ACTA ORTHOPAEDICA SCANDINAVICA SUPPLEMENTUM NO. 115

From the Department of Orthopaedic Surgery, University of Gothenburg, Sweden
(head: Professor Carl Hirsch)

Tensile Properties of Human Lumbar Longitudinal Ligaments

BY

HENRYK TKACZUK

MUNKSGAARD COPENHAGEN 1968

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Professor Marian Garlicki, head of the Orthopaedic Clinic, Warsaw, introduced me to the problems of low back pain and under his guidance I have investigated some of these problems over a period of years.

While visiting Poland in 1966 professor Carl Hirsch invited me to Sweden in order both to deepen my knowledge of low back pain and to conduct original research into a problem associated with this syndrome.

During the resulting stay in Gothenburg I have worked in the Biomechanics Laboratory of the Orthopaedic Clinic where I investigated the mechanical behaviour of the human longitudinal ligaments.

I would especially like to express my gratitude to professor Carl Hirsch for the privilege of studying under his direction and for the warm friendship which he has always extended to me.

I wish also to express my gratitude to: Professor Marian Garlicki for supporting my application to travel to Sweden and for his kind and valuable guidance of the first 14 years of my career. Professor of Civil Engineering, Columbia University, USA, Richard Skalak, for critical and helpful discussion of the technical aspects of this work. Fil. lic. Esbjörn Carlström for the supervision of the statistical analyses and fil. kand. Bo Eriksson for the necessary computation. Dr. Joseph Schatzker, Toronto, Canada, and mr. John Evans, M.Sc., Glasgow, Scotland, who translated this work and for their friendly advice and discussion. Ass. professors Alf Nachemson and Ian Goldie, dr. Andrus Viidik, dr. Lars Sullivan and colleagues from the Orthopaedic Clinic for the valuable discussion and the cordial hospitality they extended to me which helped to make my stay in Gothenburg most memorable. Mrs. Birgitta Pande for deciphering and typing the draft manuscript. Ing. Hans Hellström, mrs. Inga-Liza Elzer and miss Lena Johnsson for their diligent laboratory assistance and willing cooperation.

I would also like to extend my sincere thanks to my colleagues of the Orthopaedic Clinic in Warsaw who have so ably performed my clinical duties in the past year.

This work was supported by the following institutions and foundations:

The Swedish Medical Research Council

University of Gothenburg

The Medical Society of Gothenburg

The Swedish Labour Market Insurance Society

King Gustav V Jubilee Fund

I. INTRODUCTION

The ligaments of the locomotor system have as their function the joining of the bony elements where they are normally subjected to tension. Their functional prerequisite is that they act as elastic structures. Ageing as well as degenerative changes may alter the mechanical characteristics of ligaments.

For years investigators have speculated on which structures in the lumbar spine may produce low back pain. Many have focused their attention on the discs, others have investigated joints (Lewin 1964) and ligaments of the spine. Tensile properties of interspinous ligaments and of the ligamentum flavum have been tested (Åkerblom 1948, Newman 1952, Silver 1954, Nunley 1958, Rissanen 1960, Rolander 1966, Nachemson and Evans 1967), but there is a lack of information on the physical properties of the anterior and posterior longitudinal ligaments.

In this work the author has endeavoured to answer the following questions:

a) What are the tensile characteristics of the longitudinal ligaments of the lumbar spine?

b) Has age any influence on the tensile properties of the longitudinal ligaments?

c) Have degenerative changes of the lumbar spine any influence on the tensile properties of the longitudinal ligaments?

II. REVIEW OF LITERATURE

Historical review of literature

In 1845, Purkinie and, in 1858, von Luschka gave a detailed description of the disc, its embryology as well as the innervation of the posterior longitudinal ligaments through the sinuvertebral nerve (the nerve of Luschka). Virchow writing, in 1872, ascribed afflictions of the vertebral column to "asthenia" and, in 1881, Beneke included asthenia in constitutional disorders. Kretschmer, in 1907, studied the asthenic weakness of ligaments but finally asthenia was defined as the imperfect development of the connective tissue within the whole organism. Schmorl and Junghanns, in 1932, in their classic work on the vertebral column, have a detailed description of the structure of ligaments. The posterior longitudinal ligament they described as narrower and thinner than the anterior longitudinal ligament. Schmorl, referring to Fick (1904), said that the posterior longitudinal ligament is also more elastic and that on flexion, the posterior longitudinal ligament was compressed against the vertebral bodies, thus occluding all underlying vascular structures. Schmorl in his description of the vessels which enter the posterior aspect of vertebral body mentioned the nerve of Luschka and pointed out that irritation of this nerve could be a cause of pain in the region of the vertebral column.

In 1940, Roofe, using the silver stain method, demonstrated unmyelinated nerve endings in the posterior longitudinal ligament. The innervation of the posterior longitudinal ligament has also been investigated by Wiberg, (1949) Hirsch, (1951) Pedersen, Blunck and Gardner (1956). Stilwell, in 1956, gave a very detailed description of the innervation of the anterior longitudinal ligament in a monkey. Magnuson (1944) demonstrated that if one injected physiological saline into the ligament surrounding the intervertebral foramen one may produce pressure on the root-ganglia and thus cause pain. Hirsch, in 1948 and in 1963, injected physiological saline into lumbar discs and reproduced pain characteristic of clinical low back pain. Inman and Saunders (1942, 1944 and 1947) stressed the role which ligaments play in the mechanics of the spine and considered that low back pain was caused by irritation of the nerves of the longitudinal ligaments.

Willis, in 1941, considered the various possible causes of low back pain and felt that one of the most important factors was overloading of the longitudinal ligaments. This statement was based on congenital deformations of the vertebral column.

Haboush, in 1942, studied the order in which ligaments tore on forceful flexion of the vertebral column. He determined that the posterior longitudinal ligament as well as the lateral portions of the anterior longitudinal ligament burst or tore before the interspinous ligaments.

Allbrook, in 1957, analyzing the cause of "anterior lipping" felt that it formed as a result of traction at the attachment of the anterior longitudinal ligament to the vertebral bodies.

O'Connell, in 1951, felt that low back pain may be caused by partial tearing or by stretching of ligaments. The relaxation of ligaments is, in O'Connell's opinion, caused by a hormone produced by the corpus luteum (relaxin) during pregnancy which may be the cause of unremitting low back pain during pregnancy particularly if the woman has to do hard work.

Harris and MacNab (1954) link the degenerative changes in the intervertebral disc with changes taking place in the ligaments, particularly the longitudinal ligaments and ligamentum flavum.

Leikkonen, in 1959, in his discussion of disc pathology, stated that the posterior longitudinal ligament could be stretched by the bulging nucleus pulposus.

In 1962, Arutynow, basing his contentions on observations made at surgery, concluded that degenerative changes are not confined to the disc but also involve the posterior longitudinal ligament and the ligamentum flavum.

Cremonese and Tomatis, in 1963, stated that nerve impulses which originate from irritation of the posterior longitudinal ligament via the sinuvertebral nerve of Luschka cause, in severe cases, a protective muscle spasm. This enlarges the intervertebral space on the side of the disc herniation and thus diminishes the force on the disc producing, at the same time, the so called sciatic scoliosis as well as straightening of the lumbar lordosis.

Roaf, in 1960, claimed that he was not able to disrupt the normal anterior longitudinal ligaments on flexion or extension of the lumbar spine as the bone fractured. On the other hand rotation easily damages the ligaments. He felt that degenerative changes have no influence on either the posterior or anterior longitudinal ligaments.

Hackett, in 1961, felt that excessive stretching of the ligaments stimulates their sensory nerves which, via a sympathetic reflex, cause neuro-vascular disturbances in the region of the bone. This, in turn, results in decalcification and thus weakens the insertion of other ligaments. In this way a vicious circle is established.

Hirsch (1954 and later) discussed the degeneration and deformation of the disc, which causes it to interfere with the longitudinal ligaments and thus irritates their nerves, as being a possible cause of pain.

General properties of collagen and ground substance

COLLAGEN

The chief components of ligaments are collagen and ground substance. The physical properties of most ligaments are determined by the collagen component. The collagen is not only the framework of all ligaments but also of all connective

tissue. As a protein it belongs to the group of sclero-proteins and comprises 1/3 of the protein of a human being. Grassman, in 1965, gave perhaps the best characterization of collagen as a substance in his opening address at St. Andrew's when he stated: "Collagen indeed is a peaceful protein which is not as active as its prominent brother myosin which is needed to raise the sword. Collagen causes no wounds but it heals wounds".

Collagen is produced by the fibrocytes which in turn are surrounded closely by the collagen fibres. The collagen fibres are built up from amino-acids in the following order: glycine, proline, hydroxyproline, glycine (Hall 1965). The hydroxyproline, which comprises about 20 per cent of the amino-acids found in collagen, is considered to be responsible for the resistance of the collagen fibre to various chemical substances (Gross 1961, Hall 1965). The solubility properties of collagen allow collagen to be divided into three fractions (Harkness 1961 and 1966, S. F. Jackson 1964). These fractions are:

1) The saline soluble fraction
2) The acid soluble fraction
3) The insoluble fraction

The fraction which is saline soluble is the one found in young collagen or in collagen structures with a rapid metabolic turn-over, e.g. the uterus during pregnancy (Hall 1965). The acid soluble fraction also belongs to young collagen. The greatest portion of connective tissue, however, is made up of the insoluble fraction of collagen which characterizes mature connective tissue (Gallop 1964, Hall 1965, Piez 1966). The soluble fractions of collagen, with ageing, undergo a change and become insoluble (Gross 1952, Banga 1966).

The amino-acids aggregate to form molecules of collagen which are also known as tropo-collagen. The molecular weight of collagen is in the region of 350.000. The molecule is 2800 Å in length and about 15 Å in width (Hall 1965, Harkness 1966).

There is a number of theoretical models illustrating the molecular structure of collagen (Gross 1952, Gustavsson 1956, Schmitt and Hodge 1960, Harkness 1961, Ramachandran 1962). The most popular theoretical model is a helix coiled to the left which is comprised of three fractions, alfa, beta and gamma, and the whole structure in turn is in the form of a superhelix, or trihelix, coiled to the right. The forces which bind the superhelix together are not fully known. The assumption is that there are hydrogen bonds between the alfa, beta and gamma fractions (Hall 1965, Banga 1966). The molecules are joined end to end and in this way give rise to protofibrils which have the same linear orientation and are bound to each other by electrostatic forces (Schmitt and Hodge 1960). Of note, however, is the fact that the molecules are staggered, overlapping each other by one quarter of their length. Thus, in a 2800 Å section this staggering repeats itself four times, approximately 700 Å apart, and is visible in the electronmicroscope as the dark cross bands. The fibrils, which are made up of protofibrils, are within the

resolution of the electronmicroscope as they have a width of 500—1000 Å (Schmitt et al. 1942, Schmitt 1944, Wassermann 1951, Hall 1965). The fibrils, in turn, are joined to give rise to fibres which are then within the resolution of the ordinary microscope. The fibrils appear to be glued to each other by mucopolysaccharides (Banga 1966). The mucopolysaccharides can be removed by heating (about 60—70°C) and then the collagen changes into a mass of randomly coiled fibres (Banga 1966, Harkness 1966, Piez 1966). With age some properties of collagen undergo a change. Verzár, in 1957, showed that the energy of contraction, which the collagen fibres generate under the influence of temperature, is directly proportional to their age. The older the collagen the greater the energy. Banga, in 1966, found that young collagen is more extensible and has greater tensile strength than old tissue. Gross, in 1952, found that collagen fibres in the young are both thinner and shorter than in the mature. Kohn and Rollerson (1958) found that young fibres absorb fluid in greater quantities and more rapidly than old collagen. Verzár (1957) and S. F. Jackson (1964) found that, during an isotonic contraction, older collagen fibres contract more and use up more energy than young ones. Jackson considers that the changes which take place in the physical properties of collagen are the result not only of ageing but also of maturation and are the adjustment to greater stresses. It has also been established that, with ageing, the amount of ground substance diminishes in favour of an increase in the thickness of the collagen fibres (Catchpole et al. 1951, Sobel and Marmorston 1956, Gersh 1959, Hall 1966) and the ratio of the mucopolysaccharides to collagen (H/C ratio) is inversely proportional to age (Sobel and Marmorston (1956).

GROUND SUBSTANCE

The collagen fibres have a close relationship with the ground substance which is comprised chiefly of mucopolysaccharides. It is considered that the mucopolysaccharides are produced either in the fibrocytes or in the mast cells (Catchpole et al. 1951, Ragan 1952, Jorpes and Yamashima 1956). Meyer, in 1956, classified the mucopolysaccharides into sulphated mucopolysaccharides and nonsulphated mucopolysaccharides. To the sulphated mucopolysaccharides belong the following: Chondroitin sulphate A, B, C, heparine sulphate, keratosulphate. To the nonsulphated group belong: Hyaluronic acid and chondroitin.

It is felt that the structure of mucopolysaccharides is in the form of a polymer made up of repeated couplets of two sugars. Hyaluronic acid does not branch whereas chondroitin sulphate does branch through the sulphate radical. It is known that chondroitin sulphate links with protein. The combination of mucopolysaccharides with protein is in the form of large radicals which contain or trap large amounts of water within their domains. This, in turn, influences the physical properties of the connective tissue (Gustavsson 1956, Hall 1965).

Tensile characteristics of collagenous tissues

The first account of the elastic properties of different human tissue was published by Wertheim in 1847. This investigator noted that the deformation of the tissues was not proportional to the loading. The curve constructed on the basis of load and deformation had the shape of a hyperbola. In addition, Wertheim stated that the curve was described by a second degree equation $Y^2 = ax^2 + bx$. This relationship has since been substantiated by many investigators (Annovazzi 1928, Gratz 1931, Åkerblom 1948, Stucke 1950, Dick 1951, Rigby 1964, Viidik 1965—66, Kenedi, Gibson and Daly 1965) but their interpretation of the physical properties differ considerably.

It is generally agreed that ligaments exhibit visco-elastic behaviour. A material is called visco-elastic if its properties are some combination both of a solid and of a fluid (Ferry 1961, Fredrikson 1964, Frost 1967).

If one wishes to utilize a classical model of a visco-elastic structure then biological material seems to fit most closely to the Voigt-Kelvin element. This is a material "in which the stress is the sum of two parts, one proportional to the strain and the other proportional to the strain rate" (Ferry 1961, Fredrikson 1964).

In carrying out the loading of a ligament one obtains a curve in which the toe part is concave in the direction of the load axis. Subsequently the line described becomes less and less concave and approaches a straight line.

When the yield point is reached the curve changes direction into the direction of the strain axis (plastic part). Plastic deformation continues until the breaking point is finally reached.

Many authors are of the opinion that the first part of the curve, the toe part, is dependent on the wavy formation of collagen bundles (Stucke 1950, Rigby 1964, Kenedi et al. 1965, Viidik 1966). The curve becomes less concave under the influence of increasing load and, when finally the wavy formation disappears and the collagen fibres have assumed an orientation in the line of stress, a straight line is achieved that is the elastic portion of the curve. Dick (1951) and Ridge and Wright (1965), studying the elastic properties of skin, were of the opinion that the toe part is due to the presence of elastin fibres and the remainder of the curve is representative of collagen. The elastic property of collagen is evident in the section of the curve between the toe part and the beginning of the plastic range (Annovazzi 1928, Gratz 1931, Smith 1954, Wright and Rennels 1964). Wright and Rennels (1964) determined that loading a collagen sample to 60—80 per cent (1.8 Kg/mm^2) of the failure load causes a permanent elongation of the specimen. Gratz (1931) felt that the maximal loading of human fascia lata, before plastic deformation took place, was 1.4 Kg/mm^2. Smith (1954) studying the cruciate ligaments of the rabbit, found that one could attain the elastic behaviour of the tissue by loading it with the weight of the rabbit over a 5-minute period or with

momentary submaximal loads. In biological materials, such as the ligaments, two time-dependent phenomena appear; namely the creep and the relaxation phenomenon. The gradual approach to limiting extension (strain) under a constant force (stress) is called creep (Ferry 1961, Fredrikson 1964). The gradual reduction in force (stress) exerted by a body under a fixed amount of compression or extension is called relaxation (Ferry 1961, Fredrikson 1964). Both these phenomena exist in the living tissues and make the testing of the physical properties of ligaments very difficult. The curves which one obtains by consecutive loading of the ligament are not identical (Viidik 1967, Galante 1967).

COLLAGEN IN AGEING

Collagen is a protein which may be described as "old" in that sense that, with ageing, it undergoes slow changes in its metabolic turn-over (Banga 1966). Wertheim (1847) found that the "stiffness" of tissue increases with age. Annovazzi (1928) found that ligaments from young animals were more deformable than ligaments of older animals. A similar observation was made by Banga (1966) who described young collagen tissue as more stretchable and more resistant to tearing. Gustavsson (1956) stated that connective tissue of mature animals contained more collagen. Ingelmark (1948) proved that with ageing collagenous tissues lose water regardless of their function. Katzenstein and Fecher (1924) described the ligaments of the human knee as less deformable after the age of 40 years. Curtis (1963), while studying the modulus of elasticity, found that ligaments of older people were more rigid, particularly under slow rates of straining. Galante (1967), studying the physical properties of the annulus fibrosus, showed that there is an age difference in the elastic properties of the annulus. He stated that elongation, residual deformation and energy dissipation decreased progressively until the age of 26, after which they remained practically constant.

