A STUDY OF SELECTED LIGAMENTS AND TENDONS OF THE KNEES AND ANKLES OF THE RHESUS MONKEY, BABOON AND CHIMPANZEE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Metallurgy, Mechanics, and Material Science

1981

ABSTRACT

A STUDY OF SELECTED LIGAMENTS AND TENDONS
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The purpose of this research was to study the mechanical properties of the flexor hallucis longus and tendo-calcaneus tendons of the ankles, and the medial collateral and patellar ligaments of the knees from the rhesus monkey, baboon, and chimpanzee using a three hour experimental protocol which included preconditioning, constant strain rate, cyclic, and relaxation tests. The preconditioning tests, in most cases, indicated a lack of stability of the preconditioning due to a long term relaxation phenomena. Constant strain rate data were used to measure the value of the tangent modulus which correlated well with the percent of elastin found from histological observations. The maximum stress, tangent modulus, load input energy, and hysteresis area all decreased with a decrease in strain rate. Cyclic and relaxation tests showed a short term viscoelastic response of comparable nature being linear with the natural logarithm of time.

ACKNOWLEDGEMENTS

The author wishes to express his deepest appreciation and gratitude to the following people for making this phase of his graduate work possible:

To Dr. Robert Wm. Little, his major advisor, for his constant encouragement and friendship, and especially, his valuable assistance in the preparation of this thesis;

To Mr. David L. Hyler for his friendship and technical assistance in this study;

To Mr. Robert Schaeffer for his technical assistance in the smooth operation of the testing equipment used in this study;

To Ms. Jane Walsh for her friendship, and especially her technical assistance in the histological preparations used in this study;

To the United States Air Force for the financial support required for this study under contract number F33615-79-C-0514.

To his family, especially his parents, Joseph and Libia, for their understanding, encouragement, endless patience, and interest in his graduate work and academic education.

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INTRODUCTION

Tendons and ligaments play a major role in the body's support system, and have stimulated much interest and many investigations to determine their mechanical and histological properties. Tendons consist of fibrous cords of connective tissue that join muscle fibers to bones. Ligaments are also fibrous connective tissues that provide bone-to-bone junctions giving the necessary support and strength to the joints. The basic study of the connective tissues, tendons and ligaments, is fundamental to understanding the mechanical properties of collagenous tissues. A brief survey of earlier work will be presented as the various methods of testing, specimen choice and data analysis used by different researchers have led to results which are hard to compare.

One of the most comprehensive surveys of investigations on the structure and function of mammalian tendon was conducted by D.H. Elliott in 1965 [1]. He stated that besides primarily transmitting tensions, tendons had important secondary functions. These were "the removal of the bulk of a muscle from the joint over which it acts, the concentration of muscular pull on to a small area of bone, the modification of muscle action over several joints by retaining bands and the possible protection of the muscle by the buffer action of its tendon during an unexpected stress". In 1975, Evans and Barbenel [2] defined tendons as serving mainly as force transmitters, but having a second mechanical function in their ability to store energy elastically.

Elliott [1] also included a histological description of the structure of tendons in his 1965 paper. He described tendon as consisting almost entirely of collagenous tissue whose structural unit is the collagen fiber, or primary tendon bundle, and constitutes approximately 80% of the dry weight of the tendon. Primary tendon bundles are organized into secondary tendon bundles, which are organized into fasciculi, or tertiary tendon bundles. In 1973, Minns et al [3] described tendons as consisting "largely of parallel wavy bundles or sheets of collagen fibers surrounded by the interfiber matrix and a sparse network of elastic fibers. The collagen fibers provide the great strength of the tissue while the elastic fibers seem to provide elastic recovery and draw the collagen fibers back to the wavy condition in the relaxed state". They also described the structure of ligaments which differs from that of tendons. While collagen is a major constituent in most tendons and ligaments, elastic fibers were reported to be more prevalent in ligaments. When in the relaxed state, the collagen fibers in the ligaments were much less aligned than in tendons. A more complete structural hierarchy of tendon was given by Kastelic et al [4] in 1978. To briefly summarize this hierarchy, they described tendon as consisting of five units of tropocollagen that made up micro-fibrils, which form together to make sub-fibrils that generate fibrils producing fascicles, and finally constituting a tendon.

Rigby et al [5], in 1959, determined that the geometry of the collagen fiber in wet rat-tail showed a macroscopic banded or crinkly pattern when in its natural position. Their further observations indicated that the apparent helical microstructure of the tendon was an illusion resulting from the angle and type of illumination, and found the

wave-form disappeared with less stretching than the amount required to eliminate the helix. The wet rat-tail tendon was also teased into many hundreds of wavy "subfibers" which showed no evidence of inter-twining. A very detailed study was also done in 1972 by Diamant et al [6], who analyzed the periodic bands along the rat-tail tendons by using polarizing optics. They discovered that the periodic pattern along the tendon had a two-dimensional planar and sinusoidally shaped arrangement. Evans and Barbenel [2], in 1975, studied the way fibers were grouped into bundles or fasciculi and recognized that the dimensions and arrangements of these bundles vary considerably from tendon to tendon.

Much has been written about the viscoelastic properties of biological tissues. As long as the strain did not exceed approximately 4% for strain rates between 1 - 20%/minute, Rigby et al [5] found that the rat-tail tendon's mechanical behavior was reproducible, or reversible, when the tendon was allowed to rest a few minutes after each elongation. Partington and Wood [7] in 1963, showed that the stress-strain properties of rat-tail tendon fibers were reversible up to 2% strain. If fibers were stretched more than 3%, the mechanical behavior was irreversible, and the fibers did not return to their original length when released.

In the reversible region, tendons and ligaments exhibited a non-linear behavior. A typical stress-strain curve is shown in Figure 1, and can be divided into three ranges. In Region I, the response was linear due to the elastin fibers resisting extension, while the wavy collagen fibers were straightened or aligned, but do not carry the load. Therefore, the waviness of collagen determined the extent of the Region I response. The secondary range, Region II, showed gradually increasing

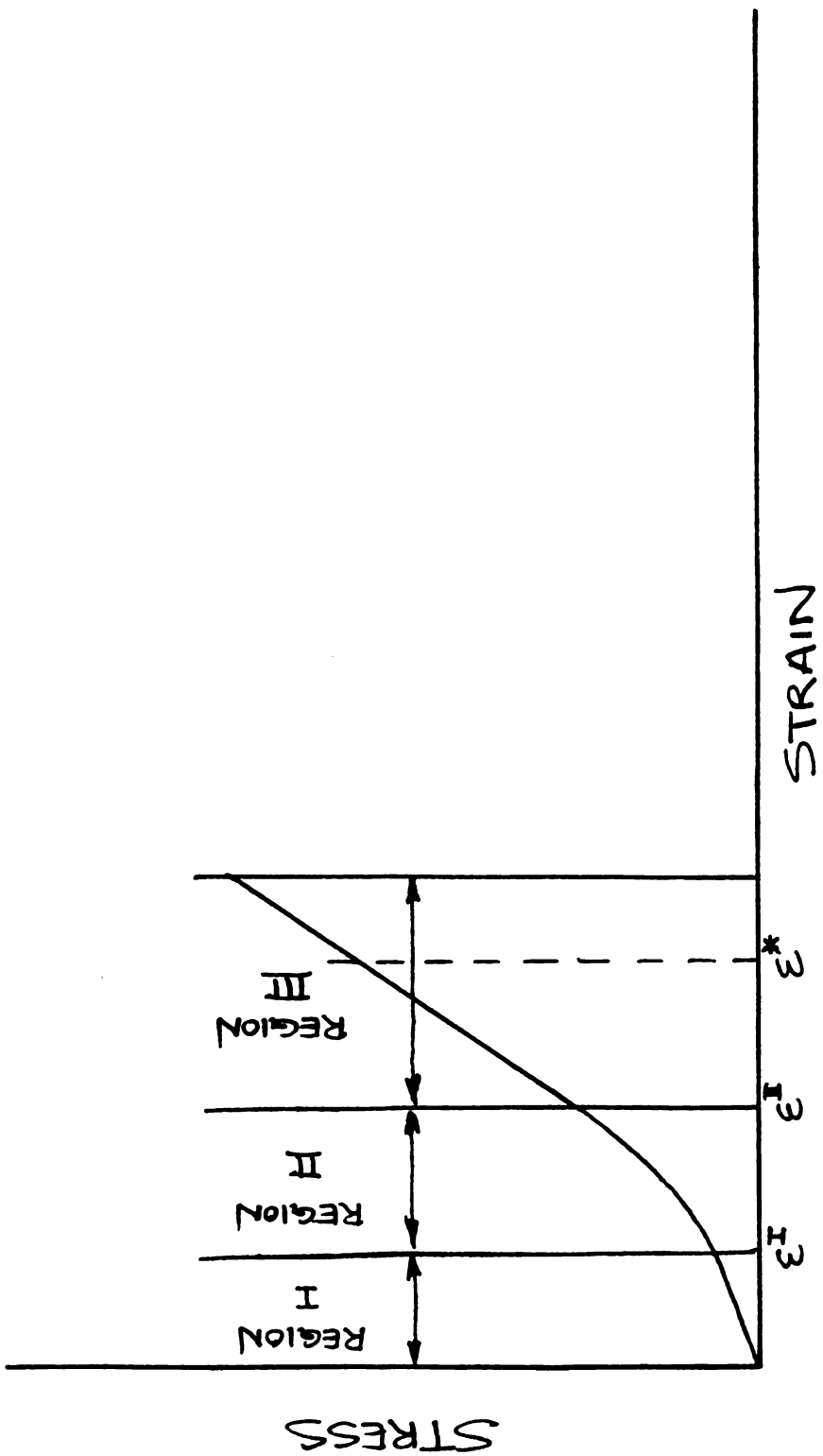

Figure 1. Typical stress-strain curve for connective tissue.

slope due to the stiffening of the tissue by the loading or the variation in length of the collagen fibers. The slope of Region II was determined by the distribution of the original waviness for individual collagen fibers. In the works of Rigby et al [5] in 1959, Millington et al [8] in 1971, and Diamant et al [6] in 1972, it was concluded that Region III exhibited a high, constant slope as all straightened collagen fibers resisted further extension. The tangent modulus or slope was determined by the quantity and structure of collagen in the tissue, and represented a measure of the stiffness.

In 1959, Rigby et al [5] computed an average maximum slope from the stress-strain curves for wet rat-tail tendon, to be $8.0 \pm 2.0 \times 10^9$ dynes/cm^2 (800 ± 200 MPa) at a strain rate of 10%/min. They also noticed that strains up to 20% could be reached without collagen fiber bundles breaking if the strain rate was sufficiently slow, less than 1%/min. When strain rate was increased, the stress-strain curves were identical, but shifted toward the stress axis. Elliott [1] in 1965, measured the mechanical tensile strength of certain tendons from different mammalian species. For example, the human tendo-calcaneus tendon had a tensile strength of 4.7 kg/mm^2 (0.48 MPa). Minns et al [3], in 1973, observed complete elastic recovery in human Achilles tendon specimens that were loaded up to 2% strain, and gave values for the failure stress for certain tendons and ligaments. The tensile strength of human Achilles tendon was given as 4950 - 8000 lbf/in^2 (1.73 - 2.79 MPa), while for the ligamentum nuchae from an adult cow was 200 - 2500 lbf/in^2 (0.07 - 0.87 MPa). Viidik [9] in 1980, reported a higher ultimate strength of tendons as 50 - 100 N/mm^2 (50 - 100 MPa) with an ultimate strain in the range of 15 - 30%.

Biological materials exhibit viscoelastic behavior in any time-dependent test. This was revealed in rate dependency for stress-strain tests at different constant strain rates, and for hysteresis under cyclic loading, cyclic stress relaxation, and stress relaxation under constant strain. In 1972, Fung [10] noted that the hysteresis loops of canine artery decreased with succeeding cycles to a steady state after a number of cycles. Torp et al [11] in 1974, recorded decreases in hysteresis loop area and maximum stress at a constant maximum strain of 2% for rat-tail tendon. They also showed that the decay in maximum stress versus the log of the number of cycles is almost a linearly decreasing function. This decay rate was similar to stress relaxation at constant strain. However, the strain level was constant for the stress relaxation, while in the cyclic tests, the deformation was cyclically applied to a constant strain level.

The researchers referenced used different methods of testing and data analysis, but all found that tendons and ligaments exhibited viscoelastic properties that could be defined from stress-strain curves, hysteresis, cyclic, and relaxation data. With these parameters a mathematical model could be developed to describe this tissue.
This thesis will present the mechanical properties and histological characteristics of the medial collateral and patellar ligaments of the knee and the flexor hallucis longus and tendo-calcaneus tendons of the ankle of the rhesus monkey, baboon, and chimpanzee, to determine parameters that could be used in the development of a mathematical model. These viscoelastic properties will be defined using data from different stress-strain rates, hysteresis, cyclic, and relaxation tests.

MATERIALS and METHODS

Special techniques in sample preparation, gripping, and testing protocol were adopted from a previous study [12]. These aspects will be discussed in detail, including a concise description of the testing equipment.

Sample Preparation

Tendo-calcaneus and flexor hallucis longus tendons and patellar and medial collateral ligaments were dissected from rhesus monkey, baboon and chimpanzee carcasses which were supplied by the Air Force Aerospace Medical Research Laboratories. These animals were medically healthy and not previously used in experimentation. After death, the primates were chilled in dry ice immediately, and transported to the testing laboratory. The lower limbs were removed from the hip socket and stored at $-5^{o}C$ until further dissections could be performed. While frozen, the lower limbs were sectioned into knee and foot regions and stored separately in identifiable plastic bags. Prior to dissection and testing, the tissue was allowed to thaw at room temperature. Dissection was performed in a humid environment using a humidifier blowing air over the tissue.

