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Function and Structure of Collagenous Tissue

Investigations on the tensile strength,
general rheology and morphology of tendons
and joint ligaments in the rabbit

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CONTENTS

This presentation gives the background to and summarizes the following publications. They will be referred to by the Roman numerals given.

I. VIIDIK, A.: Tensile strength properties of Achilles tendon systems in trained and untrained rabbits. *Acta orthop. scand.* In press.

II. VIIDIK, A.: The effect of training on the tensile strength of isolated rabbit tendons. *Scand. J. plast. reconstr. Surg. 1*: 141–147, 1967.

III. VIIDIK, A.: Elasticity and tensile strength of the anterior cruciate ligament in rabbits as influenced by training. *Acta physiol. scand.* In press.

IV. VIIDIK, A.: A rheological model for un-calcified parallel-fibred collagenous tissue. *J. Biomech. 1*: 3–11, 1968.

V. FRISÉN, M., MÄGI, M., SONNERUP, L. and VIIDIK, A.: Rheological analysis of soft collagenous tissue. Part I: Theoretical considerations. *J. Biomech.* In press.

VI. FRISÉN, M., MÄGI, M., SONNERUP, L. and VIIDIK, A.: Rheological analysis of soft collagenous tissue. Part II: Experimental evaluations and verifications. *J. Biomech.* In press.

VII. VIIDIK, A. and EKHOLM, R.: Light and electron microscopic studies of collagen fibers under strain. *Z. Anat. Entwickl.-Gesch. 127*: 154–164, 1968.

GENERAL INTRODUCTION

Connective tissue constitutes the soft skeleton of most organs and consists of fibers, mainly collagen and elastin, embedded in a matrix, which is a colloid where glycosaminoglycans dominate. The cells that produce these fibers, the fibrocytes, are characteristically distributed in different tissues.

Collagen, which is a fibrous protein, is present throughout the animal kingdom and, at least among the mammals, it exhibits little or no species variation. (PIEZ & GROSS 1959, SLACK 1959). Compared with other filamentous structures in the animal body, collagen fibers possess great strength and little extensibility. This protein plays an important part in transmitting and resisting forces in the uncalcified tissues of the body. Studies aiming at elucidation of the mechanical properties of connective tissue and their correlation to the morphological details, are therefore best performed on tendons and joint ligaments, where collagen is found in parallel-fibred bundles surrounded by scanty amounts of ground substance and fibrocytes between the bundles.

Following an introductory survey of the morphology, mechanics and some methodological problems, this account will first deal with tensile strength parameters and certain elasticity characteristics of tendons and joint ligaments in the rabbit and how they change with training of the animal (I–III). General rheology of collagenous tissue will then be dealt with using a joint ligament in the rabbit as the test specimen (IV–VI). These findings will thereafter be correlated to the morph-

ology of the tissue on light and electron microscopic levels (VII).

No attempt will be made to compose even a fairly complete survey of the vast literature on the morphology and biophysical characteristics of collagen. A survey of morphology has been compiled by WASSERMAN (1956) and functional aspects have been reviewed by HARKNESS (1961) and ELLIOTT (1965). The biophysics and biochemistry have been dealt with in the reviews by BEAR (1952), GUSTAVSON (1956), RAMACHANDRAN (1967) and BAILEY (1968). Other monographs with extensive bibliographies have been published by BANGA (1966) and CHVAPIL (1967). Therefore only papers with direct relevance to the present problems will be discussed. Also the literature on the mechanical features of collagen treated in various ways, mainly in the leather industry, is prolific. With certain exceptions, the present discussion will be confined to fresh, native collagen as found in tendons and joint ligaments.

MORPHOLOGY

The primary building unit of collagen is the tropocollagen molecule, which is composed of polypeptide chains. There are three amino acid chains coiled into left-hand helices and the composition is different in all three of them. The molecule itself (Fig. 1) is constituted by a right-handed super-helix formed by these three chains (RICH & CRICK 1955). Much discussion has been devoted to how

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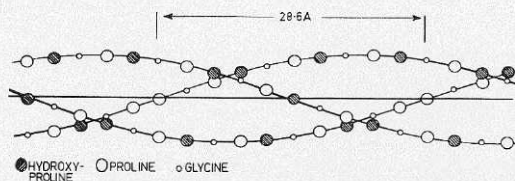
Fig. 1: A schematic representation of the three left-handed amino acid helices twined to a righthanded superhelix. Only the hydroxyproline, proline and glycine residues are indicated (after LOWTHER 1963, © and courtesy of Academic Press).

Fig. 2: A schematic two-dimensional representation of the arrangement of the tropocollagen molecules (each indicated by an arrow) into a fibril. D is the periodicity seen in the native fibril and $4.40 \times D$ is the length of the tropocollagen molecule (after HODGE, PETRUSKA & BAILEY 1965, © and courtesy of Butterworths).

these tropocollagen molecules are organized into the long cross-striated fibrils seen in the electron microscope. Since it has been demonstrated in studies on native and reconstituted aggregates of collagen that the length of the molecule is 4.4 times the length of the period of the native fibril (HODGE & PETRUSKA 1962) the older idea of parallel alignment of the molecules with displacement of adjacent molecules by multiples of one quarter of the length of the tropocollagen molecule (GROSS, HIGHBERGER & SCHMITT 1954) has been, at least partly, abandoned. This idea included also end-to-end attachment of the molecules. It has been suggested by HODGE & PETRUSKA (1962) and OLSEN (1963) that the discrepancy between native period and molecule lengths may be explained by a combination of overlaps and gaps between the molecules in addition to the normal quarter stagger thus adapting the old concept to the new results (Fig. 2). GRANT, HORNE & COX (1965) have proposed another model emphasizing cross-linking and intertwining of the molecules. Recently an idea that the tropocollagen molecule consists of five segments, four of these having the length of the period (D) seen in the native fibril and the fifth being shorter (0.4 D), has

won support. This concept includes the quarter-stagger idea for the four D segments and leaves a gap of 0.6 D between consecutive molecules which is compatible with the steric conditions found (SMITH, 1968).

The native collagen fibril exhibits an axial periodicity, which by wide angle X-ray diffraction analysis of relaxed fibers is determined to 2.86 Å. By extension of the fiber it can be increased to about 3.1 Å (COWAN, NORTH & RANDALL 1955). This pattern is considered to reflect the arrangement of amino acid residues in the helices. In low angle X-ray diffraction a macroperiodicity is exhibited instead. This is for dried fibers 640 Å, which corresponds with the periodicity of the native fibrils as reported in most electron microscopic studies, but it can by moistening of the fiber be increased to 680 Å (BEAR 1944). The periodicity seen in the electron microscope consists of alternating dense and less dense bands that can be divided into several subunits (Cf. SMITH 1968 and others). The diameters of the fibrils, however, exhibit considerable variation both in and between

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species (Cf. the compilation by HARKNESS 1961).

The fibers, composed of bundles of fibrils, are visible in the light microscope. They have diameters ranging down to 0.2μ. In polarized light birefringence is exhibited and it is positive in the direction of the longitudinal axis of the fibrils (Cf. WASSERMANN 1956). It can, by the Ambronn immersion technique, be divided into at least two types, a positive uniaxial form birefringence and an intrinsic or crystalline birefringence (PFEIFFER 1943). At the semi-microscopic level the fibers probably have a wavy course. (RIGBY, HIRAI, SPIKES & EYRING 1959) although a helical arrangement also has been advocated (VERZÀR 1965).

The tendon is part of a functional unit that consists of bone, tendon, muscle, tendon and bone. One of the tendons consists in most units of this type only of short collagenous strands. The joint ligament is part of a bone, ligament and bone complex. At the insertion of the tendon into the bone the collagen fibers go directly into the bone and merge with the fibers of it (VIS 1957, HALL 1965) (I: Fig. 8). The same is true for ligaments such as the cruciates in the knee joint (VIIDIK, SANDQVIST & MÄGI 1965). In the transitional zone between tendon or ligament and bone there are chondrocyte-like cells between the fibrocytes and osteocytes, the different types merging into each other (HALL 1965, VIIDIK et al. 1965, Fig. 10). In the musculo-tendinous junction there is a fairly large contact surface between the muscle fiber and the collagen fibril as the tendon components are invaginated into the ends of the muscle fibers in the numerous terminal indentations of the outer sarcolemmal layer (GELBER, MOORE & RUSKA 1960, SCHWARZACHER 1960).

TENSILE STRENGTH PARAMETERS

Most investigators, who have dealt with the mechanical properties of collagenous tissue, have mainly performed various forms of strength and stiffness measurements, for instance determination of breaking strength, "modulus of elasticity", the shape of the load-deformation curve in general and the energy required to reach a certain load or deformation. There are two principal approaches possible for such studies. Either the whole functional unit can be used (bone-tendon-muscle-tendon-bone or bone-ligament-bone) or the isolated tendon or a part of it. The first approach yields information on the collagenous tissue until the weakest point of the system fails, and as this generally is the bone or muscle, no information can be obtained on the behavior of tendons or ligaments beyond that point. Changes in the properties of collagen, however never physiologically utilized, cannot be investigated with this approach. The second approach yields information on the whole range of the tissue's load-deformation relationship, provided that the experiments are performed without producing artificial rupture initiation in the specimen tested. Therefore, it seems most advisable to employ both approaches to obtain adequate information.

Since the classical study by MCMASTER (1933) the complex of calcaneus, Achilles tendon, gastrocnemius muscle and femur has been used as a test specimen by e.g. FINK & WYSS (1942), STUCKE (1951), DAVIDSSON (1954, 1956) and VIIDIK (1966). Most of these investigations have been concerned with the tensile strength and rupture site registrations. It was established by MCMASTER (1933) and confirmed by later investigators that a healthy

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tendon never ruptures. Degeneration is required or the complex fails at some other point.

