RHEOLOGICAL PROPERTIES OF SOFT EXTENSIBLE ANIMAL TISSUE IN BOTH LIVING AND EXCISED STATES

Thesis by

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In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1970

(Submitted September, 1969)

ACKNOWLEDGMENTS

It is a pleasure to express my appreciation to Professor Wayland for his advice and criticism during the course of this research. I am deeply indebted to Dr. W. G. Frasher for his tutelage in developing the surgical skills, inflation clamps and physiological controls required for the handling of tissue preparations. Dr. Peter Gaehtgens has generously assisted in suggesting and monitoring the physiological controls in many of the latter experiments. The physiology lectures and many discussions with Dr. Michael Taylor have given invaluable assistance in the early stages of this work.

My heartfelt thanks are due to Professor P. J. Blatz for introducing me to the study of finite elastic strain theory and for providing encouragement and timely criticisms. Thanks are also due to Professor E. E. Sechler for many fruitful discussions and for suggesting the use of the flexure in the test apparatus.

The generous financial aid of the National Institute of Health Traineeships and Grant No. HE 08977, the Douglas Corporation, and the California Institute of Technology is gratefully acknowledged.

I am indebted to Mrs. Betty Wood for her assistance in preparing the figures and Mrs. Judy Prince for typing the manuscript.

ABSTRACT

A mechanical characterization, over a wide range of response, of a particular soft extensible animal tissue, the mesentery of the cat, is presented. The structure of the mesenteric tissue is made up of a complicated array of components and the mechanical response is influenced not only by local factors, but also by adjustments of higher control centers of the animal. Certain individual aspects of the response of living mesenteric membrane per se have been studied and contrasted with (1) membrane strongly influenced by or containing large blood vessels, (2) excised membrane and (3) membrane after circulatory collapse and accompanying sustained gut contraction.

A freely floating segment of mesentery exists in a state of tension which can be demonstrated by making an incision through the plane of the mesentery. A technique has been developed to determine the magnitude of this tension and also the corresponding stretch which is designated here as the initial configuration length. The tension level in the tissue at the initial configuration length is not unique but can vary significantly according to the activity of the components of the membrane per se as well as the state of the gut and the large blood vessels. The most nearly unique length of the tissue which can be detected by these experimental methods is a relaxed length determined by excising a piece of tissue of known dimensions and measuring the freely floating (in a physiologic solution) dimensions to which the tissue relaxes. There is no marked material anisotropy

in the plane of the membrane, i.e. the two principal dimensions in the plane of the mesentery do not vary by more than five percent even with wide history variations just prior to excision.

The temperature of the test preparation was monitored during the course of the tests and maintained at the level of the core temperature of the animal. Since this temperature could drop as much as three or four ^{o}C as a result of the anesthesia, the influence of temperature variations on the force-stretch response of the tissue was studied and was found to be less significant than the influence of mechanical degradation in successive loading cycles.

A theoretical characterization that correlates rather well with the data of the loading curves for the various tissues has been proposed. The limitations and assumptions incorporated in this treatment have been discussed and when appropriate additional experimental data are procured then the analytical treatment can likewise be extended to a more adequate characterization.

Photographic materials on pp. 53, 54, 55, 56, 58, 59, 60, 61, 63, 64, 66, 67, 71, 72, 83, 89, 90 and 93 are essential and will not reproduce clearly on Xerox copies. Photographic copies should be ordered.

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Introduction

Many animal organs and systems such as the lungs, stomach, bladder and blood vessels undergo considerable extension in the course of their normal function. For a complete understanding of the extension response and time behavior of such soft elastic tissue it is necessary to consider the morphological structure of the tissue. The response of this structure is manifested in chemical, electro-chemical, neural, thermodynamic and mechanical activity. Since an analysis incorporating all of these features does not appear feasible at this time, the purpose of this investigation is to present a mechanical characterization of a particular tissue over a wide range of response and to show how certain aspects of this response compare with other tissue or with extensible synthetic materials. Once the individual aspects of the response are better understood, then it should be possible to synthesize the composite response of the total tissue.

A schematic, showing the relationship of the present experiments on mesenteric tissue to three larger areas of animal tissue study, is presented in Table I.

A detailed description of the test tissue (cat mesentery) and its preparation will be given in Part I. The two types of test specimens and the concomitant test apparatus will be described in Part II. The surgical procedure and instrumentation calibration are outlined in Part III. Several distinct series of tests were necessary and were performed in order to obtain a mechanical characterization of the tissue and to determine some of the inter-relationships of the

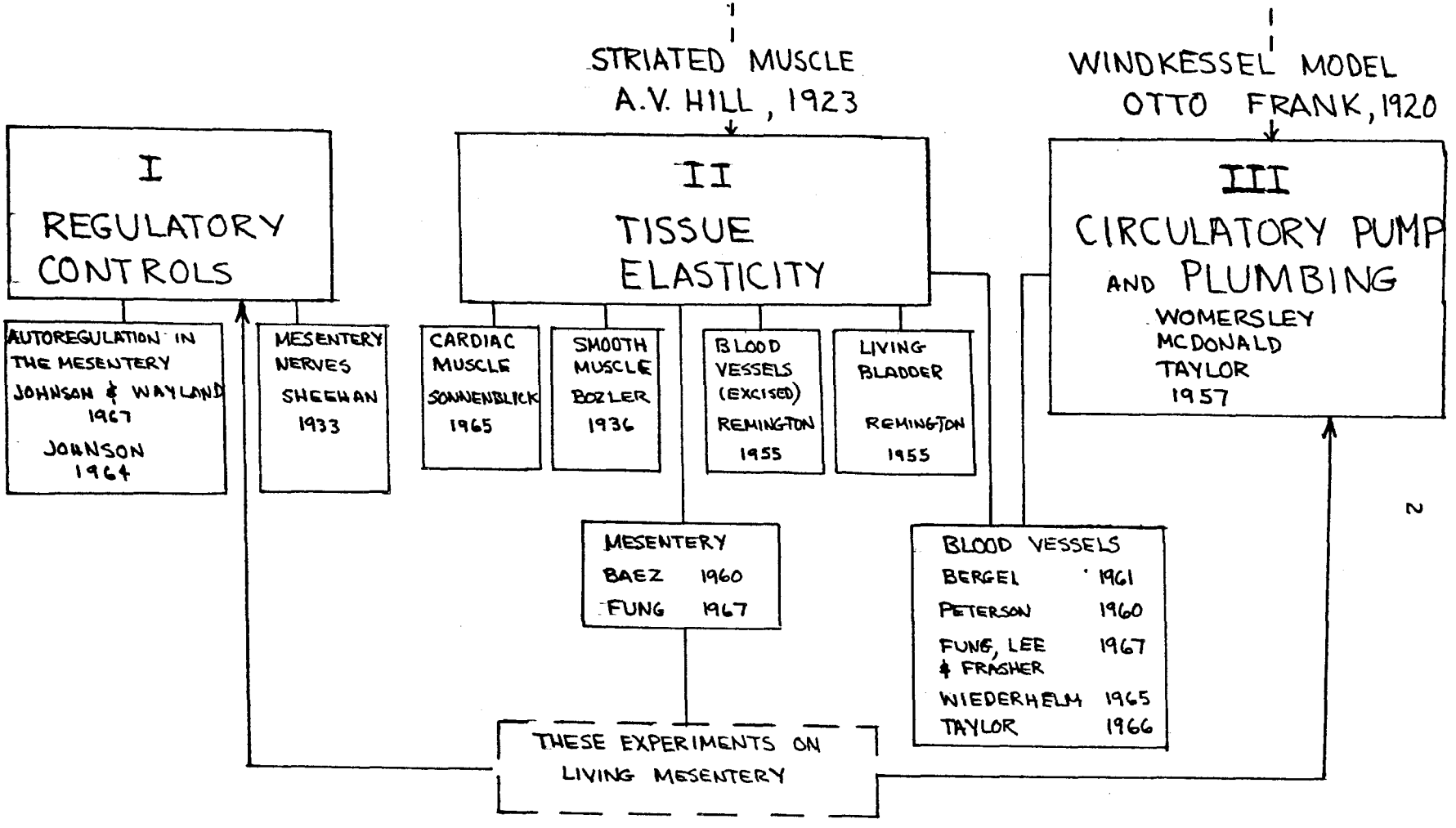

TABLE I THREE AREAS OF ANIMAL TISSUE STUDY TO WHICH THE PRESENT WORK IS RELATED AND SOME CURRENT WORKS IN THESE FIELDS

many components. In Part IV, the test results for mesenteric membrane per se (the central experiments of this research) will be discussed first in order to establish a framework for interpreting the results of the other experiments. In these membrane per se experiments the influence of other structures such as the gut and the large blood vessels was first made as weak as possible. Once the general character of the response of the membrane was known for the animal in this state of surgery, then it served as a convenient reference for the studies of the tissue in general or the excised tissue. Later series of experiments included studies of the load-bearing components of the tissue as well as failure and stability properties.

These experiments have indicated that it would be convenient to have a mathematical characterization of the mesenteric tissue over a wide range of response that is reasonably assumed to be elastic, i.e. the loading part of the response curve. In Part V, a stress-strain law, based on a power of the principal stretch ratios, is proposed that correlates rather well with the data from the mesenteric tissue and fits into the general framework of the finite elasticity theory of Valanis and Landel (21). In this and other (for instance,19) finite elasticity theories the mechanical behavior of a given class of materials can be completely represented by a strain energy density function which depends only on the principal stretch ratios and temperature. The stress field is then generated by differentiating this function with respect to the stretch ratios. When the stress field is

homogeneous, the result of this differentiation gives directly the stress-strain law such as that proposed (in Part V).

I. Test Tissue and its Preparation.

The tissue chosen for this study is cat mesentery, a wedge-shaped, thin transparent membrane attached to the inner margin of the small intestines, conveying their blood vessels and lymphatics, and anchoring them to the dorsal wall of the abdominal cavity (see Figure 1). The mesentery is areolar connective tissue (30 micra thick) the main mass of which has three parts which can be distinguished on histological examination: (1) the collageneous or white fibers which are arranged in a seemingly unorganized pattern, (2) the elastic or yellow fibers and (3) the amorphous mucopolysacchride ground substance. The two broad surfaces of the membrane are covered by pavement-like mesothelial cells 2 to 3 micra thick. Throughout the tissue there is a network of smaller blood vessels; the largest orders of these branchings can be seen in Figure 1. Also visible in the preparation, but not distinct in the photograph, are frequent clusters of Pacinian corpuscles, each of which may be as large as 1 mm. These corpuscles are the encapsulated endings of sensory nerve fibers. Other nerve fibers are also present and can be detected in stained preprations.

Since surgical intervention induces alterations in the mechanical response of elastic animal tissue which are difficult to assess, it is necessary for the experimenter to work with specimens which have been made available with a minimum of surgery. Little direct surgery is required in making a midline incision to expose the mesentery; however, the margins of the incision can apply enough pressure to the

ends of the intestines in the exposed loop to induce both contractions of the gut and petechial hemorrhage in the mesenteric membrane. Mechanical protection against these two conditions was provided by a lucite tray which could accomodate the entire small intestine (see Figure 2). The half cylindrical retractors at the front of the tray engaged and retracted the upper and lower margins of the midline incision. The two slanting sides of the tray then made it possible to insert the keyhole of the tray up to the root attachment of the mesentery at the dorsal wall of the abdominal cavity. The temperature controlled, osmotically balanced physiologic solution in the tray permitted one fan of the mesentery to float freely above the middle stage of the tray while the remainder of the loops on either side floated freely in the two deep reservoir wells.

This freely floating segment of mesentery is in a state of tension which can be demonstrated by making an incision through the plane of the nesentery. The incision opens up into a wide hole as can be seen in Figure 3. In such a freely floating state there is no indication of any marked material anisotropy in the plane of the membrane, but the level of tension in the intact mesentery, when it is clamped and stretched or contracted and returned to these floating dimensions, can vary significantly according to the activity of the components of the membrane per se as well as the state of the gut and the large blood vessels.

Since the mesentery is thin and quite slippery great care must be used in anchoring and clamping it for mechanical testing. An

ingenious tongue and groove inflation clamp devised by Dr. W. G. Frasher was used to secure the mesentery without crushing it. This clamping device and the two geometrical configurations which have been used for testing this tissue will be described in the next section.

II. Test Specimens and Test Apparatus.

A: Types of Specimen and Types of Tests

The wedge-shaped segments of mesentery described in the last section lend themselves readily to testing as intact or excised strip biaxial specimens* or as a circular membrane inflated by a hydrostatic head of physiologic solution. Each of these tests emphasize different aspects of the response of this tissue to externally applied mechanical force, to internally applied perfusing pressure, and to the initial state of tension existing in the tissue.

The strip biaxial specimens which were available for testing had a width to length ratio of about four and were tethered at the ends by the surrounding tissue when the tests were performed on the intact, anesthetized animal (Figure 4). In an effort to determine the magnitude of the mechanical tethering, from the surrounding material of a large sheet, and the positive clamping action of the grips, tests were performed on both tethered and free strips of dental dam, a latex rubber. Figure 5 shows that in the force range of 0 to 400 grams the mechanical effect of tethering by the sheet is only a few percent for this material. The "set" in the material at the end of the loading cycle is the same for both tethered and free specimens. To demonstrate that this is set, not slippage from the grips, ink marks were drawn on the specimen and monitored during the course of the experiment. In this diagram force, rather than stress, is plotted as a

*In a strip biaxial test a wide rectangular thin sheet is clamped in the width direction so that the width remains unchanged. The short wide strip is then stretched in the length (short) direction.

function of stretch ratio for ease of comparison with the mesentery data which are also presented in this form. The reason for plotting force rather than stress is that the thickness dimension of the mesentery was difficult to determine accurately; the best average values of thickness which could be determined in the experimental series was $t_o = 56\mu$ (the significance of the relaxed dimension, subscripted o, will be explained later) and $t_{\text{initial configuration}} = 30\mu$. For the dental dam specimen the thickness was 911μ but otherwise the dimensions were the same as those for the mesentery as given in Figure 4b.

In the mesentery preparation, strip biaxial specimens were clamped in the floating configuration and then were given step increments of displacement, both in contraction and extension, about the state of biaxial tension existing in the freely floating preparation in an effort to determine both this state of tension and its variation with changes in the state of the animal as well as the general character of the stress-strain response of the tissue.

The strip biaxial specimens were tested not only in the intact configuration but also as excised specimens. To insure that the excised specimens were obtained in the biaxial state of tension a lucite bench was placed between the two bottom halves of the clamps and then the bars (with their assembled tops) and the lucite bench were firmly clamped with two C clamps to maintain this configuration during the cutting operation (see Figure 9).

The second series of tests of the mesentery were the inflation tests of an initially plane, circular membrane. However, before

doing the mesentery tests similar inflation tests were performed on dental dam. The similarities and differences in response of these two materials will be given in detail in a later section.

B: Test Apparatus

The complete assembly of the test apparatus used in testing the strip biaxial specimens is depicted in Figure 14. The components of the assembly will now be discussed in some detail.

Each side of the strip biaxial clamp was a pair of stainless steel bars, with tongue and groove design, containing an inflated inner seal as shown in Figures 6 and 9. One of the bottom halves of the clamp was firmly screwed to the base of the lucite tray. The other clamp bottom, when assembled with its top half, was anchored via a yoke with two screws to a flexure as shown in Figures 7 and 8. A frame supporting the flexure was driven fore or aft by a two-way ratchet attached to a drive screw. In parallel with the flexure was a support bar for the force transducer which had a negligible displacement travel (.12 mm at 500 gm of force) in comparison with the fore and aft travel of the flexure frame. The fore and aft motion of the entire frame (which was also the fore and aft motion of the strip biaxial clamp) could be measured both by a vernier and a linear displacement transducer connected to one channel of a Sanborn recorder, permitting determination of the rise time of the step increments of 1 mm cranked in by the ratchet. The force transducer, which works in compression only, was connected to a second channel of the Sanborn recorder and gave a record of the time history of stress relaxation

following the displacement increments.

A smaller set of strip biaxial clamps was fabricated for use in some of the tests. These miniaturized clamps, essentially a scaled down version of the larger clamps, are schematically sketched in Figure 16.

Another complete set of test apparatus, designed for the inflation tests of the circular specimens, is shown in two parts in Figures 11 and 12. Figure 11 shows the plumbing and arrangement of the bottle of physiologic solution, along with the pressure transducer, which was used to measure the pressure head of the physiologic solution. An auxiliary zero water level was introduced for ease in initially adjusting the water level of the bottle to that of the inflating clamp. This zero water level was then clamped off and served as the reference water level throughout the experiment. The crown height of the membrane at the various stages of inflation was measured by the vernier on a traveling microscope (Figure 12).

The circular clamp utilized the same tongue and groove design and inflated inner seal as was used in the bar clamps as is shown in Figure 10. However, the latex rubber membrane covering the top half of the circular clamp had to be specially cast on a contoured aluminum mold and then carefully glued and anchored with surgical silk to the top lucite frame. The bottom half of the clamp had a thermocouple to monitor the temperature of the warm physiologic solution driven into the inlet of the clamp by the hydrostatic pressure head.

One or the other of the assemblies just described was used in the experiments, depending on the type of specimen which was chosen. However, the remainder of the apparatus (the lucite tray and the heat bath) was the same for all experiments. The heat bath, mounted on an ancillary table, was used to maintain the temperature of the physiologic solution entering the tray through the side inlet and also through the drip above the keyhole of the tray. One temperature probe was inserted inside the abdominal cavity of the animal, a second probe was placed in the tray and the third probe which was mounted in the circular clamp was also used in the inflation experiments. These probe temperatures were monitored and the temperature of the tray bath was adjusted to keep its level at that of the core temperature of the animal.

The protective lucite tray, which was filled with physiologic solution, was utilized in maintaining a stable environment for all the loops of the small intestine. Photographs of the tray are shown in Figure 2 and a description of its use was given in Part I.

III. Test Procedure.

Prior to preparing the tissue for the strip biaxial tests the force transducer, linear displacement transducer, flexure pivot and support frame were mounted on an auxiliary table and carefully calibrated. First the force transducer and flexure were calibrated by dead weights placed in a small basket (Figure 13a) and a calibration curve was plotted (Fiugre 13b). Next the displacement transducer was zeroed to the zero on a vernier mounted on the stationary frame, 1 mm increments of displacement were cranked in by the ratchet on either side of the zero setting, and these 1 mm readings were checked against the deflections on the Sanborn recording paper.

In the next step of the test procedure the animal was anesthetized intraperitonially using sodium pentobarbitol with a dosage of 30mg per kilo of body weight. After the midline incision was made the spleen and omentum were packed off with gauze and replaced in the upper part of the abdominal cavity. The margins of the midline incision were temporarily closed and the animal was moved to the main operating table and placed on its left side, with its back supported against a back rest. The lucite tray, filled with warm physiologic solution through a side inlet, was positioned in front of the midline incision, the retractors of the tray engaged the margins of the midline incision and the loops of the small intestines and mesentery were exposed through the keyhole of the tray. A second drip of physiologic solution was placed a few centimeters above the keyhole so it would drip just inside the tray and insure a more even tempera-

ture distribution of the physiologic solution.

The particular fan of mesentery which was chosen for the test was permitted to float above the center stage, to which the lucite bench and the bottom halves of the clamps were anchored, while the loops on either side floated in the two deep reservoir wells. Then four small incisions were made so the four pins of the clamp could be screwed into the bottom halves of the clamp. The tops of the clamp were inserted over the pins and the four screws were gently tightened. As soon as the clamp tops were inflated with air the mesentery was secured for testing as shown in Figure 4.

At this point a quick recheck was made to determine if any adjustments needed to be made in the dead weight calibration of the force transducer. The dead weights and basket were removed, the flexure support was moved to the main table, the two slots at the bottom of the flexure were dropped over the two screws in the yoke mounted to one of the clamp tops, and finally the two screws were tightened. Next the length of the tissue between the two bar tops was carefully measured with a vernier. Figure 14 shows the complete assembly.

Since the force transducer only works in compression there are three important reference lengths to measure: (1) the above initial configuration length at which the tissue is in a state of biaxial tension, (2) the particular length ℓ_*, to which the tissue is brought once the C clamps and lucite bench are removed and at which the force transducer is set to read zero force and (3) the relaxed length ℓ_o, determ-

ined by excising a piece of tissue of known dimension at the end of the experiment and measuring the freely floating dimensions to which the tissue relaxes.

After the lucite bench and C clamps were removed, the ratchet was used to contract the length of the tissue to a length estimated to be the relaxed length, the force transducer was set to read zero force, the contracted length between the bars was measured and the reference vernier reading was recorded. Depending on the prior preparation the tissue could then be tested as an intact strip biaxial specimen or as an excised strip biaxial specimen.

For the inflation tests the fan of mesentery was prepared in the same manner as for the strip biaxial tests. Then the bottom half of the circular clamp was mounted to the center stage of the lucite tray and three incisions were made for the three pins of the circular clamp. The top half of the clamp was loosely positioned, a circular focusing template was placed on top of it, and the microscope was centered and afterwards racked up a short distance to accommodate the final adjustments of the clamp top. After the focusing template was removed the water bottle circuit was connected to the circular clamp bottom and the level of the solution in the bottle was adjusted to the fluid level of the circular clamp. The circular top was gently fastened with the three screws and the membrane seal was inflated with air.

Prior to the preparation of the tissue specimen the pressure transducer was calibrated against the hydrostatic pressure head, in

cm of water, of the bottle of physiologic solution whose height was measured by a linear scale placed alongside the bottle. Now the reference water level was clamped off and recorded, the microscope was focused on the center of the flat circular membrane and this reference level was also recorded. The mesentery was then inflated by elevating the height of the water bottle and the crown height of the membrane was measured by again focusing the microscope, shown in Figure 12, on the top of the inflated membrane and noting the accompanying increment of the vernier.

IV. Data and Comparison with Other Tissue and Extensible Synthetic Materials.

Once the calibration of the test apparatus and the surgical procedure were completed, all the loops of the small intestine and the accompanying mesentery were exposed and floating in the warm physiologic solution in the protective lucite tray. An appropriate fan of mesentery was then selected and permitted to float above the middle stage of the tray. The segment most frequently chosen for study lay along the jejunum and was selected because it was the largest window of membrane free from the large arteries and veins. Some of the size and shape variations of this particular window are displayed in Figure 15. The size of the bar clamps are such that only rectangular specimens of membrane were tested for any of these fans; however, in some preparations one outer corner, or the outside edges of the clamps, did bear on large blood vessels. Thus, in order to insure that such factors as pressure on large vessels were not included in the studies of the membrane per se, the miniature bar clamps were utilized.

A log of all the animal experiments is given in Appendix I, along with some comments about each particular animal and the main objective of that experiment. The data were taken as soon as possible after the surgical procedure and the positioning of the clamps. The length of time the specimen was held at each setting of the displacement or inflation increments was 45 seconds, or is recorded on the representative graph for that series of experiments.

The structure of the mesenteric tissue is made up of a complicated array of components and the response of this structure is influenced not only by local factors, but also by adjustments of higher control centers of the animal. This experimental program has been able to detect the influence of some of these many factors, but at this stage of the work it is not possible to completely unravel the response of the tissue and associate it with the many discrete components. Certain individual aspects of the response of living mesenteric membrane per se have been studied and contrasted with (1) membrane strongly influenced by or containing large blood vessels, (2) excised membrane and (3) membrane after circulatory collapse and accompanying sustained gut contraction. Therefore, the discussions of the experiments have been organized to display the current findings and to offer some possible interpretations.

The test results for mesenteric membrane per se will be discussed first since they are the central experiments of this research and are vital in establishing a framework for interpreting the results of the other experiments.

Characteristic force-stretch diagrams for a control specimen I of mesenteric membrane per se, i.e. with no large supplying vessels in the immediate vicinity of the test specimen, are shown in Figure 17 for two complete cycles of stretch and release of stretch. As discussed in the last section, $\lambda_* = \ell_* / \ell_o$ is the stretch ratio to which the bars are contracted initially and is the position at which the force transducer is set to read zero. From this setting the tissue was given

1 mm step increments of displacement, with a rise time of approximately 0.2 sec, after which the displacement was held constant for 45 sec. before proceeding to the next step. Typical recordings of the force and displacement transducers are displayed in Figure 17a. The force in the tissue rose synchronously with the step increments of displacement to a peak value and then relaxed 10-20% during the remainder of the 45 sec. before the next loading. Most of the relaxation occurred in the first two or three seconds after which there was a slight but continuous decay for the remainder of the time. At the end of the loading cycle the direction of the ratchet motion was reversed and the tissue was given step decrements of displacement. With a decrease of stretch the force falls off rapidly and then shows a slight gradual recovery with time (5-10%). It was observed, though not shown on this particular graph, that even though the loading and unloading curves are obtained incrementally with intermediate duration up to several minutes at each point of extension, there is still evidenced a great deal of hysteresis.

In all the various series of experiments the temperature of the bath was monitored and maintained near the core temperature of the animal. Since this core temperature could drop as much as 3 or 4 ^{o}C as a result of the anesthesia, the influence of temperature variations on the force-stretch response of the tissue was studied. To do this a second similar specimen to the control one, from the adjacent fan of mesentery, was likewise tested over two loading cycles; then the temperature of the bath was lowered by 5^{o}C and a third loading cycle was

performed. Afterwards the temperature of the bath was elevated again to the level of the animal's temperature and after a few minutes, to allow for stabilization, a fourth loading cycle was performed. Figure 17 demonstrates that the response of the tissue on the third loading cycle, when the temperature was reduced by $5^{o}C$, falls between the response on the second and fourth loading cycles. This experiment demonstrates, as did two earlier such experiments, that the force-stretch response of the mesentery, when the temperature is reduced by $5^{o}C$ and also a five minute wait is incorporated between cycles, lies within the envelope of tissue response of mesenteric membrane for successive loading cycles.

When an excised strip biaxial specimen of mesentery is tested in the same manner as the intact strip there are some noticeable differences as well as similarities in response as is indicated in Figure 18. The first difference is that there is a negligible force response until a certain value of λ is reached and then there is a rapid rise in force as the stretch ratio increases. This feature of the response has led Prager (41) to characterize excised mesentery as a "locking material". A second difference in response is that the force relaxation is somewhat less in the excised tissue than in the intact tissue. One factor which may contribute to both of these differences in the intact and excised specimen is that in the living animal the circulating blood is under pressure as it courses through the vessels of the tissue. This internal pressure could contribute to the overall stress response of the tissue and also a significant part of the stress relaxa-

tion could result from the squeezing out of blood as the tissue is continuously stretched.

In these excised specimens the influence of temperature variations on the force-stretch response of the tissue was also studied. After the specimen was tested over two loading cycles, the temperature of the bath was lowered by 9°C and a third loading cycle was performed. Afterwards the temperature was elevated again to the initial level of the bath and a fourth loading cycle was performed. These experiments likewise demonstrated that, even with the 9°C change in bath temperature, the response of the tissue on the third loading cycle lies within the envelope of tissue response of excised membrane for successive loading cycles.

At this stage in the experimental work it appeared desirable to investigate the mechanical response of a specimen of mesenteric membrane with no large supplying vessels in or near the specimen and then, from the same animal, to determine the response of a specimen containing large supplying vessels. That is, since the general character of the response of mesenteric membrane per se is now known for the animal in this state of surgery, it will be useful for comparison with the response of membrane containing large supplying vessels as well as the response of excised membrane.

