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ABSTRACT

Biomechanical Comparison of Interosseous and Subperiosteal Flexor Tendon Reattachment

by
Alisa Beth Olin

This study was undertaken to biomechanically compare the interosseous and subperiosteal distal flexor tendon reattachment techniques. Twenty New Zealand white rabbits underwent division of the right second and third hind limb profundus flexor tendon distal to the insertion of the superficial flexor. All tendons were repaired using the Kleinnert-Bunnell configuration with a 5-0 nylon tied over a cotton pad on the dorsum of the digit. In half of the animals, the second profundus was reattached into a hole in the middle phalanx and the third profundus was reattached after periosteal stripping of the middle phalanx. The other animals underwent subperiosteal repair of the second flexor and interosseous repair of the third.

The limbs were immobilized for three weeks at which time 10 animals were sacrificed (Group 1), the limb disarticulated and frozen. The remaining (Group 2) were, following an additional five weeks, sacrificed and the limbs harvested and frozen. The limbs were thawed and the repaired tendons biomechanically tested to failure with an Instron, servo-hydraulic mechanical test system.

RESULTS:

	<u>Interosseous</u>	<u>Subperiosteal</u>
Group 1 (3 weeks)	17 ± 4 N	15 ± 5 N
Group 2 (8 weeks)	45 ± 16 N	58 ± 19 N

Controls (3 weeks) and controls (8 weeks) produced mean and standard deviation values of 91 ± 16 Newtons and 100 ± 20 Newtons respectively.

These results were analyzed with the ANOVA, Analysis of Variances method. No statistical differences were found between the interosseous and subperiosteal repair sites. It is concluded that both techniques were successful in attaining sufficient healing. The repaired tendon-bone interfaces were capable of withstanding physiologic loads.

**BIOMECHANICAL COMPARISON OF INTEROSSEOUS
AND SUBPERIOSTEAL FLEXOR TENDON REATTACHMENT**

by
Alisa Beth Olin

**A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biomedical Engineering**

Biomedical Engineering Committee

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This thesis is dedicated to
my beloved husband, Dr. Richard J. Olin.

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CHAPTER 1

INTRODUCTION

1.1 Overview

Movement is a primary function of the skeletomuscular system of animals. Holding together this dynamic structure is a connective tissue network chiefly composed of the collagen protein. Muscles insert into the periosteum of bone via a dense collagenous tissue called tendon. Tendons function to coordinate overall movement of the skeletal musculature. Some biomechanical properties of joints are determined by the tendon-bone interface, since the size of the tendinous insertion as well as its angulation, will alter the motion of the skeleton. The basic components of tendons are primarily tendon fibers with a small admixture of elastic tissue and a hydrated protein gel, which histologically, can best be described as collagen, fibrils, fibroblasts, elastin, and an amorphous matrix. The hierarchical organization of collagen (Kastelic et al., 1978) reveals that there are ten types, of which type I collagen forms the components of various tissues such as lung, liver and tendon.

Morphogenesis, the complex process of tendon growth and development describes the significant changes in cell morphology, macroanatomic organization and biochemical activities. During morphogenesis, tendon cells called fibroblasts differentiate from the mesenchyme and are responsible for the biosynthesis, secretion and organization of collagen. This result is a composite chiefly made up of type I collagen, which is considered relatively homeostatic once fully developed. Morphogenesis of the immature tendon is characterized by (1) a change in the cell morphology from fusiform cells exhibiting complex bifurcation's, (2) the lateral organization of groups of cells into fascicles, (3) the deposition of uniaxial collagen fibrils and fibril bundles, (4) the appearance of crimped

collagen and (5) an increased proportion of interstitial matrix (McBride et al., 1985). The growth and developmental processes require cell-cell, cell-matrix macromolecule, and matrix macromolecule-macromolecules interactions. It is the formation of its uniaxial fibrillar framework that provides tendons with their eventual unique biomechanical properties.

This investigation is a qualitative analysis of the ultimate pull-out strength of repaired flexor tendons of adult New Zealand rabbits. Two surgical techniques, *interosseous* and *subperiosteal* fixation of lacerated tendons, were utilized to measure the differences of repair strengths at the newly formed tendon-bone interfaces. In order to assess the biomechanical property of pull-out strength, it was necessary to design and test a customized fixation device. The device allowed for different forms of healing at the interface to be factored into the load experiments. Limbs from other studies provided an opportunity for preliminary investigations to take place to demonstrate the accuracy of this testing technique.

In order to explore the factors involved in the healing process at the interface repair site and their correlation to the tendon's pull-out strength, it was necessary to undertake an *in vivo* study. White, adult, male, New Zealand rabbits were selected as the subjects of this research because of the similarities of the histological and the anatomical composition of the tendon-bone complex between that of the rabbit hind limb and that of the human hand. Despite this similarity, the rabbit paw functions as a digging apparatus, whereas the human hand is more complex and flexible and has the ability to grasp objects. Two observations can be cited from this comparison. The strength of the rabbit tendon as dictated by function could be inherently greater than that of the humans. Secondly, human hand injuries are primarily caused by accidents involving the laceration of the palmar surface. Ultimately, hand surgeons who must surgically repair human hand tendon lacerations would have a study comparing these two common techniques determining their healing strengths at the newly formed tendon-bone interface. What follows is an overview

of the aforementioned subject matter detailing tendon anatomy and biomechanical properties along with flexor tendon injury and repair processes.

1.2 The Collagens

Collagen is a protein, which differentiates from its precursor, the tropocollagen molecule. This precursor molecule has been determined to be a part of the interstitial substances of tissues. It is the primary building block of connective tissue. Individual fibrils are created by fibroblasts, resulting in an intertwining complex of proteins, which in turn consist of long amino acid chains with polypeptide linkages. The diameter of the fibril varies greatly in and between species from 200-500 Å. Collagen fibers are composed of bundles of fibrils visible in light microscopy. These strands of fibrils require an amorphous, hydrophilic ground substance in which to bundle together to create the collagenous, connective tissue matter. When considering all ten distinct types of collagen (Pachence et al., 1987) it becomes apparent that their distribution is ubiquitous throughout the body. The types I, II, and III are the most common forms, exhibiting their own unique banding pattern of their fibrillar strands (Table 1.1).

Type I collagen is represented by regular dense connective tissues such as skin, lung, muscle and tendon. These tissue collagens like all connective tissues have several common chemical, physical and biomechanical properties.

<u>Collagen</u> <u>Type</u>	<u>Molecular</u> <u>Designation</u>	<u>Tissue or Organ Distribution</u>
Type I	$\alpha 1(I)_2 \alpha 2(I)$	skin, bone, tendon, cornea, annulus fibrosis, placenta lung, liver and muscle
Type I trimer	$\alpha 1(I)_3$	skin, tumor, tendon and liver
Type II	$\alpha 1(II)_3$	cartilage, annulus fibrosis, nucleus pulposus, vitreous body
Type III	$\alpha 1(III)_3$	fetal skin, aorta, uterus, placenta, synovia, heart, liver, lung, nerve
Type IV Type V	$\alpha 1(IV)_3, \alpha 1(IV)_3$ $\alpha 1(V)_2 \alpha 2(V)$ $\alpha 1(V)_2 \alpha 2(V) \alpha$ $3(V)$	basement membrane placenta, skin, bone, tendon, synovia, cornea, aorta, nerve, lung, liver, muscle, placenta villi and uterus
Type VI	$\alpha 1(VI) \alpha 2(VI) \alpha$ $3(VI)$	aortic intima, placenta, uterus, and skin
Type VII		unknown, placenta, skin
Type VIII		unknown, endothelial cultures
Type IX	$\alpha 1(IX) \alpha 2(IX) \alpha$ $3(IX)$	cartilage, inter vertebral disc
Type X		unknown, chondrocyte culture, growing cartilage

Table 1.1 The Collagens: Molecular Configuration and Source

1.3 Tendons: Anatomy and Histology

1.3.1 Ground Substance

The most important function of the interstitial matrix, or ground substance, appears to be as a transference of applied loads to the collagenous fibers (Vincent, 1982). The amount of ground substance between tendon fiber bundles appears to be small, although its function is significant. As noted, the composition of the amorphous ground substance is water filled with mucopolysaccharides and mucoproteins. Some examples of these macromolecules are glycosaminoglycans and proteoglycans which assist in the stabilization of the watery gel. Protein bound-water molecules within the hydrophilic gel provide an important functional property to tendinous tissue. The combination of the regular, dense, uniaxial fibers, plus this gelatinous matrix, provides tendons with the structural properties necessary for their function.

1.3.2 Tendon Fibers

The tendon fibers tend to be as long as the actual tendon. Fibers are assembled into primary bundles, the dimension of which varies with the anatomical location of the structure. Because they are uniaxial, the primary fiber bundles course along the long axis in either a wavy or helical pattern. Each bundle is surrounded by a connective tissue meshwork of dissimilar properties. The fibers throughout the tendon will intermittently anastomose with each other at acute angles.

Anatomically, the tendon can be visualized as a white narrow bundle of fibers that is responsible for the attachment of a skeletal muscle into the periosteum of bone or directly into cartilage. Tendinous attachments are anchored into the periosteum by means of Sharpey's fibers. Some tendons develop as flat sheet-like structures called aponeuroses.

However, for the purpose of this study, the anatomic description will be limited to that of the cord-like uniaxial structures and their relationship to bone (Figures 1.1a, 1.1b, and 1.1c).

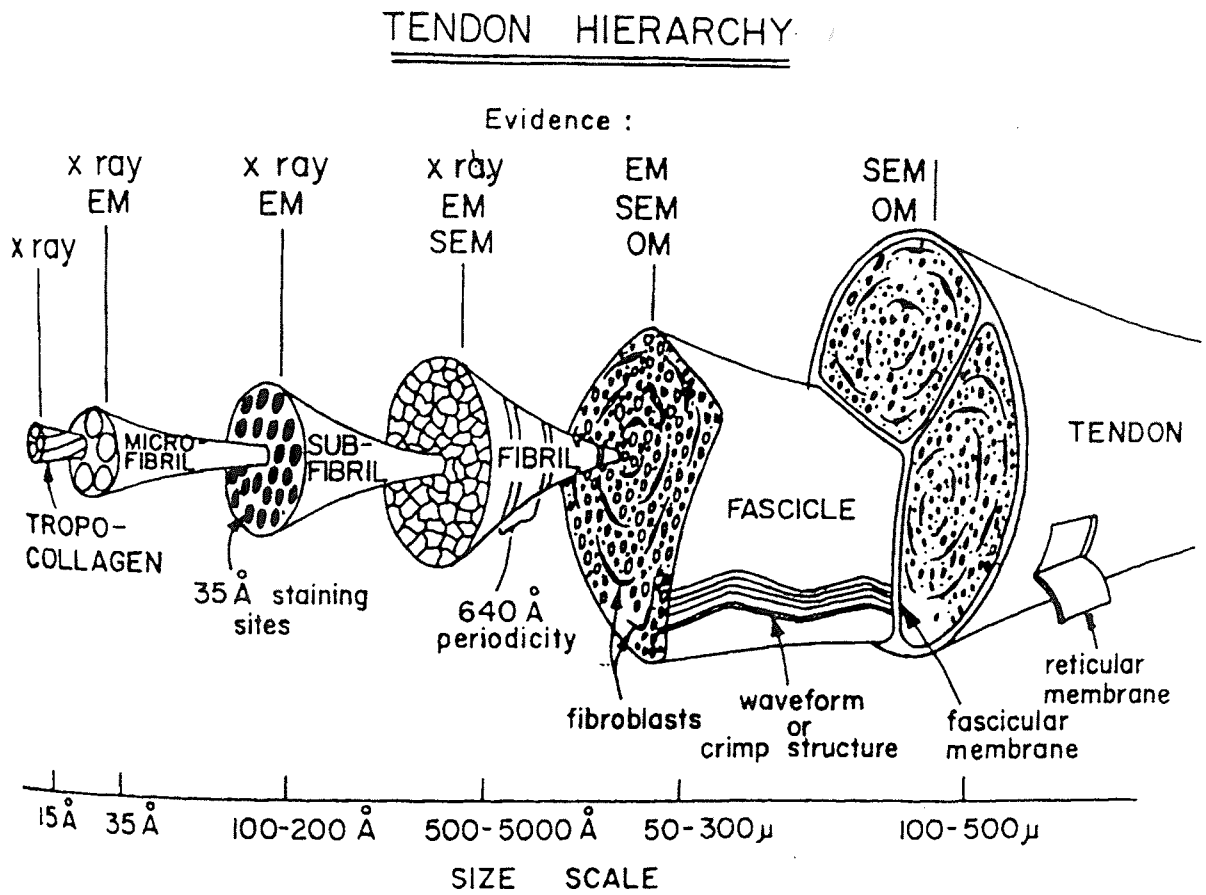


Figure 1.1a
Tendon Hierarchy.

