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RESEARCH PROJECT INITIATION

Date: July 23, 1974

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Dr. John W. Crenshaw
Biomedical Sciences Support Grant Committee
School of Biology
Campus

NOTE: Balance of Program funds (2,807) in B434

Assigned to: Textile Engineering

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RA-3 (6-71)

GEORGIA INSTITUTE OF TECHNOLOGY

OFFICE OF RESEARCH ADMINISTRATION

Post

RESEARCH PROJECT TERMINATION

Date: October 1, 1975

Project Title: Investigate Environmental-Mechanical Behavior of Elastin

Project No: E-27-624

Principal Investigator: Dr. J. L. Lundberg

Sponsor: DHEW/PHS - Biomedical Sciences Support Grant

Effective Termination Date: 5-31-75

Clearance of Accounting Charges: N/A

Grant/Contract Closeout Actions Remaining: None

Assigned to School of Textile Engineering

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Library, Technical Reports Section
Rich Electronic Computer Center
Photographic Laboratory
Terminated Project File No. _____
Other Dr. John W. Crenshaw, Jr.
File G-32-608

RA-4 (5/70)

E27-624

INVESTIGATIONS OF ENVIRONMENTAL-MECHANICAL BEHAVIOR OF ELASTIN

BY

DR. J. L. LUNDBERG
SCHOOL OF TEXTILE ENGINEERING

DR. B. R. LIVESAY
ENGINEERING EXPERIMENT STATION

GEORGIA INSTITUTE OF TECHNOLOGY

FINAL REPORT FOR THE PERIOD

1 JULY 1974 - 30 JUNE 1975

SUBMITTED TO THE

BIOMEDICAL SCIENCES SUPPORT GRANT COMMITTEE

AUGUST 8, 1975

Abstract

Investigations of the Environmental-Mechanical Behavior of Elastin

by

Dr. J. L. Lundberg
School of Textile Engineering

Dr. B. R. Livesay
Engineering Experiment Station

The mechanical behavior of elastin isolated from *bovine ligamentum nuchae* was investigated. The specimens were studied in the environment of physiological saline solutions at temperatures between 20°C and 40°C. The elastins investigated here are anisotropic, oriented, fibrous structures with well developed fibrils aligned approximately along the axes of the original *ligamenta nuchae*. The elastic constants and fracture behavior of these elastins was determined by stress strain measurements. Elastins in physiological saline solutions were found to be weak, soft, pliable materials. The stress-strain behavior to break is represented fairly well by the linearly elastic equation for incompressible, isotropic materials at finite strains. Stress-temperature coefficients of the elastins were positive with the stress increasing with temperature at constant strain. The temperature coefficients in physiological saline solutions was much greater than those predicted by the statistical theory of rubber elasticity. A mechano-chemical model should give the large, positive temperature coefficients of stress which are needed to fit the data. Elastins are viscoelastic as shown by hysteresis in the cyclical deformation experiments.

EXPERIMENTAL-MECHANICAL [illegible] OF ELASTIN

by

D. R. Livesay and J. L. Lundberg

Background

Elastins, the fibrous proteins in connective tissue in animals, usually are considered to be the most "elastic" of proteins. Elastins in various swelling media including water have been described as rubbers (1,2). Although the stress-strain-time responses of elastins are viscoelastic (1,2,3) and nonlinear (3), investigators have tried to carry out experiments so that they can fit long-time response to simple rubber elascitiy theory (1,2). This forced fit to rubber elasticity theory appears to be contrived at best. Indeed, were elastins rubbers, elastins probably could not serve in connective tissue. The objective of this investigation is to study with some care the stress-strain-time response of elastin and to begin to characterize its nonlinear viscoelastic behavior.

Experimental Methods

The elastins used in these studies were obtained from *bovine ligamenta nuchae*. The ligaments were provided by a meat packing firm.* The procedure used to isolate the elastin involved boiling the ligaments in water under pressure at 140°C for 24 hours. A dual razor blade slicer was used to prepare mechanical test specimens having cross-sections of 2-3 mm by 5 mm. The slices were made parallel to the fiber axis. Specimens were attached to fiberglass mounting tabs using Eastman 910 cement.

The bovine ligamentum nuchae material was kindly provided from freshly slaughtered cattle by Duffeys Boneless Beef Co. of Carrolton, GA. These ligaments are known as the "back strap" in the packing industry.

Mechanical [illegible] tests were conducted with specimens in the environment of [illegible]iological 0.85% saline solution. The temperature was increased by [illegible]ichrome wire coil emersed in the saline solution and chemically protected by teflon tubing. The temperature was lowered in an early arrangement by the addition of ice to the solution and later by a closed circuit cooling water circulating system. Special fixtures were assembled to adapt two instrument systems of the Micromechanics Laboratory for the mechanical tests conducted in these investigations. The tensile axis of a test specimen is horizontal for both of these mechanisms, making it convenient to submerge a specimen in a liquid for the mechanical tests. Stress-strain and stress-temperature characteristics were measured using the screw-driven test machine arrangement shown in Figure 1. Strain-temperature, strain-time (creep) and a few stress-strain measurements were conducted using the apparatus shown in Figures 2 and 3. A few torsion measurements were also made on exposed elastin specimens using the arrangement shown in Figure 4.

The screw-driven test machine shown in Figure 1 is a versatile mechanical test apparatus which is adpatable for mechanical testing of specimens ranging from integrated circuit bond wires to bulk copper crystals. A force transducer having a capacity of 450 grams was most suitable for the measurements here. A differential transformer was used for recording the specimen displacement. A pyrex dish containing the saline solution could be raised or lowered for submersion of the specimen and removed for the mounting of new specimens. The heater was supplied by a current regulated power supply and the temperature of the solution was read using a thermister probe having an analog output. A stirrer maintained uniform temperature throughout the solution.

Stress strain measurements were made by driving the stage at one of two speeds, 0.025 inch/min. or 0.25 inch/min., with the temperature stabilized at a desired value. The stress-temperature measurements were made after first extending the specimen so that a desired initial stress was obtained. With the strain maintained constant, the temperature was uniformly raised and lowered. The data was recorded on an X-Y-plotter.

The strain-temperature measurements were carried out by modifying a microtensile device designed for measurements involving relatively small specimen displacements. It turned out to be particularly convenient for recording strain-temperature behavior at constant stress and for creep measurements. This apparatus, shown in Figures 2 and 3, employs a force coil suspended in a radial magnetic field to apply stress to the specimen and a free core differential transformer to measure specimen elongation. The force coil, differential transformer core and one specimen mount are all suspended from a friction free arrangement of tungsten fibers. The saline bath for this system employed teflon protected nicrome wires for heating and stainless steel tubing through which water was circulated in a closed circuit cooling system. This apparatus was specifically designed to measure small strains for specimens where usual extensiometers would adversely influence the stress strain measurements. Although the net strains encountered for our elastin materials were relatively large, the sensitivity of the apparatus for changes in strain relative to temperature at constant stress and also to time after stressing was useful. In addition, a few stress-strain curves were plotted with this apparatus on a stepped basis by moving the fixed post supporting the specimen mounting bracket.

A few measurements were conducted on elastin specimens stressed in torsion but with the saline solution applied with an eye-dropper. The

experimental arrangement used here is shown in Figure 4. An environmental system corresponding to those used with the other two instruments could be fabricated for this apparatus as well but this effort was not made due to the additional complexity coupled with instrument range limitations and available time. The aorta of a monkey species *macaca mulatta* was obtained through the courtesy of Dr. Harold McClure of the Yerkes Primate Research Center. Mechanical property tests were conducted on specimens prepared from this material to compare with the corresponding elastin measurements. The specimen mounting configuration shown in Figure 5 was used for these tests in addition to cementing specimen ends with Eastman 910. A cross section of the aorta was simply slipped over the cylinders like a small rubber band.

Results

A typical stress strain curve for elastin stressed in tension in a physiological saline solution is illustrated in Figure 6. The upper curve shows the small hysteresis which always occured with cycling and the lower curve shows the same specimen then pulled to fracture. Figure 7 shows another specimen pulled to failure but the separation was not abrupt. The specimens of Figures 6 and 7 were at 37^{o}C while stressed. The extended failure shown in Figure 7 was not uncommon. Some specimens supported a significant load for displacement extending considerably beyond the point of failure initiation. Fracture surfaces were not at all smooth but were characterized by relatively massive jagged regions slipping past each other. The relatively low fracture stresses and the appearance of the fracture surfaces indicate that failure was not due to elastin fiber fracture as much as by the lack of strong connective material coupling the fibers. The unprocessed ligament had much greater strength.

The curves of Figure 8 illustrate the magnitude of the energy loss per cycle as a function of the number of cycles stressed. The energy loss during first cycle stressed was always noted to be significantly greater than that which occurred in subsequent cycles. As seen in the lower curve of Figure 8, the energy loss per cycle approached a minimum value after a few cycles.

The stress rates used for the stress-strain measurements were determined by an elongation of either 0.025 inch/min. or 0.25 inch/min. It was carefully established that the shape of a stress-strain curve was not significantly influenced by the particular choice of drive speed employed. Creep of these materials was also found to be insignificant at loads sufficiently removed from fracture stresses to insure that failure was not occurring.

A series of elastin specimens were stressed to failure at different temperatures between 20°C and 40°C. A temperature dependence of fracture strength could not be developed from this data. However, careful measurements on single specimens stressed to a value about half the expected fracture stress showed that the elastic modulus increases slightly with temperature over this range.

The temperature dependence of the stress of elastin specimens held at a constant strain value was studied. A specimen was initially pulled to desired stress and strain values. The temperature of the saline solution was then slowly varied from 20°C to 40°C and then back to 20°C. As seen in Figure 9 the stress increases significantly as the temperature increases. A few specimens fractured from the temperature induced stress increase so that sufficiently small low temperature initial stress values were selected to avoid this problem. The hysteresis associated with temperature cycling is seen in these curves. Generally, the first few temperature cycles were characterized by open curves. However, after about

five cycles the stress-temperature curves usually formed almost closed loops as shown in Figure 10. Figures 9 and 10 show the fourth and fifth cycles, respectively, of the same specimen.

