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THE INFLUENCE OF MECHANICAL FORCES ON THE GLYCOSAMINOGLYCAN CONTENT OF THE RABBIT FLEXOR DIGITORUM PROFUNDUS TENDON

GERALD C. GILLARD, HELEN C. REILLY, PAUL G. BELL-BOOTH and MICHAEL H. FLINT

Department of Surgery, School of Medicine, University of Auckland, Private Bag, Auckland 1, New Zealand

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The physical forces acting on the flexor digitorum profundus tendon of the rabbit were altered by anterior translocation of the tendon. The glycosaminoglycan (GAG) content was determined in regions of the tendon previously under tension or previously subjected to pressure. There was an increase in the GAG content in the original tension transmitting region. Initially this was due to increased hyaluronic acid and chondroitin sulfate content during the early remodeling phase. Later when tension was restored to the translocated tendon, the content of these two GAG decreased to normal values while the high overall GAG concentration was maintained by increased amounts of dermatan sulfate. Finally the dermatan sulfate content and the total GAG content returned to normal values.

The original pressure bearing region showed a rapid loss of total GAG. This was mainly due to a loss of chondroitin sulfate component, and eventually the region showed a GAG composition similar to that of normal tension transmitting tendon. Replacement of the translocated tendon to its normal position resulted in a slow replacement of the GAG, particularly chondroitin sulfate, in the pressure bearing region. The extent of recovery appeared to be dependent on the length of time the tendon had been left in the translocated position. Attempts to form a new pressure bearing structure in another part of the tendon also resulted in changes in the proportions of the GAG, but little change in total GAG content.

INTRODUCTION

The gross morphology and development of connective tissues and the type of extracellular macromolecules within such tissues are largely genetically controlled but little is known of the fine control of the relative concentrations of these extracellular components. The normal function of most connective tissue is to withstand or transmit physical forces and it is evident that these exert a control over the metabolism and organisation of the tissues. This has been observed where unique biochemical and morphological modifications correlate with unusual or abnormal functional requirements[1,2] or can result from the application of abnormal or modified physical forces[3–8]. The involvement of mechanical forces in control mechanisms has also been demonstrated using connective tissue cells in culture[9,10].

Previously we have postulated that collagen fibers may be involved in the control of proteoglycan synthesis. The staining affinity of collagen for the dyes of the Masson trichrome procedure, and therefore the surface charge characteristics of the collagen vary with the longitudinal tension on the collagen fibers[11–13]. Tension on collagen fibers also directly influences their axial periodicity[1,14]. We have shown a relationship between these properties of the collagen fibers and the glycosaminoglycan (GAG) content in the normal and relaxed tendoachilles (TA)[7,13] and in the flexor digitorum profundus (FDP) tendon of the rabbit hind limb[1,15].

The rabbit FDP tendon is a convenient model in which to study the effects of mechanical forces on connective tissue metabolism, for, in addition to the tensional forces which most tendons transmit, this tendon is also subject to pressure and frictional forces over a localised sesamoid-like region[16]. As the tendon curves forward below the ankle to the sole of the foot, this sesamoid-like region (located on the anterior surface of the tendon, region S, Figure 1a) is in contact with the inferior surface of the talus and calcaneum. The curvature of the

tendon induced by this directional change also maintains the tensional state on the collagen fibers on the posterior aspect of the curve (region B, Figure 1a)[16].

The investigative potential of this model system is enhanced by the fact that the tendon can be moved to the extensor aspect of the leg to relieve the pressure-bearing pad of its normal compressional forces without dividing the tendon transversely. This also relieves the tensional forces from the main body of the tendon (region A, Figure 1a).

In this paper we report changes in the glycosaminoglycan content of various regions of the FDP tendon after altering these mechanical forces by surgical manipulations.

MATERIALS AND METHODS

New Zealand white rabbits (6–8 weeks old) were subjected to one of nine surgical procedures which were performed on the left hind leg, the right hind leg acting as a paired control. All rabbits were anaesthetised by a metered fluothane/oxygen mixture administered through a face mask after initial preoperative sedation.

