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Dimensional Changes in Fibrous Macromolecules: The System α-Keratin–Lithium Bromide[1]

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Anisotropic dimensional changes in α-keratin fibers immersed in aqueous LiBr solutions have been studied. Contraction and relongation in the absence of any external force is shown to occur concomitantly with the crystal–liquid phase transformation of the keratin in accord with principles previously deduced from studies of simpler fibrous macromolecules. The transformation can be induced by variation of the temperature at constant LiBr concentration, or isothermally by varying the salt concentration at constant temperature. Processes of this type are in the general category of melting being caused by chemical reactions. Various types of reactions are considered and the available evidence favors an interaction of the LiBr at the peptide linkage with the resultant structural alteration of randomly selected amino acid residues, this hypothesis being in accord with the observed phase diagram.

Introduction

Theoretical and experimental studies have shown that anisotropic dimensional changes in axially oriented crystalline macromolecular systems accompany the crystal–liquid transformation. The dimensional changes observed on a macroscopic level are a manifestation of the distinctly different conformations that the individual chains assume in the two states. Shrinkage will usually accompany melting as a result of the diminished axial extension of a chain in the molten or amorphous state as compared to the extended conformations characteristic of the crystalline state.[2,3] In the absence of an external tensile force, spontaneous re-extension of the completely molten fiber under conditions conducive to crystallization will be observed if the system contains a sufficient number of permanent intermolecular crosslinkages that were formed in the oriented state.[4] Anisotropic dimensional changes as well as related changes in thermoelastic properties that occur in fibrous natural rubber,[5,6] fibrous polyethylene,[4] collagen[7,8] and glycerinated muscle fibers[9] have been treated and discussed from this point of view.

Since dimensional changes in such systems are a consequence of a phase transition, the process of contraction and extension will be very sensitive to changes in the intensive variables of the systems such as the temperature, pressure, stress and to the solvent medium in which a fiber is immersed. In particular, alteration of the chemical environment of the medium could affect the equilibrium between the two phases and thus induce and govern the anisotropic dimensional changes. Attention is therefore directed to systems wherein contractility can be evoked by appropriate chemical reaction. In the simpler types of polymers, such as fibrous natural rubber and fibrous polyethylene, the crystal–liquid transformation is easily brought about by a change in the temperature. In order to induce the phase transition by means of chemical processes, a polymer is required, the chain units of which are chemically reactive. The fibrous proteins fulfill this condition and also occur naturally in the crystalline axially-oriented state.[4] Thus the structural requirements for contraction to accompany melting are satisfied. Shrinkage is widely observed among the fibrous proteins as a result of reaction with a variety of chemical reagents. Despite the apparent diversity of these systems and of the reagents employed, melting of the native crystalline arrangement appears to be a common feature in the shrinkage of the fibrous proteins. Several examples described in the literature that adhere to the principles set forth have been indicated.[4]

For a systematic study of contractility in fibrous proteins, more detailed quantitative information is required to ascertain not only whether or not a given contractile process follows the prescribed mechanism, but also whether or not there are any general classes of reagents and reactions that affect the thermodynamic stability of the crystalline state in a collection of polypeptide chains. If further generalizations beyond the elucidation of the basic mechanism can be evolved as to the nature of chemical melting for this class of polymers, further information in regard to the mechanochemistry of various naturally occurring processes can be obtained.

The proteins of the keratin class occur in the oriented crystalline state[10–12] and intermolecular crosslinks are built into the system naturally by means of covalent disulfide linkages. Alexander and Hudson[13] have pointed out that two distinctly different types of contractile processes are observed in α-keratin fibers. In one of these the intermolecular disulfide crosslinks are severed by chemical means; contractility in this case is irreversible. An explanation of this process in terms of the change in length with crosslinking of oriented polyethylene and natural rubber has already been offered.[4] In contrast, the other contractile process is presumed to occur without the severance of the intermolecular crosslinks and is

(1) Presented in part before the Division of Biological Chemistry, American Chemical Society, New York, N. Y., Sept., 1960.

(2) P. J. Flory, *Science*, **124**, 53 (1956).

(3) P. J. Flory, *J. Am. Chem. Soc.*, **78**, 5222 (1956).

(4) L. Mandelkern, D. E. Roberts, A. F. Diorio and A. S. Posner, *ibid.*, **81**, 4148 (1959).

(5) D. E. Roberts, L. Mandelkern and P. J. Flory, *ibid.*, **79**, 1515 (1957).

(6) J. F. M. Oth and P. J. Flory, *ibid.*, **80**, 1297 (1958).

(7) P. J. Flory and R. R. Garrett, *ibid.*, **80**, 4836 (1958).

(8) P. J. Flory and O. K. Spurr, Jr., *ibid.*, **83**, 1308 (1961).

(9) L. Mandelkern, A. S. Posner, A. F. Diorio and K. Laki, *Proc. Natl. Acad. Sci. (U. S.)*, **45**, 814 (1959).

(10) W. T. Astbury, *Proc. Roy. Soc. (London)*, **B134**, 303 (1947).

(11) K. Bailey, W. T. Astbury and K. M. Rudall, *Nature*, **151**, 716 (1943).

(12) W. T. Astbury, *Trans. Faraday Soc.*, **34**, 378 (1948).

(13) P. Alexander and R. F. Hudson, "Wool, Its Chemistry and Physics," Reinhold Publishing Corp., New York, N. Y., 1954, p. 378.

observed when the polymer is subjected to conditions conducive to melting; re-elongation occurs concomitantly with recrystallization. Of the various reagents that can induce contractions of the latter type, aqueous solutions of LiBr seem most amenable to detailed study since the transformation can be conducted under relatively mild conditions where the integrity of the polypeptide chains and the concentration of the intermolecular crosslinks can be maintained. Various observations in the literature give indication that contractility of α-keratin from various types of hair can be carried out reversibly in this medium and that significant structural changes occur during the process.[14-18] It appears, therefore, to be a suitable system for more detailed studies from the point of view outlined above. The purpose of the present investigation, therefore, was to ascertain if the contractile process was coupled to the crystal–liquid phase transition, and if so, to consider the nature of the chemical interactions that affect the equilibrium between the two phases.

Experimental

Materials.—Fibers selected for study were Lincoln wool and horse hair. A supply of the former specimens was obtained from the U. S. Department of Agriculture Experimental Station. The latter, from a horse's tail, was kindly furnished by the Harris Research Laboratory. The specimens were cleaned by successive Soxhlet extractions with carbon tetrachloride, acetone and water and were then air dried and stored in the absence of direct light until used.