The investigators who looked into the problem of ageing of connective tissue used different techniques. Many studies were carried out on the solubility properties of collagen in different substances (Harkness 1961, Hall 1965, Banga 1966). The first one who determined the correlation between contractability of collagen under the influence of temperature and the relationship of this with ageing was Verzár (1957). The contractile property of young and old collagen, under the influence of concentrated solutions of urea, was studied by Elden (1965). Banga (1966) tried to age collagen on the basis of potassium iodide staining. Kratky, Lauer, Ratzenhofer and Sekora (1962) studied the age-dependence of human tendon collagen using X-ray diffraction. They found a difference between the X-ray diffraction patterns of young and old collagen. Rigby (1964), comparing young and old collagenous tissues, investigated their physical properties by determining the strain-stress curves, X-ray diffraction patterns and shrinkage temperatures. He determined that ligaments of older people have greater resistance to strain, their X-ray diffraction pattern showed less distinct wavy formation and

their shrinkage temperature was higher. Sylvén, Paulson, Hirsch and Snellman (1951), studying the properties of the nucleus pulposus by electronmicroscopy, found that, with ageing, the three-dimensional lattice-gel system, containing a dense network of collagen fibrils and amorphous interfibrillar substance, irregularly loses the amorphous mucoid material and the fibrils become visible. The above authors were of the opinion that this mucoid interfibrillar material contained a large quantity of water. The decrease with ageing in the amount of this mucoid material results in a decrease in the water content and thus influences the physical properties of this tissue.

POST-MORTEM CHANGES

The question of how rapidly the mechanical behaviour of collagen tissues changes post-mortem has been of great interest to many investigators and to date the question has remained unresolved. Wertheim (1847) investigated tendons, nerves and arteries of dogs immediately and five days after death. He determined that no differences in the tensile properties of the tissues took place. Annovazzi (1928) found that the physical properties of the cruciate ligaments of the canine knees change immediately after death and even more so in the next ten hours. Gratz (1931), while studying the human fascia lata preserved in physiological saline for 18 hours, concluded that it seemed to be essentially alive. Kenedi et al. (1965) using a bioengineering approach to the study of human skin could not find a noticeable difference in the tensile properties of the skin in vivo or in vitro. Viidik, Sandqvist and Mägi (1965) on the basis of detailed studies of the cruciate ligaments of the rabbit, came to the conclusion that no distinct changes in the tensile characteristics of the tissue occur within the first 96 hours after death. The authors concluded that the greatest cause of the change in the tensile properties of the tissue was dehydration.

The condition for maintaining the physical properties of the ligaments was preservation of the intact and closed knee-joint until the test was performed. Hirsch and Galante (1967) tried to determine the loss of water from the moment of death to the time of testing by determining the weight of the intervertebral disc of rabbits immediately after death and after 48 hours. They found that the difference between the two groups was not significant. It may be, however, that the change in the physical properties of ligaments takes place almost with the last heart-beat, and this may in some way change the hydromechanical property of the collagen tissue. Thereafter other changes take place really very slowly and depend chiefly on the loss of water.

III. ANATOMY OF THE LONGITUDINAL LIGAMENTS OF THE HUMAN LUMBAR SPINE

The longitudinal ligaments are situated directly on the posterior and anterior aspects of the vertebral bodies and thus join the adjacent vertebrae. (Fig. 1.) Accurate descriptions of the ligaments, if one disregards the old classical anatomical texts, were given by Fick (1904), Strasser (1908), Schmorl and Junghanns (1932), Rauber-Kopsch (1932), Hirsch and Schajowicz (1952).

ANTERIOR LIGAMENT

The anterior longitudinal ligament constitutes a strong band which covers the anterior aspect of the lumbar spine as well as some of the antero-lateral aspect. The ligament has a firm attachment to the vertebral bodies particularly at their edges. The average width of the ligaments, measured during this study in 35 autopsy specimens in the age range 20 to 67 years, was as follows:

L5....2 cm
L3....2.5 cm
L2....2.3 cm

These measurements were carried out at the level of the vertebral bodies. At the same levels the average thickness of the ligament was:

L5....1.9 mm
L3....2.1 mm
L2....0.9 mm

There is an evident thinning of the anterior longitudinal ligament at the level of the annulus fibrosus. The average thickness of the anterior longitudinal ligament, at the level of the L4—5 intervertebral disc, was 0.8 mm. The ligament is easily separated from the underlying annulus fibrosus but is connected to it by fibres which are disposed at right angles to the axis of the anterior longitudinal ligament (Schmorl and Junghanns 1932). At the level of L3 and L2 the anterior (i.e. the abdominal) surface of the ligament is usually irregular and there are accessory fibres adjacent to the main portion of the ligament. From L2 upwards the ligament becomes thinner and narrower.

POSTERIOR LIGAMENT

The posterior longitudinal ligament is narrower and weaker than the anterior longitudinal ligament. At the level of L5—S.1 it appears as an irregularly developed oval structure which varies from 2—2.25 mm in width. From L5

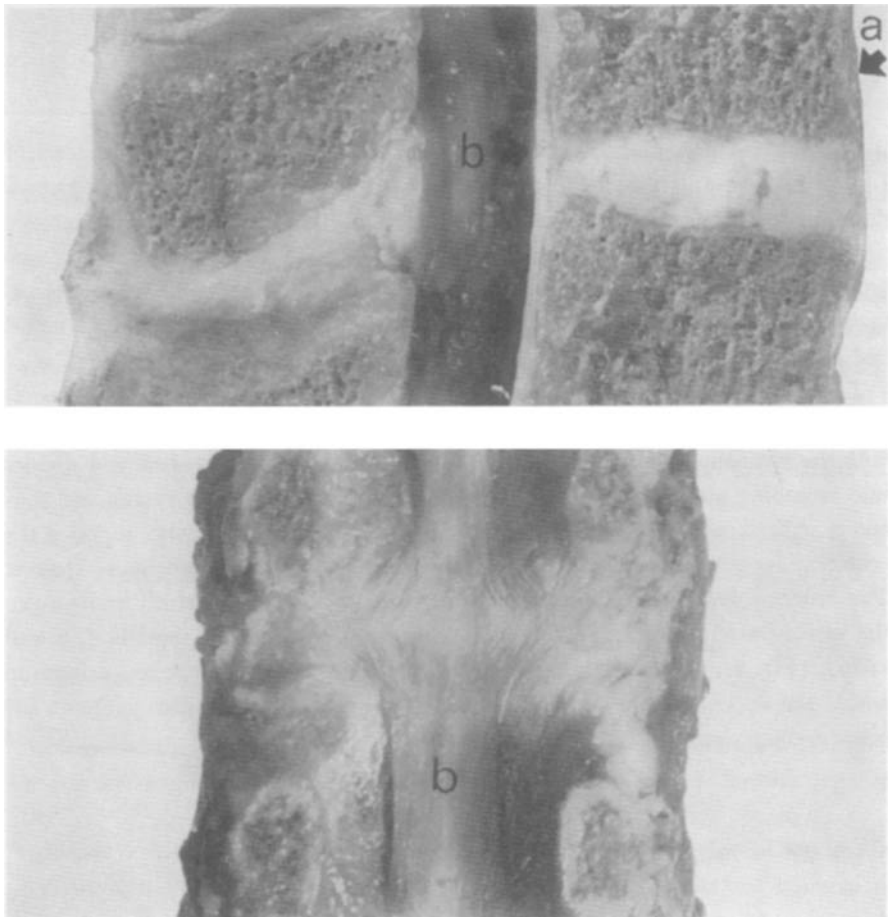


Fig. 1. The longitudinal ligaments of the lumbar spine.
a) Anterior longitudinal ligament.
b) Posterior longitudinal ligament.

upwards the ligament forms a narrow band which widens symmetrically at the level of each disc. The ligament is strongly attached to fibres of the annulus fibrosus and to the edge of the vertebral body but very poorly, and in some cases not at all, to the posterior aspect of the vertebra. The average widths of the ligament at the different levels are as follows:

L5 . . . . 1.4 cm disc level
L3 . . . . 1.5 ,, ,, ,,
L2 . . . . 1.1 ,, ,, ,,
L5 . . . . 0.7 cm vertebral body level
L3 . . . . 0.8 ,, ,, ,, ,,
L2 . . . . 0.6 ,, ,, ,, ,,

The average thickness of the ligaments are as follows:

L5....1.3 mm

L3....1.4 „

L2....0.9 „

The posterior longitudinal ligament appears to be best developed at the level of L3 and L4 where its average thickness is 1.4 mm. From L2 upwards the ligament thins markedly. Throughout the whole length of the ligament one can note a distinctly thicker middle portion approximately 2.5—4 mm in width. In cross-section the ligament has the form of an elipse. In the mid-portion of each vertebral body the posterior longitudinal ligament covers the vascular foramina (Schmorl and Junghanns 1932).

The posterior longitudinal ligament is innervated by branches of the sinuvertebral (or Luschka) nerve. This nerve has fibres which connect the nervus spinalis with the sympathetic ganglia and it enters the intervertebral foramen and divides into ascending and descending branches. It gives branches to the vessels and thus enters with them into bone through the vascular foramina. It also supplies the posterior longitudinal ligament and, to some degree, the ligamentum flavum. The anterior longitudinal ligament is supplied by branches which come from the sympathetic plexus which is connected with the nervus spinalis (Stilwell 1956). (Fig. 2.) In contradistinction to other ligaments of the vertebral column in which the nerve endings are superficial, the anterior longitudinal ligament has nerve endings deeply situated in its substance (Hollinshead 1965).

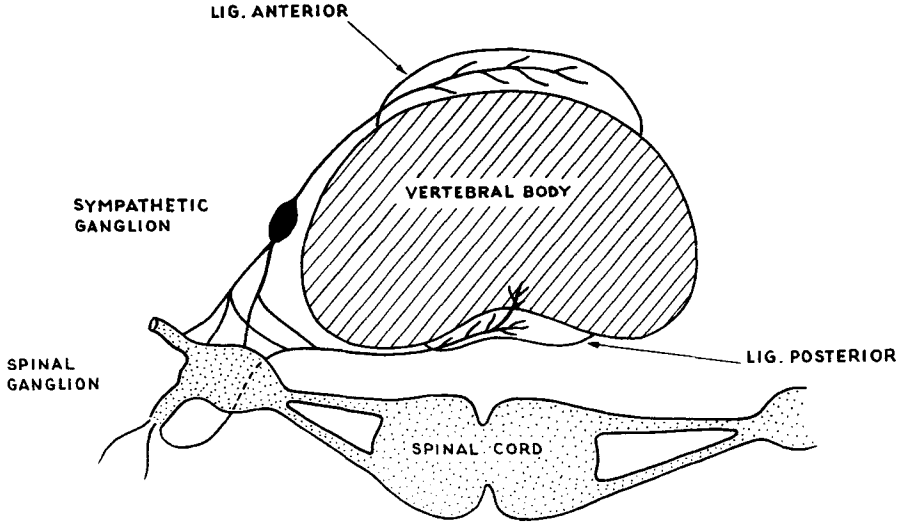


Fig. 2. Innervation of the longitudinal ligaments (after Stilwell).

IV. MATERIAL AND METHODS

Material

The anterior and posterior longitudinal ligaments were taken from lumbar vertebral columns obtained at post-mortem. The studies carried out were based on 74 vertebral columns from which 484 samples were taken. In addition to the ligaments, samples of the annulus fibrosus were obtained for histological studies. The specimens were tested within 48 hours of death. The ligaments were taken from the segments S.1 to T.12 inclusively and occasionally the 12th thoracic segment was included. The annulus fibrosus was sampled from L2, L3, L4 and L5. The condition of the intervertebral disc was assessed on the basis of its macroscopic appearance basing it on the generally accepted criteria (Friberg 1948, Friberg and Hirsch 1949, Virgin 1951, Hirsch and Schajowicz 1952, Ingelmark and Ekholm 1952) which are:

Grade 1. The normal disc: The annulus fibrosus and the nucleus pulposus are white without a distinct transition as the fibrous structure of the annulus is not visible.

Grade 2. The visibility of the fibres of the annulus determines the transition zone between the nucleus and the annulus.

Grade 3. Visible fissures in the annulus fibrosus. The border between the nucleus and the annulus is no longer distinct. The nucleus pulposus is rather dry and at times one can see distinct brown staining, the so called "brown degeneration".

Grade 4. Marked changes. Tears and sequestrations in the region of the annulus and nucleus and brown degeneration, deformation of the adjacent bone to the annulus in the form of osteophytes.

The anterior longitudinal ligament was removed in the form of a band, taken from its mid-portion and measuring 2 cm in width. The posterior longitudinal ligament was taken in its entirety with the exception that the segments between L5 and S.1 as well as those between T.12 and L.1, because of their size, (the ligament is narrow and thin) were not always suitable for testing. The anterior longitudinal ligament in the segment between L4 and L5 was frequently damaged during autopsy and was not always suitable for testing. In addition, particularly in children, the ligaments in the upper segments were too thin and did not meet our test requirements. These segments of the ligaments were not tested but were used for histological examinations. The ligaments were taken from the level of the disc as well as from the segment overlying the vertebra. Where possible the fragments of the ligaments, which were adjacent to the disc, were tested separately. Table I lists the specimens tested and Table II shows the number of the different test as well as the numbers of "preparations of the ligaments" which were used for these tests.

TABLE I

Subject number	Autopsy number	Age years	Sex	Death to testing	Cause of death
1	I- 600	77	F	1 day	Cardiac failure
2	L.S- 59	76	M	1 ,,	Bronchopneumonia
3	I- 949	60	M	2 days	Subdural haematoma
4	I- 586	45	M	1 day	Cardiac failure
5	L- 973	51	M	12 hours	Tumor cerebri
6	I- 948	42	F	2 days	Carcinoma pancreati
7	I- 984	53	F	1 day	Rupt. aneur. a. cerebr. med.
8	L.S.- 80	55	M	2 days	Acute alcoholic intoxication
9	I- 60	36	F	1 day	Tumor cerebri
10	I-1209	48	M	2 days	Tumor cerebri
11	I- 777	68	M	2 ,,	Cardiac failure
12	L.S.- 56	92	M	1 day	Circulatory failure
13	I- 739	59	M	1 ,,	Myocardial infarction
14	I- 761	62	M	10 hours	Myocardial infarction
15	I- 706	82	F	1 day	Cardiac failure
16	I- 783	64	F	1 ,,	Cardiac failure
17	I- 644	45	F	2 days	Rupt. aneur. a. carotis int.
18	I- 653	51	F	2 ,,	Myocardial infarction
19	I- 732	20	M	1 day	Contusio cerebri
20	I- 688	56	M	1 ,,	Cardiac failure
21	L- 771	63	F	2 days	Embolia pulm.
22	I- 621	38	M	1 day	Embolia cerebri
23	I- 679	73	M	2 days	Myocardial infarction
24	I- 786	56	F	1 day	Myocardial infarction
25	I- 767	58	F	1 ,,	Myocardial infarction
26	I- 759	35	F	12 hours	Embolia pulm.
27	I- 428	56	M	1 day	Aneur. art. cerebri med.
28	I- 748	70	F	10 hours	Myocardial infarction
29	I- 689	55	M	1 day	Myocardial infarction
30	II- 148	74	M	2 days	Peritonitis
31	I- 178	66	M	1 day	Circulatory failure
32	L.S.- 43	65	F	2 days	Bronchopneumonia
33	I- 61	70	M	1 day	Myocardial infarction
34	I-1214	57	M	1 ,,	Myocardial infarction
35	I-1037	48	M	12 hours	Peritonitis
36	I-1341	51	F	2 days	Intra-abdominal haemorrage
37	I-1229	24	F	12 hours	Pulmonary embolism
38	I-1176	21	F	1 day	Myocarditis
39	I-1056	46	M	1 ,,	Cardiac failure
40	I-1108	50	M	1 ,,	Respiratory failure
41	I-1054	29	M	1 ,,	Peritonitis
42	I- 909	33	F	2 days	Uremia
43	I-1178	66	M	1 day	Cardiac failure
44	I-1438	65	F	1 ,,	Cardiac failure

