Natural Protein Fibers

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ABSTRACT: Alpha keratin fibers (hairs, wools, quills, and other mammalian appendages) together with fibroin fibers such as silks and spiders webs are all highly extensible fibrous proteins for which the mechanical properties are of primary importance both to the animal from which they originate and their ultimate application by man. Similarly, the collagens are highly inextensible fibrous proteins, which form the major component of mammalian skin and connecting structures such as tendons. All these fibrous proteins are biological polymers of polypeptide chains for which the mechanical and allied physical properties, such as water absorption, relate to both their macrostructure and their molecular and near-molecular structure. Because of both their commercial application and their relatively complex structure at the molecular and near-molecular level, interpretation of the physical properties of α-keratin fibers represents the main component of this presentation. The mechanical properties of α-keratin fibers are primarily related to the two components of the elongated cortical cells, the highly ordered intermediate filaments (microfibrils) which contain the α-helices, and the matrix in which the intermediate filaments are embedded. The matrix consists of globular proteins plus water, the content of the latter being dependent on the fibers environment. The Extended Two-Phase Model (ETPM) has been developed and results in a detailed coverage of the bulk mechanical properties of α-helical fibers in terms of their known molecular and near-molecular structure. The inextensible protein fibers, the collagens and fibroins, are also briefly discussed in terms of the relationship between mechanical properties and the structure of these fibers. J Appl Polym Sci 83: 489–507, 2002

Key words: polypeptides; mechanical properties; α-keratin; molecular interactions; water

INTRODUCTION

Naturally occurring fibrous proteins relevant to this discussion fall into one of two categories. The first category is the highly extensible fibers such as the α-keratins (wools, hairs, and other mammalian external appendages such as quills and horns), and fibroin fibers (various silks and spider webs). The second category of fibrous proteins is the relatively inextensible collagen fibers. These fibers form the major component of skins of many animals together with their highly inextensible sinews. In this second category of natural proteins, it is the requirement of high stiffness and low extensibility to break which both result from the molecular and near-molecular structure of these fibers. Other fibrous proteins, in particular the muscle proteins of myosin and actin, have been completely omitted in this article, being beyond the scope relevant to this discussion.

Because of their application commercially, emphasis has been placed on the mechanical properties of α-keratins and the relationship of these properties to both macrostructure and molecular and near-molecular structure.

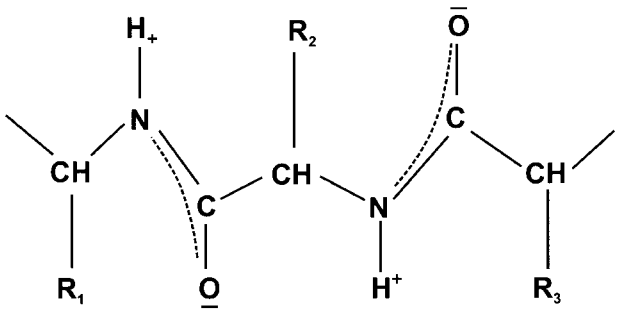

Figure 1 Diagrammatic representation of a portion of a polypeptide chain indicating the planar nature of the bonding to the amide nitrogen N. The displacement of the electrons results in the shortening of the nitrogen to carboxyl C bond restricting rotation about this bond. The displacement of the electrons (- - - - - -) results in an effective dipole as indicated by the negative and positive charges. R_1, R_2 and R_3 are the side chains of the amino acid residues forming the polypeptide chain.

Natural fibrous proteins, as with all proteins, are biological polymers consisting of polypeptide chains formed by the condensation of amino acids into chains of amino acid residues (Fig. 1). Pauling[1] has shown that the amide groups of the amino acid residues forming the polypeptide chains are planar, forming a dipole, as shown, from the positive charge on the nitrogen —NH and a negative charge on the oxygen of the —C═O group. Both the —NH and —C═O groups of the amides are able to form hydrogen bond interactions in all polypeptide chains both as inter- or intra-chain bonds. The side chains (R) may provide Coulombic, hydrogen bonds, and polar interactions, and, in the right environmental conditions, hydrophobic interactions between polypeptide chains. Because the side chains possibly consist of up to approximately 20 differing amino acid residues, the sequence of the amino acid residue with their different side chain groups provide the basis of the structures formed by the interacting polypeptide chains. The only major covalent bond between the polypeptide chains is provided by the diamino acid cystine, which on condensation can result in an inter- or intra-chain bond with each of the two amino acid residues forming a component of a polypeptide chain (Fig. 2). The presence of the cystine in α-keratin fibers is primarily responsible for the high stability of these fibers to environmental degradation by heat, cold, light, water, biological attack, and mechanical distortion. In all polymeric fibers, whether natural or synthetic, covalent bonds within the structure behave as rigid links, transferring the forces within the structure with negligible distortion to themselves. Nearly all the strains within the polymer, aside from flow between molecular chains, is produced in the hydrogen bonds, van der Waals, and Coulombic interactions. This results in a similar stiffness (Young's modulus) for small distortions for all polymeric fibers at temperatures (T_g) low enough to prevent segmental and chain movement. The value of the Young's modulus[2] obtained corresponds to the value of ice of $9.6 \times 10^9 N/m^2$. Ice is completely held together by hydrogen bonding and its Young's modulus contrasts to the value of $10^{12} N/m^2$ for diamond, a solid held completely together by a network of covalent linkages of carbon–carbon bonds (Table I).

In the pursuit of the understanding of the mechanical properties of natural fibrous proteins in terms of the microstructure of the fiber by application of various physical and chemical techniques, it is necessary to separate the factors controlling the mechanical equilibrium state of the fiber and the time-dependant or dynamical properties of the fiber. Techniques such as low- and high-angle X-ray diffraction, electron microscopy, polarized infrared absorption, and optical birefringence all primarily refer to the equilibrium state of the fiber. Dielectric absorption, electrical conductivity, nuclear magnetic resonance, and calorimetry measurements combined often with suitable structural plasticizers such as water,

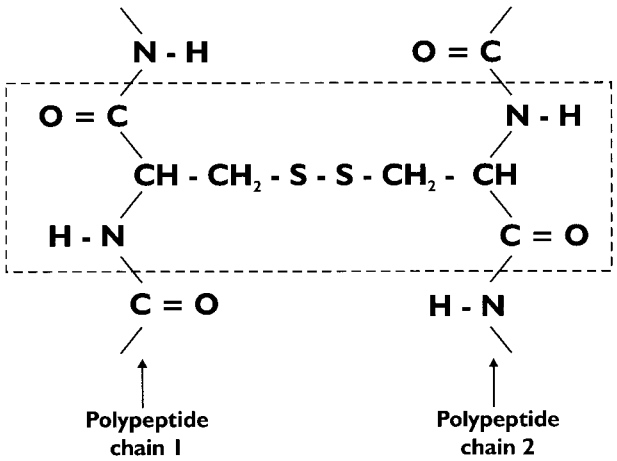

Figure 2 Diagrammatic representation of the diamino-acid cystine residue linking by covalent bonding two polypeptide chains 1 and 2. This bonding represents the main covalent interaction in α-keratin fibers and may exist as either an interchain linkage between two chains or as an intrachain linkage between two components of the same polypeptide chain.