Three specimens from the same animal were tested with the miniaturized clamp and the structural arrangement of the vascularization is shown in Figure 19. The first tissue was located along the jejunum and in the upper left corner of the test specimen there were

supplying vessels of an intermediate order of branching. The second tissue was situated at an intermediate position along the intestine between the first specimen and the ileocaecal junction and the supplying vessels of the tissue lay well beyond the clamps. The third sepcimen lay near the ileocaecal junction and a straight run of large vessels traversed the entire width of the specimen. Figure 20 shows that the force-stretch response in the range of λ's less than 2 is the flattest for the membrane of specimen II and that the force response is greatest for tissue III which contains the largest vessels. All three specimens show a steep rise in force response at $\lambda \approx 2.4$. Tissue III also shows an extremely wide hysteresis loop.

Remington (1) has made a very careful and thorough study of the stress relaxation and hysteresis loop behavior of several excised tissues. His conclusion is that

> "a hysteresis loop was not a specific characteristic of smooth muscle extensibility. Loops qualitatively similar to those given by aortic rings were seen with ligamentum nuchae (predominantly elastic tissue) and with an aortic ring allowed to putrefy for 14 days. Similar loops were also seen with a length of carotid artery (musculo-elastic) and with femoral artery (muscular). Any difference in the elastic behavior of these tissues would appear quantitative, not qualitative...
>
> The dependence of the loop on the amount of stretch, and the relative independence of the period at fixed peak length, were qualitatively similar for fresh aorta, putrefied aorta, ligamentum nuchae, and femoral artery."

In his study of the stress relaxation of these excised tissues he found that

> "the tension fell rapidly for the first 30-50 msec. from the completion of the stretch, and then more slowly. When the initial tension value was taken as that seen after 50 msec., the decay rate thereafter was fairly linear when plotted against the log of time. The tension decay earlier was not clearly linear against log-time, and much more variable."

The general form of stress relaxation in the intact mesenteric tissue is the same as Remington has found in excised tissue except that the magnitude is somewhat greater. Remington states

> "the evidence is suggestive that two different processes contributed to the tension fall at fixed length, one being quite transient and producing a relatively large tension fall, and the other continuing for a long time but producing a slower tension fall. The former was related to the (wide hysteresis) loop phenomenon, the latter not clearly so."

The primary objective of the series of tests with the miniaturized clamps was to determine the general character of the mechanical response of mesenteric membrane per se: that is to determine the magnitude of mechanical variations from one fan of mesentery to another in the same animal as well as the variations from animal to animal. A graphical presentation of the response of 14 specimens from 9 animals is given in Figure 21 along with the mean curve for the 9 animals. The encircled number at the end of each of the 9 curves indicates the number of tissue specimens from that particular animal. When more than one specimen was tested from an animal the variations in response between these specimens is indicated by the brackets on the verical lines drawn through the $\lambda_{\text{initial configuration}}$ for that individual animal. These data indicate that the variations from animal to animal is greater than the variations between speci-

mens from the same animal. When three or four specimens are tested from the same animal the location of the fan from which the specimen is taken varies all the way from the ileocaecal junction to the jejunum so that there are visible differences in the gross architecture of the fan. The mean curve for the excised tissue and also the mean curve for five specimens strongly influenced by large blood vessels are also displayed for comparison with the membrane per se.

In these preparations the gut was quiescent and relaxed in so far as could be determined by visual observation and by the time history of the force recordings. As the other series of tests are discussed more evidence will be presented to support the interpretation that the difference in response between intact tissue and excised tissue is a characteristic of the membrane and not just a test artifact. It should be noted at this point that on excision of the tissue at least four significant facts are immediately evident: (1) the mechanical tethering of the tissue by the surrounding sheet is lost, (2) a considerable amount of blood is lost from the tissue, (3) all muscle innervation from the central nervous system is severed and (4) the tissue is no longer perfused with blood operating under the systemic pressure head. Thus, with all these changes, it is reasonable to expect a difference in response between intact tissue and excised tissue.

Characteristic force-stretch diagrams for intact strip biaxial specimens tested with the larger rectangular clamps are shown in Figure 22 for two complete cycles of stretch and release of stretch. It is immediately evident that these tissues evince a great deal of

hysteresis and also that on the second loading cycle the material does not recover the initial loading response. Synthetic materials, such as a wet strip from a nylon stocking, also show this same qualitative behavior (Figure 23) when tested over two complete loading cycles. Both the mesentery and the nylon stocking show a greater distensibility on the second loading cycles; however, both unloading cycles are approximately the same.

One similarity of the response of both intact and excised tissue is that there is a continuous degradation in response on successive loading cycles if there is no wait between cycles. Figure 24 vividly displays this degradation for the excised tissue whereas Figure 22 characterizes it for the intact tissue. Remington found that some of the excised tissues he tested would degrade in response for the first three cycles and then would become stable about the response obtained on the third cycle. His resulting data are displayed in Figure 25.

Each of these four factors--(1) negligible force response for small λ's in excised tissue, (2) greater stress relaxation in intact tissue, (3) wide hysteresis loop even if considerable time is allowed for stress relaxation, and (4) degradation in tissue response on successive loading cycles, if there is no wait between cycles--will be discussed in more detail as the further tests of the mesentery are now reviewed.

In the next tests, an intact strip biaxial specimen of mesentery was tested in one complete loading and unloading cycle, then circula-

tory collapse was induced, i.e. the systemic blood pressure dropped essentially to zero, and a second loading and unloading cycle was performed on the same tissue. Figure 26 shows that this second loading cycle does not differ appreciably from the unloading curve of the intact tissue. Even though the systemic blood pressure had now been reduced almost to zero, the gut which had been quiescent began to contract quite vigorously so that the tissue response was about the same as the response that would have been obtained on a second successive loading cycle in the living animal. As soon as this second cycle was completed the tissue was excised and tested for two additional cycles. On excision of the tissue at least four significant changes are immediately evident: (1) the mechanical tethering of the tissue by the surrounding sheet is lost, (2) a considerable amount of blood is lost from the tissue, (3) all muscle innervation from the central nervous system is severed, and (4) the tissue is no longer perfused with blood operating under the systemic pressure head.

For the excised tissue the force response on the first loading cycle is much flatter than in the previous loading cycle of the tissue. However, at the large value of λ it shows the same steep rise in response as is noted in the intact tissue. The response on the second loading cycle of the tissue is about the same as the force response on the first unloading cycle. This suggests that there are probably at least two different components of the tissue that are carrying the load and one of these is no longer effective in the excised tissue. The most obvious component of the tissue which could be affected by

excision is the vascular smooth muscle structures which are filled with blood under systemic pressure and are innervated by the central nervous system.

Since Stacy (3) has shown that a carotid artery will recover its tension response in about 5 minutes, the next tests which were performed on intact strip biaxial specimens incorporated a five minute wait between loading cycles to allow for muscle recovery and a return influx of blood. In addition to waiting five minutes between loading cycles the amplitudes of the loading cycles were varied to determine if there is some critical λ beyond which the muscle suffers incomplete recovery. In the first two loading cycles the tissue was loaded to the initial tension level existing in the freely floating preparation and then in the third and fourth cycles it was loaded to a considerably higher level of tension (Figure 27). In the course of the third loading cycle the animal experienced some breathing difficulty and it is noted that on the third unloading cycle the response of the tissue has deteriorated somewhat. However, after the five minute recovery time the tissue still appears viable during the course of the fourth cycle. For this particular tissue there is no indication of incomplete recovery in this load range. In this series of tests some of the specimens (Figure 28) did show evidence of incomplete recovery (at least in the five minutes recovery time) between the third and the fourth loading cycle. For these specimens the force response (in the range of λ's less than 2) was comparatively much flatter and smaller than in tissue that recovered.

In the next experimental series, the force-stretch response of two strip biaxial specimens from the same animal were tested using the larger rectangular clamps. Even in these tests there were noticeable variations in the tissue response (as shown in Figure 29) and these variations could be correlated with the large blood vessel arrangements. Even though the actual test specimen contained only blood vessels of about the same order of branching, photographs (Figure 30) of the structural arrangement of the larger supplying arteries and veins just beyond the test segment were quite different and could influence not only the blood flow but also the relative innervation from the sympathetic nerves (which will now be discussed).

In the mesentery Sheehan (4, 5) has designated the functional aspects of the nerves as follows: (1) intestinal nerves, i.e. nerves running through the mesentery and supplying the intestinal wall, (2) vascular nerves which supply the innervation of the blood vessels, and (3) true mesentery nerves. He states

> "the first and probably also the second group are for obvious reasons both efferent and afferent: the latter group, however, is presumably only afferent, as there are as far as we know, no free muscle fibres in the mesenteric tissue proper".

These true mesentery nerve endings are of three main types: (1) Pacinian corpuscles supplied by splanchnic mylinated nerves (sympathetic), (2) free nerve endings of mylinated nerves which are rare in occurrence and probably somatic in origin, and (3) free nerve endings of a fine plexus of non-mylinated fibers that appear to be

purely sympathetic. Sheehan states

> "the fine non-mylinated nerve plexus contains ganglion cells in the mesentery and appears to be purely sympathetic. The function of this non-mylinated nerve would appear to be...the control of unstriated muscle and probably also the transmission of the impulse which gives rise to the symptom of true visceral pain. It was definitely continuous with the fine plexus of unmylinated nerves spun around the blood vessels and capillaries of the mesentery and was as well marked at the root as at the peripheral border of the mesentery. It extended out into the very thin avascular portions of the mesentery, which are composed almost entirely of the two endothelial layers of peritoneum".

The correlation of the tissue response with the structural arrangement of large vessels near the test specimen, with their potential influence on the blood supply and innervation of the tissue, suggested that the systemic blood pressure and sympathetic activity of the tissue should be monitored. In all the succeeding experiments the systemic blood pressure was monitored from the time just before the exposure of the mesentery, during the tissue preparation and clamping, and throughout the course of the tissue tests. At the time of exposure of the mesentery there was a rise in systemic blood pressure sustained for about five minutes followed by a similar rise sustained for about one minute at the time the clamp tops were fastened and inflated. The system then stabilized about this average pressure level of 140 mm Hg and in the course of loading and unloading there were no further significant pressure changes (Figure 31). Next extreme variations in systemic blood pressure and/or sympathetic activity were induced and after the system had stabilized at a

new level the loading cycles were repeated to determine the resultant effects on the tissue response. The tissue was maintained at its contracted length during this course of adjustment and stabilization of the animal's system to the new equilibrium state. The force transducer which had initially been set to read zero force at the contracted length indicated a gradual but continuous change in force reading during this time presumably from influx or efflux of blood and/or muscle recovery. As shown in Figure 32, at the beginning of each successive loading cycle the tissue was loaded from this level of force. It was noted that in the living animal the force did not reduce to a zero reading at the termination of any of the successive loading cycles, i.e. there was some residual force reading; however, after circulatory collapse the tissue did show zero force at the termination of the loading cycle.

In summary the variations in systemic blood pressure and/or sympathetic activity did influence the force response of the tissue. Since changes in any one factor in the animal's system can elicit adjustments in many others,the system appeared to stabilize at a level dependent on the resultant of the various adjustments. In previous studies it was found that a five minute recovery period was needed to permit the tissue to recover the zero force reading; in contrast the drastic systemic change here in the living animal maintained a force reading even at the termination of successive loading cycles.

The next step in the experimental series was the study of the

tissue component (or components) which is prominent in carrying the load at large stretch ratios both in the excised and intact tissue. First the intact tissue was contracted and stretched to a length slightly greater than its initial length (Figure 33). After a five minute wait at the termination of the first loading cycle, the amplitude of the second loading cycle was doubled and again the tissue was given a five minute recovery period. Then the tissue was excised and tested in a third loading cycle of even greater amplitude; the initial load response was quite flat up to stretch ratios greater than two at which length a steep rise in force response occurred. This abrupt rise in force could not be sustained by the tissue and it began to tear randomly in several small spots, but there was no catastrophic gross tearing of the tissue at these load levels. On unloading the tissue there was no appreciable recovery in the 45 seconds between the step decrements of displacement and there was a wide hysteresis loop similar to the one in the intact tissue. In some of these experiments the time between the step increments of displacement was varied and only the force reading at the end of the time increment was recorded on the graph to determine the time dependence of the hysteresis loop. In Figure 34 the time increments for the first two loading cycles were 10 seconds and those for the third and fourth loading cycle were one minute. At the end of the fourth loading cycle the tissue was maintained at this maximum length for a period of 15 minutes to determine the additional amount of stress relaxation that would occur.

Microscopic examination of the tissue structure and the arrangement of the load bearing matrix of protein fibers in this stretched state was not possible for the strip biaxial specimens; however, it was possible in the inflated circular specimens where many of the morphological details could be studied. In such inflation experiments the circular clamp was positioned on the tissue in the freely floating position and an initial photograph (Figure 35a) of the fibrous protein matrix was taken showing a kinked, random, and indistinct arrangement of fibers. The tissue was then inflated with a hydrostatic pressure head of 40 cm of water and a second photograph (Figure 35b) was taken which showed that some of the fibers had straightened out and appeared to be load bearing while many still remain kinked. On further inflation of the tissue by a head of 100 cm of water all of the fibers (Figure 35c) had straightened out in randomly oriented directions. The diameter of these fibers is of the order of 5 μ; there are also distinct globular structures which apparently adhere to the fibers. Thus, the micro-structure that determines the elastic properties of the tissue may be influenced by labile cross-links as well as the structural coiling and uncoiling of the long protein fibers. A photograph (Figure 35d) of the tissue after deflation to the original configuration indicates that the fibers are loosely coiled and have not completely regained the initial indistinct, tightly kinked form.

Inflation studies were also useful in determining the failure and stability properties of the tissue. As mentioned in Part II,

inflation tests were first performed on dental dam and the information from these tests served as a reference guide in the animal tissue inflation studies. When dental dam is inflated by a hydrostatic pressure head, the crown height of the specimen increases monotonically with increase in pressure until a certain level of inflation is achieved at which there is a sudden large increase in crown height indicating a snapping over of the specimen from one region of stable equilibrium to another region at much larger values of crown height. Figure 36 shows such a non-dimensionalized plot of the crown height-pressure relationship for dental dam. Similar inflation tests for intact mesentery specimens show a monotonic increase in crown height with increase in pressure head, however, there is no indication of instability of these specimens. Figure 37 demonstrates the crown height - pressure relationship on both inflation and deflation of an intact mesentery specimen.

Onset of failure of mesentery specimens is not easily detected when they are inflated with a pressure head of physiologic solution. If instead the specimen is inflated with air and a layer of physiologic solution is maintained on the top surface of the specimen it is easy to see the miniature air bubbles which escape when small vacuoles are torn in the tissue. Presumably these vacuoles may result from separations between the pavement-like endothelial cells on the top and bottom surfaces of the membrane. The photograph in Figure 38 pictures the random distribution of air bubbles escaping from the vacuoles which have been formed when the tissue is inflated by a

pressure head of 100 mm of Hg. Thus in this test we again see the occurrence of a random mode of local failure rather than catastrophic gross failure.

The test results from the inflation experiments were also useful in showing that the differences in response of intact and excised membrane, as summarized in Figure 21, are a characteristic of the activity of the components of the membrane and are not, therefore, just an artifact of the experimental tests. The results of this series of tests has been summarized in Figure 39 and each of the tests will now be discussed individually.

In the prototype circular clamp a small slot, cut through the tongue in the base, permitted a pair of large supplying arteries and veins to enter the test specimen without being completely occluded. Specimens from a mother and her seven month old kitten, tested with this prototype clamp, show a wide hysteresis loop indicating a much stiffer response on loading than on unloading. By visual observation of the tissue during the course of loading it was possible to note the blanching of the tissue as the blood was squeezed out and likewise the reddening of the tissue with the return influx of blood. In the later experiments, a clamp without the slot was utilized so that there was no efflux and influx of blood. When an intact mesentery specimen, without large supplying vessels, was inflated the tissue response was relatively much softer and the hysteresis loop was comparatively not as great. After the second loading cycle of this intact tissue, the specimen was excised and an additional loading cycle was performed.

(In the course of this cycle there was not much further degradation.) Following the circulatory collapse of the animal, the smooth muscle of the gut developed a firm sustained contraction (and the cross-section became circular). The pressure response of a test specimen from a second fan of mesentery, adjacent to the first excised fan, was now much stiffer than the original response of the first fan where no gut contraction occurred. However, even though the pressure response of the second specimen showed a stiffer behavior, the relaxed dimensions of the two specimens were not markedly different; the initial diameter of the two specimens was 3.7 cm. and upon excision the floating dimensions of the first membrane in two perpendicular directions were 2.25 cm and 2.30 cm and the dimensions for the second membrane were 2.25 cm and 2.35 cm.

In another animal, two specimens were prepared and tested following circulatory collapse and the accompanying sustained gut contractions. The response of both specimens was about the same and was also much stiffer than the response of the intact mesentery specimen. In the fifth experiment a segment of mesentery which had suffered severe petechial hemorrhage was clamped and tested. The response of this tissue was likewise quite stiff and the width of the hysteresis loop was relatively about the same as the loop for the intact mesentery specimen.

In summary it is noted that drastic changes induced in the mesenteric membrane can change its response and make it relatively much stiffer than the intact membrane where such changes have not

occurred. Thus, even if the handling of the tissue during the course of the experiment is the agent that induces an altered response, the test results have validity since such changes could also occur as a result of drastic or pathologic changes in the living animal. That is, the test results for such cases act as an upper bound on the magnitude of the response of the tissue; whereas, the test results from excised tissue serve as a lower bound for the tissue response.

V. Theoretical Characterization.

These experiments have indicated that it would be convenient to have a mechanical characterization of the mesenteric membrane over a wide range of response that is reasonably assumed to be elastic. When an arbitrary specimen is stretched in the laboratory, it is observed that a significant amount of hysteresis occurs upon unloading. Since hysteresis implies energy dissipation through heat and/or physiologic chemical reactions, it is probably not possible to characterize the unloading curves by a nondissipative or elastic theory. Accordingly, this discussion is limited to a characterization only of the loading curve for the various tissues.

In order to characterize elastic materials in a state of large deformation, an appropriate theory already has been developed (19). This so-called finite deformation theory has been applied by other experimenters to the characterization of some types of elastic animal tissue. For example, Fung (20) has worked with rabbit's mesentery and has chosen an exponential type of stress-strain law that correlates quite well with the experimental data obtained from such tissue stretched in the physiologic range. In what follows, we shall suggest an alternative stress-strain law based on a power of the stretch ratio which also fits quite well the mesenteric data. The advantage of introducing another function is that it fits in the more general framework of finite elasticity presented by Valanis and Landel (21).

During the past several decades theoretical mechanicians have proceeded from the assumption that a small class of materials may be

characterized as homogeneous and isotropic continua. In fact such a class of materials is well represented by most rubberlike materials - natural, synthetic, and foamed. (22,23) Under this assumption, the mechanical behavior of a given representative material may be subsumed completely in a strain energy density which depends only on the principal stretch ratios and temperature.

For mesenteric tissue, the assumption of homogeneity is strictly ad hoc inasmuch as the mesentery membrane is comprised of an elastic tissue permeated by vascular ducts of various sizes oriented primarily in the plane of the membrane. Thus, in describing such a material as homogeneous, one assumes that the properties which characterize the material are averaged through the thickness of the membrane. (23) Furthermore, the assumption of isotropy is also strictly ad hoc. It is recognized that a material perforated by oriented ducts must at best behave orthotropically if not even aeleotropically. But since our experimental data are incomplete, and for the purpose of simplifying the ensuing analytical discussion, isotropy is assumed. When appropriate additional experimental data are procured, it will be a relatively simple extension of the present work to account for anisotropy. Finally, having accepted homogeneity, the assumption of continuity follows and merely implies that there are no holes in the structure. This in turn is valid in living tissue because the membrane is actually air-tight prior to failure in the inflation tests.

The principal stretch ratios, which are the ratios of the lengths of the deformed structure relative to those of the undeformed structure,

are measured parallel to a set of Cartesian axes fixed in a body subjected to a state of homogeneous stress.

Rivlin has shown that the strain energy density must be a symmetric function of these stretch ratios and has chosen to express it in the form:

$$W=W(I_1, I_2, I_3) \tag{1}$$

where

$$I_1=\sum \lambda_i^2 \tag{2}$$

$$I_2=I_3 \sum \lambda_i^{-2} \tag{3}$$

$$I_3=\prod \lambda_i^2 \tag{4}$$

where $\{I_1\, I_2\, I_3\}$ are the invariants of the principal deformation tensor $\{\lambda_i^2\}$. In the event the material can be assumed to be incompressible, I_3 is taken to be unity.

An alternate expression for the strain energy in terms of symmetric functions has been suggested by Valanis (21) who has chosen to express it in the form:

$$W=\sum f(\ln \lambda_i) \tag{5}$$

where f is an arbitrary function of the stretch ratios, and where the logarithmic argument is admitted for algebraic convenience. In the case of incompressibility, Eq. (5) is subject to the constraint:

$$\sum \ln \lambda_i=0 \tag{6}$$

Note that in Eq. (5) there appears only <u>one</u> symmetric function, which is, therefore, an invariant of the deformation tensor, but not an irreducible invariant as are I_1 and I_2. Since Eq. (1) depends on two

invariants with $I_3=1$, and Eq. (5) depends on one invariant, the Valanis function is a special case of the more general Rivlin function. The justification for using the Valanis function will be provided by the accuracy with which it represents data.

According to the principle of virtual work, the principal stress field is generated by:

$$\sigma_i = \frac{\partial w}{\partial \lambda_i} \tag{7}$$

where σ_i is the load per unit undeformed cross section transverse to a given principal direction.

With Eqs. (5) and (6), Eq. (7) becomes

$$\sigma_i \lambda_i = f'(\ln \lambda_i) + \bar{k} \tag{8}$$

where $\bar{k}$ is a Lagrange multiplier introduced to account for incompressibility and the prime denotes differentiation with respect to its argument.

Proceeding from the observation that principal stress-stretch data obtained on mesenteric tissue are initially rather flat and then evince a rapid stiffening, a power law for the form of f is chosen and written as follows:

$$f(\ln \lambda_i) = C(\lambda_i^{\alpha} - 1) \tag{9}$$

so that

$$f'(\ln \lambda_i) = C\alpha\lambda_i^{\alpha}. \tag{10}$$

Substitution into Eq. (8) yields:

$$\sigma_i \lambda_i = C\alpha\lambda_i^{\alpha} + \bar{k} \tag{11}$$

which must agree with Hooke's law in the limit of small strain, i.e.

$$\sigma_i = 2G\epsilon_i + p. \tag{12}$$

After setting:

$$\lambda_i = 1 + \epsilon_i \tag{13}$$

in Eq. (11) and expanding to linear terms in ϵ_i, one obtains

$$C = \frac{2G}{\alpha^2} \tag{14}$$

so that Eq. (11) becomes:

$$\sigma_i \lambda_i = \frac{2G}{\alpha} \lambda_i^{\alpha} + \overline{k} \tag{15}$$

And for strip biaxial tension, we have:

$$\sigma\lambda = \frac{2G}{\alpha}\left(\lambda^{\alpha} - \frac{1}{\lambda^{\alpha}}\right) = k\left(\lambda^{\alpha} - \frac{1}{\lambda^{\alpha}}\right) \tag{16}$$

The constitutive equation of the material thus contains two parameters, k and α, which must be determined by experiments.

This constitutive relation will now be applied to two representative loading curves for mesenteric tissue from Figure 17 (mesenteric membrane per se) and Figure 22 (membrane tested with the larger clamps). In Figure 40 the data from the membrane per se experiments are indicated by the symbols and the proposed power law is indicated by the solid line. The parameters used to fit the power law expression are α=9 and k=0.069 gmf/cm^2 (0.001 psi). In Figure 41 the data from Figure 22 are likewise given by the symbols and the proposed power law is shown as a solid line. The parameters used in this fit are α=4 and k=92 gmf/cm^2 (1.3 psi). The data in each case are rather well correlated by the power law, but the material parameters are quite different and indicate that the large vessels, perfused by blood under pressure and having active properties, can

have a profound influence on the stress-strain response of the tissue.

Summary

The structure of the mesenteric tissue is made up of a complicated array of components and the mechanical response of this structure is influenced not only by local factors, but also by adjustments of higher control centers of the animal. The primary objective of the experiments was to determine the general character of the mechanical response of mesenteric membrane *per se*: that is to determine the magnitude of mechanical variations from one fan of mesenteric membrane to another in the same animal as well as the variations from animal to animal. However, the behavior of the membrane *per se* cannot be isolated from the influence of other structures such as the gut and the large blood vessels. For this reason, the experiments fall into two catergories: (1) experiments in which the influence of the gut and the large blood vessels was as weak as possible and (2) experiments in which the influence of the gut and/or the large blood vessels was quite prominent. The influences of some of these many interrelated factors have been displayed in the current findings and will now be summarized:

1. An intact freely floating segment of mesentery exists in a state of biaxial tension.
2. The stress-strain curve for mesenteric tissue is not unique, i.e., it is extremely history dependent and the level of tension in an intact specimen can vary significantly according to the activity of the components of the membrane *per se* as well as the state of the gut and the

large blood vessels.

3. The most nearly unique length of the tissue which can be detected by these experimental methods is a relaxed length determined by excising a piece of tissue of known dimensions and measuring the freely floating (in a physiologic solution) dimensions to which the tissue relaxes. There is no marked material anisotropy in the plane of the membrane per se since the variations in stretch ratio in two principal dimensions in the plane do not vary by more than five percent even with wide history variations just prior to excision of either rectangular or circular specimens.

4. The stress-strain diagram for intact strip biaxial specimens is significantly different from the diagram for excised strip biaxial specimens (i.e., specimens which are excised after the rectangular clamps have been positioned and inflated). However, in the course of a loading cycle both tissues show a wide hysteresis loop. Even though the loading and unloading curves are obtained incrementally with intermediate durations up to several minutes at each point of extension, there is still evidenced a great deal of hysteresis. Such hysteresis loop behavior is characteristic not only of various animal tissues but also many extensible synthetic materials. For instance, a wet strip from a nylon stocking shows this same qualitative

behavior when tested over a complete loading cycle.

5. Both excised and intact strip biaxial mesentery specimens show stress relaxation; however, stress relaxation is more pronounced in the intact specimens than it is in the excised specimens in a corresponding time interval.

6. For both excised and intact strip biaxial mesentery specimens there is a continuous degradation in response on successive loading cycles (for the three loading cycles in these experiments) if there is no wait between loading cycles.

7. Intact strip biaxial specimens which have large supplying vessels immediately adjacent to the specimen can completely recover in five minutes after a loading cycle and give the same force-stretch response on a second loading cycle. In other specimens, where there are no large supplying arteries and veins immediately adjacent to extensive areas of the tissue just beyond the test specimen, there appears to be a certain critical λ beyond which the tissue will not completely recover if a five minute wait is permitted between successive loading cycles in a stable state of the tissue.