Subject number	Autopsy number	Age years	Sex	Death to testing	Cause of death
45	I-1316	64	M	2 days	Myocardial infarction
46	I-1051	66	F	1 day	Myocardial infarction
47	I-1205	67	F	1 ,,	Pulmonary embolism
48	II- 279	58	F	8 hours	Carcinoma of the stomach
49	II- 243	55	M	1 day	Carcinoma of the bladder
50	I- 941	22	M	12 hours	Cardiac failure
51	I-1134	17	M	8 ,,	Circulatory failure
52	I-1170	59	F	1 day	Cerebral tumor
53	I-1091	51	F	2 days	Intracranial haemorrhage
54	I-1331	4	F	1 day	Meningitis purulenta
55	I-1276	1 day	F	8 hours	Hydronephrosis
56	I-1329	10 days	F	12 hours	Atelect. pulm.
57	I-1330	17 ,,	M	1 day	Immatur.
58	I-1228	5 years	F	12 hours	Tumor cerebri
59	I-1317	10 days	F	1 day	Pneumonia
60	I-1293	20 ,,	M	8 hours	Pneumonia
61	I-1318	30 ,,	M	10 ,,	Intracranial haemorrhage
62	I-1281	1 day	M	10 ,,	Haemorrhagia pericardii
63	I-1055	57 years	M	2 days	Haemorrhagia cerebri
64	II- 235	59 ,,	F	1 day	Embolia pulm.
65	I-1212	2,5 ,,	M	1 ,,	Bronchopneumonia
66	I-1444	55 ,,	M	2 days	Haemorrhagia cerebelli
67	I- 639	36 ,,	M	10 hours	Uremia
68	I- 925	36 ,,	F	1 day	Circulatory failure
69	I- 616	58 ,,	M	8 hours	Bronchopneumonia
70	I-1273	54 ,,	M	1 day	Carcinoma of the stomach
71	I-1343	63 ,,	M	2 days	Myocardial infarction
72	II- 79	58 ,,	M	2 ,,	Bronchopneumonia
73	I- 57	26 ,,	F	10 hours	Uremia
74	I-1417	64 ,,	M	1 day	Myocardial infarction

TABLE II

Number of experiment	Experiment	Number of samples	Specimen Number
1	The water loss of samples exposed to air.	15	1, 2
2	The swelling of the ant. and post. ligaments in different solutions	48	5, 6, 7, 8
3	The water loss from samples in the high humidity chamber.	20	3, 4
4	The influence of rapid freezing and thawing on physical properties of long. ligaments.	28	9, 10, 11
5	Studies on the loading capacity of segments of long. ligaments of full thickness.	22	67, 68, 72
6	Studies on the loading capacity of samples of equal dimensions from the ant. and post. long. ligaments.	30	66, 73, 74
7	Repetitive loading.	116	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33
8	Studies to obtain preloading point for the long. ligaments.	—	65, 29, 51, 47, 48, 50, 43, 54, 42, 73, 70, 9, 71, 69
9	Microscopic investigation of ligaments subjected to loading.	—	12, 51, 61
10	Effect of age and degeneration on tensile properties	206	34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64,

Methods

At first the ligaments were removed with the aid of a dissection microscope (Zeiss) using 15-fold magnification. Experience later showed this to be unnecessary. In addition, refinements in the preparation technique of the samples minimized damage to the surface of the preparation. In order to determine the orientation of the collagen bundles in both the anterior and posterior longitudinal ligaments histological studies were carried out. We detected a marked wavy formation but nevertheless the mean orientation of bundles was parallel to the longitudinal axis of the lumbar spine. Histological studies of ligaments placed under a certain amount of tensile stress revealed that the collagen bundles straightened and assumed a relationship parallel with one another. The anterior

longitudinal ligament as well as the posterior were cut into segments which corresponded to the levels of L1, L2, L3, L4 and L5. Thereafter these were put in clamps and were loaded to the point of tearing. These studies showed that the ligaments were visco-elastic. The load/strain curves were typical of a collagenous structure (Viidik 1966, Galante 1967). It was also possible to conclude that, for comparable tests, identical-sized samples should be used.

PREPARATION OF THE TEST SAMPLE

The ligaments, after removal from the vertebral column, were cut into segments which corresponded to the levels of the lumbar spine. These were then frozen in a carbon dioxide snow stream and were cut on the Jung freezing microtome. Special calibration of the microtome allowed us to cut sheets of 0.5 mm thickness. During the sectioning, the knife of the microtome was so positioned that it was parallel to the direction of the collagen bundles.

MEASUREMENTS OF THE THICKNESS OF THE TEST SAMPLE

Great difficulties were encountered in determining the thickness of the test samples because of the visco-elastic nature of the tissue. At first we tried to measure the frozen samples with a micrometer screw gauge but found that this was very inaccurate as the samples thawed very rapidly and changed in consistency. We finally based our measurements on the system introduced by Galante (1967) during his studies of the physical properties of the annulus fibrosus. This method consists of a dial displacement gauge with an accuracy of + or — 0.01 mm made by the firm "Metron". This gives measurements of the thickness of the ligament placed between two metal plates. The weight of the plates as well as the pressure of the micrometer piston does not exceed 0.13 gm/mm^2 which results in a minimal creep of no more than 0.03 mm. If the thickness of the test sample showed a difference greater than 5 per cent from the standard thickness, or if the thickness of the test sample was not uniform, the sample was discarded.

METHOD OF OBTAINING IDENTICAL SAMPLES

In order to obtain identical bands of uniform width from the sheets, we at first employed a dissection microscope. The samples were cut from the sheet under a 15-fold magnification with the aid of a grid graduated in mm which was placed under the sheet. We found that the accuracy which one could obtain in this way was not adequate and, in addition, the time taken to obtain samples was far too long. The sample dried, a very significant factor, as we found in our later experiments. We again made use of an instrument introduced by Galante, the so called "stamping press" which employs two types of dies. For experiments in which we tried to determine the breaking point, dies with a narrowed central segment were employed. For all other determinations dies of size 30×2 mm were

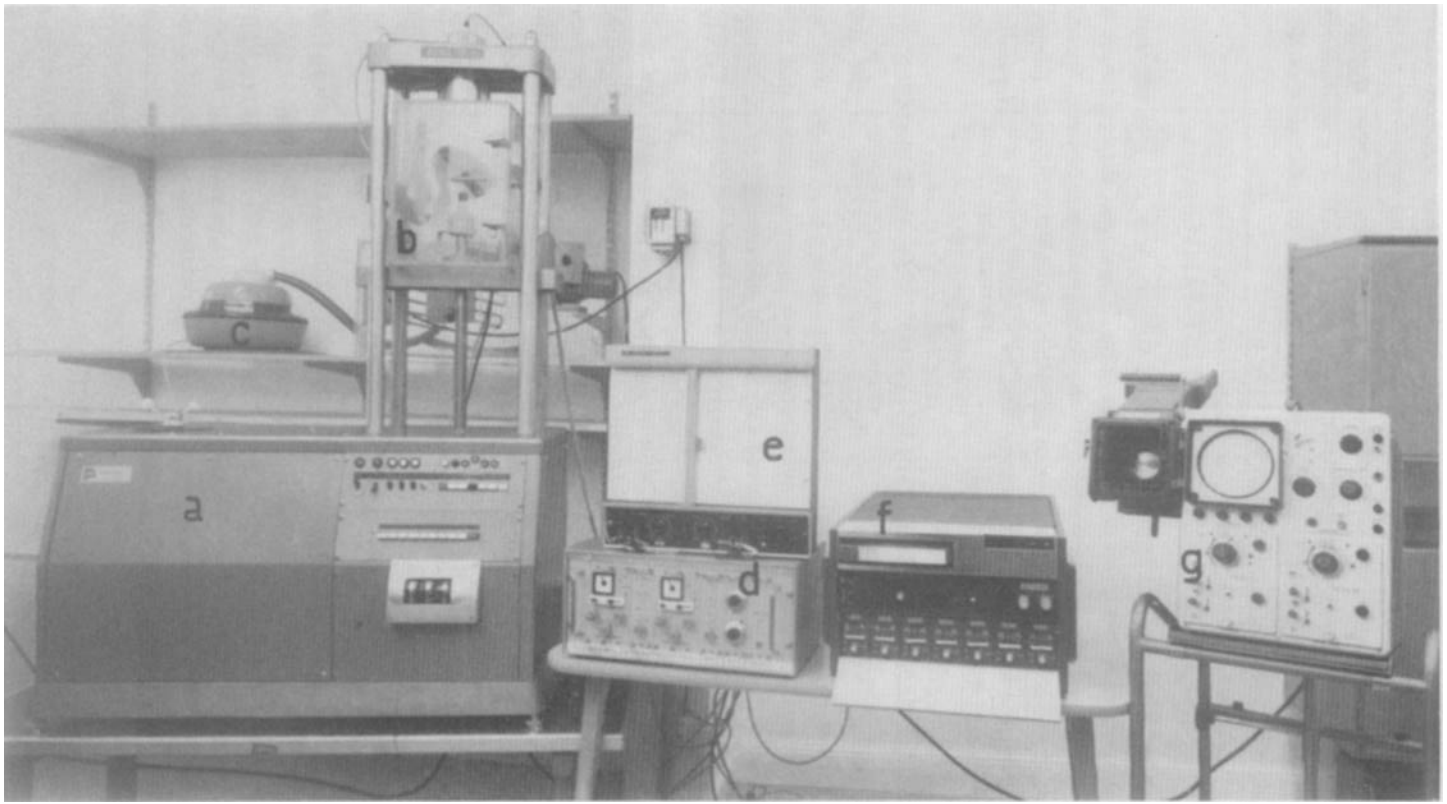


Fig. 3. Testing and recording equipment. (according to Hirsch et al.)
a) "Alwetron" test machine.
b) Test chamber with humidity and temperature sensors.
c) "Defensor" humidifier.
d) Amplifiers
e) XY-recorder.
f) Tape recorder.
g) Oscillograph

employed. A soft plastic material was placed under the sample which was then stamped with the die. A goniometer attached to the base of a rotating wooden disc allowed us to cut the ligaments parallel to the course of the collagen fibres. The cutting dies were machined out of steel which were subsequently hardened. The cutting edges were sharpened at an angle of 15°. In this way we obtained samples of 30 mm length, occasionally a little shorter, of 0.5 mm in thickness and 2 mm in width. The length of the sample was not critical as the distance between the testing clamps during the test was always 9 mm, the length-width ratio being 4.5 : 1.

TESTING EQUIPMENT

Two types of testing systems were employed in our tests. In the initial determinations of the whole ligament we employed the "Alwetron" Electronic Universal Testing Machine, T-2000, coupled to an XY-recorder unit, Philips Y-type. We found, however, that the recording device was inadequate. In all other tests we used the following system: Alwetron T-2000 coupled to a Hewlett Packard Moseley recorder model AM 7001 or AM 7030. In order to determine the accuracy of the recording device we coupled the system with an oscilloscope (Textronix type I AG) equipped with a Polaroid camera. (Fig. 3 and 4.) The curves obtained with the recorder were compared to those obtained with the oscilloscope and they were found to be identical. In trying to determine the breaking point we employed a 50 Kg load cell with a 0.1 per cent accuracy. In the other experiments we used a 1 Kg load cell with a 005. per cent accuracy. The rate of loading was varied from 0.5 mm/min. to 50 mm/min. Most tests were conducted at a strain rate of 5 mm/min.

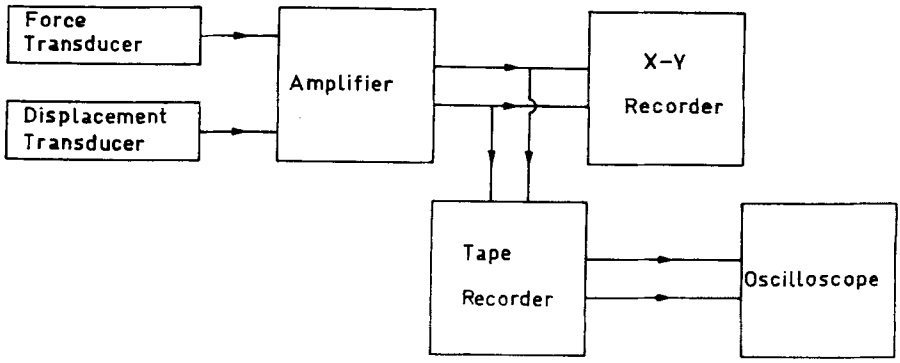


Fig. 4. Block diagram of the recording system.

HIGH HUMIDITY CHAMBER

After preliminary tests on ligaments exposed to air we decided that, in order to minimize the water loss which affects the physical properties of the tissue, it would be necessary to provide a high humidity environment. Thus we had a special humidity chamber constructed out of Plexiglass which surrounded that part of the testing machine in which the test sample was placed. This humidity chamber was connected to a "Defensor" humidifier which can produce 80 m^3 of saturated air per hour. This machine raises the relative humidity in the chamber to 100 per cent within 5 minutes. The humidity, as well as the temperature, of the air within the chamber was measured at the level of the test sample with a hygrometer and a thermometer. Although condensation can sometimes be observed on the chamber walls, experiments (see page 36) have shown that this does not result in up-take of water by the sample. A second high-humidity chamber, which was equipped with a high output humidifier ("Defensor"), was used during the preparation of samples. The front wall of this chamber was made of glass and this allowed us to prepare the ligaments under visual control with an environmental humidity of 100 per cent. The humidity was measured with a hygrometer (Inor) which was suspended at the level of the preparations. During the transfer of the ligaments from the high humidity chamber to the microtome and from the microtome to the testing machine, the ligaments were wrapped in a polyethylene sheet.

CLAMPS

Ligaments have a tendency to slip out of clamps and thus it was necessary to investigate different clamping procedures. After carrying out experiments with a number of clamps, we finally adopted a clamp which is closed by means of a

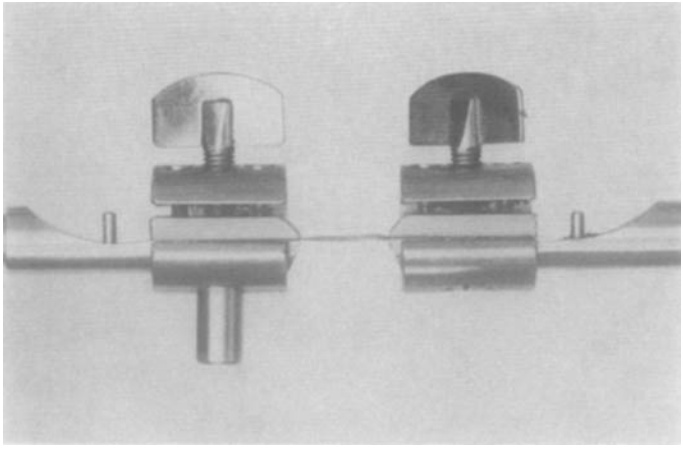


Fig. 5. The test clamps.

butterfly screw on the side. (Fig. 5.) The samples were examined with the dissection microscope both while they were being loaded and also after loading. The analysis of the load/deformation curves did not show any slipping of the samples and loading of the samples to the point of rupture resulted in the rupture of the ligaments in the free space between the clamps.

METHODS OF TESTING

We carried out the following investigations in order to describe the tensile characteristics of the longitudinal ligaments:

a) Determination of the breaking point of segments of ligaments of full dimension as well as samples of identical size.
b) Repetitive loading of samples of identical size.
c) The determination of the preloading point.
d) Determination of the elastic properties of the ligament and their relationship to deformation changes in the lumbar spine, the level from which the ligament was taken and the age.

Experiments in which the samples were exposed to room air, were carried out at a temperature between 19—24°C and at a relative humidity between 60—70 per cent. The studies in the high humidity chamber were performed in saturated air at 23—28°C.

In the determination of the preloading point, specimens were loaded at a rate of 0.5 to 50 mm/min. in the range 10—1000 gm.

In the load tests carried out with the samples of identical size, we loaded and unloaded the samples successively with 500 gm at 35-second intervals. We obtained three significantly differing curves (see page 40). In the studies designed to determine the elastic properties of the ligaments in relation to degenerative changes and age, the samples were placed in a high humidity chamber and loaded to 500 gm. After one minute we measured the stress relaxation, i.e. the decrease in stress at a constant deformation. Then, after waiting a further three minutes, we loaded the sample again. After a certain amount of time, which was arbitrarily chosen to be one minute, the sample was loaded again, then immediately unloaded and in this way we obtained curves which showed very small residual deformation. The determination was repeated once more to see whether the curves were repeatable and whether all the parameters of the curve were identical. In order to determine whether one could rely on the results obtained from the sample, we carried out the same test on one or two more samples taken from the same segment of the ligament. The results were compared and, if the curves did not correlate, this particular study of the test sample was discarded and not included for analysis in our material.

EVALUATION OF THE CURVES

The following parameters were evaluated from the load/deformation curves:

The total deformation during loading, the residual deformation in the sample and the energy dissipation. The deformation was calculated from the curve on the basis of simple measurement and expressed in mm. Energy dissipation was calculated with the aid of a planimeter (Amsler, accuracy of 1/10th cm^2) from the area contained between the loading and the unloading curve. The resulting energy loss is calculated in gm-mm. The failure load of the samples was measured from the graphs and expressed in Kg.