Table I Young's Modulus and the Bonds Extended for Various Solids Below Their T_g (see text)

Solid	Temperature of Measurement (°C)	Initial Young's Modulus (GPa)	Main Bonds Extended
Ice	−16	9.6	Hydrogen bonds
Wool	−190	9.7	Hydrogen bonds and Coulombic interactions
Diamond	−20	1000.0	Covalent C—C bonds
Nylon (6:6)	−57	11.0	Hydrogen bonds and van der Waals interactions
Dacron	−57	20.0	Polar and van der Waals interactions
Orlon	−57	14.0	Polar and van der Waals interactions

various alcohols, etc., reveal the structural dynamics of the fiber. X-ray diffraction data, for example, basically relate to the state of the organized crystalline regions of the fiber with only minor information about the presence of amorphous structures. In the consideration of mechanical properties in terms of a fiber's micro- and macromolecular structure, a complete integration is necessary of all the data available from various techniques.

THE α-KERATIN FIBERS

Morphology

Of the natural fibrous proteins, the α-keratin fibers of the wools and hairs of various mammals have provided the materials from which cloth has been woven or felts manufactured since prehistoric times. The modification of the shape and general appearance of human hair has been a topic of cosmetic interest for mankind, also for a similar period. All hairs and wools consist of three basic components: the external cuticle, the cortex, and the medulla. The cuticle consists of a scale structure over the surface of the main shaft of the hair or wool fiber (the cortex) (Fig. 3). In the case of wools, the layers of scales each about 1 μm thick just overlap, providing a covering for the fiber of the thickness of one scale. In the case of human hair, the scale thickness is about 0.5 μm. These latter scales overlap considerably, with a resultant cuticle structure of 6–10 scale thick-

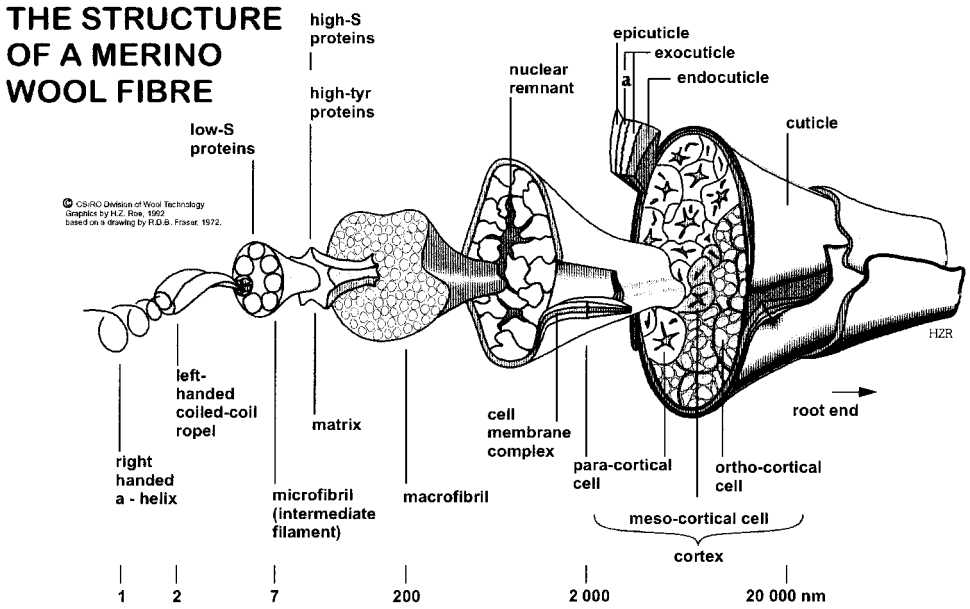

Figure 3 Cross-section diagrams of a merino wool fiber showing the structure at progressive magnifications. The mesocortex shown at the boundary of the bilateral structure is a highly ordered component,[68] differentiated from the paracortex primarily in low-crimped merino wools. (Produced by H. Roe from a drawing by Dr. R. D. B. Fraser.)

ness. In both hairs and wools, the scales form a ratchet-like structure resulting in a directional friction effect, the fibers being smoother in the direction of growth. This scale structure provides the basis of felting in wool and other keratin fibers. The cuticle cells structurally are amorphous, as is indicated by their lack of optical birefringence.[3] Their physical presence provides a barrier to the absorption of larger molecular structures such as dyes. In the consideration of the mechanical properties, the presence of the scales are of marginal effect as shown by tests on fibers with no cuticle such as porcupine quill.[4] Further, tests on fibers from α-keratin sources such as rhinoceros horn, which do not have a cuticle, indicate similar mechanical properties as that of wools and hairs.

Mechanically, the most important component of any α-keratin fiber is the cortex, the main shaft of the fiber, which is covered by the scale structure of the cuticle. The cortex of the fully formed fiber consists of elongated cells of length of approximately 100 μm and of an irregular cross-section of a few micrometers. A cell membrane complex (CMC) of approximately 25-nm thickness separates each cell from its neighbor and provides the only continuous material phase along the fiber. Knowledge of the arrangement of the components within the CMC is still a matter of speculation (Leeder[5]). The CMC plays an important role mechanically, because it provides the adhesion between cortical cells and hence transmits applied external stress to these cells. Because the CMC is only about 6% of the cortex structure,[5] the bulk mechanical properties of the cortex of wool and other α-keratin fibers correspond in their behavior in terms of the prime parameters of moisture content, temperature, and time to a continuum of cortical cells.

Under an electron microscope, cortical cells are seen to consist of long filaments, originally known as microfibrils. These microfibrils, in conformity with similar units of other cell structures, are now referred to as intermediate filaments (IFs). These filaments are oriented parallel to the axis of a wool or hair fiber. The IFs are uniform, with a diameter of about 7.3 nm, and in a wet fiber are set about 11 nm apart.[6] In any cortical cell, the IFs are grouped together in units of about 0.5 μm in diameter called macrofibrils. Within each macrofibril, the IFs are separated by a sulphur-rich protein matrix. Whereas IFs do not appear to vary in dimensions from one α-keratin to another, the matrix varies both in quantity and chemical composition. In any macrofibril, the IFs are spatially related to each other, and are in close interaction, the whole macrofibril acting as a single mechanical unit. The IFs represent approximately 50–60% by mass of the cortex and contain the α-helical material responsible for the characteristic high-angle X-ray diffraction pattern diagnostic for α-keratins.

