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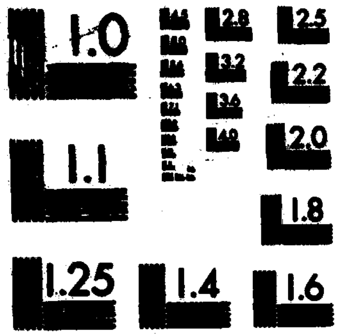

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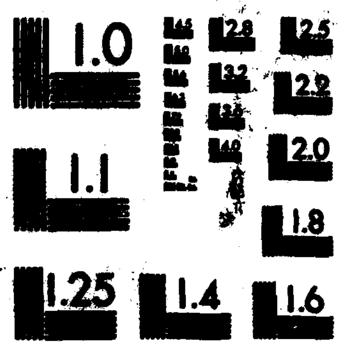

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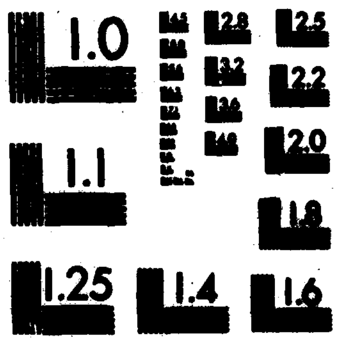

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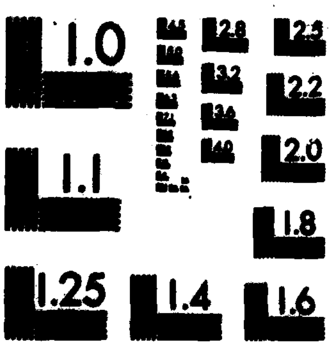

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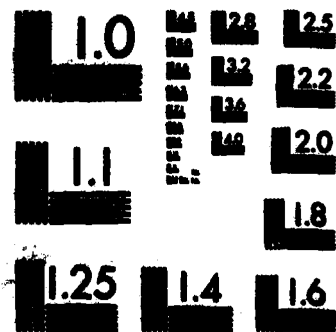

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MECHANICAL PROPERTIES OF LOWER LIMB TENDONS AND LIGAMENTS IN PRIMATES

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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-82-56

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report covers a study of the material property characteristics of four ligaments and tendons from the lower limbs of primates and is part of a three year study of the mechanical properties of soft connective tissues. The mechanical properties 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 were tested. The mechanical test program included establishment of initial tissue geometry, relaxation, constant strain rate, hysteresis, and cyclic relaxation tests. Long term

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) preconditioning stability was monitored throughout the test program. Information on the mechanical properties of the ligaments and tendons is essential to the understanding of injuries that result from escape and crash episodes. Different primate species data will aid in the selection of animal models and interspecies scaling techniques.

SUMMARY

This report presents mechanical test data for two tendons and two ligaments of the lower limb in three primate species. The research is being conducted in order to gain insight into the mechanism of extremity injuries encountered during egress from high performance aircraft.

Both the ligaments and tendons exhibit viscoelastic effects over both short and long range test programs. These viscoelastic effects may be correlated with the percent of elastin in the tissues and show variations between species. The mechanical properties 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 are presented and specific parameters are determined to be used in the development of mathematical models. These models will serve as the basis for human injury studies.

PREFACE

This study was conducted in the Department of Biomechanics, College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan, 48824, under AF Contract No. F33615-79-C-0514. Dr. Robert William Little, Professor and Chairman of the Department, was the Principal Investigator; Dr. Robert P. Hubbard was the Co-investigator. The experiments, part of a three-year effort, were conducted in support of Work Unit 72311409, "Mechanical Stress on Soft Tissue Material Properties." Dr. Arnold R. Slonim, Biodynamic Effects Branch, Biodynamics and Bioengineering Division, Air Force Aerospace Medical Research Laboratory, was the project scientist and contract monitor. Mr. Daniel J. Selke based his Master of Science dissertation on this portion of the research.

The cooperation and assistance of Colonel A.R. Bankneider and Major John G. Golden of the Veterinary Sciences Division, Air Force Aerospace Medical Research Laboratory, and of Dr. Charles E. Graham, Deputy Director, Primate Research Institute, New Mexico State University at Holloman AFB, New Mexico, in providing primate cadaveric specimens vital to this study are very much appreciated.

The authors gratefully acknowledge the assistance of Laura Hayes for help in typing this report.

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Mechanical Properties of Lower Limb Tendons and Ligaments in Primates

INTRODUCTION

The transmission of force and the control of motion in the skeletal system are influenced by the mechanical properties of ligaments and tendons. Tendons are mostly collagenous connective tissue and connect muscle to bone. Ligaments provide bone to bone connections and are composed of both collagen and elastin. Because of their importance to the understanding of body mechanics, these tissues have been the subject of many investigations. In 1965, D.H. Elliot (2) published a comprehensive survey of the literature on the structure and function of mammalian tendon. He described tendon as consisting almost entirely of collagenous tissue (80% of dry weight) with the collagen fibers in parallel wavy bundles. Elastic fibers are more prevalent in ligaments and the collagen fibers are much less aligned than in tendons. This fiber composition and structure greatly influence the mechanical response of these soft connective tissues.

The viscoelastic nature of biological tissue has been considered in detail during the last two decades. As long as the strain did not exceed approximately 4% for strain rates between 1-20%/minute, Rigby *et al.*(8) found that the rat-tail tendon's mechanical behavior was reproducible, or reversible, if the tendon was allowed to rest a few minutes after each elongation. Partingon 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 exhibit a non-linear behavior. A typical stress-strain curve is shown in Figure 1, and can be divided into three ranges. In Region I, it is felt that the response is linear due to the elastin fibers resisting extension, and the wavy collagen fibers straightening. The secondary range, Region II, shows gradually increasing slope due to the loading of the collagen fibers. In the works of Rigby *et al.*(8) in 1959, Millington *et al.*(5) in 1971, and Diamant *et al.* (1) in 1972, it was concluded that Region III exhibited a high, constant slope as all straightened collagen fibers resisted further extension.

Rigby *et al.* (8) 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 appeared to be identical, but shifted toward the stress axis. Elliott (2) in 1965 measured the mechanical tensile strength of certain tendons from different mammalian species, with the human tendo-calcaneus tendon having a tensile strength of 4.7 kg/mm^2 (0.48 MPa). Minns *et al.* (6), 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 lbs/in^2 (1.73-2.79 MPa); while the tensile strength was 200-2500 lbs/in^2 (0.07-0.87 MPa) for the ligamentum nuchae from an adult cow.

Viidik (12) in 1980 reported a higher ultimate strength for tendons of 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 as is revealed in the rate dependency for stress-strain tests at different constant strain rates, the hysteresis under cyclic loading, the cyclic stress relaxation, and the stress relaxation under constant strain. In 1972, Fung (3) 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 under cyclic strain to 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 was almost a linearly decreasing function similar to stress relaxation at constant strain.

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 the tissue. This paper will present the mechanical properties 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.

MATERIAL AND METHODS

Techniques in sample preparation and gripping and the test protocol were adopted from a previous study (4). The dissection procedure had also been discussed in a report on the ligament and tendon morphology and histology (9). Additional histological studies were made of the longitudinal sections to estimate the percent of elastin present in the tissues. These were visual inspections with use of a microscope, and the elastin was found to be more prominent in the peripheral portion than in the central area, which was composed of dense collagen fiber bundles. No precise measurement could be made due to the elastic fiber waviness which exhibited eccentric length and amplitude. The percent of elastin in the different tissues is summarized in Table 1.

TABLE 1. ESTIMATED PERCENT OF ELASTIN CONTENT

Ligament or Tendon	Rhesus Monkey	Baboon	Chimpanzee
Flexor Hallucis Longus Tendon	5-10%	5%	<1%
Tendo-Calcaneus Tendon	20%	30%	5-10%
Medial Collateral Ligament	25-30%	25-30%	25-30%
Patellar Ligament	+	25-30%	10-15%

+No value was estimated since 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.

Testing Protocol:

Preliminary testing was used for grip design refinement and the confirmation of the general viscoelastic nature of the tissues. This testing showed that the tissues exhibited a typical soft tissue load-extension response with increasing stiffness at low elongations and an apparent linear region at higher elongation. Each tissue was ramped slowly to the point where the load deflection response was in the linear region, and this maximum extension 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 minutes.
8) Determine initial unloaded length .
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 grips 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

An initial adjustment of biological tissues to load and deformation was suggested by Fung (3), and initial testing was suggested to obtain reproducibility of test responses. This adjustment was termed preconditioning, and although observable it is not conceptually understood. The adopted test protocol incorporated an initial preconditioning and constant strain rate tests throughout the balance of the testing program to check stability of the preconditioning. In general, it was found that the material could not be preconditioned in a manner to yield reproducible 1% per second constant strain rate responses throughout the program. The peak stress at maximum strain during these checks was normalized against the post-preconditioned peak, and the results are shown in Figures 1-4 for each tissue and different species.

