

Some Fundamental Molecular Mechanisms of Contractility in Fibrous Macromolecules

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ABSTRACT The fundamental molecular mechanisms of contractility and tension development in fibrous macromolecules are developed from the point of view of the principles of polymer physical chemistry. The problem is treated in a general manner to encompass the behavior of all macromolecular systems irrespective of their detailed chemical structure and particular function, if any. Primary attention is given to the contractile process which accompanies the crystal-liquid transition in axially oriented macromolecular systems. The theoretical nature of the process is discussed, and many experimental examples are given from the literature which demonstrate the expected behavior. Experimental attention is focused on the contraction of fibrous proteins, and the same underlying molecular mechanism is shown to be operative for a variety of different systems.

Contractility and the corollary or associated phenomena of tension development are important phenomena that are observed in many natural occurring processes. These processes invariably involve the utilization and interaction of macromolecules. In the present paper these phenomena and related problems are discussed from a very general point of view. Emphasis is placed on the principles that have been developed from studies of the properties of long-chain molecules without any direct initial concern with any specific functioning system. Primary attention is therefore focused on the pertinent physical-chemical properties of macromolecules and the molecular events that are involved in contraction. This is an interesting endeavor by itself, even without concern for the biological implications. The formulation of the appropriate principles and their demonstration, in laboratory experiments with model systems, could serve as a basis for investigating similar phenomena in natural functioning systems and direct attention to possible events that could be occurring on a molecular level. The hope obviously exists that such an approach will eventually prove to be a fruitful one and will help to elucidate the general

principles that govern the more complex contractile phenomena of natural processes. The question as to whether the approach being taken, namely, from the point of view of the physicochemical properties of macromolecules, will be a fruitful one in explaining biological processes remains to be established and is not of immediate concern here.

Alternatively, study in great detail can be made of specific contractile systems, treating each as an individual and unique problem. In this approach any unity that might exist among the different systems does not become readily apparent. A great deal of effort and study has been devoted to microscopic and morphological study of the striated muscle system (1) and as a result certain contractile mechanisms have been proposed. Morales (2, 3) has recently reviewed and discussed the incompatibility that appears to exist between the morphological and physicochemical approaches. Eventually, of course, both these and other approaches that might be brought to bear on the problems of living systems must become reconciled and unified. We may, perhaps, be just at the beginning of such an endeavor.

The general physical-chemical framework within which to examine the problem comes from thermodynamics, which, although not concerned with molecular detail, sets forth the necessary criteria that must be fulfilled. These conditions come directly from the work of J. W. Gibbs (4), and there is no necessity to formulate any new potential functions or new concepts for the problems under present discussion. According to the laws of thermodynamics, for a reversible, isothermal, isobaric process in a closed system, the change in the Gibbs free energy is given by

$$dG = -w \quad (1)$$

where w is the work done by a system on its surroundings. For any cyclical process, wherein the system returns to its initial state, the change in the Gibbs free energy must be zero by virtue of the fact that it is a state function. Hence, no net work can be performed during a cyclical process in a closed system. This unhappy circumstance is alleviated by open systems wherein there can be an exchange of matter between the system and its surrounding. For this case,

$$dG = -w + \sum_i \mu_i dn_i \quad (2)$$

where μ_i is the chemical potential of species i and dn_i is the number of moles that enter or leave the system, the summation being extended over all species present. For a cyclical process, net work can now be performed. Since $dG = 0$, the net work becomes equal to

$$w = \sum_i \mu_i dn_i \quad (3)$$

where the change in mole number and chemical potential must now be evaluated over the complete cycle. Equation 3 simply states that for an isothermal-isobaric process, although no change is experienced by the working substance, net work can be performed during the cycle if there is transfer of dn_i moles of at least one species between two different chemical potentials. Equation 3 sets forth rather succinctly the requirements for an isothermal-work cycle. It has a simple analogy to a Carnot cycle operating nonisothermally, wherein, although the working substance (a gas in this instance) experiences no change at the completion of the cycle, net work has been performed as a result of the transfer of some mass from one thermal potential (temperature) to another. The detailed thermodynamic formalism for various hypothetical isothermal cyclic processes involving fibrous systems has been described (5-7).

Although the thermodynamic analysis is important and has far-reaching consequences, our major concern at present involves understanding the molecular nature of the working substance. For the class of problems under study, the working substance is macromolecular. We wish to elucidate the nature of its structure and molecular properties which allow such substances to support an applied force and to undergo anisotropic dimensional changes. It is these properties which enable a macromolecular substance to participate in isothermal processes in which chemical energy is converted into mechanical work. Macromolecules contain many atoms covalently linked together, and it is this feature which endows polymers with their unique structure and gives rise to a variety of properties not found in monomeric substances. It is necessary to examine these structural features in more detail and, in particular, to distinguish between two states in which polymer molecules can exist and to discuss the nature of the transformation between these two states.

One of the states of long-chain molecules is the liquid or amorphous state, although it most often possesses a very high viscosity. This state is characterized by the lack of any long-range molecular order, which results from the fact that the orientations of the bonds connecting the chain atoms of a given molecule are not correlated one with another and the molecular centers are located in random positions. This is thus a state of molecular disorder which arises primarily from the conformation taken by the individual molecules. In this state a linear dimension of the individual molecules, conveniently represented by the root mean square distance between chain ends $\langle r^2 \rangle^{1/2}$, is proportional to $n^{1/2}$, the square root of the number of bonds comprising the chain.

The other macromolecular state of interest is one in which three-dimensional long-range order exists. In this case the chain bonds adopt and perpetuate a preferred set of bond orientations for the major portion of the chain, and the molecular axes of the chains are aligned one with another. The thermodynamic, physical, and mechanical properties are vastly different in these two states (8). In the ordered or crystalline state a linear molecular

dimension is proportional to n . Hence, for long-chain molecules where n becomes large, a linear dimension in the ordered or crystalline state will greatly exceed that in the disordered state. A further distinction has to be made in regard to the crystalline state to determine whether the ordered regions are randomly arranged relative to one another or are preferentially oriented. When the molecular axes of the ordered chains are oriented along a macroscopic dimension in space, one has a fibrous substance, i.e. a system which is both crystalline and axially oriented. There are two major contractile processes on a molecular level with which we will be primarily concerned. In one of these the molecular events are restricted to the liquid or amorphous state, while the other involves changes that take place during the liquid to crystal transformation.

CONTRACTILITY IN THE AMORPHOUS STATE

As has been indicated above, the amorphous state of polymers is characterized by a great conformational versatility among chain bonds, which allows the individual molecules to adopt an extraordinarily large number of spatial configurations. This configurational versatility is the underlying reason why, in this state, high polymeric substances possess the ability to sustain large deformations and to return to their original dimensions when the applied stress is removed. When deformed, the number of configurations that are available to the macromolecular system is decreased, and there is a concomitant decrease in the entropy (9, 10). This decrease in entropy makes a significant and major contribution to the retractive force. As long as irreversible flow processes are retarded, by the introduction of intermolecular cross-links, the system will return to its initial or configurationally most favored state upon the removal of the imposed stress. Although this phenomenon is observed in all disordered polymers above their glass temperature, such mechanical behavior has been popularly termed rubber elasticity. By analogy to a Carnot cycle, which utilizes a gas as a working substance, a deformable material as described above can also serve as the working substance in a reversible thermal engine (8). The general thermodynamic requirement for the stability of phases requires that the force-length adiabatic coefficient be steeper than the corresponding isothermal one. Hence, the completion of a closed cycle is ensured. An actual engine operating on this principle and using natural rubber as a working substance was described many years ago by Wiegand (11).

Closely related to the rubber elastic deformation is the swelling of an amorphous network (cross-linked) in an open thermodynamic system, i.e. when immersed in a large excess of a supernatant fluid. The deformation in this case, which is manifested by an isotropic swelling or dilation, results from the free energy of interaction between the chain units of the network and the monomeric species in which it is immersed, rather than from an applied

force. The amount of swelling that occurs is controlled by details of the network structure and by the thermodynamic and chemical environment of the immersion medium. Changes in the amount of swelling can be controlled by alteration of the temperature, pressure, composition, pH, and ionic strength of the medium. A detailed discussion of the thermodynamics and statistical mechanics of the swelling process and its dependence on network structure can be found elsewhere (9, 10). In contrast to the anisotropic deformation observed when an external force is applied, swelling results only in a uniform dilation. However, swelling and a rubber elastic deformation can be combined by the imposition of an applied force to the network while it is immersed in a fluid. The deformation and resulting dimensional changes will then be made anisotropic and will also respond to changes in the thermodynamic environment of the medium, as has been discussed above. A very important characteristic of the type of dimensional change just described is that there are no abrupt changes in the force-length relations, since all the molecular processes are restricted to the amorphous state. Hence, for a rubber elastic deformation or for swelling, the extensive thermodynamic variables are continuous functions of the intensive variables.