To explore the properties of bone-ligament-bone complexes most often knee joint specimens have been used. Anterior cruciate ligaments were used by SMITH (1954), VIIDIK et al. (1965) and VIIDIK (1966) while collateral ligaments were investigated by CLAYTON & WEIR (1959), ADAMS (1966) and TIPTON, SCHILD & FLATT (1967). From these studies it is evident that healthy collagenous tissue also as joint ligaments is not the weakest point of the functional unit. When failure load is reached, rupture occurs as a tear-off fracture of a bony insertion.

The mechanical properties of isolated tendons were first studied by WERTHEIM (1847). Whole tendons (CRONKITE 1936, ROLLHÄUSER 1950a, STUCKE 1950, WALKER, HARRIS & BENEDICT 1964, VAN BROCKLIN & ELLIS 1965, ELLIOTT 1967, VIIDIK 1967) as well as teased out fibers (COMPTON 1949, STUCKE 1950) and cut sections (GALANTE 1967) have been used. Also fasciae as fascia lata (GRATZ 1931) and fascia plantaris (WRIGHT & RENNELS 1964) have been investigated. There are also many reports on the mechanical properties of skin; the geometry of the collagen fibers in that tissue, however, makes it less suitable for studies of the protein per se (TREGEAR 1966).

The tensile properties of collagenous tissues are commonly described by the relation between load and deformation. This may be adequate for comparisons between geometrically congruent specimens. But a specimen with half the thickness of another fails at half the load required to fail the other one although both of them are of the same quality. The case with the deformation of specimens of various original lengths is similar. To overcome the scatter caused by these conditions when comparing geometrically dissimilar specimens, stress (load per unit cross-sectional area) versus strain (deformation per unit original length) should be calculated.

The tensile strength of "fresh" tendons has been found to be in the range of 46 N (STUCKE 1950) to 125 N (CRONKITE 1936) per square millimeter.[1] From isolated fibers assuming the material to be purely collagen and having the specific gravity of 1.4 HARKNESS (1961) calculated the breaking strength for pure collagen to be in the range of 100–500 N/mm^2. The corresponding calculation for whole tendons, assuming the collagen content to be 30 per cent, gave 150 to 300 N/mm^2, a figure only a little below that for isolated fibers. On the other hand the figure for skin collagen was found to be only 100 N/mm^2. This discrepancy can be explained by the different geometrical orientation of the fibers (HARKNESS 1961, TREGEAR 1966). The stress-bearing structures on the molecular level are the collagen protein chains. It has been calculated (GUSTAVSON 1956, p. 147) that it would require a stress in the order of 3000 N/mm^2 to break a molecular chain at its weakest point, the -C-N-bond, while the actual breaking strength is less than one tenth of that value. Therefore, it is more probable that the failure occurs in the transversal dimension, where mainly cohesive forces exist (HIGHBERGER 1947).

More information is obtained if not only the ultimate tensile strength but also the load-deformation relationship towards that point is recorded. When testing geometrically fairly uniform specimens it is sufficient to record load versus elongation, which is the

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[1]) N = newton, internationally used force unit, $1\ N \simeq 0.102$ Kp (kilogram-force) $\simeq 0.225$ lb$_f$.

type of deformation in tensile tests, to get useful curves and sometimes this is the only approach possible. Knee joint specimens with a cruciate or collateral ligament do not lend themselves to measurements of original length and cross-sectional areas of the ligaments. In most cases as with isolated tendons of reasonable length and diameter the dimensions vary within groups and are measurable. Then stress versus strain should be calculated.

Such load-deformation or stress-strain curves start with a "toe" part, i.e. a convexity towards the strain or deformation axis (e.g. REUTERWALL 1921, STUCKE 1950, ROLL-HÄUSER 1950a, RIGBY et al. 1959, LaBAN 1962, ELLIOTT 1967) after which a fairly linear section ensues. This is indicated by A in Fig. 3 showing a load-deformation curve for a tendon. The rupture may occur in this region (MORGAN 1960, WRIGHT & RENNELS 1964, VIIDIK et al. 1965) or the

Fig. 3: Drawing after an oscilloscope tracing of a tensile strength experiment on an isolated tendon until failure. Load (*F*) is recorded versus deformation (*X*). *A* is the start of the linear part of the curve, and *B* the end of it. α indicates the angle measured as "elastic stiffness" of the linear part. *C* indicates the maximum load.

curve may bend off towards the deformation axis (B in Fig. 3) before rupture occurs; the load value may even decrease below the maximum load value (C in Fig. 3) before rupture. The area between the load-deformation or stress-strain curve and the deformation/strain axis corresponds to the energy used for breaking the structure or bringing it to a certain point of the curve.

As the concept "modulus of elasticity" is based on a linear relationship between the load and deformation, it can not be applied to the curves of collagenous tissue, which are sigmoid in shape. However, "elastic stiffness" can be measured for the fairly linear part of the curve (in Fig. 3 the angle α), and of course, the tangent to the curve at any point chosen.

ELASTICITY AND PLASTICITY

So far the load-deformation relationship for tendons and ligaments when loaded at constant speed until failure has been discussed. It has, however, long been evident that there are non-elastic components in the mechanical properties of collagenous tissue. GRATZ (1931) showed there to be a plastic component involved even when stresses less than one third of maximum stress were applied. Both ROLLHÄUSER (1950a) and STUCKE (1950) found that consecutive stress-strain curves for a tendon shifted to the right indicating the presence of a plastic component. SMITH (1954) found this not to be the case when the load applied was of short duration, suggesting the presence of a viscous component, which was supported by the fact that a creep phenomenon could be demonstrated when the load was applied for longer periods. The creep phenomenon is a gradual change of deformation at constant
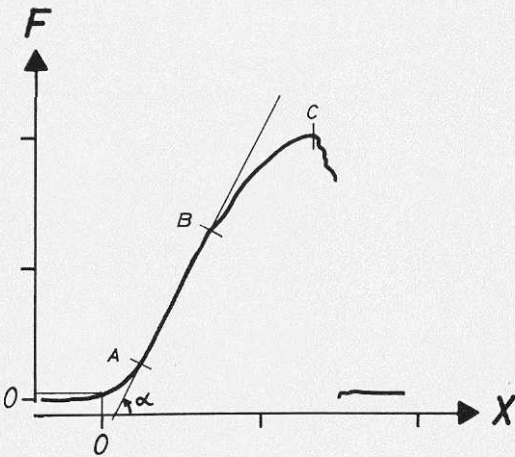
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load, asymptotically tending towards a certain value. On the other hand, if the deformation is kept constant after a certain load/stress value is reached, the load/stress decreases asymptotically towards a certain value (load/stress-relaxation). RIGBY *et al.* (1959) demonstrated these phenomena, i.e. the creep and the shift to the right, to be most prominent during the first cycle of loading and called this "conditioning". Permanent deformation depending on the amount of previous loading was demonstrated by LABAN (1962) and he also reported a viscous effect, which he called time-dependent phase of plastic-like change. A hysteresis between the curves for loading and unloading and a moderate shift to the right for consecutive load-deformation curves for cyclic loading-unloading were also demonstrated by VANBROCKLIN & ELLIS (1965). From these reports it can be concluded that collagenous tissue is not simply elastic and that its load-deformation or stress-strain curve is not linear except for a certain fairly linear midportion. Therefore no "modulus of elasticity" exists; the stiffness not only changes for different parts of the curve but it is also dependent on the testing history of the specimen prior to the actual experiment. Further, there is a certain amount of plastic and viscous deformation, some of which is recoverable [by ROLLHÄUSER (1950a) and SMITH (1954) called elastic after-effect].

Some investigators, however, have reported more or less perfect elasticity for collagenous tissue. Thus, ANNOVAZZI (1928) found ligaments from dogs elastic within certain limits, so did SMITH (1954) for loads in the magnitude of the animals body weight. The measuring techniques used in these cases may not have been fully adequate.

In order to measure the degree of elasticity with precision a backlash-free screw arrangement was constructed and equipped with electronic recording equipment for load and deformation (VIIDIK 1966). With this set-up it was demonstrated that the anterior cruciate ligament in the rabbit's knee joint exhibited plasticity even when subjected to loads of 30 N, which is about one tenth of the failure load. This plasticity was most evident during the first loading-unloading cycle. There was also a clear difference between the slopes of the linear portions of the load-deformation curves for the first and second cycles. Load-relaxation was also demonstrated and it was greatest in the first cycle. Both the plasticity and load-relaxation were found to be relatively of the same order of magnitude whether the load used was one third or one tenth of failure load. The elastic after-effect phase was found to be completed in 5 minutes.

Also more complicated methods of evaluating the mechanical properties have been advocated. Empirical equations had been put up and some constants evaluated (e.g. KING 1957, MORGAN 1960, RIDGE & WRIGHT 1964) but no useful interpretations had been forwarded. Likewise, mechanical analogies had been suggested for synthetic polymers (ALFREY & GURNEE 1957) and later for bone by SEDLIN (1965). However no quantitative interpretations had yet been shown possible. Therefore, these methods did not offer more for the evaluation of changes in the physical properties of tendons and joint ligaments with altered functional states than the experimental programme as described by VIIDIK (1966).

REMARKS ON EXPERIMENTAL TECHNIQUES

The techniques used when investigating the mechanical properties of tendons and joint ligaments have varied to a very great

extent. Various types of set-ups from the suspending of one end of the specimen from a fixed point and pouring water or sand into a bucket tied to the other end of it and then recording the amount of water or sand in the bucket at failure, down to modern material testing machines with electronic recording systems have been used.

