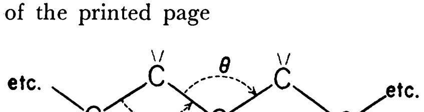
13 July 1956, Volume 124, Number 3211 SCIENCE

Role of Crystallization in Polymers and Proteins

Paul J. Flory

High polymeric structure confers on a substance a set of physical properties which combine the capacity for long-range deformation with high strength. This unique combination of properties is unknown among other types of molecular organization. The distinguishing feature of the polymer molecule brought to prominence by attempts to establish interpretations of these properties is its remarkable configurational versatility. The possibility of rotations about the valence bonds that join successive atoms of the polymer chain gives rise to a vast array of molecular configurations. This fact assumes more than passing interest when it is realized that molecular configurations and macroscopic dimensions of a polymeric substance frequently may be interrelated. Before proceeding with the discussion of mechanical and elastic properties of polymers from this point of view, it is appropriate to consider briefly the configurational character of the long-chain polymer molecule.

A chain of $n + 1$ carbon atoms connected in series by n single valence bonds, each of length l and at an angle θ to its predecessor, is conveniently represented as follows, within the restrictions imposed by the two-dimensionality of the printed page



While this manner of representation is satisfactory for the purpose of conveying the chemical structure, it is a grossly erroneous portrayal, in general, of the spatial configuration of the chain. Rotations about chain bonds, the angle θ between successive bonds being preserved, destroy the planarity of the configuration implied by the foregoing representation. An almost unlimited variety of three-dimensional configurations differing in the values of the angles of rotation about each bond is thus possible. This multitude of irregularly coiled configurations must be summarized in statistical terms if progress beyond this point is to be made.

It is customary for this purpose to adopt the distance r separating the ends of the chain as a suitable measure of the spatial "size" of a given configuration. The planar configuration depicted in the previous paragraph corresponds to the maximum extension of the chain consistent with fixed values of l and θ. We thus have

$$r_{\max.} = nl \sin(\theta/2) \qquad (1)$$

for the distance of separation of the end units in this form. In the irregularly coiled forms, such as those that arise from rotations about bond angles as discussed in the previous paragraph, the value of r varies widely from one configuration to another; generally $r << r_{\max.}$ for such configurations. The average size of the polymer molecules may then be characterized by the root-mean-square value of r, which we designate by $(\overline{r^2})^{1/2}$, averaged over all configurations.

Theoretical calculation of the average distance between chain ends for the randomly coiled molecule recalls the classical problem of random flights treated by Lord Rayleigh, and encountered also in the diffusion of particles that are subject only to Brownian motion. In complete analogy with the displacement of a diffusing particle after tracing a path of specified length (nl), one finds that the root-mean-square of r should be proportional to $n^{1/2}$. If θ equals the tetrahedral angle 109.5° and rotation about valence bonds is completely unrestricted, it is found that

$$\sqrt{\overline{r^2}} = \sqrt{2}\, l \sqrt{n} \qquad (2)$$

Hence, the ratio of the root-mean-square of r to $r_{\max.}$ should approximate $1/n^{1/2}$; if the chain is very long, the average distance r should therefore be only a small fraction of $r_{\max.}$

Experiments on dilute solutions of polymers, on the whole, bear out these predictions. A polystyrene of molecular weight 1 million, for example, for which $n \cong 2 \times 10^4$, exhibits an experimentally determined end-to-end distance of about 700 angstrom units (after correction for effects of solvent-polymer interaction) (*1*). At full extension, we calculate $r_{\max.} = 24{,}000$ angstroms, and for free rotation, $(\overline{r^2})^{1/2} = 300$ angstroms. The observed value is much closer to the latter figure, though significantly larger than it. This discrepancy is attributable to steric hindrance of rotation about the valence bonds. Configurations of most other linear polymers correlate fairly well with those calculated for a random coil. The molecules of cellulose derivatives, however, are considerably more extended, indicating a characteristic stiffness of the chain. More striking departures from the random coil configuration are found in the case of proteins such as collagen and in some synthetic polypeptides as well. Mounting evidence (*2–5*) seems to indicate persistence of rodlike, helical configurations of molecules of these substances in certain solvents even at high dilution. We shall dwell later on the consequences of this peculiarity of the protein molecular chain.

Amorphous and Crystalline States

In the bulk polymer, in contrast to the dilute solution, the chain molecules are obliged to coexist in close proximity.

The author is professor of chemistry at Cornell University. This article is based on the Harrison E. Howe memorial lecture delivered by Dr. Flory before the Rochester Section of the American Chemical Society, Rochester, N. Y., 19 Nov. 1955.

Their geometrical requirements and configurational habits must be reconciled accordingly with the limited space available. Two distinct states of the bulk polymer are clearly distinguishable: (i) the amorphous state in which the polymer chains are irregularly coiled and (ii) the crystalline state in which the chains are arranged to lie parallel to one another. If the molecules are sufficiently flexible, they may readily assert their proclivity for configurational disorder by adopting the former state. Each individual molecule in the amorphous polymer will be entangled in an utterly haphazard fashion with its many neighbors, whose configurations are similarly disordered. Indeed, it may be shown, subject to the foregoing condition of sufficient flexibility, that the configurations of individual chain molecules in the amorphous state should be equivalent to those which they would adopt in a dilute solution (*6*) (aside from effects specifically dependent on the interaction with the solvent). In other words, if the life history of one molecule of the bulk amorphous polymer could be followed photographically, it would be observed to wander, in the course of time, through a series of configurations not unlike those it would assume in a dilute solution (although the rate of transformation from one form to another would be very different). The ability of a high polymer to sustain a very large deformation when in this amorphous state (that is, noncrystalline state), is an immediate consequence of the configurational potentialities of its molecules. The randomly coiled chain molecule may comply with the applied stress merely through a readjustment of bond rotation angles. Macroscopic dimensions and molecular configurations are often directly related one to the other in polymeric substances, as I shall have occasion to emphasize repeatedly in this article.