The other minor components that may be present in α-keratin fibers are the medulla, melanin, and nuclear remnants. The medulla is a group of specialized cells, vacuolated and present in continuous, discontinuous, or fragmented state along the axis of coarser α-keratin fibers. Mechanically these cells represent empty space, and fibers containing these cells are avoided in the textile industry because of their coarseness and their irregular dye uptake. Melanin is granules of pigment which provide the natural coloring material in α-keratin fibers, and mechanically act as small hard intrusions dimensionally of the order 1 μ. Again, in the textile industry, such fibers are normally avoided. The melanin does not represent any part of the molecular structure of α-keratin and is not considered further in this discussion. Nuclear remnants from the original cortical cells represent such a very minor component volumetrically, that they are omitted in any consideration of the mechanical properties of α-keratin fibers. Basically, as far as bulk mechanical properties of an α-keratin fiber are concerned, the fiber dry is regarded as consisting of long IFs oriented parallel to the fiber axis and embedded in a sulphur-rich matrix protein.

The Two-Phase Model of α-Keratin Fibers

Speakman[7] was the first to note that, for small distortion ($\sim$ 1% strain), the longitudinal stiffness of wool fibers was sensitive to the moisture content of the fibers. He found that over the range from dry ($\sim$ 0% relative) humidity to wet (100% relative humidity) within the time scale of his experiments the longitudinal stiffness changed by less than 3 to 1. Over the same range of moisture change, the torsional rigidity changed by more than 10 to 1. Further, he noted that the longitudinal swelling of wool fibers from dry to wet was only about 1.2%, whereas the diametral swelling was about 16% over the same change of moisture content. Speakman suggested that both the swelling and mechanical changes by considering the long molecular chains of the fibers to be preferentially oriented in the fiber direction with a high proportion of water-weakened interactions such

as hydrogen bonds acting at right angles to the fiber direction. The observation that the diameter of wool fibers in an environment of 65% relative humidity is increased by 10% if placed in water, whereas the longitudinal swelling over the same environment range was almost negligible (~ 0.1%), suggested that the wool fibers were made of a structure more rigid than molecular chains swelling laterally relative to each other. The two-phase model[8] of α-keratin fibers was proposed for which long, water-impenetrable relatively rigid cylindrical rods (phase C in Fig. 4) were present in the fiber, set parallel to the fiber axis and embedded in a water-absorbing matrix (phase M). The absorption of water by phase M mechanically weakens this phase, whereas phase C is water-impenetrable, thus is mechanically unaffected by the presence of water. X-ray diffraction studies showed that the swelling diametrally for a wet fiber by the crystalline component is almost negligible (<2%) (Bendit, E. G., private communication). Further, polarized infrared absorption measurements indicate that in heavy water (D_2O), the exchange of hydrogen to deuterium of the amide —NH does not occur at room temperature for the ordered —NH groups.[9] Both of these results identify a water-impenetrable phase with ordered crystalline α-keratin components of the IFs in the cortex. It is this crystalline material in the long axially oriented IFs that corresponds to the phase C cylinders of the proposed two-phase model.

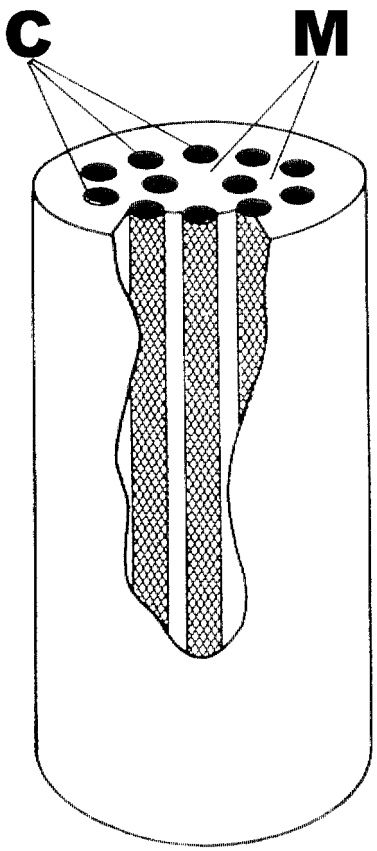

Figure 4 Cylindrical two-phase model of an α-keratin fiber consisting of rigid water-impenetrable rods C set parallel to the fiber axis embedded in the matrix phase M which is available to and weakened by water.

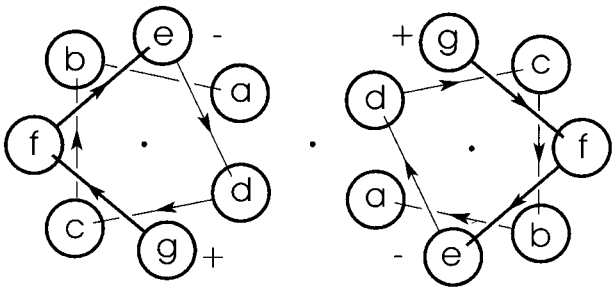

Figure 5 The α-helices form into two-strand ropes with interactions between the helices a result of the heptad repeats of residues (a b c d e f g). The side chains of the residues "e" and "g" are oppositely charged, and "a" and "d" are apolar. The result is a combined Coulombic and hydrophobic interaction between the α-helices. The figure shows two heptad segments of each α-helix viewed in the direction of the axes of the helices.

Astbury et al.[10,11] first suggested from high-angle X-ray diffraction data that the unextended α-keratin fibers consist of organized folded polypeptide chains which on high extension transformed into extended polypeptide chains forming the β-keratin structure. Pauling[12] proposed that the folded structure of polypeptide chains in α-keratins corresponded to the α-helices formed by the interaction of hydrogen bonds between the amide NH and carboxylic CO groups of successive turns of each helix. The polypeptide chains form the α-helix with 3.7 residues of amino acids per turn of the helix. Mechanical and X-ray diffraction measurements by Bendit[13,14] showed that the unfolding of the α-helical structure commences above a few percent longitudinal strain on an α-keratin fiber, suggesting that the α-helical regions extend the full length of the IFs.

Breakdown of the disulfide bonds in the diamino acid residues of cystine by reduction results in the protein fractions forming the α-keratin structure becoming soluble. Three distinct protein fractions[15] are produced: the low-sulfur S-carboxymethyl kerateine A referred to as SC-

MKA, the high-sulfur S-carboxymethyl kerateine B referred to as SCMKB, and in some α-keratin fibers, high-glycine tyrosine proteins rich in the residues glycine and tyrosine. The low-sulfur proteins SCMKA have a helical content of around 50% and have been associated with the IFs, which contain the α-helices forming the crystalline regions. The high-sulfur proteins SCMKB and the high-glycine tyrosine proteins are both associated with the matrix component of the α-keratin fiber cortex.