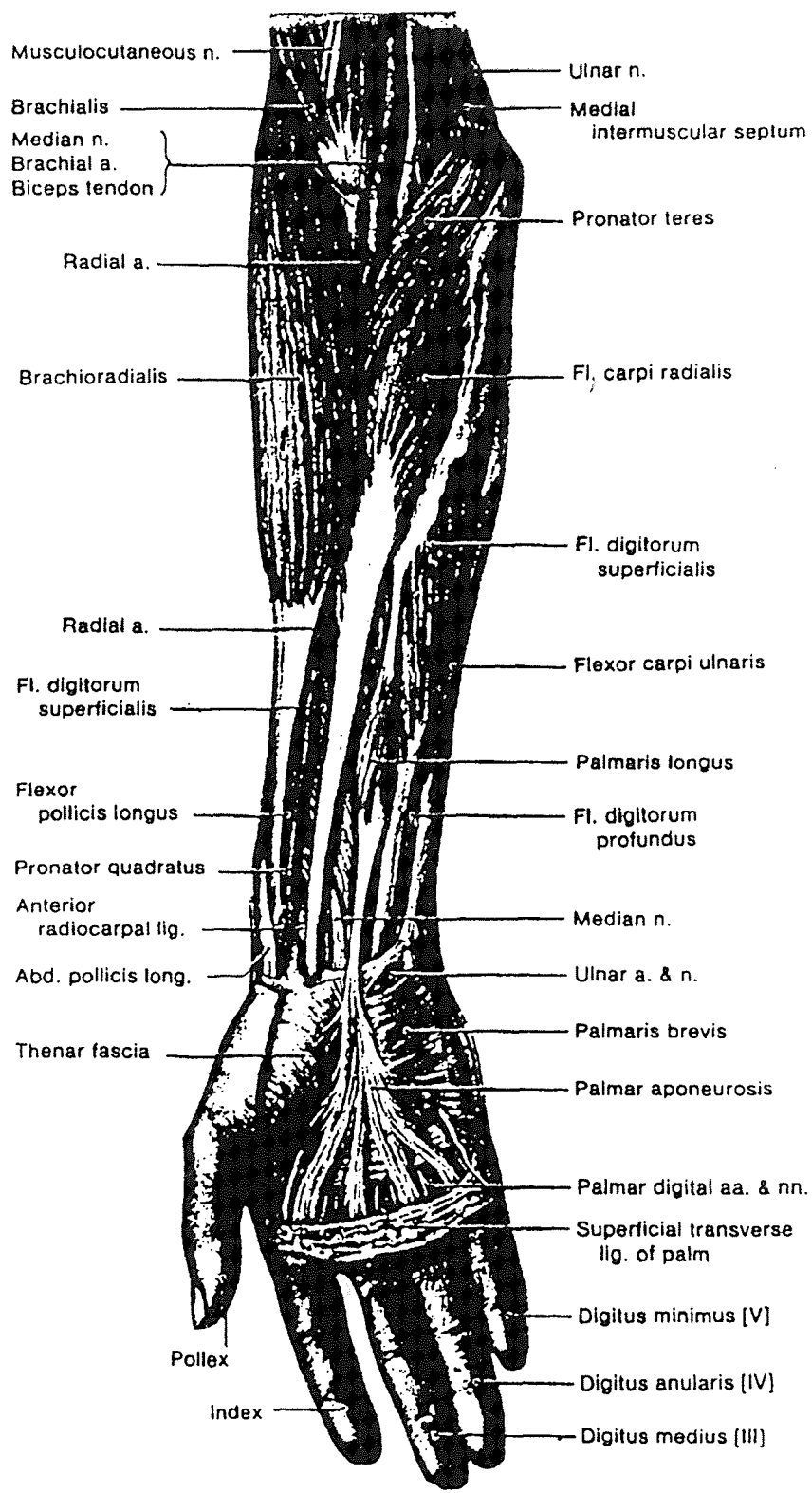


Figure 1.1b
Anatomy of the Human Hand.

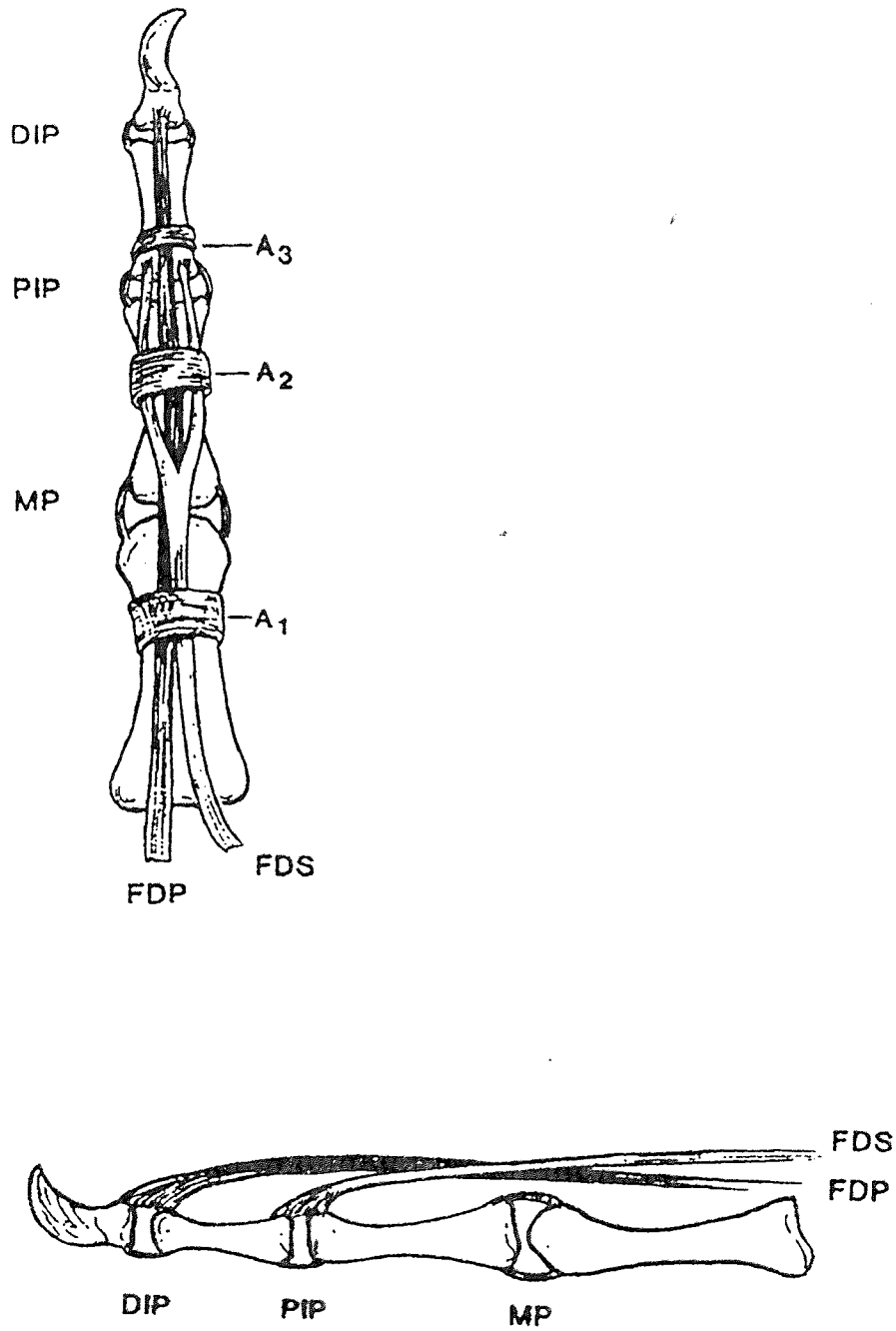


Figure 1.1c
Dorsal and Lateral Views of the Rabbit Limb and Toe.

1.4 The Tendon Bone Complex

1.4.1 Histology of the Tendon Bone Complex

Tendons serve to attach specific muscles to their points of insertion in bone, functioning to transmit contractile forces, thereby creating skeletal movement. Experiments as far back as Ranvier in 1880 have shown that the interface of the tendon bone complex consists of tendinous collagen fibers invaginating into the periosteal matrix. The delineation between the two structures is usually quite marked, unless the tendinous fibers enter the bone as a narrow bundle, whereby they become quickly indistinguishable among the bone's fibrous outer layer. Normally, the tendon inserts at the interface in multiple fibrous bundles ending in clumps known as fibers of Sharpey, which histologically are easily distinguishable. Morphologically, as bones grow they tend to progressively engulf the tendon bundles. During the later maturation stages calcification hardens the tendon-bone interface, beginning some distance under the bony surface and continuing partially into the tendons' attachments. Amprino and Cattaneo consider this to be the precursors for the true Haversian lacunae. The tendon fibers eventually are fully incorporated into the organized bone. On dried bone samples these attachment sites appear macroscopically as being elevated and rugose.

1.4.2 Biomechanics of the Tendon-Bone Interface

Alteration of the physical characteristics of tendon can vary their subsequent biomechanical properties. These physical constituents subsequently are categorized as (1) the arrangement and thickness of the tendon fibrils; (2) water bound proteins; (3) the

thickness of the fibrous attachment as it enters the periosteum; and (4) the angle of the tendinous insertion into the periosteum. The tendon bundles course in a long wavy and helical pattern along their long axis. The assemblage of fibers in parallel bundles provides tendons with their inherent strength to resist forces of deformation. It expediates load forces from muscle to bone in the direction of its axis. As the number of fiber bundles increases so does the diameter of the tendon structure or unit. This, in turn, will increase the ability of the tendon to transfer larger loads before failure. Surrounding the fiber bundles is a connective tissue meshwork that allows occasional molecular crosslinks to take place. The covalent crosslinks increase the tendon's ability to resist deformation and displacement.

