$-\log C_A$, according to equation 1, yields a straight line. The value of p is calculated from the slope of the line to be 3.04, so that the highest order complex is Cd(glycinate)$_3$. The electrode reaction is reversible, and the average value of $\log k_f$ is calculated by means of equation 1 to be 9.94.

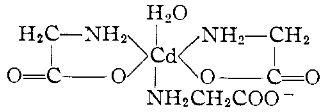
TABLE V

POLAROGRAPHIC RESULTS FOR CADMIUM–GLYCINE COMPLEX

Each solution contains 5×10^{-4} M $Cd(NO_3)_2$, glycine half neutralized with KOH, KNO_3 to keep $\mu = 0.15$, 25°.

$C_{glycinate}$	$-E_{1/2}$	$-\log C_A$	$\log k_f$ ($k_f = k_1k_2k_3$)
0.000	0.583		
.020	.726	1.699	9.93
.030	.743	1.523	9.96
.040	.753	1.398	9.93
.050	.762	1.301	9.97
.060	.769	1.222	9.93
		Av.	9.94

The value of $\log k_1k_2$ for the cadmium–glycine complex is reported as 8.1,[18] obtained by the pH method, so that $\log k_3$ becomes 1.8. It is obvious from these values that the tendency to form the highest order complex is extremely weak, and a possible structure for the complex is therefore



in which two glycinate ions are chelated but the third is not chelated. Douglas, Laitinen and Bailar[20] note that monodentate groups usually give a coördination number of four for the cadmium ion, but the coördination number of six seems to be more common for polydentate groups. In the structure postulated above, the coördination number is six, with one of the positions occupied by the solvent.

We have also investigated polarographically the cadmium complexes of leucine, isoleucine and norleucine and found that the highest order complex in each case is CdA_3^-, where A is the amino acid ion. These complexes are less stable than the glycinate complex, presumably because of steric hindrance. However, since the polarographic waves for these complexes are not strictly reversible, no quantitative values for the formation constants were calculated.

Acknowledgment.—The authors are indebted to Professor Henry S. Frank, University of Pittsburgh, for suggesting the entropy studies and for helpful discussions, to Mr. R. A. Manning for carrying out some of the experiments. This research was sponsored by the National Science Foundation.

(20) B. E. Douglas, H. A. Laitinen and J. C. Bailar, Jr., THIS JOURNAL, **72**, 2484 (1950).

PITTSBURGH, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

Theory of Elastic Mechanisms in Fibrous Proteins

BY PAUL J. FLORY

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This paper is concerned with problems relating to dimensional changes in systems comprising long chain molecules so constituted as to occur, under suitable conditions, in the state of high order characteristic of native fibrous proteins. Particular attention is given to the process of disordering of the molecular chains, which is treated as a reversible phase change between crystalline and amorphous states. Thermodynamic relations between the force f, the temperature T and the length L are developed for fibers of uniform constitution, for fibers whose properties vary axially, and for systems containing a second component (diluent). The hitherto unexplained thermoelastic characteristics of typical fibrous proteins are readily accounted for by the hypothesis that decrease in length signifies melting of crystalline regions. Network structures formed by cross-linking polymeric chains in the oriented (crystalline) state are considered from the point of view of the statistical mechanical theory of elasticity of polymeric systems. Significant differences as compared to networks formed by cross-linking disordered chains in the usual manner are noted: the length to which the cross-linked fiber will shrink on melting in the absence of a force is predicted to increase approximately as the square root of the degree of cross-linking; at extensions substantially greater than this relaxed length, the force of retraction should be independent of the degree of cross-linking. By combining these results of the statistical theory of elasticity with the thermodynamic relationship $\partial(f/T)/\partial(1/T) = \Delta H/\Delta L$, where ΔH and ΔL are the latent changes in heat and length accompanying melting, the force, length and temperature may be related over ranges which include the phase change. The elevation of the melting point which should result from cross-linking in the ordered state is treated theoretically.

Introduction

The physical structures of fibrous proteins have been extensively investigated by the techniques of X-ray diffraction, electron microscopy, optical birefringence and polarized infrared absorption. The principal features of the arrangement of the polypeptide chains consequently are reasonably well established, although certain details remain obscure. A state of molecular organization appropriately designated as crystalline is the dominant structural characteristic brought to light by these studies.

In contrast to the comparatively advanced state of knowledge concerning the structures of fibrous proteins, little progress has been achieved toward an understanding of the physical properties of proteins. In particular, the mechanism by which protein fibers undergo major changes in length has not been satisfactorily explained. Analogies to the deformation of rubber have been suggested,[1,2] but with no more than limited success. This is not

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(1) K. H. Meyer, *Proc. Roy. Soc.* (*London*), **B139**, 498 (1952); K. H. Meyer, A. J. A. van der Wyk, W. Gonon and C. Haselbach, *Trans. Faraday Soc.*, **48**, 669 (1952).

(2) E. Guth, *Ann. N. Y. Acad. Sci.*, **47**, 715 (1947).

surprising in view of the prevalence of an ordered crystalline arrangement of the polypeptide chains in proteins. Crystallinity, and processes of melting and crystallization, must markedly influence these properties, and especially those involved in transformations such as denaturation, swelling and deformation. The fact that present structural information has not been brought to bear on these phenomena, may be attributed to the lack of suitable theories relating to the mechanical properties of crystalline, and especially semi-crystalline, polymers.[3]

From studies of the thermoelastic behavior of collagen, elastin and muscle, Wöhlisch[4] pioneered the concept of melting during shrinkage, and crystallization on stretching. A. V. Hill[5] and E. Ernst and co-workers[6] reached similar conclusions from studies of the thermal and volume changes accompanying contraction and stretching of muscle fibers. These authors, however, have been disinclined to regard melting and recrystallization as the processes primarily responsible for the dimensional changes in muscle. Ernst[6] states emphatically that these processes are merely incidental to the deformation mechanism, and not the basis for the mechanism itself. Other authors do not consider that melting and crystallization have any special relevance to the problem.

The situation is somewhat clearer in the case of collagen. The shrinkage of collagen is essentially a melting phenomenon,[4,7,8] and it has been shown recently that the process may properly be regarded as a phase transition.[8] Wiederhorn and co-workers[7] attempted to treat the influence of the stress developed in collagen fibers held at fixed length on the melting point according to an approximate theory[9] of crystallization in stretched rubber networks—a theory which is inappropriate for a highly oriented fiber. In spite of records of these views in the literature, it would be erroneous to conclude that designation of the shrinkage of collagen as a melting process has gained widespread acceptance at the present time.

It is a premise of the present paper that dimensional changes in fibrous proteins are integrally related to changes in crystallinity.[10] The force of retraction manifested under conditions favoring contraction is considered to originate in a shift in the crystalline–amorphous equilibrium. Polypeptide chains freed by melting from the ordered and relatively extended configuration characteristic of the crystalline state adopt random configurations, except as restricted by the applied stress. For any stress likely to be borne by amorphous chains, the length of the random chain molecule projected on the fiber axis will be considerably less than its length in the crystalline state. Thus, melting must bring about contraction.

The foregoing viewpoint is manifestly plausible in the light of knowledge of the behavior of long chain molecules in general. It is substantiated moreover by various observations on processes of dimensional change in fibrous proteins, as has been discussed elsewhere.[3] We shall be concerned therefore with the formulation of suitable relationships by means of which to proceed with application of the stated hypothesis to deformation phenomena. In attempting to achieve this objective we find that current theories of elastic deformation and crystallization, developed for other, less organized, linear polymers, are not immediately applicable. It is necessary therefore to consider carefully the morphological features which distinguish the structures of fibrous proteins from other polymers.

The presence of a well developed fibrillar structure is of foremost importance in this connection. Long fibrils, or filaments, with diameters of 80 to 200 Å., or only about one order of magnitude greater than the diameters of the individual molecular chains, can be distinguished in silk fibroin, myosin, collagen and elastin. Near perfect axial alignment of chain molecules prevails within the fibrils according to wide angle X-ray diffraction. Fibrillar order resembling that in proteins is sometimes observed in highly oriented synthetic polymers.[11] A considerable proportion of amorphous material usually co-exists with these oriented fibrils, hence it seems justifiable to make the generalization that fibrillar organization in proteins exceeds considerably that to be found in mechanically oriented synthetic polymers.

The prevalence of *intra*molecularly hydrogen bonded chain configurations in most native fibrous proteins (β-forms excepted) seems well established. Those of the k-m-e-f group probably occur in the α-helical form proposed by Pauling and Corey.[12] The "protofibrillar" element of collagen probably consists of three chains wound together helically,[13] and not of a single chain molecule. In both instances the stability of the structure is derived from *internal* hydrogen bonds; such bonding as may occur between adjacent fibrillar elements must be very weak. Experiments on dilute solutions of collagen[14–16] and of synthetic polypeptides[17] indicate that individual protofibrillar elements, comprising either a single molecule or several (three) of them wound together, are capable of stable existence independently of one another.[18] Collagen[14,15]

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

(4) E. Wöhlisch, *Biochem. Z.*, **247**, 329 (1932); *Kolloid Z.*, **89**, 239 (1939); *Naturw.*, **28**, 305, 326 (1940); E. Wöhlisch, H. Weitnauer, W. Grüning and R. Rohrbach, *Kolloid Z.*, **104**, 14 (1943).

(5) A. V. Hill, *Proc. Roy. Soc.* (*London*), **B139**, 464 (1952).

(6) E. Ernst, J. Balog, J. Tigyi and A. Sebes, *Acta Physiol.*, **2**, 243 (1951); E. Ernst, G. Lodanyi and J. Tigyi, *ibid.*, **2**, 271 (1951).

(7) B. A. Wright and N. M. Wiederhorn, *J. Polymer Sci.*, **7**, 105 (1951); N. M. Wiederhorn and G. V. Reardon, *ibid.*, **9**, 315 (1952).

(8) R. R. Garrett and P. J. Flory, *Nature*, **177**, 176 (1956).

(9) P. J. Flory, *J. Chem. Phys.*, **15**, 397 (1947).

(10) See also reference 3.

(11) K. Hess, "La Recerca Scientifica," Supplemento 1955 . Vol. **25**

(12) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 272 (1951).

(13) G. N. Ramachandran and G. Kartha, *Nature*, **176**, 593 (1955); A. Rich and F. H. C. Crick, *ibid.*, **176**, 915 (1955); P. M. Cowan, S. McGavin and A. C. T. North, *ibid.*, **176**, 1062 (1955).

(14) M. B. Mathews, E. Kulonen and A. Dorfman, *Arch. Biochem. Biophys.*, **52**, 247 (1954).

(15) P. M. Gallop, *ibid.*, **54**, 486 (1955).

(16) H. Boedtker and P. Doty, THIS JOURNAL, **77**, 248 (1955).

(17) E. R. Blout and M. Idelson, *ibid.*, **78**, 497 (1956); P. Doty and J. T. Yang, *ibid.*, **78**, 498 (1956); P. Doty, J. H. Bradbury and A. M. Holtzer, *ibid.*, **78**, 947 (1956).

(18) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Serie Chim.*, **29**, 223 (1955).

and gelatin[19] molecules, even in dilute solution, appear to transform from the random-coil form present at elevated temperatures to the protofibrillar form when cooled.