8. Since the core temperature of the animal could drop as much as 3 or 4^{o}C as a result of the anesthesia, the influence of this amount of temperature variation on the force-stretch response of mesenteric membrane <u>per se</u> was

investigated. In those strip biaxial experiments where the temperature of the bath was reduced by $5^{\circ}C$ or more, the force-stretch response of the mesentery still lay within the envelope of tissue response of the membrane for successive loading cycles.

9. Not only is there a significant difference in response of excised and intact strip biaxial mesentery specimens, but there is also a wide variation in the magnitude of the mechanical response of intact specimens having different patterns and orientation of vascularization.

10. There are at least two different components of the wedge-shaped fan of mesentery which are prominent in carrying the load. At the smaller stretch ratios the vascular smooth muscle structures, innervated by the central nervous system and perfused by blood under systemic pressure, are the most prominent load carrying structure whereas at the larger stretch ratios the collageneous fiber matrix, and perhaps the two layers of endothelial cells, are recruited as the prominent load-carrying component.

11. Large variations in systemic blood pressure and/or sympathetic activity of the nervous system do influence the force-stretch response of strip biaxial specimens strongly influenced by large blood vessels. Since changes in any one factor in the animal's system can elicit adjustments in many others the system appears to stabilize at a level

dependent on the resultant of the various adjustments and at the current state of our studies it is not possible to completely determine what this level will be. If the tissue is maintained at a contracted length during the course of such adjustments and system stabilization, there can be a gradual and continuous change in the tension level of the tissue and this tension level may not be reduced to the pre-existing level even at the termination of successive external loading cycles.

12. In the strip biaxial tests, when specimens were stretched to lengths at which failure could be detected, it was noted that the tissue began to tear randomly in several minute spots, but there was no catastrophic gross tearing of the tissue at these load levels. The inflated specimens likewise failed locally by the rupture of the tissue at random, discrete points. Catastrophic gross tearing only occurred after a further increase in the loading.

13. On inflation by a hydrostatic pressure head the mesenteric tissue shows a stable, monotonically increasing relation between inflating pressure and crown height of the tissue up to the onset of local failure. In contrast, dental dam exhibits a monotonically increasing crown height-pressure relationship up to a certain level of inflation, after which there is a sudden large increase in crown height indicating a snapping over of the specimen from one region of stable

equilibrium to another region at much larger values of the crown height.

14. The test results from the inflation experiments were useful in showing that drastic changes such as sustained gut contractions, petechial hemorrhage and compression of large blood vessels can change the response of mesenteric membrane and make it relatively much stiffer than the intact membrane where such changes have not occurred. Thus, even if the handling of the tissue during the course of the experiment is the agent that induces an altered response, the test results have validity since such changes could also occur as a result of drastic or pathologic changes in the living animal. That is, the test results for such cases act as an upper bound on the magnitude of the response of the tissue;whereas, the test results from excised tissue serve as a lower bound for the tissue response.

15. A theoretical characterization that correlates rather well with the data of the loading curves for the various tissues has been proposed. The limitations and assumptions incorporated in this treatment have been discussed and when appropriate additional experimental data are procured then the analytical treatment can likewise be extended to a more adequate characterization.

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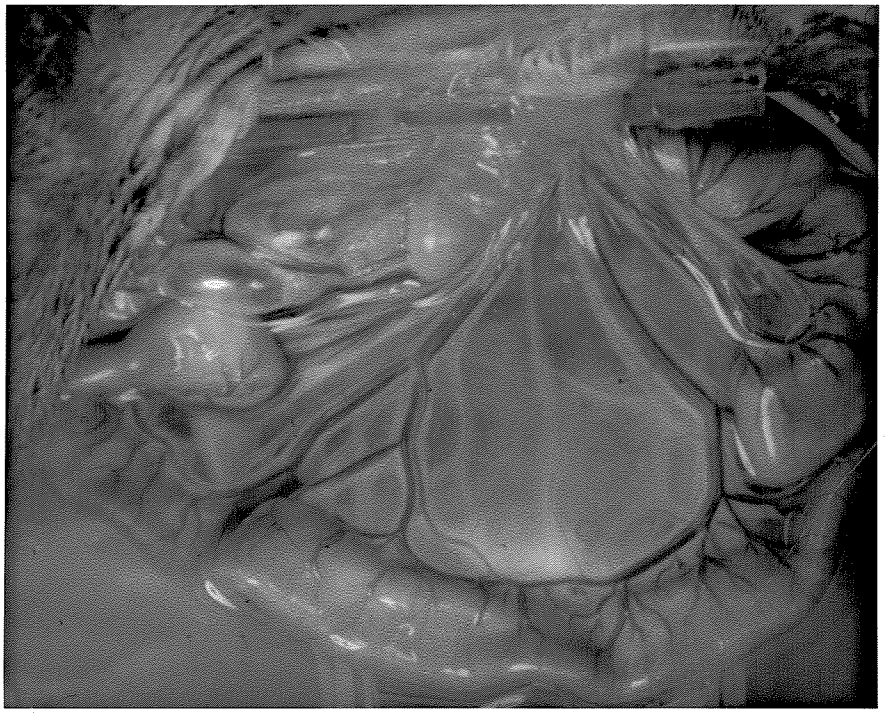


Figure 1 . Wedge-shaped fan of mesentery showing the structural arrangement of the blood vessels and the small intestines.

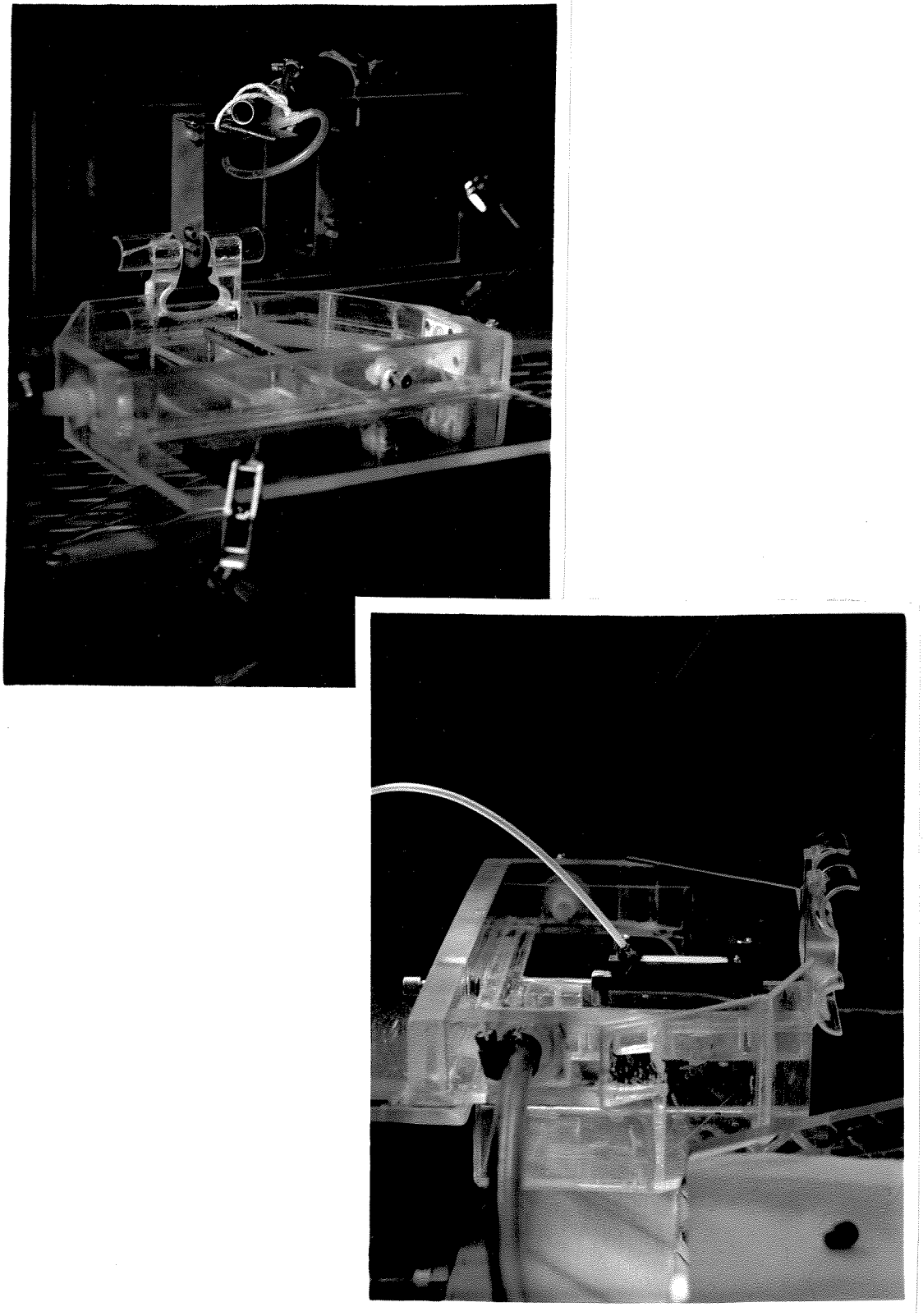


Figure 2. Lucite tray used for testing mesentery specimens.

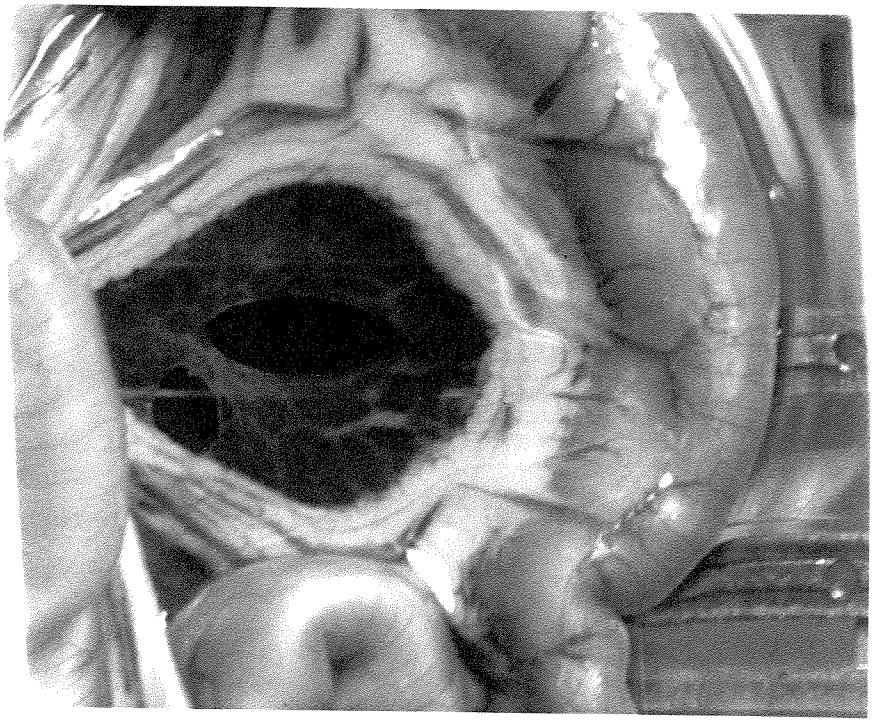
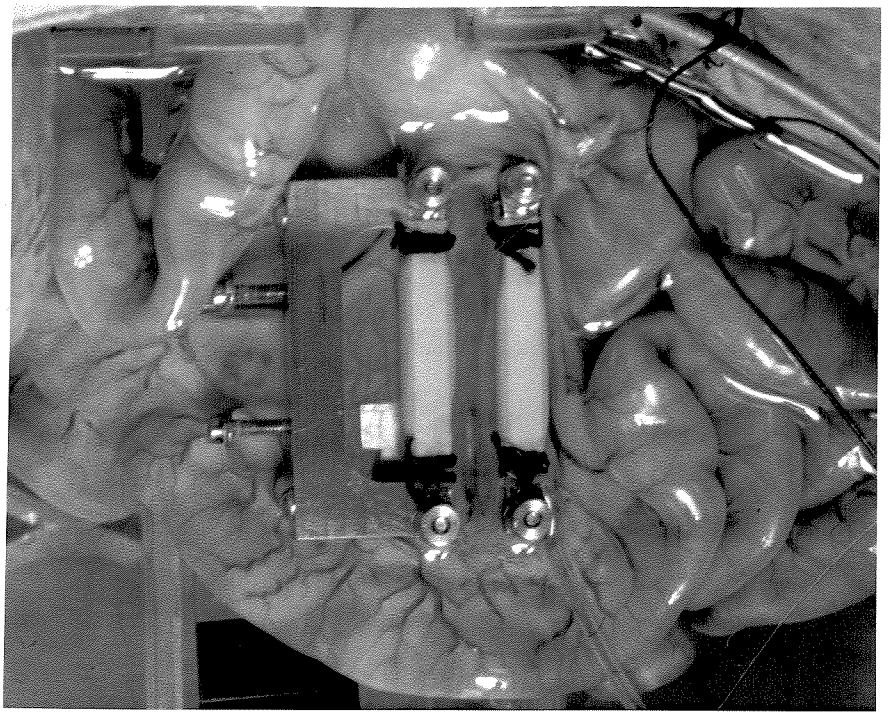
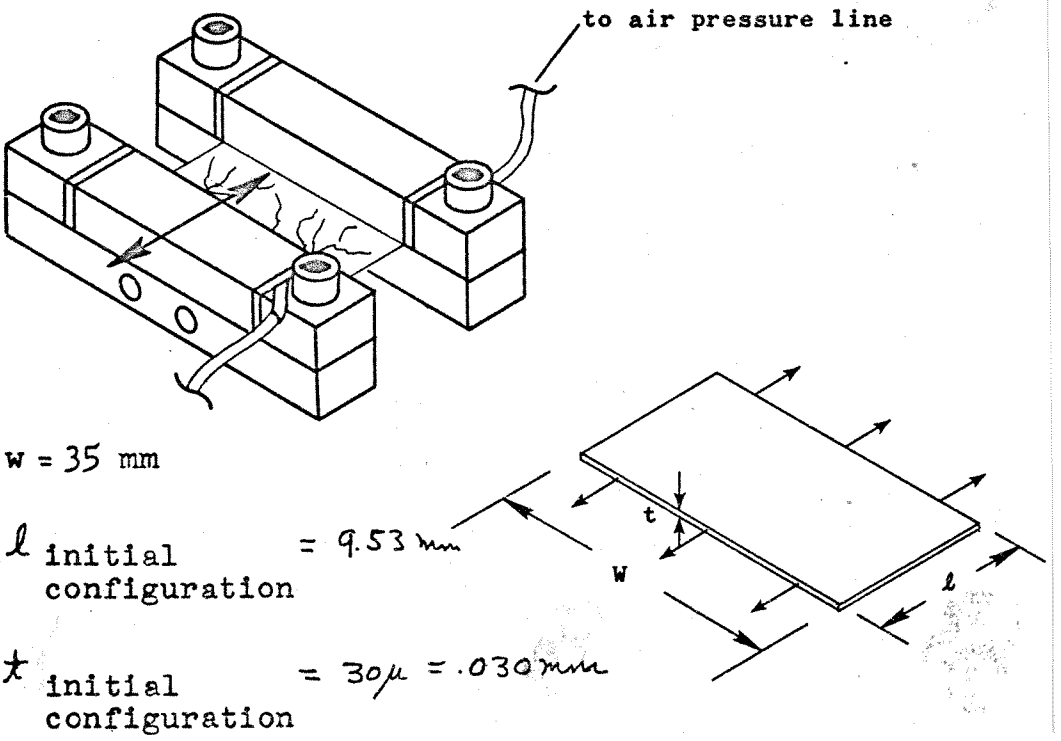


Figure 3. A state of biaxial tension exists in the freely floating mesentery as is evidenced by the gaping holes which form when an incision is made through the plane of the membrane.

Figure 4. a. The assembled clamps on the initially freely floating fan of mesentery.



b. Excised specimen



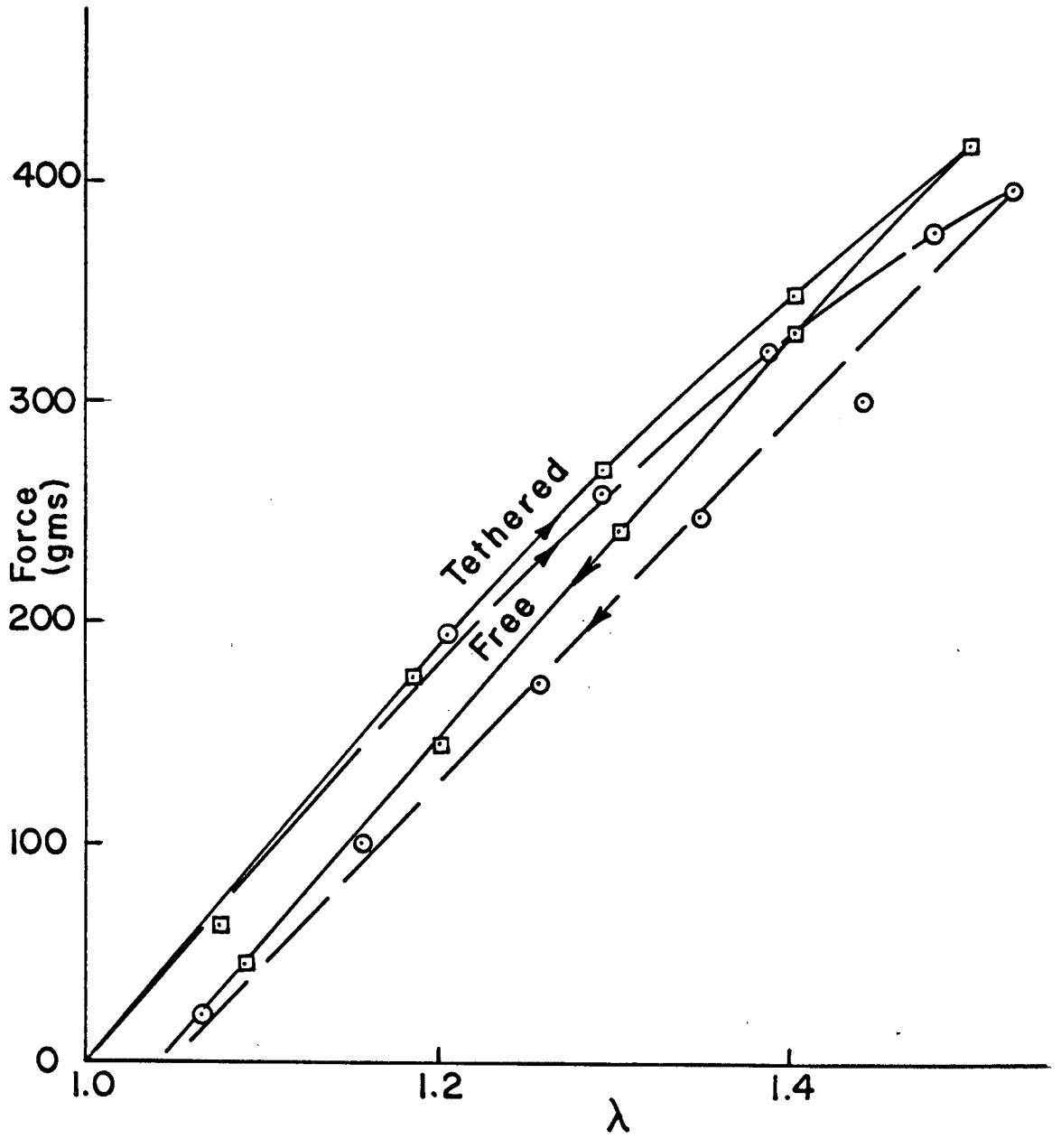
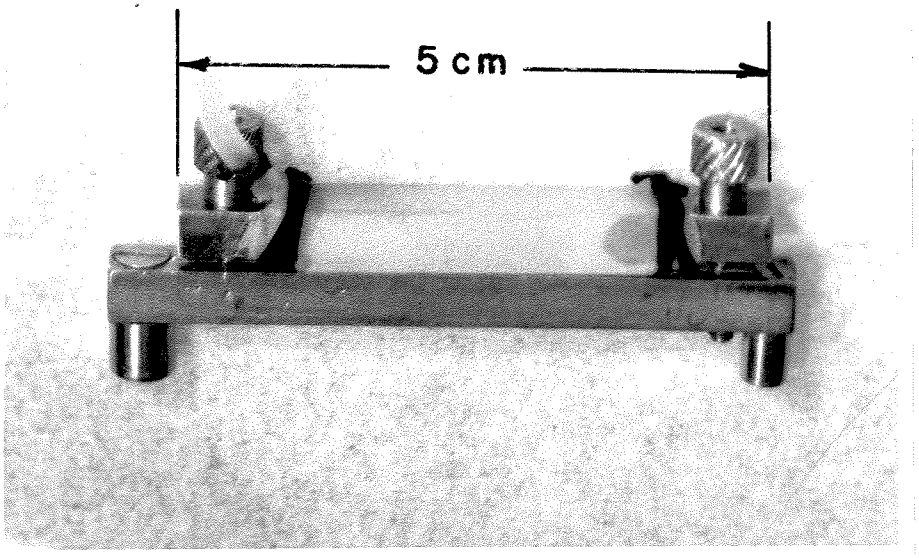
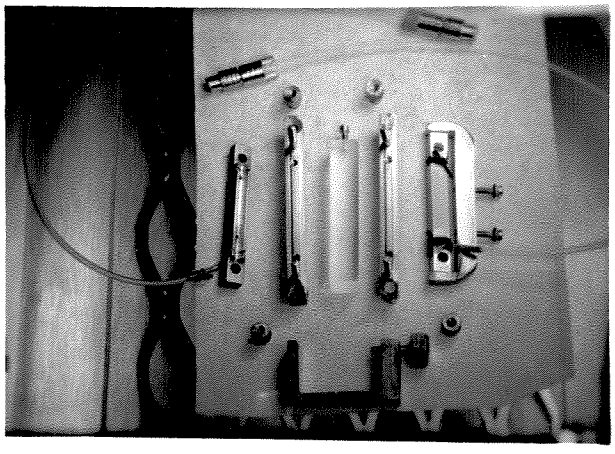


FIG. 5 FORCE - STRETCH DIAGRAMS FOR TETHERED AND FREE STRIP BIAXIAL SPECIMENS OF DENTAL DAM





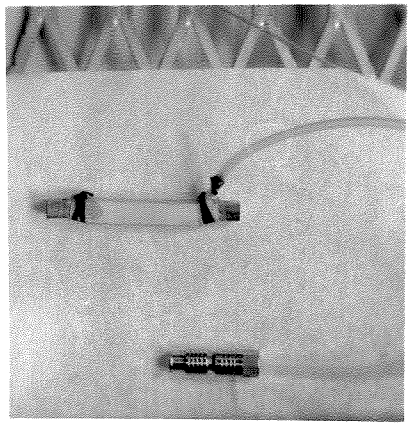


Figure 6. Strip biaxial clamps.

Figure 7. Flexure, force transducer, linear transducer and support frame.

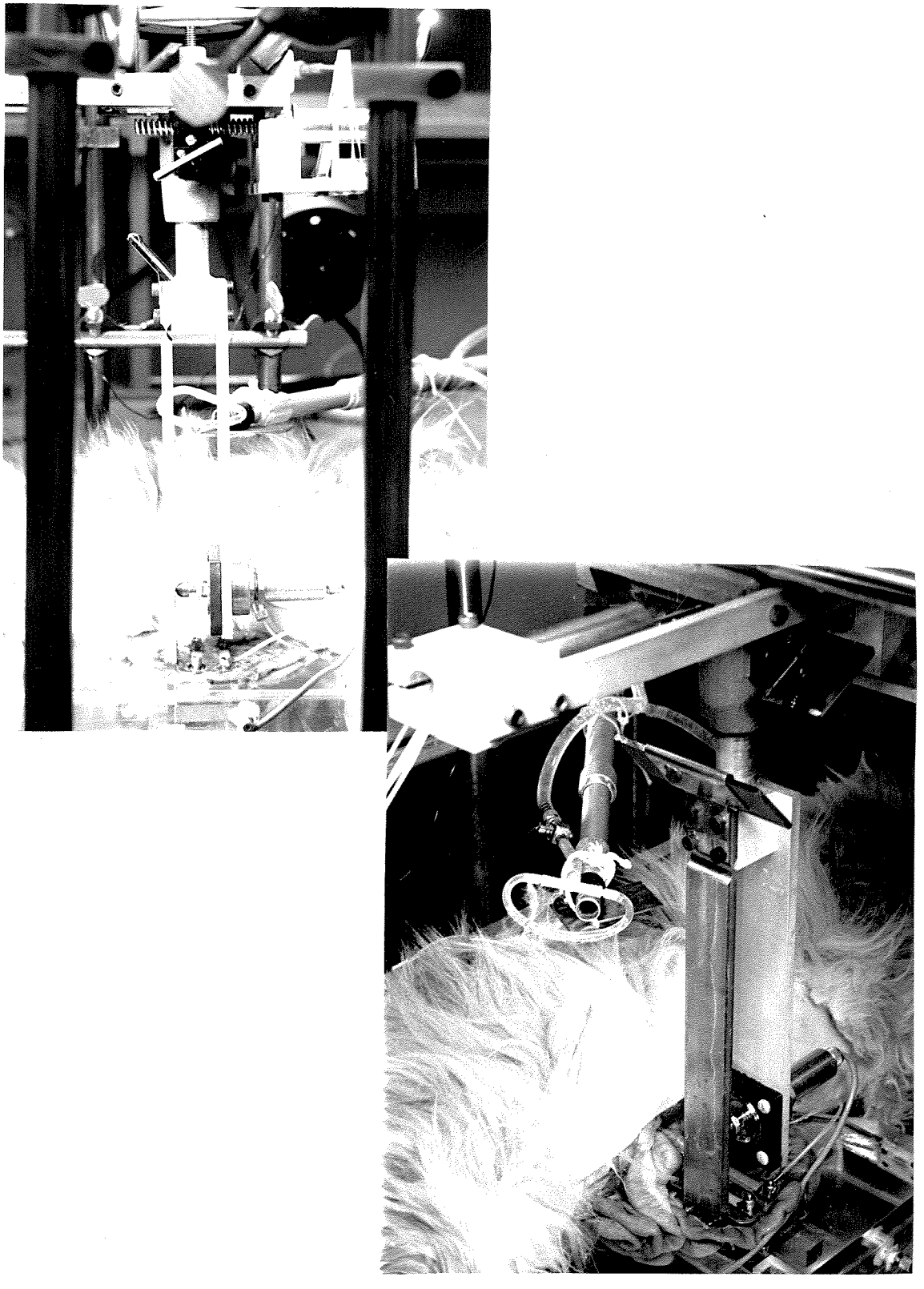


Figure 8. Flexure assembly.

