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**TENSILE PROPERTIES
OF THE HUMAN LUMBAR
ANNULUS FIBROSUS**

BY

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*To my wife
and my parents*

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I. INTRODUCTION

Motion in the vertebral joint complex occurs as a function of deformations in the intervertebral disc. The magnitude of these deformations determines the extent of vertebral excursion. When degenerative changes appear in the disc it is possible to demonstrate abnormalities in this mechanical response. In terms of magnitude however, severe structural changes cause only insignificant disturbances in functional behavior. For that reason it is difficult to correlate symptoms arising from the lumbar spine with alterations in the load response of the intervertebral discs.

The annulus fibrosus is an essential constituent of the fibrous intervertebral joint. Its collagen fibers resist forces and provide most of the stability necessary during motions of the vertebrae. Ruptures of the annulus, a prerequisite of nuclear prolapse, are common in disc degeneration. Their occurrence can be the result of changes in the physical properties of the material with a decrease in the tensile strength of the fibers. They can also be the product of alterations in the distribution of forces leading to local concentrations of stress. Nerve endings have been reported in the most peripheral layers of the annulus (Fick 1904, Wiberg 1949, Hirsch, Ingelmark and Miller 1963, Jackson, Winkelman and Bickel 1966) pointing to a link between abnormal disc behavior and low back pain.

The load deformation properties of the annulus fibrosus and particularly its response to tensile forces are evidently important determinants in the normal and pathologic function of the intervertebral disc. The present study was set to answer specific questions brought forwards by these considerations:

- 1) What is the response of the lumbar annulus fibrosus to tensile forces and how is it related to the fiber framework?
- 2) Is this behavior uniform or does it vary in different areas of the annulus?
- 3) What is the tensile strength of the tissue?
- 4) How are these characteristics affected by age?
- 5) Do morphological or biochemical changes in the tissue due to degeneration processes affect its load-extension response?

The present investigation

In this investigation the tensile properties of the lumbar annulus fibrosus were studied in human postmortem specimens.

As a primary step the variables incident to the methods of preparation and testing had to be controlled. Changes induced in the water content of the samples

were thought to be of primary importance. An attempt was made to control this source of variation and the first series of experiments approached this problem. The methodology was further completed in regard to the sectioning and testing of the samples.

After defining the in vitro laboratory conditions a study of tensile properties of the lumbar annulus fibrosus was made in relation to: 1) Orientation of fibers. 2) Location of samples in different areas. 3) Time dependent effects. 4) Tensile strength. 5) Influence of age and degeneration; morphological and biochemical correlations were used to evaluate these effects.

The findings are discussed in the general context of the mechanics of the intervertebral disc.

II. REVIEW OF THE LITERATURE

Morphological considerations

The normal disc

The intervertebral disc consists of three parts: the annulus fibrosus in the periphery, the cartilage plates above and below and the nucleus pulposus in the center.

The annulus fibrosus in the adult lumbar spine is formed by a series of concentric encircling lamellae (Fick 1904, Beadle 1931). It is thicker anteriorly where the lamellae are more numerous than posteriorly. Towards the sides the lamellae spread over a far wider area. The peripheral lamellae attach themselves to the bony edge of the vertebral body in the manner of Sharpey fibers (Erdheim 1931) while the remaining continue into the cartilaginous plates (Fick 1904, Beadle 1931, Hirsch and Schajowicz 1952). In the front intimate connections exist with the anterior longitudinal ligament, while the posterior longitudinal ligament is not so firmly attached to the annulus (Beadle 1931, Hirsch and Schajowicz 1952).

The collagen fibers run a uniform course in each lamellae, the fibers of adjoining lamellae cross each other in opposite directions (Fick 1904, Strasser 1908—13). The annulus is basophilic, a property which increases towards the nucleus. The more superficial layers stain acidophilic as do the longitudinal ligaments. Here the round cartilage-like cells disappear and fibrocytes are encountered. The fibers are thinner and less aggregated towards the midline and in the posterior area (Fick 1904, Hirsch and Schajowicz 1952), the amount of amorphous ground substance is greater here. The hyaline cartilage endplates delimit the disc from the vertebral body above and below, peripherally they come in contact with the bony epiphyseal ring (Beadle 1931).

The nucleus pulposus centrally situated consists of a three dimensional network of collagen fibrils enmeshed in a mucoprotein gel (Hirsch, Paulson, Sylvén and Snellman 1952). It occupies about 30—50 per cent of the disc's cross-section area (Perey 1957, Nachemson 1960, Eie 1966).

Changes induced by age and degeneration

During adult life the intervertebral disc undergoes a series of morphological changes of varying severity. Beadle (1931) remarks, that after the middle

decades of life, the finding of well preserved discs is the exception rather than the rule. All of the anatomical components of the disc are involved.

In the annulus fibrosus (Hirsch and Schajowicz 1952) areas of "so called mucous degeneration" appear after the second decade. The fibers in the lamellae become hyalinized and foci of cartilaginous metaplasia appear. Concentric cracks and cavities may be present as early as the 15th year of life. These concentric tears become common later and are most often seen anteriorly. Radiating ruptures are common in the posterior part of the annulus. They run from the center to the periphery and granulation tissue may be found within them (Hirsch and Schajowicz 1952, Goldie 1958).

While in the young annulus the extracellular ground substance stains diffusely with PAS and Alcian Blue, a disordered pattern of PAS reactive granules is seen in older subjects (van den Hooff 1964). These granules were thought to consist of a protein carbohydrate component which had become free from its binding to collagen.

Changes occur in the cartilage plates in the form of intraspongy nuclear herniations, erosions, ingrowth of granulation tissue, and cartilage hyperplasia (Saunders and Inman 1940, Coventry, Ghormley and Kernohan 1945). In the nucleus pulposus the gel like consistency is lost and progressive fibrosis and cavitation occur. Anterior or posterior nuclear protrusions might be present.

Electron microscopic studies of samples from annulus and nucleus pulposus (Sylvén, Paulson, Hirsch and Snellman 1951, Dahmen 1963) showed that degeneration induced irregular arrangement and thinning of the collagen fibrils, shortening and loss of differentiation of the period of transverse striation and appearance of granules in the tissue matrix.

Biochemical characteristics

The biochemical characteristics of the intervertebral disc show changes parallel to the morphological alterations.

Püschel (1930) found that the water content of the disc was highest at birth, 88 per cent in the nucleus and 78 per cent in the annulus fibrosus. With age, water content decreased to values of about 70 per cent in both areas. A correlation between degeneration and loss of water was evident. Observation of the data reveals that in the annulus fibrosus the decrease in values occurred during the first and second decade, while in the nucleus it progressed during all age groups.

The ground substances of both nucleus pulposus and annulus fibrosus contain mucopolysaccharides; these are present in larger amounts in the nucleus (Sylvén 1951, Hirsch, Paulson, Sylvén and Snellman 1952, Malinsky 1958), chondroitin

sulfate was evident (Sylvén 1951, Malinsky 1958) and identified by Orr (1954) as chondroitin 6-sulfate or chondroitin sulfate C.

Kerato-sulfate was shown in the nucleus (Gardell and Rastgeldi 1954) and later hyaluronic acid and chondroitin sulfate A and C were demonstrated (Hall, Lloyd, Happey, Horton and Naylor 1957, Bernardi, Happey and Naylor 1957). Sulfur and dry weight determinations in human nuclei pulposi indicated that the polysaccharides remained at a stationary level or slightly decreased with age. A large decrease in sulfur values was evident with gross degeneration (Hirsch, Paulson, Sylvén and Snellman 1952). Sulfated acid mucopolysaccharides in the annulus fibrosus (Malinsky 1958) increased with age and accumulated in the clefts apparent in the structure with degeneration. Non sulfated acid mucopolysaccharides decreased with age. Similar changes occurred in the nucleus pulposus.

An increase in the ratio keratosulfate — chondroitin sulfate of the nucleus pulposus with age was concluded by Hallén (1958), and Gardell and Hansen (1959) from hexosamine and sulfate determinations. According to Hallén (1958) total hexosamine content decreased with age from 14 per cent of dry weight at 15 years to 6 per cent at 90 years.

Findings of similar nature were evident in herniated disc material (Davidson and Woodhall 1959); the total polysaccharide content was reduced and the collagen content increased. The chondroitin sulfate moiety of the polysaccharide fraction was reduced to a larger extent than the keratosulfate. In degenerated discs the polysaccharide content was lower than in normal samples of the same age group (Naylor 1962).

Mitchell, Hendry and Billewicz (1961) reported that the highest polysaccharide levels were reached in the 30—40 age group declining to its lowest values in later years. Concomitantly with the increase in hexosamines, slightly higher values of collagen were observed. The nitrogen content was constant. In prolapsed discs the hexosamines were decreased, collagen and total protein were elevated. No separation between annulus and nucleus was made in this investigation.

In rabbits Hansen and Ullberg (1960) examined the uptake of S^{35} by the nucleus pulposus and found that cell uptake, indicating chondroitin sulfate synthesis, was highest at the annulus — nucleus junction. With age this activity decreased.

Buddecke and Sziegoleit (1964) identified chondroitin-4-sulfate, chondroitin-6-sulfate, keratosulfate and hyaluronic acid from human intervertebral discs. The total content of these acid mucopolysaccharides decreased with age. In newborn infants chondroitin sulfate constituted over 80 per cent of the acid mucopolysaccharides. It decreased progressively while keratosulfate increased. Between one and four years of age the chondroitin-4-sulfate / chondroitin-6-sulfate

ratio was 2 to 1. At 60 to 80 years of age, chondroitin-6-sulfate was the main component of this fraction.

In fractionation studies of glycosaminoglycans of the human nucleus pulposus (Antonopoulos 1965) a decrease with age in total content of glycosaminoglycans and chondroitin-6-sulfate was found. Keratosulfate and hyaluronic acid remained constant.

All of these investigations have approached the study of the intervertebral disc as a whole or have isolated the nucleus pulposus. No analytical data is available on the changes exhibited by the annulus fibrosus.

Differential thermal analysis studies (Dickson 1966) have shown that the peak temperature of both nuclear and annular samples was higher in young (below 10 years) than in older specimens (above 50 years). Samples of annular tissue obtained at surgery tended to shrink slightly below the expected values for postmortem tissue.

The mechanical response of the disc and its alterations

Most experiments concerned with the mechanical response of intervertebral discs have been performed on segments of the spine including at least, two vertebrae or part of them and the intervening discs (Fessler cit. by Fick 1911, Göcke 1932, Petter 1933, Hirsch 1951, Virgin 1951, Ingelmark and Ekholm 1952, Hirsch and Nachemson 1954, Hirsch 1955, Bartelink 1957, Perey 1957, Brown, Hansen and Yorra 1957, Hardy, Lissner, Webster and Gurdjian 1958, Evans and Lissner 1959, Nachemson 1960, Eie 1966, Rolander 1966). The specimens were placed between the jaws of a loading machine and were subjected to compression. The load deformation curves obtained when plotting the results were convex to the deformation axis at moderate stress levels, as the load rose the rigidity of the specimen increased until a yield point was reached (Göcke 1932, Virgin 1951, Ingelmark and Ekholm 1952, Hirsch and Nachemson 1954, Brown, Hansen and Yorra 1957, Rolander 1966). On removal of the load, Göcke (1932) showed the presence of residual deformation, its magnitude depending on the load reached and the age of the specimen. Residual deformation was larger in young samples and after swelling in 0.9 per cent NaCl solution. When performing load cycles, hysteresis and residual deformation were evident (Virgin 1951, Ingelmark and Ekholm 1952, Hirsch and Nachemson 1954, Brown, Hansen and Yorra 1957, Rolander 1966). Both phenomena were larger in the first cycle than in the following ones.

Discs from old subjects exhibited greater stiffness and decreased residual deformation than young specimens (Göcke 1932, Virgin 1951). Hysteresis was

larger in the young material (Virgin 1951). Decreased compressibility in old discs was also shown by Ingelmark and Ekholm (1952). Hirsch (1951) used an elastometer to determine pressures in the outer surface of the annulus. In degenerated discs considerable variations were found in pressure distribution.

Hirsch and Nachemson (1954) measured the anterior and posterior expansions of the annulus fibrosus when compressing intervertebral discs and reported average figures of 0.5 mm expansion for 50 kg loads and 0.75 mm for 100 kg loads. Degenerated discs exhibited 30 per cent more expansion than normal ones. Horton (1958), Naylor (1962), believed that the deformation characteristics of the annulus, "its elasticity", were related to changes in the angle between alternating sheets of fibers. This property showed no changes with age. Horton (1958) calculated that the expansion values reported by Hirsch and Nachemson (1954) indicated a change of interstriation angle of 1° . Brown, Hansen and Yorra (1957) reported that the expansion of the annulus varied in different areas of the disc periphery.

Intradiscal pressures (Nachemson 1960) in normal autopsy specimens were found to be on the average 30 to 50 per cent higher than the applied load per unit area. Hydrostatic behavior of the nucleus was reported. It was concluded that the nucleus pulposus transformed the vertical compressive forces into tangential stresses in the annulus fibrosus. These stresses were estimated to be three to five times the applied load per unit area. The vertical pressures on the annulus were thought to be low. The weight bearing capacity of the posterior joints and ligamentous structures was found to be minimal, at least for loads up to 220 Kp (Nachemson 1963). In degenerated discs the nucleus did not show hydrostatic behavior. In general the pressures were lower than in normal discs. It was concluded that the annulus was subjected to higher vertical stresses and lower tangential forces in degenerated specimens. These findings were confirmed in intravital measurements (Nachemson 1966).

In the compression experiments previously discussed, vertical displacements were measured between the jaws of the loading machines. Rolander (1966) during motion studies of vertebral segments used direct extensometer readings. He reported in normal discs values of vertical displacement of 2.6 per cent at 0.05 to 0.06 Kp/mm² loads; the previous investigators obtained corresponding values of 10 to 15 per cent displacement for the same loads. The differences could be accounted for mainly by deformations in the vertebrae at higher stress levels. In the same investigation (Rolander 1966), removal of the intervertebral joints and posterior ligaments did not lead to any increase in the range of movement; severance of the ligament flava had a small effect; the annulus fibrosus was found to be the main stabilizing factor in the vertebral segment. With excentric loading it was found that the disc's resistance to a change of angle was greatest in extension, less in lateral flexion and least in forward flexion.

Degenerated discs exhibited increased vertical deformation and less resistance to flexion. No horizontal instability was found in the material investigated.

In roentgenographic studies of vertebral motion in vivo, increased horizontal displacement in disc degeneration was reported by Knutsson (1944), Gianturco (1944), Fletcher (1947) and Schalimtzek (1958).

Brown, Hansen and Yorra (1957) performed tension tests to failure in samples from the annulus fibrosus of two intervertebral discs. Considerable variation was found and tensile strength values of up to 0.42 Kp/mm^2 were reported. The most peripheral areas of the annulus anteriorly and posteriorly exhibited the highest strength values.

The strength of the vertebral body has been the subject of many investigations (Rauber 1876, Messerer 1880, Lange 1902, Göcke 1926, 1931, Bartelink 1957, Perey 1957, Decoulx and Rieunau 1958, Eie 1966, Weaver and Chalmers 1966).

In cubes of fresh bone from lumbar vertebrae tested parallel with the long axis of the bone, Rauber (1876) found an average compressive strength of 0.84 Kp/mm^2 . In whole vertebrae, Messerer (1880) reported values between 0.22 — 0.78 Kp/mm^2 . Lange (1902), who loaded specimens consisting of three vertebral bodies and intermediate discs and performed direct extensometer readings of deformation, reported strength values of 0.15 — 0.56 Kp/mm^2 . In lumbar vertebrae tested in an Amsler machine, Göcke (1926, 1931) found failures occurring at loads between 0.57 — 0.70 Kp/mm^2 . The values reported by modern workers are in complete agreement with these previous investigations. In cubes obtained from the center of vertebral bodies Weaver and Chalmers (1966) found average values for failure in compression of 0.32 Kp/mm^2 . They reported a decrease in strength and mineral content of the samples with increasing age.

In axial compression tests of vertebrae and discs, failure occurs first at the end-plate as pointed out by several authors (Perey 1957, Brown, Hansen and Yorra 1957, Decoulx and Rieunau 1958, Rolander 1966). Perey (1957) reported average values for the resistance of the end-plate of 1.09 Kp/mm^2 or 600 Kp total load in the 20—30 years age group and 0.43 Kp/mm^2 or 260 Kp total load in the group over 60 years of age. These figures are in the same range as those found by other investigators. Rolander (1966) demonstrated that fracture of the end-plate occurs at the load at which deformation curves for disc and vertebral body tend towards one another.

Theoretical calculations of the forces acting on the lumbar discs based on simple lever principles are in complete disagreement with the known physical properties of the individual spine components. For example, Bradford and Spurling (1945) calculated that a load of 1000 Kp would be exerted in the lumbar discs when lifting a 160 Kp weight from the floor in a position of forward bending.

Davis (1956, 1959), Bartelink (1957), Morris, Lucas and Bresler (1961), Eie and Wehn (1962), found by direct measurements that an increase in pressures occurs within the thorax and abdomen in the early stages of lifting. It is thought that this mechanism decreases the forces acting on the spine.

During moderate and heavy exercise the axial pressures acting on the lumbo-sacral spine were calculated to be within the range of 0.06 to 0.15 Kp/mm² (Eie 1966). The abdominal musculature was said to have a relieving effect and to represent approximately 40 per cent of the contraction force of the erector spinae.

EMG studies have shown that in the early stages of lifting, when the forces acting on the spine are presumably the highest, the activity of the erector spinae muscles is very low, while the abdominal muscles are very active (Floyd and Silver 1955, Bartelink 1957, Morris, Lucas and Bresler 1961). In full trunk flexion the erector spinae muscles are completely relaxed (Floyd and Silver 1955, Carlsöö 1961). Davis, Troup and Burnard (1965) in chrono-cyclophotographic studies of spine motion, demonstrated that there was little intrinsic movement of the lumbar spine during the early phase of lifting. Extension of the lumbar spine was delayed until the weight was raised for about a third of the distance, when the occurrence of maximal intervertebral compression forces was no longer likely.

Nachemson (1966) has performed intradiscal pressure measurements in living subjects. The approximate load acting on the third lumbar vertebra in a 70 kg individual in the sitting position was calculated to be 142 Kp. Standing the load was 99 Kp, supine under anaesthesia 20 Kp. The highest values occurred with the subject sitting, leaning forwards and lifting a weight and were in the range of 270 Kp. Tangential tensile stresses in the posterior annulus fibrosus were calculated and the highest value recorded in the forward tilting and weight bearing position was 0.73 Kp/mm².

General properties of collagen and ground substance

Collagen

The mechanical properties of connective tissues depend in large proportion upon collagen, their structural protein. The collagen molecule or tropocollagen can be represented as a rodlike cylinder 3000 Å long and about 14 Å in diameter. It consists of three left handed helical polypeptide chains wound around a common axis to form a right handed superhelix. The aminoacid composition is characterized by a content of 1/3 glycine and a large proline plus hydroxyproline content (of the order of 2/9) (Piez 1966). This last feature provides a

specific method for detecting and estimating collagen on the basis of hydroxyproline determination (Neuman and Logan 1950). Proline and hydroxyproline prevent easy rotation of the regions in which they are located, thus imparting rigidity and stability to the collagen molecule (Gross 1961). Collagen properties are not only related to its chemical composition but also depend on the physical arrangement of its individual molecules and interactions between chains. In the native collagen fibril the molecules are lined up facing in the same direction and overlapping by about one fourth of their length. This overlapping creates the characteristic periodicity (600 to 700 Å) seen in electron microscopy (Gross 1961). This arrangement has been denied by Smith (1965), who instead proposed a ring disposition around a central molecule, the first ring containing six molecules and successive rings increasing by additional six molecules each.