HISTOLOGICAL STUDIES

Histological studies were carried out on longitudinal and transverse sections of the anterior and posterior portions of the annulus fibrosus. Sections of the anterior and posterior longitudinal ligaments were obtained at the disc level as well as at the level of the vertebral bodies. In order to prevent buckling of the ligaments on removal from the spine they were fastened to a rigid base. Ligaments which were previously tested by loading were similarly fastened and fixed in 10 per cent formalin. After fixation the disc material, as well as the sections from the ligaments, were treated by standard histological methods and stained with Eosin and hematoxyline. In those experiments in which we studied the behaviour of collagen bundles under the influence of different loads, we used very thin sections of unstained tissue. These ligaments were stretched under the microscope by means of special clamps which were loaded with weights varying from 10—100 gm. In subsequent experiments ligaments were stretched in the "Alwetron" machine to a predetermined load and then compressed between two glass plates before being photographed under the microscope.

RADIOLOGICAL EXAMINATIONS

All lumbar spines obtained were X-rayed in both the anteroposterior and lateral projections. The roentgenograms so obtained were analyzed and graded according to the degeneration. For this analysis the disc height and lipping were taken as criteria. Occasionally we found spina bifida, spondylosis and old fractures in the vertebral bodies. The radiological grading was subsequently correlated with the results obtained by histological methods.

COLLECTION AND RECORDING OF DATA

All experimental data were tabulated and subsequently analyzed statistically employing standard statistical methods. Where data were being collected in order to determine the correlation between the elasticity and the degenerative changes

and ageing, we employed punch-cards. On these cards we recorded the results obtained from our curves, radiological examinations, as well as histological studies of the disc and of the ligaments before and after loading.

Statistical methods

The statistical methods employed in this work are the following:

1. In studying samples exposed to air and samples placed in different solutions sign tests have been performed.

2. In comparing samples from anterior and posterior ligaments and in comparing samples from degenerated and non-degenerated specimens Students t-test has been applied to the differences between measurements from samples that have been considered as paired. This technique has also been employed in the comparison between frozen and control samples and in comparing the sample weights before and after placing them in a high humidity chamber.

3. In order to investigate the influence of age and level, regression equations have been fitted to the data by means of the "method of least squares".

4. Graphical illustrations in air-exposure experiments and "swelling curves" are fitted to the computed means but the age dependence graphs are products of the "method of least squares".

The sign test and t-test, when applied to differences, both serve as tools to test whether there are different means in two groups or not. Both methods require every observation in one group to be matched to an observation in the other group.

In the air-exposure and swelling experiments the observations in the two groups are actually from the same samples before and after "treatment" and clearly paired. In the other comparisons observations have been considered paired. The t-test performed on differences from such pairs then shows whether the mean difference should be considered as zero.

The sign test does not take into account the value of the difference but only its sign. Under the necessary assumption of differences being normally distributed, the t-test always shows a significant difference between group means if a sign test does (the converse not being true). Consequently when a sign test showed a significant difference the more involved computation work of the t-test was not performed.

The "method of least squares" is the method of fitting a line to given data so as to minimize the sum of squared deviations from this line, e.g. assuming a model:

$$Y = C_0 + C_1 X_1 + C_2 X_2 + \ldots\ldots$$

determine the coefficients C_0, C_1, C_2 . . . . . .

so that the sum of all squared deviations

$$(Y-C_0-C_1X_1-C_2X_2-\ldots\ldots)^2$$

has a minimum value.

To test whether the coefficients differ significantly from zero or not, t-tests are carried out.

All computation work involving the "method of least squares" has been performed in a computer.

V. DISCUSSION OF METHODS

Water loss of samples exposed to air

In our initial investigations of the tensile properties of the longitudinal ligaments of the lumbar spine we observed that the physical properties of these ligaments could change rapidly with dehydration. When we introduced samples of small dimensions (1 mm^2 in cross section), this led to an even more rapid change in their physical properties as the rate of water loss was greater. We observed that samples exposed to air for 8 minutes gave different curves from those obtained with fresh tissue. In order to determine the rate and the amount of water loss we carried out the following experiment:

From two lumbar spines (nos. 1,2 Table I), which were in a high humidity chamber, we obtained 14 samples of the following dimensions; $20 \times 2 \times 0.5$ mm. Each sample was immediately enveloped tightly in polyethylene and stored in this fashion until it was weighed. The samples were weighed on a precision balance (E. Mettler type H 16). These were subsequently exposed to air. During the exposure the relative humidity was 60—70 per cent and the air-temperature 20°C. Six samples were weighed at 3 minute intervals during a period of 30 minutes (Table III). Eight samples were weighed at 5, 10 and 30 minutes after initial exposure (Table IV).

Results:

TABLE III

Series of 6 samples

Minutes	0	3	6	9	12	15
Mean weight (gm)	0.0446	0.0432	0.0421	0.0408	0.0396	0.0388
Standard deviation	0.0010	0.0014	0.0015	0.0015	0.0018	0.0014

Minutes	18	21	24	27	30	
Mean weight (gm)	0.0375	0.0367	0.0359	0.0350	0.0341	
Standard deviation	0.0013	0.0013	0.0014	0.0015	0.0016	

TABLE IV

Series of 8 samples

Minutes	0	5	10	30
Mean weight (gm)	0.0424	0.0388	0.0369	0.0320
Standard deviation	0.0010	0.0016	0.0012	0.0012

To test the hypothesis that there is no effect of exposing the samples to air, a sign test was performed. This was applied on the initial weight and on the first weight after exposure to air.

Since every sample in both series has decreased in weight the hypothesis of no effect is rejected in both cases. Water loss of samples is presented graphically (Fig. 6).

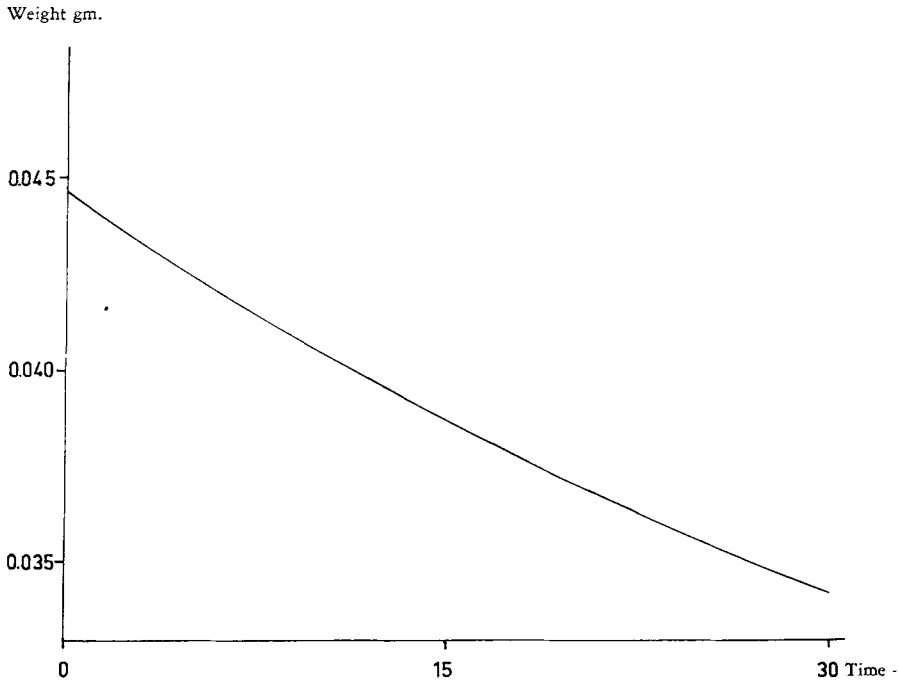


Fig. 6. Loss of weight of samples in air at 64 per cent relative humidity and 20°C temperature.

CONCLUSION

The drying of small samples, which we employed in the testing of the tensile properties of ligaments, takes place very rapidly. During the test procedure, prolonged exposure to air at 60—70 per cent relative humidity and 20°C temperature led to a much more significant water loss from the samples.

BEHAVIOUR OF THE ANTERIOR AND POSTERIOR LONGITUDINAL LIGAMENTS IN DIFFERENT SOLUTIONS

In order to find out if the water loss from the samples could be prevented by means of immersion in different solutions such as distilled water, Ringer solution, human plasma and Macrodex we carried out the following experiments.

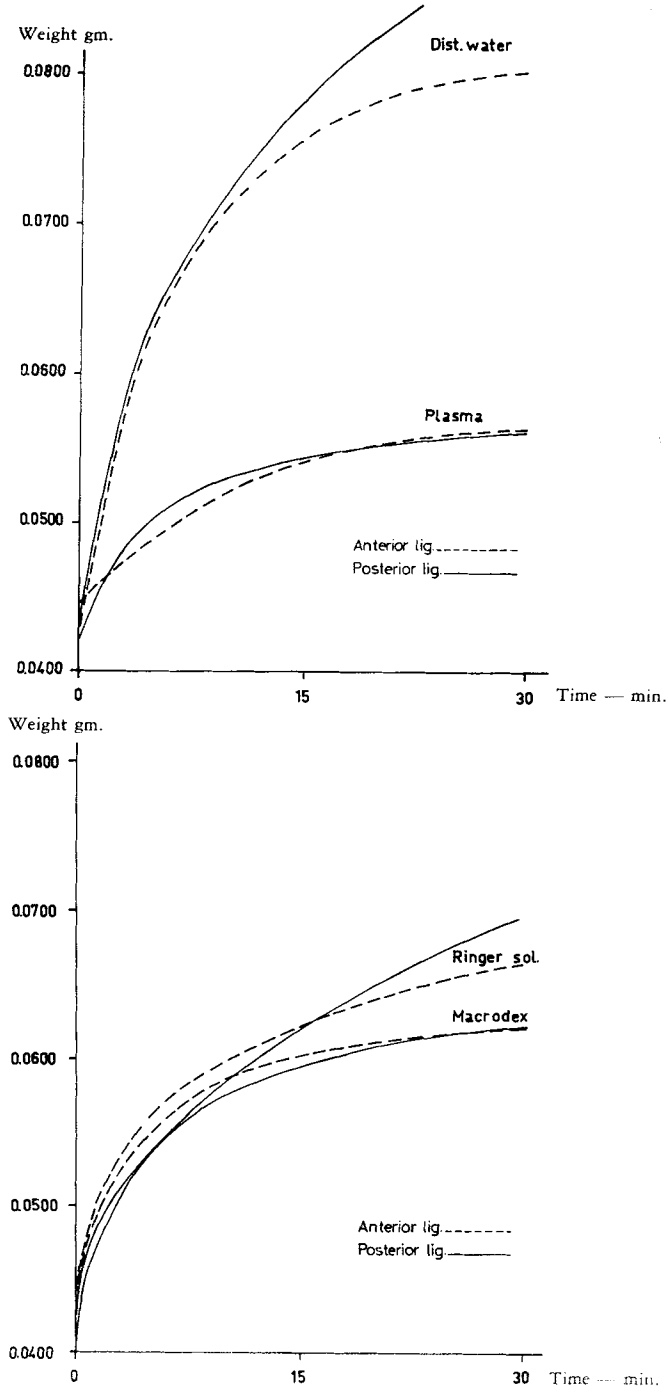


Fig. 7. Swelling curves of samples of anterior and posterior longitudinal ligaments in different solutions.

We obtained 48 samples of dimension 20×2×0.5 mm from four lumbar spines (nos. 5, 6, 7, 8; Table I). The samples were divided into two groups of 24, one group consisting of samples taken from the anterior longitudinal ligament and the second group of samples consisting of those taken from the posterior longitudinal ligament. In order to have objective results and not rely on data obtained from one lumbar spine, we then mixed the samples (within the groups anterior and posterior) and divided them into eight groups of six. This gave us four groups each consisting of six samples from the anterior longitudinal ligament and four groups consisting of six of the posterior longitudinal ligament. These units of six we shall refer to as sub-groups. Each sub-group was then tested in the following solutions: Ringer solution, human plasma, Macrodex and distilled water. The experiment was carried out separately on the sub-groups comprising the anterior longitudinal ligament and those comprising the posterior longitudinal ligament. A sample, after it was removed from the solution, was placed between two sheets of Munktell filter paper no. 3 for five seconds in order to remove the superficial solution and these were subsequently weighed at intervals of 3, 6, 9, 12, 30 and 60 minutes. Immediately after weighing the samples were reimmersed in their respective solutions. The results obtained after three minutes of immersion were analyzed statistically and compared with the initial weight. A graphical correlation was also carried out between the results obtained in samples from the anterior longitudinal ligament and the posterior longitudinal ligament. The fluid uptake of the samples was plotted on a graph which gave us "swelling curves" (Fig. 7).

Results:

TABLE V

Means (M) and Standard deviations (S.D.) for samples in different solutions.
All figures computed from data of six samples and expressed in gm.
All figures are to be multiplied by 10^{-4}.

Water

	0 min.		3 min.		6 min.		9 min.		12 min.		30 min.	
	M	S.D	M	S.D	M	S.D	M	S.D	M	S.D	M	S.D
ant.	428	12	499	17	602	30	645	32	693	27	803	24
post.	432	7	524	17	619	17	657	16	689	13	886	22

Ringer

ant.	433	13	514	18	540	15	569	17	578	17	658	21
post.	418	13	498	12	518	13	549	15	563	17	685	17

Plasma

ant.	440	14	462	13	472	12	505	13	516	12	557	19
post.	413	11	460	12	489	12	511	15	519	12	558	12

Macrodex

ant.	430	15	504	15	530	19	553	17	569	16	619	16
post.	410	11	474	17	518	17	553	23	563	23	621	27

To test whether there is a significant difference between mean weight at the beginning of the experiment and after three minutes in a particular solution, a sign test has been performed.

Since every sample in every experiment has increased in weight the sign test shows a significant difference between the weight of the sample before and after placing in the solution.

CONCLUSION

There was no difference between the fluid uptake of samples obtained from the anterior longitudinal ligament and from the posterior longitudinal ligament. A significant increase in the weight of the samples takes place after three minutes of immersion in distilled water, Ringer solution, human plasma or Macrodex.

Distilled water was most rapidly taken up by the samples; human plasma and Macrodex showed the lowest uptake (see Fig. 7). The fluid uptake was so rapid and so great that storage of ligament samples by wrapping them in gauze previously soaked in some solution could not be employed.

Water loss from samples in the high humidity chamber

The experiments which were carried out in order to determine the water loss from samples exposed to air (at 60—70 per cent relative humidy and 20—24°C) as well as the experiments on the solution uptake of samples led to the conclusion that environmental conditions must be created which would lead to the following:

1. No water loss from the sample during the test procedure.
2. No fluid uptake of the sample during the test procedure.

Such environmental conditions can be created if the test is carried out in air which is saturated with water vapour. In order to prove that such environmental conditions existed in a high humidity chamber and thus would meet our two test requirements the following experiment was carried out.

From two lumbar spine (Table I nos. 3, 4) ten samples were obtained from the anterior longitudinal ligament and ten from the posterior longitudinal ligament.

After complete mixing, ten of these were placed in the high humidity test chamber and ten in the high humidity preparation chamber. The humidity in these chambers was continuously monitored during these experiments and the environment immediately surrounding the sample was maintained at 100 per cent relative humidity. In the test chamber the temperature was 26°C and, in the preparation chamber, 24°C. The samples were maintained in each chamber for a period of one hour. After 10 minutes in the test chamber we noticed considerable condensation occurring on the walls of the chamber. We thought that similar condensation could be occurring on the samples which might lead to a subsequent uptake of water. In order to check whether this phenomenon was taking place as well as to determine whether any water loss occurred while the samples were in the humidity chamber, all samples were removed after one hour and immediately weighed.

RESULTS

For each sample the weights before and after the experiment were determined and the results were analyzed by means of a t-test.
For the 20 differences it was found that:

Mean difference = 0.000015

Standard deviation of differences = 0.000080

This yields a t-value of 0.83 which is obviously not significant.

CONCLUSION

In a high humidity chamber containing air of 100 per cent relative humidity at a temperature of approximately 25°C, both water loss and water uptake by the sample can be prevented. The condensation on the walls of the chamber did not lead to a similar condensation on the sample.

Influence of rapid freezing and thawing on the tensile properties of the ligaments

In order to obtain sheets of uniform thickness our material was frozen at a temperature of —60°C. As we felt that this sudden freezing and thawing might influence the physical properties of the longitudinal ligaments we performed the following experiments:

Experiment 1

The anterior and posterior longitudinal ligaments of two lumbar spines (Table I, nos. 9, 10) were prepared in a high humidity chamber. These were then divided into segments corresponding to lumbar levels. Each sample was carefully divided

(along the longitudinal axis) into two halves. One half was kept in a high humidity chamber whilst the other half was immediately transferred to the Cryo-microtome where it was frozen to —60°C with carbon dioxide snow. Subsequently it was taken back to the high humidity chamber and allowed to thaw. The thickness of the corresponding halves was then measured. Once it was established that both halves had the same thickness we obtained two samples from each half. In this way we had two samples from both the frozen and control halves of the anterior longitudinal ligaments at the levels of L5, L4, L3 and L2. From the posterior longitudinal ligaments we were able to get only four samples, two from the level of L3 and two from L4 respectively, as the other levels were not wide enough to allow for sampling. Samples thus obtained were then mechanically tested in a high humidity chamber and the resulting data were compared. The parameters of the load/deformation curves from corresponding levels of the lumbar spine were very similar. The curves differed systematically and this was related to the difference in the thickness of the ligament at the different levels of the lumbar spine.