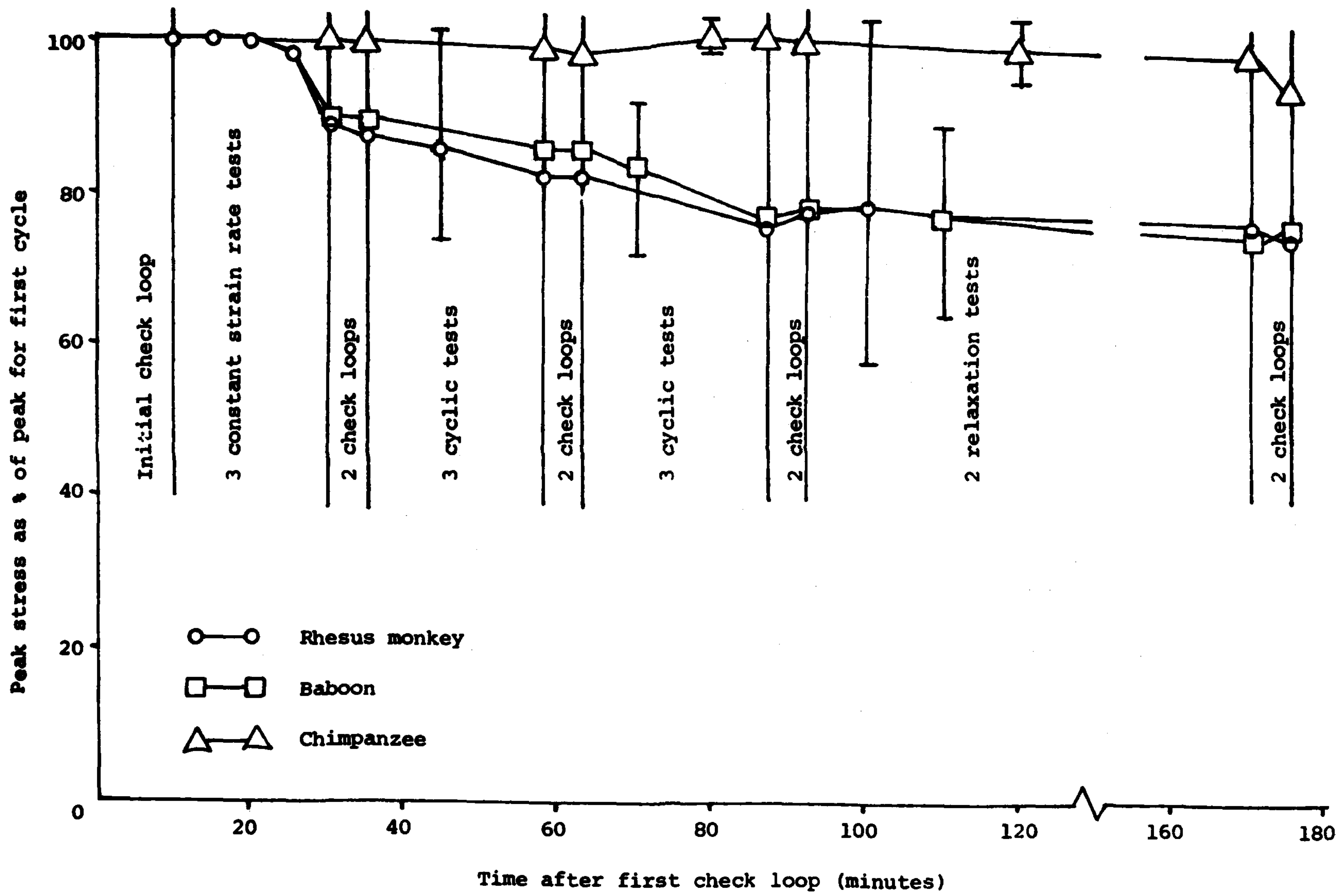

Figure 1: Preconditioning Stability of Flexor Hallucis Longus Tendon

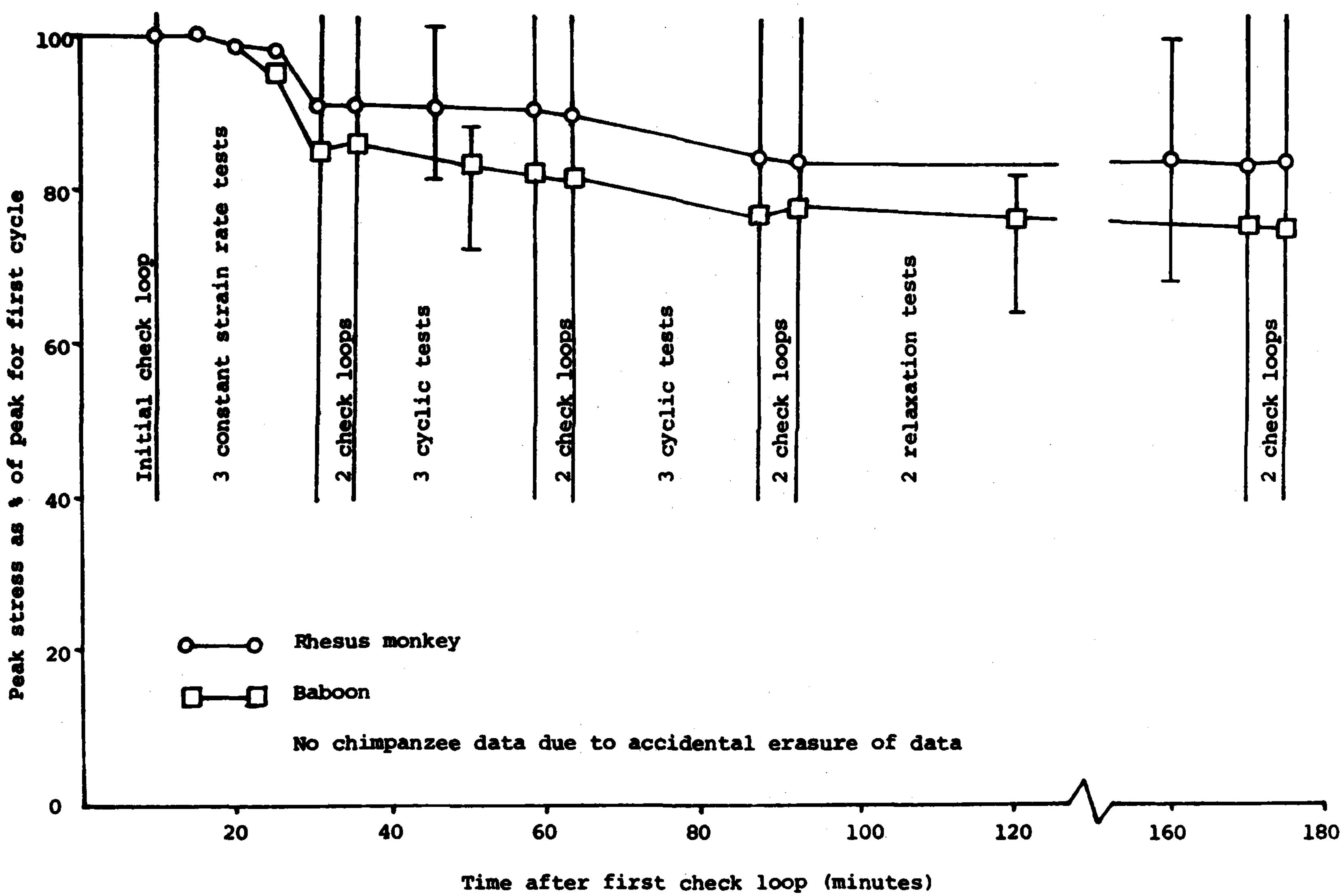

Figure 2: Preconditioning Stability of Tendo-calcaneus Tendon

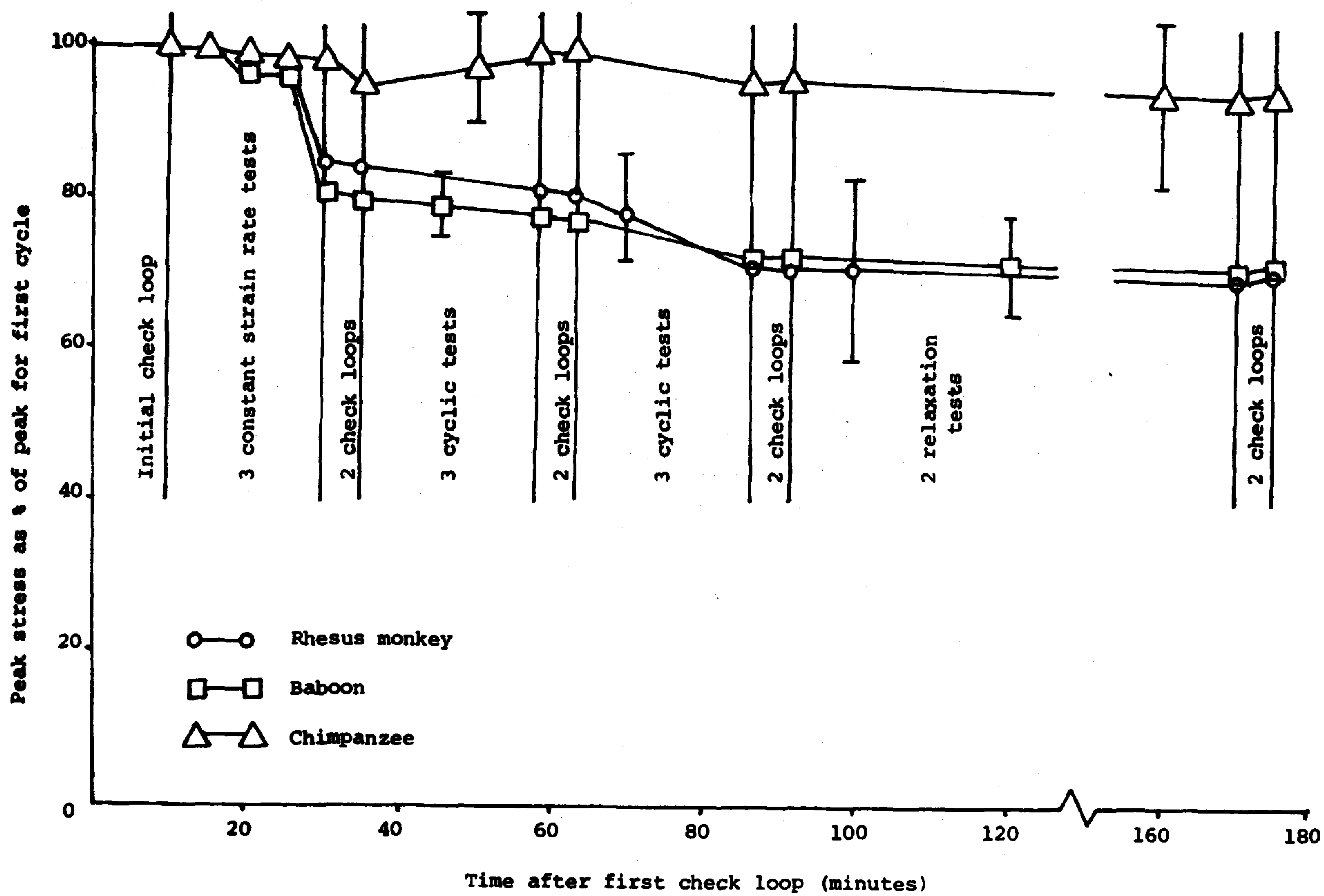

Figure 3: Preconditioning Stability of Medial Collateral Ligament

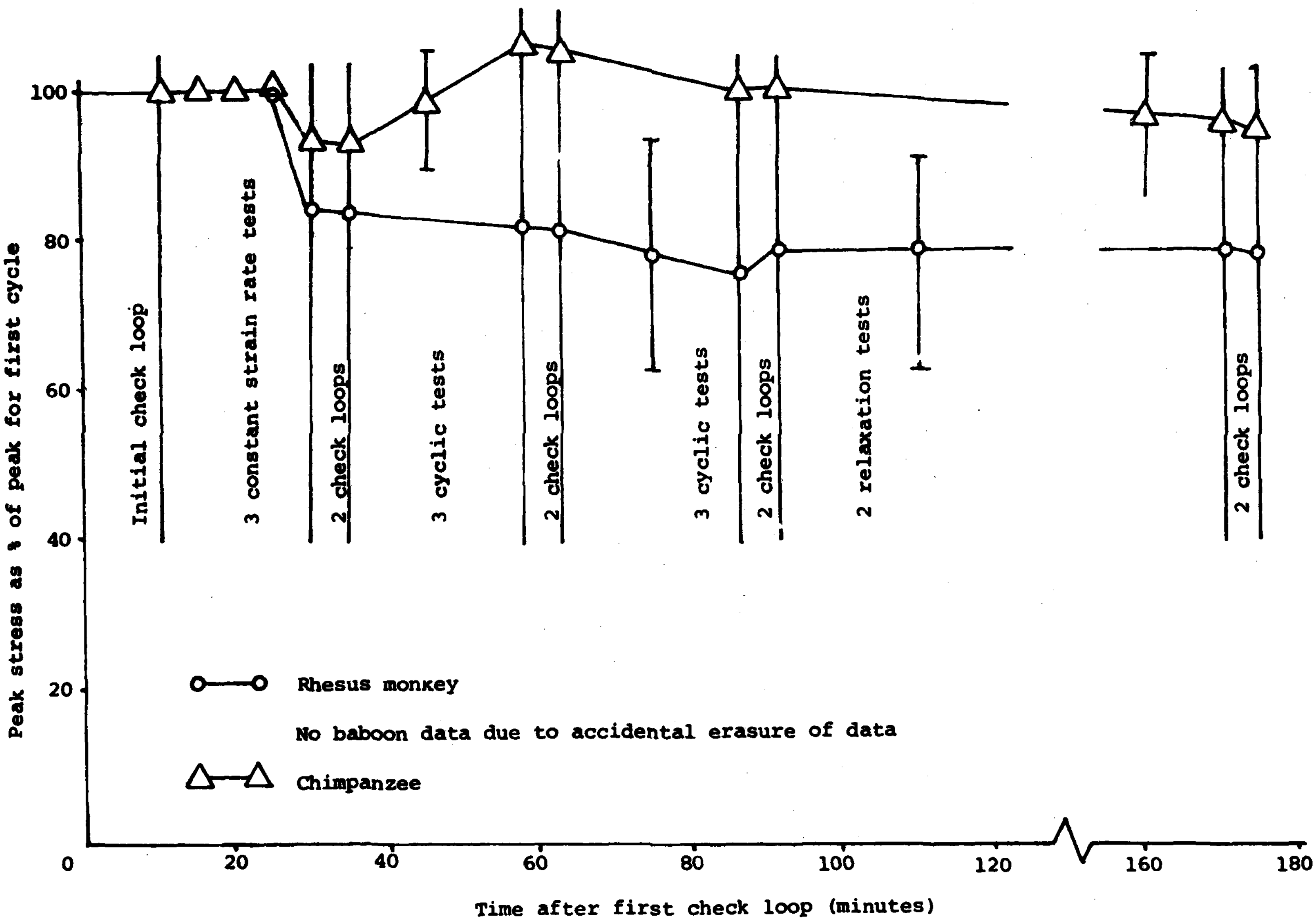

Figure 4: Preconditioning Stability of Patellar Ligament

It is not known whether these preconditioning or long term viscoelastic effects are independent of the short term viscoelastic responses nor their exact cause. It has been suggested that they may be due to changes in the state of cross-linking, alteration in the state of hydration, or realignment of the fiber matrix. Figures 1-4 indicate that the peak check loop stress declined or relaxed to 70-90% of its initial value during the test sequence. This lack of stability presents difficulties when one attempts to compare the response to different tests within the test protocol due to the continuous long term relaxation. However, comparisons may be made between specimens for any given tests due to the standard protocol. The observation that ligaments and tendons do not reach a stable preconditioned state also raises questions about direct comparisons between one study and another when different test protocols are used.

The mechanical properties of the 4 tissues from the different species are summarized in Table 2 for a constant strain rate of 100%/sec. These data are based upon averaged curves for each of the tissues shown in Figures 5-8. The stiffness or tangent modulus of the tendons were within the range of 300 to 1000 MPa as reported by Swanson (10) for mammalian tendon. In general the tissue from the chimpanzee was stiffer than that from either the baboon or rhesus monkey. This coincided with the smaller percent of elastin in these tissues for the chimpanzee. This variation in elastin content between species may represent a functional adaptation reflecting different lower limb usage by the various primates.

TABLE 2. 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 Tendon	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- 37.70	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 5 to 8.
** 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 5 to 8.