Complete collagen molecules are synthesized by cells and extruded into the extracellular space where they polymerize into fibrils and fibers at increasing levels of organization (Jackson 1956, Gross 1961). At this point, the three helical chains in the molecules are held together mainly by hydrogen bonds established between the amine of glycine and the carboxyl of the aminoacids (Gross 1961). The individual chains may be composed of smaller subunits attached by non-covalent bonds (Piez 1966).

A most important property of collagen is that after synthesis and aggregation into fibrils, covalent cross-links are formed. These thermo-stable cross-links may be intra- or intermolecular and provide further stabilization to the fibril structure (Piez 1966). This process leads eventually to the formation of a three dimensionally, covalently, cross-linked lattice, which is insoluble to anything other than the most severe extraction procedures (gelatinization) or by breaking the cross-links by chemical or enzymatic methods (Gallop 1964). The nature and location of these covalent bonds is the subject of continuing discussion (Bornstein 1966).

There is an increasing tendency to explain many of the physical changes that occur with age in the collagen structure with these covalent cross-links (Verzár 1963, Elden 1965 a). During growth the proportion of collagen which is soluble in aqueous solutions of salts and acids decreases (Gross 1957, Wirtschafter and Bentley 1962). The swelling capacity of collagenous tissues diminishes with increasing age (Kohn and Rollerson 1958, 1959). When collagen fibers are heated in the presence of water or are exposed to certain compounds they contract to about one third of their original length (Verzár 1957, 1963, Banga, Baló and Szabó 1956, Elden 1963). Contraction takes place when the energy provided is sufficient to break up the ordered structure of collagen to a state of random disorder. Covalent cross-links are not affected, only hydrogen bonds and salt-like cross-links are disrupted (Jackson 1965). In isotonic contraction, old tissues contract more than young ones. In isometric tests old tissues develop

higher tensions (Verzár 1963). Similarly the amount of collagen that goes into solution during contraction decreases with age.

According to Jackson (1965) these changes are mostly related to maturation rather than to the effects of aging and reflect the requirement that collagen must be insoluble and relatively inert to reach its optimal functional capacity.

Ground substance

The fiber framework is closely associated with the amorphous material known as ground substance. Besides the usual crystalloids of extracellular fluid this is basically a mucopolysaccharideprotein complex where chondroitin sulfate A, B, C, keratosulfate, hyaluronic acid and heparin sulfate can be identified (Meyer 1966). The majority of these mucopolysaccharides appear to be nonbranched polymers composed of alternating hexuronidic and N-acetylhexosaminidic groups. In keratosulfate, the uronidic group is replaced by D-galactose.

The polysaccharides are covalently bound to protein in the tissue (Laurent 1966), the resulting compounds are called protein polysaccharides (Schubert 1966). According to Meyer (1966), the mucopolysaccharides isolated from proteolytic digest of connective tissue or after extraction with alkali are really artefacts, breakdown products of protein complexes in part free of peptides, in part still covalently linked to peptide chains of variable lengths. These high molecular weight polymers trap large amounts of water within their domain, an important factor in determining their physical and mechanical properties (Ogston 1966, Schubert 1966).

Variables affecting the determination of tensile properties of collagenous tissues

When performing mechanical investigations in samples from connective tissues a number of variables must be taken into consideration. The tissue is not alive and a time lapse has occurred after death. After sampling, the specimens are often preserved for varying periods until preparation and testing are performed and variables can be introduced by the methods of preservation. The environmental factors to which the samples are exposed during preparation and testing are critical in relation to their influence on water content. When tissues are exposed to air they dry; consequently, in a large number of the investigations to be reviewed, the samples were immersed in aqueous solutions prior or during testing procedures.

Postmortem changes

Evidence points to the fact that postmortem changes do not affect mechanical

properties of some tissues within a certain time after death. Wertheim (1847) found no changes in stiffness and strength of tendon, nerve and artery of one dog five days after death.

Gratz (1931) reported that fascia lata behaved as "essentially alive" within 18 hours following removal from the body. Rigby, Hirai, Spikes and Eyring (1959) stored tendons in saline solution at 2° to 3° C and found no changes in properties "for as long as 1 to 2 months". Ridge and Wright (1965) reported no changes in tensile characteristics of skin within 48 hours after death.

Viidik, Sandqvist and Mägi (1965) concluded from a study on rabbit knee ligaments, that no consistent changes in tensile characteristics were evident up to 96 hours following death. The animals were stored intact at 18° to 20° C until shortly before tests, and the experiments were performed in air.

Contradictory statements have been advanced for ligaments. Katzenstein and Fecher (1924) found increased stiffness and higher elasticity in ligaments 48 hours postmortem but admitted that the effect could be partly due to dehydration. Annovazzi (1928) found the same effects in dog ligaments 10 hours after death and Smith (1954) claimed the same changes occurred within one hour but reported no figures.

Preservation methods

Preservation in formalin has been reported to alter the mechanical characteristics of soft tissues (Stucke 1950, Elden 1964 a, Viidik and Lewin 1966) and bone (Carothers, Smith and Calabrisi 1949, Calabrisi and Smith 1951, McElhaney, Fogle, Byars and Weaver 1964, Evans 1964, Sedlin 1965, Sedlin and Hirsch 1966). The effect is the result of an increase in collagen cross-linking induced by formaldehyde.

Deep freezing has been said not to alter mechanical characteristics of tendons (Rigby, Hirai, Spikes and Eyring 1959, Van Brocklin and Ellis 1965), bone (Frankel 1960, Sedlin 1965, Sedlin and Hirsch 1966), skin (Ridge and Wright 1965) and intervertebral disc segments (Bartelink 1957, Hardy, Lissner, Webster and Gurdjian 1958, Nachemson 1960). Rabbit ligaments, however, exhibited significant differences in failure energy and dip incidence of the curves following deep freezing (Viidik and Lewin 1966).

The formation of ice from water is the most important physical change occurring when tissues are carried to low temperatures. The freezing injury has both a mechanical and a chemical background (Meryman 1960). The first one is related to the size of the ice crystals, a factor depending on the rate at which freezing proceeded and the temperature reached. The chemical injury is produced as the growing ice crystals remove water from the tissues leaving behind an increased concentration of solutes; the length of freezing state is of

importance in this connection. In general temperatures of storage must be quite low, at any rate below -39°C which is considered to be the limit for super cooling.

Air drying

Loss of tissue water by air drying is well known to affect mechanical properties of biological materials. Wertheim (1847) showed that dehydration increased the stiffness and strength in tension of tendons, muscles, arteries and nerves. The load elongation curves approached a straight line. Essentially similar effects were shown later by Katzenstein and Fecher (1924) and Annovazzi (1928) in ligaments, and by Stucke (1950) and Rollhäuser (1950 b) in tendons.

The effect is also present in bones; Wertheim (1847) showed that elongation was more proportional to loads in dry bone tissue. Rauber (1876) reported increased elasticity in bending of femoral samples and increased breaking strength in femoral and humeral samples after drying.

Evans and Lebow (1951), Dempster and Liddicoat (1952), Smith and Walmsley (1959), Evans (1964), Sedlin (1965) and Sedlin and Hirsch (1966) found increase in elasticity modules and strength of cortical bone after drying.

Immersion in aqueous solutions

When in contact with aqueous solutions collagenous tissues swell (Schade 1913, Kaye and Lloyd 1924, Lloyd, Marriott and Pleass 1932, Küntzel and Pranke 1933, Gustavsson 1956).

In solutions free from salts, swelling is minimal at neutral pH, and shows a well defined maximum in acid solutions and a poorly defined maximum in alkaline solutions. Both maxima are repressible in the presence of salts (Lloyd, Marriott and Pleass 1932). The nature of this process is complex. Osmotic swelling occurs in solutions of acids and bases and is associated with the ionic protein groups. Lyotropic swelling predominates in neutral solutions and is due to interactions of ions and molecules with the nonionic bonds of the protein (Gustavsson 1956).

The magnitude of swelling in a tissue is also related to its degree of organization and the amount of internal stabilization of its proteins (Kaye and Lloyd 1924, Gustavsson 1956). The isolated fiber bundle of ox-hide takes up more water than the whole hide. A gelatin gel with the same protein content as goat skin, exhibits much greater swelling.

The ground substance protein polysaccharides exhibit important interactions with water (Fessler 1957, Ogston 1966, Laurent 1966) and their amount and characteristics are expected to affect the equilibrium swelling volume of connective tissues (Milch 1965).

The process is also age dependent; in tendons, swelling at neutral pH has been shown to decrease with age (Rollhäuser 1950 a, Elden 1964 b).

Swelling of intervertebral disc samples in 0.9 per cent NaCl solution was shown by Göcke (1932) who also studied the swelling process in acid, alkaline and salt solutions and showed decrease of water uptake with age. Swelling in NaCl solution or water was illustrated by Naylor and Smare (1953), Bush, Horton, Smare and Naylor (1956), Hendry (1958). The annulus fibrosus exhibited less water affinity than the nucleus pulposus (Naylor and Smare 1953, Hendry 1958).

The process of swelling has been shown to alter the mechanical properties of certain collagenous structures. Viidik and Lewin (1966) showed changes in tensile strength characteristics of rabbit knee ligaments after swelling in saline solution. The load, elongation and energy at failure were larger after four hours in saline solution than in control samples.

Göcke (1932) showed increased deformation and reduced recovery in compression, in intervertebral discs after swelling for 24 hours in 0.9 per cent NaCl solution. However, fluid was reported to improve recovery after compression tests in intervertebral discs by Ingelmark and Ekholm (1952), who perfused their samples with horse serum, and Virgin (1951), who immersed the specimens in Ringer's solution.

Tensile properties of collagenous tissues

Stress-strain curves

Wertheim (1847) was the first modern scientist to investigate the tensile properties of body tissues. In tests of human tendon, muscle, nerve and arteries he studied the characteristics of the load elongation diagrams. Elongations were not proportional to loads, the hyperbole type of curves were convex to the load axis. An equation was fitted to the curves with the form: $Y^2 = ax^2 + bx$, and coefficients of elasticity derived from it. Wertheim also illustrated the effects of air drying and postmortem changes.

All successive investigations have shown the same general characteristics of the load elongation diagrams with an initial toe of varying magnitude followed in some cases by a straight line before plastic flow occurs.

Different interpretations have been advanced to explain the shape of the load elongation diagram. In tendons, Reuterwall (1921) and Rigby, Hirai, Spikes and Eyring (1959) related it to the stretching of the wave pattern seen with polarized light.

In arteries it has thought to be due to the distribution of collagen and elastin

in the arterial wall. Krafska (1939) determined elastic moduli of aortas, ligamentum nuchae, smooth muscle from human intestine, and tendon, and concluded that the collagen fibers were responsible for the straight line character of the upper half of the curve. Reuterwall (1921), Burton (1954) attributed the initial part of the curve to the elastin component and the final slope to the collagen fibers of the arterial wall. A more complex relation including smooth muscle has been presented by Apter (1964) and Apter, Rabinowitz and Cummings (1966).

Dick (1951) also concluded from studies on skin that the elastic fibers were responsible for the initial resistance to elongation. Kenedi, Gibson and Daly (1965) related the appearance of the stress-strain diagrams to a progressive orientation of the collagen fibers in the tissue. They interpreted their stress-strain curves in power law form a method used for collagen fibers by Morgan (1960) and discussed earlier by Scott-Blair (1944).

Ridge and Wright (1965) who also performed tension studies in human skin found that the initial part of their curves could be fitted to a mathematical function that described the straightening and orientation of the fibrous material. The second stage of extension was interpreted in power law form and characterized the stretching of orientated collagen.

General rheological properties

It is now generally accepted that the mechanical properties of collagenous tissues are complex and that important viscous and perhaps plastic effects are present (Harkness 1966).

Roy (1880) in tensile studies on arterial strips defined an elastic and a viscous component and illustrated time dependent effects. The presence of residual deformation after loading in cartilage and its time dependance was reported by Göcke in 1928, and by Hirsch in 1944.

Gratz (1931) illustrated in fascia lata a sigmoid curve, hysteresis loop behavior and residual deformation after loading. The same characteristics were shown by Stucke (1950) in tendons.

Smith (1954) in rabbit ligaments found elasticity to submaximal loads of short duration. A viscous body type of response was found with the same loads during a longer period.

Rigby, Hirai, Spikes and Eyring (1959) showed that in tendons mechanical behavior was reproducible as long as strain did not exceed 4 per cent values, otherwise the tendons became progressively easier to extend, and values up to 35 per cent could be reached. With repeat tension cycles (Rigby 1964) rat tendons became increasingly stiffer and exhibited less hysteresis and slight

increase in length. Complete recovery of mechanical properties of the samples occurred after a ten minute period of rest between extensions.

In repeat stress relaxation tests in rabbit ligaments Viidik (1966) showed the phenomena to be larger on the first test, no changes occurred in subsequent tests independently of the resting period between them. In joints, stress relaxation was illustrated by Johns and Wright (1964), in skin by Ridge and Wright (1965), in bone by Smith and Walmsley (1959) and by Sedlin (1965).

McElhaney (1965) and Sedlin (1965) described time dependent effects in the deformation properties of bone and showed that deformation was a function of the rate at which it was induced.

The complex rheological properties of tissues including time dependent and plastic effects have been expressed in mechanical model form in recent years. Appropriate combinations of three basic elements, elastic, viscous and plastic, in series or parallel, with linear or exponential characteristics can be used to define the rheological properties of most materials (Reiner 1958). It is difficult, however, to ascribe specific anatomical elements to the constants of such models (Sedlin 1965).

Effect of fiber orientation

The geometrical disposition of the collagen fibrils is of primary importance in determining the mechanical properties of connective tissues. In tendons where the bundles of fibers lie in close relation with each other and parallel to the long axis of the structure, extensibility is low and tensile strength high (Elliott 1965). In skin where a loose three dimensional network with a less evident symmetry is present, extensibility is of high magnitude and tensile strength lower than in tendon (Harkness 1961).

Evidence shows that collagenous structures are anisotropic; their response to stress varies in the different axis, a property related to the orientation of the fibrillar structures.

In compact bone according to Gebhardt (1905) and Weidenreich (1923) the collagen fibers are organized in lamellae alternating in a circumferential or longitudinal direction, arranged around the longitudinal axis of the Haversian system. Rauber (1876) showed differences in bending properties between longitudinal and transverse sections of cortical bone. Maj and Toajari (1937) found that bending strength was largest in the longitudinal axis of the bone and concluded that this characteristic mechanical anisotropy was the result of the arrangement and number of the collagen fibers. Dempster and Liddicoat (1952) found that the compressive strength of cortical bone was largest along the longitudinal axis, while no differences were found between the radial and

tangential directions. Hirsch and Da Silva (1967) illustrated the close relation between small changes in fiber direction and strength of cortical bone.

Gratz (1931) stated that fascia lata is resistant to stress in the longitudinal direction of the fibers while strength is low in the transverse direction.

Harkness, Harkness and McDonald (1957) pointed to the importance of the arrangement of the fibrous connective tissue fibers in determining mechanical properties of the arterial wall. Collagen fibers in the media of blood vessels may be organized in two sets of helices one right handed and one left handed arranged in alternating layers, a pattern seen in most cylindrical organs (Harkness 1961).

Wolinsky and Glagov (1964) studied the structure of the arterial wall of rabbits at different distending pressures. A progressive orientation of the fibrillar components was evident with increasing pressure, from a random distribution in the relaxed state to a tight helical configuration of small pitch at pressures values close to the mean arterial pressure. Distinct differences in tensile properties of specimens cut in the longitudinal or transverse axis of arteries has been reported by Roy (1880), Reuterwall (1921), Wilens (1937), Hierton and Jordan (1956), and Milch (1965).

Skin has directional effects in its tensile strength (Mendoza and Milch 1965). Tensile strength in rat skin is largest parallel to the wrinkle lines (Berard, Woodward, Herrmann and Pulaski 1964). Human skin is more extensible across Langer's line than parallel to them (Ridge and Wright 1965). Abdominal skin shows more elongation at low loads in the longitudinal than in the circumferential direction of the body (Kenedi, Gibson and Daly 1965). The elastic properties of leather vary with the principal direction of the weave in the skin (Gustavsson 1956). Studies of stretched skin in vitro showed that with increasing extension, increasing orientation of the collagen fibers in the line of force was evident until all fibers were parallel to the direction of the load (Gibson, Kenedi and Craik 1965). It was concluded that variations in the collagen and elastic fiber networks, probably determine the variations due to age, location and direction rather than the characteristics of the collagen material itself.

Aging effects

Wertheim (1847) found that the coefficient of elasticity of bones, tendons and nerves increased, while the cohesion of the tissues decreased with advancing age.

Katzenstein and Fecher (1924) in human knee joint ligaments concluded that elastic behavior was most efficient in the 20 to 40 years old group. Above and below this age extensibility and residual deformation were larger. Annovazzi (1928) found that ligaments from young rabbits were easier to deform and exhibited less elasticity, than those from adult animals.

Rollhäuser (1950 a) reported tensile strength values of 3 to 4.5 Kp/mm² in newborn tendon against 9 Kp/mm² in adult tissue. With age elongation was larger in the young group.

Curtis (1963) reported that old tendons have a higher elastic modulus than young ones using slow rate of straining. No differences were found at rapid straining rates (Elden 1964 a). In later reports (Elden 1966) the changes in stress-strain behavior were found to be related to growth but not to senescence.

Rigby (1964) found similarities between the effect of repeat cycling of tendons and the aging effect in mechanical properties, X-ray diffraction pattern and shrinkage temperature. The tendons became stiffer, the residual deformation smaller, a more oriented pattern was evident with X-ray diffraction and the shrinkage temperature was higher.

Dick (1951) reported deformations of large magnitude in skin from old subjects and related this to changes in the elastic fiber network. Elden (1965 b) found no differences in stress-strain behavior of aging rat dermis. The transverse to longitudinal strain ratio was found to be age dependent and rise rapidly in old skin (Kenedi, Gibson and Daly 1965). Changes in tissue constants with age were reported by Ridge and Wright (1965). The normal tensile strength of Wistar rats was found to increase with age (Mendoza and Milch 1965).

Little or no differences were found as a function of age either in the deformation or recovery of adult articular cartilage in indentation elastometric studies (Sokoloff 1966). In fibrillated cartilage significant differences in elastic and recovery responses were earlier reported by Hirsch (1944).

Loss of extensibility with age, well in advance of macro or microscopic changes in the structure of the arterial wall was shown by Roy in 1880. Essentially the same increasing stiffness has later been shown by many investigators (Wilens 1937, Krafka 1940 and others). Yater and Birkeland (1929—30) indicated in addition an increase in aortic extensibility up to the age of 27 years.

III. MATERIALS AND METHODS

Material

This study was based on human lumbar spines obtained at routine postmortem examinations. A total number of 68 lumbar spines, and 592 testing samples were investigated.