In the light of these observations it seems plausible to assume that fibrogenesis *in vitro* is preceded by formation of protofibrillar elements.[20] These are stabilized by intramolecular hydrogen bonds. They subsequently aggregate from the dilute solution in which they are initially dispersed. The resulting aggregates assume fibrillar form as an obvious consequence of geometrical requirements. It is possible to show, moreover, that the intramolecular attractive energy required for aggregation of such highly asymmetric particles is very small.[21] The formation of protofibrils prior to aggregation and the occurrence of aggregation in a medium of low concentration are factors favoring a high degree of parallel order such as is observed in typical fibrous proteins.

Formation of fibers from synthetic polymers contrasts markedly with the process of fibrogenesis envisioned above. In the first place, the crystallinity encountered in synthetic polymers ordinarily derives much of its stability from *interchain* forces. No stable protofibrillar precursor is therefore to be expected; even if such elements were present, the high viscosity of the medium—melt or fairly concentrated solution—would hamper systematic aggregation of them. The dimensions of the crystallites which form are consequently much smaller than the length of a molecule. (The degree of crystallinity usually is comparatively low, *i.e.*, around 50% or less.) Macroscopic fiber orientation is not spontaneously generated; it is developed only by externally induced orientation of the crystalline regions.

If cross linkages[22] occur in the fibrous proteins, they may be presumed to be formed subsequent to generation of the fiber. They must therefore be superimposed on, or in, a *previously ordered structure.* It is essential to recognize how this circumstance contrasts with the cross-linking of a polymer as ordinarily carried out in the amorphous state in which the molecular chains are disordered and randomly coiled. Theories previously developed for cross-linked polymers may not, therefore, be directly applicable to fibrous proteins (see Part II).

An attempt to treat the elastic properties of ordered structures such as seem to be involved in fibrous proteins will be presented in this paper. Part I is concerned with the formulation of suitable thermodynamic relations for application to ordered fibrils in which the crystal–amorphous phase transition is coupled with dimensional changes. The theory of elasticity of a system for molecules initially in parallel array but subsequently converted to the amorphous state is presented in Part II. The necessary modification of the elastic equation of state (*i.e.*, the force–length–temperature relation) by incidence of crystallization is developed in Part III.

Glossary of Principal Notations

L	length of sample
L^a, S^a, etc.	length, entropy, etc., of sample when totally amorphous
L^c, S^c, etc.	length, entropy, etc., of sample when totally crystalline
L_i	length of sample in isotropic (amorphous) state
α	relative length L/L_i
ΔH, ΔS, ΔL	total changes in heat content, entropy and length upon melting crystalline sample
Δs	entropy change per repeating unit
$\Delta h'$	heat of fusion per mole of *statistical segments*
λ	fraction of sample in the amorphous state
f	force of retraction
α_T^a, α_T^c	linear thermal expansion coefficients, amorphous and crystalline
N	no. of units in *one* molecular chain extending the length of the fiber (Part I)
N_s	total no. of *statistical segments* in the entire sample (Part III)
ν	total no. of chains in the network structure
σ, σ_j	no. of chains, or chain vectors, in a cross-section
$\overline{r^2}$, $\overline{r_0^2}$	mean square end-to-end length of a chain, and the same quantity for unconstrained chains
x, y, z	components of the end-to-end chain vector r
n', l'	no. of *statistical segments* per chain, and length of each
$<\alpha>$	linear dilation factor $(\overline{v^2}/\overline{v_0^2})^{1/2}$
V	total volume
v	molar volume
$<\alpha>_0$, V_0, L_{i0}	values of corresponding quantities in absence of diluent
T_m, T_m^i, T_m^c	melting temp., melting temp. at $f = 0$, ($L = L_i$), and melting temp. for $L = L^c$

Part I. Thermodynamics of Deformation of Semicrystalline Fibers

The differential of the Gibbs free energy F of a fiber subject to a uniform tensile force f acting in the direction of the fiber length L may be written

$$dF = -S\,dT + V\,dP + f dL \qquad (1)$$

where S and V are the entropy and volume of the fiber, and T and P are the temperature and pressure. For equilibrium to prevail at constant T, P and L, the free energy F must be a minimum with respect to all permissible displacements of the system, *i.e.*

$$\delta F \geqq 0 \qquad (2)$$

for every virtual displacement consistent with the restraints on the system. In particular, the free energy must be a minimum with respect to changes in the fraction $1 - \lambda$ of the fiber which is crystalline, *i.e.*

$$(\partial F/\partial\lambda)_{P,T,L} = 0 \qquad (3)$$

The function $F - fL$ may be used to advantage when P, T and f are chosen as independent variables. Thus

$$d(F - fL) = -S\,dT + V\,dP - L df \qquad (4)$$

and the necessary and sufficient condition for equilib-

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(19) C. Robinson, "Nature and Structure of Collagen," Edited by J. T. Randall, Butterworths, London, 1953, p. 96; C. Cohen, *Nature*, **175**, 129 (1955).

(20) Protofibrils of collagen are discussed by R. S. Bear, "Advances in Protein Chemistry," Vol. VII, 1952, p. 69. J. Gross, J. H. Highberger and F. O. Schmitt, *Proc. Natl. Acad. Sci.*, **40**, 679 (1954), refer to a tropocollagen particle in this connection.

(21) P. J. Flory, *Proc. Roy. Soc. (London)*, **A234**, 73 (1956).

(22) The term cross linkage as here used includes only those bonds between adjacent molecular chains which are of sufficient permanence to survive melting and deformation, or swelling, without rupture or exchange with other cross-linking sites. Intermolecular hydrogen bonds would not ordinarily be included as cross linkages as here defined.

rium at constant P, T and f is expressed by

$$\delta(F - fL) \geqq 0 \qquad (5)$$

with respect to all permissible variations of the system. For systems in which tensile deformation is involved, the function $F - fL$ occupies a role analogous to that of the free energy F for systems wherein isotropic changes in volume are the only dimensional changes permitted.

Uniform Fibers.—We first consider fibers which are homogeneous and uniform in chemical composition, structure and cross-section throughout, apart from such differences in cross-section as may arise from differences in crystallinity along the length of the fiber. As we shall be primarily concerned with fibers of very small cross-section (*i.e.*, fibrils), it will be justified to consider that all polymer chains within any given cross-section occur either in the crystalline or in the amorphous state. The fraction λ of the fiber which is amorphous may therefore be identified with the ratio of the length of that portion of the fiber which is amorphous to the length of the entire fiber when totally amorphous at the same P, T and f.

Under the foregoing conditions each element of the fiber, or fibril, which is amorphous will be characterized by the same intensive variables, *e.g.*, specific volume, specific entropy and specific length; the same applies to the crystalline regions. We may therefore formulate the total free energy as

$$F = \lambda F^{\mathrm{a}} + (1 - \lambda)F^{\mathrm{c}} \qquad (6)$$

where F^{a} and F^{c} are the free energies of the fiber when totally amorphous and totally crystalline, respectively, under the given conditions specified by the values of P, T and f. Other extensive properties (S, L, H, etc.) may be expressed in a corresponding manner. In order for crystalline and amorphous regions to coexist (*i.e.*, $0 < \lambda < 1$) at equilibrium the necessary and sufficient condition (5) requires that $[\partial(F - fL)/\partial\lambda]_{P,T,f} = 0$, and hence according to (6) that

$$(F^{\mathrm{a}} - fL^{\mathrm{a}}) = (F^{\mathrm{c}} - fL^{\mathrm{c}}) \qquad (7)$$

The change in the equilibrium retractive force f with temperature when amorphous and crystalline regions coexist may be transformed as

$$\begin{aligned}(\partial f/\partial T)_{P,\,\mathrm{eq}} &\equiv (\partial f/\partial T)_{P,\,\Delta(F-fL)=0} \\ &= -[\partial\Delta(F - fL)/\partial T]_{P,\,f}\,[\partial\Delta(F - fL)/\partial f]^{-1}{}_{P,T}\end{aligned}$$

where the subscript eq stands for amorphous–crystalline equilibrium, and Δ signifies the difference between the values for the subsequently designated quantity in the amorphous and crystalline states at the same P, T and f. Upon evaluating the derivatives through use of eq. 4, we have

$$(\partial f/\partial T)_P = -\Delta S/\Delta L \qquad (8)$$

where ΔS and ΔL are the changes in entropy and length on fusion of the entire fiber at constant T, P and f. As this equation holds for any value of λ (for the one component system under consideration), provided only that $0 < \lambda < 1$, it must hold also for any value of L so chosen as to be consistent with the coexistence of amorphous and crystalline polymer. Hence, we could have written

$$(\partial f/\partial T)_{P,\lambda} \equiv (\partial f/\partial T)_{P,L} = -\Delta S/\Delta L \qquad (8')$$

which may be deduced alternatively from the well known thermoelastic relation

$$(\partial f/\partial T)_{P,L} = -(\partial S/\partial L)_{T,P} \qquad (9)$$

Equation 9 follows directly from eq. 1.

The entropy change on melting the crystalline fiber at constant P, T and f is given by

$$\begin{aligned}\Delta S = Q_{\mathrm{rev}}/T &= (\Delta E + P\Delta V - f\Delta L)/T \\ &= (\Delta H - f\Delta L)/T\end{aligned}$$

where Q_{rev} is the heat absorbed in reversible melting, and ΔE and ΔH are the concurrent changes in internal energy and enthalpy, respectively. Substitution in eq. 8 yields the relationship previously

$$(\partial f/\partial T)_P = f/T - \Delta H/T\Delta L \qquad (10)$$

derived by Gee[23] using a similar method.[24,25] Gee's equation is expressed in more compact form as

$$[\partial(f/T)/\partial(1/T)]_P = \Delta H/\Delta L \qquad (11)$$

Ordinarily $\Delta L < 0$ whereas $\Delta H > 0$; hence, f/T will increase with T when the fiber contains both crystalline and amorphous zones.

Equations 10 and 11 are obvious analogs of the Clapeyron equation. In the former equations $-f$ and L occupy roles corresponding to those of P and V in the latter. The temperature T may be regarded as the melting temperature T_{m} under a force f and a pressure P. It is implicit in the foregoing formulation for a system of one component, with uniform properties throughout as specified above, that the equilibrium force f must be independent of the length over the two-phase range at constant T and P, in further analogy to the relation of P to V in the two-phase region of an ordinary one-component system at constant temperature.