Measurements of strain-temperature characteristics for constant stress were carried out on the electromagnetic loaded microtensile device. The behavior of these curves was consistent with that observed above. However, after several cycles some of these specimens demonstrated no hysteresis as shown in Figure 11. This curve was reproduced on subsequent temperature cycles and was fitted by the expression:

$$\Delta L = - 110 \ln \Delta T + 420$$

Similar strain-temperature curves at constant stress on the unprocessed *ligamenta nuchae* also contracted with increased temperature but the magnitude of the negative elongation was much smaller than that obtained for the pure elastin.

Stress-strain curves on a monkey aorta had a very different behavior as seen in the hysteresis loop of Figure 12 and the subsequent stress to failure shown in Figure 13. The strain temperature curve shown in Figure 14 for a specimen from the aorta demonstrated an opposite temperature behavior to that found from the *bovine ligamenta nuchae*. The aorta should have a much smaller elastin content. Torsion measurements made on a few elastin specimens yielded a linear torsional stress-strain curve. These specimens were too large to stress to failure on the microtorsion apparatus. They also were not submerged in the saline solution but were wetted by an eye-dropper so that these particular data were not considered significant.

Discussion

The elastins investigated in this study are anisotropic, oriented, fibrous structures with well developed fibrils aligned approximately along the axes of the *bovine ligamenta nuchae* from which the elastins were isolated (see Figure 15). Thus, for purposes of interpreting results and comparisons with other studies, we must consider elastins to be complicated, composite structures. This is consistent with recent investigations of the separation and characterization of elastic fibers (4).

The compositions, structures, morphologies, and properties of elastins depend upon sources and methods used for separating other materials (5). The method for separating collagens, mucopolysaccharides, and other materials in "ground substance" from elastins used in these studies, by hydrolysis with water at 140°C for 24 hours, is one of the more drastic methods. Therefore, the elastins used in this study probably are as pure as those used by other investigators (2). Of course, we must expect that the separation methods must affect to some degree the stress-strain-time behavior of the specimens.

The stress-strain curves for elastins measured at constant strain rates of of $7 \cdot 10^{-3}$ and $7 \cdot 10^{-4} sec^{-1}$ are not far from neo-Hookean in their behavior. Curves are concave toward the strain axis reminiscient of the equation for the restoring force, f, of an incompressible, isotropic material with modulus of elasticity, E, (6a)

$$f = \frac{E}{3}\left(\alpha - \frac{1}{\alpha^2}\right) = \frac{E}{3}\left[1 + \varepsilon - \frac{1}{(1+\varepsilon)^2}\right] \qquad (1)$$

where $\alpha \equiv$ extension ratio

$\varepsilon \equiv$ strain.

The restoring forces as functions of strain are low and are of reasonable magnitude compared to those predicted by the simplest, statistical theory of elasticity for isotropic rubbers. The restoring force given by statistical theory of rubber elasticity is (6b)

$$f = kTN\left(\alpha - \frac{1}{\alpha^2}\right) = kTN\left[1 + \varepsilon - \frac{1}{(1+\varepsilon)^2}\right] \qquad (2)$$

where k ≡ Planck - Boltzmann constant,

T ≡ absolute temperature,

N ≡ number of molecular segments between crosslinks per unit volume.

The magnitudes of N calculated from stress-strain curves are reasonable, of the order of 10^{-5} to 10^{-4} g·mol/cc corresponding to segment molecular weights of 10^5 to 10^4 g/mol. Segments of this size and weight are large enough to satisfy the requirement of liquid-like mobility of molecular segments between crosslinks in the statistical theory of rubber elasticity.

The average stress at break, $(1.6 \pm 0.4)\cdot 10^6$ dyn/cm^2, and the average elongation at break, $\varepsilon = 0.54 \pm 0.11$, are low compared to ordinary rubbers. These breaking strengths, 23 ± 6 psi or $(1.2 \pm 0.4)\cdot 10^{-3}$ g/denier, are characteristic of weak materials. In tension, the elastins seem to fail by tearing and separation of fibrils. The materials removed from *ligamentum nuchae* contribute substantially to its strength. Minns and colleagues have demonstrated this (7). The elastins used in this study are disappointingly weak.

The initial slopes of the stress-strain curves, which are frequently and often incorrectly called initial moduli of elasticity, are low in value, about $(5.1 \pm 0.8)\cdot 10^6$ dyn/cm^2 (73 ± 12 psi or $(3.8 \pm 0.6)\cdot 10^{-3}$ g/denier). Such low slopes are characteristic of soft, pliable materials. Using initial slope data for E in equation 1 and the average strain at break of $\varepsilon = 0.54$, the stress at break calculatied by equation 1 is

$f = 1.9 \cdot 10^6$ dyn/cm^2 which is within 20 percent of the observed average stress at break. Because failure occurs at relatively low extensions, equation 1 for the restoring force of an incompressible, isotropic material represents stress-strain behavior quite well. No recourse to rubber elasticity is necessary to explain stress-strain diagrams for our elastins.

The stress-temperature coefficients of elastins are positive; stress increase with temperature at constant strains. This observation has led investigators to regard elastins as rubbers (1,2). Evident in descriptions of earlier experiments is that elastins are not simply rubbers; they behave like a viscoelastic material requiring waits for relatively long periods to obtain stable stress-strain data. Stress-temperature coefficients at constant strains for elastins reported by Flory and Hoeve (1) and by Ciferi and colleagues (2) are reasonable in magnitude for rubbers as predicted by equation 2. These investigators immersed elastins in water ethylene glycol, water-glycol mixtures, and dimethyl sulfoxide.

Our rather careful measurements of stress at constant strain as elastins immersed in saline solution is slowly warmed and cooled in cycles over two or more hours show (1) that stress rises with temperature with slope decreasing with increasing temperature, (2) that stress falls sufficiently rapidly with decreasing temperature that a hysteresis loop is formed, and (3) that the stress-temperature hysteresis loop is closed only after several stress-temperature cycles have been completed. Further, our stress-temperature coefficients are about 100 times greater than that predicted by equation 2 at 20°C, about 16 times greater at 30°C, and about four times greater at 40°C. This indicates that simple,

statistical theory of rubber elasticity is inadequate for elastins in saline solutions, media similar to those in which they function in nature.

At constant stress, strain ε decreased as temperature increased, this approximately in accord with the empirical equation

$$\varepsilon = - A \ln T + B \qquad (3)$$

where A and B are constants.

We attach no significance to this equation except to observe that equation 3 is in accord with the principle of corresponding states.

In cyclical extension, hysteresis loss was observed. As we would expect, hysteresis decreased on successive cycling. At constant stress, creep was negligible over periods of 10^4 seconds. Similarly at constant strain, stress relaxation was negligible over like periods.

Our results are different from those reported to date: First, we carried out mechanical measurements on elastins in physiological saline solutions. Second, in these solutions, we found negligible creep and relaxation. Third, slopes of stress-strain curves, breaking strengths, and elongations at break are relatively low characteristic of weak, pliable materials. Fourth, temperature coefficients of stress at constant strain are much greater than can be predicted from the statistical theory of rubber elasticity. Fifth, elastins are viscoelastic; in cyclical extension and cycling with temperature at constant strain and at constant stress, hysteresis loops are obtained.

Measurements of strain as a function of temperature for *ligamentum nuchae* showed smaller decreases in strain with increasing temperature than did elastins. The strain at constant stress of an aorta of a monkey increases with temperature. Also the stress-strain curve at constant rate of extension is concave toward the stress axis for bands cut from the aorta of a monkey. This shows that as elastin content decreases

the stress-strain behavior of elastins is masked by the behavior of other components in these complex composite structures.

Conclusions

Elastins in physiological saline solutions are weak, soft, pliable materials.

The stress-strain behavior to break for elastins is represented fairly well by the linearly elastic equation for incompressible, isotropic materials at finite strains.

The temperature coefficients of stress at constant strain for elastins in physiological saline solutions are much greater than those predicted by the statistical theory of rubber elasticity.

Elastins are not rubbers in saline solutions. A mechano-chemical model can give the large, positive temperature coefficients of stress which are needed to satisfy observations.

Elastins are viscoelastic as shown by hysteresis in cyclic experiments.

Elastins are not isotropic, amorphous or rubberlike materials. They are fibrous, anisotropic, oriented, composite structures with fibrils somewhat aligned parallel to the axes of the *ligamenta nuchae* from which the elastins were extracted.

Relatively pure elastins and the composite structures to which elastins contribute should be investigated in some detail with measurements of stress-strain-time behavior and structure and morphology. We intend to focus our attentions on mechanical properties.

Projections

The work under this program accomplished a primary goal of establishing that the cooperating laboratories have the capability for making significant technical contributions in research areas involving the mechanical behavior of biological materials. The data generated on this program will be refined for publication. We feel that the results of this work are sufficiently significant to serve as a foundation for the preparation of a proposal to NIH for a much more comprehensive program.

Acknowledgments

The authors wish to express their sincere appreciation to the Committee on Biomechanical Sciences Support Grants for providing the funds which made this work possible. We also wish to acknowledge the assistance of Mr. Kenneston Carr, Mr. Robert Newsom and Miss Connie Livesay in the mechanical property measurements and of Mr. Frank Ko for isolating the elastin specimen materials.

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3. Unpublished work of F. K. Ko, B. R. Livesay and J. L. Lundberg.

4. Ross, R. and P. Bornstein, J. Cell Biology 40, 366 (1969) Sci. Am., June 1971, p. 44.

5. Steven, F. S., R. J. Minns and H. Thomas, Connective Tissue Res. 2, 85 (1974).

(6a). See for example: Ward, I. M., Mechanical Properties of Solid Polymers, (Wiley-Interscience, London, 1971), p. 40.

(6b). Reference 6a, p. 69.