Sampling was limited to cases where the autopsy procedure was performed within 48 hours following death. The specimens were obtained as indicated by the experiments to be performed, no specific sampling system was used. The spine segments included from the twelfth thoracic to the first sacral vertebrae, so that six intervertebral discs were obtained in every case. When possible all levels were used but occasionally discs had to be discarded because of damage at the autopsy procedure, or extreme degeneration.

The study group is described in table number I. It includes spines from both sexes and different age groups. The experiments in which the different spines were used are listed in table II.

TABLE I. *The study group.*

Subject number	Autopsy number	Age (years)	Sex	Time in hospital (days)	Death to testing	Cause of death
1	I 1296	5	F	1	1 day	Bronchopneumonia
2	I 954	14	M	12	2 days	Head injury
3	I 1222	17	M	23	1 day	Cardiac failure
4	II 185	18	F	1	1 day	Malignant lymphogranuloma
5	I 976	19	M	16	1 day	Chronic glomerulonephritis
6	I 29	20	M	1	1 day	Circulatory failure
7	I 601	20	F	3	2 days	Cerebral contusion
8	V 10	21	M	—	2 days	Multiple trauma
9	I 428	22	M	113	2 days	Cardiac failure, pulmonary hypertension
10	V 547	23	M	—	1 day	Multiple trauma
11	I 891	25	M	1	1 day	Uremia
12	I 1152	25	M	1	2 days	Electrocution
13	I 984	26	M	1	1 day	Poisoning
14	I 781	31	M	1	1 day	Respiratory failure
15	I 1395	32	F	60	10 hours	Uremia, carcinoma cervix uretus
16	I 485	33	M	7	2 days	Rupture aneurysm internal carotid artery
17	II 86	34	M	46	1 day	Carcinoma testicle
18	I 1014	35	M	1	1 day	Subdural hemorrhage
19	I 962	35	M	63	1 day	Cerebral tumor
20	I 986	36	M	6	1 day	Pulmonary edema

Subject number	Autopsy number	Age (years)	Sex	Time in hospital (days)	Death to testing	Cause of death
21	II 106	36	F	63	1 day	Malignant lymphogranuloma
22	I 540	37	M	17	2 days	Uremia
23	V 410	38	M	—	1 day	Multiple trauma
24	II 233	38	F	16	2 days	Carcinoma of the stomach
25	II 124	40	M	1	1 day	Perforated duodenal ulcer
26	I 552	41	M	2	2 days	Pneumonia, pulmonary edema
27	I 1106	44	M	9	2 days	Cerebral tumor
28	I 1183	44	M	9	1 day	Cardiac failure
29	II 659	44	M	2	1 day	Pulmonary edema, epidural hematoma
30	LS 6	44	F	—	2 days	Respiratory failure, chronic bronchitis
31	I 191	45	F	150	1 day	Burns
32	I 1411	46	M	5	2 days	Gastro intestinal bleeding
33	I 1197	46	M	71	2 days	Arachnoiditis, post op. hydrocephalus
34	I 149	46	M	—	10 hours	Myocardial infarction
35	II 19	46	M	18	1 day	Intra-abdominal bleeding
36	I 523	47	F	21	1 day	Pulmonary embolism
37	I 290	47	F	26	1 day	Carcinoma ovarian tube
38	I 134	47	M	101	2 days	Carcinoma of the lung
39	I 706	48	M	44	2 days	Chronic glomerulonephritis
40	I 850	48	F	7	1 day	Uremia
41	I 547	48	M	29	2 days	Mediastinal tumor
42	I 1182	49	M	3	2 days	Cerebral tumor
43	II 131	50	F	66	1 day	Pulmonary embolism, carcinoma uterus
44	I 684	51	F	92	8 hours	Pulmonary edema, atelectasis, chronic nephritis
45	I 1264	54	M	1	2 days	Bronchopneumonia
46	I 1158	54	F	5	12 hours	Carcinoma cervix uteri
47	I 872	54	F	16	1 day	Cerebral malacia
48	II 5	55	M	34	1 day	Cardiac failure
49	I 1244	56	F	1	10 hours	Myocardial infarction
50	I 1053	56	M	27	1 day	Post op. internal hydrocephalus
51	I 72	57	F	5	1 day	Cerebral hemorrhage, pulmonary embolism
52	I 1245	61	M	2	12 hours	Pulmonary infarction
53	I 840	62	M	1	1 day	Cardiac failure
54	I 925	62	M	1	1 day	Cardiac failure
55	II 241	63	M	7	1 day	Gastric bleeding
56	I 1081	63	M	5	1 day	Myocardial infarction
57	I 1144	64	M	—	2 days	Portal thrombosis, gastrointestinal bleeding
58	II 165	64	F	8	2 days	Cardiac failure
59	I 1169	66	M	10	8 hours	Cerebral hemorrhage
60	I 817	67	M	150	1 day	Cardiac failure
61	II 242	68	M	25	1 day	Uremia
62	I 1005	68	M	2	2 days	Cardiac failure
63	II 232	69	M	13	2 days	Congestive heart failure
64	I 1151	69	F	6	2 days	Congestive heart failure
65	I 1409	70	F	2	1 day	Myocardial infarction
66	I 758	71	M	1	1 day	Pulmonary embolism
67	I 1175	77	M	6	8 hours	Congestive heart failure
68	I 818	78	M	4	1 day	Congestive heart failure

TABLE II. *The Experiments*

Experiment Number	Title of experiment	Number of samples	Subjects contributing
1	Variations in water content within 48 hours after death	20	Rabbit experiment
2	Swelling of the annulus in different solutions	40	12, 24, 34, 55
3	The effect of immersion in 0.9 % NaCl on tensile properties	11	39, 56
4	Air exposure experiments	45	3, 28, 57, 62
5	The effect of air exposure on tensile properties	16	33, 47
6	Repetitive loading	16	40, 55
7	The effect of rapid freezing and thawing	16	32, 37
8	Tensile properties in different directions	114	7, 9, 16, 17, 21, 25, 31, 36, 38, 56
9	Tensile properties at the horizontal axis and along the fiber direction axis in 1.5 mm thick specimens		
10	Tensile properties in the two fiber direction axis	18	22, 26, 29
11	Tensile properties in different depths in the anterior and posterior annulus fibrosus	16	41, 43, 44
12	Behavior of samples under constant deformation	81	8, 15, 19, 20, 35, 45, 50
13	Tensile properties at different rates of deformation	10	48
14	Determinations of tensile strength	7	42
15	Effect of age and degeneration on tensile properties	38	4, 6, 27, 30, 35, 51
		124	1, 2, 5, 10, 11, 13, 14, 18, 19, 20, 23, 33, 39, 45, 46, 49, 50, 52, 53, 54, 58, 59, 60, 61, 63, 64, 65, 66, 67, 68
16	Effect of age and degeneration on some biochemical characteristics	40	1, 2, 5, 13, 18, 19, 20, 46, 50, 54, 58, 59, 61, 63, 67, 68

Evaluation of the material

The spines were radiographically examined for signs of intervertebral disc degeneration (Knutsson 1944). Attention was paid to narrowing of the disc, presence of osteophytes, and subchondral bone changes and accordingly the discs were classified as normal, moderately or severely degenerated.

All specimens were examined macroscopically and the degree of degeneration assessed in agreement with accepted standards (Lindblom 1944, Friberg and Hirsch 1950, Lindblom 1951, Virgin 1951, Hirsch and Schajowicz 1952, Ingelmark and Ekholm 1952, Naylor and Smare 1953). (Figs. 1, 2, 3, and 4.)

Grade 1. Normal discs. Annulus free from ruptures and shiny white; shiny white gelatinous nucleus.

Grade 2. The appearance is normal but the nucleus exhibits a more fibrous structure. A clear boundary is present between annulus and nucleus.

Grade 3. Isolated fissures in the annulus. The nucleus is dry and occasionally discolored. The boundary between the nucleus and annulus is no longer distinct.

Grade 4. Severe changes. Ruptures and sequestrae in both annulus and nucleus. Marginal osteophytes often found.

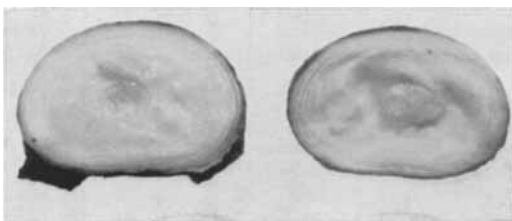


Figure 1. Macroscopic grade one.

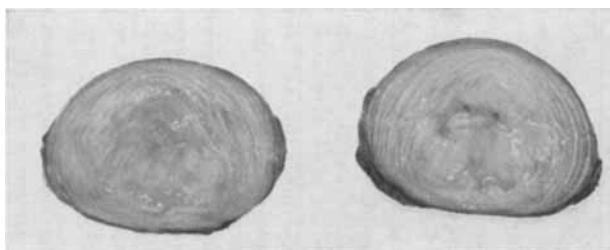


Figure 2. Macroscopic grade two.

In agreement with earlier studies (Friberg and Hirsch 1950, Lindblom 1951), it was found that some discs with normal roentgenograms had undergone severe changes on macroscopic examination. Intervertebral discs with a macroscopic grading of 1 or 2 were used in the experiments dealing with the standardization

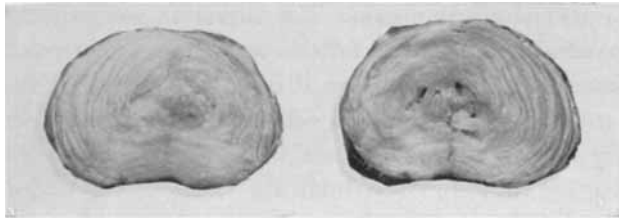


Figure 3. Macroscopic grade three.

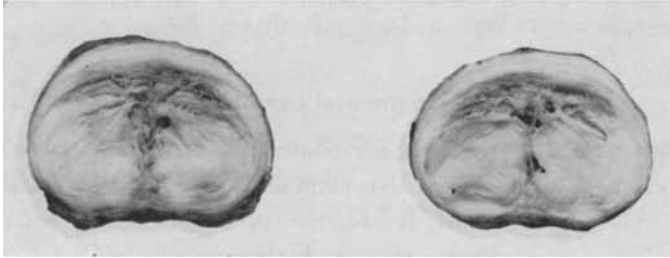


Figure 4. Macroscopic grade four.

of methods and with the evaluation of the general tensile characteristics of the tissue. When studying the effects of age and degeneration, discs from all ages and degeneration grades were included. In these particular experiments, the samples were examined histologically after the tensile tests were performed. To evaluate the specimens, the incidence and magnitude of several morphological changes were taken into account. These alterations as described by Hirsch and Schajowicz (1952) were: cavities filled with granular material, cartilage metaplasia, fissures, presence of vascular or fibrous tissue. An arbitrary grading system was used and accordingly the samples were divided in three groups: 1) Normal or minimal degenerative changes. 2) Moderate degeneration. 3) Severe degeneration.

Site of sampling

The general design of this investigation called for small specimens of uniform size and configuration. Taking into account the morphology of the annulus fibrosus it was considered necessary to cut the samples parallel to the direction of its lamellae. For these reasons the anatomical shape of the annulus limited the possibilities of sampling. Anteriorly the annulus is thickest, highest and least curved, a feature that facilitated the preparation of specimens. In most experiments the anterior annulus area was used as a sampling site and the samples were taken at a depth between 1 and 2 mm from the periphery. A limited number of posterior annulus samples could be obtained and were used in the

evaluation of the tensile properties. The lateral segment of the annulus is markedly curved in lumbar discs; for this reason it was not possible to obtain satisfactory lateral specimens.

Methods

The specimens were prepared and tested within two hours of sampling to avoid the variables involved with the use of preservation methods. When not directly handled the spines were kept in closed polyethylene bags.

Environmental conditions

Sample preparation was done under controlled environmental conditions. The intention was to maintain a relative humidity of 100 per cent whenever the specimens were exposed to air. It had been previously determined that under these conditions loss of tissue water could be controlled. All the procedures were performed in a room with a total volume of 14 m³ approximately, equipped with insulated double doors and special electrical outlets. The room temperature varied between 20 to 23° C. Humidity was produced with a commercial humidifier (Defensor 3001) with a vaporizing capacity of 4 liters of water per hour. Two standard hair-type hygrometers (Inor) were used for control of relative humidity values. The humidifier was kept functioning constantly during the entire procedure and within 10 minutes the hygrometer readings showed 100 per cent relative humidity. A permanent foglike atmosphere and condensation everywhere in the room indicated almost constant saturation conditions.

Section of samples

After removing the longitudinal ligaments the intervertebral discs were dissected free and rectangular blocks were obtained from the anterior or posterior annulus. The blocks had 25 to 30 mm in length, the height of the disc in width and the thickness of the annulus. They were cut by sharp dissection following the contour of the inner part of the annulus. From these blocks it was intended to obtain sheets of constant thickness cut parallel to the fiber layers of the annulus. In the initial experiments these sheets were removed by sharp dissection using a Zeiss stereo microscope during the procedure. It was attempted to obtain a thickness of 1 mm, but the dimensions of the samples were far from constant with this procedure.

In later experiments the specimens were cut with a Jung freezing microtome. This instrument is designed to cut very thin sections, for histological

purposes. A calibration procedure was necessary to adapt it for the thick sections needed in this investigation. A dial displacement micrometer (Metron) measuring with an accuracy of ± 0.01 mm was used for this purpose. It was attached to the frame of the microtome and placed perpendicular to the table to measure its relative displacements. The feeding wheel of the microtome was then calibrated at 0.5 mm intervals, and reference marks attached to it to provide reproducible sections.

The blocks were flattened and frozen in the microtome table with a carbon dioxide snow stream. They were sectioned in sheets of 1.5 mm or 1 mm depending on the experiments to be performed.

A control was made to insure that sectioning was made parallel to the layers of the annulus later in the procedure, when the final testing samples were ready. They were examined under a dissecting microscope and those not fulfilling this requirement were discarded.

Thickness measurements

In preliminary trials a micrometer eye piece was attached to a stereomicroscope and used as a measuring device. Although theoretically the accuracy of the system was within ± 0.01 mm with the magnification used, errors could be easily induced by slight changes in the position of the specimen or movements of the microscope.

A thickness gage was developed to permit contact measurements with a constant minimal force acting on the specimen distributed over a known surface area (fig 5). These requirements were essential as the material was not rigid and would creep under the influence of a load.

A dial displacement gage with an accuracy of ± 0.01 mm (Metron) was used as a measuring device. It was attached in a vertical position to a metal stand and kept balanced at a fixed distance from the measuring table by means of two thin flexible steel plates. This distance could be changed to allow measuring samples of different dimensions. When not in use the system was kept unloaded with a spring device. A 2 mm thickness gage was used to zero the dial micrometer. The specimen was measured with a 1 mm thickness plate on top of it, the zero position represented in that way 1 mm thickness in the specimen. The combined weight of the plate and the force exerted by the micrometer represented a constant reproducible load of 0.13 p/mm^2 applied to the specimen during measurements. A small amount of creep was noticeable, seldom over 0.01 mm. The measurements obtained in this way represented the average thickness of the sheets.



Figure 5. The contact thickness micrometer.



Figure 6. The modified press with a cutting die mounted.

Stamping press

From the sheets, final testing samples were made in a press and die assembly (fig. 6). A punch press was modified to mount the cutting dies. A round stamping table was manufactured of wood and covered with a layer of plexiglas first and soft plastic next to protect the sharp edges of the cutting devices. The table could rotate around a central axis and a goniometer was incorporated in its base to allow die-cutting of samples at varying angles. Unless it is specifically stated all specimens were stamped along the long axis of the sheets.

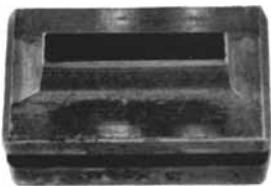


Figure 7. 25×5 mm cutting die used in the early experiments.



Figure 8. 30×2 mm cutting die used for most experiments.

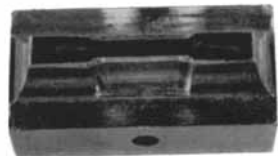


Figure 9. The cutting die with a reduced central section used in determinations of tensile strength.



Figure 10. A detail of the Instron floor model tensile tester. The recorder is at the left, the control panel at the right. The air compression clamps and jaws faces are mounted.

Cutting dies and specimen size

The specimens were stamped with specially designed cutting dies. These instruments were machined of steel subsequently hardened. The cutting edges were sharpened at an angle of 15 degrees. Three different dies were used. A 25×5 mm die was arbitrarily chosen for the initial experiments related to the development of methods such as weight determinations during air exposure or swelling (fig. 7).

For later experiments where tensile tests were performed, two dies were designed. The first one 30×2 mm in dimensions was used in the usual tensile tests (fig. 8). The second one, a die with a reduced central area of 10×2 mm and expanded end segments of 7.5×3 mm was employed for determinations of tensile strength (fig. 9). When using the rectangular shaped die, if one of the

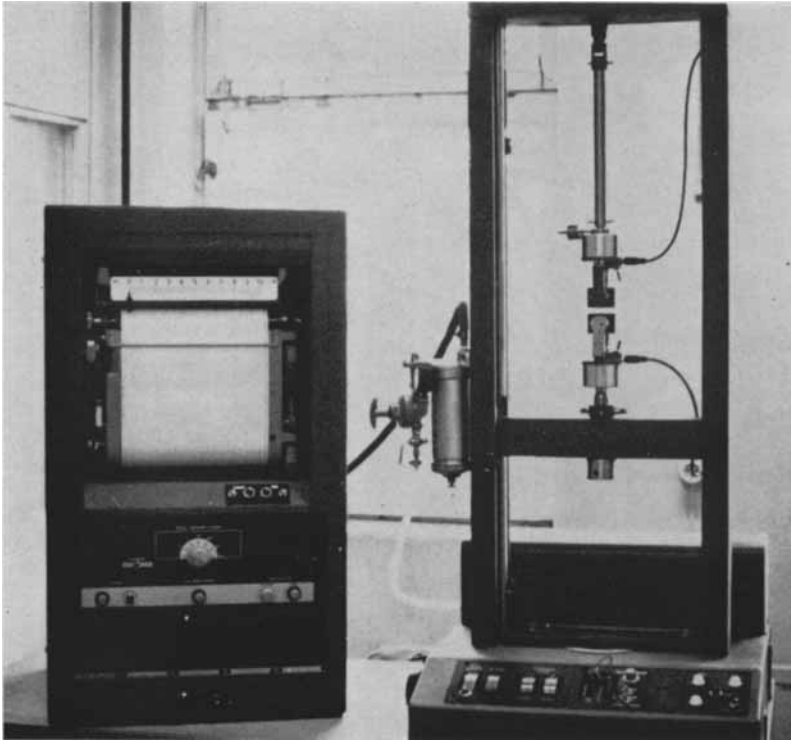


Figure 11. The Instron table unit with the air compression clamps.

ends of a specimen was found not parallel to the sheets of the annulus, that segment could be discarded, provided that it was small and left a length of at least 20 mm. This was not possible with the reduced section die and the number of samples that was necessary to discard when using it was high.

The width of the specimens obtained with the last two dies was 2 mm and the testing length adopted in all cases, that is to say the distance between the clamps of the testing machine, was 10 mm; the length—width ratio was 5:1. It represented the best compromise that could be reached between ideal sample size from a technical viewpoint and available material.

