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**Hierarchical structure and mechanical properties of collagen in
the intervertebral disc**

Cassidy, James Joseph, Ph.D.

Case Western Reserve University, 1990

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**HIERARCHICAL STRUCTURE AND MECHANICAL PROPERTIES OF
COLLAGEN IN THE INTERVERTEBRAL DISC**

by

JAMES JOSEPH CASSIDY

**Submitted in partial fulfillment of the requirements for
the Degree of Doctor of Philosophy**

Thesis Advisors: Drs. Anne Hiltner & Eric Baer

Academic Advisor: Dr. Janie Fouke

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CASE WESTERN RESERVE UNIVERSITY

January 1990

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James J. Cassidy

HIERARCHICAL STRUCTURE AND MECHANICAL PROPERTIES OF
COLLAGEN IN
THE INTERVERTEBRAL DISC.

Abstract

by

JAMES JOSEPH CASSIDY

The hierarchical organization of collagen fibrils in the intervertebral disc is characterized by structural gradients. In the annulus fibrosus, the thickness of lamellae increases abruptly 2 mm inward from the edge of the disc, dividing the annulus into peripheral and transitional regions. Lamellae in the lateral and posterior annulus have a broad distribution of lamellar thicknesses. In alternate lamellae, fibrils are inclined with respect to the vertical axis of the spine in a layup structure. From the edge of the disc inward to the nucleus, this interlamellar angle decreases from 62 to 45 degrees. Within lamellae, collagen fibrils exhibit a planar crimped morphology. The plane of the waveform is inclined with respect to the spinal axis by the interlamellar angle. From the edge of the disc inward, the crimp angle increases from 20 to 45 degrees and the crimp period decreases from 26 to 20 μm . A hierarchical

model of the disc is presented that incorporates these morphological gradients.

Mechanical testing in load-deflection, stress relaxation, and creep modes reveals the response at each level of the hierarchy to compression. The stress-strain curve of the disc in compression contains toe, linear elastic, and yield regions similar to other collagenous tissues in tension. This demonstrates that while the disc is loaded in compression, the fibrils of the annulus are loaded in tension.

The role water transport plays in determining the mechanical properties of the disc is also established. During constant compressive strain stress relaxation experiments, the volume of the disc decreases with time by an amount equal to the macroscopic strain. A model is developed which hypothesizes that the relaxation and creep responses are due to transport of water out of the disc as the result of the pressure gradient across the cartilage endplates caused by an externally applied stress. The model considers cases analogous to the 3- and 4-parameter viscoelastic models, but contains elements which have a physical significance.

DEDICATION

My dissertation is dedicated to all those who faithfully supported and encouraged me in my academic endeavors, particularly my father, James W. Cassidy, and Katherine L. Farley, who did not live long enough to share in the fruits of these labors.

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LIST OF SYMBOLS

D	- strain dependency parameter, Nm^{-2}
E_1	- modulus, Nm^{-2}
E_2	- modulus, Nm^{-2}
ϵ_0	- strain at $t = 0$, $m m^{-1}$
$\epsilon(t)$	- strain, $m m^{-1}$
ϵ_{int}	- intercept strain, $m m^{-1}$
ϵ_∞	- asymptotic strain, $m m^{-1}$
G	- time dependency parameter, $Nm^{-2}s^{-1}$
h_1	- height of the disc before testing, m
K	- permeability constant, $m^4N^{-1}s^{-1}$
L	- thickness of the cartilage endplate, m
l	- crimp length, m
n_1	- viscosity, $N sm^{-2}$
P_0	- osmotic pressure, Nm^{-2}
P_d	- pressure in disc, Nm^{-2}
p	- crimp period, m
ϕ	- crimp angle, degrees
q	- volume flux, $m^3m^{-2}s^{-1}$
σ_0	- applied stress, Nm^{-2}
t	- time, s
τ	- creep time constant, s
θ	- interlamellar angle, degrees

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CHAPTER 1

HIERARCHICAL STRUCTURE OF THE INTERVERTEBRAL DISC

1.1 Introduction

The intervertebral disc is a complex hierarchical structure composed of the annulus fibrosus and nucleus pulposus which are anchored to the vertebral bodies by cartilaginous endplates. The annulus is made up of fibers of collagen type I arranged in concentric lamellae around the nucleus.¹ The fibers are organized in an alternating layup structure at an angle of about 65 degrees with respect to the spinal axis.² The ends of the fibers are bound to the cartilage endplates, thus anchoring the lamellae.¹ The nucleus pulposus is an amorphous gel of collagen type II, proteoglycans, and contains up to 88% water.³ From the peripheral annulus to the nucleus there is a smooth gradient of collagen types. At the edge of the annulus more than 95% of the collagen is type I. This percentage decreases along an approximately linear gradient to less than 5% in the nucleus. An opposing gradient exists for collagen type II, which comprises more than 95% of the collagen of the nucleus and less than 5% of the annulus.⁴ The morphology

of these two collagen types and their possible interaction is not understood.

The intervertebral disc in vivo serves to absorb shock and permit motion in the spinal column. It is loaded primarily in compression with elements of torsion, flexure, and extension. Mechanical testing in compression yields a non-linear stress-strain curve. Bulging of the disc during compressive loading indicates that axial loads are transmitted into radial and tangential tensile stresses in the lamellae of the annulus.⁵ This supports the hypothesis that the disc behaves in compression as an incompressible fluid contained in a thick-walled cylinder.⁶ More recently, investigators have explored the relationships among disc geometry, arrangement of collagen fibers in the annulus fibrosus, and compressive mechanical properties. A simple model based upon this work correlates mechanical properties with the arrangement of collagen fibers in the annulus by treating them as tendons and relating fiber extension to stress.⁷

Previous experience in this laboratory with other collagenous tissues has demonstrated the importance of understanding the mechanical response of connective tissues in terms of their hierarchical structure. Collagen fibers in the tendon have a planar crimped

geometry.⁸ This fiber morphology is reflected in the shape of the stress-strain curve.⁹ The curve has three distinct regions corresponding to the state of deformation in the collagen fibers. These are a toe region of increasing modulus where the fiber crimp is gradually straightened, a region of constant modulus where collagen fibers are stretched elastically, and a yield region of decreasing modulus where fibers are irreversibly deformed and damaged.

In the intestine, a more complex biaxially loaded tissue, crimped collagen fibers are arranged in layers. With the fiber direction in one layer at an angle of approximately 60 degrees to the fiber orientation in the adjacent layers, the wavy fibers form helices of opposite sense as they wind around the lumen.¹⁰ This organization results in highly anisotropic mechanical properties.¹¹ As the intestine is stretched, the initial response is uncrimping of the collagen fibers and a limited change in angle between fibers followed by extension of the straight fibers.¹² In both the tendon and intestine, the arrangement of collagen fibers produces a highly anisotropic mechanical response that accommodates the physiological loading conditions of the tissue in vivo.

The object of this study is to characterize the hierarchical structure of the collagen in the

intervertebral disc at the resolution of the optical microscope. These observations are incorporated into a model that correlates macroscopic mechanical behavior with the response of the disc structure to compressive deformation.

1.2 Methods

Five fresh human lumbar spinal segments were removed at autopsy by transverse sectioning through the vertebral bodies. The subjects were ages 31, 36, 56, 59, and 80 years. The cause of death in all cases was unrelated to bone or connective tissue disease.

Specimens were double wrapped in plastic to maintain an environment of approximately 100 percent relative humidity. If specimens were not used immediately, they were refrigerated at 5°C for not more than 24 hours. Individual discs were isolated by cutting through the vertebral bodies above and below using a bandsaw. Posterior articulating facets and pedicles were removed so that the remaining test specimen consisted of an isolated intervertebral disc with approximately 1 cm of viable vertebral bone above and below. This specimen preparation involved exposing the disc to the air for a period of not more than 30 seconds which minimized water loss. The anterior-posterior length was approximately

30 mm and the lateral width was 40 mm in all cases and the height ranged from 9.5 to 11.0 mm at the anterior periphery.

Discs for histomorphometry were immersed in formalin (40% formaldehyde solution) for 48 hours. The disc was separated from the vertebral bodies by making a transverse cut through the cartilage endplate with a razor blade. Slices were taken along orthogonal planes related to the cylindrical geometry of the disc. These were in the transverse and radial (sagittal) anatomic directions and in the circumferential direction by cutting a chord across the periphery of the disc. In addition, slices were cut through the annulus fibrosus parallel to the long axis of the collagen fibers. The slices were mounted in paraffin, sectioned on a microtome, and mounted on slides. Three histological stains were employed: hemotoxylin and eosin for connective tissue, trichrome for collagen, and sirius red for collagen birefringence measurements. The L1-L2 disc from each of four subjects was used to measure lamellar thickness. The L2-L3 disc was used to measure the interlamellar angle. The L3-L4 disc from each of two subjects was used to examine the morphology of the collagen fibers in the annulus fibrosus.

Transmission optical microscopy was used to examine the disc specimens over a range of magnifications from 10X to 1000X. A qualitative examination of the transverse, radial, and circumferential anatomy of the disc was made using brightfield and Nomarski differential interference contrast techniques. Quantitative measurements of the characteristic parameters of the collagen fibers were made using planar crossed polarized light as per the previously reported methodology.⁸

1.3 Results

A radial section through the disc, viewed between crossed polarizers in the optical microscope, reveals the relationship between the annulus fibrosus, nucleus pulposus, and cartilage endplates, Figure 1. While the nucleus and cartilages exhibit little optical activity, the dense fibrous lamellae are highly active. The lamellae of the annulus fibrosus are separate and distinct from one another with no interconnections visible at the optical microscope level.