7. Minns, R. J., P. D. Soden and D. S. Jackson, J. Biomechanics 6, 153 (1973).

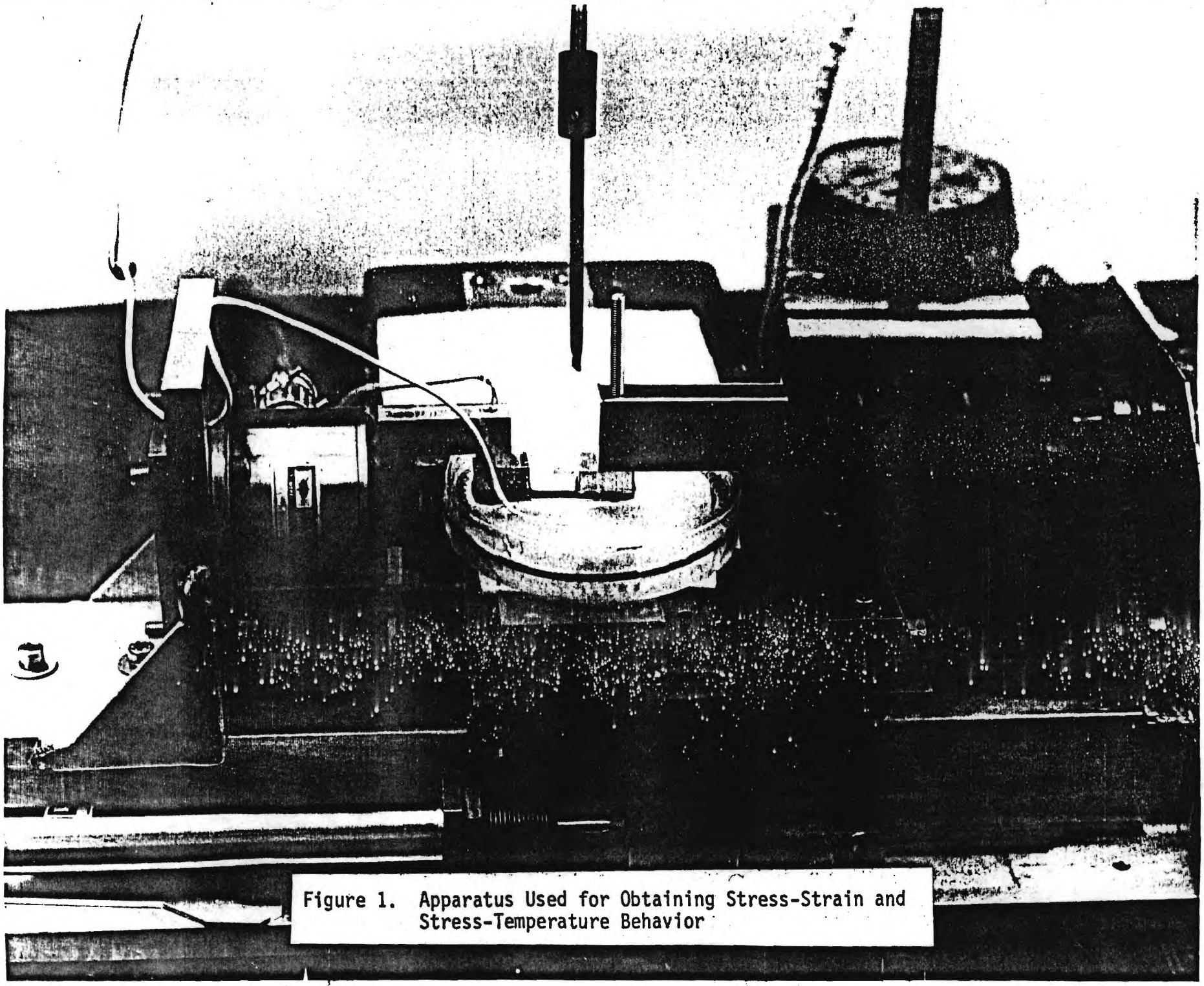


Figure 1. Apparatus Used for Obtaining Stress-Strain and Stress-Temperature Behavior