Dissection of the Knee Region:

Skin and extraneous tissues were removed to expose the ligaments of interest. The patellar ligament was removed first due to its accessibil-

ity. The test specimen ran from the apex of the patella bone to the anterior tibia below the condyle. A surgical chisel was used with moderate blows from a rubber mallet against the condyle of the anterior tibia to insure a bone-ligament-bone specimen. Next, the medial collateral ligament was dissected from the medial epicondyle of the femur to the medial tibial shaft, again yielding a bone-ligament-bone subject with use of a chisel and mallet. The bony attachments of both ligaments were sanded smooth on a sander to facilitate mechanical gripping.

Dissection of the Foot Region:

Skin and superficial tissue of the plantar aspect of the foot along with the posterior side of the leg was removed to expose the inner structures. First, the tendo-calcaneus tendon was dissected after being freed of fascia from the gastrocnemius muscle to the calcaneum. The tendon was severed below the muscle and separated from underlying tissue all the way to the calcaneum where the bony attachment was preserved. Next, the flexor hallucis longus muscle and tendon were exposed by removing the soleus muscle, and extraneous muscles and tendons. The part of the tendon used for testing was severed before the osseo-fibrous tunnel and at the midpoint of the plantar exposure. After the dissections were completed, all tissues were wrapped in moist paper towels and stored at $4^{o}C$ for not more than 24 hours, at which time mechanical testing was performed.

After all mechanical testing was completed, the tissues were fixed in 10% buffered formalin for several days. A post-fixation in Bouin's

fluid for 24 hours followed. Samples were then sectioned in transverse and longitudinal planes and processed according to standard paraffin embedding methods. The tissue blocks were cut at 7μ thickness on a rotary microtome and stained. The stains routinely used were Hematoxylin-Eosin for general morphology [13], Davenport's modification of Halmi's for collagen and elastin [14], and Frankel's Orcein for elastic fibers [15]. Histological samples were taken from ligaments and tendons of both the right and left limbs of the three primates. Cross-sectional areas were measured by placing metric graph paper underneath the slides and "counting squares" using a microscope. The areas of different ligaments and tendons are given in Tables 1 to 4. Details of area measurements are described under "Geometric Properties" (See page 21).

Histological Observations

Tendo-calcaneus Tendon:

The tendo-calcaneus is a large broad tendon located on the posterior aspect of the lower limb. It attaches the gastrocnemius muscle, which composes a large portion of the back of the leg, to the calcaneum (heel bone). Where the gastrocnemius fibers unite with the tendo-calcaneus, the soleus muscle tendon integrates into the main body of the tendo-calcaneus, producing a "two-tendon" structure in the middle and proximal areas. In the distal area, there is a twisting and fanning out of the fibers providing a broad area of insertion as the tendon attaches to the calcaneum.

Table 1. Cross-sectional Areas of the Flexor Hallucis Longus Tendon from Histological Slides.

		Left Foot			Right Foot		
Species	Region†	Representative†† Cross-Sectional Area, mm^2	Total$^{\alpha}$ Range, mm^2	Total Cross-$^{\alpha\alpha}$ Sectional Area Average, mm^2	Representative†† Cross-Sectional Area, mm^2	Total$^{\alpha}$ Range, mm^2	Total Cross-$^{\alpha\alpha}$ Sectional Area Average, mm^2
	U				9.75	9.5-10.0	
Rhesus	M	6.10	4.0- 8.0		6.81	4.0- 9.0	
	L				7.00	6.0- 8.0	
				6.10			7.12
	U	13.33	11.0-15.0		11.50	10.0-12.5	
Baboon	M	5.90	4.0- 8.0		7.50	7.0- 8.0	
	L	8.00	8.0		8.29	5.0-12.0	
				9.27			8.52
	U	27.97	27.0-29.0		13.20	9.0-12.0	
Chimpanzee*	M				14.33	13.0-16.0	
	L	25.20	25.0-26.0		16.00	11.0-20.0	
				26.55			14.27

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near fibrous distal sheath
M = middle area between upper and lower region
L = lower area near osseo-fibrous tunnel.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
$\alpha\alpha$Total cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

Table 2. Cross-sectional Areas of the Tendo-calcaneus Tendon from Histological Slides.

		Left Foot			Right Foot		
Species	Region[†]	Representative[††] Cross-Sectional Area, mm^2	Total[α] Range, mm^2	Total Cross-[αα] Sectional Area Average, mm^2	Representative[††] Cross-sectional Area, mm^2	Total[α] Range, mm^2	Total Cross-[αα] Sectional Area Average, mm^2
	U				11.50	11.0-12.0	
Rhesus	M	7.50	6.5-10.0		7.54	5.0-15.0	
	L				10.67	9.5-12.0	
				7.50			9.33
	U	19.83	19.0-21.0		16.61	14.0-22.5	
Baboon	M	18.35	16.0-21.5		14.00	13.0-15.0	
	L	23.80	22.0-28.0		17.60	12.0-27.0	
				20.07			16.71
	U				32.67	32.0-34.0	
Chimpanzee*	M	29.00	26.0-32.0		40.33	39.0-42.0	
	L	59.33	55.0-63.0		62.50	56.0-69.0	
				44.17			43.00

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near gastrocnemius muscle
M = middle area between upper and lower region
L = lower area near calcaneum.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

Table 3. Cross-sectional Areas of the Medial Collateral Ligament from Histological Slides

		Left Foot			Right Foot		
Species	Region†	Representative†† Cross-Sectional Area, mm²	Total^α Range, mm²	Total Cross-^αα Sectional Area Average, mm²	Representative†† Cross-Sectional Area, mm²	Total^α Range, mm²	Total Cross-^αα Sectional Area Average, mm²
	U	5.75	4.0- 8.0		8.29	6.5-10.0	
Rhesus*	M	4.88	3.0- 5.0		4.75	3.0- 5.5	
	L	5.94	5.0-7.0		6.92	5.0- 8.0	
				5.52			6.74
	U	12.00	11.0-12.5		12.00	10.0-14.0	
Baboon*	M	9.83	8.0-12.0		4.30	3.0- 5.0	
	L	10.33	8.5-11.5		5.90	4.5- 7.0	
				10.47			8.15
	U	17.33	14.0-20.0		26.33	24.0-28.0	
Chimpanzee*	M	17.17	16.0-18.0		12.67	12.0-14.0	
	L	18.00	17.0-19.0		15.00	14.0-16.0	
				17.47			18.00

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near epicondyle of femur
M = middle area between upper and lower region
L = lower area near medial tibial shaft.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

Table 4. Cross-sectional Areas of the Patellar Ligament from Histological Slides.

		Left Foot			Right Foot		
Species	Region†	Representative†† Cross-Sectional Area, mm²	Total[α] Range, mm²	Total Cross-[αα] Sectional Area Average, mm²	Representative†† Cross-Sectional Area, mm²	Total[α] Range, mm²	Total Cross-[αα] Sectional Area Average, mm²
	U	35.20	32- 39		38.33	34- 42	
Rhesus*	M	33.60	32- 35		34.50	32- 43	
	L	33.67	31- 37		34.67	23- 42	
				34.13			36.75
	U	43.25	33- 47		41.00	37- 45	
Baboon*	M	44.67	39- 59		41.00	40- 61	
	L	47.67	39- 53		58.00	48- 66	
				45.62			50.58
	U	110.00	100-116		81.00	80- 82	
Chimpanzee*	M	77.50	77- 78		80.00	80	
	L	109.33	102-115		100.00	83-110	
				101.63			87.75

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near apex of patella
M = middle area between upper and lower region
L = lower area near condyles of the tibial shaft.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

Microscopically, the tendo-calcaneus is composed predominantly of collagen with varying amounts of loose connective tissue and vasculation. Two distinct segments are usually seen in the middle and proximal regions, but are not evident in the most distal sections. Loose connective tissue and small blood vessels are seen between these segments. The collagen fiber bundles are parallel and slightly to moderately wavy in nature and elastic fibers are evident in all areas. Elastin content from histological slides ranged from approximately 5 - 10% for the chimpanzee, 20% for the rhesus monkey, and up to a value of 30% for the baboon. In areas of collagen-muscle attachment, the muscle fibers joined the collagen fibers at angles up to 30^{o}. The tendon capsule is composed of loose connective tissue with muscle fibers often seen on the anterior side. This connective tissue varies from a compact layer at the posterior surface to a broader area of loose connective tissue on the anterior surface.

Flexor Hallucis Longus Tendon:

The flexor hallucis longus tendon has an elliptical cross-section and extends distally from the flexor hallucis longus muscle in the posterior of the lower leg, through the osseo-fibrous tunnel along the plantar aspect of the foot, through the fibrous distal sheath and attaches to the bottom of the distal phalange of the first toe. At the microscopic level, it is an ovoid structure made up of compact collagen fiber bundles with small amounts of loose connective tissue between the fascicles. Areas of fasculation are also found among the groups of bundles, while muscle can be seen along the

capsule surface on the anterior aspects. Loose connective tissue and blood vessels are found on the anterior surface, while the capsule on the posterior surface is very compact, showing very little loose connective tissue or vasculation. The parallel fiber bundles are slightly to moderately wavy, and elastic fibers are evident at all levels as well as in the surrounding loose connective tissue. Elastin content ranged from approximately 5 - 10% for the rhesus monkey, and approximately 5% for the baboon, to a value of less than 1% for the chimpanzee.

Patellar Ligament:

The patellar ligament is a broad, thick ligament situated on the anterior surface of the knee. It attaches from the tibia below the condyles to the non-articulating area of the apex of the patella bone. The ligament is composed of compact collagen bundles with a broad area of loose connective tissue surrounding the joint capsule. Extensions of the capsular connective tissue invade the structure dividing the fascicles into segments. This is particularly noted at the proximal and distal ends with less marked segmentation in the middle region. The fiber bundles are broad, parallel, and slightly wavy. Large areas of loose connective tissue and vasculation are seen between groups of bundles. Elastic fibers are also seen in all areas sampled, with a high content of approximately 25 - 30% for the baboon and approximately 10 - 15% for the chimpanzee. No value for elastin content was estimated for the rhesus monkey, since the samples used for testing were so small that once histological preparations were made for determining

ligament cross-section, insufficient material was available for longitudinal sections. It is worthy to note that in the middle region on the posterior side of a longitudinal section of the patella, there exists a narrow band of parallel fibers that runs perpendicular to the fiber bundles of the main ligament. These fibers are moderately wavy and are separated from the rest of the ligament by a zone of loose connective tissue and blood vessels.

Medial Collateral Ligament:

The medial collateral ligament (also known as the tibial collateral) is a long, thin, flattened ligament. It attaches at the top of the medial epicondyle of the femur, transverses the medial aspect of the knee, and attaches to the medial tibial shaft.

Microscopically, it has an elongated, elliptic cross-sectional structure composed of round fascicles with small amounts of loose connective tissue between them. A thick capsule surrounds the ligamentous structure. Longitudinally, the fiber bundles are compact, parallel, relatively acellular and moderately wavy. Loose connective tissue with some vasculation is observed in the capsular composition. Elastic fibers are found approximately 25 - 30% for all species within the fiber bundles, and in greater amounts in the ligament capsule.

Gripping

The concept that ligaments constrain the motion of the knee was the basis of the grip design. The bone attachments for the ligaments

were held by the grips, and the axial displacements were imposed by the testing machine. By tightening the screws to secure the bony attachments within the grips, slippage of the tissue was prevented or kept at a minimum. The function of tendons is to transmit forces from muscle to bone. To eliminate slippage for all ligaments and tendons, especially at the muscle end of the tendon, a waterproof abrasive mesh (silicon carbide 120 grit "sand screen" by 3M) was epoxied to the surface of the grips. This increased the effectiveness of the grips and at the same time helped in preventing any damage in cutting or tearing of the tissue during testing. The grips were designed to be much stiffer than the samples tested so that the motion of the testing machine would be equal to the sample displacement. The same grips were used for all specimens and are shown schematically in Figure 2. The upper gripping plate was attached to the actuator of the testing machine and the lower plate to the load cell. The sample was held against these plates by stainless steel bars.

Mechanical Testing Equipment

Mechanical testing of the tissue specimens was conducted with an Instron (Model 1331) machine that is hydraulically powered and electronically controlled to produce uniaxial extensions at rates up to 1 m/s. Sample extension (grip motion) and load were recorded with a digital storage oscilloscope (Nicolet Model 201) and stored for subsequent analysis on flexible, magnetic diskettes (Verbatim mini-disks$_{TM}$). The data was finally transferred to a micro-computer (Digital pdp 11/03) for analysis.

