The Thermoelastic Behavior of Isolated Aortic Strips of the Dog

By Richard W. Lawton, M.D.

A detailed thermodynamic study of isolated strips of dog aorta is basic to the interpretation of the nonlinear and apparently rubber-like elastic behavior of such specimens. Experimental data indicate that the retractive force in isolated aortic strips of the dog is entropic. Volume changes with elongation in such strips are negligible. However, a large negative internal energy contribution to the total force is present at small elongations. Studies of aortic strips extracted with alkali suggest that the latter force arises from the elastic tissue itself.

The highly important elastic properties of the aorta and large arteries are due primarily to the presence of elastic tissue. Throughout the arterial tree, however, the elastic tissue is admixed with varying amounts of smooth muscle and collagen fibers resulting in varying amounts of distensibility. These vessels show a striking nonlinearity in their elastic behavior, partly because of this mixture of fiber types and the sequence with which they participate in the retractive force, partly because of variable smooth muscle tone, and partly because of the basic nonlinearity of the mechanical behavior of each fiber type. Remington and associates, in decomposition experiments on aortic rings, have demonstrated this sequential participation of the various elements during stretching. The retractive force at equilibrium under moderate loads arises from the elastic tissue and smooth muscle network. At progressively larger loads, more and more collagen fibers participate in the retractive force until all the load is borne by them. The sequence with which the collagen fibers come into play and the amount of smooth muscle tone, however, may be expected to alter the elastic behavior of the aortic specimen as a whole.

The smooth muscle-elastic tissue network responsible for the aortic retractive force over a rather wide range of distension shows mechanical and thermal properties often associated with rubber. Although extensive mechanical studies of the aorta have been performed, there does not exist, to our knowledge, a detailed thermoelastic study of the aorta. Because such a study is basic to the interpretation of the mechanics of the aorta, thermoelastic observations were made during the course of an investigation of the mechanics of isolated aortic strips and are presented below. Using classic thermodynamic relations, the approximate entropic and internal energy contributions to the total retractive force over a wide range of lengths were computed. The data indicate that the retractive force in aortic strips is primarily the result of a change in entropy.

Thermodynamics

For an elastic system undergoing a reversible stretching process, in passing from one equilibrium state to another, the internal energy of the system may be altered by work done on it, or by heat transferred to it. This may be written

$$d\mathcal{U} = TdS + FdL$$

where $d\mathcal{U}$ is an infinitesimal change in internal energy, $TdS$ represents the heat transferred; it is the product of $T$, the absolute temperature, and $dS$, the change in entropy. The work performed on the system by stretching it equals the force times the change in length, $FdL$. Actually the work may be more complex and additional terms may be added. For instance, if there is a volume change in the system during stretching, then

$$d\mathcal{U} = TdS - PdV + FdL$$
where \( P \) is the pressure and \( V \) the volume of the system. At constant temperature the total retractile force under these conditions is given by

\[
F = \left( \frac{dU}{dL} \right)_T - T \left( \frac{dS}{dT} \right)_T + P \left( \frac{dV}{dL} \right)_T
\]  

(3)

The retractile force is divided, then, into three components; that related to a change in entropy, that related to a change in internal energy and that related to a change in volume of the specimen with stretch. These three components may be associated with several different elastic mechanisms. Such an association must be based of necessity on non-thermodynamic evidence because of the highly general nature of the thermodynamic approach.

The internal energy term is the major term in substances obeying Hooke’s Law and reflects the slight separation of adjacent atoms or the distortion of valence angles with stretch. For these substances the entropy term may be negligible and the total retractile force then would equal the sum of the internal energy and the volume-pressure fractions. The entropy term, on the other hand, is associated, especially in the case of rubber, with the reorientation and unkinking of long molecular chains which may take place without altering either interatomic distances or valence angles. The molecular chains are believed to become less randomly arranged during the stretching process, their thermal motions become restricted, energy is lost by thermal processes and there results a decrease in the entropy of the specimen. In gum rubber the entropy term makes up most of the retractile force although in some rubbers there may be small contributions by the internal energy and the volume-pressure terms.