The amorphous state of random disorder requires for its existence a fairly high minimum tortuosity of the participating polymer chains (*6*). The space cannot be filled to high density with long, semirigid rods, or even with gently curving ones. If the polymer chains concerned cannot submit to the required flexibility without intolerable strain, a state of parallel order will be preferred. In this state, the molecules may be presumed to occur either as (nearly) fully extended planar zigzag chains, or in helical forms as dictated by the stereochemical requirements of their chain substituents (and in the case of proteins by intramolecular hydrogen bonding). Thus, a state of parallel order *could* arise without the aid of intermolecular forces (*6*). In actual instances where such order occurs, forces operating between the chain molecules may be expected, however, to further the ordering process. If the chain possesses a regularly repeating structure with polar groups or substituents occurring at definite intervals, such forces will tend to align adjacent chains so that polar groups and/or substituents occur in regularly spaced planes intersecting the molecular axes. We may thus construct a theoretical basis for the well-known phenomenon of crystallization in polymers. Crystallization of a polymer may therefore be attributed to two factors: chain inflexibility and intermolecular forces. The extent to which these factors are separately responsible for the crystallinity in a given polymer remains largely in the realm of conjecture and need not concern us at this point. It is sufficient to note that x-ray diffraction studies, supported by other observations, demonstrate the occurrence of states of order in polymers having structures which are repeated with regularity along the polymer chains and that in such states the chains lie parallel with their axes adjusted longitudinally as required to bring corresponding groups of adjacent chains into alignment.

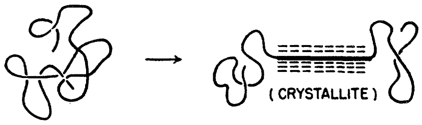


Fig. 1. Configurational change in crystallite formation.

Further investigations involving a variety of techniques, including infrared absorption spectra and density determinations as well as x-ray diffraction, reveal that crystalline polymers contain a proportion of polymer in the amorphous state which may comprise from 10 to 50 percent or even more of the sample. The line breadths of the comparatively diffuse wide-angle x-ray reflections are indicative, moreover, of crystalline regions, or crystallites, having dimensions only of the order of 100 angstroms. This result is supported by low-angle x-ray diffraction. The crystallites evidently are interspersed with a matrix of amorphous polymer. The crystalline regions being much smaller than the lengths of the chain molecules, each of the latter must traverse a number of the former. The participation of a portion of an irregularly coiled polymer chain in the formation of a crystallite is depicted schematically in Fig. 1.

Failure of the degree of crystallinity to rise to higher levels seems to be due to the intractability of the long polymer chain, especially when a portion of it is imbedded in a crystallite. Entangled sections of chain molecules trapped in the amorphous regions between crystallites are unable to undergo the configurational rearrangements required for deposition on the surface of a neighboring crystallite.

Crystallinity disappears above a characteristic temperature that is referred to as the melting point. Melting is accompanied by a latent volume change, a latent heat, increase in transparency (usually), and a marked decrease in elastic modulus and strength. Some investigators have nevertheless been reluctant to regard the transformation in a polymer system between crystalline and amorphous states, or its reverse, as a phase transition. This reluctance has been inspired by the submicroscopic polycrystalline structure, on the one hand, and by the rather broad melting ranges sometimes observed on the other. It may be shown, however, that the former fact does not in principle disqualify the transformation from classification as a phase transition. The breadth of the melting range may be greatly reduced by adopting a slow heating schedule which assures ample opportunity for annealing before completion of the melting process. The final disappearance of crystallinity then occurs abruptly at a reproducible temperature, provided that the chain consists of a sequence of regularly repeating identical units. This melting point is depressed by diluents in strict accord with the dictates of thermodynamic equilibrium as applied to a phase transition (*7, 8*). Crystallization of the supercooled melt proceeds by growth about nuclei which may be generated spontaneously in the pure melt or may develop about foreign heterogeneities (*9, 10*). The crystallization process conforms exceedingly well with nucleation theory of phase changes in general and hence would not be explicable as a gradual homogeneous improvement in molecular order. In the following discussion we adopt the thesis that the amorphous-crystalline transition and its reverse are properly regarded as phase transitions, notwithstanding the small dimensions of the crystallite phases.

Transformation between the amorphous and crystalline states may—under suitable circumstances, and especially at high deformation (elongation)—exert a

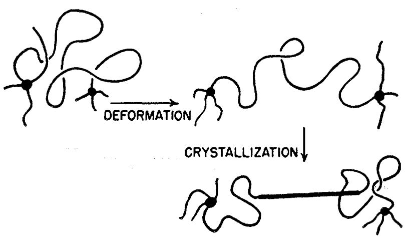


Fig. 2. Deformation of a randomly coiled chain of a network structure, followed by incorporation of part of the chain in an oriented crystallite. The crystallized portion is indicated by a heavy straight line, and cross-linked points are indicated by heavy dots.

marked effect on the elastic characteristics of the polymer. Specifically, a change in the length of the polymer may induce the development of oriented crystallites; conversely, growth of oriented crystallites may cause an increase in length. This coupling of a phase transition with macroscopic dimensional changes presents various interesting features whose importance contrasts sharply with the scant attention given to them.

Mechanism of Elastic Response in Amorphous Polymers

Let us first consider the underlying mechanism which endows a chain polymer in the amorphous state of random coiling with the capacity to translate dimensional change into stress. If we choose to characterize each random coil molecule by the vector **r** joining its ends, the lengths and directions of these vectors will vary statistically from one molecule to another. In the absence of an externally applied restraint, such as a stress which may in one way or another be transmitted to the molecules, the most probable situation for an individual molecule will be that characterized by the lowest possible value of the scalar end-to-end distance r, which will be realized when the end units of the chain lie adjacent to one another. In common with related statistical problems, however, this most probable state will be of comparatively rare occurrence owing to the competition of numerous only slightly less probable states in which the vector **r** assumes various values greater than zero though small compared with $r_{\text{max.}}$ for full extension. These deviations from the most probable $r = 0$ are statistical fluctuations, and they may be represented mathematically by the normal error, or Gaussian, function. We have already expressed the root-mean-square value of the magnitude of r in Eq. 2 for the case of a tetrahedrally bonded chain. This relationship should hold quite generally, although the numerical constant ($2^{\frac{1}{2}}$) will need to be adjusted somewhat as required by bond angles (θ) and hindrances to rotation about them.