Testing equipment

Two different testing set-ups were employed in this investigation. An Instron TTCM floor model tensile testing machine (fig. 10) and an Instron TM table model unit (fig. 11). Experiments dealing with tensile strength and the age and degeneration study were performed with this last equipment; all other tests were made on the floor model machine. Both units have the same basic charac-

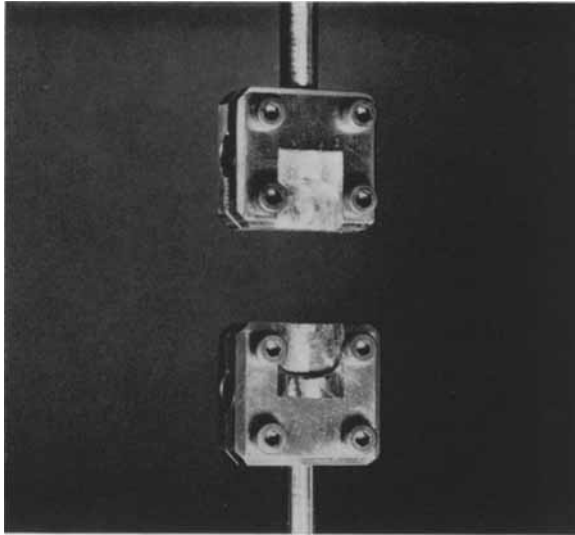


Figure 12. Detail of the alternate set of clamps.

teristics, allowing tests under many different conditions. The force measuring system uses load cells with an accuracy of $\pm 1/4$ per cent. The crossheads provide a constant rate of specimen deformation, independent of the load with speeds varying from 0.02 cm/min. to 40 cm/min. Like the crosshead, the chart of the recorder is driven synchronously over a wide range of speeds. The correspondence between the motions of the chart and crosshead, provides accurate records of sample extension. The ratio of chart to crosshead speed can be selected to give a large choice of extension magnifications.

Clamps

The characteristics of the material are such that gliding during application of tension can represent a problem. Special jaw faces were developed with matched blunt serrations at 0.75 mm intervals and were fitted to the Instron air compression clamps. With these clamps a constant known pressure can be maintained between the jaws. The ideal pressure was found to be 1.5 Kp/cm². At this level no gliding occurred and in tensile strength experiments "free space" ruptures were obtained. As these clamps are heavy and could not be adapted to the small capacity tension cell used when small loads were required, an alternate set of clamps was designed (fig. 12). The jaws faces had the same 0.75 mm interval matching serrations and were tightened by means of four screws.

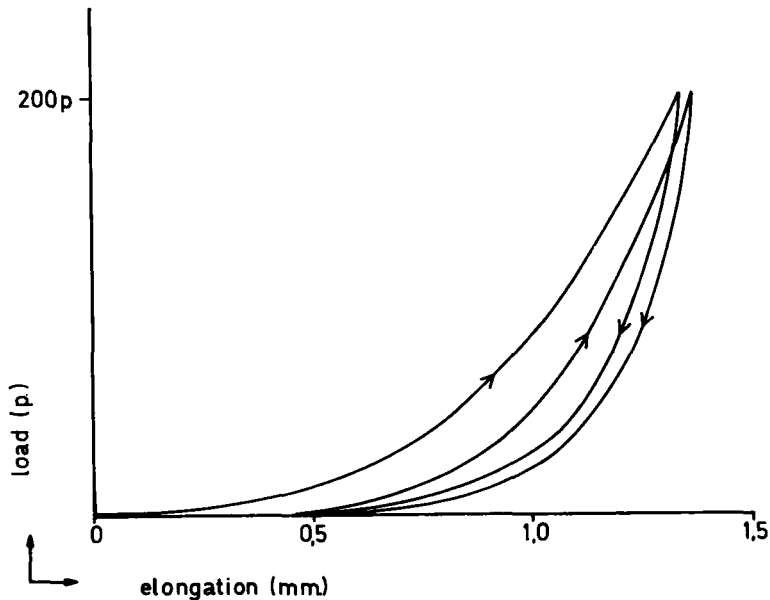


Figure 13. Load elongation diagram from a sample cycled twice to a load of 200 p.

Methods of testing

All tests were performed in air in rooms where the temperature and relative humidity were controlled. The room conditions in the experiments with the Instron floor model unit were 65 per cent relative humidity, 21 degrees C temperature. A relative humidity of 90 per cent and temperature 21 degrees C was employed when using the table model. These conditions were accurate to ± 2 per cent. The tests were accomplished within one minute of exposing the samples to air. Humidity saturation conditions would have been desirable as discussed later. However, the testing machines belonged to and were located in other institutions where they were intensively used for other purposes a fact that made unpractical the development and installation of a suitable humidity chamber.

Most tests were performed at a rate of deformation of 0.5 cm/min. and a recorder paper speed of 50 cm/min. providing a magnification factor of one hundred for readings of specimen elongation. Due to the characteristics of the recorder this was the fastest rate of loading that allowed magnifications suitable to the size of the deformations occurring in the specimens.

In most experiments the samples were cycled twice between zero and a given load (fig. 13). In determinations of tensile strength the specimens were loaded to failure. In stress relaxation experiments, the distance between clamps was maintained constant after reaching a given value, and the load was measured as a function of time.

Parameters

The following parameters were evaluated from the load elongation diagrams: 1) Elongation to a given load. 2) Residual deformation. 3) Energy dissipation. The first two were read directly from the curves, and expressed in mm. Energy dissipation was determined by planimetry reading of the area under the loading and unloading curves. Appropriate corrections for chart magnification and paper speed were made. The energy values in p.mm were normalized for specimen volume in mm³ (p = pond). In the air drying and swelling experiments where the dimensions of the specimens changed after the original measurements, and in the freezing tests where the samples were cut by hand, the thickness of the specimens could not be determined with accuracy. In these cases energy values were expressed in p.mm.

Elongation values provided information on the elastic stiffness of the material. The residual deformation and energy dissipation reflected viscous damping effects.

In the tensile strength experiments, where the tests were carried to failure two parameters are reported: ultimate strength in Kp/mm² and elongation to failure in mm.

Statistical methods

Standard statistical methods were used (Steel and Torrie, 1960). Means and standard errors were employed as measures of central tendency and dispersion respectively. Analysis of variance or Student's t test were applied to evaluate significance of differences between treatments. When using analysis of variance, Sheffe's contrasts method was applied to evaluate independent differences between means in multiple comparisons (Sheffe' 1953). Product moment correlation was used in evaluation of relations between biochemical data and age.

Linear regression analysis was employed to evaluate loss of weight as a function of time in samples exposed to air. It was also applied in the study of relations between tensile properties and age.

The 5 per cent level of probability was chosen as the criterion of statistical significance.

Significance at the 5 per cent level is indicated by one asterisk* and at the 1 per cent level by three asterisks***.

IV. DISCUSSION OF THE METHODS AND RELATED EXPERIMENTS

In an investigation of this nature there are many factors that can influence the characteristics of the samples and modify their load deformation response. The methods finally adopted were based on a series of experimental procedures, where these variables were evaluated. Consideration was given to the effect of postmortem changes, the environmental factors and the use of specific techniques for preparing or testing the samples. In evaluating postmortem and environmental influences the main subject discussed here is the water content of the tissues and the variations occurring due to these factors. In the study of specific techniques repetitive tests and the use of a freezing microtome are considered.

Postmortem changes

The most evasive of the factors mentioned are of course the changes that occur after death. When using autopsy material there is a time lag between death and necropsy examination that escapes the control of the investigator. It appears, however, from a review of the experimental evidence available in relation to other tissues (Chapter II) that within a certain time following death, at least three to four days, the tensile properties are not affected. In the publications where opposite conclusions were drawn, the effects of dehydration were possibly added or the experimental evidence was incomplete. The 48 hours postmortem sampling limit adopted in this investigation was empirically chosen based on this previous evidence.

In devising methods, one of the main objectives was to maintain the water content of the samples at the same level as when removed from the body. It was of interest to know whether changes in the water content had occurred in the interval between death and sampling. This question was answered in the following experiment.

Variations in water content within 48 hours after death

For obvious reasons animals were used in this experiment. Two rabbits of similar age and weight were chosen. They were sacrificed with a heavy occipital blow and immediately after, five intervertebral discs from each animal were chosen in a random fashion and removed. The incisions were closed and the animals stored at 4 degrees C for 48 hours. At the end of this period another group of ten discs was

sampled. In a saturation humidity atmosphere the annulus fibrosus was dissected from each disc. The specimens were weighed in an analytical balance with an accuracy of ± 0.01 mg. Subsequently the samples were vacuum dried at 2×10^{-3} mm Hg for 48 hours. Weighing was repeated and wet weight - dry weight differences calculated. Water content was estimated as the ratio between this difference and the wet weight of the samples:

$$\frac{\text{Wet weight} - \text{dry weight}}{\text{wet weight}}$$

Student's t test was used to evaluate the significance of differences in water content between the two groups.

Results. The data is summarized in table III.

TABLE III. *Water content of annulus fibrosus immediately after and 48 hrs. following death.*

Condition of samples	$\frac{\text{Wet-dry weight}}{\text{Wet weight}}$	n
Fresh	0.736 ± 0.003	10
48 hours	0.737 ± 0.011	10

t = 0.028 non significant.

The differences between the two groups were not significant indicating that the water content of annulus fibrosus samples remained constant for at least 48 hours following death.

Environmental influences

When tissues are removed from the body and are exposed to a foreign environment their water content is the most labile factor to be influenced. To avoid loss of water in air the method adopted by many investigators is to immerse or expose the samples to aqueous solutions; in these circumstances collagenous tissues swell. Evidence shows that tensile properties are significantly affected in both cases pointing to the necessity of a different method. In the next series of experiments the effects of environmental influences on annulus fibrosus samples was studied and a solution to the problem was proposed.

Swelling of the annulus in different solutions

The following experiment was designed to study swelling characteristics of the annulus in four different media: distilled water, 0.9 per cent sodium chloride solution, rheomacrodex (10 per cent dextran) and human plasma. The last two solutions were chosen on the assumption that due to their osmotic pressure they would tend to minimize the amount of water uptake in the tissue.

40 samples $20 \times 5 \times 1$ mm in dimensions were obtained from the anterior annulus fibrosus of four different lumbar spines. They were divided in a random fashion in 4 groups of ten samples each. The samples were weighed fresh in an analytical balance to an accuracy of ± 0.01 mg. They were subsequently placed in beakers containing the different solutions employed, at room temperature. They were removed at 5 min., 30 min., 1 hour, 2, 3, 5, 7, 24 and 32 hours and weighed. The surfaces were blotted for five seconds in filter paper prior to each weighing, to remove excess surface liquid. (Elden 1964 b). Swelling curves were obtained, plotting weight vs. accumulated time of swelling. Original weights and fully swollen weights were used as variables in the statistical analysis.

Results. The samples exhibited considerable swelling in the four media investigated. (fig 14). Water was accumulated at a rapid rate in the beginning until the process reached an equilibrium phase. In distilled water the uptake was higher and equilibrium was reached in 2 hours. In the other solutions equilibrium swelling was established within five hours, and was of similar magnitude.

The data is summarized in table IV.

TABLE IV. *Swelling of samples in different solutions.*

Solution	Average original weight in gr.	Average weight after swelling in gr.	n	t
Distilled water	0.1066 ± 0.0054	0.2554 ± 0.0180	10	10.083***
0.9 % NaCl	0.1017 ± 0.0020	0.1824 ± 0.0030	10	25.544***
Human plasma	0.0968 ± 0.0061	0.1702 ± 0.0090	10	8.528***
Rheomacrodex (10 % Dextran)	0.0920 ± 0.0020	0.1927 ± 0.0066	10	20.170***

The differences in weight before and after swelling were highly significant in all instances. The shape and dimensions of the samples changed making cross-section measurements inaccurate.

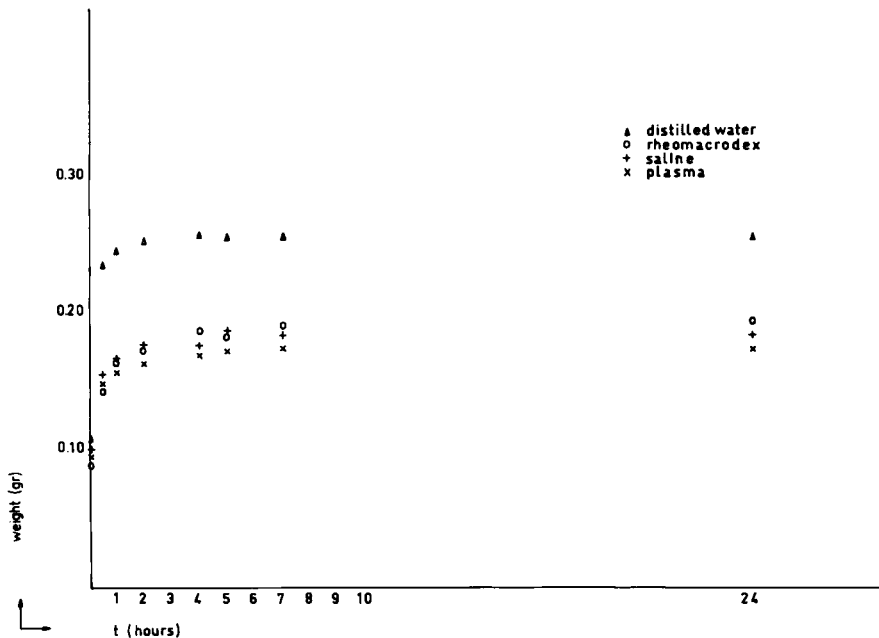


Figure 14. Swelling curves of anterior annulus fibrosus samples in different solutions.

The effect of immersion in 0.9 per cent NaCl solution on tensile properties

Water uptake of considerable magnitude was induced by all the solutions previously studied. 0.9 per cent sodium chloride was arbitrarily chosen to investigate the effects of swelling on tensile properties.

Eleven anterior annulus samples 2×1 mm in cross-section were prepared. They were tested in tension and cycled twice to 200 p in air at 65 per cent relative humidity and 21 degrees C temperature. They were then immersed in 0.9 per cent NaCl solution for five hours. After swelling was completed, they were retested as before. Elongation to 200 p, residual deformation and energy dissipation were calculated from the curves. The data for the first cycle is presented here. Student's t test was used to evaluate the significance of differences before and after swelling.

Results. The evaluated data is summarized in table V.

TABLE V. *The effect of swelling in 0.9 per cent NaCl on tensile properties.*

	Before swelling	After swelling in 0.9 % NaCl	n	t
Elongation mm	1.13 ± 0.06	1.86 ± 0.10	11	11.152***
Residual deformation mm	0.27 ± 0.02	0.56 ± 0.04	11	6.455***
Energy dissipation p. mm	26.357 ± 1.254	61.332 ± 6.483	11	5.811***

Significant differences occurred in all the evaluated parameters. The samples became more extensible, the residual deformation and the energy dissipation were significantly larger. (Fig. 15).

THE EFFECT OF IMMERSION IN 0.9% NaCl

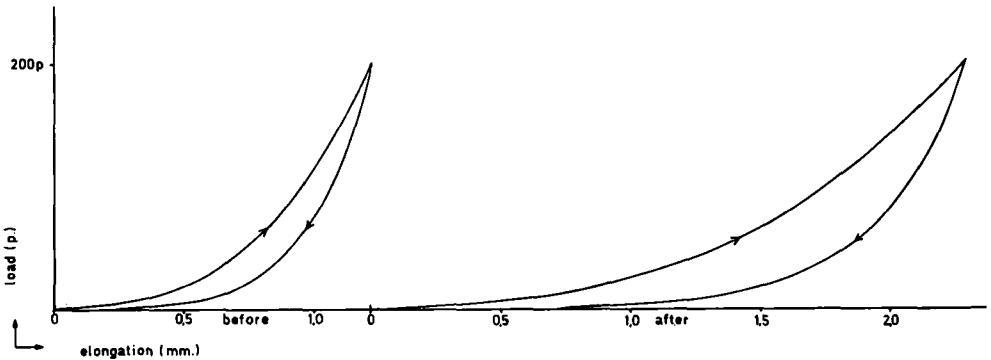


Figure 15. First tension cycle in one sample before and after swelling in 0.9% sodium chloride solution.

From these experiments it is concluded that immersion techniques are inadequate regardless of the solution used. To expose the samples to aqueous media without immersing them as for example wrap them in a compress soaked in 0.9 per cent sodium chloride solution will bring about the same process of swelling although a steady state of equilibrium may not be reached.

Air exposure experiments

To study the process of water loss in air, samples were exposed to atmospheres of controlled relative humidity and temperature. The experimental conditions were produced in a specially designed box provided with automatic temperature and humidity control (Van der Valk 1965). The box was furnished with double walls made of plexiglas to provide dead space insulation and unobstructed view within the box, and openings fitted with plastic gloves to allow work inside of the chamber without inducing humidity changes. The dimensions of the box were $120 \times 60 \times 60$ cm³. Humidity was produced by blowing air with a fan over a water container electrically heated to facilitate evaporation. Drying was accomplished using a separate fan blowing over silica gel. A psychrometer constructed with temperature sensitive diodes was used for automatic humidity control. Changes in temperature were induced with an electric heater or a cooling coil which used circulating tap water. Automatic temperature control was provided by means of a contact thermometer and a relay system. A torsion balance weighing to an accuracy of ± 0.2 mg was placed inside the box. (Figs. 16 and 17).

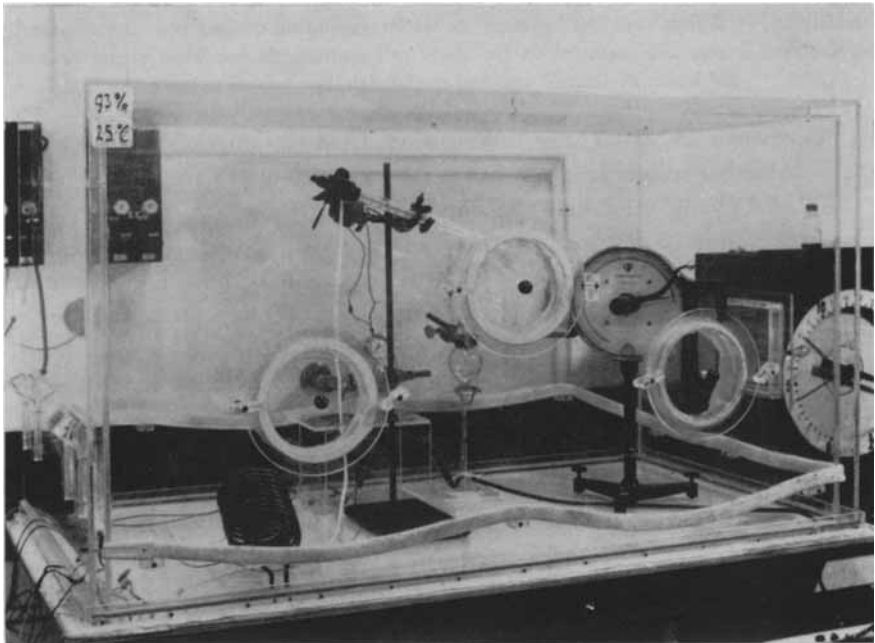


Figure 16. The plastic box used to expose samples to air with varying temperature and relative humidity.