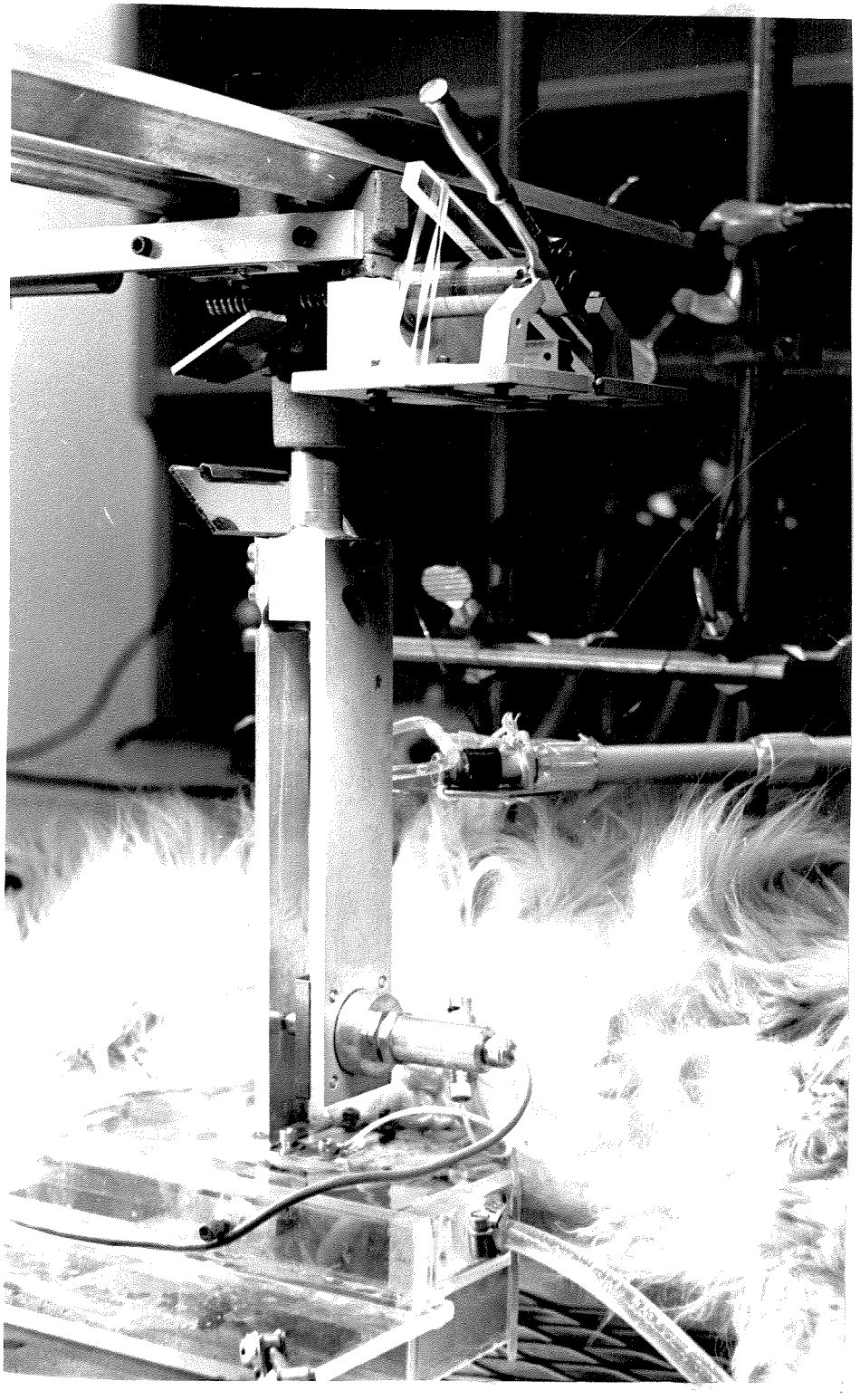


Figure 9. Clamp bottoms, lucite bench, two C clamps.

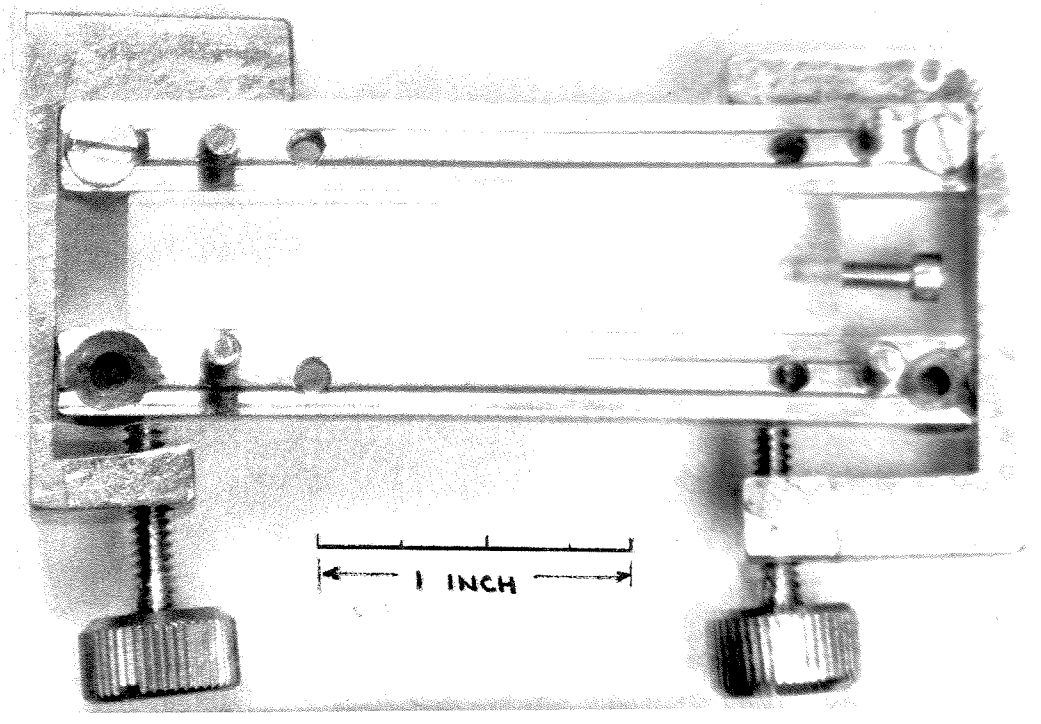
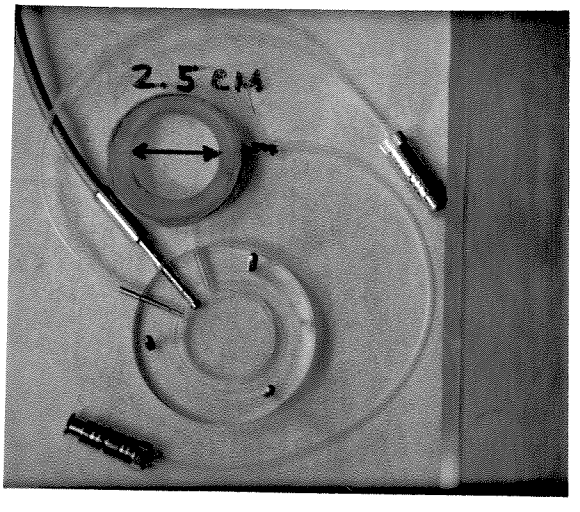


Figure 10. Circular clamp with temperature probe.



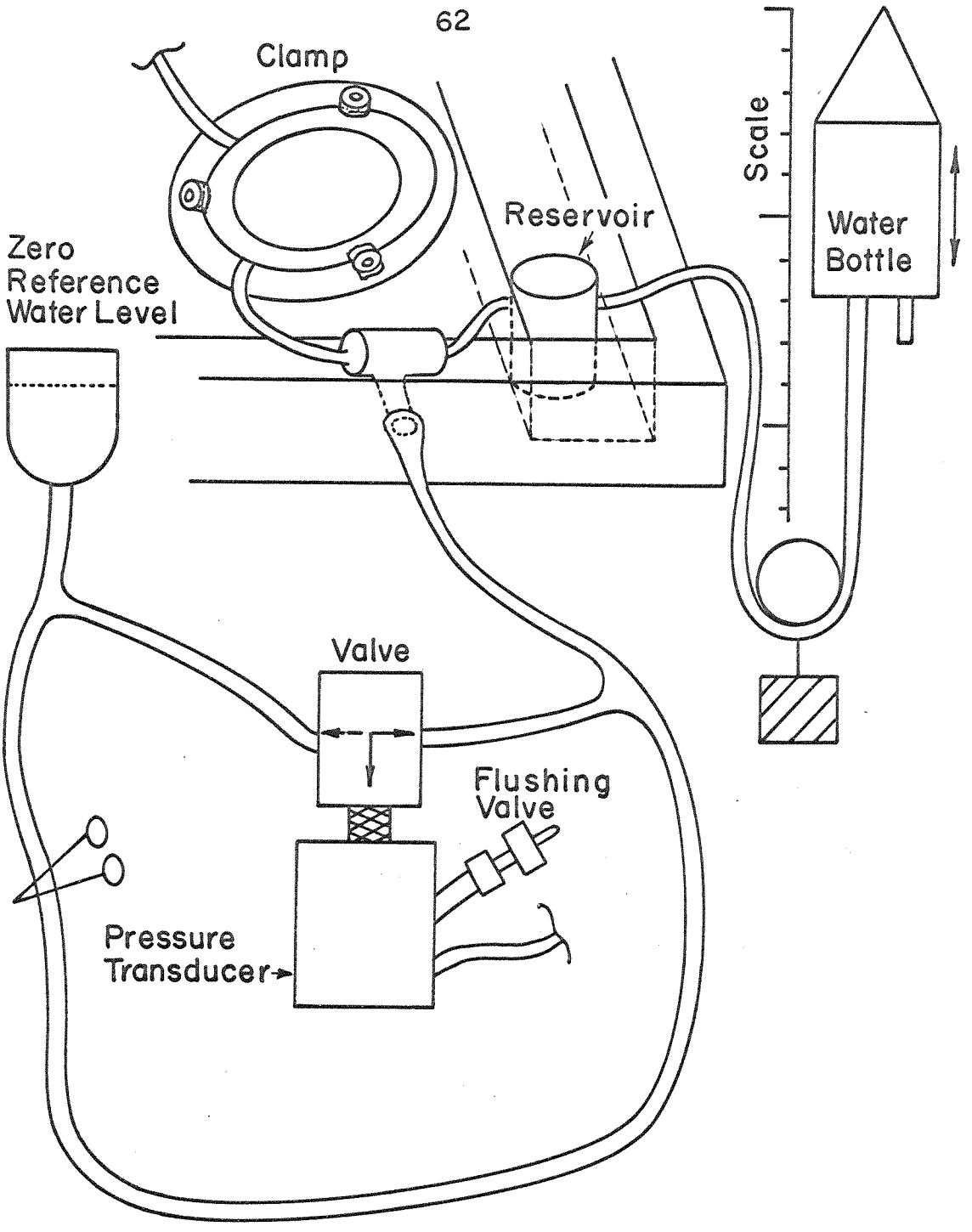


FIG. II SCHEMATIC OF THE PLUMBING AND PRESSURE TRANSDUCER USED IN THE INFLATION EXPERIMENTS

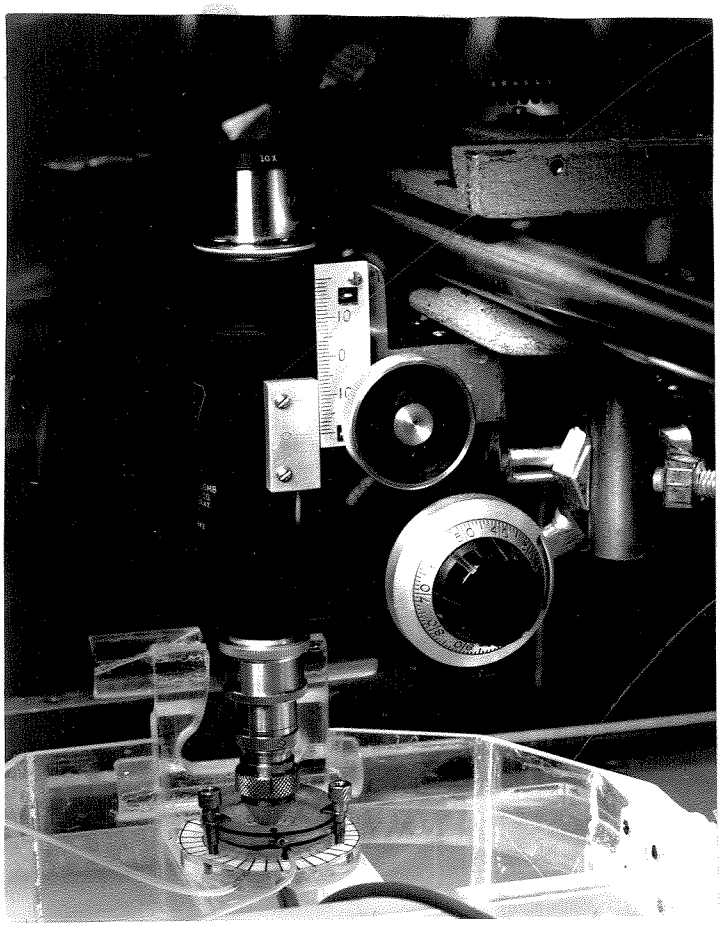
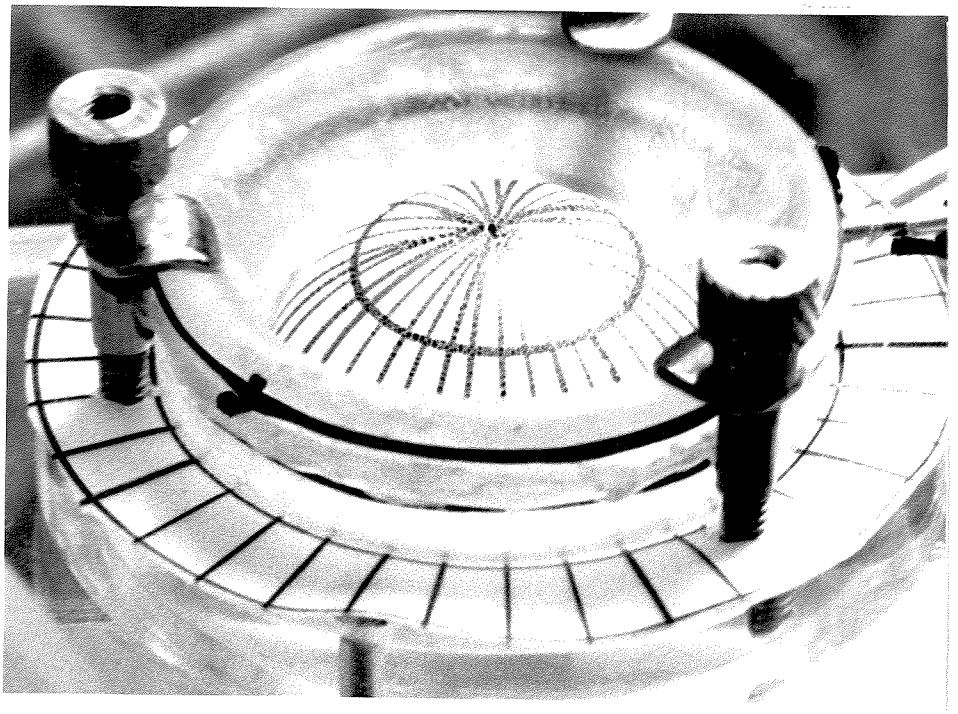


Figure 12

Traveling microscope with vernier attachment



Inflated rubber membrane

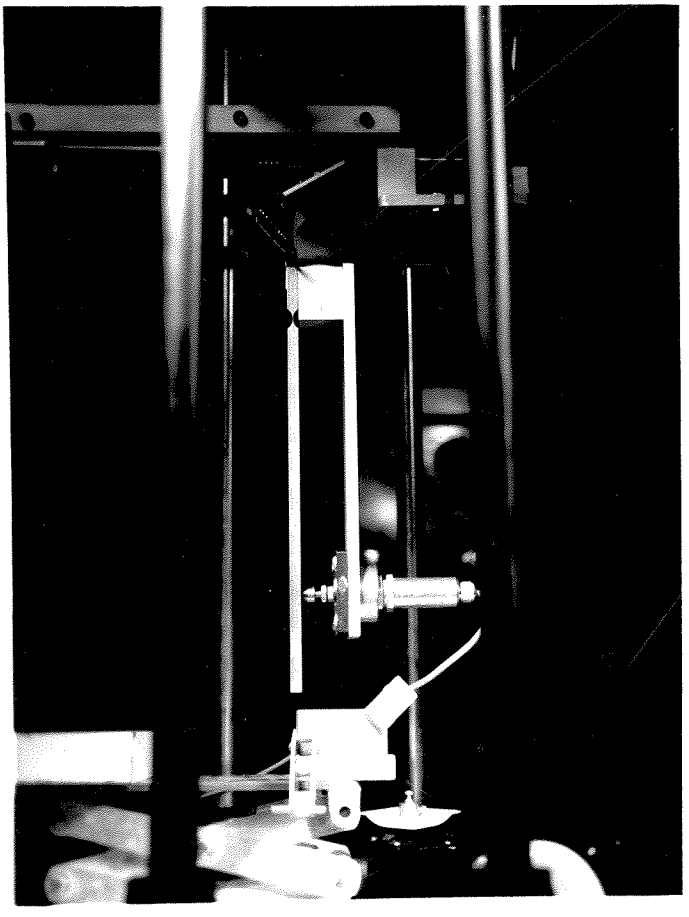


Figure 13 a. Force transducer calibration using dead weights.

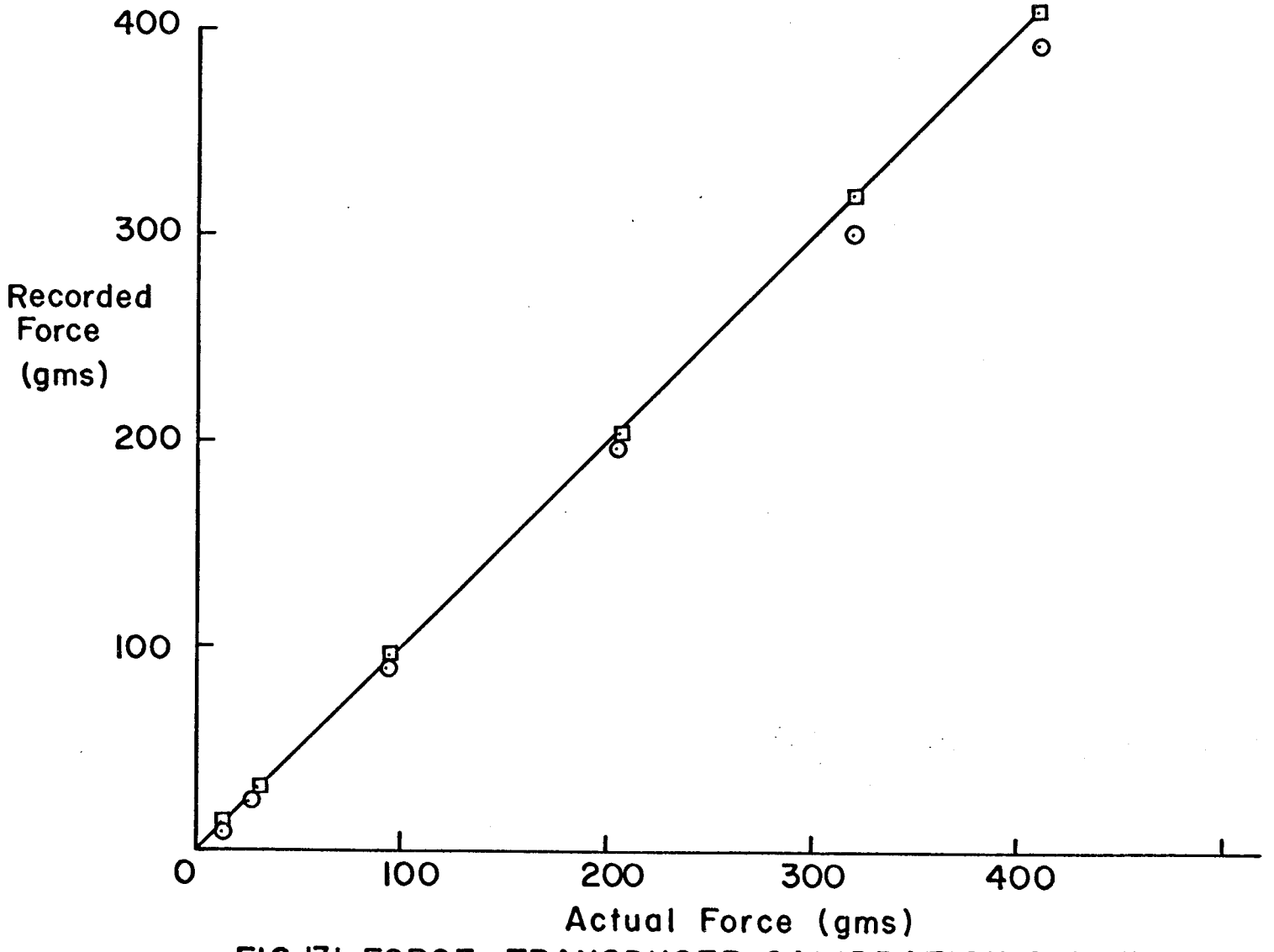


FIG.13b FORCE TRANSDUCER CALIBRATION CURVES

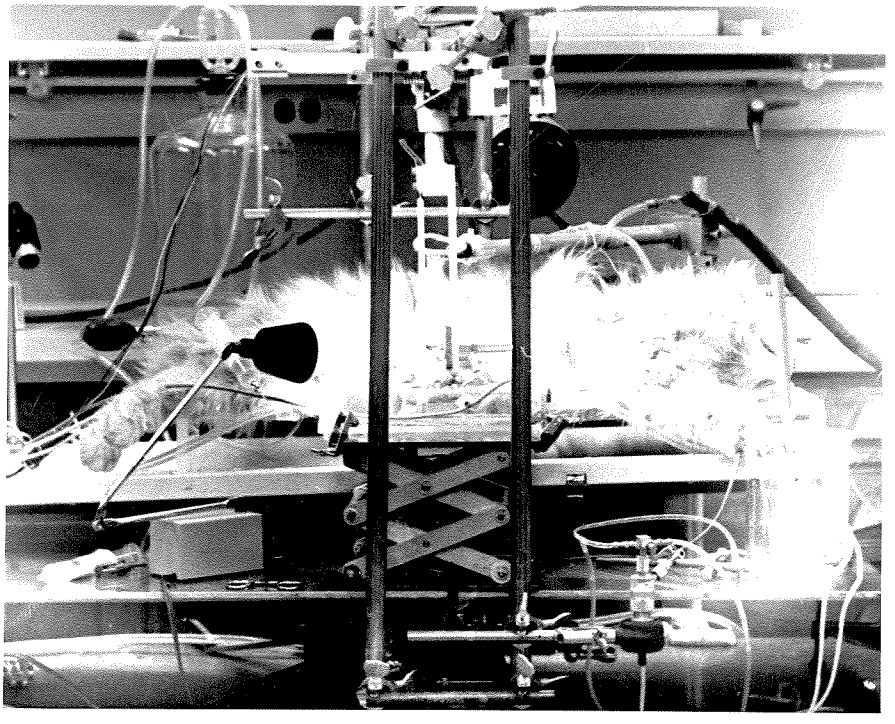


Figure 14. Main operating table after the tissue has been prepared, the two physiologic drips are operational, the strip biaxial clamps have been positioned and the flexure pivot frame has been assembled.

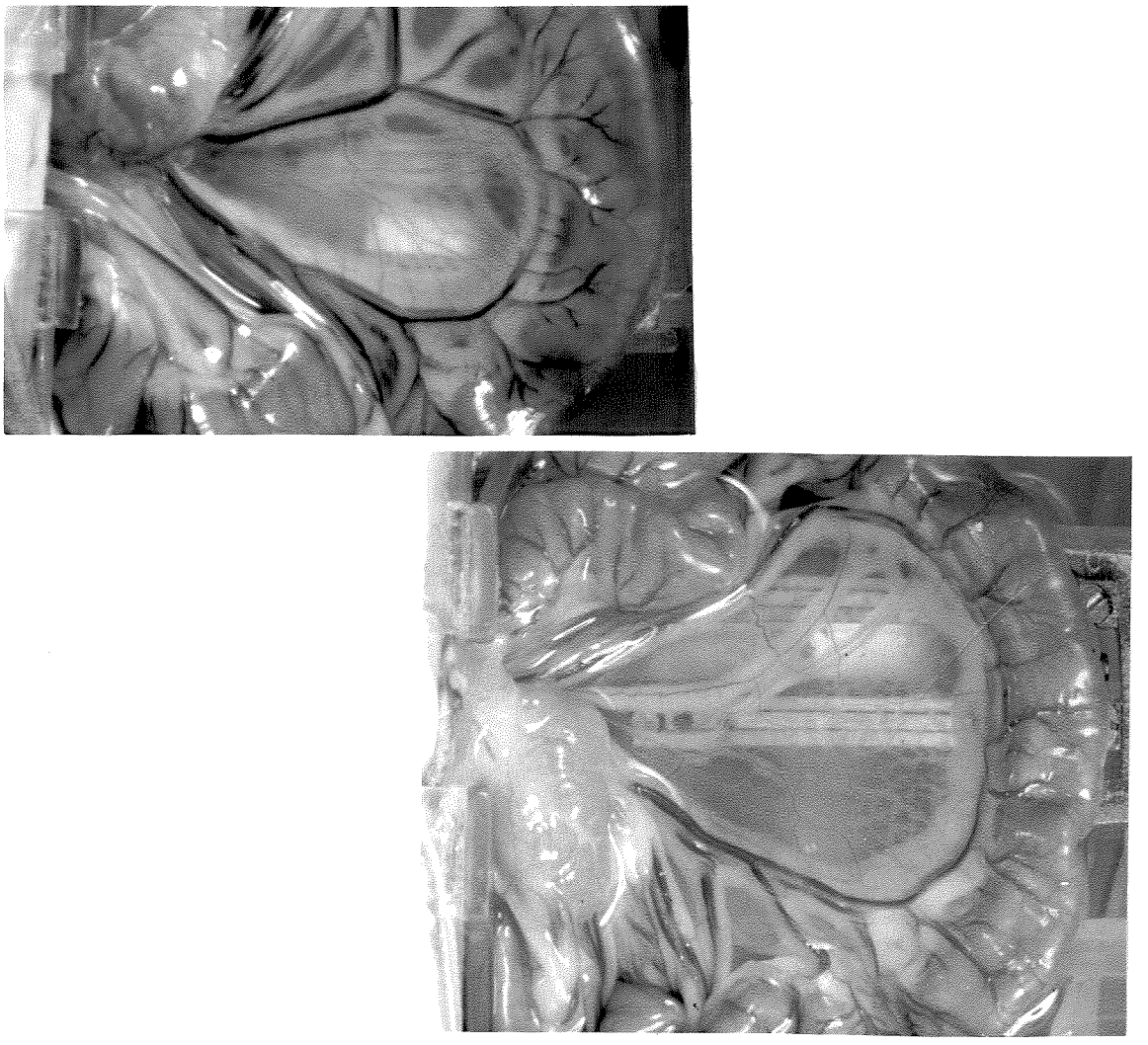
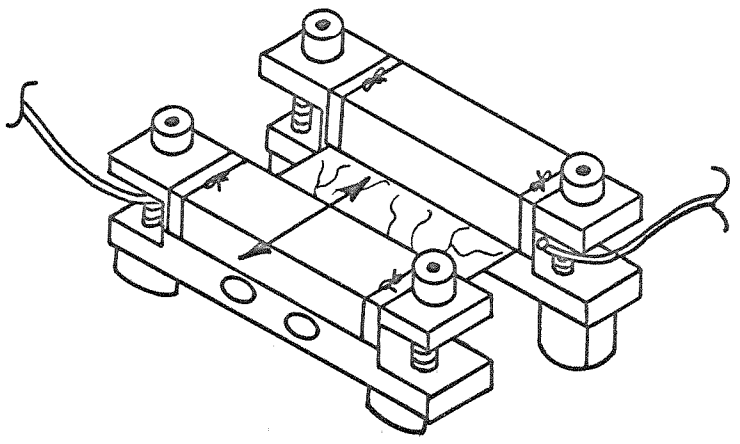
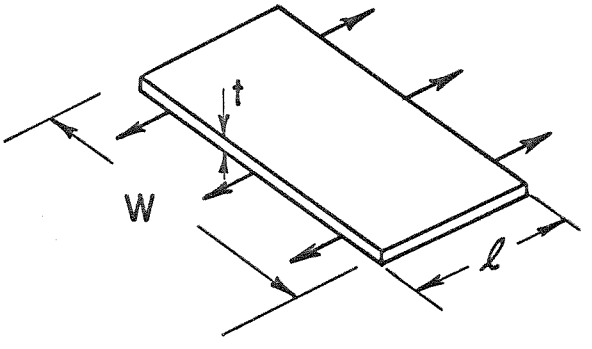


Figure 15. Three different tissue specimens with different arrangements of the large supplying arteries and veins.