The lithium bromide used was of reagent grade. Saturated solutions were prepared with distilled water and filtered to remove extraneous dirt. The concentrations were determined by means of a Mohr titration for bromide ions. The concentration of the saturated solutions of LiBr at 25° was found to be 12.06 ± 0.04 moles per liter, in agreement with the previous report of Gibson and Kincaid.[19] Solutions of a given concentration of LiBr were prepared by diluting aliquots of the saturated solution at 25°; caution was exercised to allow for the large heat of dilution. The accuracy of the dilutions was periodically checked by a direct determination of the bromide ion concentration, and the procedure outlined above was found to be adequate.

Length Measurements.—Lengths of individual, single fibers were determined as a function of temperature while the fibers were immersed in LiBr solutions of a given concentration. The solution and the fibers were contained within a tube having a ground glass cap. One end of the fiber was tied to a loop of Nichrome wire which in turn was suspended from a glass hook joined to the cap. A small weight, of approximately 10 mg., was tied to the bottom of the fiber to keep it from rising to the surface of the solution. Thus the entire fiber was immersed in a large excess of solution. The glass tube was then mounted in a thermostat bath, wherein the temperature was maintained constant to within ± 0.1°. Lengths were measured by means of a Gaertner cathetometer, accurate to 0.001 mm. The initial lengths of the fibers were about 20 mm.; however, since the fibers invariably contained kinks, an uncertainty of about 1% exists in the initial length.

In order to determine reliable melting points of polymers in general, and the α-keratin–LiBr system in particular, conditions as close as possible to those for equilibrium must be established. It was found that the adoption of a slow heating schedule was necessary to obtain reproducible melting temperatures. Melting points were first roughly determined with rapid heating. The experiment was then repeated with new fibers and fresh solutions, starting approximately 10° below the expected melting temperature. For solutions greater than 5.5 M LiBr, heating rates of 1° per 24 hr. were utilized. Reproducible melting points were obtained when this procedure was followed, and the melting temperatures were invariably significantly lower than those obtained on fast heating. It would appear that adequate time must be allowed for the diffusion of the ionic species into the fiber. When the concentration of LiBr is reduced below 5.5 M, complications develop as a consequence of thermal degradation at the temperatures required to observe melting. Hence, in this concentration range the heating rates were increased to 1° per 3 hr. so that the melting temperatures reported may be somewhat high. At temperatures above 110° degradative processes take place so rapidly that reliable observations of the length–temperature relations could not be made. This precludes studies in LiBr solutions less than 3.75 M.

X-Ray Diffraction.—Wide-angle X-ray diffraction patterns of single α-keratin fibers were obtained using a Micro Camera with a pinhole collimating system. Nickel filtered Cu radiation was employed and the X-ray generating tube was operated at 20 kv. and 30 ma. The incident X-ray beam was directed normal to the macroscopic fiber axis of the specimen. The fiber-to-film distance was 15 mm. and the exposure time was approximately 24 hr.

Swelling.—The weight fraction of LiBr solution imbibed by molten horse hair fibers was determined by gravimetric methods. Small snips of fiber, weighing approximately 0.5 mg., were immersed in a large excess of an appropriate LiBr solution at temperatures 2–4° above the measured melting temperatures. Swelling equilibrium was established in 24–48 hr.; at this time the fibers were removed from the solution, blotted to remove solution adhering to their surfaces and inserted into a weighed cylindrical capsule of aluminum foil, sealed at one end. After sealing the other end, the capsule and its contents were weighed, all weighings being performed on a semi-micro balance. The composition of the swollen network could then be calculated with an estimated error of about 10%. The weight fraction of polymer in this phase was converted to volume fraction by utilizing the known densities of LiBr solution[19] and of α-keratin.[20]

Results

Melting and Recrystallization.—Some typical results obtained for the dependence of the length on the temperature are illustrated in Fig. 1. Of

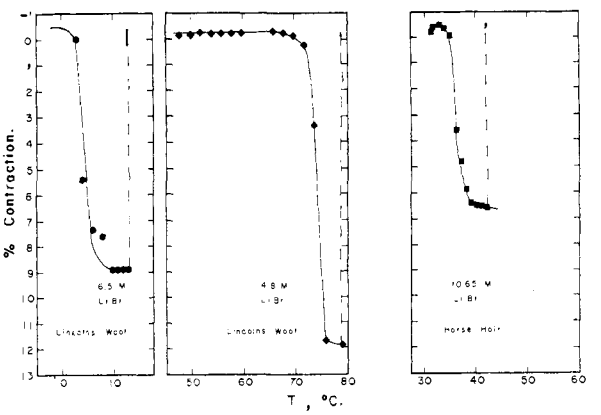


Fig. 1.—Length–temperature relations for α-keratin fibers immersed in aqueous LiBr solutions of indicated molarity. Solid lines and points indicate heating cycle; dashed vertical line indicates regeneration of original length upon immersion in pure water.

(14) P. Alexander, *Ann. N. Y. Acad. Sci.*, **53**, 653 (1951).

(15) J. Griffith and A. G. Alexander, *Textile Research Journal*, **27**, 755 (1957).

(16) A. R. Haly and J. Griffith, *ibid.*, **23**, 32 (1953).

(17) E. Hambraeus and R. Steele, "International Congress of Sciences, Applied to the Textile Industry" (collected papers), Ghent, 1951, p. 119.

(18) A. E. Brown and L. G. Beauregard in "Sulfur in Proteins," Academic Press, Inc., New York, N. Y., 1959, p. 959.

(19) R. E. Gibson and J. F. Kincaid, *J. Am. Chem. Soc.*, **59**, 25 (1937).

(20) R. D. B. Fraser and T. P. MacRae, *Textile Research Journal*, **27**, 867 (1957).

major significance in these plots is the fact that contraction or shrinkage occurs over a very narrow temperature interval. The temperature at which the contractile process is complete is well defined, and on either side of this temperature interval the linear expansion coefficients are normal. Plots of this type are characteristic of a first-order transition between two distinct macroscopic phases. The length therefore can be assigned the role of an extensive thermodynamic variable which undergoes a finite discontinuity at the transformation temperature. This is in complete harmony with the changes usually observed in other extensive thermodynamic quantities, such as the enthalpy and volume, during a transformation of this type. The temperature at which the transformation occurs depends both on the nature of the fiber (Lincoln wool or horse hair) and the LiBr composition. It is not limited to a fixed LiBr composition, as has been implied by previous work.[14-16,18,21] Contractility in a given type fiber is observed over a wide range in the composition of the liquid medium. The transformation temperature is, however, determined by the LiBr concentration. Transition temperatures below 25° are observed which are much lower than would be anticipated from consideration of previously published reports.[14,18] This can be attributed to the slow heating rates employed in the present work.