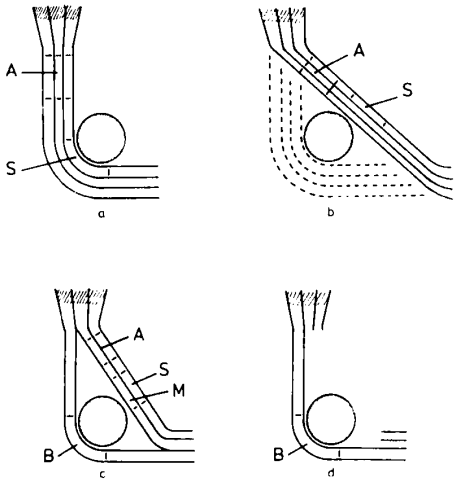

FIGURE 1 Diagrammatic representation of the surgical manipulations of the flexor digitorum profundus tendon. A, B: regions normally under tension *in situ*. S: region abutting on the talus and calcaneum and under pressure. M: intermediate segment between S and B. (a) Normal position, (b) translocated position, (c) partial translocation of tendon segments containing the S and M regions with the B containing segment left in position behind the talus and calcaneum, (d) surgical excision of the S and M containing segments with the B containing segment left in position. The shaded circle represents the talus and the calcaneum.

Surgical Procedures

Procedure 1 and the translocation part of 2 were aimed at releasing pressure and frictional forces from the S region of the FDP and also the tension forces from the A region. Procedures 2 and 3 were designed to induce pressure on a tendon segment previously under tension (region B). Procedure 4 re-applied frictional and pressure forces onto the S region. A partial release of pressure on the FDP S region without direct surgical intervention on the tendon was obtained with procedures 5, 6 and 7, while 8 and 9 were designed to assess the influence of operative trauma and postoperative inflammation without directly affecting the FDP tendon. Procedures 5, 8 and 9 involved operations on the TA which lies adjacent to the FDP tendon.

1. *Anterior translocation and fixation of the FDP tendon (Figure 1b).* FDP tendon translocation was accomplished by exposure of the medial side of the lower leg and foot through a longitudinal incision in the groove between the TA and tibia. Using optical magnification, all the anterior branches of the posterior tibial vessels and nerves were located and divided to permit posterior displacement of the neurovascular bundles. This allowed the anterior translocation of the FDP and its covering paratenon sheath without the need for transverse division of the tendon. All small divided paratenon vessels were coagulated before the tendon was slipped forward from its groove behind the talus and calcaneum onto the extensor aspect of the leg. It was maintained there by a 3/0 silk stitch loop passed through the fibrocartilagenous ring of the extensor retinaculum which provided a suitable anchor point.

After examining the FDP tendon to determine the size of its sesamoid-like thickening, its position was marked by a 6/0 silk suture passed through the posterior paratenon. In earlier experiments, the skin was immediately closed in layers with interrupted sutures. In later experiments before skin closure, the bed from which the FDP had been translocated was filled with a 2 mm diameter rod of medical grade silastic to prevent obliteration of the bed by granulation tissue and to allow the

subsequent replacement of the tendon into its correct anatomical position (see procedure 4). This rod was sutured at its ends to prevent its migration.

2. *Anterior translocation of the anterior two-thirds of the FDP tendon leaving the posterior one-third* in situ (*Figure* 1*c*). After exposure of the leg and freeing the FDP tendon as in 1, the tendon was split longitudinally into its composite musculotendonous segments to allow anterior displacement of the anterior two-thirds of the tendon (containing region S and a region (M in Figure 1), intermediate between S and B). The posterior tension transmitting segment (containing region B) was replaced *in situ*.

3. *Excision of the anterior two-thirds of the FDP tendon* (*Figure* 1*d*). The FDP tendon was freed as in 2 but the anterior two-thirds of the FDP tendon was excised completely. Procedures 2 and 3 necessitate incisions through the paratenon of the FDP tendon.

4. *Relocation of the FDP tendon translocated by procedure* 1 (*Figure* 1*a*→*b*→*a*). After a predetermined period of time in the translocated position the medial side of the leg was re-explored and the anteriorly translocated tendon slipped back into its groove behind the talus and calcaneum which, in the later animals, had been maintained by the silastic rod. In earlier experiments it was necessary to remove the granulation tissue which had developed in the channel. Care was taken to ensure that the posterior aspect of the sesamoid-like region was still identifiable by a marker stitch.

5. *Tendoachilles tenotomy combined with sciatic neurectomy*. After exposure of the leg as before, the TA was severed just above its insertion at the calcaneum. The sciatic nerve was also exposed in the thigh and a segment of approximately 1 cm was removed as previously described[12].