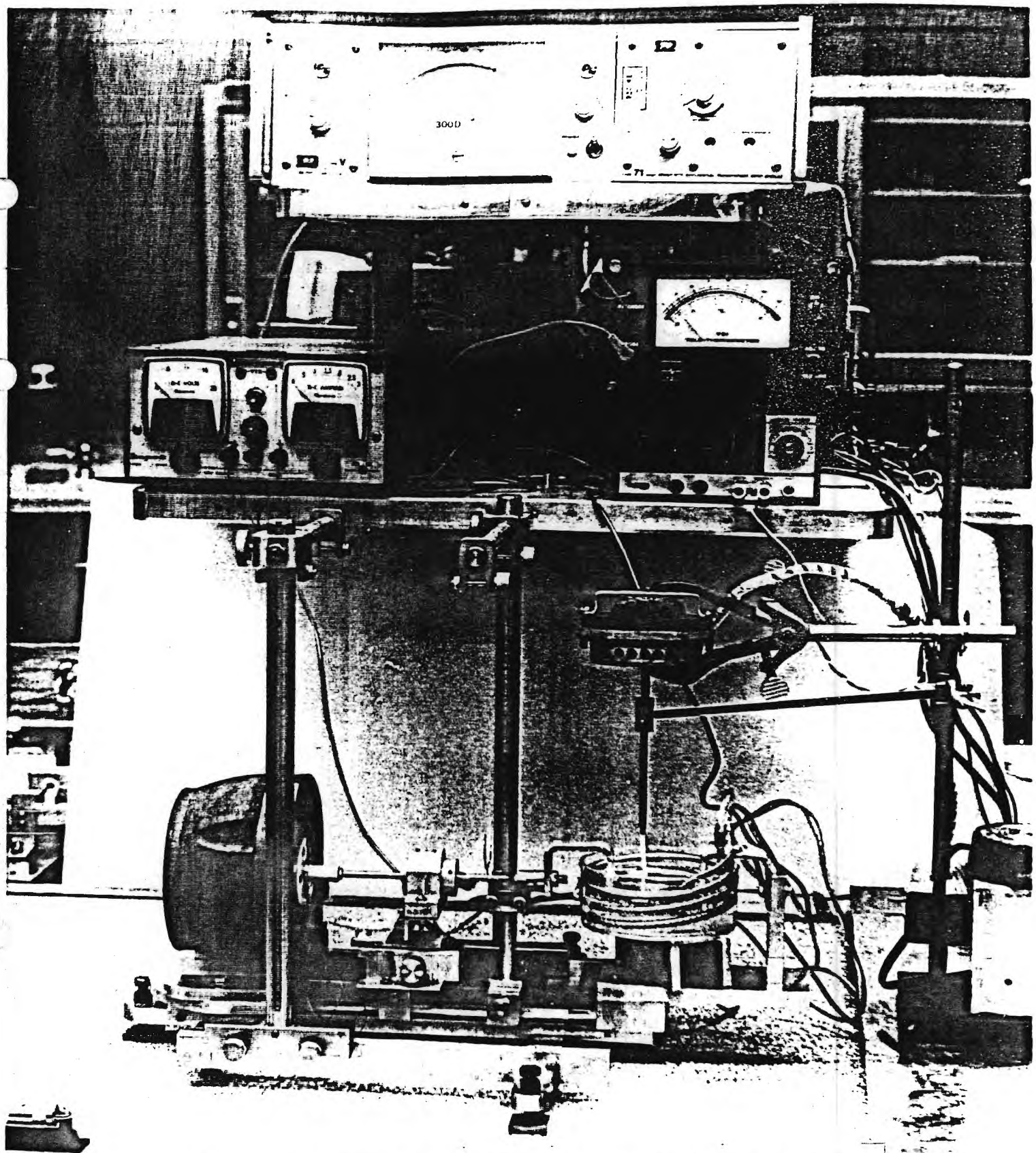


Figure 2. Apparatus Used for Obtaining Strain-Temperature and Creep Behavior

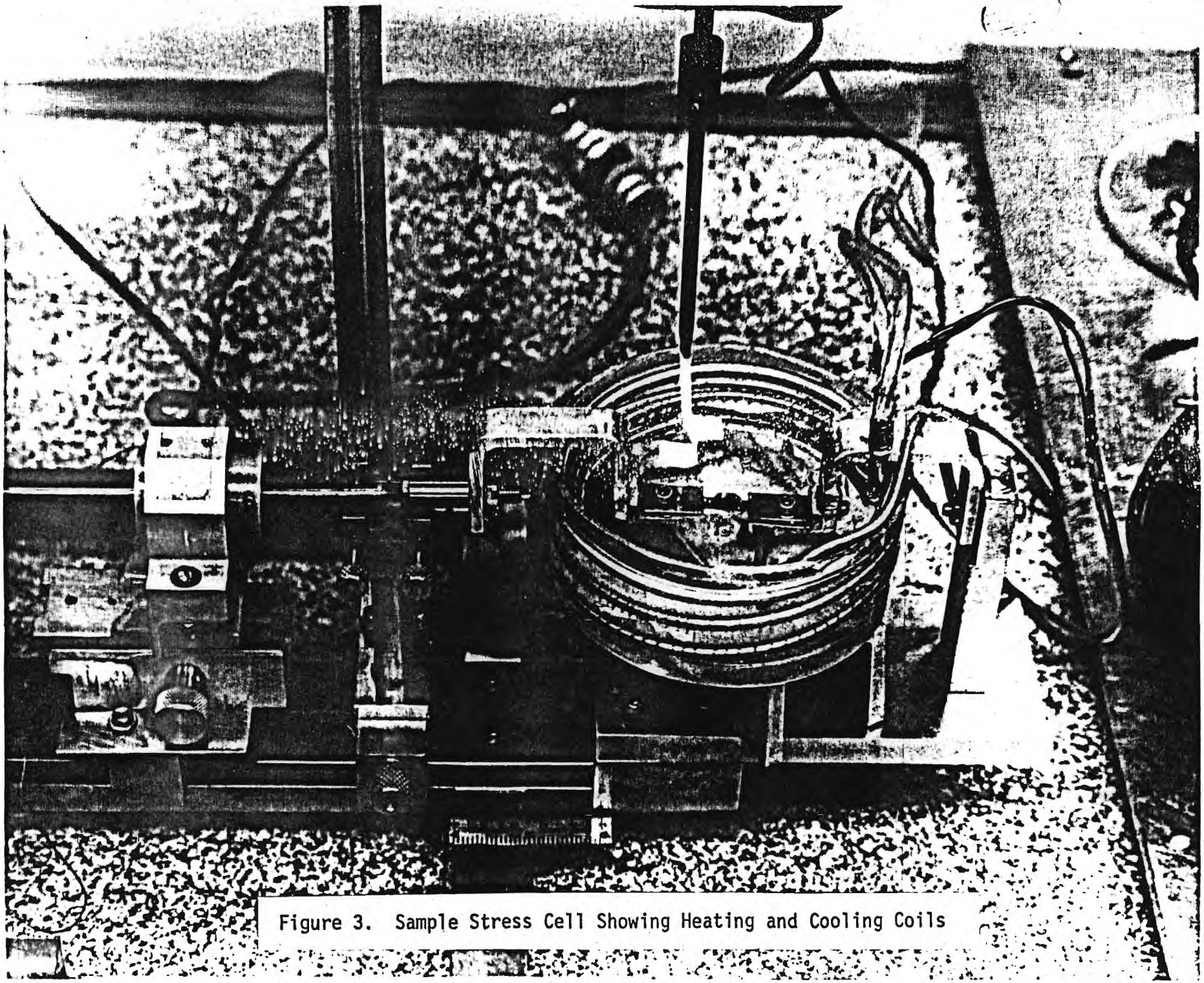


Figure 3. Sample Stress Cell Showing Heating and Cooling Coils

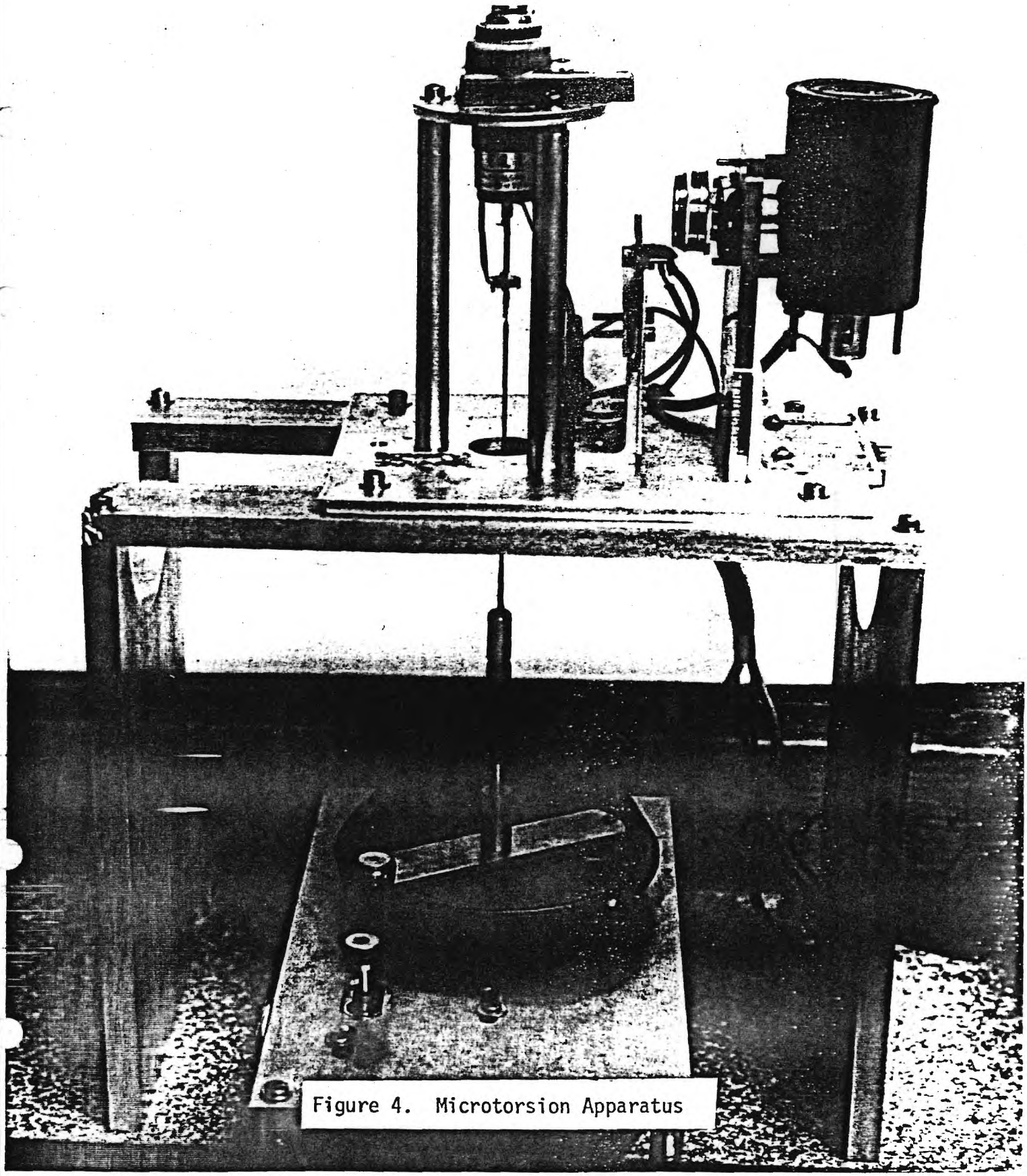


Figure 4. Microtorsion Apparatus

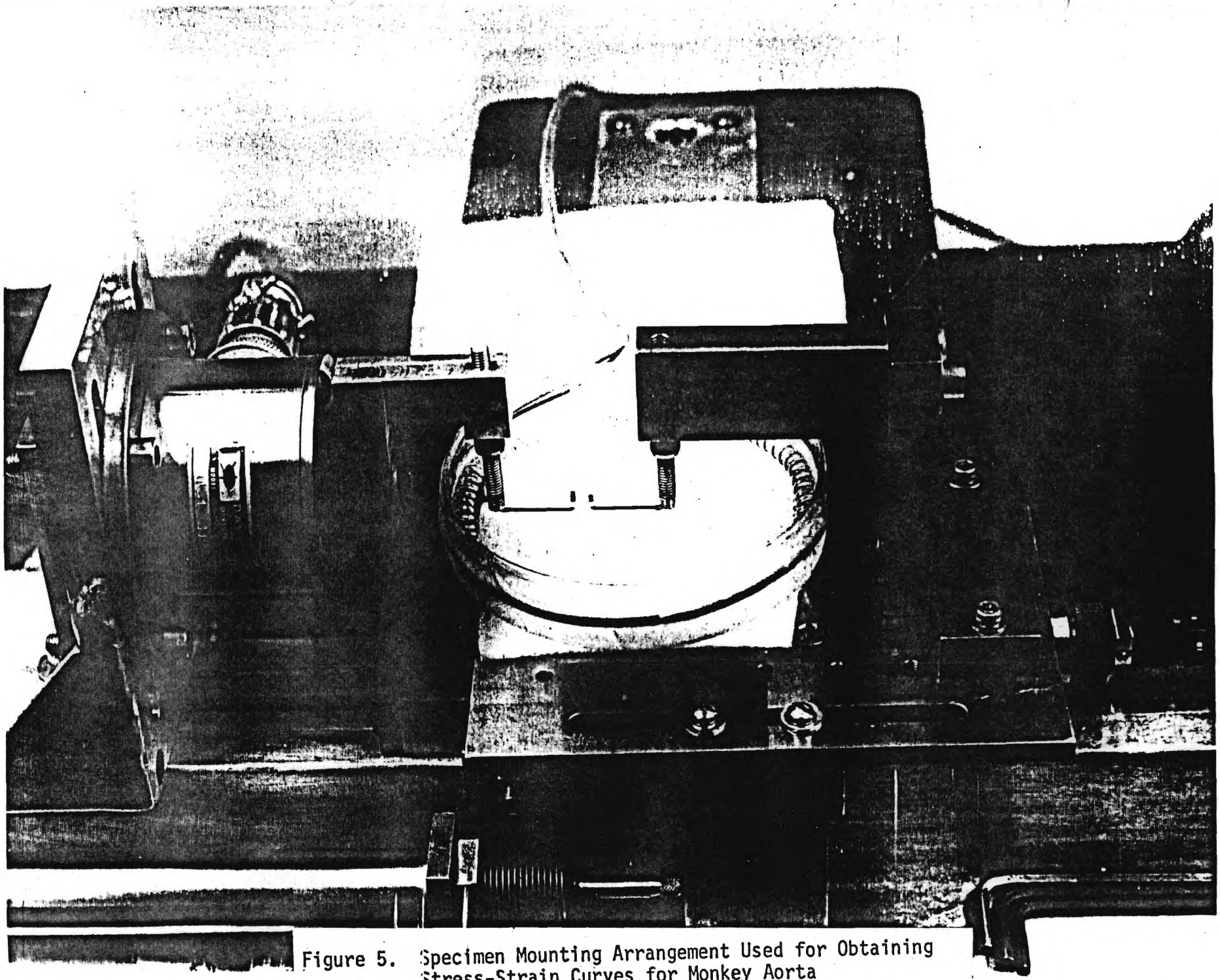


Figure 5. Specimen Mounting Arrangement Used for Obtaining Stress-Strain Curves for Monkey Aorta