W = 28 mm

ℓ initial configuration = 7.0 mm

t initial configuration = 30μ = 0.03 mm

FIG. 16 EXCISED SPECIMEN WITH THE MINIATURIZED RECTANGULAR CLAMPS

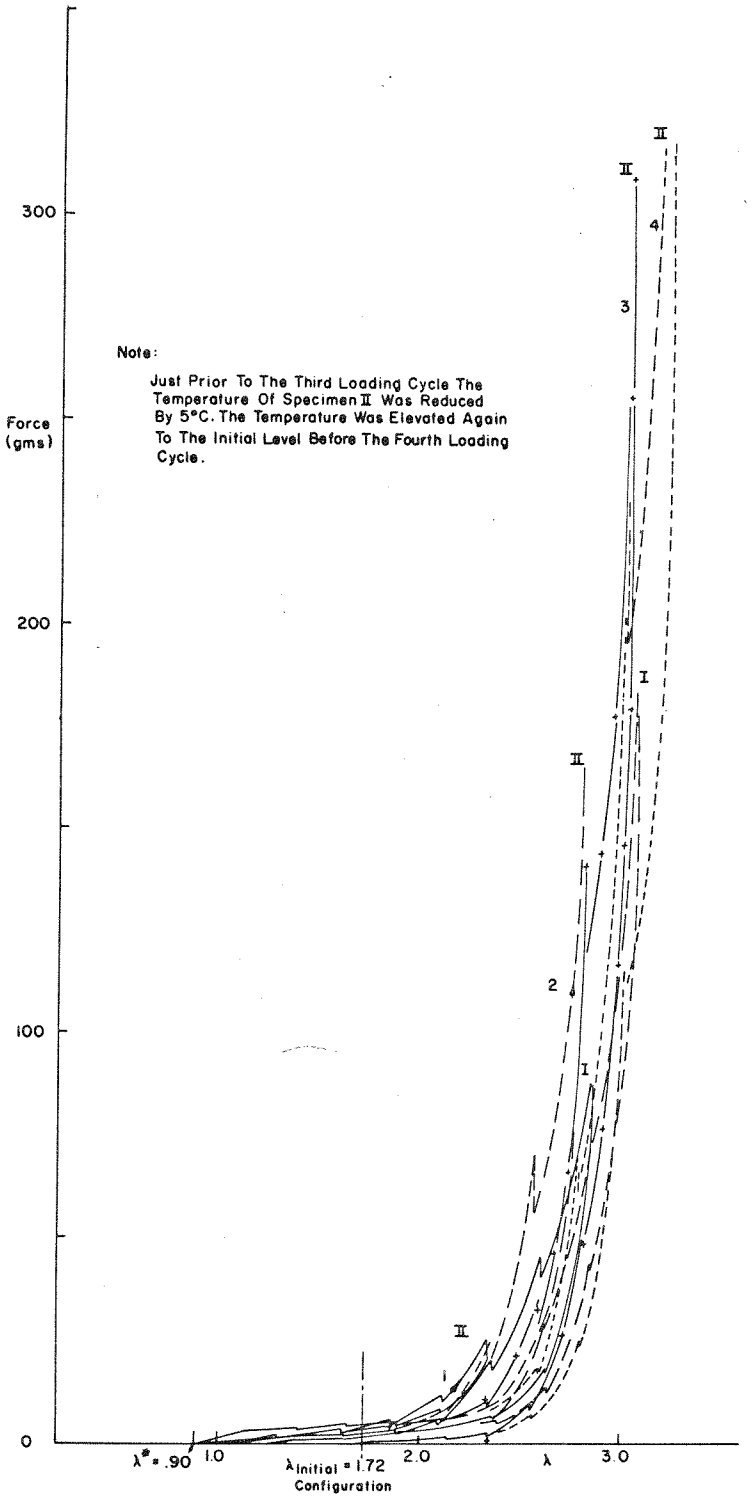


FIG. 17 FORCE-STRETCH DIAGRAMS OF TWO MESENTERIC MEMBRANE SPECIMENS PER SE TESTED WITH THE MINIATURIZED CLAMPS. FIVE MINUTE WAIT BETWEEN LOADING CYCLES.

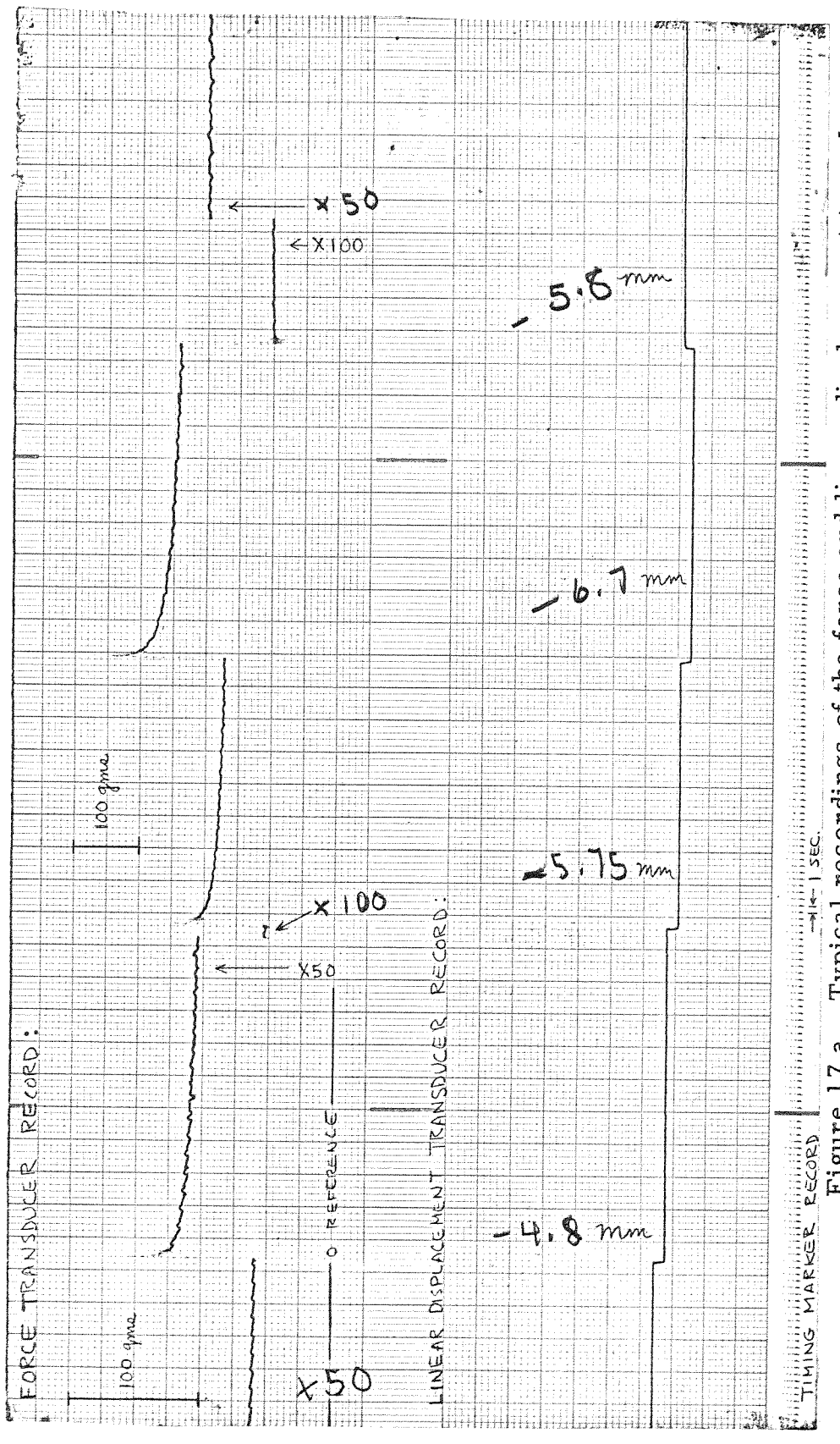


Figure 17 a. Typical recordings of the force and linear displacement transducers

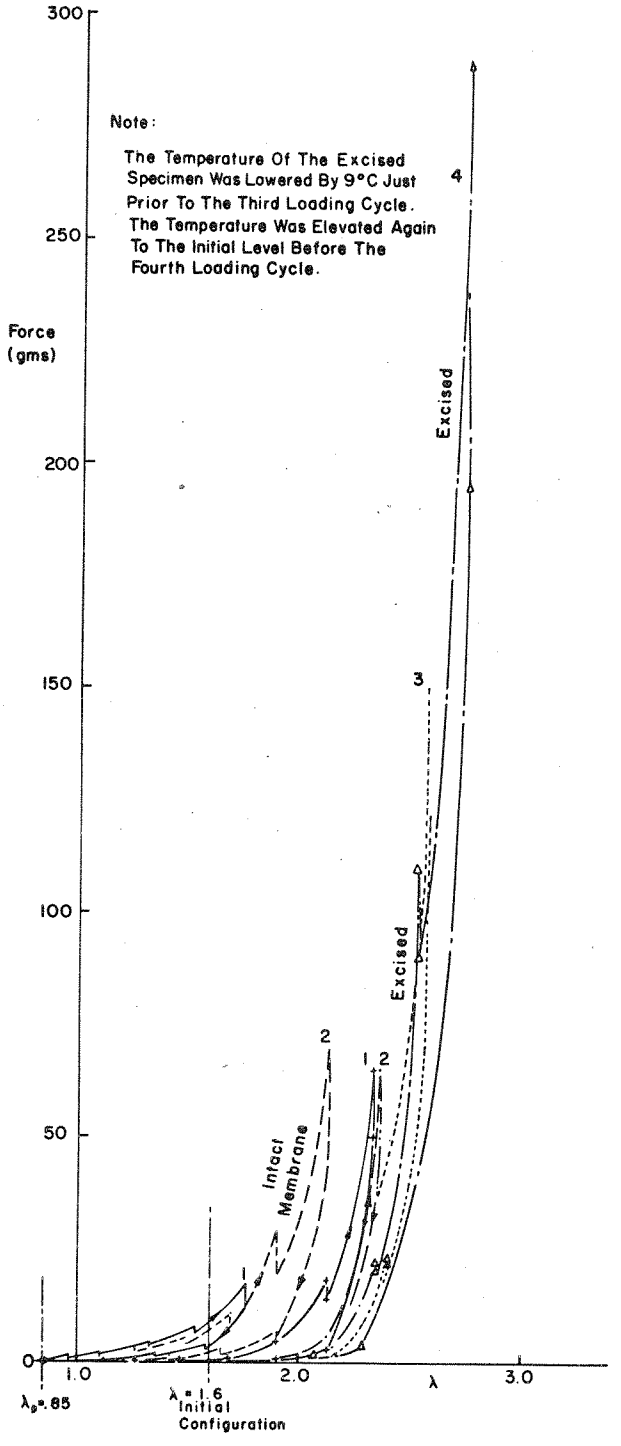
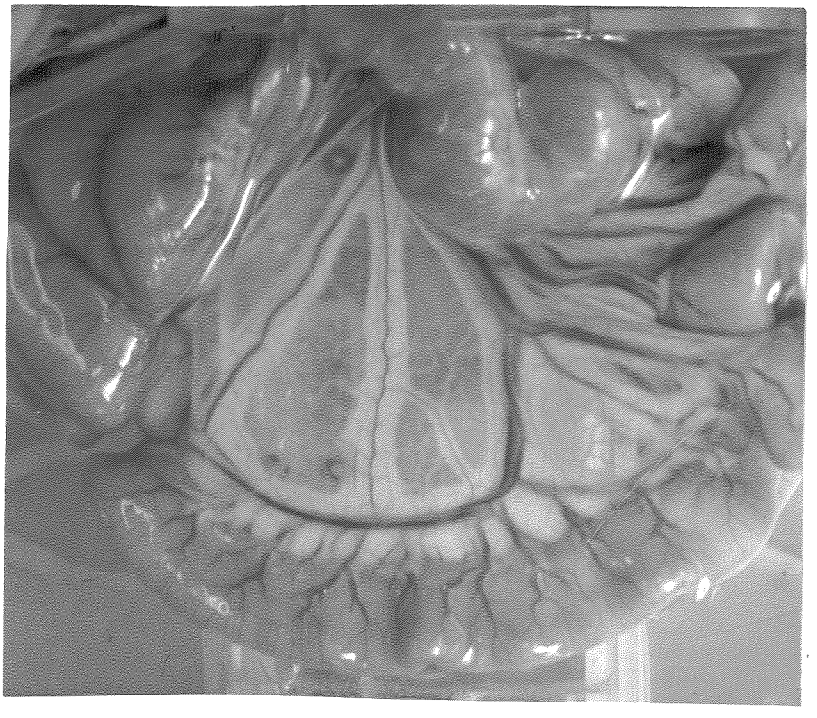


FIG. 18 FORCE-STRETCH DIAGRAMS FOR AN INTACT MESENTERIC MEMBRANE AND ALSO THE SAME SPECIMEN AFTER EXCISION. THE MINIATURIZED CLAMPS WERE USED AND A FIVE MINUTE WAIT WAS INCORPORATED BETWEEN LOADING CYCLES.

Figure 19. Three different specimens of mesentery from the same animal.

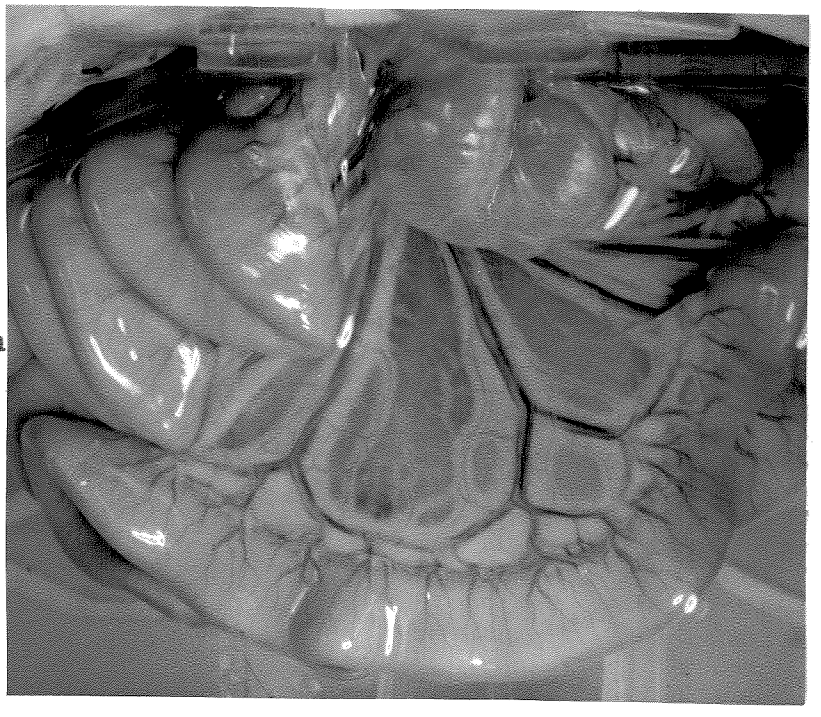
a. Specimen I

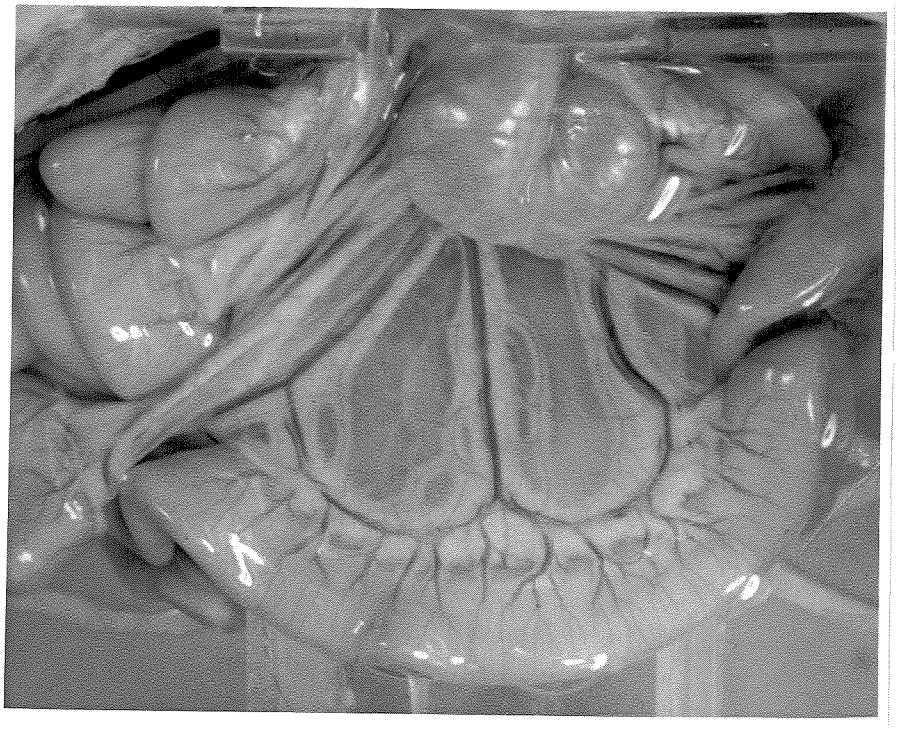
Situated along the jejunum.



b. Specimen II

Located at an intermediate position between specimen I and the ileocaecal junction.





c. Specimen III

Situated near the ileocaecal junction.

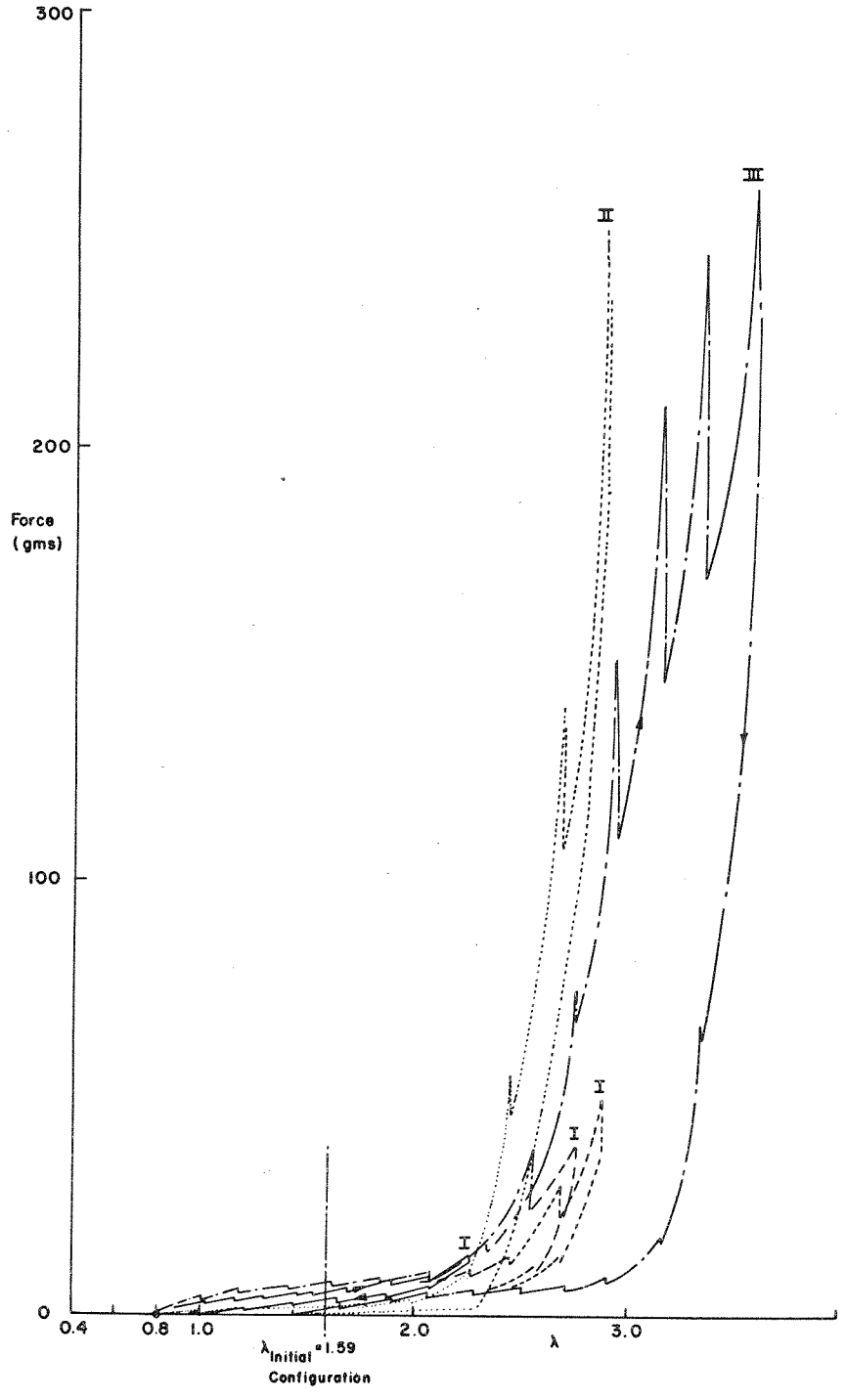


FIG. 20 FORCE-STRETCH DIAGRAMS OF THREE INTACT MESENTERY SPECIMENS WITH DIFFERENT ORDERS OF LARGE VASCULARIZATION. THE MINIATURIZED CLAMPS WERE USED AND A 5 MINUTE WAIT WAS INCORPORATED BETWEEN LOADING CYCLES.

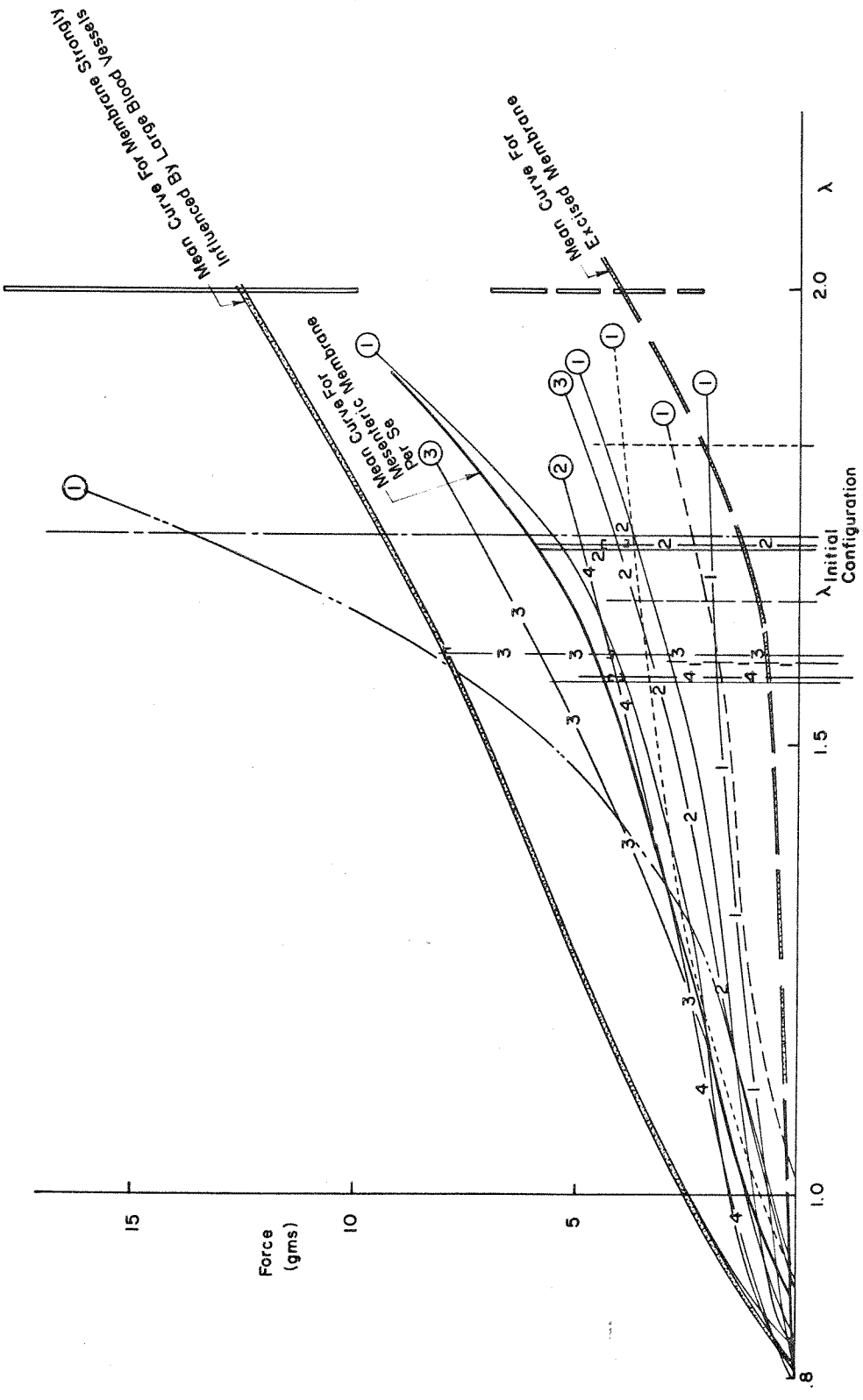


FIG. 21 NINE FORCE-STRETCH DIAGRAMS FOR FOURTEEN SPECIMENS OF MESENTERIC MEMBRANE PER SE AND THE MEAN CURVE FOR THESE NINE DIAGRAMS. THE MEAN FOR THE EXCISED MEMBRANE AND THE MEAN FOR FIVE SPECIMENS STRONGLY INFLUENCED BY LARGE BLOOD VESSELS ARE GIVEN FOR COMPARISON.