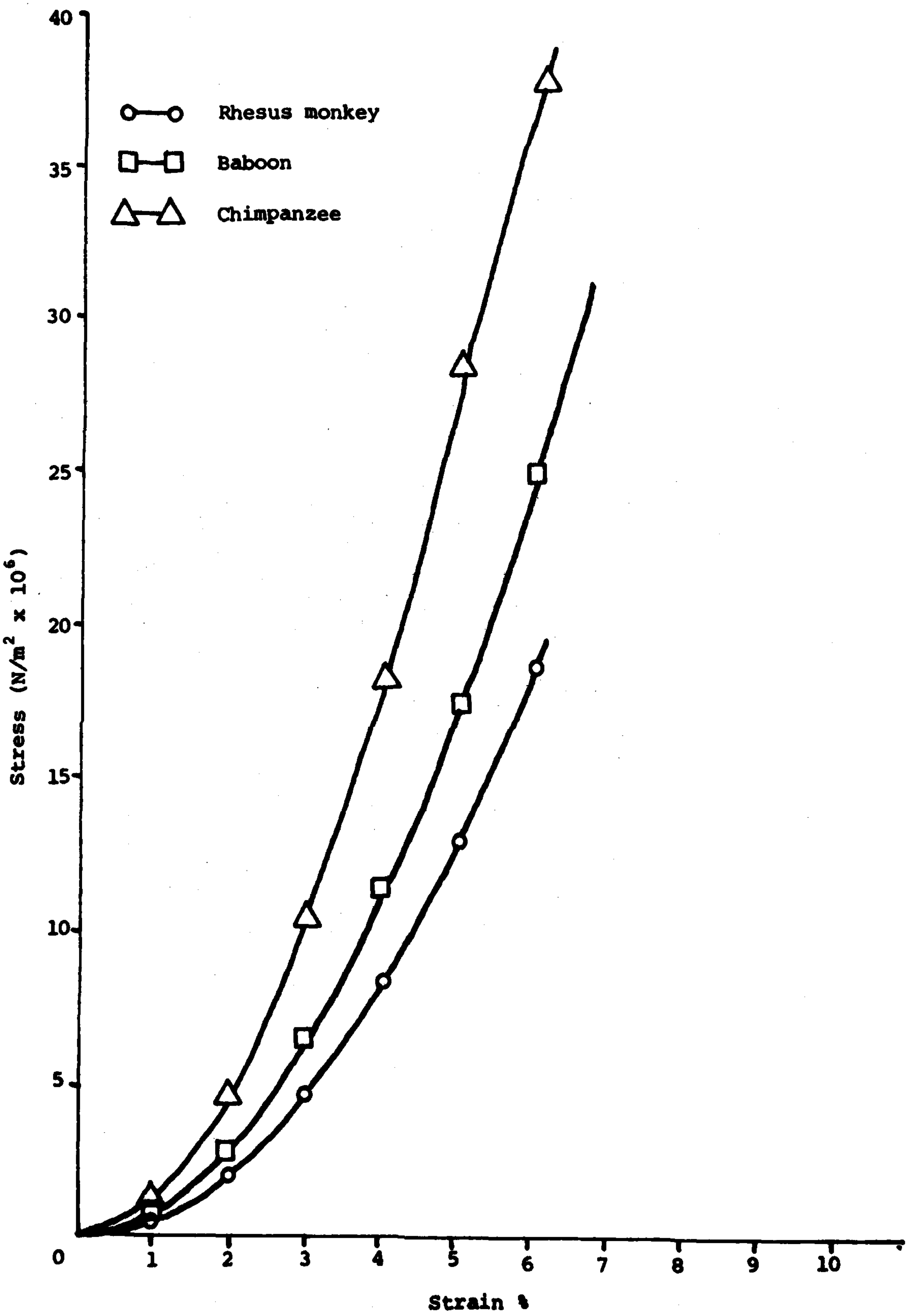

Figure 5: Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Flexor Hallucis Longus Tendon

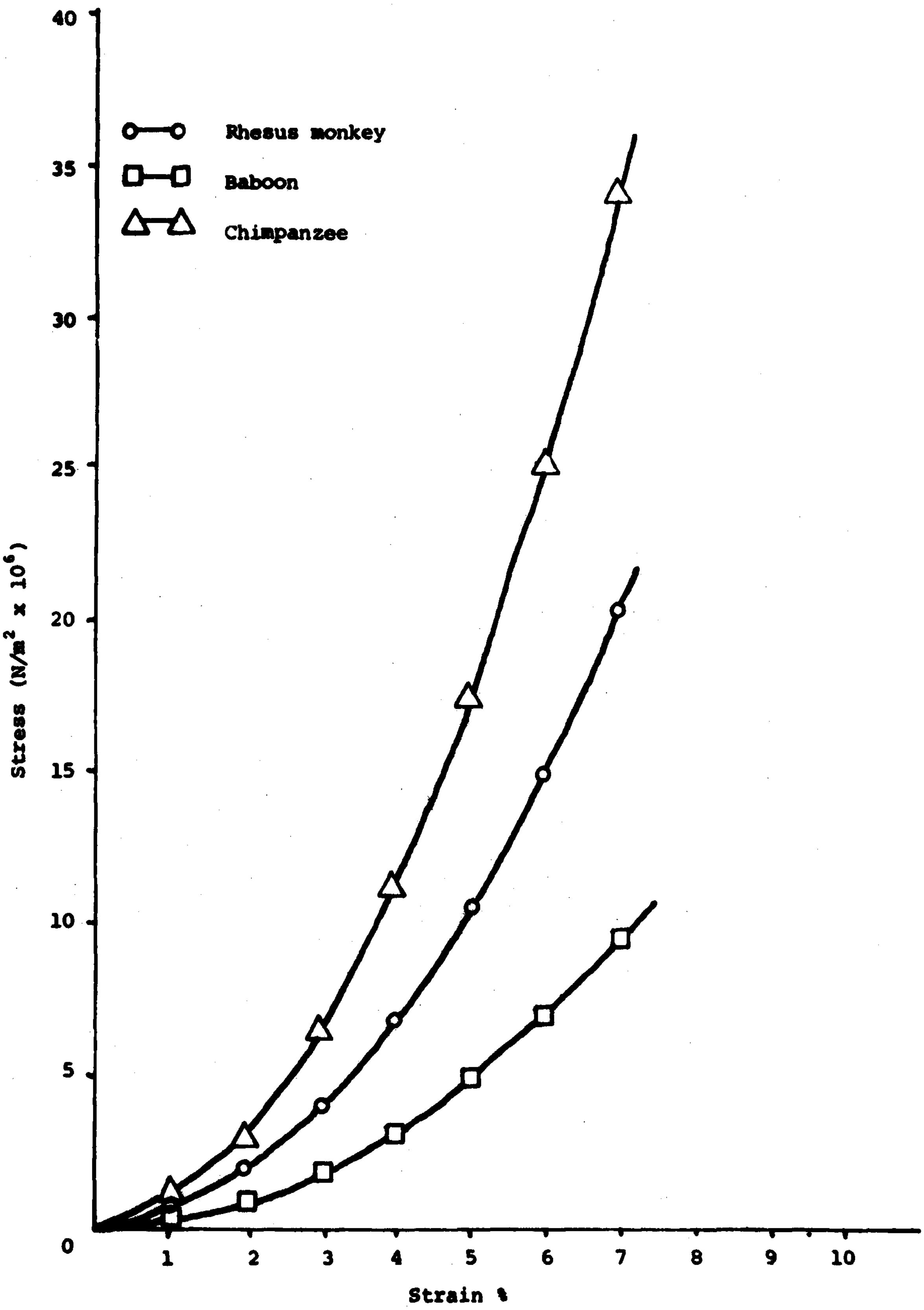

Figure 6: Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Tendo-calcaneus Tendon

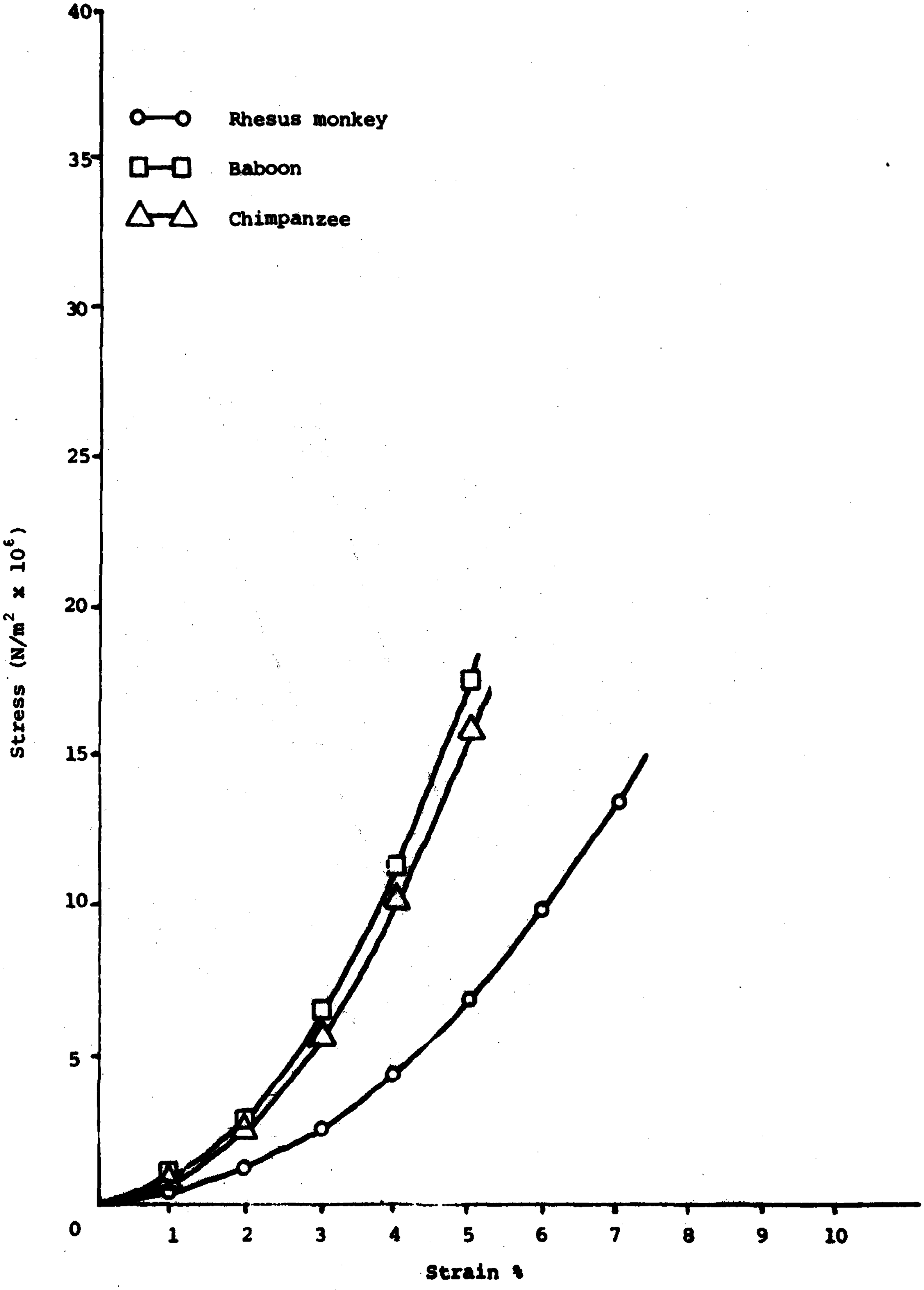

Figure 7: Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Medial Collateral Ligament