In sections taken through the anterior annulus fibrosus, the thickness of the lamellae varies with radial location, Figure 2. Each point represents the average of three measurements with a standard deviation of $\pm 10 \mu\text{m}$. At the periphery of the disc, lamellae

Fig. 1. Radial section through the disc showing the annulus fibrosus, nucleus pulposus, and cartilage endplates. Note change in lamellar thickness between peripheral and transitional annulus fibrosus. (Sirius Red stain)

Fig. 2. The lamellar thickness gradient in the anterior annulus fibrosus: Plot of lamellar thickness vs. radius. Standard deviation of each point is within the size of the point.

average approximately 130 μm in thickness although at the very edge of the disc, many lamellae less than 100 μm in thickness were found. This region extends from the edge of the disc inwards for 2.2 mm and represents the outermost 18 lamellae. At this point, there is a sharp increase in lamellar thickness to approximately 260 μm . This thickness is maintained through the balance of the annulus inward to the nucleus pulposus from 2.2 to 7.5 mm in depth and comprises about 20 lamellae. The increase in lamellar thickness has a high correlation with depth having an r value of 0.80. This two part distribution in lamellar thickness divides the anterior annulus fibrosus into a peripheral region and a transitional region. In the vicinity of the nucleus, the boundaries between lamellae become poorly defined and the lamellae gradually are replaced by nuclear material. There is no clear demarcation between the annulus fibrosus and nucleus pulposus.

Lamellar thickness was also measured at the lateral, Figure 3, and posterior, Figure 4, aspects of the intervertebral disc. In sections through the lateral annulus, thickness values are widely scattered over a range from 80 to 400 μm with an average of 200 μm . In the posterior annulus the lamellar thickness is also scattered, however lamellae are generally thinner than in

Fig. 3. The lamellar thickness gradient in the lateral annulus fibrosus: Plot of lamellar thickness vs. radius. Standard deviation of each point is within the size of the point.

Fig. 4. The lamellar thickness gradient in the posterior annulus fibrosus: Plot of lamellar thickness vs. radius. Standard deviation of each point is within the size of the point.

the lateral annulus with thicknesses ranging from 50 to 250 μm with an average of 135 μm . In both the lateral and posterior regions no change in the lamellar thickness is seen through the depth of the annulus fibrosus. This observation is confirmed by statistical analysis using correlation coefficients which shows r values of 0.30 for the lateral and 0.15 for the posterior respectively. A distinction between peripheral and transitional regions, found in the anterior annulus is not observed.

A circumferential section cut through the annulus fibrosus shows the fibrous nature of the layers, Figure 5. In successive lamellae, the orientation of the fibers alternates with respect to the spinal axis. This interlamellar angle is not constant through the thickness of the annulus, as reported previously.¹ The angle formed between the collagen fibers in the lamellae and the axis of the spinal column decreases from the edge of annulus inward.

Measurements of the fiber orientation were made on sections cut through the annulus at a point halfway between the anterior and lateral aspects on the disc, Figure 6, and halfway between the lateral and posterior, Figure 7. In both positions, the angle of fiber orientation decreased from 62 ± 2 degrees at a depth of 0.5 mm to 47 ± 5 degrees close to the nucleus. Since the

Fig. 5. Circumferential section through the annulus fibrosus showing orientation of collagen fibers in successive lamellae. (Sirius Red stain)

Fig. 6. The interlamellar angle gradient at the anterior-lateral aspect of the annulus fibrosus: Plot of interlamellar angle vs. radius.

Fig. 7. The interlamellar angle gradient at the posterior-lateral aspect of the annulus fibrosus: Plot of interlamellar angle vs. radius.

lamellae in the anterior-lateral region are wider than in the posterior-lateral region, the 47 degree fiber orientation was observed at a depth of 7 mm in the former and 5 mm in the latter. Close to the nucleus pulposus it is difficult to differentiate between lamellae, and data points may be off by one lamella or approximately 300 μ m. Nevertheless, it appears that the angle of fiber orientation does not vary within a single lamellae as it circumscribes the disc, although the angle varies systematically from one lamella to the next. Through the depth of the annulus the decrease in fiber angle is gradual and no sharp transition is observed. A statistical analysis of this trend showed a high degree of correlation between interlamellar angle and depth with an r value of 0.97. The data are remarkably reproducible from specimen to specimen.

A section through the intervertebral disc cut parallel to the fibers in one set of alternating lamellae shows the concentric fibrous lamellae of the annulus fibrosus surrounding the nucleus, Figure 8. Viewed at higher magnification between crossed polarizers, characteristic extinction bands are observed along the collagen fibers, Figure 9. This pattern is similar to that observed in tendon and was analyzed using the same technique employed by previous investigators.* Periodic

Fig. 8. Transverse section through the annulus fibrosus cut parallel to collagen fibers in one set of lamellae. Note wavy conformation of fibers in alternate layers. (Sirius Red stain)

Fig. 9. Crimped collagen fibers within a single lamella stained with sirius red and viewed in crossed polarized light.

- a. +24 degree rotation
- b. 0 degree
- c. -20 degree rotation

light and dark banding when viewed between crossed polarizers is indicative of periodic variation in orientation within a birefringent fiber. Dark bands are produced in those regions of the wavy fibers where the tangent to the wave is parallel to one of the polarization directions. When the fibers are rotated about an axis normal to the microscope stage, the banding pattern will shift. With the crossed polarizers oriented parallel and perpendicular to the overall fiber directions, the dark bands are evenly spaced along the fiber, Figure 9b. Upon rotation of the microscope stage, pairs of dark bands approach each other producing alternate narrow and wide light intervals. Upon further rotation to a particular angle 2ϕ , the dark bands bordering the narrow interval merge into a single dark band, Figure 9a. Further rotation causes the bands to become lighter until the fiber is uniformly bright when the polarizers are oriented at 45 degrees. Rotation of the microscope stage in the opposite direction yields the same effect in reverse, Figure 9c, and the dark bands are now located at positions where the collagen fibers were bright previously. This behavior denotes a planar crimped waveform configuration of the collagen fibers for which the crimp angle ϕ is readily obtained from the polarizer angle.*

The crimp parameters at the anterior-lateral location were measured through the depth of two disc specimens. The crimp angle, ϕ , and crimp period, p , were measured and from these data, the crimp length, l , was calculated. The crimp angle increases from about 20 degrees at the periphery to 45 degrees in the lamellae closest to the nucleus while the period appears to decrease linearly through the depth from 26 to 20 μm , Figure 10. A statistical analysis of this trend showed a high degree of correlation between the crimp angle and depth with an r value of 0.95. The calculated crimp length is constant through the depth of the annulus at about 13.5 μm . This suggests that the length is the fundamental parameter of the crimp.

The suggested functional relationships are shown with solid lines in Figure 10: l is constant at 13.5 μm , the period decreases linearly through the depth of the annulus according to $p = -0.70 \times (\text{depth}) + 26.5$, and the crimp angle increases as $\cos^{-1} (p/2l)$. This function expanded is:

$$\phi = \cos^{-1} \{0.30 \times (\text{depth}) + 1\} \quad (1)$$

Fig. 10. The crimp angle and crimp period gradients in the annulus fibrosus: Scatterplot of crimp angle, period, and length vs. radius with suggested functional relationships highlighted with solid lines. Open points represent data from subject #1, age 31. Closed points represent data from subject #2, age 36.

Deviation of the data from this curve near the periphery of the disc indicates that p is not precisely linear with depth.

1.4 Discussion

The intervertebral disc is a two part structure composed of the gelatinous nucleus pulposus surrounded by the concentric fibrous lamellae of the annulus fibrosus. A model for the fibrous organization of the annulus fibrosus is proposed based upon optical microscope analysis of the morphology of the connective tissue structure. This model, shown in Figure 11, incorporates the structural hierarchy of the collagen fibers on the macro- and microscopic scales. At the anterior aspect of the disc, the annulus fibrosus is divided into peripheral and transitional regions by the thickness of the lamellae. In the peripheral region, the lamellae are about 130 μm or one-half the thickness of lamellae in the transitional region. The two regions are clearly delineated. At the lateral and posterior aspects of the disc, the lamellar thicknesses are distributed over a broad range through the depth of the annulus and the peripheral and transitional regions cannot be discerned. The average lamellar thickness is greater at the lateral aspect of the disc than at the posterior. Since the

Fig. 11. Proposed hierarchical model of the intervertebral disc showing lamellar structure, fiber orientation, and crimp morphology in the annulus fibrosus.

number of lamellae is the same at all locations, variations in lamellar thickness account for the annulus being thickest at the anterior region and thinnest at the posterior.

The lamellae that comprise the annulus fibrosus are continuous layers that form cylindrical walls around the nucleus pulposus. In each layer, the collagen fibers are arranged parallel to one another. In successive layers, the orientation of the fibers alternates with respect to the axis of the spinal column. For each lamellae, this interlamellar angle, θ , remains constant around the circumference of the disc. From the edge of the disc inward through the annulus, the interlamellar angle decreases in a linear manner.