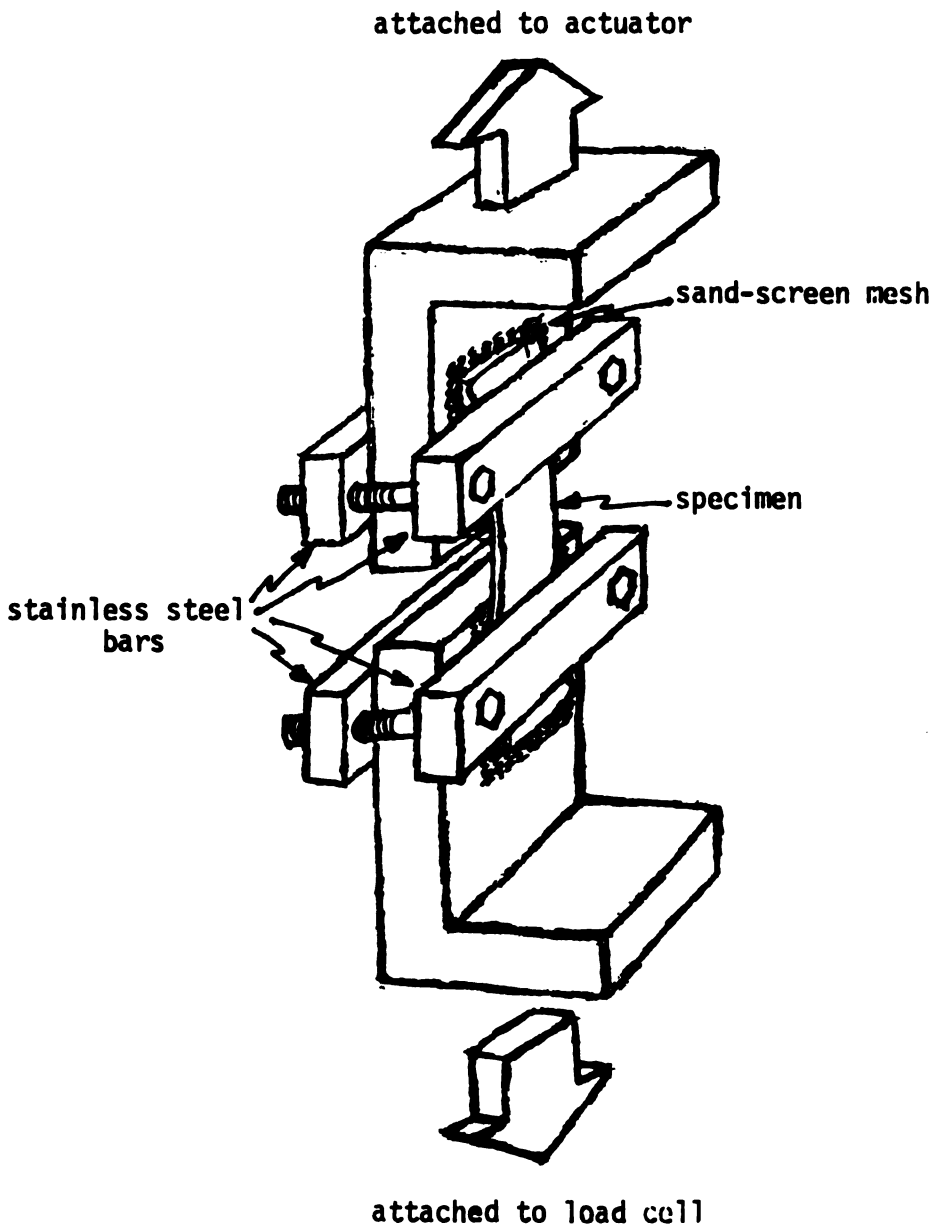

Figure 2 Schematic Drawing of Testing Grips

All ligaments and tendons were tested at room temperature ($27^{o}C$) in a chamber which is supplied with water-saturated air. An additional physiological saline (0.9%) drip was used over the samples to sustain tissue moisture. A stereo-microscope (WILD M5A) and camera (WILD MPS 51) were attached to the chamber base and used to observe samples during testing. The chamber and microscope could be rotated to view the specimen at different angles.

Mechanical Testing Protocol

Preliminary testing was used for grip design refinement and the confirmation of the general viscoelastic nature of the tissues. Current literature does not establish any definitive physiological limit on load and deflection of these tissues. Each tissue was ramped slowly to the point where the load deflection response appeared linear. This maximum extension at which the specimen was ramped established the maximum strain, E*. Initial testing was used to establish the following protocol for examination of the response to successive extensions, the relaxation of load, and the load response to haversine extensions at various frequencies.

A. Preconditioning

1) Thaw test sample, wet with normal saline.
2) Mount sample and tighten grips.
3) Ramp slowly to establish a strain level E* well into linear region III, which will be the maximum non-destructive strain.
4) Hold at E* for 2 minutes and tighten grips.
5) Unload and wait 10 minutes.
6) Ten constant rate cycles of 1% per second to E*.
7) Wait 5 minues.
8) Determine initial unloaded length ℓ.
9) Three tests to E* at 1% per second with 5 minute wait after each test.

B. Constant Strain Rate Loading and Unloading

1) One test at 100% per second to E* followed by 5 minute wait.
2) One test at 1% per second to E* followed by 5 minute wait.
3) One test at 0.01% per second to E* followed by 5 minute wait.
4) Two tests to E* at 1% per second with 5 minute wait after each to check preconditioning stability.

C. Cyclic Tests

Examination of the data from the last test in B.4 will establish the strain, E^{II}, at the transition from the non-linear toe region, region II, to the linear region III.

1) Cycle strain from $0.4E^{II}$ to E^{II} at 10 Hertz for 40 seconds followed by a 5 minute wait.
2) Using the same minimum and maximum strains as test C.1, cycle 40 seconds at 1 Hertz followed by a 5 minute wait.
3) Using same minimum and maximum strains as test C.1, cycle 40 seconds at 0.1 Hertz followed by a 5 minute wait.
4) Check preconditioning stability by test B.4.
5) Using a strain equal to $E^{II} + 0.2 (E^* - E^{II})$ as a minimum and E* as the maximum level, cycle 40 seconds at 10 Hertz followed by a 5 minute wait.
6) Using the minimum and maximum strains from C.5, cycle 40 seconds at 1 Hertz followed by a 5 minute wait.
7) Using the minimum and maximum strains from C.5, cycle 40 seconds at 0.1 Hertz followed by a 5 minute wait.
8) Check preconditioning stability by test B.4.

D. Relaxation

From the second test in C.8, determine new E^{II} transition strain or confirm E^{II} from test series C.

1) Ramp at 100% per second to $0.7E^{II}$ and hold until relaxation approaches zero (approximately 10 minutes).
2) Return to zero strain and wait an equal time as relaxation time in D.1.
3) Ramp at 100% per second to E* and hold until relaxation approaches zero (approximately 25 minutes).
4) Return to zero strain and wait an equal time as relaxation time in D.3.
5) Check preconditioning stability by test B.4.

Geometric Properties:

The initial length of a tendon or ligament was defined as the distance between "grip-to-grip" and was measured with the unloaded tissue in place in the testing machine. Crude cross-sectional areas were taken at this time, width times thickness, but the areas used in data analysis were obtained by measurements from histological slides made after testing. The cross-sectional areas calculated did not include the surrounding connective tissue having a large elastin content in the peripheral area. Any discrepancy of the cross-sectional areas of the tendons and ligaments recorded between right and left leg can be explained by the fact that the primates tested were of different sex and body weight. Thus, the chimpanzee's left and right patellar ligament and flexor hallucis longus tendon were taken from two physically different specimens, one weighing nearly three times more than the other.

RESULTS and DISCUSSION

Constant strain rate loading and unloading data were collected in digital form with the Nicolet digital oscilloscope, and then transferred to the pdp 11/03 computer for analysis. A three-degree polynomial least square technique was employed to smooth this constant strain rate data for noise elimination. These polynomial curves were used to calculate smooth stress-strain curves, hysteresis and energy areas, and the tangent moduli from data obtained in the experimental program.

The experimental protocol was constructed to collect data that would allow for the development of a mathematical model using a hereditary integral and/or structural analysis. This thesis is concerned with finding those parameters needed for the hereditary integral analysis. A qualitative discussion of these physical parameters is included, but no attempt was made to put them back into the hereditary model so as to discern whether or not this analysis could serve as a predictive model. Although this predictive model will not be discussed here, it is the subject of future Ph.D. work.

The concept of an initial material adjustment of biological tissues undergoing a series of mechanical tests was termed "preconditioning" by Fung [5]. The precise definition of preconditioning is not possible since it is not exactly known nor fully understood what has happened in the tissue during this initial period. Therefore a rigid,

experimental protocol (See page 19) was established to help stabilize and record any short or long time dependency effects of the tissue's viscoelastic nature.

In order to help stabilize the material response, the tissue was initially exposed to a preconditioning process that focused on the repeatability of tests at a certain level of strain equal to or greater than those used in the test program. It is not known whether or not the initial or preconditioning changes which occur are independent of the other observed viscoelastic effects. As suggested in the current literature, these preconditioning effects may be due to changes in the state of cross-linking, alteration in the state of hydration, or realignment of the fiber matrix. Constant strain rate loading and unloading, cyclic, and relaxation tests were performed sequentially to record the tissue's mechanical properties. Overall preconditioning stability was measured by conducting a constant strain rate test at 1% per second after each testing sequence (See Figures 3 to 6). As seen from these graphs, the maximum stress levels of subsequent check loops dropped during this test program to approximately 75 to 95% of the value attained on the initial cycle for the tendons (flexor hallucis longus and tendo-calcaneus), and approximately 70 to 95% for the ligaments (medial collateral and patellar). There was no preconditioning data recorded for the patellar ligament of the baboon and the tendo-calcaneus tendon of the chimpanzee due to the accidental erasure of data. The check loops throughout the protocol showed the lack of stability of the preconditioning response due to a long term relaxation phenomena during the testing sequence, and therefore comparisons of one test with another