Systems Containing a Diluent.—If the fiber, or fibril, comprises more than one component, *e.g.*, if it contains a diluent in addition to the polymer, equilibrium between crystalline and amorphous phases requires that $F - fL$ shall retain its minimum value when δn_{i} moles (or moles of structural units) of component i are transferred from the crystalline to the amorphous phase at constant P, T and f, and hence that

$$\delta(F - fL) = \delta n_{\mathrm{i}}\mu_{\mathrm{i}}{}^{\mathrm{a}} - \delta n_{\mathrm{i}}\mu_{\mathrm{i}}{}^{\mathrm{c}} = 0$$

or

$$\mu_{\mathrm{i}}{}^{\mathrm{a}} = \mu_{\mathrm{i}}{}^{\mathrm{c}} \qquad (12)$$

where $\mu_{\mathrm{i}}{}^{\mathrm{a}}$ and $\mu_{\mathrm{i}}{}^{\mathrm{c}}$ are the chemical potentials of component i in the amorphous and crystalline regions, respectively, *i.e.*

$$\mu_{\mathrm{i}}{}^{\mathrm{a}} = (\partial F^{\mathrm{a}}/\partial n_{\mathrm{i}})_{P,T,L,n_{j \neq \mathrm{i}}} = [\partial(F^{\mathrm{a}} - fL^{\mathrm{a}})/\partial n_{\mathrm{i}}]_{P,\,T,\,f,\,n_{j \neq \mathrm{i}}} \qquad (13)$$

with analogous definition of $\mu_{\mathrm{i}}{}^{\mathrm{c}}$. Here F^{a} and F^{c} may be regarded as the free energies of the totally amorphous and totally crystalline fiber, respectively, at the same compositions as the amorphous and crystalline regions in the partially crystalline fiber, and at the same P, T and f.

If the system consists only of the two phases crystalline and amorphous (*i.e.*, if there is no super-

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(23) G. Gee, *Quart. Rev.*, **1**, 265 (1947).

(24) G. Gee, private communication.

(25) E. Wöhlisch, *Naturw.*, **28**, 305 (1940), has given the incorrect relationship

$$(\partial f/\partial T)_{P,L} = -(\partial\Delta H/\partial\Delta L)_{P,T}/T$$

(in present notation) for the force–temperature coefficient, apparently obtained by substituting $\Delta S = \Delta H/T$, instead of $\Delta S = (\Delta H - f\Delta L)/T$, in eq. 9.

natant solvent phase), then the necessary and sufficient conditions for equilibrium are expressed by a set of equations like (12) for each component. We shall be concerned primarily with the case of a single polymer constituent in the presence of a diluent (*e.g.*, water), and it will be assumed, as an approximation, that the diluent occurs only in the amorphous phase, the crystalline phase being unaffected by the diluent. The more general conditions for phase equilibrium then reduce to the single requirement

$$\mu_2{}^{\mathrm{a}} = \mu_2{}^{\mathrm{c}} \qquad (14)$$

where the subscript 2 signifies polymer; or

$$\mu_{\mathrm{u}}{}^{\mathrm{a}} = \mu_{\mathrm{u}}{}^{\mathrm{c}} \qquad (14')$$

where μ_{u} is the chemical potential per mole of structural units. While the foregoing assumption may not be fully justified for fibrous proteins, the complications necessitated by incorporation of the additional condition $\mu_1{}^{\mathrm{a}} = \mu_1{}^{\mathrm{c}}$ (where the subscript 1 refers to the diluent) seem not to be justified by the greater rigor and generality thus made possible.

Proceeding as in the preceding section, we find

$$(\partial f/\partial T)_{P,n} = -[\partial(\mu_{\mathrm{u}}{}^{\mathrm{a}} - \mu_{\mathrm{u}}{}^{\mathrm{c}})/\partial T]_{P,f,n}/ [\partial(\mu_{\mathrm{u}}{}^{\mathrm{a}} - \mu_{\mathrm{u}}{}^{\mathrm{c}})/\partial f]_{P,T,n}$$

where the subscript n denotes constancy of composition in the amorphous phase; the crystalline phase is assumed pure and therefore of fixed composition. The numerator may be evaluated as the difference between the partial molar entropy in the amorphous phase and the molar entropy in the crystalline phase; the denominator must then be expressed as the difference between a partial molar length (amorphous) and a molar length (crystalline). The awkwardness of the latter quantities may be avoided through multiplication of both numerator and denominator by the total number of structural units in the fiber. The result can then be expressed as

$$(\partial f/\partial T)_{P,n} = -\Delta\bar{S}/\Delta\bar{L} \qquad (15)$$

where

$$\Delta\bar{S} = \bar{S}^{\mathrm{a}} - S^{\mathrm{c}}$$
$$\Delta\bar{L} = \bar{L}^{\mathrm{a}} - L^{\mathrm{c}}$$

Here $\bar{S}^{\mathrm{a}}$ and $\bar{L}^{\mathrm{a}}$ are partial derivatives of the total entropy and length, respectively, of the amorphous phase with respect to the fraction (λ) of the polymer in that phase. The crystalline phase being assumed pure, S^{c} and L^{c} are the entropy and length of the totally crystalline fiber at the same T, P and f, in accordance with notation employed in the preceding section.

It follows also that

$$[\partial(f/T)/\partial(1/T)]_{P,n} = \Delta\bar{H}/\Delta\bar{L} \qquad (16)$$

where $\Delta\bar{H}$ is similarly defined; it comprises the heat of fusion ΔH and the *differential* heat of dilution.

Two cases will be considered. In the first the total quantity of solvent associated with the polymer is fixed; *i.e.*, the fiber and contents operate as a closed system. The concentration of polymer in the amorphous phase (*i.e.*, solution) must consequently increase as melting progresses. Constancy of composition of the amorphous phase then implies constancy of λ and we therefore re-express eq. 15 and 16 as

$$(\partial f/\partial T)_{P,\lambda} = -\Delta\bar{S}/\Delta\bar{L} \qquad (15')$$
$$[\partial(f/T)/\partial(1/T)]_{P,\lambda} = \Delta\bar{H}/\Delta\bar{L} \qquad (16')$$

The relative partial molar quantities $\Delta\bar{S}$, $\Delta\bar{L}$ and $\Delta\bar{H}$ will vary with composition, hence the force–temperature derivative will depend on λ. It is to be noted that under these circumstances $[\partial(f/T)/\partial(1/T)]_{P,\lambda}$ is not to be identified exactly with $[\partial(f/T)/\partial(1/T)]_{P,L}$, for

$$[\partial(f/T)/\partial(1/T)]_{P,\lambda} = [\partial(f/T)/\partial(1/T)]_{P,L} + (1/T)(\partial f/\partial L)_{P,T}\,[\partial L/\partial(1/T)]_{P,\lambda} \qquad (17)$$

The force being dependent upon the length, the second term on the right does not equate to zero.

In the second case to be considered the amorphous portion of the fiber is in equilibrium with an excess of diluent present in a separate (supernatant) phase. Fulfillment of equilibrium then requires, in addition to the condition expressed by eq. 14, that

$$\mu_1{}^{\mathrm{a}} = \mu_1{}^{\mathrm{s}}$$

where $\mu_1{}^{\mathrm{s}}$ is the chemical potential of the diluent in this phase. The mixed phase is in equilibrium with two pure phases, and the system is univariant at constant pressure. The degree of swelling of the amorphous (mixed) phase may be considered to be controlled by cross linkages or other factors whose effect is constant, in conformity with the assumption of uniformity of the fiber. The composition of the amorphous phase will then be independent of λ. Since the system is univariant, the force will be uniquely determined by the temperature (at constant pressure), hence total melting should occur at constant force as in the case of the one-component system considered in the preceding section.[26] It follows that

$$(\partial f/\partial L)_{P,T} = 0$$

for $0 < \lambda < 1$, and hence that

$$[\partial(f/T)/\partial(1/T)]_{P;\lambda} = [\partial(f/T)/\partial(1/T)]_{P,L}$$

Under the foregoing conditions, eq. 15 and 16 require revision as

$$(\partial f/\partial T)_{P,\lambda} \equiv (\partial f/\partial T)_{P,L} \equiv -\Delta\bar{\bar{S}}/\Delta\bar{\bar{L}} \qquad (18)$$
$$[\partial(f/T)/\partial(1/T)_{P,\lambda} \equiv [\partial(f/T)/\partial(1/T)]_{P,L} = \Delta\bar{\bar{H}}/\Delta\bar{\bar{L}} \qquad (19)$$

where the double barred quantities represent changes for the process:

crystalline fiber + diluent = soln. of vol. fraction v_2

In other words, the double barred quantities refer to the sums of latent changes on melting plus *integral* changes for mixing with solvent to the equilibrium concentration in the mixed phase. According to the assumptions, these quantities remain constant throughout melting, in contrast to the situation for two phases where the *amount* of diluent in the mixed phase is fixed.

Non-uniform Fibers.—Variations in the chemical structure and cross-section of the fiber along its length will broaden the transition between crystalline and amorphous forms. The primary effect of a variation in chemical structure will be manifested

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[26] The system considered is formally analogous to the three-phase, two-component system consisting of a liquid solution simultaneously in equilibrium with the solid solute and the solvent vapor.

in a change of the melting point at a given force, or in the critical force for a given temperature, the pressure being constant. A variation in cross-section will be reflected in the stress, and as melting equilibrium must depend directly on the stress, such variations will lead similarly to different values for the critical stress in various cross-sections.[27] The transition may thus occur over a range of tensile force at constant T and P, instead of at a unique force, in systems which otherwise would be univariant at constant pressure as previously discussed.

In accordance with these considerations it is appropriate to characterize various sections of the fiber, or fibril of small cross-section, by their critical forces f_j at the given T and P. Let p_j designate the fraction of the length of the fiber *in its reference state*, conveniently taken to be the totally crystalline fiber, which may be characterized by a critical force f_j to $f_j + df_j$; *i.e.*

$$p_j = -d\lambda/df_j \tag{20}$$

where λ is specified as a fraction of the *reference length* which is amorphous at T, P and $f = f_j$. The total length of the fiber under a force f will then be

$$L = \int_{-\infty}^{f} p_j L_j^c df_j + \int_{f}^{\infty} p_j L_j^a df_j \tag{21}$$

where L_j^c is the total length for the corresponding uniform fibril made up exclusively of elements identical with element j when totally crystalline under the conditions specified by T, P and f; L_j^a is the length of this hypothetical uniform fibril in the amorphous state under the specified conditions. In other words, if N is the total number of structural (peptide) units along one molecular chain extending throughout the fibril, then L_j^c/N and L_j^a/N are the (average) components along the fiber axis of structural units in element j when crystalline and amorphous, respectively. If the fiber is sufficiently uniform in cross-section to allow replacement of L_j^c and L_j^a for all j by average values, L^c and L^a, the foregoing equation reduces to

$$L \cong (1 - \lambda)L^c + \lambda L^a \tag{22}$$

which is an analog of eq. 6.

