Z. Anat. Entwickl.-Gesch. 136, 204—212 (1972)

Simultaneous Mechanical and Light Microscopic Studies of Collagen Fibers

A. Viidik

Department of Human Anatomy, University of Göteborg, Sweden

Received December 4, 1971

Summary. An apparatus that enables simultaneous stress-strain recording and morphological observation in incidental light microscopy of a specimen surface during tensile loading is described. Results from experiments on parallel-fibred collagenous specimens (tendons) are reported and variations between behavior during the first tensile loading cycle and those in the steady-state cycles (when the plasticity and irreversible viscosity are exhausted) are described. It is found that the "toe" part of the stress-strain curve is longer from a morphological point of view than can be mechanically measured, which means that the waviness of the collagenous bundles is diminished before any measurable load is applied. The steady state means an even longer such "toe" part and a better parallel arrangement of the fibers but the waviness in the relaxed specimen cannot be disturbed by repeated loadings. The functional range of a tendon is also discussed as are the phenomenon of spraining and the possibility of pre-stress.

Key words: Biomechanics — Collagen — Connective tissue — Polarizing microscopy — Tendons.

Introduction

As collagen is the force-resisting element in soft structures in the mammalian body, considerable interest has focused on the mechanical properties of this fibrous protein. In various structures it is arranged in different geometrical patterns on fiber and fiber bundle dimension, ranging from the parallel-fibred one in the muscle tendons to the three-dimensional meshwork in the dermis. To study the behavior of this fibrous protein per se, it is advantageous to use the parallel-fibred tendons in order to eliminate geometrical complicity and to minimize the influence of ground substance, as in this tissue collagen constitutes about 85 per cent of the dry weight.

The mechanical properties of tendons have been the subject of extensive investigations. Reviews have been written recently by Harkness (1961, 1968) and Viidik (1968). Collagen has traditionally been compared with elastin and considered as inextensible when subjected to tensile forces, although already Wertheim (1847) had described a stress-strain curve for tendons. Recently it has become evident that the mechanical properties of collagen contain elastic, viscous and plastic elements (e.g. Stucke, 1950; Rigby, Hiray, Spikes and Eyring, 1959; Laban, 1962). The total mechanical behavior of collagenous tissue is strongly non-linear and Fung (1967) emphasized the futility to try to characterize the tissue by the modulus of elasticity and proposed a more complex mathematical description. In order to visualize the mechanical properties of materials mechanical analogies can be composed of idealized elements of elasticity, viscosity and plasticity (dry friction) coupled in series and/or in parallel with each other to satisfy the behavior of the test material. In order to overcome the difficulty of the nonlinearity of collagenous tissue Viidik and Mägi (1967) proposed a nonlinear arrangement of linear springs, where the springs come gradually into action with increasing deformation causing a gradual stiffening. This corresponds well to the beginning of the stress-strain relationship of parallel-fibred collagenous tissue, which starts with a "toe" part, i.e. a convexity towards the strain axis, after which a fairly linear portion ensues (cf. Fig. 3a–b). If the straining is halted before failure of the specimen, the stress reached decreases asymptotically towards a somewhat lower value, i.e. a stress-relaxation occurs. This is due to the viscous properties of the material. To account for this in the mechanical analogy a Kelvin element (i.e. elastic and viscous elements in parallel) was coupled in series with the non-linear spring arrangement (Viidik, 1968). As the stress-strain curve shifts to the right and becomes somewhat steeper (cf. Fig. 3b) while the stress-relaxation decreases with repeated cyclic loading-unloading experiments until a steady state is reached after some six cycles, an irreversible part was included into the mechanical analogy and consisted of a viscous element and a series of dry-frictional elements (strain-hardening). This analogy was then analysed mathematically and verified experimentally by Frisén, Mägi, Sonnerup and Viidik (1969a, b).