TABLE VI

(Figures are shown ± S.D.)

	before freezing	after freezing	n	t
Elongation mm	0.59 ± 0.037	0.59 ± 0.037	20	0.68
Residual deformation mm	0.16 ± 0.033	0.16 ± 0.43	20	0.57
Energy dissipation gm. mm	4.18 ± 0.51	4.17 ± 0.49	20	1.0

CONCLUSION

Rapid freezing of the ligaments to —60°C with subsequent thawing does not influence the tensile property of these ligaments.

Experiment 2

Using our routine method we prepared six samples from both the anterior and posterior ligaments of a lumbar spine (Table I, no. 11). The samples were then tested. In this way we got repeatable curves. Subsequently, without removing the sample from the clamps it was frozen with the dioxide snow and immediately thawed (the whole manoeuvre took about three minutes to carry out). The sample, together with the clamps, was then reinserted in the test machine and two successive loadings were carried out. The curves thus obtained did not differ from the previous curves.

CONCLUSION

Rapid freezing of ligaments to a temperature of —60°C and subsequent thawing does not influence their tensile properties.

VI. TENSILE CHARACTERISTICS OF LONGITUDINAL LIGAMENTS

Studies on the loading capacity of ligaments of full dimensions

From three lumbar spines (Table I. nos. 67, 68, 72) the longitudinal ligaments were obtained. The ligaments were then divided into segments corresponding to the vertebral body or disc levels. These samples were then studied to determine their loading capacity. The clamps were so adjusted that the distance between them was 10 mm. The ligaments were loaded until they tore and a continuous record was made using a Philips XY-recorder. Although 22 segments of ligaments were tested only 14 were suitable for analysis. The remainder had to be rejected because the ligaments slipped out of the clamps or tore at the point where they were held in the clamps. The results are shown in table VII.

TABLE VII

No. of specimen (Table I)	Age Years	Level	Anterior ligament		Posterior ligament	
			Yield load Kg	Failure load Kg	Yield load Kg	Failure load Kg
67	36	L5	50	64	15.5	16
67	36	L4 disc	23	23	—	—
67	36	L3 disc	—	—	10	11
68	36	L5	46	54	—	—
68	36	L4 disc	15	22	18	20
68	36	L3	—	—	22	25
72	58	L5	20	32	20	22
72	58	L4 disc	10	20	15.5	16
72	58	L3 disc	18	21	14	17

CONCLUSION

Because of the difference in the dimensions of the ligaments we obtained results which differed greatly. We could assume that the anterior and posterior longitudinal ligaments are slightly weaker at the level of the disc. It also appears that there is a greater difference between the yield-point and the failure-point for the anterior longitudinal ligament than for the posterior longitudinal ligament. The posterior longitudinal ligament ruptures almost immediately after the yield-point.

Studies on the loading capacity of samples of equal dimensions

From three lumbar spines (Table I, nos. 66, 73, 74) we obtained samples of standard dimensions. The samples were wider at their ends ("dumb-bell-shaped") to facilitate their insertion into the clamps. The significant dimensions were thickness (0.5 mm) and width (2 mm) at the mid-section. The distance between the clamps was always 9 mm. The samples were subsequently loaded to failure. Of 30 studies 26 were suitable for analysis (see Table VIII). The remainder, either because of slippage from the clamps or tearing at the clamps, were considered unsuitable for analysis and were rejected. These experiments were carried out in order to assess the load level to be used in our subsequent experiments on the elastic properties of the ligaments.

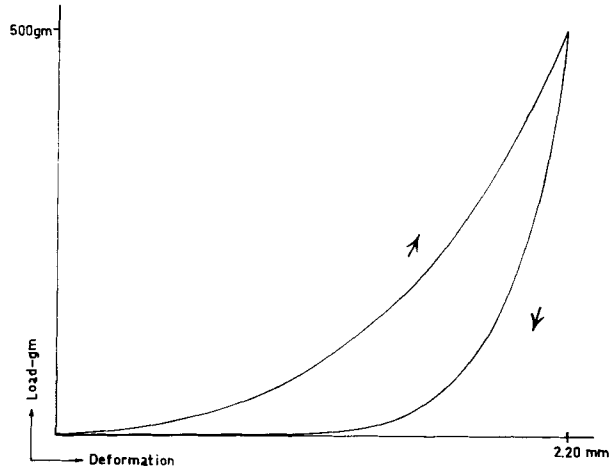
TABLE VIII

Number of specimen (Table I)	Age Years	Level	Lig. ant.		Lig. post.	
			Yield Kg	Failure load Kg	Yield Kg	Failure load Kg
66	55	L2disc	1.6	1.9	1.6	2.1
66	55	L3	1.7	2.1	2.1	2.2
66	55	L4	1.7	2.3	1.8	1.9
66	55	L5	2.1	2.3	2.0	1.8
			1.77	2.15	1.87	2.0
74	64	L1	1.7	1.8	1.6	1.7
74	64	L2disc	1.9	2.1	1.9	1.9
74	64	L4	1.7	1.8	1.7	1.8
74	64	L5	1.8	1.9	1.8	1.9
			1.77	1.90	1.75	1.83
73	26	L1	1.7	2.1	1.6	2.0
73	26	L2disc	1.8	2.0	1.7	1.9
73	26	L3	1.8	2.3	1.9	2.1
73	26	L4	2.0	2.5	1.8	2.3
73	26	L5	1.9	2.6	2.1	2.3
			1.84	2.30	1.82	2.12
		Sample average:	1.79	2.12	1.81	1.98

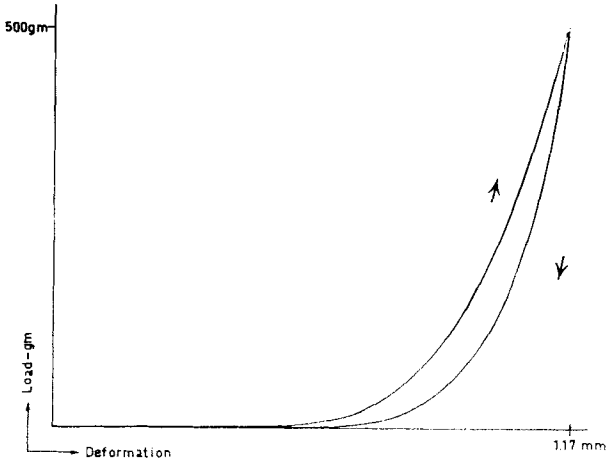
CONCLUSION

The average failure load of standard samples of the anterior longitudinal ligaments was 2.12 Kg and, of the posterior longitudinal ligaments, 1.98 Kg. The lowest failure load for the anterior longitudinal ligament was 1.8 Kg and, for the posterior, 1.7 Kg. From this we concluded that, for subsequent experiments, a loading of 0.5 Kg should be used which represented about 30 per cent of the failure load of the weakest sample used in the previous analysis and only 25 per cent of the average failure load of both the anterior and posterior longitudinal ligaments. There was an obvious difference between specimens from young and

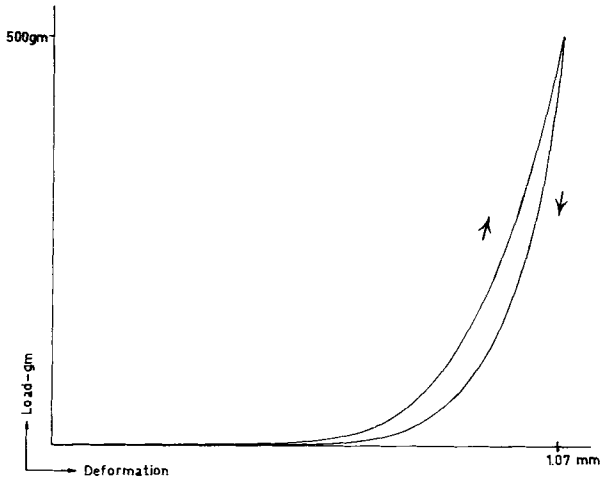
Fig. 8. Load cycling. Sample load/deformation curves obtained at 35-second intervals.



a) First cycle.



b) Second cycle.



c) Third cycle.

old spines. The failure loads for a young spine (26 years) were 2.30 Kg for the anterior ligament and 2.12 Kg for the posterior ligament, whereas, for an old spine (64 years) the corresponding values were 1.90 Kg and 1.83 Kg respectively. There is no difference between the loading capacity of samples taken from the body and disc levels.

Load tests repeated at 35-second intervals

It is generally known that repeated loading of ligaments, tendons or segments of the annulus fibrosus produces, with each repeated loading, a more elastic response (Virgin 1951, Rigby 1964, Rolander 1966, Viidik 1966, Galante 1967). In order to determine the effect of repeated loading on the ligaments, all samples were cyclically loaded three times between 0 and 500 gm at 35-second intervals. For these determinations a total of 50 samples of the anterior longitudinal ligament and 55 samples of the posterior longitudinal ligament were prepared from spines nos. 13—33, Table I.

RESULTS

The results show that there was a great difference between the curves obtained from each of the three load cycles. (Fig. 8 a, b, c.) Both the residual deformation and maximum deformation were seen to decrease with each successive load cycle. In order to assist comparison we have forced the mean values of the parameters, obtained during the third load cycle, to be unity and the following data have resulted:

TABLE IX

Comparison between mean parameters (105 samples)

	1st load cycle	2nd load cycle	3rd load cycle
Deformation			
Anterior	1.865	1.113	1.00
Posterior	1.815	1.107	1.00
Residual deformation			
Anterior	3.501	1.264	1.00
Posterior	3.864	1.242	1.00
Energy dissipation			
Anterior	2.766	1.199	1.00
Posterior	2.824	1.207	1.00

To compare the tensile properties of samples from anterior and posterior ligaments the following analysis was carried out. 20 pairs of ligaments were tested, each pair comprising a sample from the posterior and anterior ligaments of the same segment. The differences betweeen measurements from anterior and posterior samples were calculated. These differences were analyzed by means of a t-test to investigate whether the mean difference could be considered as zero. Such a comparison has been made for maximum deformation, residual deformation and energy dissipation for each of the three load cycles.

TABLE X

Deformation (mm)

	1st load cycle	2nd load cycle	3rd load cycle
Mean difference between lig. ant. and post. (paired observ.)	0.1640	0.0105	0.0490
Standard error of differences	0.25	0.17	0.18
Computed t-value	2.67	0.24	1.01

TABLE XI

Residual deformation (mm)

	1st load cycle	2nd load cycle	3rd load cycle
Mean difference between lig. ant. and post. (paired observ.)	0.1485	0.0815	0.0590
Standard error of differences	0.27	0.09	0.06
Computed t-value	2.23	3.39	2.96

TABLE XII

Energy dissipation (gm-mm)

	1st load cycle	2nd load cycle	3rd load cycle
Mean difference between lig. ant. and post. (paired observ.)	2.1650	0.9700	0.9300
Standard error of differences	3.7	2.0	1.4
Computed t-value	2.40	1.88	2.40

CONCLUSION

The results show that there are significant differences between the parameters obtained from the anterior and posterior longitudinal ligaments although these differences decrease in the second and third load cycles.

Comparing the mean parameters of each load cycle for the anterior and posterior ligaments showed that there was a similar proportional change between cycles.

VII. STUDIES TO OBTAIN THE PRELOADING POINT FOR LONGITUDINAL LIGAMENTS OF THE LUMBAR SPINE

Investigation of shrinkage of the ligaments

The parameters which we obtained from repeated load tests led us to doubt if a material which exhibited such significant residual deformation and large energy dissipation could perform a physiological function. For example, a residual strain greater than 10 per cent would make the ligament mechanically useless in the living organism. These results, and the fact that the ligaments shrank on removal from the spine, suggested that a more comprehensive study should be made. A whole series of queries arises but the most obvious is the influence of death on the mechanical properties of the ligaments. At the moment this question can only be answered hypothetically as we are in no position to prove our points. However, if we consider the ligament as a visco-elastic substance and if we analyze it from a theoretical point of view then, within the ligament there must exist a certain fluid pressure during life. This pressure is maintained by means of a complex equilibration system with the pressure within the circulatory system. The moment the heart stops beating and the circulatory pressure drops to zero the system reequilibrates and the internal pressure within the ligament may also drop, thus affecting the mechanical properties of the tissue. The fact that a ligament shrinks or shortens immediately after it is obtained from the lumbar spine indicates that this ligaments exists in a state of prestress. To what extent the prestress of the ligament changes with death or preparation is difficult to assess, however, it is possible to estimate this prestress by performing experiments on fresh autopsy specimens.

Experiment 1

Ten randomly selected lumbar spines were tested in the following manner. On the surface of the ligaments at different lumbar levels, pairs of points were marked and the distance between each pair of points was then carefully measured. In each segment no attention was paid to the position of the gauge length relative to the disc and vertebral body. The ligaments were then removed from the lumbar spine and the separation of the previously marked points was again measured. In every case the ligaments shortened by a few mm.

Results: See Table XIII.

TABLE XIII

Amount of shrinkage

No.	Lig.	Age	Level	Dimension before shrinkage mm	Dimension after shrinkage mm	Shrinkage per cent	Average for each ligament	
							mm	per cent
1	Ant.	64	L-5	30	28	6.7		
1	,,	64	L-4	30	28	6.7		
1	,,	64	L-3	30	27.5	8.3		
1	,,	64	L-2	30	27	10,0	2,4	7,9
1	Post.	64	L-5	30	28	6.7		
1	,,	64	L-4	30	28	6.7		
1	,,	64	L-2	30	27	10.0	2.3	7.8
2	Ant.	2.5	L-5	20	17	15.0		
2	,,	2.5	L-3	20	17	15.0		
2	,,	2.5	L-2	20	18	10.0	2.7	13.3
2	Post.	2.5	L-5	20	16	20.0		
2	,,	2.5	L-3	20	16	20.0		
2	,,	2.5	L-2	20	17	15.0	3.7	18.3
3	Ant.	55	L-5	30	28	6.7		
3	,,	55	L-4	30	28	6.7		
3	,,	55	L-3	30	27.5	8.3		
3	,,	55	L-2	30	27	10.0	2.4	7.9
3	Post.	55	L-5	30	27	10.0		
3	,,	55	L-4	30	27.5	8.3		
3	,,	55	L-3	30	26	13.3		
3	,,	55	L-2	30	27	10.0	3.1	10.4
4	Ant.	17	L-4	30	27	10.0		
4	,,	17	L-3	30	27	10.0		
4	,,	17	L-2	30	26	13.3	3.3	11.1
4	Post.	17	L-4	30	26	13.3		
4	,,	17	L-3	30	26	13.3		
4	,,	17	L-2	30	26	13.3	4.0	13.3
5	Ant.	66	L-5	30	29	3.3		
5	,,	66	L-4	30	28	6.7		
5	,,	66	L-3	30	28	6.7		
5	,,	66	L-2	30	27.5	8.3	1.9	6.3
5	Post.	66	L-5	30	28	6.7		
5	,,	66	L-4	30	28	6.7		
5	,,	66	L-3	30	27	10.0		
5	,,	66	L-2	30	27	10.0	2.5	8.4
6	Ant.	58	L-5	30	27	10.0		
6	,,	58	L-4	30	28	6.7		
6	,,	58	L-3	30	28	6.7	2.3	7.8
6	Post.	58	L-5	30	27	10.0		
6	,,	58	L-4	30	26.5	11.7		
6	,,	58	L-3	30	27	10.0		
6	,,	58	L-2	30	27	10.0	3.1	10.4

No.	Lig.	Age	Level	Dimension before shrinkage mm	Dimension after shrinkage mm	Shrinkage per cent	Average for each ligament	
							mm	per cent
7	Ant.	22	L-5	30	27	10.0		
7	,,	22	L-4	30	27	10.0		
7	,,	22	L-3	30	27	10.0		
7	,,	22	L-2	30	28	6.7	2.8	9.2
7	Post.	22	L-5	30	26	13.3		
7	,,	22	L-4	30	26	13.3		
7	,,	22	L-3	30	26	13.3		
7	,,	22	L-2	30	28	6.7	3.5	11.7
8	Ant.	66	L-4	30	29	3.3		
8	,,	66	L-3	30	28	6.7		
8	,,	66	L-2	30	28	6.7	1.7	5.6
8	Post.	66	L-4	30	28	6.7		
8	,,	66	L-3	30	28	6.7		
8	,,	66	L-2	30	27	10.0	2.3	7.8
9	Ant.	4,5	L-5	20	17	15.0		
9	,,	4,5	L-3	20	18	10.0	2.5	12.5
9	Post.	4,5	L-5	20	17	15.0		
9	,,	4.5	L-3	20	17	15.0	3.0	15.0
10	Ant.	35	L-5	20	18	10.0		
10	,,	35	L-3	20	18	10.0		
10	,,	35	L-2	20	19	5.0	1.7	8.3
10	Post.	35	L-5	30	28	6.7		
10	,,	35	L-3	30	27	10.0		
10	,,	35	L-2	30	27	10.0	2.7	8.9

Average shrinkage for young without degenerative changes lig. ant. 10,9%
lig. post 13,4%
Average shrinkage for old with degenerative changes lig. ant. 7,1%
lig. post. 9,0%

Experiment 2

Two lumbar spines were used (nos. 42, 70, Table I). Two metal wires were inserted into both the anterior and posterior longitudinal ligaments in a direction perpendicular to the longitudinal axis of the fibres. This was done at the disc level of L3—2 and L5—4 and the spines were then X-rayed. The ligaments were sectioned above and below the wires and with the spine and the X-ray tube in the same relative position, a second radiogram was made. From the roentgenograms we obtained the changes in separation of the wires.
Results: See Table XIV.