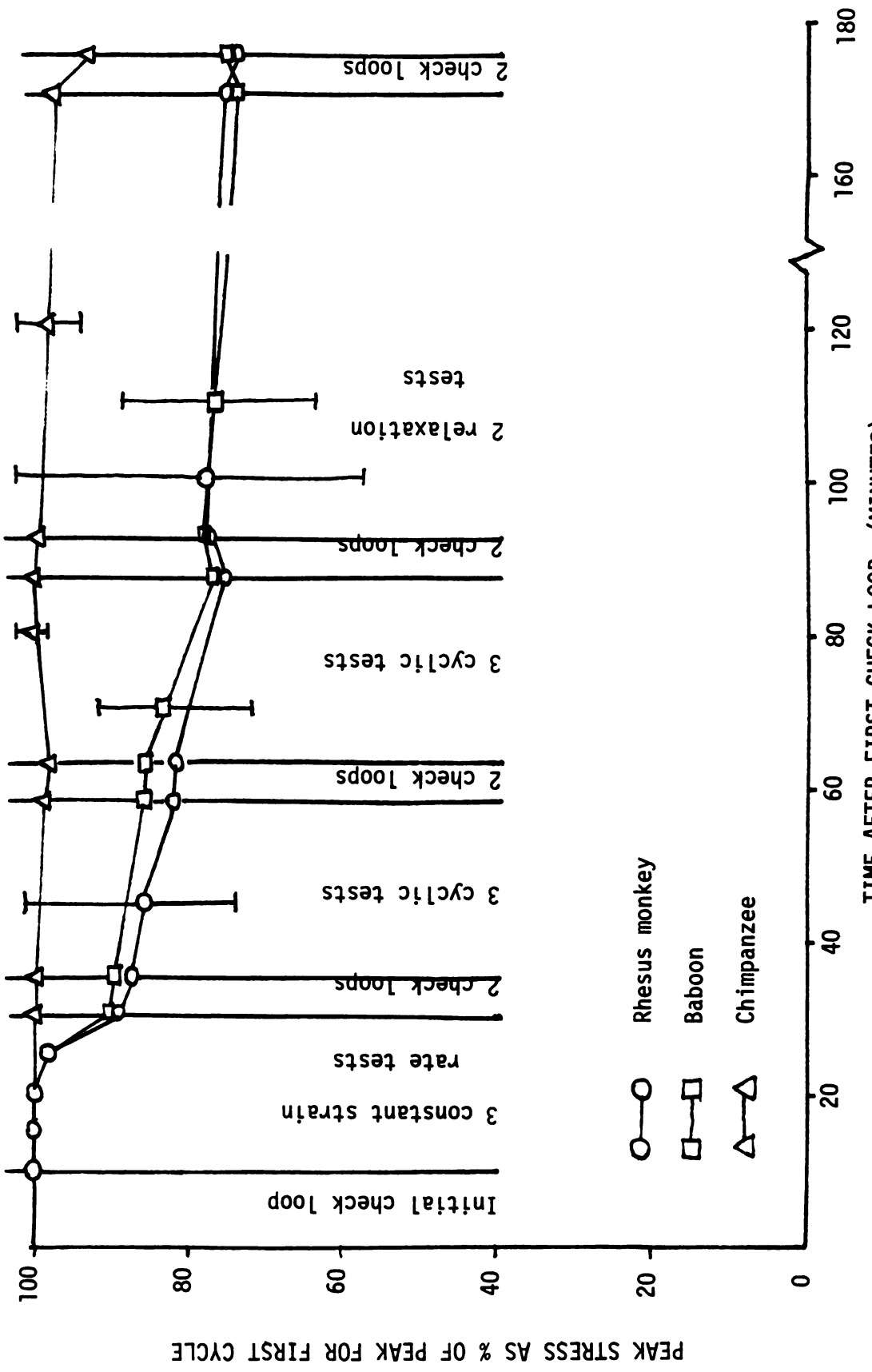

Figure 3. Preconditioning Stability of Flexor Hallucis Longus Tendon

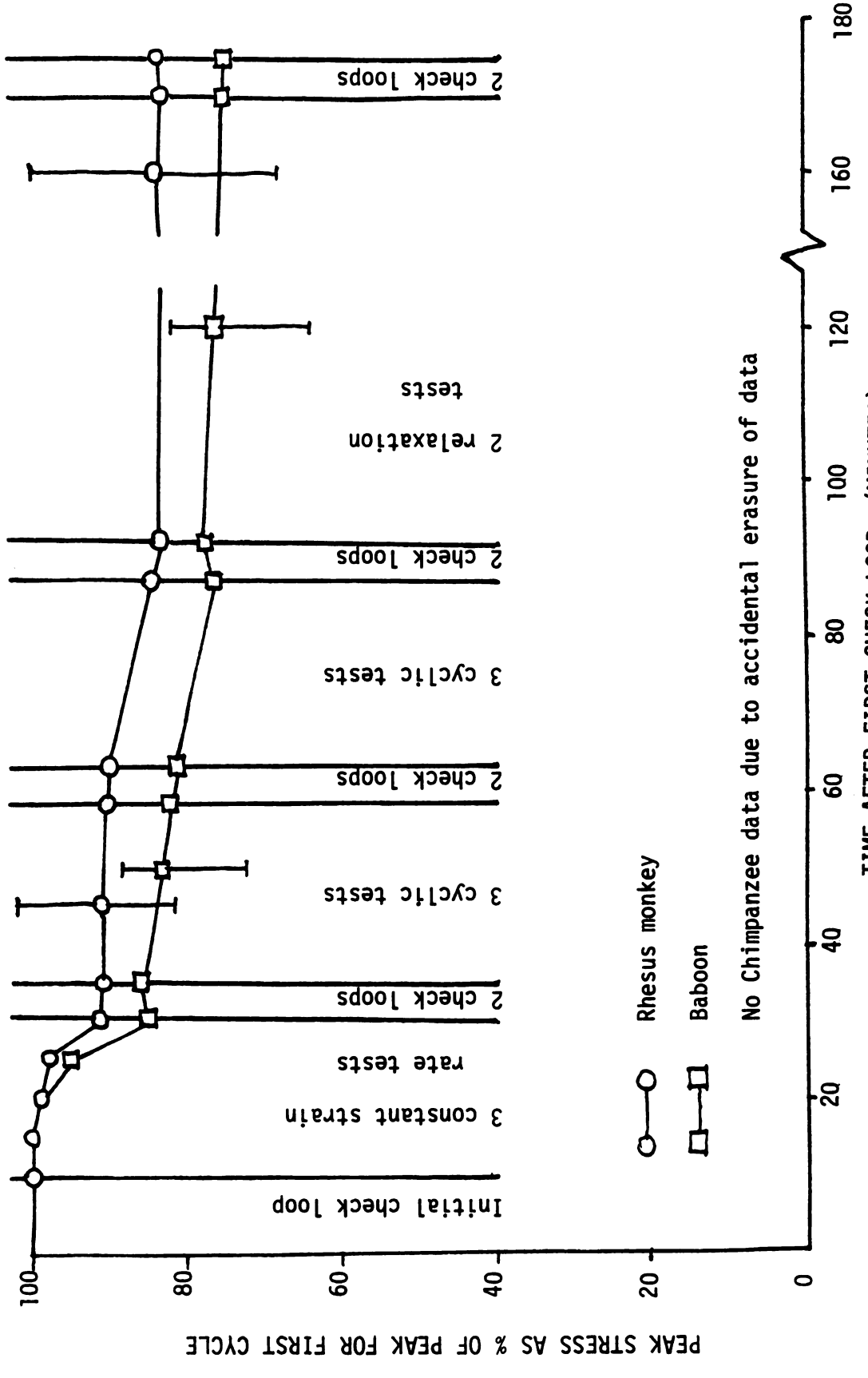

Figure 4. Preconditioning Stability of Tendo-calcaneus Tendon

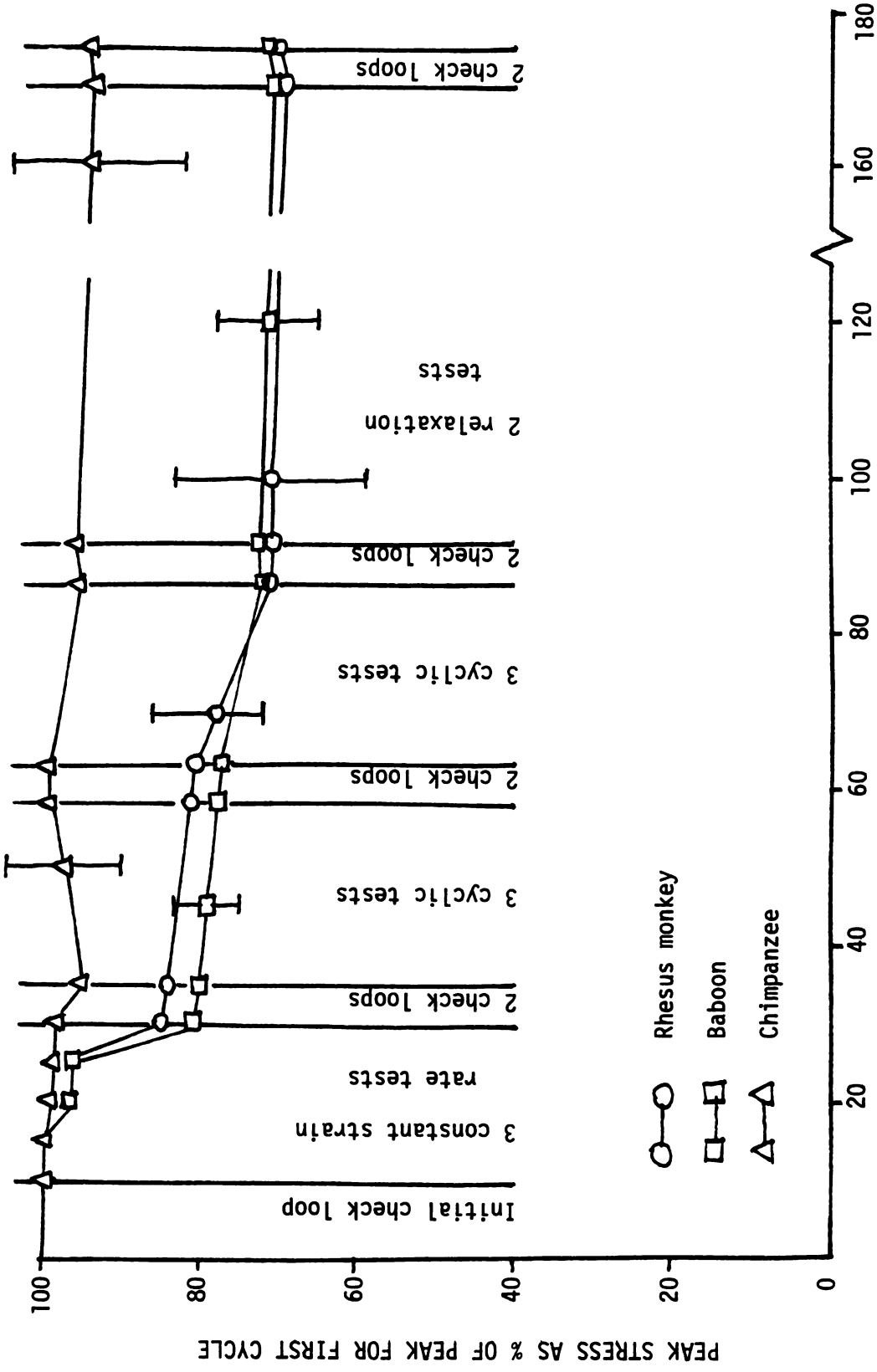

Figure 5. Preconditioning Stability of Medial Collateral Ligament

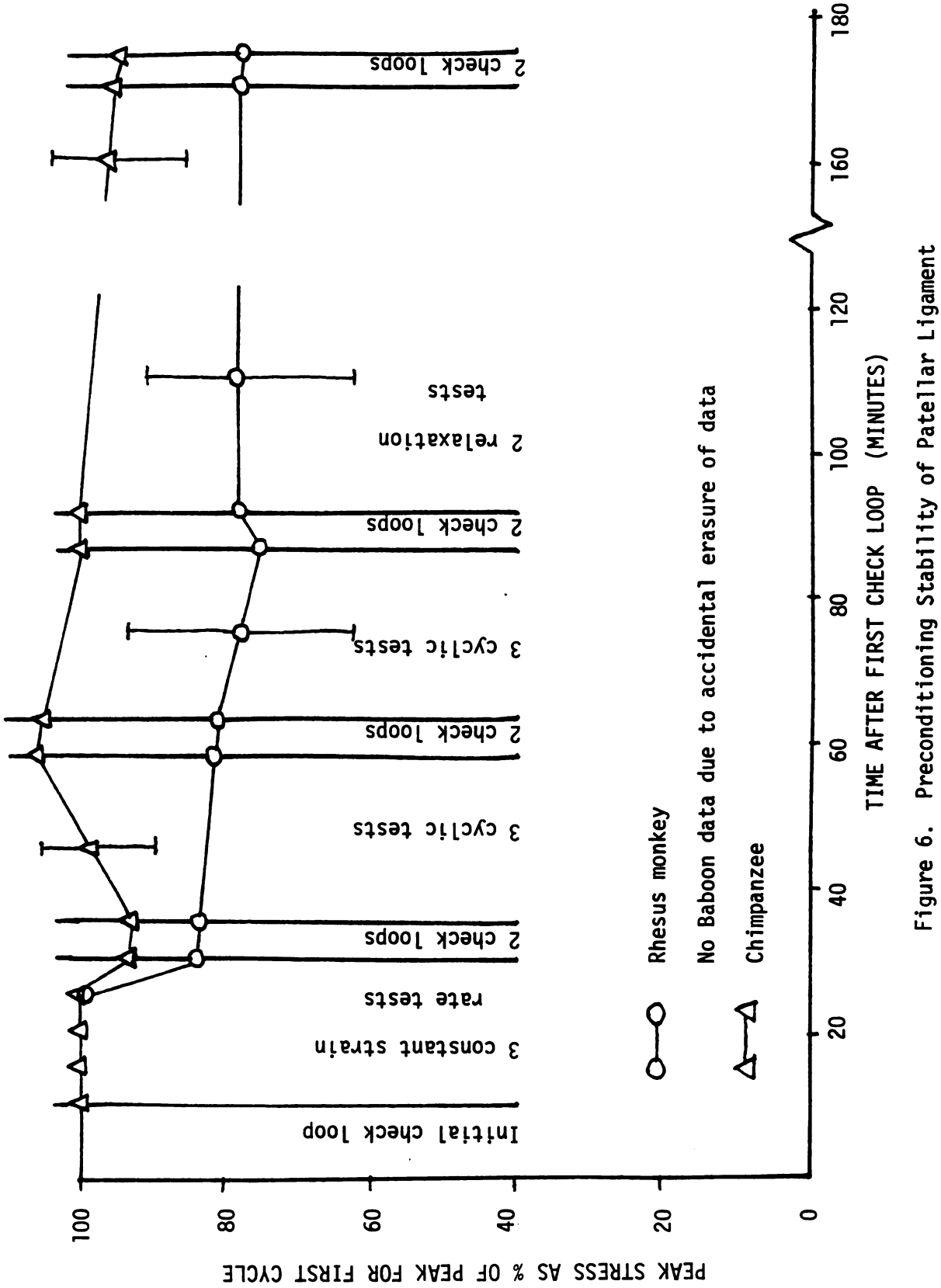

Figure 6. Preconditioning Stability of Patellar Ligament

in the testing sequence cannot be made, with exception of the chimpanzee, which showed a relatively stable response. The scatter of the data did not allow for significant variation between species.

Constant strain rate data were also used to determine the relative stiffness, or tangent modulus, of the tendons and ligaments for the three primates (See Table 5). The results recorded in Table 5 were all tabulated for a strain rate of 100% per second. Figures 7 to 10 illustrate for the specimens tested average constant strain rate curves, which were used to compute the tangent moduli, defined as the slope in the linear region of these curves. Comparisons were made between the primates for each tissue. The chimpanzee had the largest value of tangent moduli of 1060 MPa for the tendo-calcaneus tendon, while for the same tissue, the rhesus monkey had a value of 670 MPa and the baboon a value of 280 MPa. Except for the rhesus monkey's patellar ligament, which indicated a low value of 75 MPa, the tangent moduli values recorded in Table 5 were within the range of 300-1000 MPa as reported by Swanson [16] in 1971 for tendon. As seen from the histology and morphology for all four tissues (See pages 9 to 16), elastin was more prominent in the peripheral connective tissues than in the area with dense collagen fiber bundles. An estimated value of the amount of elastin was given for each primate tissue from the stained longitudinal sections used in histological studies. No precise measurement could be made due to the elastic fiber waviness exhibiting eccentric length and amplitude. The collagen fiber bundles which carry the load are oriented in the direction of the tensile load, while the function of the surrounding matrix was assumed by Torp et al [13] in 1974, to be that of

Table 5. A Summary of Averages for Specific Mechanical Properties at a Constant Strain Rate of 100%/Sec.

Ligament or Tendon	Species	Number of Samples	Maximum Strain, %		Average* Stress at 5% Strain, MPa	Range of** Max. Stress MPa	% Hysteresis Energy to Loading Energy		Tangent# Modulus MPa
			Average+	Range++			Average@	Range@@	
Flexor Hallucis Longus	Rhesus	9	8.39	4.76-17.87	13.12	65.50-212.11	18.68	5.57-29.49	600
	Baboon	4	8.63	4.82-14.38	17.50	3.46-100.25	20.81	5.86-37.46	950
	Chimp	2	8.46	8.12, 8.79	28.50	62.08,346.57	30.48	23.41,37.55	1050
Tendo-Calcaneus Tendon	Rhesus	8	7.96	4.12-11.63	10.50	3.44- 37.14	32.87	16.84-60.17	670
	Baboon	4	7.88	5.98-9.18	4.80	0.63- 24.05	24.90	8.00-53.23	280
	Chimp	2	6.80	5.52,8.08	17.30	13.48, 37.70	37.81	35.50,40.12	1060
Medial Collateral Ligament	Rhesus	2	9.15	8.38,9.92	6.80	17.78, 26.88	33.30	26.15,40.44	500
	Baboon	2	4.56	4.12,4.99	17.40	12.05, 14.01	43.32	42.42,44.22	640
	Chimp	2	5.80	5.56,6.04	15.80	13.78, 22.55	19.07	0.00,38.13	610
Patellar Ligament	Rhesus	2	9.64	9.40,9.87	1.00	2.87, 4.35	44.29	40.92,47.66	75
	Baboon	2	6.00	4.04,7.95	8.20	1.18, 9.71	47.26	44.94,49.58	360
	Chimp	2	4.28	3.62,4.93	19.20	16.71, 69.56	20.85	13.70,28.00	690

+ Total Average of All Maximum Test Strains for All Samples.
++ Range of Strain Values That Occurred at Different Test Strains for Each Specimen.
* Values Were Recorded at 5% Strain from the Stress-Strain Curves in Figures 7 to 10.
** Range of Stress Values That Occurred at Different Peak Strains for Each Specimen.
@ Total Average of All % Hysteresis Energy to the Loading Energy for All Samples.
@@ Range of the % Hysteresis Energy to the Loading Energy for All Samples.
# Values Were Taken as the Slopes of the Average Constant Stress-Strain Curves in Figures 7 to 10.