There are many examples of quantitative studies of swelling in the absence of an applied stress to be found in the literature (9, 10). There have also been described various experimental demonstrations of cyclic, reversible, mechanochemical systems which are based on the swelling of a polymeric network subject to an applied tensile force. These examples involve the immersion of a deformed network into a supernatant fluid where at least one of the monomeric components can have strong chemical interactions with the chain units. Then, when the composition of the supernatant fluid is altered, an anisotropic dimensional change, either a contraction or an elongation, at constant force, will result as a consequence of the change in the equilibrium swelling of the network. Typical among the systems that have been described are polyelectrolyte networks (12–15), whose swelling capacity depends on the degree of ionization and thus responds to pH changes in the external medium. A network comprised of a copolymer of polyvinyl alcohol and allylalloxan displays reversible changes in length as a consequence of oxidation-reduction reactions (16). In a polyvinyl alcohol network dimensional changes are incurred as a consequence of complexing with either copper or calcium ions (17). In a unique system, constructed by Kuhn et al. (18), which consists of alternate layers of films of polyvinyl alcohol and polyacrylic acid, the network is mechanically constrained to swell anisotropically even in the absence of an applied tensile stress. All the systems just described have as a common property the continuous variation of dimensional changes with compositional changes in the supernatant fluid. Thus, there are no abrupt changes in the network dimensions, or, for processes carried out at fixed length, no abrupt changes in the applied force are observed.

LIQUID-CRYSTAL TRANSITION

The properties of the crystalline or ordered state in polymers do not present in themselves any special interest with respect to contractile processes. However, the transformation between the crystalline and liquid state is of major importance in this connection. There are two principal reasons for this. One of these, which has already been referred to, is the large difference in the linear dimensions of a chain (or portion of a chain) that exists between the two states. These molecular dimensional changes can, under appropriate circumstances, manifest themselves as macroscopic changes in length during the transformation from one state to another. The other item of importance is related to the nature of the transformation itself. In macromolecular systems, the liquid-crystal transition has been amply demonstrated, both theoretically and experimentally, to be a first-order phase transition (8). A discontinuity in the free energy surface of the system must therefore exist at the transition point. Consequently, an abrupt change will occur in at least one of the derivatives of the free energy, or a related quantity, such as the entropy, volume, enthalpy, force, or length. A very important and easily recognized distinction exists between processes restricted to the liquid or amorphous state and those involving a change between the two states.

The changes that occur in the different thermodynamic variables can be illustrated by a number of different experimental cases. In Fig. 1, for example, is plotted the specific volume-temperature curve for the crystalline homopolymer linear polyethylene (19). The fusion process illustrated is very similar to the melting of monomeric systems. The melting range is limited to a few degrees at most,¹ the abrupt change in the specific volume is quite apparent, and a well-defined melting temperature exists at 138.5°C for this particular sample. The abrupt change in the specific volume over a very narrow temperature interval is a consequence of the process being a first-order phase transition involving a homopolymer of regular structure. Similar changes in other extensive variables with a small variation of the corresponding intensive variables are to be expected.

If units are incorporated into the polymer chain which differ either chemically or stereochemically from the major constituent, so that they cannot enter into the crystal lattice, the character of the fusion process will be altered. The melting temperature is lowered and the amount of the depression will depend on the concentration and sequential distribution of the foreign ingredients (22, 23). The fusion process also will become much broader. The breadth of fusion results from the lowering of the free energy of the melt by the presence of noncrystallizing units, and from the decreased availability of

¹ The data illustrated are for a sample which is polydisperse with respect to molecular weight. Even sharper fusion is reported using molecular weight fractions (20, 21).

long crystallizable sequences for participation in the crystallization process (22, 23). Despite the broad fusion processes that are observed in copolymers as compared to homopolymers, a first-order phase transition still occurs. The melting temperature is well defined theoretically (22, 23) and is capable of experimental determination (8).

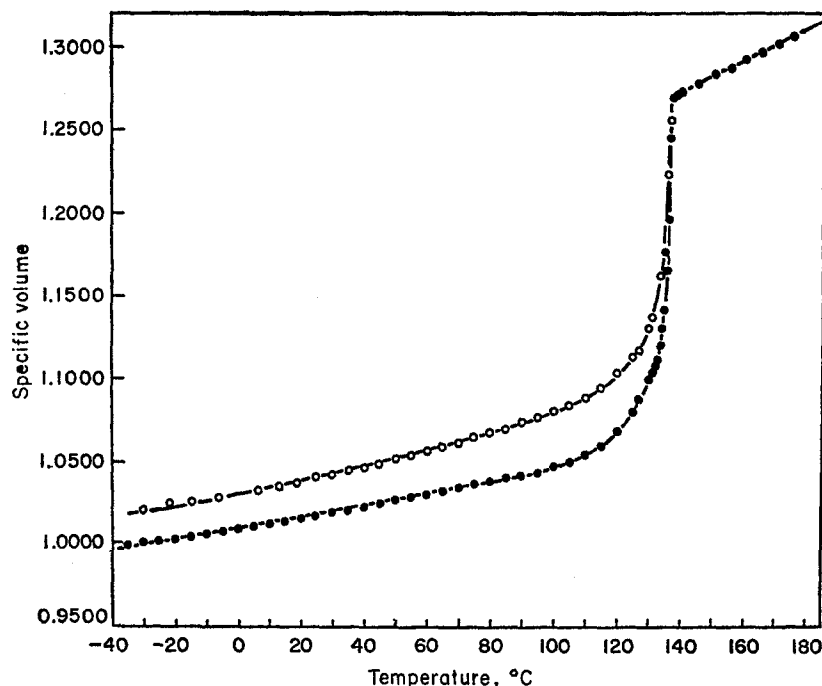


FIGURE 1. Plot of specific volume-temperature relations for unfractionated linear polyethylene. The different curves are for different crystallization conditions. *Figure reprinted by permission from Rubber Chemistry and Technology, 1959, 32:1392.*

When a crystalline homopolymer is mixed with a monomeric liquid or diluent which does not enter into the crystal lattice, the melting temperature will be depressed according to well-known thermodynamic relations (8, 22). The fusion process will again become broadened, depending on the type and amount of diluent added. The melting temperature, however, is still well defined. Melting can also be brought about, isothermally and isobarically, by chemical interaction and reaction between groups on the polymer chain and monomeric species in the surrounding medium.

Of particular interest to the problem of contractility is the set of variables force and length. If the crystalline regions of a sample are randomly arranged relative to one another, the changes in length that occur on a molecular level, upon melting at constant force, will not be macroscopically observed. This is because the changes in length in the different regions will average out to no

net dimensional change. However, if the crystalline regions were rendered fibrous, so that the molecular chain axis of the different crystalline regions were preferentially aligned along a macroscopic axis, the molecular or micro-dimensional changes that accompany the change in state should be experimentally realized. The changes in length with temperature, at constant force, that would be expected in such a fibrous system undergoing a crystal-liquid transition are schematically indicated in Fig. 2 (24). Fig. 2 *a* is for an idealized fiber homogeneous in chemical composition and geometric cross-section. At low temperature and large length L , a small positive thermal expansion coefficient typical of a crystalline solid is expected. The discontinuity in length that is indicated is a result of melting and is completely analogous in a formal

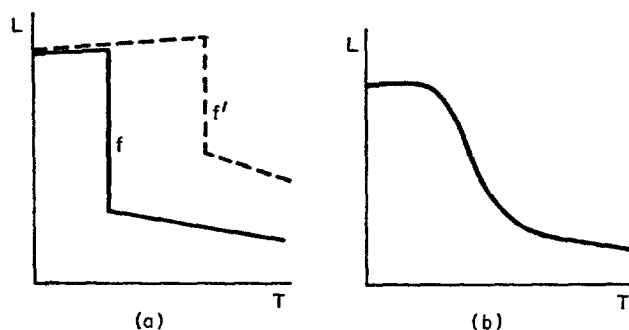


FIGURE 2. Schematic length-temperature relations for crystalline fibers; $f' > f$: *a*, for an idealized homogeneous fiber; *b*, for an inhomogeneous fiber. Figure reprinted by permission from *Science*, 1956, 124:53.

thermodynamic manner to the change in specific volume observed in Fig. 1. The diminution in the length results from the differences in chain structure in the two states. The molten or amorphous fiber displays a negative thermal expansion coefficient characteristic of a rubber elastic body (9). For an inhomogeneous fiber, the length-temperature properties are schematically illustrated in Fig. 2 *b*. As to be expected from the preceding discussion, the melting range will now become more diffuse, so that the extremely sharp discontinuity no longer exists. However, melting is still occurring; the melting temperature is still well defined, and there is a discontinuity in the length-temperature plot.