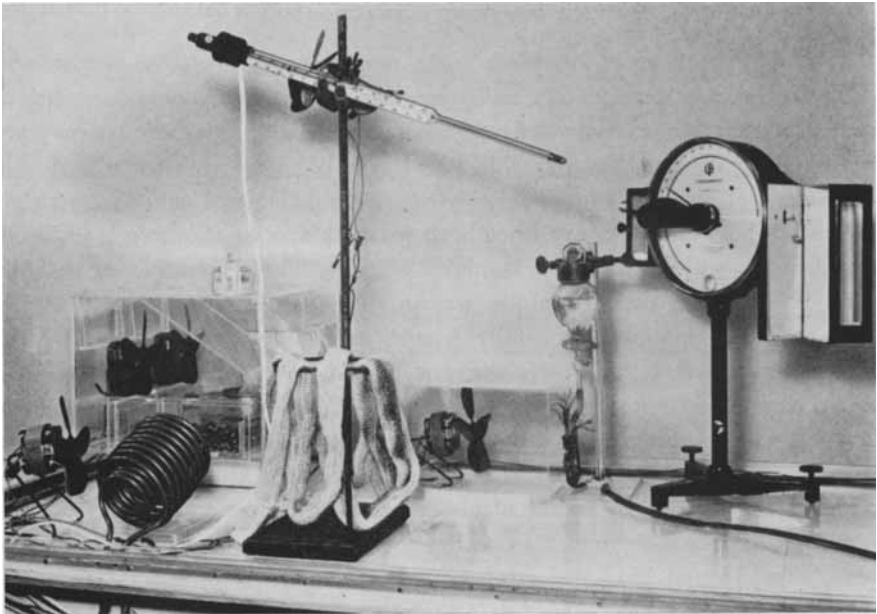


Figure 17. A detail of the contents of the box showing in the left posterior corner the humidifying — drying unit, left anterior the water circulating cooling coil, center anterior the electrical heater and mounted on the stand the contract thermometer, center posterior the humidity control unit and to the right the torsion balance.

45 samples $25 \times 5 \times 1$ mm in dimensions from the anterior annulus of four lumbar spines were divided at random in nine groups, each to be exposed to one of the following conditions:

Temperature 25 degrees C, relative humidity 70 per cent, 80 per cent, and 90 per cent. Temperature 30 degrees C, relative humidity 70 per cent, 80 per cent, and 90 per cent. Temperature 37 degrees C, relative humidity 60 per cent, 70 per cent, and 80 per cent. The capacity of the humidifying unit did not allow to obtain higher relative humidities than 80 per cent at 37 degrees C.

Five samples were used for each setting of constant humidity and temperature. They were introduced in the chamber at one minute interval and weighed initially and every fifteen minutes for a minimum of two hours.

Results. Progressive loss of weight occurred in all instances. Plotting weight versus time in air a curvilinear relation is obtained, but a straight line function can be assumed for the first 60 minutes interval (fig. 18). A regression coefficient was calculated for each curve following the method of least squares and used as a variable in an analysis of variance for relative humidities at a given

temperature. Independent comparisons between mean differences were made using Sheffe's contrasts method.

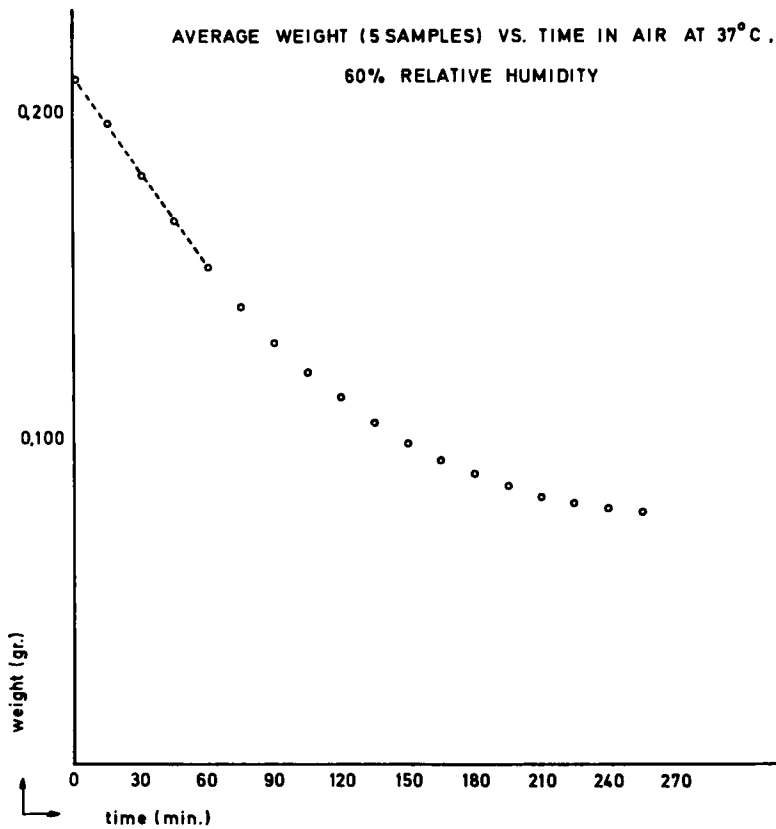


Figure 18. Loss of weight in air at 60% relative humidity 37° C temperature, illustrating a straight line relation during the first hour.

The results are summarized in Tables VI, VII, and VIII, and the statistical analysis follows each table.

TABLE VI. *Loss of weight in air at 25° C temperature.*

Relative humidity %	Regression coefficient	n
70	0.415 ± 0.017	5
80	0.303 ± 0.004	5
90	0.133 ± 0.006	5

ANOVA for 25° C.

V.S.	D.F.	S.S.	M.S.	F.
Between relative humidities	2	0.2009	0.1004	179.32***
Within groups	12	0.0068	0.0006	
Total	14	0.2077		

Average differences.

Relative humidity %	80	90
70	0.111*	0.281*
80		0.170*

TABLE VII. Loss of weight in air at 30° C temperature.

Relative humidity %	Regression coefficient	n
70	0.412 ± 0.013	5
80	0.300 ± 0.002	5
90	0.131 ± 0.006	5

ANOVA for 30° C.

V.S.	D.F.	S.S.	M.S.	F.
Between relative humidities	2	0.2001	0.1000	285.85***
Within groups	12	0.0043	0.0004	
Total	14	0.2044		

Average differences.

Relative humidity %	80	90
70	0.112	0.281*
80		0.169*

TABLE VIII. *Loss of weight in air at 37° C temperature.*

Relative humidity %	Regression coefficient	n
60	1.042 ± 0.020	5
70	0.612 ± 0.035	5
80	0.327 ± 0.012	5

ANOVA for 37° C.

V.S.	D.F.	S.S.	M.S.	F.
Between relative humidities	2	1.2981	0.6491	218.54***
Within groups	12	0.0357	0.0030	
Total	14	1.3338		

Average differences.

Relative humidity %	70	80
60	0.431*	0.716*
70		0.285*

The differences between regression coefficients of relative humidities at constant temperature were highly significant. If we plot these regression coefficients against relative humidity, (fig. 19), it is evident that loss of tissue water is similar at 25 degrees C and at 30 degrees C. The water loss gradient is larger at 37 degrees C for comparable relative humidity values. All curves approach the zero value at a relative humidity of 100 per cent, indicating that at that point no loss of water would occur. Annulus fibrosus samples have a high content of loosely bound water. Water vapor diffuses to the specimen surface along a pressure gradient, and the slope of the gradient determines the rate of transfer. When the gradient is eliminated (relative humidity 100 per cent) no loss of water occurs.

Following these conclusions the samples in the subsequent experiments of this investigation were prepared and handled in a room provided with a saturation relative humidity atmosphere. All the tensile tests performed in this study were of short duration; the samples were never exposed to air more than one minute. This implied minimal loss of water and consequently it was thought that humidity

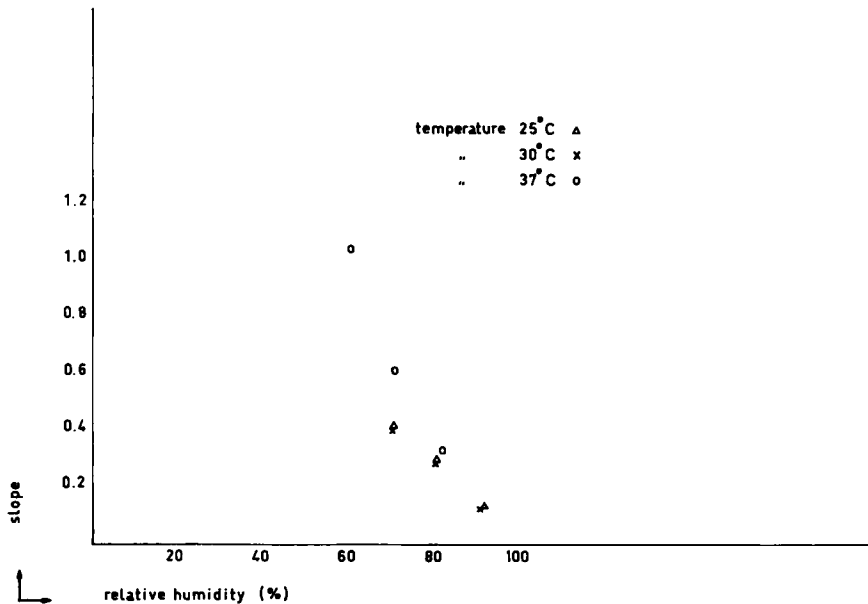


Figure 19. Average regression coefficients (slope) for curves of weight versus time during 60 minutes of air exposure plotted as a function of relative humidity.

conditions lower than 100 per cent for such a short time should have negligible effects on tensile properties. This assumption was confirmed in the following experiment.

The effect of air exposure on tensile properties

This experiment was designed to answer the following questions: 1) What is the effect of loss of water on tensile properties of annulus samples? 2) Do short periods of air exposure at 65 per cent relative humidity and 21 degrees C induce significant alterations in these same properties?

Sixteen samples 2×1 mm in cross-section from the anterior annulus of two lumbar spines were prepared. They were cycled twice to 200 p load at 65 per cent relative humidity and 21 degrees C temperature within one minute of being exposed to air. The experiment was repeated after ten and sixty minutes of air exposure. Elongation, residual deformation and energy dissipation were calculated. Data for the first cycle is presented here. Analysis of variance and Sheffe's contrasts method were used to evaluate the differences between means.

Results. The data is summarized in table IX and illustrated in fig. 20.

TABLE IX. *Effect of air exposure at 65 per cent RH and 21° C on tensile properties.*

	Time of air exposure.		
	1 minute	10 minutes	60 minutes
Elongation mm	1.05 ± 0.07	1.04 ± 0.06	0.89 ± 0.11
Residual deformation mm	0.25 ± 0.03	0.29 ± 0.02	0.59 ± 0.11
Energy dissipation p mm	24.22 ± 1.80	25.86 ± 1.18	36.82 ± 3.03

The statistical analysis was as follows:

ANOVA elongation.

V.S.	D.F.	S.S.	M.S.	F.
Between times	2	0.1885	0.0942	7.39***
Between samples	9	1.5077		
Within groups	18	0.2295	0.0127	
Total	29	1.9257		

ANOVA Residual deformation.

V.S.	D.F.	S.S.	M.S.	F.
Between times	2	0.6691	0.3345	8.73***
Between samples	9	0.4818		
Within groups	18	0.6891	0.0382	
Total	29	1.8400		

ANOVA Energy dissipation.

V.S.	D.F.	S.S.	M.S.	F.
Between times	2	939.759	469.880	16.94***
Between samples	9	743.327		
Within groups	18	499.226	27.735	
Total	29	2182.312		

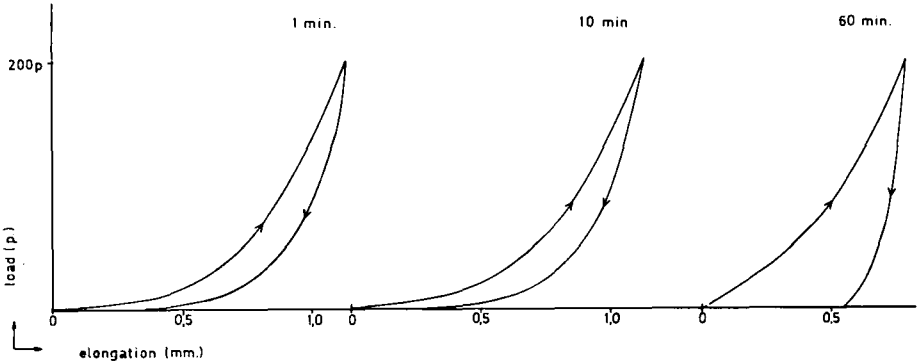


Figure 20. First tension cycle in one sample after one minute, ten minutes and sixty minutes of exposure to air at 65 per cent relative humidity, 21° C temperature.

The differences between 1 minute and 10 minutes were not significant for all the evaluated parameters. The 60 minutes data were significantly different from the other two.

Conclusion. Differences in the evaluated parameters were not significant after ten minutes of air exposure at 65 per cent relative humidity and 21 per cent C temperature. After one hour of air exposure in the same environmental conditions the samples became stiffer, exhibited increased residual deformation and increased energy dissipation.

The general effects of drying were similar to those shown for other tissues with relation to the stiffness of the sample. Energy dissipation and residual deformation increased, however, indicating larger internal damping or plastic effects.

It is evident that the conditions adopted for tensile tests, one minute of air exposure at 65 per cent or 90 per cent relative humidity and 21 degrees C, were within acceptable limits as water loss during that period is minimal. Actually at 90 per cent relative humidity the water loss gradient is smaller and tests of longer duration could probably be performed.

Repetitive loading

Repeat tests on the same specimen before and after a given treatment were used to increase experimental efficiency, and reduce the number of samples. It is known that with consecutive loading cycles in ligaments, tendons (Gratz 1931, Stucke 1950, Rigby 1964, Viidik 1966), and intervertebral discs (Virgin 1951,

Brown, Hansen and Yorra 1957, Rolander 1966), the structures become stiffer, residual deformation and hysteresis decrease. These effects are primarily of a viscous nature and recovery is expected with time.

It was assumed here that recovery after the initial loading sequence should take place after a given interval. Arbitrarily a ten minutes period was chosen between tests.

Repeat test after ten minutes interval

Sixteen samples 2×1 mm in cross-section were cycled twice in tension to 200 p at 90 per cent humidity and 21 degrees C temperature. They were removed from the testing machine, wrapped in plastic film and after ten minutes retested as previously. Elongation, residual deformation and energy dissipation were calculated. Student's t test was used in the statistical analysis.

Results. Similar values were obtained after the ten minutes interval.

TABLE X. *The effect of repeat cycles after a ten minutes recovery period.*

	Original cycle	Repeat cycle after 10 minutes	n	t
Elongation mm	1.13 ± 0.05	1.10 ± 0.05	16	0.690 non signif.
Residual deformation mm	0.33 ± 0.02	0.34 ± 0.01	16	0.595 non signif.
Energy dissipation p mm/mm ³	1.06 ± 0.09	1.08 ± 0.09	16	0.050 non signif.

The differences between the two groups are not significant. A minimal period of ten minutes between tests was consequently used when evaluating the effects of treatment on the same sample.

The effects of rapid freezing and thawing

To obtain quantitative information in tensile tests, it is essential to have testing samples of reproducible constant dimensions. Specimens of constant thickness can be cut with a freezing microtome; the following experiment was performed to determine if this procedure induced alterations in tensile properties.

Sixteen specimens were obtained from two lumbar spines and cut by sharp dissection with the control of a stereo microscope. With this

method the thickness of the samples was not very constant. Final dimensions were approximately $25 \times 5 \times 1$ mm. The samples were tested in tension and cycled twice to 200 p at 65 per cent relative humidity and 21 degrees C temperature. They were then frozen under a carbon dioxide snow stream, allowed to thaw and tested again under the same conditions. Elongation, residual deformation and energy dissipation for the first curve are reported here.

Results. In table XI the data is summarized.

TABLE XI. *The effect of rapid freezing and thawing on tensile properties.*

	Before freezing	After freezing	n	t
Elongation mm	1.30 ± 0.07	1.29 ± 0.10	16	0.019 non signif.
Residual deformation mm	0.40 ± 0.05	0.45 ± 0.04	16	1.320 non signif.
Energy dissipation p mm	30.16 ± 2.69	30.85 ± 2.80	16	0.295 non signif.

The differences before and after freezing were not significant. Rapid freezing to approximately -60 degrees C and immediate thawing did not alter the tensile properties of the samples.

This experiment justified the use of a freezing microtome. The conclusions on the effects of freezing are specifically not extended to other cases, such as the freezing method used commonly for preservation where the time element and temperature involved are of an entirely different magnitude (see chapter II).

V. TENSILE PROPERTIES OF THE NORMAL ANNULUS FIBROSUS

Effect of fiber orientation

The geometrical arrangement of the collagen fibers is a major factor determining the tensile properties of collagenous structures. In most tissues it is possible to find a correlation between their response to stresses acting in the different axis and the spacial organization and orientation of the fibrillar structure (see chapter II). The fibers in the annulus fibrosus have two well defined axis of orientation. In the lumbar spine in each alternating lamellae the fibers run in a single direction opposite to the previous one and forming an angle with the horizontal axis of 30 degrees according to Rouvière (1921) or varying between 24 to 37 degrees according to Horton (1958).

In the following series of experiments the effects of fiber orientation on the tensile properties of the annulus fibrosus were investigated.

Tensile properties in different directions

A total of 114 specimens obtained from the annulus fibrosus of ten lumbar spines were used. As described previously, blocks from the anterior annulus were dissected free. Sheets 1 mm in thickness were cut with a freezing microtome in the direction of the lamellae at 1 and 2 mm depth from the periphery of the blocks. From these sheets testing samples were die-cut at the following angles: 0, 15, 30, 50, 70 and 90 degrees.

The samples were 2×1 mm in cross-section, the length varied from 25 to 15 mm, the testing length was always 10 mm. The samples were cycled twice in tension to a 50 p load the first cycle and 100 p load on the second at 65 per cent relative humidity and 21 degrees C room temperature. Only values for 50 p loads are reported here as many specimens from the 70 and 90 degrees groups failed at the higher loads. Although 50 p represents a low stress level (0.025 Kp/mm²) it was necessarily used to compare samples with large differences in their physical properties.

Elongation, residual deformation and energy dissipation were measured from the curves and the difference between groups evaluated by analysis of variance and Sheffe's contrasts method.

Results. The data is summarized in table XII and the statistical analysis follows.