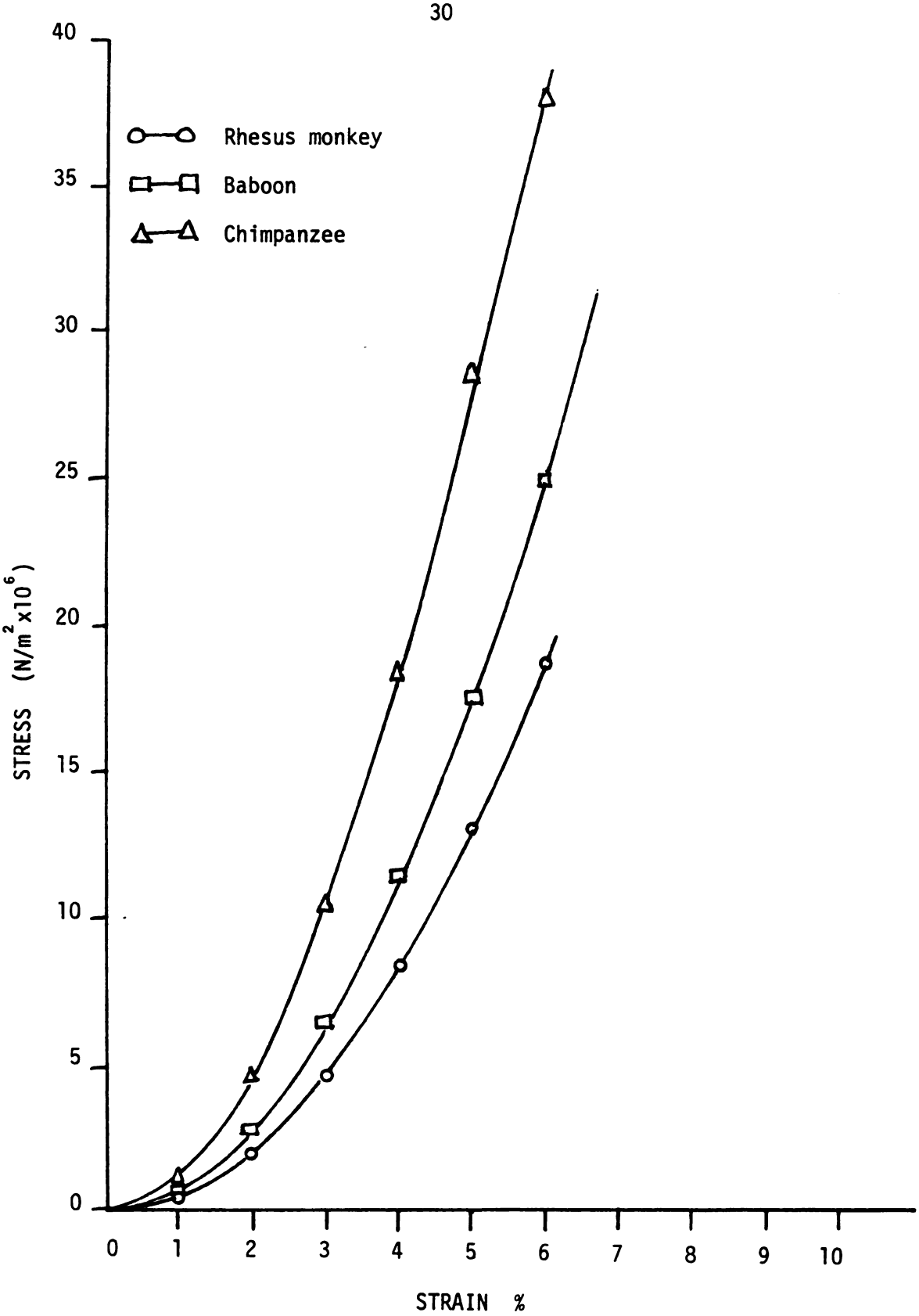

Figure 7. Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Flexor Hallucis Longus Tendon

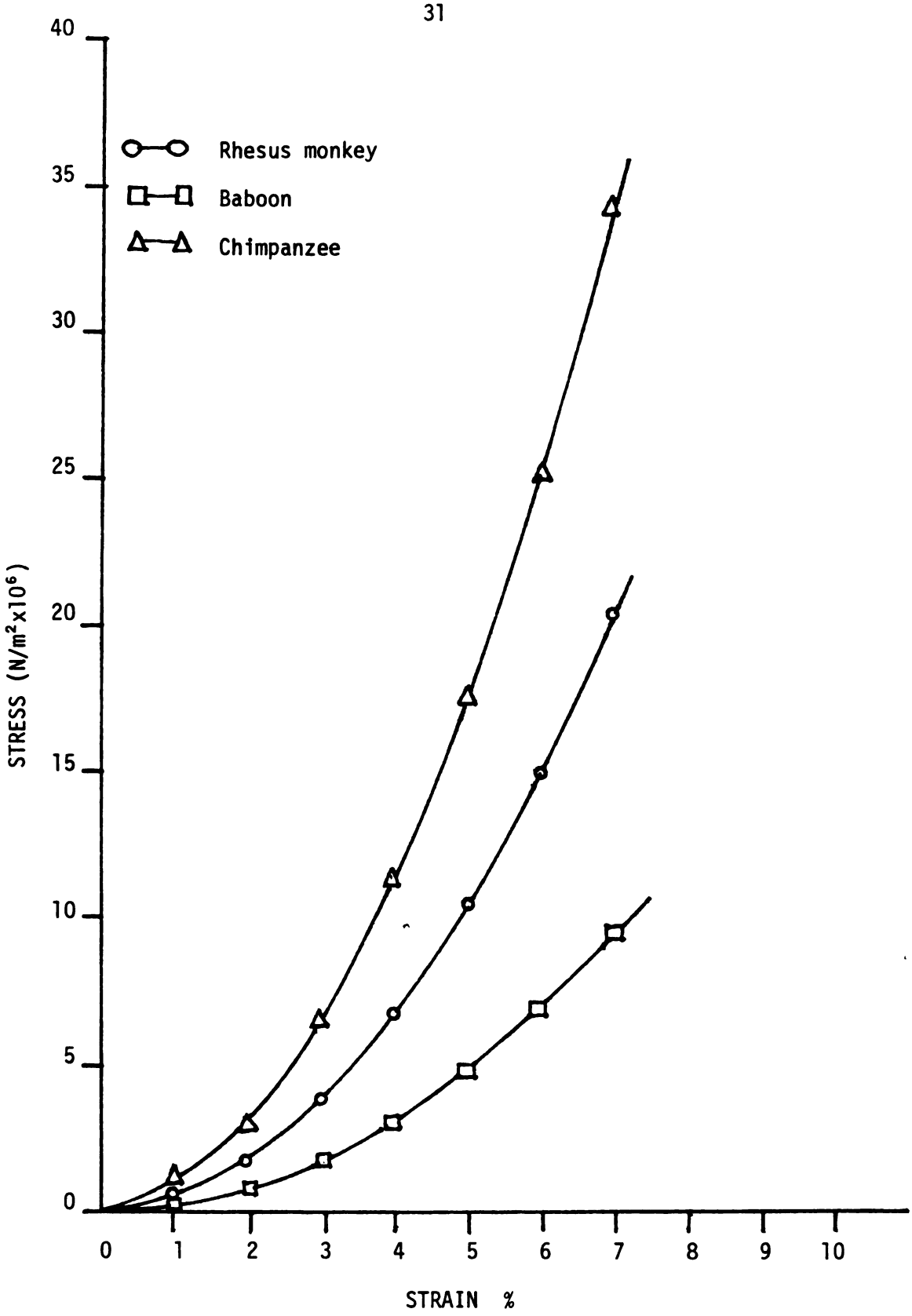

Figure 8. Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Tendo-calcaneus Tendon

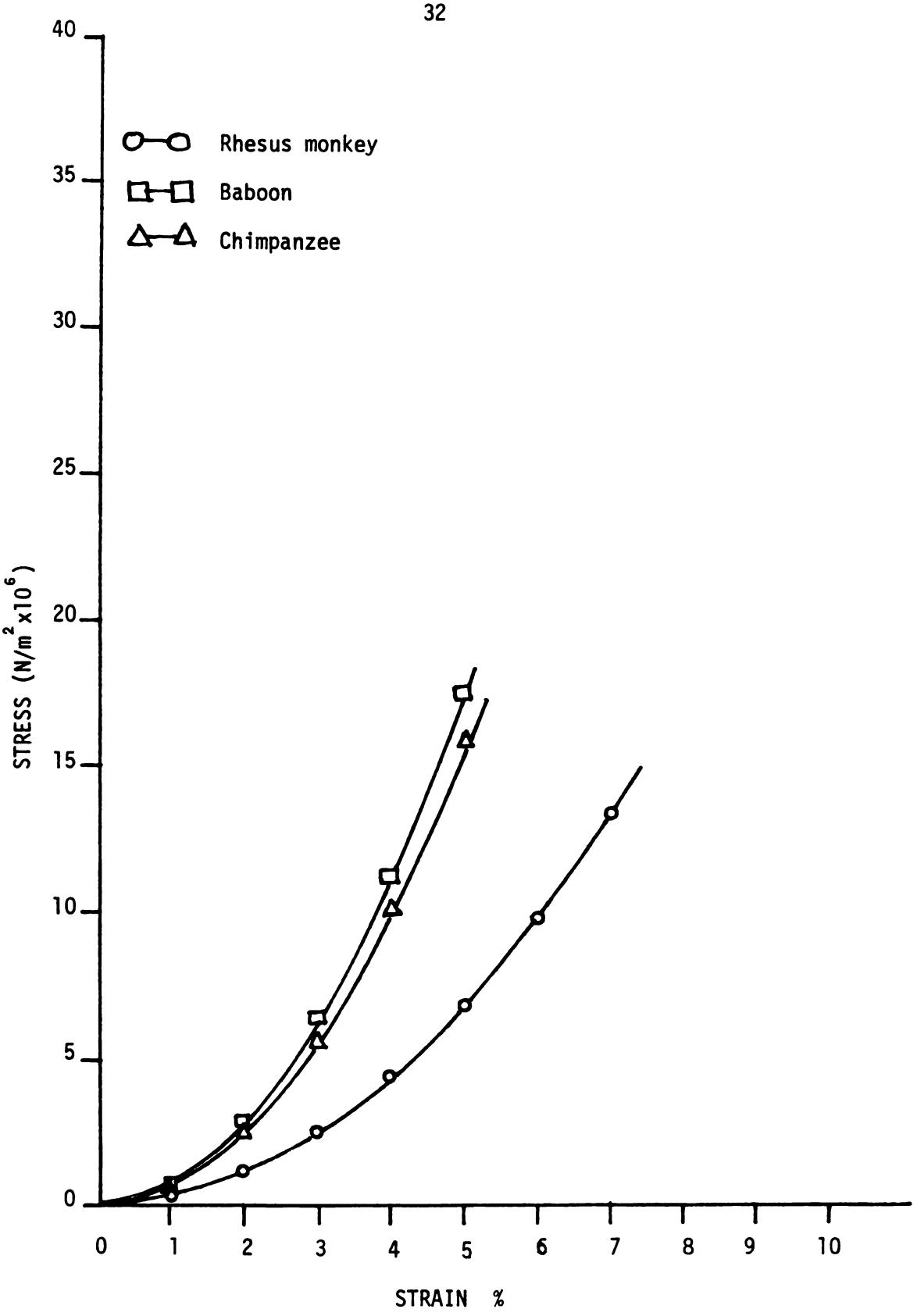

Figure 9. Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Medial Collateral Ligament

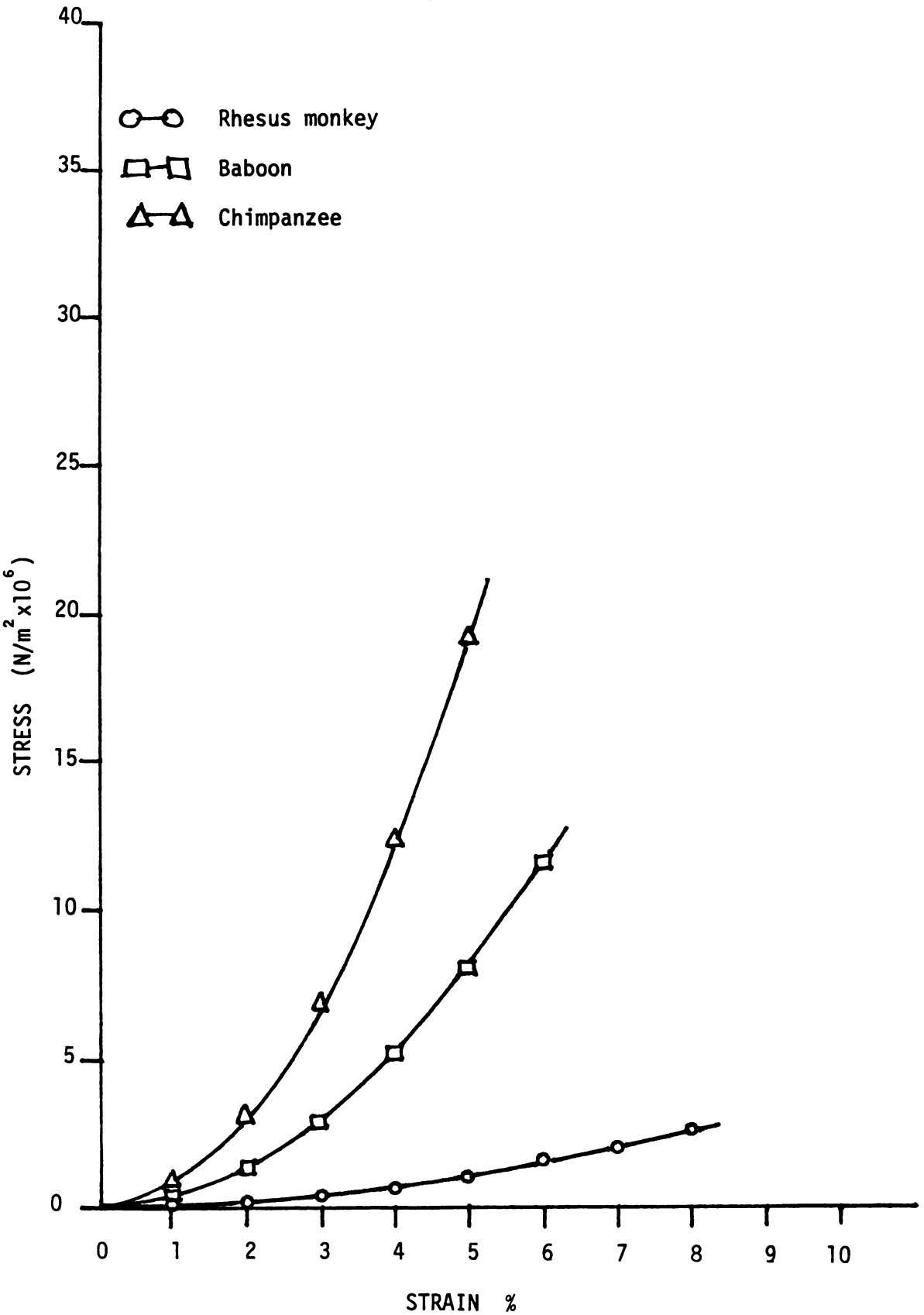

Figure 10. Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Patellar Ligament

holding the fibers together. Therefore, the larger the amounts of elastin present among the collagen fiber bundles, the smaller the tangent modulus. A variation in elastin content was observed between species and may represent a functional adaptation reflecting different lower limb usage by the various species. For example, the elastin content in the tendo-calcaneus tendon was 5 - 10% for the chimpanzee with a tangent modulus of 1060 MPa, while the rhesus and baboon had corresponding values of 20% and 670 MPa, and 30% and 280 MPa, respectively.