Even though ground substance constitutes a small portion of the tendon's dry tissue weight, it becomes significant because of its hydrophilic characteristic. Water represents a large percentage of tendon's total weight and greatly affects its biomechanical properties. Both water and proteoglycans provide the fibers with lubrication and separation crucial to their gliding function. Without this "slippage" the tendinous structure would remain so rigid that normal loads would cause frequent ruptures.

The greater the diameter of the tendinous insertion into the bony surface, the greater the load before fracture, and the greater the insertion will resist rupturing. Thicker tendon attachments tend to "fan" out at the bone interface so that the force being transmitted spreads in to move the bone, allowing for an increase in the transfer of load. The thicker the attachment of the tendon, the less likely it is to avulse.

The direction of insertion and pull of the tendon on the bone can determine the bone's architecture at that point. When the tendinous insertion angle is acute and the pull relatively even with the bone's surface, the lamellae of bone are roughly parallel to the surface as well. The opposite is true if the tendon inserts at right angles; then the bone's lamellae also develop perpendicular to the surface. Since the tendon transmits applied forces along its long axis, it then follows that when the insertion is parallel, so is the

mechanical force applied. The converse also holds true: when the insertion is located at 90°, or perpendicular to the bone, the corresponding force will also be applied at the same angle.

1.5 Injury to the Tendon Bone Complex

The last quarter of this century has shown few studies of tendons. The previous studies have proven what clinicians have observed - that the strength of tendon is great. This strength is such that the tendon will usually rupture from its corresponding muscle or avulse from its bony attachment before it ruptures. The avulsion from the tendon bone interface, in many instances, is a fracturing of the tendinous insertion along with osseous components, leaving the calcified tendon end intact. Therefore, the bone tends to fracture before the tendon itself fails.

1.6 Complications in Tendon Bone Research

Most research of tendons has taken place, at least partially, *in vitro*, since complete *in vivo* testing is impossible to perform. When testing the tendon bone complex in the laboratory, changes in mechanical properties of tendon can occur depending upon several variables. The possibility of injury to the tendon becomes a factor during dissection. Rupture could occur at the injury site providing for false strength results. Furthermore, there are differences of biomechanical properties between fresh and thawed specimens. Testing of fresh specimens provides the investigator with tendons that are less rigid and have better flow (viscosity) properties when clamped. Also, the fresh weight of the tendon cannot be obtained if the specimens still remain attached to with the tendon bone complex. A major

difficulty with testing fresh tendon specimens, generally, is the lack of time to perform the experiments once the dissection has been completed. That is why most investigators will freeze the specimens when large numbers are involved in order to perform the tests on future dates.

Freezing specimens can cause its own set of problems. Changes in the biomechanical properties can occur depending upon the size of the specimen being frozen, the speed of freezing, and the speed of thawing. The critical factor appears to be the postmortem changes of the water content within the tendon. This can be influenced by the *in vitro* milieu of barometric pressure and temperature, as well as those variables already mentioned. Specifically, as the water content of the specimen decreases, the tissue will begin to dry and increase in stiffness and rigidity. There have been observations in previous experiments which show that a specimen increases in stiffness as time lapses between sacrifice and testing (Viidik, 1973). During the thawing process, the collagen can swell if too much water, or physiologic saline, comes in contact with the specimen. Air exposure of the thawing tendons could dehydrate the specimens. Therefore, great care is needed for the tendons once completely thawed.

The studying of the intact tendon bone complex, similar to the technique applied in this experiment, created additional difficulties. In general, it has been shown to be easier to test individual tendon specimens. The dissected tendon can be accurately measured for original length, displacement, fresh weight, and cross-sectional area. However when testing the tendon bone complex unintentional weakening can occur from fixation wires and clamps attaching the bone to the Instron machine. Measurement of the tendon's original length becomes impossible if it is still affixed to the bony complex. Also, the tendon's cross-sectional area cannot be determined for the very same reasons. The cross-sectional area would be used to determine tensile stress. Even if the cross-section could be obtained, it would vary greatly depending on the section of tendon studied, as the thickness of the tendon varies at different lengths.

CHAPTER 2

METHODS AND MATERIALS

2.1 Group Assignments

Twenty, skeletally mature (5-6 pound), adult, male, New Zealand White Rabbits (Pasturella and Coccidia free) from the HARE Marlanz Company in Hewlitt, New Jersey were used. The first surgery date was set for five days from receipt of the animals, which minimized the time the rabbits spent in their cages. This was in compliance with the Animal Rights Activists and the Committee on Scientific Animal Activities at Beth Israel Medical Center. It had been stipulated in the previous protocol, written to obtain approval for this project, that the rabbits would require a minimum of three days to adjust and become oriented with their new environment, prior to undergoing and procedures. Prior to performing surgeries, extensive practice sessions of the two surgical techniques were attended by all participants in order to coordinate the skills of both teams. During the surgeries, each rabbit underwent a laceration and repair of its flexor digitorum profundus tendon to both the second and third digits of its right hind limb (Figure 2.1).

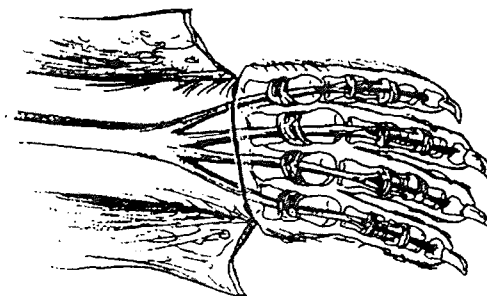


Figure 2.1

Overview of the anatomy of the second and third digits of the rabbit hind limb.

Two rabbit groups were established, and labeled groups 1 and 2 (Table 2.1). In this table those subjects in group number 1, survived for only three weeks and those in group 2 survived an eight week period. In reading the chart below, note that the "B" represents those subjects receiving the interosseous technique, and those labeled "P" underwent the subperiosteal repair.

<u>GROUP NUMBER</u>	<u>DIGIT II</u>	<u>DIGIT III</u>
1a	B	P
1b	P	B
2a	B	P
2b	P	B

Table 2.1

Table deliniating the variables studied in this experiment.

Each group contained five specimens, allowing for a total of ten subjects for both of the three and eight week periods. As each rabbit underwent two techniques per right hind limb, this amounted to forty experimental specimens (twenty interosseous repairs and twenty subperiosteal repairs as well). The reason for dividing the procedures between the two digits, was only to eliminate any later questions of there being a difference in strength between the digits.

2.2 Surgical Procedure

The surgeries took place at Beth Israel Medical Center. The animals were anesthetized with Ketamine (100mg/mL) at a dose of 40 mg/mL and Xylazine (5-15 mg/kg) via intramuscular injections prior to surgery. Also at that time, each of the twenty rabbits were tattooed with a number (1 through 20) on the inside of their left ear. The animals

labeled numbers 1 through 10 were the animals that were to live for eight weeks, and those numbered 11 through 20 were designated as three week subjects.

The animals were then placed in the prone position onto the operating table after the right hind limb had been shaved. Once the surgical field had been prepared and draped to maintain sterility, an injection of 1% lidocaine was infiltrated subcutaneously. A volar, longitudinal incision was then carried down through the subcutaneous tissue to expose the flexor digitorum profundus of the II and II digits. The tendon was then identified noting its insertion site to the base of the distal phalanx and the relationship to the distal interphalangeal joint. With a #15 scalpel this portion of the flexor digitorum profundus was detached from the insertion. After copious irrigation with sterile saline solution, the tendon was re-attached to the distal portion of the correlating II or III digit, just distal to the middle phalanx joint via one of the two surgical repair methods (Figures 2.2a and 2.2b).



Figure 2.2a

Unaltered view of the hind limbs anatomical structures following the initial incision.



Figure 2.2b

Following initial incision, the tendon has been "freed" from its protective sheath and is being lifted for clearer viewing.

For those rabbits receiving the interosseous attachment technique, a hole was made through the middle phalanx by use of a bone awl. Next a 5-0 nylon suture was placed into the distal end on the severed profundus tendon. The two ends of the suture were guided through the bony hole and the dorsal skin of the digit via a Keith needle. Using the standard Kleinert-Bunnell technique the suture was then tied over a cotton plug and fixed at the dorsal aspect of the digit (Figures 2.3a, 2.3b, 2.3c and 2.3d).

Bony Technique

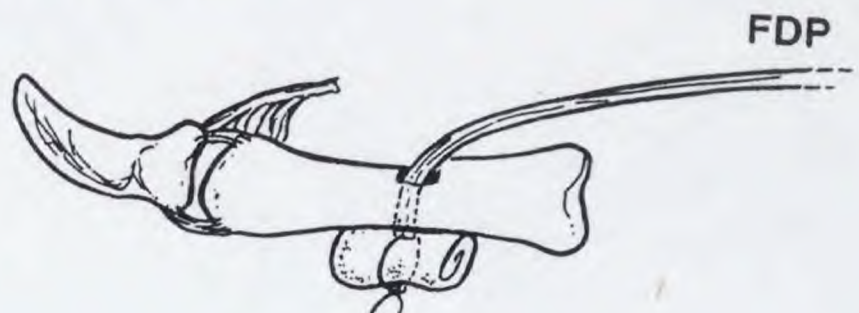


Figure 2.3a

A diagrammatic view of the interosseous repair technique.

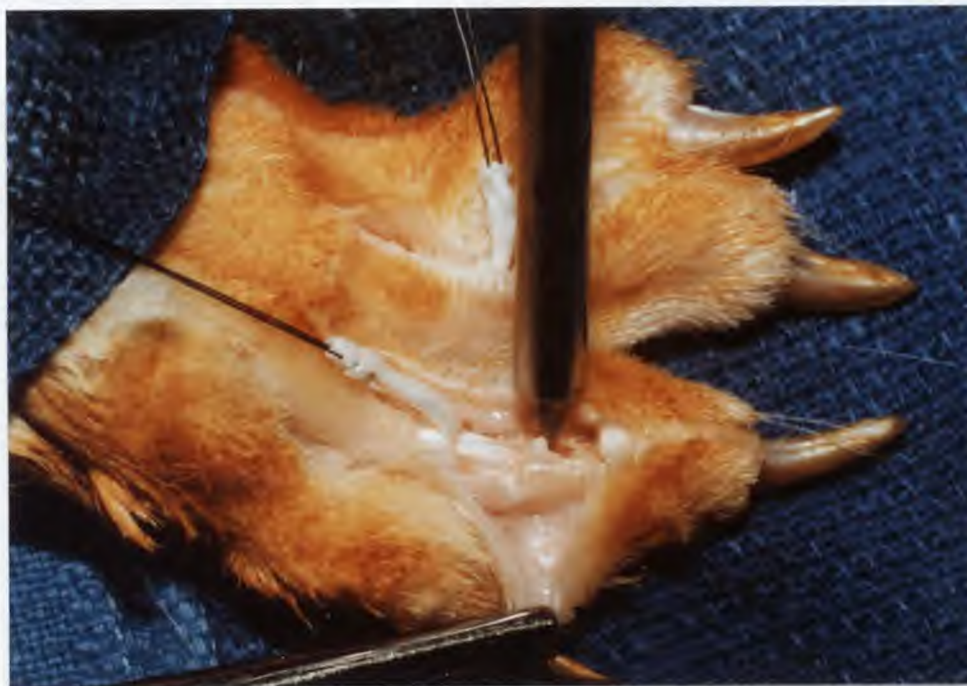


Figure 2.3b

The bone awl is being used to create the hole required for fixation.



Figure 2.3c

A Keith needle is being used to "feed" the tendon through the hole made by the bone awl. Fixation can now occur on the dorsum of the paw.