TABLE XII. *The effect of angle of section.*

Angle	Elongation mm	Residual deformation mm	Energy dissipation p mm/mm ³	n
0	0.68 ± 0.03	0.23 ± 0.02	0.25 ± 0.02	26
15	0.62 ± 0.03	0.17 ± 0.02	0.22 ± 0.01	24
30	0.93 ± 0.07*	0.29 ± 0.06*	0.32 ± 0.04*	19
50	2.37 ± 0.02*	0.51 ± 0.08*	0.88 ± 0.10*	16
70	3.99 ± 0.22*	1.33 ± 0.18*	1.91 ± 0.23*	13
90	5.03 ± 0.17*	1.57 ± 0.16*	2.35 ± 0.18*	16 (a)

ANOVA Elongation

V.S.	D.F.	S.S.	M.S.	F.
Between angles	5	312.993	62.599	254.35***
Within groups	108	26.580	0.246	
Total	113	339.574		

ANOVA Residual deformation

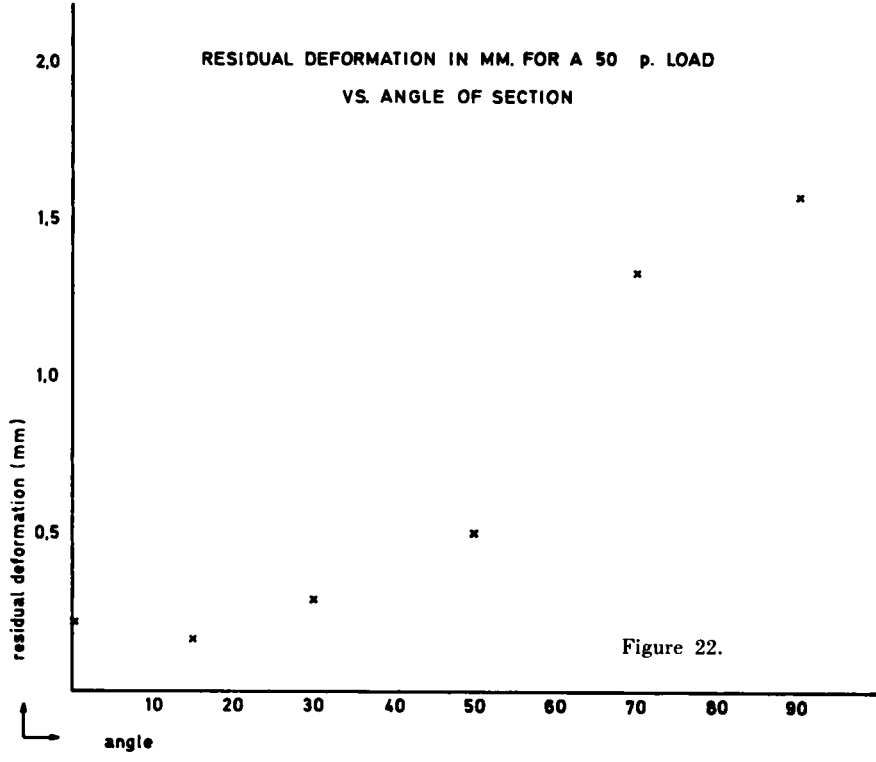
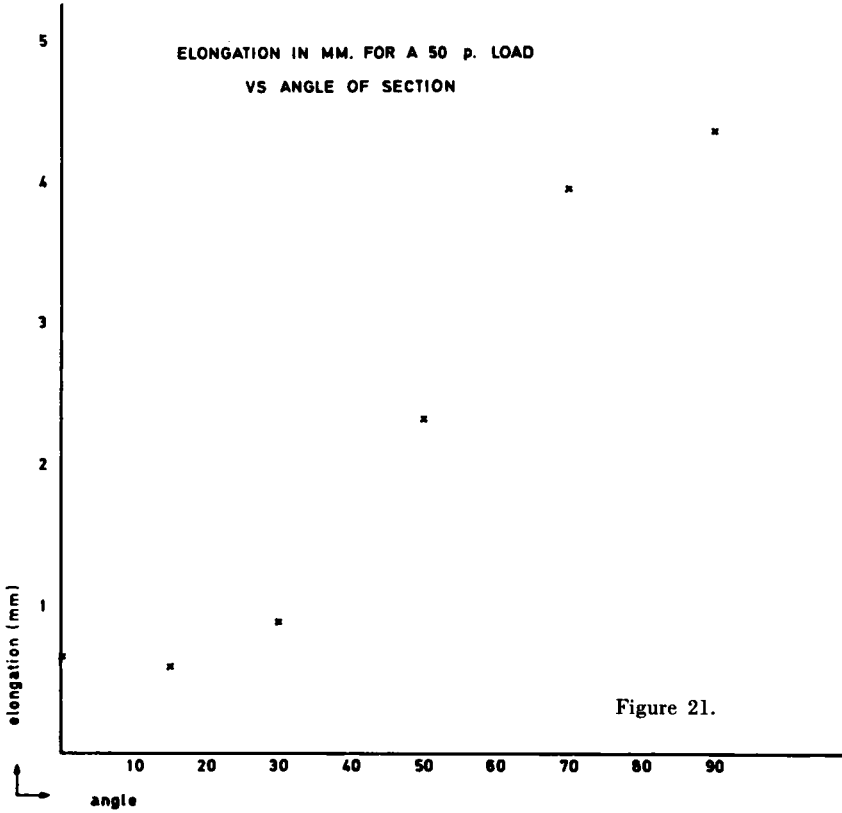
V.S.	D.F.	S.S.	M.S.	F.
Between angles	5	32.021	6.404	48.44***
Within groups	108	14.278	0.132	
Total	113	46.299		

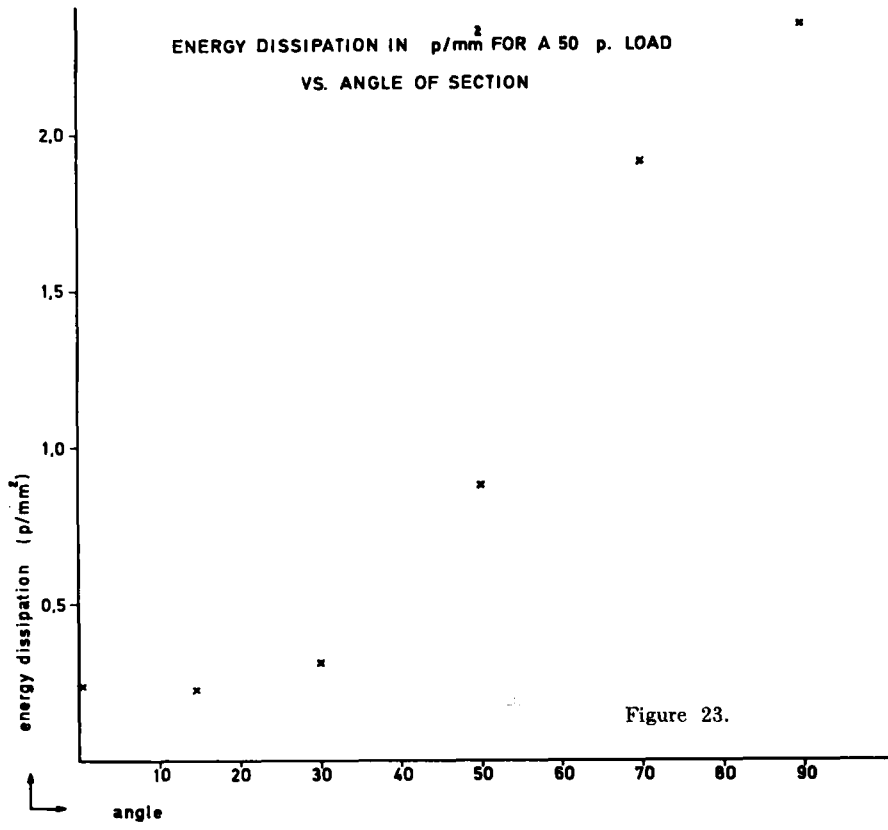
ANOVA Energy dissipation

V.S.	D.F.	S.S.	M.S.	F.
Between angles	5	70.105	14.021	80.34***
Within groups	106	18.498	0.175	
Total	111	88.602		

*) denotes significant differences from the value at 0 degrees.

(a) only 14 values in the energy dissipation group.





Small differences were present between the 0 and 15 degrees groups. The samples cut at 15 degrees tended to be stiffer and showed decreased elongation, residual deformation and energy dissipation. These differences were not significant. From 30 degrees on, the samples became progressively more extensible and exhibited increasing residual deformation and energy dissipation, reaching the highest values for the three parameters along the vertical axis (fig. 21, 22 and 23).

Tensile properties at the horizontal axis and along the fiber direction axis in 1.5 mm thick specimens

In 1 mm thick specimens small differences were present between horizontal samples and those cut close to one of the main fiber direction axis. This was thought to be a function of the geometry of the tissue. In a thin sample where

a limited number of layers is present, overrepresentation of one fiber direction may occur. If the sample is cut along that axis it will be stiffer and exhibit better recovery properties. The opposite situation may also arise. In the following experiment the thickness was increased in order to augment the number of alternating sheets of fibers.

18 specimens 2×1.5 mm in cross-section, from 3 lumbar spines, were prepared in 2 groups of 9 each, one in the horizontal axis and the other at one of the fiber directions respectively. They were cycled twice to 400 p at 65 per cent relative humidity and 21 degrees C temperature. A higher stress level was used in this experiment, to avoid variations that might exist when using the low part of the load-elongation curve.

Results. The results are reported in table XIII.

TABLE XIII. *Tensile properties in horizontal samples and fiber direction samples.*

	Horizontal	Fiber direction	t
Elongation mm	1.11 ± 0.04	1.15 ± 0.04	0.68 non significant
Residual deformation mm	0.31 ± 0.03	0.36 ± 0.03	1.04 non significant
Energy dissipation p mm/mm ³	2.19 ± 0.06	2.24 ± 0.12	0.38 non significant

The differences between the two groups were not significant. In 1.5 mm thick samples, where there are more alternating sheets of fibers than in 1 mm thick samples, no differences exist between the horizontal and the fiber direction axis at the levels of stress applied in these experiments.

Tensile properties in the two fiber direction axis

In the previous experiments the angles of section were taken at random in relation to the right or left axis of symmetry of the specimens. It was of interest to determine if the tensile properties follow a mirror distribution and both fiber direction axis have the same characteristics.

16 samples 1.5 mm thick were obtained from three lumbar spines. They were divided in two groups of eight samples, and each group die-cut in one of the fiber directions. They were cycled twice to a load of 400 p at 65 per cent relative humidity and 21 degrees C temperature.

Results.

TABLE XIV. *Tensile properties in the right and left fiber direction axis.*

	Right axis	Left axis	t
Elongation mm	1.15 ± 0.06	1.22 ± 0.07	0.81 non significant
Residual deformation mm	0.34 ± 0.04	0.32 ± 0.03	0.40 non significant
Energy dissipation p mm/mm ³	2.31 ± 0.11	2.14 ± 0.09	1.17 non significant

No significant differences were evident between the two groups. Tensile characteristics follow a mirror distribution in relation to the two axis of symmetry present.

Conclusions: The annulus fibrosus is stiffer and has better recovery properties between the horizontal and the two fiber direction axis. It is most extensible along the vertical direction. These relations are true in the range of stress in which these experiments were performed. If we accept that the stiffness in tension of the material reflects the distribution of tensile stresses to which it is exposed we can conclude that the annulus is subjected primarily to tensile stresses acting between 0 and ± 30 degrees. Tensile stresses along the vertical axis are expected to be of small magnitude because samples cut along that axis are very extensible and have very low tensile strength.

Effect of location

The annulus fibrosus is not a homogeneous structure. Different areas have different anatomical characteristics (Fick 1904, Beadle 1931, Hirsch and Schajowicz 1952, Van den Hooff 1964). Morphologically the anterior and lateral parts are higher and thicker than the posterior. Here the lamellae are narrow, few in number and the collagen fibers are thinner than in the anterior annulus. From the periphery to the midline both anteriorly and posteriorly the lamellae become less demarcated, and the collagen fibers appear to be thinner. The basophilia of the tissue increases towards the midline. The most peripheral lamellae or annulus lamellosus are acidophilic and show poor histochemical reactions (Malinsky 1958). The cells are numerous and have the appearance of fibrocytes.

It is of interest to determine if the mechanical properties change in the different areas of the annulus as this factor will provide information on the distribution of stresses in the structure under normal conditions. As differences in tensile properties could be accounted for by changes in the orientation of fibers, histological examinations and measurements of the interstriation angle were performed concomitantly with this experiment.

**Tensile properties in different depths in the anterior
and posterior annulus fibrosus**

81 samples, 2×1 mm in cross-section cut along the horizontal axis were obtained from the anterior and posterior annulus of discs from seven subjects. It was not feasible to get specimens from the lateral segments due to the extreme curvature of the structure in this area. For the anterior annulus the samples were taken at the periphery (group 0) and at a depth of 1, 2, 3, 4 and 5 mm from it. The most superficial group of samples included only those showing the typical histological characteristics of the annulus lamellosus. For the posterior annulus the samples were cut at 1, 2, 3 and 4 mm from the periphery. The samples were cycled twice to a load of 200 p at 90 per cent relative humidity and 21° C temperature. Elongation, residual deformation and energy dissipation were evaluated from the curves. Data from the first cycles is presented here. Immediately adjacent to each sample a histological section was cut at 12 microns and stained with hemotoxilin eosin. Measurements of angles between layers of fibers were made on microphotographs.

A block design was planned originally for this experiment, where all the levels anteriorly and posteriorly would be represented in each disc. As it was difficult to obtain samples from the posterior annulus in all discs and many unsatisfactory specimens had to be discarded, a different design was adopted for the evaluation of the data.

In the final statistical analysis the most peripheral sheets of the anterior annulus, sheet 0, and the deepest, sheet 5, were eliminated so that 4 levels remained anteriorly and posteriorly. Five determinations were randomized out for each group and the data fitted into a two-factor hierarchal type of analysis of variance. Sheffe's contrasts were used for independent comparisons.

Results. The results are summarized in tables XV and XVI and are illustrated in figures 24, 25 and 26.

TABLE XV. *The effect of location on tensile properties of anterior annulus samples.*

Depth from periphery mm	Elongation mm	Residual deformation mm	Energy dissipation p mm/mm ²	n
0	1.48 ± 0.06	0.62 ± 0.05	2.16 ± 0.31	7
1	1.07 ± 0.05	0.25 ± 0.02	1.07 ± 0.13	10
2	1.10 ± 0.06	0.27 ± 0.02	1.09 ± 0.11	10
3	1.26 ± 0.05	0.30 ± 0.03	1.30 ± 0.09	10
4	1.72 ± 0.13	0.55 ± 0.08	1.91 ± 0.22	10
5	1.85 ± 0.13	0.65 ± 0.09	2.44 ± 0.25	10

TABLE XVI. *The effect of location on tensile properties of posterior annulus samples.*

Depth from periphery mm	Elongation mm	Residual deformation mm	Energy dissipation p mm/mm ³	n
1	1.36 ± 0.09	0.27 ± 0.04	1.07 ± 0.21	7
2	1.52 ± 0.12	0.32 ± 0.08	1.61 ± 0.24	6
3	1.73 ± 0.16	0.67 ± 0.11	1.93 ± 0.37	6
4	2.07 ± 0.31	0.91 ± 0.12	2.00 ± 0.46	5

The statistical analysis was as follows:

ANOVA Elongation

V.S.	D.F.	S.S.	M.S.	F.
Between 1—4	3	7.2509	2.417	3.73*
Between A—P within 1—4	4	12.9640	3.241	4.98***
Residual	32	20.7039	0.647	
Total	39	40.9189		

ANOVA Residual deformation

V.S.	D.F.	S.S.	M.S.	F.
Between 1—4	3	1.2696	0.4232	17.34***
Between A—P within 1—4	4	1.5503	0.3876	15.83***
Residual	32	0.7807	0.0244	
Total	39	3.6007		

ANOVA Energy dissipation

V.S.	D.F.	S.S.	M.S.	F.
Between 1—4	3	2.4516	0.8172	5.48***
Between A—P within 1—4	4	1.3656	0.3414	2.29*
Residual	32	4.7680	0.1490	
Total	39	8.5853		

In the anterior annulus the most peripheral layer or layer 0 (annulus lamellosus) was more extensible and exhibited higher residual deformation and energy dissipation than the immediately adjacent one (layer 1). From layer 2 towards the midline the samples became progressively more extensible and showed increased residual deformation and energy dissipation. The differences between layers 1 and 2 were not significant. A similar pattern was evident posteriorly. The differences were significant in all layers. Elongation, residual deformation

ELONGATION VS. DEPTH FROM PERIPHERY

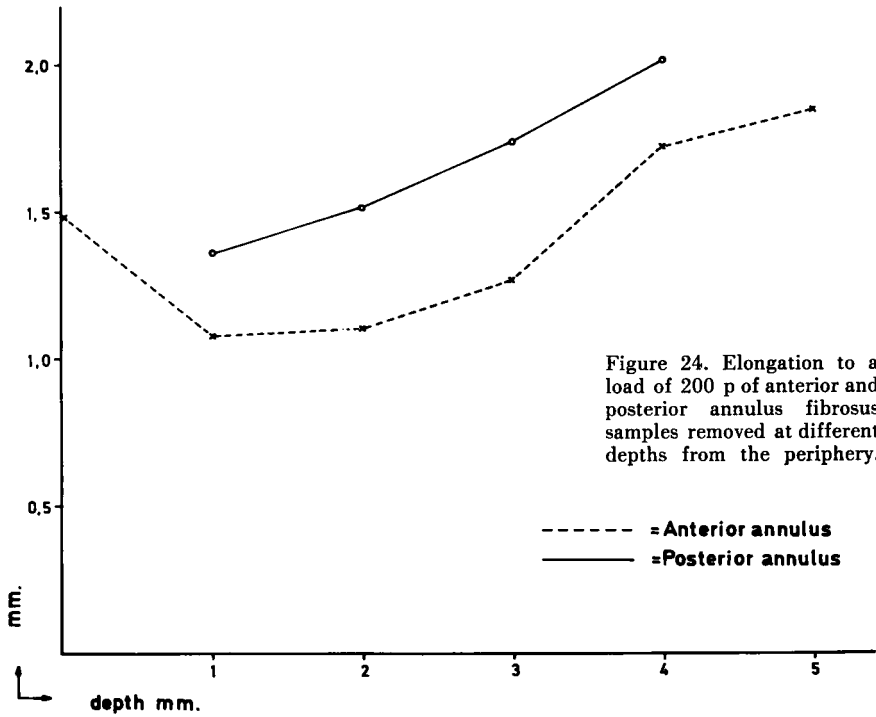


Figure 24. Elongation to a load of 200 p of anterior and posterior annulus fibrosus samples removed at different depths from the periphery.

RESIDUAL DEFORMATION VS. DEPTH FROM PERIPHERY

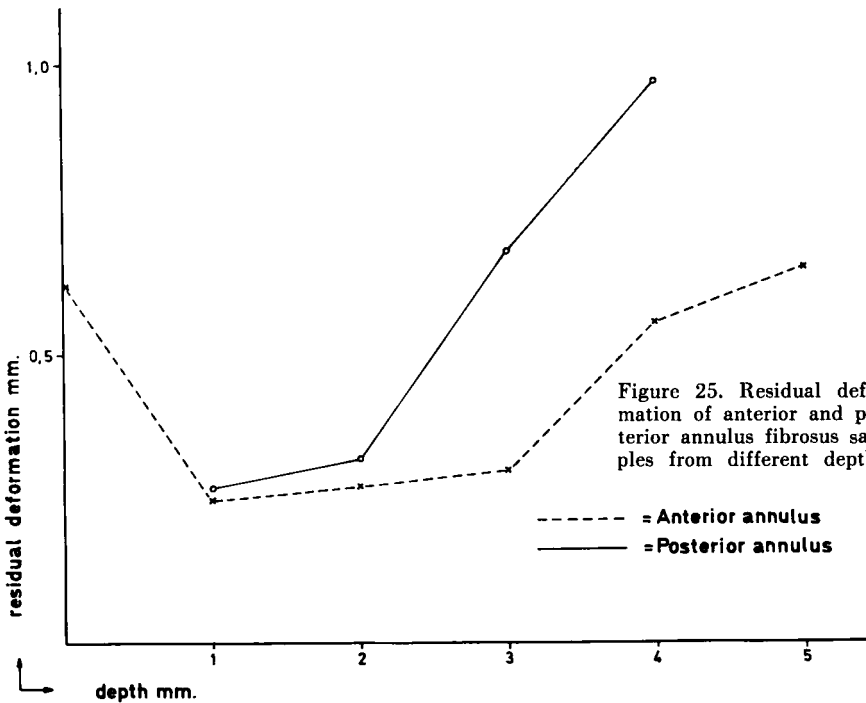


Figure 25. Residual deformation of anterior and posterior annulus fibrosus samples from different depths.