The differences seen in Table 5 in maximum test strains among primate tissues were due to the protocol in establishing E^*, defined as the maximum test extension of the linear region on the stress-strain curve. The maximum test strain values were directly dependent upon the initial test length of the ligament or tendon which varied between species and tests. Individual fibers had different points of attachment to specific bones or muscle bodies, but the initial length was defined as the "grip-to-grip" distance and was measured with the unloaded tissue in place in the testing machine. The average maximum test strain for the tendons was between 6.80 - 8.63% for all the primates, while for the ligaments the values were between 4.28 - 9.64%. No correlation between tendons and ligaments can be made at maximum test strains, so comparisons for the tissues were made at a strain level of 5.0%, using extrapolated data from the average constant stress-strain curves in some cases (See Figures 7 to 10). The average stress at a strain level of 5.0% was 4.80 - 28.50 MPa for the tendons, and 1.00 - 19.20 MPa for the ligaments, for all three primates. A pattern was noticed for the tendons between the average maximum stress and the % hysteresis energy

to loading energy. When the average maximum stress increased, the % hysteresis energy to loading energy also increased. No pattern was found for the ligaments, suggesting a larger number of tissue samples should be tested before conclusions can be drawn about hysteresis areas. Since few viscoelastic tests of this nature have been conducted on soft connective tissue, hysteresis data cannot be compared to other investigators' works.

Strain rate effects, hysteresis and energy areas were also determined from constant strain rate data (See Table 6). Each primate tissue was tested consecutively at three different strain rates; 100, 1, and 0.01% per second. For each specimen, the maximum stress, tangent modulus, load input energy, and hysteresis area all decreased with decrease in strain rate implying a strain rate dependency. An attempt was made to select a parameter which reflected the energy expended in the deformation of the tissue. This parameter was determined by taking the percentage of the hysteresis area at a particular strain rate relative to the hysteresis area at a strain rate of 100% per second. When strain rate decreases, the greatest drop in hysteresis area occurred for the ligaments of the baboon. The medial collateral recorded a decrease to 28.9% at a strain rate of 0.01% per second, while the patellar ligament had a value of 44.7% at a strain rate of 0.01% per second. The largest drop in hysteresis area for the tendons occurred for the chimpanzee to a value of 52.9% at a strain rate of 0.01% per second for the flexor hallucis longus, and a value of 58.7% at a strain rate of 0.01% per second for the tendo-calcaneus.

Graphs of cyclic relaxation data can be found in Figures 11 to 14.

Table 6. A Summary of Percentages for Specific Mechanical Properties at Variable Strain Rates

Ligament or Tendon	Species	Strain Rate %/Sec.	Percent of 100%/Sec. Strain Rate Values: Maximum* Stress %	Tangent+ Modulus %	Loaded@ Input Energy,%	Hysteresis# Area %
Flexor Hallucis Longus Tendon	Rhesus	100.00	100.0	100.0	100.0	100.0
		1.00	97.1	91.0	87.5	91.5
		0.01	83.0	79.0	73.0	87.7
	Baboon	100.00	100.0	100.0	100.0	100.0
		1.00	98.2	93.5	87.4	85.7
		0.01	86.3	82.0	74.8	79.5
	Chimp	100.00	100.0	100.0	100.0	100.0
		1.00	93.0	91.5	85.5	64.9
		0.01	83.6	77.1	78.9	52.9
Tendo-Calcaneus Tendon	Rhesus	100.00	100.0	100.0	100.0	100.0
		1.00	97.3	92.4	83.5	68.7
		0.01	80.2	77.7	67.1	66.9
	Baboon	100.00	100.0	100.0	100.0	100.0
		1.00	98.4	94.5	84.3	72.8
		0.01	89.5	79.9	69.8	69.1
	Chimp	100.00	100.0	100.0	100.0	100.0
		1.00	88.9	85.3	82.5	80.3
		0.01	83.0	65.1	79.8	58.7
Medial Collateral Ligament	Rhesus	100.00	100.0	100.0	100.0	100.0
		1.00	91.4	91.7	86.5	59.9
		0.01	80.3	81.3	75.6	55.5
	Baboon	100.00	100.0	100.0	100.0	100.0
		1.00	91.2	89.9	73.8	30.7
		0.01	78.0	82.0	61.6	28.9
	Chimp	100.00	100.0	100.0	100.0	100.0
		1.00	90.4	90.9	96.4	31.1
		0.01	79.6	81.6	85.6	30.4
Patellar Ligament	Rhesus	100.00	100.0	100.0	100.0	100.0
		1.00	91.4	88.6	82.5	53.9
		0.01	65.4	64.2	57.4	48.9
	Baboon	100.00	100.0	100.0	100.0	100.0
		1.00	94.1	93.0	80.9	52.9
		0.01	73.1	76.7	61.0	44.7
	Chimp	100.00	100.0	100.0	100.0	100.0
		1.00	97.1	93.4	92.1	86.1
		0.01	92.5	78.2	82.3	82.4

* Average % of Maximum Stress for All Samples.
\+ Average % of Tangent Modulus for All Samples.
@ Average % of Loaded Energy of Hysteresis Area for All Samples.
\# Average % of Total Hysteresis Area for All Samples.

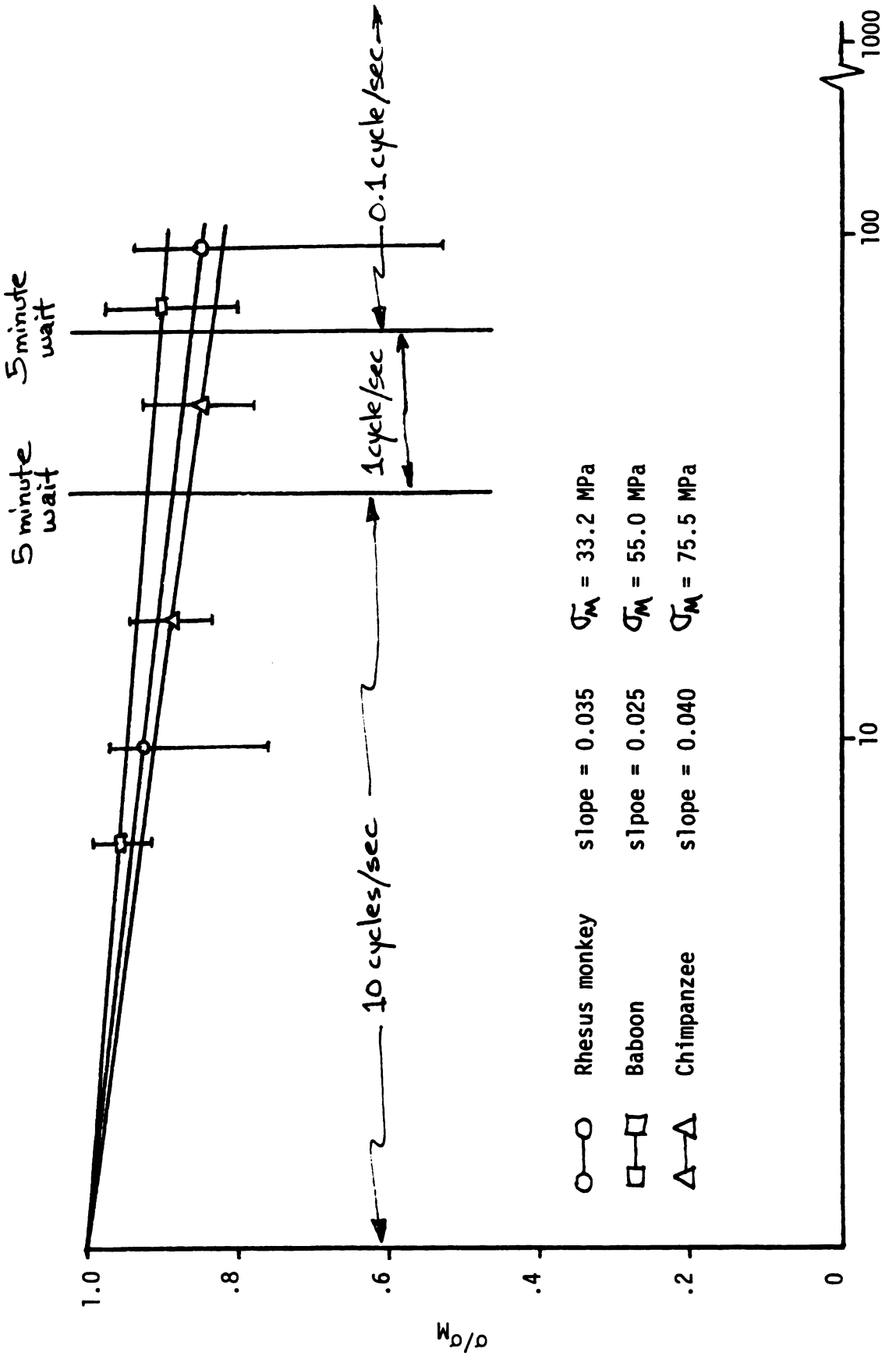

Figure 11. Average Cyclic Relaxation Curves for the Flexor Hallucis Longus Tendon