When testing whole functional systems (i.e. bone-tendon-muscle-tendon-bone or bone-ligament-bone) the bones have been the natural points for fixation, either by clamping or fixing via pins in drilled holes. In the latter case quite often the bone has been reported to fail through one drilled hole. On the other hand, when testing isolated tendons or parts of them, the adequate clamping of the specimen into the testing machine has offered many difficulties. The evaluation of results has often been troubled by slipping of the specimen or breaking it in the clamp jaw.

A thorough analysis of these problems was performed by COMPTON (1949). He found no significantly lower tensile strength values in the group of specimens that failed in a clamp jaw compared with those that failed in the free space between the two clamps. His clamps had flat surfaces and were operated by screws. However, his testing material was collagen bundles from kangaroo tail tendons that had first been dried and then pre-treated in various ways and finally exposed to air at constant temperature and certain humidity. It is therefore difficult to apply his method to fresh material, which is less rigid and tends to "flow" when clamped. Less successful results have been reported later. WALKER et al. (1964) reported only 11 ruptures out of 30 in the free space and of their remaining specimens 16 slipped from the clamps, 2 ruptured at the clamp and one ruptured at a previously damaged point. In a later study the same group refrained from studying the failure load (HARRIS, WALKER & BASS 1966). All the experiments of WRIGHT & RENNELS (1964) ended in ruptures in the serrated jaws and therefore no conclusions on tensile strength could be drawn from their study.

Another type of clamp has proved advantageous (VIIDIK 1967). These clamps were made of hard aluminium and the contact surfaces were smooth with rounded edges towards the free space between the two clamps. Plain cotton cloth, the thickness of which diminished towards the free space between the clamps, was glued to the contact surfaces, and on the top of this water proof abrasive paper. The clamps were operated by screws which were adjusted with a torque wrench i.e. a screwdriver equipped with a built-in spring and scale to indicate the amount of force applied to the screws and hence the compression between the contact surfaces of the clamps. The suitable tensions to be applied to the different tendon ends fastened into the clamps were found empirically. Analysis of results revealed that there was no tendency towards lower values in the jaw rupture groups than in the free space rupture groups.

When evaluating the tensile strength of geometrically non-congruent specimens it is necessary to know the cross-sectional areas of them. Many difficulties are encountered when trying to measure these areas. The tendon is compressible to a certain extent and its cross-sectional area is not constant throughout its length. Many of the discrepancies reported on tensile strength measurements may be due to this. Enlarged images of tendon sections have been used for estimation of the cross-sectional areas (CRONKITE 1936), so have the principle of Archimedes

(ROLLHÄUSER 1950a), devices with calipers (WALKER et al. 1964), dry weight per unit length (ELLIOTT & CRAWFORD 1965) and fresh weight per unit length (VIIDIK 1967) determinations. The values obtained by the last mentioned method proved to have a good correlation to tensile strength for tendons uniform with regard to functional state and age. This was probably due to the facts that a mean value for the whole tendon was obtained instead of values at arbitrary measuring points and that the procedure of keeping the tendon moist, which was performed by keeping it between two layers of blotting paper moistened with physiological saline, was satisfactorily standardized. The assumption that weight per unit length is directly proportional to the cross-sectional area is valid when the specific gravity is unchanged throughout the material used.

Testing of tendons and joint ligament in completely intravital conditions is impossible. Consequently, considerable attention has been paid to possible post mortem changes. The water content of the tissue is of main importance, since with drying the stiffness increases (WERTHEIM 1847, KATZENSTEIN & FECHER 1924, ANNOVAZZI 1928, STUCKE 1950, ROLLHÄUSER 1950b, MORGAN 1960). The changes caused by storing post mortem per se are less evident. ROY (1880/82) could not find any changes in the mechanical properties until putrifaction started. No consistent changes have been observed in various tensile strength characteristics when storing intact knee joint specimens for as long as 3 days (VIIDIK et al. 1965, TIPTON et al. 1967), results in agreement with less extensive studies (WERTHEIM 1847, GRATZ 1931). This agrees also with the results of experiments performed on skin (RIDGE & WRIGHT 1965). There are, however, some observations that the tissue becomes stiffer with increasing time lapse between sacrifice and testing (KATZENSTEIN & FECHER 1924, SMITH 1954), which may be due to drying.

It can therefore be concluded that collagenous tissue stays "fresh" for at least 24 hours and shows then no significant change in its water content provided that it has not been removed from its normal surroundings (HIRSCH & GALANTE 1967, GALANTE 1967). On the other hand methods to preserve the tissue after being excised from the body or intended for storage for longer periods of time have been less successful. Fixation in formaldehyde, which is common in histological techniques, definitely alters the mechanical properties (STUCKE 1950, CURTIS 1963, ELDEN 1964, VIIDIK & LEWIN 1965) as the aldehyde increases the number of crosslinkages between the amino acid chains in collagen (CURTIS 1963). A previously heterogeneous material after formaldehyde treatment exhibits no differences between specimens (STUCKE 1950). Deep freezing of the tissue has been employed quite frequently (RIGBY et al. 1959, VANBROCKLIN & ELLIS 1965, RIDGE & WRIGHT 1965) without evident changes in the mechanical properties observed. However, VIIDIK & LEWIN (1965) questioned the suitability of this method on the basis of observations in experiments on intact rabbit hind limbs. On the other hand, HIRSCH & GALANTE (1967) demonstrated that thin specimens of collagenous tissue could be rapidly frozen and thawed in a freezing microtome without affecting the mechanical properties. Also immersion in various fluids to protect the specimen from water loss or gain have been attempted. Collagenous tissue swells not only in water, to the least extent at a neutral pH (LLOYD, MARRIOTT & PLEASS 1932), but also in "physiological"

saline (VIIDIK & LEWIN 1965) and exhibits subsequent changes in its mechanical properties. These include larger deformation at failure, higher failure energy and in loading-unloading experiments larger residual deformation (VIIDIK & LEWIN 1965, GALANTE 1967). When the swelling is enhanced by acid, the stiffness of the material decreases markedly (ROLLHÄUSER 1950b). Also 10 per cent dextran solution and human plasma have been tried and found to increase the water content of collagenous tissue (GALANTE 1967).

Exposure to air causes progressive water loss from the tissue which is greatest in the beginning. This loss decreases when the relative humidity is brought towards 100 per cent. Already 65 per cent relative humidity at 21°C is sufficient to prevent changes in the water content for 10 minutes (GALANTE 1967). An alternative to a chamber with a relative humidity of 95–100 per cent, which can be used for small specimens like the anterior cruciate ligament in the rabbit's knee joint, is to wrap it in saline-moistened gauze. It has been demonstrated (VIIDIK 1966) that this technique prevents changes in the mechanical properties for more than four hours.

EFFECTS OF TRAINING ON TENSILE STRENGTH PARAMETERS OF COLLAGENOUS TISSUE (I–III)

INTRODUCTION

Tendons and joint ligaments, once their collagen is mature, are among the tissues with the lowest metabolic turn-over in the mammalian body (NEUBERGER & SLACK 1953, THOMPSON & BALLOU 1956). The incorporation rate of amino acids into connective tissue is comparatively slow and decreases with increasing age (NEUBERGER, PERRONE & SLACK 1951, NEUBERGER & SLACK 1953). In spite of this and considering that both tendons and joint ligaments are subjected to repetitive stresses throughout life, a healthy tendon almost never fails (MCMASTER 1933) and degeneration, with certain exceptions, is rare. Opinions had frequently been expressed both that exercise improved the tensile strength and that weight-bearing activities could be injurous.

In morphological studies on the effects of training, INGELMARK (1945) found that the cross-sectional areas of the primary bundles in the tendons of growing rabbits enlarged more than the number of cells increased. In a later study he found that training, while enlarging both tendons and muscles in young white mice, enlarged only the muscles in mature animals (INGELMARK 1948).

When the studies to be described here were performed (the training was performed in two groups, in 1963/64 and 1964/65, and mechanical testing in 1964 and 1965 respectively) no information was available on whether the mechanical properties of tendons and joint ligaments reacted to increased functional demands. In order to investigate some of these problems, the present studies (I–III) were undertaken.

PRESENT INVESTIGATIONS

The whole range of the collagenous tissue's possible mechanical performance cannot be utilized *in vivo* because some other part of the functional unit fails before the breaking load of the collagenous tissue is reached. However, from a theoretical point of view, it is of interest to investigate the load-deformation behavior of collagenous tissue as it is usable in a functional unit and as it is an isolated tissue *per se*. Therefore it was chosen to test both a complete functional unit (calcaneus, Achilles tendon, gastrocnemius muscle and femur) and isolated specimens of collagenous tendons, for which purpose certain hind limb tendons proved suitable. The anterior cruciate ligament from the knee joint was selected for additional mechanical experiments, in order to record mechanical changes in the soft supporting tissues of the joint in training.

The rabbit was chosen as a conveniently large animal that is relatively easy to exercise in a running-machine and as an animal that is kept comparatively easily in laboratory conditions for considerable time periods without large losses in intercurrent diseases. Its tendons are also of convenient size for this type of mechanical testing. So is its knee joint, the anterior cruciate ligament of which is easily protected from drying while being

tested for mechanical properties. As the tendons are rather long structures of small diameter, they are difficult to protect from changes in long-time experiments. It was therefore decided to confine the experiments on these to tensile strength testing that could be performed within a safe time period. Also the studies on the Achilles tendon complex were confined to tensile strength testing as autolytic changes in the muscles prevented long-time experimental schedules.