Since the force-temperature and length-temperature coefficients are related to each other by the mathematical identity

$$\left(\frac{\partial f}{\partial T}\right)_{P,L} = -\left(\frac{\partial f}{\partial L}\right)_{P,T} \left(\frac{\partial L}{\partial T}\right)_{P,f} \quad (4)$$

the force-temperature diagrams can be easily constructed from the length-temperature plots. Therefore, upon melting, a sharp increase in the applied

force will be required to maintain the length constant for a homogeneous fiber. For an inhomogeneous fiber, the stress will increase over a much wider range of temperature or the other appropriate intensive variables.

A large number of fibrous polymers (crystalline, axially oriented), including natural rubber (25, 26), polyethylene (27), polyamides (28), and collagen (29, 30), display the expected shrinkage upon melting. Two typical examples are illustrated in Figs. 3 and 4 for fibrous natural rubber (31) and for the fibrous protein collagen immersed in water (32). Plotted in these figures are the length, or relative length, as a function of temperature when no external force is applied to the sample. In both these cases, for such chemically different polymers, a very large axial contraction is observed over a narrow tempera-

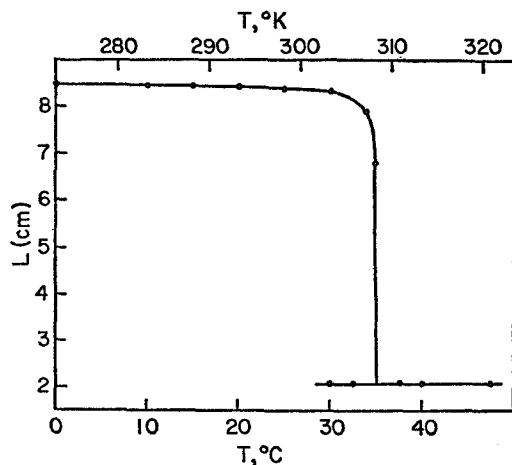


FIGURE 3. Length as a function of temperature, under zero force, for fibrous natural rubber. Figure reprinted by permission from *The Journal of the American Chemical Society*, 1958, 80:1297.

ture interval. All the thermodynamic requirements for a cooperative phase transition can be seen in these plots. The discontinuous nature of the process and the abrupt change in length that occurs are clearly demonstrated. The shrinkage is also accompanied by changes in other properties, such as the disappearance of the discrete X-ray diffraction reflections which is typical of the crystalline state, the loss of optical birefringence, and the development of rubber elastic properties. It is thus well demonstrated that this cooperative transition involves the change from the crystalline ordered state to the disordered one. The change in properties that is observed clearly results from the chain conformational differences in the two states. However, when the specimens illustrated in Figs. 3 and 4 are cooled following melting in the absence of an external force, the original state at the lower temperature is not regenerated. Instead of a crystalline, axially oriented state, the crystallites which are formed are randomly arranged relative to one another. Therefore, if the temperature is again raised, although melting will occur, the large axial

contraction will not be observed. Although, in the experiments just described, the expectations previously set forth of large anisotropic dimensional changes accompanying the change of state can be realized for the initial fusion process, the process is noncyclic as carried out so far. A major problem that presents itself, therefore, is the development and understanding of the conditions which

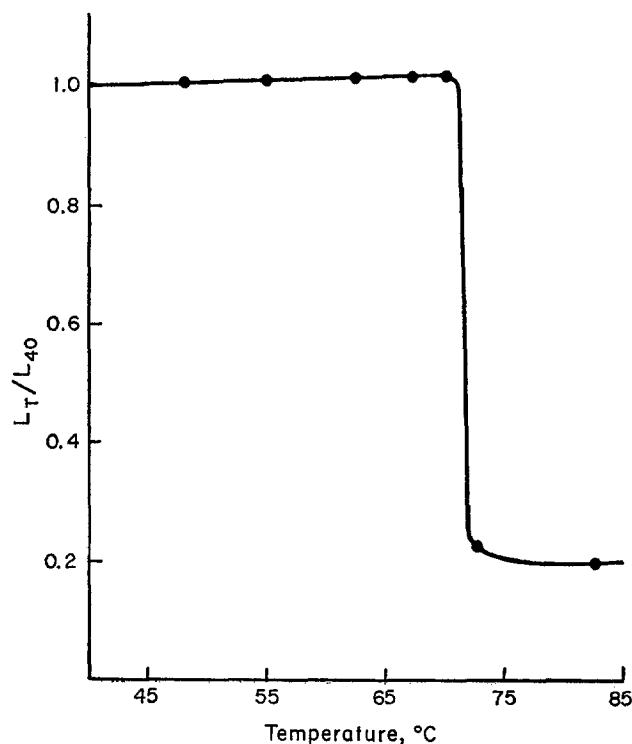


FIGURE 4. Relative length as a function of temperature, under zero force, for collagen from rat tail tendon. Figure reprinted by permission from *Journal of Cellular and Comparative Physiology*, 1957, 49 (Suppl. 1):175.

will allow the aforementioned process to be carried out reversibly and cyclically.

REVERSIBLE CONTRACTILE PROCESSES

One method by which the transformation between the oriented crystalline state and the liquid state can be carried out reversibly is by imposing an appropriate tensile force so that the two phases are maintained in equilibrium. An analysis of this problem has been given by Gee (33) and by Flory (34). The simplest case to treat first (33, 34) is a one-component system of pure polymer which is homogeneous and uniform with respect to chemical composition chain structure, and geometric cross-section. The fiber is cross-

linked and composed of highly axially oriented crystalline regions coexisting with completely amorphous zones. This fibrous system is subject to a uniform tensile force f acting in the direction of its axis. By straightforward thermodynamic methods, it is demonstrated (33, 34) that, for the two phases to be in equilibrium, it is required that

$$\left(\frac{\partial f}{\partial T}\right)_{p, \text{eq}} = -\frac{\Delta S}{\Delta L} \quad (5)$$

Here $\Delta L = L^a - L^c$ is the difference in length between the completely amorphous and completely crystalline fibers under the specified conditions of stress temperature and pressure. ΔS is the corresponding entropy change. Equation 5 is a one-dimensional analogue of the usual Clapeyron equation (in pressure-volume variables) appropriate to problems of phase equilibrium. Since for a reversible process

$$\Delta S = \frac{\Delta H - f\Delta L}{T} \quad (6)$$

equation 5 can be rewritten in more compact form as

$$\left[\frac{\partial(f/T)}{\partial(1/T)}\right]_{p, \text{eq}} = \frac{\Delta H}{\Delta L} \quad (7)$$

In the above equations, the temperature T can be identified with the melting temperature T_m for the given force f and pressure p . There is a complete analogy between the problem under discussion and the vapor-liquid and solid-liquid equilibria of monomeric substances. This connection becomes clear when it is realized that $-f$ and L correspond to the pressure and volume in the more conventional problem. It therefore follows immediately that for the one-component fibrous system, which has uniform properties throughout, the equilibrium force must be independent of length over the two-phase region at constant T and p . It will be expected under ordinary conditions that $\Delta L < 0$ while $\Delta H > 0$. Hence, from equation 7, f/T will increase with T , or the melting temperature should increase with an increase in the applied tensile force.

When the elastic equations of state are introduced into the problem the necessary integrations of equation 7 can be performed (34). The results are best illustrated graphically, and are schematically represented in Fig. 5 (24) as a plot of the force against the length for different temperatures. The origin in the diagram corresponds to the amorphous state. For amorphous networks, formed from perfectly ordered chains, the length will increase linearly with the applied force (34). The horizontal line represents the two-phase region where the force is independent of the length. The junction point between this

line and the rubber elastic (amorphous) stress-strain curve represents the equilibrium melting temperature for a given force. The sharp vertical increase in the force-length curve, after the two-phase region is traversed, represents the equation of state of the crystalline polymer with its characteristic very high modulus of elasticity. The separation of the horizontal lines in this figure is a consequence of the markedly different elastic equations of state between the crystalline and amorphous polymer. For a sufficiently high temperature, a critical point will be reached, corresponding to $L^a = L^c$, and the horizontal line representing the coexistence region will merge to a point.²

The experimental investigation of Oth and Flory (31) on fibrous natural rubber has given strong support to the major conclusions set forth above for a pure, homogeneous, one-component system. In accord with theory, it was

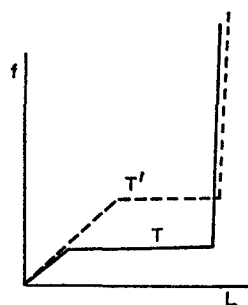


FIGURE 5. Schematic force-length diagram for an idealized homogeneous fiber; $T' > T$. Figure reprinted by permission from *Science*, 1956, 124:53.

found that, as long as the two phases coexist, f_{eq} is independent of the specimen length and increases in magnitude with increasing temperature. A compilation of their results is presented graphically in Fig. 6. The equilibrium force is plotted as a function of length for different temperatures above the isotropic melting temperature, the isotropic melting temperature being the observed melting temperature in the absence of an external force. The horizontal solid lines represent the stresses necessary to maintain the two phases in equilibrium. The length of the sample upon the completion of melting, for a given force and temperature, is indicated by the solid circles. The dashed lines represent the force-length relations in the amorphous state. In the crystalline state, the force-length relation is indicated by the vertically rising straight lines. There is indeed a very strong similarity between the experimental observations as embodied in Fig. 6 and the theoretical expectations of Fig. 5.