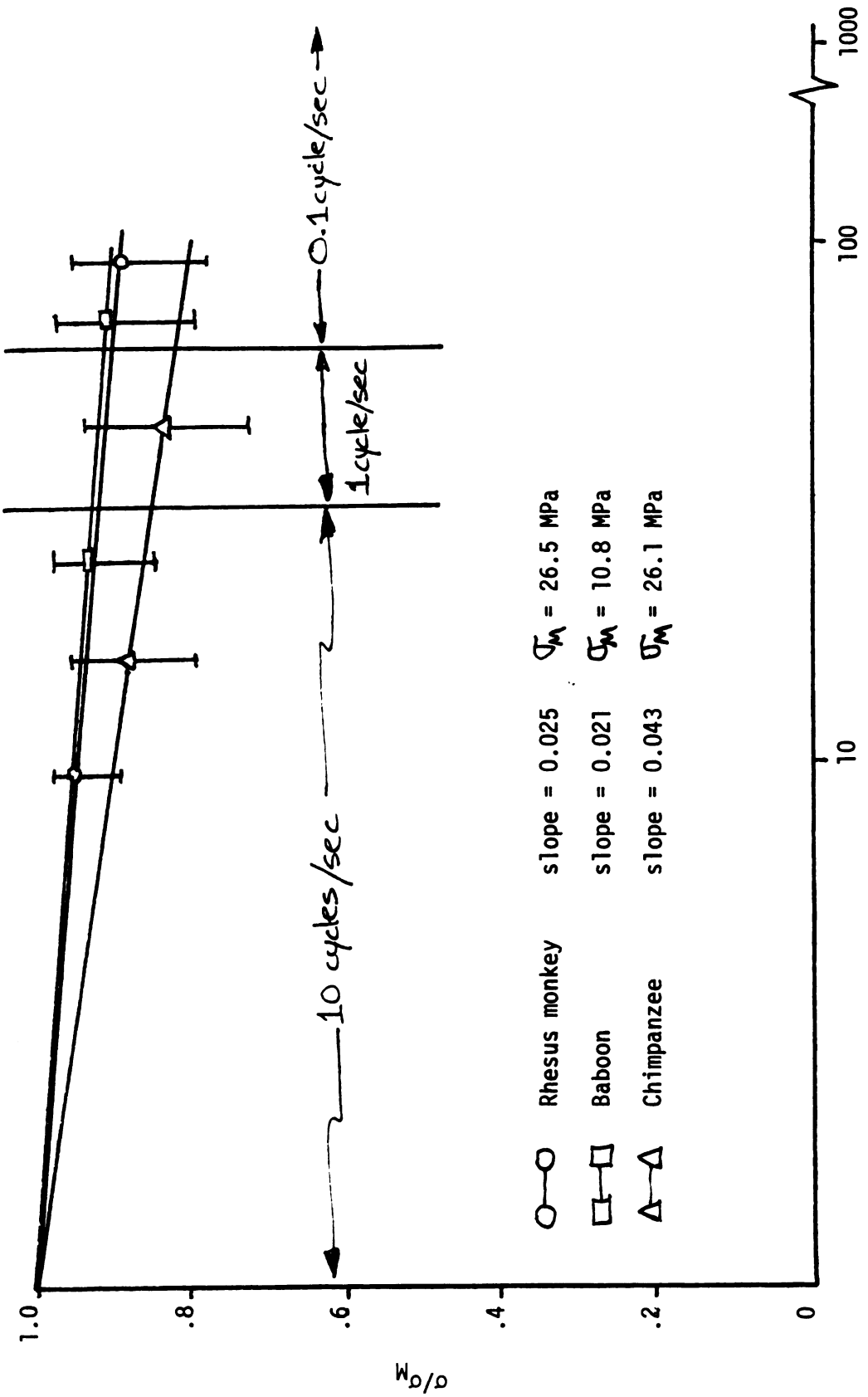

Figure 12. Average Cyclic Relaxation Curves for the Tendo-calcaneus Tendon

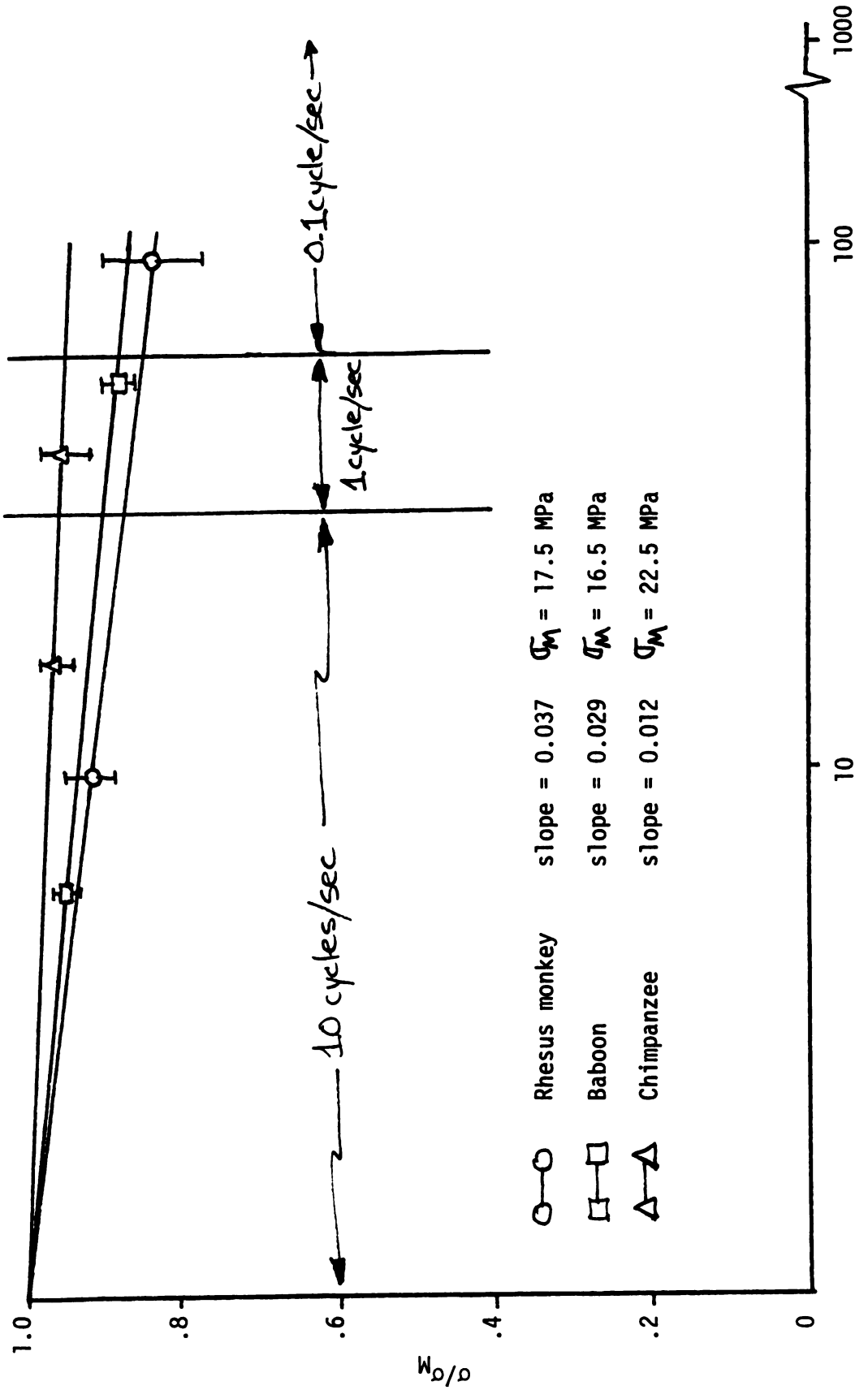

Figure 13. Average Cyclic Relaxation Curves for the Medial Collateral Ligament

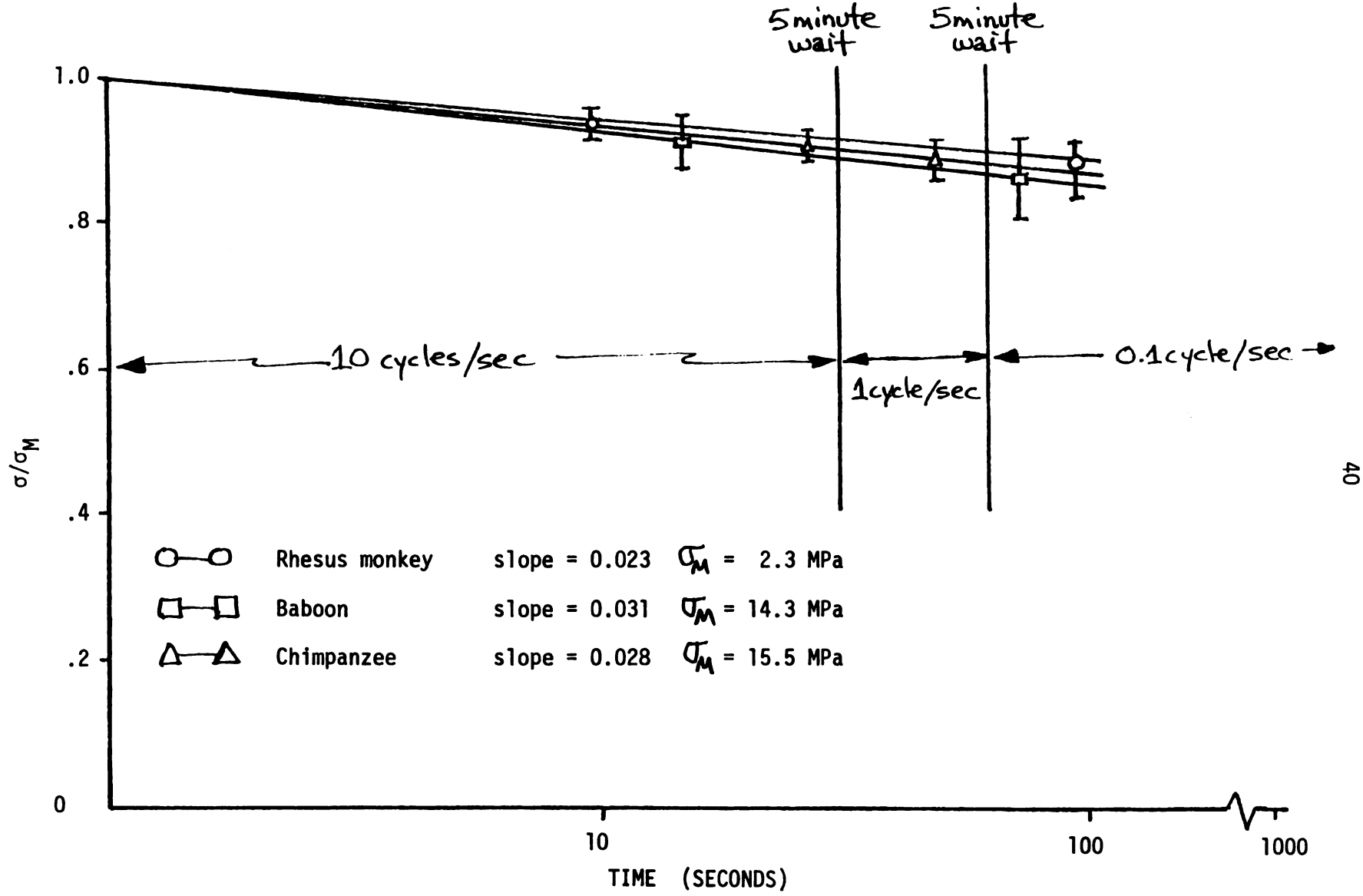

Figure 14. Average Cyclic Relaxation Curves for the Patellar Ligament

Each primate tissue was tested at three different frequency levels; 10, 1, and 0.1 cycles per second, and data was then plotted with all three frequencies on the same graph. The cyclic data curves represent the normalized stress at maximum amplitude versus the natural logarithm of time, instead of versus the number of cycles as frequently done in the literature. All stress values were normalized by the initial peak stress which occurred at maximum amplitude during the first cycle. A smooth transition between the different frequencies, with tests separated by a five minute wait, was observed on all tests. The scatter of the cyclic data did not allow for significant variation between species. Comparisons between low to high frequencies cannot be made because the three different frequencies tests were conducted at different times in the testing protocol. The only way such a comparison could be made would be to either (1) vary the order of test frequencies or (2) run single frequency tests on many different specimens. The smooth transition between frequencies may not indicate lack of frequency dependence, but instead represented a simple time response, dependent upon the total time of cyclic testing. The negative slope of the linear region for the tendons ranged between 0.021 to 0.043, while for the ligaments the slope ranged between 0.012 to 0.037. The initial drop in cyclic relaxation showed a short term relaxation phenomena, similar to that observed in standard relaxation tests.

Two types of short term viscoelastic effects were examined and compared. The first type of viscoelastic effect was the short term relaxation phenomena represented by the change of the percent of peak stress at maximum strain amplitude from cyclic relaxation data.

A second examination of short term viscoelastic effects used standard relaxation tests (See Figures 15 to 18), to measure short time (10 - 30 minutes) response. The normalized relaxation function, G(t), can be approximated as a linear function of the logarithm of time.

$$G(t) = 1 - \mu \ln(t+1)$$

G(t) is a reduced relaxation function as defined by Fung [5], in a hereditary integral formulation. A measure of the tissue's viscoelasticity is given by the relaxation coefficient, μ, and is calculated by finding the slope of the linear region of these curves. The coefficient varied from 0.031 to 0.045 for the tendons, and from 0.022 to 0.038 for the ligaments. The scatter of the relaxation data did not allow for significant variation between species. When the μ values from the relaxation data were compared to the slope values from the cyclic data for each primate tissue, it was seen that the values were approximately the same. This would indicate that the tissues seem to relax at the same rate. However, it is difficult to make a comparison of these two tests, since these tests were performed at two different time periods in the test sequence.

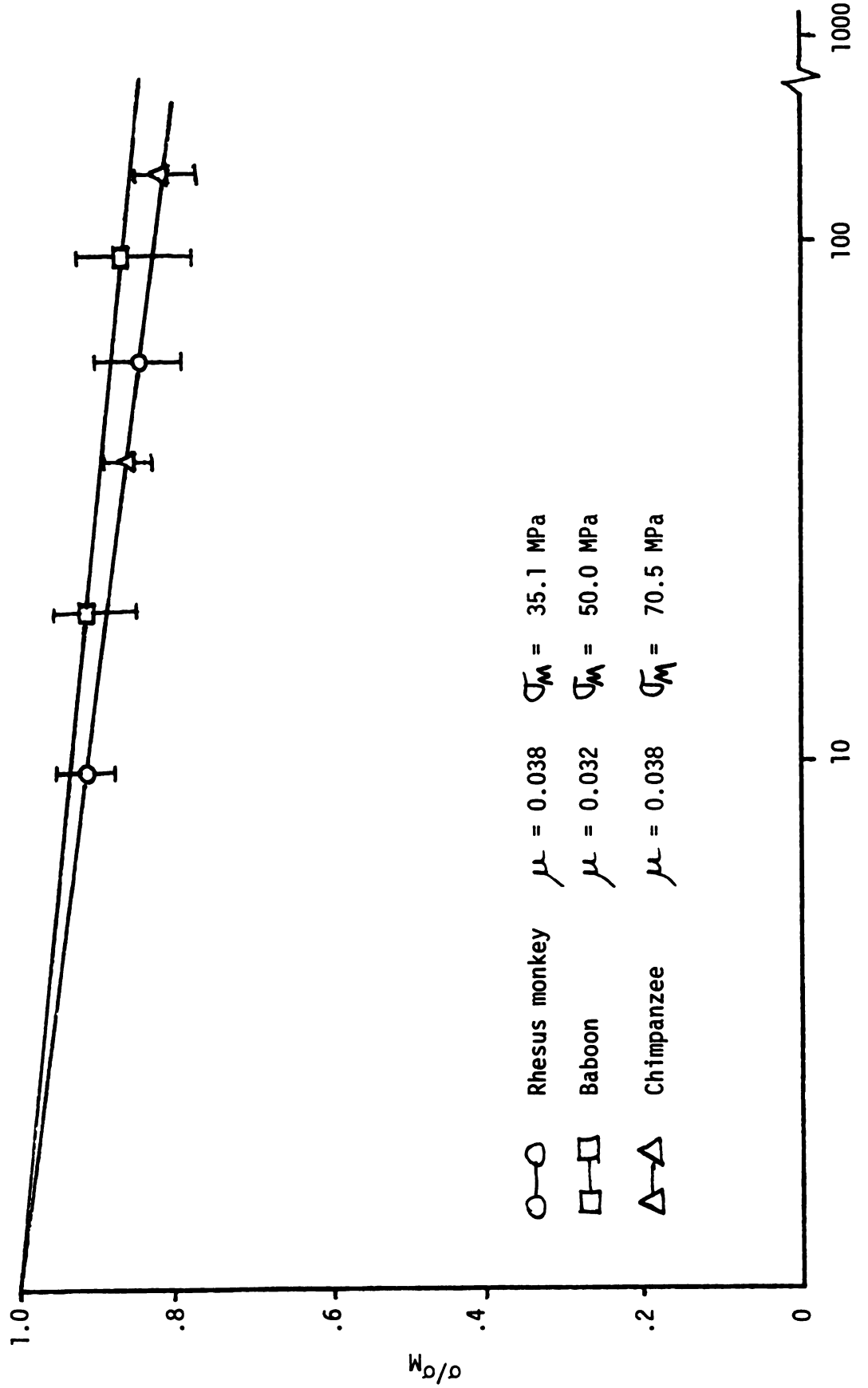

Figure 15. Average Standard Relaxation Curves for the Flexor Hallucis Longus Tendon