The training of the animals was performed in a running-machine for 40 weeks three times daily five days a week. The speed of the machine and the duration of each running session were adjusted to the maximum capacity of the animal (Cf. Fig. I: 1). This increased throughout the 40 weeks. During this time rabbits of the same stock were kept as controls in cages similar to those of the trained animals. The weight of the animals was recorded throughout the training period. When starting the period the animals of both groups were about three months old and weighed 2.4 ± 0.1 kg (mean $\pm$ S.E.). At the time of mechanical testing forty weeks later the trained animals weighed 3.4 ± 0.1 kg while the controls weighed 3.6 ± 0.1 kg. This difference in weight was not statistically significant (Cf. Fig. I: 2). Both groups were fed ad libitum. At the time of the mechanical testing the rabbits were skeletally mature in their hind limbs. The criterion of this has been the closure of the distal femoral and proximal tibial epiphyses (Cf. roentgenograms in Fig. I: 3–4). This is of importance, as uncalcified epiphyses influence the load-deformation curve (VIIDIK et al. 1965).

Achilles tendon complexes (I)

When testing the functional unit of calcaneus, Achilles tendon, gastrocnemius mus-

cle and femur previous investigators have confined themselves to record the failure load. Quite often the loading has then resulted in a rupture of a muscle belly or fracture of a bone through a drilled hole. Therefore, adequate techniques had to be developed to enable recording load versus deformation for the tendon and to prevent fracture through any artificially pre-weakened bony component. Preliminary studies (VIIDIK 1966) revealed that the muscle tissue was very sensitive to any time lapse between sacrifice and testing, a feature augmented by a history of violent muscle contractions. It was found that ruptures in the muscle belly could be avoided at low loads if the mode of sacrificing the rabbit was asphyxia induced by a muscle paralyzing drug (tubocurarine) while in general narcosis (urethane and barbiturate).

Contour-shaped clamps were manufactured for the femur and calcaneus and care was then taken to avoid sharp edges and surfaces so that concentration of forces on small areas of bone were minimized. A thin layer of polyethylene tubing was placed into the calcaneal clamp to improve the fit and to make the distribution of clamping forces more uniform.

The calcaneal clamp was fastened to the immobile end of a material testing machine, where the load was recorded by a tensile force transducer. The femoral clamp was fastened to the piston. As only the deformation of the Achilles tendon, i.e. the distance between the calcaneus and gastrocnemius muscle was of interest, a ring-shaped marker was fastened at the musculo-tendinous junction without slipping or producing failure initiation at that point. The motion of this marker in relation to the immobile end of the machine was measured as the bending of a steel blade, the U-shaped end of which

was placed against the marker and was bent by this (Cf. Fig. I: 5). The electronic part consisted of two strain gauges cemented on the steel blade and coupled in a half bridge. Both this "transducer" and the force transducer were coupled to measuring bridges and recorded on an ink jet recorder as load and deformation respectively versus time, but also photographed from an oscilloscope screen as load versus deformation. The specimen was brought to failure in a rapid loading.

Of the total 70 specimens tested in this series 62 failed as tear-off fractures from the calcaneus, one as a tear-off fracture from the femur and the remaining 7 with more complicated patterns of failure, all of them including transitions between different tissues. This confirms that the healthy tendon is not the weakest point in the functional unit. The results also show that the muscle tissue, provided it is "fresh" and not exhausted, can resist considerable forces. The weakest component is the bony insertion. It can be noted here that the tensile strength of compact bone is less than its compressive strength (PEDERSEN, EVANS & LISSNER 1949) and probably less than that of ligaments (BARNETT, DAVIES & MACCONAIL 1961). Furthermore, the Achilles tendon of the rabbit inserts into a rather small part of the dorso-plantar section of the calcaneus, where the layer of cortical bone is thin (Cf. Fig. I: 8).

No difference in failure site or mode was found between trained and controls. In both groups the load-deformation curve started with a "toe" part after which a fairly linear portion ensued. The failure occurred instantaneously or in a few steps rapidly following each other after the curve had tended to level off towards the deformation axis, no differences being found between the two groups.

Training did not increase the tensile strength of the tendon's insertion into the calcaneus to any measurable degree. The elongation at failure, the failure energy and the elastic stiffness of the linear portion of the curve did not increase significantly either. Nor did the linear range of load-deformation curve increase. However, as the mean values seemed to be higher for the parameters measured for the trained group and as there was considerable intra-group variation, the various parameters were normalized to a standard variable and then summated. The distributions in the two groups were compared by χ^2-test and a significant difference was found. This, however, was mainly due to a considerable scatter of the values in the trained group.

Isolated hind limb tendons (II)

In these experiments certain hind limb tendons of sufficient length and appropriate diameter were studied with a technique described by VIIDIK (1967) and discussed above. The tendons were fastened by two clamps into a material testing machine and loaded rapidly until failure. The time during which the tendon was exposed to air was less than 2 minutes. The deformation was measured by two strain gauges cemented on a steel blade spring that was bent by the movement of the piston. The rest of the recording was performed as described in the previous section.

A total of 212 tendons were tested. The load-deformation curves displayed a "toe" part with an insidious start, a linear portion and, before failure that occurred instantaneously, a tendency to level off towards the deformation axis. No differences were found between the trained and control animals in this respect.

When crude load and deformation readings were used, considerable scatter was exhibited

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in the various parameter values. The load values were reduced to units of fresh weight per unit length as it had been demonstrated that there is a good correlation between this value and the failure load value for a tendon (VIIDIK 1967). The figures thus calculated (in newton-millimeter per milligram) are directly proportional to stress as the fresh weight per unit length equals cross-sectional area times a factor (these figures are referred to as "stress"). This factor is constant provided the specific gravity of the tissue is unaltered. There are no reasons, as will be discussed below, to question that this is the case. The deformation values were reduced to units of original length, i.e. to strain. For all parameters these corrections reduced the variation coefficient considerably (Cf. Table II: I a versus II: I b).

To enable both a summation of a parameter for all tendon types and to summate the various parameters eliminating all sources of variation but the possible effects of training the parameter values were by classification normalized to a standard variable with the mean value of zero and the standard deviation of unity. This standard variable was based on the various mean values and standard deviations for the tendon groups from the control animals. Comparisons between the distributions for the trained and control animals were then performed by χ^2-tests.

Analysis of the material revealed no differences that could be attributed to failure site, i.e. in a clamp jaw or in the free space between the two clamps. The "elastic stiffness" for the linear part of the "stress"-strain curve in the overall material was not affected by training. An analysis of the various tendon types, however, revealed a profound difference between them. The elastic stiffness decreased significantly with training if the tendons of the peroneus longus, tertius and quartus muscles were pooled into one group ($2P<0.05$, $n=159$). The tendons of the tibialis posterior muscles, on the other hand, exhibited a tendency to stiffen when trained ($2P<0.10$, $n=52$). It can be noted that the tibialis posterior tendons had a fresh weight per unit length that was about double that for the other type of tendons. This difference was taken into account as the calculations were performed on the reduced values. No such differences between tendon types could be found for the other parameters analysed.

The maximum load and the length of the linear part of the "stress"-strain curve increased significantly with training while the failure energy and strain at maximum load remained unaffected.

Tendons of the peroneus brevis muscles from these rabbits were analyzed with respect to water and collagen content. For the determination of collagen content, analysis of hydroxyproline was performed according to the modification of STEGEMANN's (1958) method as described by BERGMAN & LOXLEY (1963), assuming the amount of collagen to be 7.46 times the amount of hydroxyproline recovered from a hydrolyzed specimen (NEUMAN & LOGAN 1950). The water content was determined as the difference between the weights of the specimen when fresh and when dried in vacuum. Training was not found to have any influence on these parameters.

Anterior cruciate ligament complexes (III)

The functional unit of the anterior cruciate ligament with its femoral and tibial insertions has been used by several previous investigators. The fixation into the testing machine has been effected with pins in holes drilled

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through the condyles, in some instances resulting in "artificial" fractures. Loading has quite often been performed by the "bucket method" and only the failure load, sometimes called separation force, recorded. In order to achieve the maximum amount of information the material testing machine was used as in the previous section (II). The proximal tibial and distal femoral ends were used as convenient sites for application of clamps. These were contour-shaped and hinged in two axes at right angles to each other. Motion was thus permitted in all directions except around the longitudinal axis of the leg. It is important that motion in this plane is prevented as the ligament in situ is twisted 90° and an unwinding of it would result in a false deformation recording.

The *tensile strength* testing was performed so that the specimen was brought to failure rapidly. This was recorded as described in the previous section. Load versus deformation was also recorded. No attempts at correlating these to cross-sectional area or weight per unit length were made nor any measurements of original length as this ligament is short and of uneven thickness throughout its course. Any attempt at correlation would, because of the large measuring errors (compared with the small dimension measured), have failed to improve the intra-group homogeneity. The load versus deformation curves started with a "toe" part, after which a fairly linear portion ensued. Then, after a certain amount of levelling off towards the deformation axis, failure occurred in one step or a few rapidly successive steps. No differences could be detected between the curves from the experiments on the trained and the control animals.

Of the total 44 specimens tested in this way 40 failed as tear-off fractures (36 from the tibia and 4 from the femur) and 4 in the ligament itself. No difference in distribution was found between the trained and control groups.

The elastic stiffness showed no significant change with training. On the other hand, the failure energy increased significantly ($2P<0.05$) with training and the maximum load and elongation at maximum load also tended ($2P<0.10$) to increase with training (Cf. Table III: I).