Changes in specimen volume with stretch may arise from changes in interatomic distances, from fundamental structural changes such as crystallization or work hardening, from ordinary thermal expansion, or, in the case of swollen specimens, from swelling and deswelling during stretching. Isolated aortic specimens, particularly those stored in Ringer’s or normal saline, should be considered as highly swollen. For rubber in equilibrium with a solvent, swelling and deswelling play an important role in the mechanical behavior of the system, because the solvent penetrates the molecular chain network. For the aorta, on the other hand, while the water of swelling appears to penetrate the fibrous elements of the aortic wall it is not clear whether this alters the molecular relationships within the fibers. Critical experiments of swelling and deswelling in aortic strips with stretch have not been made. However, the studies of Botts, Johnson and Morales have shown that for free-weighted native aortomyosin threads at small elongations the pressure-volume term is negligible as compared to the other terms. Recently Pate and Sawyer have investigated the

volume changes in dog aortic strips with stretch. In their system of aortic strip plus immersion fluid negligible volume changes occurred with elongation. Our experiments, reported below, confirm this observation for the immersed specimen. However, such studies do not rule out fluid shifts with stretch.

The entropic contribution to the total retractile force may be found by means of the Maxwell identity,

\[
\left( \frac{dS}{dT} \right)_T = - \left( \frac{dF}{dT} \right)_L
\]  

(4)

If the volume-pressure term in equation 3 can be neglected, then

\[
F = \left( \frac{dU}{dL} \right)_T + T \left( \frac{dP}{dT} \right)_L
\]  

(5)

The entropy contribution can thus be found from the slope of the isometric force-temperature curve and the internal energy contribution found by difference. If the force-temperature slope is positive, the contribution \( T \left( \frac{dP}{dT} \right)_L \) to the total retractile force is positive. Also by equation 4 \( \left( \frac{dS}{dT} \right)_T \) is negative; a decrease in the entropy has occurred during the stretching process. It is important to make the distinction between the entropic contribution to the force \( T \left( \frac{dS}{dT} \right)_T \) and the entropy itself.

In many cases it is convenient to divide equation 5 by the total force to give

\[
1 = \frac{1}{F} \left( \frac{dU}{dL} \right)_T + T \left( \frac{dT}{dT} \right)_L
\]  

(6)

This has the advantage in biologic work of eliminating the cross-sectional area from calculations when comparing a number of specimens. Thus, for the case of an ideal rubber-like material \( \frac{T}{F} \left( \frac{dF}{dT} \right)_L = 1 \) over a wide range of lengths.

**Experimental Procedures and Results**

Two methods have been employed for the determination of the isometric force-temperature slope of isolated strips of dog aorta. In one method the slope is found directly by varying the temperature and measuring the change in force while holding the length constant. Alternatively force-length curves for the specimen are obtained at two temperatures, and the change in force with temperature at any one length is determined graphically.
This latter method facilitates measurements over a wide range of lengths, which then can be correlated with the mechanical behavior of the strip. The method, however, is dependent upon a linear relation between force and temperature.

**Isometric Force-Temperature Studies.** Dog aortas were excised post mortem and immediately immersed in Ringer's solution. Some specimens were obtained immediately after death and used promptly, but the majority were obtained at various periods up to 12 hours after death and then stored for 24 to 48 hours in Ringer's solution at 5 C. in the icebox. During this period the swelling process in Ringer's solution reached equilibrium. At the time of use the aorta was opened and its circumference and thickness measured at various points. Strips from different areas were cut in several directions and their resting lengths measured. The specimen was mounted between spring clamps in a beaker containing the storage immersion fluid and the whole placed in a large regulated water bath. The lower end of the specimen was affixed by means of its clamp at the bottom of the beaker. The upper end of the specimen was fastened to a sensitive calibrated linear spring (Jolly balance) of the type used in the determination of specific gravity and surface tension or to a sensitive strain gauge (Statham Model G1-8-350). In the case of the spring as the tension changed the length of the specimen was held constant by raising and lowering the upper end of the spring by means of its vernier-operated mounting. A 1 cm. change in length of the spring equalled 0.89 Gm. Although the tension could be determined to the nearest 0.01 Gm. nearly continuous observation for constant specimen length by means of an ocular micrometer was necessary. In experiments performed with the strain gauge isometric conditions were easily preserved. The input to the strain gauge was adjusted so that its output could be conveniently determined on the same potentiometer-galvanometer apparatus used for thermocouple temperature measurements.