On merely cooling the specimen, however, the original state is not regenerated. For example, contracted specimens of both Lincoln wool and horse hair were held in their respective LiBr solutions at various temperatures below the transition temperatures for periods up to two months. No perceptible increase in length or other evidence of the reversal of the transformation was observed. If, however, the shrunken fiber is removed from the LiBr solution after melting and immersed in pure water, the original dimensions are regained. This reversion process is schematically illustrated by the dashed lines of Fig. 1. The irreversibility of the contractile process is thus only apparent. The time required for the regain of the original length is one of minutes. Reversion to the initial state can be accomplished by this procedure in the absence of any external tensile force at all temperatures from 0 to 100°, independent of the initial transformation temperature and the concentration of LiBr.

If after the return to the initial state the fiber is reimmersed in the large excess of LiBr solution, the same length–temperature curve and transformation temperature are obtained as initially. This is demonstrated by the data in Fig. 2. The initial transformation of a Lincoln wool fiber in 12.05 *M* LiBr is shown by the circles. After regeneration of the original state by immersion in pure water, a second transformation can be induced by returning the fiber to the original LiBr solution. The course of the second transformation, indicated by the squares, follows essentially the same pattern as the original transformation. Thus, in the α-keratin–aqueous LiBr system a reproducible and

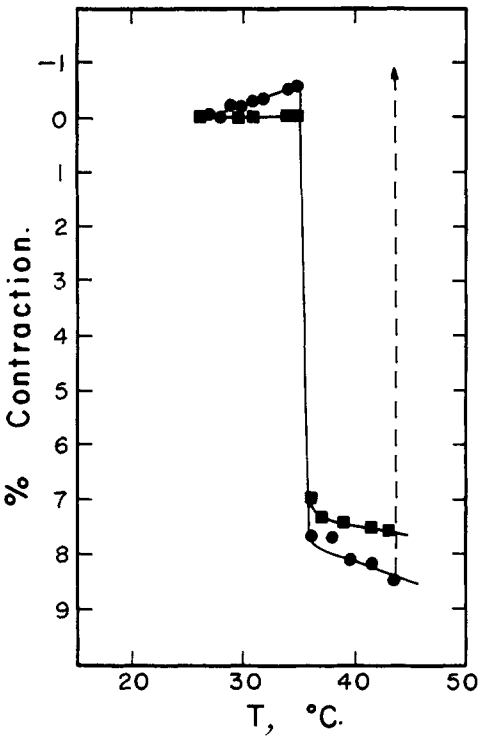


Fig. 2.—Length-temperature relations of Lincoln wool fiber immersed in 12.0 *M* aqueous LiBr solution: ●, original heating cycle; - - - -, regeneration of length in pure water; ■, second heating cycle in LiBr solution.

reversible contractile system can be developed. The return to the original state occurs, however, only by dilution.

If, however, the shrunken or molten samples are brought to temperatures greatly exceeding T_m, the melting temperature, a further contraction is observed. The amount of this additional contraction depends not only on the time and temperature, but also on the number of degrees above T_m at which the sample is held. The total contractile process becomes irreversible after such treatment, even after transfer of the specimen to pure water, so that neither the original length nor the X-ray diffraction pattern is regained. This behavior is similar to the so-called "second stage contraction" previously reported.[22,23]

The dependence of the melting temperature of both types of fibers on the LiBr concentration in the supernatant fluid is given in Fig. 3. A similar plot is observed for both the Lincoln wool and horse hair. As the LiBr concentration is increased the melting temperature is depressed until a minimum is reached at about 7 to 7.5 *M* LiBr. The melting temperatures for Lincoln wool and horse hair are 7 and 22°, respectively, at this point. As the concentration of LiBr is further increased to the saturation value (at room temperature), an almost linear increase in the melting temperature with concentration is observed. The results for the two fibers parallel one another in this range,

(21) A. R. Haly and J. W. Snaith, *Biochim. Biophys. Acta*, **44**, 180 (1960).

(22) A. R. Haly and M. Fenghelman, *Textile Research Journal*, **27**, 919 (1957).

(23) M. Fenghelman, A. R. Haly and T. W. Mitchell, *ibid.*, **28**, 655 (1958).

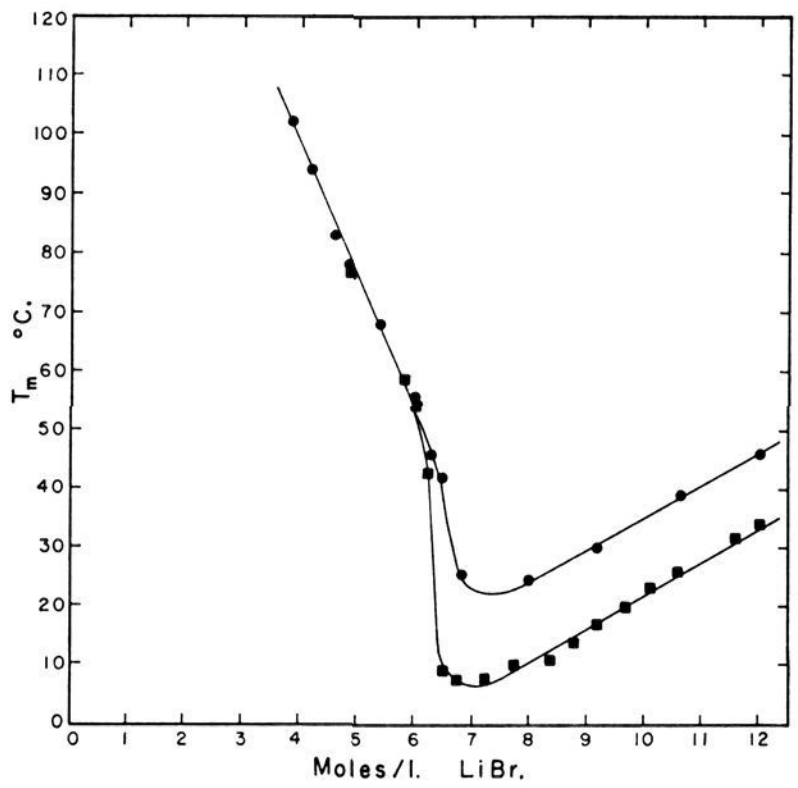


Fig. 3.—Plot of melting temperature T_m (contraction temperature) of α-keratin fibers against the molarity of the LiBr solution: ■, Lincoln wool; ●, horse hair.

with the melting temperatures of the horse hair being the greater. A great deal of attention has been paid heretofore to the contraction of α-keratin fibers in 7 to 8 M LiBr solution.[14-18,21] It is in the vicinity of these concentrations that shrinkage occurs at room temperature or below, thus making the observation and detection of the phenomena quite obvious. However, the concept has been engendered that contractility is limited to this concentration range. This is clearly erroneous and could lead to misinterpretation.