In a study with incidental and transmitted polarized light microscopy Viidik and Ekholm (1968) confirmed the observations of Rigby *et al.* (1959) and of Abrahams (1967a, b) that relaxed tendons have a wavy course and that this is straightened out during the first part of straining. They, however, had before performing their observations subjected their specimens to previous "conditioning" stress-strain cycles, thus probably eliminating most of the initial phase. Viidik and Ekholm (1968) showed that this straightening corresponds well with the "toe" part of the stress-strain curve and that this part actually is longer than the mechanical experiments indicate, i.e. some fiber bundles are straightened out before any measurable load has been caused by tensile deformation of the tendon specimen. They also found that practically all waves had disappeared when the linear portion of the curve begins.

However, the microscopical observations had to be performed after the mechanical ones as it was then not possible to record the loading during microscopy. Therefore only the steady state behavior could be investigated after the irreversible phenomena in the initial phase had been exhausted.

To be able to observe the morphological events during the initial phase of the stress-strain behavior of collagenous tissue, the mechanical and microscopical recordings must be made simultaneously. Therefore, the materials testing machine and the incidental light microscope must be integrated. Such an apparatus is described in this paper and the results from experiments with it are reported here.

Materials and Methods

For this investigation Achilles and other hind limb tendons from skeletally mature rabbits were used. The animals were sacrificed with an overdose of aether and care was taken to avoid agonal straining of the tendons. They were sectioned in a freezing microtome in parallel with the main direction of the fiber bundles. Specimens with an even surface displaying the various bundles and with a thickness of 0.5 mm were thus obtained. Care was taken to perform the freezing and thawing rapidly (cf. Galante, 1967) and no specimens, where bundles could be seen to emerge from the surface, were used. When brought to failure by gradually increasing strain these specimens behaved mechanically as intact tendons.

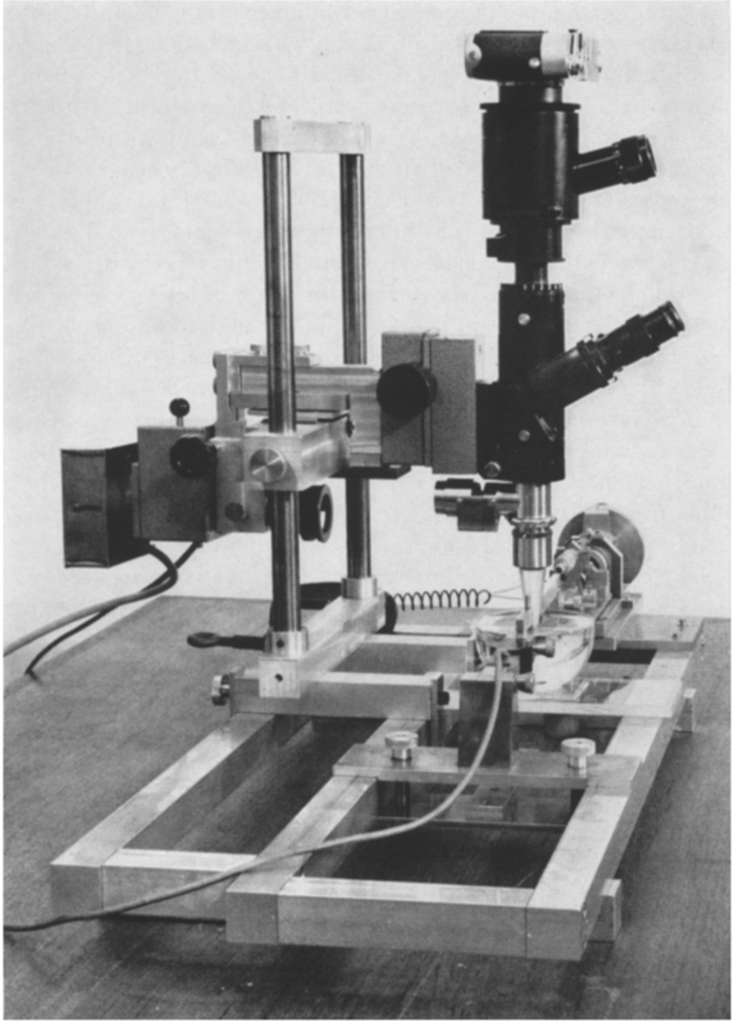

Fig. 1. The whole set-up on three rails connected by cross bars. The microphotographic equipment is fastened to the two-column stand on the two leftmost rails. The microscope with the camera equipment is seen to the right and the electronic flash unit to the left. The mechanical part of the set-up is fastened to the two rightmost rails