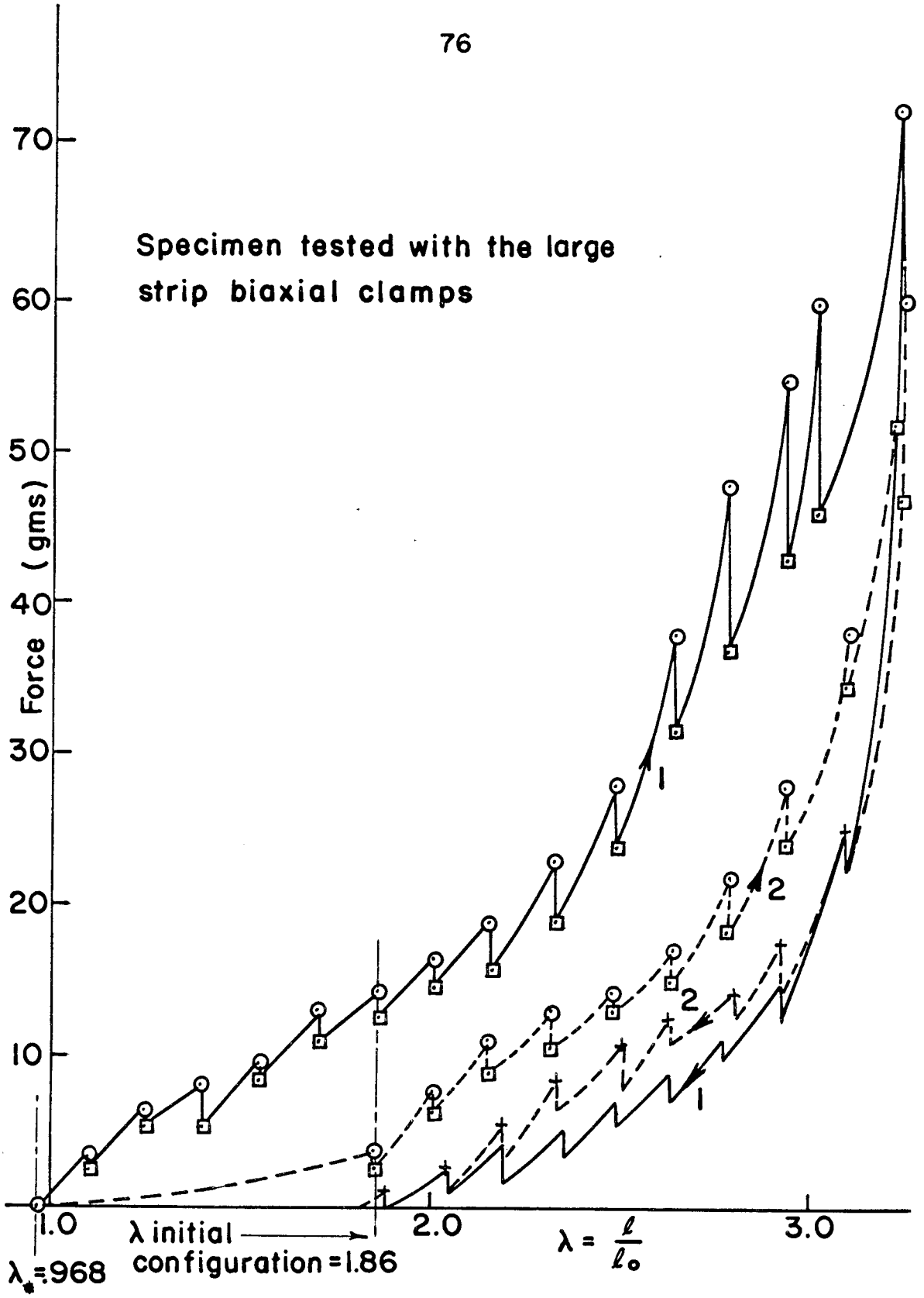


FIG. 22 FORCE STRETCH DIAGRAMS FOR AN INTACT STRIP BIAXIAL MESENTERY SPECIMEN

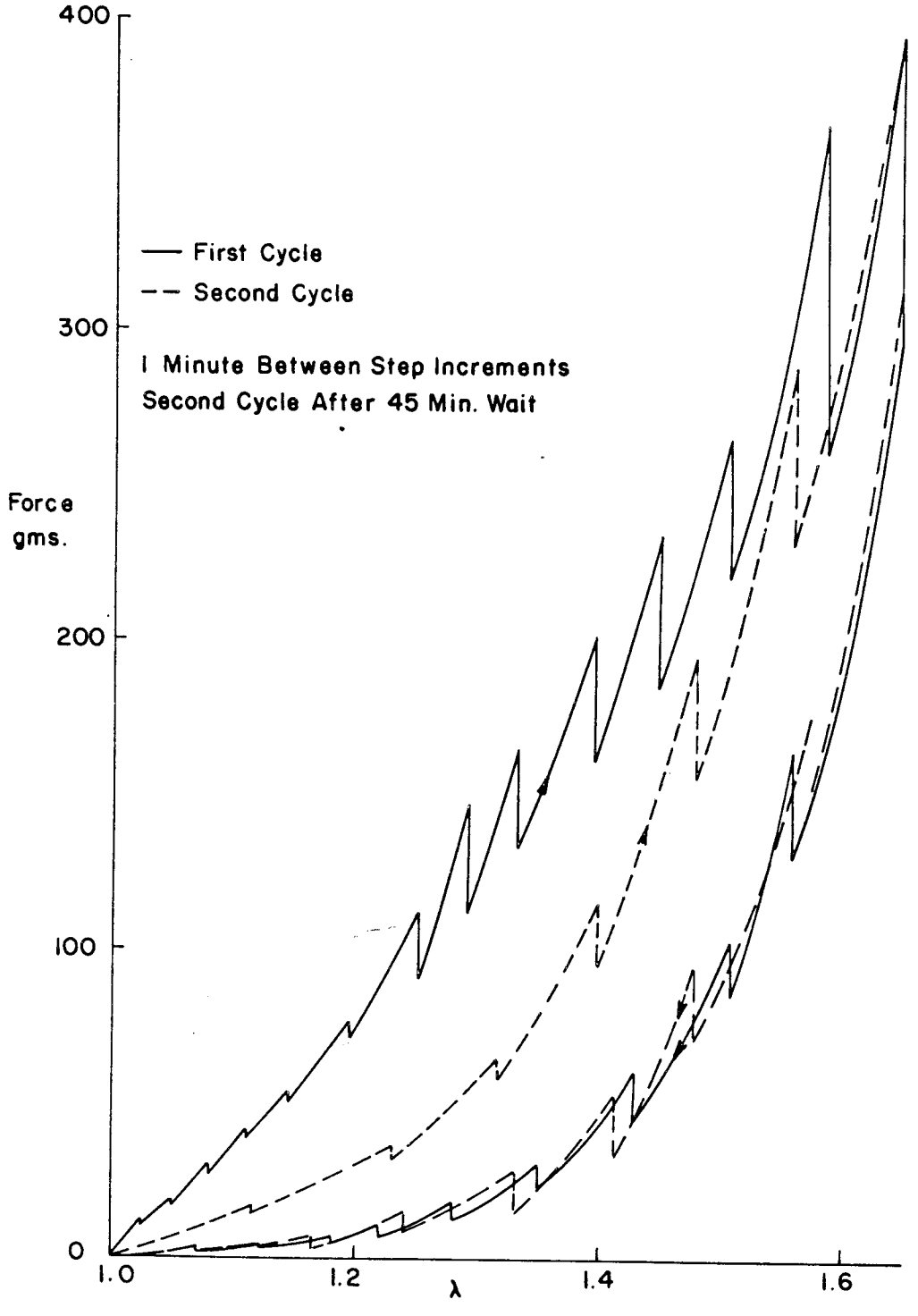


FIG. 23 FORCE-STRETCH DIAGRAMS FOR A WET NYLON STOCKING STRIP BIAXIAL SPECIMEN

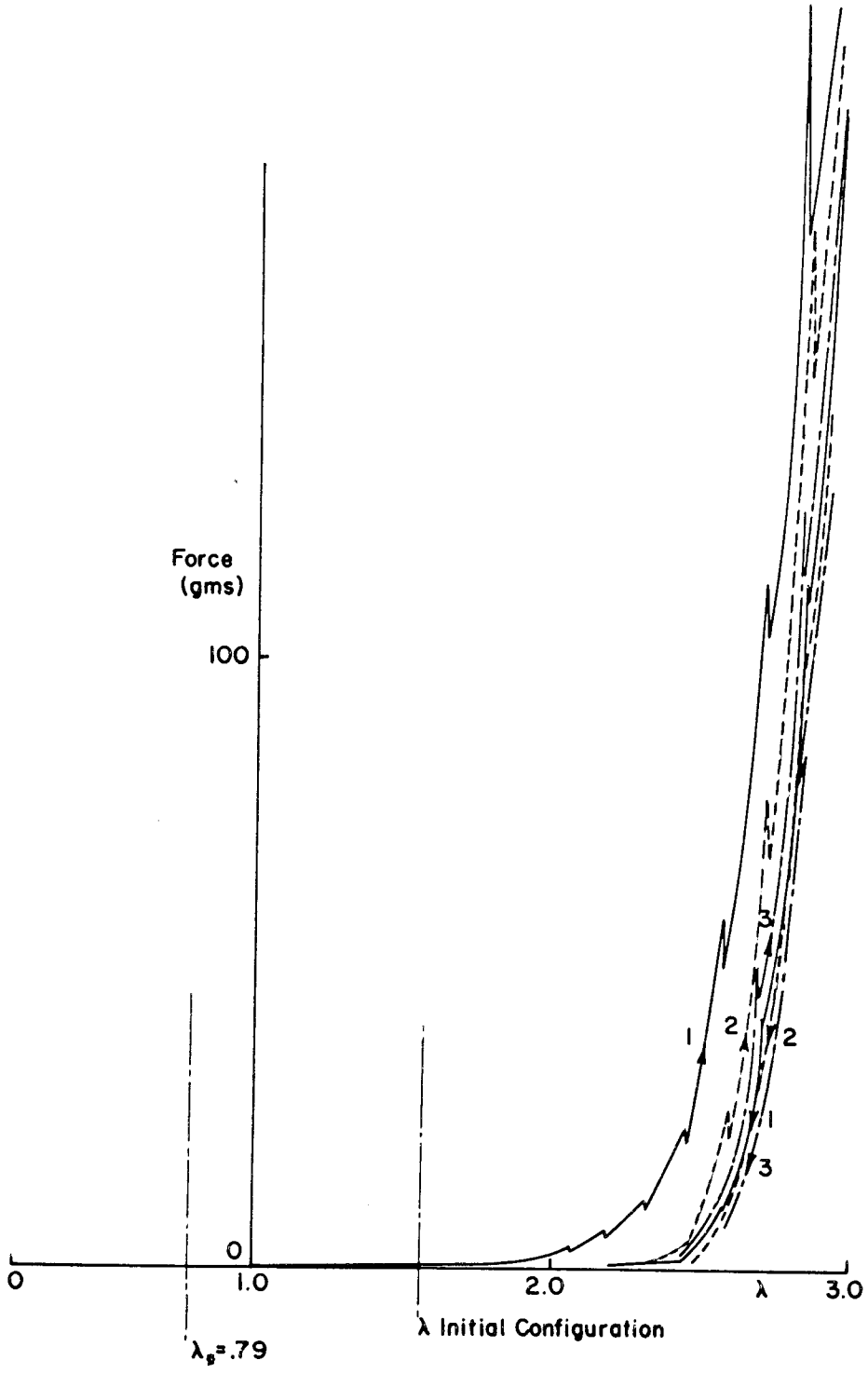


FIG. 24 FORCE-STRETCH DIAGRAMS FOR AN EXCISED STRIP BIAXIAL SPECIMEN OF MESENTERY

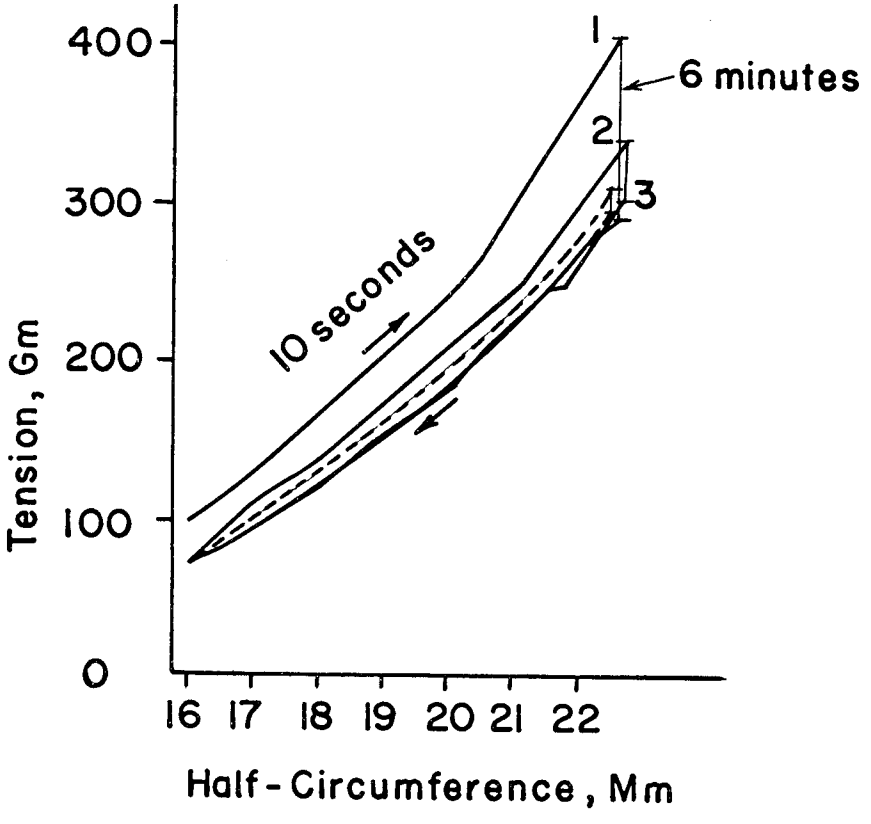


FIG. 25 EFFECT OF SUCCESSIVE STRETCHES ON THE HYSTERESIS LOOP OF A RING OF ASCENDING AORTA. (From J.W. Remington, American Journal of Physiology)

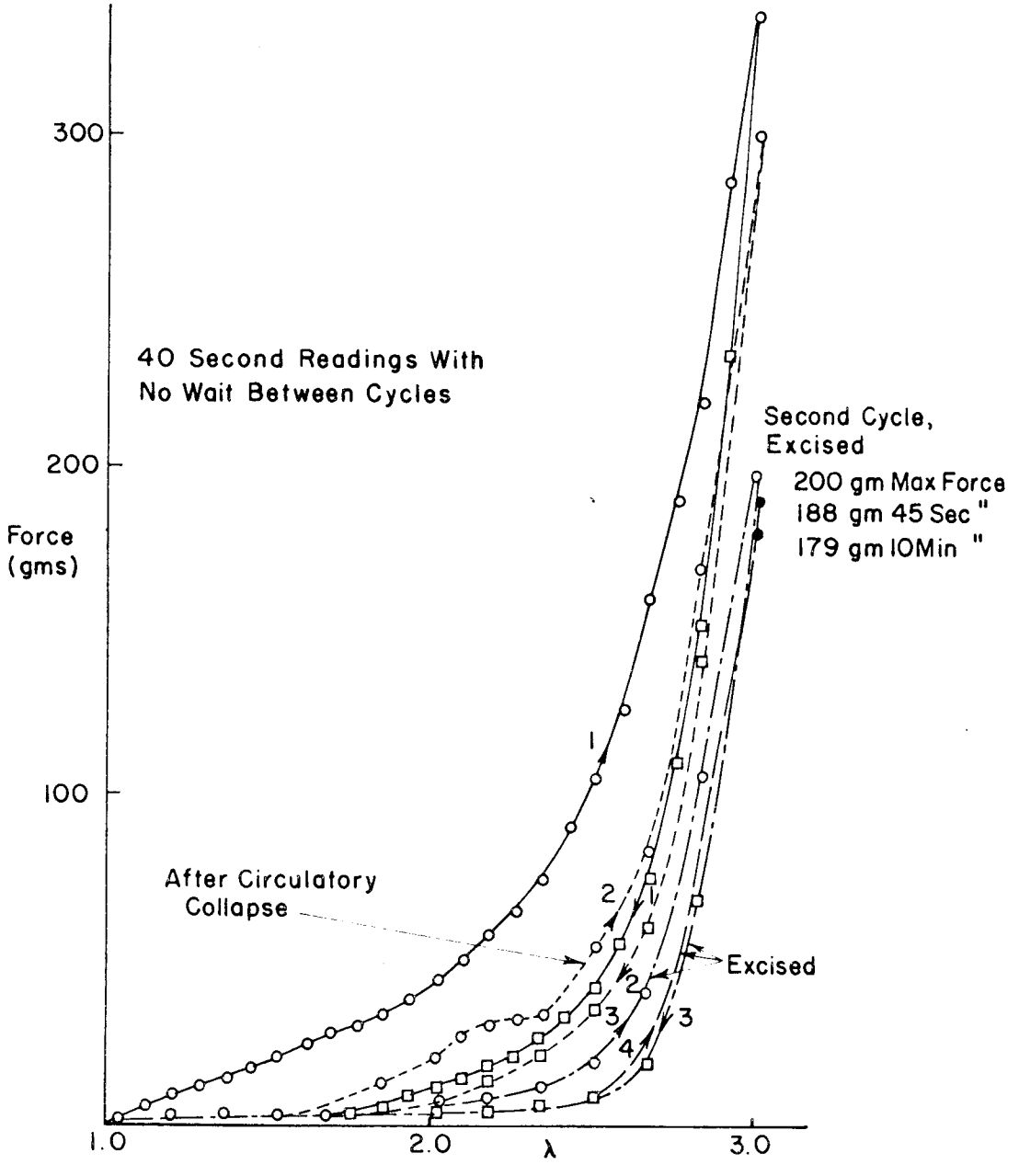


FIG. 26 INTACT MESENTERY, MESENTERY AFTER CIRCULATORY COLLAPSE AND EXCISED MESENTERY

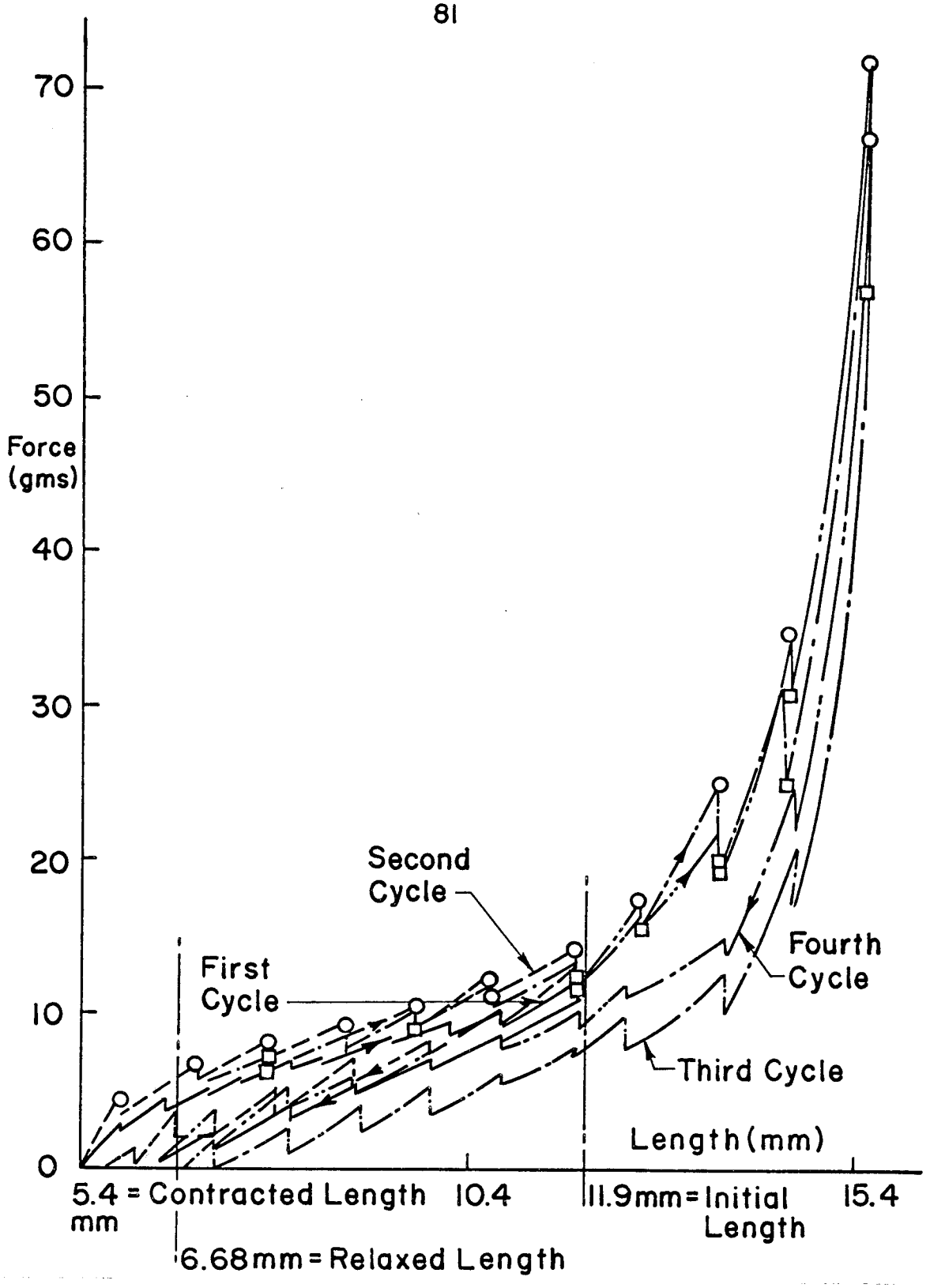


FIG. 27 LOADING CYCLES OF DIFFERENT AMPLITUDES FOR INTACT MESENTERY SPECIMEN. 5 MINUTE WAIT BETWEEN CYCLES.

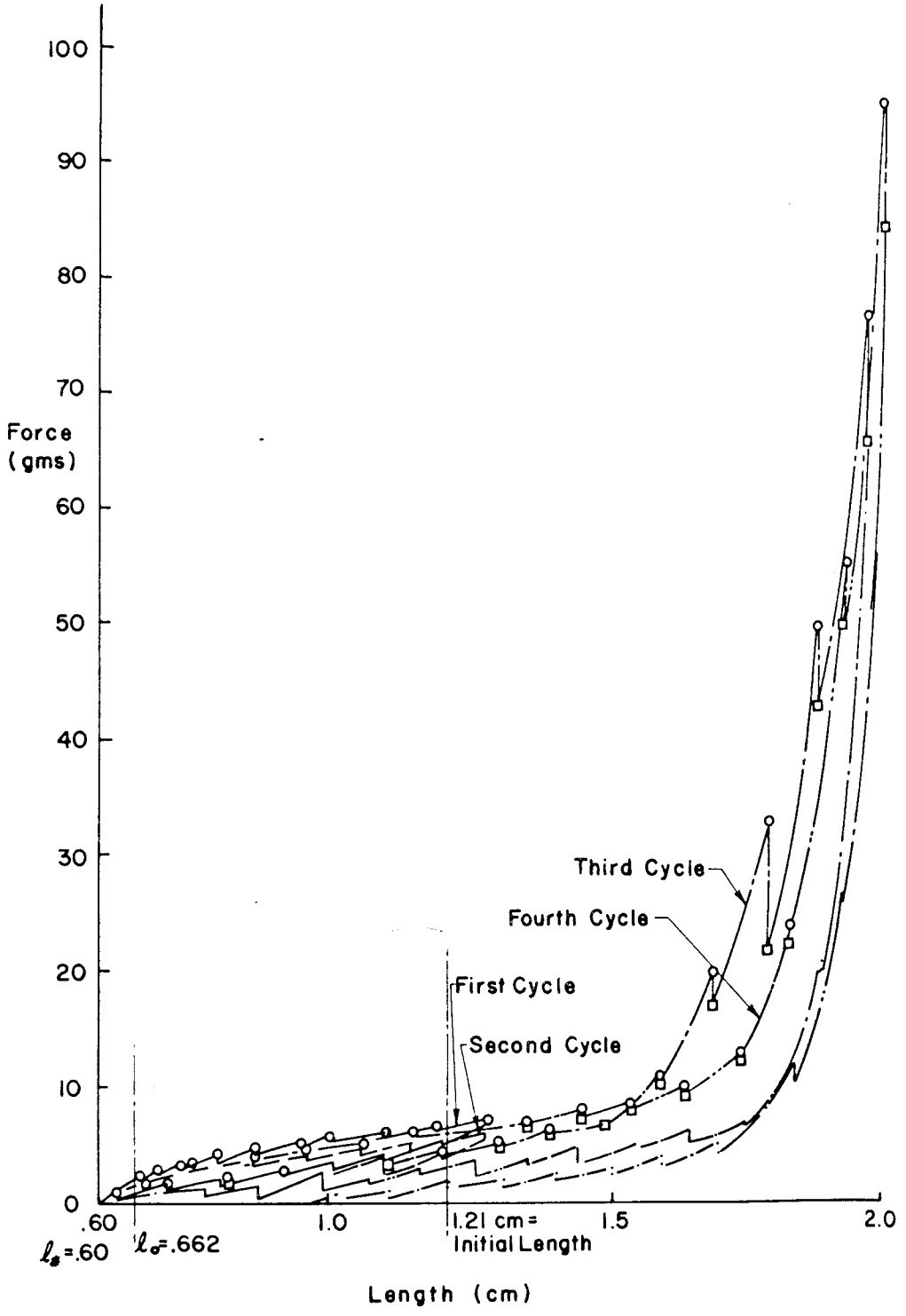


FIG. 28 LOADING CYCLES OF DIFFERENT AMPLITUDE. 5 MINUTE WAIT AT λ = 1.31 IN THIRD CYCLE

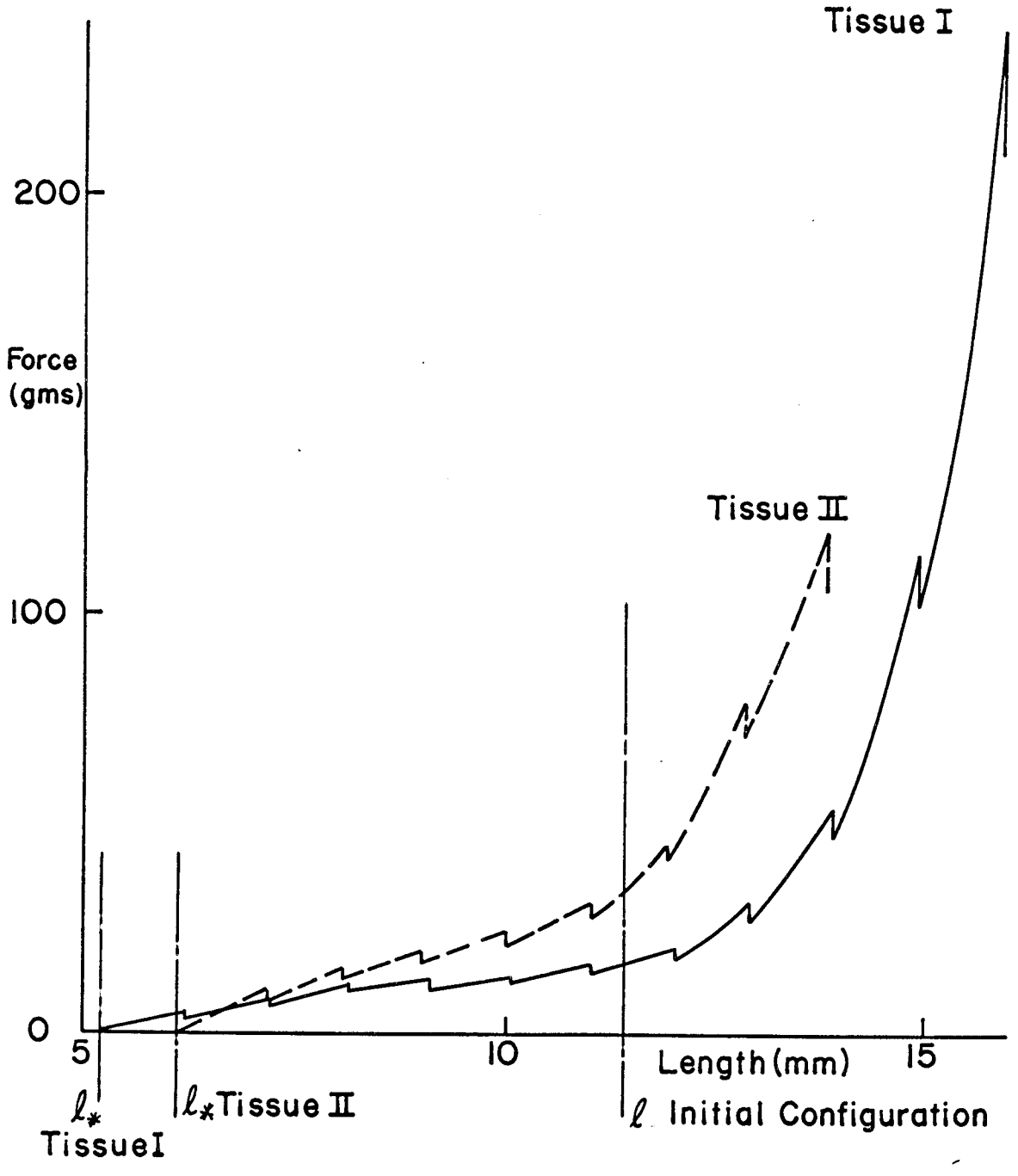


FIG. 29 FORCE STRETCH DIAGRAMS FOR TWO DIFFERENT TISSUE SPECIMENS FROM THE SAME ANIMAL

Figure 30. Two different tissue specimens with different arrangements of the large supplying arteries and veins with respect to the test specimen.