Wide-angle X-Ray Diffraction.—More detailed elucidation of the contractile mechanism requires the identification of the two phases involved in the transformation. Wide-angle X-ray diffraction patterns were obtained in each series of experiments on the original fiber, on the fiber after transformation, and on the fiber after immersion in pure water. Figure 4A illustrates the wide-angle pattern obtained for native Lincoln wool fiber with the characteristic meridional reflection at 5.1Å and the equatorial reflection at 9.8Å. The pattern for the transformed, shrunken fiber (Fig. 4B)

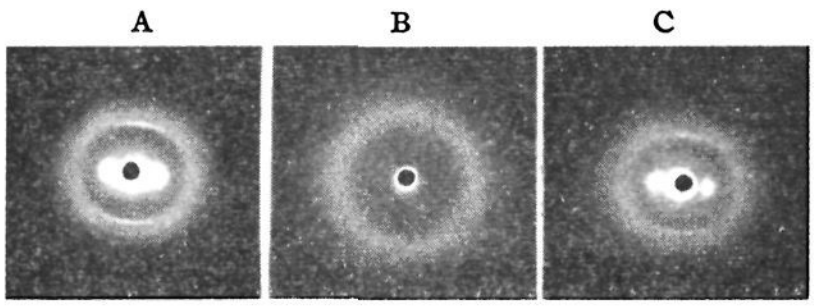


Fig. 4.—Wide-angle X-ray diffraction patterns for Lincoln wool: (A) native fiber; (B) after contraction in 6.5 M LiBr; (C) after immersion of contracted fiber in pure water.

contains no discrete reflections; the vicinity of 9–10Å. is transparent, and a broad amorphous halo exists in the range 3–4Å. After immersion in water, concomitantly with the regain of the original length, the X-ray diffraction pattern typical of the native state is restored, as can be seen in Fig. 4C. Thus the reversibility in thermodynamic properties is accompanied by a recovery of the structural characteristics. Clearly, then, the transformation involves the change from the crystalline to amorphous phases of the constituent polypeptide chains.

Isothermal Contractility.—From a consideration of the T_m-concentration curve in Fig. 3, it is apparent that if the temperature is held constant, isothermal contractility should be observed by appropriate variation of the LiBr concentration. Moreover, if the temperature and concentration range are so chosen that they encompass both sides of the minimum, then a dual contraction–re-elongation cycle should be achieved by monotonically varying the composition of the surrounding medium. As a matter of convenience, 24° was selected as a temperature of experiment. In the first set of experiments a Lincoln wool fiber was successively immersed in solutions of decreasing LiBr concentration starting with 12 M. From Fig. 3 we can deduce that the fiber is initially in the crystalline state under these conditions. The results of this experiment are summarized in Fig. 5. At about 10 M LiBr, a sharp contraction

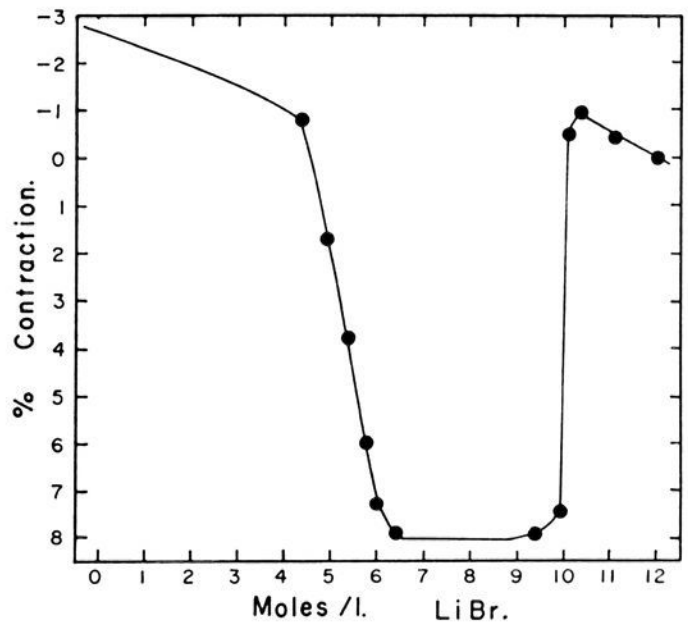


Fig. 5.—Isothermal length–concentration relations for Lincoln wool fiber immersed in aqueous LiBr solution at 24°. Measurements were conducted as the concentration of LiBr was decreased.

is observed as a consequence of the melting. This is consistent with the results of the pseudo phase diagram of Fig. 3 which was determined independently. As the concentration of LiBr is lowered, the amorphous state is traversed without any significant change in dimension. However, starting at 6.5 M LiBr re-elongation occurs, indicative of recrystallization, and eventually the original dimensions are recovered. This observation again is in agreement with the data of Fig. 3. Wide-angle X-ray patterns confirm the conclusion that melting and recrystallization have occurred as the LiBr concentration is progressively lowered. The observed dimensional changes are thus a consequence of an isothermal change of phase.