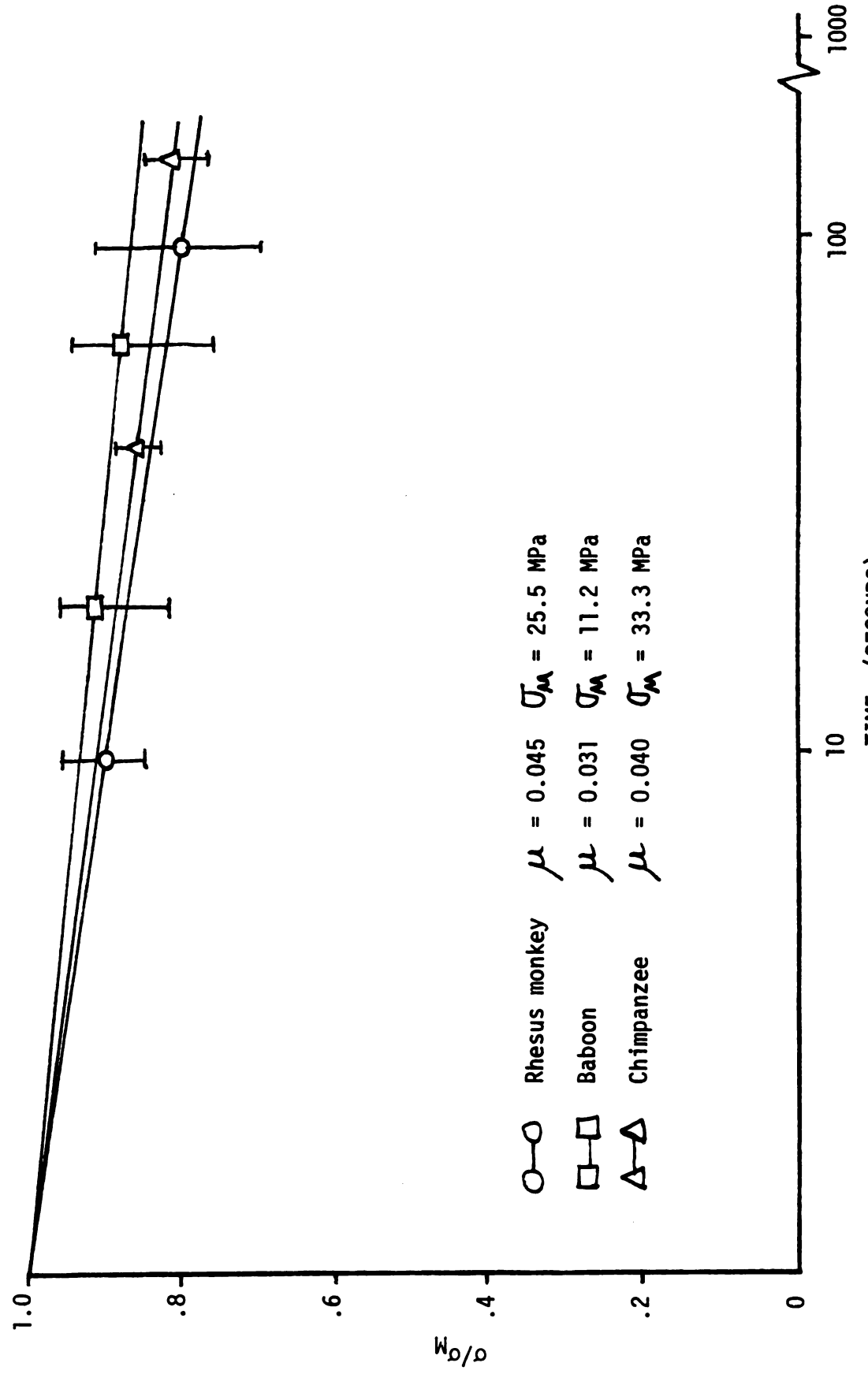

Figure 16. Average Standard Relaxation Curves for the Tendo-calcaneus Tendon

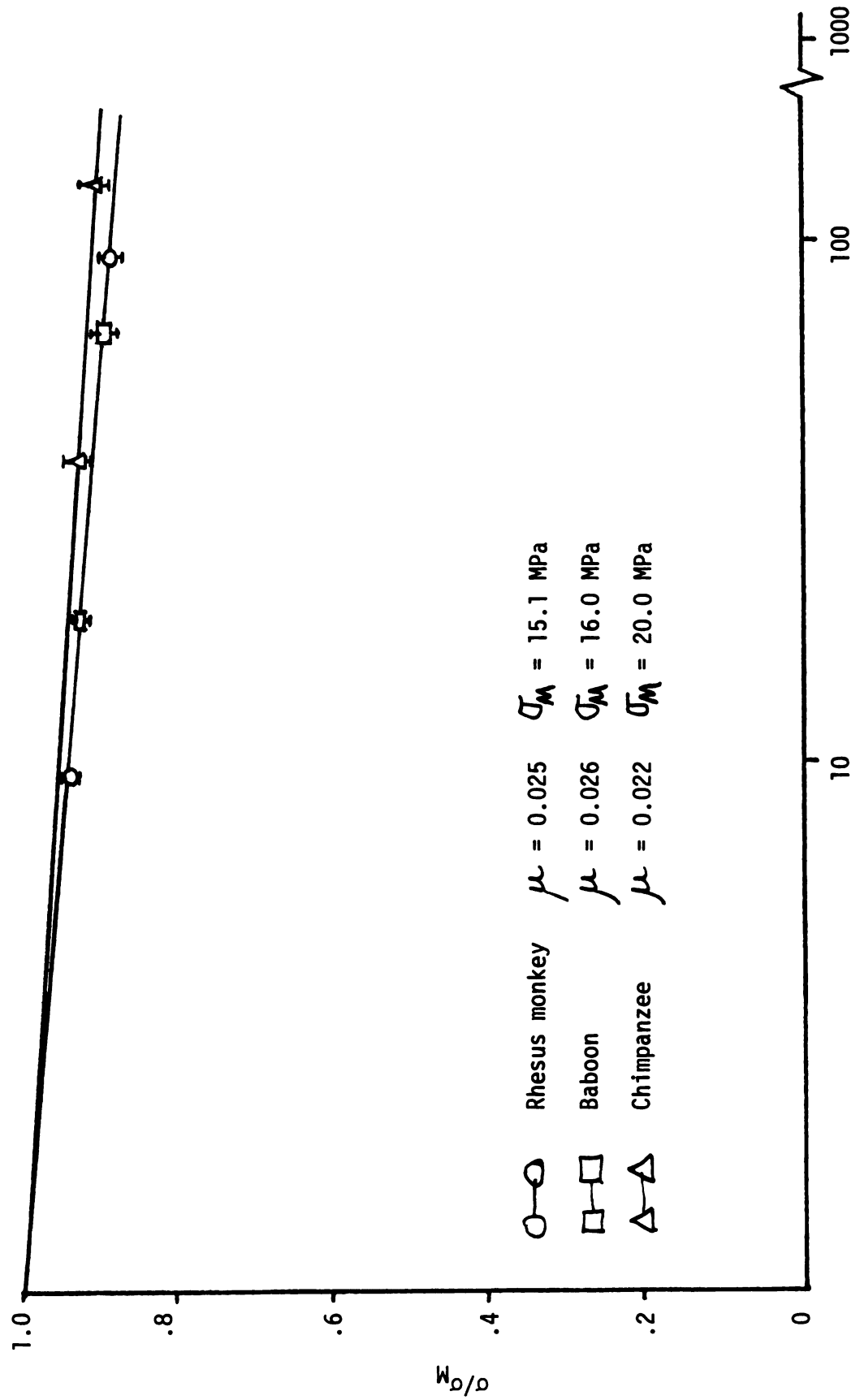

Figure 17. Average Standard Relaxation Curves for the Medial Collateral Ligament

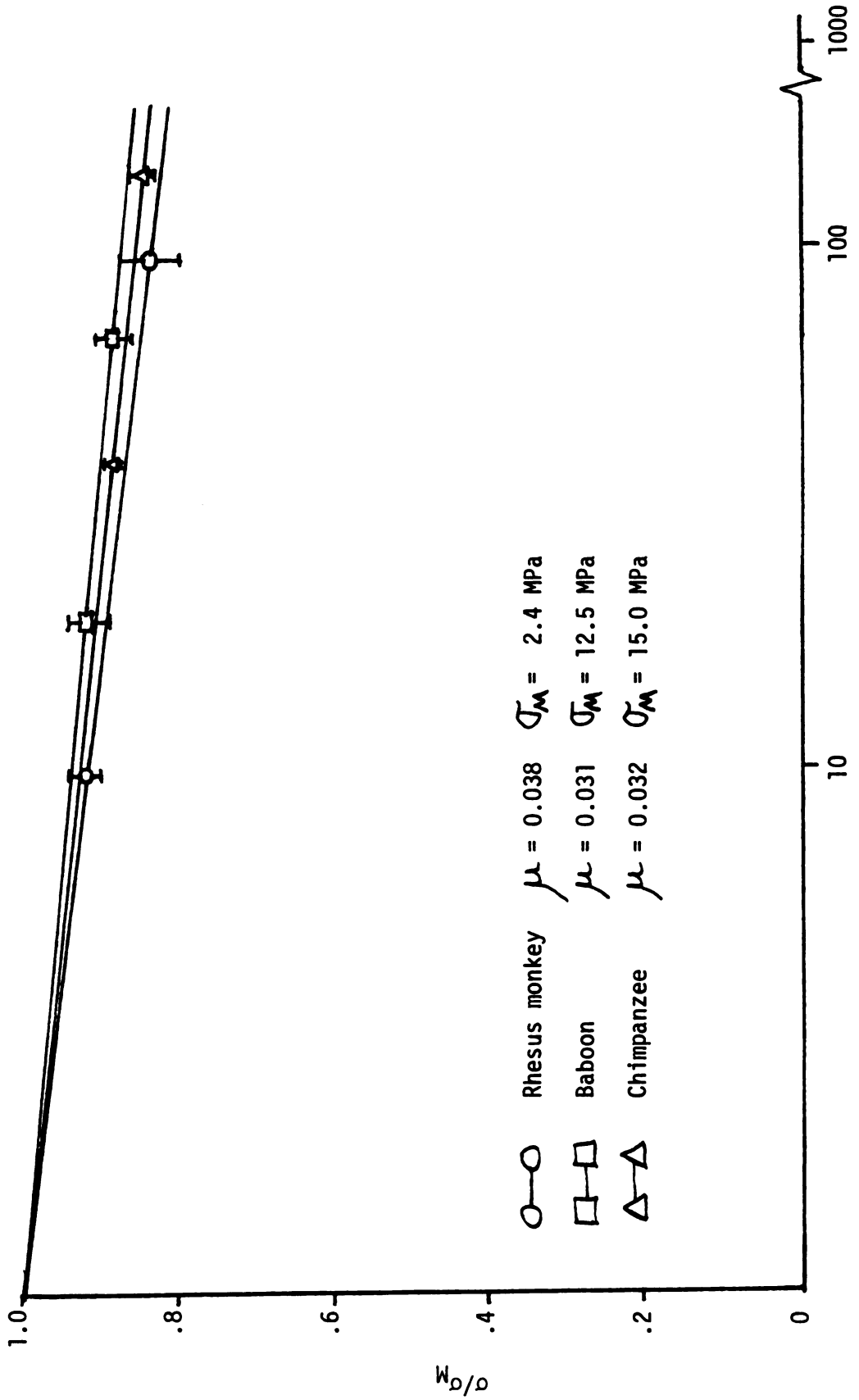

Figure 18. Average Standard Relaxation Curves for the Patellar Ligament

CONCLUSIONS

The results presented emphasize the importance of a controlled test protocol. The check loops throughout the test program showed a lack of stability of the preconditioning response due to a long term relaxation phenomenon, except for the chimpanzee, which showed a relatively stable response. Therefore, comparisons of one test with another in the testing sequence cannot be made. The scatter in the data did not allow for significant variation between species.

Constant strain rate data were used to determine the relative stiffness measured by the tangent modulus, for all three primates' tendons and ligaments. The tangent moduli values recorded were within the range of 300 - 1000 MPa reported in the literature for collagenous tissue. The value of the tangent modulus for each tissue correlated well with the percent of elastin. The higher the elastin content, the lower the value of the tangent modulus. Large variations in the percent of elastin were observed histologically between the species for the same tissue.

Differences in maximum test strains, due to the definition of the maximum test extension in the linear region on the stress-strain curve, made comparisons of properties at maximum test strains impossible, so comparisons for the tissues were made at a strain level of 5.0%. A pattern was noticed for the tendons between the average maximum stress

and the % hysteresis energy to loading energy. When average maximum stress increased, the % hysteresis energy to loading energy also increased. No pattern was found for the ligaments, but a larger number of tissue samples should be tested before conclusions can be drawn about hysteresis areas.

Strain rate effects, hysteresis and energy areas were also determined from constant strain rate data. The maximum stress, tangent modulus, load input energy, and hysteresis area all decreased with decreasing strain rate. Since few viscoelastic tests of this nature have been conducted on soft connective tissue, hysteresis data could not be compared to other investigators' works.

Cyclic strain data showed a short term viscoelastic response of comparable nature to that observed for standard relaxation tests. No frequency dependency could be observed and the data showed a continued smooth decay through frequency change. The long term preconditioning instability prevented comparison of one frequency response to another.

A second examination of viscoelastic effects used the standard relaxation tests to measure short time effects. The normalized relaxation function was approximated as a linear function of the logarithm of time. The μ value from the relaxation data was approximately the same as the slope value from the cyclic data, indicating the relaxation of the tissue was the same.

The tissue tested in this thesis were the ligaments (medial collateral and patellar) from the knees, and tendons (flexor hallucis longus and tendo-calcaneus) from the ankles of the rhesus monkey,

baboon, and chimpanzee. The physical parameters needed for the hereditary integral analysis were found from the experimental protocol. No attempt was made to put these parameters back into the hereditary analysis, but is the subject of future Ph.D. work to see if this analysis could be used as a predictive model.

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