Within each lamellae, collagen fibers are arranged in parallel arrays. The fibers display a planar crimped waveform with all fibers crimped in register. The plane of the waveform is perpendicular to the lamellar plane and inclined with respect to the spinal axis by the interlamellar angle. A gradient of crimp geometry exists in the annulus. The crimp angle, ϕ , increases and the crimp period, p , decreases linearly from the periphery of the disc inward. The length of the crimp, l , remains constant.

The significance of this model and the complex gradient structure it represents can be appreciated when examining the mechanical response of the intervertebral disc to compression. Bulging of the disc during compression indicates that axial loads are translated to radial and tangential tensile stresses in the lamellae of the annulus fibrosus.⁸ In this case, the expected response of the collagen fibers is reorientation in the radial direction and uncrimping of the waveform as is seen in the tendon and intestine.^{9, 11} X-ray diffraction of the annulus under compression has demonstrated an increase in the interlamellar angle.¹² Preliminary experiments in this laboratory have shown a decrease in crimp angle and increase in crimp period as well as an increase in the interlamellar angle when the disc was fixed under a compressive load. This response of the hierarchical structure predicts a compressive stress-strain curve with a low modulus toe region during crimp straightening and reorientation, a linear elastic region corresponding to extension of the fibers, and finally, a yield region as the structure undergoes irreversible damage. Compression of an isolated intervertebral disc specimen does show the three-part stress-strain curve characteristic of collagenous tissues in tension. As in the intestine, the lamellae will also

be most resistant to tensile stress in the directions parallel to the collagen fibers. Uniaxial tensile tests of specimens cut through several layers of the peripheral annulus show maximum stiffness along the two fiber axes.¹⁴

As a result of structural gradients through the annulus, the lamellae are not equally deformable. Peripheral lamellae, with a larger interlamellar angle and a smaller crimp angle, are less deformable than those closer to the nucleus. One study has attempted to measure the mechanical properties of the lamellae as a function of depth through the annulus. It was reported that specimens cut from peripheral lamellae were stiffer and had lower energy dissipation and residual deformation than those of more central lamellae.¹⁴

1.5 Conclusions

In conclusion:

1. The structure of the intervertebral disc at the lamellar and fibrillar levels is characterized by gradients as lamellar thickness, interlamellar angle, and crimp angle vary systematically through the depth of the annulus fibrosus. A hierarchical model of the

intervertebral disc that incorporates these observations has been devised.

2. The collagen fibers that comprise the lamellae of the annulus have a planar crimped configuration similar to that seen previously in other collagenous tissues.

3. The mechanical behavior predicted by this model is in qualitative agreement with experimental observations where the disc is tested in compression.

CHAPTER 2

RESPONSE OF THE HIERARCHICAL STRUCTURE OF THE INTERVERTEBRAL DISC TO UNIAXIAL COMPRESSION

2.1 Introduction

It was recently shown that the annulus fibrosus, the outer component of the intervertebral disc that is made up of discrete layers or lamellae of fibrous collagen, has a hierarchical structure with gradient characteristics at the various levels of organization.¹⁵ It is well known that the fibers of the lamellae are tilted with respect to the spinal axis; it is not so well known that the fibers possess a planar crimped waveform similar to that of tendon⁸ and intestine¹². Relationships between the hierarchical structure and mechanical function in these connective tissues have been developed by analyzing the response at the various levels of organization under the appropriate stress state.

Axial compression, in addition to torsion and bending, is a mode of deformation normally experienced by the disc. Many aspects of the isolated lumbar segment mechanical behavior in compression have been reported^{16, 17}, including the time-dependent viscoelastic response^{5, 18}. Differences in the

mechanical response between thoracic and lumbar discs have been reported ¹⁹ but scatter among individual discs sometimes overshadowed the differences along the spine ²⁰.

Attempts have been made to relate the mechanical response of the disc to some aspects of the structure. At the macroscopic level, radial bulging of the disc around its circumference has been measured under various loading conditions primarily to characterize biomechanical aspects of pathological conditions and therapeutic measures ^{21, 22}.

It is generally believed that compressive forces are transmitted across the disc to the fibers of the outer lamellae which are held in tension. Although the nucleus is thought to play a major role in the transmission of forces, the restoring force of the stretched fibers is considered to balance the effects of nuclear pressure ^{23, 24}. The contribution of water to the compressive properties of the disc has also been discussed. Water is expressed from the disc when it is compressed and is reimbibed when the load is removed ²⁵. Creep and stress relaxation are often attributed to squeezing out of fluid, but the relative influence of the annulus to the viscoelastic properties is seldom considered in conjunction with fluid loss ²⁶.

The purpose of the present investigation is to examine the role of the various levels of structural organization in the response of the isolated disc to compressive loading. Stress-strain measurements over a range of loading rates, in addition to stress relaxation experiments to characterize the viscoelastic behavior, are performed. Fluid loss is also determined and related to the response of the annulus and the mechanical behavior of the disc.

2.2 Experimental Methods

Spinal segments were harvested from adult mongrel canines. Discs from six spines were used to determine the effect of spinal location on mechanical properties. Unless noted, all further tests were done on discs in the thoraco-lumbar and lumbar regions and, when appropriate, adjacent discs were used to minimize variation due to spinal location. Two spines were used to determine the rate dependency of mechanical properties. Four spines were used in the stress relaxation tests and measurements were carried out at two loads on adjacent discs from two locations, thoracic and lumbar. Water content and disc geometry was examined in additional spines not used in the mechanical test procedures.

Isolated disc specimens were prepared by cutting transversely through the vertebral bones above and below the disc. The posterior articulating facets and pedicles were removed. The resulting test specimens consisted of a disc with approximately 10 mm of viable bone above and below. Discs were enclosed in plastic until tested to maintain 100% relative humidity and specimens were used fresh or kept refrigerated at 4 °C. All were tested within 72 hours of removal. Prior to testing, specimens were equilibrated at room temperature. The discs were kept sealed in plastic throughout the tests.

Disc height was measured at the periphery. The area of the disc was determined by measuring the maximum width of the disc and the length along the sagittal plane, then applying the area formula for an ellipse. An empirically determined correction factor was used to account for deviation of the disc from this idealized geometry and added 10 percent to the cross-sectional area calculated. The disc volume was calculated as the volume of an elliptical cylinder with height equal to the disc height at the periphery. A correction factor for that portion of the cylindrical volume occupied by vertebral bone was determined empirically. This equalled a 12.5 percent reduction in the initial volume.

Low strain rate mechanical tests were conducted using a screw-driven Instron 1123 mechanical testing machine. For high strain rate tests, a servo-hydraulic Instron 1331 mechanical testing machine was used. Specimens were placed between solid loading platens and preloaded to a stress of 0.1 MNm^{-2} . Disc specimens used to study the effect of location were compressed at a rate of 5 mm/min , a maximum stress was achieved and a decrease in stress observed. Specimens for stress-relaxation were compressed at a rate of 200 mm/sec to the desired strain and maintained at that strain for 30 minutes while the stress was monitored continuously.

Water content in the disc was measured by dissecting the disc away from the bone before it was freeze-dried. A weight percentage was calculated as the difference in weight before and after freeze drying divided by the initial weight. Water loss from the disc under strain was measured by compressing a test specimen and holding it in a clamp at 100 percent humidity and room temperature. After 2 hours, the disc was dissected out and freeze dried to measure the water content. A total of approximately 60 discs from six spines were dissected into anterior, lateral, and posterior regions of the annulus fibrosus and the nucleus pulposus to determine the regional water content of the disc.

Preserved disc specimens were sectioned for histological examination. Some specimens were clamped in compression before immersion in formalin (40 percent formaldehyde solution) for 48 hours. It is anticipated that fixation occurred sufficiently slowly that these specimens were fixed in the stress relaxed state. Slices were taken along the transverse and sagittal anatomic directions and in the circumferential direction by cutting a chord through the annulus fibrosus. Additional slices were cut through the annulus parallel to the collagen fibers to measure the parameters of the crimp waveform. Transmission optical microscopy was used to examine the disc specimens over a range of magnifications from 10X to 1000X. The crimp angle, period, and length were measured using crossed polarized light as per the previously reported methodology²⁴.

2.3 Results and Discussion

2.3.1 Stress-Strain Behavior

The stress-strain curve in compression of a representative intervertebral disc specimen is shown in Figure 12. The S-shaped curve may be divided into three regions. The initial response is characterized by a gradually increasing modulus with increasing strain; this toe region extends to approximately 0.10 strain. It is

Fig. 12. Stress-strain curve of intervertebral disc in compression showing toe, linear, and yield regions.

followed by a linear region of constant modulus that extends from the end of the toe region to about 0.20 strain. Finally, at high strains, the modulus begins to decrease gradually with increasing strain; this yield region continues until the disc fails.

Two modes of failure are observed in the intervertebral disc specimens. In the yield region of the stress strain curve, the specimen may attain a maximum stress value then drop sharply to a lower stress (Mode I failure) or it may simply decline gradually with increasing strain (Mode II failure). In Mode I failure, an audible popping or cracking sound often accompanies the drop in stress. This condition is seen in about 66 percent of the discs tested. No sound is heard from those specimens exhibiting the gradual decrease in stress. When specimens are dissected and examined grossly in the optical microscope, discs exhibiting a sharp stress drop usually have ruptured the cartilage endplates and material from the nucleus pulposus has been forced into the vertebral bodies. In those discs where a gradual decrease in stress is noted, fissures appear in the fibrous lamellae of the annulus fibrosus allowing nuclear material to move laterally through the disc. In some cases, gel from the nucleus has penetrated the full thickness of the annulus and appears on the outer surface

of the disc. This usually occurs at the posterior aspect of the disc where the lamellae are thinnest.