The converse process, obtained by starting with the fiber immersed in pure water at 24° and increasing the LiBr concentration, is illustrated in Fig. 6. A sharp contraction corresponding to melting is noted in the vicinity of 6–6.5 *M* LiBr. However, as the concentration is further increased the length remains essentially constant. The recrystallization demanded by the data of Fig. 3 does not occur even if the sample is held for a period of several weeks under the appropriate condition. X-Ray diffraction patterns substantiate the fact that the amorphous state is maintained. This seeming lack of reversibility from the amorphous to crystalline states as the LiBr concentration is increased is similar to that observed for a fixed salt concentration and a variation of the temperature. The same factors, therefore, appear to be operative. If, however, the concentration of LiBr is reduced, crystallization and re-elongation occur, the initial state is regenerated, and a cyclic reversible contractile system can be maintained. This latter process is similar to that recently reported by Haly and Snaith.[21]

Swelling.—The volume fraction v_2 of polymer in the swollen, completely amorphous horse hair is plotted in Fig. 7 as a function of the LiBr concentration of the supernatant phase. For each LiBr concentration, swelling equilibrium was established at temperatures just above the melting temperatures, so that comparison is not being made at the same temperature in this figure. The polymer concentration in the completely molten network increases as the LiBr concentration increases until a maximum at $v_2 = 0.5$ is reached at 6 *M* LiBr. As the concentration of LiBr is further increased to 12 *M*, a steady decrease in the polymer concentration (increase in swelling ratio) is observed, and v_2 decreases to approximately 0.4. Concomitantly, the melting temperatures are increasing.

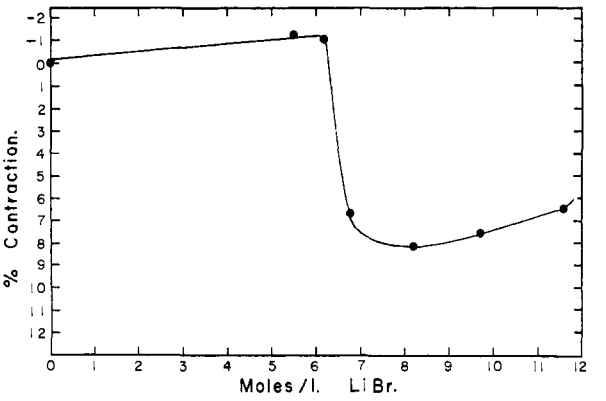


Fig. 6.—Isothermal length–concentration relations for Lincoln wool fiber immersed in aqueous LiBr solution at 24°. Measurements were conducted as the concentration of LiBr was increased.

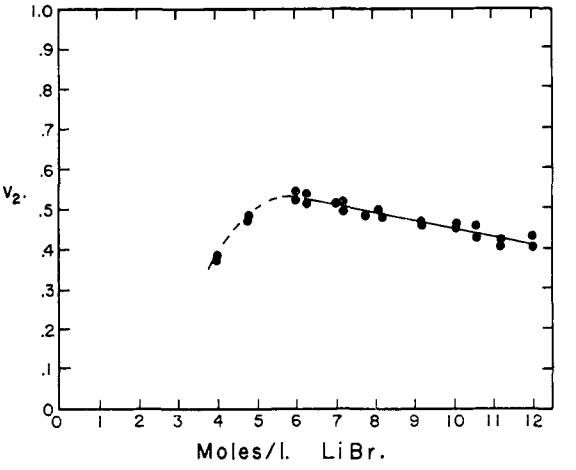


Fig. 7.—Equilibrium swelling of completely amorphous horsehair as a function of LiBr concentration of supernatant phase. Plot of volume fraction polymer in amorphous phase against LiBr concentration. Temperature in each case is two degrees above melting temperature.

Discussion

The Contractile Process.—The experimental observations summarized in Fig. 1 demonstrate that contraction and re-elongation occur concomitantly with a phase transition between two macroscopic phases. This conclusion is supported by the change observed in the X-ray diffraction patterns at various stages of the process. Moreover, it is shown from these latter observations that the native state, characterized by its oriented partial crystallinity, is transformed to the amorphous state during contraction. Thus the two phases involved can be clearly identified. It is also shown that the native state is regenerated during re-elongation. Changes in other physical properties have been reported as well. Thus on contraction there is a marked decrease in the birefringence of the fiber[16,23] and a change in the ultraviolet absorption spectrum.[24] In addition, a large decrease in the modulus of elasticity is observed after contraction, and the stress–temperature coefficient is in qualitative accord with the predications of rubber elasticity theory.[22] These observations are all consistent with a melting process and a significant change occurring in chain conformation. Therefore, the principles deduced and set forth from studies of other fibrous macromolecules[2–9] also govern contractility in the α-keratin–LiBr system. Shrinkage accompanies the melting process, re-elongation the crystallization process; and the temperature at which the transformation occurs can be deemed a melting temperature.

In the absence of any applied tensile force, the native state can be regenerated from the shrunken state merely by removing the transforming reagent. Thus, in analogy with the results reported for fibrous polyethylene,[4] the presence of intermolecular crosslinks, imposed on the initially oriented state, ensures oriented recrystallization from the melt even for this more complex polypeptide system.

When the shrunken keratin fibers are heated above the melting temperature, a further substantial contraction occurs and the contractile process becomes completely irreversible with respect to

(24) E. G. Bendit, *J. Textile Institute*, **51**, T544 (1960).

both dimensions and structure. Based upon the analysis of the results previously obtained for polyethylene, these observations would be consistent with the view that a severance of intermolecular crosslinks has now occurred. A diminution of the amorphous length would result, and oriented recrystallization without the application of an external tensile force would be prevented. Any intermolecular crosslinks reformed in the isotropic molten state for this labile system would not aid in regenerating the oriented crystalline state nor contribute to the isotropic length. Evidence has recently been offered[25] that subsequent to melting in LiBr, at temperatures above the melting temperature, interchange reactions occur between sulfhydril and disulfide groups. By this mechanism intermolecular crosslinks would be converted to intramolecular S–S linkages. According to the above hypothesis, therefore, a further contraction in the amorphous state would result, and oriented recrystallization would be prevented. Thus a simple explanation is offered of the irreversible contractility of α-keratin in aqueous LiBr consistent with the behavior of the simpler polymers. The introduction of complex structural models to explain the phenomenon does not appear warranted.[26,27]

In contrast to the simpler polymer systems, in which the crystal–liquid transition is usually accomplished by variations in temperature, melting in α-keratin is demonstrated to take place isothermally by appropriate alterations of the concentration of reactants in the supernatant liquid. The expected dimensional changes accompany the isothermal transition so that, in principle, systems of the type illustrated can be used as the working substance of an engine that isothermally converts chemical energy to mechanical work.

Although the fundamental molecular basis for contractility in this system can be established in terms of the general principles applicable to fibrous macromolecules, the nature of the chemical processes that control the phase transition must also be considered. In particular, attention must be given to the nature of the interactions of LiBr with the polypeptide chains in an effort to explain the unique melting temperature–LiBr concentration relation of Fig. 3.