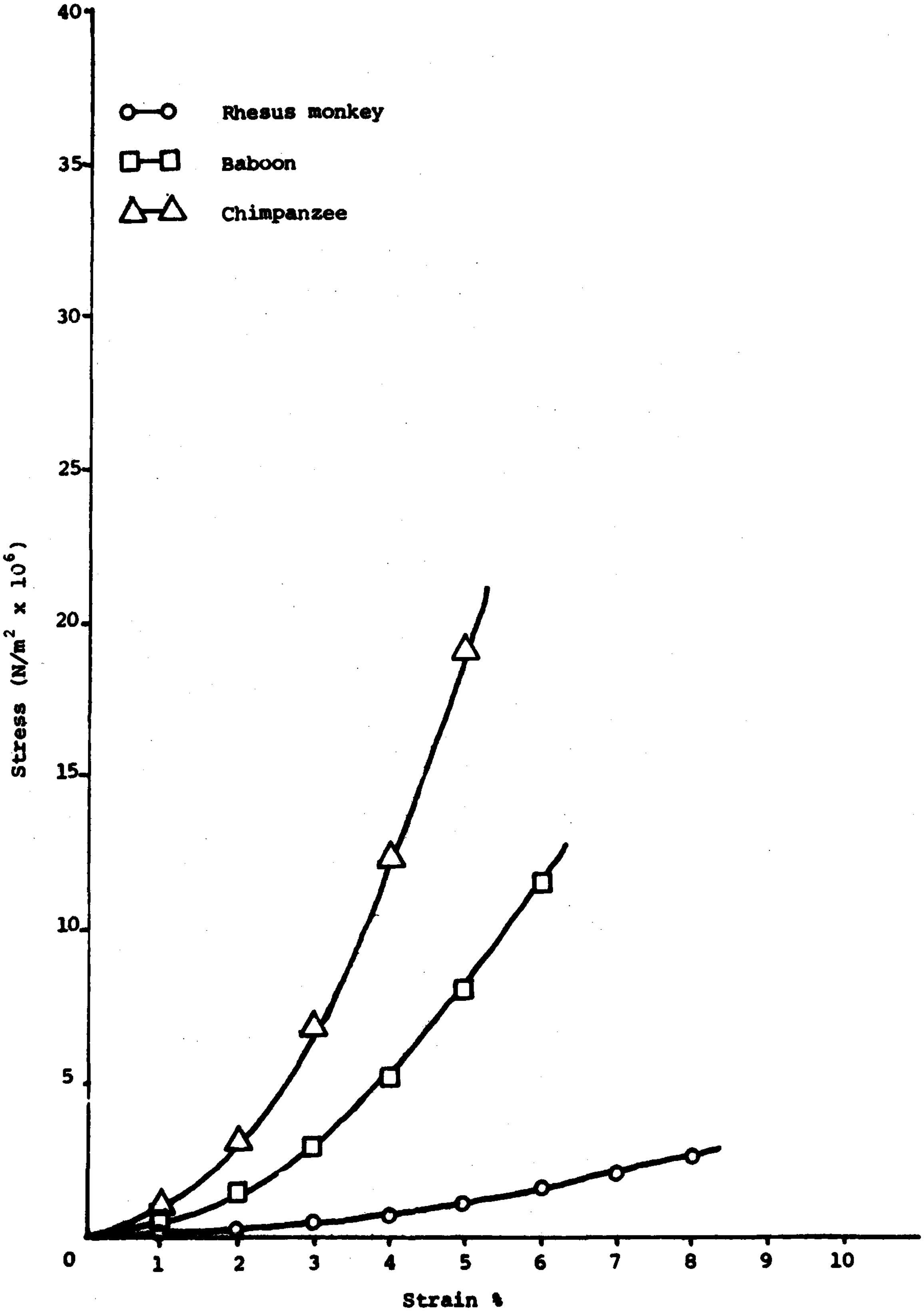

Figure 8: Average Stress-Strain Curves at a Constant Strain Rate of 100%/Sec. for the Patellar Ligament

Large variations occurred in the maximum test strain E*, and therefore stress comparisons were made at a strain of 5%. Hysteresis is defined as the percent of energy dissipated of the input energy during a constant strain rate cycle.

TABLE 3. 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	90.0	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.

Table 3 shows the strain rate sensitivity of maximum stress, maximum tangent modulus, input energy, and hysteresis energy for the tissues from different primates. Each of these parameters decreases with decreasing strain rates. The strain rates spanned four orders of magnitude from 100%/sec to 0.01%/sec. The hysteresis energy showed the largest rate sensitivity, but when compared with the corresponding input energy, the percent hysteresis (defined as percent of hysteresis area to input energy) did not exhibit high rate sensitivity for the tendon material. The peak stress values at 100%/sec were lower than anticipated due to the fact that the testing machine did not consistently follow the command function at high strain rates. This resulted in a rounding of the extension-time profile with a lower than desired extension peak. Therefore, in most cases the maximum stress and tangent modulus results show greater sensitivity between 1.0%/sec and 0.01%/sec, which may be attributable to test conditions.

Each primate tissue was tested in cyclic strain at frequencies of 10, 1, and 0.1 cps between the maximum and minimum amplitudes indicated in the test protocol. The decrease in peak stress was plotted against the logarithm of time with the three tests plotted continuously. These data, in Figures 9-12, show a continuing stress relaxation under cyclic strain. 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 was too large for identification of significant variation between species. Comparisons between low to high frequencies cannot be made because the three different frequency tests were conducted at different times in a consistent sequence 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 a simple time response, dependent upon the total time of cyclic testing. The negative slope of the linear region for the ligaments ranged between 0.012 to 0.037, while for the tendons ranged between 0.021 to 0.043. The initial 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 13 to 16) to measure the short time (10-30 minutes) response. A 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 defined by Fung (3) in a hereditary integral formulation. A measure of tissue 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 0.022 to 0.038 for the ligaments and from 0.031 to 0.045 for the tendons. The scatter of the relaxation data did not allow for comparison between species. When the values from the relaxation data were compared to the slope values from the cyclic data for each primate tissue, the values were approximately the same. This would seem to indicate that the tissues relax at the same rate under the two dissimilar strain conditions. However, direct comparison between these two tests is difficult since they were performed at two different time periods in the test sequence.