Residue sequence analysis of the low sulfur SCMKA proteins[16–18] has shown that the central portion of these purified proteins consist of rod-like predominantly helical domains. Each helical domain consists of 88% of purely helical regions with three short non-helical regions between the purely helical groups. The residue sequence of the purely helical segments forms into heptad repeats (a b c d e f g) in which the residues "a" and "d" are apolar, and "e" and "g" oppositely charged (Fig. 5). The helical structures of the SCMKA molecules form into two strand coiled-coil ropes stabilized by the hydrophobic and Coulombic interactions as indicated (Fig. 5). In the presence of water molecules, the hydrophobic interactions produced between "a" and "d" by the water network "protect" the Coulombic interactions between the residues "e" and "g" from the approach of water molecules. The Coulombic interactions reinforce the binding of the two-strand helical rope. The two-strand ropes form the rod-like domains and are the precursors of the IFs in the growing cortical cells of the α-keratin fibers. The α-helices of these rod-like domains with their non-helical tails present in the SCMKA proteins form the polypeptide chains of the IFs. Mechanical, infrared and X-ray diffraction data[19] indicate the presence in α-keratin fibers such as wool and hairs of a water-impenetrable and highly organized component consisting of 25–30% of the whole dry fiber. The water-impenetrable component C of the two-phase model is identified with these water-impenetrable α-helical domains of the IFs in the cortical cells of the cortex structure. In the wet α-keratin fiber, the high-sulfur protein fractions SCMKB, high-glycine tyrosine proteins, and the non-helical water-penetrable material of the IFs, such as the "tails" protein of the SCMKA proteins, together with the absorbed water, are all considered part of the M phase of the two-phase model. X-ray diffraction studies[20] do indicate that the IFs swell for a fiber in water with a diameter change from dry to wet of $\sim$ 6%. Earlier measurements of the movement of the α-helices apart due to fiber swelling in water was obtained from X-ray diffraction data.[21] This data, when corrected for scattering by the presence of water in the fiber resulted in a negligible value for the swelling between the α-helices in the α-helical ropes ($<$2%). This is understandable because of the hydrophobic bonding between the individual α-helices of the α-helical ropes. The swelling of the IFs by about 6% diametrally in water must occur external to the α-helical ropes, and is probably a movement apart of the tetramers, the two pairs of α-helices that are the basic subunits forming the individual IFs. The hydrogen bonding between turns of the α-helices is unaffected by the presence of water and provides the high-longitudinal stability of the α-keratin fibers. The length of a fiber is maintained from the fiber in water to a fiber in equilibrium with an environment at 65% relative humidity. From 65% relative humidity to complete dryness ($\sim$ 0% relative humidity) the length change of 1.2% is produced, as will be

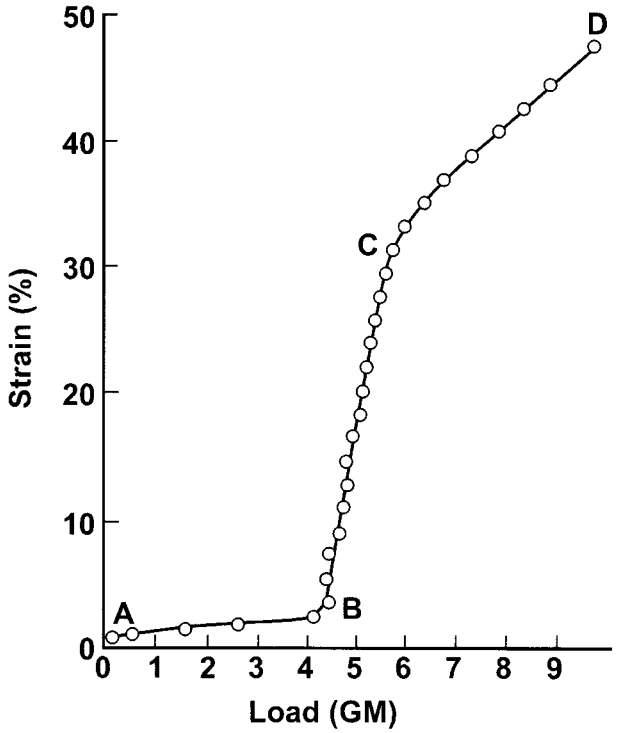

Figure 6 Typical load-extension curve in water at 20°C for a Corriedale wool fiber extended over a period of 25 days. The rate of extension from the origin (0) to the end of the Hookean region (B) was ⅜% per hour and that beyond the Hookean region (BCD) was 1 ⅞% per day. The slow rate of extension reduces the time-dependent component of stress at each extension.

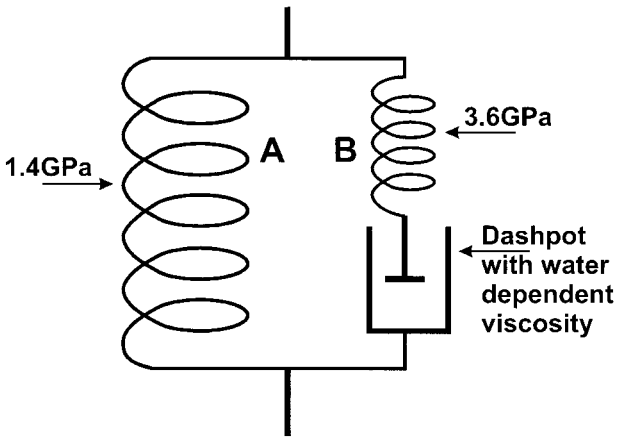

Figure 7 The spring-dashpot model of the Hookean region mechanical behavior of wool and other α-keratin fibers at around 20°C. The quantities indicated are the contributions by spring A and spring B to the Hookean modulus of the fiber. Spring A corresponds to the mechanical behavior of phase C of the two-phase model, and spring B in series with linear dashpot to the water-penetrable phase M.

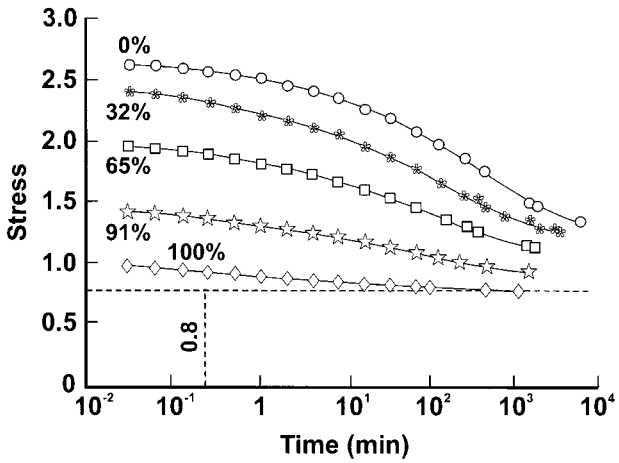

Figure 8 Stress-relaxation of a wool fiber extended at a fixed 0.8% strain at 20°C for different relative humidities of 0%, 32%, 65%, 91% and 100% (wet). The stress in all cases is calculated on the basis of the wet cross-sectional area, and the initial stress in water at 0.8% is taken as unity. Data are for fibers at 20°C and the rate of extension up to 0.8% strain is 10%/min.

shown, by slight distortion of the IFs. The α-helical ropes act mechanically as units the full length of the IFs in the cortical cells.