Tests on the degree of *elasticity* of a tissue can be performed in various ways. The results of earlier investigators have, at times, been conflicting. This may be due to lack of precision in the recordings or the difficulties in comparing studies with quite different experimental procedures. For the present experiments a backlash-free screw arrangement was used together with electronic measuring equipment for load and deformation measurements (Cf. VIIDIK 1966, and III). The loading was performed to 98 N. This value was chosen as it is a considerable load, yet safely below the failure load, which is about three times this value. The range selected contains a part of the linear portion of the load-deformation curve and partial failures of ligamentous tissue ("dips" in the curve) are seldom recorded here. The specimen, clamped as for tensile strength testing, was deformed (i.e. elongated) until the load of 98 N was reached. The deformation was then halted and the load-relaxation phenomenon was recorded for 120 seconds. Then the deformation was continued until the 98 N load was reached again, whereafter the load-relaxation was recorded for 60 seconds. Finally, after bringing the load to 98 N once more and recording the thereafter following load-relaxation for another 60 seconds, the

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specimen was brought back to zero deformation. These times were sufficient for the load-time curve to approach the asymptote. With resting intervals of 10 minutes each, a time interval sufficient for completion of the elastic after-effect, this whole procedure was performed twice more. (Cf. the schematic Figures III: 3–4).

The load-deformation curves for loading and unloading exhibited the characteristic "toe" parts and after that the fairly linear part. The unloading curve was displaced a distinct distance to the right of the one for loading, it was steeper and reached zero load when there was still some deformation left (Fig. 4). As the start of the load-deformation curve is very insidious, it is difficult to establish a zero deformation point. To avoid this source of error the point of deformation at 1 N load for the first loading of a specimen was selected as zero deformation point. After halting the deformation when the load of 98 N ($=F_0$) was reached, a load-relaxation occurred. This tended with time asymptotically towards a load value (F_A), the load decreasing most rapidly in the beginning and then more and more slowly (Fig. 5). It was found that a mathematical model described this phenomenon adequately:

$$F(t) = F_0 \exp(-\beta t) + F_A \{1 - \exp(-\beta t)\}$$

where β is the shape parameter, which controls the speed with which the load tends towards the asymptote with time. The adaptation of the equation to the experimental data was performed by a computer, F_A and β being evaluated and the correlation was found very good.

From the load-deformation curves of loading and unloading the inclinations of the linear parts were evaluated, i.e. the "elastic stiffness". Also the deformation required to reach 98 N load (B_1) for the various cycles and the deformation at the end of a testing cycle (B_2) were calculated.

It was found that successively more deformation was required to reach the 98 N load in consecutive cycles, i.e. B_1 increased. This
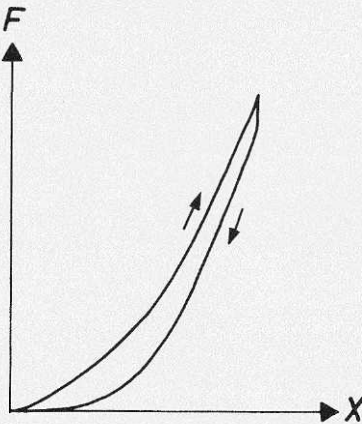

Fig. 4: A schematic load (F) versus deformation (X) diagram for a loading-unloading cycle on a ligament specimen.
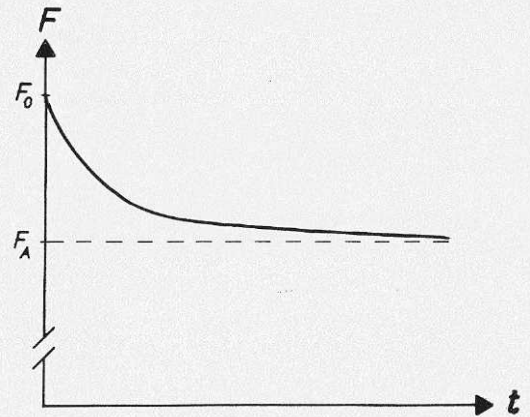

Fig. 5: A schematic diagram for load (F) relaxation of a ligament specimen from an initial value (F_0) asymptotically towards the value of F_A when time (t) tends to infinity at constant deformation.

increase was greatest from the first to the second cycle. Also B_2 increased, but to a smaller extent. These parameters, B_1 and B_2, and the difference between them, $(B_2 - B_1)$, were not influenced by training. The elastic stiffness for loading increased with consecutive cycles, mostly from the first to the second. The elastic stiffness for unloading was almost unaltered through the cycles. No changes with training were observed in these parameters either.

The asymptote for the load-relaxations, F_A, increased with consecutive cycles, i.e. the relaxation was less pronounced. F_A was found to be significantly lower, i.e. the relaxation was augmented, with training. No major differences nor changes in the shape parameter were found.

CONCLUDING REMARKS

From these experiments (I–III) it is concluded that in a functional unit including collagenous tissue, here bone-tendon-muscle-tendon-bone and bone-ligament-bone, some other tissue is the weakest point of the chain. In the cases investigated here it has been the insertions of the tendon with the smaller insertion area (i.e. the Achilles tendon into the calcaneus) and the ligament for both the trained and control animals. While the insertion of the Achilles tendon into the calcaneus is not strengthened by training, the insertion of the anterior cruciate ligament into the tibia is. It might be pointed out here that the cortical bone is much thicker in the proximal end of the tibia than in the calcaneus. These results are confirmed by those of TIPTON, SCHILD & TOMANEK (1967), who found that training increased the separation force of collateral knee ligaments in rats. The amount of dry weight or collagen per unit length of tendon is not increased in training when performed in the way reported here. This is probably due to the fact that the training was performed in the middle and later part of the growth period of the rabbits and into their skeletal maturity (i.e. from about 3 to 13 months of age) and to achieve quantitative differences the training must start at an earlier age (Cf. INGELMARK 1945, 1948). However, the tendons exhibited significantly increased tensile strength by training and the length of the linear part for their "stress"-strain curves increased too. The effect on their "elastic stiffness" is less clear; for certain tendon types it decreased, for one type it increased. This may be due to differences in function. These changes must be attributed to qualitative differences in the tissue. This is supported by the fact that the elastic properties of the anterior cruciate ligament also changed with training; its load-relaxation phenomenon increased significantly.

When determining the degree of elasticity both true elasticity, viscosity, plasticity and the parameters derived from the shape of the load-deformation curve for the tissue were studied. From these experiments it cannot be decided which components have been changed as they interact with each other. It seems probable, however, that the main effect of training has affected a combination of elastic and viscous properties. To enable a detailed analysis of such problems a comprehensive knowledge of the interaction of these properties would be required. Such studies will be presented below (IV–VI).

INTRODUCTION

Besides the previously described investigations on tensile strength and some "elasticity" parameters, the most obvious way to describe the mechanical properties of collagen is to express the stress-strain relationship by a mathematical equation and determine its constants. This has been done by KING (1957), MORGAN (1960), RIDGE & WRIGHT (1964) and KENEDI, GIBSON & DALY (1965) among others. No meaningful interpretations of the constants thus evaluated have been offered. It was shown by RIDGE & WRIGHT (1966) that one constant was dependent on the dimensions of the specimen and that another one was changed by aging. Such equations can, however, only satisfy the experimental conditions from which they are empirically derived and with changed testing patterns other equations are required to satisfy the behavior. Therefore, a comprehensive description of the mechanical behavior of collagen with these methods would necessitate very complicated sets of mathematical expressions that would be practically impossible to handle and to interpret.

In classical mechanics idealized elements of elasticity, viscosity and plasticity have been used, generally for illustrative purposes, and symbolized with the Hooke element (perfectly elastic spring), the Newton element (a dash-pot or viscous damper with a linearly viscous fluid) and the Coulomb element (dry friction body) respectively. If these single elements are combined to multi-element mechanical analogies, more complex mechanical properties can be described, and still the mathematical expressions describing these properties will be possible to handle. If the elements are combined in parallel with each other, they are equally deformed and the total load is the sum of the loads on the individual elements. A spring and a dash-pot in parallel constitute the Kelvin element. With a combination of elements in series with each other, all are subjected to the same load, which also is the same as the total load. The total deformation, in this case, is the sum of deformation of the individual elements. The Maxwell element is built up of a spring and a dash-pot in series. For each of the elements simple equations satisfy the mechanical behavior. This is discussed at length in IV.

This analogy approach has been used qualitatively by ALEXANDER (1962), who described the body wall of the sea anemone with a spring and dash-pot in parallel, i.e. the Kelvin element and this in series with a spring. Earlier the mechanical properties of synthetic high polymers have been described by various combinations of such elements (ALFREY & GURNEE 1957). Such model analysis has also been applied to more complex tissues than pure collagen, as arterial wall (APTER 1964 and 1966) and bone (SEDLIN 1965). No evaluation of data (e.g. element constants) has been demonstrated so far. To be of any use, a mechanical analogy must enable both prediction of experiment types, clarification of properties of special interest, and quantification of properties. It is also desirable to

be able to correlate mechanical properties to various morphological components of the tissue investigated.

PRESENT INVESTIGATIONS

Qualitative analogy (IV)

The stress-strain or load-deformation relationship for collagenous tissue starts with a "toe" part that is convex towards the strain/deformation axis. This cannot be simulated by any combination of the idealized elements without deviating from their linear properties. Therefore a non-linear spring arrangement, where linearly elastic springs successively come into action with increasing deformation, i.e. the elastic stiffness of the analogy increases with deformation, was proposed by VIIDIK & MÄGI (1967). With this additional "element" behavior similar to that of ligamentous tissue could be composed into an analogy.