The stress-strain response of the disc to compression is examined at each level in the spinal column from C2-C3 through L6-L7 in six spines. The upper limit of the toe region of the stress-strain curve does not change with location in the spinal column, having a mean value of 0.15 ± 0.02 . The toe strain intercept, as determined by extrapolating the linear portion of the stress-strain curve to the strain axis, likewise does not vary with location having a mean value of 0.09 ± 0.02 , Figure 13.

The modulus in the linear region increases by a factor of three over the length of the spinal column from 32 MNm^{-2} at C2-C3 to 115 MNm^{-2} at L6-L7, Figure 14. Variability in the data reflects the differences among spines from different canines. Although a linear regression fits the data with r equal to 0.95, the plot suggests that a more complex relationship exists between location and modulus. In particular, there appears to be a sharp increase in modulus from the thoraco-lumbar to the lumbar region.

The maximum stress in compression more than doubled over the length of the spinal column from 8 MNm^{-2} at C2-C3 to 19 MNm^{-2} at L5-L6 while the maximum stress of the

Fig. 13. Compressive toe intercept strain vs. location in the spinal column. The loading rate is 5 mm/min. The bars indicate the standard deviation.

Fig. 14. Compressive modulus vs. location in the spinal column. The loading rate is 5 mm/min. The bars indicate the standard deviation.

L6-L7 disc is markedly lower than that of the other lumbar discs, Figure 15. Again, a linear regression describes the data with a r value of 0.95, but like the modulus, the maximum stress appears to increase sharply from the thoraco-lumbar to the lumbar region.

2.3.2 Rate Dependence of Mechanical Properties

The experiments on the variation in properties with location in the spinal column are carried out at a moderate loading rate of 5 mm/min. Additional specimens are tested to failure at slower loading rates of 0.005, 0.05, and 0.5 mm/min and at higher loading rates of 50 and 500 mm/min. These produce a range of strain rates from approximately 0.001 to 100 min⁻¹. Specimens for these experiments are taken from the thoraco-lumbar and lumbar regions where the dependence of the mechanical properties on location was minimal. The data points on the following plots usually represent results from compression of two discs; the standard deviation is within the area of the marker. The toe strain of the disc does not change with loading rate in these tests, having a mean value of 0.12 ± 0.01 . The toe intercept strain likewise does not change with loading rate, Figure 16, with a mean value of 0.07 ± 0.01 . These values are somewhat lower than the strains reported for

Fig. 15. Compressive maximum stress vs. location in the spinal column. The loading rate is 5 mm/min. The bars indicate the standard deviation.

Fig. 16. Compressive toe intercept strain vs. strain rate.

the previous experiments on mechanical property variation with location in the spinal column. Apparently, these tests were conducted on discs from two animals whose toe strains represent the lower limit of the normal range in the canine.

The compressive modulus, E , of the disc increases with increasing loading rate from about 40 MNm^{-2} at 0.005 mm/min to 135 MNm^{-2} at 500 mm/min , Figure 17. This greater than three-fold increase in modulus is linear with loading rate, regression analysis ($r = 0.99$) of the data indicates a functional relationship of:

$$E = 17 \times \text{Log(Rate)} + 93$$

The maximum compressive stress, $\sigma_{\max}$, achieved during testing also increases with increasing loading rate from about 5 MNm^{-2} at a rate of 0.005 mm/min to 19 MNm^{-2} at 500 mm/min , Figure 18. This change is also linear with loading rate, regression analysis ($r = 0.99$) of the data yields the relationship:

$$\sigma_{\max} = 2.6 \times \text{Log(Rate)} + 13$$

Fig. 17. Compressive modulus vs. strain rate.

Fig. 18. Compressive maximum stress vs. strain rate.

The strong rate dependency of the linear and failure regions is not observed in the toe region. That the latter is characterized by low stresses over a significant range of strain supports a conclusion that the time-dependency is stress, rather than strain, dependent.

2.3.3 Stress Relaxation

Stress relaxation response of the disc deformed to a constant macroscopic strain is examined using specimens prepared according to the same protocol described for discs tested to failure. Macroscopic strains of 0.05 and 0.15 are selected because they represent the center of the toe and linear regions of the stress-strain curve respectively. In addition, two locations in the spine, thoracic and lumbar, are examined. Discs deformed to both 0.05 and 0.15 strain relax to less than 10 percent of the initial stress in approximately 30 minutes. At both 0.05 strain and 0.15 strain, there is no significant difference in the response of thoracic and lumbar discs. If the experimental data are normalized to the initial stress, all the stress relaxation curves can be superimposed to create a master curve describing the relaxation response. The master curve in Figure 19 is drawn through the mean normalized stress at various

Fig. 19. Master relaxation curve of normalized stress vs. time.

points on the relaxation curve. The shape of the stress vs. time curve does not fit a simple exponential relationship; the shape of the relaxation curve is discussed in the companion paper ¹⁸.

During the stress relaxation experiments, outward deformation of the disc is measured at the anterior, lateral, and posterior aspects of the disc using dial gauges. Outward bulging is recorded as a function of time during the test and normalized to the initial dimensions of the specimen. A large variability among individual discs is encountered. However, some qualitative conclusions may be drawn. Bulging is not symmetrical around the periphery of the disc. In general, at 0.05 strain the anterior and lateral aspects of the disc bulge about equally while the posterior bulges slightly less. This bulge is 0.10 to 0.25 mm at the anterior and lateral and 0.05 to 0.20 mm at the posterior for discs that consistently measure about 17 mm by 12 mm. At 0.15 strain, the anterior bulges slightly more than the lateral. Both bulge to a much greater extent than the posterior annulus, where there is no increase over the 0.05 strain. The anterior bulges 0.50 to 1.00 mm, the lateral 0.30 to 0.70 mm, and the posterior 0.05 to 0.20 mm. The bulge decreases with time as the stress relaxes. At 0.05 compression, the bulge is

recovered almost entirely after 30 minutes and the cross-sectional area returns to its initial dimensions. At 0.15 compression, some residual bulge remains after 30 minutes. This is 0.01 mm or less for the anterior and lateral. The posterior recovers only slightly, if at all.

The decrease in the volume of the disc with time is calculated using these dimensional changes, Figure 20. The volume lost by the disc at 30 minutes at 0.05 strain is about 5.1 percent. At 0.15 strain, the volume lost is 15.0 percent. Within experimental error, the volume change compensates almost entirely for the imposed strain.

2.3.4 Water Content

The highest water content is in the nucleus pulposus which is 82 ± 3 percent water by weight. In contrast, the annulus fibrosus averages 62 ± 4 percent water at the anterior position and the lateral and posterior regions of the annulus both average 68 ± 2 percent water. Further subdivision of these regions shows a variation in water content through the annulus. It is always lowest at the periphery, Table I, and increases from the periphery to the center of the annulus. In the posterior and lateral positions, further changes in the water

Fig. 20. Decrease in disc volume with time as calculated from bulge measurements during stress relaxation.

TABLE I: Water Content (weight %) of the Disc at Various Locations

Location	Periphery	Center	Inner	Overall
0.00 Compressive Strain (mm/mm)				
Anterior annulus	57 $\pm$ 3	74 $\pm$ 3	64 $\pm$ 4	62 $\pm$ 4
Lateral annulus	60 $\pm$ 3	77 $\pm$ 2	74 $\pm$ 3	68 $\pm$ 2
Posterior annulus	59 $\pm$ 3	67 $\pm$ 4	71 $\pm$ 3	68 $\pm$ 2
Nucleus pulposus				82 $\pm$ 3
0.20 Compressive Strain (mm/mm)				
Anterior annulus	53 $\pm$ 3	60 $\pm$ 5	54 $\pm$ 4	50 $\pm$ 4
Lateral annulus	54 $\pm$ 4	57 $\pm$ 6	59 $\pm$ 5	54 $\pm$ 5
Posterior annulus	52 $\pm$ 4	50 $\pm$ 5	48 $\pm$ 4	53 $\pm$ 4
Nucleus pulposus				71 $\pm$ 4

content between the center of the annulus and the nucleus are small. The anterior position differs in this regard and the water content decreases near the nucleus. The region of highest water content coincides with an acute narrowing in the anterior of the disc near the center of the annulus.

The water content in all regions of the disc is lower after the disc has been held in compression for a period of time. The water content decreases gradually as the strain increases so that when compressed to 0.20 strain, the overall water content of the annulus is close to 50 percent and that of the nucleus about 70 percent, Figure 21. Further subdivision of the annulus showed that the least water is lost at the periphery, Table I, while water is preferentially lost from regions in the center of the annulus or near the nucleus that initially have the highest water content. This has the effect of creating a more uniform distribution of water through the annulus so that at 0.20 compressive strain, the water content in most regions is in the range of 50 to 60 percent.

The water content is used to estimate the weight change during compression, and is reported in Table II as the fractional change in the initial weight of the disc. If the weight fractions are taken to be approximately

Fig. 21. Water content of the disc vs. compressive strain.