The important relations that exist among the force, length, and temperature in the two-phase region can be easily discerned by reference to Fig. 6. If, for

² From a purely formal point of view, a detailed analysis reveals the theoretical possibility of attaining a state where $L^a > L^c$. It is highly unlikely, however, that in any real experiment the polymer chain could sustain the large deformation required for L^a to exceed L^c .

example, the fiber is initially held at point *A* in Fig. 6, and we consider processes where the temperature is increased while the length is held constant, a path described by a vertical line upward from point *A* must be followed. In order to maintain the length constant, it becomes necessary to apply a further external force to balance the retractive force developed by the crys-

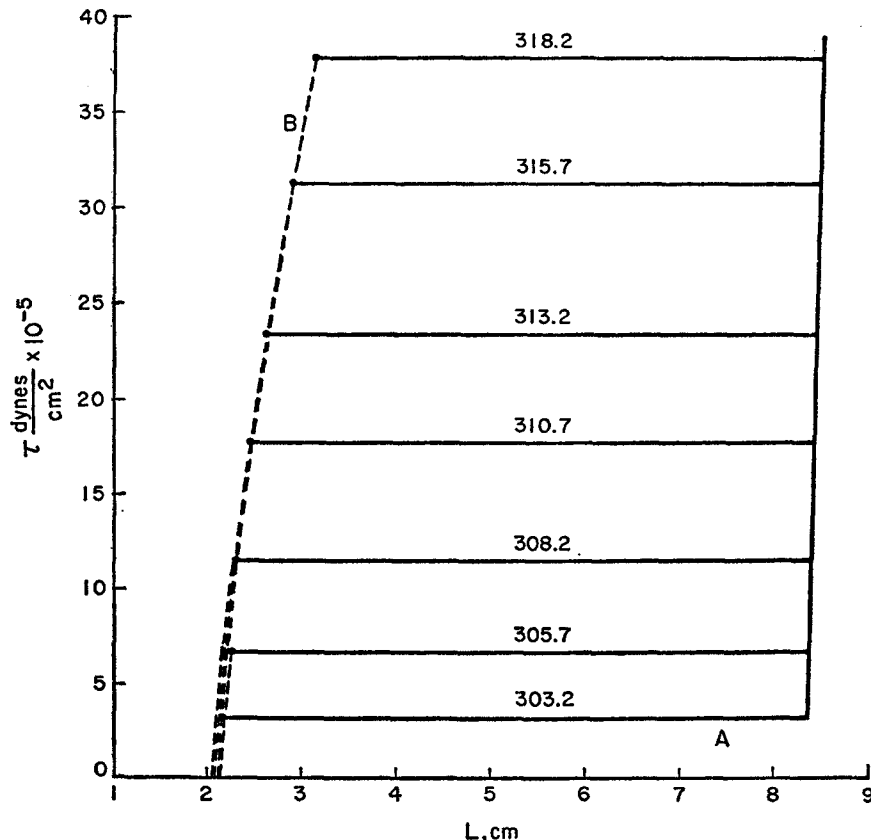


FIGURE 6. Composite plot of equilibrium stress-length relations at different temperatures for fibrous natural rubber. Data from Oth and Flory (31).

talline network. To put the matter another way, the additional stress is required to prevent melting, for upon melting the original length at *A* would not be maintained. As the temperature is raised the stresses required to maintain the two phases become very large. At 318.2°K, only 16°K above the isotropic melting temperature, a stress of 4 kg/cm² is developed. Still greater stresses could be developed by merely raising the temperature as long as the two-phase region is maintained. We thus see a demonstration of a fundamental mechanism by which large tensions can be developed in a fibrous system by traversing the two-phase region.

If the stress, rather than the length, is held constant and the temperature is raised, a horizontal path will be followed from point *A* which terminates at the appropriate dashed curve, which represents the completely amorphous state. A 4-fold diminution in length results, in the example cited, because of the transformation that occurs. This process and the accompanying dimensional change are reversible as long as the equilibrium stress is maintained. The experimental results summarized in Fig. 6 thus demonstrate that the problem of irreversibility, previously cited, can be overcome. Thus, reversible anisotropic dimensional changes and tension development can occur when the equilibrium stress is imposed and maintained so that the process can be carried out cyclically.

The above analysis and example have been for a one-component system which was uniform in both cross-section and chemical composition along the

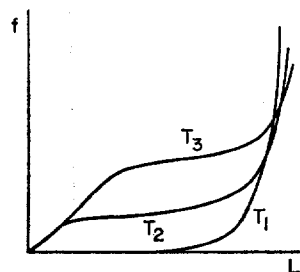


FIGURE 7. Schematic force-length diagram for an inhomogeneous fiber; $T_3 > T_2 > T_1$. Figure reprinted by permission from *Science*, 1956, 124:53.

chain. The results of the analyses can be extended to include the properties and behavior of inhomogeneous fibers. A fiber with nonuniform chemical composition can be treated as a copolymer. As has been indicated previously, the melting range of a copolymer is much broader when compared to the extremely sharp melting of a homopolymer. The length-temperature diagram schematically illustrated for inhomogeneous fiber in Fig. 7 (24) is a manifestation of this property. The broader melting range characteristic of copolymers will also be maintained when an external force is imposed on the system. Therefore, at a fixed temperature, in the melting range, fusion must take place over a range of forces, rather than at a fixed force as in the case of homopolymers. This theoretical expectation is indicated schematically in Fig. 7, where temperature $T_3 > T_2$ is in the melting range (two phases in equilibrium) and temperature T_1 is below the isotropic melting temperature. The major difference in the two-phase region between this case and the pure homopolymer is that the sharp discontinuities of Fig. 5 have become smoothed out into continuous curves, although with greatly differing slopes. However, the three major regions of interest are clearly discernible. The force-length curve corresponding to temperature T_1 is for a partially crystalline polymer below

its melting temperature. The characteristics of this curve are quite different from those for a completely crystalline polymer or for a completely amorphous one.

For a fiber possessing a varying geometric cross-section (but homogeneous in chemical chain structure), the stress will necessarily be nonuniform along the length of the fiber. Since the equilibrium will depend directly on the stress, different values of the stress corresponding to melting will occur in the different cross-sections. Therefore, at constant temperature and pressure, the transition will occur over a range in tensile forces. The force-length diagrams in the two-phase region will then be similar in character to those in Fig. 7 for temperatures T_2 and T_3 .

The fusion of oriented crystalline systems (as in the case of the nonoriented state) is not limited to pure polymer but can also be carried out with additional monomeric components present. For fibrous macromolecules of biological interest, it is virtually mandatory that the fibers be immersed in a liquid medium. The melting temperature will be lowered by this additional component, and experiments can be conducted without the dangers of thermal degradation and chemical decomposition. The formal thermodynamic analysis follows the same principles as the pure systems (34). However, it is important in dealing with a multicomponent system that two different cases be carefully distinguished. In one case, the fiber is allowed to be in contact with a large excess of the monomeric species, which constitutes a supernatant phase. There can, therefore, be an exchange of matter between the species in the supernatant phase and the polymer phases. For the other case, the total quantity of the nonpolymeric components is fixed so that the fiber and its surroundings act as a closed system.

For simplicity in the analysis, but by no means necessarily, the additional monomeric components are restricted to the noncrystalline amorphous polymer phase. For a binary closed system (one monomeric liquid component) the system is bivariant at constant pressure, and thus equilibrium force is not uniquely determined by the temperature. Total melting will, therefore, not occur at a constant force. As melting progresses or, conversely, as crystallization ensues, the composition of the amorphous phase must change since the amount of diluent available is fixed. The appropriate equilibrium requirement at a given composition of the amorphous phase becomes (34)

$$\left[\frac{\partial(f/T)}{\partial(1/T)} \right]_{p,n} = \frac{\Delta\bar{H}}{\Delta\bar{L}} \quad (8)$$

Here $\Delta\bar{H}$ represents the heat of fusion plus the differential heat of dilution, and the quantity $\Delta\bar{L}$ is similarly defined. These quantities again refer to the completely crystalline and completely amorphous fibers. The subscript n

denotes the requirement in equation 8 of constant composition in the amorphous phase. Hence the force-temperature derivatives, and thus the force, will depend on the extent of melting. The coexistence curve connecting the two phases will again be sigmoidal in character, although in this case one is dealing with a homopolymer of uniform properties.