Figure 2.3d

The cotton plug fixation utilized following both repair techniques.

Those rabbits undergoing the periosteal technique had their periosteum scraped with a scalpel which roughened the surface of the distal phalanx bone. Then, a 5-0 nylon suture was placed through the free end of the profundus tendon, in the same manner as in the bony technique. The two ends of the suture did not pass through a hole in the bone of the middle phalanx. Instead, the ends were guided separately via two Keith needles, passing around the middle phalanx through the dorsal skin of the digit. The suture was then tied and fixed in the same manner as the previous technique. The distal end of the tendon was placed in contact with the bone's surface, to allow for proper attachment and healing (Figures 2.4a, 2.4b, 2.4c, and 2.4d).

Periosteal Technique

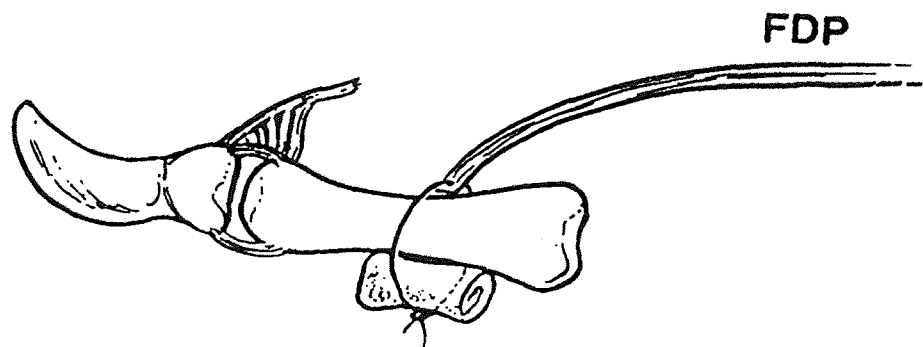


Figure 2.4a

A diagrammatic view of the subperiosteal repair technique.

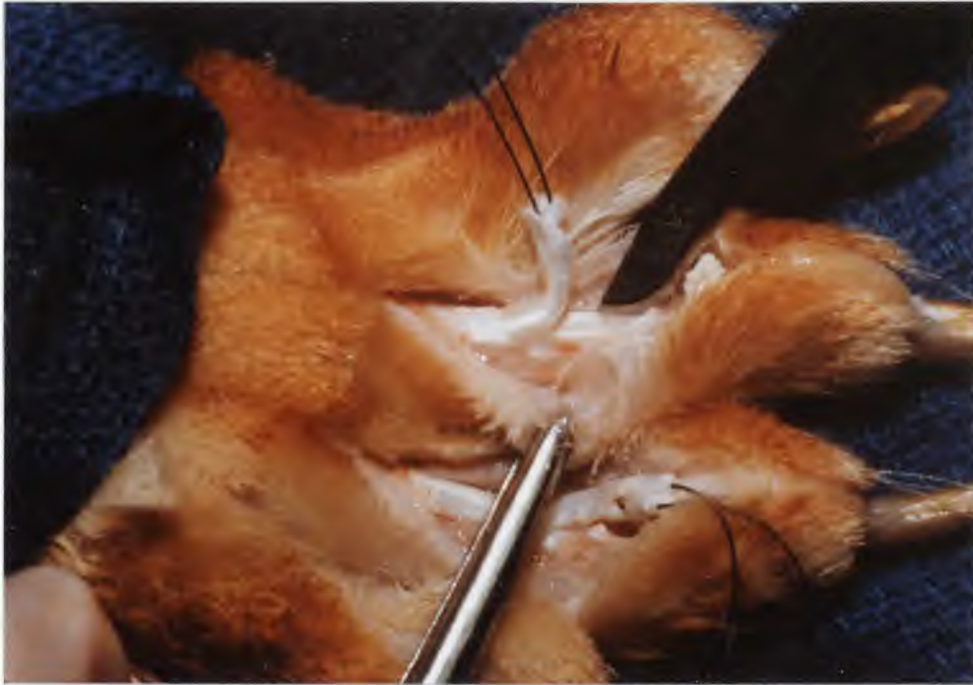


Figure 2.4b

The scalpel is "roughening" the periosteal layer of the bone in preparation of the repair site.



Figure 2.4c

A demonstration of how the two Keith needles were used to insert the suture and thereby complete the repair.



Figure 2.4d

The tendon once secured in place following the completion of the subperiosteal repair.

The skin incisions were then re-approximated using a 5-0 nylon suture. Each surgery site was then covered using sterile cling bandage wrapped as a figure-eight around each digit. A second layer was then applied to encompass the entire hind limb, prior to applying a thick layer of fiberglass casting (Figure 2.5). It allowed for a comfortable, natural resting position of 90° of flexion at the level of the hock joint. The edges of the cast proximal to the rabbit's body were smoothed, in order to avoid any unnecessary irritation (Figure 2.6).



Figure 2.5

The sterile "cling" bandage applied prior to the fiberglass cast.



Figure 2.6

The cast material, as investigated by the rabbits as they awakened from the anesthesia.

2.3 Postoperative Management

The animals were then returned to their individual cages and provided with their regular food and water. Post-operatively the animals received a 5 day course of antibiotics (Kefzol) at 5mg/kg via intramuscular injection. Based on the rules set forth by the Animal Rights Association, should any of the rabbits display signs of distress or pain, they would be given intramuscular analgesia, in order to minimize any unnecessary stress. To decrease both the costs of housing the rabbits and fatigue of the surgeons, the surgeries and the sacrifices were divided among three dates. The casts remained intact for a three week period, after which time the three week subjects were sacrificed. The eight week subjects had their casts removed at the three week mark and were allowed to move freely within their caged environment for the next five weeks (Figures 2.7a, and 2.7b).

Following this five week healing period, the ten remaining subjects were sacrificed. Both the repaired right limbs, as well as, the left control limbs, were harvested and prepared for freezing.



Figure 2.7a

Posterior view of the palmar paw, post cast removal. Notice the large amount hair regrowth.



Figure 2.7b

The dorsal paw post cast removal.

2.4 Experiment Schedule and Notes

Rabbit #1 suffered from minimal nerve damage in his left ear. The nerve was responsible for keeping his ears erect. The damage occurred when he was being labeled (tattooed). This had no effect on the surgical procedures performed.

Period of Day 1-10:

Day 1: The first surgery date. Rabbits #1-4. Eight week subjects. The animals were fed the normal dry feed along with unlimited water. They received their prescribed five day course of antibiotics. No additional pain relievers were needed.

Day 1-10 Postoperative Care:

Each rabbit was observed daily, for about one hour. All of them were fully mobile within their individual cages. Their weight bearing appeared to shift forwards toward their forelimbs, to compensate for the added weight of the casts. The subjects appeared to drag both hind limbs on the "hop" component of their "walk", as their balance and mobility was affected by the casts. The rabbits licked and cleansed the casts, as if they were their body parts, and did not appear disturbed by the noise of the casts banging on the metal cages. The veterinary staff cared for the animals on a daily basis, changing the cages and purifying the air to assure the subject remained Coccidia and Pasturella free, as well as attending to their feedings. Staff was provided to service the animals' needs on the weekends as well.

Day 10: Rabbit #2 gnawed a hole in the cast and required reinforcement of the fiberglass outer coating.

Period of Days 11 through 21:

Day 14: Rabbit #1 required reinforcement of his cast.

Day 22: Second surgery date. Rabbits #5-10. Final Number of eight week subjects.

During surgery, Rabbits # 7-9 both had very thick tendons and sheaths. Rabbit #7 had an additional A₁ pulley as an anomaly. Today the casts for rabbits #1-4 were removed, as it was three weeks post the first surgery date. A call was received to reinforce the cast on rabbit #3; however, it was not repaired as that subject was scheduled to have the cast removed late that afternoon. Hair growth upon cast removal, was approximately 75% (Figure 2.7a).

Period of Days 22-32 -Postoperative Care:

The basic procedures followed were the same as the first postoperative schedule described above. The results and observations were also similar to what was noted above. Rabbits #1-4 were adjusting to their newly mobilized right hind limb freed from the casts. All, but rabbit #1 appeared tentative and fearful and sat still for approximately two hours before attempting to move on the repaired limb.

Day 35: A call was received to repair the cast of Rabbit #10. The cast repair was postponed by 24 hours, when the team was scheduled to reassemble again.

Day 36: Third surgery date. Rabbits #11-20. All of the three week subjects.

Rabbit # 16 was difficult to sedate prior to surgery, and required additional milligrams (< 15mg.) of Xylazine. He also hit his head on the hard cement floor during this time, while struggling to avoid sedation.

Day 39: Repairs were done to the casts of rabbit # 14 and 16.

Day 43: Removal of casts rabbits #5-10: Last of the eight week subjects. Full mobility, and weight bearing occurred for all six subjects, within 30 minutes of their casts removal.

Day 57: First day of sacrificing. Rabbits #1-4 (first eight week group) and #11-20 (all of the three week subjects).

Sacrifice occurred, following the taking of photos and slides of the animals without the casts. Following necropsy, the right and left hind limbs were harvested from the hip socket, labeled twice, and prepared for transport and subsequent deep freeze. The casts were removed following necropsy.

Day 71: Sacrifice day #2. All other eight week subjects were sacrificed (Rabbits #5-10).

They were labeled twice and prepared for transport and deep freeze. The mock surgery was performed for the purposes of photography which eliminated one control subject. Rabbit #5 was the control subject that was lost for the photography purposes. It was later learned that some of the labels used to identify some of the eight week controls were lost in the freezer. Therefore, those rabbit specimens were re-labeled into a generic grouping of eight week controls. Again, this had no effect on the results, since the control group was reported as a group consisting of nineteen animals.

Day 78: Practicing the method of fixation for the biomechanical testing occurred, using specimens not involved within this study. All of the repaired and control specimens remained in deep freeze.

Day 85: Biomechanical testing occurred on the repaired specimens #'s 1-4, 5-10 and 11-20. No controls were tested. All the testing commenced on the same day, under the same conditions (temperature and barometric pressure), to minimize variation of the Instron.

Day 120: Biomechanical testing of all the control specimens.

Nineteen animals, thirty-eight tendon repairs in total.

2.5 Necropsy Protocol

All rabbits were euthenized via a Phenobarbital overdose at a concentration of 60mg/kg, administered intravenously via an ear vein. Then, both hind limbs were disarticulated at the hip joint and appropriately labeled. They were wrapped in sterile-saline soaked gauze, prepared and transported to the Hospital for Joint Diseases - Biomechanical Engineering Laboratory for cold storage at -15 to -20 °C. The repaired digit was left intact and frozen within the limb, so as to not dehydrate or change the biochemical makeup of the tendon. All specimens were frozen, and set to be biomechanically tested on the same day.

2.6 Biomechanical Testing

2.6.1 Specimen Preparation

All of the twenty surgically repaired limbs were defrosted 24 hours prior to dissection (day 85) in the cold room at approximately 4 °C. Only nineteen of the twenty control limbs were defrosted (day 120); however they were done so at room temperature, approximately 20.5 °C, over a 15 hour period. The twentieth control limb was strictly used for photographic reasons and then properly discarded. This had no effect on the statistical results, leaving enough specimens for the biomechanical testing. Dissection and mounting techniques were utilized, which were standard methods developed from previous rabbit-limb experiments as stated in the protocol. The controls were dissected and tested on the same day under uniform conditions. In total 39 rabbit limbs were studied, including both the repaired and control limbs.