TABLE II: Total Water Loss vs. Compressive Strain

Strain (mm/mm)	Weight Loss as Water ^a (g/g)	Volume Change from Bulging ^b (cm ³ /cm ³)
0.05	0.045 ± 0.002	0.051 ± 0.003
0.10	0.088 ± 0.003	-
0.15	0.130 ± 0.005	0.150 ± 0.013
0.20	0.177 ± 0.007	-
^a relaxed 2 hours		
^b relaxed 30 minutes		

equal to volume fractions, the comparison in Table II with the volume change measured directly from the bulging shows that water loss compensates for essentially the entire volume change. Although the environment is maintained at 100 percent relative humidity to minimize evaporation, no condensed water could be detected on the peripheral surfaces of the discs during compression. When the compressed specimens are removed from the test fixture, however, it is observed that a mixture of fluids had been exuded from the cut ends of the vertebral bodies and deposited on the loading platens. These two observations suggest that the principal pathway by which water is lost from the disc is through the cartilage endplates into the vertebral bodies, rather than transversely through the disc surface.

It is proposed that the mechanism responsible for the viscoelastic character of the disc response in compression, as manifested in the stress relaxation behavior and the rate dependency of modulus and strength, is transport of water out of the disc through the cartilage endplates into the vertebral bone. This hypothesis is supported by experimental data on the dimensional changes in the disc during stress relaxation and confirmed by direct measurement of the water content of compressed discs. Under physiological conditions,

instead of being exuded through the cut ends of the vertebral bodies, the water transported through the endplates is seen as being absorbed into the vascular system from which it could be restored to the disc when the stress is removed. This hypothesis accommodates the requirement for reversibility although the recovery could not be confirmed experimentally with the excised disc system.

2.3.5 Response of the Hierarchical Structure to Compression

The structural elements of the disc hierarchy could not be observed directly during compressive deformation. Instead, discs that had been fixed as they were held in compression are sectioned and examined histologically. The shape of the disc in cross-section varies with location in the spinal column. In the cervical region, the discs are elliptical and the nucleus pulposus is slightly off-center, being closer to the posterior than the anterior. In the thoracic, thoraco-lumbar, and lumbar regions, the discs are kidney-bean shaped, Figure 22a. The nucleus is also kidney-bean shaped and is found posterior to the center of the disc so that the annulus fibrosus is thicker at the anterior and lateral aspects and thinner at the posterior. This corresponds

Fig. 22. Schematic diagram showing the shape of the disc and the vertebral interface.

- a. Disc isolated from vertebral bodies.
- b. Anterior-posterior section.
- c. Lateral section.

with the relative thickness of the lamellae since the number of lamellae is the same at all positions around the circumference of the disc ¹⁵.

In sagittal sections, the interface between the vertebral bodies and the disc has a complex shape. Considering first the anterior aspect, the interface is curved so that the disc height gradually decreases from the periphery to a point halfway between the periphery of the disc and the center of the nucleus where the disc height is approximately one-half the height at the periphery. The disc height gradually increases again further inward toward the center of the nucleus but does not regain the original height at the periphery, Figure 22b. The region of the cartilage endplate to which lamellae of the annulus are anchored distinctly extends inward past the narrowest part of the disc. Lamellae in the anterior aspect are straight up-and-down or bulged slightly outward. By comparing the photomicrograph of the unloaded disc in Figure 23a with those in Figure 23b-d of discs loaded to 0.20 strain, it is seen that the lamellae peripheral to the narrowest part of the disc have a distinct outward bulge in the compressed state. Closer to the nucleus, the orientation of the compressed lamellae varies from one disc to another; the lamellae can be bulged outward, Figure 23b,

Fig. 23. Photomicrographs obtained with the stereo optical microscope of the anterior annulus showing lamellar bulging and buckling. Magnification 35X.

- a. With no load.
- b. Loaded to 0.20 compressive strain.

Fig. 23. Photomicrographs obtained with the stereo optical microscope of the anterior annulus showing lamellar bulging and buckling. Magnification 35X.

c.- d. Loaded to 0.20 compressive strain.

Fig. 24. Schematic representation of lamellar bulging and buckling at the anterior aspect of the annulus.

a. With no load.

b.- d. Loaded to 0.20 compressive strain.

bulged inward, Figure 23c, or buckled, Figure 23d. Deformation of the lamellae in this inner region of the anterior aspect is emphasized by the relatively high compressive strain that must be accommodated by the narrowest part of the disc. The variety of orientations assumed by the compressed lamellae, shown schematically in Figure 24, is attributable to several factors. For example, the curvature of the vertebral interface in this region encourages the lamellae to bulge inward as they are compressed but spreading of the nucleus pushes them outward, while the straight up-and-down orientation in the unloaded disc does not predispose the lamellae to bend preferentially in either direction. As a result, several possible modes of deformation are observed.

The vertebral interface at the posterior aspect is also curved and in this case the curvature is more severe than at the anterior aspect because the annulus is thinner, Figure 22b. Lamellae of the annulus extend only from the periphery to the narrowest part of the disc and are bulged slightly outward. When photomicrographs of the posterior region are compared, one from a disc with no load applied and the other from an adjacent disc that was fixed at a strain of 0.20, Figure 25, the outward bulge is seen to become more severe as the disc is compressed. The change in lamellar orientation at the

Fig. 25. Photomicrographs obtained with the stereo optical microscope of the posterior annulus showing lamellar bulging. Magnification 35X.

- a. With no load.
- b. Loaded to 0.20 compressive strain.

Fig. 26 Schematic representation of lamellar bulging at the posterior aspect of the annulus.

- a.** With no load.
- b.** Loaded to 0.20 compressive strain.

posterior aspect following compression is shown schematically in Figure 26.

The disc is much flatter at the lateral aspect than at either the anterior or posterior aspects, although there is enough curvature of the vertebral interface that the disc is distinctly thicker at the periphery, Figure 22c. Lamellae at the periphery are straight while those close to the nucleus bulge outward; when compressed, outer lamellae buckle while inner lamellae bulge sharply outward, Figure 27. Typical orientations of the lamellae at the lateral aspect are shown in Figure 28.

Fibers of the lamellae are oriented at an angle to the spinal axis and have a planar crimped waveform²⁸. The crimped waveform, seen in transverse sections cut parallel to the interlamellar angle, Figure 29, progressively straightens as the disc is compressed, although completely uncrimped fibers are not observed even at a strain of 0.20. The parameters that characterize the crimp are measured at various positions of the annulus at increasing strains. The data for the eighth lamella from the periphery at the anterior-lateral aspect are typical, Figure 30; they show the gradual decrease in crimp angle and increase in crimp period as the fibers uncrimp with increasing strain. Additionally,

Fig. 27. Photomicrographs obtained with the stereo optical microscope of the lateral annulus showing lamellar bulging and buckling. Magnification 35X.

- a. With no load.**
- b. Loaded to 0.20 compressive strain.**

Fig. 28. Schematic representation of lamellar bulging and buckling at the lateral aspect of the annulus.

- a. With no load.**
- b. Loaded to 0.20 compressive strain.**

Fig. 29. The crimped collagen fibers of the peripheral annulus fibrosus at various compressive strains as viewed in the polarizing optical microscope.

Fig. 30. Crimp parameters and interlamellar angle as a function of compressive strain.

the slight increase in interlamellar angle at this position in the annulus, from about 63 degrees at rest to 70 degrees when the strain is 0.20, reveals some reorientation of the fibers away from the spinal axis.

Previous studies have described a hierarchical structure for the annulus fibrosus of the human disc with gradients through the thickness at the various levels of organization¹⁵. Lumbar discs of the canine are about one-third smaller in cross-sectional area but the kidney-bean shape resembles that of the human disc and the 4:1 volume ratio of annulus to nucleus is also the same. The average number of lamellae is about 40 in both human and canine discs; lamellae are distinctly thinner at the posterior in both species and the interlamellar fiber orientation is the same. The fibers of the canine lamellae also exhibit the planar crimped waveform that was described recently¹⁵, and the characteristic parameters of crimp angle and period are similar to those of the human. It is anticipated that the deformation modes observed with the readily available canine disc will be relevant to understanding the behavior of the human disc because of the close resemblance between the hierarchical structures of the human and canine lumbar discs.

It is now possible to suggest how the three dimensional architecture of the disc responds to compressive loading. Although direct observations of the hierarchical structure are made on relaxed discs that have experienced significant reduction in volume due to water loss, the process by which this relaxed state is achieved can be imagined to occur as follows. Initially, before the volume is altered by transport of water out of the disc, lateral spreading of the nucleus forces the lamellae to deflect outward. The disc is not isotropic in cross-section and the amount of bulging is distinctly less at the posterior aspect than at either the lateral or anterior aspects. Since the fibers of the lamellae are anchored in the cartilage endplates, the lamellae accommodate the transverse strain by bulging outward. Proceeding outward from the nucleus, a cumulative increase in the amount of bulging is required by the compressive strain so that if the disc were of uniform height, peripheral lamellae would be required to bulge considerably more than inner lamellae. The curved shape of the vertebral interface which causes the disc to be narrower near the nucleus has the effect of reducing the volume displacement that must be accommodated by the outermost lamellae.

