MECHANICAL BEHAVIOUR OF TENDON *IN VITRO**

A PRELIMINARY REPORT

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Abstract-The mechanical behaviour of horse and human tendon, as characterised by the stress-strain curve, has been examined with respect to load-strain cycling and strain rate. It was found that the tendon stress-strain curve for successive cycles was reproducible provided that strain on the specimen did not exceed $2.0-4.0\%$. If this strain level was exceeded, a permanent deformation occurred. This phenomenon was verified by histological studies on strained tendon which showed that some of the collagen fibres did not return to their original orientation. Variation in the rate of strain was found to affect both the magnitude and the shape of the stress-strain curve. Additionally, it was found that the stress relaxation phenomenon for tendon was essentially the same as that found for other connective tissues.

1. INTRODUCTION

THE STRUCTURAL importance of connective tissues for transmission of forces within the human body has been realized for some time. Information regarding the response of these tissues to sudden increases in externally applied forces has become increasingly important because of the advent of high speed transportation.

The extra-cellular constituents of soft connective tissues are mainly collagen and elastin embedded in a matrix, or ground substance, of mucopolysaccharides. Nearly all the connective tissues, i.e. skin, bone, cartilage, tendon, and ligament, can be regarded as viscoelastic materials.

A viscoelastic material is a combination of an elastic solid and a viscous fluid. Measurements of viscoelasticity are dependent upon three variables: load, deformation, and time. Viscoelasticity can be recognized either by stress relaxation or by creep phenomena. For measurement of stress relaxation, a given extension is applied to a test specimen and maintained constant; the relaxing load is then measured as a function of time. For measurement of creep, a constant load is applied and the increase in length is then measured as a function of time. Creep and relaxation can be observed in most biological tissues; therefore, knowledge of viscoelasticity can lead to a better understanding of the mechanical behaviour of connective tissues.

This paper reports on a preliminary study of the mechanical behaviour of tendon *in vitro* with particular reference to the response of the collagen fibres to externally applied force. Over the last 30 years or so, several papers have been published on the mechanical behaviour of tendon; these have been discussed in a review by ELLIOTT (1965). With the exception of PARTING-TON and WOOD (1963), RIGBY (1964), ELDON (1964) , and VANBROCKLIN and ELLIS (1965) , the papers reviewed by Elliott were, in this author's opinion, of an elementary nature. The papers listed above are discussed later in the text.

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The physical terms used in this paper to describe the mechanical behaviour of tendon are defined as follows.

Stress:	Load	lb	kg
Stress:	Area of cross section;	in ² or $\frac{kg}{cm^2}$	
Strain:	Increasing length		
(a nondimensional ratio:	in or $\frac{cm}{cm}$)		

Strain rate: Change in strain per unit time; $\frac{1}{200}$.

"Elastic limit" for tendon: the point at which residual or permanent strain occurs.

Throughout the text, the measured strain has been expressed as a percentage, i.e.

Increase in length $\times 100$.

2. GENERAL METHODS OF PROCEDURE

All tests on human tendon were carried out at 37° C within 36 hr of autopsy; horse tendon was tested at 38°C within 48 hr of autopsy. All specimens were refrigerated in Ringer's solution from time of detachment until time of testing. Each specimen was immersed in Ringer's solution at the temperature used for testing for 15 min before the start of each test, unless otherwise indicated.

All experimental testing was carried out on a floor model Instron Testor, Type T.C., which is shown in Fig. 1. The machine was modified slightly so that the tendon specimens could be tested in Ringer's solution at body temperature. The immersion tank (shown in detail in Fig. 2) is mounted on the stool below the large crosshead. Experimental load and specimen strain can be recorded continuously on a time-base chart by the two-pen recorder which is contained in the control console shown on the left side of Fig. 1.

In Fig. 2, a specimen of horse extensor tendon is held in the specially designed grips. Each grip is made from stainless steel and consists of a block which encloses two self-tightening springloaded wedge-shaped jaws. The grips were successful up to the $4.0-5.0\%$ strain level;

thereafter, damage to the tendon fibres within the jaw faces occurred, and in some cases the specimen slipped from the grips.

The upper grip is connected to the Instron load cell and the lower grip is connected to the large crosshead by means of the two vertical stainless steel bars. As the crosshead is driven down, only the lower grip moves; thus the specimen is loaded in tension.