Each limb had a longitudinal volar incision along the foot down to the metacarpalphalangeal (MCP) joint. Next the flexor digitorum superficialis (FDS) muscle and associated tendon was located and harvested, leaving the flexor digitorum profundus (FDP) muscle exposed. At the level of the hock joint the distal end of the flexor digitorum profundus (FDP) muscle leads into its tendon which, bifurcates into four separate tendons. These tendinous branches insert individually into each of the four digits. The tendons of the first (I) and fourth (IV) digits were cut at the bifurcation sites, leaving only the tendons of the second (II) and third (III) digits intact with the main tendon trunk proximally. The tendon of the FDP was then split longitudinally down its midline creating two parts, whereby one half is connected to the tendinous branch of the second (II) digit and the other half is connected to the tendinous branch of the third (III) digit (Figure 2.8).

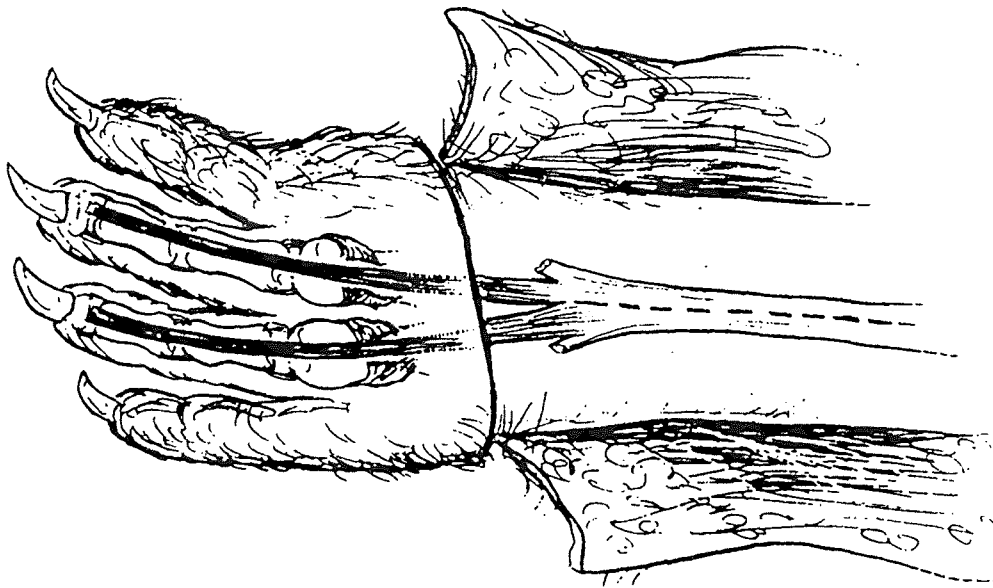


Figure 2.8

A diagrammatic view of the dissection procedure used on the FDP. The FDS has been removed and no exploration has occurred distal to the wrist. A transparent view distal to the wrist is shown for clarification of the tendinous insertions.

All surrounding connective and adipose tissue, including the lumbricals was excised proximal to the muscle-tendinous junction of the proximal FDP. The A₁ pulley of the second and third digits were removed allowing for increased freedom of the FDP tendon. At this point, there was no further dissection distal to the MCP level. It was felt that any further dissection might interfere with the state of the tendon-bone repair site and, in turn, alter the results.

Each limb was then dislocated at the hock joint leaving all of the distal structures, as described above, intact. Subsequently, the dissected FDP tendons were at this point ready for the experimental, mechanical testing. After each tendon specimen was dissected, it was placed back into sterile, saline-saturated (moistened gauze) and then stored in the cold room until they were able to undergo testing.

2.6.2 Testing Set-Up

Each tendon specimen underwent testing on an Instron servo-hydraulic testing machine which was digitally linked to a microprocessor computer. The unit at the Hospital for Joint Diseases is a custom unit designed for the purposes of biological studies. This necessitated that the servo-hydraulic actuator was located on the top leaving the load cell to be relocated to the bottom. The load cell is from Eaton Lebow in Troy, Michigan. It is a 10 kN/Nm full scale biaxial load cell. It functions using three types of Servo Controllers which compare the transducer's (load cell) input with the signal from the manually adjusted command control. The Servo Controller reacts to changes in the polarity (\pm) and magnitude (height). The Load Controller is used to provide excitation for, and process the feedback signals from the load cell transducer. A Strain Controller provides feedback to control the amount and rate of strain of a specimen under testing through the extensometer or strain transducer. Both of these controllers provide DC excitation voltage to their respective transducers. The third type, the Stroke Controller, provides AC excitation voltage to the

actuator position transducer, the Linear Variable Differential Transformer or LVDT. The LVDT is mounted in the actuator to provide an electrical feedback signal proportional to piston displacement. The Stroke controller also contains a Servo Amplifier which processes feedback signals for all three controllers and the actuator's servo valve. It is the actuator that houses the piston supplying the force that controls the piston's displacement and therefore controls the net effect on the variables affecting the specimens.

The system may be operated in any one of three modes: Load Control, Stroke Control, or Strain Control. They function simultaneously, but depend upon which parameter (load, stroke, or strain) is chosen to be the controlled variable. An internal power supply in each controller provides an excitation to the respective transducers. These voltages are modified during the operation of the system by the transducers, and the resulting feedback voltages are proportional to the magnitude of the physical motion of the transducer. The feedback signal is amplified, weighted, and trimmed in the preamplifier and range select stages of the controller and then fed to a summing junction to be compared to the command signal.

The command signal is generated by the function generator module whose peak amplitude is a constant 10v, regardless of the wave form being generated. This signal is then fed into an input circuit which consists primarily of a calibrated, ten turn potentiometer, used to scale the amplitude of the command signal. For dynamic testing, a function generator is used to provide a cyclic input signals (wave) to command cyclic changes to the load, strain and/or stroke. The function generator can provide periodic sine, triangulate, square and ramp signals. The sine and triangle wave forms can be either bipolar (normal cyclic function) or unit-polar (halver function). In this study a halver triangle wave form was used. The Instron also has a cycle counter on the hydraulic control unit which counts the number of test cycles and stops the cyclic testing when a predetermined number of cycles have been completed (Figures 2.9a and 2.9b).

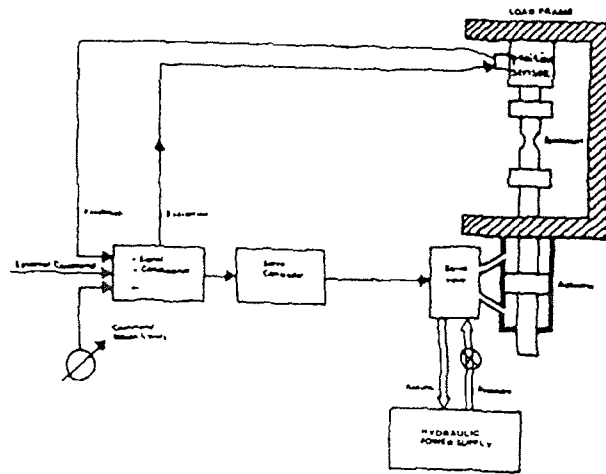


Figure 2.9a

A basic Closed-loop system with a simulated test specimen.

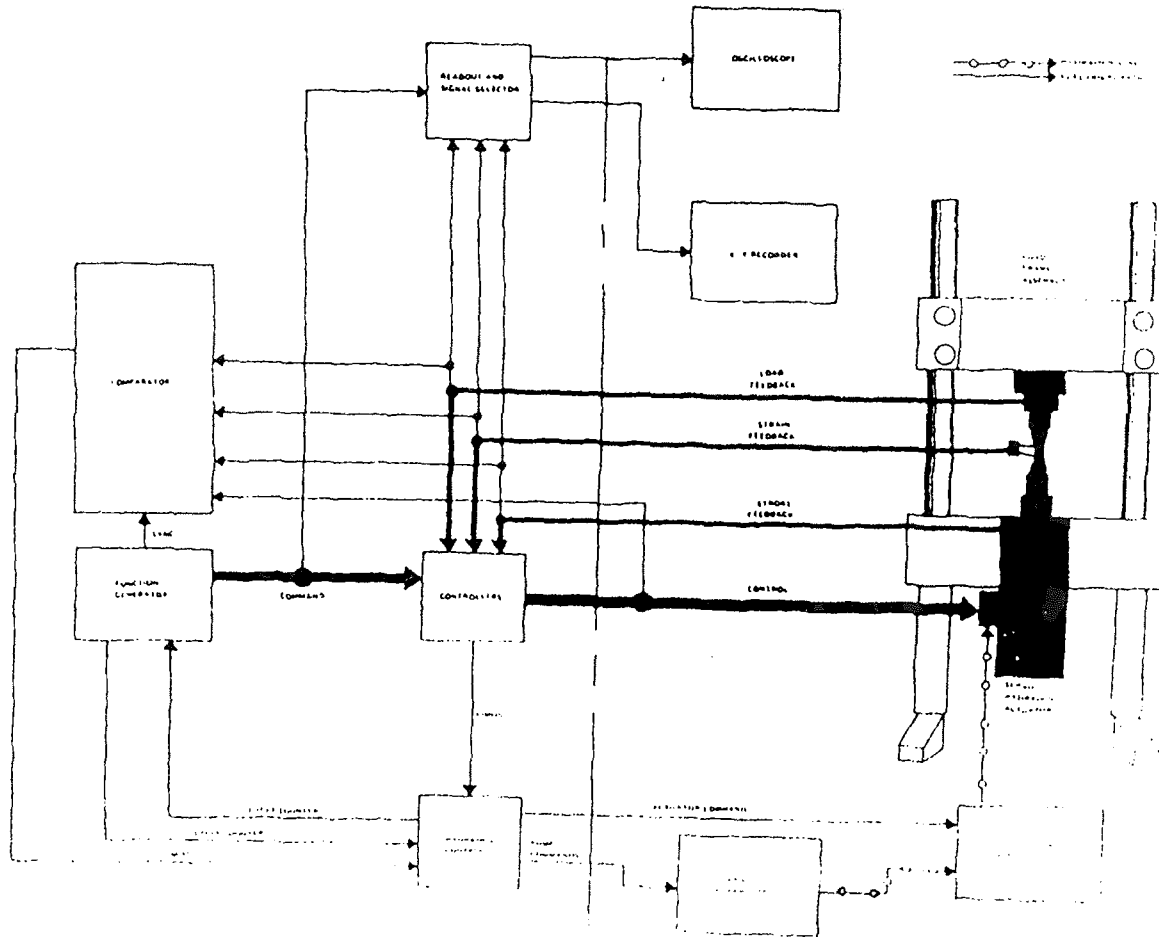


Figure 2.9b

A block diagram of the Servo-Hydraulic Control System.

The Instron's full operational capability is ± 100 mm and ± 10 kN, however, for this experiment the system was optimized for a ± 100 mm and ± 2 kN full scale output. Due to a problem with the original function generator, the Instron was run using a back-up function generator from another material testing system. Excess noise at the interface of the function generator cable with the Instron made it necessary to increase the acquisition rate to 20 Hz. The reason for this adjustment was to ensure that the rupture point was accurately recorded by the microprocessor. Curve smoothing was performed on the load displacement graph using a 4-point moving average. Other Instron operational parameters of importance were: (1) a 10 second loading period was used for a 50 mm full scale deflection of 5 mm/sec; and (2) the machine was set to use 100% of its capability; however, only 50% of its maximum range was utilized, even though the full range was always monitored (Table 2.2). In this experiment data was collected using the Instron's axial load cell, providing for uniaxial loading at the flexor digitorum profundus (FDP) tendon-bone interface (TBI). The resultant stroke on the tendons' longitudinal displacement was considered the independent variable which was recorded along the X-axis upon graphing the data. The resulting load at the TBI was considered the dependent variable and recorded along the Y-axis (figure 2.10).