When water flows out of the disc through the cartilage endplates into the vertebral bone, the outward bulge of the annulus is relieved. It is significant that the water content is highest in the nucleus and the inner regions of the annulus for these regions are observed to lose more water, and hence undergo a larger volume decrease than the outer regions of the annulus during stress relaxation. As the volume is reduced, the lamellae are no longer forced to bulge outward and they buckle to conform to the smaller separation of the vertebral bodies.

The fibers of the lamellae are constrained at the cartilage endplates so they must extend in length to accommodate the bulging. Even though the disc undergoes macroscopic compression, the fibers of the lamellae are loaded in tension and their mode of deformation can be compared to other connective tissues such as tendon⁹ and intestine¹¹ that have been analyzed in tension. In both these tissues, the initial low modulus toe region of the stress-strain curve corresponds to the progressive straightening of the planar crimped waveform of the collagen fibers. If the fibers are in a biaxial arrangement, as in the intestine, reorientation of the fibers into the stress direction occurs simultaneously. Although it is not possible to observe the fibers

directly during deformation of the disc as was done in the tendon and intestine, it can be imagined that the initial toe region of the stress-strain curve is similarly accompanied by uncrimping of the fibers of the lamellae and reorientation into the transverse direction. Presumably once the stress has relaxed and the lamellae have been restored at least partially to their original orientation, some recrimping of the fibers occurs. This would explain why only partial uncrimping and reorientation are observed when the relaxed disc is fixed and sectioned for histological examination.

It is also imagined that when the fibers are fully uncrimped, further bulging of the lamellae requires the fibers to extend; this produces the linear region of the stress-strain curve. The strong dependence of the apparent modulus on loading rate is attributed primarily to water transport out of the disc as it is being compressed. At slower loading rates this occurs fast enough that the volume loss is significant on the time scale of the experiment; less bulging is then required to accommodate the compressive displacement of the vertebral bodies and the local tensile strain experienced by the fibers is reduced. However, the well-known viscoelastic nature of the collagen fibers may also contribute to the rate dependency.

2.4 Conclusions

This study of the relationship between the measured mechanical properties of the intervertebral disc and the hierarchical structure has led to the following conclusions regarding the response of the disc system to compressive deformation:

1. It is proposed that time-dependent features of the mechanical response, specifically the effect of loading rate on the stress-strain relationship and the stress relaxation behavior, are due to a decrease in volume of the disc caused by stress-driven transport of water out of the disc through the cartilage endplates into the vertebral bone.
2. Lateral bulging of the nucleus forces the disc to bulge outward when compressed. Bulging of the peripheral lamellae is minimized by the curvature of the vertebral interface, which causes the disc height to be greatest at the periphery, and by the gradient of water content which causes water to be lost preferentially from the inner lamellae and nucleus when the disc is compressed.
3. When the disc bulges, the fibers of the lamellae are loaded in tension and their mode of deformation

is similar to that of other connective tissues such as tendon and intestine that have been analyzed in tension. The initial low modulus region of the stress-strain curve is associated with straightening of the crimped waveform and reorientation of fibers into the transverse direction, while the linear modulus region is identified with extension of the straightened fibers.

CHAPTER 3

A WATER TRANSPORT MODEL FOR THE CREEP RESPONSE OF THE INTERVERTEBRAL DISC

3.1 Introduction

Viscoelastic models employing springs and dashpots are frequently used to describe time-dependent relaxation processes in biological materials. However, the elements of these models are not directly related to the structure of the material. In order to understand the relationship of the macroscopically measured mechanical properties to the hierarchical structure of the biological system, it is necessary to develop physiologically meaningful models.

It was shown previously that compressive stress-relaxation of the intervertebral disc occurs by loss of water from the disc structure.²⁶ This mechanism differs fundamentally from the molecular reorganization normally associated with relaxation behavior of materials, but is similar to that of articular cartilage which also relaxes by means of water transport through cartilaginous material.²⁷ Numerous studies have characterized the compressive creep behavior of articular cartilage in terms of a transport coefficient, usually the

permeability.^{28, 29, 30} The disc and articular cartilage systems are not completely analogous, however. Indentation testing of cartilage results in compaction of the cartilage which decreases in thickness as water is squeezed out at the free surface, much as stepping on wet sand forces water to the surface.³¹ In comparison, the disc is tested in compression as a system; the cartilage endplates are considered to be essentially rigid, porous structures that do not deform while the compressed disc acts as a pump to force water through the pores of the cartilage into the cancellous bone of the vertebral bodies. Consequently, in order to describe the time-dependent response of the disc, it is necessary to formulate new models that are derived specifically from the structure of the intervertebral disc system.

3.2 Creep Models

3.2.1 Anatomical Basis

The intervertebral disc is modeled as a system consisting of the disc together with the cartilage endplates attached to the vertebral bodies.¹⁵ The disc is deformable and when a compressive load is applied, the annulus fibrosus bulges outward. A restoring force resisting this bulging is provided by the collagen fibers

of the lamellae that comprise the annulus. These fibers are loaded in tension when the disc is compressed.¹⁶ This compression creates a pressure within the disc which is the driving force for water to flow through the cartilage endplates and into the vertebral bodies. From there, the water is transported by the circulatory system away from the vertebrae. The strain in the lamellae of the annulus is relieved by the decrease in volume of the disc as water flows out. This model is shown schematically in Figure 31. In the following analysis, the cartilage endplates and vertebral bone are assumed to be incompressible. It is also assumed that the permeability of the cartilage controls the rate at which water is expressed from the compressed disc.

3.2.2 Analytical Model

It is assumed that compressive creep is due to flow of water out of the disc as the result of the pressure gradient across the cartilage endplates caused by an externally applied stress. The hydrostatic pressure within the disc, P_d , is not necessarily equal to the externally applied stress, σ_0 , nor is it necessarily a constant. Both strain- and time-dependencies of the pressure gradient across the cartilage will be

Fig. 31 Creep model of the intervertebral disc.

considered. Within the disc, the hydrostatic pressure is assumed to be homogeneous.

The relationship between the volumetric flux and the pressure gradient is modeled by Darcy flow in a quasi-steady-state system. Laminar flow and a linear pressure gradient across the cartilage endplate are assumed. The Darcy equation is applied to the total volume flux from the disc through two cartilage endplates (above and below the disc) having a permeability coefficient, K . The relationship between the hydrostatic pressure, P_d , and the strain, $\epsilon(t)$, is given in equation (1).

$$q = h_1 [d(\epsilon(t) - \epsilon_0) / dt] = (2 K / L) P_d \quad (1)$$

where: h_1 - height of the disc before testing, m
 $\epsilon(t)$ - strain, $m\ m^{-1}$
 q - volume flux, $m^3 m^{-2} s^{-1}$
 t - time, s
 ϵ_0 - strain at $t = 0$, $m\ m^{-1}$
 P_d - pressure in disc, Nm^{-2}
 L - thickness of barrier layer (in this case, the cartilage), m
 K - permeability constant, $m^4 N^{-1} s^{-1}$

Three cases are considered for the hydrostatic pressure in the disc:

Case I: Constant hydrostatic pressure

Case II: Strain-dependent hydrostatic pressure

Case III: Strain- and time-dependent hydrostatic pressure

3.2.2.1 Case I: Constant Pressure

In Case I, the hydrostatic pressure within the disc, P_d , is assumed to be constant for the duration of the creep experiment and equal to the difference between the applied stress, σ_0 (Nm^{-2}), and the osmotic pressure in the disc, P_0 (Nm^{-2}), as shown in equation (2).

$$P_d = \sigma_0 - P_0 \quad (2)$$

This expression for the hydrostatic pressure is substituted into equation (1) and upon integrating gives a linear relationship between strain and time as shown in equation (3).

$$\epsilon(t) = \epsilon_0 + [2 (\sigma_0 - P_0) K / (h_1 L)] t \quad (3)$$

Equation (3) predicts a linear creep curve, Figure 32a, and the permeability, K , can be calculated from the slope. Since, the experimental creep data are not linear, it is necessary to consider cases where the hydrostatic pressure is not constant.

3.2.2.2 Case II: Strain-Dependent Pressure

Case II considers the increase in osmotic pressure as the amount of water in the disc decreases. This increase in osmotic pressure is assumed to be linear with strain and equation (4) gives the hydrostatic pressure within the disc where the system parameter, D (Nm^{-2}), represents the magnitude of the strain-dependency.

$$P_d = \sigma_o - [P_o + D (\epsilon(t) - \epsilon_o)] \quad (4)$$

Substituting of this relationship into Equation (1) and integrating yields the strain as a function of exponential time as in equation (5).

$$\epsilon(t) = \epsilon_o + [(\sigma_o - P_o) / D] \times \{1 - \exp [-2 K D t / (h_i L)]\} \quad (5)$$

According to this equation, the strain reaches an asymptotic value, ϵ_o , as the exponential term becomes

CASE I: CONSTANT PRESSURE GRADIENT

CASE II: STRAIN DEPENDENT PRESSURE GRADIENT

CASE III: STRAIN AND TIME DEPENDENT PRESSURE GRADIENT

Fig. 32 Three cases of the creep model.

negligible at long times, Figure 32b, and equation (5) reduces to

$$D = (\sigma_0 - P_0) / (\epsilon_0 - \epsilon_\infty) \quad (6)$$

The system parameter D can be calculated from equation (6). Equation (6) is substituted into equation (5) to yield equation (7).

$$-\ln[(\epsilon(t) - \epsilon_\infty) / (\epsilon_0 - \epsilon_\infty)] = [2 K D / (h_1 L)] t \quad (7)$$

Using the value of D obtained from equation (6), the permeability, K, is calculated from the slope of the plot of $-\ln[(\epsilon(t) - \epsilon_\infty) / (\epsilon_0 - \epsilon_\infty)]$ versus t.