Chemical Melting.—The ability of aqueous LiBr solutions to induce contractility in fibrous macromolecules is not limited to the α-keratins. At appropriate temperatures and concentrations melting and contractility also occur in feather keratin[28] (one of the naturally occurring keratins possessing a β crystallographic structure), collagen,[29] elastoidin[29] and glycerinated muscle fibers.[29] Crosslinked silk fibers have also been shown to undergo contraction in this medium.[30] Hence the action of LiBr is not dependent on any specific crystallographic structure of the polypeptide chains since the above proteins encompass all the known major crystalline categories. Moreover, since these proteins contain widely diverse amino acid compositions, the action of LiBr would not appear to be specific to a particular type residue. One can conclude, therefore, that aqueous LiBr solutions can cause the complete disruption of the ordered crystalline structure of the fibrous proteins.

This conclusion would appear to be in contradiction to that reached by Harrington and Schellman.[31] These investigators studied the optical rotatory power of dilute solutions of various globular proteins and concluded that the addition of LiBr to the system favored the stabilization of the native ordered helical structures. The basis for this deduction was that the optical rotation invariably became less levorotatory. When interpreted in terms of the known behavior of the simple homopolypeptides such as polyglutamic acid[32] and poly-γ-benzyl-L-glutamate,[33] this would indicate the formation of further α-helical structures. Consequently, the suggestion has been made that the action of LiBr is drastically different in dilute solution as compared to the more concentrated systems typical of the fibrous proteins.[21]

The interpretation of Harrington and Schellman has been seriously questioned recently as a result of a reanalysis of optical rotation data at one temperature.[34] It can also be shown,[35] by measuring the optical rotation as a function of temperature of dilute ribonuclease–LiBr solutions, that despite the specific optical rotation becoming more positive with the addition of LiBr, the transformation temperature is in fact progressively and substantially lowered. Caution must therefore be exercised in the interpretation of optical rotatory data in this instance. We can conclude, however, that LiBr acts as a universal transformer over the complete polymer concentration range in disordering the organized structure of proteins and polypeptides.

The unique nature of the T_m-LiBr concentration diagram of Fig. 3 receives confirmation from mechanical measurements.[36] The modulus of elasticity at room temperature of an α-keratin fiber immersed in aqueous LiBr solution decreases as the LiBr concentration is increased. A sharp minimum is observed at $7M$ LiBr, at which concentration the modulus has been decreased by a factor of three in comparison with that in pure water. As the LiBr concentration is increased beyond this point, the modulus increases, reaching values greater than that observed in the absence of LiBr. Structural changes within the constituent polypeptide chains are clearly indicated, consistent with the pseudo phase diagrams of Fig. 3, and the occurrence of an isothermal phase transition at the stipulated temperature.

(25) W. J. Crewther and L. M. Dowling, *Biochim. Biophys. Acta*, **46**, 605 (1961).
(26) M. Feughelman and A. R. Haly, *ibid.*, **32**, 596 (1959).
(27) M. Feughelman and A. R. Haly, *Kolloid. Z.*, **168**, 107 (1960).
(28) L. Mandelkern, J. C. Halpin and A. F. Diorio, in preparation.
(29) L. Mandelkern and W. T. Meyer, unpublished results.
(30) H. Zahn, H. Zuker, W. Ditscher, D. Wegerle and J. Meinhofer, *Chem. Ber.*, **89**, 407 (1956).
(31) W. F. Harrington and J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, **30**, 21 (1956).
(32) P. Doty, *Rev. Modern Physics*, **31**, 107 (1959).
(33) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **78**, 498 (1956); *ibid.*, **79**, 761 (1957).
(34) C. C. Bigelow and I. I. Geschurud, *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, **32**, 89 (1961).
(35) L. Mandelkern and D. E. Roberts, *J. Am. Chem. Soc.*, **83**, 4292 (1961).
(36) R. Steele, *J. Soc. Cosmetic Chemists*, **3**, 99 (1952).

Differential thermal analysis experiments on various undiluted α-keratin fibers give evidence for a melting transition in the range of 250–300°.[37] Extrapolation of the data of Fig. 3 is consistent with the above observation.

To treat formally the results as a problem in heterogeneous equilibrium, stipulation must be made of the phases and components that are involved. Since the polymeric fiber is immersed in a large excess of liquid, we are dealing with an open system. At the melting temperature three phases coexist in equilibrium.[3] These are the crystalline polymer phase, the amorphous polymer phase and the supernatant aqueous solution. The amorphous polymer phase is a swollen network at a given polymer concentration. Being an open system, the composition of this phase, particularly the polymer concentration, is not fixed. It can thus be altered as the equilibrium melting temperature changes. From studies of simpler polymer–diluent mixtures[38] and of polyethylene networks immersed in a large excess of solvent,[39] it has been found that the melting temperature in these cases depends solely on the polymer concentration in the molten phase. If this effect predominates in the system presently under consideration, then the change in the melting temperature with LiBr concentration should be reflected in a systematic variation of the swelling ratio of the amorphous phase with changing salt concentration.

According to the results of Fig. 7, the volume fraction of polymer in the molten phase increases slightly until the LiBr concentration corresponding to the minimum in the melting temperature of Fig. 3 is reached. With further increase in LiBr concentration a small but steady decrease in polymer concentration is observed. If the polymer concentration in the mixed phase were the sole determining factor, then an initial increase in melting temperature to a maximum, followed by a decrease, should be observed with increasing LiBr concentration. This is exactly opposite to what is in fact observed. Hence, though in principle the composition of the amorphous phase cannot be neglected in a formal analysis of problems of this type, there must be other factors which predominate in determining the melting temperature–LiBr concentration curve.