Owing to Brownian motions of the chain elements, the configuration of the polymer molecule will be subject to incessant rearrangements. Its end-to-end length r consequently will undergo frequent and unpredictable alterations, so that in the course of time r will wander, as it were, over the entire range of the statistical distribution. If, however, the end points of the chain were fixed in space, they would experience impulses arising from the thermally stimulated Brownian motions of the chain segments, which tend to alter the configuration. The direction of these impulses will be variable, some of them tending to increase r and others to decrease it, but averaged over a sufficient period of time their resultant will be in the direction which would, if it were permitted, change r toward its most probable value —that is, $r = 0$. The intensity of the impulses will depend on the temperature. A complete analysis of the problem shows that the average force delivered by these impulses will be proportional to the absolute temperature T and to the distance r, provided that it is less than about half of $r_{\text{max.}}$ The theoretical equation for the average force f in a single chain is

$$f = 3kTn(r/r^2_{\text{max.}}) \qquad (3)$$

where k is the Boltzmann constant, n is the number of statistical units in a chain between cross linkages, and $r_{\text{max.}}$ (see Eq. 1) is the maximum extension of the chain.

It remains to consider how the distance r of an individual chain may be coupled with the macroscopic dimensions of the sample. This will depend of course on the structure of the polymer. The cross-linked network structures, such as occur in vulcanized rubbers, are illustrative. Polymeric networks of this nature may be formed by the introduction of cross linkages at random points of contact between the randomly entangled chains of the amorphous mass. These cross linkages usually occur only at intervals of 100 or so units along a given molecule. Although intervening portions of the molecules may vary their configurations, the points of cross linkage confer a permanence on the structure such that any change of configuration (r) for one chain will entail coordinated configurational changes among those to which it is connected. If the sample is deformed, the cross linkages must assume new relative positions in space as dictated by the deformation, hence the r values for the chains running between successive cross linkages will be altered by the deformation. One may, in fact, consider the molecular chains connecting cross linkages to act like Hooke's law springs, each of which exerts a retractive force (averaged over time) which is proportional to its length r, as expressed by Eq. 3. (The actual molecular mechanism, however, is very different from that responsible for the retraction of a mechanical spring.)

When a polymer having a random network structure (for example, vulcanized rubber) is subjected to simple elongation, for example, components of the end-to-end lengths of the chains in the direction of the elongation will be increased. The resulting alteration of chain configurations produces a force tending to restore the sample to its original length. This force of retraction can thus be related to the changes in molecular configuration resulting from the deformation.

Crystallization and Dimensional Changes

Let us now examine how crystallization, under the influence of an externally applied stress, should be expected to affect the elastic behavior of a polymer. Some sort of network will again be assumed; otherwise relaxations of the chains would lead to permanent displacements of them relative to one another, and hence to nonrecoverable deformation. Consider a chain of this network situated as shown in Fig. 2 with the end-to-end vector approximately parallel to the direction of elongation produced by a fixed tensile force. It is an experimental fact that crystallites produced by stretching generally occur with chain axes rather well oriented parallel to the axis of elongation. If a portion of the chain depicted in Fig. 2 is incorporated in such a crystallite in the manner indicated, the average stress exerted by the chain at its end points will be reduced. This conclusion may be reached merely by applying the principle of LeChatelier, or from more detailed considerations as follows. The units acquired by the crystallite being relegated to fixed positions, only the residual amorphous chain units are capable of undergoing alterations of positions inspired by thermal motions; hence the retractive force is to be attributed to them only. While the remaining amorphous units are reduced in number, the distance which they must span (that is, the vector difference between **r** and the length of the crystallite) is reduced much more owing to the disproportionately greater distance taken up by those units which have crystallized. The mean degree of extension $r/r_{\text{max.}}$ for the amorphous sections will therefore have been reduced due to the nearly perfect alignment of those units which have crystallized; hence the force delivered to the cross linkages will be diminished as a result of crystallization.

Considerations such as these lead to the conclusion that orientation imposed by stretching should promote crystallization and, conversely, that crystallization in a previously oriented (for example, elongated) polymer should diminish the stress. These expectations are amply confirmed by experiments on extensible polymers that are capable of undergoing crystallization. The melting point is increased by elongation; occurrence of crystallization while the sample is held at fixed length is accompanied by a decrease in stress. If in the latter experiment the temperature is lowered below the melting point for the undeformed polymer, the stress may, during the course of crystallization, fall to zero, and the length may subsequently increase spontaneously beyond the stretched length (*11*).

Having discussed the mechanism by which a stress is generated in an amorphous polymer as a result of deformation and how this stress may be modified by crystallization, we direct attention now to the inferences which may be drawn concerning the molecular state in a substance of unknown constitution from its elastic and thermoelastic behavior. If this substance is capable of sustaining a large elastic deformation, the presence of long, irregularly coiled polymer molecules is unequivocally indicated. If the polymer recovers its original dimensions after release of the stress, the presence of cross linkages, or physically equivalent interchain connections, may be assumed as well. Crystallization may or may not be induced during the deformation. If it is not, the deformation produces a directly calculable alteration in the statistical distribution of the lengths (r) of the chains between cross linkages. The deformed distribution will represent a less probable state and hence one of lower entropy. Assuming that the force thus generated is to be attributed solely to this change in entropy, the force at fixed sample length should *increase* directly with the absolute temperature T (see Eq. 3). If we choose to observe the length at constant force, it should *decrease* approximately (except at low elongation) as $1/T$—that is, the linear thermal expansion coefficient should be negative. Curve *a* in Fig. 3 is illustrative of this case, which applies to amorphous rubberlike polymers generally.

Consider again the elongated sample held at fixed length. If the polymer has partially crystallized following elongation, its crystallites being oriented in compliance with the deformation, the stress will have been diminished as a consequence of crystallization as previously noted. An increase in temperature will promote melting—that is, the reverse of the process depicted in Fig. 2. The release of chain units from the state of high orientation in the crystallites increases the over-all extensions of the amorphous chains. Thus, in addition to the increase in tension due to the greater Brownian motion at the higher temperature (to adopt an alternative manner of expression of the effect described in the previous paragraph), there will be a further—and probably considerably greater—increase in tension due to melting. At constant force, this will be observed as an enhanced thermal contraction, as depicted by curve *b* in Fig. 3.

Finally, if the polymer were brought to a high degree of crystallinity which remains substantially unchanged throughout the temperature interval, the material should then exhibit normal, (that is, positive), thermal expansion (see curve *c* in Fig. 3) characteristic of the crystalline state of matter generally.

The first two types of thermoelastic behavior are observed in vulcanized rubber. At moderate elongations in the range from 1.5 to 3 times the relaxed length, ideal rubber behavior (curve *a*) prevails. At somewhat higher elongations, where the rubber is partially crystalline, the melting which accompanies an increase in temperature causes the negative thermal expansion to be considerably greater in magnitude (*12*). Highly oriented fibers at temperatures well below their melting points sometimes fall in the third classification, which is better illustrated, however, by the fibrous proteins to be discussed in the second section following.