The Mechanical Properties of α-Keratin Fibers

The mechanical properties of α-keratin fibers are best discussed in terms of the three distinct regions of longitudinal behavior of these fibers with the changing parameters of temperature, time, and moisture content, the latter depending on the relative humidity of the fiber's environment. These three regions of extension versus force applied to the fiber become quite distinct in water. Respectively, the three regions are 1. the Hookean region, 2. the Yield, and 3. Post-Yield regions, which range from 0 to 2%, 2% to 25–30%, and beyond 30% strain. Within these ranges, the stiffness of the fibers is in the approximate ratio of 100:1:10 (Fig. 6). Astbury and Haggith[22] showed that in the initial Hookean region the strain in the fiber corresponds to the distortion of the α-keratin X-ray diffraction spacings in the fiber direction.

The longitudinal behavior of the α-crystalline component of the fiber is as expected on the basis of the two-phase model. For longitudinal extension of the fibers at room temperature, the stiffness changes from a dry (0% relative humidity) to wet (100% relative humidity) environment within the time frame from seconds to minutes by a factor of 2.7 to 1. However, mechanical measurement taken to equilibrium of the Young's modulus of α-keratin fibers shows no difference in the value whether measured wet or dry.[23] Further, even if the fiber is progressively swollen[24] in concentrated aqueous LiBr solution up to a concentration 5M for which the crystallinity of the α-keratin is intact, the stiffness of the α-keratin fibers at equilibrium is unchanged. For all these measurements, the wet diameter of the fiber was applied in the calculation of the Young's modulus. A value of the longitudinal mechanical equilibrium Young's modulus of 1.4 GPa was obtained for wool fibers. This value was found to be maintained for temperatures less than 50°C. The α-keratin fibers behaves in the Hookean region mechanically as a fixed Hookean spring contributing 1.4 GPa to the

Table II Time-Dependent Behavior of Wool Fibers in Stress Relaxation (Fig. 8)

Relative Humidity (%)	Time Constant in Minutes
0.0	5.2×10^{2}
14.4	2.4×10^{2}
32.0	$1.8_{5} \times 10^{2}$
65.0	$1.2_{5} \times 10^{2}$
91.0	4.2×10^{1}
100.0	6.6×10^{-1}

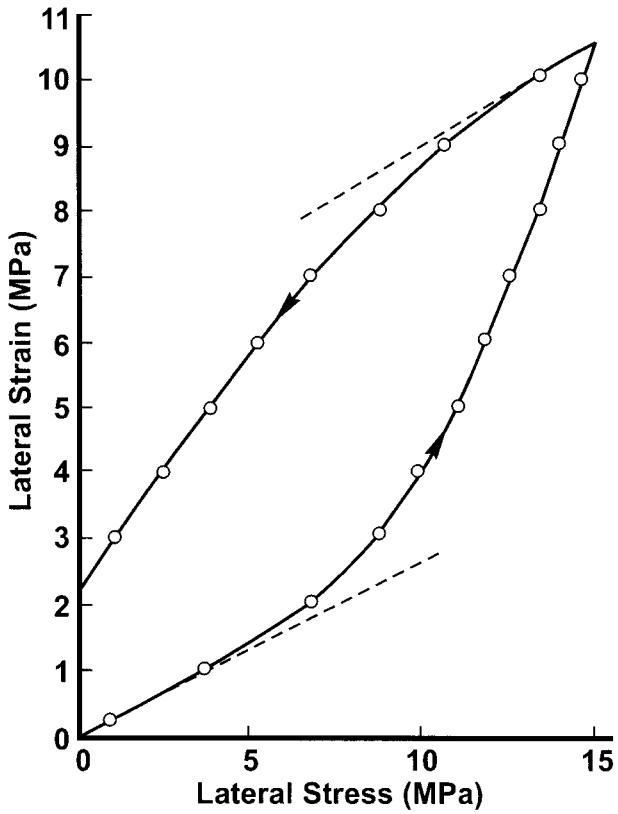

Figure 9 The stress–strain hysteresis curve obtained perpendicular to the growth direction for an African porcupine quill sample in distilled water at 20°C.

Young's modulus, in parallel with a spring and dash pot in series (Fig. 7). The viscosity of the dash pot is moisture- and temperature-dependent and is related to the mobility of the molecular segments, main chains, and side chains of the M phase of the two-phase model. The 1.4 GPa fixed contribution to the Young's modulus corresponds to the water-impenetrable C phase. The stiffness is produced by the opposition to the extension of the ordered α-helical ropes forming the C phase. The time-dependent behavior of the wool fibers[24] is presented in the stress relaxation curves of Fig. 8 and Table II. The M phase is the molecular structure responsible for the time-dependent component of the longitudinal Young's modulus of a fiber. Mechanically, for longitudinal strains phase C and phase M operate in parallel. This is why the phases are equally strained, and the longitudinal forces in each phase are additive for the whole fiber when longitudianlly extended. In water, in particular, because of the relatively much higher stiffness of the C phase, torsional and lateral mechanical properties are more relevant for obtaining information with regard to the M phase. In the latter two cases, the mechanical effect of the phases is essentially to act in series with the changes of properties of M phase dominant. The increase of the initial incremental torsional rigidity of fibers for dry as opposed to wet of 10 to 1 corresponds to a change in the M phase of the order of 15 to 1 or more assuming that the phase represents an estimated 25–30% of the dry fiber volume.

Torsional measurements of single wool fibers in water indicate a highly time-dependent rigidity. Mechanical measurements perpendicular to the fiber direction for rhinoceros horn and porcupine quill in water completely parallel the torsional results obtained for wet wool fibers.[4] The nonlinear visco-elastic behavior of the wet M phase is best described as that of a weak "gel" structure, which with extension at a fixed rate is in progressively weakened by bond breakdown. If the extension ceases, the broken bonds re-form rapidly in equilibrium with the extended state, and on contraction the initial stress–strain behavior mirrors the stress–strain behavior during extension (Fig. 9). This mechanical behavior corresponds to "thixotropy," in which weak bonds in a "gel" structure during extension progressively break down to an extremely mobile state, the "sol" state. On cessation of extension, the bonds reform and the structure is again in a gel state. Measurement on porcupine quill sections in water show an initial incremental Young's modulus of 0.3 GPa which beyond 2–3% strain drops rapidly to $\sim$ 0.01 GPa with extension up to 35–40% strain. A more detailed discussion of the molecular mechanisms involved in this proposed thixotropic behavior for phase M of the two-phase model of α-keratin fibers is left to the final comments on the mechanical properties of α-keratin fibers.

The Yield Region

When an α-keratin fiber, such as wool or hair, is stressed in water beyond the initial Hookean region, extension of the fiber increases rapidly with little change in the applied stress. This low incremental stiffness persists up to about a strain of 25 to 30%, at which the fiber stiffens. The region of strain between the end of the Hookean region and the stiffening of the fiber around 25 to 30% strain defines the Yield region. Extension CD (Fig. 6) beyond the Yield region with the mechanically stiffer behavior of the fiber up to break defines the

Post-Yield region. Speakman[25] showed that, for extensions within the Yield region at room temperature, the mechanical properties of the fibers were completely recovered after release of these fibers in water for a period of approximately 20 h.