The length–temperature coefficient at constant force may be obtained readily from an expression for the entropy analogous to eq. 21, thus

$$S = N\int_{-\infty}^{f} p_j \sigma_j s_j^c df_j + N\int_{f}^{\infty} p_j \sigma_j s_j^a df_j \tag{23}$$

where s_j^c and s_j^a are entropies per structural unit in element j, and σ_j is the number of chains passing through section j. By application of the relationship $(\partial S/\partial f)_{P,T} = (\partial L/\partial T)_{P,f}$, we obtain from eq. 23

$$(\partial L/\partial T)_{P,f} = \int_{-\infty}^{f} p_j(\partial L_j^c/\partial T)_{P,f}\, df_j + \int_{f}^{\infty} p_j(\partial L^a{}_j/\partial T)_{P,f}\, df_j - Np_f\sigma_f\Delta s_f \tag{24}$$

where p_f, σ_f and Δs_f refer to values of the respective quantities in the cross-sectional element for which crystalline and liquid phases are in equilibrium at the force f, *i.e.*, at the finite limits of the integrations. The linear thermal expansion coefficients

$$\alpha_{Tj}{}^c = (L_j^c)^{-1}(\partial L_j^c/\partial T)_{P,f}$$
$$\alpha_{Tj}^a = (L_j^a)^{-1}(\partial L_j^a/\partial T)_{P,f}$$

should be comparatively insensitive to the stress and to minor structural variations along the fiber. Hence, with negligible error they may be replaced by α_T^c and α_T^a for all j. Equation 24 then becomes

$$(\partial L/\partial T)_{P,f} = \alpha_T{}^c \int_{-\infty}^{f} p_j L_j^c\, df_j + \alpha_T{}^a \int_{f}^{\infty} p_j L_j^a df_j - N\sigma_f p_f \Delta s_f \tag{25}$$

With the introduction of the approximations used above in eq. 22, we have

$$(\partial L/\partial T)_{P,f} \cong (1 - \lambda)L^c\alpha_T{}^c + \lambda L^a\alpha_T{}^a - Np_f\sigma\Delta s \tag{26}$$

where σ_f and Δs_f, in accordance with the other approximations, are replaced by average values.

Now the linear thermal expansion coefficient α_T^c for the crystalline phase may be presumed to possess a positive value similar to that for crystalline organic compounds, *i.e.*, 1 to 2 × 10^{-4} °C.$^{-1}$. The values of α_T^a, on the other hand, should be negative, (except at very low forces[28]), and, according to elasticity theory for rubber-like polymers it should be approximately equal to $-1/T$, *i.e.*, *ca.* 3 × 10^{-3} °C.$^{-1}$ at ordinary temperatures. In comparing the first and second terms it is to be noted however that L^a will be considerably smaller than L^c, except at very high stresses. Hence $L^a\alpha_T{}^a$ may not be much greater in magnitude than $L^c\alpha_T{}^c$. The sign of the sum of the first two terms will depend on the degree of crystallinity; for low degrees $(1 - \lambda)$, their sum will be negative, and for greater $1 - \lambda$ it will be positive.

The third term in eq. 26 represents the contribution—always negative—to the length–temperature coefficient due to increase in crystallinity with stretching. Its magnitude depends directly on the fraction p_f of the fiber which undergoes the amorphous–crystalline transformation per unit increase in force at P, T and f. At intermediate degrees of crystallinity, this term may dominate others on the right-hand side of eq. 26, with the result that $(\partial L/\partial T)_{P,f}$ will be strongly negative. At sufficiently high degrees of crystallinity, p_f will necessarily diminish toward zero, and the first term, representing the positive thermal expansion of the crystalline fiber, will be dominant.

Thus, the totally amorphous fiber $(\lambda = 1)$ should exhibit moderate negative thermal expansion (rubber-like), except at very low deformations (see footnote above). At intermediate degrees of crystallinity, $(\partial L/\partial T)_{P,f}$ should be strongly negative. As the degree of crystallinity is increased (*i.e.*, with increase in length) it should reach a minimum value (maximum negative value), then increase, becoming positive at high degrees of crystallinity as already noted.

The more frequently measured force–tempera-

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(27) Our choice of the force rather than the stress as the variable in the thermodynamic analysis is dictated by the uniformity of the former throughout the length of the fiber, irrespective of the variations considered.

(28) When $f = 0$, the amorphous, or liquid, fraction may display a normal, *i.e.*, positive thermal expansion coefficient. In analogy with rubber, "thermoelastic inversion" of $\alpha_T{}^a$ should occur at the force required for a comparatively small deformation; for larger forces $\alpha_T{}^a$ will be negative, approaching $-1/T$ asymptotically at large f.

ture coefficient $(\partial f/\partial T)_{P,L}$ may be derived from $(\partial L/\partial T)_{P,f}$ by using the relation

$$(\partial f/\partial T)_{P,L} = -(\partial f/\partial L)_{P,T}\,(\partial L/\partial T)_{P,f} \qquad (27)$$

Since $(\partial f/\partial L)_{P,T}$ is always positive, the sign of $(\partial f/\partial T)_{P,L}$ will be opposite to that of $(\partial L/\partial T)_{P,f}$, and it will reach zero at the same length or force, as that at which the latter coefficient is zero. The foregoing conclusions may therefore be applied to the interpretation of $(\partial f/\partial L)_{P,T}$. This coefficient should be positive for the totally amorphous polymer, increase with crystallization to a maximum, then decrease to zero, becoming negative at high degrees of crystallinity. The behavior here predicted is substantiated by observations on various fibrous proteins,[29-31] whose peculiar thermoelastic characteristics have not heretofore received satisfactory explanation.[3]

Part II. Statistical Theory of Elasticity in Oriented Non-crystalline Fibers

In the usual statistical theories of elasticity of cross-linked polymers, network structures are considered to have been formed by cross-linking a system of random coiling, long chain molecules while in an *equilibrium set of configurations*. The initial system is necessarily *isotropic* if this condition is fulfilled. We now undertake to derive an elastic equation of state for an amorphous (*i.e.*, non-crystalline) network structure formed under conditions such that the chain molecules were not in equilibrium configurations at the moment of generation of the network (although the random situation will be included as a special case). Particular attention will be devoted to network structures formed by imposing cross linkages, or permanent interconnections of any other nature, between adjacent chain molecules while in a state of orientation. This orientation may have been induced by an externally applied stress, or it may have arisen spontaneously in the process of forming fibrils from highly asymmetric protofibrillar particles (see Introduction). The polymer may have been crystalline, or partially so, when the interconnections were superimposed on it. We would by no means exclude the possibility of forming cross linkages within (oriented) crystalline regions; this would, in fact, seem to be properly descriptive of the circumstances attending cross-linking in fibrous proteins. The elasticity relations to be derived in this section of the paper apply, however, only to the polymer in the *amorphous state* irrespective of the state of the polymer during cross-linking. We may, for example, consider the elastic properties of a network structure which was formed by cross-linking oriented and crystalline chains and subsequently converted to the amorphous state by melting.

Besides ordinary valence cross linkages between polymer chains, the interconnections may conceivably be imposed by interfibrillar material, possibly occurring at fairly definite intervals along the fiber axis as in the case of the Z-bands in muscle. The interconnections may even be provided by crystalline zones bounding the amorphous zone under consideration, which thereby fix the relative positions of the ends of the chains running along irregular paths through this intervening amorphous region. In this latter case we shall be obliged, *for the purposes of this section of the paper*, to assume that the content of the amorphous regions remains unchanged during deformation, the boundaries with the crystalline zones neither advancing by further crystallization into the amorphous region nor receding by melting when the sample elongates or contracts. The conditions of phase equilibrium which govern the situation when this restriction is removed will be dealt with in Part III.

In conformity with procedures previously adopted in dealing with network structures,[32,33] we define a chain as that portion of a molecule extending from one cross-linked unit to the next along the given molecule. Each such chain may be characterized by a vector r connecting the average positions of its ends (*i.e.*, the bounding cross-linked units). The state of the system as a whole is specified by its chain vector distribution. If the cross linkages are formed within crystalline regions, or in amorphous zones neighboring on crystalline regions, this distribution may be altered somewhat on melting at fixed external dimensions of the sample. Such alteration should be small, and in any case is unimportant, as we shall be concerned only with the vector distribution *in the amorphous state*. It will be assumed that this distribution is altered directly as the macroscopic dimensions when the sample is deformed; *i.e.*, an affine transformation of the average positions of the cross-linked units is assumed.

Finally, we shall assume gaussian polymer chains.[32] The probability that a given chain, free from all constraints, exists momentarily in a configuration such that the cartesian components of its end-to-end vector are in the ranges x to $x + \mathrm{d}x$, y to $y + \mathrm{d}y$, z to $z + \mathrm{d}z$, is therefore expressed as

$$W(x, y, z)\,\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z = (3/2\pi\overline{r_0^2})\exp[-(3/2\overline{r_0^2})(x^2 + y^2 + z^2)]\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z \qquad (28)$$

where $\overline{r_0^2}$ is the mean square of the end-to-end distance of the free chain. For gaussian chains $\overline{r_0^2}$ is proportional to the (average) number of units in a chain. It will be convenient for later purposes to express this relationship as

$$\overline{r_0^2} = n'l'^2 \qquad (29)$$

where n' is the number of statistical elements in the so-called "equivalent statistical chain"[34] and l' is the length of one element. At maximum extension $r = r_\mathrm{m}$ where

$$r_\mathrm{m} = n'l' \qquad (30)$$

Equations 29 and 30 serve to define the parameters n' and l' in terms of quantities $\overline{r_0^2}$ and r_m, which are susceptible to evaluation.

The configuration entropy of the system characterized by a given set of vectors $\mathbf{r}_\mathrm{j}$, one for each chain, will contain a term

$$k\sum_\mathrm{j}\ln W(\mathbf{r}_\mathrm{j}) = (k\nu/2)[-(3/\overline{r_0^2})(\overline{x^2} + \overline{y^2} + \overline{z^2})]$$

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(29) H. B. Bull, This Journal, **67**, 533 (1945).

(30) A. Weber and H. Weber, *Biochem. Biophys. Acta*, **7**, 214, 339 (1951).

(31) M. Morales and J. Botts, *Disc. Faraday Soc.*, **13**, 125 (1953).

(32) P. J. Flory, "Principles of Polymer Chemistry," Cornell Univ. Press, Ithaca, N. Y., 1953, pp. 458–470.

(33) L. R. G. Treloar, "The Physics of Rubber Elasticity," Oxford Univ. Press, New York, N. Y., 1949.

(34) See ref. 29, pp. 411–413.

for the internal configurations of the chains, where ν is the total number of chains; $\overline{x^2}$, $\overline{y^2}$ and $\overline{z^2}$ are the average squares of the components of the chains in the specified state, and k is the Boltzmann constant. The sum of the components, *i.e.*, the quantity $\overline{x^2} + \overline{y^2} + \overline{z^2}$, equates to the mean-square end-to-end distance designated by $\overline{r^2}$. The number ν of chains must necessarily equal the number of intermolecularly cross-linked units.

The contribution to the entropy from the random distribution of the interconnections over the total volume V must be considered in addition to the chain configuration expressed above. If the interconnections consist of tetrafunctional cross linkages (*i.e.*, cross linkages between two molecules, which therefore join the ends of four chains, defined as above), the number of cross linkages will be half the number of chains, or $\nu/2$. Hence, the resulting entropy term may be written[32,35,36]

$$(k\nu/2) \ln V + \text{Constant}$$

The network configuration entropy is therefore of the form[32,35]

$$(k\nu/2)[-3(\overline{x^2} + \overline{y^2} + \overline{z^2})/\overline{r_0^2} + \ln V + \text{Const.}]$$

Since the transformation of average chain coördinates is assumed to be affine for all deformations (in the amorphous state) including dilation through swelling by a solvent, it is evident that the volume V will be proportional to $(\overline{x^2}\,\overline{y^2}\,\overline{z^2})^{1/2}$. The reference state is conveniently taken as the isotropic network with mean-square vector components $\overline{x_0^2} = \overline{y_0^2} = \overline{z_0^2} = \overline{r_0^2}/3$. Thus, in addition to the stipulation of isotropy, the last equality requires that the dilation in the reference state shall be such that the resultant mean-square chain vector length will equal its value for the unconstrained free chains (*i.e.*, $\overline{r_0^2}$). If for example the network was formed in the presence of a diluent, the mean-square chain displacement length $\overline{r^2}$ in the isotropic and unswollen state may be less than $\overline{r_0^2}$, hence an increase in volume will be required to reach this reference state. In other instances, where for example $\overline{r_0^2}$ has been diminished by a change of temperature subsequent to formation of the network, shrinkage of the network to a volume less than that of the polymer itself might be required. The physical impossibility of realization of the reference state in such a case does not, of course, detract from its usefulness as a reference state.