A binary open system, where only a pure liquid species is present, is univariant at constant pressure. Hence, at phase equilibrium the force is uniquely determined by the temperature and will be independent of sample length, in analogy to the case of the pure homogeneous one-component system. In this case, as melting progresses, the composition of the amorphous phase is governed by the conditions of swelling equilibrium by virtue of the large excess of liquid in the supernatant phase. Consequently, the composition of the amorphous phase is independent of the extent of melting. It is then found (34) that

$$\left[\frac{\partial(f/T)}{\partial(1/T)} \right]_{p,L} = \frac{\Delta\bar{H}}{\Delta\bar{L}} \quad (9)$$

$\Delta\bar{H}$ and $\Delta\bar{L}$ here represent the sum of fusion of the pure species plus the integral change that occurs on mixing the requisite amount of each species to arrive at the composition of the swollen amorphous phase. Since this composition is fixed through the melting region, the right-hand side of equation 9 is constant during melting.

The phenomena described above can be generalized to include multicomponent systems. When another monomeric species is added, an open system now has two degrees of freedom while the closed system has three at constant pressure. It is now possible to develop coexistence curves, completely analogous to those illustrated in Figs. 5, 6, and 7, but at constant temperature in this instance. The variance in each case is now reduced by one, and melting can take place isothermally simply by varying the chemical potential of one of the monomeric species. Instead of having families of curves representing different temperatures as in the figures cited, the curves now will be at different chemical potentials for one of the monomeric species. Thus, by varying the concentration of a monomeric species, which affects the melting temperature, melting, reversible contractility, and tension development can take place isothermally. The analysis for the isothermal case is similar to that given above. In multicomponent systems the crystal-liquid equilibrium can also be regulated by chemical reactions between functional groups on the fiber and the monomeric species.

Detailed analytical experiments for systems of two or more components that are presently available exist mainly for the fibrous proteins, particularly cross-linked collagen (35). It is found that the major theoretical expectations discussed above are confirmed by experiment. For collagen fibers immersed

in a large excess of water the equilibrium force required to maintain the two phases in equilibrium, at a fixed temperature, is independent of the extent of the transformation and, hence, the length of the fiber. This observation is in accord with the univariance of the system. The stress required increases substantially with increasing temperature. Extremely large stresses, of the order of 50–100 kg/cm², are developed in the two-phase region (35). Studies involving collagen fibers immersed in aqueous KCNS solutions have also been reported (35). The isotropic melting temperature of collagen is lowered by the addition of KCNS to the supernatant fluid (35). Hence two-phase equilibrium can be obtained at lower temperatures than with pure water. Large stresses are again required to maintain the equilibrium, but surprisingly this system also behaves as a univariant one. Pryor (36) has reported that when a collagen fiber is immersed in a 2 M mercury–potassium iodide solution, another medium which is known to induce melting in collagen (37), tensions as high as 100 kg/cm² are required to maintain the length constant. Other examples of melting the fibrous proteins by chemical interactions will be discussed in more detail subsequently. It becomes clear, however, even at this juncture of the discussion, that tension development and contractility can be developed isothermally by appropriate variation of the composition of monomeric species present. Reversibility, for all the cases cited above, is obtained by the imposition of an equilibrium force which maintains the crystalline and liquid phases in equilibrium.

As has been described above, the complete melting of an axially oriented system, in the absence of an external stress, usually results in an irreversible contraction. A special circumstance exists, however, where this irreversibility can be avoided and the melting-crystallization process, and the concomitant dimensional changes, can be carried out cyclically. This situation results from the introduction of intermolecular cross-links into a system of highly axially oriented chains. In the usual cross-linking procedure from which a conventional type of network results, the cross-links are randomly introduced into a system of randomly arranged chain units; i.e. the polymer is in the amorphous state. In this case the network is necessarily isotropic in the state in which it is formed. No significant dimensional changes are incurred, and the isotropic length is constant and independent of the cross-linking density. In contrast, when highly oriented chains are cross-linked and then are rendered isotropic either by melting or by the relaxation of the imposed stress, a significant increase in the isotropic length is observed (27, 30, 31, 38–40). It is found that the ratio of the isotropic length, after cross-linking to the initial isotropic length, increases in proportion to the square root of the number of intermolecular cross-links that are introduced. The proportionality factor depends very strongly on the state of chain orientation at the time the cross-links are introduced. The increases in length result from the fact that the

macroscopic length of a sample can be related directly to the number of chains comprising the sample and their root mean square end-to-end distance, and inversely to the number of chains in cross-section (34). For a highly ordered system the theoretical expectation is that the isotropic length should increase directly with the square root of the number of cross-links introduced.

Increases in the isotropic length by factors of 2 or 3 are easily achieved (30, 31, 38-40). For a highly oriented polyethylene fiber an increase in the ratio of the isotropic length of about 23 (in this case measured above the melting temperature at 140°C) has been reported (27). These large extensions are observed without the application of an external stress and cannot usually be obtained by the mechanical deformation of an amorphous network since either rupture or crystallization will intervene. We might parenthetically note that if in the isotropic state some of these cross-links were severed an irreversible axial contraction would ensue.

In the present context, the major interest lies in the large extension that is achieved in the liquid state, by the cross-linking procedure indicated, without an external force being applied. When such a system is cooled below its melting temperature crystallization will occur. The large extension in the molten state should establish a preferential axis for the crystallization. It will therefore be expected that the crystallites formed will be axially oriented. This expectation is indeed confirmed by the wide angle X-ray diffraction patterns of samples treated in the manner described (27). The melting of this axially oriented system should therefore be accompanied by a contraction, and the crystallization by reelongation, and this process should be capable of being carried out cyclically without the presence of an external force as long as the covalent intermolecular cross-links are maintained during the process. Not all networks are expected to behave in this manner, but only those formed by the cross-linking of highly oriented chains. Fig. 8 demonstrates that the dimensional changes that are expected for the crystallization and melting of such systems are indeed observed for a polyethylene network (27). At the lower temperatures the fiber is in the crystalline state (obtained after melting the initially cross-linked fiber and recrystallization by cooling), and a slight positive thermal expansion coefficient, typical of crystalline polymers, is observed during the initial heating. A sharply defined axial contraction occurs concomitantly with melting over the expected narrow temperature interval. Above the melting temperature, the thermal expansion coefficient is slightly positive, as expected in the liquid state in the absence of a force. On cooling, the fiber regains its original dimensions as recrystallization occurs. The heating-cooling process can be repeated cyclically with the same dimensional changes being observed. When the cooling is carried out slowly, as is illustrated in Fig. 8, supercooling is observed, as is reflected in the dimensional lag. Supercooling is characteristic

of the crystallization from the melt of all polymeric systems and can be minimized by rapid cooling. Fig. 8 demonstrates that, coupled with the liquid-crystal transformation, a reversible contractile system can be obtained which is completely cyclic and does not require the imposition of an external stress. The sharpness of the contraction in this case is a consequence of the melting of a homopolymer in accord with the fact that the process is a first-order phase transition. For a copolymer fiber or for a fiber immersed in a diluent, the swelling and contraction range will be broadened for the reasons previously discussed. If a force were imposed on this system the same characteristic features would still be observed; the only difference would be that

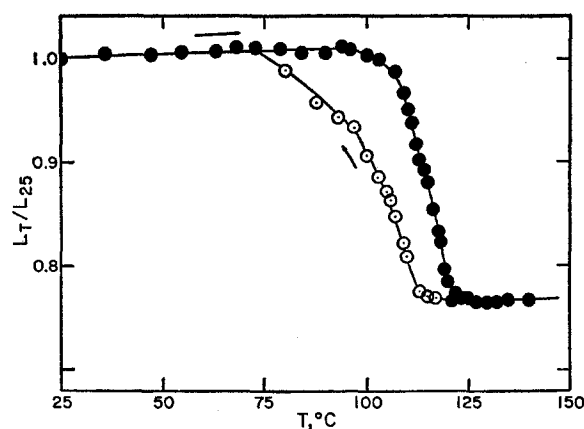


FIGURE 8. Plot of relative length against temperature for reversibly contractile polyethylene fiber. ●, heating; ○, cooling. Figure reprinted by permission from *The Journal of the American Chemical Society*, 1959, 81:4148.

the melting temperatures would be raised in accord with equation 7. Thus, fibers such as the one just described can serve as the working substance of an engine that converts thermal energy into mechanical work (27). The thermodynamic analysis and theoretical efficiency of such an engine operating in a closed cycle are identical with those of a Carnot cycle utilizing a gas as the working substance.