Astbury[26] concluded from his high-angle x-ray diffraction measurements that the Yield region was the result of extension in an amorphous region of the α-keratin structure. He proposed that the stiffening of the fiber, when extended into Post-Yield region, was due to the unfolding of the organized crystalline structure from the folded α-configuration to the extended β-state. Creep and stress-relaxation measurements of fibers in water have shown that the mechanical behavior of fibers in the Yield region correspond to the theoretical behavior of units in a State A extending into longer units in State B. This model behavior was first considered by Burte and Halsey,[27] and was shown by Feughelman and Rigby[28,29] to correspond completely to the creep and stress-relaxation properties for wool fibers in water both for a range of temperatures and different loads and extensions. Bendit,[30] using more sensitive X-ray diffraction techniques, showed that the α-keratin fibers begin to unfold into the extended β-state at strains just above the Hookean region and that, by the end of the Yield region, about 30% of the α-keratin had been converted to the extended β-state. This latter observation is completely compatible with the proposal that the C phase contained in the IFs of the α-keratin structure effectively extends the full length of the fiber structure. The conversion of α-crystallites to extended β-units for extensions in the Yield region occurs at a nearly constant stress level and represents thermodynamically a first-order transition from α-crystallites $\rightarrow$ β-units. The restiffening of the fibers extended into the Post-Yield region has to be considered in terms of structural events within the α-keratin fibers other than those proposed by Astbury. It should be noted that the M phase for an extending fiber in water contributes only a small level of a nearly constant force, because the phase in the Yield region is in a "sol" state. For a fiber extended into the Yield region at a fixed strain, only the time constant of the relaxation is affected by phase M and not the level of force attained at equilibrium. For a fiber held at lower moisture contents in a drier environment, the level of stress in the Yield region increases because of the increased viscosity of the M phase with the contribution due to the C phase remaining a constant. This latter conclusion is derived from observation of the mechanical properties of the same fiber, before and after a setting treatment of the fiber which destroyed the α-crystalline structure in the fiber.[31]

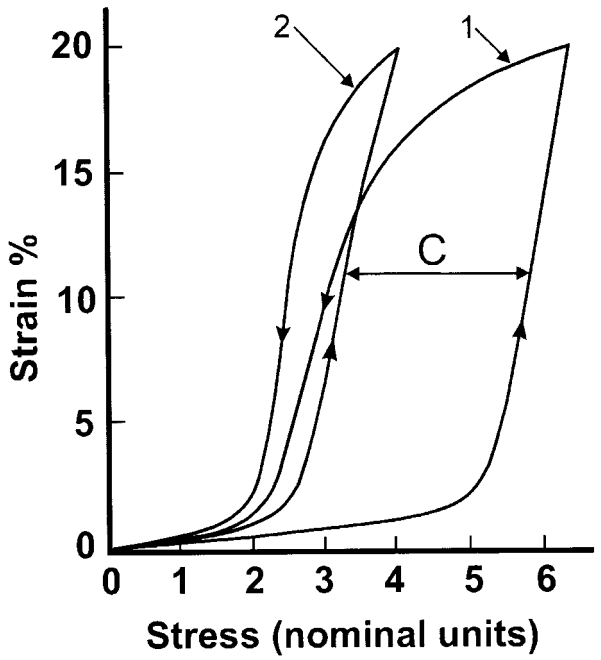

Figure 10 The hysteresis loops for the same fiber in distilled water at 20°C (curve 1) and in acidified water at pH 1 (curve 2). The rate of extension and retraction is 0.1%/min between zero and 20% strain. Repetition of the extension and contraction of the fiber as per curve 1 results in a rapid weakening of the extension curve. Similar repetition of curve 2 shows a complete reproducibility of mechanical properties per cycle. The result arises from the extremely slow recovery of the Coulombic interactions for the fiber in water after breakdown during extension. The level of stress during retraction of the fibre in curve 1 is also at the same level as curve 2, due to the absence of Coulombic interactions reforming in the time scale of the experiment for curve 1. All Coulombic interactions are broken in the acidified water for curve 2.

Mechanical hysteresis measurements[32] on wool fibers in water at 20°C between 0 and 20% strain, when compared with the same measurements for the same fiber in aqueous HCl solution at pH 1, show that the mechanical properties of the fiber are reversible in the solution at pH 1 in which the salt-links are all neutralized because the —COO^- has been converted to COOH (see Fig. 10). In water during extension, the force level is much higher than the level in the fiber for the Yield region at pH 1. During contraction, the force level in water is close to that for pH 1, strongly

suggesting that the extension in water for an α-keratin fiber the α-helices transforming to β extended chains involves the breakdown of both hydrophobic interactions and Coulombic interactions (salt-links), and on contraction when the β structures revert to the α-helices the hydrophobic interactions are reformed. However, the Coulombic interactions, because of their interaction with water, only re-form slowly. It is this slow re-formation of the Coulombic interactions that make it necessary to relax wool fibers in water for approximately 20 h to recover at 20°C the mechanical properties of the unextended fiber. Estimates of the contribution of entropy[33] to the force level in water of a fiber extended in the Yield region show this contribution is so small that the effect of internal energy is overwhelming in determining the level of force. This means that during extension of the fiber in water α-helices $\rightarrow$ β extended state and on contraction the procedure is reversed without the re-formation of the Coulombic interactions. During the contraction, it is the free-energy change produced by the hydrophobic interactions primarily that provides the forces of contraction.

In summary, the mechanical properties of the Yield region of α-keratin fibers in water is primarily dependent on the unfolding of α-helices in the C phase of the two-phase model during longitudinal extension. On contraction the mechanical properties are dependent on the refolding of the α-helices of the C phase with the level of force reduced by the slow re-formation of the Coulombic interactions in part responsible for the stability of the α-helices. Mechanically in parallel for longitudinal extensions, the M phase provides a viscous opposition to both folding and unfolding of the α-helices of the C phase. Essentially the M phase is in a "sol" state during extension and contraction, whose viscosity is moisture-content dependent.

Post Yield Region

For a wool or hair fiber in water at room temperature extended beyond the Yield region (25–30% strain), the stress–strain relationship stiffens rapidly to a linear relationship (see CD in Fig. 6). This linear relationship for a normal wool fiber in water at 20°C persists up to a few percent strain prior to the fiber breakage at about 50% strain. The incremental Young's modulus in the Post-Yield region for a normal wool fiber in water at 20°C is about 0.3 to 0.4 GPa. This value is independent of the fiber's moisture content (based on the wet fiber diameter). However, it varies rapidly with temperature reducing by a factor of 2 to 3 at 70°C.[32] Extension of the fibers into the Post-Yield region quite sharply and progressively increases the irrecoverability of the original mechanical properties of the fibers.[34] It was Speakman[35] who first suggested that mechanical behavior of the α-keratin fibers in the Post-Yield region resulted from the involvement of a covalently bonded network including disulphide bond crosslinking. This involvement of the disulfide bonds in the form of cystine residues bridging polypeptide chains has been extensively covered and confirmed in the literature, and has a critical role in the phenomena of setting in α-keratin fibers.