During the experiment both the beaker and...
the water bath were stirred constantly. The temperature was determined by means of a B.S. #30 copper-constantin thermocouple in the beaker. The aortic strip, together with its original immersion fluid, was allowed to equilibrate for at least an hour at the highest temperature and tension to be used in the experiment. This precaution allowed such irreversible processes as plastic flow to go nearly to completion and, therefore, to contribute negligibly during the time interval of subsequent heating and cooling cycles. If the initial equilibration period was omitted the first complete temperature cycle was irreversible and, therefore, discarded. The use of the original immersion fluid assured continued equilibrium of the swelling process.

At the end of the equilibration period the specimen was carried through several cycles of cooling and heating. The rates of all cycles were approximately the same, 0.5 °C. per minute or less, and led to good reproducibility. In figure 1 are shown the results of such a force-temperature experiment in which an isolated longitudinal aortic strip of the dog was studied at two elongations. The slope is steeper for the greater extension of the specimen so that the entropic contribution to the total force is larger while the entropy itself decreases (equation 4). That the slope depends to some extent upon the direction in which the strip is taken is shown in figure 2 where a longitudinal, a diagonal and two circumferential strips of comparable cross section, resting length and elongation, taken from the same region of the thoracic aorta of a single dog, are compared. These curves are for strips at small elongations. They show a gentle curvature that is not surprising considering the heterogeneous nature of the tissue of the aortic wall. Perhaps the collagen of the adventitia, whose force-temperature slope is negative, contributes to the curvature.

In table 1 are compared the values of $T \left( \frac{dF}{dT} \right)_L$, computed from the slopes of tangents drawn at 20, 30 and 40 °C, along the curves of figure 2. The circumferential strips were cut just above and just below the others so that they were averaged for comparison with the longitudinal and diagonally cut specimens. The values obtained by drawing the chord between 20 and 40 degrees compares favorably in each case with the value obtained from the tangent drawn at 30 °C. The data indicate the order of magnitude of error resulting from the calculation of the entropic contribution from the stress-strain curves obtained at two constant temperatures. 

**Table 1.**

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<thead>
<tr>
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<tbody>
<tr>
<td>$T \left( \frac{dF}{dT} \right)_L$</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tan @ 20C.</td>
<td>7.36</td>
<td>4.40</td>
<td>4.65</td>
</tr>
<tr>
<td>Tan @ 30C.</td>
<td>4.31*</td>
<td>3.67*</td>
<td>3.60*</td>
</tr>
<tr>
<td>Tan @ 40C.</td>
<td>3.34</td>
<td>2.18</td>
<td>0.98</td>
</tr>
<tr>
<td>Av. (20 &amp; 40C.)</td>
<td>5.35</td>
<td>3.34</td>
<td>2.82</td>
</tr>
<tr>
<td>Chord (20–40C.)</td>
<td>4.41*</td>
<td>3.28*</td>
<td>3.23*</td>
</tr>
</tbody>
</table>

$\Delta^* = +2.3% -10.6% -10.3%$

These experiments were performed in air as a part of a study of certain dynamic mechanical properties of aortic strips. Although the conditions are not ideal for thermodynamic measurements, such measurements can be correlated with the mechanical properties of the strip. In addition, variabilities due to swelling of the specimen were reduced. The numerical data agree satisfactorily with those obtained on immersed specimens.