6. *Sciatic neurectomy alone*. A 1 cm segment of sciatic nerve was removed as in 5 but without exposure or interference with the lower limb.

7. *Plaster-of-Paris immobilisation*. One leg was immobilised in a below-knee plaster cast enclosing the toes with the foot and ankle in a plantigrade position.

8. *Longitudinal incisions in the tendoachilles*. The TA was exposed as before and four incisions were made in the TA from just above its insertion to just below the musculotendonous junction.

9. *Loose transverse suturing of the tendoachilles*. The TA was exposed as before and four loose sutures of 3/0 silk were passed through the TA.

At various time intervals after the surgical procedures were performed, animals were killed in groups of three by intravenous overdose of nembutal. The FDP tendons were dissected from normal and experimental legs, the paratenon was removed and the tendons divided into their discrete anatomical segments as described previously[1]. For biochemical determinations, similar regions of tendons from experimental legs were pooled as were those from the normal legs. For statistical purposes, the results at various time periods on the graphs which follow were combined and compared to suitable controls by the Student *t* test applicable to small numbers of samples.

Reagents Used

Hyaluronic acid (Grade I), glucuronolactone, Dowex 1 (×2, Cl^- form, 200–400 mesh), *Proteus vulgaris* chondroitin ABC lyase (E.C. 4.2.2.4) and bovine testicular hyaluronidase (E.C. 3.2.1.35, Type V) were obtained from Sigma Chemical Co., St Louis, MO, USA. Pigskin dermatan sulfate was obtained from Hoffman-La Roche, Basel, Switzerland. Chondroitin sulfate was prepared from bovine nasal cartilage by papain digestion and cetyl pyridinium chloride precipitation[17]. All other reagents used were of analytical grade. Silastic rod was obtained from Dow Corning Australia Ltd., Blacktown, N.S.W., Australia.

Analytical Methods

After drying the tissue samples to constant weight at 105–110°C, the GAG were isolated by digestion with papain (E.C. 3.4.22.2), TCA precipitation and dialysis. They were then fractionated on columns (2 ml bed volume) of Dowex 1 (×2; Cl^- form; 200–400 mesh) into non-sulfated (eluted with 0.75 M NaCl) and sulfated components (eluted with 3.0 M NaCl). The GAG in the fractions were qualitatively analysed by electrophoresis on cellulose acetate membranes in 0.2 M $ZnSO_4$ by the method of Breen *et al.*[18] before and after digestion with testicular hyaluronidase or *Proteus vulgaris* chondroitin ABC lyase. Quantitation of the fraction contents by assay for hexuronic acid was by

the method of Bitter and Muir[19] using glucuronolactone as standard. Total hexuronolactone content was determined by summation of the fraction contents. In some cases hexosamine analyses were also undertaken. The proportions of chondroitin sulfate and dermatan sulfate in the sulfated GAG fractions were determined by assay for dermatan sulfate by the method of Di Ferrante *et al.*[20] Pigskin dermatan sulfate was used as standard and it was assumed that the standard gave the same color yield as the material isolated from rabbit tendon. Based on the hexuronolactone content of the dermatan sulfate standard, the dermatan sulfate contents have been expressed in the Tables and Figures as hexuronolactone attributable to dermatan sulfate. Hexuronolactone attributed to chondroitin sulfate was determined by difference and these ratios of components were cross-checked by electrophoresis. These methods have been outlined in full in a previous publication[1].

RESULTS

In a previous publication[1], chondroitin sulfate, dermatan sulfate and hyaluronic acid were shown to be the only GAG detectable in measurable quantities in the FDP and TA tendons by the analytical methods used. This conclusion was based on hexosamine analyses and electrophoresis