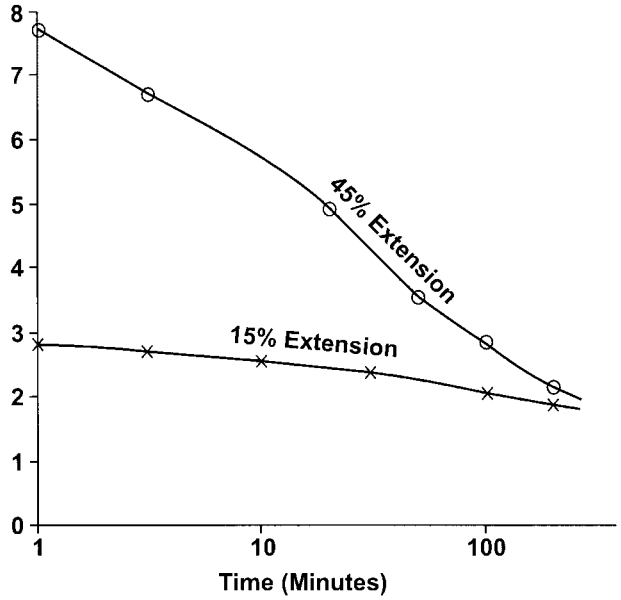

Figure 11 Stress-time behavior of wool fibers in water at 45°C in the Yield (15%) and Post-Yield regions (45%).

Comparison of stress-relaxation for Corriedale wool[36] extended in distilled water at 45°C to 15% and 45% strain (see Fig. 11) clearly separates out the component of mechanical behavior responsible for the Post-Yield region. The initial levels of stress at each extension of nearly 3 and 8×10^7 Pa, respectively at the 1-min level, were both at near equilibrium at the 200-min level at a stress of just under 2×10^7 Pa. The stress relaxation for strains in the Post-Yield region contains a component of stress above that in the Yield region with a half amplitude time of about 10 min at 45°C. In the stress relaxation in both the Yield and Post-Yield regions, the stress component re-

Table III Results of Mechanical Tests Performed on Four Groups of Wool Fibers

Wool Type	PY GPa	η/PY	S_{15}/PY	H/PY
Lincoln (pen grown)	0.37	0.40	0.115	5.3
Lincoln (field grown)	0.42	0.44	0.110	4.7
Corriedale (pen grown)	0.38	0.42	0.098	3.6
Merino (field grown)	0.29	0.43	0.132	4.3

sponsible for the unfolding of the α-helical structures into the extended β-units is present. The value of just under 2×10^7 Pa is the stress required to maintain equilibrium between the α and extended β-units. In terms of the two-phase model, the stress relaxation in the Post-Yield results from continued unfolding of the α-helices of the C phase together with distortion in parallel of a highly crosslinked non-water absorbing structural component responsible for the mechanical stiffening in the Post-Yield region. Discussion of the placing of the latter component is left to later in this work.

It is worth noting that although change in moisture content has no significant effect on the Post-Yield region stiffness, it does affect the turnover strain between the Yield and Post-Yield regions. For Corriedale wool fibers from dry to 65% relative humidity no significant change in the turnover strain was observed. From 65% relative humidity to wet (100% relative humidity) a consistent progressive increase in the turnover strain occurred, reaching 6.6% above the value at 65% relative humidity.

Collins and Chaikin[37] performed measurements on four groups of wool fibers, two from pen-grown sheep, Lincoln and Corriedale, and two groups from field-grown sheep, Lincoln and Merino. Mechanical tests[38] were performed on these fibers at room temperature in water for their incremental Young's modulus in the Post-Yield region. A comparison was performed for each group of fibers between the Post-Yield incremental Young's modulus (PY) and the torsional modulus (η), Hookean modulus (H), and stress (S_{15}), the stress on the fiber at 15% strain (typical of the Yield region). All the tests were performed in the water at room temperature and the comparison is set out in Table III.

An examination of these measurements clearly indicates the consistent relationship between the torsional modulus in water of each of the wool groups and the corresponding incremental Young's Modulus of the Post-Yield region. The result certainly points to a common component of the fiber's structure responsible for the measurements of PY and η for all the four quite diverse wool types. Here again, the structural significance of this observation is left to the discussion of models for the mechanical behavior of the α-keratin fibers.

The Keratin–Water System

Water plays a basic role in the time-dependent mechanical properties of α-keratin fibers. Present within the α-keratin structure are sites of attachment for water molecules by hydrogen bonding, polar and electrical charge interaction. Many charged groups, such as Coulombic interactions, are shielded from interacting with water by the presence of hydrophobic interactions. Many of the potential interacting sites are on the surface of the highly ordered regions, such as the IFs, and on the surface of globular proteins. In the latter case, the formation of the globules during the fiber growth occurs in the moist environment of the cortical cell. This would inevitably result in hydrophobic interactions being internal in the globular structure and groups, which interact with water, on the surface of the globules. Nuclear (proton) magnetic resonance (NMR)[39] clearly separates protons attached to the keratin structure from protons attached to water molecules. The result of these NMR measurements show that the water molecules are considerably more mobile than the components of the keratin structure. Also, over the range of relative humidities 0 to 90%, there is a progressive increase in the mobility of all the water molecules with increasing water content. The data shows that any water increase changes the binding energy of all the water already absorbed in the keratin structure. There is no indication of separation of water into different energies of bonding, rather, average bonding energy exists, with a resulting water mobility considerably less than liquid water. Algie and Gamble[40] obtained data on the dielectric properties of the keratin–water system at various water contents from complete dryness to near 100% relative humidity. They concluded that with the exception of a very small amount of water ($\sim$ 2%) for a system near dryness, there was no specific differentiation of the water present at any relative humidity into specific binding states. Rather, as the water in the keratin–water system is increased there is a general change in the binding of all the water molecules.

The electrical conductivity[41] of keratin-water has been shown to correspond to that of a proton-semiconductor. Water, because of its ability to form up to four hydrogen bonding interactions, forms a three-dimensional network of hydrogen bonds. Similar but more organized bonding in ice, and also in nylon–water and cellulose–water, have been shown to act as proton semiconductor in which protons move through a three-dimensional network by a series of water molecule rotations, accompanied by proton jumps between the two equilibrium positions for a proton, that exist between the two oxygen atoms of a hydrogen bond in the water network.

The NMR, electrical and dielectric data presented, all point to a model of water present in the keratin structure consisting of a continuous three-dimensional mass of hydrogen-bonded water molecules. This water mass at its boundary or interface with the keratin structure interacts with the hydrophilic groups on the surface of the keratin, such as the surface of the globular proteins of the matrix and the surface of the IFs.

