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INFLUENCE OF POSTMORTAL STORAGE ON TENSILE STRENGTH CHARACTERISTICS AND HISTOLOGY OF RABBIT LIGAMENTS

by

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INTRODUCTION

The mechanics of the human locomotor system, as revealed by purely mechanical techniques, have attracted increasing interest during recent decades. Because of the difficulty in obtaining fresh material, most studies have been performed on autopsy material which has been stored for varying lengths of time. In order to evaluate the results of such studies, one must determine whether postmortal changes affect the mechanical properties of various tissues.

Many authors have mentioned the problem, but only a few have investigated it. The results are contradictory, great differences having been recorded concerning mechanical properties of tendons and ligaments. The number of tests made has often been inadequate and the terminology used confusing, thus making comparisons difficult. The authors, therefore, felt that it would be useful to determine how long a period of time must elapse after death before one must consider the influence of postmortal changes on results.

The aims of the study have been (1) to investigate qualitatively the mechanical characteristics, especially tensile strength, of the anterior cruciate ligament of rabbits, (2) to determine how these physical properties are influenced by postmortem changes and (3) correlate the results with the histological picture.

The following symbolics and units of recording are used throughout the text:

Load, P, in kp (9,80665 kg m/s^2)

Elongation, Δl , in mm

Area, A, in mm^2

Tensile stress, σ, in kp/mm^2

Strain, ε, in $\frac{\Delta l}{l_0}$ (dimensionless)

Energy, W, in kpmm

All through the statistical analysis a level of significance of 5 per cent has been applied.

SURVEY OF THE LITERATURE

Mechanical properties of ligaments.

Reutervall (1921) performed experiments on the ligamentum nuchae from human cadavers and found an elastic after-effect of 4 per cent. He also found similar properties in tendons. He noted that collagenous and elastic fibres in a relaxed preparation lay in a wavy formation and considered this to be of importance to the distensibility curve.

Investigating knee joint ligaments in the dog, Annovazzi (1928) came to the conclusion that they were extensible when loaded and, within certain limits, elastic. He showed that ligaments containing elastic fibres had a lower elastic limit than those consisting solely of collagenous fibres.

Hardy (1951), in a study on the ligamentum patellae of the cat and the human ligamentum flavum and calcaneo-navicular ligament, found no extensibility in »collagenous« ligaments but 140 per cent extensibility (of its unstretched length) in »elastic« ligaments. As his methods and results are not presented, his observations are difficult to evaluate.

Smith (1954) experimented on the anterior cruciate ligament of the rabbit and concluded (1) that the failure load of the ligament was related to the weight of the animal, (2) that the temporary extensibility was 20 per cent of the original length of the ligament, and (3) that the ligament was elastic to a load in the order of body weight but to submaximal loads only when of short duration. He found a relationship between body weight and failure load but did not consider it uniform enough to permit calculation of an individual ligament's failure load.

Steindler (1955) reviewed physical properties of the locomotor system. He believed that those ligaments, which under normal motion are especially exposed to tensile stresses, should be largely composed of elastic fibres. According to this believe, the ligamenta flava, for example, should be hypertrophied when the spine is unstable. If the stress is excessive or too prolonged a ligament should become relaxed and loose its elasticity. He thought that tendons and fascia might react similarly.

Mechanical properties of other soft connective tissue structures.

Wertheim (1847) investigated the mechanical properties of tendons, among other parts of the human body. He used fresh preparations from bodies aged one to seventy-four years. He found that the load-elongation curves of the tendons resembled a hyperbola with the vertex in the origin. For bone the curve was a straight line through the origin. With drying the modulus of elasticity increased and the shape of the curve approached that of bone.

Gratz (1931) found that human fascia lata had »high elastic properties«, and claimed that the presence of excessive fatty tissue might interfere with their elasticity as well as their viability.

A classical clinical and experimental study on the biomechanics was made by McMaster in 1933. In the experimental part of his work he loaded the calcaneus — tendo Achilles — m. gastrocnemius — femur — complex. Only when half of the tendon was cut through he did obtain a tendon rupture, in the other cases a tendon-muscle-junction rupture or a bone fracture occurred. He concluded that the normal tendon was not the weakest point of the system investigated.

Cronkite (1936) tested the tensile strength of a great number of human tendons. Each tendon was held by a pair of clamps, designed to prevent slipping, and was fixed in a standard testing machine of the type used by engineers. The results showed very great variations in the tensile strength of different tendons from the same body, and of the same tendon from different cadavers. The tensile strength ranged from 8700 to 18000 pounds per sq. inch in different cadavers, and in the case of individual cadavers the maximum value was as much as three times the minimum value. Hence Cronkite stated that it was futile to establish a norm of tensile strength for tendons in general.

Stucke (1950) studied human Achilles tendons. He found that this tendon had its highest elasticity in the third decade of life. The degree of elasticity, however, showed an obvious fall with age after this period. He reproduced stress-strain curves and found a tendency to decreased extensibility at 0.89 kp/mm^2 and 41 per cent of the maximal extension. He gave the maximum figures of 4.67 kp/mm^2 and 7.34 per cent elongation.

Hill (1951) stated that tendons of muscles were extensible and elastic but did not specify his methods or results.

Dick (1951) tested the reaction of human skin under pressure and studied the histological picture. He concluded that »it is reasonable to correlate the initial tensions in the skin with the condition of the yellow elastic fibres«. In an experiment on fascia lata and dura mater he compared them with rubber and suggested that »white collagenous fibres by themselves respond to distension as does rubber«.

The tensile strength of the special connective tissue developing in a healing skin wound has been studied by Sandblom (1944), and Sandblom, Petersen and Muren (1955). More recently studies have been made by Sandberg and Zederfeldt (1963).

Postmortal changes of mechanical properties of ligaments.

Wertheim (1847) tested a tendon from a dog immediately after death and five days later its remaning counterpart, which had been stored, and found no difference in the modulus of elasticity.

Annovazzi (1931) found that the physical properties of knee joint ligaments of dogs changed rapidly after death.

Gratz (1931) tested human fascia lata, obtained at operations, after two to eighteen hours' storage in salinemoistened gauze. He noted that after eighteen hours' delay the material »was essentially alive«. No figures are given and there is no mention of any differences or similarities.

Cronkite (1936) tested the tensile strength of human tendons, a few of them twenty-four to forty-eight hours after death and un-embalmed, the remainder embalmed and ten to eighteen months old. He noted that the old tendons differed little in strength from the others.

Åkerblom (1948) found that the extensibility and elasticity of human ligamenta flava were practically unaltered after unprotected storage in a refrigerator for an unspecified time. However, he based his statement on a single specimen.

Stucke (1950), in his investigation on the elasticity of human Achilles tendons, compared relatively fresh amputation and autopsy material with material fixed for some time in 10 per cent formalin, finding every individual peculiarity lost in the latter group. To eliminate the time factor his tests were performed within six hours after removal from the body.