The crystal-liquid phase transition could, in principle, be used to convert thermal into mechanical energy in a heat engine in which the working substance—a crystallizing polymer—contracts at high temperatures due to melting and lengthens due to oriented crystallization as the temperature is lowered. [The Wiegand demonstration machine (*13*) is similarly actuated by the thermoelastic properties of amorphous rubber; however, crystallization probably is not involved over the range of its operation.] The elastic force and sample length in such a system would perform as analogs of the pressure and volume in a steam engine, where the phase change is one of vaporization rather than melting.

A crystallizing polymer having functional groups of such a nature as to be readily altered by a change in the chemical environment could be used as the working substance of a machine for converting chemical into mechanical energy. Alteration of the chemical environment in such a way, for example, as to promote ionization of the functional groups should encourage melting—that is, lower the melting point—and hence bring about contraction. Lengthening would be produced by an opposite change. Such a mechanical-chemical system, since it depends on a phase transition, should be a far more sensitive converter of chemical to mechanical energy than the much-discussed polyelectrolyte systems which give rise to relatively low isotropic forces of osmotic origin.

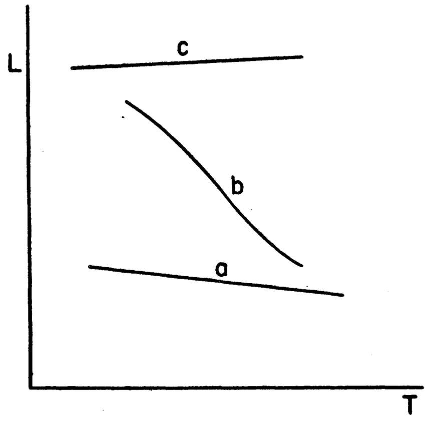


Fig. 3 Relation of length to temperature for (*a*) moderately extended ideal rubber, (*b*) oriented, partially crystalline sample, and (*c*) fully crystalline fiber.

Structure of Fibrous Proteins

Wide-angle x-ray diffraction offers abundant evidence for ordered, or crystalline, arrangements of the polypeptide chains of native proteins. The degree of crystallinity is often high, the fraction of truly amorphous, irregularly coiled chains outside crystalline regions being relatively low. On the other hand, the degree of perfection of order within the crystalline regions generally is rather low—as evidenced by the breadth and small number of x-ray reflections observed—even by comparison with the crystallinity found in other polymers. The imperfect organization of the crystalline regions probably is due to the bulkiness of the substituents on the amino acids units and to the differences between these substituents on the various peptide units.

The polypeptide chains in the fibrous proteins α-keratin, myosin, epidermin, and fibrinogen appear to be arranged in their native states in the α-helical configuration of Pauling and Corey (*14*). These configurations are stabilized by intramolecular hydrogen bonds between amide groups occurring in one turn of the helix with those of the preceding and succeeding turns. The individual polypeptide chains thus assume the forms of rigid rods having diameters (about 10 angstroms) which are small compared with their lengths. The composite fiber is made up of aggregates, or fibrils, in which the rodlike helices are aligned parallel to one another in regular (hexagonal) transverse arrangement. Intermolecular interactions between adjacent helices are weak; the structure derives its stability predominantly from the intramolecular hydrogen bonding within each chain molecule.

The configuration of the polypeptide chains in native collagen has not been established unequivocally (*15*). The N—H bonds of the amide groups, according to polarized infrared studies, are directed approximately perpendicular to the fiber axis, suggesting the possibility of intermolecular hydrogen bonding. Other evidence points to the presence of more or less discrete "protofibrillar" structural entities (*16*) consisting of either a single chain wound in a shallow helix, or of three chains wound together in a triple helix (*15*), or possibly a "coiled-coil" (*17*), of greater pitch. Whatever the structure of the protofibrillar element of collagen may be, it seems unlikely that these elements are bound together by strong hydrogen bonds; hence the primary molecular entity for present purposes may also be regarded as an internally bonded rodlike structure.

Upon stretching under suitable conditions, the α-keratins can be converted to their β forms in which the polypeptide chains are nearly fully extended and hydrogen bonding occurs between amide groups of adjacent chains. (Silk fibroin and feather keratin possess the β-structure in their native states.) The β-form resembles more closely the structures generally found for polar fiber-forming synthetic polymers, in which the crystal structure derives its stability primarily from interchain forces. Synthetic polypeptides may similarly occur in either the α or β modifications, and in some instances either form may be obtained by casting from solutions in different solvents (*18*).

A protein can be rendered noncrystalline by subjecting it to suitable conditions of solvent environment and temperature. It then dissolves or swells; in the case of a fibrous protein, it also shrinks. The most thoroughly investigated transformation of this sort is the shrinkage of collagen that occurs in water in the vicinity of 60°C or in ethylene glycol at approximately 68°C (*19*). The sample shrinks to about 20 percent of its initial length, and the x-ray diffraction pattern which is characteristic of crystalline native collagen simultaneously disappears. In the swollen, contracted state, the material exhibits typical rubberlike behavior; it is reversibly extensible, and it responds to extension with a retractive force which, for a given extension, is approximately proportional to the absolute temperature. It must be concluded that in the shrinkage of collagen the polypeptide chains abandon the ordered arrangement of the crystal for the greater configurational freedom offered by the random coil state.

The shrinkage process in collagen occurs abruptly within a temperature interval of a few degrees, and it is accompanied by an increase in total volume, by the absorption of a latent heat, and, as mentioned previously, by a "latent-length" change. Under appropriate conditions, the melting point can be determined within a degree or two. It is increased by tensile stress and depressed by diluents in a systematic fashion. It has been shown recently (*19*) that collagen, previously rendered amorphous by heating in ethylene glycol and subsequently allowed to remain for a period of hours at a temperature some 20° below the initial melting point, gradually undergoes a spontaneous recrystallization as indicated by an unmistakable, though small, decrease in volume. When the temperature is raised, the re-formed crystalline material melts at the same temperature as previously observed for initial melting.