The anterior cruciate ligament with its bony attachments from the rabbit's knee joint was chosen as experimental specimen. This structure has been the subject of several investigations and its tensile strength behavior is well-known (publ. III). Its bony attachments offer points for clamping without any possibility of slipping and the load-deformation curve is typical of that for collagenous tissue. In these experiments, animals which had roentgenologically closed distal femoral and proximal tibial epiphyses were used to avoid any influence from epiphyseal cartilages (Cf. VIIDIK et al. 1965). To enable a perfectly constant predetermined rate of deformation an Alwetron electronic universal testing machine (described in IV & VI) was used. Besides the precautions to avoid drying as discussed above the specimen in the clamps was confined in a high humidity chamber (about 95 per cent relative humidity).

The load was recorded by a tensile force transducer and the deformation by a differential transformer, each coupled to a measuring bridge; the signals from these were recorded on an x-y-recorder and photographed from the screen of an x-y-coupled oscilloscope. The possible error in the measurements was found to be less than one per cent and the long-time recording stability of the x-y-recorder was found to have no measurable drift.

The loading to $98\ N$ in the loading-unloading cycles was selected and resting intervals of 10 minutes were used according to the discussion above (p. 18). For the first consecutive loading-unloading cycles of a specimen the "toe" part of the load-deformation curve became more pronounced and the curve shifted to the right in diagram. If the specimen was deformed at a constant speed until the load reached a value selected (designated F_0 and here $98\ N$) and the deformation then kept constant, the load relaxed asymptotically towards a certain load value (F_A). The $F_0 - F_A$ difference was greatest in the first experiment on a specimen and diminished to some extent in the following ones. After a certain number of experimental cycles the thereafter following ones were identical with each other. The same was the case with the load-deformation diagrams of the loading-unloading experiments. From these observations it was concluded that the behavior of collagenous tissue consisted of an initial phase and when that was exhausted a stationary phase. The analogy for the stationary phase was considered first.

From these and previous (publ. III) experiments it was evident that the mechanical analogy must contain elastic and viscous ele-

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ments and, to avoid any non-linear elements, the spring arrangement according to VIIDIK & MÄGI (1967). The dash-pot must be coupled in parallel with at least one spring, as the dash-pot is recoverable; although with a certain time lapse. The site of the non-linear spring arrangement could be in parallel or in series with the dash-pot. If it were placed in parallel with it, the "toe" part would disappear in experiments with very high deformation speeds. However, this was not the case. Therefore it was placed in series with the dash-pot, in parallel with which a linear spring was placed. When the deformation in a loading-unloading cycle was reversed, an instantaneous drop in the load occurred without any corresponding decrease of the deformation. This indicated the presence of a dry friction element. The analogy for the stationary phase of the collagenous tissue's behavior (Fig. 6) will then be: a Kelvin element (the spring c_K and the dash-pot k_K) in series with an array of elastic springs $(c_0, c_1 .. c_n)$ coming into action after certain amounts of deformation $(0, \Delta_1 \ldots \Delta_n)$ resulting in a non-linear spring action (ϕ) and in series with both these sets a dry friction element (s).

To account for the properties of the collagenous tissue during the first experimental cycles an addition of irrecoverable viscous and plastic elements was required. To account for the shift to the right of the load-deformation curve, the analogy, as presented above, was coupled in series with a set of dry friction elements coupled to each other by strings that initially were loose. The load-deformation coordinates for this set were designated (F_C, X_C). In this arrangement the elements come into action gradually, starting with the one coupled directly to the stationary phase analogy. The others come into action
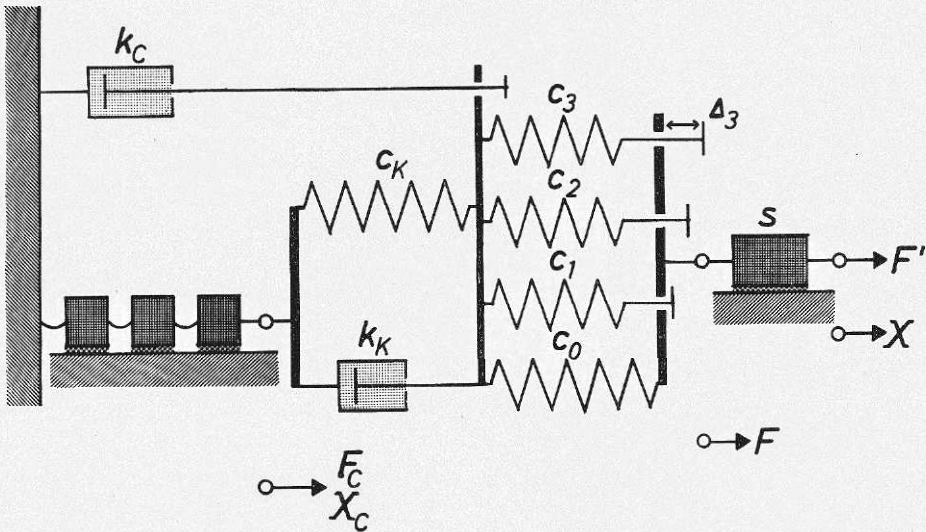

Fig. 6: The mechanical analogy for collagenous tissue. k indicates a dash-pot, c a spring and s indicates a dry friction element. Sub-index c indicates the part of the analogy belonging to the initial phase of material behavior, while the remaining elements belong to the stationary phase.

gradually with increasing yield force. The action is irreversible and, as there should be no sharp bends, this set of elements was coupled in parallel with the irreversible dash-pot (k_c). To enlarge the influence of this dash-pot, it was coupled in series with the Kelvin element of the stationary phase analogy too.

The stationary phase of this analogy remains stationary, i.e. the results of loading-unloading or load-relaxation are unaltered in consecutive experiments, provided the maximum load during the initial phase is not exceeded. Otherwise, a new initial phase for a higher load level occurs. The same is true for the collagenous tissue.

Mathematical analysis of the analogy (V)

The purpose of the analogy described above was to design appropriate experimental schemes to evaluate tissue properties of special interest and to quantify them. To be able to perform this, a knowledge of the analogy behavior is necessary. This is achieved by deriving the constitutive equations for it and by investigating the characteristic properties of these equations under certain conditions. This derivation and analysis is also necessary for the verification of the analogy, which so far appears to satisfy qualitatively the experiments performed. The verification must, of course, be performed before using the analogy.

The stationary phase analogy was considered first. Let the deformation for the whole model be X, for the Kelvin element x and for the spring array z. The load for the analogy (without the dry friction element) is designated F. Time derivatives are marked with a dot, (e.g. $dX/dt = \dot{X}$). Spring constants

are designated c and dash-pot constants k, with appropriate indices. The equation for the Kelvin element is well-known and reads with lettering as described above

$$F = c_K x + k_K \dot{x}$$

The corresponding equation for the spring array is more complicated. It can be written

$$F = z\phi(z) - \psi(z)$$

where ϕ is the spring constant of the array and increases with deformation in steps and ψ is another step function. These two equations combine to form the constitutive equation for the hypothetic model material after elimination of x and z as $X = (z + x)$:

$$\dot{F} + F(\phi + c_K)/k_K$$
$$= \dot{X}\phi + Xc_K\phi/k_K - c_K\phi/k_K$$

The solution of this equation is discussed in publ. V. One problem here of main interest is the time-dependent step function causing the "constant" of integration to assume different values in the different intervals. The alternative with a continuous non-linear spring with the instantaneous stiffness at deformation X equal to $\varphi(X)$ is also discussed in publ. V.

Of special interest for the design of experimental schemes is the behavior of the analogy at very low ($\dot{X} \simeq 0$) and very high ($\dot{X} \simeq \infty$) deformation speeds. For very low speeds the constitutive equation gives ($\dot{X} \simeq 0$, $\dot{F} \simeq 0$)

$$F = (\phi X - \psi)c_K/(\phi + c_K)$$

i.e. the dash-pot is without influence and the model behaves like the two springs, ϕ and c_K, coupled in series with each other. On the other hand at very high speeds the Kelvin element in the analogy is rigid and all deformation occurs in the spring array, ϕ. Thus

$$F = X\phi(X) - \psi(X)$$

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which is the same as the constitutive equation for the non-linear spring array but with z substituted by X.

These evaluations and the analogy behavior at definite constant speeds are discussed at length in publ. V.

The initial phase of the analogy lends itself less easily to fruitful mathematical analysis because the series of dry-frictional elements and the irreversible dash-pot interact with each other. During loading the load is divided between the two of them. During load-relaxation, however, the total decreasing load on the analogy is re-distributed from the dash-pots (k_C and k_K) to the spring (c_K) and the series of dry-frictional elements, which results in a continuous increase of the force, F_C, up to the value F_A. This means that a certain amount of sliding occurs in the series of dry-frictional elements during load-relaxation in the initial phase of testing, which prevents the explicit evaluation of k_C without exact knowledge of the relation $F_C = f(X_C)$. It can be noted that, as both the series of dry-frictional elements and the dash-pot k_C are irreversible, unloading parts of the deformation cycles are never influenced by the initial phenomena in other respects than a shift to the right in the load-deformation diagram.

Model operation and determination of element constants (VI)

In the previous section the behavior of the analogy in certain conditions was analysed and on the basis of this, some operations with the model can be performed and certain element constants evaluated.

(i) Deformation at a very high speed yields the behavior of the spring array directly, and for the linear part of the curve $\phi = $ constant $=\tan \alpha_1$. (ii) deformation at a very low speed yields for the linear part of the curve $\phi c_K/(\phi + c_K) = $ constant $=\tan \alpha_2$. (iii) load-relaxation at constant deformation yields $-(\phi + c_K)/k_K = \tan \beta$, where $\tan \beta$ is the slope of $\ln (F - F_A) = f(t)$. [Cf. VI: p. 2, where it is shown that $\ln (F - F_A) = f(t)$ is a straight line]. From these three schemes the explicit values of ϕ in the linear range, c_K and k_K can be evaluated.