Hardy (1951) stated that an »elastic ligament« lost its characteristic physical properties within one hour after death and that after five hours no extension occurred on a load of fifty-six pounds. The statement was based on one human calcaneo-navicular ligament from an amputated leg fixed in formol-saline. It is considered that his conclusions from the presented data are not fully justified.

Smith (1954) stated that as soon as one hour after death ligaments became less readily extensible, i. e. the modulus of elasticity increased, and reacted elastically to greater loads. The changes then became progressive. In his investigations he set the limit for fresh ligaments at thirty minutes. He gave no figures on the postmortal changes.

The stress-strain relationship of human embalmed plantaris tendons was investigated by Walker, Harris and Benedict (1964). They found the tensile strength of dried tendons to be higher than that of moist ones.

Some work on the mechanical properties of tendons subjected to irradiation has been done. Braams (1961) found that the presence of water reduced the sensibility of the material to radiation, i. e. decrease of the tensile strength was less. Ashley and co-workers (1964) found that irradiated tendon grafts functioned as satisfactorily as the non-irradiated ones.

In some works on allied structures postmortal changes are mentioned.

Evans and Lissner (1959), in a study on biomechanics of the human lumbar spine and pelvis obtained at autopsy, noted that embalming tended to harden and stiffen the specimen and to increase the average maximum load.

Hardy, Lissner, Webster and Gurdjian (1959), in their study on loading tests on the lumbar spine, used refrigerated, embalmed and deepfrozen specimens but noted no apparent effect due to the varying storage methods.

Studying lumbar intradiscal pressure, Nachemson (1960) stored the specimens in a refrigerator and a deep-freezer. He stated that it was impossible to ascertain whether any postmortal changes had occurred and, if so, the changes were not uniform.

MATERIALS AND METHODS

Materials.

The material in the present study has been obtained from the cruciate ligaments of the knees of rabbits weighing between 2050 and 4200 gms. Only adult animals have been used to avoid influence of uncalcified epiphysis upon the shape of the load-elongation curve. Closure of the distal femoral and proximal tibial epiphyses has been checked at X-ray examination.

The anterior cruciate ligament has been used for the mechanical tests. The anterior cruciate ligament before and after testing as well as the corresponding posterior ligament have been taken for the histological studies. A test specimen consists of the ligament with its bony attachment at each end, thus constituting a complete functional unit (bone-ligament-bone). The test specimens are arranged in six groups according to the length of time between killing of the animal and testing. These are a »fresh« group and groups which have been stored for 2, 6, 24, 48 and 96 hours between killing and testing. Each group contains both knees of four animals. In addition there are six single knees in the »fresh« group coming from succesful preliminary experiments and they are included in the material when calculating the mean values given in Table 2. 8 further knees from 4 rabbits are included in the 6-hour group in order to elucidate a marginal case in the statistical analysis (cf. p. 26).

The rabbits in the »fresh« group have been anesthetized with four ml 25 per cent urethane subcutaneously and 0.5 ml 10 per cent allypropymalum intravenously per kg body weight to enable both knees to be removed in vivo. The soft tissues around the knee joint have heen removed, leaving only the capsule intact. The femoral and tibial shafts are then severed at an appropriate distance from the joint and the capsule with the adjoining ligaments, except the anterior cruciate ligament, are excised. The time lapse between ligation of the femoral artery and the end of the experiment ranges from eight to eleven minutes. The tests have been made approximately two minutes after exposure of the ligament to air. This group is subsequently called the 0-hour group.

The animals in the other groups have been sacrificed by means of a heavy occipital blow and stored at 18-20 ° C until used.

Testing and recording equipment.

The materials testing machine used (Fig. 1) consists of a truck on two horizontal rails which is pushed or pulled by the plunger of the hydraulic cylinder. The fixed end of the testing system is a steel cylinder anchored to the rails. The force is applied to the plunger by an electrically powered oil pump. The activation and increase of the force are controlled by a system of valves. By using the same valve settings for each experiment the application of load on the system is approximately uniform per time unit.

The test specimen is attached to contoured clamps (Figs. 1 and 2) capable of vertical and horizontal movement but not rotation. The clamps are formed after the bone shape in order to prevent all slipping phenomena by the load application. It is important to avoid rotation because the anterior cruciate ligament is twisted in situ giving the im-

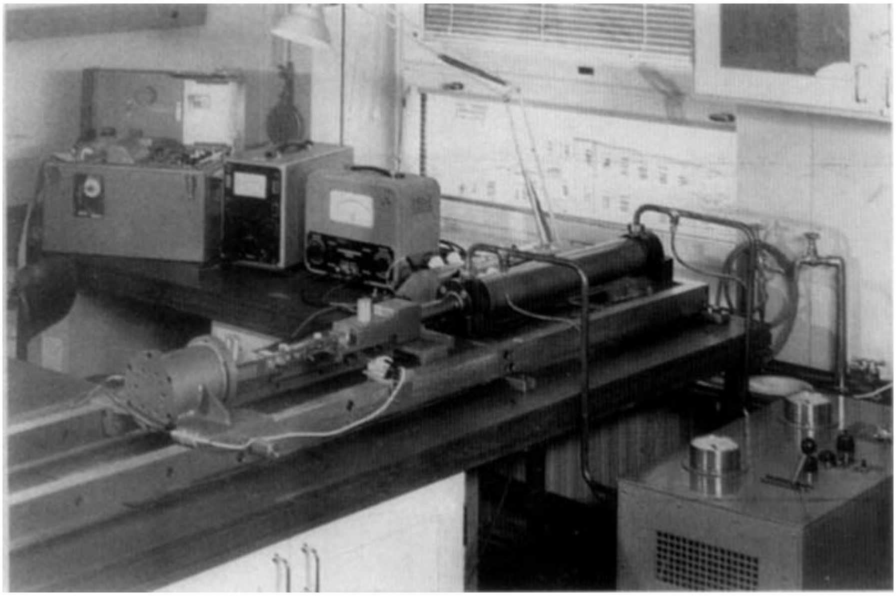

Fig. 1. The strength testing apparatus. On the table in the background there are from left to right the directwriting recorder and two bridges (one for load, the other for elongation). From the left, on the table in the foreground, there are the load pick-up, the clamps holding the specimen, the plunger and hydraulic cylinder. To the right of this table, there are the oil pump and its control panel.

pression that the tibial head has been rotated medially 90° in relation to the femur. If the ligament is allowed to unwind under stress a false elongation reading would be recorded. The clamps are attached to the fixed steel cylinder and the moving plunger, respectively. Thus, any movement of the plunger away from the cylinder would produce tensile strain and stress in the ligament between the clamps.

The load is recorded by the pick-up consisting of four strain gauges coupled in complete bridge mounted inside a steel cylinder at the fixed end of the test bench, the pick-up manufactured by Dietz. The distorsion of the cylinder is caused by the actual tensile force, and the effect on the strain gauges is indicated on a direct reading measuring bridge (Philips PT 1200).

