histology **A**, **B** and immunohistochemical detection of collagens (**C**–**H**) in the transverse ligament of the atlas. **A** The sesamoid fibrocartilage (*SF*) underlying the articulating surface exhibits a strong metachromasia which contrasts to the almost unstained part of the ligament. (*; toluidine blue, ×40). **B** Higher magnification of the sesamoid fibrocartilage showing typical rounded cells (*FK*) and a basket-weave-like orientation of the collagen fiber bundles. Note the thin layer of orthochromatic material at the articular surface of the ligament (*; toluidine blue, ×250). **C** Widespread, diffuse labeling for collagen I in the sesamoid fibrocartilage (×160). **D** Widespread, diffuse labeling for collagen III in the sesamoid fibrocartilage (×160) **E** Patchy pericellular labeling for collagen VI in the sesamoid fibrocartilage (×200). **F** Strong pericellular labeling for type VI collagen near the articular surface of the sesamoid fibrocartilage in a region corresponding to the layer of orthochromatic material shown in an adjacent section illustrated in **B** (×200). **G** Low-power view of the entire transverse ligament, showing a continuous band of labeling for collagen II extending from one enthesis (*E*) to the other, through the sesamoid fibrocartilage (*SF*) (×4.5). **H** High-power view of immunohistochemical labeling for collagen II in the sesamoid fibrocartilage. Note the pronounced pericellular distribution in the region corresponding to the layer of orthochromatic material shown in an adjacent section illustrated in **B** (*; fibrocartilage cells; ×190). (From Milz et al. 2001a, with permission of Lippincott Williams and Wilkins)

Table 10 Summary of immunohistochemical labeling patterns in the various regions of the transverse ligament (lig. transversum atlantis; the first number counts for the positive markings found in the regions investigated, second number)

Region	Matrix molecule							
	Collagen II	KS	DS, C4S	C4S	C6S	Aggrecan	Link protein	Versican
Sesamoid fibrocartilage	13/13	11/13	13/13	7/13	13/13	7/7	6/7	3/7
Enthesis fibrocartilage	7/7	6/7	7/7	5/7	7/7	7/7	5/7	2/7
Fibrous zone	0/13	10/13	13/13	3/13	13/13	4/13	5/13	6/13
Dura mater	1/13	10/13	13/13	10/13	3/13	0/13	4/13	6/13

C6S, chondroitin 6 sulfate; C4S, chondroitin 4 sulfate; DS, dermatan sulfate; KS, keratan sulfate.

5.3.2 Immunohistochemical Findings

5.3.2.1 Collagens

Type I, II, and VI collagens are detected in all investigated regions of the transverse ligament of the atlas (Fig. 11) and in the adjacent spinal dura mater. Although no significant differences concerning the intensity of labeling for type VI collagen were detectable, there are significant differences with regard to the labeling pattern of this type of collagen between fibrocartilaginous and non-fibrocartilaginous regions of the ligament. In the fibrocartilaginous parts, labeling is predominantly pericellular, with this pattern especially pronounced in the regions adjacent to the articular surface (Fig. 11).

Pronounced labeling for type II collagen is characteristic of the sesamoid and of the enthesis fibrocartilages. In some cases, labeling extended from one fibrocartilaginous region to the other, so that the transverse ligament of the atlas is positively labeled for type II collagen virtually in its entirety (Fig. 11). In the region of the fibrocartilages, type II collagen occurs in the shape of labeled fiber bundles, whereas intense pericellular labeling dominates in the vicinity of the articular surface (Fig. 11).

5.3.2.2 Glycosaminoglycans and Proteoglycans

Keratan, dermatan, and chondroitin 4 sulfates are detectable in the entire transverse ligament and in the dura mater (Fig. 12): Although labeling of the dura mater is occasionally observable, labeling for chondroitin 6 sulfate is consistently detectable

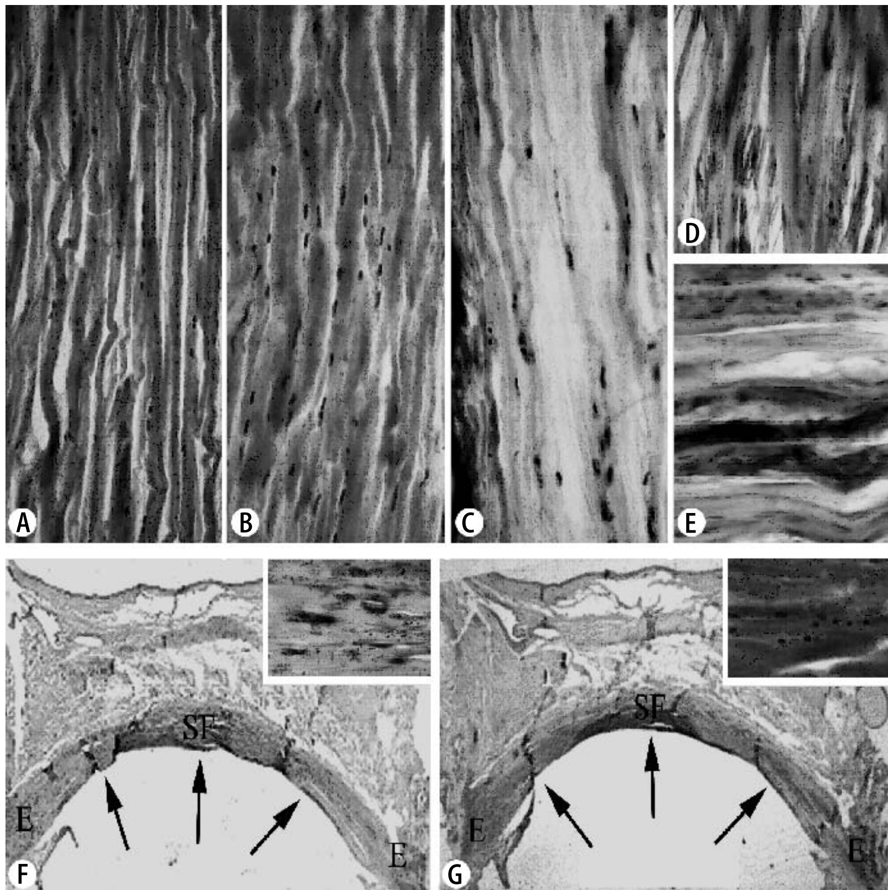


Fig. 12A–G Immunohistochemical labeling patterns for glycosaminoglycans, proteoglycans, and link protein in the transverse ligament of the atlas. **A**. Widespread and diffuse labeling for keratan sulfate in the sesamoid fibrocartilage (×170). **B, C**. Labeling for chondroitin 4 and dermatan sulfates with antibody 2B6 + chondroitinase ABC **B**, and labeling for chondroitin 4 sulfate alone, with antibody 2B6 + chondroitinase AC **C** in the sesamoid fibrocartilage. The weaker labeling in **C** indicates that much of the labeling in **B** is associated with dermatan sulfate (both ×180). **D** Patchy, pericellular labeling for chondroitin 6 sulfate (×270). **E** Patchy labeling for versican in the fibrous regions of the ligament (×220). **F** Low-power view of the whole ligament immunolabeled for link protein. Note the continuous band of labeling near the articular surface (*arrows*) extending from the sesamoid fibrocartilage (*SF*) to the entheses (*E*) at either end of the ligament (×3.5). *Inset* is a high-power view of the patchy and pericellular labeling for link protein around the sesamoid fibrocartilage cells (×70). **G** Low-power view of an adjacent section to that shown in **E** labeled for aggrecan. The labeling also extends from one enthesis (*E*) to the other (*arrows*), but is strongest near the articular surface of the sesamoid fibrocartilage (*SF*; ×3.5). *Inset* is a high-power view of the diffuse labeling in the ECM (×130). (From Milz et al. 2001a, with permission of Lippincott Williams and Wilkins)

only in the fibrocartilaginous regions of the ligament. The intensity of labeling varies slightly between different individuals and is rather strong in some individual cases (Fig. 12). Labeling is predominantly pericellular and emphasizes the basket weave arrangement of fiber bundles in the sesamoid fibrocartilages.

Patchy labeling for versican occurs in the fibrous regions of the ligament, but not in the fibrocartilaginous ones (Fig. 12). Aggrecan and link protein are detectable both in all sesamoid fibrocartilages (Fig. 12) and in four of seven investigated enthesis fibrocartilages. Labeling for aggrecan and link protein is homogeneously distributed throughout the entire ECM; in a number of fibrocartilages, the distribution pattern is largely pericellular and closely resembles the pattern of distribution of chondroitin 6 sulfate. Occasionally, zones with positive labeling extend from the sesamoid fibrocartilage toward the neighboring enthesis.

5.4 The Transverse Ligament of the Acetabulum

5.4.1 Histological Findings

A thin layer of cartilage-like cells, which constitute a sesamoid fibrocartilage, is consistently present in the center of each investigated transverse ligament near its inner surface, where it faces the femoral head (Fig. 13). Additionally, a strong and pronounced enthesis fibrocartilage is present at both bony attachments (Fig. 13). The remaining regions of the ligament show fibrous tissue, which is usually typical of ligaments. Figure 4 offers a diagrammatical representation of the distribution of the various tissues. Although the size of the sesamoid fibrocartilage varies considerably from specimen to specimen, it is always characterized by cartilage-like cells, which are distributed throughout metachromatic ECM.

The immunohistochemical labeling characteristics of the various parts of the transverse ligament of the acetabulum are summarized in Table 11. They will be described at length in ensuing paragraphs.