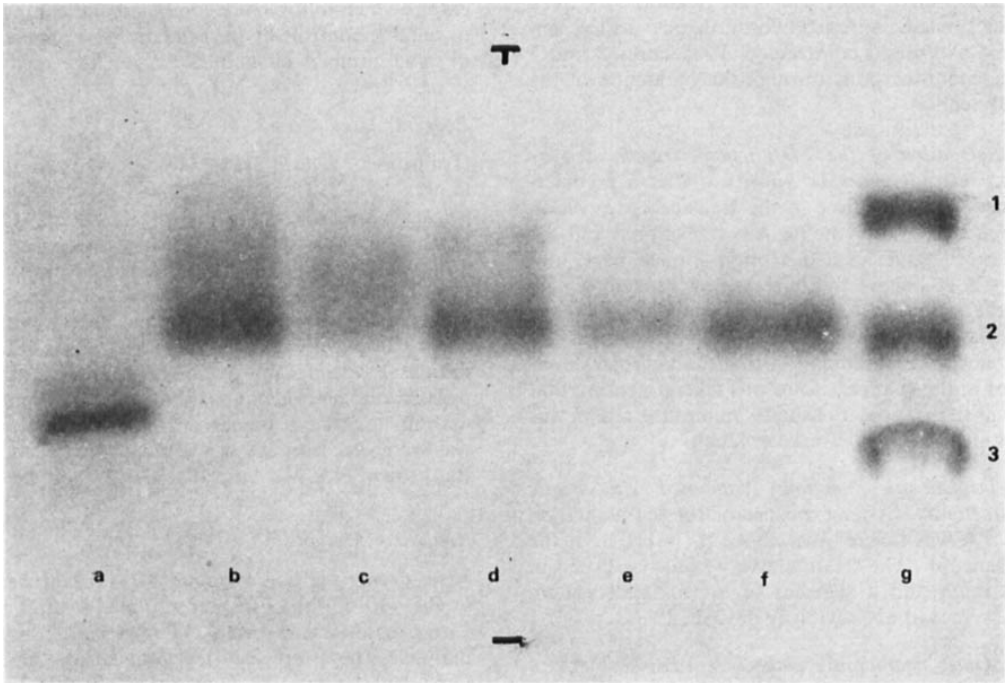

FIGURE 2 Electrophoretogram of the GAG from regions of rabbit tendons. The samples were prepared from tendon regions obtained from a group of 2 rabbits whose FDP tendons had been translocated for 14 days (Figure 1b). GAG were isolated after papain digestion and eluted from Dowex 1 (×2, Cl^- form). Each sample or standard GAG (0.2–0.3 μg) was applied to cellulose acetate strips and electrophoresis was carried out in 0.2 M $ZnSO_4$ in a Beckman microzone system. Position of the GAG were detected with 1% Alcian Blue. Samples were eluted from Dowex 1 (×2) with (a) 0.75 M NaCl and (b–f) 3.0 M NaCl after 0.75 M NaCl. (a) A region, translocated FDP tendon, (b) S region, translocated FDP tendon, (c) S region, control FDP tendon, (d) A region, translocated FDP tendon, (e) A region, control FDP tendon, (f) TA, control leg, (g) standards: 1, chondroitin sulfate; 2, dermatan sulfate; 3, hyaluronic acid. The quantities loaded are not quantitatively related to the concentrations of GAG in the original samples. The quantitative results obtained on these samples are included in Figures 3 and 4.

TABLE I

The GAG contents of the A and S regions of the FDP tendon after control operations. Groups of rabbits, each of three animals, were operated on by procedures 6, 7, 8 and 9 (see Materials and Methods, Surgical Procedures). The A and S regions (see Figure 1a) of the FDP tendon were obtained after the stated time period. Control tissue was obtained from the unoperated leg. The GAG were prepared by papain digestion and fractionated by chromatography on Dowex 1 (×2)

Operation	Period after operation	†Sample	Total hexuronolactone (μg/mg dry wt.)	Percentage of hexuronolactone attributable to: Hyaluronic acid	Dermatan sulfate	Chondroitin sulfate
Longitudinal incisions in TA (Procedure 8)	8 days	Ao	0.51	26	55	19
		Ac	0.38	27	67	6
		So	7.28	19	23	58
		Sc	7.48	20	24	56
Sutures in TA (Procedure 9)	12 days	Ao	0.40	22	78	5
		Ac	0.37	20	69	11
		So	6.61	19	22	60
		Sc	5.47	19	26	55
Neurectomy only (Procedure 6)	8 days	Ao	0.37	27	73	
		Ac	0.55	44	49	7
		So	2.08	36	41	23
		Sc	5.46	28	23	49
Plaster cast immobilisation (Procedure 7)	30 days	Ao	0.59	22	54	24
		Ac	0.48	15	58	27
		So	2.71	25	42	33
		Sc	6.40	17	21	62

† See Figure 1a. o = operated leg; c = control leg.

before and after specific digestion of the preparations with bovine testicular hyaluronidase or *Proteus vulgaris* chondroitin ABC lyase. Similar procedures carried out on many of the samples used in this present study have demonstrated no additional GAG components after surgical manipulation. A typical electrophoretogram before digestion with the specific enzymes has been included (Figure 2) but detailed results of the other qualitative and quantitative procedures have been omitted.