Since the melting temperature–LiBr concentration relation cannot be formally explained by the composition of the polymer phases, chemical interactions between the components must also be considered. One consequence of a chemical reaction will be to alter the value of the chemical potential of the polymer unit in either or both the crystalline and amorphous phases. This will by necessity shift the crystal–liquid equilibrium and affect the melting temperature. In principle, therefore, melting or crystallization can be brought about solely as a result of chemical processes involving the chain repeating unit. Though many types of reaction can be envisaged,[2,4] one of the simplest is that of complexing, wherein the resulting complex for steric reasons can no longer be accommodated in the crystal lattice. Melting must therefore ensue. For this situation, only the chemical potential of the polymer unit in the amorphous phase is affected and equation 1 is applicable.[40]

$$1/T_m - 1/T_m^0 = (R/\Delta H_u)(V_u/V_1)(v_1 - \chi_1 v_1^2) + RN_A/\Delta H_u \ln (1 + Ka) \quad (1)$$

In this equation T_m^0 is the melting point of the pure undiluted polymer and T_m the melting point of the system when the volume fraction of the polymer in the mixed phase is $1 - v_1$. ΔH_u is the heat of fusion per mole of structural units, N_A is the mole fraction of units capable of undergoing the complexing reaction, the quantity (V_u/V_1) represents the molar volume ratio of polymer unit to diluent molecules and χ_1 is the thermodynamic interaction parameter for the system. The equilibrium constant for the complexing reaction is given by K, and a is the activity of the complexing reagent. (As originally derived, equation 1 is applicable to the equilibrium for a two-component two-phase (crystalline and amorphous) closed system. It is easily extended and is of the same form for the open system of three phases presently under consideration when only one nonpolymeric component is present. In the present context, where the supernatant phase is multicomponent, the use of equation 1 involves the tacit assumption that the concentrations of the nonpolymeric constituents are the same in both the mixed and supernatant phases. The utilization of the more rigorous variant of this equation does not appear warranted in terms of the analysis that can be made with the present experimental data.)

According to this equation, the melting temperature (temperature of contraction) will be dependent primarily on the polymer concentration in the mixed phase, the activity of the monomeric reactant and the equilibrium constant for binding. (When the polymer concentration is fixed, as in a dilute protein solution for example, redefinition of T_m^0 to account for the effect of dilution on the melting temperature will allow for the consideration of only the last term in equation 1. However, this procedure, which is commonly adopted, could lead to an erroneous analysis if the aforementioned concentration conditions are not rigidly adhered to.) For a fixed polymer concentration the variation of the equilibrium temperature will be governed mainly by the term Ka. Thus, if K is large, only a small change in the activity of the reactant will suffice to shift the equilibrium. Conversely, even with a small K, if the activity of the reacting reagent were large, melting would occur.

It is well-known[34,36,41] that the activity of LiBr in aqueous solution is abnormally high (the activity of the water being necessarily low) and consequently marked effects could be expected, irrespective of the value of K. Thus the thesis that melting is caused by the simple binding of LiBr would appear

(37) R. F. Schwerner, Jr., and J. H. Dusenbury, *Textile Research Journal*, **30**, 800 (1960).

(38) L. Mandelkern, *Rubber Chemistry and Technology*, **32**, 1392 (1959).

(39) L. Mandelkern, D. E. Roberts, J. C. Halpin and F. P. Price, *J. Am. Chem. Soc.*, **82**, 46 (1960).

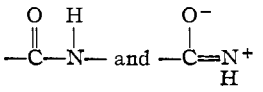
(40) P. J. Flory, *J. Cellular Comparative Physiol.*, **49**, Suppl. 1, 175 (1957).

(41) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworth, London, 1955.

to be particularly attractive in the present case. However, two fundamental interpretive difficulties present themselves. According to (1) a monotonic depression of the melting temperature should be observed with increasing activity of LiBr. Though this is observed for the initial addition of LiBr, the observation of a minimum in T_m and its subsequent increase would not be explicable on this basis. Moreover, according to this scheme the simple binding mechanism should be reversible, Consequently, recrystallization should occur merely on cooling the molten system. As has been indicated, this is not observed, even when allowance is made for retardation of the crystallization rate for kinetic reasons. Recrystallization is observed almost instantly, however, merely by decreasing the LiBr concentration of the supernatant phase. The nature of the phase diagram of Fig. 3 and the unattainability of the crystalline state merely by cooling the melt argue against the transformation being induced by a simple binding process.

Alternatively, the effect of the reactant in altering the structural characteristics of the chain repeating unit can be considered in seeking the basis for the chemical melting. If the units so altered by the interaction with LiBr are selected at random, the resulting polymer will have the melting or crystallization properties of a random copolymer,[42,43] the altered units acting as noncrystallizing coingredient. A systematic depression of the melting temperature would then be expected. As the concentration of the coingredient became sufficiently high (with increasing concentration of reactant) so that it became the major component, it could itself undergo crystallization, and the melting temperature-composition curve would change in order to conform to the melting of the new crystalline structure. The curves in Fig. 3 are reminiscent of the melting of the simpler type of random copolymers as, for example, copolyesters and copolyamides.[38] Thus, some measure of support is given to the hypothesis outlined. Moreover, it is known that the crystallization of copolymers from the melt is a relatively slow and protracted process, which would be consistent with the observations for t α-keratin system.

The plausibility of this explanation depends on the specification of the structural changes, so that a thermodynamic analysis of the melting point–composition relations can be made, as well as requiring the direct observation of the new crystal structure. The universal action of aqueous LiBr solutions in causing the disruption of the ordered structure in a variety of proteins and polypeptides focuses attention on the common structural feature, the peptide linkage. The C–N bond in the –C(O)–NH– group possesses a large amount of double bond character which results from the resonance between the structures[44]



The partial double bond character of the C–N linkage severely restricts rotations about this bond so that the peptide group assumes a coplanar configuration with the choice of either a *cis* or *trans* structure. For the ordered structures which proteins and polypeptides assume, the *trans* structure is presumed to be favored.[44,45] Thus, a disruption of the *trans* configuration in a succession of amino acid residues will render the crystalline state less stable. This could be accomplished by chemical interactions with the peptide group resulting in either a transitory or permanent loss of the double bond character of the C–N bond. In the former case, the net result would be the introduction of *cis* configurations into the chain, while the loss of resonance stability could lead to a single bonded C–N linkage, as long as the reactant were bound to the peptide group. In either case the ordered structure would be disrupted, the melting temperature lowered, and melting behavior resembling that of copolymers should be observed.

The work of Katchalski and collaborators[46–48] in studying the properties of dilute solutions of poly-L-proline give support to the general concept that interactions with the amide group (or in the case of proline polymers the imide group) can lead to structural transformations of an existing ordered form. In particular, mineral acids such as perchloric acid and neutral salts such as aqueous solutions of LiBr are particularly effective. In the case of perchloric acid the conclusion has been reached, based on dilute solution properties, particularly optical rotation, that *cis–trans* isomerism is affected. In other words, a transitory state allowing free rotation about the C–N bond is achieved so that an equilibrium distribution between *cis* and *trans* configurations is eventually reached. Although aqueous LiBr solutions affect the conformation of proline-type polymers, a detailed analysis of the resulting configurational changes has not as yet been made to ascertain as to whether or not the planar character of the peptide group is still maintained.