The set-up for the combined mechanical and morphological study is shown in Fig. 1. The basic mechanical unit was a back-lash-free screwing device according to Viidik (1966) operated by an electric motor, the speed of which was coupled to and regulated by a tyristor unit to achieve a torque independent of the speed. Instantaneous halting was achieved by reversing the polarity for a short moment. The screwing device was placed on two rails of a three-rail-system united by cross bars. The deformation of the specimen was recorded as the movement of the moving part of the screwing device by a differential transformer (Bofors RLK-1-S, ± 6 mm). The immobile end of the testing system was fixed via a force transducer (Bofors KRK-1, 200 N) to the rails. Both transducers were coupled to measuring bridges (Philips

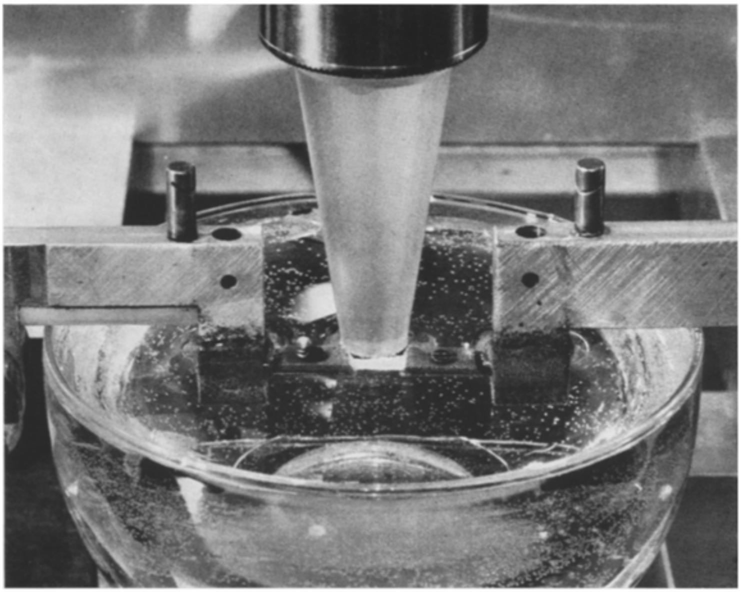

Fig. 2. The specimen in clamps and immersed into Ringer's solution. The water immersion hood of the microscope objective is immersed into the solution between the clamps and right above the specimen

PT 1200) and the signals from these were recorded as load versus deformation on an x-y-recorder (Hewlett-Packard Moseley 7004 A). For both systems the recorded signals were directly proportional to the load and deformation and calibrations proved fully reproducible.

Parts of a Leitz Ortholux microscope were fastened via a cross bar onto a two-column stand on two of the rails. The horizontal movements in two directions could be performed by rack gears and were guided by slide blocks enabling the tracing of the same part of the specimen throughout the test series. The gross movement in the vertical direction was performed by moving the cross bar with the microscope and the fine movement by a precision microscope focusing gear.

The two clamps and the specimen immersed in Ringer's solution buffered to pH 7.4 are shown in Fig. 2. An objective 3.8/0.12 together with a water immersion hood were used. Illumination was provided by an Ultrapak incidental light equipment together with a polarizing system. Microphotographs were taken with a Leica MDa camera on a micro adapter and exposure light was provided by flash illumination of short duration (1/1000 sec) from a Braun F-650 straight discharge tube coupled to a F-800 electronic flash unit and adapted to a Leitz microflash optical system to achieve maximum intensity and optimal refraction. The synchronising contact of the camera for flashbulbs was coupled to the y-axis of the recorder in order to mark the position on the load-deformation diagram, where photomicrographs were made.