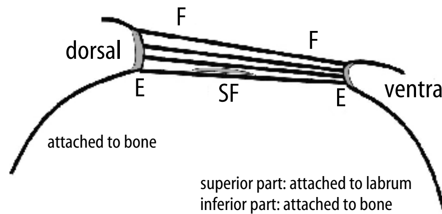


Fig. 13 Schematic drawing of the transverse ligament of the acetabulum to show its topographical situation: (*E*) the attachments of the ligament to the ventral and posterior horn of the lunate surface; fibrous region (*F*) of the ligament; sesamoid fibrocartilage (*SF*). Note that the femoral head presses against the inner surface of the ligament where the sesamoid fibrocartilage is situated

Table 11 Each entry shows the number of ligaments in which positive labeling was seen (out of the eight ligaments examined)

Region	Matrix molecule								
	Collagen II	KS	DS, C4S	C4S	C6S	Aggrecan	Link protein	VS	Tenascin
Ventral enthesis	7/8	8/8	8/8	7/8	7/8	5/8	5/8	5/8	5/8
Dorsal enthesis	8/8	8/8	8/8	7/8	8/8	7/8	8/8	1/8	5/8
Inner part of the ligament (close to articular surface)	3/8	8/8	8/8	7/8	6/8	4/8	4/8	6/8	7/8
Outer part of the ligament	0/8	8/8	8/8	6/8	0/8	1/8	0/8	7/8	7/8

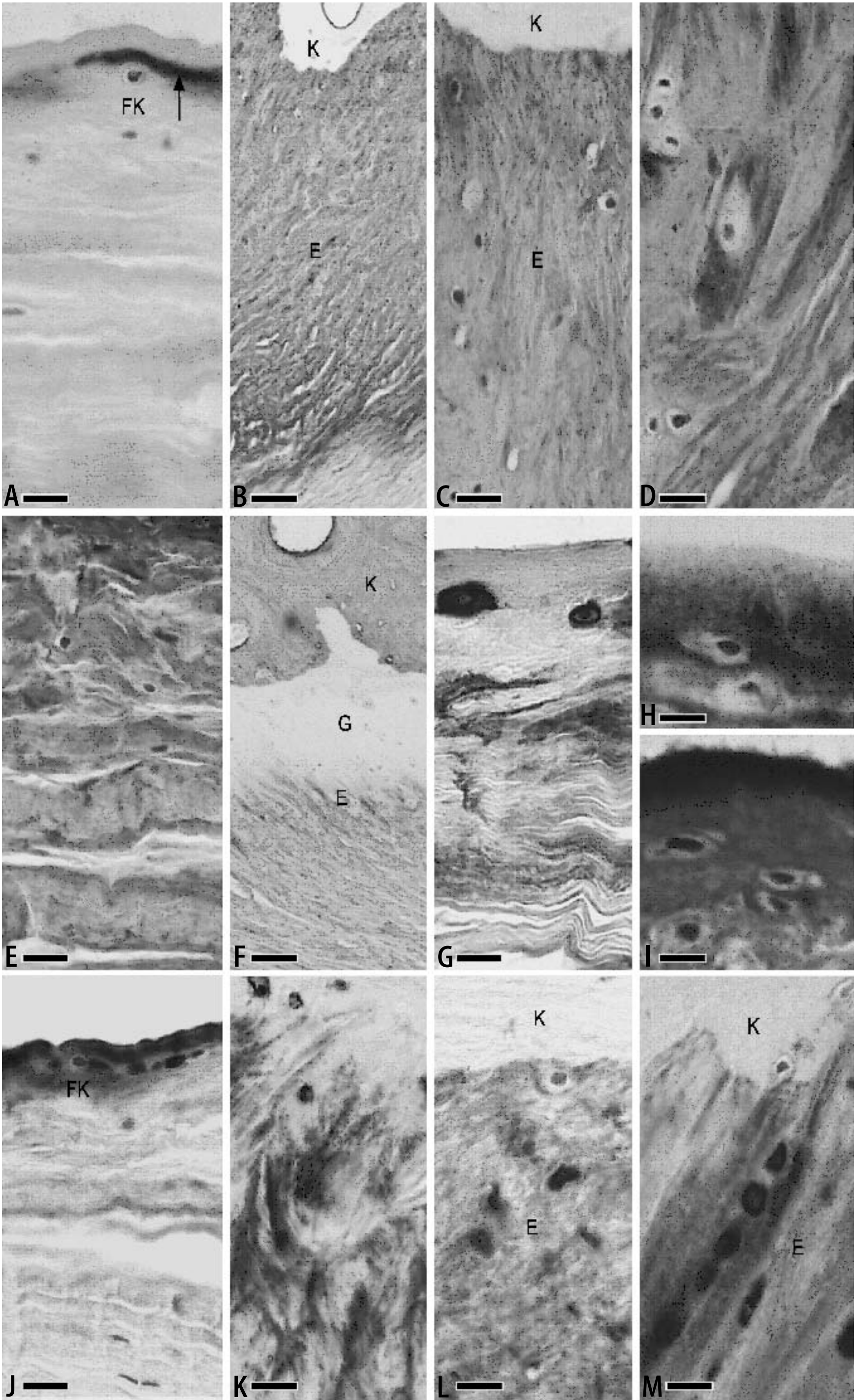
C6S, chondroitin 6 sulfate; C4S, chondroitin 4 sulfate; DS, dermatan sulfate; KS, keratan sulfate; VS, versican.

5.4.2 Immunohistochemical Findings

5.4.2.1 Collagens

Labeling for type I, III, and V collagens is found in all regions of the ligament (Fig. 14), although in a number of enthesis fibrocartilages have a zone without labeling for type I collagen. While there are no differences concerning the intensity

Fig. 14 **A** High-power view of the sesamoid fibrocartilage showing large fibrocartilage cells (*FK*) under the inner surface of the ligament. The immunohistochemical labeling for type II collagen shows a thin but distinct pericellular marking of collagen fibers. *Bar*, 20 µm. **B** Low-power view of an enthesis labeling for type II collagen. (*K*) bone, (*E*) enthesis. *Bar*, 80 µm. **C** Strong, pericellular labeling for type III collagen in an enthesis fibrocartilage. (*K*) bone, (*E*) enthesis. *Bar*, 20 µm. **D** High-power view of a prominent enthesis fibrocartilage with a strong pericellular labeling for type II collagen. *Bar*, 20 µm. **E** Widespread, diffuse labeling for type III collagen in an sesamoid fibrocartilage. *Bar*, 20 µm. **F** Enthesis labeling for type I collagen with a non-labeled gap between bone and the rest of the enthesis fibrocartilage. (*K*) bone, (*E*) enthesis, (*G*) gap. *Bar*, 80 µm. **G** Patchy pericellular labeling for type VI collagen in the sesamoid fibrocartilage. *Bar*, 20 µm. **H, I** Labeling for chondroitin 4 and dermatan sulfates with antibody 2B6 + chondroitinase ABC **I**, and labeling for chondroitin 4 sulfate alone, with antibody 2B6 + chondroitinase AC **H** in the sesamoid fibrocartilage. The weaker labeling in **H** indicates that much of the labeling in **I** is associated with dermatan sulfate. *Bar*, 10 µm for both. **J** Distinct, pericellular labeling for chondroitin 6 sulfate at the inner surface of the transverse ligament. Note the cells (*FK*) of the sesamoid fibrocartilage. *Bar*, 20 µm. **K** Staining of chondroitin 6 sulfate in the enthesis fibrocartilages is largely pericellular and highlights the interwoven arrangement of fiber bundles that characterize this region. *Bar*, 20 µm. **L, M** Enthesis with an extracellular matrix labeling for aggrecan **L** and link protein **M.** (*K*) bone, (*E*) enthesis. *Bar*, 20 µm for both. (From Milz et al. 2001b, with permission of Blackwell Publishing Ltd.)


FK
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of labeling for collagen VI detectable in the various parts of the ligament, a predominantly pericellular labeling can be observed primarily in the sesamoid and enthesis fibrocartilages. In the sesamoid fibrocartilage, the pronounced pericellular labeling is found in the immediate vicinity of the articular surface (Fig. 14).

Pronounced labeling for type II collagen is characteristic of both the ventral and the dorsal enthesis fibrocartilages of all ligaments investigated (Fig. 14). No difference in the labeling could be detected between one end (attachment) of the ligament and the other. Labeling for collagen II is present only in three of eight investigated sesamoid fibrocartilages. Moreover, it is definitely more limited there than at the attachments (Fig. 14).

5.4.2.2
Glycosaminoglycans and Proteoglycans

Labeling for keratan sulfate, dermatan sulfate, and chondroitin 4 sulfate are present in all regions of the ligament, whereas chondroitin 6 sulfate is detectable only in sesamoid and enthesis fibrocartilages, where the intensity of its labeling varies from relatively weak to strong. Labeling for chondroitin 6 sulfate in the sesamoid fibrocartilages is predominantly pericellular, which emphasizes the interwoven arrangement of collagen fiber bundles typical of that region (Fig. 14).

Labeling for versican is predominantly present in the fibrous, less cartilaginous regions of the ligament (Fig. 14). In contrast, aggrecan and link protein may be detected in the fibrocartilaginous regions only (Fig. 14). Generally, labeling for aggrecan and link protein is distributed throughout the ECM relatively diffusely, but in several fibrocartilages, a strong pericellular labeling pattern is seen, which resembles the distribution of chondroitin 6 sulfate.