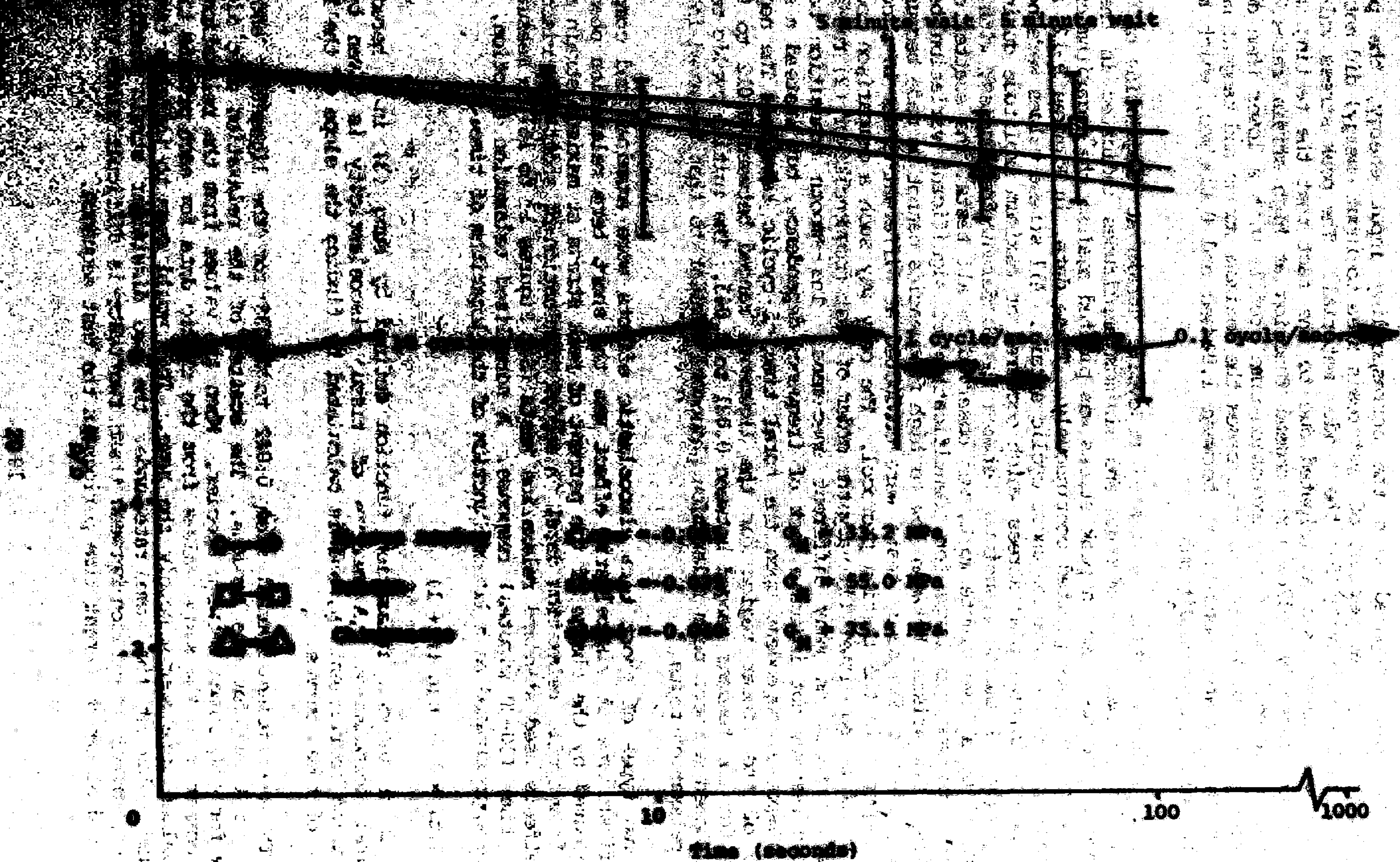

Figure 9: Average Cyclic Relaxation Curves for the Flexor Hallucis Longus Tendon

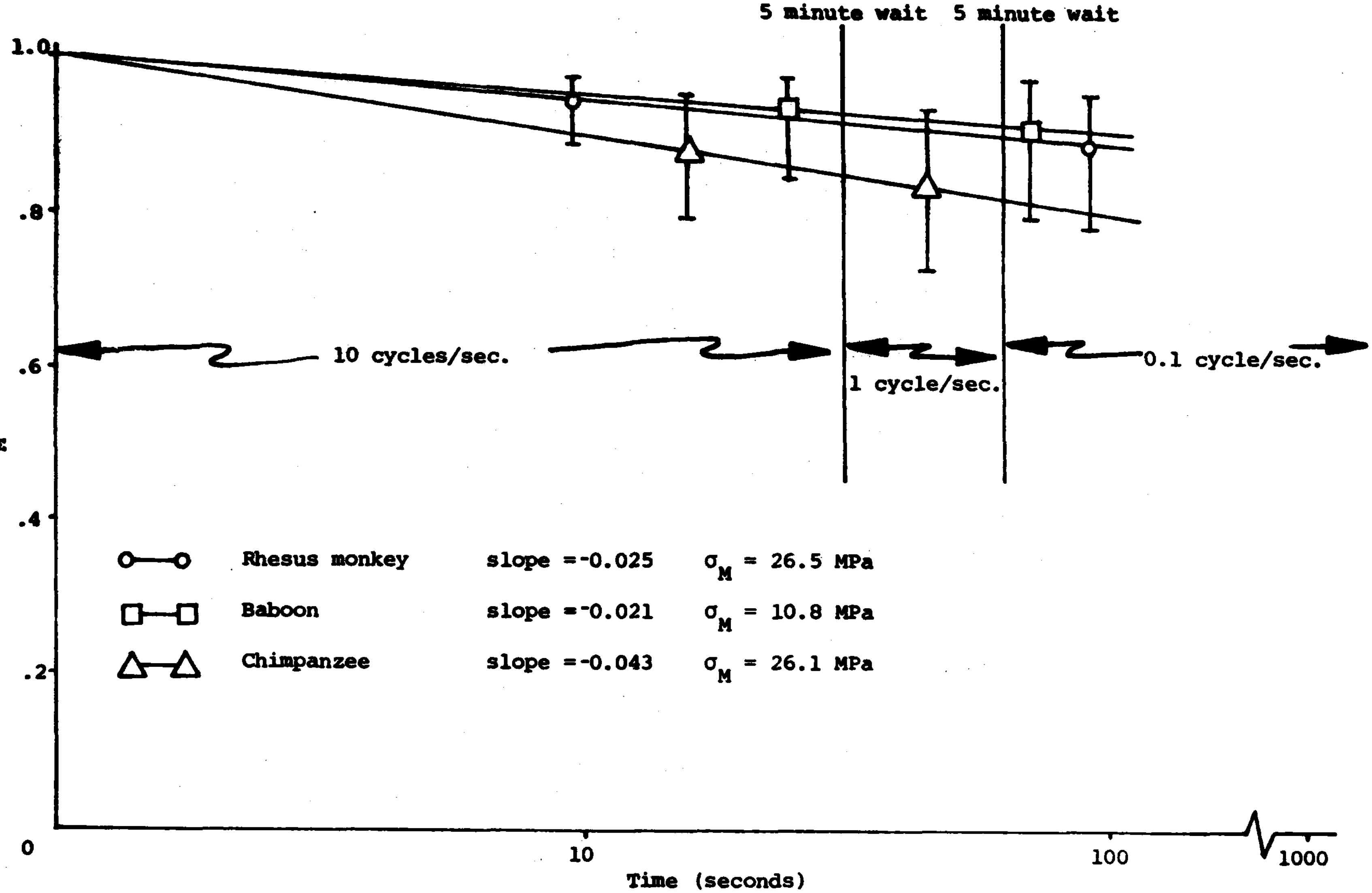

Figure 10: Average Cyclic Relaxation Curves for the Tendo-calcaneus Tendon

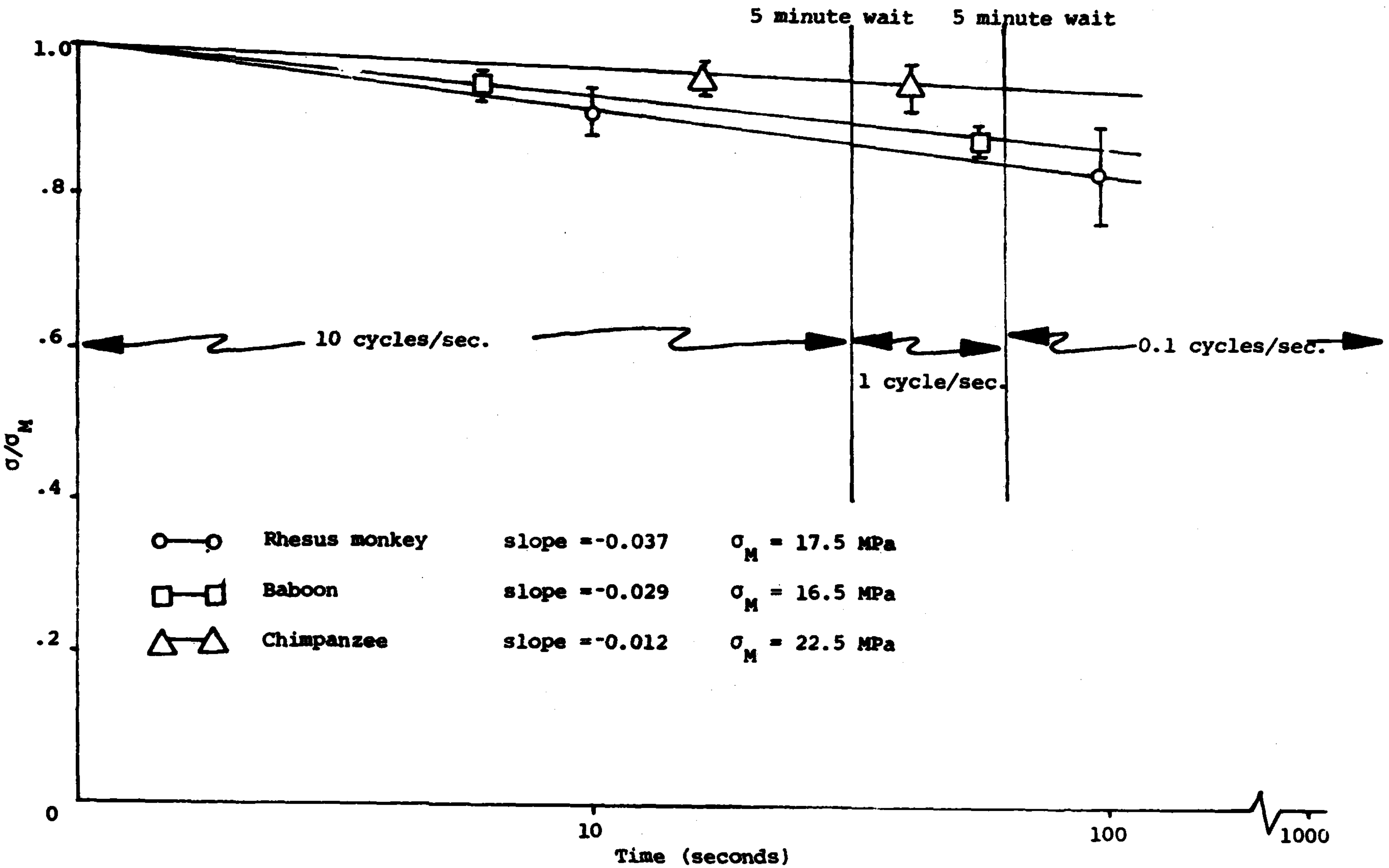