To obtain these curves, the specimen was suspended in air between spring clamps within a tubular, lucite, moist chamber fitted on one side with an optical face. Relatively stable air temperatures and high humidity were obtained by means of a reflux condenser which opened into one side of the chamber. Water flowed through the outer jacket of the condenser at a relatively constant temperature from the hot and cold water taps. Air from the compressed air line bubbled through the inner tube of the condenser which was partially filled with water. At high temperatures (38 °C.) and humidity the chamber fogged making optical measurements of specimen length impossible. Therefore, radiation from a thermostatically controlled soldering iron was directed by means
of a reflector at the surface through which these measurements were made, dissipating the condensed vapor. Although these provisions for temperature and humidity control were crude, a reasonably constant temperature was obtained within the chamber when equilibrium of the various components was reached (approximately ±0.5 C.), presumably because of the excellent insulating properties of the lucite which attained and maintained a relatively constant temperature. Measurements of air temperature within the chamber were made by means of two parallel B.S. #30 copper-constantin thermocouples projecting from the wall of the chamber. The temperature of the specimen was measured by means of a copper-constantin thermocouple inserted in its substance just above the upper spring clamp.

Length measurements were carried out by means of a precision cathetometer. The distance between two ink dots on the specimen was determined with an error of less than 1 per cent. The ink dots were placed in the middle section of the strip to reduce end effects on the measurements. Longitudinal strips were frequently used in these experiments because their extra length allowed for more accurate length determinations.

Before beginning any stress-strain experiment the specimen was stretched rapidly by hand up to 50 to 75 per cent of its resting length and then released. About 10 cycles of such prestretching enhances the reversibility of subsequent stress-strain measurements. Initially the largest weight to be used was hung on the specimen which was allowed to elongate to constant length or until the rate of elongation became sufficiently small to introduce little error. The specimen was then progressively unloaded. Before measuring the length for a given load the specimen was subjected to a few cycles of free longitudinal vibration and then allowed to reach equilibrium length and temperature as the vibration damped out. Such preliminary vibration has often been used in the study of rubber in an effort to bring about a more ideal measure of length for any particular force. After all the weights were removed, the specimen was progressively unloaded again and oscillated before each length measurement. With care, little hysteresis was introduced in most experiments. Using the above procedure the force-length curves were largely reversible, the specimen frequently returning to within 1 per cent of its initial length. Finally the specimen was completely unloaded and the resting length determined. Under these conditions stress-strain curves were obtained at two temperatures, usually room temperature and approximately 38 C. The curves were plotted, the best smooth line drawn and \( \frac{dF}{dT} \) measured graphically. Two such curves are shown in figure 3.

In a favorable, highly extensible dog aortic strip, resolution of the stress into internal energy and entropic contributions by equation 5 results in the curves shown in figure 4. At rest length there is a large positive entropic contribution, \( F_e \), balanced by an equal and opposite internal energy contribution, \( F_u \). As the specimen is stretched, the entropic con-
RELATIVE LENGTH (L/L₀)

**FIG. 4.** Resolution of forces in an extensible dog aortic strip. F = total force on specimen; Fₑ = entropic contribution; Fᵢ = internal energy contribution. Calculations from stress-strain curves at two temperatures by equation 5.

The entropic contribution rises paralleling the stress-strain curve. It is apparent that in this case the shape of the stress-strain curve is determined primarily by the entropic contribution to the total force. Inasmuch as \( \frac{dF}{dT} \) is becoming increasingly positive throughout this range, the entropy itself, by equation 4, is progressively decreasing.

In figure 4 the internal energy contribution remains negative and essentially constant. In a less extensible specimen and in specimens at large elongations, the internal energy contribution becomes positive as shown in figure 5. At approximately 50 per cent extension the internal energy contribution, \( Fᵢ \), rises sharply, eventually to be the major positive component of the stress. Considerable variability is found among specimens. These are related to the region of the aorta from which the specimen is obtained, the age of the aorta, in the case of human specimens, and the storage and handling of the specimen. Because the force-temperature curve is not linear the shape of the curves of the entropic and internal energy contributions shown in figures 4 and 5 should be interpreted with caution. On the whole, however, the entropic contribution to the total retractile force is positive at small elongations, while the internal energy contribution, which is initially negative, becomes the major positive component of the force at large elongations. The results on any one specimen are reasonably reproducible despite errors introduced by the curvature of the force-temperature curves. In figure 6 determinations of the entropic and internal energy contributions for an aortic strip from the dog were made daily for four days and then on the seventh day post mortem. When not experimented upon, the specimen was kept in icebox storage. In figure 6, the data is presented in the modified form of equation 6. As a result of the curvature of force-temperature data, the entropic contribution calculated in the neighborhood of 37 C. should more closely approach unity than is shown in figure 6 where the chord was drawn between temperature extremes.