The elongation of the whole system, i. e. the increase of the distance between the fixed steel cylinder and the plunger, stretching the ligament (the only extensible structure), is registered by strain gauges cemented on a steel plate deflected by a vertical steel pin on the plunger (Fig. 1).

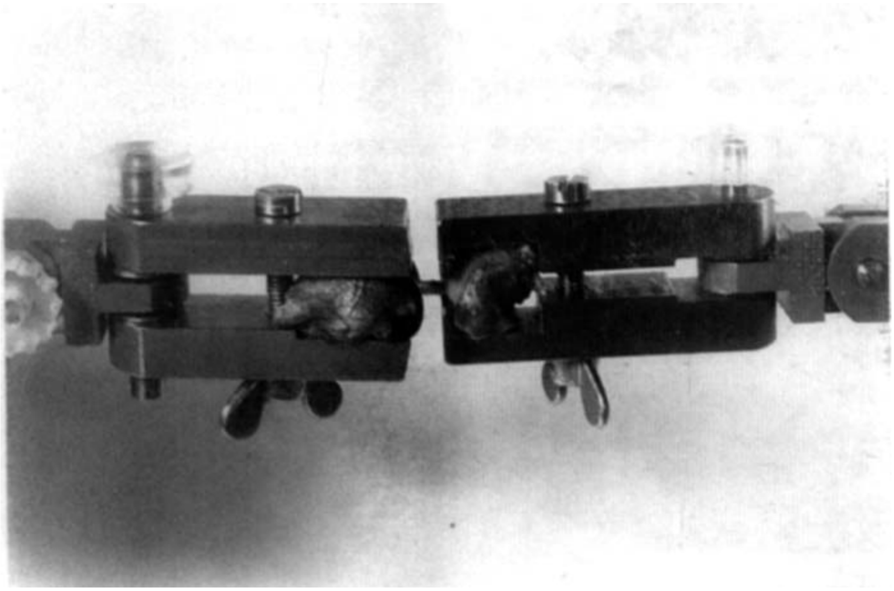

Fig. 2. Close-up of the clamps and test specimen, showing, from the left, the distal end of the femur, the anterior cruciate ligament and the proximal end of the tibia.

The stretching of the strain gauges is indicated on a direct reading measuring bridge (Philips GM 5536).

Both bridges are connected with the Oscillomink which recorded load and elongation simultaneously on separate channels. Fig. 3.

The whole system has been calibrated once prior to the commencement of the experimental series with the aid of tensiometers (Vibro-Meter GM 11/S) for load and by a micrometer gauge for elongation. The mechanical calibration has been checked from time to time and found to be correct.

The electronic calibration of the system by means of built in electronic calibration signals has been carried out before each experiment. Checks have been carried out immediately after the experiments on several occasions and the calibration has been found correct.

The gain of the amplifiers in the recorder has been set to give one mm deflection for one kp load and five mm deflection for one mm elongation. The recordings have been read under a lowpower dissection microscope. For each kilopond load change the corresponding elongation has been estimated at one quarter of a millimeter on the

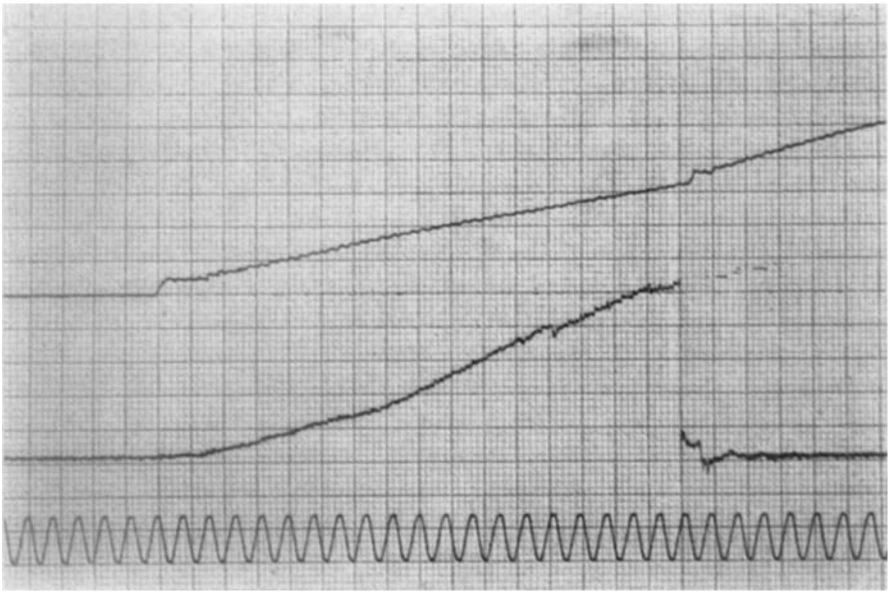

Fig. 3. Curves obtained from the recorder in one test, with elongation at top, load in the middle and fifty c/s time markers at bottom.

recording. Thus, eliminating the time axis, tensile load-elongation diagrams have been plotted where the zero point of elongation axis is set at five kp load except for the calculations of areas beneath the curve where zero is set at 0 kp load (Fig. 4). At five kp load the structures

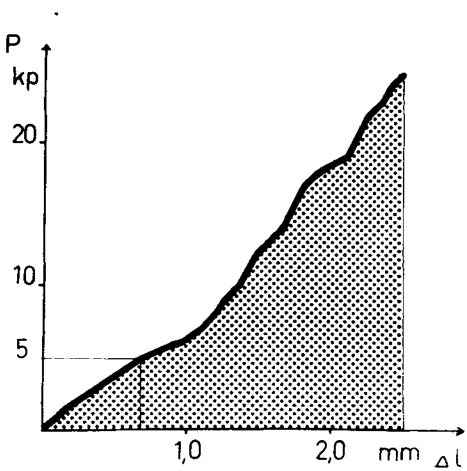

Fig. 4. The load-elongation diagram plotted from the recording illustrated in Fig. 3, with load (P) in kp along the ordinate and elongation ($\triangle l$) in mm along the abscissa. The shaded area represents the amount of energy required to rupture the specimen. The outlined coordinates represent the »five-kp-level«.

except the actual ligament are considered stretched and »fixed in tension«. This has proved to be true. Looking at the diagrams the curves of the »fresh« group are parallel and using the »five kp load equals 0 mm elongation« —point the curves coincide.

The error in the measurements have been studied by multiple measurements, 16 in all. Table 1 gives Karl Pearson's coefficient of variation (Kendall and Stuart, 1958), which has been calculated from the series of measurements of the different components of the equipment. The figures give the errors found in per cent. The tensile strength pick-up has been subjected to a standard load of 42 kp. The elongation pick-up has been deflected 4 mm and the pointer instrument has been read. Both bridges have been unbalanced with a standard amount of resistance change and the pointer instruments have been read. Both channels of the recorder have been subjected to standard variation of the input voltage and the recordings have been read as described before. A constant area has been measured by the planimeter.