Results

A typical load-strain diagram of a first loading-unloading cycle of a tendon specimen is shown in Fig. 3a and corresponding photomicrographs are shown in Figs. 4a–d. The specimen was brought to eight per cent strain (tensile deformation i.e. elongation in per cent of original length). The starting point of the test is indicated by "a" on Fig. 3a and the point where load was first recordable by "b". The corresponding photomicrographs are Figs. 4a and b. No difference could be

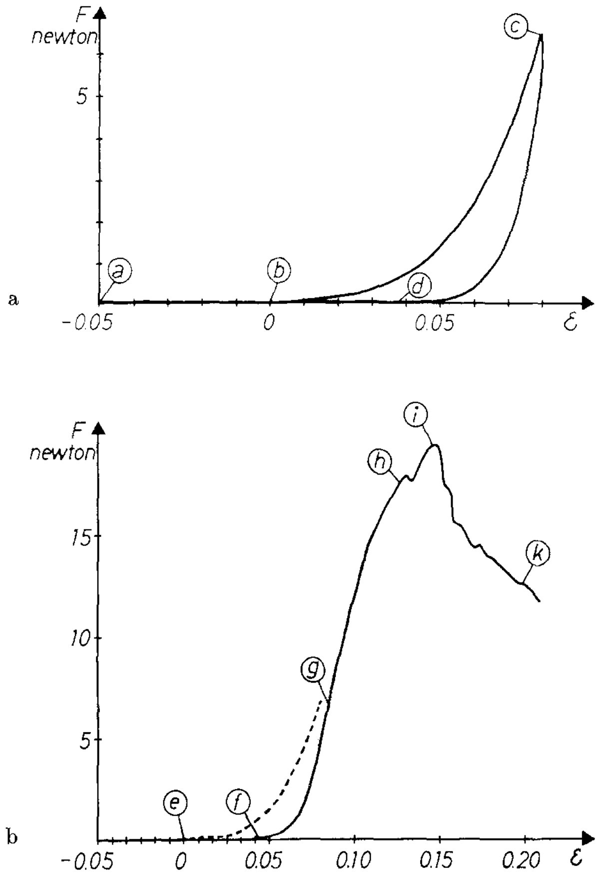

Fig. 3.a The first loading-unloading cycle for a rabbit hind limb tendon specimen to eight per cent strain. The load is given in newton on the F axis and strain in per cent of original length on the abscissa. b A load-strain diagram for the same specimen as in (a) after the irreversible phenomena had been exhausted at the load level reached in the first cycle. when it is strained until failure. The loading curve for the first cycle is indicated by a dotted line

found between these two stages of straining, except that some of the waves in the first picture were deeper. Then, during the mechanical toe part the waves were straightened out gradually, although the waves in a fiber bundle disappeared simultaneously in the whole length of the bundle. "c" indicates the point of eight per cent strain, where the load corresponded to about one third of the maximum load. This was clearly within the linear part of the load-strain curve. Here all fiber bundles were straightened out except two minor strands that exhibited waviness. Such small wavy strands were quite often seen on this stage and were probably

Fig. 4a—k. Photomicrographs of the specimen of Fig. 3. Magnification 32×, incidental light and polarizing system. a–d correspond to the letters in Fig. 3a and e–k to Fig. 3b