The load is recorded directly by the load cell, and its electrical output drives the load pen across the recording chart. At the same time, the specimen extension is measured by the standard 1 in. Instron extensometer attached directly to the specimen (Fig. 2). The extensometer (No. $G.51.12$) weighs approximately 0.1 lb and has a maximum clamp spring force of 0.025 lb. The electrical output from the extensometer is fed to the servomotor which drives the strain-recording pen across the chart. The extensometer, which was waterproofed with a nonbrittle electrical insulating paint, was calibrated before the start of each test by means of a graduated calibration bar which was immersed in the Ringer's solution. Because of such factors as grip slippage, the actual crosshead displacement should never be used for determining extension.

A pump is used to circulate Ringer's solution through the immersion tank via a heat exchanger which maintains a given temperature to within $+0.5$ °C.

The cross sections of the specimens tested were determined from density and length measurements taken after the test. The average area of the cross section was obtained for the portion within the 1-in. gauge length. Since the specimen strains did not exceed 4.0% in the cycling and strain rate experiments, decreases in cross section were very small; thus the errors involved in using the nominal stress values (i.e. the load per unit cross section of specimen after test) are, for practical purposes, insignificant.

3. EXPERIMENTAL STUDIES

The experimental studies on tendon have been divided into five sections:

FI6. 1. Instron Testor. Control console is on the left and crosshead assembly is on the right. The clear plastic tank is placed below the crosshead so that the tendon specimen can be immersed in Ringer's solution.

FiG. 2. Tendon specimen immersed in Ringer's solution. The waterproofed strain gauge extensometer is attached to the horse extensor tendon before the assembly is immersed. The Ringer's solution is circulated through a heat exchanger which maintains a given temperature to within $\pm 0.5^{\circ}$ C. The specimen is held in the stainless steel grips by the action of the self-tightening spring-loaded wedge-shaped jaws.

FIG. 8. Unstrained horse extensor tendon (H & E stain, \times 200). The wave formation of the unstrained collagen fibres is noticeable.

FIG. 9. Horse extensor tendon, strained to 3.5% and clamped (H & E stain, \times 200). The collagen fibres now have a parallel arrangement.

Fig. 10. Horse extensor tendon, strained to 3.5% , unloaded, and allowed to relax (H & E stain, \times 200). The collagen fibres in the right half of this photograph have not returned to the unstrained wave pattern shown in Fig. 8.

(1) Determination of standard stress-strain curve

(2) Effects of cycling and determination of elastic limit

- (3) Effect of strain rate
- (4) Determination of stress relaxation

(5) Histological studies of strained tendon Each section includes a description of the specimens used, the special adjustments of the apparatus required, and the results obtained.

3.1. *Determination of standard stress-strain curve*

Figure 3 shows the stress-strain curve for Achilles tendon from a 54-year-old female. The experimental points were obtained from the continuous recordings of load and strain on the time-base chart. The applied strain rate in this case was $100\frac{\gamma}{6}$ /min, or crosshead speed of 1 in./min, but the measured rate varied from 18% /min to 34% /min because of viscoelastic effects and grip slippage.

The curve in Fig. 3 has the characteristic shape found for other biological tissues; it describes three distinct regions before rupture of the specimen occurs (KENEDI *et al.,* 1966).

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The primary region is that of $0-1.5\%$ strain, where there is considerable increase in extension with a small increase in tension. In this region, histological studies (discussed in Section 3.5) show that the wavy formation of the collagen fibres straightens out.

The secondary region of the curve is that of $1.5-3.0\%$ strain, where the fibres become fully oriented in the direction of the load and begin to take up more tension.

In the final region, that of $3.0-5.0\%$ strain, there is a linear relationship between stress and strain, and it is assumed that the amount of extension is now governed fully by the behaviour of the collagen itself in tension. Physical rupture of the fibres commenced at the $5.0-6.0\%$ strain level; at this level, the tendon was unable to tolerate any further increase in load.