TABLE XIV

		Distance between wires		Contraction Per cent
		Before separation mm	After separation mm	
Spec. No. 42	Lig. ant.	30	27	10
	Lig. post.	30	26	13.3
Spec. No. 70	Lig. ant.	30	28	6.6
	Lig. post.	30	27	10

CONCLUSION

The average amount of shrinkage obtained on sectioning ligaments at the disc level, in normal conditions, is the same as the shrinkage at the body vertebra level.

Experiment 3

For this experiment lumbar spine no. 73 (Table I) was used. This lumbar spine showed no degenerative changes as determined by means of radiological technique and microscopic and macroscopic examinations. A needle was inserted into the nucleus pulposus between L3—L2 and L4—L3 levels and about 0.3 cm^3 of water was injected into each. Employing the intradiscal pressure measuring device of Nachemson, it was determined that the intradiscal pressure was raised, by the injection of water, from its resting pressure of 0.5 Kg/cm^2 to 7 Kg/cm^2 at the level between L3—L2 and 5 Kg/cm^2 at the level between L4—L3. Two points were marked on each ligament corresponding to the discs and vertebral bodies at the above-mentioned levels. The distance between these two points was then measured. Subsequently the ligaments were cut and the distance between these points was again measured.

Results: See Table XV.

TABLE XV

	Distance between points		Difference mm	Per cent
	Before cut mm	After cut mm		
Lig. anterior				
L3-L2 disc level	23	16	7	30.4
L4-L3	20	15	5	25
L3 body vert. level	25	20	5	20
Lig. posterior				
L3-L2 disc level	20	10	10	50
L4-L3 ,, ,,	20	12	8	40
L3 body vert. level	25	15	10	40

In order to determine the shrinkage of ligaments from a segment of the lumbar spine where intradiscal pressure was not raised artificially, the ligaments were cut in similar fashion at the levels of the S.1—L5 disc and L5 body vertebra.

Results:

TABLE XVI

	Distance between points		Difference mm	Per cent
	Before cut mm	After cut mm		
Lig. anterior				
S1-L5 disc level	25	23	2	8
L5 body vert. level	25	23	2	8
Lig. posterior				
S1-L5 disc level	10	8.5	1.5	15
L5 body vert. level	25	22	3	12

The shrinkage of the ligaments from segments of this vertebral column, where the intradiscal pressure was not artificially raised, did not differ from the shrinkage observed in the samples of ligaments studied from segments of other vertebral columns in previous experiments.

CONCLUSION

Increase in the intradiscal pressure causes an increase in the prestress of the longitudinal ligaments of the vertebral column.

Experiment 4

Specimen no. 9, Table I.

Using a thick needle and instruments used routinely for the extirpation of the nucleus pulposus, the nuclei of the discs L4—L3 and L3—L2 were removed. At a distance of 3 cm two points were marked on both the anterior and posterior longitudinal ligaments at the level of the L3 vertebral body. In addition, two points were marked at 2 cm separation on both ligaments between the levels of L4—L3 and L3—L2. The ligaments were then cut and the distance between the points was again measured.

Results:

TABLE XVII

	Distance between points		Difference mm	Per cent
	Before cut mm	After cut mm		
Lig. anterior				
L3-L2 disc level	20	19	1	5
L4-L3 ,, ,,	20	19.5	0.5	2.5
L3 body ,,	20	19.5	0.5	2.5
Lig. posterior				
L3-L2 disc level	20	18.5	1.5	7.5
L4-L3 ,, ,,	20	19	1	5

On the level of S.1—L5, where the intradiscal pressure was not decreased artificially, the ligaments were cut and the amount of shrinkage was measured.

TABLE XVIII

	Distance between points		Difference mm	Per cent
	Before cut mm	After cut mm		
Lig. anterior				
S.1-L5 disc level	20	18.5	1.5	7.5
L5 body vert. level	30	28	2	6.6
Lig. posterior				
S.1-L5 disc level	20	17.5	2.5	12.5
L5 body vert. level	30	27	3	10

Decrease in the intradiscal pressure causes a decrease in the preload in the longitudinal ligaments.

General conclusions

The above experiments lead to the conclusion that the longitudinal ligaments exist in a prestressed state due to the condition of the intervertebral discs. On removal from the vertebral column, the ligaments shrink as they are no longer subjected to this tensile force. The amount of shrinkage or shortening following removal depends on the amount of prestress and this varies with the state of both the intervertebral disc and the annulus fibrosus. In young people, in whom the intervertebral disc as well as the annulus fibrosus is intact, the prestress is greater and thus the subsequent shrinkage or shortening is greater. As degenerative changes occur, with the concomitant destruction of the intervertebral disc, the prestress becomes lower which follows the known reduction in nuclear pressure.

Approximate prestress of the anterior and posterior longitudinal ligaments

Experiments carried out on the shortening of ligaments following their removal from the spine showed that the anterior and posterior longitudinal ligaments did not shorten equally. The anterior longitudinal ligaments shortened less than the posterior. In addition, we noted that degenerative changes of the intervertebral disc had a definite bearing on the subsequent shortening of the ligaments. Ligaments obtained from spines without degenerative changes, or those of grade 2 degeneration, shortened significantly more than those obtained from spines where the intervertebral discs were destroyed.

It was observed that, in the group of healthy or slightly degenerated spines, the anterior longitudinal ligament shortened following preparation by 10.9 per cent, and the posterior, by 13.4 per cent. Similarly, in the second group, that is those with degenerative changes of grade 3 or 4, the anterior longitudinal ligaments shortened by 7.1 per cent, and the posterior, by 9.0 per cent.

On the curves obtained from loading experiments the loads, which corresponded respectively to 10.9, 13.4, 7.1 and 9.0 per cent stretching of the samples, were determined (see Table XIX).

TABLE XIX

Samples of equal dimensions (1 mm²)

	Average shortening of lig. %		Corresponding load gm	
	Anterior	Posterior	Anterior	Posterior
Non-degenerated	10.9	13.4	20–25	50–65
Degenerated	7.1	9.0	10–20	45–50

As the above values of preload were determined for small samples of the ligaments, approximate calculations of total ligament preload were performed. These calculations, based on the average dimensions of the ligaments, gave us values for group 1 of 200—250 gm for the anterior longitudinal ligament and 225—350 gm for the posterior longitudinal ligament. Similarly, in group 2, 150—200 gm for the anterior and 225—250 gm for the posterior longitudinal ligament were the values obtained.

In order to check the above calculations, 12 samples of full ligament cross-section were loaded to 5 Kg. From two lumbar spines (nos. 42, 44, Table I) 6 samples were obtained from the anterior and 6 from the posterior longitudinal ligaments. (This represented three samples from each of the ligaments of each of the spines.) In one lumbar spine there were no degenerative changes present whereas, in the other, both macroscopic and microscopic changes were of the order of grades 3 or 4. From the curves obtained during the loading of these samples it was then possible to calculate the average value of force corresponding to 10.9, 13.4, 7.1 and 9.0 per cent strain respectively and these values were as follows:

TABLE XX

Samples of full cross-section

	Average shortening of lig. %		Corresponding load gm	
	Anterior	Posterior	Anterior	Posterior
Non-degenerated	10.9	13.4	180	300
Degenerated	7.1	9.0	120	180

CONCLUSION

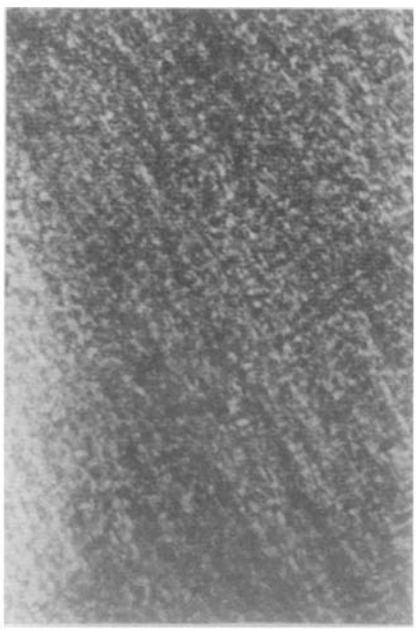
The exact determination of the preload value for the longitudinal ligaments is very complicated. Results may differ markedly since the dimensions of the ligaments vary. It is, however, possible to demonstrate that prestress exists and has a value between 120—350 gm. It appears that the prestress is dependent on the pressure within the nucleus pulposus and on the elasticity of the annulus fibrosus.

VIII. MICROSCOPIC INVESTIGATION OF THE LONGITUDINAL LIGAMENTS SUBJECTED TO LOADING

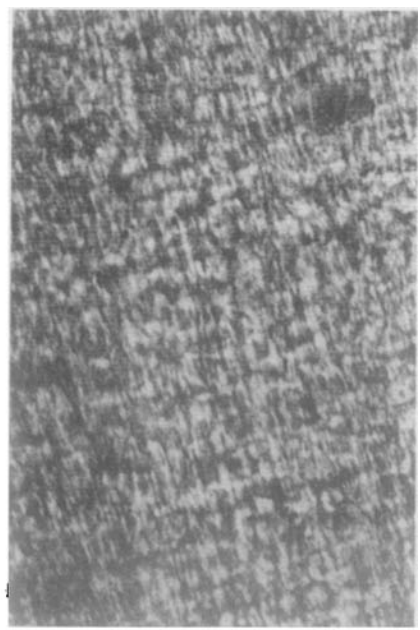
In order to determine what happens to the collagenous fibres of the ligaments during loading, the following investigations was made. Samples of 0.05 mm thickness were obtained from both the anterior and posterior longitudinal ligaments (specimen nos. 12, 51, 61, Table I). They were placed under a microscope and the following observations were recorded photographically using 100-fold magnification. The samples were subsequently stretched using small hooks arranged in such a way that the loading could be increased by the addition of weights. With each load increment a photograph of the specimen was taken.

It was noted that the collagenous fibres of fully relaxed ligaments were not visible. However, rows of light and dark spots were observed and these appeared to be arranged into regular stripes (Fig. 9 a, b, c, d). On initial loading the outlines of the fibres appeared and the light and dark spots began to vanish. With increased loading the outline of the collagenous fibres became more distinct and

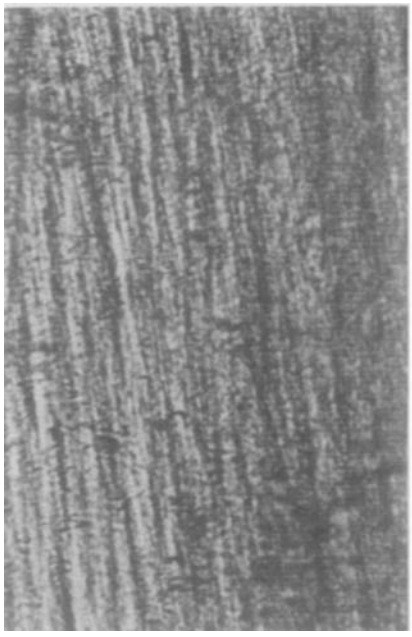
Fig. 9. Micrographs of the ligament subjected to uniaxial load (x 100)



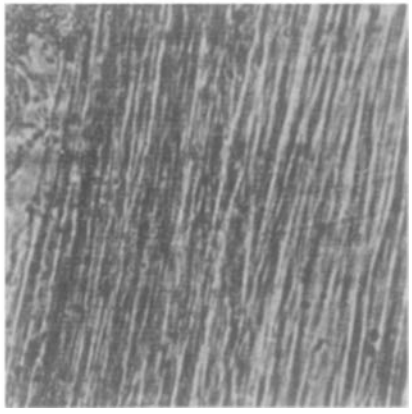
a/unloaded



b/approx. 15% failure stress



c/approx. 30% failure stress



d/approx. 60% failure stress

the light and dark spots vanished completely. As the loading is further increased one could see a distinct reorientation of the fibres into the line of the force. The fibres then began to glide upon one another until the sample tore.

CONCLUSION

It is possible to study, under an ordinary microscope, collagenous fibres without using stains which change the tensile properties of these fibres. Employing a system which allows for increments in loading, one can observe the behaviour of collagenous fibres subjected to increasing load. During loading the collagenous fibres rearrange themselves parallel to the axis of stress. The greater the tensile stress the more complete the rearrangement of the fibres. As the failure load is approached there is a relative gliding of fibres and this represents the breaking of the forces which hold the fibres together. It is only when these forces are broken that the fibres tear completely.

IX. STUDY OF THE TENSILE PROPERTIES OF THE LIGAMENTS AS RELATED TO DEGENERATIVE CHANGES, AGE AND LEVEL

The degeneration of the lumbar spine is a common process occurring in adult life. The earliest changes possible to find occur in the discs. Hirsch (1951, 1954), Nachemson (1960), Rolander (1966) and Galante (1967) have demonstrated that changes in the physical properties of the disc are a function of the degenerative process. In order to investigate the influence of degenerative processes and age on the tensile properties of the anterior and posterior longitudinal ligaments the following studies were performed on samples from cadavers 1 day to 67 years of age (Table I, nos. 34—64).

Effect of degeneration, age and level

206 samples from 30 lumbar spines were investigated.

In order to describe the degree of degeneration the material was graded macroscopically according to the criteria described under "Materials and Methods" (see page 27). Subsequently the roentgenograms were analyzed and a histological study was made of both the annulus fibrosus and the ligaments. The material was then divided into three groups using the following criteria.

Group 1. Those without degenerative changes.

Group 2. Those with degenerative changes qualifying for group 2 according to macroscopic criteria (see page 19) and without radiographic changes. Histologically, degenerative changes such as cavities filled with granulation material and partial cartilagenous metaplasia.

Group 3. Marked degenerative changes visible macroscopically qualifying for group 3 and 4 (see page 19). Radiologically; osteophytes as well as narrowing of intervertebral discs. Histologically; fissures in the annulus which are filled with vascularized fibrous tissue and pronounced cartilage metaplasia (Hirsch and Schajowicz 1952).

To analyze the tensile properties in samples from non-degenerated and degenerated specimens the following computations have been made. 143 non-degenerated samples and 63 degenerated samples have been subjected to multiple regression analysis.

This analysis yields an equation of the form:

$$Y = C_0 + C_1 A + C_2 A^2 + C_3 L$$

with regression coefficients C_1, C_2, C_3 showing the influence of age (A) and level (L).

To detect discrepances resulting from the linear influence of age, a term consisting of the square of A has been put into the model.
The dependent variable Y has been subsequently calculated from values of deformation, residual deformation and energy dissipation.
The results are as follows:

TABLE XXI

Deformation mm

Non-degenerated $Y = 0.64 - \underline{0.0072}\ A + \underline{0.00007}\ A^2 + \underline{0.0259}\ L$

Degenerated $Y = 8.68 - \underline{0.26}\ A + \underline{0.0002}\ A^2 + 0.01\ L$

Residual deformation mm

Non-degenerated $Y = 0.17 - \underline{0.0018}\ A + 0.00001\ A^2 + \underline{0.0066}\ L$

Degenerated $Y = 1.82 - 0.05\ A + 0.0004\ A^2 + \underline{0.005}\ L$

Energy dissipation gm-mm

Non-degenerated $Y = 4.12 - \underline{0.04}\ A + 0.0002\ A^2 + \underline{0.17}\ L$

Degenerated $Y - 35.76 - 1.04\ A + 0{,}008\ A^2 + \underline{0.15}\ L$

CONCLUSION

The underlined regression coefficients are significantly different from zero at the 5 per cent level. In the non-degenerated material the coefficients for age and level are always significant and the coefficient for A^2 is just significant for deformation.

An analysis of variance for the whole regression model, that is comparing the sum of squares due to regression with the sum of squared deviations from the estimated regression equation, showed that the variance is significant for every measurement in the non-degenerated material but not in the degenerated material. This is due to the spread in the measurements and the fact that a very restricted interval of age was considered.

Effect of degeneration

TABLE XXII

Means and standard errors of the results from degenerated and non-degenerated samples in the age range 45—70 years.

	Degenerated	Non-degenerated
Deformation mm		
Mean	0.46	0.53
S.E.	0.068	0.076
Residual deformation mm		
Mean	0.12	0.13
S.E.	0.026	0.024
Energy dissipation gm-mm		
Mean	3.11	3.33
S.E.	0.68	0.61

These are the means and standard errors calculated with no attention paid to differences between anterior and posterior ligaments, age or level. They make one suspect that there are differences between degenerated and non-degenerated samples. To analyze such a difference, six samples from degenerated specimens were selected. These were paired with six samples from the non-degenerated material. The purposes of pairing are as follows:

The samples were from specimens of the same age and the same level and the anterior and posterior ligaments corresponded.