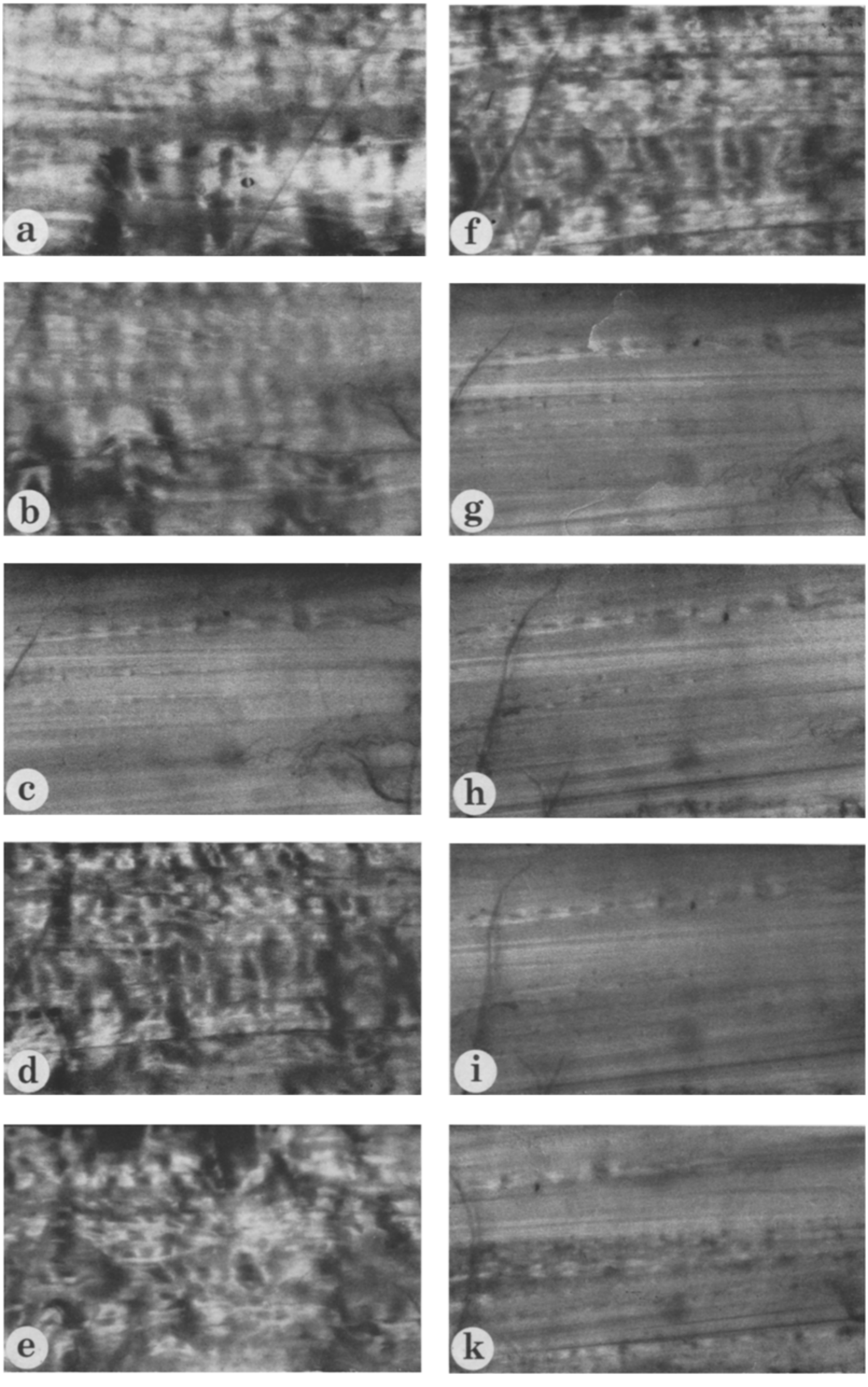

Fig. 4a–k

caused by partial ruptures, as they were not present during the later section of the toe part. However, these are more frequent above the one third maximum load level and show, if large enough, as vertical dips in the load-strain curve (cf. Viidik, Sandqvist and Mägi, 1965; Viidik and Lewin, 1966). When the deformation was reversed, the unloading curve was steeper and reached the zero stress level to the right of the corresponding loading curve. This point is marked with "d". The corresponding photomicrograph is Fig. 4d. This point showed a picture similar to the "b" picture, although "b" is to the left of "d".

After the first loading-unloading cycle of the specimens to eight per cent strain the irreversible mechanical phenomena were exhausted at the load level reached in the first cycle by keeping the specimen at that load with gradually increasing the deformation until the load value became constant without requiring additional deformation adjustment. Then after ten minutes resting period at the completely relaxed state (point "a" in Fig. 3a) to recover the reversible viscous properties completely the specimen was deformed at the same constant strain rate until failure occurred.