As the final strain (30.0%) was large, errors made by use of nominal stress values are significant. If it is assumed that tendon is a material which maintains a constant volume under stress, the ratio of the lateral contraction to the longitudinal extension $= 0.5$. This was the method used to make an estimate of the true

FIG. 3. Stress-strain curve for Achilles tendon from a 54-year-old woman. Specimen was tested in Ringer's solution at 37° C 24-hr post mortem.

area and, therefore, the true stress, in Fig. 3.

This result is typical for sixty samples of horse and human tendon tested so far, in that the the general shape of the stress-strain curve is similar, although in this case the maximum stress obtained is somewhat low. Results have been obtained for other human Achilles tendons, human toe extensor tendons, and horse extensor and flexor tendons. The highest stress value recorded for human tendon was $5000 \, lb/in^2$ (345 kg/cm^2) while the highest value recorded for horse tendon was 8000 lb/in² (552 kg/cm²). It is interesting to note that stress-strain curves of all tendons tested to date show the same characteristic secondary region, with a rapid change in curvature at about the $1.5-3.0\%$ strain level.

Because of the nonlinear shape of the stressstrain curve, it is incorrect to use a simple Young's modulus value (the stress-strain ratio) to describe the overall mechanical behaviour of tendon.

3.2. *Effects of cycling and determination of 9 "elastic limit"*

RIGBY *et al.* (1959), PARTINGTON and WOOD (1963), and RIGBY (1964) all found that if rat-tail tendon was strained beyond a $2.0-4.0\%$ level, is suffered a permanent deformation. Partington and Wood also observed that load extension curves for successive cycles beyond the 2.0% level were displaced along the extension axis. To see if this overstrain phenomenon 9 occurred in horse and in human tendon, and to ascertain the effects of repeated cycling, the following experimental procedure was adopted.

The load and extension cams of the Instron pen drive mechanism were set to cycle the test specimen between a given strain and zero load. In effect, the crosshead moves at a fixed rate, pulls the specimen to the predetermined strain, stops, reverses, and unloads the sample until the load cam registers zero.

The pen drive mechanism requires at least 3 see to travel full scale in order to achieve maximum sensitivity in recording. If the load increases too rapidly, errors can be introduced in the cycling tests because the load pen may lag behind the load signal. To resolve this difficulty, a Honeywell galvanometer recorder was connected to the load cell terminals to monitor the load signal. The sensitivity of the strain pen was unaffected since the maximum strain in the cycling tests did not exceed 5.0% , and therefore, only half the scale of the chart was utilised (the strain pen selector switch was set for 10.0% strain for the full scale of the chart). Before commencement of the actual test, the specimens were mechanically preconditioned by 10 cycles to the 1.5 or 2.0% strain level and then allowed to rest for 10 min. This preconditioning allows the wedge grips to become fully embedded in the test specimen.

The results of repeated strain cycling to different strain levels on one sample of horse extensor tendon are shown in Fig. 4. This specimen was cycled 10 times to the 2.0% strain level and then allowed to rest for 5 min while the strain cam was reset to produce a 3.0% strain. It was then cycled 10 times to the 3.0% strain level, allowed to rest and the cam reset to give a 4.0% strain. No rest period was allowed between cycles of the same strain magnitude.

Curves nos. 1 and 10 are, therefore, the first and last cycles to the 2.0% strain level, plotted as nominal stress against percentage strain. The curves are, for practical purposes, identical, because complete recovery was obtained after each of the 10 cycles. The crosshead speed selected, 2 in./min, produced an average specimen strain rate of $45\frac{\cancel{0}}{0}$ min \pm 5%/min. The total time for 1 cycle to the 2.0% strain level was about 5.3 sec. The stress obtained at the maximum level was approximately 1600 lb/in² (110 kg/cm^2) .

Curves nos. 11 and 20 are, respectively, the first and last cycles to the 3.0% strain level. After no. 11, the tendon did not return to the initial gauge length of 1.0 in., but acquired a 0.5% "residual" strain. This "residual" strain progressively increased to 1.0% after cycle no. 20. The strain cam was not reset to zero, hence the strains are still based on the initial gauge

FIG. 4. Effect of repeated cycling--10 cycles to $2.0 \frac{\%}{\%}$, 10 cycles to $3.0 \frac{\%}{\%}$, 10 cycles to $4.0 \frac{\%}{\%}$ -on horse extensor tendon. The specimen, obtained from the left front leg of a 2-year-old thoroughbred, was preconditioned (by 10 cycles to 2.0% strain) and tested in Ringer's solution at 38°C. $(Area = 0.032 in²)$

length of 1.0 in. As the "residual" strain, at zero load, increased, the nominal stress fell from 3250 lb/in² (224 kg/cm²) to 2650 lb/in² (185 kg/ cm'), partly because the time for each complete cycle had become progressively shorter (from 7-0 sec for no. 11 to 5.5 sec for no. 20).