5.5
Tendon and Trochlea of the Superior Oblique Muscle

5.5.1
Histological Findings

A narrow layer of cells with relatively large nuclei and clearly visible layers of cytoplasm forms a small sesamoid fibrocartilage, which is situated at the central part of the tendon where it faces the trochlea (Fig. 15). An additional fibrocartilage, which is much more distinct, is formed by the outer layer of the trochlea. The trochlea's fibrocartilage surrounds a central region, which, due to the absence of type I collagen, shows a composition of ECM similar to that of hyaline cartilage (Fig. 15). The remaining regions of the tendon show features typical of fibrous tendinous tissue. The topographical layout of the various tissues is diagrammatically presented in Fig. 5. Although the size of the sesamoid fibrocartilage in the region of redirection of the tendon varies considerably from specimen to specimen, the fibrocartilaginous phenotype is always characterized by cartilage-like

cell morphology. The immunohistochemical findings of the various investigated tissues are summarized in Table 12.

5.5.2 Immunohistochemical Findings

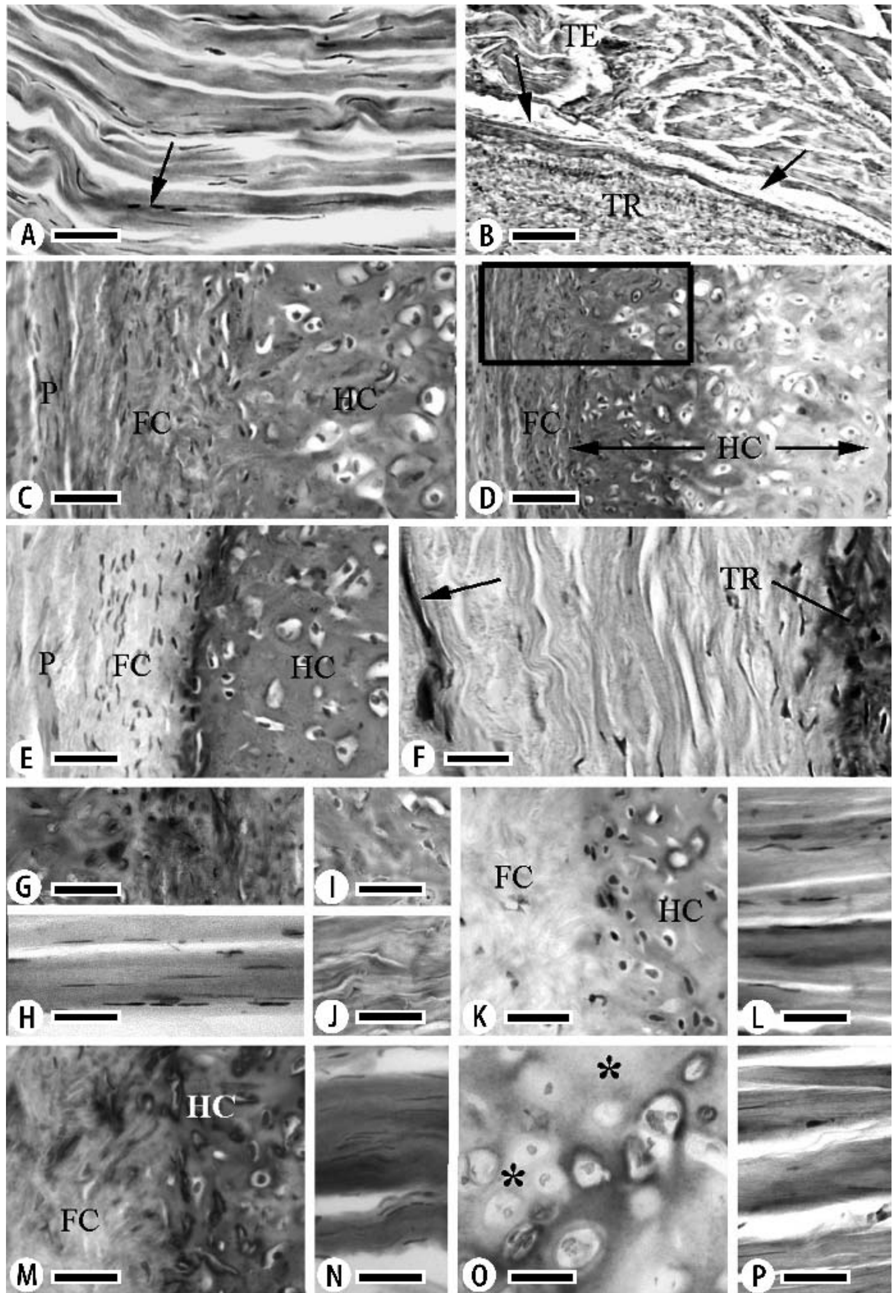
5.5.2.1 Collagens

Labeling for type I, III, and VI collagens is detectable in all parts of the tendon of the superior oblique muscle (Fig. 15). In the center of each trochlea there is a zone in which labeling for type I collagen is absent and in which especially large cells with distinct cytoplasm layers can be seen (Fig. 15). Although there are no differences concerning the intensity of labeling for collagen VI detectable in the various parts of the tendon, a predominantly pericellular labeling can be observed in the sesamoid fibrocartilage more distinctly than in the remaining tendon. This pericellular labeling is most prominent in the region of the surfaces of tendon and trochlea (Fig. 15).

A particularly strong and pronounced labeling for type II collagen characterizes the entire trochlea—especially the central parts. This positive zone is occasionally surrounded by a layer of tissue in which labeling for type II collagen is absent (Fig. 15). In the tendon of the superior oblique muscle, labeling for type II collagen is present in 7 of 11 specimens. Labeling is discernible only in the immediate

Fig. 15A–P Immunohistochemistry of the trochlea and superior oblique tendon. **A** Uniform labeling for type VI collagen in the tendon. Note from the elongated shape of the nuclei (*arrow*) that the tendon lacks fibrocartilage cells. *Scale bar*, 40 µm. **B** The bursa (*arrows*) between the trochlea (*TR*) and the tendon (*TE*) in a section labeled for type VI collagen. Note the pronounced pericellular staining in the trochlea. *Scale bar*, 80 µm. **C, D**) High- **C** and low-power **D** views of the trochlea showing the presence of type I collagen in both the hyaline cartilage (*HC*) and fibrocartilage (*FC*) regions. Part **D** highlights the variability in labeling within the zone of hyaline cartilage; the region in the rectangle in **D** corresponds to the entire field shown in **C**. *P*, perichondrium. *Scale bars*, **C** 30 µm; **D** 60 µm. **E** Type II collagen labeling in the trochlea in a neighboring section to that illustrated in **C** and taken at the same magnification. Note the complementary pattern of staining in **C** and **E**; only the hyaline cartilage (*HC*) zone labels for type II collagen and the fibrocartilage (*FC*) is unlabelled. *P*, perichondrium. *Scale bar*, 30 µm. **F** Localized labeling for type II collagen in the tendon (*arrow*), near to the contact zone with the trochlea (*TR*). *Scale bar*, 20 µm. **G, H** Chondroitin 6 sulfate in the trochlea **G** and tendon **H**. *Scale bars*, 30 µm. **I, J** Keratan sulfate labeling in the trochlea **I** and tendon **J**. *Scale bars*, 30 µm. **K, L** Aggrecan in the trochlea **K** and tendon **L**. Note the more pronounced labeling in the hyaline cartilage region (*HC*) of the trochlea than in the peripheral fibrocartilage (*FC*) zone. *Scale bars*, 30 µm. **M, N** Link protein in the trochlea **M** and tendon **N**. As with aggrecan, labeling is more pronounced in the former. *Scale bars*, 30 µm. **O, P** Versican labeling in the trochlea **P** and tendon **P**. Note the patchy labeling in the trochlea; versican is absent from parts of the territorial and interterritorial matrices (*asterisks*). *Scale bars*, 30 µm. (From Milz et al. 2002, with permission of Investigative Ophthalmology and Visual Science)

vicinity of the sliding/articulating surfaces and is situated close to the articulating side of the small sesamoid fibrocartilage (Fig. 15).



5.5.2.2
Glycosaminoglycans and Proteoglycans

Keratan sulfate, dermatan sulfate, and chondroitin 4 sulfate are detectable in all investigated parts of the tendon (Fig. 15). However, the complete set of antigens was not always detectable in all investigated specimens. The trochlea shows labeling for those glycosaminoglycans in all cases. Chondroitin 6 sulfate is present in all trochleae and in 9 out of 11 sesamoid fibrocartilages. In these fibrocartilages, intensity varies from strong to moderately distinct immunohistochemical labelings, which are generally arranged pericellularly (Fig. 15).

Labeling for versican and tenascin is present in all parts of the tendon and in all of the trochleae. In certain cases, where cells with distinctly cartilaginous morphology are present in the center of the trochlea, labeling for versican and keratan sulfate is absent in both the territorial and the interterritorial matrices. Aggrecan and link protein can also be detected in all parts of the tendon and in the trochlea (Fig. 15) Labeling is distributed homogeneously throughout the tendon's ECM. However, labeling in the trochlea is predominantly pericellular and tends to be more pronounced toward the central region.

Table 12 A summary of the immunolabeling characteristics of the tendon of the superior oblique muscle and the trochlea. Each column entry shows the number out of the total of 11 specimens examined in which positive labeling was seen

Antigen	Region				
	Proximal tendon	Midpart of tendon	Distal tendon	Sliding surface opposite trochlea	Trochlea
Collagen I	11	11	11	11	11
Collagen II	0	1	1	8	11
Collagen III	11	11	11	11	11
Collagen V	8	9	8	9	11
Collagen VI	11	11	11	11	11
DS and C4S	11	11	11	11	11
C4S	8	9	8	11	11
C6S	8	8	8	9	11
KS	3	3	3	5	10
C6S-os. (7D4)	1	2	1	6	11
Versican	9	9	9	9	11
Tenascin	10	10	10	10	11
Aggrecan	2	10	10	10	11
Link Protein	11	11	11	11	11

ChAC, chondroitinase AC; ChABC, chondroitinase ABC; C6S, chondroitin 6 sulfate; C4S, chondroitin 4 sulfate; DS, dermatan sulfate; KS, keratan sulfate.