This second stress-strain curve for the same specimen as in Fig. 3a is shown in Fig. 3b, where the position of the loading curve for the first cycle is indicated by a dotted line. The corresponding photomicrographs are Figs. 4e–k. There was a clear shift to the right, the curve became steeper and the "toe part without load" longer. The specimen could usually be strained 4–5 per cent from the starting point of the toe part of the first cycle without getting any load reading. The starting point for the initial curve is indicated by "e" in Fig. 3b and the corresponding photomicrograph of the second experiment on the specimen is shown in Fig. 4e. The waves in this position tended to be deeper than "b" but the bundles seemed to be more parallelly arranged than in "a", where no load had been applied at all. "f" is the point where some load could be recorded in the second experiment (Fig. 4f) and here the picture was similar to "b". "g" indicates the same load level as reached at the peak in the first experiment and the photomicrograph in Fig. 4g is similar to Fig. 4c. At the "h" level, which is just before a major dip in the curve the same fibers were still straightened out as in "c", i.e. all except a few strands, which already had failed. At "i", which is the maximum load point, always more fiber bundles had regained a wavy appearance (Fig. 4i). At "k" the load value had dropped considerably and a pronounced part of the fibers displayed waviness and gliding apart occurred (Fig. 4k). Later on the fiber bundles regained their appearance as in Fig. 4e, although splitting up and gliding apart were very evident. In some instances the failure was abrupt at "i" level and afterwards only a few fiber bundles bridged over the gap. This was more pronounced in the specimens that failed in one of the clamp yaws than those that failed in the free space between the yaws.

Discussion

Here an apparatus is described that enables simultaneous microscopic observation/recording of a specimen surface and mechanical testing of the specimen. A polarizing system is used for the "shadowing" of the surface and for the elimination of reflexes on it. On the other hand, the birefringence of the collagen fibers cannot be displayed by this incidental light technique.

The functional morphology of rabbit hind limb tendons during initial phase mechanical behavior and its transition into a steady state behavior were also investigated. It was found that in the loading-unloading cycles of a specimen the mechanical toe part started later in the cycles following exhaustion of the initial phase phenomena than in the first cycle. This was found to be due to a rearrangement of the fiber bundles to a more parallel orientation during the first cycles. The wavy formation of the bundles, however, could not be diminished by repeated mechanical straining cycles and was present even after failure except where the bundles were splitted up. Failures of minor strands occurred even at rather low load levels, i.e. below one third maximum load.

It is not yet established where the "physiological" working range of the muscle tendon ends. These experiments indicate that it should be considerably below the one third maximum load limit investigated here as partial ruptures, although very small, occurred already at this level. It is probable that when spraining of tendons occurs, i.e. morphologically partial ruptures and clinically soreness, the load is most often brought to somewhere between the one third and two third maximum load levels. At higher load levels an avulsion of a bony insertion would occur, as the tendon never is the weakest point in the functional unit of bone, tendon, muscle, tendon and bone (McMaster, 1933; Viidik, 1969). Furthermore, it was calculated by Harkness (1968) on the basis of the investigation by Elliott and Crawford (1965) that the tensile strength of a tendon is about four times the maximum isometric tension its muscle can create. The relation between the tensile strength of muscle fibers in contraction and that of its tendon, which is of interest for e.g. the analysis of the influence of externally applied torque, remains to be investigated. However, it is probable that the maximum stress in the tendon is never reached as the injuries encountered in orthopaedic surgery are mostly tear-off fractures.

It was pointed out by Elliott (1965) that the waviness of tendon fiber bundles was straightened out at one tenth of the maximum tension its muscle was able to create. This is well in agreement with the present investigation that the waviness is straightened out within the toe part, the most of it being within 5 per cent of the maximum load of the tendon, cf. Fig. 3b and the calculations of Harkness (1968). It can therefore be concluded that the functional activity in the tendon occurs within the toe part of its stress-strain curve. The early part of it may be at most times in activity, although no specific information is available on whether there is a pre-stress in the relaxed system of bone-tendon-muscle-tendon-bone in vivo or not.