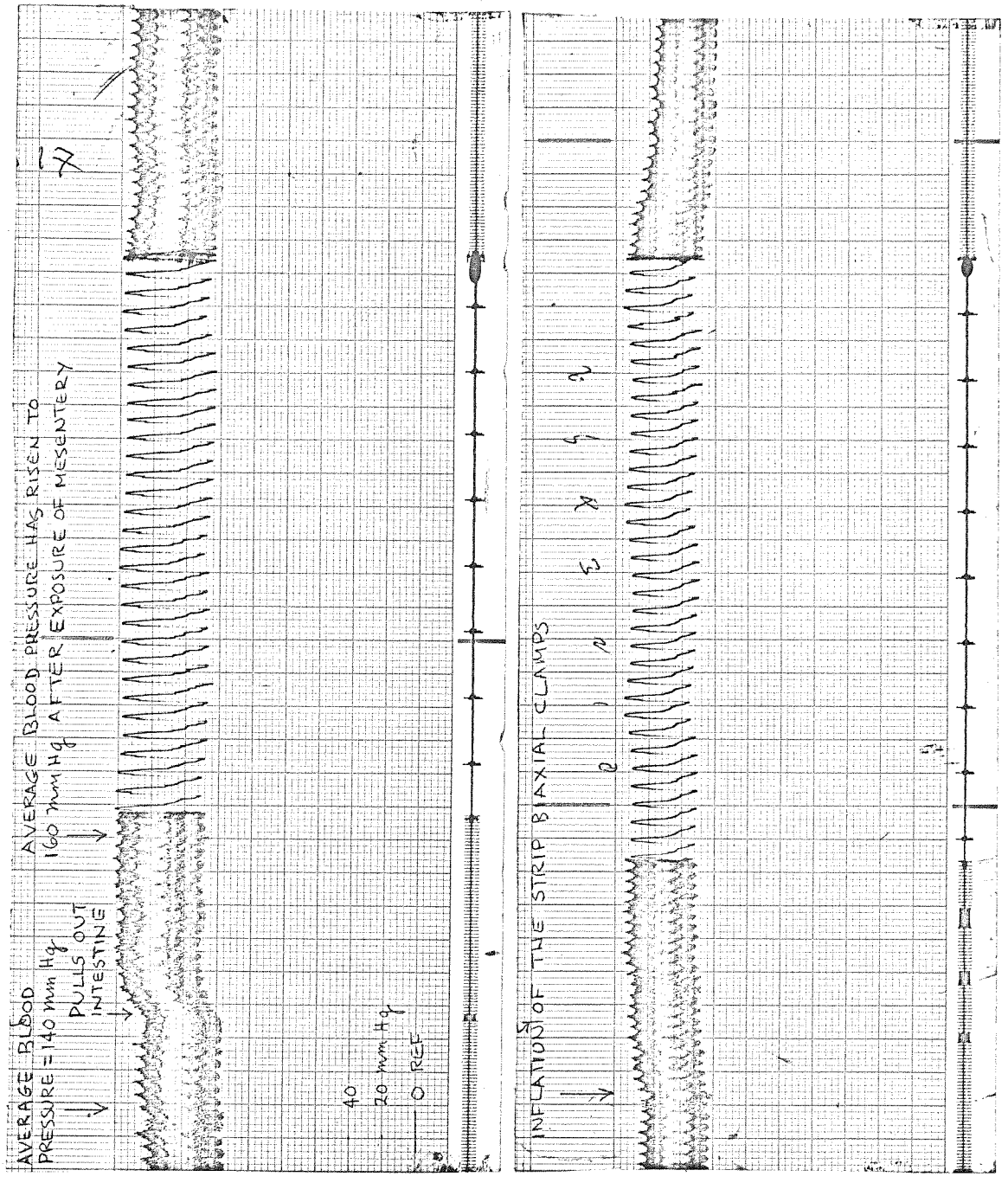

AVERAGE BLOOD PRESSURE = 140 mm Hg
PULLS OUT INTESTINE
AVERAGE BLOOD PRESSURE HAS RISEN TO 160 mm Hg AFTER EXPOSURE OF MESENTERY
40
20 mm Hg
0 REF
INFLATION OF THE STRIP BIAXIAL CLAMPS

Figure 31 a

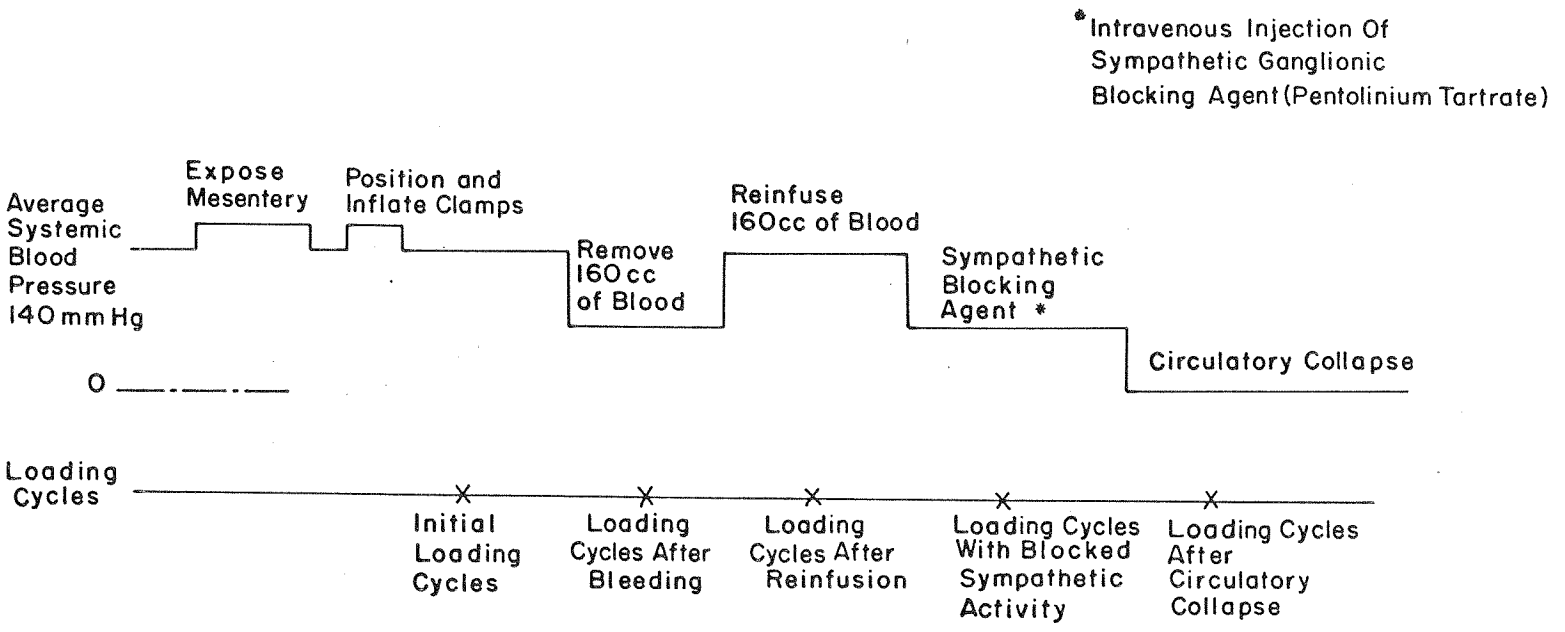


FIG. 31 b TIME HISTORY OF AVERAGE SYSTEMIC BLOOD PRESSURE

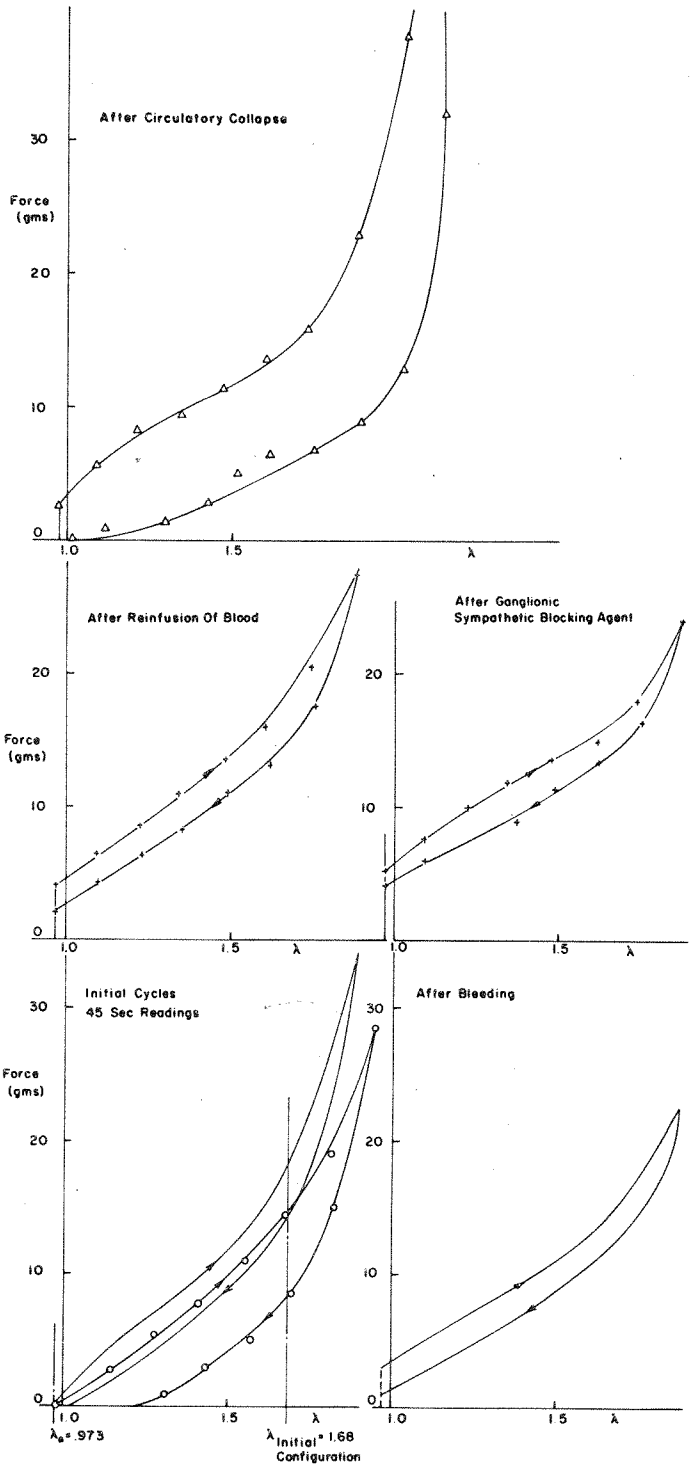


FIG. 32 DRASTIC VARIATIONS IN THE SYMPATHETIC ACTIVITY OF THE CENTRAL NERVOUS SYSTEM

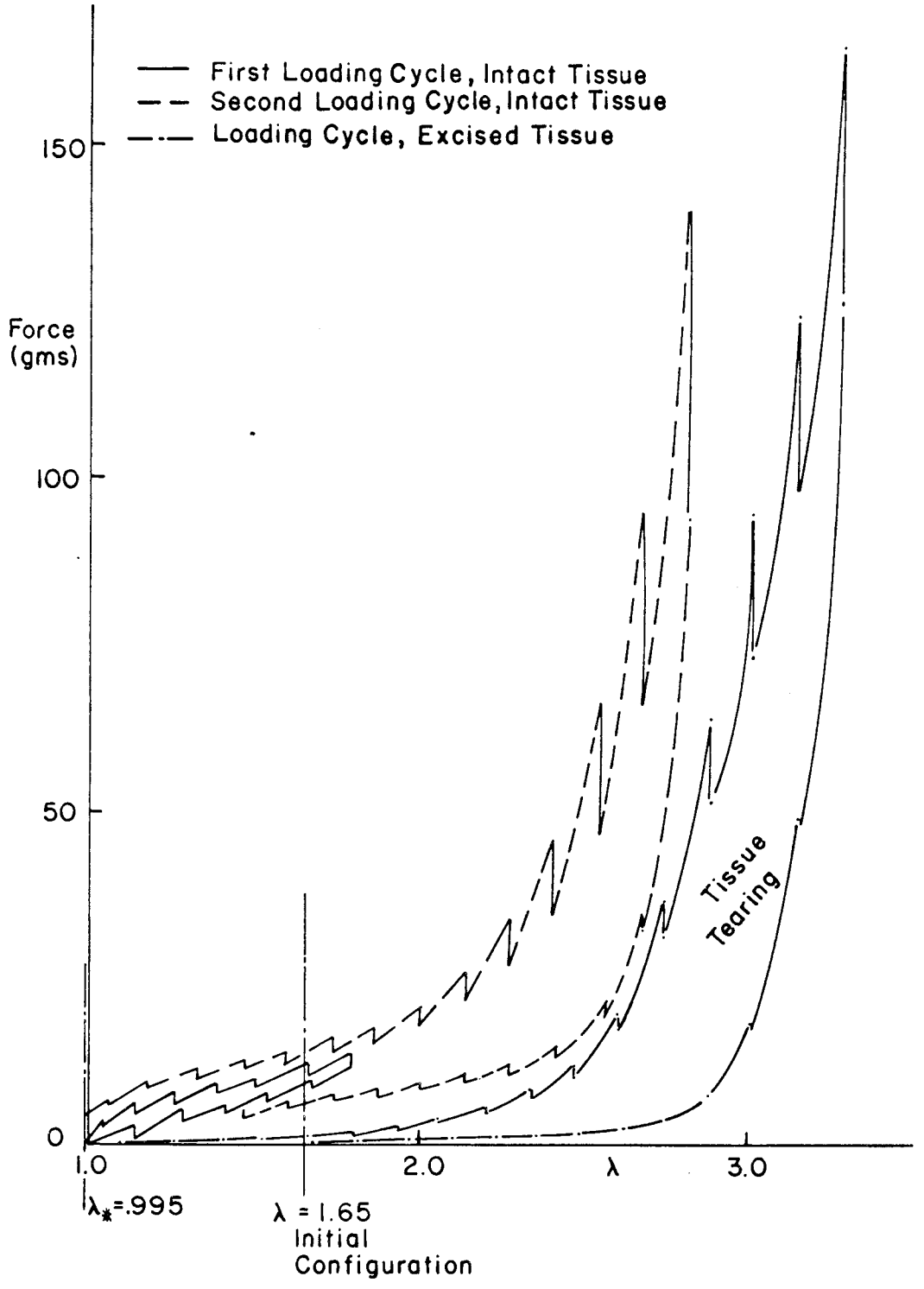


FIG. 33 LOADING CYCLES

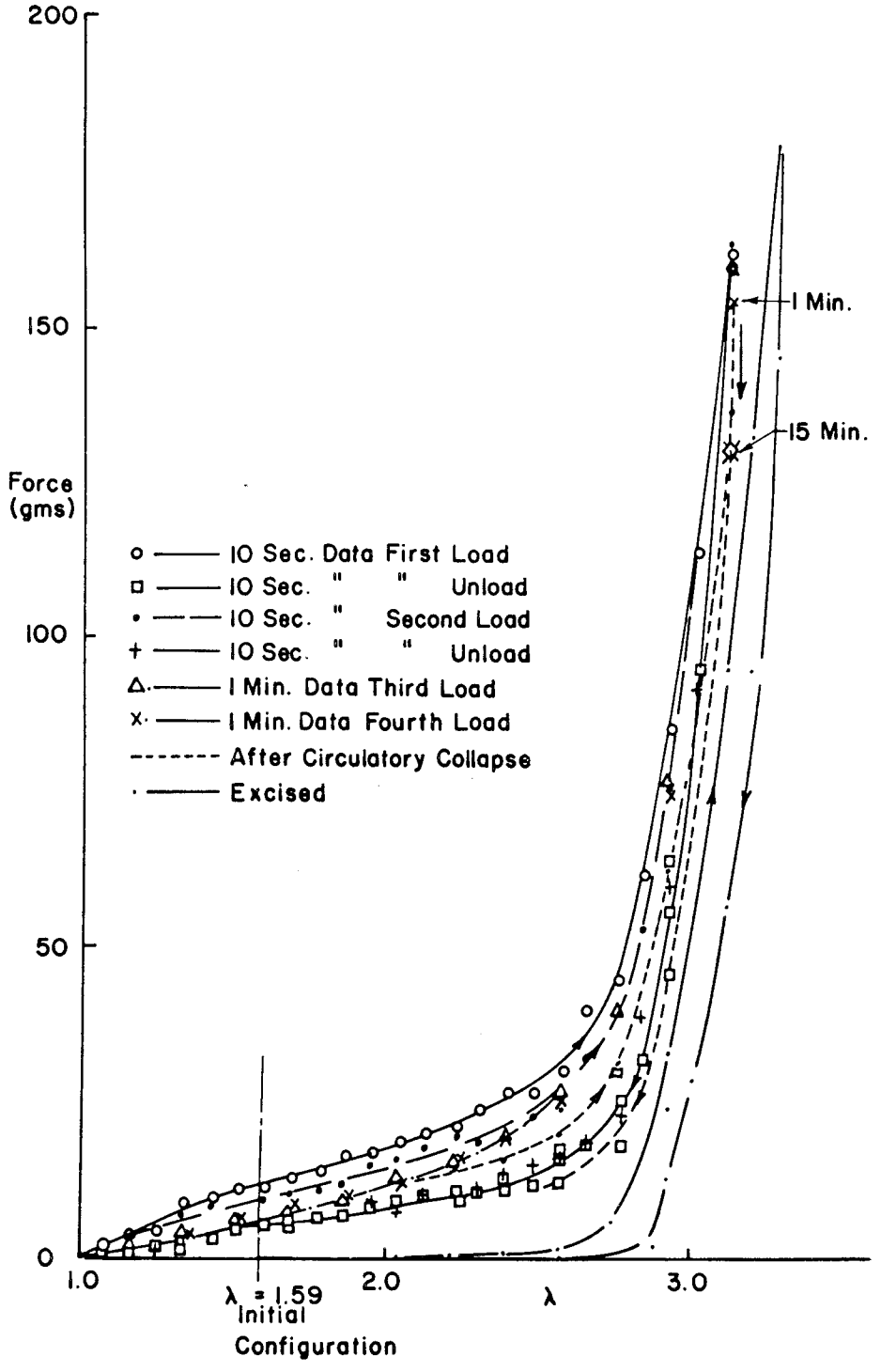


FIG. 34 INFLUENCE OF THE TIME DURATION OF THE STEP INCREMENTS OF DISPLACEMENT ON THE WIDTH OF THE HYSTERESIS LOOP. NO WAIT BETWEEN LOADING CYCLES.

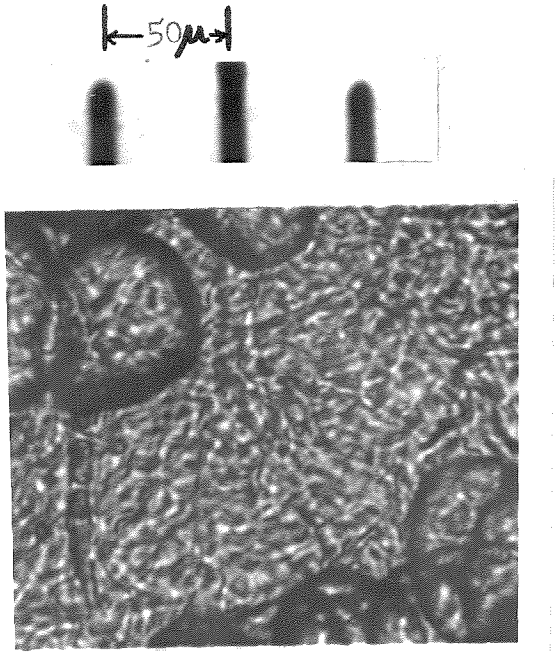


Figure 35. a. Kinked, random, indistinct arrangement of the fibrous protein matrix.

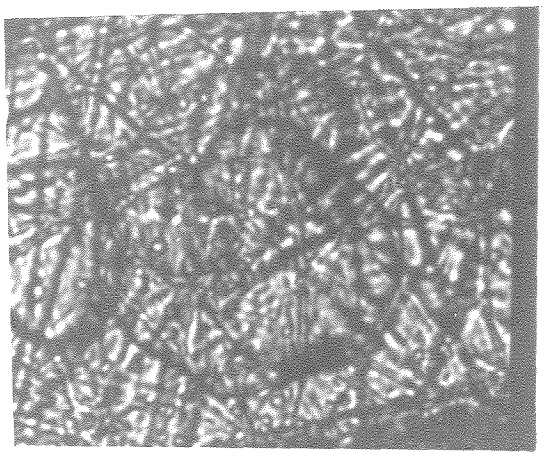


Figure 35. b. On inflation of the tissue with a hydrostatic head of 40 cm of water some of the protein fibers have straightened out and appear to be load-bearing.

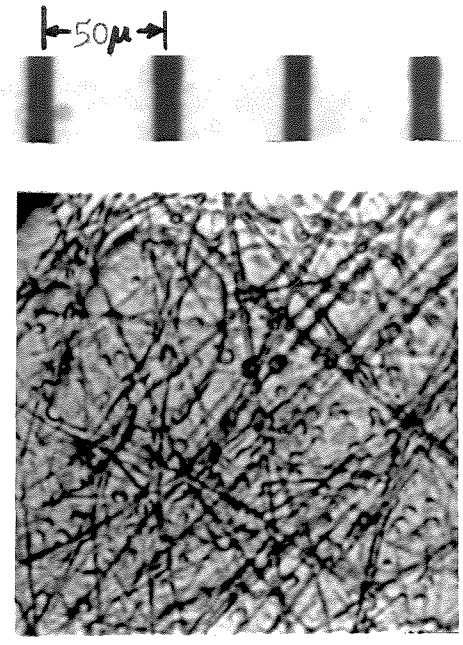


Figure 35. c. On further inflation of the tissue with a hydrostatic pressure head of 100 cm of water all the fibers have straightened out in randomly oriented directions.

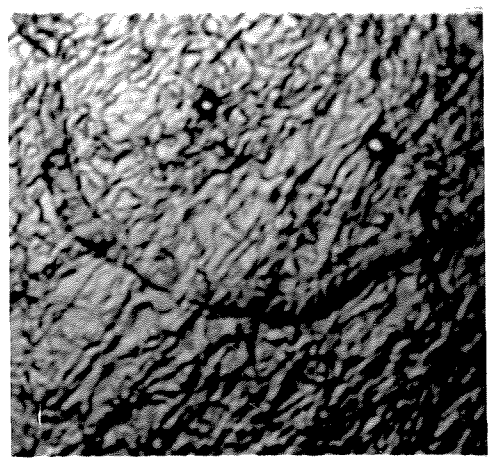


Figure 35. d. On deflation of the tissue the fibers are loosely coiled, but have not regained the original, tightly kinked configuration.