Control Operations

The results of analysis of the GAG content of the A and S region of the FDP tendon (Figure 1a) of animals in which the TA had been damaged by longitudinal incision (procedure 8) or by the introduction of sutures (procedure 9) are reported in Table I. There was no major change in the GAG content or the relative proportions of the three GAG in either of these FDP regions when compared with the same regions from the tendon of the unoperated leg. The results after sciatic neurectomy (procedure 6) or plaster cast immobilisation (procedure 7) are also included in Table I. The glycosaminoglycan composition of the A region was not markedly affected by procedures 6 and 7 when compared to the translocation experiments reported later but a loss of glycosaminoglycan was apparent in the S region.

The results obtained after TA tenotomy and sciatic neurectomy (procedure 5) are given in Figure 3. In the A region of the FDP tendon there was again no change in the total GAG content or in the relative proportions of the three components ($p>0.2$). In the S region there was a marked loss of GAG ($p<0.01$) which was mainly due to a loss of the chondroitin sulfate component ($p<0.01$) but the slight decrease in hyaluronic acid and dermatan sulfate contents was significant ($p<0.05$).

FDP Translocation

In five groups of animals, the FDP tendon was slipped from its channel behind the calcaneum and talus and translocated onto the extensor aspect of the leg without dividing the tendon transversely

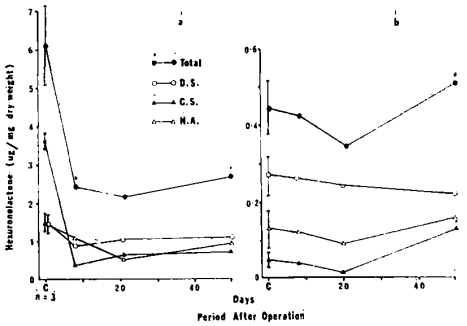

FIGURE 3 The effect of TA tenotomy and sciatic nerve neurectomy on the GAG content of the S region (a) and A region (b) of the FDP tendon (see Figure 1a). The GAG were isolated by papain digestion and chromatography on Dowex 1 (×2). C, mean value±S.D. of the GAG content of control tendon regions obtained from the unoperated legs; n = number of determinations in obtaining C. For further details see Figure 2.

(procedure 1 and Figure 1b). In two other groups the FDP tendon was split longitudinally and the anterior two-thirds of the tendon (containing S and M segments) were translocated. The remaining third of the tendon (containing the B segment) was left *in situ* (procedure 2 and Figure 1c). The A and S segments (Figure 1b, c) were analysed for GAG content after various periods of time in the translocated position. There was no significant difference between the results after the two translocation procedures. Results are shown in Figure 4 (A region) and Figure 5 (S region). At the longer periods (4.75 and 9.5 months) it was not possible to separate S and M containing segments and the analysis figures for these samples in Figure 5 are for the S and M regions combined. There were only two animals in the group killed 14 days post-operation.

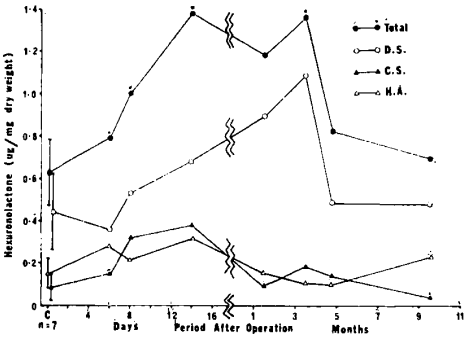

FIGURE 4 The GAG content of the A region of the FDP tendon after translocation. Tendon regions were obtained after partial translocation (time periods 3.5 and 9.5 months, Figure 1c) or total translocation (remaining time periods, Figure 1b). D.S., dermatan sulphate; C.S., chondroitin sulphate; H.A., hyaluronic acid. For further details, see Figure 2.