The value of ϕ can also be calculated at the point where reversed deformation is started (and the instantaneous load drop finished) as the deformation curve is not yet influenced by the Kelvin element, thus $\tan \alpha_3 = \phi$. It can also be shown that the spring in the Kelvin element, c_K, can be evaluated from $F = f(X_{\dot{X}=0} - X_{\dot{X}=\infty})$ (Cf. Fig. V: 6), which means that the difference between deformation for a certain load value in the load-deformation curves for very low and very high speeds is the deformation of c_K for that load value. Information on possible speed dependence of k_K can be obtained from load-deformation curves at definite constant deformation speeds as the curve levels off towards an asymptote when $X \rightarrow \infty$.

Some evaluation of the initial phase phenomena is also possible. The sliding of the dry frictional elements can be demonstrated by consecutive first and second loading of the analogy for the initial behavior to a certain load value with very low speed. (Cf. Fig. VI: 8). No explicit evaluation of k_C is possible without previous knowledge of $F_C = f(X_C)$; it is not possible to perform both investigations on the same specimen as it would require two first loadings.

Some alternative models are also discussed in publ. VI. In these the linear properties of some elements are changed or series of elements replace single elements. None of these alternatives could describe the behavior of

the collagenous tissue qualitatively as satis-factorily as the analogy presented in publ. IV.

For the experimental verification specimens consisting of femur, anterior cruciate liga-ment and tibia were used. The tests were performed in the Alwetron material testing machine. The evaluations required, theore-tically, deformation speeds $\dot{X}=0$ and $\dot{X}=\infty$, both of which are impossible to achieve. The maximum speed of the machine, 500 mm/min. was used, which means that the specimen failed after about a quarter of a second and the range measured on the oscilloscope photograph was passed within 0.15 seconds. Instead of $\dot{X}=0$ the minimum speed of 0.5 mm/min. was used with stops for 120 seconds at every 10 N load, during which time a certain load-relaxation was completed (the dash-pot unloaded). This procedure cor-responds to a deformation speed of 0.06 mm/min. The $F=f(X)$ curve for $\dot{X}\simeq0$ was then constructed from the bottom points of the serrated curve.

ϕ for the linear part of the specimen be-havior was calculated in the two ways de-scribed. No significant difference was found between these two types of evaluation and there was a good correlation between the two evaluation methods. c_K was evaluated from $F=f(X_{\dot{X}=0}-X_{\dot{X}=\infty})$ and was found to be constant, i.e. the spring in the Kelvin element is linear. It was also evaluated from the set of equations utilizing measurements of tan α_1 and tan α_2. Between these two modes of evaluation a significant difference was found in that the latter method yielded lower values. The correlation between the two evaluation methods, however, was good.

The dash-pot in the Kelvin element, k_K, was investigated by analysis of the load-relaxation phenomenon in the stationary phase and within the linear range of ϕ. Thus ln $(F-F_A)=f(t)$ was found linear and the correlation of experimental data to the equa-tion very good. The influence of deformation speed when loading up to F_0 was also in-vestigated and, although there seemed to be a slight change in tan β, but it was not significant. Also $\{F(X_{\dot{X}=V})-F(X_{\dot{X}=0})\}=f(X)$ was calculated. According to the analogy (Cf. Fig. V: 6) this function should reach a constant value if, after the last step of ϕ has come into action, $X\to\infty$. This was never reached probably due to the fact that speci-men fibers started to fail before a sufficient part of the linear section was passed. This was seen as "dips" in the load increase, when loads of about 150 N were reached.

Experiments of the sliding of dry-frictional element series is given in Fig. VI: 11. Numer-ical values of model constants are also given in publ. VI.

CONCLUDING REMARKS

An analogy for the mechanical behavior of collagenous tissue has been presented. It satisfies qualitatively the experimental be-havior of joint ligaments as investigated here (IV–VI) and previously (III). It can be noted that the equation for load-relaxation found empirically in that study (III: p. 5) is, if rewritten with other symbols, identical with the one found theoretically for load-relaxa-tion in the stationary phase of the analogy (VI: p. 2). Thus the shape parameter of III (β) is identical with tan $\beta=-(c_K+\phi)/k_K$ in VI. It was observed in the analysis of the anal-ogy that unloading of the analogy was not influenced by the initial phenomena. This means that the unloading parameters for the three cycles in the "elasticity" studies in III should be unaltered with the exception of a

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certain increase of the deformation values. This is also the case (Cf. Table III: II).

The analogy presented is at least qualitatively compatible also with the results of other investigators on the elasticity, viscosity and plasticity of collagenous tissue (e.g. GRATZ 1931, ROLLHÄUSER 1950a, STUCKE 1950, LABAN 1962 and VAN BROCKLIN & ELLIS 1965).

In the experimental control of the model no decisive differences between the behavior of the biological tissue and the mechanical analogy have been found. The significant difference between the two methods of evaluating c_K can be explained by the calculation methods used. When calculating from $F = f(X_{\dot{X}=0} - X_{\dot{X}=\infty})$ the c_K is of first order influence and all the distance between $F = 10\ N$ and $F = 100\ N$ has been used. When calculating from the readings of tan α_1 and tan α_2 at $F = 100\ N$, ϕ is of first order influence and a small error in the reading produces a small error in the ϕ value but a considerable error in the c_K value as this is of second order influence. This is reflected in the larger scatter around the mean value of the c_K measured in the latter way (Cf. the variation coefficients). Furthermore, the latter estimation method depends on the assumption that tan α_1 is measured in the asymptotic region of $F = f(X)$ at $\dot{X} = \infty$ but at the measuring point of $100\ N$ load some curvature in the curve might prevail as the theoretical $\dot{X} = \infty$ can never be obtained experimentally. These findings do not contradict the validity of the analogy and the quantifications of it.

Thus an analogy is arrived at and experimental schemes presented that can be used for evaluating the rheology of collagenous tissue.

INTRODUCTION

In the publications previously discussed (IV–VI) an analogy for the mechanical behavior of collagenous tissue was proposed and analysed. This analogy consisted of a Kelvin element and an array of linear springs resulting in a non-linear spring action, thus giving the "toe" part of the stress-strain curve. In this array the springs come into action gradually with deformation. When relaxed the collagen fibers in a tendon lie in a wavy formation, which becomes straightened out, when the tendon is strained and this phenomenon could correspond to the "toe" part of the stress-strain curve (RIGBY et al. 1959, ABRAHAMS 1967). The building units of the fibers are the tropocollagen molecule chains, which are polymers. The mechanical behavior of such molecular arrangements is usually described by series of Kelvin elements (Cf. ALFREY & GURNEE 1957). The "toe" part of the stress-strain curve cannot be explained by such a mechanical analogy (Cf. VI) and therefore it should be of interest to try to correlate the ultrastructure of the specimens with the linear part of the stress-strain curve. This is particularly so, as it has been demonstrated by X-ray diffraction studies that the periodicity of collagen can be enlarged from 2.86 to about 3.1 Å by straining of the fibril about 10 per cent (COWAN et al. 1955) thus indicating that the molecular chains lend themselves to straining.

A comparative study of the collagenous tissue with incidental and transmitted polarized light microscopic techniques together with electron microscopy was therefore undertaken to try to correlate the mechanical behavior with the morphology. Hind limbs tendons from skeletally mature rabbits were used as test specimens.

PRESENT INVESTIGATIONS (VII)

Incidental light microscopy

For this investigation longitudinal sections through the Achilles tendons were made in a freezing microtome (ad modum GALANTE 1967) and load-strain curves were produced according to the stationary phase behavior of IV. The specimens were then strained in an extensometer operated by a micrometer screw and mounted on the incidental light microscope while immersed in saline. A polarizing system was used to extinguish reflexes in the surface and to shadow it. Photographs were taken with certain intervals in flash illumination. The photographs were correlated to points on the load-strain curve.

It was found that the toe part started very insidiously and already, when the first recordable load was produced by gradual straining some of the waves seen in the fully relaxed preparation (Fig. VII: 3a) had disappeared. The waves seen in a fiber bundle vanished practically simultaneously in the length of the bundle seen. When the linear part of the load-strain curve was reached, all waves had vanished and no further changes could be detected with additional straining (Fig. VII: 3c).

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Polarized light microscopy

For these studies rabbit hind limb tendons were used. The tendons were strained to various degrees and while in tension each clamped between the two halves of a ring thus preventing the tendons regaining their original length during histological processing. Some specimens were processed without rings and others were fixed in crotonaldehyde prior to straining and ring clamping.

All specimens exhibited birefringence. The unclamped specimens and those fixed prior to clamping exhibited a periodic change in the direction of the birefringence thus giving the impression of a cross-striation. In the strained specimens the birefringence was uniform in direction and independent of the degree of strain. It was noted that no "cross-striation" was present in the unstrained specimens processed in rings (Fig. VII: 4).

Electron microscopy

The specimens were similar to those in the previous part and also processed in clamping rings. The possible error in the measurement of the period length of the cross-striation was studied thoroughly when developing the methods. A measuring error of less than one per cent was found and the error due to sectioning at some angle to the longitudinal axis of the fibril was found to be even less.

It was found that the fibril cross-striation period length was 681 ± 2 Å (mean $\pm$ S.E.) for the preparations fastened unstrained in rings and processed thereafter. The measurement of the specimens processed without rings and unstrained exhibited no significant difference but a larger variation. Straining 2, 4, 6, 8 and 10 per cent caused a gradual increase in the period length of some fibrils. Some other fibrils exhibited very short period lengths. When divided into groups by classification of period lengths (Cf. VII: Table) the distribution in the 2, 4 and 6 per cent groups was found to be significantly different from the one in the unstrained group. The period length of individual periods in fibrils exhibiting "normal" and increased period lengths were measured and analysis revealed that the increase was homogeneous in the periods in one fibril. No nodose period parts could be observed even in the most strained fibrils.