6 Discussion

6.1 Discussion of the Method

Immunohistochemical methods of detection rely on the particular affinity of antibody and antigen. Affinity in this context means the force of attraction between two macromolecules, namely the antibody and the antigen. This force is responsible for the binding and its maintenance. By their nature, these bindings are neither covalent nor purely electrostatic (Kiernan 1999). Rather, they are based upon a complex three-dimensional lock-and-key principle. In general, antigen antibody binding depends upon a mixture of ion-based attraction forces, and covalent and hydrophobic interaction (Kiernan 1999).

Due to the high specificity with which the antibody recognizes the antigen (Kiernan 1999, Lehninger et al. 1994), immunohistochemical methods are very appropriate for the labeling of macromolecules, in this case especially of proteins, and for the investigation of their distribution throughout tissue.

Corresponding to their origin and to the way they are obtained, antibodies are separated into two essential groups, namely monoclonal and polyclonal antibodies. Polyclonal antibodies are obtained from the serum of immunized animals (mostly rabbits, goats, or sheep): Ideally, they are directed against various epitopes of one and the same macromolecule. A polyclonal antibody preparation, therefore, is a mixture of various antibody populations, which, however, if the preparation is of high quality, are able to recognize one and the same macromolecule.

In contrast, monoclonal antibodies bind to a certain antigenic determinant (epitope), since they descend from the same hybridized B-cell clone. Thus, the specificity of a monoclonal antibody usually surpasses that of a polyclonal antibody. In the present case, we worked with only monoclonal mouse antibodies in all but the first investigations for type I and III collagens.

Antibody molecules consist of various subunits, which functionally differ considerably. In each immunoglobulin G (IgG) molecule, one can distinguish two short and two long molecule chains (long, heavy H chain; short, light L chain), which are connected by disulfide bridges. This results in a Y-shaped molecule possessing a variable region at the Y's two arms. This region is responsible for the antigen–antibody reaction. Therefore, it is a bivalent molecule. The composition of the remaining parts of the molecule is constant and allows a practical distinction of IgG molecules originating from different species. By enzyme splitting, e.g., with papain, further subunits can be produced, such as the F_{ab} and F_c fragments. The F_{ab} fragment is able to bind monovalently and is also utilized for detecting reactions.

In case an antibody is directed against a relatively small molecule, it is possible that the variable regions will recognize different, similar molecules as well. This is referred to as cross-reactivity. If this phenomenon occurs, it considerably impairs specificity. Thus, it is imperative to undertake an exact biochemical characterization of an antibody before it is used for immunohistochemical labeling.

Care was taken in such characterizations in the antibodies utilized in the course of the present project. This applies particularly to the antibodies against the various glycosaminoglycans and proteoglycans, which were generously provided by the connective tissue laboratory of the School of Biosciences, University of Wales (Prof. B. Caterson).

Detection of bound primary antibodies is possible by various methods. The common aim is to produce labeling detectable under the light microscope. In the present case, we have chosen the ABC peroxidase method, since it provides the most reliable results in tissues of the locomotor system and therefore is frequently utilized for similar studies worldwide. According to various authors (Kiernan 1999), this method leads to the cleanest preparations with the least interference by non-specific background labeling. The use of special optimized detection systems (Vector "Elite" ABC peroxidase kit) ensures a reliable, specific, and sensitive detection of the respective primary antibodies.

The detection system works according to the following principle. A biotinilated secondary antibody—in this case, an antibody made in horse—binds to the primary antibody and, in a second step, reacts with an avidin-biotin-peroxidase complex. After an appropriate chromogen has been added (in this case, DAB), visible dye deposition occurs. A benefit of this technique is that the dye deposits are resistant to organic solvents, which in turn allows permanent mounting with DPX resin. In the detection kits used for the present project, a certain avidin subpopulation (avidin-D) is utilized. According to the producer (Vector Labs., Burlingame, CA, USA), this avidin-D reacts with biotin about 10–100 times more sensitively than the avidin usually employed. Furthermore, the reaction locally amplifies the resulting color signal by producing complexes, which contain a number of peroxidase molecules.

6.2 Discussion of the Present Study's Structure

Since immunohistochemical methods were first introduced, numerous efforts have been undertaken to utilize the technique for quantifying statements. But since the principle type of detection signal it allows is either positive or negative, the method cannot be used for quantification, such as for the amount of antigen at a given localization. Either, a certain antigen can be detected, or it is not present in the section. Peroxidase detection reaction allows no quantification, since the amount of chromogen depositing does not correlate stoichiometrically with the amount of antigen detected.

In consideration of these difficulties, we have attempted to use the extension of the reaction zone of select target molecules of the ECM in order to obtain an indication of the area covered by a certain mechanical compressive force. We have started out from the consideration—as outlined in the introduction—that the ECM reacts very sensitively to alterations of the local mechanical situation. Our hypothesis was that especially in such specialized and strictly organized tissue as

the matrix of tensile tendons, molecules that are an expression of compression forces ought to be clearly discernible. We assumed that a molecular front of sorts ought to be describable that corresponds exactly to the threshold value of compressive force provoking occurrence of the molecule in tissue. Thus, we should be able to describe rather exactly what total part of the surface of the tendon within the area of redirection of pull is subjected to certain mechanical conditions relevant for reaction.

By a comparison with previous investigations that have been able to demonstrate the presence of various molecules in parts of wrap-around tendons, suspicion has risen that the molecules characteristic of compression zones do not all react identically to local mechanical stimulus. From preliminary results, we had to assume the existence of ECM molecules already present because of low compressive strain, and of others that are formed on more intense mechanical stimulation. We may, as it were, thus assume a zone for each "marker molecule" that is characteristic of a certain limited area of compressive strain.

In the course of the experiment, however, it was seen that the individual zones could not be distinguished as clearly as desired. On the other hand, a clear picture was obtained for numerous mechanical situations, which allows further interpretation.

6.3 Discussion of Results

6.3.1 Extensor Tendons of the Toes

In all investigated toes, a metachromatic "sesamoid" fibrocartilage, as observed previously in the extensor tendons of the fingers, in various different tendons, and in the enthesis area (Lewis 1996, Benjamin et al. 1995, Carvalho 1995), is present on the deep surface of the extensor tendon. Detection of a metachromatic zone is a sign of an increased adsorption of polyanions, i.e., acidic carbon hydrates (Prento et al. 1991). The metachromatic reaction's relative stability to dehydration allows us to conclude that the polyanions are, at least partly, strongly sulfated glycoconjugates (e.g., glycosaminoglycans or proteoglycans). However, a particularly alcohol-resistant form of metachromasia is not present. Usually, such a type of metachromasia occurs only if the tissue contains large quantities of strongly sulfated proteoglycans (Prento et al. 1991).

The enthesis of the toe extensor tendons shows basically the same staining pattern as that of the finger extensor tendons (Lewis 1996). The only difference between the two entheses is that those areas of the toe entheses that are metachromatic and bear a positive labeling for type II collagen are considerably larger than the corresponding areas in the fingers.

In contrast to the findings of Kumagai et al (1994), who investigated the insertion of the tendon of m. supraspinatus in rabbits, type II collagen was also detected in the region of the tidemark in our specimens. Labeling for type III collagen could

be detected in the entire tendon tissue, also in contrast to the findings of Kumagai et al. (1994).

The accumulation of glycosaminoglycans in the region of the sesamoid fibrocartilage, which is detectable with toluidine blue, is congruent with the findings of various authors in entirely divergent tissues. For instance Flint et al. (1974) describe a higher concentration of proteoglycans in articular cartilage subjected to static compressive strain, whereas Parkkinen et al. (1992) describe an increases synthesis of proteoglycans under dynamic compressive strain. Gillard et al. (1977a,b) observed considerably more glycosaminoglycans in areas of the skin as well as of the flexor tendons of rabbits that are subject to stronger compressive strain than in areas that are not. In addition to these findings, the labeling for dermatan sulfate detectable in all investigated tendon parts may be interpreted as analogous to the findings of Gillard et al. (1977b) in rabbits' flexor tendons. There, the occurrence of dermatan sulfate was related to the ability of the tissue to sustain tensile strain.

According to Watanabe et al. (1994), the ubiquitous labeling for dermatan sulfate detectable in the investigated part of the tendon can be interpreted as an indication of tensile strain that affects the entire tendon homogeneously. This in turn results in the production of large complexes of hyaluronic acid and dermatan sulfate that are relatively resistant to traction (Watanabe et al. 1994). This interpretation is further backed by findings in other human fibrocartilaginous tissues subjected to both compressive and tensile forces. The human meniscus, for instance, shows a high concentration of dermatan sulfate (Roughley and White 1992). In accordance with the findings of Gillard et al. (1977b), Parry et al. (1982) describe an occurrence of dermatan sulfate especially in those parts of rabbit flexor tendons which are subject to tensile force, whereas chondroitin sulfate occurs particularly in those parts of the tendon under direct compressive strain. In these parts, an increased concentration of dermatan sulfate, as opposed to those parts which are under tensile strain exclusively, may frequently be observed (Gillard et al. 1977b).