The system just described and illustrated in Fig. 8 could also be operated over a very small temperature interval, so that only partial melting occurred and the level of crystallinity changed only slightly. In this case the full diminution in length would not be observed. For small changes in length, it would be extremely difficult to detect any major morphological changes by present techniques. One might even be tempted to conclude, based solely on morphological observations, that there has been no change in molecular conformation. However, the molecular events that occur are established by

studying the complete process. Observations which are restricted to a small portion of the melting curve are recognized to be part of an over-all or continuous process and not an isolated event. In this manner the thermodynamic variables L and T , the wide angle X-ray diffraction and birefringence before and after melting, and the change in elastic properties demonstrate that the shortening is a consequence of a phase transition with major changes in molecular conformation. Any relatively small and isolated length-temperature or tension-temperature observations can be given a proper physical-chemical description by investigating the complete range of variables involved.

At this point it appears appropriate to summarize the conclusions that have been reached from the analysis of the simpler types of polymers. Both experiment and theory demonstrate that crystallization under a tensile force results in an elongation while contraction will occur during the melting of an axially oriented system. There are corresponding effects on the tension for experiments that are carried out at constant length. These phenomena are reversible and cyclic either when an equilibrium stress is imposed or when intermolecular covalent cross-links are introduced into an originally oriented system. The changes in length or in tension that are observed occur concomitantly with a first-order phase transition and reflect the conformational changes in the constituent macromolecules that are involved. From the general approach to the problem being taken here, it becomes conceptually important to separate the contractile process, or the molecular mechanism of contraction, from the chemical events that may be governing the contraction. Since the generalization has already been made that the phase equilibrium can be governed by isothermal chemical reactions, this distinction becomes important in trying to elucidate the molecular mechanism of contraction in more complex systems. With the molecular principles that have been outlined here and the approach just described, we next examine a variety of contractile processes which involve the fibrous proteins.

FIBROUS PROTEINS

Bailey and Astbury (41) and Astbury (42, 43) established in their classical work that a number of proteins, termed the fibrous proteins, occur naturally in the crystalline state and also possess a high degree of axial orientation. These include the α - and β -keratins, collagen, elastoidin, fibrin, and muscle fibers. Despite the large differences in the detailed crystallographic structure that exist among these species, they all have in common the primary structural features that are necessary to display contractility by means of a crystal-liquid transition. Contraction or supercontraction of the fibrous proteins has been a well-known and widely reported phenomena (e.g. reference 44). The contractility of all the fibrous proteins in a variety of media and under

diverse experimental conditions has been shown to be a consequence of the phase transition cited. The large number of experimental observations upon which this conclusion is based are detailed elsewhere (8, 45). For present purposes specific examples have been selected to illustrate further the principles involved and to demonstrate how a unified approach to the problem delineates the common molecular origin of contraction or supercontraction in the fibrous proteins.

We examine first the role of intermolecular covalent cross-links. If such cross-links are known from chemical analysis to be present naturally in a given fibrous protein, then it can be presumed that they are formed subsequent to fiber formation. They are, therefore, imposed as a previously axially oriented system. The contractile process would then be expected to be reversible, in the absence of an external force, irrespective of the mode by which melting is induced. Conversely, for those fibrous proteins in which the cross-links are absent or severed, contractility (in the absence of an applied stress) will be irreversible unless stable cross-links are introduced into the native crystalline, oriented state. The hydrothermal shrinkage of native collagen, without any external force, which has been previously shown to be the result of complete melting and the destruction of all ordered structure, is not reversed merely by cooling the fiber. This is in harmony with the fact that the amino acid analyses of this protein do not indicate the presence of covalent intermolecular cross-links. However, if the native collagen is cross-linked (tanned), the process becomes reversible (46), with the regeneration after melting of the original length and ordered structure.

The contractility of the α - and β -keratins immersed in various media has been extensively studied (8, 45, 47). It was recognized that two distinctly different types of contractile processes exist for these proteins (47). They are either reversible or irreversible. Those in the latter category are now recognized as occurring in media which sever the intermolecular disulfide cross-links, while for the reversible contractile processes the cross-links are maintained. Contractility in the keratins can result by interaction with a variety of reagents (47). Aqueous LiBr solutions are one system that have been amenable to detailed quantitative studies, since the transformation can be conducted under relatively mild conditions. The integrity of the polypeptide chains and of the cross-links is thus maintained. The melting (contraction) occurs sharply as the temperature is increased, for a fixed LiBr concentration, or at a well-defined concentration at constant temperature. This kind of observation has been experimentally demonstrated for all of the fibrous proteins when immersed in a variety of solutions (8, 45). A typical example of the contraction of α -keratin, when immersed in solutions of differing concentrations of LiBr, is illustrated in Fig. 9 (48). The sharp decrease in length that occurs over a narrow temperature interval is readily apparent.

The regeneration of the length, as indicated by the dashed lines, is accomplished by dilution. The thermodynamic evidence that is illustrated for melting is supported by the changes in other properties, such as the wide angle X-ray diffraction patterns (48), the birefringence (49), and the elastic properties (50). Lithium bromide is one member of a class of salts whose aqueous

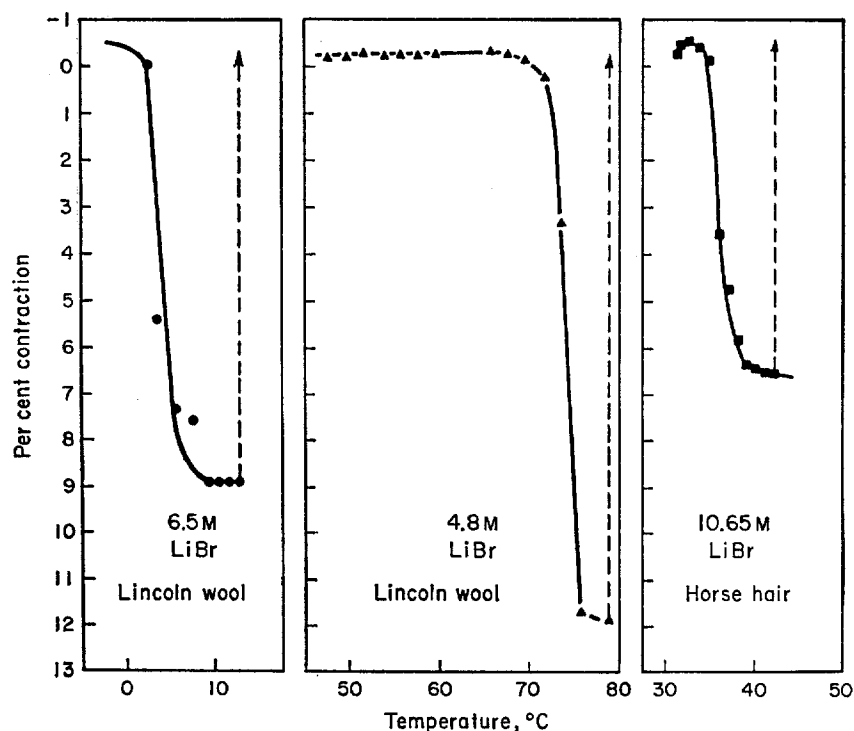


FIGURE 9. Length-temperature relations for α -keratin fibers immersed in aqueous LiBr solutions of indicated molarity. —, heating; ---, regeneration of original length upon immersion in pure water. Figure reprinted by permission from *The Journal of the American Chemical Society*, 1962, 84:1383.

solutions act as a universal contracting (melting) medium for all of the fibrous proteins (8, 45, 48).

From the data in Fig. 9 it can be deduced that there is a characteristic melting temperature for each concentration of LiBr. Hence it should be possible to induce melting isothermally in this system by appropriately fixing the temperature and varying the salt concentration in the supernatant phase. This process should also occur reversibly with change in the salt concentration. This expectation has been confirmed for the α -keratin-LiBr system (48) and demonstrates in a simple manner how fibrous macromolecules can be

utilized as the working substance of an engine that isothermally converts chemical energy to mechanical work.

The other fibrous proteins, including glycerinated muscle fibers, also undergo contraction as a consequence of melting in both LiBr and other solutions containing monomeric reagents. A typical example is given in Fig.