Letting ΔS_{el} represent the difference between the ("elastic") entropy of the state characterized by $\overline{x^2}$, $\overline{y^2}$ and $\overline{z^2}$ and the reference state, we thus obtain

$$\Delta S_{\text{el}} = (3k\nu/2)[-(\overline{x^2} + \overline{y^2} + \overline{z^2})/\overline{r_0^2} + 1 + \ln <\alpha>] \tag{31}$$

where

$$<\alpha> = (\overline{x^2}\,\overline{y^2}\,\overline{z^2}/\overline{x_0^2}\,\overline{y_0^2}\,\overline{z_0^2})^{1/6} \tag{32}$$

If $\overline{x_i^2} = \overline{y_i^2} = \overline{z_i^2} = \overline{r_i^2}/3$ represents the *isotropic* state of the *same volume* as corresponds to $\overline{x_2}$, $\overline{y_2}$, $\overline{z_2}$, then

$$<\alpha> = (\overline{r_i^2}/\overline{r_o^2})^{1/2} \tag{32'}$$

The parameter $<\alpha>$ measures the geometric mean of the linear dilation relative to the reference state, hence we shall refer to $<\alpha>$ as the dilation factor. It varies as the cube-root of the volume of the network, and must therefore depend on the degree of swelling by a diluent. If v_2 represents the volume fraction of polymer in the swollen network, it follows that

$$<\alpha> = <\alpha>_0 v_2^{-1/3} \tag{33}$$

$<\alpha>_0$ being the value of the dilation factor in complete absence of diluent. Diluent may have been present during cross-linking, hence $<\alpha>_0$ is not necessarily the value of the linear dilation of the network at the time of its formation. Moreover, since in general $\overline{r_0^2}$ will depend on the temperature, the value of $<\alpha>$ at constant volume (and the value of $<\alpha>_0$ also) may vary with the temperature.

If the network is formed by cross-linking polymer molecules in the configurationally random isotropic state, then at the same network volume and temperature as prevailed during cross-linking $<\alpha> = 1$. If cross-linking was performed in this manner in absence of diluent, then at the temperature of network formation $<\alpha>_0 = 1$; upon subsequently swelling the network $<\alpha> = v_2^{-1/3}$. The value of $<\alpha>$ for a network formed by cross-linking oriented chain molecules will depend on details pertaining to the dimensions of the molecules and their arrangement; $<\alpha>$ for the network at its initial volume and temperature may be either less than or greater than unity, as is evident from the definition of $<\alpha>$ according to eq. 32. Equations 31, 32, 32′ and 33 appear to be generally applicable to any situation provided only that the deformations are affine and the polymer is amorphous.

We restrict our treatment to simple elongations, in which a single coördinate (x) is varied, while the remaining coördinates (y and z) change equally as required by the designated dilation. Thus, $\overline{y^2} = \overline{z^2}$, and according to eq. 32

$$\overline{y^2} = \overline{z^2} = <\alpha>^3(\overline{r_0^2})^{3/2}/3^{3/2}(\overline{x^2})^{1/2} \tag{34}$$

Substitution of eq. 34 in 31 yields

$$\Delta S_{\text{el}} = (k\nu/2)[-3\overline{x^2}/\overline{r_0^2} - 2<\alpha>^3(\overline{r_0^2}/3\overline{x})^{1/2} + 3 + 3\ln<\alpha>] \tag{35}$$

In order to relate the root-mean-square axial component $(\overline{x^2})^{1/2}$ to the length L of the sample and the number ν of chains which it contains, we introduce a quantity σ defined as the number of chain vectors passing through a plane transverse to the axis of the sample. For simplicity, let each vector be assigned the direction which causes its x-component to be positive. The number of vectors originating per unit length is ν/L, L being the macroscopic length of the sample. Let $P_x\,\mathrm{d}x$ be the fraction of the vectors having components with magnitude x to $x + \mathrm{d}x$ along the fiber axis. Vectors of this group which originate within the distance x preceding a given transverse plane will pass through that plane. The number of such vectors is $(\nu x/L)P_x\,\mathrm{d}x$. The total number of vectors passing through the plane is therefore

$$\sigma = (\nu/L)\int_0^\infty xP_x\mathrm{d}x$$

or

$$\sigma = (\nu/L)\bar{x} \tag{36}$$

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(35) P. J. Flory, *J. Chem. Phys.*, **18**, 108 (1950).

(36) If the chains are joined by interconnections of higher functionality, the factor $^1/_2$ is to be replaced by $2/f$ where f is the functionality. See ref. 35.

where $\bar{x}$ is the mean value of the *magnitude* (*i.e.*, the modulus) of the x-components of the chain vectors. It will be convenient to use instead

$$\sigma' = \sigma\overline{x}/(\overline{x^2})^{1/2} = (\nu/L)(\overline{x^2})^{1/2} \qquad (37)$$

The ratio $(\overline{x^2})^{1/2}/\overline{x}$ will differ from unity accordingly as the distribution of axial components is variable (assuming all chains to be of equal contour length, *i.e.*, to contain the same number of units[37]). For randomly cross-linked chains $(\overline{x^2})^{1/2}/\overline{x} = (\pi/2)^{1/2} = 1.25$; for networks formed by cross-linking ordered chains, this ratio should closely approach unity.

Both σ and σ' must *in any case* be invariant to deformation, provided only that the transformation of the vectors by the deformation is affine. It is permissible therefore to substitute for $\bar{x}^2$ and L in eq. 37 the values, $\overline{x_i^2} = \overline{r_i^2}/3$ and L_i, of these quantities in the isotropic state of identical volume (*i.e.*, the same $<\alpha>$). We thus obtain

$$L_i = (\nu/\sigma')(\overline{r_i^2}/3)^{1/2} = (\nu/\sigma')(\overline{r_o^2}/3)^{1/2} <\alpha> \qquad (38)$$

which defines the length L_i of the sample in the isotropic state specified above. From eq. 33

$$L_i = (\nu/\sigma')(\overline{r_o^2}/3)^{1/2}<\alpha>_o v_2^{-1/3} \qquad (38')$$

$$L_i = L_{io} v_2^{-1/3} \qquad (38'')$$

where L_{io} is the isotropic length when all diluent has been removed.

The essential distinction between the cross-linking of oriented molecules on the one hand and of random coiled molecules on the other becomes apparent upon relating σ (or σ') to the total number of chains ν in the two cases. If the chains are *sufficiently oriented* along the fiber axis, then σ may at once be identified with the number of macromolecules in a cross-section. Hence σ (and σ') must *be independent of the degree of cross-linking.* To consider the matter from the point of view of eq. 36 and 37 which define σ and σ', we note that the average projected distance $\bar{x}$ between cross-linked units along a given chain (sufficiently oriented, *cf.* seq.) must vary inversely as the degree of cross-linking, and hence inversely as ν. The products $\nu\ \bar{x}$ in eq. 36 and $\nu\ (\bar{x}^2)^{1/2}$ in (37) must therefore be independent of ν, hence σ is invariant with ν.

Whereas in the case under consideration σ is independent of ν, L_i increases with ν. This may be shown as follows. According to eq. 29, $\overline{r_o^2}$ increases proportionally to the number of units per chain; it must therefore vary as ν^{-1}. In view of eq. 38 and the constancy of σ' the *isotropic length* L_i *must increase as* $\nu^{1/2}$ $<\alpha>$ in the course of cross-linking sufficiently oriented chains. Depending on details of the chain packing and orientation which evade generalizations, $<\alpha>$ may either increase or decrease with ν. The change should however be very small, hence it is justified to conclude that L_i will increase very nearly as $\nu^{1/2}$.

The network formed by cross-linking (gaussian) polymer chains in the unoriented state is necessarily isotropic. L_i may therefore be identified with the length of the specimen when cross-linked, its value being independent of ν. To be sure, the value of L_i may be altered by swelling or de-swelling subsequent to creation of the network, as is manifest in eq. 36′ and 36″. However, for any specified degree of swelling, L_i will be invariant with cross-linking for networks formed in the manner stated. Letting $L = L_i$ = constant in eq. 37 and observing that $\overline{x_i^2} = \overline{r_o^2}/3$ varies as ν^{-1}, we find at once that σ' (and σ) *must increase as* $\nu^{1/2}$.

This increase in the number of chain vectors in a cross section comes about because a given random coiling chain may penetrate the cross section several times in the course of its path from one cross linkage to the next. Consider, for example the molecule having the configuration indicated in Fig. 1. Let it be cross-linked initially at the points 1, 2 and 3. One of its chain vectors, namely, that between 2 and 3, then passes through the cross-section indicated by the broken line. If a further cross linkage is added at point 4, the plane will be penetrated by three successive vectors asssociated with the molecule considered, namely, the vectors (1,4), (4,2) and (2,3). The possibilities for increasing σ in this manner must vanish as backward looping of the chains is suppressed by orientation. The physical basis for the distinction between cases considered is thus obvious.

Elimination of $\overline{x^2}$ and $\overline{r_o^2}/3$ from eq. 35 by use of eq. 37 and 38, respectively, gives

$$\Delta S_{el} = -(k\nu/2)[(<\alpha>/L_i)^2(L^2 + 2L_i^3/L) - 3 - 3\ln<\alpha>] \qquad (39)$$

or

$$\Delta S_{el} = -(k\nu/2)[(<\alpha>_o/L_{io})^2(L^2 + 2L^3_{io}/Lv_2) - 3 + \ln v_2 - 3\ln<\alpha>_o] \qquad (39')$$

The force of retraction is given by

$$f = (\partial\Delta F_{el}/\partial L)_{P,T,v_2} = (\partial\Delta F_{el}/\partial L)_{P,T,<\alpha>} \qquad (40)$$

We consider for the present that the degree of swelling $(1/v_2)$ remains constant in deformation. Under the assumption that the enthalpy change with deformation is negligible (in accordance with studies on various polymers), $\Delta F_{el} = -T\Delta S_{el}$. It follows from eq. 39 that

$$f = BTL(1 - L_i^3/L^3) \qquad (41)$$

where

$$B = k\nu(<\alpha>/L_i)^2 = k\nu(<\alpha>_o/L_{io})^2 \qquad (42)$$

Substitution of $\alpha = L/L_i$ in these equations leads to the alternative relation

$$f = (kT\nu<\alpha>^2/L_i)(\alpha - 1/\alpha^2) \qquad (43)$$

$$= (kT\nu<\alpha>_o^2/v_2^{1/3}L_{io})(\alpha - 1/\alpha^2) \qquad (43')$$

The stress τ referred to the cross-section of area A_i in the isotropic state, given by f/A_i, is

$$\tau = (kT\nu/V)<\alpha>^2(\alpha - 1/\alpha^2) \qquad (44)$$

where $V = A_iL_i$ is the volume at the dilation $<\alpha>$. The stress τ_o referred to the isotropic cross section in absence of diluent is

$$\tau_0 = (kT\nu/V_0)<\alpha>_0^2 v_2^{-1/3}(\alpha - 1/\alpha^2) \qquad (44')$$

where V_o is the unswollen volume.