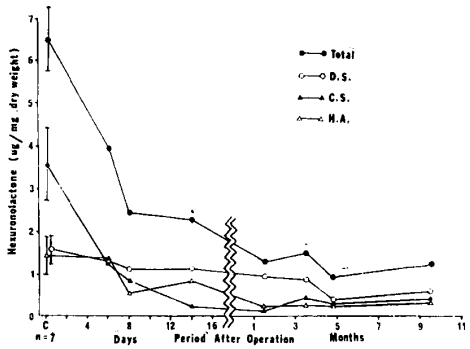

FIGURE 5 The GAG content of the S region of the FDP tendon after translocation. For details, see Figures 2 and 3.

In the A region, FDP translocation caused an increase in total GAG content during the period of remodelling. Up to 14 days after translocation while the tendon was slack, the rise in GAG content was due to increases in the amounts of chondroitin sulfate and hyaluronic acid, while the quantity of dermatan sulfate remained relatively constant. During the time period of 1 to 3.5 months post-operation, when tension was regained, the proportions of chondroitin sulfate and hyaluronic acid decreased, while the elevated GAG content was maintained by increased amounts of dermatan sulfate. Compared to the zero time controls, the increased total GAG content was significant at the time periods 8 days to 3.5 months ($p<0.01$); increased hyaluronic acid content was significant at time periods 6–14 days ($p<0.05$); increased chondroitin sulfate content was significant at 8–14 days ($p<0.02$); the increased dermatan sulfate content was significant at time periods 14 days to 3.5 months ($p<0.02$). After longer time periods the relative proportions of the three GAG and total GAG content returned to normal values.

In the S region, there was a rapid loss of GAG during the first 8 days after translocation, mainly

TABLE II

The GAG content of the A region of the FDP tendon after translocation and replacement of the tendon. The GAG were isolated by papain digestion and chromatography on Dowex 1 (×2). The control samples were obtained from the unoperated legs and control values have been combined ($n = 3$). For details see Figures 2 and 3

Period translocated (days)	Period replaced (days)	Total hexuronolactone (μg/mg dry wt.) (±S.D.)	Percentage of hexuronolactone attributable to:		
			Hyaluronic acid	Dermatan sulfate	Chondroitin sulfate
0	0	0.60±0.12	24±11	70±19	16±11
40	183	0.54	24	69	7
90	165	0.69	25	68	7
150	270	0.73	35	54	11

in the chondroitin sulfate fraction; such that dermatan sulfate became the predominant GAG. Throughout the time period statistical significance was seen in the differences in total GAG content ($p<0.01$), chondroitin sulfate ($p<0.01$), dermatan sulfate ($p<0.05$) and hyaluronic acid ($p<0.05$) compared to the controls.

FDP Translocation and Replacement

After translocation of the whole of the FDP tendon to the anterior aspect of the ankle for a period, it was replaced in its fibro-osseus groove for a further time period (procedure 4 and Figure 1a→b→a) before samples were obtained from the A region (Table II) and the S region (Figure 6). In the A region there was little change in total GAG content ($p>0.2$) or in the relative proportions of the three components ($p>0.2$). Figure 6 also includes, for comparison, the results from the S region of tendons which had been translocated for the same time period as those which were subsequently replaced for a further period. As noted previously (Figure 5), the initial translocation resulted in a loss of GAG and dermatan sulfate became the predominant component. After relocation of the tendon there was a trend towards a replacement of previously lost GAG. After the two shorter periods in the translocated position, the increases in total GAG and in chondroitin sulfate contents was significant ($p<0.02$) while there was no change in the hyaluronic acid and dermatan sulfate contents ($p>0.2$).

Application of Pressure to a Tensional Part of the Tendon

The posterior (B) region of the FDP tendon was brought directly into contact with the calcaneum and the talus either by translocation of the S and M containing segments to the extensor aspect of the ankle (procedure 2 and Figure 1c), or by complete removal of the S and M containing segments (procedure 3 and Figure 1d). After various time periods, the GAG content of the B region was determined and the results are shown in Figure 7. The results obtained after either complete removal or anterior translocation of the S and M containing segments were not significantly different and all results have been grouped together. There was no statistically significant change in the total GAG content of the B region up to a period of 9.5 months after operation ($p>0.2$). However, the proportion of hyaluronic acid decreased ($p<0.02$) while that of dermatan sulfate increased ($p<0.05$). This had taken place at the earliest period studied (3.5 months). There was little change in the proportion of chondroitin sulfate throughout ($p>0.2$).