Flint et al. (1982) interpret an increased concentration of chondroitin sulfate in the hand's flexor tendons as a sign of local compressive strain, which affects the tendon in addition to the regular tensile strain. One may directly apply this interpretation to our findings in the extensor tendons, since it is mostly chondroitin 6 sulfate that is found in those areas of the extensor tendons of the 2nd through 5th toes subject to compression. These parts of the tendons can also be well distinguished from the remaining tendinous tissue, as they contain sesamoid fibrocartilages. The sesamoid fibrocartilages of the toes' extensor tendons immunohistochemically show similar labeling patterns to those of the fingers for type I, III, and VI collagens(Ralphs and Benjamin 1994, Lewis 1996).

In contrast to the extensor tendons of the fingers, most sesamoid fibrocartilages of the extensor tendons of the toes show positive labeling for type II collagen. This labeling is particularly typical of the 2nd through 5th toes. It appears only logical to correlate this regular occurrence of type II collagen in the sesamoid fibrocartilages with the mechanical strain on these tendons. It is generally higher and is exerted over a longer period of time than in the finger tendons. Obviously, the deep aspect

of the toes' extensor tendons are also subjected to frequent local compressive strains, resulting in a more pronounced reactive production of type II collagen in the sesamoid fibrocartilages. However, the compressive force affecting the extensor tendon of the 1st toe does not seem to be sufficiently strong in all cases to stimulate production of type II collagen. This seems all the more plausible if one takes into account the movement pattern of the 1st toe's IP joint, especially in the walking cycle. This IP joint is flexed considerably less frequently than those of the 2nd through 5th toes.

Since type I, II, and III collagens occur in the shape of fibrils of various diameter, which are regularly composed of more than one type of collagen (Silver 1983, Burgeson and Nimni 1992, Keene et al. 1987, 1995), and since, besides local pH, it is the concentration of glycosaminoglycans that significantly influences the latitudinal growth of such fibrils—in vitro, chondroitin 6 sulfate causes the formation of fibrils of 30 nm diameter (Silver 1983)—we assume that glycosaminoglycans may be expected to be the first to react to mechanical alterations of the tissue. In this context it is interesting to observe that in chondrocyte cultures the pericellular collagen fibrils—mostly type II collagen—have a diameter of 15 nm (Ruggiero et al. 1988). Therefore, we may well correlate our findings, and the resulting chronological pattern of tissue adaptation, with the findings of Merrilees and Flint (1980), who described loosely arranged collagen fibrils of narrow diameter (average approximately 30 nm) in parts of tendons subjected to compressive force and even in areas of the skin subjected to comparable types of strain (Flint et al. 1984), whereas tight, parallel fibrils of larger diameter (average between 30 nm and 150 nm) predominated in regions subject to tensile force.

Bray et al. (1993) are of the opinion that type VI collagen serves as an anchoring structure between chondroitin sulfate/hyaluronic acid complexes, on the one hand, and type I collagen fibrils, on the other. This arrangement would surround the collagen fibrils with a strongly hydrated layer that would make easier the transportation of fluids between the fibrils. According to Burgeson and Nimni (1992), type VI collagen favorably influences the attachment of fibroblasts to the collagenous network. The distribution of labeling for type VI collagen observed here could well be correlated with such a model. However, our findings contradict the strictly pericellular distribution of type VI collagen postulated as normal in human tissue by Roberts et al. (1993).The predominantly pericellular labeling that they described is only correct, in our observations, around the osteocytes of the bone in the enthesis area, at most.

The regular occurrence of type II collagen in the sesamoid fibrocartilages of the extensor tendons can be interpreted as a sign of adaptation to compressive force. The PIP joints of the 2nd through 5th toes are usually flexed more strongly in the walking cycle than the IP joint of the 1st toe. If the joint is in a flexed position, the tendon can articulate with the distal articular face of the proximal phalanx, which is covered with hyaline cartilage. A more acute flexion angle of the joint results in a higher compressive force on the undersurface of the extensor tendon, where it slides over the distal articular face of the proximal phalanx. If the foot is in

a normal position this means that the local compressive force in the 2nd through 5th toes is larger than in the extensor tendon of the 1st toe.

Burton-Wurster et al. (1993) detected that pressure of longer duration (18 h) impeded the synthesis of proteoglycans in tissue blocks of explanted hyaline cartilage, and that intermittent pressure did not influence the synthesis rate. Other investigations, however, have shown that in certain load-bearing cycles, intermittent compressive force is appropriate for increasing the synthesis rate of proteoglycans (Evanko and Vogel 1993; Robbins et al. 1997).

Combining these findings with our knowledge about the walking cycle, the conclusion seems justified that intermittent local compressive load caused fibrocartilaginous regions to develop in the extensor tendons. In this sense our findings concerning metachromasia, which signify varying concentrations of glycosaminoglycans and proteoglycans, may also be explained. The regionally varying distribution of the various glycosaminoglycans throughout the investigated part of the tendon provides further support for this assumption.

Labeling for 3B3- and 7D4, i.e., for two different epitopes of chondroitin 6 sulfate that are usually rare in adult tissue, have hitherto been evaluated as an indication of degenerative alterations of intervertebral disc tissue or of articular cartilage (Caterson et al. 1990, Carney et al. 1992, Roberts et al. 1994, Slater et al. 1995). Besides that, pronounced labeling for 3B3- regularly occurs in embryonic opossum articular cartilage up to day 19 postpartum and disappears in the adult animal (Archer et al. 1996). It is therefore difficult to estimate, whether our specimens may perhaps represent an early stadium of degeneration of these parts of the tendons that cannot be diagnosed with conventional morphological methods. Strikingly, it is predominantly the fibrocartilage cells that are positive, whereas the remaining parts of the tendons always are free from any labeling. If one applies the interpretation of labeling for 3B3- that has been outlined above, this might signify that degenerative alterations become first manifest in the fibrocartilages and only do so much later in the remaining tendinous tissue.

According to Flint et al. (1984), high concentration of dermatan sulfate in skin is a sign of mature tissue. This means that the presence of this labeling signifies not only the mechanical function of the tissue, but also the age of the donor. A similar explanation of the uniform labeling for keratan sulfate throughout the entire tendinous tissue offers itself, since Bishop and Pearce (1993) demonstrated that the relative amount of keratan sulfate in the cartilaginous end plates of the vertebral bodies increases with advancing age and possibly increasing degeneration of the intervertebral disc. Applying this interpretation to the toes' extensor tendons, we may also assume that the presence of labeling results from the advanced age of the donors. Such a view, however, contradicts Flint (1974), who interpreted higher concentrations of keratan sulfate as a sign of tensile strain. We conclude that both factors may be causal for the labeling for keratan sulfate that we have observed.

From our results we deduce that evidently all cells of the fibrocartilages of the extensor tendons are capable of producing type II collagen, but that they only do

so if the mechanical pressure stimulus rises above a certain threshold. This would explain why we have found type II collagen in all sesamoid fibrocartilages of the 2nd through 5th toes and in all of the investigated enthesis fibrocartilages, but only in three out of six fibrocartilages of the 1st toe. The occurrence of type II collagen and/or chondroitin-6-sulfate-positive fibrocartilage in a tendon thus seems to be a morphological indicator of a significant local compressive force affecting the tissue in addition to the regular tensile force.

According to our interpretation, these results are a further proof of the claim that the process of functional adaptation to mechanical strain not only leads to measurable alterations in mineralized tissues or in hyaline articular cartilage, but that such reactions occur likewise in those tendons and ligaments that cross the synovial joints.

6.3.2
Extensor Tendons of the Hands in the Region of the MCP Joint

The present results show unambiguously that there is a distinct fibrocartilage present in the extensor tendons in the area of the MCP joints, which is comparable to that of the extensor tendons in the area of the proximal IP joints (PIP joints; Lewis et al. 1998). Furthermore, it is shown that the fibrocartilage's immunohistochemical labeling pattern for glycosaminoglycans and collagens is mostly identical with that which has been described in the fibrocartilage of the extensor tendons of the PIP joints (Lewis et al. 1998). At both sites, chondroitin 6 sulfate and type II collagen are typical markers of the fibrocartilaginous phenotype; and in either joint the occurrence of type II collagen is particularly characteristic of the extensor tendon of the index finger. Despite the higher torque (due to the longer lever) of the extensor tendons in the region of the MCP joints (Littler and Thompson 1987) and despite the relative independence of movement of the MCP joints in various gripping functions of the hand, there is no more distinct fibrocartilage present in the extensor tendons than there is in the region of the PIP joints. The potentially higher compressive forces, which affect the extensor tendons in the region of the MCP joints, are possibly counterbalanced by the tendon's independent layout outside the articular capsule, not forming part of it. In the region of the PIP joints, the extensor tendon completely replaces a part of the articular capsule; therefore it can be pressed directly against the head of the proximal phalanx if the joint is in a flexed position (Lewis et al. 1998). In contrast, the deep peritendinous connective tissue is interposed in the region of the MCP joints (Landsmeer 1955). This tissue is potentially capable of intercepting part of the compressive forces and homogeneously distributing it throughout the entire tendon. This interpretation is backed by occasional occurrences of chondroitin 6 sulfate in the deep peritendinous connective tissue. Possibly, the maintenance of a comparable degree of fibrocartilaginous differentiation at two sites along the course of an extensor tendon may also be explained by the similarity of the resistance to traction and to compression at either site, which prevents occurrence of a weaker link in the chain

of force transmission that might be caused either by too extensive cartilaginous differentiation or else by the absence of differentiation.