The foregoing equations for the retractive force and stress are general, within the limitations of the gaussian approximation at any rate. They apply equally to networks formed from molecules in ran-

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(37) This assumption has been employed in rubber elasticity theory.[32,33] It could be eliminated by introducing a further averaging process. The results would not, however, be affected.

dom configurations and from highly oriented molecules. It follows that the elastic behavior of variously formed networks should be indistinguishable insofar as dependence of stress on the relative length α is concerned. The equations given in the preceding paragraph correspond fully to those obtained in previous theories of rubber elasticity,[32,33] except for the presence of the dilation factors $<\alpha>$ and $<\alpha>_o$. The correspondence is exact if we set $<\alpha>_o = 1$ and hence $<\alpha> = v_2^{-1/3}$. This situation should apply if the network is formed in the absence of diluent and if the chain configuration parameter $\overline{r_o^2}$ is not altered by a change of temperature subsequent to network formation (see eq. 32′). The present equations are more general than those previously given in that account is taken of circumstances in which $<\alpha>_o \neq 1$ owing to non-compliance with either of the conditions mentioned.

A most important difference between the two cases considered is apparent on inspection of the parameters B and L_i in eq. 41. Whereas according to eq. 42 B is proportional to ν for cross-linking of random chains, it is *independent* of ν in networks formed by cross-linking well oriented chains, L_i being then proportional to $\nu^{1/2}$ $<\alpha>$ (see above). Thus, in the former case L_i is independent of ν but B increases directly as ν; in the latter case L_i increases approximately as $\nu^{1/2}$ but B is independent of ν.

Equation 41 can be expressed in a form which may prove more useful for comparison with experiments on networks formed from well oriented chain molecules by introducing the length L_m of the fiber at maximum extension—a quantity amenable to estimation from the structure of the molecules comprising the fiber. The maximum length of one chain between cross-linkages is $n'l'$ according to eq. 30, and the mean number of chains in one linear sequence throughout the length of the fiber is ν/σ. Hence

$$L_m = (\nu/\sigma)n'l' \qquad (45)$$

Recalling eq. 29 and 38, and ignoring the difference between σ and σ' in this case, we then obtain the alternative expressions

$$B = (3k\sigma/l'L_m) = 3k\nu n'/L_m^2 \qquad (46)$$

The equivalent segment length (see eq. 29 and 30) may be taken as the ratio $\overline{r_o^2}/r_m$, which may be evaluated from studies of molecular configuration and fiber structure. The retractive force to be expected at a given fiber extension may thus be calculated. For L sufficiently greater than L_i, we have by eq. 41 and 46

$$f \cong (3kT\sigma/l')(L/L_m) = 3kT\nu n'(L/L_m^2) \qquad (47)$$

When this condition applies the retractive force at a given length is independent of the number of cross linkages.

Elastic Properties at Swelling Equilibrium.—Thus far in Part II, the polymer network has been treated as a closed system, *i.e.*, the quantity of diluent, if any, in the network has been assumed to be fixed during deformation. We now treat the network as an open system in equilibrium with a surrounding liquid phase. For simplicity, the diluent absorbed by the polymer is assumed to consist of a single component. The following considerations would apply equally to a diluent mixture provided mole ratios of the substances partitioned between the two phases are the same in each.

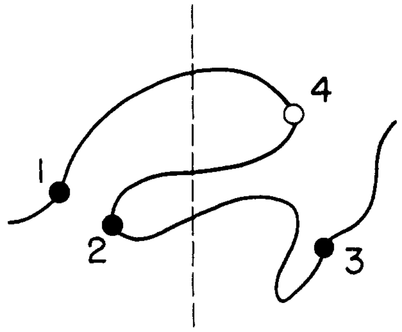

Fig. 1.

In accordance with procedures previously used in treating swelling equilibrium,[32,35] we express the free energy of the system as

$$\Delta F = \Delta F_{el} + \Delta F_M \qquad (48)$$

where ΔF_M is the free energy of mixing polymer and diluent. The elastic free energy ΔF_{el} may again be equated to $-T\Delta S_{el}$. The free energy of mixing is considered to depend on the composition of the network phase, but not on its deformation; ΔS_{el} depends on both. At swelling equilibrium $(\partial\Delta F/\partial n_1)_{P,T,L} = 0$, where n_1 is the number of moles of diluent contained in the network phase. Hence

$$T(\partial\Delta S_{el}/\partial v_2)_{T,P,L}\,(\partial v_2/\partial n_1)_{T,P,L} = (\partial\Delta F_M/\partial n_1)_{T,P}$$

The first of the partial derivatives on the left is readily evaluated by differentiating eq. 39′, L_{io} being a constant of the structure. The second of the partial derivatives reduces to $-v_2^2\mathrm{v}_1/V_o$ where v_1 is the molar volume of the diluent and V_o is the volume of the network in absence of diluent. For the derivative on the right, we adopt an expression of the form given by polymer solution theory. Presuming the external phase to consist of pure diluent, this can be expressed as

$$(\partial\Delta F_M/\partial n_1)_{T,P} = RT[\ln(1 - v_2) + v_2 + \chi_1 v_2^2]$$

where χ_1 is an interaction parameter.[38] Combining these expressions, we have at equilibrium

$$(\nu\mathrm{v}_1/N_a V_o)[<\alpha>_o^2(L_{io}/L) - v_2/2] = -[\ln(1 - v_2) + v_2 + \chi_1 v_2^2] \qquad (49)^{39}$$

where N_a is Avogadro's number.

If the retractive force f is zero, $L = L_i = v_2^{-1/3} L_{io}$, and eq. 49 becomes

$$(\nu\mathrm{v}_1/N_a V_o)[<\alpha>_o^2 v_2^{1/3} - v_2/2] = -[\ln(1 - v_2) + v_2 + \chi_1 v_2^2] \qquad (50)$$

Upon setting $<\alpha>_o = 1$, this relationship becomes identical with the standard expression for swelling equilibrium in a network under no external constraint.[32,35]

The force of retraction exerted by a deformed, swollen network which remains at equilibrium with an external liquid phase is given by

$$f = (\partial\Delta F/\partial L)_{P,T,v_2}$$

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(38) See ref. 32, Chapter XII.

(39) Eq. 49 with $<\alpha>_o = 1$ is identical with eq. 15 of ref. 35.

which reduces at once to eq. 40 inasmuch as ΔF_M of eq. 48 is independent of L. The previous relations for the retractive force, deduced for the closed system, are immediately applicable to the present case. We now require however that v_2 be assigned its equilibrium value dictated by eq. 49. The concentration v_2 thus becomes a function of L, and therefore of f, for the open system. If, therefore, eq. 41 is used for the force expressed as a function of the length, L_i given by (38″) is also a function of the swelling ratio (v_2^{-1}). It is expedient to rewrite eq. 41 as

$$f = BTL(1 - L_{io}^3/v_2L^3) \qquad (41')$$

where B according to eq. 42 is independent of v_2. Since v_2 is a function of L according to eq. 49, the functional dependence of f on L for the open system is obtained by simultaneous solution of eq. 41′ and and 49. The change of v_2 with L is small, however.

Part III. Crystallization Equilibrium

Amorphous–Crystalline Transition under Stress.—The equilibrium between crystalline and amorphous phases under a tensile force may be treated by combining the results of Parts I and II. Specifically, the results of Part II may be used to furnish the "equation of state" relating ΔL of eq. 11 to the force f. A relationship between f and the transition temperature, or melting point, T may then be obtained by integration. The procedure is analogous to integration of the Clausius–Clapeyron equation for liquid-ideal vapor equilibrium.

By definition, $\Delta L = L^a - L^c$ where the length L^a of the *totally* amorphous sample may be identified with L of Part II. (The superscript, omitted in Part II since only the amorphous phase was under consideration, is here restored.) Upon substituting eq. 41 in 11 we thus obtain

$$(L^a - L^c)\mathrm{d}[L^a - L_i^3/(L^a)^2] = (\Delta H/B)\mathrm{d}(1/T) \qquad (51)$$

Integration between the limits L^c and L^a, B, L_i and L^c treated as constants, yields

$$(L^c - L^a)^2[1 + 2L_i^3/L^c(L^a)^2] = 2(\Delta H/B)(1/T_m - 1/T_m^c) \qquad (52)$$

where T_m is the melting point, or transition temperature, at a force such that the amorphous length is L^a, and T_m^c is the melting point when $L^a = L^c$. Substitution from eq. 46 for B provides the alternative expression

$$[(L^c - L^a)/L_m]^2[1 + 2L_i^3/L^c(L^a)^2] = (2\Delta h'/3R)(1/T_m - 1/T_m^c) \qquad (53)$$

where $\Delta h'$ is the heat of fusion per mole of equivalent elastic elements; *i.e.*, $\Delta h' = \Delta HN_a/\nu n'$. For $T_m < T_m^c$ eq. 52, or 53, yields two solutions L^a, one less than and the other greater than L^c. There are no real solutions for $T_m > T_m^c$. Thus, T_m^c appears to be a critical temperature above which the crystalline phase cannot exist.

It is to be noted, however, that at the critical length $L^a = L^c$ the chain extension may be near its maximum L_m; in any case this limit is likely to lie beyond the range within which the gaussian approximation is legitimate. The equations given above will accordingly be in error for L^a values approaching L_m or L^c, and the literal interpretation of T_m^c given above will be quantitatively in error. Since L^c and T_m^c occur merely as reference quantities in eq. 52 and 53, their presence does not vitiate use of the relationship for values of L^a within the gaussian range. Amorphous polymers invariably suffer rupture at lengths considerably less than L_m, hence the restriction on L^a will not be serious.

Integration of eq. 51 between the limits L_i and L^a leads to the relationship

$$2(L^a - L^c)[L^a - L_i^3/(L^a)^2] - [(L^a)^2 + 2L_i^3/L^a - 3L_i^2] = (2\Delta H/B)(1/T_m - 1/T_m^i) = 2L_m^2(\Delta h'/3R)(1/T_m - 1/T_m^i) \qquad (54)$$

where T_m^i is the equilibrium temperature for melting to an isotropic amorphous phase. We thus obtain the reciprocal melting temperature T_m relative to $1/T_m^i$ as a function of the amorphous length L^a. The relationship of T_m to the tensile force f may be obtained by simultaneous solution of eq. 41 and 54.[40] It is to be understood that equations 52 to 54, as well as others which follow, refer to states of phase equilibrium; subscripts to this effect have been omitted for simplicity.