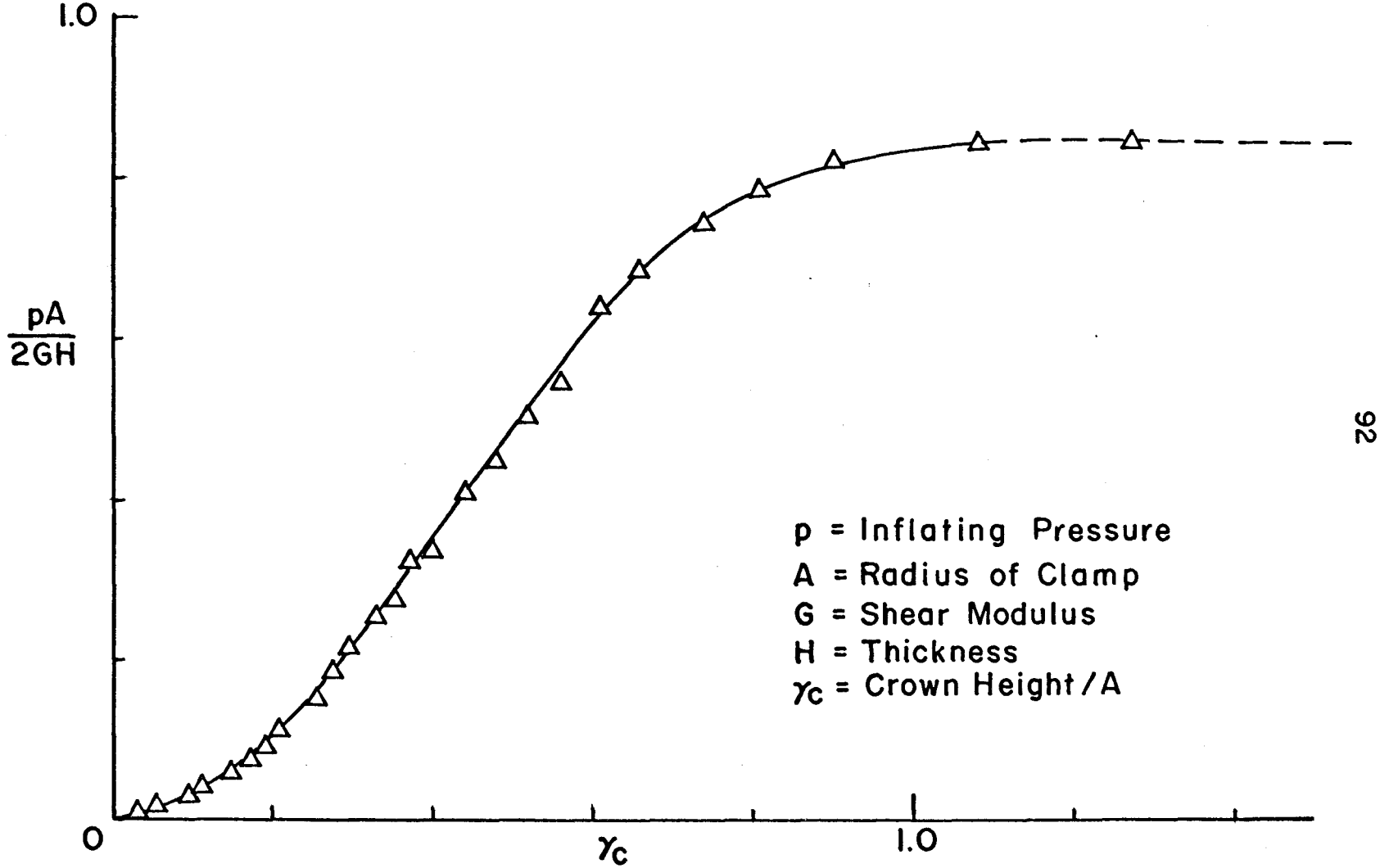


FIG. 36 NON-DIMENSIONAL PLOT OF CROWN HEIGHT-INFLATING PRESSURE FOR A DENTAL DAM SPECIMEN

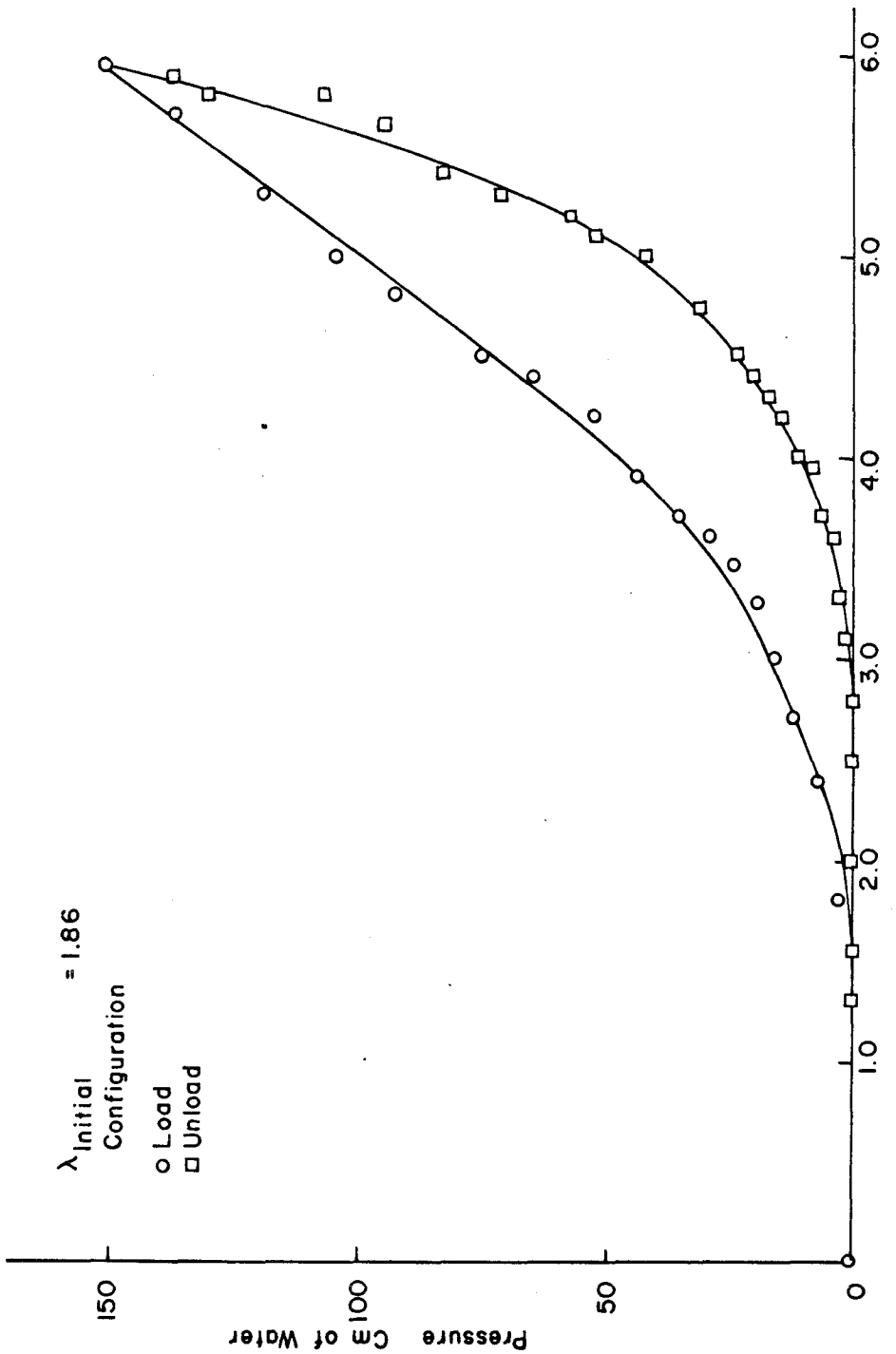


FIG.37 INFLATION OF AN INTACT MESENTERY SPECIMEN

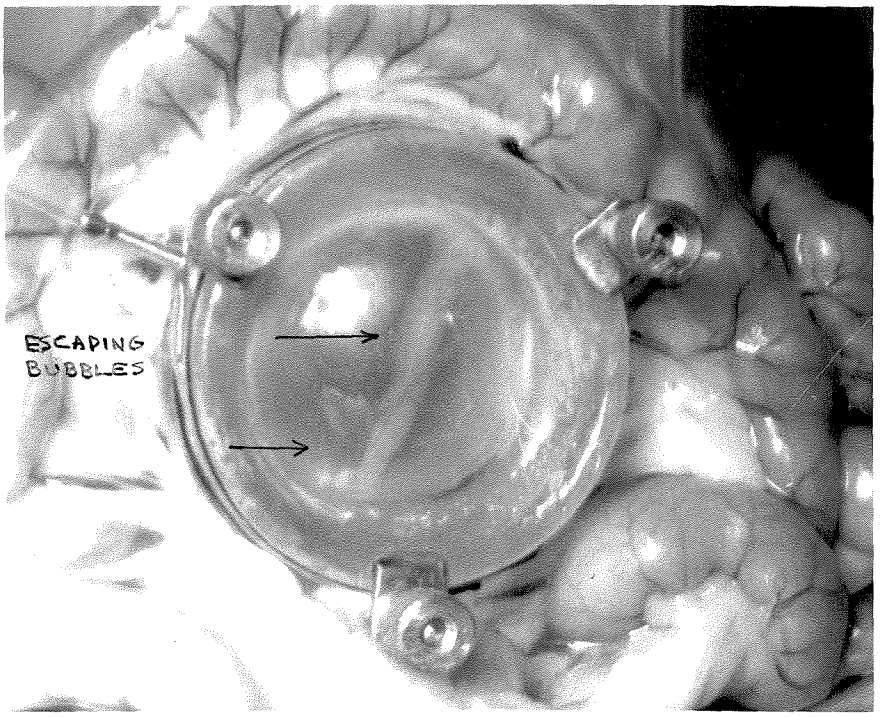


Figure 38. Inflated specimen failure mode.

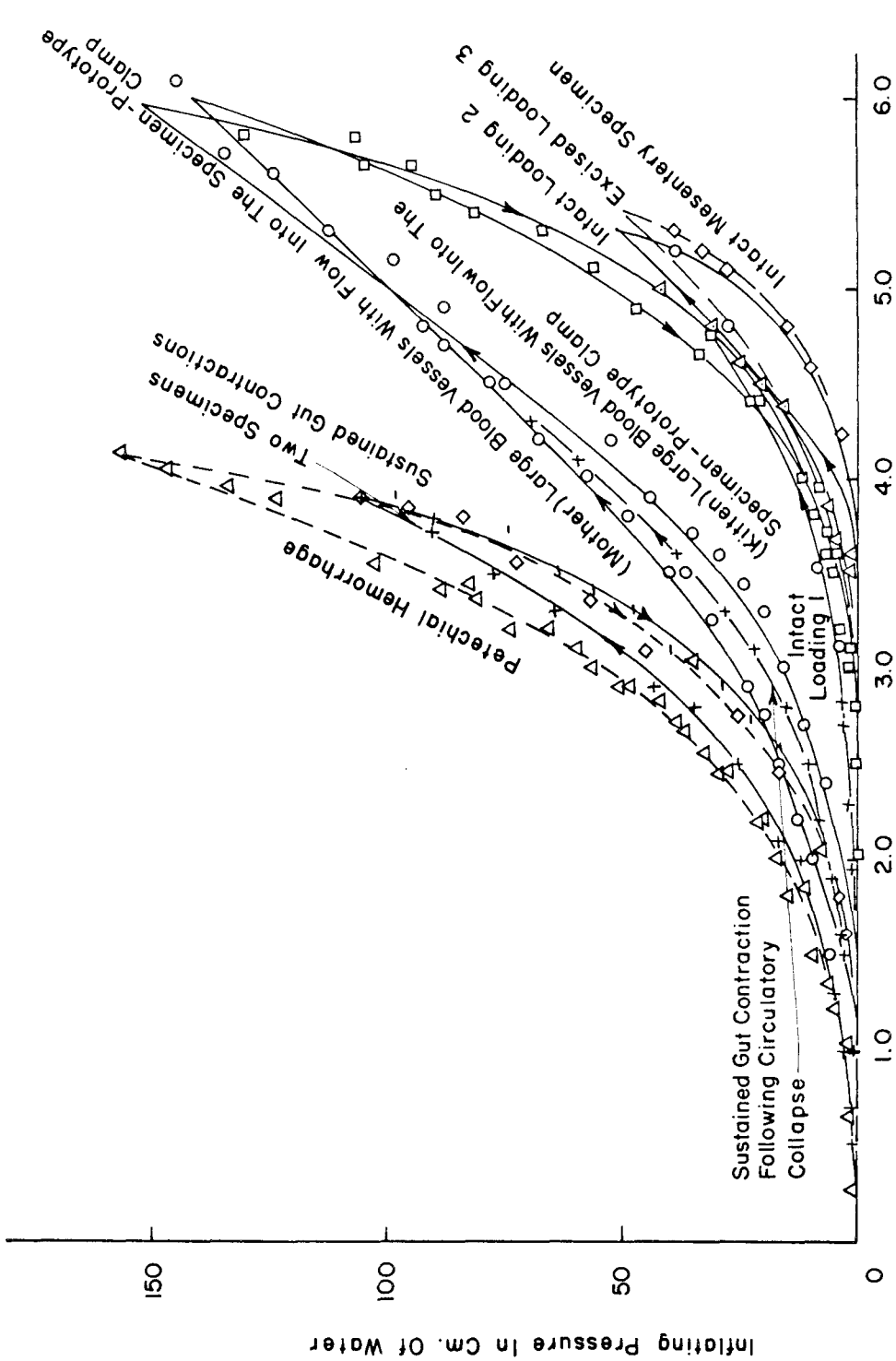


FIG. 39 INFLATION OF MESENTERY SPECIMENS BY A PRESSURE HEAD OF PHYSIOLOGIC SOLUTION

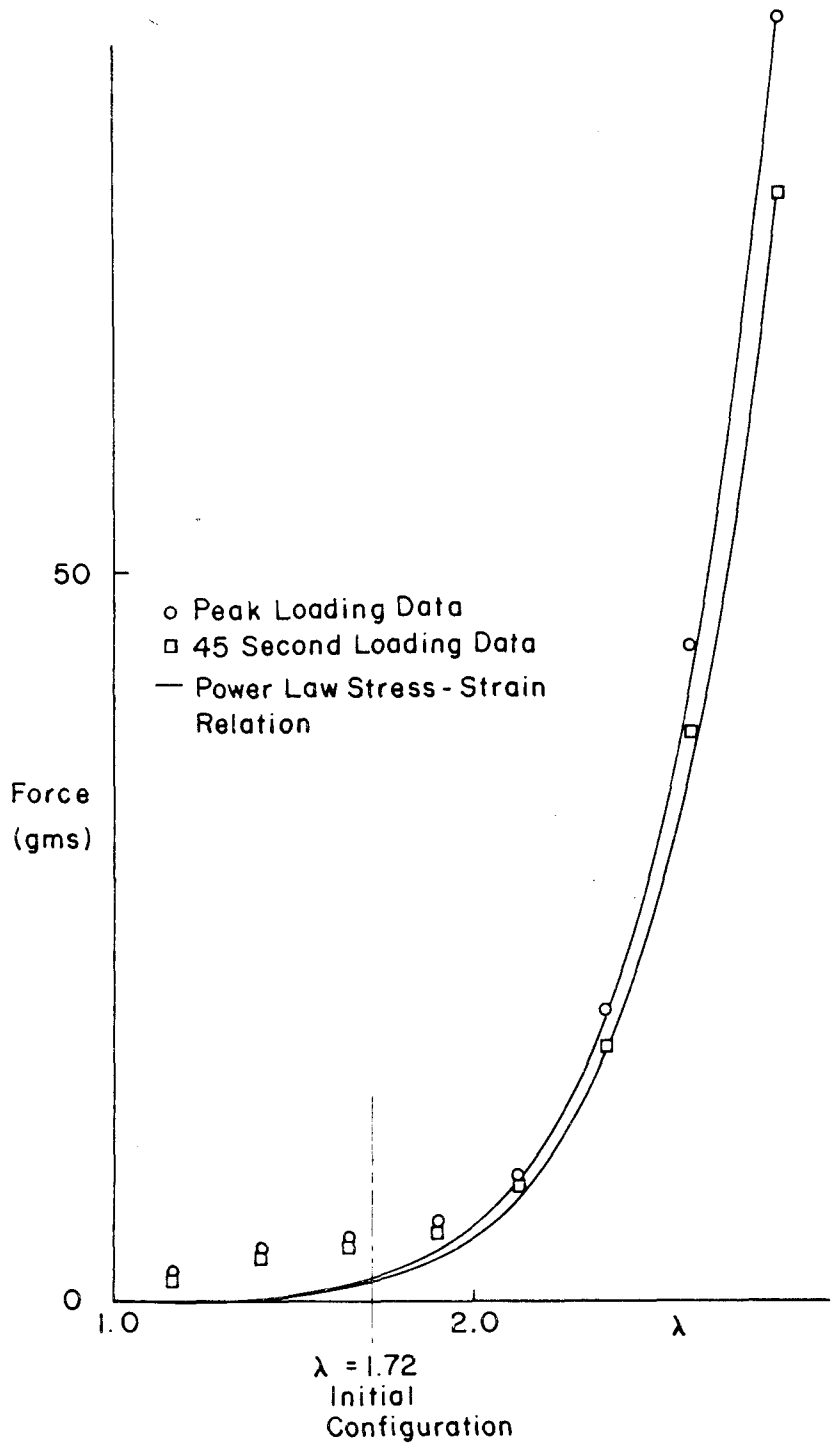


FIG.40 POWER LAW STRESS-STRAIN RELATION AND ITS CORRELATION WITH EXPERIMENTAL LOADING DATA FROM MESENTERIC MEMBRANE PER SE

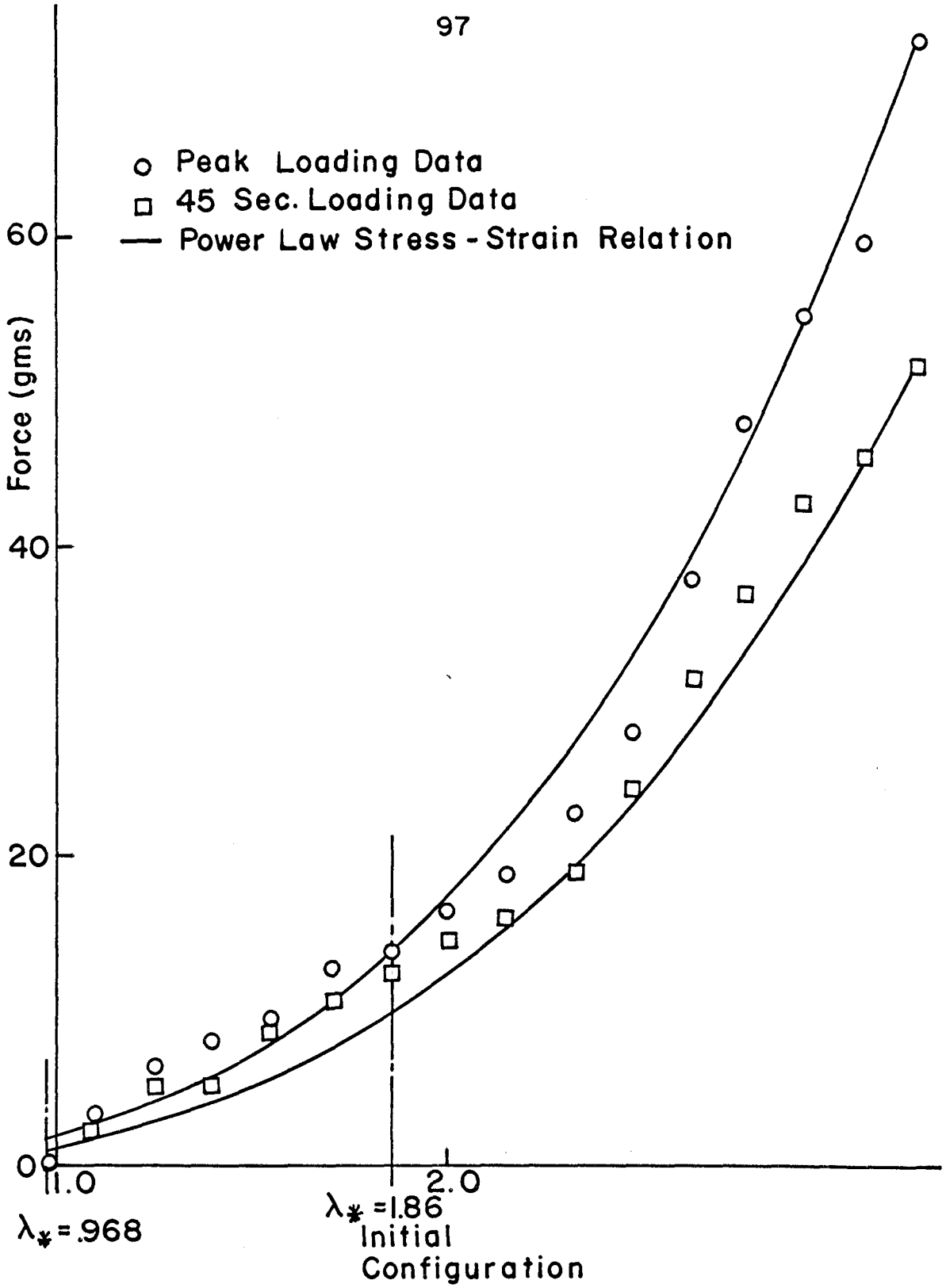


FIG. 41 POWER LAW STRESS-STRAIN RELATION AND ITS CORRELATION WITH EXPERIMENTAL LOADING DATA FROM FIG. 22

APPENDIX I

Log of Animal Experiments

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
1	4	1/11 1967		Learning surgical procedure and cannulation for wedge shaped clamp.		
2	4	1/20 1967		"		
3	3	1/25 1967		"		
4	4.8	2/8 1967		"		
5	5.5	2/22 1967		"		
6	4.1	3/1 1967		"		
7	2.3	6/9 1967		Rectangular clamp, dead weights		x
8	3.2	7/10 1967		"		x
9	3.9	8/15 1967		Outer clamp design, tissue layout and handling procedure		
10	2.3	8/23 1967		Atropine application for gut paralysis		
11	2.6	9/8 1967		"		
12	2.8	9/14 1967		" , dead weights		x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
13	6.1	10/30 1967		Rectangular clamp, force transducer and flexure. Tissue ruptured at a loading in excess of 500 grams.		
14	4.2	11/2 1967		Rectangular clamp, force transducer and flexure. At 400 gms of loading the tissue began to tear.		x
15	5.2	12/30 1967		Rectangular clamp.		
16	5.1	1/3 1968		Rectangular clamp. The tissue began to tear at a loading of 340 gms.		
17	3.9	1/18 1968		Perfused strip biaxial specimen		x
18	4.3	1/19 1968		Excised strip biaxial specimen		x
19	4.4	2/15 1968	1.68	Perfused strip biaxial specimen, excised tissue tore at a loading of 114 gms.		x
20	4.4	2/19 1968	1.62	Perfused strip biaxial specimen, excised tissue tore at a loading of 84 gms.		x
21	4.6	2/23 1968	1.82	Perfused specimen, circulatory collapse, excised.		x
22	5.5	3/4 1968	1.95	"		x
23	3.0	3/11 1968	1.35	Excised strip biaxial and uniaxial specimen.		x
24	4.6	3/14 1968	1.90	"		x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
25	3.9	4/4 1968	1.40	Perfused specimen, circulatory collapse, excised.		x
26	4.7	4/15 1968	1.59	10 sec., 1 min. readings, circulatory collapse, excised.	33	x
27	2.9	4/16 1968	1.61	Young animal, heparinized perfused strip biaxial specimen, circulatory collapse, excised.	25	x
28	5.4	5/6 1968	2.0	Excised strip biaxial and uniaxial specimen.		x
29	3.2	5/8 1968	1.83	"		x
30	4.6	5/15 1968	1.59	2 minute readings, strip biaxial specimen.		x
31	4.2	5/23 1968	1.88	30 sec. readings, strip biaxial specimen, excised.		x
32	4.0	7/22 1968	2.08	Strip biaxial specimen, need to make new tray		x
33	4.5	8/12 1968		Need to modify tray and key hole position, inlet for solution, and retractors at front of tray		
34	3.9	8/27 1968		Need adjustable backrest, skin margin restraint, gauze restraint for excess gut, omentum, and spleen.		
35	2.6	8/31 1968	1.79	Make reservoir wells deeper, change angle of front wall of tray, change mounting position of stationary specimen clamp, make animal bench wider and longer.		x
36	4.0	12/10 1968	1.64	(Mother) Strip biaxial specimen, inflation test.		x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
37	2.5	12/19 1968	1.91	Mother cat, strip biaxial specimen, inflated specimen.		x
38	1.9	12/20 1968	1.86	Kitten, strip biaxial specimen, inflated specimen	21, 36, 39	x
39	4.9	12/23 1968	1.78	Two tissue specimens from the same animal, thickness measurement, inflated specimen.	28, 29	x
40	5.0	12/30 1968	1.73	Two tissue specimens, excised spec., thickness meas., 5 min. wait between cycles.	26	x
41	4.0	1/6 1969	1.83	Two tissue specimens, circulatory collapse, excised spec., thickness meas., no large supplying. vessels near clamp, 5 minute wait between cycles.	27	x
42	5.2	1/8 1969	1.72	Two tissue specimens, excised spec., 5 minute wait between cycles.		x
43	5.7	1/22 1969	1.56	Cyanide injection but still had gut contractions after circulatory collapse, intact & excised strip biaxial specimens, 5 minute wait between cycles.	7, 8, 14, 23	x
44	4.1	1/28 1969	1.57	Intravital microscope pictures, 4 loading cycles with 5 minute wait between cycles for intact strip specimens.		x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
45	3.4	2/3 1969	1.65	Strip biaxial specimen, different amplitude loading cycles with 5 minute wait between cycles, excised specimen, intravital microscope pictures.	32	x
46	2.7	2/6 1969	1.74	Intravi. micros. pictures	3, 34	
47	5.2	2/26 1969	1.68	Strip biaxial spec., intact, after bleeding, after reinfusion of blood, after blocking agent, after ciculatory collapse. 5 min. wait between cycles.	30, 31	x
48	3.8	3/3 1969	1.66	Strip biaxial spec., intact after bleeding. Animal died. Excised specimen. 5 minute wait between cycles.		x
49	3.6	3/10 1969	1.73	Strip biaxial spec., intact, after bleeding, after reinfusion of blood, after blocking agent, after circulatory collapse. 5 minute wait between cycles. Inflation test to determine failure mode.	37, 15	x
50	4.4	3/25 1969	1.59	Three strip biaxial specimens from the same animal. One tissue contained a large supplying artery and vein; a second tissue contained an intermediate order of vessel branching; the third tissue contained no large vessel in or near the specimen. The miniaturized rectangular clamps were used. 5 minute wait between cycles.	19, 20	x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
51	3.4	3/28 1969	1.72	Two strip biaxial specimens from the same animal. The first (control) specimen was tested over two loading cycles and contained no large supplying vessels The second similar membrane specimen was tested over 2 loading cycles, the temperature of the bath was reduced by 5°C, then a third loading cycle was performed. The bath temperature was raised by 5°C and a fourth loading cycle was performed. 5 min. wait between loading cycles. The miniaturized rectangular clamps were used.	17, 1	x
52	5.1	4/2 1969	1.6	Strip biaxial specimen, intact and excised. The intact specimen was tested over two loading cycles. The tissue was then excised and tested over two additional loading cycles. The temperature of the bath was reduced by 9°C and a third loading cycle was performed. The bath temperature was returned to the original level and a fourth loading cycle was performed. The miniaturized clamps were used and a 5 minute wait was incorporated between cycles.	18, 21, 4	x

Animal	Weight kilos	Date	λ_2	Comments	Fig. No.	Data
53	3.4	4/14 1969	1.57	Three strip biaxial specimens from the same animal. The miniaturized clamps were used and a 5 minute wait was incorporated between loading cycles. The third specimen was excised and tested for two more loading cycles.	21	x
54	3.0	4/21 1969	1.43	Four strip biaxial specimens from the same animal. The miniaturized clamps were used and a five minute wait was incorporated between loading cycles. The fourth specimen was excised and tested for an additional cycle. The animal's average blood pressure was 100 mm Hg.	21	x
55		5/1 1969		Used Dr. Johnson's preparation showing sustained gut contraction. Two specimens were tested using the circular inflation clamp. The first remained attached to the surrounding fans; the second was excised following clamping.	39	
56	3.7	5/2 1969	1.62	An intact specimen was tested for two cycles of pressurization, using the circular clamp. The tissue was excised and then tested for a third cycle. After circulatory collapse and accompanying sustained gut contraction, a second specimen was clamped and subjected to a pressurization loading.	39	x