DISCUSSION

The normal flexor digitorum profundus (FDP) tendon of the rabbit hind limb has a region subject to tension and another region subject to pressure (Figure 1a, A and S respectively). A previous report[1] has shown that in the region subject to tensional force, the tissue GAG content is of the order of 0.2% of the dry weight with dermatan sulfate the predominant polymer, while in the region subject to compression there may be 15–20 times as much GAG with chondroitin sulfate the major fraction. This latter is comparable in function to an articular cartilage. In a third region (the B region posterior to the S, Figure 1a) the major GAG component is hyaluronic acid. The GAG content of the segments of control tendons from

the unoperated rabbit hind limbs used in this study followed the same pattern as in the previous publication, although on this occasion there was less GAG in the S region. This may be related to the effect of operative intervention on the general mobility of the animals, or to differences in age.

We believe that the changes in glycosaminoglycan type and content which we have reported in the various segments of the tendon following the surgical manipulations are due to alterations of the mechanical forces acting on the tendon and not due to inflammatory changes associated with the operations. This belief is based on the fact that the changes in the S region were only found following procedures which directly or indirectly affected the function of the FDP tendon, i.e., FDP translocation, sciatic neurectomy, TA tenotomy and neurectomy or lower limb immobilisation. Procedures designed to produce comparable operative trauma or inflammatory response of the lower leg

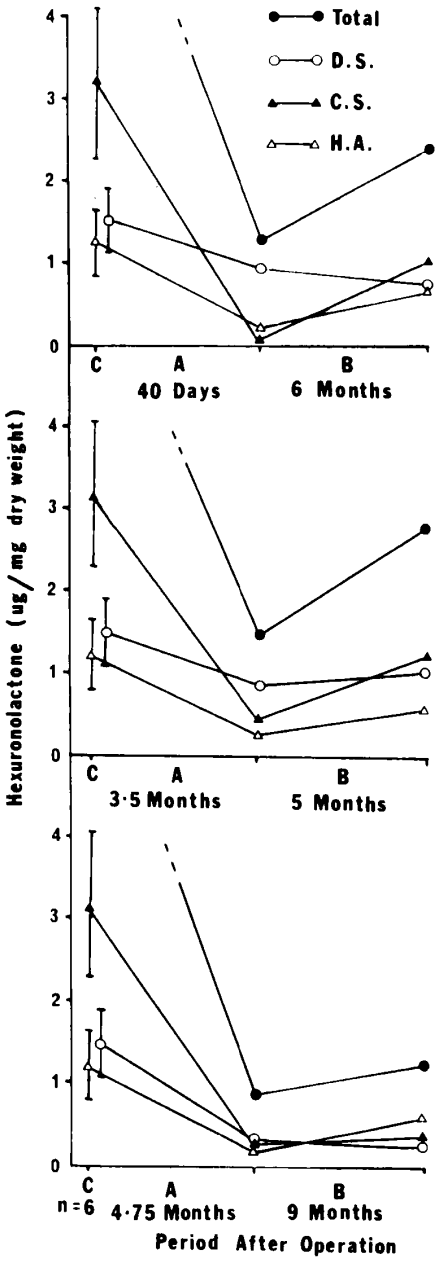

FIGURE 6 The GAG content of the S region of the FDP tendon after translocation and replacement. After a period (A) in the translocated position (Figure 1b), the tendons were replaced in the groove behind the talus and calcaneum (Figure 1a) for a further time period (B). The GAG were then isolated and analysed from the S region of the tendon as before (see Figures 2 and 3). The figure also shows for comparison, the analytical results of the GAG of the S regions of tendons translocated for the time period A but not subsequently replaced. For clarity the total hexuronolactone figures have not been included for the control tissue samples.

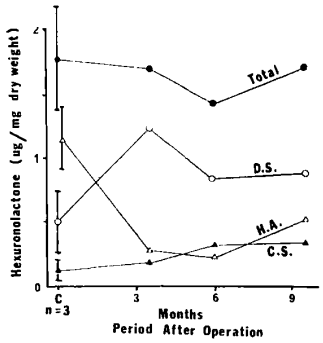

FIGURE 7 The GAG content after application of transverse pressure to the B region of the FDP tendon. The B region of the FDP tendon was brought into direct contact with the talus and calcaneum by excision of the tendon segments containing the S and M regions (6 month figures, see Figure 1d) or by translocation of these segments (3.5 and 9.5 months, see Figure 1c). The GAG content of the B regions were then determined (see Figures 2 and 3).

without interfering with the FDP tendon function, i.e., the longitudinal incisions and the transverse suturing of the tendoachilles, produced no changes in the FDP.