3.2.2.3 Case III: Time- and Strain-Dependent Pressure

Although it will be seen that Case II adequately describes the experimental observations, it does not take into consideration the probability that the retractile force exerted by the collagen fibers in the lamellae of the annulus fibrosis is time-dependent. The well-known viscoelastic character of collagen fibers would introduce certain features to the creep curve that could not be confirmed in this study due to the limitations of the experiment.⁹ As indicated above, Case II requires that

the strain reach an asymptotic value at long times. It is possible that the disc does not exhibit such behavior. It is also possible that the disc may exhibit non-recoverable strain when unloaded, although this could not be determined from in vitro measurements on the open system. Both these phenomena can be accommodated by adding a linear, time-dependent term to the hydrostatic pressure. This is illustrated in equation (8) where the system parameter, G ($\text{Nm}^{-2}\text{s}^{-1}$), represents the magnitude of the time-dependency.

$$P_d = \sigma_o - [P_o + D (\epsilon(t) - \epsilon_o) - G t] \quad (8)$$

Substituting equation (8) into equation (1) and integrating yields equation (9), which has both an exponential and a linear dependence of strain on time, Figure 32c.

$$\epsilon(t) = \epsilon_o + \left\{ \left[(\sigma_o - P_o) / D \right] - \left[(h_i L G) / (2 K D^2) \right] \right\} \times \\ \{ 1 - \exp [-2 K D t / (h_i L)] \} + (G / D) t \quad (9)$$

At long times, the exponential term in equation (9) is negligible and the relationship between strain and time is linear with a slope of G/D . The intercept of this linear region extrapolated to the strain axis, ϵ_{int} ,

is given by equation (10) and is a function of the system parameters K, D, and G.

$$\epsilon_{int} = \epsilon_0 + [(\sigma_0 - P_0) / D] - [(h_i L G) / (2 K D^2)] \quad (10)$$

Equation (11) is obtained when equation (10) is substituted into equation (9).

$$-\ln[(\epsilon(t) - \epsilon_{int} - (G / D) t) / (\epsilon_0 - \epsilon_{int})] = [2 K D / (h_i L)] t \quad (11)$$

Using the value of G/D obtained from the slope of the linear, long time region of the creep curve, a plot of the quantity on the left side of equation (11) versus t should be linear with a slope equal to 2KD/(h_iL). Equations (10) and (11) can then be solved simultaneously to calculate the values of K and D.

3.3 Verification of the Models

3.3.1 Experimental Methods

Spinal segments were harvested from adult mongrel canines. Discs from two different animals were used in the tests. The discs selected were from levels T11-T12 through L6-L7. Spinal segments were divided into groups

of three adjacent discs with each disc in a group tested at a different stress level. Isolated disc specimens were prepared by cutting transversely through the vertebral bones above and below the disc. These cuts were made parallel to the disc. The posterior articulating facets and bony processes were removed. The resulting test specimens consisted of a disc with approximately 10 mm of viable bone above and below. The area of the disc was determined by measuring the maximum and minimum diameters of the disc and applying the area formula for an ellipse. A correction factor was used to account for deviation of the disc from this idealized geometry.²⁶ Discs were enclosed in plastic to maintain 100% relative humidity. Discs were used fresh or kept refrigerated at 4 °C until tested. All were tested within 72 hours of removal. Prior to testing, specimens were equilibrated at room temperature. The discs were kept sealed in plastic throughout the test.

A servo-hydraulic Instron 1331 mechanical testing machine was used. Specimens were placed between solid loading platens and preloaded to a stress of 0.1 MNm^{-2} . This tare stress was five percent or less of the creep stress in any of the tests. A stress of 2, 5, 7, or 10 MNm^{-2} was applied by driving the crosshead at a rate of 70 kNs^{-1} until the creep stress was achieved, then

maintaining that stress for the 60 minute duration of the experiment. These stress levels were chosen because they fall within the linear region of the stress-strain curve of the disc in compression.²⁶ Displacement vs. time curves were recorded and converted to strain vs. time by normalizing to the height of the disc measured at the periphery at rest. Two of three disc specimens loaded to 10 MNm^{-2} failed in creep. Data from these tests were not considered in the following analysis. During the course of the test, no water was seen seeping through the outer surface of the disc. However, upon removing the specimens at the conclusion of the test, a mixture of water, blood, and marrow was observed on the surfaces of the loading platens.

3.3.2 Results and Discussion

The data from creep experiments at stress levels of 2, 5, 7, and 10 MNm^{-2} are shown in Figures 33a - d. Case I predicts a linear creep curve which is not observed experimentally. The experimental data do not differentiate between the models described in Cases II and III. Case III is the more comprehensive model because it can account for a non-asymptotic creep curve and irreversible deformation of the disc following unloading. Data for the typical creep experiment shown

Fig. 33a Creep data for all specimens tested at 2 MNm^{-2} .

Fig. 33b Creep data for all specimens tested at 5 MNm^{-2} .

Fig. 33c Creep data for all specimens tested at 7 MNm^{-2} .

Fig. 33d Creep data for all specimens tested at 10 MNm^{-2} .

in Figure 34 are plotted according to Case II, equation (7), in Figure 35 and according to Case III, equation (11), in Figure 36. The osmotic pressure of the disc, P_0 , as obtained from the literature, was assumed to be 0.2 MNm^{-2} .³² The thickness of the cartilage endplates was determined from sagittal sections through the disc to be 0.3 mm. The system parameters K , D , and G were calculated for Case II and Case III at four stress levels and the results are summarized in Table III.

The contribution of the cancellous bone of the vertebral bodies to the permeability, K , was tested by increasing and decreasing the height of the bone left attached to the disc specimen. In tests using bone heights of 7 and 12.5 mm, no significant change in the parameters, K , D , or G was observed. This supports the hypothesis that the cartilage endplate is the structure that controls flow of water from the disc during creep.

The permeability, K , was higher in Case III than in Case II. For the Case II analysis, not all the creep curves leveled off after 60 minutes, so ϵ_0 was taken as the strain at 60 minutes when the experiment was terminated. If the experiment had been continued for a longer time, a larger value of ϵ_0 would probably have been indicated and this would affect the value of the permeability constant obtained from the Case II analysis.

Fig. 34 Creep data for a typical specimen at 7 MNm^{-2} .

Fig. 35 Creep data from Fig. 4 plotted according to Case II, equation (7).

Fig. 36 Creep data from Fig. 4 plotted according to Case III, equation (11).

TABLE III: Analytical Creep Model Parameters

	2 MNm ⁻² (n = 3)	5 MNm ⁻² (n = 3)	7 MNm ⁻² (n = 2)	10 MNm ⁻² (n = 1)
Permeability, K (x10 ⁻¹⁷ m ⁴ N ⁻¹ s ⁻¹)				
Case II	0.64 (0.02)	0.33 (0.03)	0.20 (0.01)	0.21
Case III	0.85 (0.14)	0.39 (0.02)	0.27 (0.08)	0.26
Strain Dependency Parameter, D (MNm ⁻²)				
Case II	10.6 (0.5)	22.8 (4.8)	37.4 (2.1)	35.5
Case III	18.2 (0.9)	34.5 (13.0)	51.1 (4.2)	52.2
Time Dependency Parameter, G (x10 ⁻⁶ MNm ⁻² s ⁻¹)				
Case III	421 (56)	795 (360)	853 (4)	1609

The permeabilities reported here are lower than the permeability of articular cartilage which is reported in the literature to be in the range of $2 - 8 \times 10^{-17} \text{ m}^4 \text{N}^{-1} \text{s}^{-1}$.³⁰ This is at least in part the result of the lower water content of endplate cartilage compared to articular cartilage. Endplate cartilage was found to contain 60 - 65 percent water by weight as determined by freeze-drying excised cartilage specimens. Adult articular cartilage is reported to be 85 percent water by weight at the superficial surface and decreases to 70 percent at the subchondral bone.³¹ It has been shown in articular cartilage that permeability decreases with decreasing water content in a non-linear manner.^{34, 35}

Permeability in the disc was observed to decrease with applied stress. This is also observed in articular cartilage, where it is attributed to compaction of the cartilage matrix. This compaction in a decrease in the size and number of pores available for water to flow through the cartilage. In the disc, it could mean that the assumption that the cartilage endplate is incompressible may not hold at the stress levels tested or, alternatively, that the model proposed does not accommodate all the complexities of water movement in and from the disc.

The strain-dependency parameter, D , was higher in Case III than in Case II due to the introduction of the time-dependency parameter, G , into the model. The value of D in Case III must be greater in order to compensate for this additional term. Both the strain- and time-dependency parameters increased in magnitude with stress. The variation with time of the pressure in the disc, P_d , is plotted according to Case II, equation (4), and Case III, equation (8), in Figure 37 for two values of the applied stress, 2 and 7 MNm⁻². The initial pressure is less than the applied stress by an amount equal to the osmotic pressure of the disc. Initially, P_d decreases more rapidly for Case III because the magnitude of the strain dependency, represented by the parameter D , is larger. At longer times the effect of the time-dependency parameter G causes P_d for Case III to level off and the curves cross as the pressure must approach zero at long times for Case II.