<u>Machine Specifications</u>	<u>Explanation</u>
[CTRL: STRK: 100% R: 50% A: 100%M] [LOAD: 20% R: 100% M]	Stroke represents the control variable, at 100% of the machines capability. One half (50%) of the maximum capacity was utilized. The load was dependent at 2 kN.
[CTRL: ROT: 100% R: 0% A: 0% M] [TRQ: 100% R: 0% M]	The rotational component was set at $\pm 100\%$, and the torque at ± 100 NM.
[Load cell: 10 kN: full scale =2000N]	The load cell was set to operate as 10kN within the full scale range of 2kN.
[Halver triangle used = 10 sec period] The load cell is from Canton, Massachusetts. It's model # is 6467-119.	A halver triangle was used with a 10 second period The Instron is from Eaton Lebow in Troy, Michigan.

Table 2.2

Further Instron specification used in this experiment.

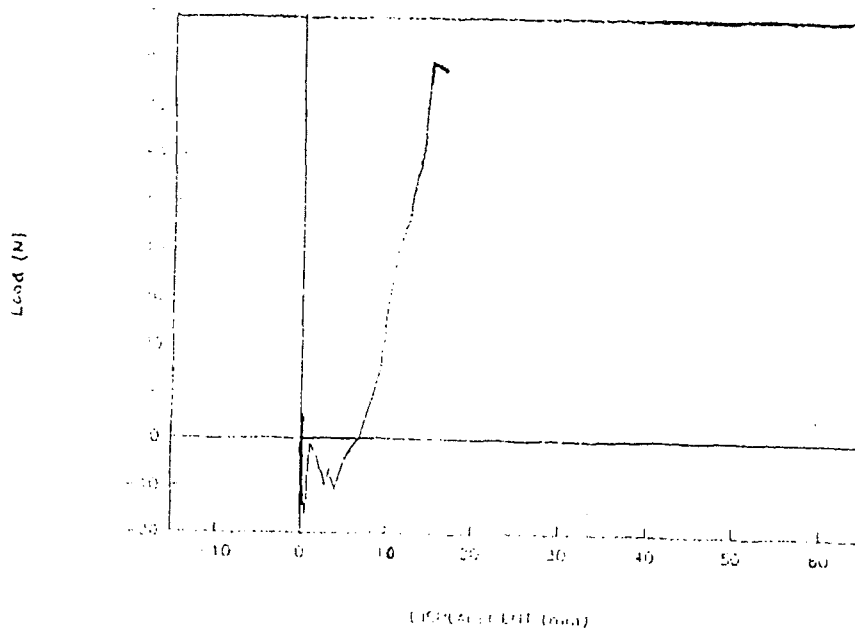


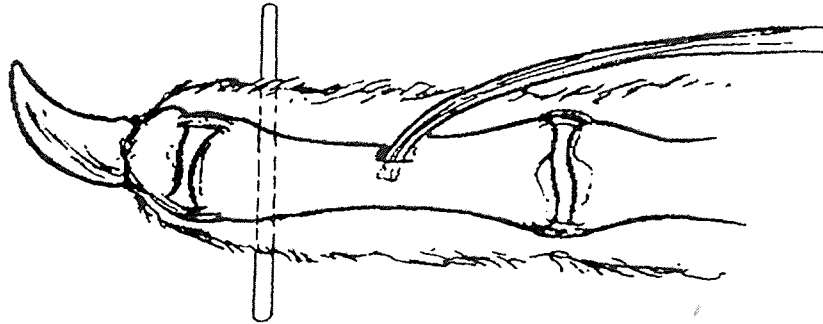
Figure 2.10

An example of the Load vs. Displacement output as shown on the Instron's microcomputer.

The load was recorded as the maximum load value associated with the point of failure at the rupture site on the tendon. Since the Instron is capable of biaxial testing, the rotational axis was locked in place to limit the recording to a single axis. It is important to note that by using a 10 kN load cell within the Instron, the testing was being done within a 20% range of its total capacity. An electronic gain of 2.5 was used making the effective testing range 2 kN.

Due to the variability of the clinical tendon lengths, the distance between the upper and lower Instron grips, or grip length, also varied ranging from 2.9 cm to 6 cm. In determining the grip length, the maximum obtainable length was utilized, which averaged between 5 and 6 cm. This length was optional, since the longer the length of tendon specimen made available, the greater the affinity for its displacement. Applying load to the longitudinal axis of the tendon demanded that the tendon's biomechanical properties remain intact during the testing of the tendon-bone repair site. These properties included elasticity and plasticity, thus allowing for displacement measurements. Since the intent of this study was to look at the maximum tendon failure strength, the discrepancy in grip length was not important. A unique coding system for the specimens was developed for microcomputer input and processing. Each specimen was labeled according to its animal number and digit number followed by the suffix "ALI". For example: 7-D2.ALI identified animal number seven and digit number two.

Prior to testing, each tendon was removed from its gauze wrap and placed on the counter. A hand drill was used to place a 2 inch length of 0.062 inch diameter Kirshner wire (K-wire) into the distal end. This was made possible because the surgical procedure removed the tendon from its original insertion location, and re-attached it to the same phalanx, but at a more proximal location. Of importance was the accuracy when palpating for this fixation point, because poor placement would interfere with the surgical repair site (Figure 2.11).



K-Wire

Figure 2.11

The application of the K-wire.

Once the tendon was prepared, the Kirshner wire and bone construct were placed into the upper grip of a custom designed testing fixture. The upper grip of this fixture (Figure 2.12a) was hollow and hemi-cylindrical in shape, with a slotted base which acted as a shelf to hold the Kirshner wire/bone-tendon unit stable, while allowing the proximal end of the tendon to be fed through the lower grips.

The lower part of the fixture was comprised of a pneumatic grip with two parallel plates. The plates were covered with surgical tape, to soften the surfaces, thereby decreasing potential damage to the tendon. The proximal end of the tendon was centered and then clamped in this grip so that as large a gauge or grip length (grip to grip distance) as possible was created (Figures 2.12b, 2.12c, and 2.12d).



Figure 2.12a



Figure 2.12b



Figure 2.12c



Figure 2.12d

The Instron set-up.

(a) the entire tendon-bone complex is awaiting testing. (b) The Instron
 (c) The initial starting position prior to distraction. The proximal or "free" end
 is in the lower grip. (d) A lateral view of the unit and specimen prior to testing.

The tendon was then distracted at a slow loading rate of 5 mm/second until each tendon reached its failure point. Some specimens required repeat testing when failure of the grip apparatus occurred prior to rupture of the tendon. Specific causes of grip failure are elucidated within the next few chapters. The data was then statistically analyzed via a program entitled SPSS, Release 5.0, for Windows. In order to carry out a statistical test, it is necessary to begin by stating the hypothesis presumed within the study. This hypothesis is termed the null hypothesis and is denoted by H_0 (pronounced "H naught"). Also required is an alternate hypothesis, which is the hypothesis accepted when the null hypothesis is rejected. This alternate hypothesis is abbreviated as H_a (pronounced "H sub a").

For example: $H_0 : p = 0.60$

$H_a : p \neq 0.60$

If the null hypothesis is rejected, the alternate hypothesis is accepted, suggesting that H_a does not equal 0.60.

A null hypothesis about a population proportion states that the population proportion is equal to some specific number p_0 (pronounced "p naught"). The number p_0 is specified by the investigator and is determined by the question needing to be answered. To test the null hypothesis, the one-sample z-test is used. This test is based on the one-sample z statistic given by the formula

$$z = \frac{\hat{p} - p_0}{s_{prop}} \quad (2.1)$$

where p is the sample proportion and s_{prop} is

$$s_{prop} = \sqrt{\frac{\hat{p}(1 - \hat{p})}{n}} \quad ; \quad (2.2)$$

again " \hat{p} " represents the sample size.

When a two sided test is done the probability is the p-value for the calculated z statistic, but only if the null hypothesis is true. Since the null hypothesis is rejected when the p-value is too small, it can be stated that this p-value is what determines whether the hypothesis is rejected or accepted. It is up to the individual to decide prior to testing that p-values less than a particular value warrant rejection of the alternate hypothesis, and p-values greater than or equal to that value that warrants rejection of the null-hypothesis. This cut-off value is called the significance level and is denoted statistically, by α . The most common significance levels are 0.10, 0.05, 0.01 and 0.001. In this study a value of 0.05 was utilized. Using the same significance level as in this study, that the p-value obtained is 0.038. The null hypothesis would be rejected, since it is less than 0.05. Conversely, if the p-value is 0.057, the null hypothesis holds true - since it is greater than 0.05. The computer's statistical analysis program - SPSS for Windows, is concerned with the question of whether the two population means are equal, not the sample means. Therefore, its calculations are determined by

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (2.3)$$

where "x", is the sample mean, "s", is the variance and "N", is the sample size. The subscripts "1" and "2" indicate the group numbers respectively.

Based on the sampling distribution, the program is able to calculate the probability that a difference would occur if the two population means (μ_1 and μ_2) are equal. This probability is called the observed significance level. If this level is small enough (usually less than 0.05) the hypothesis that the population means are equal is rejected.

Another statistic based on the "t" distribution can be used to test the equality of means hypothesis. This statistic is known as the pooled variance t- test. It is based on the assumption that the population variance in the two groups are equal and is obtained using a pooled estimate of that common variance. The test statistic is identical to the equation for

"t" given previously, except that the independent group variances are replaced by a pooled estimate, s_p^2 ,

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_p^2}{n_1} + \frac{s_p^2}{n_2}}} \quad (2.4)$$

where for s_p^2 , the pooled variance is a weighted average of the independent variances. This is calculated as

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \quad (2.5)$$

The purpose of performing Levene's tests is to test the hypothesis that the two population variances are indeed equal. This test is less dependent on the assumption of normality than other tests for the equality of variances. It is obtained by computing for each comparison the absolute difference from its group mean and then performing a one-way analysis of variance on the differences. If the observed significance level for this test is small, the hypothesis that the population variances are equal is rejected. SPSS, the software used, would generate an output for the Levene's test as seen in Table 2.3.

<u>Variable</u>	<u>Number of Cases</u>	<u>Mean</u>	<u>Standard Error of Mean</u>	<u>Standard Deviation</u>
pxd2w3	5	14	2	5
pxd3w3	5	15	3	6

Mean Difference = -1.0

Levene's Test for Equality of Variances: F=0.242 p= 0.636

t-test for Equality of Means 95%

<u>Variances</u>	<u>t-value</u>	<u>DOF</u>	<u>p-value</u>	<u>SED</u>
equal	-0.3	8	0.8	4
unequal	-0.3	8	0.8	4

Table 2.3

An example of the computer output from the SPSS software.

DOF = Degrees of Freedom,
SED = Standard Error of Difference.