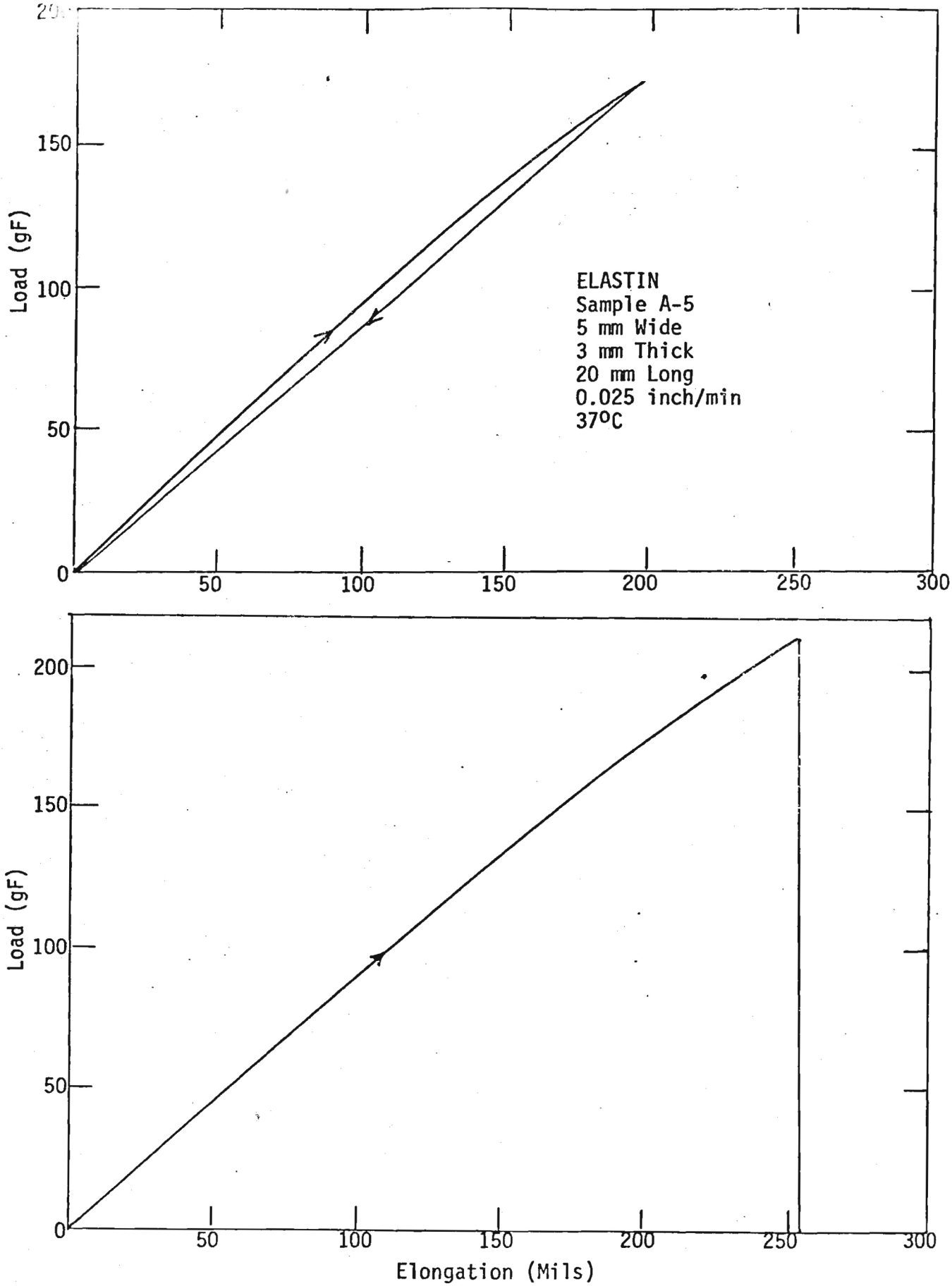


Figure 6. Stress-Strain Curves for Elastin Specimen

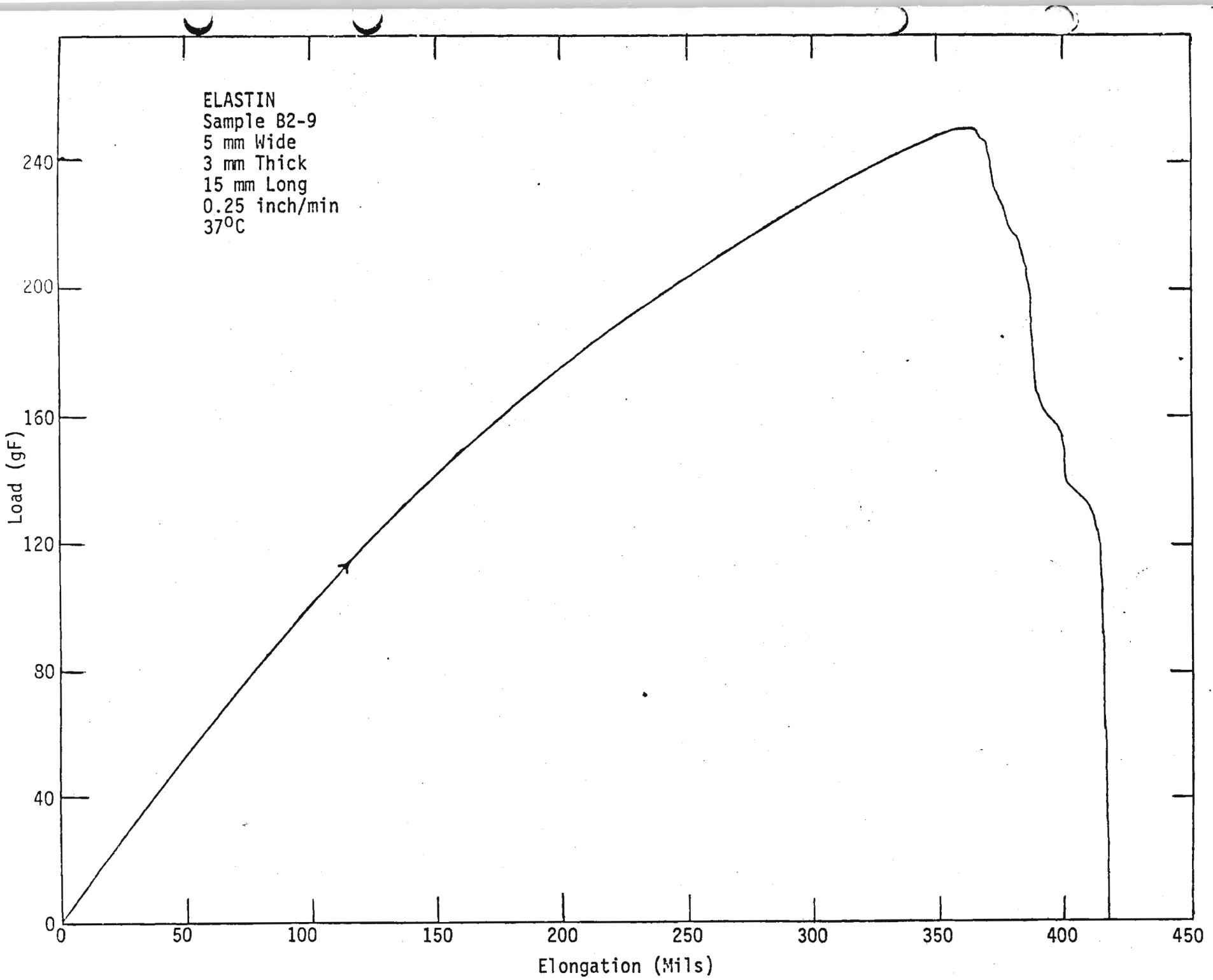
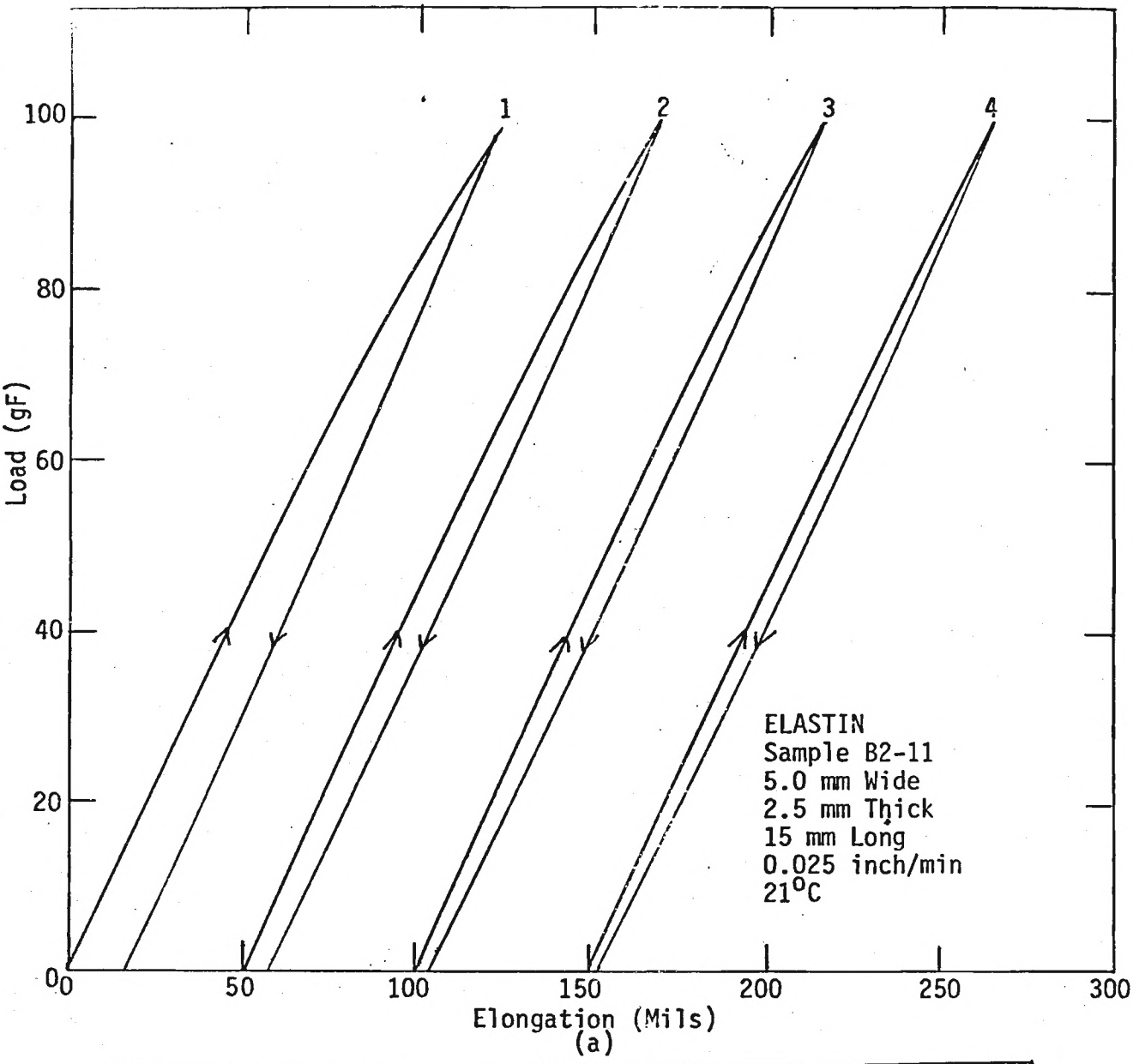