The relative magnitude of the strain- and time-dependent stress terms in equation (8) for the Case III analysis varies with time since the strain-dependent term initially increases rapidly then approaches an asymptote while the time-dependent term increases linearly. Although the magnitude of the strain-dependency is larger throughout the duration of the experiment, its relative

Fig. 37 Pressure in the disc as a function of time for Case II and Case III.

influence is reduced at longer times as the effect of the time-dependency parameter increases, as shown in Figure 38.

The Case II analytical model was tested with stress relaxation data at a strain of 0.15 obtained previously on identically prepared disc specimens.²⁶ Values of K , D , ϵ_0 , and ϵ_∞ for creep at 2, 5, and 7 MNm⁻² were used to calculate the time at which $\epsilon(t)$ was equal to 0.15 from equation (7) of the Case II creep model. These values of t lie on the stress relaxation curve for a constant strain of 0.15 in Figure 39 and thus provide confirmation that the proposed model describes the stress relaxation response of the disc as well as the creep response. This indicates that the same mechanism is responsible for the behavior of the disc under both loading conditions.

3.4 Viscoelastic Models

Viscoelastic models composed of ideal elastic and viscous components are frequently used to describe a creep response such as that seen in the disc. These models cannot address physical mechanisms responsible for time-dependent behavior in complex hierarchical systems such as the intervertebral disc. However, comparisons between these models and the analytical models of the disc do reveal the relationship between the elements of

Fig. 38 Ratio of the magnitudes of the strain- and time-dependency terms for Case III as a function of time.

Fig. 39 Stress relaxation data for a compressive strain of 0.15 from Reference 1 and calculated points from the creep model assuming Case II behavior.

the viscoelastic models and the system parameters K , D , and G .

3.4.1 3-Parameter Model

The relationship between strain and time in the 3-parameter creep model, Figure 40a, is given by equation (12).

$$\epsilon(t) = \sigma_0/E_1 + \sigma_0/E_2 (1 - \exp(-t/\tau)) \quad (12)$$

The creep time constant is obtained from the slope when equation (12) is plotted in the form given in equation (13).

$$-\ln [(\sigma_0/E_1 + \sigma_0/E_2 - \epsilon(t)) / (\sigma_0/E_2)] = t/\tau \quad (13)$$

The 3-parameter viscoelastic model is equivalent to the Case II flow model for upon inspection equation (12) has the same form as equation (5). When the terms are equated, the modulus, E_2 , is related to the strain-dependency parameter, D , by equation (14).

$$E_2 = D\sigma_0 / (\sigma_0 - P_0) \quad (14)$$

3 PARAMETER VISCOELASTIC MODEL

4 PARAMETER VISCOELASTIC MODEL

Fig. 40 Viscoelastic solid models:

- a. 3-parameter model.
- b. 4-parameter model.

Equating the exponential terms in equations (5) and (15), the creep time constant is related to both the permeability, K , and the strain-dependency parameter, D .

$$\tau = h_1 L / (2KD) \quad (15)$$

3.4.2 4-Parameter Model

The relationship between strain and time in the 4-parameter creep model, Figure 40b, is given by equation (16).

$$\epsilon(t) = \sigma_0/E_1 + \sigma_0/E_2 (1 - \exp(-t/\tau)) + (\sigma_0/\eta_1) t \quad (16)$$

At long times, the exponential term in equation (16) becomes negligible and the creep curve is linear as in equation (17).

$$\epsilon(t) = \sigma_0/E_1 + \sigma_0/E_2 + (\sigma_0/\eta_1) t \quad (17)$$

Since $\sigma_0/E_1 = \epsilon_0$, the intercept of the linear, long time region of the creep curve with the strain axis is equal to $(\sigma_0/E_2 + \epsilon_0)$ and the slope is σ_0/η_1 .

The creep time constant is obtained by plotting equation (16) in the form given by equation (18).

$$-\ln [(\sigma_0/E_1 + \sigma_0/E_2 + (\sigma_0/\eta_1) t - \epsilon(t)) / (\sigma_0/E_2)] = t/\tau \quad (18)$$

Comparison of equation (16) with equation (9) reveals that the 4-parameter viscoelastic model is equivalent to the Case III flow model. By equating terms, the modulus, E_2 , of the 4-parameter model is related to the strain-dependency parameter, D , and the time-dependency parameter, G , by equation (19).

$$E_2 = 2KD^2\sigma_0 / [(2KD(\sigma_0 - P_0) - h_1 LG] \quad (19)$$

Equating the exponential terms of equations (9) and (20), the time constant, τ , is related to both the permeability, K , and the strain-dependency parameter, D .

$$\tau = h_1 L / (2KD) \quad (20)$$

The viscosity, η_1 , which represents the non-recoverable strain, is related to the strain- and time-dependency parameters by equation (21).

$$\eta_1 = \sigma_0 D / G \quad (21)$$

3.4.3 Results and Discussion

Values for the elements of the 3- and 4-parameter models were calculated from experimental data using plots of the form given by equation (13) and equation (18) and are summarized in Table IV. The time constant, τ , is independent of applied stress in both the 3- and 4-parameter models which means from equations (15) and (20) that the product of K and D is a constant for both Cases II and III. The viscoelastic parameters E_1 , E_2 , and η_1 all increase with applied stress with the possible exception of η_1 from the single 10 MNm^{-2} test. Comparison of a typical creep experiment with the calculated curves for the 3-parameter model, equation (12), and the 4-parameter model, equation (16), shows that both models fit the data, Figure 41. The 4-parameter model is more attractive because it can accommodate an irreversible component of deformation.

Kaleps, Kazarian, and Burns fit a 3-parameter viscoelastic model to experimental creep data from intervertebral discs harvested from rhesus monkeys and humans.^{36, 37} They employed an interpolation and optimization scheme to obtain values for E_1 , E_2 , and η_1 . All the human discs were loaded at stress levels well below those employed in this study ($0.1 - 0.4 \text{ MNm}^{-2}$). However, a limited number of monkey specimens were loaded

TABLE IV: Viscoelastic Model Parameters

	2 MNm ⁻² (n = 3)	5 MNm ⁻² (n = 3)	7 MNm ⁻² (n = 2)	10 MNm ⁻² (n = 1)
3 Parameter Model Elements				
$\tau(\text{sec})$	1416 (88)	1342 (94)	1224 (133)	1297
$E_1(\text{MNm}^{-2})$	22.4 (2.5)	37.5 (4.4)	44.9 (0.1)	63.3
$E_2(\text{MNm}^{-2})$	11.8 (0.6)	23.8 (5.0)	38.6 (2.2)	36.2
4 Parameter Model Elements				
$\tau(\text{sec})$	544 (191)	811 (268)	703 (246)	719
$E_1(\text{MNm}^{-2})$	22.4 (2.5)	37.5 (4.4)	44.9 (0.1)	63.3
$E_2(\text{MNm}^{-2})$	23.7 (1.2)	41.0 (15.8)	57.6 (2.8)	60.5
$\eta_1(\times 10^3 \text{ MN sm}^{-2})$	78.5 (18.6)	226 (39)	419 (33)	324

Fig. 41 Creep data for a typical specimen compared with the 3-parameter model, equation (12), and the 4-parameter model, equation (16).

at levels of 2 MNm^{-2} and these results are compared in Table V. The creep curve calculated with Kaleps' values for a 3-parameter model has a much higher initial strain and a flatter creep curve. These differences probably result from species differences as well as from specimen preparation and test conditions. In Kaleps' work, discs were deep frozen prior to testing and the posterior articulations were left intact during the test. It is known that freezing the disc prior to testing causes ice crystals to form in the disc structure that can disrupt the collagenous architecture of the disc and alter the water content.¹⁴ The presence of intact articulating facets would alter the configuration of the compressive load, particularly at higher strain, from a uniaxial compression to a combination of compression and shear stresses. The effect of varying the applied compressive stress on the values for elements in the viscoelastic model is also addressed in the Kaleps data. Although the variability in results between discs at similar stress levels is high, the trends observed are similar to those in this study. Specifically the moduli increase with increasing applied stress, while the time constant appears to remain essentially constant.

TABLE V: Comparison of Viscoelastic Model Parameters for $\sigma_0 = 2 \text{ MNm}^{-2}$

	This work	Kaleps, et.al.
τ (sec)	1416 (88)	4812 (1761)
E_1 (MNm^{-2})	22.4 (2.5)	9.7 (4.7)
E_2 (MNm^{-2})	11.8 (0.6)	8.7 (2.5)

3.5 Conclusions

Models for the compressive creep response of intervertebral disc have been developed. These are based on previous observations that the time-dependent mechanical response arises from transport of water out of the disc through the cartilage endplates into the vertebrae. Assuming that the driving force for water transport is a pressure gradient across the cartilage between the disc and the vertebral bodies caused by an external stress, the following conclusions can be made:

1. The experimental creep data fit models which consider the strain- or strain- and time-dependency of the pressure in the disc. System parameters obtained from analysis of creep data also correctly predict the stress relaxation.
2. These models are analogous to the 3- and 4-parameter viscoelastic models but contain elements directly relatable to the physical structure.
3. The permeability of the endplate cartilage calculated from these models is slightly lower than that reported for articular cartilage; differences in the water content of the cartilages may account for this.

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