Finally, curve no. 21 is the first of the 10 cycles to the 4.0% strain level. Here the "residual" strain increased from 1.0% to 1.2% . Again, the strain values are based on the initial

gauge length of 1.0 in. The maximum stress at the 4.0% strain level was 4500 lb/in^2 (310) $kg/cm²$).

Other similar experiments on tendon where the "residual" strain was measured as a function of time, indicate that this "overstraining" effect is permanent.

To date, the following number of cycling experiments have been performed on different. samples of tendon.

The above results show that if tendon is strained beyond the $2.0-3.0\%$ level, permanent deformation will result. The tests also show that if tendon is not strained beyond the $2.0-2.5\%$ strain level, then test results from one sample are reproducible and, therefore, one sample can be used for a series of tests as long as this level is not exceeded.

The point at which "residual" or permanent strain occurs is henceforth referred to as the "elastic limit".

3.3. *Effect of strain rate*

With many materials, an increase in the strain rate produces an increase in the stress magnitude for any one strain level. This phenomenon is very noticeable in the behaviour of viscoelastic materials (SMITH, 1956). As the behaviour of tendon is viscoelastic, it would be expected that the variation in strain rate would affect both the magnitude and the shape of the stress-strain curve.

RIGBY *et al.* (1959) reported that an increase in the rate of strain did not significantly alter the shape of the reproducible region of the stress-strain curve. They found that the main effect was an increase in the load required to produce a given strain. The latter was partly confirmed by VANBROCKLIN and ELLIS (1965) who found that the modulus of elasticity for human toe extensor tendon was a function not only of stress but also the rate at which the stress was applied. VanBrocklin and Ellis further indicated that the shape of the stress-strain curve is also affected by variation in strain rate. However, they maintained a minimum stress level in each cycle and the maximum strains were between

 $4.0-6.0\%$; they exceeded the "elastic limit" for tendon determined in the present study.

In view of these two possibly conflicting reports and in order to find out whether there is a relationship between stress magnitude and strain rate, experiments were performed on five horse extensor tendons, five human Achilles tendons, and one human extensor tendon.

Some of the difficulties inherent in this type of experiment are worth mentioning. A constant crosshead speed does not necessarily mean that the measured strain rate will be constant, because several other factors must be considered. As the crosshead speed is decreased the viscous components of the tendon matrix have more time to come into play; this factor, coupled with movement of the grip jaws and possible grip slippage, can result in a nonlinear rate of strain. It is, therefore, necessary to increase the crosshead speed during a test if any of the above factors become apparent.

On the Instron, this is a fairly simple procedure provided a strain pacing control has been fitted in the apparatus. This pacing control isolates the constant speed gear box, thus allowing manual control of the crosshead drive servomechanism. The manual pacing control, unfortunately, is only effective up to strain rates of $20.0\frac{\gamma}{6}$ /min. Thereafter it is necessary to revert to a constant crosshead speed and to select only the results which give a fairly good linear line on the time-base chart.

Figure 5 is the result of several tests on the one specimen of Achilles tendon. Each test was carried out at a different rate of strain-from 1.0% /min to 50.0%/min. The effect of increasing the rate of strain is immediately apparent. Not only is the stress magnitude increased but also the shape and position of the stress-strain curve are altered as the slope of the linear portion of the curve increases with increasing strain rate. For each different strain rate the strain level was not allowed to exceed 2.0% . The spacing of the curves suggests that there might be a relationship between the stress magnitude and the strain rate. If tendon were a linear viscoelastic material an exponential

FIG. 5. Effect of an increasing strain rate on the stress-strain curve for one sample of human Achilles tendon. Specimen, from a 54-year-old woman, was tested in Ringer's solution 24-hr post mortem.

relationship would exist between the stress and the strain rate.*

The results from Fig. 5 have been replotted in Fig. 6 as stress-against-strain rate on a double logarithmic plot. It can be seen that the possibility of an approximate relationship between stress magnitude and strain rate does exist. Only one of the specimens in this series, a horse extensor tendon, did not show the type of relationship between stress magnitude and strain rate described in Fig. 6. As yet, no comparative mathematical analysis has been made of the double logarithmic plots because additional data are still being gathered. It is hoped that an equation will be produced which will define the stress-strain curve for tendon in terms of strain rate.