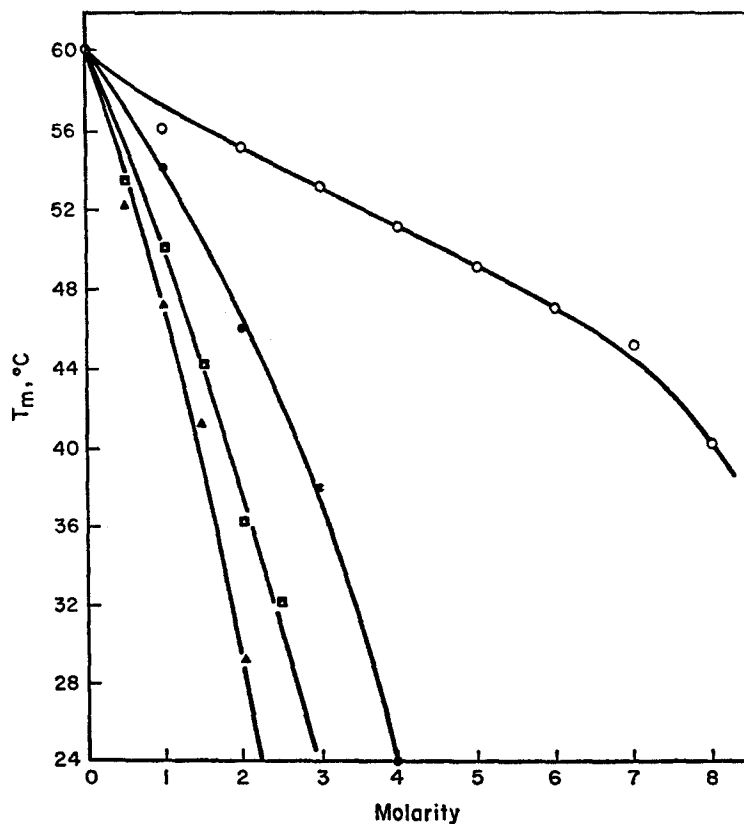


FIGURE 10. Plot of melting temperature of elastoidin fibers as a function of molarity of monomeric reagent in supernatant phase. \circ , urea; \square , CaCl_2 ; \blacktriangle , KCNS ; \triangle , KI ; \bullet , LiBr . Figure reprinted by permission from *The Journal of Physical Chemistry*, 1962, 66:375.

10 for the variation of the melting point (contraction temperatures) of cross-linked elastoidin fibers with concentration when immersed in aqueous solutions of various monomeric reagents (37). The contraction temperature is systematically lowered by the addition of the third component (salt), and the relative amount of the depression depends on the nature of the salt. At a given concentration, contraction similar to that illustrated in Fig. 9 for the α -keratin-LiBr system is observed. It becomes obvious, therefore, that, for

any of the situations illustrated in Fig. 10, contraction and reelongation can be accomplished isothermally by appropriately varying the concentrations of the added third species. If one is so inclined, machines can be built, utilizing a fibrous protein such as collagen as the working substance, which isothermally convert chemical energy into mechanical work. The principle of operation of such a machine is based on a phase transition being monitored by chemical interactions. Such machines have been constructed. Pryor (51) built one based on the collagen-KI system, and more recently another machine, based on the melting of collagen by LiBr solutions, has been reported (52). These machines serve as very nice experimental demonstrations of the principles being discussed.

The melting or contractile medium is not limited to aqueous salt solutions. Very similar observations are noted in nonaqueous salt solutions (53), in water-alcohol mixtures,³ and in other pure liquids.³ Contraction can also be obtained with collagen and elastoidin fiber by varying the pH (8). The presence of certain divalent ions in the supernatant fluid actually raises the melting temperature of the fiber. However, despite the diversity of the media that can cause contraction, the same underlying molecular mechanism still prevails. This is the reason for developing the conceptual distinction between the chemical interactions governing the phase transition and the molecular contractile mechanism.

As has been indicated above, glycerinated muscle fibers behave in the various salt solutions in a manner very similar to the other fibrous proteins (54, 55). It is, of course, well known that muscle fibers will contract in ATP solutions, and hence it is of some interest to study this process in some detail (56). The relative change in length of the glycerinated muscle fiber that is observed isothermally (25°C) with increasing ATP concentration is plotted in Fig. 11. The data in this plot give a clear indication that a cooperative phase transition is occurring. Most of the contraction occurs over a very small change in the ATP concentration. This is similar to the other melting curves that have been presented in which the temperature is the intensive variable. The melting process for this system is supported by the change in the wide angle X-ray patterns (56). Recrystallization and reelongation after complete melting are not induced in this system by either dilution or cooling. This is indicative of the absence of permanent covalent intermolecular cross-links. Some reelongation is observed upon dilution after only very limited amounts of contraction have been allowed. Small amounts of contraction resulting from partial melting will not be detected by X-ray diffraction, as was discussed previously in connection with the melting of polyethylene fibers. Although the experimental results illustrated in Fig. 11 are clearly

³ L. Mandelkern and E. Villarico. Unpublished observations.

not of any direct physiological validity, they do demonstrate that the contraction of the glycerinated muscle-ATP system occurs by the same molecular mechanism as contraction of the other fibrous polymers that have been discussed. Evidence for contraction accompanying melting has also been given by glycerinated muscle fibers immersed in ATP-glycerol-water mixtures and ATP-ethylene glycol-water mixtures (57). Again, large changes in length are observed with small changes in solvent composition or temperature.

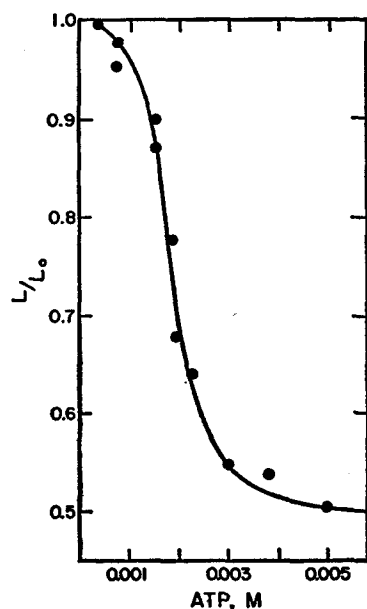


FIGURE 11. Plot of relative change in length of glycerinated muscle fibers at 25°C as function of ATP concentration in supernatant phase. Figure reprinted by permission from *Proceedings of the National Academy of Sciences*, 1959, 45:814.

The selected examples that have been discussed above make amply clear that in laboratory experiments the contractile behavior of the fibrous proteins adheres to the principles that have been set forth, which were deduced primarily from the studies of the structurally simpler polymers. Thus, the same basic underlying mechanism exists for all fibrous macromolecules irrespective of origin, chemical or crystallographic structure, or the particular manner in which melting has been induced.

The role of the added component in altering the transition temperature lends itself to formal analysis by considering the melting or freezing point of a ternary system. In this case one has the macromolecule, the major liquid component, and the added reagent as the third species. Straightforward thermodynamic consideration (58) indicates that, in order for a change to occur in the melting temperature (either a decrease or increase), there must be a preferential interaction between the added reagent and the macro-

molecular species in one of the two polymer phases. If this preferential interaction does not exist, the melting temperature will not change with the addition of and increasing concentration of the third species. An extreme manifestation of the required preferential interaction would be actual binding in one of the phases. The cooperative conformational change induced in dilute collagen solutions by the same type of monomeric reagents discussed above has been quantitatively shown to be consistent with a binding mechanism (59). Other direct evidence for the binding of the salts in one of the phases has also been given (60–62). In the general context of phase transitions in ternary systems, the detailed role of ATP, or its decomposition products, in causing the transformation of glycerinated muscle fibers is not a priori obvious.

For all the fibrous systems discussed, the contraction observed must be a result of conformational changes in at least one of the constituent macromolecules present. As has been indicated, this manifests itself not only in dimensional changes but in changes in other properties as well. Hence one should expect to find that the isolated macromolecular species, i.e. in dilute solution, would also undergo a cooperative conformational change in a similar environment and under conditions similar to those in which contraction in the fiber is observed. This expectation is indeed realized for cases where the comparison can be made. The constituent macromolecules of the fibers undergo cooperative structural changes in dilute solution under similar conditions of solvent composition. For example, soluble collagen in dilute aqueous solution undergoes cooperative conformational changes when a variety of salts are added. These same additives influence the contraction temperature of collagen and elastoidin. It has been shown by Tonomura et al. (63) that dilute solutions of myosin in aqueous LiBr undergo a cooperative structural transition. Similarly, both glycerinated muscle fibers and myosin fibers contract in this medium (54). Brahms and Kay (64, 65) have shown that myosin undergoes a cooperative structural transition in ethylene glycol–water mixtures (in the absence of ATP). Glycerinated muscle fibers show a contraction by the same mechanism, with changes in the banded pattern, when immersed in the same mixture.⁴ In addition to this qualitative correlation, a quantitative comparison can also be made.³ When allowance is made for the influence of the protein concentration on the melting temperature, then the effect of the added third component is found to be independent of the protein concentration and, in many cases, even the nature of the protein itself.³ Not only, therefore, can contraction be coupled with the formalism of a phase transition, but a quantitative description in terms of the detailed molecular conformational changes that are involved can be developed.