Grounded on the results of previous investigations, we have also clearly shown that aggrecan is a characteristic component of the sesamoid fibrocartilages of all fingers. Aggrecan is a large proteoglycan that occurs typically in hyaline articular cartilage (Jackson et al. 1991; Heinegård and Oldberg 1993), and that is presumably associated with most of the chondroitin 6 sulfate detected in the extensor tendons. Thus, the situation is analogous to that described in those regions of the deep flexor tendons of adult cows that are subject to compressive force (Vogel 1995; Robbins et al 1997). A similar connection has been shown for the sesamoid fibrocartilage of the human Achilles tendon (Waggett et al. 1998) and for the tendons of the rotator cuff (Berenson et al. 1996). The fact that aggrecan typically occurs in the sesamoid fibrocartilage of fingers allows the assumption that fibrocartilage cells may increase the proteoglycan synthesis more easily than the type II collagen synthesis. Such a phenomenon is further highlighted by experimental studies of Robbins et al. (1997) and earlier of Evanko and Vogel (1993), who detected a comparable behavior in the region of the bony pulleys (hypomochlia) of the deep flexor tendons of cows. Tendon segments from these regions were subjected to a cyclical compressive force in vitro. The aggrecan synthesis significantly increased during the first three days of the culture. A further observation to support this assumption may be made in the course of development of the suprapatella (a fibrocartilaginous region in the quadriceps tendon of rats, rabbits, and other small mammals; Tischer et al. 2002). Here, proteoglycans accumulate in the ECM long before type II collagen first occurs (Benjamin et al. 1991). Besides, there are no hints in the literature indicating that, in response to a change of mechanical strain, the synthesis of type II collagen may be increased as fast as the synthesis of proteoglycans. In the region of a surgically effected bony attachment of a tendon (enthesis), a gradual occurrence of type II collagen may be observed (Liu et al. 1997).

Furthermore, it seems important to be aware that compressive force on the extensor tendons of the fingers always requires a contraction of the flexor muscles. If the fingers are not flexed, a tightening of the extensor muscles alone is not capable of effecting increased pressure on the extensor tendons in the area of the MCP or the PIP joints. The two flexor muscles of each finger (m. flexor digitorum superficialis and profundus) are together about four times stronger than the corresponding extensors (Lanz and Wachsmuth 1935). This is significant in the context of maximal force applied in the so-called “power grip” and the maximal compressive strain on the extensor tendons which results from this. As opposed to the middle and ring fingers, the index finger has a further extensor muscle (m. extensor indicis), whose tendon fuses with that of m. extensor digitorum in the region of the MCP joints. From the fact that the maximal force of m. extensor indicis roughly corresponds to one third of the force of the entire m. extensor digitorum (Lanz and Wachsmuth 1935), one may conclude that in the case of an equal contraction of the extensor and flexor muscles (maximal “power grip”), the extensor tendon of the index is probably subjected to a higher compressive strain in the region of the MCP joint

than are the remaining tendons. Although the small finger also has an additional extensor muscle (m. extensor digiti minimi), the finger as a whole is considerably weaker and cannot sustain such maximal force. This difference is likewise reflected by the quality of the sesamoid fibrocartilages. Although the muscle forces are considerably lower in fine-tuned grasping ("precision grip"), the musculature of the index is more frequently activated than that of the other fingers due to the frequent opposition of index and thumb. Together with the wider range of motion of the index, these factors can account for the observation that the phenotype of the sesamoid fibrocartilage of the 2nd extensor tendon is "more cartilaginous" than the fibrocartilages of the other extensor tendons. The difference may not have anything to do with a wider tendon excursion, since there exist only minute differences between the fingers with regard to this parameter (Pahnke 1987). Particularly in the region of the MCP joints the differences are virtually non-existent; rather, the tendon excursion is identical in the 2nd, 3rd, and 4th fingers (Elliot and McGrouther 1986). Likewise, it is improbable that differences between the fingers' resting positions are significant, although the MCP joint of the index typically shows a less acute angle of flexion than the other fingers' MCP joints (Jones 1941).

6.3.3 The Transverse Ligament of the Atlas

The immunohistochemical labeling patterns for glycosaminoglycans and collagens found in the transverse ligament of the atlas are essentially comparable to the distribution patterns previously described in other fibrocartilages (Lewis et al. 1998; Milz et al. 1998, 1999). In all parts of the ligament, typical markers of the fibrocartilaginous phenotype are chondroitin 6 sulfate and type II collagen, which are found both at the enthesis and in the sesamoid fibrocartilage. The pericellular basketweave arrangement of type VI collagen fibers is another characteristic of human fibrocartilage. The layer of orthochromatic extracellular matrix situated directly beneath the articular facet toward the dens of the axis resembles a morphologically similar boundary layer, namely, that covering the sesamoid fibrocartilage at the undersurface of the Achilles tendon of rats (Rufai et al. 1996). The exact function of this boundary layer is still unknown. Speculation is that it protects the sesamoid fibrocartilage at those sites where it is exposed to direct compressive and shear forces. Another opinion is that it might only be rudimentary degenerative material (Rufai et al. 1995, 1996).

Possibly the most interesting finding of the present study is the consistent presence of aggrecan and link protein in the sesamoid fibrocartilage of all ligaments investigated. Aggrecan is a large proteoglycan, which is considered to be a characteristic feature of hyaline articular cartilage (Heinegård and Oldberg 1993). It is presumably associated with the main mass of the chondroitin 6 sulfate detected in the ligament, as it has been described in those regions of the deep flexor tendons of adult cows that are subjected to compressive force (Vogel 1995; Robbins et al

1997). Similar occurrences of chondroitin 6 sulfate are described in the sesamoid fibrocartilage of the human Achilles tendon (Waggett et al. 1998), in the sesamoid fibrocartilages in the region of human MCP joints (Milz et al. 1999), and in the tendons of the rotator cuff (Berenson et al. 1996).

The consistent presence of aggrecan and chondroitin 6 sulfate in the transverse ligament of the atlas permits the conclusion that the ligament is subjected to a considerable compressive force by the dens of the axis. Link protein is an extracellular glycoprotein, which is responsible for the stabilization of the interaction between aggrecan and hyaluronic acid and thus significantly contributes to the formation and maintenance of juvenile and adult cartilaginous extracellular matrix (Watanabe and Yamada 1999). The two epitopes that are recognized by the antibody 8A4 utilized in the current investigation are situated within the "tandem-repeat domain" of link protein and belong to that part of the molecule which participates in the interaction with hyaluronic acid (Doege et al. 1986; Goetnick 1993). To our knowledge, the results presented here are the first description of such a pronounced occurrence of link protein in a human ligament.

From a clinical point of view, the detection of a distinct fibrocartilage which shows considerable occurrences of aggrecan and link protein is significant both for cases of acute ligament injuries and for those of chronic inflammatory diseases. In those rare cases in which an isolated rupture of the transverse ligament of the atlas is reported (e.g., Segal et al. 1987), it may be assumed that one of the fibrocartilaginous regions is directly affected by the rupture. Since the potential of healing is limited, as in other fibrocartilages, and since such ruptures have to be stabilized, the therapy chosen is usually a surgical fusion of atlas and axis (Lipson 1977; Levine and Edwards 1989). Incomplete ruptures or other injuries of the transverse ligament of the atlas can result in an altered mechanical situation that may lead to an ossification or calcification of the ligament (Perera et al.1995; Constantin and Bouteiller 1998, Hayashi et al. 1998). Due to our findings we assume that in these cases a metamorphosis to mineral tissue may be caused, or at least favorably influenced, by the existence of such a prominent fibrocartilage in the transverse ligament of the atlas.

In about 20% of patients with chronic rheumatoid arthritis, the transverse ligament of the atlas gradually degenerates, which increasingly impairs the mechanical stability of the atlanto–axial complex (Zeidman and Ducker 1994; Fujiwara et al. 1998; 1999). Since aggrecan cleavage by aggrecanase in hyaline articular cartilage has been described as a characteristic of rheumatoid arthritis (Caterson et al. 1999; Lohmander et al. 1993), it seems possible that such cleavage occurs in the transverse ligament of the atlas, thus contributing to its degeneration. In this context it is remarkable that link protein—as discussed at length in Sect. 6.3.3—is nowadays considered to be one of the important antigenic targets in the autoimmune processes in the course of juvenile and adult forms of rheumatoid arthritis (Guerassimov et al. 1997, 1998; Zhang et al. 1998). Since rheumatoid arthritis is a systemic disease which mostly affects tissue of the cartilaginous phenotype, and since the transverse ligament of the atlas shows especially pronounced labeling

patterns for link protein, the ligament may be seen as a preferred target of such destruction. Therefore, it is to be expected that the transverse ligament of the atlas will lose its mechanical function more rapidly in the case of disease than would be expected of a ligament that contains no fibrocartilaginous tissue and is subjected to an exclusively tensile strain.

6.3.4 The Transverse Ligament of the Acetabulum

Our findings show that the central part of the transverse ligament of the acetabulum, which faces the articular surface, may be classified as a "moderately cartilaginous" sesamoid fibrocartilage. The results of the immunohistochemical investigation thus support the conclusions that may be drawn form the biomechanical data provided by Löhe et al (1994, 1996), Lazennec et al (1997), and Vandenbussche et al. (1999). Accordingly, the transverse ligament of the acetabulum is subject to the combination of local compressive and tensile stress during load bearing of the hip joint. This stress is caused by moving apart of the horns of the lunate articular surface of the acetabulum and/or is the consequence of the slight incongruence of the articulating surfaces of the hip joint. In either case, the articular surface of the transverse ligament of the acetabulum is pressed against the femoral head.