Figure 7. Stress-Strain Curve to Fracture for Elastin Specimen Stressed in Tension



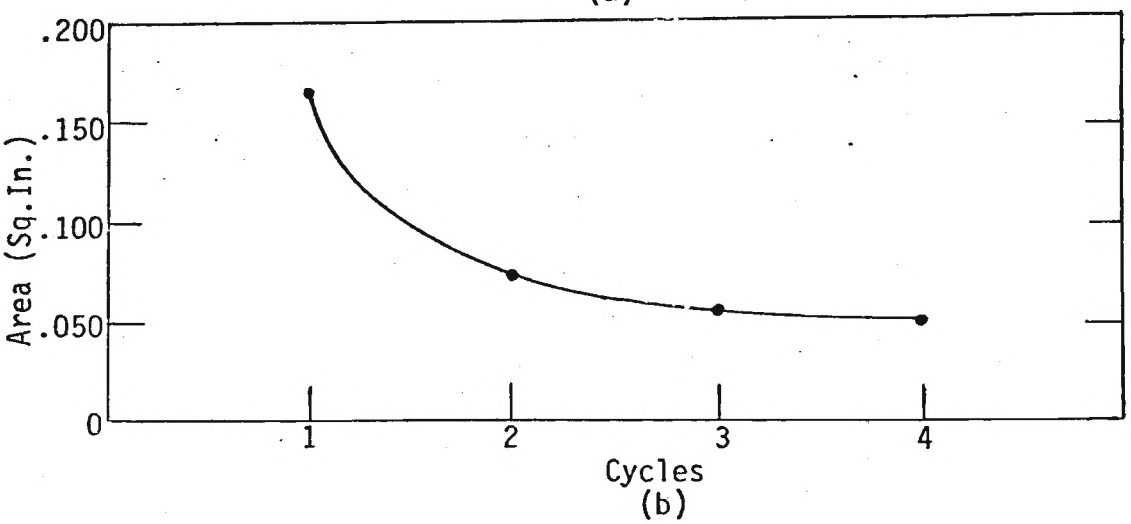


Figure 8. (a) Successive Stress-Strain Curves for Elastin Specimen
(b) Hysteresis Loss per Cycle vs Number of Cycles

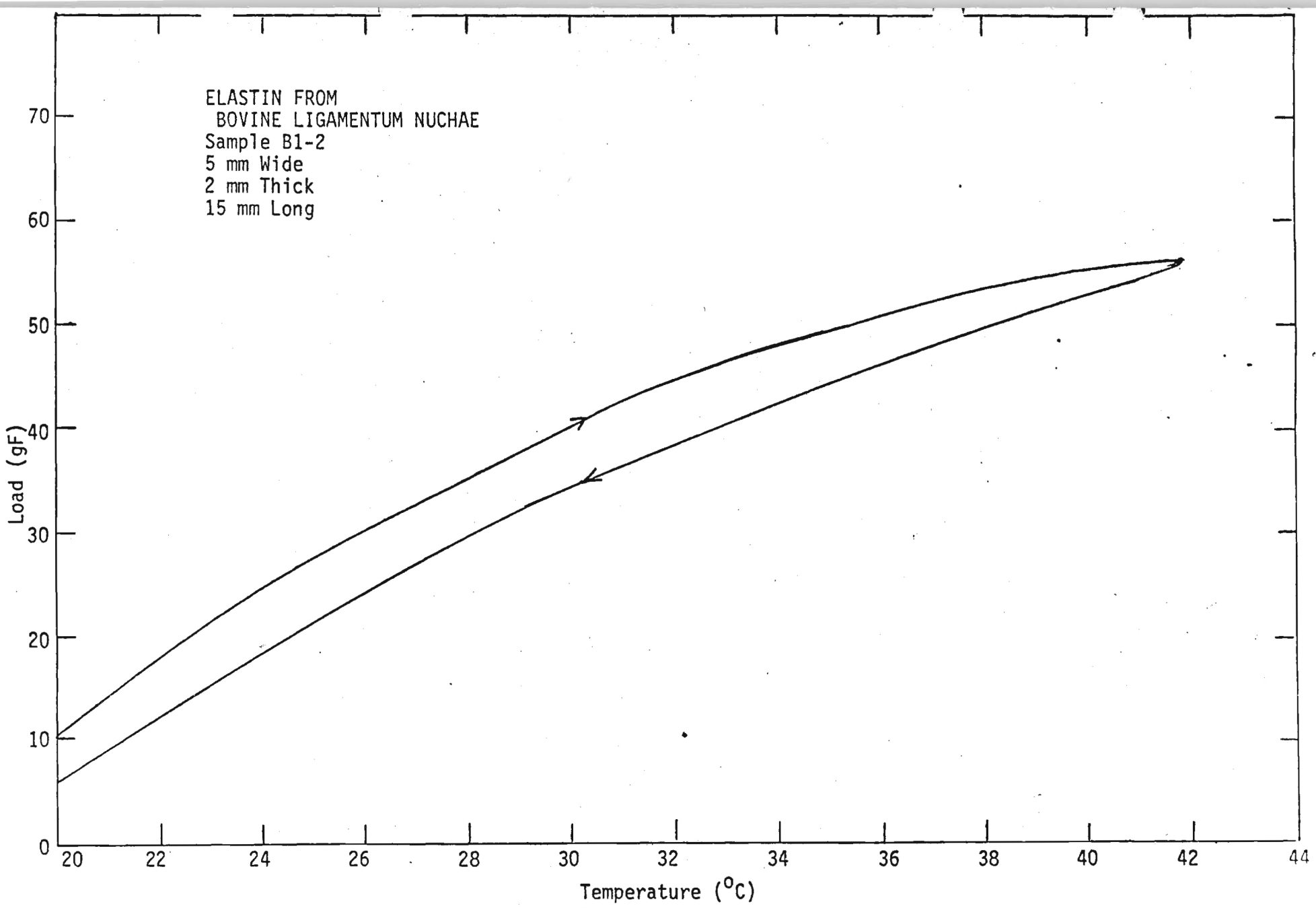


Figure 9. Stress-Temperature Curve for Elastin at Constant Strain (Fourth Cycle)

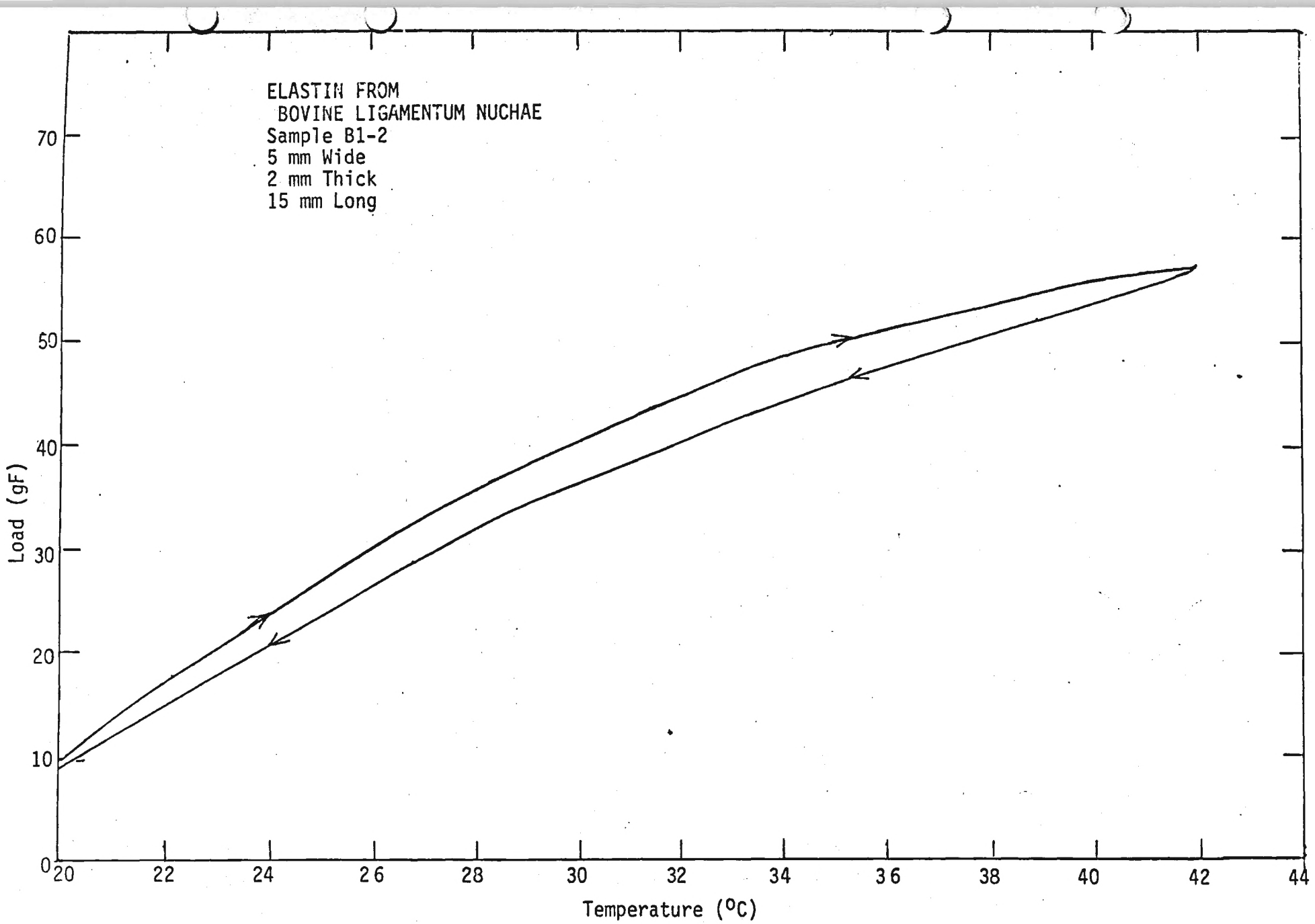


Figure 10. Stress-Temperature Curve for Elastin at Constant Strain (Fifth Cycle)