The array of evidence cited admits of no other interpretation than that which attributes the shrinkage of collagen to a phase transition. Nearly two decades ago, Wöhlisch (*20, 21*) applied the term *melting* to the shrinkage of collagen and published a series of papers upholding this view not only for collagen but for other fibrous proteins as well. His pioneering work has been largely ignored in recent reviews of the subject, and few investigators have pursued his ideas further. [See, however, the work of Wiederhorn and coworkers (*22*), who have treated shrinkage of collagen from this point of view with a considerable measure of success, and of Pryor (*22a*), who has given a similar qualitative account of dimensional changes in collagen and muscle.] No satisfactory alternative explanation for the pronounced dimensional changes in fibrous proteins has been offered. Whereas crystallinity in proteins has been extensively investigated, the possibility that this crystalline order should, under suitable conditions, be susceptible to melting does not seem to have received the attention it deserves (*15, 16*). The explanation for this surprising oversight probably lies in the general reluctance of investigators to apply principles governing phase equilibria to the melting, or dissolving, of a polymeric substance. Meanwhile, evidence has accumulated which fully justifies the contention that melting in synthetic polymers, natural rubber, and cellulose derivatives is in fact a phase transition (*8*). The melting of proteins obviously should now be treated from the same point of view. In so doing, we shall circumvent the perfunctory classification of the phenomenon of shrinkage in collagen and other fibrous proteins as a denaturation—a nebulous term meaning many things, but more often than not it may well be taken to signify melting.

Lack of strong interchain forces between adjacent molecules in the internally hydrogen-bonded helical form might seem to weigh against the case for a phase transition. This supposition is incorrect, however. As we have seen, melting (to be distinguished from dissolving *without* disruption of the helix) in fibrous proteins involves conversion of the polymer chains to the random coil state. Such processes must necessarily be cooperative in nature; hence they should exhibit the characteristics of a phase transition. One may, in fact, show that transformation to this random state should manifest itself as a first-order transition even if interchain forces contributed not at all to the stability of the crystal (*6*). The helical structure confers a rigidity on the molecule which dictates parallel arrangement of the molecules except at very low protein concentrations. The transition should be cooperative and of the first order, regardless of whether stabilization of the crystalline form depends primarily on intermolecular or on intramolecular forces (*6*). Moreover, even if molecularly dispersed at high dilution, transformation of a polypeptide chain from the internally hydrogen-bonded helical configuration to a random coil should occur within a narrow temperature interval, which would become vanishingly small if the chain length were increased indefinitely while maintaining constancy of composition and structure. This conclusion follows at once from considerations, which were presented recently by Schellman (*23*), of the internal energy and entropy changes associated with the transformation of individual polypeptide chains.

Recent investigations on solutions of collagen (*4, 5, 24, 25*), gelatin (*3*), and several synthetic polypeptides as well (*5*) indicate that particles of rodlike form may persist in certain solvent media, even at very low concentrations where molecular dispersion should prevail. Evidence for this conclusion rests primarily on the high viscosities of such solutions, on dimensions indicated by light-scattering measurements, and on optical rotation. Such behavior is, of course, consistent with the predominantly intramolecular character of the forces responsible for stabilization of the helical molecular configuration. Statistical mechanical considerations of the properties of solutions of highly asymmetric rodlike particles show that only a very small lateral attraction between the particles should suffice to bring about parallel aggregation of the rods (*6*).

Electron-microscope observations on fibrous proteins such as collagen, elastin, and myosin reveal the presence of fibrils having diameters down to about 100 angstroms or less. These fibrils in turn aggregate to larger filaments. The foregoing considerations suggest a mechanism of fibrogenesis involving primary formation of single molecules (protofibrils, *16*) in the rodlike helical form, which then, depending sensitively on the effect of the surrounding solvent medium on the intermolecular interaction, may aggregate to fibrils. It thus seems likely that ordered arrangement of the individual molecules precedes aggregation.

The process of formation of ordered molecular arrangements—that is, fibrils—in proteins evidently departs significantly from the corresponding generation in synthetic polymers of ordered regions—that is, crystallites. Intermolecular forces usually are assumed to contribute substantially to the latter process (although their importance probably has been overemphasized, *6*). Successive units of the molecular chains consequently adopt the ordered configuration required for the crystalline state concurrently with, and not prior to, their deposition on the growing crystallite. The molecule as a whole, or large sections thereof, ordinarily will not assume the rodlike configuration without the incentive offered by interchain forces prevailing in the crystalline arrangement. Ordering of the molecular chains may therefore be looked upon as a consequence of crystallization. In the formation of fibrillar proteins, on the other hand, the intraparticle forces operating within the helical configuration may be sufficient alone to produce the ordered molecular configuration. Very small intermolecular attractions should then fulfill the conditions necessary for the generation of crystalline fibrillar material. Regardless of whether or not independent particles in the rodlike configuration occur as actual intermediates which subsequently aggregate to fibrils, the occurrence of crystalline order in fibrous proteins may thus be attributed predominantly to the propensity of the polypeptide chain for its characteristic rodlike helical form. It may be significant also that fibrogenesis of proteins takes place in media of very low protein concentration, whereas synthetic fibers crystallize from a concentrated solution or melt.

There is ample evidence for the formation of microfibrillar structural organization also in highly drawn synthetic fibers (*26*). However, a considerable fraction of amorphous material usually remains interspersed with the oriented crystallites, and the extent of fibrillar development appears to be much inferior to that occurring in the fibrous proteins. Irrespective of the adequacy of the foregoing interpretation, the effect of the characteristic morphology of the protein fiber on its physical properties should not be obscured by attempts to draw close analogies with synthetic polymers (other than the synthetic polypeptides, of course) and rubber (*21, 27, 28*). It may readily be shown that theories of elasticity and swelling developed for more random systems should not be directly applicable to fibrous proteins, for example (*29*). Accordingly, it becomes necessary to adapt current theories of polymer behavior to the circumstances prevailing in fibrous proteins.

Elastic Properties of Fibrous Proteins

The partial melting of a fibril consisting of a parallel bundle of polypeptide chains is diagramed in Fig. 4. In accordance with the concept of the transformation as a phase transition, the crystalline regions, or zones, are depicted as remaining highly ordered, though coexisting with an amorphous zone wherein crystalline order has disappeared altogether. The latter should therefore be rubberlike and readily extensible under tension. It is possible to show that for any tension which the amorphous fibril is likely to withstand without rupture, its length will be less than that for either the α or the γ helix of Pauling and Corey (*14*). The fibril length must decrease, therefore, as melting proceeds.