The present investigation allows no quantification of the magnitude of local compressive stress on the ligament. Due to its immunohistochemical profile, however, it is possible to rank the transverse ligament of the acetabulum within the wide spectrum of dense connective tissues that reaches from entirely fibrous to distinctly cartilage-like. The transverse ligament of the acetabulum is clearly more fibrous than, e.g., the transverse ligament of the atlas (Milz et al. 2001); however, it is more cartilaginous than the tendon of m. extensor pollicis longus in the region where it is redirected by the dorsal tubercle of the radius (Benjamin et al. 1995). The transverse ligament of the acetabulum contains a small number of, as it were, strategically positioned fibrocartilage cells at its articulating inner surface where it is in direct contact with the femoral head. This observation refutes previous authors' claims to the contrary (Williams et al. 1998).

Furthermore, at least in some specimens, weak labeling for aggrecan and type II collagen (i.e. for those molecules that typically occur in articular cartilage) was present where they are responsible for enabling the tissue to be resistant to compressive stress (Jackson et al. 1991). In articular cartilage, the high density of negatively charged sulfated glycosaminoglycans, which are a major part of aggrecan, leads to a high water-binding capacity in the tissue. The aggrecan-and-water complex is at least temporarily held in position by the fibrous network of the type II collagen molecules.

In addition to the sesamoid fibrocartilage in the center of the ligament, distinct fibrocartilages are present at either bony attachment of the transverse ligament. This is interesting, since unlike numerous other ligaments, this one does not cross

a joint, and is only connecting two regions of the same bone. Accordingly, articular movements, as they usually result in an alteration of direction of the collagen fibers at the enthesis region, cannot occur in this ligament. Thus, a mechanism seems to be absent which is usually connected with the production and presence of a fibrocartilage due to increased shear forces at the tendon's attachment site (review concerning this topic: Benjamin and Ralphs 1995). However, the transverse ligament of the acetabulum may to some extent alter its direction if it is subjected to strong tensile stress due to direct pressure of the femoral head on the central part of the ligament during load bearing of the hip joint.

In other parts of the body, articular flexions can cause tendon and ligament fibers to alter their direction rather strongly. Together with the usual tensile stress, these suffice to explain the presence of a prominent enthesis fibrocartilage (Benjamin et al. 1986; Lewis et al. 1998). A sound rule is: the more acute the angle of flexion, the more fibrocartilage can be expected (Evans et al. 1991, Benjamin and Ralphs 1995). A factor for the presence of such as strong enthesis fibrocartilage in the transverse ligament of the acetabulum is, we assume, the unusually rapid increase of shear strain caused by the quickly increasing tensile stress in the ligament. Although the degree of maximal tensile tension in the transverse ligament of the acetabulum is yet unknown, it is expected to be relatively high if the measured longitudinal differences in length resulting from load bearing of the hip joint is considered (Löhe et al. 1994, 1996; Lazennec et al. 1997, Vandenbussche et al. 1999). In this case it could be possible to assume that, despite a relatively insignificant alteration of the fiber course's angle, a biologically relevant shear stress may occur at the attachments due to a rapid increase of tensile stress, and that this shear stress is the actual mechanical stimulus that causes the development of enthesis fibrocartilage. Furthermore, cyclical longitudinal changes of the ligament during static and dynamic load of the hip joint (walking, running, jumping) certainly contribute to the development of fibrocartilage as well (Löhe et al. 1994, 1996; Lazennec et al. 1997, Vandenbussche et al. 1999).

A further phenomenon that can contribute to the development of enthesis fibrocartilage is to be seen in the fact that any tendon reduces its diameter with elongation. Such a change of shape, however, must necessarily be avoided in the region of the bony attachment, because it would inevitably result in extraordinarily high stress—especially by shear strain. This "stretching brake" theory of the enthesis fibrocartilage was first outlined by Knese and Biermann (1958); however, it has found little resonance up to this day. Yet our results correlate well with these assumptions, since the increased presence of aggrecan and link protein in the transverse ligament of the acetabulum could explain why at this site the ligament's diameter does not change even under severe stress. Aggrecan resists any deformation by compressive force because it can bind large quantities of water. Since water cannot be compressed, the aggrecan rich tissue can likewise not be deformed at this site. Thus there is no possibility of a change in the ligament's diameter.

6.3.5
Tendon and Trochlea of the Superior Oblique Muscle

In Sect. 3.5 we described at length the mechanical situation of the tendon in the trochlea region where its direction of pull is altered. The morphological findings, however, allow no quantification of the intensity of the local compressive strain to which the tendon is subjected at this site. Yet it may be said that the tendon of the superior oblique muscle occupies a marginal position in the spectrum of tendinous and ligamentous tissues that stretches from purely fibrous to cartilaginous. The tendon contains distinctly fewer fibrocartilage cells than, e.g., the transverse ligament of the atlas described above, and even fewer than the tendon of m. extensor pollicis longus in the region where it passes over the dorsal tubercle of the radius (Benjamin et al. 1995). On the gliding surface of the tendon of the superior oblique muscle only isolated, strategically positioned, fibrocartilage cells are present in the area of direct contact with the trochlea. Only in one case is immunohistochemical labeling for type II collagen detectable, whereas aggrecan is regularly detectable. Aggrecan is a macromolecule typical of articular cartilage, with whose resistance against compressive force it is correlated (Jackson et al. 1991). In the articular cartilage, water is bound in the tissue by the high loading density of the sulfated glycosaminoglycans, which were also detectable in the investigated tendon. The water-and-aggrecan (hyaluronic acid-) complexes thus formed subsequently interact with the fibrous networks of type II collagen and—if the latter is absent, as in our case—also with type I collagen molecules (Hedlund et al. 1999).

In contrast, a distinct fibrocartilage is detectable histologically and immunobiochemically in the trochlea. In its center, it shows characteristics of hyaline cartilage. This is emphasized by the consistent presence of type II collagen and chondroitin 6 sulfate.

Tendon and trochlea of the superior oblique muscle are an example of partners involved in a gliding situation reacting differently. While the compressive stresses in the tendon do not reach the intensity of mechanical stimulation necessary for the full adaptation, they clearly do so in the trochlea. The detection of aggrecan in the tendon proves that compressive strain is present, as may be expected. However, it is not intense enough to also provoke the production of type II collagen.

This finding is not as contradictory as it seems at first. A comparable situation is seen in the subchondral mineralization of the large joints. Here, too, the density is higher in the area of the lesser articular partner than in that which is more extensive. The reason for this is undoubtedly the different duration of compressive stress in the course of motion. Whereas the lesser surface remains in constant contact in the course of the excursion, the contact zone migrates across the entirety of the larger articular surface during the excursion.

A comparable situation is that in the region of the trochlea. The excursion of the tendon up to 5.6 mm ensures a certain “exoneration” in the peripheral zones. The central segment alone remains in constant contact in any case. Therefore,

compressive stress sufficient to provoke the production of fibrocartilage may occur here alone.

A clinical picture caused by the loss of mobility of the tendon of the superior oblique muscle in the region of the trochlea is referred to in medical literature as "superior oblique tendon sheath syndrome" or "Brown's syndrome" (Brown 1950; Catford and Hart 1971; Wilson et al. 1989; Hadjadj et al. 1998). This mobility disorder of the tendon can have a number of causes and frequently results in surgical intervention (Stein and Pabst 1969; Haworth 1970; Scott and Knapp 1972, Sprunger et al. 1991). Although the etiology of the various forms of this functional disorder is controversially discussed, it has long been suspected that several of the acquired and frequently intermittent forms of Brown's syndrome may be associated with inflammatory processes (Bielschowsky 1904; Girard 1956, Roper-Hall and Roper-Hall 1971; Jacobi 1972, Sandford-Smith 1975), in particular with the clinical picture of rheumatoid arthritis (for a review see Pittke 1986; Wilson et al. 1989).

It has been long known that in Grave's disease (*Morbus Basedow*), autoantibodies against a 23 kDa protein of human fibroblasts are a very probable reason for ophthalmopathy (Bahn et al. 1989). However, only a few signs of a manifestation of a further autoimmune disease in the region of the external eye muscles have been established. Several authors have pointed out that there might possibly exist a coincidence of acquired forms of Brown's syndrome and rheumatoid arthritis (Mortensen et al. 1998; Olivares et al. 1988; Roifman et al. 1985; Beck and Hickling 1980; Sandford 1969, 1975) This coincidence has been described for both juvenile (Kemp et al. 1984; Roifman et al. 1985) and adult (Mortensen et al. 1998; Olivares et al. 1988) forms of rheumatoid arthritis. In this context, it is interesting to note that Brown's syndrome is occasionally regarded as stenotic tenosynovitis or tenovaginitis. A thickening of the tendon of the superior oblique muscle is described, which impedes the free excursion of the tendon in the region of the trochlea (Sandford-Smith 1973): However, its etiology is not further expanded.

Bearing in mind the assumption that functional adaptation determines the distribution of the investigated molecules, it is striking that aggrecan and link protein typically occur in the fibrocartilaginous regions of the trochlea and, at least in several cases, also in the tendon, whereas versican, which is considered to be the large proteoglycan typical of connective tissue (Zimmermann 1993), is characteristic of the more fibrous regions and—accordingly—is absent in the centers of individual trochleae that appear to be at the chondroid end of the spectrum of tissues. Link protein is an extracellular glycoprotein which is necessary for stabilizing the interaction between aggrecan and hyaluronic acid. Its reactive groups are situated in so-called tandem-repeat domains that are responsible for this interaction. The monoclonal antibody 8A4 is able to recognize two epitopes in these domains (Doege et al. 1986; Goetinck 1993), allowing one to conclude that the link protein labeled here may be functionally active.