The results to date confirm that if tendon is stretched rapidly, high stresses will be induced in the tendon which allow it effectively to transmit large forces to the bones and joints with a small increase in tendon length. However, very occasionally the rate of load application will be sufficient to rupture the tendon. This, of course, also occurs in muscle, but muscle generally has a much greater cross section; hence, the induced stresses will be lower.

3.4. Determination o]stress relaxation

Stress relaxation is measured as follows:

^{*} If in a material the stress-strain ratio is a function of time alone and not of stress magnitude, then the material is said to exhibit linear viscoelastic behaviour.

FIG. 6. Double logarithmic plot of stress magnitude against strain rate replotted from the experimental results in Fig. 5 to show the effect of strain rate on stress magnitude for any given strain.

A specimen is strained to a given extension, the extension is held constant and the relaxing stress (load) is measured as a function of time. The relaxation effect in human toe extensor tendon is shown in Fig. 7. This specimen was strained to 2.4% in 5 sec, the crosshead was stopped, and the relaxing load was measured as a function of time. The nominal stress fell from 1500 lb/in 2 (106 kg/cm²) to 335 lb/in² (23 kg/cm²) in 16 min. For convenience, a semi-logarithmic graph has been used for presenting the results. From such a plot it is possible to develop an exponential equation of several terms to describe the behaviour of the material. Such an equation can be represented by a mechanical model consisting of a large number of springs and dashpots.

Other results on tendon" indicate that the relaxing stress approaches an equilibrium value after 50-60 min, after which the stress becomes fairly stable. This work is being continued.

3.5. *Histological studies of strained tendon*

The following experiment was designed to permit observation of the behaviour of tendon fibres at different levels of strain. Specimens of horse extensor tendon were cut into three parts-two tensile test specimens and one control. The first tensile specimen was strained to a predetermined level; then the extensometer was removed and replaced by a clamp which was fixed securely over the 1.0 -in. gauge length. The specimen was

unloaded and removed from the grips; however, the center section remained strained in the clamps. The clamped tendon was then placed in formalin. The second tensile specimen was strained to the same level as the first, unloaded, and allowed to relax before placement in the formalin. The unstrained control was also placed in formalin. All three specimens were fixed in formalin for at least 48 hr.

After fixation, the specimens were stained with hematoxylin and eosin $(H & E)$ by means of standard laboratory histological procedures. No recovery was observed when the clamp was removed from the first specimen after fixation in formalin. This technique allows examination of portions of one tendon in which the collagen fibres are (a) in the strained state, (b) in the strained and relaxed state, and (c) in the unstrained state.

To date, eight extensor tendons from four horses have been studied in this fashion. Examination of the unstrained controls shows that, in general, the collagen fibres have a clearly defined wave pattern. Figure 8 is an

excellent illustration of an unstrained tendon. After the tendon is strained, this wave pattern gradually disappears. At about the $1.0-1.5\%$ strain level the wave pattern has completely disappeared, and the collagen fibres have become parallel and oriented in the direction of applied load.

In Fig. 9, the specimen has been strained and clamped at the 3.5% strain level; parallel rows of collagen fibres are now very apparent.

In Fig. 10, the specimen was strained to the 3.5% strain level, unloaded, and allowed to relax. In the right half of this figure some straight rows of collagen fibres can be seen. This would appear to indicate that not all of the collagen fibres in this particular specimen have returned to their original unstrained orientation.

The histological studies on specimens strained to the 1.0, 2.0, 3.0 and 4.0% level indicate that above the $2.0-3.0\%$ strain level not all the fibres return to their original unstrained wave pattern. It appears, therefore, that some permanent structural change may have occured either in the collagen network or in the bonding of the

FIG. 7. Effect of stress relaxation in human toe extensor tendon. Sample, from a 57-year-old woman, was tested in Ringer's solution at 37°C 24-hr postmortem.

mucopolysaccharides within the collagen network.