This table compares the subperiosteal technique on digit 2 at three weeks to the subperiosteal technique done on digit 3 at three weeks. The cut-off value for determining significance within this study is $p < 0.05$. In this example $p = 0.636$ which is greater than 0.05, which means that the null-hypothesis cannot be rejected. Also, because this value is greater than 0.05, you assume the variances are equal and therefore, the equal variances line is utilized to obtain the t-value, df(degrees of freedom), 2-tailed significance (p-value), and standard error of differences. Ultimately, the 2-tailed significance (p-value) is what is needed to determine if there is a difference between the populations. In this example the two-tailed significance value is $p = 0.8$. This is compared to $p < 0.05$, and is shown to be larger. This "new" $p > 0.05$ leads to the conclusion that there is no significant difference between digit II and digit III after healing three weeks from the periosteal technique used for flexor tendon repairs. Therefore, the researcher is able to reject the null hypothesis, and subsequently state that a difference between digits is highly improbable.

CHAPTER 3

RESULTS

3.1 Observations and Information

	<u>Number of specimens</u>
Ruptured at the repair site	38
Avulsion at the TBI	2

Table 3.1

Results of the repaired tendons.

	<u>Number of Specimens</u>
Fixation Method Failed First	1
Tendon Itself Failed	3
Avulsion at the TBI	27
Rupture at the Lower Instron Grips	2
Fracture of the Bone, Resulting in Tendon Rupture	5

Table 3.2

Results of the control tendons.

In Table 3.1, the results of the repaired tendons rupture sites were reported. It is believed that one of the two tendons which avulsed from the bone, because of a weakening caused

by the fixation method. It was necessary to re-test this specimen, as the fixation method failed, prior to obtaining a value significant. The results in Table 3.2 represent the results obtained from testing the controls. Both the twenty-seven specimens reported from the control reading and the thirty- eight failures reported from the repaired specimens proved what has been previously reported about the ultimate strength of tendons.

In order to further analyze the data it was necessary to engage in the use of an ANOVA, or Analysis of Variances Test. In order to do so the variables studied needed to be limited to that of the two surgical procedures (interosseous vs. subperiosteal) versus the two time periods (three vs. eight weeks). Therefore, it was necessary to prove that there was no significance between the two digits (II vs II), so as to eliminate this variable. This led to the use of the Levene's Test for the Equality of Variances (Table 3.3a, 3.3b, 3.3c, and 3.3d).

<u>Variable</u>	<u>Number of Cases</u>	<u>Mean</u>	<u>Standard Error of Mean</u>	<u>Standard Deviation</u>
pxd2w3	5	14	5	2
pxd3w3	5	15	6	3

Mean Difference = -1.0

Levene's Test for Equality of Variances: $F=0.242$ $p= 0.636$

t-test for Equality of Means 95%

<u>Variances</u>	<u>t-value</u>	<u>DOF</u>	<u>p-value</u>	<u>SED</u>
equal	-0.3	8	0.8	4
unequal	-0.3	8	0.8	4

Table 3.3a

Comparison of digit II vs. III following the subperiosteal technique and three weeks.

DOF = Degrees of Freedom; SED = Standard Error of Difference

<u>Variable</u>	<u>Number of Cases</u>	<u>Mean</u>	<u>Standard Error of Mean</u>	<u>Standard Deviation</u>
pxd2w3	5	17	3	1
pxd3w3	5	16	4	2

Mean Difference = 0.8

Levene's Test for Equality of Variances: $F= 0.2$ $p= 0.7$

t-test for Equality of Means 95%

<u>Variances</u>	<u>t-value</u>	<u>DOF</u>	<u>p-value</u>	<u>SED</u>
equal	0.4	8	0.7	2
unequal	0.4	8	0.7	2

Table 3.3b

Comparison of digit II vs. III following the interosseous technique and three weeks.

DOF = Degrees of Freedom; SED = Standard Error of Difference

<u>Variable</u>	<u>Number of Cases</u>	<u>Mean</u>	<u>Standard Error of Mean</u>	<u>Standard Deviation</u>
pxd2w8	5	52	11	5
pxd3w8	5	63	25	11

Mean Difference = -10

Levene's Test for Equality of Variances: $F= 3$ $p= 0.1$

t-test for Equality of Means 95%

<u>Variances</u>	<u>t-value</u>	<u>DOF</u>	<u>p-value</u>	<u>SED</u>
equal	-0.8	8	0.4	12
unequal	-0.8	5	0.4	12

Table 3.3c

Comparison of digit II vs. III following the subperiosteal technique and eight weeks.

DOF = Degrees of Freedom; SED = Standard Error of Difference

<u>Variable</u>	<u>Number of Cases</u>	<u>Mean</u>	<u>Standard Error of Mean</u>	<u>Standard Deviation</u>
bx2w8	5	38	9	4
bx3w8	5	53	19	9

Mean Difference = -16

Levene's Test for Equality of Variances: $F = 5$ $p = 0.06$

t-test for Equality of Means 95%

<u>Variances</u>	<u>t-value</u>	<u>DOF</u>	<u>p-value</u>	<u>SED</u>
equal	-2	8	0.1	9
unequal	-2	6	0.2	9

Table 3.3d

Comparison of digit II vs III following the interosseous technique and eight weeks.

DOF = Degrees of Freedom

SED = Standard Error of Difference

In summary, it was confirmed by the null-hypothesis being rejected that there was no significant difference between the two digits (II and II), regardless of the type of surgical repair performed, or the healing times allotted (Table 3.4).

bx2w3 vs. bx3w3 where $p = .07$; therefore $p > 0.05$
px2w3 vs. px3w3 where $p = 0.8$; therefore $p > 0.05$
bx2w8 vs. bx3w8 where $p = 0.1$; therefore $p > 0.05$
px2w8 vs. px3w8 where $p = 0.4$; therefore $p > 0.05$

Table 3.4

Summary of the comparison made between digit II and digit III.

By eliminating this variable it was possible to perform an ANOVA, which provided the means and standard deviations for the entire populations, both repaired and control specimens. In order to input the values into the computer the program demanded that "codes" be established. They are as follows:

- Code: 1 represents the subperiosteal repair at three weeks
 2 represents the interosseous repair at three weeks
 3 represents the subperiosteal repair at eight weeks.
 4 represents the interosseous repair at eight weeks
 5 represents the control group for three weeks
 6 represents the control group for eight weeks

Variable Label	Mean	Standard Deviation (\pm)	# of Specimens
Total Population	64	37	78
Code 1	15	5	10
Code 2	17	3	10
Code 3	58	19	10
Code 4	45	16	10
Code 5	91	16	20
Code 6	100	20	18

Table 3.5

Results from the ANOVA.

Graphing of these values, provides a clearer picture, that the means obtained at three weeks were less than those obtained for the eight weeks specimens. The eight week specimens, in contrast to the control groups, also demonstrated smaller mean values (Figure 3.1).

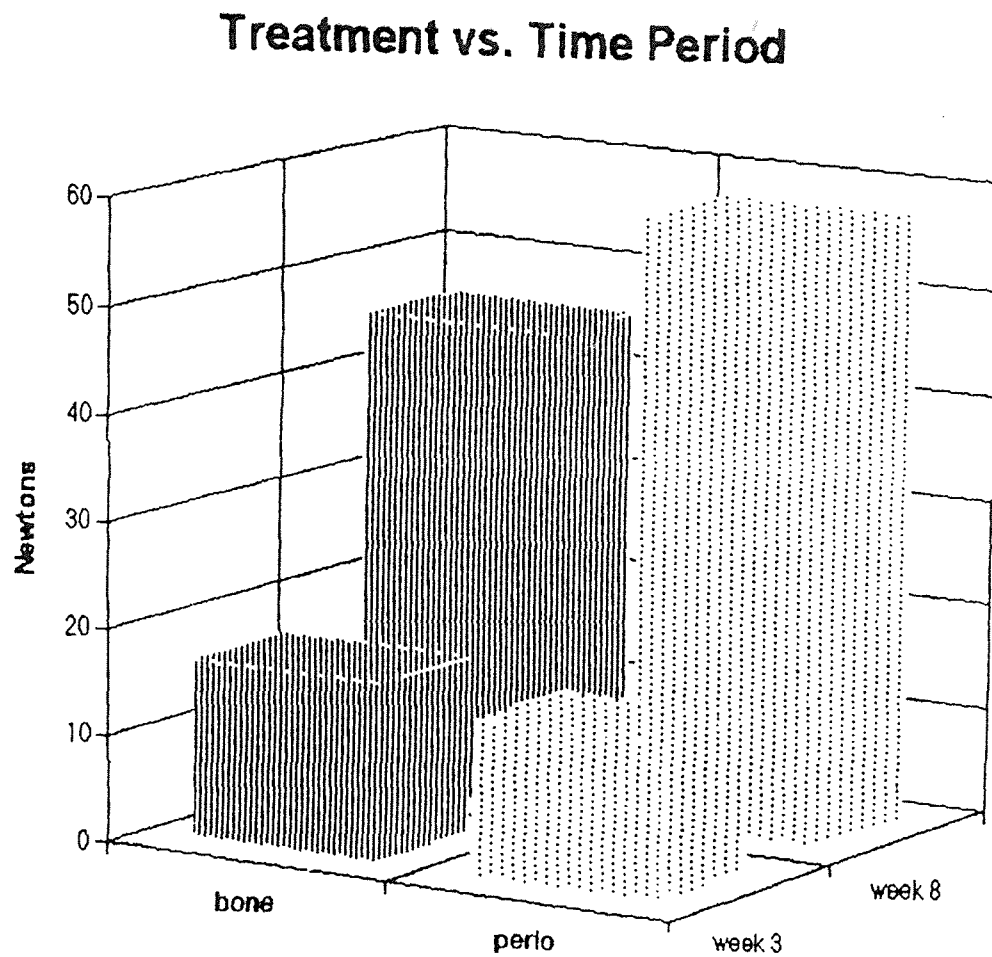


Figure 3.1

A bar graph of the results.

The Control values reported were 91N for the three week specimens and 100N for the eight week specimens.

CHAPTER 4

DISCUSSION

The pull out strength of the tendon from the bony interface is a function of how much load can be applied prior to failure at a tissue site. Previous research has indicated that the tendon is so strong that it will fail only after either the attachment or the bone fails. The tendon that ruptures at the insertion will usually contain a bulb of periosteum, making the distinction between rupture of the attachment or bone difficult to discern. Histologic sections could differentiate, yet for the purposes of this study it was not necessary. The parameters identified were: (1) whether the repair site tendon bone interfaces healed well enough at three weeks and eight weeks and (2) whether there was a significant difference between the two surgical techniques used to repair the lacerated tendons. The question examined by this study was to determine which of the two surgical repair techniques was superior. The resultant data, showed quantitatively, that the average pull-out strengths of the repaired tendon-bone interface for both techniques were statistically equivalent. Both resulted in completely healed interfaces that were physiologically capable of withstanding normal loads. Controls were used to establish the baselines for the pullout strength of normal rabbit tendon-bone interfaces. Comparing these baselines with the experimental data, it was observed that the repaired interfaces were less able to resist load. Many reasons exist for this difference as will be explained later.

The results of the ANOVA, Analysis of Variance, tests were used to compare the two surgical techniques with the time periods of three and eight weeks (Table 3.5 and Figure 3.1). The three week specimens for both techniques revealed markedly lower pull-out strength relative to the eight week specimens. The basic nature of tendon bone repair dictates that the longer the period of healing allowed, i.e. eight weeks, the better the quality

of the strength of the repair. At the three week mark (Group 1) it was demonstrated that the interosseous technique ($17 \text{ N} \pm 3$) resulted in a higher mean value than that of the subperiosteal ($15 \text{ N} \pm 5$). However, following eight weeks of healing (Group 2), the subperiosteal technique ($58 \text{ N} \pm 19$) was recorded at a higher mean value than the interosseous technique ($45 \text{ N} \pm 17$).