**FIG. 6.** Resolution of forces in a less extensible dog aortic specimen. F = total force on specimen; Fₑ = entropic contribution; Fᵢ = internal energy contribution. Calculations from stress-strain curves at two temperatures by equation 5.
Volume Change with Stretch. Before the internal energy contribution can be evaluated it is necessary to consider other factors which might contribute to the total retractile force. Of considerable importance is the volume-pressure term appearing in equation 3 which we have neglected in the foregoing analysis. Holt and McPherson have shown this term to be negligible in rubber up to 400 per cent extension. We have used a modification of their apparatus to study the volume change in aortic strips with stretch. The apparatus, shown diagrammatically in figure 7, consisted of a tubular brass chamber which communicated via a side arm with a reservoir and with a Kahan-type serologic pipet graduated in 0.001 ml. over a range of 0.2 ml. which measured volume changes within the system. The dilatometer chamber was closed at either end by small shredded lead “stuffing boxes” through which passed the ends of a long brass drill rod, 1 mm. in diameter. The drill rod was a continuous piece so that when 1 cm. of the rod was pulled out the top, an equal length entered from below. When the drill rod was drawn through the empty chamber a volume change of less than $2 \times 10^{-4}$ ml per centimeter occurred. The dilatometer was calibrated by moving the rod in and out of the chamber at only one end.

It was desirable to employ aortic specimens of as large a volume as possible. Usually longitudinal strips about 12 cm. long and 1 cm. wide with a total volume of approximately 1 ml. or more were satisfactory. The strip was passed through a brass loop on the drill rod and through another brass loop soldered to the chamber wall. The two ends of the specimen were sewn together to give a double length about 6 cm. in the long axis. The apparatus was carefully filled with saline. The fitting which adapted the pipet to the apparatus was designed to minimize the trapping
of air bubbles. The entire apparatus was immersed in a large water bath at constant temperature. Volume changes in the system with stretch were easily measured to the nearest 0.001 ml in the pipet with a small buret magnifier.

For the above specimen a 50 per cent extension, i.e., \( dL = 6 \text{ cm.} \), total force approximately 100 Gm., the largest volume change we have observed was of the order \(-0.003 \text{ ml.} \). For \( P = 1033 \text{ Gm. per square centimeter} \)

\[
P \left( \frac{dV}{dL} \right) = 1033 \times -0.003 \frac{6}{6} = -0.5 \text{ Gm.}
\]

The contribution by the pressure-volume term to the total force is thus less than 1 per cent. Ordinarily the volume changes observed were well within the range of experimental error so that moderate extension of aortic strips appears to be essentially an isovolumetric process.

**DISCUSSION**

The actual thermodynamic changes observed in any experiment cannot be related, per se, to any one or combination of elastic mechanisms. Such a relation can only be established by nonthermodynamic evidence. In addition, in the experiments cited above, the experimentally determined quantities were the entropic and pressure-volume contributions to the total stress; the internal energy contribution was found by simple subtraction. Therefore, what we have called the internal energy contribution is the algebraic sum of all other forces, both positive and negative, operating on the specimen. Finally, positive forces, i.e., the entropic contribution, are retractile forces tending to shorten the specimen or to resist extension while negative forces, i.e., the internal energy contribution, are forces operating to extend the specimen in the direction of stress. It would appear then that in the case of aortic strips, we are dealing with a balance of such forces operating to bring about a given length under a given load.