Figure 11: Average Cyclic Relaxation Curves for the Medial Collateral Ligament

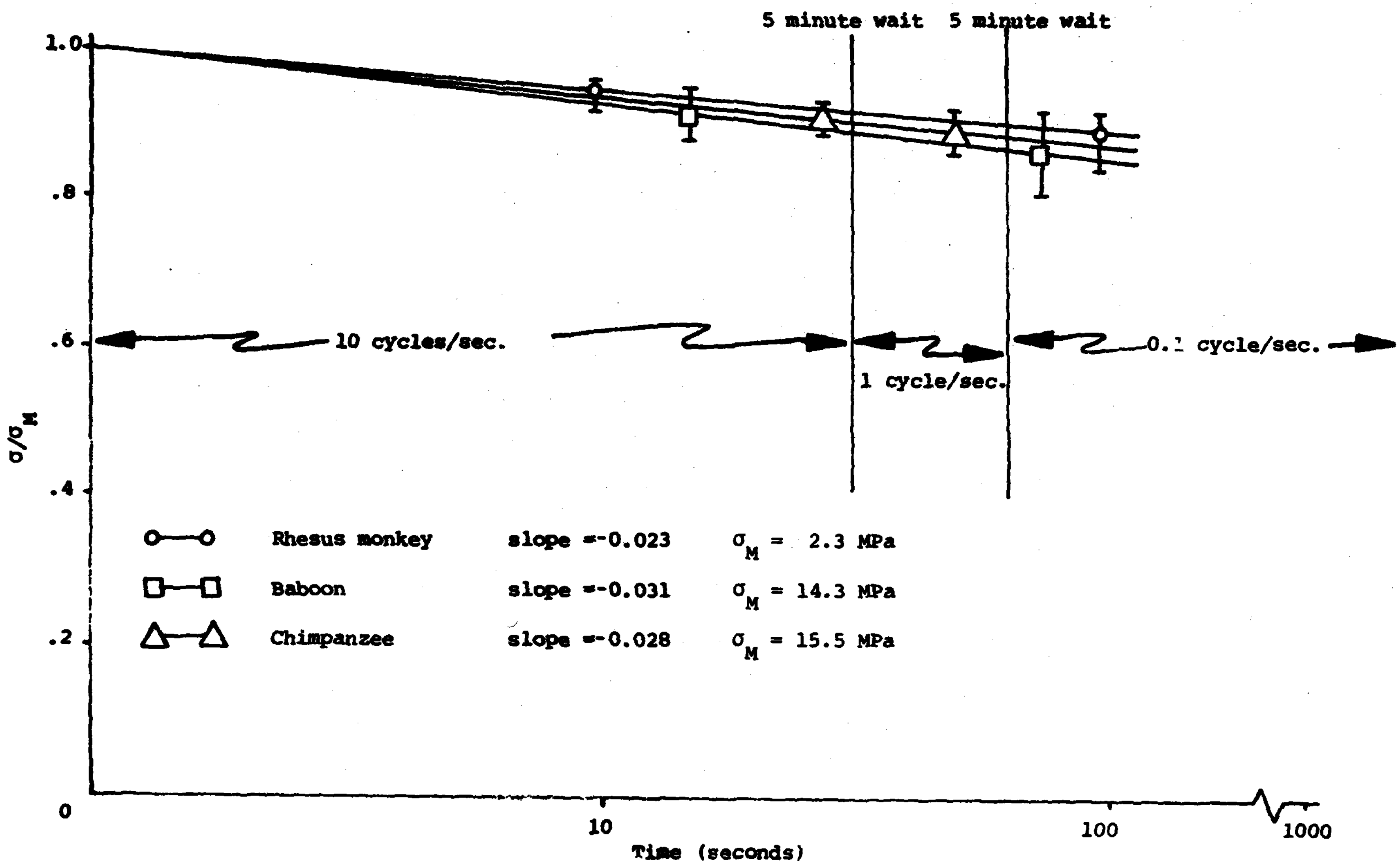

Figure 12: Average Cyclic Relaxation Curves for the Patellar Ligament

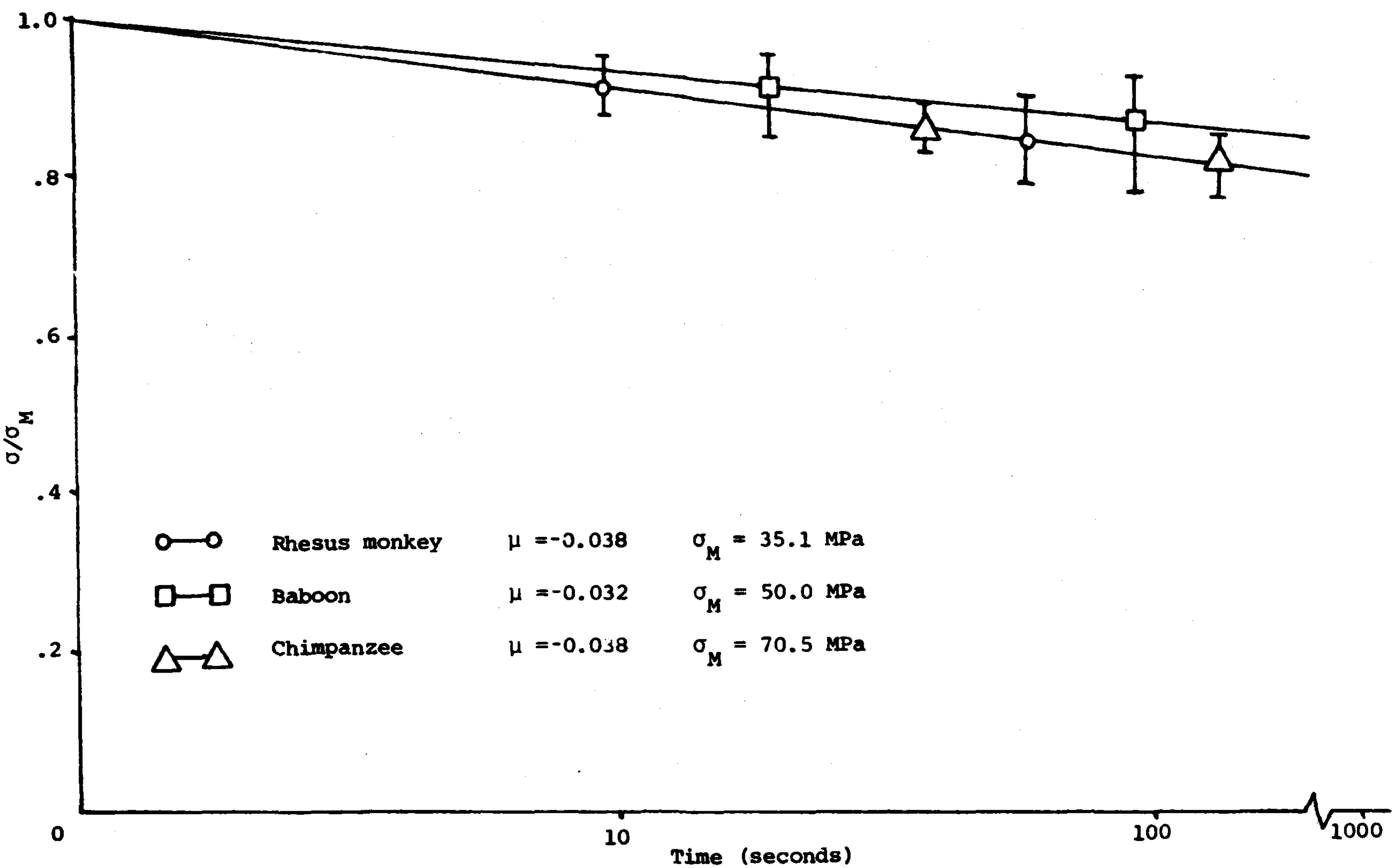

Figure 13: Average Standard Relaxation Curves for the Flexor Hallucis Longus Tendon