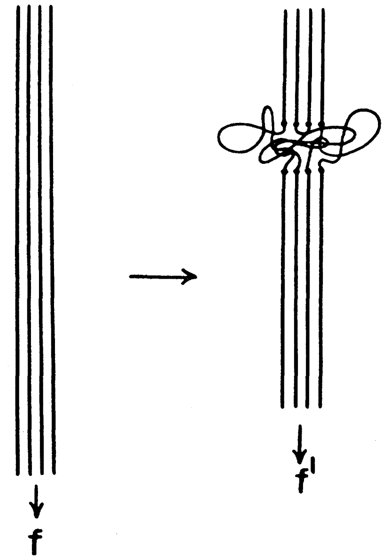


Fig. 4. Partial melting of a highly oriented fibril brought about either by elevation of the temperature or by diminishing the tensile force from f to $f' < f$.

We pause at this point to consider the force-length relation for the amorphous zone in a partially melted fibril. The configuration of the section of a molecular chain which extends through this zone will be constrained by the adjoining crystalline regions, which fix the positions of its ends. The crystalline boundaries perform the role of cross linkages in a network structure. The analogy is by no means exact, and grave errors might result if one were to adopt the tempting conclusion that conventional rubber elasticity theory should apply literally to the amorphous zone.

An important distinction arises owing to the axial orientation of the end-to-end vectors **r** for the chains which traverse the amorphous zone. Since we assume perfect parallel order of polymer molecules in the initial crystalline fibril, and since partial melting of a section of the fibril does not dislodge the bounding units, these must necessarily define a vector which is parallel to the fiber axis. (If the fibril melts completely, yet the molecules are constrained by cross linkages of one sort or another, the same considerations should apply approximately, provided that the cross linkages are separated on the average by distances large compared with the fibril diameter.) This situation contrasts sharply with that existing in a random network structure whose chain vectors are distributed uniformly over all directions in the relaxed state.

The elastic retractive force generated from elongation of the amorphous zone of the fibril should, under the circumstances described, be given merely by the sum of the forces for each of the σ chains making up the cross section, or, according to Eq. 3, by

$$f = 3kT\sigma nr/r^2_{\max}. \qquad (4)$$

where n is the number of statistical segments within the length of the amorphous section of one chain, r is the axial length of the amorphous zone of the fibril, and $r_{\max}$ is its length at full extension of the chains. Equation 4 predicts a retractive force proportional to the length. It also predicts that the length of the amorphous zone should vanish under a load of zero. Imperfections of order, disregarded under the foregoing idealized assumptions, should produce (small) transverse components in the vectors **r**, and these will mitigate the physically unpalatable prediction of complete collapse in the axial dimension. Extraneous structural matter restraining transverse expansion of the fiber would act likewise. Although vitiated somewhat on these accounts, Eq. 4 may be adopted as an approximate "equation of state"; it differs from, and is simpler than, that for a random rubberlike network (*7*).

If permanent (that is, covalent) cross linkages occur between substituents belonging to adjacent chains of the protein fibril, these may be presumed to have been formed subsequent to generation of the fibril. The cross linkages are therefore superimposed on a previously ordered structure, in contrast to the conventional cross-linking of more or less randomly disordered polymer chains. Cross-linking should produce effects which are strikingly different in the two cases. Those introduced in the random state increase the modulus of elasticity in direct proportion to their number, and they diminish the stability of subsequently developed crystallites. Cross-linking of parallel chains, on the other hand, should increase the stability of crystallites (that is, raise their melting point) and have no effect on the modulus of elasticity of the amorphous polymer obtained by melting. This somewhat surprising result follows rigorously from the requirement that the end-to-end vectors for each chain lie parallel to the fiber axis (*29*). The assumed absolute parallelism will be justified only to the extent that cross-linking is confined to perfectly ordered fibrils in which the length of a cross linkage is negligible compared with the distance between cross linkages. The qualification should not be serious, hence Eq. 4 may be expected to serve as a satisfactory approximation for the retractive force in the amorphous polymer irrespective of the degree of cross-linking.

If the fibril is uniform throughout its length, melting should take place at a sharply defined temperature which will depend on the environment (that is, the surrounding liquid medium) and on the force f. At a temperature such that the fibril is amorphous at $f=0$, the length L should first increase continuously with f until the critical force for crystallization is reached, whereupon the sample should elongate at constant f until it is totally crystalline. This behavior, illustrated in Fig. 5, is analogous to vapor-liquid condensation, with f and L replacing P and V as variables. The value of the critical force will of course depend on the temperature and may in fact be negative.

The dependence of the length on the temperature at constant force is illustrated in the right-hand diagram of Fig. 5. At high elongations, the small positive thermal expansion characteristic of a crystalline solid is indicated. This line is interrupted by sudden shrinkage at the melting point; thereafter, the length decreases gradually with temperature as for an ideal rubber (see Fig. 3). The melting point appears as a discontinuity in the diagram and should increase with the force f. The thermal behavior of collagen in the vicinity of its shrinkage temperature conforms remarkably well to this rather idealized scheme.

Melting seems to occur over an appreciably wider range of temperature in other fibrous proteins. Such diffuse melting may be due to variations in chemical structure along the fiber axis, or to nonuniformities in the cross-sectional area. These variations should broaden the melting range, with the result that the discontinuities indicated in Fig. 5 are smoothed to continuous curves. Force-length and length-temperature diagrams for such a fiber are indicated schematically in Fig. 6. Thermoelastic behavior of the type indicated has been found experimentally for myosin (*21, 28*), collagen (*20, 21*), elastin (*30*), and keratin (*31*). (Usually the force-temperature coefficient, rather than the length-temperature coefficient, is reported. These two coefficients are, however, directly related, though of opposite sign.) The thermal expansion coefficients are moderately negative (that is, rubberlike) for the highly shrunken fibers, strongly negative at intermediate lengths, and positive (that is, "normal") at high extensions (*27*). No adequate explanation has heretofore been given for this peculiar pattern of thermoelastic behavior. The wide variation of the thermoelastic coefficient over the extension range has led some writers to discount altogether the significance of thermoelastic measurements on protein fibers (*32*); others have drawn conflicting conclusions arising from differences in the state of the fiber under the experimental conditions (*33*). The superficially peculiar changes exhibited by the thermoelastic coefficients find straightforward explanation in the hypothesis of a phase change.