	Measurement	
	load	elongation
Pick-up + bridge	1.41	1.09
bridge	0.48	0.22
recorder	0.45	0.32
planimeter	0.11	

Table 1. The coefficient of variation for multiple measurements with the components of the equipment.

Mechanical basis of the stress-strain diagram.

It is essential to discuss a material's mechanical properties in terms of a coherent system of well-defined units. The metric unit of length (mm) and the unit of force kp (kiloponds)*) have been adopted.

*) The force acting on the mass of one kilogram subjected to a gravity of 9.80665 m/sec^2 = one kilopond.

The stress-strain diagram is a useful means to illustrate a material's strength characteristics in both elastic and plastic ranges (Fig. 5). Such a diagram should also be suitable for determining the mechanical properties of the anterior cruciate ligament studied here as well as the extent to which these may change with time after death.

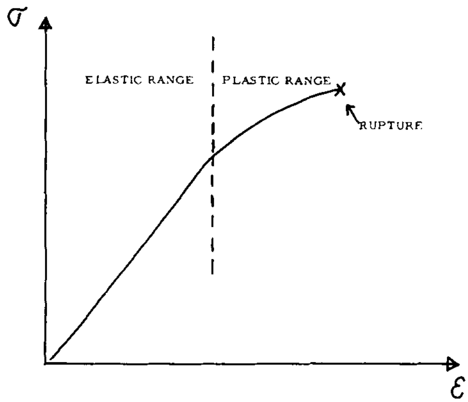

Fig. 5. The type of stress-strain diagram for an elastoplastic material.

Such a diagram is generally valid when the material is stretched, whatever its geometrical shape, because the loading is defined by the stress (σ), which equals force per unit area, and the strain (ε), which is the longitudinal deformation ($\triangle l$), per unit length (l_0). This diagram can be used for predicting a variety of properties of a body of any known shape, such as its failure load, elongation and the energy required to cause rupture.

In order to construct such a diagram for a particular ligament, one must be able to stretch it in the direction of its fibres, determine corresponding values of load (P) and elongation ($\triangle l$), and know the original area (A) and length (l_0) of the ligament. The relationship between load and elongation for the bone-ligament-bone complex may be found without undue difficulty in accordance with Fig. 6. But, owing to the fact that the ligament is disposed obliquely in relation to direction of the force, the true force acting on the ligament will be the measured load times a factor greater than unity and the true elongation of the ligament will be the measured elongation times a factor less than unity. In the case of the ligament studied here, the elongation of the bone is negligibly small compared with the elongation of the ligament; and, on account of the uniform appearance of the preparations, the multiplying factors may be assumed constant between preparations at corresponding stages of the tests.

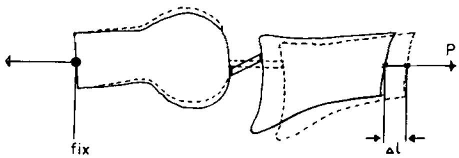

Fig. 6. Sketch of the specimen before (whole lines) and during testing (dotted lines).

The original length and area of the ligament are less readily evaluated, however, for the transitions from bone to ligament are poorly defined and the true effective area can scarcely be determined without harming the ligament. Hence, since it is not feasible to derive an adequate stress-strain diagram for the ligaments in the series under study, a so-called load-elongation diagram has been constructed which, as shown, is a special case of the more general stress-strain diagram. The load-elongation diagram fails to reveal variations due to geometric dissimilarities between preparations.

As in the present work the main interest is put to detect variations caused by postmortal storage, the possible geometric dissimilarities are included in the »fresh« as well as the stored groups.

If the preparations are assumed absolutely uniform and a scale factor based on the rabbit's weight or a skeletal dimension is applied, then it can be determined whether the family of load-elongation curves becomes less dispersed after such correction.

Another justification for using the load-elongation diagram is the fact that the bone-ligament-bone chain is a normal functional system whose weakest link determines its functional strength. This aspect of the matter admittedly deviates somewhat from the method of treating the ligament strictly in isolation.

And this is in accordance with earlier investigators. Smith (1954) found that the rupture of the anterior cruciate ligament of rabbit always occurred at the attachment to the tibia, a flake of bone being torn away. Investigators of similar structures have come to the same conclusion. In his experiments on healthy Achilles tendons from cats and rabbits, Davidsson (1954 and 1956) never obtained a tendon rupture but found that the tendon became detached from its origin or its insertion, or a femoral fracture occurred. Rupture of the Achilles tendon was not obtained in normal frogs (Fink and Wyss 1942) nor in the corresponding tendons of dogs (Stucke 1951).

With the aid of the load-elongation diagram one can observe the following characteristic quantities:

1. The *ratio* $\frac{P}{\triangle l}$ which should be constant within the elastic range and, if so, equals the slope or tan a (corresponds to the modulus of elasticity $E = \frac{\sigma}{\varepsilon}$ in the stress-strain diagram);
2. The *failure load,* P_{max};
3. The *elongation at rupture,* $\triangle l_{max}$; and
4. The *failure energy,* W_f, corresponding to the area between the curve and the $\triangle l$ axis or, expressed mathematically,

$$W_f = \int_{o}^{failure} P \, . \, d(\triangle l)$$

In the case of a ligament, tan a is the most significant quantity because a ligament probably normally works in the elastic range and never approaches the failure point. Nevertheless, if it is desired to evaluate the risk of overstraining the ligament, the quantities P_{max}, $\triangle l_{max}$ and W_f are most useful.

Abrupt deflections of the curve denote that discontinuous processes are taking place in the ligament i. e. that some fibres are rupturing and forcing the remaining intact fibres to carry the load. Accordingly a smooth load-elongation curve means that the individual fibres of the ligament are of uniform quality with respect to tensile strength.

Another explanation of the deflections would be slip phenomena at the ends of the specimen, but slip of that order is avoided by using contoured clamps.

Yielding, i. e. structural changes, do not cause load decrease, but longer or shorter periods of constant load.

RESULTS

Gross shape of load-elongation curves.

Roughly three types of load-elongation curves could be distinguished according to their shape. The largest group comprised more or less

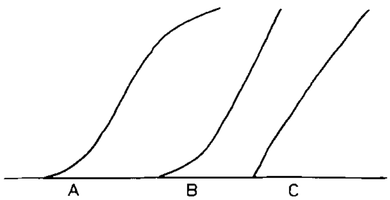

Fig. 7. Three typical shapes of the load-elongation curve.

S-shaped (sigmoid) curves having a rather shallow or flat initial portion, a steeper and linear intermediate portion and a more level final portion, the latter sometimes exhibiting a marked curvature (Fig. 7 A). A somewhat smaller group commenced similarly but continued almost linearly of the failure point (Fig. 7 B). The curves of the last group were linear throughout or convex to the left (Fig. 7 C). In the load range from 0 to five kp there was, with few exceptions, good agreement with the extended course and gradient of the curve. Hence this portion of the curve was taken into account in classifying the types of curve shape.

Slope of load-elongation curves.