The reproducibility of thermoelastic data on aortic strips during prolonged icebox storage indicates that the smooth muscle does not participate appreciably in this response. For the case of small elongations, the retractile forces appear to arise primarily in the elastic tissue. The polymeric nature of the elastic tissue protein, elastin, the absence of a definite x-ray diffraction pattern and of electron microscopic banding of fibers in prepared unstretched elastic tissue specimens, its relative apolar nature as indicated by its resistance to dilute acids, alkalis and boiling, and its long range reversible extensibility have lead to its recognition as a rubber-like material.\(^\text{10}\) This nonthermodynamic evidence for the rubber-like state correlates with the finding of a decrease in entropy with stretch. Such a decrease in entropy may be interpreted, in accordance with statistical mechanical concepts of polymer networks, as configurational changes in the network, i.e., progressive alignment and orientation of the polymer chains in the direction of stretch due to free rotation of the molecular chain segments.

Recent microscopic studies by Lansing\(^\text{11}\) indicate, however, the extraordinary complexity of the elastic tissue fiber. Following the digestion with elastase of a surrounding amorphous material, a single fiber is revealed to be made up of many filaments wound in helical fashion. This elaborate organization would indeed set the elastic tissue fiber apart from an idealized rubber wherein the total force is the result of a change in entropy alone.

![Figure 8](http://circres.ahajournals.org/)

**FIG. 8.** Comparison of the entropic contribution to the force in fresh and NaOH extracted aortic specimens using equation 6. Circles = dog 27; triangles = dog 28. Open circles and triangles = fresh specimens; solid circles and triangles = the same strips following 0.1 normal NaOH extraction for one hour at 93 to 98 C.
It seems likely that this orderly arrangement of filaments contributes to the negative value of the internal energy contribution. Perhaps in the isolated specimen the helical arrangement behaves as a compressed spring. Indeed, Meyer has suggested for the ligamentum nuchae a model composed of a steel spring held in compression by a rubber band. On warming, the tension of the rubber would increase by an entropic mechanism while that of the spring remains nearly constant. He postulated that transverse bands of collagen, which occur in the ligamentum nuchae, or transverse linkages between parallel primary valence chains might account for such an extending force.

On the basis of microscopic observations of single elastic tissue fibers during digestion Lansing has suggested that actually the helical filaments of such fibers are wound under tension. The filaments appear to spring apart as the digestion proceeds. We have studied aortic elastic tissue specimens extracted by Lansing's method. Such specimens at small elongations show essentially the same thermoelastic behavior as the original unextracted aortic strips. In figure 8, two specimens, each in the fresh and extracted state, are compared. Although only the entropic contribution \( T F / F T \) is shown, the internal energy contributions, by equation 6, are similarly equal but negative. Experiments on extracted elastic tissue must be interpreted with caution, however, because the extraction procedure may very well result in the denaturation of the tissue.

It does not seem likely that a negative internal energy contribution can arise from a mechanism such as appears to exist for native actomyosin threads. Here such an extending force is believed the result of like charges along the protein and is found to decay away rapidly with stretch as the charges are separated. In synthetic systems such electrostatic effects can be demonstrated. However, the apolar nature of elastic tissues and the absence of a rapid decay with stretch are against an electrostatic explanation of a negative internal energy contribution in aortic strips. There remains to be investigated whether swelling and deswelling contributes in an important way to the thermoelastic behavior of aortic strips during elongation.

The change in force-temperature slope at large elongations presumably arises from the collagen network for collagen is known to have a negative temperature coefficient. The fibers, which are shown histologically to have a highly coiled configuration, are believed to uncoil passively and to bear the load only when completely straightened out. Changes, such as crystallization and work hardening, which are observed in other polymers and which might occur in the elastic tissue with repeated stretch and with age, are obscured because of the presence of collagen. It is probable that the collagenous adventitia of the aorta protects the vessel in some measure against such possible changes.

**Summary**

Resolution of the force in isolated aortic strips of the dog into entropic and internal energy contributions has been carried out. For small elongations the positive retractive force is entropic; the internal energy contribution is negative.

The contribution resulting from volume changes in the specimen with elongation is negligible.

The thermoelastic behavior of aortic strips is interpreted in the light of recent studies of extracted elastic tissue fibers.

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