LiBr is also known to form complexes with simpler compounds containing some of the characteristic features of the peptide group.[49–51] For example, complexes of the general empirical formula $LiX \cdot n\ Co(NH_2)_2$ with $n = 1$, 2 or 3 have been established for aqueous solutions of LiBr or LiCl with urea.[49,50] In addition, complex formation of this same general formula between LiBr and N-methylacetamide or dimethyl acetamide has also been demonstrated.[51] Contrary explanations of the nature of the complex have been offered. In one case the bonding has been attributed to an ion–dipole attraction between the metal ion and the nitrogen atom of urea[49]; in the

(42) P. J. Flory, *J. Chem. Phys.*, **17**, 223 (1949).
(43) P. J. Flory, *Trans. Faraday Soc.*, **51**, 848 (1955).
(44) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci. (U. S.)*, **37**, 205 (1951).
(45) S. Mizushima, *Advances in Protein Chem.*, **9**, 299 (1954).
(46) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela and G. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960).
(47) W. F. Harrington and M. Sela, *Biochim. Biophys. Acta*, **27**, 24 (1956).
(48) I. Z. Steinberg, A. Berger and G. Katchalski, *ibid.*, **28**, 647 (1958).
(49) W. G. McGavock, J. M. Bryant and W. W. Wendlant, *Science*, **123**, 897 (1956).
(50) I. I. Geschwind, *Nature*, **187**, 324 (1960).
(51) J. Bello and H. R. Bello, *ibid.*, **190**, 440 (1961).

other, bonding between the carbonyl oxygen with either the Li ion or the water of the Li hydration shell has been postulated.[41] It should therefore be expected that a similar type of complexing will occur with the peptide group along a polymer chain. Clarification of the details of the binding will allow an understanding of the resulting bond structure. The possibility of the formation of cyclic structures between successive pairs of amino acid residues resulting from Li ion binding can also be given consideration.

Thus, a basis exists for the expectation of structural alteration of the repeating units as a result of interactions with aqueous LiBr solutions. When present in sufficient concentration, the newly formed structure could crystallize in a new type lattice. In this connection it is important to note that polyglycine can exist in a crystallographic structure, where X-ray diffraction and infrared studies show that the conformation of the polypeptide chain differs significantly from either that of the α-helix or the normal β forms.[52] Pertinent to the present study is the fact that one of the ways in which this form of polyglycine is prepared is by precipitation of the polymer with water from an aqueous LiBr solution, polyglycine being normally insoluble in pure water. For the case of α-keratin immersed in LiBr solutions, Hambraeus and Steele[17] claim that at room temperature for solutions of $5M$ concentration and above, a new X-ray diagram of the fiber was obtained. We have not, however, been able to unequivocally substantiate the latter report. Thus, some evidence exists, as yet inconclusive, which supports the concept of the formation of a new crystallographic structure and which is consistent with the postulated mechanism of chemical melting.

It is clear, however, that a quantitative understanding of the chemical processes involved in the melting is incomplete. Despite this shortcoming, it has been demonstrated that the crystal–liquid transformation in the fibrous proteins can be controlled by chemical reactions. The distinction made between the contractile process and the reactions which induce melting thus appear to be justified. Contractility in the fibrous proteins by this general mechanism, resulting from interaction with other species, therefore can be expected.

(52) C. H. Bamford, L. Brown, E. M. Cant, A. Elliot, W. E. Hanky and B. R. Malcolm, *Nature*, **176**, 396 (1955).

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Initiation of Methyl Methacrylate by Aromatic Radical-anions

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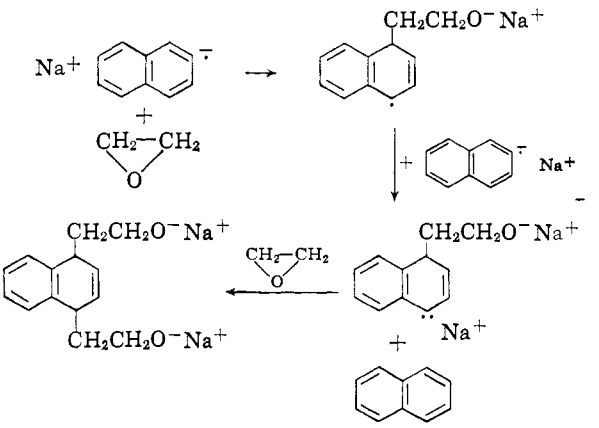
Methyl methacrylate has been polymerized by a variety of aromatic hydrocarbon radical-ions. Some initiators were found to induce polymerization by electron transfer, others by bond formation with the methacrylate. The mechanism of initiation appears to be governed to a large extent by the orbital energy of the initiator and to be more or less independent of the counterion.

Introduction

The importance of radical-ions as initiators of anionic polymerization has been recognized since the realization by Szwarc[1,2] that the initiation of styrene by sodium naphthalene proceeds by an electron transfer mechanism regenerating free naphthalene. Sodium naphthalene also initiates α-methyl styrene by electron transfer and these monomers have also been polymerized by other radical-anions.

It has recently been demonstrated by Tobolsky, Rembaum and Eisenberg[3] that in the polymerization of α-methyl styrene by monosodium diphenyl acetylene a bond is formed between the initiating molecule and the polymer. The mechanism of initiation in this particular case is obviously quite different from a mere electron transfer.

Bond formation in the initiation of other monomers by radical-ions is not unknown—this is the manner in which sodium naphthalene for example opens siloxane rings.[4] Equally striking is the mechanism by means of which the same complex initiates polymerization in ethylene oxide.[4,5] The reaction schemes for these two cases are similar and are represented by



and the 1,2- addition product.

These examples together are sufficient indication that the mode of initiation by a radical-ion depends on both initiator and monomer. It cannot

(1) M. Szwarc, *Nature*, **178**, 1168 (1956).

(2) M. Szwarc, M. Levy and R. Milkovich, *J. Am. Chem. Soc.*, **78**, 2656 (1956).

(3) A. V. Tobolsky, A. Rembaum and A. Eisenberg, *J. Polymer Sci.*, **45**, 347 (1960).

(4) M. Morton, A. Rembaum and E. E. Bostick, *ibid.*, **32**, 530 (1958).

(5) D. H. Richards and M. Szwarc, *Trans. Faraday Soc.*, **55**, 1644 (1959).