The slope of the load-elongation curve, tan α, was a qualitative, but not quantitative, characteristic of the material tested. The tan α was calculated from a minimum applied load of five kp (reasons for this choice of starting point have been given previously) along the linear portion of the curve to the load applied at the point where the curve begins to level off. In about 60 per cent of the diagrams the change in the slope of the curve occurred at a load of five kp but in the remaining 40 per cent it occurred at a slightly greater load.

tan α was computed from the formula:

$$\tan \alpha = \frac{\Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}},$$

where x is the numerical value of $\triangle l$ in mm $\times$ 10 and y is that of P in kp for each point of measurement.

The means of tan α values for the stored groups and the controls, i. e. the 0-hour group, are given in table 2. The statistical analysis was carried out as follows. The means in the stored groups were compared by analysis of variance with the difference between groups, and the difference between right and left knee assumed as important variates (Hald 1952). The results are given in table 3 and show that there are no significant differences between stored groups, nor are the differences between right- and left-knee-measurements significant. The comparison of the mean of all stored groups with the mean of the controls by Student's t-test showed that there were no significant differences in these means.

Failure energy.

The shaded area beneath the curve (Fig. 4) represents the energy required to rupture the ligament. The areas of these roughly triangular figures were measured with an Amsler planimeter (type 612).

Statistical analysis performed similarly to those applied to tan α shows no significant differences, cf. tables 2 and 3.

A decline in failure energy in the series could be masked by an increase in body weights in the groups tested. It is seen in tables 2 and 3 that there are no significant differences in body weights and this possibility was rejected as an explanation for the lack of change in the failure energy values.

Failure load.

The occurrence of an immediately total rupture of the test specimen is shown in the original recording (Fig. 3) by an instantaneous drop of the load to 0 kp. This occurred in most of the cases. In some cases (30 per cent) the 0 kp level was restored in a succession of steps. This difference in the shape of the curve beyond the rupture point bears no relation to the rupture site. Mean absolute failure loads are shown in Table 2. No correlation with the weight of the animal was found in the

0-hour group (Kendall and Stuart, 1958). Statistical analysis of the failure load performed in the same way as those applied to tan a reveals

GROUP	n	MEAN VALUES (± standard error)				
		tan a	Failure energy kpmm	Failure load kp	Failure elongation mm	Body weight kg
Stored 2 hrs	8	1.40 ± 0.10	44.5 ± 4.6	30.6 ± 1.3	2.06 ± 0.15	3.57
6	8	1.37 ± 0.14	65.7 ± 11.9	31.1 ± 2.9	2.77 ± 0.34	3.73
24	8	1.24 ± 0.16	47.0 ± 8.6	28.6 ± 1.7	2.38 ± 0.34	3.48
48	8	1.42 ± 0.15	38.2 ± 4.1	30.4 ± 2.1	2.03 ± 0.15	3.41
96	8	1.37 ± 0.09	49.5 ± 8.0	30.4 ± 1.5	2.19 ± 0.22	2.67
All stored groups	40	1.36 ± 0.05	49.0 ± 3.5	30.2 ± 1.0	2.29 ± 0.12	3.37
Controls (fresh)	14	1.53 ± 0.07	36.0 ± 5.9	26.9 ± 1.1	1.74 ± 0.07	2.80
Diff. all stored and contr.		- 0.17	13.0	3.3	0.55	0.57
t-test of diff.		- 1.46	1.83	1.74	1.25	1.72

Table 2. Main table for tan a, failure energy, failure load and elongation at failure.

Parameter	Source of var.	df.	Sum of squares	Mean square	F
tan α	Between groups	4	0.3595	0.0719	1.08
	Right vs. left knee	5	0.3998	0.0666	< 1
	Remainder	30	4.2912	0.1192	-
	TOTAL	39	5.0505	-	-
Failure energy (kpmm)	Between groups	4	3359.88	839.96	1.92
	Right vs. left knee	5	2189.57	437.92	< 1
	Remainder	30	15525.73	517.52	-
	TOTAL	39	21075.18	-	-
Failure load (kp)	Between groups	4	29.09	7.27	< 1
	Right vs. left knee	5	48.13	9.63	< 1
	Remainder	30	1059.75	39.90	-
	TOTAL	39	1136.97	-	-
Failure elongation (mm)	Between groups	4	2.9818	0.7455	1.71
	Right vs. left knee	5	2.1794	0.4359	< 1
	Remainder	30	16.0025	0.5334	-
	TOTAL	39	21.1637	-	-
Body weight (kg) all groups	Between groups	5	3.7724	0.7545	2.47
	Within groups	18	5.4895	0.3050	-
	TOTAL	23	9.2619	-	-

Table 3. Analysis of variance to the parameters in table 2.

no indication of any relationship between this load and time of storage, cf. tables 2 and 3.

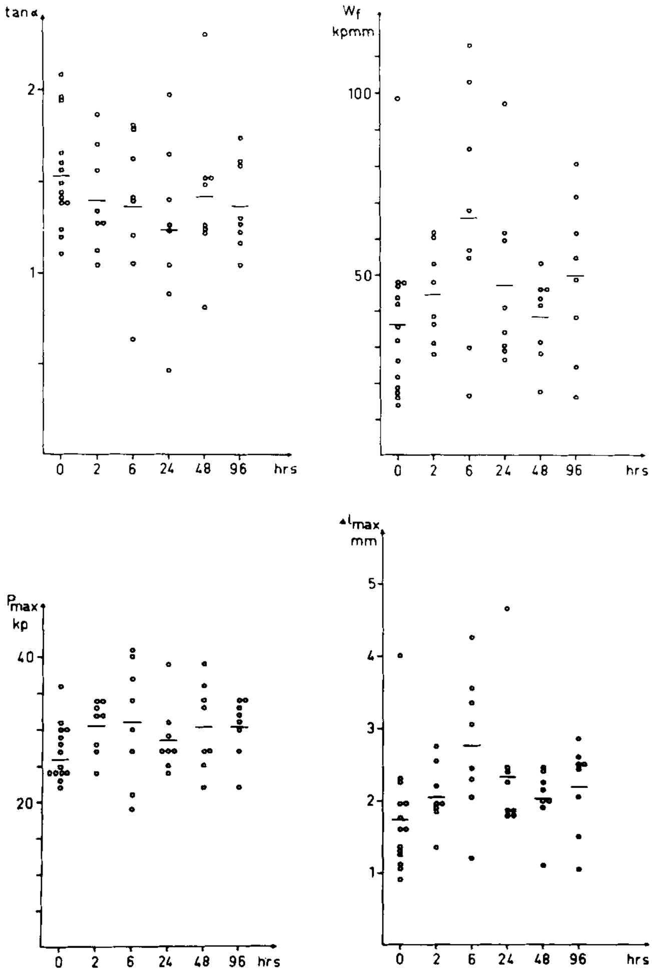

Fig. 8. The individual values of tan α, failure energy, failure load and elongation at failure in the different time groups. — denotes the mean value of a parameter in a time group.

Elongation at rupture.