Acknowledgement. This work was supported by the Swedish Medical Research Council (Project No. B70-12X-2883-01) and by Carin Trygger Memorial Fund.

References

Abrahams, M.: The mechanical behaviour of tendon collagen under tension in vitro. In: Digest 7th Int. Conf. Medical Biological Engineering (ed. B. Jacobson), p. 509. Stockholm: Almqvist&Wiksell 1967a.

Abrahams, M.: Mechanical behaviour of tendon in vitro. A preliminary report. Med. biol. Engng **5**, 433–443 (1967).

Elliott, D. H.: Structure and function of mammalian tendon. Biol. Rev. **40**, 392–421 (1965)

Elliott, D. H., Crawford, G. N. C.: The thickness and collagen content of tendon relative to the strength and cross-sectional area of muscle. Proc. roy. Soc. B **162**, 137–146 (1965).

Frisén, M., Mägi, M., Sonnerup, L., Viidik, A.: Rheological analysis of soft collagenous tissue. Part I: Theoretical considerations. J. Biomech. **2**, 13–20 (1969a).

Frisén, M., Mägi, M., Sonnerup, L., Viidik, A.: Rheological analysis of soft collagenous tissue. Part II: Experimental evaluations and verifications. J. Biomech. **2**, 21–28 (1969b).

Fung, Y. C. B.: Elasticity of soft tissues in simple elongation. Amer. J. Physiol. **213**, 1532–1543 (1967).

Galante, J. O.: Tensile properties of the human lumbar annulus fibrosus. Acta orthop. scand., Suppl. 100 (1967).

Harkness, R. D.: Biological functions of collagen. Biol. Rev. **36**, 399–463 (1961).

Harkness, R. D.: Mechanical properties of collagenous tissues. In: Treatise on Collagen, vol. II A (ed. B. S. Gould), p. 247–310. New York: Academic Press 1968.

Laban, M. M.: Collagen tissue: Implications of its response to stress in vitro. Arch. phys. Med. **43**, 461–466 (1962).

McMaster, P. E.: Tendon and muscle ruptures. J. Bone Jt Surg. **15**, 705–722 (1933).

Rigby, B. J., Hirai, N., Spikes, J. D., Eyring, H.: The mechanical properties of rat tail tendon. J. gen. Physiol. **43**, 265–283 (1959).

Stucke, K.: Über das elastische Verhalten der Achillessehne im Belastungsversuch. Langenbecks Arch. klin. Chir. **265**, 579–599 (1950).

Viidik, A.: Biomechanics and functional adaption of tendons and joint ligaments. In: Studies on the anatomy and function of bone and joints, (ed. F. G. Evans), p. 17–39. Berlin-Heidelberg-New York: Springer 1966.

Viidik, A.: Function and structure of collagenous tissue. Göteborg: Elanders 1968.

Viidik, A.: Tensile strength properties of Achilles tendon systems in trained and untrained rabbits. Acta orthop. scand. **40**, 261–272 (1969).

Viidik, A., Ekholm, R.: Light and electron microscopic studies of collagen fibers under strain. Z. Anat. Entwickl.-Gesch. **127**, 154–164 (1968).

Viidik, A., Lewin, T.: Changes in tensile strength characteristics and histology of rabbit ligaments induced by different modes of postmortal storage. Acta orthop. scand. **37**, 141–155 (1966).

Viidik, A., Mägi, M.: Visco-elastic properties of ligaments. In: Digest 7th Int. Conf. Medical Biological Engineering (ed. B. Jacobson), p. 507. Stockholm: Almqvist & Wiksell 1967.

Viidik, A., Sandqvist, L., Mägi, M.: Influence of postmortal storage on tensile strength characteristics and histology of rabbit ligaments. Acta orthop. scand., Suppl. 79 (1965).

Wertheim, M. G.: Mémoire sur l'élasticité et la cohésion des principaux tissus du corps humain. Ann. Chim. Phys. **21**, 385–414 (1847).

Prof. Dr. med. A. Viidik
Anatomisches Institut
Aarhus Universität
DK-8000 Aarhus C
Dänemark