If the deformation is sufficient to justify use of eq. 47 instead of (41), *i.e.*, if $(L_i/L^a)^3 < < 1$ (see eq. 41), eq. 53 and 54 simplify to

$$(L^c - L^a)^2/L_m^2 \cong (2\Delta h'/3R)(1/T_m - 1/T_m^c) \qquad (55)$$

and

$$[(L^a)^2 - 2L^aL^c]/L_m^2 \cong (2\Delta h'/3R)(1/T_m - 1/T_m^i) \qquad (56)$$

Elimination of L^a/L_m from the approximate equations 55 and 56 through use of eq. 47 then yields

$$(f/T)_{eq} = (3k\nu n'/L_m)[(L^c/L_m) \pm \sqrt{(2\Delta h'/3R)(1/T_m - 1/T_m^c)}] \qquad (57)$$

and

$$(f/T)_{eq} \cong (3k\nu n'/L_m)[(L^c/L_m) \pm \sqrt{(L^c/L_m)^2 + (2\Delta h'/3R)(1/T_m - 1/T_m^i)}] \qquad (58)$$

where the subscript designating phase equilibrium has been restored.

These approximate results admit of simple graphical representation. Consider the case of a homogeneous fiber whose elastic behavior in the amorphous state is satisfactorily described by eq. 47. As shown in Fig. 2, f/T at the temperature T should increase linearly with L from O to A. At A crystallization sets in, and the length then increases without increase in f until the phase transformation is complete at B. The value of f/T over this region corresponds to the lower root of eq. 57, or of eq. 58. In consideration of the relatively high rigidity to be expected for the crystalline state, the stress has been assumed to rise almost vertically with elongation beyond point B.

If the aforementioned limitation of the gaussian approximation is ignored, and we assume for the moment that the polymer chains are capable of accommodating an extension well beyond the length L^c, then according to eq. 57 and 58 the amorphous phase should be regenerated along the line DE. The force during this (hypothetical) transition corresponds to the larger root given by eq. 57 or 58. The line connecting A and E is the continuation of the stress–strain relation (OA) for

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[40] Equations 52, 53 and 54 may be derived alternatively by application of the condition $\Delta(F - fL) = 0$ for phase equilibrium at constant P, T and f. For this purpose ΔF is resolved into a free energy of fusion in the reference state defined previously and the elastic free energy ΔF_{el}.

the amorphous phase. With increase in temperature, points A and E are displaced toward point C. The horizontal segments AB and DE consequently must diminish in length with rise in temperature, finally vanishing at the critical point C.

The predicted remelting of the crystalline phase can scarcely be expected to be encountered in actual fibers. Even if L^c were appreciably less than L_m, rupture of the amorphous phase would immediately follow its regeneration.

Use of the complete equations 54 and 41 would bring about replacement of the linear stress–strain curve running through the origin in Fig. 2 by a curve beginning at $L = L_i > 0$. This curve is asymptotic to a line through the origin. Other features of the diagram would be unchanged.

If the fiber is immersed in an excess of a diluent and the amorphous regions are swollen to equilibrium with the diluent, then $\Delta h'$ is to be replaced in the preceding equations by $\Delta\bar{\bar{h}}'$, the heat of fusion plus the integral heat of dilution per equivalent elastic element (see Part I). For a uniform fiber, $\Delta\bar{\bar{h}}'$ should remain constant throughout the phase transformation.

If, however, the fiber contains a fixed amount of diluent, and this is confined to the amorphous regions, then, in accordance with the previous consideration of this case in Part I, $\Delta h'$ must be replaced by $\Delta\bar{h}'$, the heat of fusion plus the partial heat of dilution. Since the composition of the amorphous phase must then vary with λ, $\Delta\bar{h}'$ will vary, and the horizontal straight lines AB and A′B′ in Fig. 2 must be replaced by curves with positive slopes and curvatures.

Relationships corresponding to those given above may be shown to hold for an inhomogeneous fiber (Part I), provided, however, that they are interpreted to apply to a particular element (j) of the fiber which is in phase equilibrium at the point designated by T and f_j, or T and L_j^a. The line AB and BD must, of course, be replaced by a sigmoid curve, whose form will depend on the distribution of elements according to their characteristic transition temperatures ($T^i_{m,j}$)

Effect of Cross-linking on the Melting Temperature.—The melting point T_m^i of the unstressed, isotropic fiber has been introduced above merely as a constant of integration. The manner of dependence of T_m^i on the degree of cross-linking will now be examined.

For this purpose we regard T_m^i as the ratio of the heat of fusion to the entropy of fusion. It is expedient to separate ΔS into terms ΔS°, ΔS°_X and ΔS_{el}^i, representing, respectively, the entropy of fusion in the absence of constraints imposed by cross linkages on the chain configurations, the alteration of the chain configurational entropy due to the presence of the cross linkages in the hypothetical reference state (amorphous; see Part II) for which $\alpha = <\alpha> = 1$ and hence $\overline{r^2} = \overline{r_0^2}$, and the entropy change in passing from this state to the real isotropic state wherein $\alpha = 1$ but $<\alpha>$ assumes its value characteristic of the given network structure. We write

$$1/T_m^i = (\Delta S^\circ + \Delta S^\circ_X + \Delta S_{el}^i)/\Delta H$$

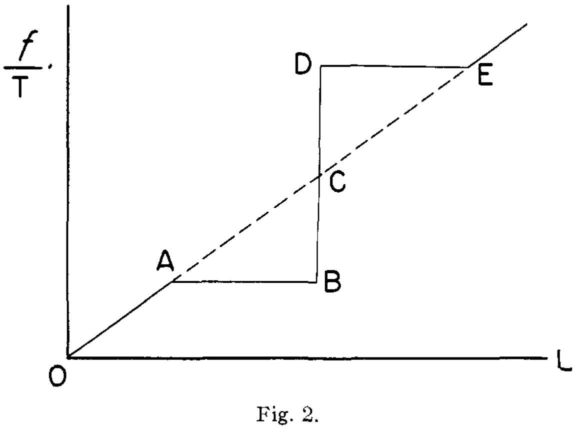

Fig. 2.

To the extent that the formation of cross-linkages does not interfere directly with crystallization of the units involved in cross-linkages, both ΔH and ΔS° may be taken to be independent of the degree of cross-linking. This seems a reasonable assumption for the fibrous proteins under consideration.[41] Hence, ΔS° may be identified with the entropy of fusion for the polymer containing no cross linkages, and

$$1/T_m^i = (1/T_m^\circ) + (\Delta S_X^\circ + \Delta S_{el}^i)/\Delta H \qquad (59)$$

where T_m° is the melting point for the polymer without cross-linkages, or, perhaps more accurately, in the absence of the chain configurational restraints imposed by the cross-linkages.

If $<\alpha>$ is known, ΔS_{el}^i may be obtained from eq. 39 with $L = L_i$; its contribution will be very small. Attention centers therefore on ΔS_X°, which, neglecting ΔS_{el}^i, represents the alteration of the entropy of fusion which results from the presence of the cross-linkages. It is to be observed that the ordinary entropy changes associated with the chemical reaction responsible for cross-linking are of no importance, inasmuch as they affect the crystalline and the amorphous states equally.

For the cross-linking of random chains, ΔS_X° is essentially zero[42,43] for the reason that units are paired at random in the formation of the cross linkages. Hence, cross-linking does not effect a decrease in the configurational disorder characteristic of random chains.[43] In the cross-linking of polymer chains arranged in parallel array, an element of the order existing in this state is imposed on the network structure thus generated. When a given unit of a particular molecule is to be cross-linked, *it must join with one of its immediate neighbors in the ordered array.* Its possibilities are thus limited to cross-linking with one of several

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(41) In other polymers, cross-linked units are likely to be excluded from crystalline regions by their steric requirements, with the result that the entropy of fusion (*i.e.*, ΔS°) will be *increased* by an amount $R\nu/N_u$ per mole of units,[38] where ν is the number of cross-linked units and N_u is the total number of units. The melting point should therefore be depressed on this account. Observed depressions of the melting point by cross linkages in vulcanized rubber (L. Mandelkern, private communication) are considerably greater than would be predicted in this way, possibly owing to further restraints on crystal development arising from the cross linkages (see A. W. Gent, *J. Polymer Sci.*, **18**, 321 (1955)). The *increase* in melting point here predicted will be modified, and possibly outweighed, by these factors.

(42) P. J. Flory and J. Rehner, Jr., *J. Chem. Phys.*, **11**, 512 (1943).

(43) F. T. Wall and P. J. Flory, *ibid.*, **19**, 1435 (1951).

chains. More important, the unit in the chain involved is uniquely specified, for it must occur in the adjacent position in the highly oriented (crystalline) state. This uniqueness is maintained throughout subsequent transformations, such as melting, provided merely that the original network structure remains intact.

This situation is depicted in Fig. 3, where unit A of one chain is to be cross-linked to adjacent unit A′ of another. Preceding and succeeding cross-linked units along the respective chains are designated by C_1, C_2, C_3 and C_4. The given unit may in each case cross-link with any one of its immediate neighbors (six for hexagonal packing), hence the cross linkage will not in general unite the same pair of chains joined by the cross linkage at A.

The alteration of the entropy of fusion ($\Delta S_X{}^\circ$) by the cross linkage in question may be deduced by computing the probability that the two units involved occur in suitable juxtaposition. The probability of fulfillment of this condition in the crystalline state may be expressed by a ratio of volume elements $\Delta\tau_X/\Delta\tau$, where $\Delta\tau_X$ is the range of coordinates available to one unit relative to the other *after* the cross-linkage is formed, and $\Delta\tau$ refers to the corresponding range for neighboring units *in the crystalline state prior to cross-linking.*

The probability that such a pair of units "find each other" in the amorphous state will be designated by $\omega_A\ \Delta\tau_X$. (In the ordinary cross-linking of random chains, this calculation becomes superfluous for the reason that unit A may combine with *any other* eligible unit of the system.[42,43]) The cross-linkages $C_1, \ldots C_4$ are considered to have been established previously. The contribution of cross-linkage A to $\Delta S^0{}_X$ will therefore be given by

$$k \ln[(\omega_A\Delta\tau_X)(\Delta\tau/\Delta\tau_X)^{-1}] = k\ln(\omega_A\Delta\tau) \qquad (60)$$

where k is the Boltzmann constant.