Mean absolute elongation values at the rupture point are shown in table 2. Statistical analysis was performed in the same way as those applied to tan α and revealed no indication of any relationship beween this elongation and time of storage, cf. tables 2 and 3.

Dips in curve.

Some of the load curves recorded during the tests show, at various intervals, a slight drop in the load after which the curve resumes its previous slope. Such drops of 0.5 kp or more were designated »dips«. The frequency of dips and their mean magnitude in each time group are given in table 4.

Analysis of variance is applicable to the dip frequency only with caution (Scheffé 1959). If the analysis is performed (cf. tables 4-5) it reveals no significant difference between the stored groups, but the mean of all stored groups is significantly higher than that of the con-

Time group (hours postmortem)	No. of curves	No. of dips	Frequency per curve	Magnitude in kp		
				min.	mean	max.
2	8	6	1.1	0.5	1.9	5
6	8	12	1.5	0.5	1.9	9
6 (augmented)	16	23	1.4	0.5	1.4	6
24	8	10	1.3	0.5	2.1	9
48	8	8	1.0	0.5	1.3	4
96	8	5	0.8	0.5	1.1	2
All stored groups	40	44	1.1	0.5	1.8	9
Controls	14	9	0.4	0.5	1.1	2
Diff. all stored and controls			0.7	-		
t-test of diff.			2.06*	-		

Table 4. Frequency of dips in the curves and their magnitude.
* denotes significancy.

trols. Even though this difference exists, comparison of the individual stored groups to the control group by Wilcoxen's method (1945) shows that only the 6-hour group value is significantly higher than that of the controls. This significance persists when the 6-hour group is doubled. This set of significant and non-significant results cannot definitively be evaluated here. It is probable that the difference between the stored groups and the controls was due to chance, since no trend could be established.

The mean dip magnitude is still less suitable for a stastitical analysis.

Parameter	Source of var	df.	Sum of squares	Mean square	F
Frequency of dips	Between groups	4	3.35	0.84	< 1
	Right vs. left knee	5	7.75	1.55	< 1
	Remainder	30	38.50	0.84	-
	TOTAL	39	-	-	-

Table 5. Analysis of variance to the frequency of dips.

Site of rupture.

The bone-ligament-bone complexes usually (in about 80 per cent of the cases) ruptured either as a result of the tearing away of a piece of bone, varying in size from a tiny chip to the greater part of the femoral or tibial condyle, or failure of the ligament itself. Whilst the former type actually are fractures — usually called tear-off fractures — the latter constitute true ligament ruptures. The rupture site distribution within time groups is shown in Fig. 9 and table 6.

Here the Fischer-Irwin test (Lehmann 1959) was used, to compare the frequency of the ligament ruptures in the stored groups, one by one, to the control group. It is noteworthy that there are many ligament ruptures in the original 6-hour group, but this is not the case with the enlarged 6-hour group. Yet no significant differences could be found between the 0-hour group and the other groups even if the observations from the same animal were considered independent.

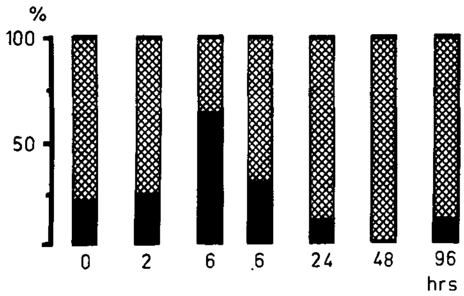

Fig. 9. Distribution of rupture sites within time groups. Black shading denotes ligament rupture and cross-hatching bone fracture. The two columns for the 6-hour group represent the original and the augmented series of preparations (cf. p. 12,26).

Group	Lig.	Bone			Frequency ligament ruptures
		femur	tibia	Total	
Stored 2 hrs	2	0	6	6	0.25
6	5	1	2	3	0.63
6 (aug.)	5	1	10	11	0.31
24	1	0	7	7	0.13
48	0	1	7	8	0.00
96	1	3	4	7	0.13
Controls	3	1	10	11	0.13

Table 6. Rupture sites in the femur-anterior cruciate ligament-tibia preparations.

HISTOLOGY

Literature.

It was established long ago that ligaments and tendons are composed of collagenous fibres, disposed in bundles paralleling the direction of stretch which are separated by rows of fibrocytes. In transverse sections through a ligament, the fibrocytes are endowed with a stellate shape by their processes which insinuate themselves between the bundles of fibres. Near the attachments to bone the fibrocytes become rounded and encapsulated and the amount of amorphous intercellular substance increases considerably. Such views have been published by Hardy (1951), Smith (1954) and Vis (1959). Ham and Leeson (1961) review the subject in their textbook of histology.

Methods.

Immediately after their excision the ligaments were fixed in 10 per cent formalin solution. If in the case of anterior cruciate ligaments subjected to tensile strength testing, bone fragments had been detached together with the ligament, the entire preparation was decalcified in a mixture of equal volumes of concentrated formic acid and 7 per cent sodium formate solution. The formalin-fixed and decalcified preparation was rinsed in water and dehydrated by passing through solutions of 70 per cent to 100 per cent ethanol and lastly into methyl benzoate. After pre-treatment with xylene, the preparation was embedded in paraffin and cut into five μ sections which were stained by Delafield's haematoxylineosine procedure.

Results.

The histological part of the present investigation was commenced with a comparison between the posterior and anterior cruciate ligaments in the 0-hour group. Their histological pictures exhibit no conclusive differences. The following account of the normal appearances refers to the posterior ligament which has not been subjected to tensile strength tests.

The histological appearance of the ligaments in the various time groups is not always unequivocal, the degree of the changes varying widely both between ligaments within groups and between groups. Thus, for example, sections of almost normal appearance are sometimes encountered in ligaments fixed long after death. The comments given below summarize those changes encountered in the majority of sections in each time group.

The ligaments in the *0-hour group* are composed of collagenous fibres disposed in parallel bundles separated by rows of fibrocytes. Towards its attachments the ligament contains a higher proportion of ground substance, the cells are more rounded and apparently constitute a stage intermediate between fibrocytes and chondrocytes (Fig. 10). In transverse sections the fibrocytes lodge between closely spaced collagenous bundles and present a punctate or stellate appearance (Fig. 11). This agrees well with observations reported by previous workers.

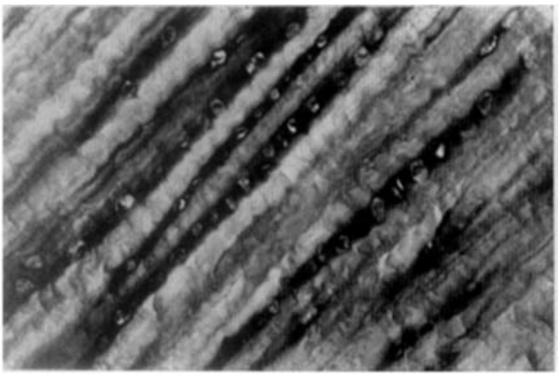

Fig. 10. Longitudinal section from normal ligamentous tisue near a bony attachment, presenting fibrocytes that apparently constitute a stage intermediate between fibrocytes and chondrocytes. (240x).