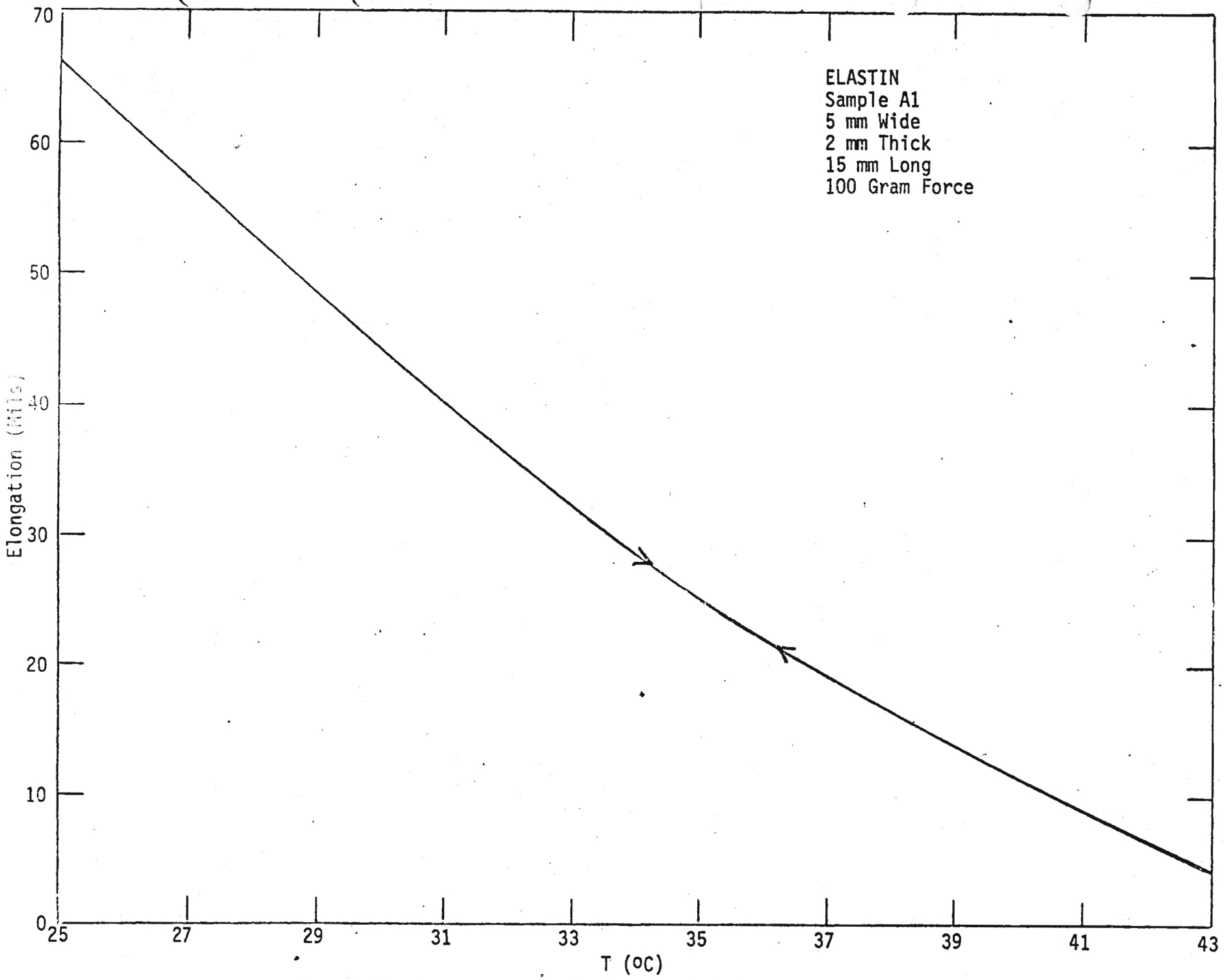


Figure 11. Stress-Temperature Curve for Elastin at Constant Stress

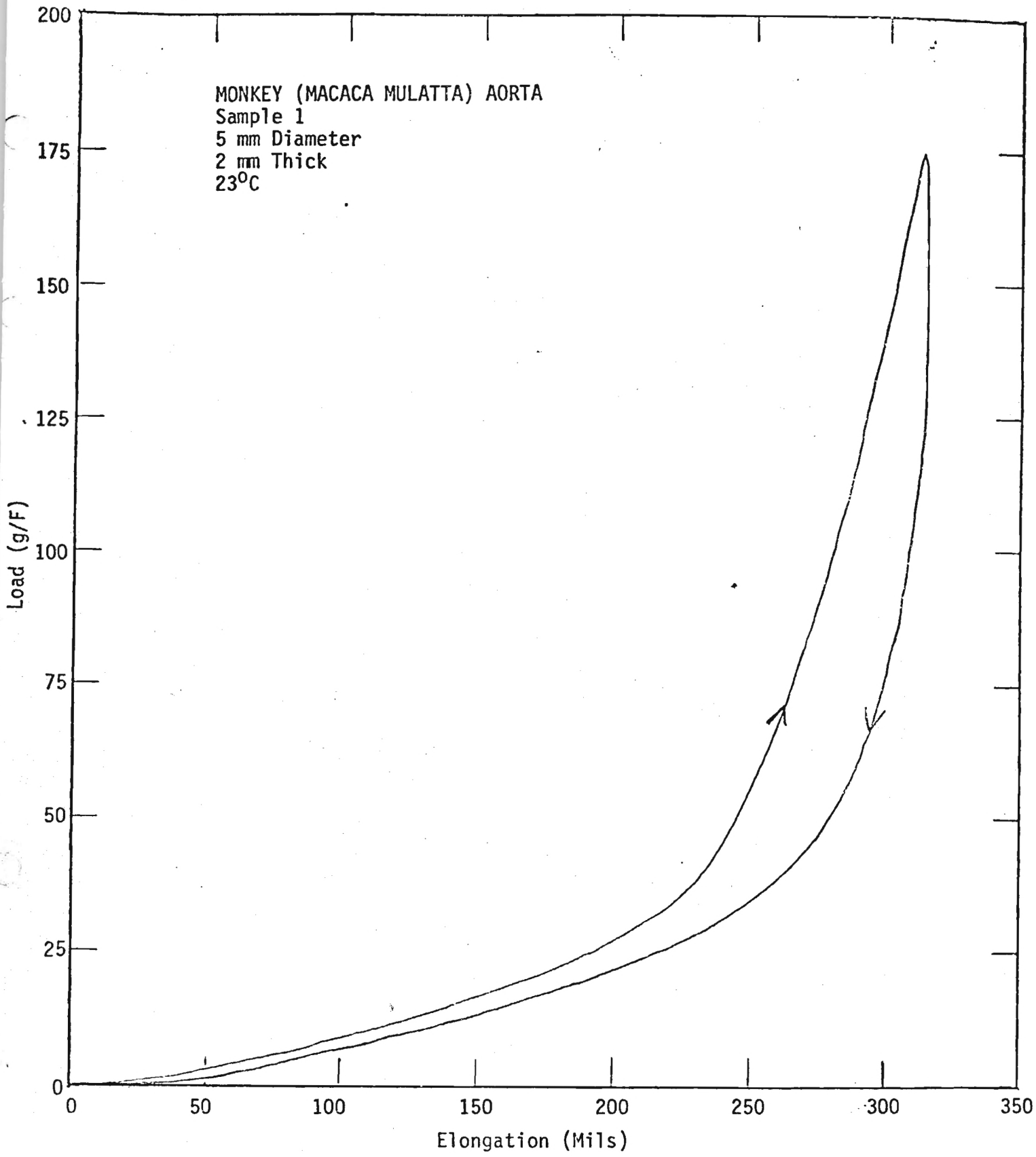


Figure 12. Stress-Strain Characteristics of Monkey Aorta Showing Hysteresis Loss

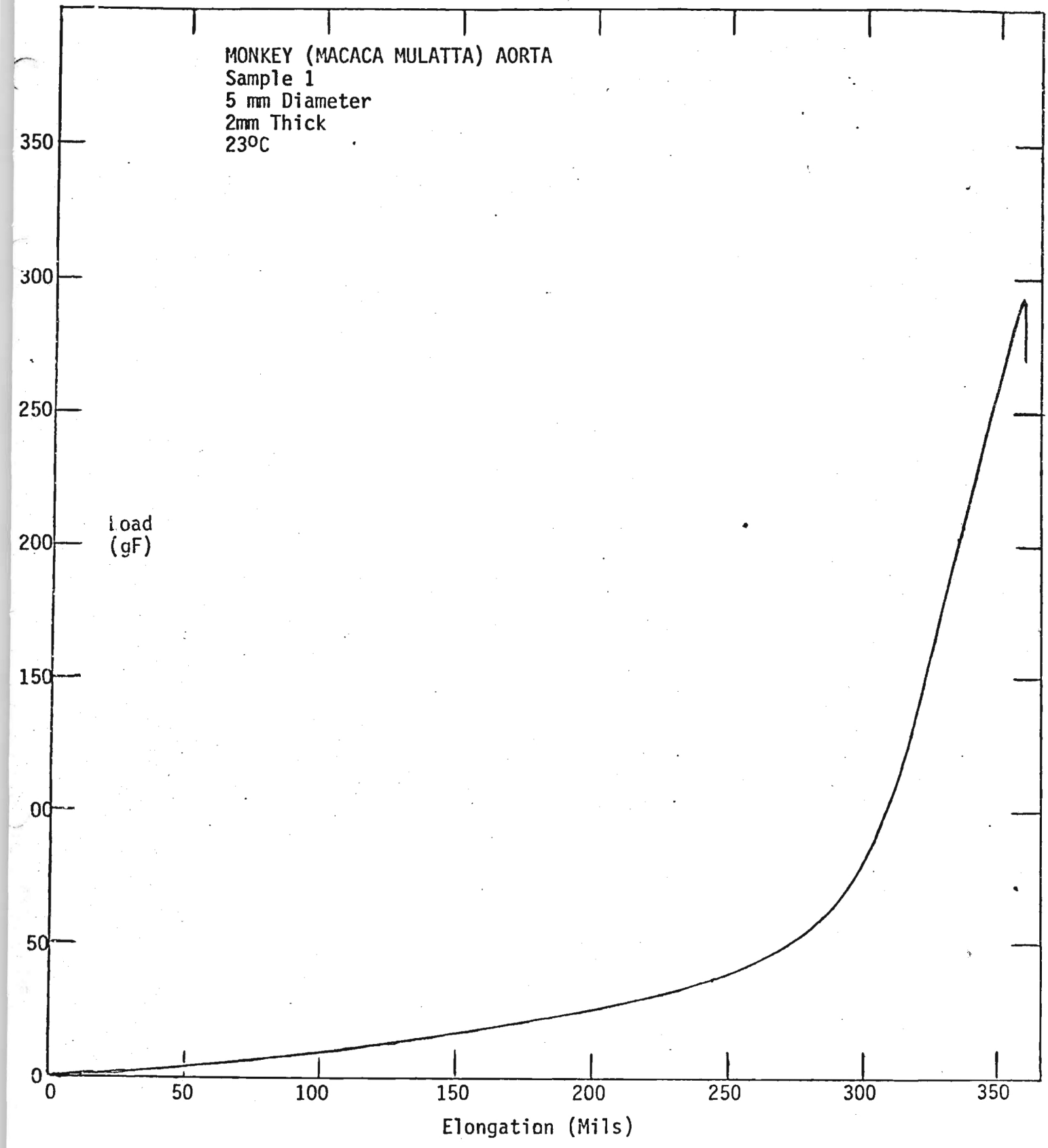