The neighboring cross linkages $C_1, \ldots C_4$ may, without error, be treated as if they are situated at their most probable positions in space. For the purpose of evaluating $\omega_A\Delta\tau$ it is convenient to consider the chain from C_1 to C_2 to be severed at A, and that from C_3 to C_4 at A′. Then $\omega_A\Delta\tau$ may be equated to the probability that the ends of all four chains meet in $\Delta\tau$ divided by the product of the probabilities that the pairs of severed ends are suitably situated to be re-united with restoration of the initial structure. Assuming gaussian chains

$$\omega_A\Delta\tau = \frac{\Delta\tau \int W_1(\mathrm{r}_1)W_2(\mathrm{r}_2)W_3(\mathrm{r}_3)W_4(\mathrm{r}_4)\mathrm{d}\tau}{\int W_1(\mathrm{r}_1)W_2(\mathrm{r}_2)\mathrm{d}\tau \int W_3(\mathrm{r}_3)W_4(\mathrm{r}_4)\mathrm{d}\tau}$$

where W_1, W_2, etc., are gaussian functions (eq. 28) for the four chains extending, respectively, from C_1, C_2, etc., and $\mathbf{r}_1$, r_2, etc., are the corresponding vectors reaching from the mean positions of the respective cross linkages C_1, C_2, etc., to the volume element $\mathrm{d}\tau$. The integrations extend over all space. If $\mathbf{r}$ represents the vector from an arbitrary origin to the volume element $\mathrm{d}\tau$, and $\mathbf{R}_1$, $\mathbf{R}_2$, etc., the vectors to the points C_1, C_2, etc., then $\mathrm{r}_1 = \mathbf{r} - \mathbf{R}_1$, $\mathrm{r}_2 = \mathbf{r} - \mathbf{R}_2$, etc. Using cartesian coördinates and separating the integrals over the components x, y and z of $\mathbf{r}$, we obtain ω_A as the product of three factors like

$$\omega_{A,x} = \frac{\int \exp[-\beta_1{}^2(x - X_1)^2 \ldots - \beta_4{}^2(x - X_4)^2]\mathrm{d}\tau}{\int \exp[-\beta_1{}^2(x - X_1)^2 - \beta_2{}^2(x - X_2)^2]\mathrm{d}\tau \int \exp[-\beta_3{}^2(x - X_3)^2 - \beta_4{}^2(x - X_4)^2]\mathrm{d}\tau}$$

where $\beta_1{}^2$, etc., represent the values of $3/2\ \overline{r_0{}^2}$ for the respective chains (see eq. 28) and X_1, etc., are the components on the x-axis of the vectors R_1, etc.

Evaluation of the integrals yields

$$\omega_{A,x} = \left[\frac{(\beta_1{}^2 + \beta_2{}^2)(\beta_3{}^2 + \beta_4{}^2)}{\pi(\beta_1{}^2 + \beta_2{}^2 + \beta_3{}^2 + \beta_4{}^2)}\right]^{1/2} \exp\left[\frac{(\beta_1{}^2X_1 + \beta_2{}^2X_2 + \beta_3{}^2X_3 + \beta_4{}^2X_4)^2}{\beta_1{}^2 + \beta_2{}^2 + \beta_3{}^2 + \beta_4{}^2} - \frac{(\beta_1{}^2X_1 + \beta_2{}^2X_2)^2}{\beta_1{}^2 + \beta_2{}^2} - \frac{(\beta_3{}^2X_3 + \beta_4{}^2X_4)^2}{\beta_3{}^2 + \beta_4{}^2}\right] \qquad (61)$$

By choosing as origin of coördinates the most probable position of junction A, the first term of the exponent reduces to zero. The remaining terms in the exponent may be evaluated as averages taken over similar pairs of severed chains, each characterized by the same β_j and β_{j+1} (*i.e.*, by the same number of units n_j and n_{j+1}). In the isotropic reference state X_j and X_{j+1}, for a set of equivalent chains will be distributed over random values, obeying the characteristic gaussian functions for these chains. These terms may be evaluated therefore as their averages over the pair of gaussian distributions of the X's. The result is

$$<(\beta_j{}^2X_j + \beta_j{}^2{}_{-1}X_{j+1})^2> = (\beta_j{}^2 + \beta_j{}^2{}_{+1})/2$$

Substitution into eq. 61 yields

$$\bar{\omega}_{A,x} = [(\beta_1{}^2 + \beta_2{}^2)(\beta_3{}^2 + \beta_4{}^2)/\pi(\beta_1{}^2 + \beta_2{}^2 + \beta_3{}^2 + \beta_4{}^2)]^{1/2}\, e^{-1} \qquad (62)$$

as the mean value of $\omega_{A,x}$ to be expected for cross-linking of units connecting chains having the given set of β values. The product of three such expressions yields $\bar{\omega}_A = \bar{\omega}_{A,x}\ \bar{\omega}_{A,y}\ \bar{\omega}_{A,z}$. Without serious error, each $\beta_j{}^2$ in eq. 62 may be replaced by the average $\bar{\beta}^2 = 3/2\ \overline{r_o{}^2} = 3/2\ \overline{n'}\ l'^2$ (see eq. 29); the corresponding expression for $\omega_{A,y}$ and $\omega_{A,z}$ may be treated likewise. Thus

$$\bar{\omega}_A\Delta\tau = (\bar{\beta}^2/\pi e^2)^{3/2}\Delta\tau \qquad (63)$$

$$= (3/2\pi e^2 l'^2\overline{n'})^{3/2}\Delta\tau \qquad (63')$$

We thus obtain the mean factor by which the relative "configurational probability" of the amorphous state is diminished as the result of forming one cross linkage between units situated as neighbors in the crystalline array. If the distribution of cross-linked units along any given chain molecule is random, then the average number of statistical units between cross linkages will be the *expected number* of such units extending from a unit selected at random to the next cross-linked unit along the given chain. This is the ordinary *number average* per chain between cross linkages. We may therefore take

$$\overline{n'} = N_s/\nu = N_s/2\kappa$$

where N_s is the total number of statistical segments, or units, in the system, ν is the number of cross-linked units, and $\kappa = \nu/2$ is the number of cross linkages. Hence

$$\bar{\omega}_A\Delta\tau = (3/\pi l'^2)^{3/2}(\kappa/e^2N_s)^{3/2}\Delta\tau \qquad (64)$$

The appropriate factor for a total of $\nu/2$ cross linkages is given by the product

$$\Omega_\kappa(\Delta\tau)^{\nu/2} = \prod_{\kappa=1}^{\nu/2} \bar{\omega}\Delta\tau$$

$$= C^{\nu/2}(e^2N_s)^{-3\nu/4}[2^{\nu/2}(\nu/2)!]^{3/2} \qquad (65)$$

where

$$C = (3/2\pi l'^2)^{3/2}\Delta\tau$$

is a dimensionless quantity of the order of unity. The entropy change ΔS_X is given accordingly as $k\ln[\Omega_\kappa(\Delta\tau)^{\nu/2}]$, or

$$\Delta S_x = k\nu[(1/2)lnC - 9/4 + (3/4)\ln(\nu/N_s)] \quad (66)$$

This result probably is to be preferred over a previous estimate[42] which, in the present notation and with neglect of the term corresponding to $\ln C$, is

$$\Delta S_x = k\nu[(9/8)ln(\nu/N_s) - 3.4]$$

Schellman[18] recently treated the effect of intramolecular cross linkages in stabilizing the configuration of an individual (folded) polypeptide chain, with a result not unlike that given in eq. 66.

According to eq. 59 with neglect of $\Delta S_{el}{}^i$, and eq. 66

$$(1/T^\circ{}_m - 1/T^i{}_m) = (R\nu/N_s\Delta h')(A - (3/4)ln(\nu/N_s)) \quad (67)$$

where $A = 9/4 - (1/2)\ln C$. For moderate or low degrees of cross-linking, such that the fraction N_s/ν of segments cross-linked is small, the logarithmic term in eq. 67 should exceed A. We thus conclude that cross-linking of oriented fibers should result in an increase in melting temperature $T^i{}_m$. This is indeed true of collagen fibers cross-linked with various tanning agents. Ordinary vulcanization of rubber, on the other hand, depresses its melting point somewhat. This again is in accord with theoretical prediction, as set forth above.

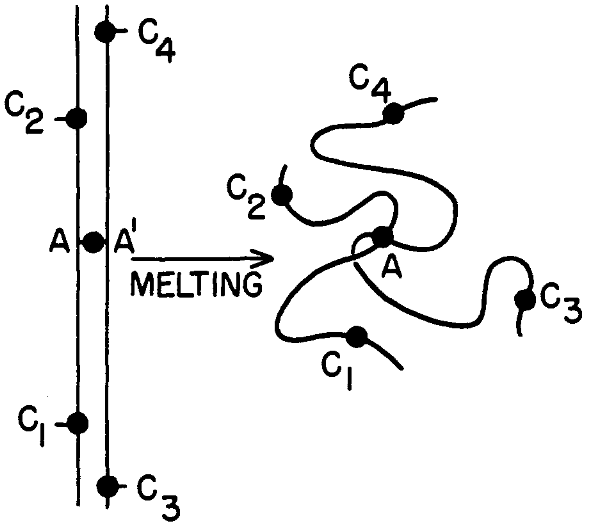

Fig. 3.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Flow Dichroism and its Application to the Study of Deoxyribonucleic Acid Structure[1]

BY LIEBE F. CAVALIERI, BARBARA H. ROSENBERG AND MORTON ROSOFF

RECEIVED APRIL 6, 1956

An apparatus has been constructed which enables dichroism measurements to be made on flowing solutions. Viscosities can also be determined using a manometer in the system. An analytical treatment has been developed which permits the calculation of apparent rotary diffusion constants and dichroism values at complete orientation. The method has been applied to solutions of deoxyribonucleic acid and the results are discussed in terms of its structure.

Introduction

The dichroism of flowing solutions has been measured in a number of cases[2,3] but the technique has not been fully developed as an analytical tool. In the course of an investigation dealing with the effects of salt and *p*H changes on the macrostructure of DNA, we have found dichroism measurements, using plane-polarized ultraviolet light, to be extremely sensitive to changes in structure. In general, the sensitivity to changes in shape is approximately equal to that of viscosity measurements. Furthermore, although the method is analogous in some respects to flow birefringence, it is unique in that it yields information about specific parts of the molecule. In this paper we present an analytical treatment which leads to apparent rotary diffusion constants and dichroism values for complete orientation, thus providing information concerning both the shape and the "internal" structure of the DNA molecule.

Experimental

Description of the Flow Dichroism Apparatus. The Cell.—The cell[4] was constructed of clear fused quartz according to the following specifications: it was rectangular with inner dimensions of 0.238 × 12 × 120 mm., the optical path being 0.238 mm. The thickness of the quartz walls was 2 mm. Quartz tubing (outer diameter 6 mm.) was fused at either end of this section, providing a facile inlet and outlet. The tubes were gently tapered at the junction of the rectangular section to minimize pressure losses in the flowing solutions.

The housing for the cell was constructed in the Sloan-Kettering Institute Machine Shop. Appropriate guides were inserted to ensure that the rectangular faces were parallel to the Beckman spectrophotometer housing block. Provision was also made for sliding the cell away from the light path in order to balance the spectrophotometer.

The Driving Mechanism.—The solutions were placed in a 25-ml. medical syringe driven by a worm gear actuated by a synchronous Bodine motor (output = 6 r.p.m.). A set of gears between the motor and the worm gear provided about 15 speeds. At the end of the path of the syringe the worm gear was reversed automatically by means of a microswitch. The rate of discharge from the rectangular section was easily calculated from the volume discharged from the calibrated syringe.

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[1] This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. C-471) and from the Atomic Energy Commission (Contract No. AT(30-1)-910).

[2] A. Butenandt, H. Freidrich-Frickson, G. Hartung and G. Scheibe, *Z. physiol. Chem.*, **274**, 276 (1942).

[3] (a) W. E. Seeds and M. H. F. Wilkins, *Disc. Faraday Soc.*, **9**, 417 (1950); (b) D. Zucker, J. F. Foster and G. H. Miller, *J. Phys. Chem.*, **56**, 170 (1952).

[4] Made by the Amersil Company, Hillside, New Jersey.