A hypothesis that explains this phenomena views the histologic changes as a function of time of healing. As previously explained, the normal tendon bone interface integrates within the outer layer of the bone, the periosteum. In the initial healing stage, the tendinous callus consisting of a hematoma and inflammatory exudate changes to that of a more fibrous material (Ketchum, 1977 and Lindsay, 1987). Periosteum, at the three week mark, consisting of these collagen fibers and fibroblasts, is relatively weaker than the interosseous technique which initially binds the tendon securely. However, biochemical changes, as the healing phase continues towards eight weeks, reveals enough collagenous integration and ossification that the subperiosteal tendon more naturally integrates relative to the interosseous counterpart. The formation of fibers of Sharpey eventually begin solidifying the tendinous insertion, as the calcification of the interface completes the initial healing stage.

The histologic, biochemical and metabolic criteria demonstrate tendon's intrinsic capacity to heal (Greenwald, et al., 1991). At least three weeks of immobilization, which is traditional postoperative management, allows for sufficient healing to withstand minimum load forces. The results, therefore, indicate that this minimum load is less than 14 N and 10 N for the interosseous and subperiosteal repairs, respectively. Research suggests that active tendon mobilization is usually begun after a three week period of postoperative immobilization with a cast. Furthermore, during the subsequent period of 7-10 days following cast removal, physiologic, early controlled movement, has been demonstrated to have no effect on tendon rupture (Feehan, et al., 1990). Upon eight weeks of post operative healing, the results indicate that significant further integration of the tendon bone

interface has occurred. Minimum pull-out strengths of 37 N for the subperiosteal and 29 N for the interosseous repair support this observation. Even though histologically, the bony repair is still ossifying, biomechanically, the site must be able to withstand normal functional limits of load. Consideration of a physiologic parameter indicating repair site healing is a qualitative analysis common to many studies. Quantitatively, it has been shown that the two surgeries succeeded in repairing the tendon-bone interfaces sufficient to withstand these normal, physiologic forces.

Controls were necessary to establish a baseline for the pull-out strength of tendon under similar experimental conditions. Of the thirty-eight control specimens, the majority failed at the tendon-bone interface usually as a result of avulsion of the tendinous end. Others failed when the bone fractured or splintered, but only three controls failed due to the tendon itself rupturing. (Table 3.1). Similarly, the results of the study revealed the repaired tendon-bone interfaces, as well as the surrounding osseous structures, were the main sites of failure. Biomechanically, due to its dense, regular uniaxial bundled collagen fibers the tendon, is considered one of the strongest soft tissue structures in the body. Research has shown that before the tendon itself ruptures, the other tissues failed first, which supported comparable results widely accepted to date (Viidik, 1973).

The pull-out strength of the control specimens were $91 \text{ N} \pm 16$ for three week specimens and $100 \text{ N} \pm 20$ for the respective eight week specimens (Table 3.5). As can be readily observed, the controls were recorded as having a much greater resistance to load relative to their experimental counterparts. Several hypothesis explain this phenomena factoring in biomechanical and experimental technique variables. Earlier studies have indicated that a devascularization occurring at the surgery site tend to decrease its resistance to resultant forces. Devascularization could therefore alter the already relatively inert metabolic tendon. Collagen has been demonstrated as having a half life of only 300-500 days. Due to its low metabolic rate, collagen has less ability to adapt to trauma and change (i.e. force) than most tissues. Secondly, the repaired tendon procedure called for a

shortening of the tendon length in order to lacerate and subsequently reattach the distal end to bone. This shortening effect may have altered the tendon's normal displacement characteristics, as well as the ability to flow normally under a given force. This study allowed loads to be applied by increasing the displacement by 5 mm/second increments. The ratio of original length to its displacement (strain) is greater for the control specimens than it was for those repaired. Similarly, the effects on the elasticity and viscosity could have negatively impacted, resulting in a repaired tendon-bone interface and associated tendon that was more rigid and liable to rupture (Viidik, 1973). Lastly, other studies have concluded that following eight weeks post surgery, another twelve months of activity is needed for tendons to return to their normal strength (Loitz et.al., 1989). Extrapolating from this observation, a hypothesis supposes a continuing ossification transpiring at the repaired interface. Similar to the morphogenesis of the original site, the repair continues to slowly calcify proximally along the tendon insertion anchoring the tissue to the bone's periosteum. The initial eight week healing phase would continue to strengthen with increased solidification of the collagen interface during the following year.

The application of load along the tendon's long axis is similar to the forces found in nature. The main function of tendon is to transfer muscular forces to bone, which it insert. In order to study how much load the tendon-bone complex could withstand and at what location failure occurred, it was necessary to maintain its integrity, as well as some of the surrounding tissues. As indicated in the introduction, previous research has shown many of the difficulties that arise when testing tendon still attached to its contiguous bony complex. However, this arrangement was necessary due to the questions investigated in this study. As mentioned, the two surgical techniques involved repair of this interface. The repairs were all the more significant and applicable to their natural controls since their associated tissues remained intact during testing. This eliminated the variable of an altered environment surrounding the interface from affecting the results.

Biomechanical modeling is unique when testing the entire tendon-bone complex, since it is not possible to measure the tendon's original length, as well as, its fresh weight and cross-sectional area. Certain assumptions are made using comparative analyses, in order to eliminate extraneous variables (Greenwald, et al., 1991). The variables incorporated within this study were the healing periods of three versus eight weeks, the two surgical techniques -interosseous versus subperiosteal - and the two digits - toe II versus toe III. The purpose for investigating the difference between digits was to eliminate the possibility that toe II contained a stronger tendon than toe III. Through the use of the Levene's Tests for the Equality of Variances, as well as, individual t-tests performed on groups one and two, this study demonstrated that there was no significant difference between the digits (Table 3.4). Therefore, the null hypothesis, that a difference exists between the two digits, is rejected and the alternate hypothesis is accepted ($p > 0.05$). Essentially, this allowed for the elimination of this variable so that an ANOVA test (both Bonferroni and Scheffe) could be examined.

Utilizing the resultant data, a statistical analysis, called an ANOVA, was performed. This was followed by a post-hoc analysis. These provided this study with the mean and standard deviation values, which demonstrated that there was no significant difference between the two surgical procedures ($SD > \pm 0.05$). Therefore, it can be stated that both surgical techniques -interosseous and subperiosteal -resulted in relatively similar healing strengths at the repaired tendon-bone interfaces. In other words, this study has quantitatively shown that the pull-out strengths of both repaired interfaces were comparably successful and physiologically functional after three and eight week periods of healing.

CHAPTER 5

CONCLUSION

This study compared the two surgical techniques -interosseous and subperiosteal- for the repair of lacerated tendons at the tendon-bone interface. Upon testing these sites against controls it was necessary to utilize the subjects of twenty white, adult, male New Zealand rabbits. The resultant dissected specimens were entire tendon-bone complexes of both the left and right hind limbs. Variables, such as the differences between toe II and III, were eliminated through quantitative analysis and extrapolating the data to fit basic standard biomechanical modeling assumptions. Following a statistical analysis called and ANOVA, or Analysis of Variances test, the resultant data revealed significant results. It was shown that statistically, both techniques were equally capable of creating the necessary tendon-bone repairs. They showed the ability to heal successfully, when measured in terms of pull-out strength. Test data further revealed that both techniques resulted in the ability to withstand normal physiologic forces.

Appendix A

Raw Data provided to the Instron

<u>Specimen #</u>	<u>Group</u>	<u>Code</u>	<u>Digit</u>	<u>Load</u>
1	bxd2w8	4	2	49
2	bxd2w8	4	2	39
3	bxd2w8	4	2	36
4	bxd2w8	4	2	39
5	bxd2w8	4	2	25
6	bxd3w8	4	3	56
7	bxd3w8	4	3	35
8	bxd3w8	4	3	33
9	bxd3w8	4	3	65
10	bxd3w8	4	3	77
11	pxd2w8	3	2	57
12	pxd2w8	3	2	61
13	pxd2w8	3	2	44
14	pxd2w8	3	2	62
15	pxd2w8	3	2	38
16	pxd3w8	3	3	43
17	pxd3w8	3	3	97
18	pxd3w8	3	3	68
19	pxd3w8	3	3	70
20	pxd3w8	3	3	35

21	bx _d 2w ₃	2	2	17
22	bx _d 2w ₃	2	2	15
23	bx _d 2w ₃	2	2	13
24	bx _d 2w ₃	2	2	21
25	bx _d 2w ₃	2	2	19
26	bx _d 2w ₃	2	3	17
27	bx _d 3w ₃	2	3	21
28	bx _d 3w ₃	2	3	10
29	bx _d 3w ₃	2	3	15
30	bx _d 3w ₃	2	3	18
31	px _d 2w ₃	1	2	10
32	px _d 2w ₃	1	2	17
33	px _d 2w ₃	1	2	12
34	px _d 2w ₃	1	2	10
35	px _d 2w ₃	1	2	21
36	px _d 3w ₃	1	3	8
37	px _d 3w ₃	1	3	19
38	px _d 3w ₃	1	3	15
39	px _d 3w ₃	1	3	23
40	px _d 3w ₃	1	3	10
41	cx _d 2w ₈	6	2	94
42	cx _d 2w ₈	6	2	64
43	cx _d 2w ₈	6	2	110
44	cx _d 2w ₈	6	2	102
45	cx _d 2w ₈	6	2	107

46	cxd2w8	6	2	93
47	cxd2w8	6	2	113
48	cxd2w8	6	2	113
49	cxd2w8	6	2	77
50	cxd3w8	6	3	114
51	cxd3w8	6	3	125
52	cxd3w8	6	3	93
53	cxd3w8	6	3	76
54	cxd3w8	6	3	113
55	cxd3w8	6	3	123
56	cxd3w8	6	3	56
57	cxd3w8	6	3	120
58	cxd3w8	6	3	114
59	cxd2w3	5	2	110
60	cxd2w3	5	2	116
61	cxd2w3	5	2	98
62	cxd2w3	5	2	67
63	cxd2w3	5	2	65
64	cxd2w3	5	2	117
65	cxd2w3	5	2	101
66	cxd2w3	5	2	89
67	cxd2w3	5	2	76
68	cxd2w3	5	3	101
69	cxd3w3	5	3	101
70	cxd3w3	5	3	91

71	cx3w3	5	3	94
72	cx3w3	5	3	97
73	cx3w3	5	3	72
74	cx3w3	5	3	114
75	cx3w3	5	3	73
76	cx3w3	5	3	87
77	cx3w3	5	3	82
78	cx3w3	5	3	85

Table A.1

Raw data as analyzed by the statistical software.

Appendix B

Duplicate Photographs in Black and White



Figure A.1
Black and White for Figure 2.2a



Figure A.2
Black and White for Figure 2.2b



Figure A.3
Black and White for Figure 2.3b



Figure A.4
Black and White for Figure 2.3c



Figure A.5
Black and White for Figure 2.3d



Figure A.6
Black and White for Figure 2.4b



Figure A.7
Black and White for Figure 2.4c



Figure A.8
Black and White for Figure 2.4d



Figure A.9

Black and White for Figure 2.5

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