The sesamoid-like S region in particular appears to be very susceptible to alterations of the physical forces acting upon it. Removal of pressure from this region results in rapid loss of >60% of the GAG within 8 days. The major component which is lost is chondroitin sulfate such that dermatan sulfate becomes the predominant remaining glycosaminoglycan. A similar loss of chondroitin sulfate with less effect on dermatan sulfate content has also been reported in the periarticular tissues of immobilised legs of dogs[4]. Proteo-dermatan sulfate is less readily extractable from skin, tendon and heart valves than is proteo-chondroitin sulfate[21-23] and it would appear that dermatan sulfate is intimately associated with thicker collagen fibers and perhaps less susceptible to degradative enzymes. Eventually the GAG composition of the S region becomes similar to that of normal tension transmitting tendon.

Replacement of the translocated FDP tendon into its channel behind the talus and calcaneum results in an increase in GAG content of the depleted S region and chondroitin sulfate tends to become the major GAG component. The recovery of GAG in the S region is very much slower than the initial loss observed after translocation and it would appear that if the tendon is left for too long a period (greater than 3.5 months) in the translocated position, this reversal is more difficult.

Whereas all the procedures which affected the normal function of the FDP tendon affected the GAG content of the S region, only those procedures which markedly reduced the tensional state of the FDP (i.e., translocation) affected the GAG content of the tensional A segment of the tendon as well. In this region of the translocated tendon, while the tendon is initially slack, there is a significant increase in total GAG content and this appears to be due mainly to an increased dermatan sulfate content. This is coincident with remodelling of the slack tendon as previously observed in Achilles tendon relaxed by tenotomy[7]. In the translocated FDP tendon, the tendon later becomes taut, presumably by remodelling of the tendon and also of the muscle[24] and is accompanied by a return to normal GAG content. Relocating the taut tendon did not produce any changes in the glycosaminoglycan content in the A region. This second operation is potentially more damaging and traumatic than the initial translocation procedure. This again emphasises that mechanical factors are more important than trauma or inflammation in the changes we have reported.

In order to gain some insight into the relative importance of genetic and environmental factors in the development of the S region, attempts were made to induce a new S-like region in another part of the tendon. Experimentally, this was brought about by removal or translocation of the S and M containing regions (Figure 1c, d) such that the B region abutted directly onto the calcaneum. There was no change in the total GAG content of the region, but the proportion of hyaluronic acid decreased, the proportion of dermatan sulfate increased while the proportion of chondroitin sulfate remained relatively constant. We have no obvious explanation for the change in dermatan sulfate/hyaluronic acid ratio, but it may be related to the altered function and mechanical properties of the residual strip of tendon. The tensional force per unit cross section on this would be greater than normal and, since it appears that dermatan sulfate is the predominant GAG in tensional situations, this would lead to an increased content of dermatan sulfate. As there would be less relative movement between collagen fibers in this region, there would be less requirement for hyaluronic acid.

The changes we have observed which restore the tissue to a chemical composition appropriate to the new functional demands indicate that the cells are able to translate the mechanical forces into chemical events but the mechanisms by which this is attained are still uncertain. Mechanical deformation of soft connective tissues causes changes in the charge distribution of the proteoglycans due to transient alterations in Donnan equilibria, diffusion potentials and streaming potentials[25,26]. However, collagen fibers under different tensional states also show variations in their physical properties. Changes in their affinity for the negatively charged dyes of the Masson trichrome procedure[11,13,14] and also variations in the length of the D axial period have already been described[1,14]. The changes in staining are related to the relative availability of positive charges on the collagen fibril surface as the result of minor conformational changes in the collagen under different tensional states.

We have proposed previously that in tendon the charge on the collagen fibrils is a major component in an electrochemical mechanism which controls proteoglycan synthesis by the adjacent cells[1]. We

believe that the maintenance of the low GAG content in the tensional segments of the tendon could be related to the increased positive charge on the collagen fibrils under tension. Recent demonstration of the effects of a synthetic polycation on GAG synthesis by chick fibroblasts in culture lends support to this concept[27]. On the other hand, the higher GAG content of pressure bearing regions such as the S region of the FDP tendon or articular cartilage may be maintained by a second and independent control mechanism associated with the effect of intermittent pressure on the electrochemical properties of the matrix proteoglycan.

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