For each parameter the differences between the non-degenerated samples and the degenerated samples were calculated.

To test whether the mean difference could be considered as zero a t-test was performed.

The results of these test procedures are as follows:

TABLE XXIII

	Deformation mm	Residual deformation mm	Energy dissipation gm-mm
Mean difference	0.14	0.03	0.6
Standard error of difference	0.07	0.01	0.95
Computed t-value	5.12	8.85	1.47

CONCLUSION

Deformation and residual deformation were significantly larger in the non-degenerated group of samples. No significant differences were observed in the energy dissipation.

Effect of age

In order to study the influence of age from degenerated samples, the mean level has been put into the equations giving the following:

TABLE XXIV

Deformation: mm	$Y=0.73-0.0072\,A+0.00007\,A^2$
Residual deformation: mm	$Y=0.19-0.0018\,A+0.00001\,A^2$
Energy dissipation: gm-mm	$Y=4.67-0.0361\,A+0.00020\,A^2$

The regression coefficients are still the same.
The equations are illustrated graphically. (Fig. 10, 11, 12.)

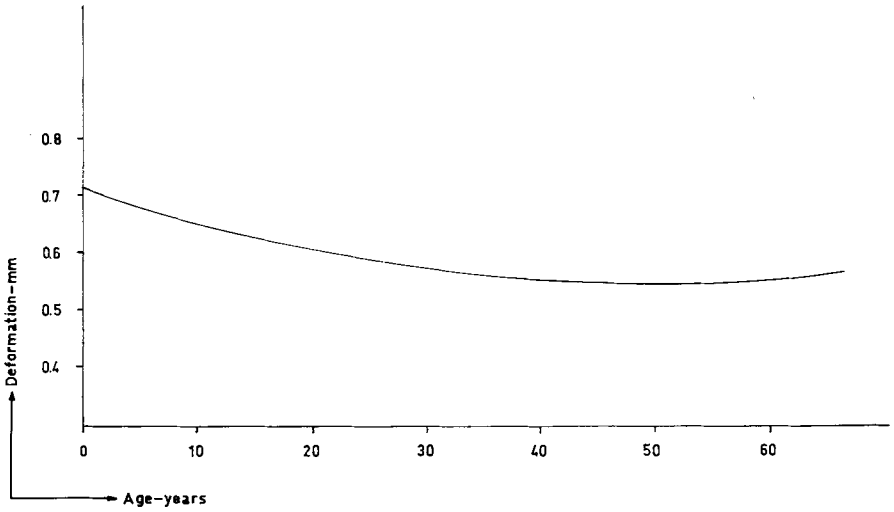


Fig. 10. Mean level of total deformation as a function of age. Non-degenerated samples.

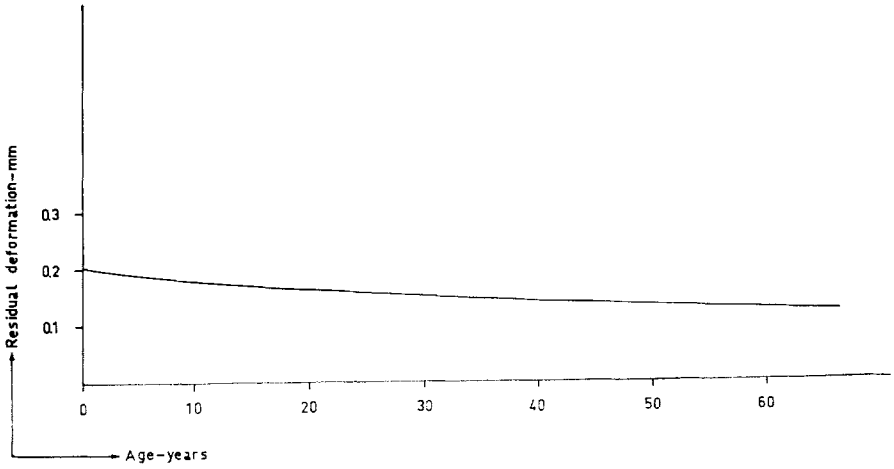


Fig. 11. Mean level of residual deformation as a function of age. Non-degenerated samples.

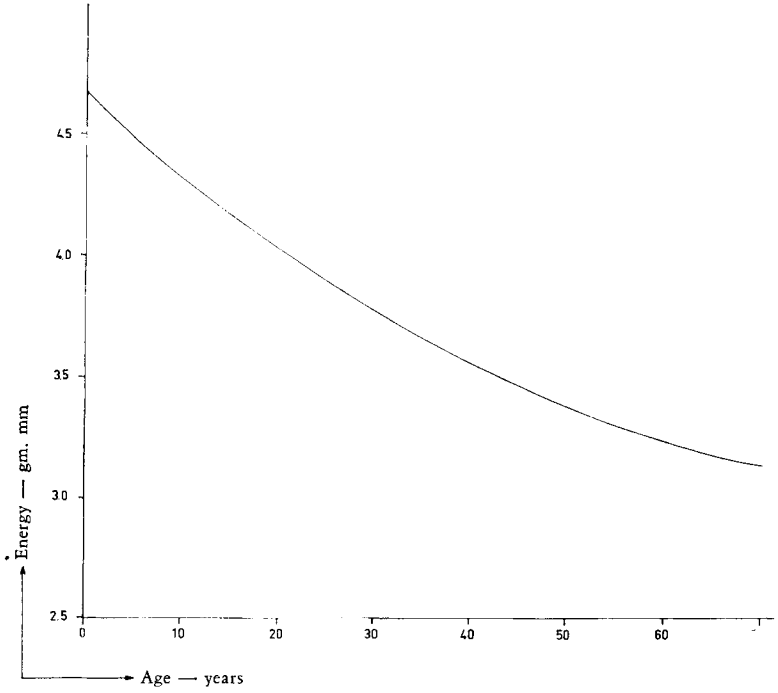


Fig. 12. Mean level of energy dissipation as a function of age. Non-degenerated samples.

CONCLUSION

In the young, elongation and residual deformation decreased progressively with age. After 20—30 years of age, changes occur more slowly. The rate of change in energy dissipation decreases after the age of 40 years.

X. DISCUSSION

The anterior and posterior longitudinal ligaments lie on the anterior and posterior surfaces of the intervertebral discs and are attached to both the discs and the bodies. In view of this relation it is evident that these ligaments will deform with any structural deformation of the disc which can be the result of forces acting on the disc or of structural changes. These ligaments are richly innervated and thus their stimulation may give rise to the sensation of pain (Roofe 1940, Inman and Saunders 1944, Hirsch 1948, Wiberg 1949, Stilwell 1956, Pedersen, Blunck and Gardner 1956, Hirsch, Ingelmark and Miller 1963, Hollinshead 1965, Jackson, Winkelman and Bickel 1966).

The longitudinal ligaments are in a state of prestress, as is evidenced by their shrinkage following removal from the spine. This state of prestress is maintained by the conditions of the discs.

A histological study of unstressed ligaments has revealed a wave-like configuration of the fibrous components which makes it difficult to distinguish separate fibres. On loading, the fibres become progressively polarized and eventually slide upon one another, thus causing rupture of the tissue.

During mechanical testing of the ligaments it was observed that the material stabilized in approximately 3 minutes of loading, after which time it behaved elastically. This time-dependent phenomenon may depend on the fibres and their arrangement and on the properties of the ground substance.

The interrelationship between the disc and the ligaments must be considered both statically and dynamically. If we use certain simplifications in a statical analysis we can consider the relation between the disc and ligaments in two distinct cases, i.e. when the disc-faces of the vertebral bodies are parallel or angulated. In order to determine the change in a force acting on a segment of the ligament adjacent to a disc and how this force is dependent on the angle that the adjacent vertebrae form with each other, the following system was analyzed (see Fig. 13).

The cartilaginous end-plate of L5 was designated A and the cartilaginous end-plate of S.1 was designated B. The anterior and posterior segments of the ligaments within the limits of the disc were designated a and b respectively and the theoretical analysis was carried out by making the following assumptions:

1) That this is a system consisting of the fibres of the ligaments connected to those of the annulus fibrosus which, in turn, enclose the semi-fluid gelatinous substance of the nucleus pulposus.

2) That the disc is normal. A force analysis was carried out on the segment between L5 and S.1 but such a technique may be employed at all other levels provided the variable, angle alfa, is altered accordingly (see diagram).

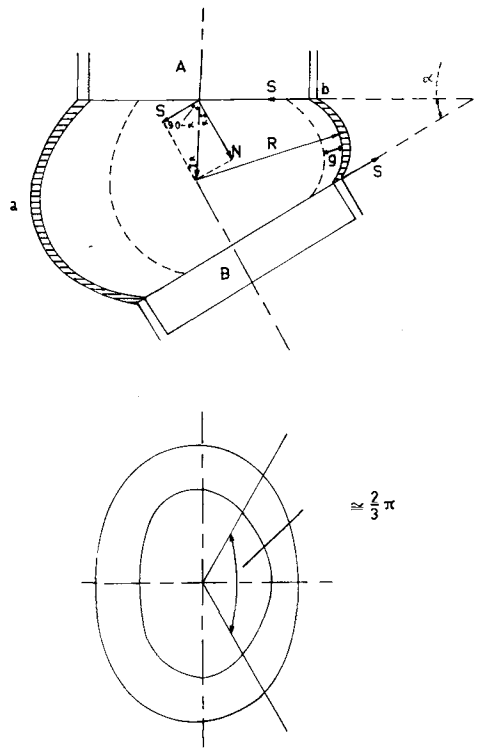


Fig. 13.

Description of theoretical model

We shall assume that the disc is a distorted sphere with a radius R and is loaded from above by the element A, which is the surface of the vertebral body exerting a force G at right angles to the surface A. The deformed sphere (disc) rests on the surface B, which is the surface of the vertebral body below, where surface area is not parallel to A but makes an acute angle alfa with A. Any force, which acts at right angles to the tangent drawn at a point either on the outer or inner surface of the sphere (nucleus pulposus), is transmitted through the outer layer of this sphere. The pressure within the system we shall designate P and the thickness of the wall of the sphere (annulus fibrosus) g. We shall assume that the angle alfa does not exceed 45°.

In the analysis of this system, the force G, acting on this sphere and on the element B, its support, may be resolved into its components N, which is perpendicular to the surface B, and the tangential component S. These two component forces S and N, acting on A, produce a shear and compression with respect to B. Disregarding the accuracy of force transmission, by the shear on B, one can conclude that the stresses in the walls of the container, where they come

in contact with the elements A and B, will be dependent on the Force S. The magnitude of this force S is G· sin α. The force N exerts a pressure on B equal to G· cos α. The force N diminishes as the angle α becomes greater. If the two elements A and B are parallel to each other then the angle α is zero and the pressure P is dependent upon the force G and is given by $P = \frac{G}{F}$, where F is the contact area of the element with the sphere.

The stress within the wall is assumed to be $\sigma_r = \frac{P \cdot R}{2g}$ where R is the radius of the wall and g the thickness of the wall. As changes take place in the angle α, there is effective a variable angle β of the wall considered to be approximately 1/3 of the circumference (see diagram). There is a further stress which is brought about by the force S in this segment as $\sigma_d \cong \frac{S}{g \cdot \frac{2}{3}\pi R}$ which is in addition to the stresses brought about by the pressure P. Although the stress σr and σd do not add directly because they act in different directions, their combined effect may be expected to be more severe than either one acting alone. In order to obtain numerical values we must then get an average value in every case for the radius R, the thickness of the wall g (annulus fibrosus) as well as the area of contact of the sphere with the vertebral body F.

When the angle α is zero or has a very small value, the annulus fibrosus can tolerate a high loading and the ligaments in contact with the annulus are exposed to small tensile stresses. In the lower segments, where the angle alfa is relatively large, the tolerance of the annulus is much lower and one can reach the

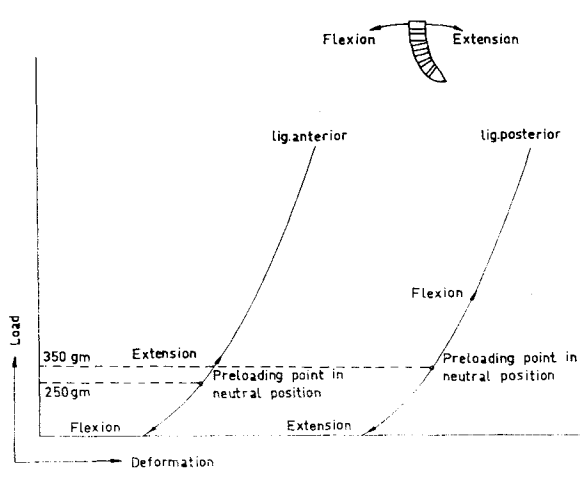


Fig. 14.

point of rupture much sooner. In this case the ligament is subjected to much higher forces and strains. Once degenerative changes take place, the annulus thins and undergoes much greater deformation which causes excessive stretching of the posterior longitudinal ligament. At the moment when the annulus ruptures the above described analysis ceases to hold.

During flexion and extension of the lumbar spine the longitudinal ligaments adjust to the position of the vertebral column and thus either lengthen or shorten. It is probable that the ligaments only work during a certain phase of loading which may be illustrated by the following load/deformation curve (see Fig. 14).

As the disc space decreases the ligaments have to function in the toe part of the curve. When the ligaments are subjected to rapid deformation during movement of the spine then the peak forces induced could be larger than those developed in a wholly elastic material.

XI. SUMMARY

The aim of this programme was to investigate "in vitro" the tensile characteristics of the longitudinal ligaments of the human lumbar spine and to determine the influence, on these properties, of age and degeneration. The material for this work was obtained from 74 lumbar spines taken from cadavers in the age range 1 day to 92 years. In all, 484 samples of the longitudinal ligaments were tested.

Preliminary experiments were performed in order to evaluate and develop the proposed experimental techniques.

While preparing ligament samples of standard dimension it was observed that a significant water loss occurred during 3 minutes of exposure to ambient air (60—70 per cent relative humidity and 20—28°C). In an attempt to prevent this dehydration a number of ligament samples were immersed in distilled water, Ringer solution, human plasma or Macrodex. Within 3 minutes all samples were found to have absorbed a significant amount of fluid, the greatest uptake occurring in distilled water and the least in human blood plasma.

A similar experiment was performed on samples exposed for one hour to water-saturated air and no significant change in weight was observed.

In the above experiments there was no significant difference between the behaviour of the anterior and posterior ligaments.

Subsequent experiments were performed in a high humidity chamber containing air at 100 per cent relative humidity and 25—28°C and a similar environment was employed during sample preparation.

In order to obtain samples of ligaments of uniform thickness, sheets from the ligaments were cut on a Cryo-microtome, the ligaments having been frozen to a temperature of —60°C. Investigations showed that rapid freezing and subsequent thawing had no significant effect on the tensile properties. Once the experimental techniques had been established, the test programme proper, was initiated.

The first experiment was to determine the failure load of the ligaments and it was observed that the anterior ligament is stronger than the posterior. These results are scrattered as the dimensions of ligaments from each lumbar spine showed a considerable variation. In order to obtain comparable results, standard samples of the ligaments (of dimension 0.5×2 mm and 9 mm distance between the test clamps) were introduced. Such samples were found to have average failure loads of 2.12 Kg and 1.98 Kg for the anterior and posterior ligaments respectively and this was independent of the level of the lumbar spine from which the samples were obtained. In samples from severely degenerated spines, failure occurred at substantially lower loads. In the investigation of all the other parameters a maximum load of 0.5 Kg was employed as this approximates to 30 per cent of the average failure load.

Subsequent cyclic loading experiments (35-second intervals) were conducted to determine the difference between the tensile properties of the anterior and posterior ligaments as well as the dependence of these properties on the level of the lumbar spine from which the samples were obtained.

A statistical analysis of the results obtained from the experiments disclosed the following:

There is a significant difference between the tensile properties of the anterior and posterior ligaments. In the second and third load cycles the difference between the deformation of these ligaments is not significant.

The tensile properties of both ligaments are dependent on the level of the lumbar spine. The shrinkage of ligaments occurring on separation from the lumbar spine suggested that these ligaments are in a state of prestress and experiments, conducted with increased and diminished intradiscal pressure, led to the conclusion that this prestress is dependent on the condition of the disc.

Values of the prestressing were calculated from experiments performed on samples of standard dimensions and were substantiated by similar experiments on whole ligaments. In non-degenerated specimens the preload was 200—250 gm in the anterior ligament and 225—350 gm in the posterior. However, in degenerated specimens the values of preload were 120—200 gm and 225—250 gm for the anterior and posterior ligaments respectively.

Histological investigation on unstained ligament sections showed that there is a progressive polarization of the collagen fibre bundles with increasing stress. No orderly fibre structure was evident in unstressed samples but, with the progressive application of load, an increasing proportion of the fibres was reorientated parallel to the axis of stress.

The extensibility of both the anterior and posterior ligaments was found to decrease rapidly with ageing but, after 20—30 years, this progression of stiffening is less marked.