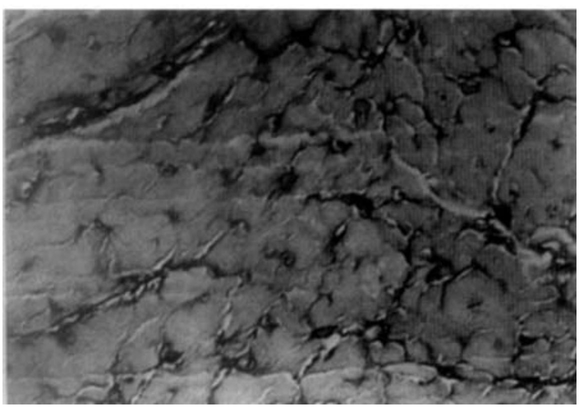

Fig. 11. Transverse section of normal ligamentous tissue, showing collagenous fibres disposed in regular bundles with firbocytes between them. (288x).

In the *2-hour group* the collagenous bundles seem swollen and exhibit numerous patches with, especially in transverse sections, a structureless, hyalinized appearance. Less sharply demarcated from one another than in the 0-hour group, the bundles seem less coherent. Though the majority of cells have a normal appearance, some of them are larger and rounder than those in the 0-hour group (Fig. 12).

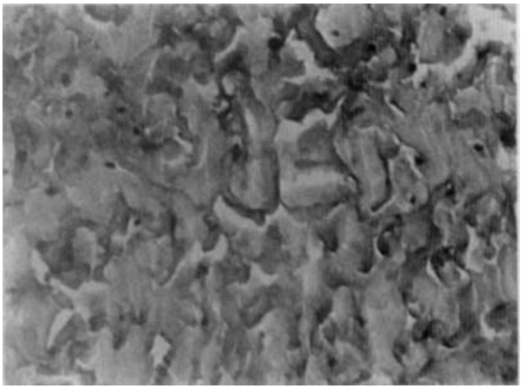

Fig. 12. Transverse section of a ligament in the 2-hour group, illustrating the structureless and hyalinized appearance of the collagenous bundles and some larger fibrocytes in addition to those of normal size and shape (288x).

The *6-hour group* is not conspicuously different from the 2-hour group. In addition to similar structureless patches, the collagenous fibres are more evident.

In the *24-hour group* the changes continue with blurring of the collagenous bundles which are spaced farther apart. Some cell nuclei in occasional sections exhibit abnormally poor staining properties, but others are heavily stained. A few nuclei disintegrate into even more heavily stained fragments (Fig. 13).

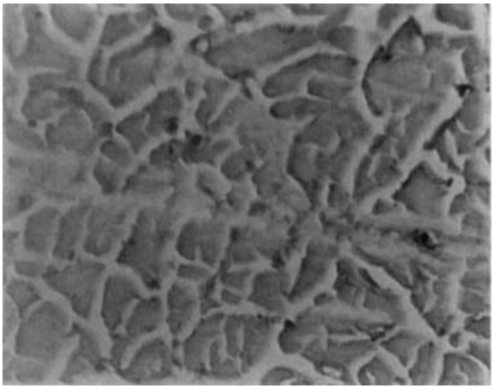

Fig. 13. Transverse section of a ligament in the 24-hour group, showing poor cohesion between collagenous bundles and varying stainability of fibrocyte nuclei. (288x).

The most characteristic feature in the *48-hour group* is the low nuclei content in extensive parts of the preparation. This is particularly apparent in transverse sections which occasionally present no cell structures other than small, heavily stained nuclear remnants (Fig. 14).

Fig. 14. Transverse section of a ligament in the 48-hour group, illustrating paucity of cell nuclei and varying nuclear stainability. (288x).

The most extensive changes, with patches of totally decomposed and completely structureless tissue, a few deformed nuclear remnants and large spaces traversing the preparation, are encountered in the *96-hour group.*

Summing up, the changes seen in the several groups increase with time after death. In the main these changes are apparently due to autolysis, which gives rise to progressive blurring of structures in the collagenous bundles and decomposition of the cells. The autolytic changes in the ligamentous ground substance also seem to impair the cohesion of the collagenous bundles. So far as could be determined by the technique employed the individual fibres, on the other hand, are resistant to autolysis and consequently maintain their shape and structure throughout the period of observation.

This agrees with the result of investigations on the preservation of tendon grafts. Thus, Herzog (1963) found the degeneration to begin with the cell nuclei and to proceed later in the interstitial tissue, the collagenous fibres remaining intact.

DISCUSSION

When and how the mechanical properties of tendons and ligaments undergo postmortal changes has not been established previously. Not having studied the problem systematically previous workers have reported highly contradictory observations. In those cases where observations on the action of postmortal changes were reported, only few specimens were studied.

In the present investigation series of similar ligament preparations from rabbits were tested under standardized conditions to ascertain whether postmortal changes occur, the ultimate aim being to appraise the suitability of autopsy ligamentous material for biomechanical studies.

Theoretically there may be postmortal morphological, chemical and mechanical changes, which usually interact with one another. Here it was elected to study certain mechanical properties, among which the results of strength tests serve as criteria characterizing a material and from which conclusions may be drawn regarding changes that may occur. The strength of a material is a concept with wide implications, in which both elastic and plastic as well as rheologic (time-depedent) factors are involved. Here the load-elongation diagram of ligamentous tissue was studied in experiments where the load was increased at a constant rate until reaching the rupture point.

To provide absolutely unambiguous values independent of the inner structure of the preparation, one should use a specimen of uniform length and cross-sectional area and apply the load in the direction of the long axis of the specimen. This is not feasible for studying ligaments because ligaments are of different lengths and have cross-section areas which are not uniform through-out the ligament. Furthermore, there is no reliable method for measuring their characteristic quantities. It is also impossible to measure the force acting in the exact lengthwise direction of the fibres. It should be kept in mind that the specimen tested is not the ligament alone but the whole bone-ligament-bone complex.

The method when using the load-elongation diagram, doesn't give any quantitative results concerning the strength of the ligament, but is a convenient way to fix possible qualitative deviations from the behaviour of a control group (i. e. here the 0-hour group), which, indeed, also has been the aim of the present investigation.

The procedure of choice would seem to be to apply to the ligament a load increasing at a uniform and fairly high rate (not interrupted for measurements), and to obtain continuous recordings. The elasticity of the ligament was not studied in the present investigation, since that would have called for repeated returns to zero loading from varying applied loads. Such a procedure would have been relatively timeconsuming especially regarding exposure to air.

The load-elongation curve is usually sigmoid in shape, its gross appearance agreeing with the diagrams published by Gratz (1931), who related the force to unit cross-sectional area and expressed strain as a function of the original length. Like Annovazzi (1928) and Smith (1954), he found that the collagenous ligament was extensible under load.