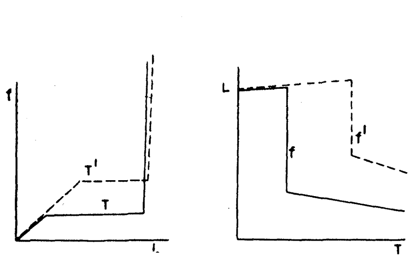


Fig. 5. Force-length and length-temperature relations for an ideal homogeneous fibril, $T' > T$ and $f' > f$.

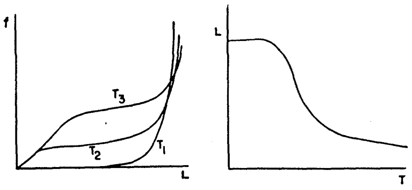


Fig. 6. Force-length and length-temperature diagrams for an inhomogeneous fibril, $T_3 > T_2 > T_1$.

The stress-strain curve for resting muscle (*34*) shown in Fig. 7, corresponds approximately to the curve for T_1 in Fig. 6. Even at a force of zero, under the particular conditions involved, the fiber is predominantly crystalline, although a substantial portion of it may be presumed to be amorphous. With further elongation, the stress-strain curve turns rapidly upwards, and the differential elastic modulus approaches the high value characteristic of complete crystallinity. Virtual elimination of rubberlike elastic elements at the highest elongations attainable is indicated by reversal of the sign of the thermoelastic coefficient to a positive ("normal") value (*28*).

When resting muscle (in contrast to stimulated muscle) is stretched, heat is evolved (*34*) and the volume of the system decreases slightly (*35*). The former of these observations is a thermodynamically required concomitant of the pronounced increase in stress observed with rise in temperature (*21, 28*). Both observations provide compelling evidence for crystallization upon stretching, as was recognized by the investigators responsible for the observations cited (*21, 28, 34, 35*). Whether or not the crystal-liquid transition (as here considered) is an integral part of the mechanism of dimensional change was not, however, explicitly answered (*34, 35*). Other authors have been inclined to dismiss the thermoelastic effects and indicated changes in crystallinity as matters of secondary importance.

The evidence is less clear in the case of stimulated muscle. Thermal and volume changes are actually the reverse of those cited for resting muscle, heat being evolved (*36*) and the volume decreasing (*37*) when the excised muscle, or muscle fiber, is stimulated under either isometric or isotonic conditions. These results might seem to rule out melting during contraction in active muscle. It must be borne in mind, however, that the complex chemical changes, brought on by stimulation and occurring in the muscle and in the associated sarcoplasmic fluid, may well obscure the thermal and volume effects due to partial melting of the muscle fibrils.

Absence of positive evidence notwithstanding, it is tempting to speculate that partial melting of crystalline fibrils may also provide the key to the mechanism whereby muscle transforms chemical energy to mechanical energy with such remarkable facility. On this view (see Pryor, *22a*), contraction of active muscle is due to melting brought on in some manner by stimulation; lengthening during relaxation signifies recrystallization. It remains to consider how chemical energy is brought to bear on the phase transition during the period of nonequilibrium of the muscle fiber and its environment resulting from stimulation.

Now a change in the chemical environment will, in general, alter the chemical potential of a polymer in the liquid, that is, solution, state. The melting point, or range, will be shifted by such a change. The degree of crystallinity, and hence the external dimension, will reflect this change most sensitively if the fiber is in-

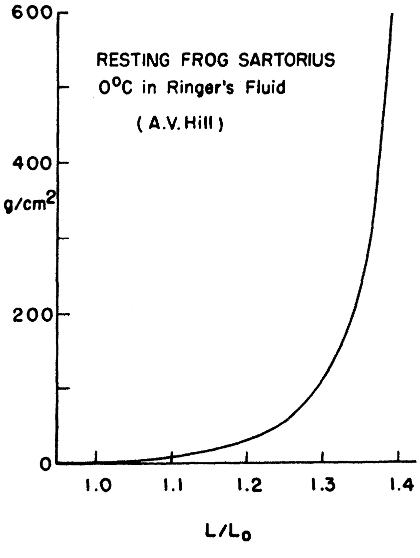


Fig. 7. Stress-strain curve for resting muscle according to A. V. Hill (*34*).

termediate in degree of crystallinity. The "razor edge" character of the dimensional change in muscle may thus be explained.

As for a more precise description of how the chemical environment may affect the crystal-liquid equilibrium, we can only surmise that a reagent generated during stimulation, and presumably a product of myosin adenosine triphosphatase activity, is operative in this connection. It may tend to form complexes with functional groups of the protein or to ionize the latter by proton transfer. One may suppose that accommodation of the complex, or of the ionic group plus its gegenion, is sterically difficult, if not impossible, within the crystalline phase, but that no corresponding difficulty arises in the liquid state. The postulated reagent should therefore tend to shift the equilibrium in favor of the amorphous state, thereby promoting shortening. Its dissipation should allow recrystallization during relaxation, and hence a restoration of the initial (rest) length.

The foregoing explanation seems to be free from a number of objections which may be leveled at the current view that dimensional changes in muscle are inspired by alterations of the charge on the protein molecule considered as a random coil polyelectrolyte. The "electrostatic" theory disregards the crystalline morphology of muscle and leads inevitably to the prediction of more or less isotropic changes in dimensions (that is, shrinkage and swelling, rather than contraction and elongation). It offers no satisfactory basis for the peculiar thermoelastic effects observed. Moreover, it is extremely doubtful that the forces generated in tetanic contraction could be held in abeyance by electrostatic repulsions between charges situated on the resting muscle fibril immersed in the sarcoplasmic fluid.