The significance of the strong labeling for this functionally remarkable molecule becomes clear when one correlates it with those clinical observations that describe

a coincidence between the transient appearance of Brown's syndrome and the existence of juvenile and adult forms of chronic rheumatoid arthritis (Beck and Hickling 1980; Kemp et al. 1984). Since the enzyme degradation of aggrecan by aggrecanase is a typical sign of degeneration of articular cartilage in rheumatoid arthritis and other arthropathies (Caterson et al. 1999; Lohmander et al. 1993), it seems probable that a comparable process of degeneration occurs in the trochlea, and possibly in certain parts of the tendon, that in turn results in degeneration, or at least in a local inflammatory reaction with swelling and thickening of the tendon.

In this context, it is an important circumstance that by now link protein is considered to be one of the essential antigens recognized in the setting of an autoimmune response in juvenile and adult forms of rheumatoid arthritis (Guerassimov et al. 1997, 1998; Zhang et al. 1998). As the trochlea and, depending on the region and the molecular parameter, also certain sites within the tendon have a very prominent fibrocartilage and a strong labeling for link protein, aggrecan, and type II collagen (which itself is an essential antigen in rheumatoid arthritis; Falta and Kotzin 1998), it becomes clear why this region is a preferred target for manifestations of inflammatory reactions. After all, rheumatoid arthritis is a systematic disease that on principle affects all tissues of cartilaginous phenotype. It has to be expected that in the course of disease, the trochlear region will lose its mechanical function—i.e., to ensure free gliding mobility of the superior oblique tendon—earlier than it would if only fibrous tissue were present at that site. This assumption is grounded upon previous reports of partial disappearance of enthesis fibrocartilage at the attachments of the finger extensor tendons on the distal phalanges of patients with rheumatoid arthritis (Benjamin et al. 1993).

These findings, therefore, allow the assumption that large amounts of aggrecan, link protein, and type II collagen in the trochlea and parts of the tendon are a natural target for (auto)immune reactions, such as have previously been reported in patients with rheumatoid arthritis (Guerassimov et al. 1997, 1998; Zhang et al. 1998).

6.4 Conclusions

Using select examples, the present study attempts to contribute to the understanding of the connection between local compressive stress and the occurrence of molecules typical of cartilage in the ECM of tendons and ligaments.

The extension of the molecular adaptation zone roughly corresponds with the zone subjected to compression; merely tensile stress without accompanying compressive stress does not result in the production of fibrocartilage. This applies to the investigated tendons and ligaments where they cross a bony pulley (i.e., hypomochlion), or where they are subjected to direct pressure by another articular component. The occurrence of fibrocartilage at the bony attachment site (enthesis) can be explained analogously. Where flexion at the site of attachment causes shear

Table 13 Relation between the occurrence of the different components of the extracellular matrix and the relative magnitude of tensional and compressive stress. Note that compressive stress can occur also in tissues which are at the same time subjected to tensional tress

Mechanical situation	GAGs	Molecular marker PGs	Collagens	Tissue morphology (partly from literature)
Pure tensional stress	DS, KS	Tenascin, Versican	+++ Collagen I, Collagen III, VI	Fibroblasts
Low compressive stress	DS, KS	Tenascin, Versican + Aggrecan	++ Collagen I, Collagen III, VI	Diminishing of cellular processes
Increasing compressive stress	DS, KS, C4S	Tenascin, Versican ++ Aggrecan, + Link protein	+ Collagen I, Collagen III, VI + Collagen II	Oval cells, often arranged in rows
High compressive stress	DS, KS, C4S, C6S	Tenascin, +++ Aggrecan, ++ Link protein Versican is negative	Collagen I (only traces), Collagen III, VI ++ Collagen II	Rounded, chondrocyte-like cells
Very high compressive stress	DS, KS, C4S, C6S (++)	+++ Aggrecan, +++ Link Protein Tenascin is negative	+++ Collagen II ++ Collagen III, VI Collagen I is negative	Hyaline-like Cell morphology, formation of chondrones

this also results in local compressive stress and the ECM reacts by a characteristic molecular adaptation (Table 13).

Therefore, an analysis of the molecular components of the ECM allows one to make conclusions about the tissue's local mechanical situation. Since these "molecular parameters of strain" are highly sensitive indicators, they significantly help in estimation of both the expansion of the adaptation zones and the degree of local compressive stress.

Adaptation may also be detected histomorphologically from the increasingly changing shapes of fibroblasts. They withdraw their processes and become cells typical of cartilage, which are also referred to as fibrocartilage cells. This change-of-shape characteristic occurs regularly from the outside to the inside. A series of sections will clearly show that this restructuring process is directly correlated with the locally effective compressive strain. We assume that this process of change could also imply a chronological order, but as we rely on human material, we are not yet able to corroborate this suggestion.

With regard to the composition of the ECM, there is also a typical order detectable that depends on the intensity and duration of the local mechanical situation, especially the compressive force. If local compression increases, the gly-

cosaminoglycans react before the proteoglycans, which in turn tend to react earlier than the collagens.

Among the glycosaminoglycans, the order in which they occur is roughly the following. The first low local compressive strain results in the appearance of dermatan sulfate and keratan sulfate. Such a mechanical situation is achieved in nearly all investigated tendons and ligaments, even by the deformation of the tissue from tensile stress alone. Thus, we find these molecules virtually throughout all investigated specimens. Even relatively minor stress seems to be sufficient to cause these molecules to form. If local compressive stress increases, chondroitin 4 sulfate is the next to occur. Finally, the last glycosaminoglycan to occur in the morphologically distinctly fibrocartilaginous tissue—in case now of significant local compression—is chondroitin 6 sulfate.

With regard to proteoglycans, the order is somewhat different. Versican and, to a lesser extent, tenascin are typical markers of dense connective tissue with an abundance of collagen fibers. These proteoglycans decrease and eventually vanish locally, indirectly proportionate to the increase of compressive stress and resulting fibrocartilage formation. In contrast, aggrecan and link protein expression becomes more and more prominent. In tissues of particularly cartilaginous phenotype (hyaline-like tissue phenotype), type I collagen eventually disappears and is replaced by type II collagen.

The ECM's molecular composition is significant also with regard to various forms of rheumatoid arthritis. In the literature, hints are increasing that part of the inflammatory destruction may potentially and at least indirectly be ascribed to autoimmune processes against fibrocartilaginous components of ECM. Most likely target molecules appear to be aggrecan and link protein. This pathological process applies particularly to such zones that—due to hydrostatic intermittent compressive strain—have undergone significant cartilaginous, i.e., hyaline-like, restructuring. Such reorganization is particularly distinct in the region of the transverse ligament of the atlas. One is led to the conclusion that the prominent fibrocartilages investigated in the course of the present study are sites prone to exhibit an extraarticular manifestation of rheumatoid arthritis.

7 Summary

The present study pursues the hypothesis that local compressive force and the occurrence of cartilage-specific transformation processes within the extracellular matrix of tendons and ligaments are directly correlated. We compare the pattern of certain marker molecules typical of (fibro)cartilage in select examples. Investigations are carried out of the extensor tendons of toes and fingers, the transverse ligament of the atlas, the transverse ligament of the acetabulum, and of the tendon of the superior oblique muscle and its trochlea. The marker molecules are detected with standardized immunohistochemical methods.

The results show that certain molecules only occur under conditions of (relatively high) compressive stress, others being the result of tensile stress. The molecular spectrum of the molecules of the ECM allows qualifying conclusions as to the mechanical situation of a given part of the tissue. A quantifying statement about the intensity of compressive stress is not possible to make thus far, but the extension of the restructuring areas corresponds to the area of compressive stress.

Depending on the intensity and duration of the local compressive strain, the molecules involved may be ordered chronologically according to their occurrence in the ECM. The glycosaminoglycans react at lower stress levels than the proteoglycans, which in turn react earlier than the collagens, especially with regard to the vanishing of type I collagen and the first occurrence of type II collagen. Of the glycosaminoglycans, dermatan sulfate and keratan sulfate occur first. These are detectable in virtually all cases. They are followed by chondroitin 4 sulfate. The last glycosaminoglycan, which occurs in already significantly fibrocartilaginous tissue, is chondroitin 6 sulfate.

Under chronologically intensifying compressive stress in the increasingly fibrocartilaginous tissues, the proteoglycans versican and, to a lesser extent, tenascin—characteristic markers of fibrous tissue—can still be detected. However, their spatial expansion steadily decreases until they finally vanish. Contrastingly, aggrecan and link protein expression becomes increasingly prominent in such tissues.

The spatial expansion of the adaptation zones in tendons and ligaments roughly corresponds with the zones subjected to compressive force; tensile stress alone does not result in a production of fibrocartilage.

The questions asked at the beginning may thus be answered as follows: The molecular composition of the various fibrous connective tissues, such as tendons and ligaments, can be directly correlated with the respective tissue's mechanical function. As an expression of this regular interrelation, a ranking of certain ECM molecules may be set up that corresponds to the type, intensity, and duration of the mechanical stress. Grounded on this, it seems possible to prognosticate the occurrence of certain components in the ECM depending on the nature of the mechanical stress.

The occurrence of certain molecules within the fibrocartilaginous tissue is of clinical importance in connection with various forms of rheumatoid arthritis and

perhaps other diseases with an autoimmune-related etiology. Since a considerable part of the inflammatory destructions involved may at least indirectly result from autoimmune processes directed against the cartilage-type components of the ECM, every fibrocartilage constitutes a potential target to a certain degree. This applies particularly to those fibrocartilages whose ECM has a molecular composition closely resembling that of hyaline articular cartilage. Therefore, knowledge of the regional molecular composition allows a prediction of sites where clinical symptoms may occur in the course of rheumatoid arthritis.

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