Neither the value of tan α representing the ligament's modulus of elasticity nor the failure energy exhibit any changes with time after death. Nor does the failure load and elongation show any variations between time groups. Moreover, unlike Smith (1954), we have found no association between absolute failure load and body weight, most likely because our animals show a much lower body weight variation.

Probably the dips indicate successive failure of overstrained fibre strands, shifting the load to previously unstretched strands until they in turn fail.

The frequency of dips in the load curve of the recording is higher in the 6-hour group than in the other, mutually similar time groups. This persists when the group is doubled.

The discrepancies exhibited by the 6-hour group cannot be accounted for on mechanical grounds because the later time groups exhibit no accentuation of the tendencies in the earlier ones. Whether chemical changes could have been responsible must remain an open question since no chemical analyses have been carried out.

The mechanical results correspond with what one would expect on morphological grounds. It would be unlikely that the collagenous fibres, the functional element in the preparations, would undergo rapid postmortal structural changes within the period of the experiments.

The evidence from the experiments does not completely eliminate the possibility of postmortal ligament change within ninety-six hours. No time group differs significantly from any other. Naturally, in postmortal periods longer than four days autolytic processes would be likely to affect the mechanical properties of the tissue.

The majority of previous workers have stated that ligaments preserve their mechanical properties for a short post-mortem period, seldom longer than an hour or two. However, most of those workers who have described their techniques mentioned that the ligaments were exposed to air, unlike the case in the present investigation where they have remained in the intact and closed knee joint until the test was due to commence. And in such circumstances, as disclosed by our findings, no changes of significance take place even after four day's storage at room temperature.

SUMMARY

The present investigation was designed to reveal whether the mechanical properties and histological appearance of ligaments undergo postmortal changes by storage. Such knowledge is of importance in mechanical studies on human autopsy material.

The literature on the mechanical properties of ligaments and related tissues is surveyed, particular attention being paid to published information concerning postmortal changes.

In the present investigation sixty-two intact anterior cruciate ligaments from rabbits were subjected to strength tests. The test specimens, consisting of the ligament with its femoral and tibial attachments, were divided into six groups according to the number of hours between the death of the animal and testing. Methods and apparatus are described and illustrated.

The mechanical basis for the experiments and the analysis of the results is discussed.

The results have been analysed with respect to the following factors: (i) the gross shape of the load-elongation curve, (ii) the slope of these curves, (iii) the area beneath these curves as a measure of the failure energy, (iv) the failure load, (v) elongation at failure, (vi) dips in the curves, and (vii) the rupture site on the bone-ligament-bone preparations.

The analysis of the numerical material does not reveal evidence for any consistent changes with time.

The ligaments were also studied histologically using standard methods. The histological study of the ligaments discloses progressive blurring of the structure of the collagenous bundles combined with decomposition of cells and ground substance. These changes are ascribed mainly to autolysis. On the other hand, the collagenous fibres proved highly resistant to autolytic changes.

ZUSAMMENFASSUNG

In dieser Arbeit wurde untersucht, inwieweit bei Verwahrung postmortale Veränderungen eintreten, die die mechanischen Eigenschaften und das histologische Bild des Ligaments betreffen. Das Wissen über dieses Verhalten ist von fundamentaler Bedeutung, da man mechanische Studien an humanem Sektionsmaterial ausführen will.

Eine Uebersicht der Literatur über die mechanischen Eigenschaften der Ligamente und der naheliegenden Gewebe wird gegeben. Besondere Beachtung wird dem, was frühere Verfasser betreffs postmortaler Veränderungen gefunden haben, geschenkt.

In dieser Arbeit wurde intaktes Ligamentum cruciatum anterius mit Femur- und Tibiaansats von erwachsenen Kaninchen studiert. Das Material umfasst sechs Gruppen mit Aufbewahrung von null bis sechs und neunzig Stunden von der Tötung an bis zur Ausführung des Versuchs. Die Methodik und Apparatur wird beschrieben.

Die grundlegenden Prinzipien und der mechanische Hintergrund zu den Experimenten, sowie die Analysen der Resultate werden diskutiert.

Folgende Faktoren wurden analysiert: 1) die allgemeine Form der erhaltenen Spannungsdehnungskurven (load-elongation), 2) die Inklinationskoeffizienten dieser Kurven, 3) das Feld unter diesen Kurven sowie ein Mass von der Brech-energie, 4) Bruchlast, 5) Dehnung beim Bruch, 6) Zacken (dips) in den Kurven und 7) die Rupturstelle des Knochen-Ligament-Knochen-Präparats.

Die Analyse des Zahlenmaterials hat kein Vorkommen von systematischen Veränderungen gezeigt, die während der Zeit entstanden sind.

Eine histologische Untersuchung der Ligamente wurde bei Verwendung von Standardmethoden ausgeführt. Das mikroskopische Bild innerhalb der verschiedenen Zeitgruppen wird beschrieben. Es zeigt eine fortschreitende Verschwommenheit in der Struktur der kollagenen Bündel, sowie Auflösung der Zellen und Grundsubstanz. Diese Veränderungen könnten hauptsächlich mit Autolyse erklärt werden. Die kollagenen Fibern dagegen haben sich als sehr resistent gegen autolytische Veränderungen erwiesen.

RÉSUMÉ

L'étude présente était destinée à faire voir si les propriétés mécaniques et la nature histologique des ligaments subissent des changements post mortem durant un procès de conservation. De telles connaissances sont importantes dans les études mécaniques sur la matière d'autopsie humaine.

La bibliographie sur les propriétés mécaniques des ligaments et des tissus apparentés a été étudiée en prêtant spécialement attention aux informations publiées concernant les changements post mortem.

Dans l'étude présente, 62 ligaments cruciformes antérieurs intacts de lapins furent soumis à des épreuves de résistance. Les spécimens de l'étude, formés du ligament et de ses liens fémoraux et tibiaux, furent répartis en six groupes d'après le nombre d'heures écoulées entre la mort de l'animal et l'épreuve. Les méthodes et les appareils sont décrits et illustrés.

La base mécanique des expériences et l'analyse des résultats sont décrites.

Les résultats furent analysés en tenant compte des facteurs suivants:

1) la forme élémentaire de la courbe charge-allongement,
2) l'inclinaison de ces courbes,
3) la surface au-dessous de ces courbes comme mesure de l'énergie de défaut,
4) la charge de défaut,
5) l'allongement à la rupture,
6) les points minimums des courbes,
7) le point de rupture sur les préparations os-ligament-os.

L'analyse du matériel numérique ne révèle aucune preuve de changements qui tiennent au temps.

Les changements furent aussi étudiés d'un point de vue histologique en utilisant des méthodes traditionnelles. L'étude histologique des ligaments révèle un obscurcissement progressif de la structure des faisceaux collagènes, combiné avec une décomposition des cellules et de la substance de base. Ces changements sont surtout attribués à l'autolyse. D'un autre côté, les fibres collagènes se montrèrent très résistantes aux changements autolytiques.

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