⁴ L. Mandelkern and S. S. Dubey. Unpublished observations.

The discussion so far clearly has not involved contractility or tension development in any actual physiological or biological functioning system. Nor has any been implied. It could be viewed as an exercise in the physical chemistry of contractility which demonstrates certain ideas. One has, however, been concerned with the contractile properties of macromolecules which are intimately involved in natural functioning systems. The understanding that has come forth from the study of nonphysiological processes lends some promise that the same concepts might apply to natural processes. The validity of this approach must obviously await further detailed experimentation, on actual functioning systems, that is guided by the principles set forth above. We wish, however, to point out that the classical observations of A. V. Hill (66), which quantitatively relate the shortening velocity of stimulated muscle to the applied force, find their counterpart in the model systems discussed. Pryor (36) has reported that the shortening velocity of collagen fibers, when immersed in KI solution, obeys the identical quantitative relations that are applicable to stimulated muscle. For the collagen system, shortening as a consequence of melting has been unequivocally demonstrated (35, 37). A connection between melting and muscle contraction can thus be made. A discussion of Hill's relation, in terms of melting kinetics, will be presented shortly.⁶

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⁶ L. Mandelkern. Unpublished observations.

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Discussion

Dr. Huxley: I'd like to make a sort of general comment to start with. It seems to me, when one is formulating a theory, that one is generally allowed, and often obliged, to ignore certain parts of the experimental evidence. The experiments may be wrong or our interpretation of them may be wrong; but it seems to me extremely dangerous and wasteful to ignore the whole of the experimental evidence. To a large extent, it seems that that's what is being done here, when one tries to derive things like the A. V. Hill equation from considerations of conformational changes of macromolecules which are arranged in a fiber in series, and to justify this by pointing to the length-tension diagram of a glycerinated muscle, and saying that there may be some significance in the fact that this length-tension diagram resembles that obtained from other fibers in other solvents.

There is a great deal of evidence now with which, I think, the large majority of people in the field agree, that when muscles shorten, and this includes glycerinated muscles in ATP, there is not an over-all change in the length of the filaments. Obviously, there is some configurational change going on somewhere in the system, and this is most likely taking place in the cross-bridges; so, naturally, we are interested in the mechanics and the energetics and the thermodynamics, and so on, of configurational changes. If people would only apply the analysis to repetitive changes in the cross-bridges then we might really get somewhere; but to totally ignore the (I think) quite obvious experimental facts, on the basis that they are structural matters, that it is somebody else's field, that they're not really prepared to take seriously, is a course of action that is likely to lead to a tremendous waste of effort.

In particular, there seems to be a case of ignoring the experimental findings even in the case of the length-tension diagram itself. We have seen these length-tension diagrams of muscles shortening, presumably (from their shape) from below rest length, but there are also available perfectly valid length-tension diagrams of glycerinated muscles and of live muscles beyond rest length, and these show that you get another descending limb on that side of equilibrium length too, and that when you stretch the muscle to sarcomere lengths between 3.5 and 4 μ , the tension becomes zero.

This is very adequately explained on the basis of the tension being generated by cross-bridges; it seems to me not at all obvious that it can be explained on the basis of configurational changes in structures arranged in series, and it certainly shouldn't be something that can just be ignored.

So, I would say, of course we're interested in configurational changes, but why not work out the theory in terms of the structure that most people now think exists? Then we might get nearer the truth instead of having these long controversies about whether or not there is an analogy.

Dr. Mandelkern: The major portion of the talk that we presented was concerned with the very general or academic aspect of the contractility problem. This was made clear in the introductory section. Now this happens to be an extraordinarily interesting set of phenomena in physical chemistry which holds the attention of at least a few people. I don't think that I was invited to this Symposium to discuss contraction in living muscle. At least I hope I was not. One certainly has the right to adopt the view that the approach taken will not be fruitful when living systems are studied. There still, however, seem to be some problems that are in need of resolution.

On the other hand, we have been able to make a connection between the approach taken and properties of living muscle in the derivation of Hill's relation. This comes from a very simple premise and results in constants having physical meaning. The least that we have achieved is a consistency; certainly there is no proof or claim for uniqueness. If one looks at the basis for the derivation, then one can quickly conclude that the velocity-force relations must be quite different above resting length, as is observed experimentally. I would again like to point out that the constants have well-defined physical meaning and can be subject to further theoretical and experimental test.

Dr. R. E. Davies: These physicochemical systems are really fascinating. I am always amazed at the ingenuity of the people who are working with them and how nice and precise the answers are when you can work out the thermodynamics and do all the proper measurements.

Professor Mandelkern said that perhaps he is a victim of a unique set of coincidences which have allowed him to arrive at the Hill equation. The assumption is that perhaps he got the right answer from false premises. Unfortunately, it is not a unique set of coincidences. About 12 years ago, Dr. Wilkie wrote a paper in *Progress in Biophysics and Biophysical Chemistry* (4:228, 1954), as it was then named, called "Facts and theories about muscle," and even then he showed that five mutually exclusive theories of muscle contraction all could account for the Hill equation. It is necessary to account for the Hill equation before you start answering the 50 or 60 or more other facts that have to be accounted for in muscle.

The information about the change in A. V. Hill's constant a to α is correct; the latter constant depends on the load lifted (see *Proc. Roy. Soc. (London)*, Ser. B. 159:297, 1964). Your difficulty here again is that the basis of your calculation comes from something that just isn't so in muscle. The Δl term for muscle is not related to the particular energetic state. If you let a muscle contract, it will stay contracted, and there is no special force or energy difference between the contracted state and the original rest length. It needs virtually zero force, an almost immeasurably small force, to pull it back again to its rest length.

This fact is interpreted as showing that there are no cross-linked bridges in the muscle between the thick and thin filaments in resting muscle, and so the very basis of the theory, just as Dr. Huxley said, cannot apply to the system in living muscle. It doesn't happen that way; there must be a cyclic mechanism repeating itself at the cross-bridges, and as soon as you have cyclic mechanisms, then the whole of these formulations collapse because, for the cyclic one, you get $\Delta G = 0$. The whole thing really depends on the transformation of chemical potential which, some distance from the actual contractile event, follows from the breakdown of ATP.

Dr. Mandelkern: I fail to see the pertinency of Dr. Davies' last few remarks to the paper that we have presented. It is quite clear that the general approach we are taking to the problem of contractility is either not being understood or not being accepted. Either way, one has been able to show that Hill's relation can be derived from very general considerations. This may be upsetting to some because there is no need to invoke any specific properties of the living muscle system, at least as far as this phenomenon is concerned. To others this may be a very comforting result. Pryor (*Progr. Biophys. Biophys. Chem.* 1:216, 1950) has already shown that collagen fibers during melting obey very similar velocity-force relations.

It is, of course, true that there have been a large number of derivations of Hill's relation. These all have as a common feature the invoking of a unique set of starting assumptions, which have no counterpart in other physical phenomena. In the derivation that has been presented here, the constants are related to well-defined physical quantities. They therefore can be subject to independent study to test further the validity and appropriateness of the derivation. It is in this way that scientific progress can be made.

Question from the Floor: I have no comment on the muscle part of the talk. I would just like to call the attention of the audience to a concept that has been developed by Dr. Carolyn Cohen with reference to the structure of fibrous proteins, based on the monumental work of Astbury. Astbury categorized the fibrous proteins in the collagen class, and the α - and β -fibrous proteins in the *K-m-e-f* class. Dr. Cohen has shown further differences in the fibrous proteins (1966. Architecture of the α -class of fibrous proteins. In *Molecular Architecture in Cell Physiology*. T. Hayashi and A. G. Szent-Györgyi, editors. Prentice-Hall, Inc., Englewood Cliffs, N. J. 169). The large fibrous α -proteins are made up of two distinct domains: one α -helical coiled coil and the other globular in structure. Some α -proteins are very rigid and seem to be made up almost totally of the α -helical coiled coil domain, whereas there are other proteins, such as myosin, which seems to contain about 60% coiled coil portion. I do not remember the exact figures of Dr. Cohen as to the percentage contribution of the coiled

coil and globular domain in the structure of myosin. Dr. Cohen points out in her review that conformational changes would occur in the globular region of the myosin molecule.

The main point I wish to make concerning Dr. Mandelkern's presentation is that one has to consider the kind of fibrous protein one is dealing with. It seems to me that one would observe quite different conformational changes depending on the type of fibrous protein.