CONCLUDING REMARKS

In the study utilizing incidental and transmitted light microscopy the previous observations that relaxed tendons have a wavy course (RIGBY et al. 1959, ABRAHAMS 1967) has been confirmed and also that by straining this wavy formation vanishes. It was demonstrated here that the "toe" part of the load-strain curve actually is longer than that deemed from mechanical experiments only, as the waviness of some fiber bundles vanishes before any measurable load has been applied to the specimen. All waves disappear before the linear portion of the curve begins. This morphological behavior corresponds well with the spring array in the mechanical analogy. In the transmitted light polarized microscopy no difference was seen in the uniform birefringence in the strained specimens. It might have been possible to demonstrate some difference between various strain degrees, if the Ambronn technique had been utilized. This was deemed not worthwhile, as it had been demonstrated that the various fiber bundles in a specimen come under tension at various intervals (VII). Consequently, the Ambronn technique would have measured a mixture of different degrees of strain.

The electron microscopic study demonstrated that in the linear part of the load-strain curve, and even to some extent before, the collagen fibrils are elongated. The elongations found lie well within the values found for strained fibers in X-ray diffraction analysis (COWAN et al. 1955). No indication of nodosity as described by SCHMITT, HALL & JAKUS (1942) when straining isolated fibrils could be found. As the straining was performed to quite near the failure strain, it may be concluded that some part of the fiber fails before the extensibility of the fibrils is fully utilized. The small period length values measured at times in the higher degree strain groups are probably due to rupture of fibrils followed by recoiling in its components. The period length measured in the unstrained group corresponds well with the macro-periodicity calculated for moist collagen in X-ray diffraction studies (BEAR 1944).

It could not in this study definitely be established that the Kelvin element corresponds to the collagen molecule itself. It seems, however, probable and methods were suggested for its further elucidation (VII).

GENERAL CONCLUDING REMARKS AND SUMMARY

Collagen is a fibrous protein that possesses great strength in combination with little extensibility. It plays an important role in transmitting and resisting forces in most organs, where it constitutes a soft skeleton. In tendons and joint ligaments collagen is found in parallel-fibred bundles. Therefore such tissues are suitable for the investigations of the tensile behavior of these fibers. This presentation gives the background to and summarizes the present studies (I–VII) on some mechanical properties of collagenous tissue and their relation to morphology.

The structure of collagenous tissue down to the molecular level, as found with light and electron microscopic and X-ray diffraction techniques, is briefly reviewed. Previous investigations on the tensile strength and the degree of elasticity are discussed. Particular emphasis is laid on the experimental techniques used and possible sources of artifacts in tissue behavior. It is known that collagenous tissue, as in tendons and joint ligaments, is to a limited degree extensible, has a breaking strength of about 50–100 N/mm^2 and shows a sigmoid stress (load per unit cross-sectional area)—strain (deformation per unit original length) relationship with a "toe" part in the beginning (i.e. initially relatively large deformation with small loads). There is also a viscous component and this shows itself as load-relaxation (decreasing load when the deformation has been performed at any strain rate $[v \neq 0]$ to a stress level and halted) or creep (increasing deformation when a constant load is applied). There is also an elastic after-effect, that is completed within 5 minutes, but some permanent deformation (plasticity) occurs also even after application of moderate loads. The tissue is sensitive to loss of water, which results in a stiffening, but may be kept "fresh" for more than 24 hours.

In the present studies the interest was first focused on the reaction of tendons and joint ligaments to increased functional demands. As tendons and ligaments are parts of functional units (bone-tendon-muscle-tendon-bone and bone-ligament-bone), techniques to perform experiments on both such complete functional units and isolated tendons had to be developed. The following tensile strength parameters were studied: (i) "elastic stiffness" (tangent for the linear part of the load-deformation or stress-strain curve), (ii) failure energy for the system tested, (iii) maximum load/stress, (iv) deformation/strain at the load/stress at which the linear part of the curve ends.

To investigate the reaction to increased functional demands, rabbits were trained in a running-machine for forty weeks. Animals of the same stock were kept as controls in the same laboratory conditions during the same time (I). At the time of the mechanical testing the animals were skeletally mature. Tensile strength characteristics of the Achilles tendon as part of a functional unit were tested to the point of failure of the unit's weakest point. The load and deformation of the tendon were recorded continuously. The weakest point in the system of calcaneus-

Achilles tendon-gastrocnemius muscle-femur was, for both animal groups, the insertion of the Achilles tendon into the calcaneus. None of the tensile strength parameters measured was altered significantly by training.

The whole range of tensile loading of the tendon could not be investigated on the complete functional unit, as failure load of the system was first reached at some other point than the tendon. Isolated hind limb tendons were therefore also studied (II). In these experiments the tendons were fastened with clamps, that did not produce artificial failure initiations (i.e. jaw breaks did not occur at lower load/stress values than the free space breaks). As the cross-sectional area of a tendon is difficult to measure accurately, the load per unit fresh weight per unit length was calculated and designated "stress". This characteristic is directly proportional to stress (load per unit cross-sectional area). It was found that the maximum "stress" and the length of the linear part of the "stress"-strain curve increased significantly with training. The collagen and water content of hind limb tendons were also determined and no changes found between the tendons of the trained and control animals.

From these studies it can be concluded, that training does not alter the amount of tendon tissue, if the training is performed in the middle and later part of the animal's growing period. However, some qualitative changes occur in the tissue. These cannot be detected when testing complete functional units as they are in the range beyond the failure point of the weakest component in the unit.

In the same animal groups also the anterior cruciate ligament with its bony attachments was studied for tensile strength characteristics and degree of elasticity (III). The testing was performed with the specimen clamped in contour-shaped clamps for the tibia and the femur. It was found for both trained and control animals that failure occurred in the bony insertion of the ligament. In the tensile strength experiments the maximum load and elongation at maximum load tended to increase and the failure energy became significantly higher with training. These results show that the bony insertions of the ligament, i.e. the region where the collagen fibers of the ligament intermingle with those of the bone becomes stronger, but no explicit information is obtained on the properties of the ligament itself. Therefore, tests on the degree of elasticity were also performed.

In consecutive loading-unloading experiments on a specimen the "elastic stiffness" for loading phase increased but the one for unloading phase remained unchanged. The deformation required to achieve a certain load value increased with consecutive tests, i.e. plasticity was present. After loading with a definite deformation speed up to a certain load value and then halted the deformation, the load decreased asymptotically towards a certain value. That is, the viscosity manifested itself in a load-relaxation. It was found that the parameter that was altered with training was the load-relaxation. This was greater in the ligaments of the trained animals. This finding supports the conclusion from the study on isolated tendons (II) that a qualitative change occurs with training of the animal.

As it was impossible to analyse the nature of the load-relaxation phenomenon without knowledge of the interaction between the elastic and viscous properties, a mechanical analogy for the tensile behavior of collagenous tissue was developed (IV). Such an analogy is composed of idealized elements of elasticity (springs), viscosity (dash-pots) and plasticity (dry frictional elements) coupled

in series and/or parallel with each other and it makes possible the derivation of mathematical expressions for composite mechanical behavior, which are possible to handle and to survey. The behavior of collagenous tissue could be described qualitatively by an analogy consisting of two parts, one of which accounted for irreversible changes during the first several loading-unloading cycles, and another which accounted for the stationary behavior observed in the subsequent, identical cycles. To enable description of the "toe" part of the load-deformation curve, a non-linear spring array of linear springs coming into action with certain intervals was developed. The analogy for the stationary behavior consists of a spring and a dash-pot in parallel (constituting a Kelvin element) and in series with them the non-linear spring array and a dry-frictional element. For the initial behavior there are coupled to this analogy another dash-pot and a series of dry-frictional elements. The analogy thus described satisfies the behavior of collagenous tissue as found both by the author and previous investigators.

In the next two publications (V and VI) the constitutive equation for the analogy was developed and its properties in specific cases analysed. Some alternative solutions for the description of the collagenous tissue's mechanical behavior were found less satisfactory. No decisive differences between the behavior of the experimental specimens and the proposed analogy could be found. Certain pertinent element constants were determined in suitably designed experiments, some of them in two different ways thus verifying the analogy experimentally. To make possible the evaluation of element constants in the stationary phase, three experiments are needed: (i) a deformation at a very low speed, (ii) a load-relaxation and (iii) a deformation at a very high speed. For the initial phase some additional experiments are required. (Cf. VI).

The morphological picture of tendons during strain was studied by light and electron microscopy (VII). The waviness of the tendon fiber bundles seen in the light microscope in a relaxed specimen was found to disappear with the start of the "toe" part of the stress-strain curve. It was also observed that the various bundles were strained gradually. By electron microscopy it was demonstrated that the period length of the collagen fibrils in relaxed tendons was about 680 Å and that this measurement could be influenced significantly by straining of the tendon. Some fibrils from strained tendons were found to have increased period lengths, while others had shortened lengths. It was suggested that the shortening was due to rupture and recoiling. It was concluded that a tendon's mechanical behavior during the "toe" part of its stress-strain relationship is explainable by decreasing waviness of the fiber bundles, whilst the linear part of the stress-strain curve is related to changes in the molecular chains. The waviness and its disappearance thus corresponds well to the non-linear spring array of the analogy. The molecular chains, however, can not by this study definitely be linked to the Kelvin element of the analogy.

These investigations provide a basis for rational analysis of the biomechanics of collagenous tissue and point out the close correlation between the function and the structural arrangement of the tissue.

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