Both deformation and residual deformation were significantly smaller in samples obtained from the degenerated spines than in the normal samples.

REFERENCES

Åkerblom, B.: Standing and sitting posture with special reference to the construction of chairs. Nordiska bokhandeln, Stockholm, 1948.

Allbrook, D.: Movements of the lumbar spinal column. J. Bone Jt Surg. 39-B:339, 1957.

Annovazzi, G.: Osservazioni sulla elasticità dei legamenti. Arch. Sci. biol. (Bologna) 11:467, 1928.

Arutynow, A. J.: [Basic problems of the pathology and surgical treatment of proplapsed intravertebral discs.] Vop. Neirokhir. 4:21, 1962.

Banga, I., Baló, J. and Szabó, D.: Submicroscopic structure of collagen fibres; their contraction and relaxation. Acta morph. Acad. Sci. hung. 6:391, 1956.

Banga, I.: Structure and function of elastin and collagen. Akadémiai Kiadó, Budapest, 1966.

Beneke, F. W.: Constitution und constitutionelles Kranksein des Menschen. Marburg, 1881.

Catchpole, H. R., Joseph, N. R. and Engel, M. B.: Electrochemical state of symphyseal connective tissue under hormonal stimulation. Amer. J. Physiol. 167:774, 1951.

Craik, I. E. and McNeil, I. R.: Histological studies of stressed skin. In: Biomechanics and related bio-engineering topics. Symposium, Glasgow, 1964. Pergamon Press, Oxford, 1965:159.

Cremonese, M. and Tomatis, G.: Considerazioni sulla patogenesi della deviazioni della colonna lombare in corso di lombosciatalgia da ernia discale. Clin ortop. 15:483, 1963.

Curtis, D. H.: The effect of chemical crosslinking agents on the mechanical properties of rat-tail tendon. Ann Arbor University Microfilms Inc., 1963.

Dick, J. C.: The tension and resistance to stretching of human skin and other membranes, with results from a series of normal and oedematous cases. J. Physiol. 112:102, 1951.

Elden, H. R.: Biophysical aspects of aging in connective tissue. Advances Biol. Skin 6:229, 1965.

Elliott, D. H.: Structure and function of mammalian tendon. Biol. Rev. 40:392, 1965.

Ferry, J. D.: Viscoelastic properties of polymers. Wiley, New York, 1961.

Fick, R.: Handbuch der Anatomie und Mechanik der Gelenke unter Berücksichtigung der bewegenden Muskeln. Bd 1—3. Fischer, Jena, 1904—11.

Fredrickson, A. G.: Principles and applications of rheology. Prentice-Hall, Englewood Cliffs, N. J., 1964.

Friberg, S.: Anatomical studies on lumbar disc degeneration. Acta orthop. scand. 17:224, 1948.

Friberg, S. and Hirsch, C.: Anatomical and clinical studies on lumbar disc degeneration. Acta orthop. scand. 19:222, 1949/50.

Frost, H. M.: An introduction to biomechanics. Thomas, Springfield, III., 1967.

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

Gallop, P. M.: Concerning some special structural features of the collagen molecule. Biophys. J. Suppl. 1:78, 1964.

Gardner, D. L.: Pathology of the connective tissue diseases. E. Arnold, London, 1965:322.

Gersh, I.: Aging and ground substance of connective tissue. V. A. Prospectus, Research in aging. Veterans Administration, Washington, 1959.

Gibson, T., Kenedi, R. M. and Craik, J. E.: The mobile micro-architecture of dermal collagen. A bio-engineering study. Brit. J. Surg. 52: 764, 1965.

Grassman, W.: Opening remarks to: Structure and chemistry of collagen. In: Structure and function of connective and skeletal tissue. Butterworths, London, 1964:1.

Gratz, C. M.: Tensile strength and elasticity tests on human fascia lata. J. Bone Jt Surg. 13:334, 1931.

Gross, J., ed. : Aging of connective tissues. Problem identification. J. Geront. 7:584, 1952.

Gross, J.: Collagen. Sci.Ameri. 204:121, 1961.

Gustavson, K. H.: The chemistry and reactivity of collagen. Academic Press, New York, 1956.

Göcke, C.: Elastizitätsstudien am jungen und alten Glenkknorpel. Verh. dtsch. orthop. Ges. 22:130, 1927.

Haboush, E. J.: An anatomical explanation of traumatic low-back pain. J. Bone Jt Surg. 24:123, 1942.

Hackett, G. S.: Prolotherapy in low back pain from ligament and bone distrophy. Canton Clin. Med. 7:2551, 1961.

Hall, M. C.: The locomotor system: functional histology. Thomas, Springfield, Ill., 1965.

Harkness, R. D.: Biological functions of collagen. Biol. Rev. 36:399, 1961.

Harkness, R. D.: Rheological problems of collagenous tissues. Lab. Pract. 15:166, 1966.

Harris, R. I. and MacNab, I.: Structural changes in the lumbar intervertebral discs. Their relationship to low back pain and sciatica. J. Bone Jt Surg. 36B:304, 1954.

Hirsch, C.: An attempt to diagnose the level of a disc lesion clinically by disc puncture. Acta orthop. scand. 18:132, 1948/49.

Hirsch, C.: Studies on the mechanism of low back pain. Acta orthop. scand. 20:261, 1950/51.

Hirsch, C., Paulson, S., Sylvén, B. and Snellman, O.: Biophysical and physiological investigations on cartilage and other mesenchymal tissues. VI. Characteristics of human nuclei pulposi during aging. Acta orthop. scand. 22:175, 1952.

Hirsch, C. and Schajowicz, F.: Studies on structural changes in the lumbar annulus fibrosus. Acta orthop. scand. 22:184, 1952.

Hirsch, C. and Nachemson, A.: New observations on the mechanical behavior of lumbar discs. Acta orthop. scand. 23:254, 1954.

Hirsch, C.: The reaction of intervertebral discs to compression forces. J. Bone Jt Surg. 37A:1188, 1955.

Hirsch, C.: The mechanical response in normal and degenerated lumbar discs. J. Bone Jt Surg. 38A:242, 1956.

Hirsch, C., Ingelmark, B. E. and Miller, M.: The anatomical basis for low back pain. Acta orthop. scand. 33:1, 1963.

Hirsch, C.: Etiology and pathogenesis of low back pain. Israel J. med. Sci. 2:362, 1966.

Hirsch, C. and Galante, J.: Laboratory conditions for tensile tests in annulus fibrosus from human intervertebral discs. Acta orthop. scand. 38:148, 1967.

Hirsch, C., Sonnerup, L. and Viidik, A.: Investigation of mechanical properties in biological tissues. J. Biomechanics, 1968. (In press.)

Hollinshead, W. H.: Anatomy of the spine: points of interest to orthopaedic surgeons. J. Bone Jt Surg. 47A:209, 1965.

Ingelmark, B. E.: The structure of tendons at various ages and under different functional conditions. II. An electron-microscopic investigation of Achilles tendons from white rats. Acta anat. (Basel) 6:193, 1948.

Ingelmark, B. E. and Ekholm, R.: Über die Kompressibilität der Intervertebralscheiben. Acta Soc. Med. upsalien. N. S. 57:202, 1951/52.

Ingelmark, B. E.: De funktionellt anatomiska förhållandena i ryggraden med särskild hänsyn till dess småleder. Acta Univ. gothoburg. 62:1956:1.

Inman, V. T. and Saunders, J. B. de C. M.: The clinico-anatomical aspects of the lumboscaral region. Radiology 38:669, 1942.

Inman, V. T. and Saunders, J. B. de C. M.: Referred pain from skeletal structures. J. nerv. ment. Dis. 99:660, 1944.

Inman, V. T. and Saunders, J. B. de C. M.: Anatomicophysiological aspects of injuries to the intervertebral disc. J. Bone Jt Surg. 29:461, 1947.

Jackson, H. C., 2d, Winkelmann, R. K. and Bickel, W. H.: Nerve endings in the human lumbar spinal column and related structures. J. Bone Jt Surg. 48A:1272, 1966.

Jackson, S. F., Partridge, S. M., Harkness, R. D. and Tristram, G. R., eds.,: Structure and function of connective and skeletal tissue. Butterworth, London, 1964.

Jorpes, E. and Yamashina, I.: Die Mukopolisaccharide und Glykoproteide des Bindegewebes. Chemie und Stoffwechsel von Binde und Knochengewebe. Springer, Berlin, 1956.

Katzenstein, M. and Fecher, K.: Über die Elastizität der Kapsel und der Ligamenta collateralia des menschlichen Kniegelenkes. Langenbecks Arch.klin. Chir. 130:1, 1924.

Kenedi, R. M., Gibson, T. and Daly, C. H.: Bio-engineering studies of the human skin. II. In: Biomechanics and related bio-engineering topics. Symposium, Glasgow, 1964. Pergamon Press, Oxford, 1965:147.

Kohn R. R. and Rollerson, E.: Relationship of age to swelling properties of human diaphragm tendon in acid and alkaline solutions. J. Geront. 13:241, 1958.

Kratky, O., Lauer, M., Ratzenhofer, M. and Sekora, A.: Dependence on age of the X-ray diagram of human tendon collagen. In: Collagen. Interscience, New York, 1962:227.

Kretschmer, E.: Körperbau und Charakter. Untersuchungen zum Konstitutionsproblem und zur Lehre von den Temperamenten. 21. u. 22 Aufl. Springer, Berlin, 1955.

LaBan, M. M.: Collagen tissue: implications of its response to stress in vitro, Arch. phys. Med. 43:461, 1962.

Lange, C.: Untersuchungen über Elasticitätsverhältnisse in den menschlichen Rückenwirbeln mit Bemerkungen über die Pathogenese der Deformitäten. Z. orthop. Chir. 10:47, 1902.

Leikkonen, O.: Low back pain and sciatica. Acta orthop. scand. Suppl. 40, 1959.

Lewin, T.: Osteoarthritis in lumbar synovial joints. A morphologic study. Acta orthop. scand. Suppl. 73, 1964.

Magnuson, P. B.: Differential diagnosis of causes of pain in lower back accompanied by sciatic pain. Ann. Surg. 119:878, 1944.

Meyer, K.: The chemistry of the mesodermal ground substances. Harvey Lect. 51:88, 1955/56.

Nachemson, A.: Lumbar intradiscal pressure. Experimental studies on post-mortem material. Acta orthop. scand. Suppl. 43, 1960.

Nachemson, A.: The influence of spinal movements on the lumbar intradiscal pressure and on the tensile stresses in the annulus fibrosus. Acta orthop. scand. 33:183, 1963.

Nachemson, A.: In vivo discometry in lumbar discs with irregular nucleograms. Some differences in stress distribution between normal and moderately degerated discs. Acta orthop. scand. 36:418, 1965.

Nachemson, A.: The load on lumbar disks in different positions of the body. Clin. Orhtop. 45:107, 1966.

Nachemson, A. and Evans, J.: Some mechanical properties of the third human lumbar interlaminar ligament (ligamentum flavum). 1968. In print.

Nemetschek, T. Grassmann, W. and Hofmann, U.: Über die hochunterteilte Querstreifung des Kollagens. Z. Naturforsch. 10:61, 1955.

Newman, P. H.: Sprung back. J. Bone Jt Surg. 34B:30, 1952.

Nunley, R. L.: The ligamenta flava of the dog. A study of tensile and physical properties. Amer J.phys.Med. 37:256, 1958.

O'Connell, J. E. A.: Sciatica and the mechanism of the production of the clinical syndrome in protrusions of the lumbar intervertebral discs. Brit. J. Surg. 30:315, 1942/43.

O'Connell, J. E. A.: Protrusions of the lumbar intervertebral discs. J. Bone Jt. Surg. 33B:8, 1951.

Pedersen, H. E., Blunck, C. F. H. and Gardner, E.: The anatomy of lumbosacral posterior rami and meningeal branches of spinal nerves (sinuvertebral nerves). J. Bone Jt. Surg. 38A:377, 1956.

Piez, K. A.: Crosslinking of collagen. Birth Def. Orig. 2:5, 1966.

Ragan, C.: The physiology of the connective tissue (loose areolar). Ann. Rev. Physiol. 14:51, 1952.

Ramachandran, G. N., Sasisekharan, V. and Thathachari, Y. T.: Structure of collagen at the molecular level. In: Collagen. Interscience, New York, 1962:81.

Randall, J. E.: Elements of biophysics. 2 ed Year Book Medical Publishers, Chicago, 1962.

Rauber, A. and Kopsch, F.: Lehrbuch und Atlas der Anatomie des Menschen. Bd 1—2. 19. Aufl. Thieme, Stuttgart, 1955.

Ridge, M. D. and Wright, V.: A rheological study of skin. In: Biomechanics and related bioengineering topics. Symposium, Glasgow, 1964, Pergamon Press, Oxford, 1965:165.

Rigby, B. J.: Effect of cyclic extension on the physical properties of tendon collagen and its possible relation to biological ageing of collagen. Nature (London) 202:1072, 1964.

Rissanen, P. M.: The surgical anatomy and pathology of the supraspinous and intraspinous ligaments of the lumbar spine with special reference to ligament ruptures. Acta orthop. scand. Suppl. 46, 1960.

Roaf, R.: A study of the mechanics of spinal injuries. J. Bone Jt Surg. 42B:810, 1960.

Rolander, S. D.: Motion of the lumbar spine with special reference to the stabilizing effect of posterior fusion. Acta orthop. scand. Suppl. 90, 1966.

Roofe, P. G.: Innervation of annulus fibrosus and posterior longitudinal ligament; fourth and fifth lumbar level. Arch. Neurol. Psychiat. (Chic.) 44:100, 1940.

Schmitt, F. O., Hall, C. E. and Jakus, M. A.: Electron microscope investigations of the structure of collagen. J. cell. comp. Physiol. 20: 11, 1942.

Schmitt, F. O.: X-ray and electron microscope studies of structure of collagen fibres. J. Amer. Leath. Chem. Ass. 39:431, 1944.

Schmitt, F. O. and Hodge, A. J.: The tropocollagen macromolecule and its properties of ordered interaction. J. Soc. Leath. Trades Chem. 44:217, 1960.

Schmorl, G. and Junghanns, H.: Die gesunde und kranke Wirbelsäule im Röntgenbild. Thieme, Leipzig, 1932. Fortschr. Röntgenstr. Erg.-Bd 43.

Silver, P. H. S.: Direct observation of changes in tension in the supraspinous and interspinous ligaments during flexion and extension of the vertebral column in man. J. Anat. (Lond.) 88:550, 1954.

Skalak, R.: Personal communication. 1968.

Smith), J. W.: The elastic properties of the anterior cruciate ligament of the rabbit. J. Anat. (Lond.) 88:369, 1954.

Sobel, H. and Marmorston, J.: The possible role of the gel-fiber ratio of connective tissue in aging process. J. Geront. 11:2, 1956.

Sobel, H. and Marmorston, J.: Hormonal influences upon connective tissue changes of aging. Recent Progr. Hormone Res. 14:457, 1958.

Stilwell, D. L., Jr.: The nerve supply of the vertebral column and its associated structures in the monkey. Anat. Rec. 125:139, 1956.

Strasser, H.: Lehrbuch der Muskel- und Gelenkmechanik. Bd 1—2. Springer, Berlin, 1908—13.

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

Sylvén, B., Paulson, S., Hirsch, C. and Snellman, O.: Biophysical and physiological investigations on cartilage and other mesenchymal tissues. II. The ultrastructure of bovineand human nuclei pulposi. J. Bone Jt Surg. 33A:333, 1951.

Verzár, F.: The ageing of connective tissue. Gerontologia (Basel) 1:363, 1957.

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

Viidik, A.: Biomechanics and functional adaption of tendons and joint ligaments. In: Studies on the anatomy and function of bone and joints. Springer, Berlin: 1966:17.

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

Viidik, A.: A rheological model for uncalcified parallel-fibred collagenous tissue. J. Biomechanics 1:3, 1968.

Virchow, R.: Über die Chlorose und die damit zuzammenhängenden Anomalien im Gefäss-Apparate, insbesondere über Endocarditis puerperalis. Beitr. Geburtsh. Gynäk. 1:323, 1870/72.

Virgin, W. J.: Experimental investigations into the physical properties of the intervertebral disc. J. Bone Jt Surg. 33B:607, 1951.

Wassermann, F.: Electron microscopic study of the submicroscopic network of fibrils as a component of connective tissue. Anat. Rec. 111:145, 1951.

Wertheim, M. G.: Mémoire sur l'élasticité et la cohésion des principaux tissus du corps humain. Ann. Chim. Physique. 21:385, 1847.

Wiberg, G.: Back pain in relation to the nerve supply of the intervertebral disc. Acta orthop. scand. 19:211, 1949/50.

Williams, P. C.: The lumboscaral spine; emphasizing conservative management. McGraw-Hill, New York, 1965.

Willis, T. A.: Anatomical variations and roentgenographic appearance of the low back in relation to sciatic pain. J Bone Jt Surg. 23:410, 1941.

Wright, D. G. and Rennels, D. C.: A study of the elastic properties of plantar fascia. J. Bone Jt Surg. 46A:482, 1964.