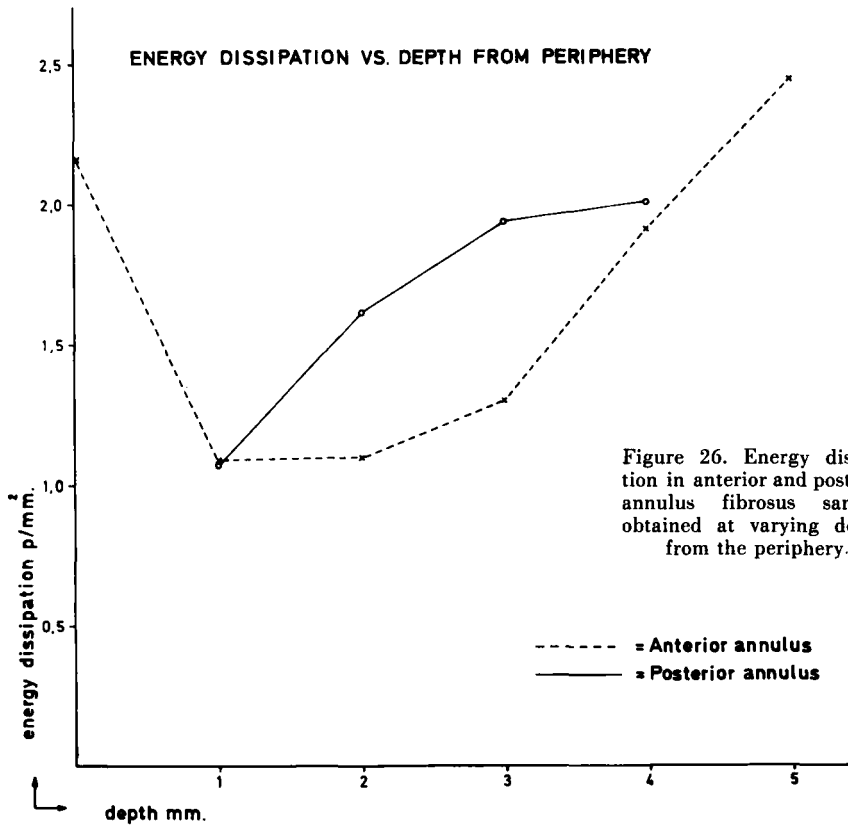


Figure 26. Energy dissipation in anterior and posterior annulus fibrosus samples obtained at varying depths from the periphery.

and energy dissipation were significantly higher in the posterior than the anterior annulus.

The average values for the angle between fibers was 52 ± 3 degrees in the anterior annulus and 58 ± 5 in the posterior annulus. The differences were not significant ($t_{(40)} = 0.58$).

In summary, samples from the anterior annulus were stiffer and had better recovery properties than posterior annulus samples. Both anteriorly and posteriorly stiffness and recovery characteristics increased towards the periphery.

Time dependent effects

The general characteristics of the tension cycles presented in this investigation, showing hysteresis, residual deformation and changes with succeeding cycles indicate a complex rheological behavior. In biological materials elastic and viscous effects are well known to exist and plastic properties can be present.

Combinations of these basic elements have been used to express behavior in model form. Although no such attempt is made here, the following two experiments were performed to illustrate viscous characteristics in the annulus fibrosus.

Behavior of samples under constant deformation

Ten samples 2×1 mm in cross-section from the anterior annulus were used. The samples were loaded in tension at a rate of elongation of 0.5 cm/min. at 90 per cent relative humidity and 21° C temperature. The crossheads of the testing machine were stopped at a load of 200 p and deformation was kept constant with the recorder running and registering load as a function of time during a period of 60 seconds.

Results. With constant length a decrease in stress occurred in all the samples. The slope of the relaxation curve was calculated at its origin and again at 60 seconds. It was expressed as, $\tan \alpha = -\frac{dP}{dt}$ where P is the load in ponds and t the time in seconds. The average initial slope was, $\tan \alpha_{t=0} = -31.85 \pm 1.26$ p/sec. The average slope after 60 seconds was, $\tan \alpha_{t=60} = -0.34$ p/sec.

Tensile properties at different rates of deformation

7 anterior annulus samples 2×1 mm in cross-section were used. The specimens were cycled twice in tension to 200 p at 0.5 cm/min. and a recorder speed of 50 cm/min. They were allowed to recover for 10 minutes and retested to the same load at a rate of deformation of 5 cm/min. and a recorder speed of 100 cm/min. A higher rate of extension could not be used due to the speed of response of the recorder. Tests were performed at 90 per cent relative humidity and 21° C temperature.

Results. Average values for the first cycle are summarized in table XVII.

TABLE XVII. *The effect of rate of deformation on tensile properties.*

	Rate of elongation		n	t
	0.5 cm/min	5 cm/min		
Elongation mm	1.14 ± 0.04	1.16 ± 0.05	7	0.445 non significant
Residual deformation mm	0.41 ± 0.02	0.45 ± 0.04	7	1.466 non significant
Energy dissipation p mm/mm ³	1.30 ± 0.06	0.88 ± 0.11	7	3.315***

Elongation and residual deformation values showed no significant changes. The energy dissipation during the cycle was lower at the faster rate of speed.

The presence of stress-relaxation and the fact that the response of the samples to stress was a function of the rate at which deformation was induced indicates that annulus fibrosus samples exhibit viscous damping effects.

Tensile strength

Tensile strength is a fundamental rheological property of biological interest. Its significance is enhanced when the range of stress to which a structure is subjected in vivo is known. It appears in general that the safety factor of body tissues is relatively high (Harkness 1966).

Ruptures of the annulus fibrosus are a common finding in disc pathology. Their relation to the etiology of disc herniation and the symptoms of low back pain has been discussed by Hirsch and Schajowicz (1952). As some of these ruptures, for example, the radiating type, could be considered failures in tension, data on tensile strength of the annulus fibrosus is of obvious interest.

Determinations of tensile strength

The specimens in this experiment were divided in two groups: The first one die-cut in the direction of the fibers, the second along the horizontal axis. A special cutting die with a reduced central area was used. Final sample dimensions were $10 \times 2 \times 1$ mm in the central segment and $7.5 \times 3 \times 1$ mm in the expanded end sections. A total of 38 anterior annulus samples from six lumbar spines were tested. Many samples had to be discarded prior to testing. When using a parallel blade cutting die, it was possible to discard just a part of the specimen if only one of the ends was not completely parallel to the direction of the sheets of the annulus. With the reduced section die this was not feasible, and provided a limit to the number of samples tested.

The specimens were loaded in tension to failure at a rate of extension of 0.5 cm/min. in air at 90 per cent relative humidity and a 21° C temperature. Values for ultimate strength in Kp/mm^2 and elongation to failure in mm are reported.

Results. Four samples showed failure at the clamp edges in the fiber direction group and two samples in the other. They were excluded from the analysis.

The average values for 18 samples cut along of the fiber direction axis were: 0.90 ± 0.11 Kp/mm^2 for ultimate strength and 2.96 ± 0.16 mm of elongation

to failure. The variation was rather large and was attributed to overrepresentation of a given fiber direction. In four samples with low values this was evidently the case when examined under a dissecting microscope. If they are eliminated the figures for tensile strength would be 1.07 ± 0.09 Kp/mm² and elongation 3.15 ± 0.18 mm.

15 samples cut along the horizontal axis showed average values for tensile strength 0.35 ± 0.03 Kp/mm² and for elongation to failure 2.51 ± 0.17 mm. The differences between the two groups are highly significant.

VI. TENSILE PROPERTIES RELATED TO AGE AND DEGENERATION

Disc degeneration is a common process occurring in adult life. While in some cases the end result is a complete disintegration of the normal structure, it is possible to find discs of aged subjects showing minimal morphological alterations. In other patients severe disc degeneration may be present in the third decade of life. The etiological factors underlying the degenerative process are in all probabilities quite complex (Hirsch 1966). The biochemical composition of the tissue is changed, and particularly the mucopolysaccharide fractions have received considerable attention (see chapter II).

It is of interest to determine in which way these alterations affect function as it is often thought that the low back pain syndrome is related to abnormalities in the mechanical behavior of the disc. Hirsch (1951), Hirsch and Nachemson (1954), Nachemson (1960), Rolander (1966) have shown changes in the response of discs to forces as a function of degenerative processes.

The evidence indicates that the annulus has an important bearing in the production of these functional changes. A study of its tensile behavior in samples from all ages and showing all degrees of degeneration was therefore planned. Degeneration was assessed histologically in all cases. In a limited number of specimens biochemical studies were performed.

The effect of age and degeneration on tensile properties

124 samples from 30 lumbar spines were investigated. The samples were all obtained from the anterior annulus at a depth of 1 to 2 mm from the periphery. They were 2×1 mm in cross-section and cut along the horizontal axis of the discs. They were tested in tension, cycled twice to 200 p in an Instron table model testing machine at 90 per cent relative humidity and 21° C temperature. From the load elongation diagrams three parameters were evaluated: elongation, residual deformation, and energy dissipation. Data for the first cycle will be reported here. Following the tensile tests all samples were fixed in 10 per cent formalin. Sections 12 microns thick were then cut with a freezing microtome and stained with hematoxylin and eosin. A classification was made taking into account the incidence and magnitude of

the histological characteristics of degeneration in the annulus fibrosus as described by Hirsch and Schajowicz (1952); cavities filled with granular material, cartilage metaplasia, fissures, presence of vascular or fibrous tissue.

The samples were divided on microscopical basis in three groups: 1) Normal or minimal degeneration. 2) Moderate degeneration. 3) Severe degeneration.

Results.

Effects of age. Only samples with a microscopic grade 1 were considered. Elongation, residual deformation and energy dissipation decreased progressively until age 26, after which they remained practically constant (fig. 27 and 28).

Regression lines were calculated according to the method of least squares for the average values of subjects between 5 and 26 years. In the group of samples above this age a regression analysis showed no significant age effects.

The results are summarized in table XVIII and XIX.

TABLE XVIII. *Average values in samples below age 26.*

Elongation mm	Residual deformation mm	Energy dissipation p. mm/mm ³	n
1.32 ± 0.03	0.48 ± 0.04	1.31 ± 0.07	27

The regression lines were as follows: Elongation, 1.657-0.021 X; residual deformation, 0.782-0.019 X; energy dissipation, 1.978-0.040 X.

All regression coefficients were highly significant.

TABLE XIX. *Average values in normal samples above age 26.*

Elongation mm	Residual deformation mm	Energy dissipation p mm/mm ³	n
1.03 ± 0.02	0.29 ± 0.01	0.93 ± 0.02	69

The regression coefficients for each parameter were as follows: Elongation, -0.0030; residual deformation, -0.0028; energy dissipation, 0.0025. All regression coefficients were not significant, indicating that no age dependent effects were present on the parameters evaluated.

Effects of degeneration. Samples above age 26 were evaluated as it was shown that in normal tissue no age dependent effects could be demonstrated above that point. Degeneration grades 2 and 3 were pooled together and the differences

AVERAGE ELONGATION AS A FUNCTION OF AGE (NON DEGENERATED SAMPLES)

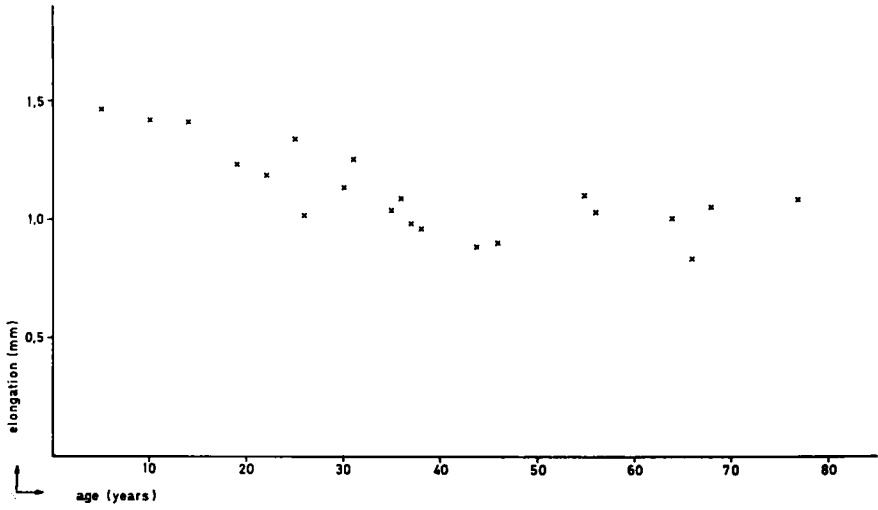


Figure 27.

AVERAGE ENERGY DISSIPATION AS A FUNCTION OF AGE (NON DEGENERATED SAMPLES)

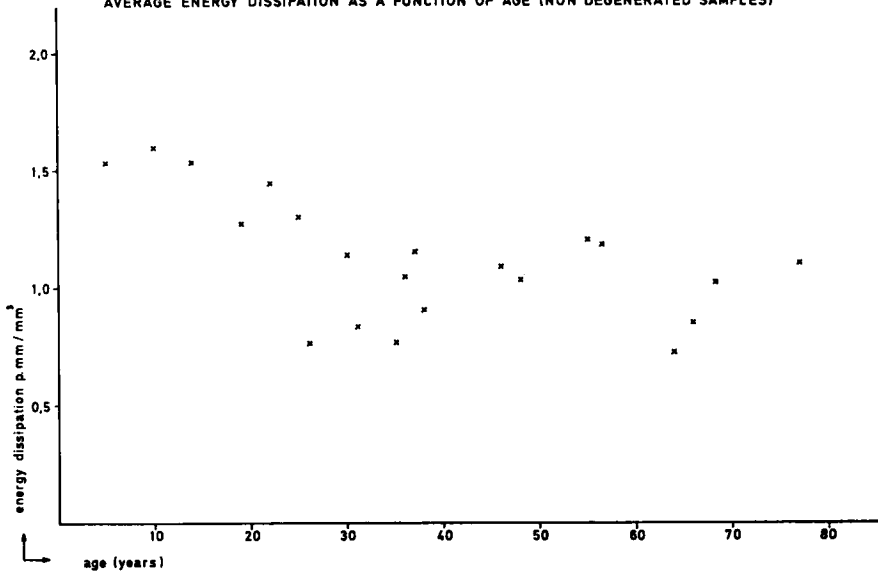


Figure 28.

with samples graded as 1 were tested. Several samples failed before reaching 200 p and had to be discarded from the analysis. Histological examination showed a grade 3 degeneration in these samples.

Elongation and energy dissipation were significantly larger in the degenerated group of samples. No changes were observed in residual deformation. (Table XX)

TABLE XX. *Effect of microscopic degeneration on tensile properties.*

Condition of samples				
	Normal	Degenerated (Grades 2+3)	n	t
Elongation mm	1.03 ± 0.02	1.34 ± 0.03	97	10***
Residual deformation mm	0.29 ± 0.01	0.30 ± 0.03	97	0.5
Energy dissipation p mm/mm ³	0.93 ± 0.02	1.61 ± 0.03	97	6.8***

The effect of age and degeneration on some biochemical characteristics

In 40 cases a specimen immediately adjacent to the sample used for tensile tests was removed and processed for analysis of: glycosaminoglycans, total hexosamine and hydroxyproline.

The content of glycosaminoglycans was determined by fractional elution of cetylpyridinium (CP) complexes as described by Antonopoulos, Gardell, Szirmai and de Tyssonsk (1964) and by a combination of this procedure with a technique recently developed by Anseth, Gardell and Heinegård (unpublished).

The specimens were freeze-dried and weighed. They were then digested with papain with the digestion mixture described by Antonopoulos, Gardell, Szirmai and de Tyssonsk (1964). 3 ml of that mixture were used for each sample. Aliquots of 50 ul were added to CPC-micro-columns; four columns were used for each specimen. They were eluted with the following sequence of solvents: 1 per cent CPC, 0.3 M NaCl and 0.6 M MgCl₂. The fractions from two of the columns were treated as described in the original procedure. The average results from the two determinations are reported here for each specimen. The CPC fractions from the two remaining columns were evaporated and the residue was dissolved in 0.25 ml of 2.5 M NaCl. This solution was extracted with 1 ml of isoamylalcohol. The tubes were centrifuged and

the isoamylalcohol was carefully removed by a capillary pipette. Another portion of isoamylalcohol was added and the procedure repeated. After the last extraction with isoamylalcohol the aqueous phase was diluted with 5 ml of distilled water and this solution was passed through a micro ECTEOLA-cellulose column (3×40 mm). This column was eluted with 0.02 N HCl followed by 6 N HCl. The 0.02 N HCl fraction was discarded while the 6 N HCl fraction was analyzed for its content of hexosamine as described by Antonopoulos, Gardell, Szirmai and de Tyssonsk (1964).

It has been shown in other experiments (Antonopoulos, Gardell, Szirmai and de Tyssonsk 1964, Antonopoulos 1965), that the 0.3 M NaCl and the 0.6 M MgCl₂ fraction from the CPC-cellulose column represent hyaluronic acid and chondroitin sulfate respectively. The 1 per cent CPC fraction contains keratosulfates and the glycoprotein splitproducts from the tissue. The 6 N HCl fraction from the ECTEOLA column represent the keratosulfates.

As a control the total amount of hexosamine in the specimen was determined after direct hydrolysis of 20 ul aliquots of the digested sample, by the Elson and Morgan reaction as modified by Antonopoulos, Gardell, Szirmai and de Tyssonsk (1964).

Hydroxyproline was determined on aliquots from the digested samples using the Grunbaum and Glick (1956) modification of the Neuman and Logan procedure.

Results.

Effects of age. The total hexosamine content showed a positive age correlation increasing up to the fifth decade and remaining then at a stationary level ($r = 0.429$). The 1 per cent CPC fraction which includes keratosulfates and glycoproteins paralleled the total hexosamine pattern but showed a decrease after the fifth decade. A positive age correlation was also present here ($r = 0.597$). The same characteristics and a positive age correlation were shown by the 0.3 M NaCl eluted fraction containing hyaluronic acid ($r = 0.518$). The keratosulfate fraction, 6 N HCl, showed also the same appearance but the drop after the fifth decade was more pronounced and consequently the correlation coefficient with age became non significant ($r = 0.250$). The chondroitin sulfate fraction eluted with 0.6 M MgCl₂ remained largely stationary, although tended to decrease with age. The correlation coefficient with age was non significant ($r = -0.070$).

These observations are illustrated in figure 29.