This work is being continued, and additional specimens of tendon will be processed for study with the electron microscope to determine the effect of strain on the individual fibre.

4. SUMMARY AND CONCLUSIONS

A preliminary study of the mechanical properties of horse and human tendon *in vitro* has been made with use of measurements of the response of tendon to externally applied tensile loads. The experimental tests and procedures were divided into five categories: (1) determination of standard stress-strain curve, (2) effects of cycling and determination of elastic limit, (3) effect of strain rate, (4) determination of stress relaxation, and (5) histological studies of strained tendon.

When considering the mechanical behaviour of tendon during normal activity, the secondary region of the stress-strain curve shown in Fig. 3 becomes most important because the experimental results indicate that the normal level of tendon activity lies below the 3.0% strain level.

The constant strain rate experiments show quite clearly that tendon is capable of transmitting fairly large forces to the skeletal frame with only a small increase in tendon length. However, because of the viscoelastic nature of the material there will always be a small time lag between muscle contraction and limb movement. Under *in vitro* experimental conditions, the fastest strain rate recorded was on the order of 1.0% strain/sec; this is obviously less than the normal rate of muscle contraction. However, an estimate of the rate of normal muscle contraction should allow use of the experimental constant strain rate results to predict the actual forces transmitted through the tendon during normal activity.

A permanent deformation, or "residual" strain, occurred when tendon was strained beyond a $2.0-3.0\%$ strain level. The point at which this "residual" strain becomes apparent in relaxed tendon has been called the "elastic

limit." When this limit is exceeded it is assumed that an irreversible structural change takes place. Histological studies indicate that the collagen fibres do not return to their original form once this elastic limit has been passed. Use of the electron microscope should provide additional information regarding the structural formation of the collagen fibres under strain, and the effect on these fibres of exceeding the elastic limit.

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COMPORTEMENT MECANIQUE D'UN TENDON *IN VITRO--* NOTE PRELIMINAIRE

Sommaire---Le comportement mécanique du tendon de l'homme ou du cheval, caractérisé par la courbe force-contrainte a été examiné en tenant compte du cycle charge-tension et de la valeur de la tension. I1 est apparu que la courbe force-contrainte pour des cycles successifs était reproductible pourvu que la contrainte sur la préparation n'excède pas 2 à 4 $\%$. Si ce taux était dépassé, on observerait une déformation permanente. Ce phénomène a été vérifié par des études histologiques sur des tendons étirés: elles ont montré que plusieurs des fibres collagènes ne sont pas revenues à leur orientation primitive. On a trouvé que la variation de la valeur de la contrainte affecte à la fois l'amplitude et la forme de la courbe force-contrainte. De plus en a trouvé que le phénomène de relaxation pour le tendon était essentiellement le même que celui d'autres tissus de liaison.

MECHANISCHES VERHALTEN VON SEHNEN *IN VITRO* (VORLAUFIGE MITTEILUNG)

Zusammenfassung--Das mechanische Verhalten von Pferde- und Menschensehnen, wie es in der 'Stress-Strain'-Kurve (Belastungs-Dehungs-Kurve) charakterisiert ist, wurde hinsichtlich des Belastungs-Dehnungs-Zyklus und der Dehnungsgeschwindigkeit untersucht. Es ergab sich, da β die Belastungs-Dehnungs-Kurve der Sehne über mehrere Zyklen reproduzierbar ist, wenn die Dehnung der Probe nicht über 2,0-4,0 % hinausging. Wurde dieser Dehnungsgrad iiberschritten, so stellte sich eine permanente Deformation ein. Dieses Phänomen wurde durch histologische Untersuchungen an gedehnten Sehnen verifiziert; es zeigte sich, daß einige der Kollagenfasern nicht zu ihrer ursprünglichen Orientierung zurückkehrten. Anderung der Dehnungsgeschwindigkeit beeinflußte sowohl Größe als auch Form der Belastungs-Dehnungs-Kurve. Außerdem wurde festgestellt, daß das Stress-Relaxations-Phänomen für die Sehne im wesentlichen gleich dem bei anderen Bindegeweben gefundenen war.