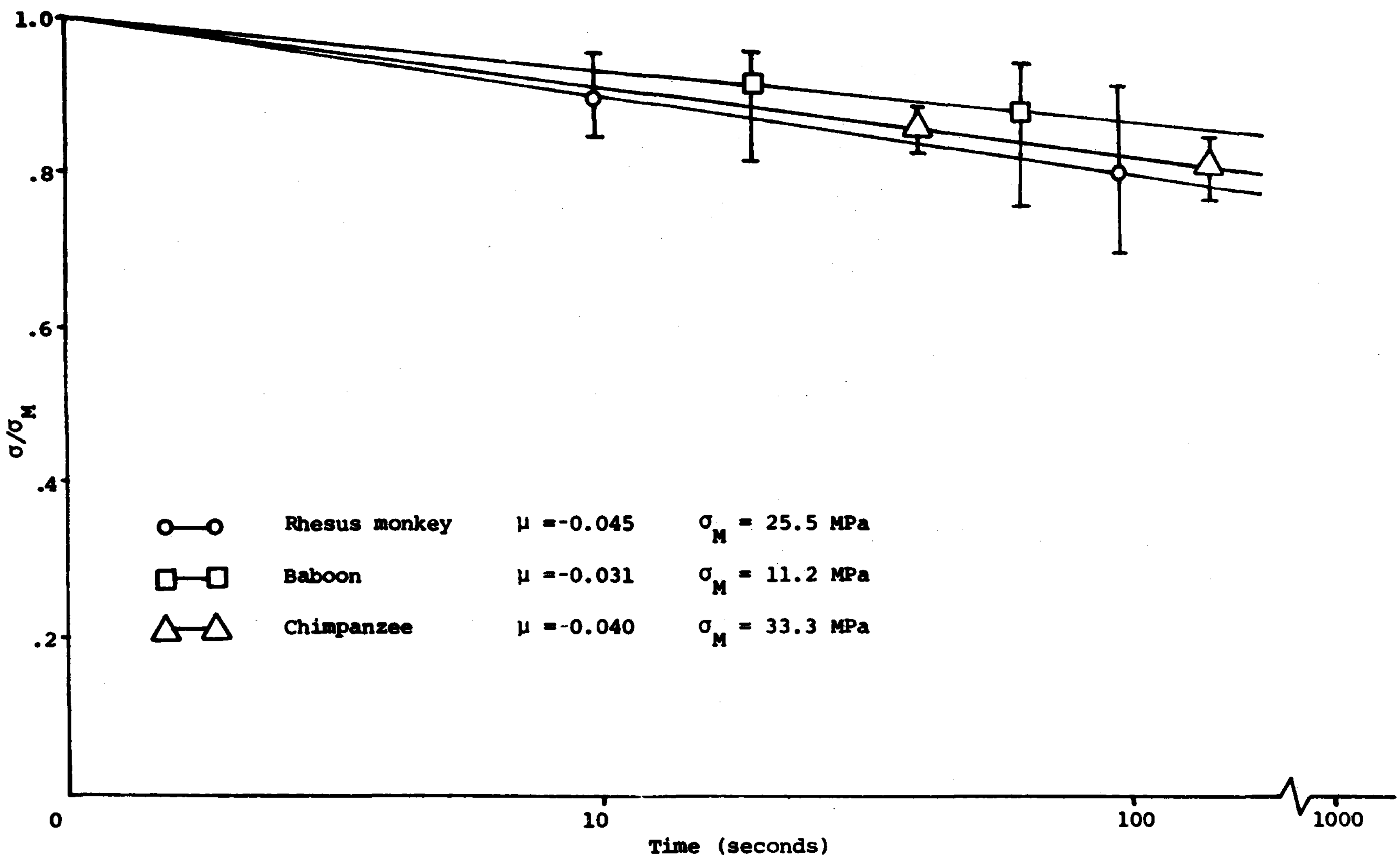

Figure 14: Average Standard Relaxation Curves for the Tendo-calcaneus Tendon

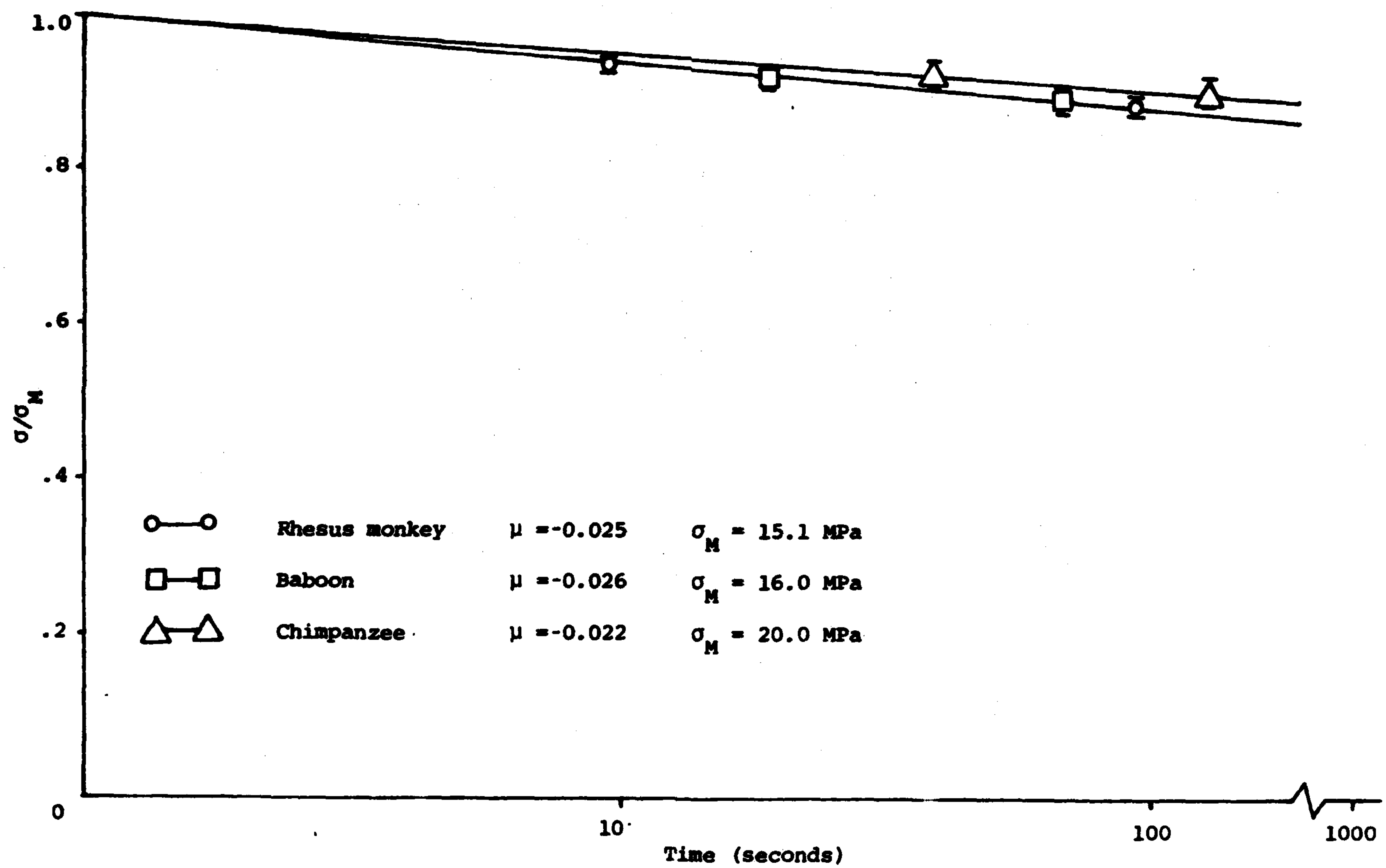

Figure 15: Average Standard Relaxation Curves for the Medial Collateral Ligament

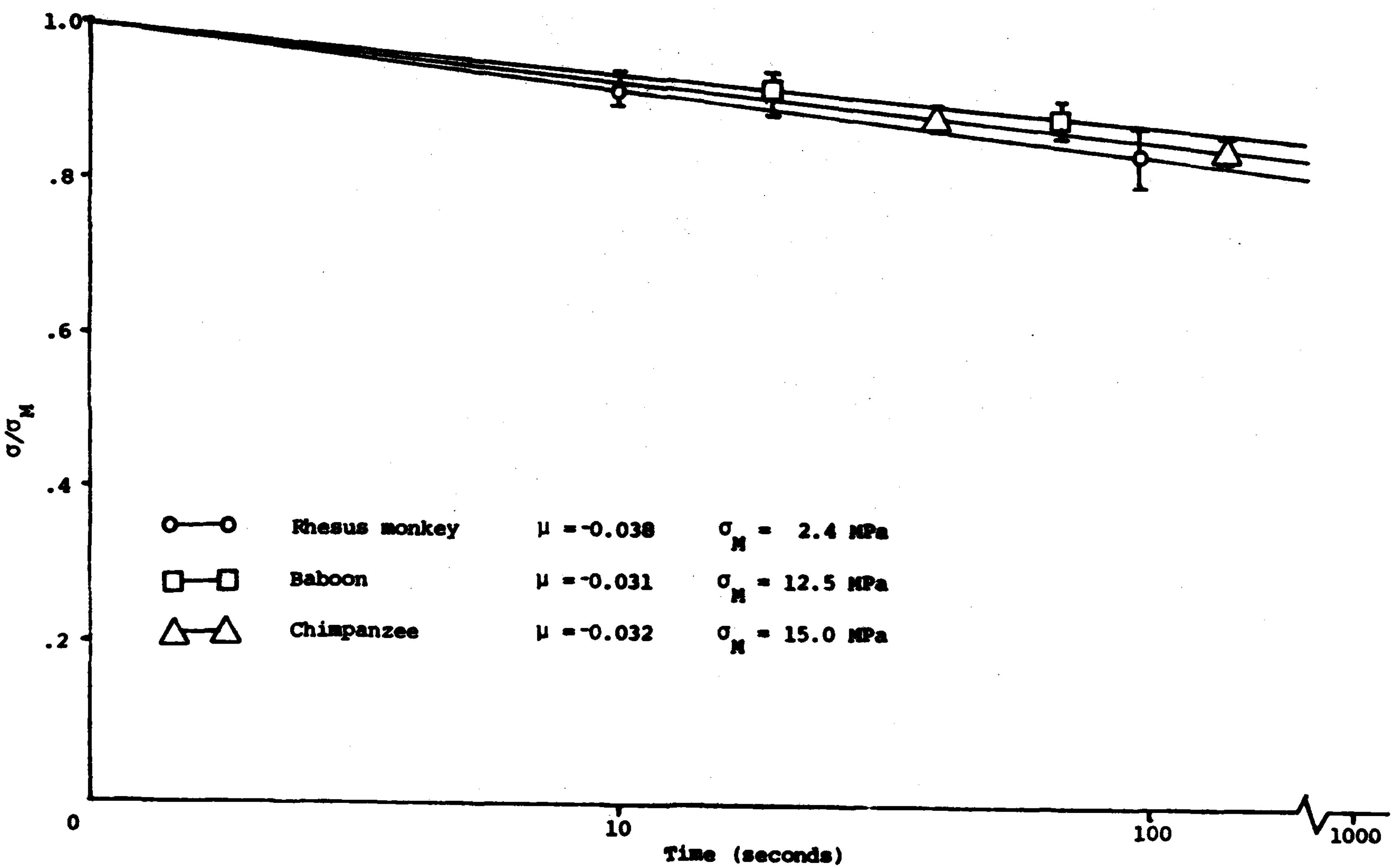

Figure 16: Average Standard Relaxation Curves for the Patellar Ligament

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