The hydroxyproline content did not show any significant age changes ($r = 0.052$).

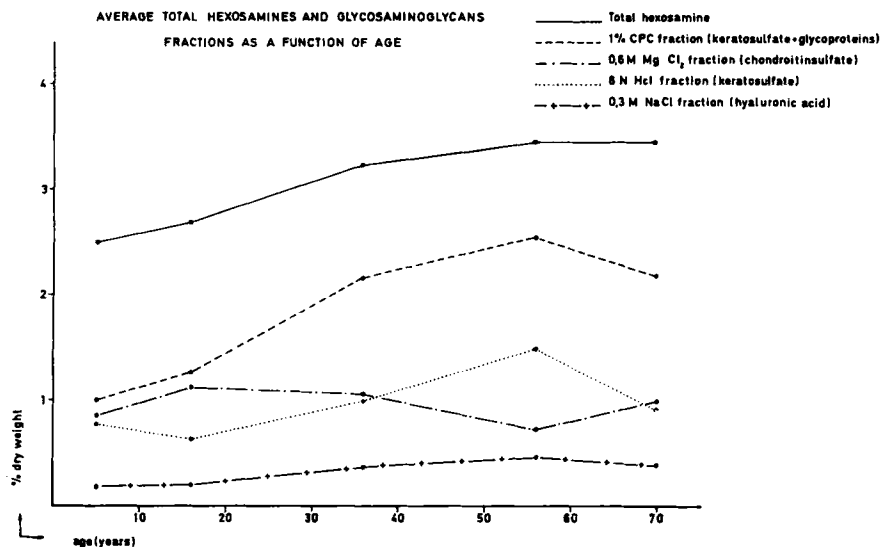


Figure 29. Average total hexosamines and glycosaminoglycans expressed in per cent dry weight as a function of age in non degenerated samples.

The average hydroxyproline value for the whole group was: 7.49 per cent dry weight or a collagen content of 55.42 per cent in dry weight. Assuming a water content of 70 per cent, we can derive 16.4 per cent of collagen in wet tissue.

The results are summarized in table XXI.

TABLE XXI. Average content in per cent dry weight of the various fractions in the different age groups.

Fractions per cent dry weight	Age in years				
	0—10	10—20	30—40	50—60	60—80
Hydroxyproline	8.00	8.13	7.77	6.00	7.60
Total hexosamines	2.50	2.68	3.23	3.44	3.43
1 % CPC	1.00	1.26	2.15	2.54	2.17
0.3 M NaCl	0.19	0.20	0.36	0.46	0.37
0.6 M MgCl ₂	0.85	1.12	1.05	0.72	0.99
6 N HCl	0.77	0.63	0.99	1.48	0.90

Effects of degeneration. As in the evaluation of tensile tests the specimens were divided in two groups, the first one including grade 1 samples and the second grade 2 and 3 specimens pooled together. All samples showing degeneration were from subjects above 55 years. They were compared with normal samples of

the same age group. A t test was used to evaluate the differences between averages. A significant decrease was observed in total hexosamine content, 1 per cent CPC, 0.3 M NaCl and 6 N HCl fractions. No significant differences occurred in hydroxyproline and chondroitin sulfate (0.6 M MgCl₂ fraction) values. (Table XXII).

TABLE XXII. Content of the different fractions in per cent dry weight in normal and degenerated samples.

Fractions per cent dry weight	Condition of samples		n	t
	Non degenerated	Degenerated		
Hydroxyproline	7.18 ± 0.40	7.04 ± 0.94	22	0.163
Total hexosamine	3.44 ± 0.24	2.65 ± 0.32	29	1.945*
1 % CPC	2.27 ± 0.13	1.52 ± 0.19	29	3.243***
0.3 M NaCl	0.43 ± 0.04	0.24 ± 0.04	28	3.012***
0.6 M MgCl ₂	0.88 ± 0.11	0.81 ± 0.10	29	0.429
6 N HCl	1.05 ± 0.10	0.56 ± 0.08	29	3.203***

In summary: Between 5 and 26 years of age the samples exhibited in tensile tests progressively increasing stiffness, improving recovery properties and decreasing internal damping effects. After the middle of the third decade no significant changes in tensile properties were present as a function of aging.

The biochemical parameters evaluated did not parallel the mechanical behavior of the samples in relation to aging. A progressive increase in total hexosamines, keratosulfate and hyaluronic acid was noticed up to the sixth decade, followed by a drop of the last two fractions afterwards. Chondroitin sulfate and hydroxyproline did not show age dependent changes.

As a result of degeneration the samples became more easily elongated and showed increased energy dissipation. Total hexosamines and glycosaminoglycans with the exception of chondroitin sulfate were significantly decreased.

VII. DISCUSSION

Some simplifications are useful when discussing the distribution of forces in the intervertebral disc. Nachemson (1960), who analyzed these stresses, used for that purpose the analogy of a thin walled cylinder subjected to internal pressure. Accordingly, the pressure of the nucleus is transmitted to the annulus in form of circumferential tensile stresses. Longitudinal tensile stresses are small or do not exist due to the axial compressive forces acting on the spine. The characteristic mechanical response of the annulus could be fitted into this concept as it will preferentially resist tensile stresses acting close to or along the horizontal axis.

The analysis was based on equilibrium considerations, assuming that the stresses are uniformly distributed across the thickness of the wall. To determine the distribution in a thick walled cylinder to which the annulus more favorably compares, the deformation properties of the wall must be considered. (Timoshenko 1955/56). The internal pressure will set radial compressive and tangential tensile stresses in the cylinder. The radial compressive stresses are highest in the inner part of the shell and decrease towards the periphery. The distribution of tangential stresses depends on the properties of the material. If the wall is homogeneous linear elastic the stresses will be highest in the inner part and decrease outwards. If the wall is plastic the opposite distribution occurs. When in an elastic cylinder the yield stress of the inner region is exceeded and the wall becomes partly plastic, the tangential stresses increase towards the periphery until the elastic region is reached and decrease afterwards. The experimental data indicates that the annulus is non homogeneous and has a complicated rheological behavior. Its stiffness and recovery properties and consequently the resistance to forces increase towards the periphery. This could be interpreted as a mechanism by which the internal layers "yield" and provide for a more uniform distribution of forces across the wall, avoiding stress concentrations in the inner regions.

The spine is constantly subjected to motions involving varying amounts of rotation around its longitudinal axis. As a result of torsional forces shear stresses will be set up on a horizontal cross-section of the annulus. If we assume a circular shape these stresses will be highest in the periphery and decrease towards the midline, a pattern followed by the properties of the annular wall.

The characteristics of the posterior annulus suggest that under normal conditions it is subjected to tangential tensile stresses of lower magnitude than the anterior annulus. As no significant differences were found in the angle at which

fibers cross between the anterior and posterior annulus, we assume that the pattern of response to forces in different directions is similar in both areas.

To understand the response of the fiber arrangement of the annulus to forces acting on one plane a simple analogy can be made. The construction can be compared to a truss where two extensible rods are attached to two rigid plates above and below (Fig. 30). The following situations are of interest:

a) Extension of the truss (Fig. 31). If the distance between the plates is increased by Δ , the corresponding elongation of the rods δ is obtained from a geometrical study. If the deformation is small i.e. $\Delta \ll L$

The result is:

$$\sin \alpha = \frac{\delta}{\Delta} \quad \Delta = \frac{\delta}{\sin \alpha}$$

From this formula it is apparent that the truss is very extensible if α is a small angle, because a relatively small elongation Δ of the rod corresponds to a large

extension δ of the truss. On the other hand if the angle is larger $\alpha \rightarrow \frac{\pi}{2}$

the truss becomes stiffer since then $\frac{\Delta}{\delta} \rightarrow 1$ and the extension of the truss could not occur without considerable deformation of the rods.

b) For rotational motion (Fig. 32) the truss makes no resistance at all if the midpoints of the plates are free to move. If on the other hand they are fixed, the resistance is small for all values of α as long as the angular motion is small since then the end points of each rod move approximately vertically and the deformation of the truss results from a translation but not a deformation of the rods. For large angular deformation with fixed midpoints the resistance increases gradually according to fig. 33.

c) For horizontal translation the resistance is always large as it results in almost direct elongation of the rods.

In the annulus similar conditions are met due to the small value of the angle between fibers. As a result, the resistance against angular motion is mainly due

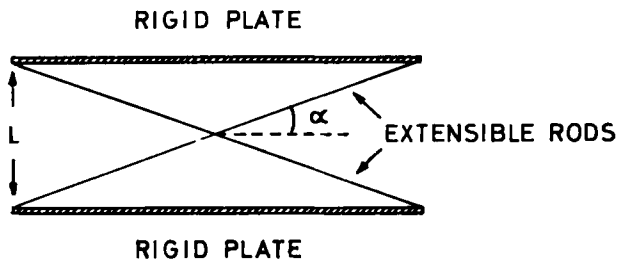
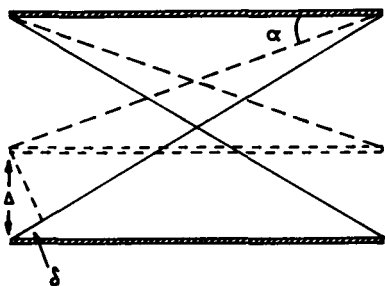
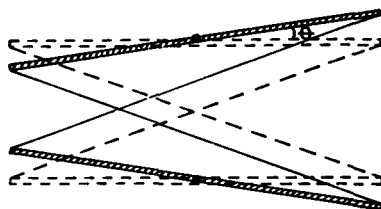


Figure 30.



EXTENSION OF THE TRUSS

Figure 31.



ROTATION OF THE TRUSS

Figure 32.

RESISTANCE TO ROTATION AS A FUNCTION OF
ANGULAR DEFORMATION ϕ

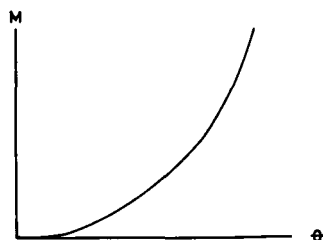


Figure 33.

to compression of the annulus in the concave side, and to ligaments or muscles on the convex side as pointed by Rolander (1966). The resistance to horizontal translation is large. The annulus fibrosus provides minimal resistance in tension to angular motions and maximal stability against horizontal displacements.

When testing samples in tension at low values of stress, no differences were found between horizontal samples and those cut along the direction of fibers. However, large differences were present in tensile strength values between the two groups. Evidently a different mechanism of deformation is responsible for the behavior of samples at high levels of stress. It is generally accepted that in most tissues the first portion of the stress-strain curves represents changes in orientation of the collagen fibers, gliding along bundles, or involves associated networks such as elastin or smooth muscle. Only at higher stress levels are the collagen fibers directly subjected to tension effects. In textiles, similar model

concepts have been derived (Olofsson 1964) where straightening of the curved fibers in the fabric accounts for the initial elongation, while direct stretching of fibers only occurs in the high portion of the load elongation diagrams.

The ultimate strength of tissues is then a property that can be directly related to the collagen framework. For that reason values are often expressed in terms of collagen cross-section area. Calculated in that manner the tensile strength of tendon would be 15 to 30 Kp/mm², of bone 50 Kp/mm², skin 10 Kp/mm², skeletal muscle 3 to 5 Kp/mm² (Harkness 1961). The differences are large and probably due to variations in orientation and linkages in the collagen network.

As pointed by Gustavsson (1956) the mechanism of rupture in collagen fibers does not involve breaking of the main polypeptide chain (covalent bonds). A load of the order of 300 Kp would be required to rupture the weakest covalent linkage, the C—N bond of 14 Å length. It is possible that fiber bundles rupture by breaking the lateral cohesive forces between them, so that fibrils glide over each other until failure occurs.

The values obtained in our experiments are actually not representative of the tensile strength of the fibers, as theoretically only one half of these are running along the axis of the specimen. If this fact is taken into account the tensile strength of the annulus samples would be around 2 Kp/mm². If we think in terms of strength per unit collagen we can derive figures of 13 Kp/mm² assuming a collagen content of 16 per cent. These values are almost in a similar range as those calculated for cross-sectional area of collagen in tendon and somewhat below than those found for isolated collagen fibers (Harkness 1961).

The tensile strength of the normal annulus is higher than the ultimate strength in compression of the endplates and vertebral body. This is in agreement with experimental and clinical observations indicating that trauma will produce fractures of the vertebrae but not disc herniations.

The same is not true in samples exhibiting morphological alterations. In that case significant differences with normal tissue were demonstrated in tensile behavior and in biochemical content. Degenerated samples showed a less efficient behavior in tension, they were easier to elongate and their recovery properties were decreased. Some samples actually failed at the loads used in the tests which represented approximately 30 per cent of the tensile strength of normal specimens. The safety factor of the degenerated disc is decreased and ruptures could occur as a result of stresses of not unusual magnitude. From a morphological viewpoint degeneration is seen first in the inner regions of the disc and progresses outwards. The same pattern is exhibited by the radiating ruptures affecting initially the deepest layers of the annulus and extending later to the more peripheral ones. This supports the concept that it is not the normal tissue that fails as a result of tangential or nearly horizontal stresses but that affected by degenerative processes.

In the vertical direction however, tensile strength is low even in normal tissue and horizontal type of ruptures could occur due to relatively small vertical tensile forces.

The collagen content of the samples was not altered by degeneration processes. The changes in load deformation response must be related to other factors, such as observed decrease in mucopolysaccharides, diminished cohesive forces between fibers and in later stages disruption of the normal framework. The protein polysaccharide compounds are considered to be of great importance in the determination of the mechanical properties of connective tissues (Schubert 1966). The observed decrease in polysaccharide fractions reflects an alteration in these protein complexes which evidently affects the behavior of the fiber construction.

As it has been pointed in relation to other connective tissues, age affected tensile properties at a stage in life where changes can be related to maturation. From the third decade on, the tensile characteristics remained unaltered and no correlations could be derived with the significant increase exhibited by some of the mucopolysaccharide fractions.

VIII. SUMMARY

Low back pain has for long been interpreted as a symptom of intervertebral disc disease, and has often been explained in mechanical terms. For that reason the response of the disc to forces has been the subject of repeated investigations. A correct interpretation of mechanical behavior requires knowledge about the load deformation characteristics of the individual components of the disc. The annulus fibrosus is an integral part of it, playing a most important role in its function and pathology. An investigation of the tensile properties of the lumbar annulus fibrosus was therefore performed in postmortem specimens. A total of 592 samples obtained from 68 lumbar spines were used.

Before undertaking the study of tensile characteristics an investigation of the laboratory conditions and methods of preparation and testing was made and the following conclusions were derived:

1) No changes in water content of samples from rabbit's annulus fibrosus could be demonstrated up to 48 hours following death.

2) Immersion of samples prior or during testing in different solutions to avoid water loss was found to be an unsatisfactory procedure. Swelling of considerable magnitude was induced in distilled water, 0.9 per cent sodium chloride solution, human plasma, and 10 per cent dextran.

The tensile properties of the specimens were significantly altered by water uptake as shown after swelling in 0.9 per cent sodium chloride solution. The samples became more extensible, residual deformation and energy dissipation were significantly larger.

3) By exposing samples to air at different relative humidities and temperatures it was determined that loss of tissue water would not occur or be minimal at a relative humidity of 100 per cent. Water loss was a function of the relative humidity in the air.

A relative humidity of 100 per cent was used whenever the samples were exposed to air during preparation procedures.

4) Loss of water by exposure for one hour to air at 65 per cent relative humidity and 21° C temperature induced significant changes in tensile properties. The samples became stiffer, but residual deformation and energy dissipation also increased. With ten minutes of exposure to the same conditions no significant differences were obtained.

Consequently, environmental conditions of 65 per cent or 90 per cent relative humidity and 21° C temperature were used in tensile tests with a duration not exceeding one minute of air exposure.

5) Ten minutes following a tensile test the samples recovered their properties justifying the performance of repetitive tests in the same specimen.

6) Rapid freezing with a carbon dioxide snow stream and immediate thawing did not affect the tensile response of the samples, enabling the use of a freezing microtome for sectioning purposes.

Methods based on these experimental conclusions were used in an investigation of the tensile behavior of the lumbar annulus fibrosus.

The response of the annulus to forces varied according to the direction in which the samples were cut. The tissue was stiffest and exhibited lowest deformation and energy dissipation between the horizontal axis and an angle of 30° . Samples cut at increasing angles were more extensible and had poor recovery properties showing a minimum along the vertical axis.

The annulus is not a homogeneous structure in its response to stress. Stiffness increased and recovery properties improved from the midline to the periphery across the thickness of the annulus in samples tested along the horizontal axis. The most peripheral anterior layer showed poor recovery properties and was more extensible than the immediately subjacent tissue. The anterior annulus was stiffer and had less residual deformation and smaller hysteresis cycles than the posterior annulus.

The tensile strength of normal annulus tissue was investigated along the horizontal axis and along one of the fiber direction axis. Average values of 0.35 Kp/mm^2 were obtained for horizontal samples and 0.90 Kp/mm^2 for specimens cut along the direction of the fibers. With the assumption that only one half of the fibers were running along the long axis of the specimen values around 2 Kp/mm^2 were estimated. The tensile strength for collagen cross section was 13 Kp/mm^2 assuming a collagen content of 16 per cent.

Annulus fibrosus samples exhibited stress relaxation when elongated to a constant deformation. The deformation was found to be a function of the rate of elongation; energy dissipation significantly decreased when the samples were tested at a higher rate of deformation. All the evaluated parameters, elongation, residual deformation and energy dissipation in samples cycled to a load of 200 p decreased with age from ages 5 to 26. After 26 years no significant differences were found between the different ages. Degenerated samples, evaluated by histological examination, exhibited significantly higher values for elongation and energy dissipation than specimens showing normal histological characteristics.

Specimens taken from areas immediately adjacent to the tested samples were used for determinations of hydroxyproline, total hexosamines and fractionation of glycosaminoglycans.

No significant changes with age were found in hydroxyproline values. The average content of hydroxyproline was 7.49 per cent dry weight of tissue.

Collagen values of 16.4 per cent wet tissue were calculated from these figures assuming a water content of 70 per cent.

Total hexosamines increased with age until the fifth decade and remained constant in normal samples. With the fractionation procedures the same pattern (although with a decrease in values after the fifth decade) was exhibited by the 1 per cent CPC fraction which includes keratosulfates and glycoproteins, the 0.3 M NaCl fraction composed of hyaluronic acid, and the 6 N HCl fraction containing keratosulfate. Chondroitin sulfate remained approximately constant.

Total hexosamines and some of the glycosaminoglycans were significantly decreased in degenerated samples. Degeneration did not affect chondroitin sulfate and hydroxyproline content per unit dry weight of tissue.

These experimental results showed a well defined pattern of response in the annulus fibrosus to tensile forces in various areas and along different axis. A simple analysis of the fiber construction confirmed the experimental conclusions. The annulus extends with ease along the vertical axis and provides little resistance against angular motion; elongation of the fibers is minimal in these circumstances. On the other hand resistance against horizontal displacement is very large. In this way the two basic properties of stability and motion are provided.

The tissue exhibited considerable tensile strength, almost in the range of tendons when the values were expressed in terms of collagen content. After the third decade of life, the tensile properties of the samples were not influenced by age. Degeneration, however, induced significant alterations in the load-deformation response of the tissue, decreasing its mechanical efficiency.

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