Figure 13. Monkey Aorta Stressed to Failure

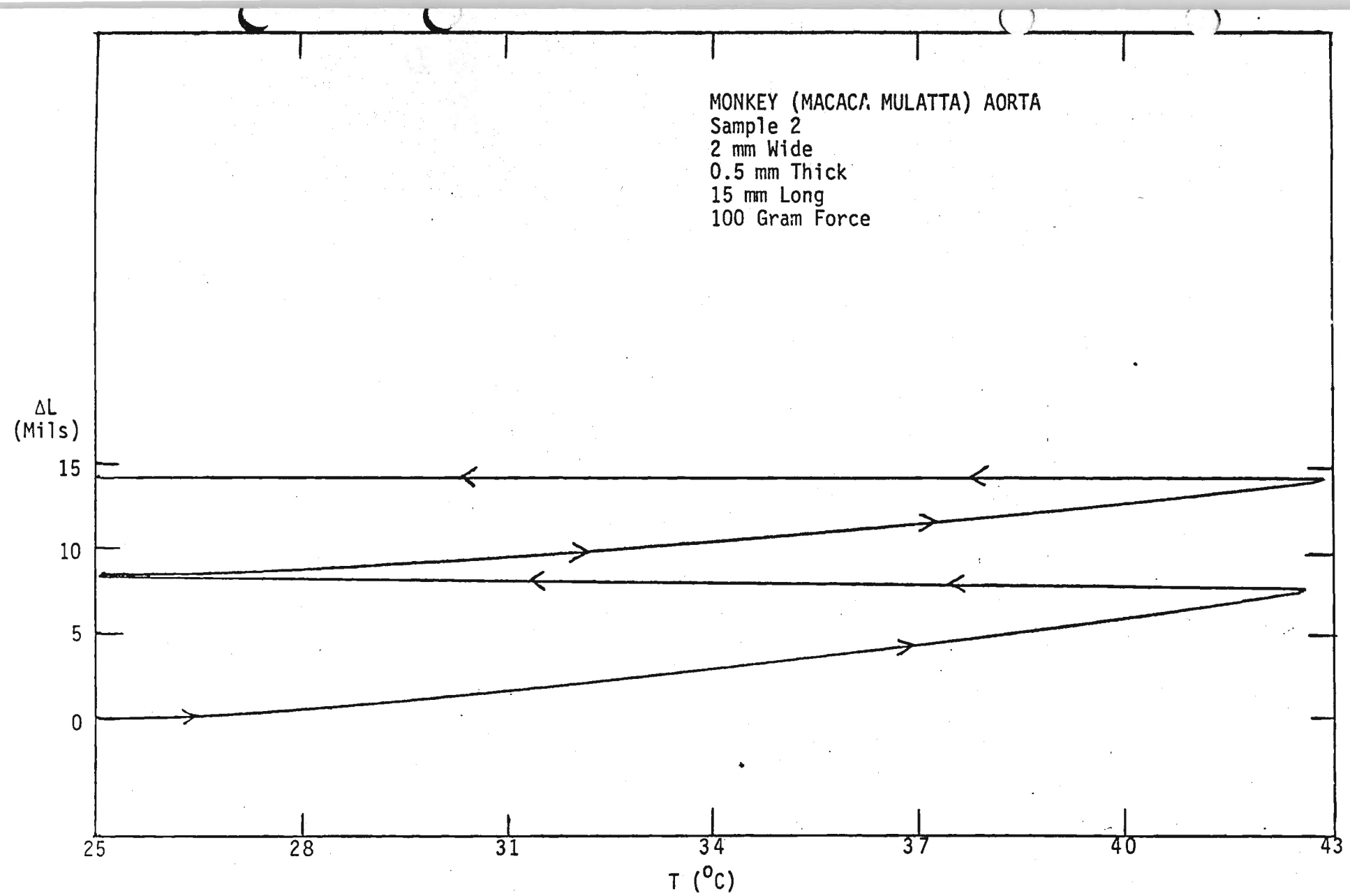


Figure 14. Stress-Temperature Behavior of Monkey Aorta at Constant Stress

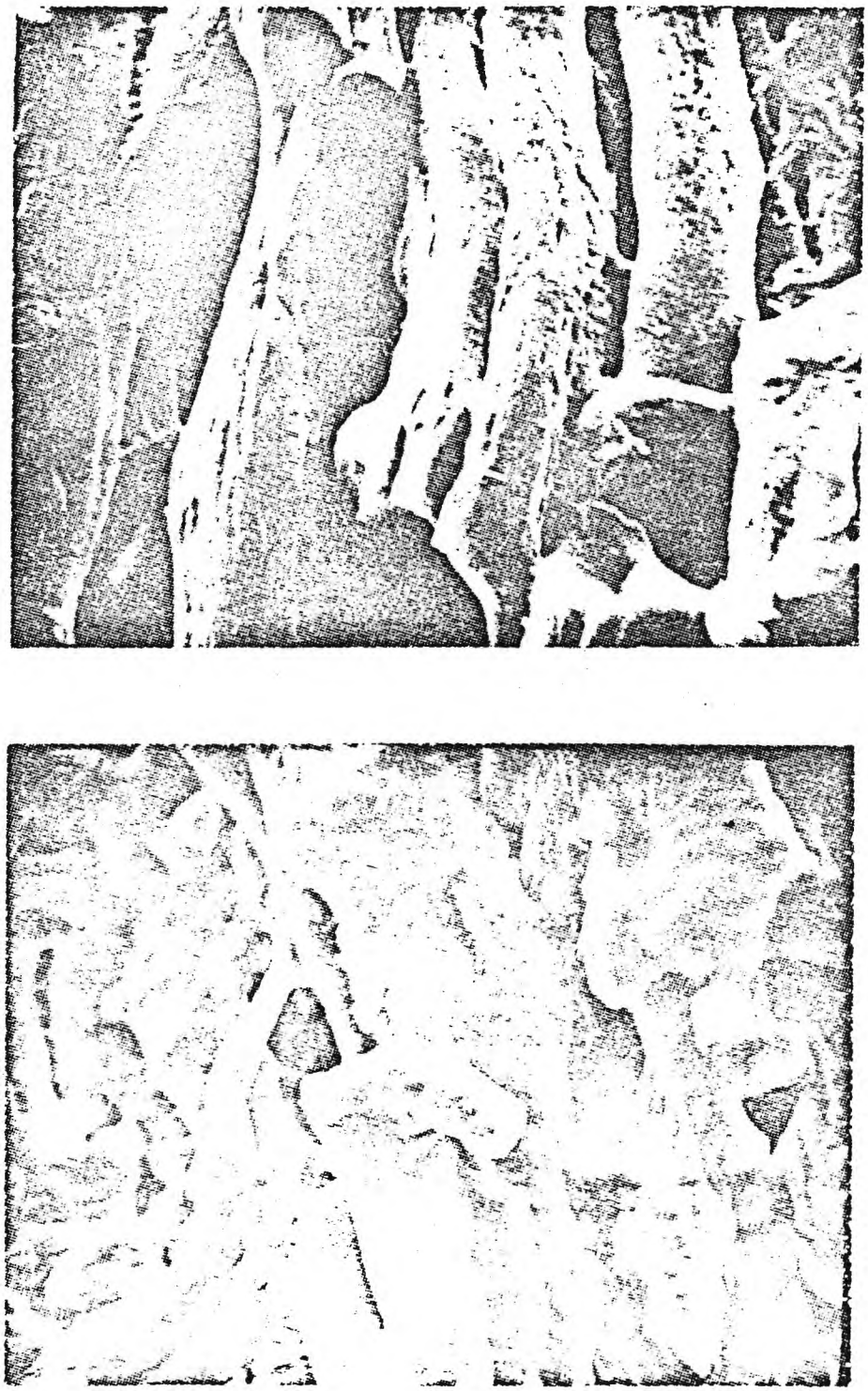


Figure 15. Scanning Electron Micrographs of Freeze Dried Elastin Material Showing Fibers (x1000)