References and Notes

1. T. G. Fox, Jr., and P. J. Flory, *J. Am. Chem. Soc.* 73, 1915 (1951).
2. A. M. Holtzer, H. Benoit, P. Doty, *J. Phys. Chem.* 58, 624 (1954); R. M. Badger and R. H. Blaker, *J. Phys. and Colloid Chem.* 53, 1056 (1949); S. Newman and P. J. Flory, *J. Polymer Sci.* 10, 121 (1953).
3. C. Robinson, in *The Nature and Structure of Collagen,* J. T. Randall, Ed. (Butterworths, London, 1953), p. 96.
4. C. Cohen, *Nature* 175, 129 (1955).
5. P. Doty *et al., J. Am. Chem. Soc.* 76, 4493 (1954); H. Boedtker and P. Doty, *J. Am. Chem. Soc.* 77, 248 (1955); P. Doty, J. H. Bradbury, A. M. Holtzer, *J. Am. Chem. Soc.* 78, 947 (1956).
6. P. J. Flory, *Proc. Roy. Soc. London* A234, 60, 73 (1956).
7. ———, *Principles of Polymer Chemistry* (Cornell Univ. Press, Ithaca, N.Y., 1953), p. 563 ff.
8. L. Mandelkern, *Chem. Revs.,* in press.
9. A. Keller, G. R. Lester, L. B. Morgan, *Trans. Roy. Soc. London* A247, 1 (1954); L. B. Morgan, *Trans. Roy. Soc. London* A247, 13 (1954); F. D. Hartley, F. W. Lord, L. B. Morgan, *Trans. Roy. Soc. London* A247, 23 (1954).
10. L. Mandelkern, F. A. Quinn, Jr., P. J. Flory, *J. Appl. Phys.* 25, 830 (1954); P. J. Flory and A. D. McIntyre, *J. Polymer Sci.* 18, 592 (1955).
11. W. H. Smith and C. P. Saylor, *J. Research Natl. Bur. Standards* 21, 257 (1938); A. V. Tobolsky and G. M. Brown, *J. Polymer Sci.* 17, 547 (1955).
12. L. A. Wood and F. L. Roth, *J. Appl. Phys.* 15, 781 (1944).
13. W. B. Wiegand, *Trans. Inst. Rubber Ind.* 1, 141 (1925).
14. L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U.S.* 37, 272 (1951).
15. J. C. Kendrew, in *The Proteins,* H. Neurath and K. Bailey, Eds. (Academic, New York, 1954), vol. 2B, pp. 931–942.
16. R. S. Bear, *Advances in Protein Chem.* 7, 69 (1952).
17. G. N. Ramachandran and G. Kartha, *Nature* 176, 593 (1955); A. Rich and F. H. C. Crick, *Nature* 176, 915 (1955); P. M. Cowan, S. McGavin, A. C. T. North, *Nature* 176, 1062 (1955).
18. C. H. Bamford, W. E. Hanby, F. Happey, *Nature* 164, 751 (1949); 166, 829 (1950); C. H. Bamford *et al., Proc. Roy. Soc. London* B141, 49 (1953).
19. R. R. Garrett and P. J. Flory, *Nature* 177, 176 (1956).
20. E. Wöhlisch, *Biochem. Z.* 247, 329 (1932); *Kolloid-Z.* 89, 239 (1939).
21. ———, *Naturwissenschaften* 28, 305 (1940).
22. B. A. Wright and N. M. Wiederhorn, *J. Polymer Sci.* 7, 105 (1951); N. M. Wiederhorn and G. V. Reardon, *J. Polymer Sci.* 9, 315 (1952).

22a. M. G. M. Pryor, in *Progress in Biophysics,* J. A. V. Butler and J. T. Randall, Eds. (Butterworths-Springer, 1950), vol. I, p. 216.

23. J. A. Schellman, *Compt. rend. trav. lab. Carlsberg Sér. chim.* 29, 223 (1955).
24. M. B. McEwen and M. I. Pratt, in *The Nature and Structure of Collagen,* J. T. Randall, Ed. (Butterworths, London, 1953), p. 158.
25. P. M. Gallop, *Arch. Biochem. and Biophys.* 54, 486 (1955); M. B. Mathews, E. Kulonen, A. Dorfman, *Arch. Biochem. and Biophys.* 52, 247 (1954).
26. K. Hess, paper presented at the International Symposium on Macromolecular Chemistry, Milan, Italy, 1954; K. Hess and H. Kiessig, *Z. physik. Chem.* A193, 196 (1944).
27. E. Guth, *Ann. N.Y. Acad. Sci.* 47, 715 (1947).
28. E. Wöhlisch, *Pflugers Arch. ges. Physiol.* 246, 469 (1942); W. Josenhans, *Z. Biol.* 103, 61 (1950).
29. P. J. Flory, *J. Am. Chem. Soc.,* in press
30. E. Wöhlisch *et al., Kolloid-Z.* 104, 14 (1943).
31. H. B. Bull. *J. Am. Chem. Soc.* 67, 533 (1945).
32. K. Bailey, in *The Proteins,* H. Neurath and K. Bailey, Eds. (Academic, New York, 1954), vol. 2B, p. 1048.
33. K. H. Meyer and C. Haselbach, *Nature* 164, 33 (1949); H. J. Woods, *Nature* 164, 34 (1949).
34. A. V. Hill, *Proc. Roy. Soc. London* B139, 464 (1952).
35. E. Ernst and J. Tigyi, *Acta Physiol.* 2, 243 (1951); E. Ernst, J. Balog, J. Tigyi, *Acta Physiol.* 2, 253 (1951).
36. A. V. Hill, *Proc. Roy. Soc. London* B136, 242 (1949); B137, 268 (1950); B141, 314 (1953).
37. E. Ernst and J. Tigyi, *Acta Physiol.* 2, 261 (1951); E. Ernst, G. Lodányi, J. Tigyi, *Acta Physiol.* 2, 271 (1951).

Pathologic Effects of Atomic Radiation

Appreciation of the pathologic effects of radiation on man has required of this committee and its subcommittees consideration of voluminous experimental work on animals, as well as such direct data on human beings as are available. When the results of controlled experimental studies are considered in the light of the human data, it is found that the sequence of pathological changes is indeed quite similar in man and in animals, although man has certain definable peculiarities of response.

The human data include: (i) results of excessive exposure to x-rays and radium in the early days; (ii) results of more moderate exposure to different forms of radiation, as experienced by cyclotron workers; (iii) results of introduction of naturally occurring radioelements into the body, notably radium preparations and thorotrast; (iv) effects of exposure at Hiroshima and Nagasaki; (v) observations on populations irradiated by fallout; [and] (vi) additional observations from clinical radiotherapy, use of artificial isotopes in therapy, a very limited number of accidents in atomic energy work, and certain statistical surveys of large groups.

Experimental work covers the whole field and includes studies of acute and chronic effects on many species of animals.

Certain human effects have to be assumed from consideration of experimental knowledge—for example, early effects of high doses to the central nervous system and results of absorption of most of the artificially produced isotopes—and it is fair to say that the lethal dosage of penetrating radiation for man is less well known than for many other species.

Radiation has been added to the means