The water and the keratin polymers form an interpenetrating network of two polymeric[42] systems which are compatible at their interface. The known sectional dimensions of the IFs (7.3 nm) and the inter-IF distance of wet α-keratin fibers ($\sim$ 11 nm) means that the water polymer phase dimension is of the order of 1 nm, when consideration is given also to the presence in the matrix between the IFs of globular proteins. The glass transition temperature T_g of the keratin–water composite should be a merger of the individual transition temperatures T_1 and T_2 of the keratin and water, and given by the Fox equation,[42]

$$\frac{1}{T_g} = \frac{W_1}{T_1} + \frac{W_2}{T_2}$$

where W_1 and W_2 are the weight fractions of the keratin and water, respectively. The presence of a single transition temperature T_g for the keratin–water system has been shown by Wortmann et al.[43] to obey the Fox equation quoted above and certainly supports the proposal of the two polymers of water and keratin forming a compatible interpenetrating network of two polymeric systems. It should be also noted that the effectiveness of the hydrophobic interactions between the α-helices of the helical rod domains in the IFs requires a continuous network of water molecules.

Structural Models of α-Keratin

As with many polymers, α-keratin fibers indicate from high-angle X-ray diffraction data[26] the presence within their structure of oriented crystalline regions together with amorphous material. The simple two-phase model of water-inpenetrable crystalline rods C oriented in the fiber direction and embedded in an amorphous water absorbing matrix M has been discussed and is widely accepted to explain the water sorption behavior and the mechanical response of fibers for small distortions. As indicated earlier, the low-sulfur protein fraction SCMKA extracted from α-keratin fibers contains the α-helical material and is associated with the C phase of the two-phase model. The high-sulfur proteins extract SCMKB together with any high glycine tyrosine proteins together with water form the embedding matrix M.

The first simple model[8,44] of the mechanical behavior of α-keratin fibers consisted of a C phase containing α-helices ordered in the fiber direction with the M phase consisting of an amorphous polypeptide chain network crosslinked by disulfide bonds formed by the diamino reduces of cystine. In extending this model into the Yield region, the α-helices are unfolded to form the extended β-state. For a fiber in water the initial extension of the M phase results with negligible opposition from the amorphous polypeptide chains as they unfold and are oriented in the fiber direction in parallel with the unfolding of the α-helices. An extension is eventually reached when the orientation and ordering of the randomized polypeptide chains of phase M can no longer proceed without the severe straining or breaking of crosslinks of the polypeptide network. This above extension was identified with the turnover strain for the fiber from the Yield to Post-Yield regions. The above model satisfies the requirement that the fibers' stiffness increase in the Post-Yield region is compatible with the proposed covalent bond breakdown with this extension.

Watt[45] has shown that cystine reduced but unalkylated wools have no significant change in equilibrium water content for fibers in water at room temperature. Wools in which 90% of the disulfide bonds had been broken by reduction had an equilibrium water content only 1% higher than that of an unreduced normal fiber. Similar results were reported by Haly.[46]

Chapman and Hearle et al.[47] have extended this model with minor addenda to explain the zonal unfolding of the α-helical structures across

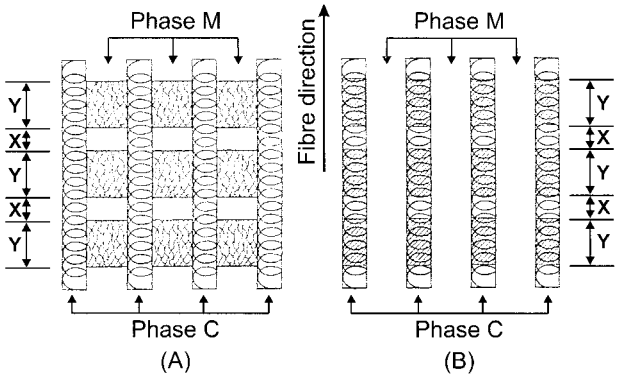

Figure 12 The relationship between phase C and phase M of the two-phase model in terms of the series-zone model: (A) with the series zones Y in the phase M, and (B) with the series zones Y in phase C.

the microfibrils in a macrofibril as observed by Haly.[49] The simplicity of the model is in itself a major attraction. However, the model fails to recognize the nature of the density of crosslinking present in the amorphous phase of the α-keratin cortex. The necessity for a concentration of crosslinkages rather than a relatively uniform dispersal has been discussed.[36] Further, Chapman et al. consider the recovery from extension in the Yield region as a result of elastomeric forces produced by the entropy change due to the fiber extension. Peters and Woods,[33] however, have shown in α-keratin fibers that the effect of internal energy in determining the load is overwhelming.

The mechanical[49] properties of wool fibers taken from the same position on the sheep before and after the administration of sulfur-containing amino acids into the sheep's abomasum resulted in no notable mechanical property changes despite a measured increase of 35% of the sulfur content. This increase of cystine content has been suggested from the work of Gillespie and Inglis[50] to be associated with the high-sulfur protein SCMKB extracted from the α-keratin structure. A similar conclusion exists for the lack of moisture change as indicated by Watt,[45] with major disulfide breakdown; that the disulfide crosslinkages are concentrated structurally in isolated units. Mechanical tests performed on sections of porcupine quill and rhinoceros horn at right angles to the fiber direction and in water at 20°C indicate completely parallel behavior to that of fibers in torsion.[4] Sections of fiber extended to break (>35% strain) showed continuous weak "sol" behavior right up to break with no indication of any stiffening due to crosslinking. Because it is the water-weakened matrix that provides more of the distortion due to the lateral extension, the simplest conclusion is that the high-sulfur protein, which exists in the matrix, must be confined to isolated units which remain undistorted by the lateral extension.

The series-zone models[51] were suggested from mechanical data for α-keratin fibers swollen and with their α-helices randomized in concentrated aqueous lithium bromide solution. In these models the disulfide crosslinks were concentrated in regular zones either in the IFs, or in the matrix alongside the IFs (see Fig. 12). Crewther[52] introduced a more detailed model, which may be considered in terms of a series-zone model, in which the high-sulfur fraction of the matrix protein (SCMKB) is in the form of a globular protein bound to the micro-fibrils by alternating polar or single disulfide bonds. Mechanical tests on wool fibers in concentrated formic acid show that the fibers behave as "filled" elastomers[36] exhibiting the "Mullin's effect." The results point to the possible presence of an internal structure or "filler" such as the globular protein proposed by Crewther.

Wortmann and Zahn[53] have developed a model based on more detailed knowledge available on the structure of IFs. Their model corresponds to a series-zone model in which the stiffening in the Post-Yield region of a wet α-keratin fiber is associated with the IFs. Disulfide bonding between α-helical ropes in the tetramers of the IFs are proposed as the cause of the stiffening.

The results in Table III of the close correlation between the wet torsional rigidity of a fiber and its Post-Yield region stiffness means that the same structural component of the α-keratin fiber responsible for opposition to shear during the application of torsion to the wet fiber is responsible for the Post-Yield region stiffness. Over the temperature range of 25°C to 70°C for two different wool types the Post-Yield region stiffness[54] decreases by a factor of 2–3. No such loss of mechanical properties occurs for the IFs of the same wool fibers, as measured by the stress level in the Yield region at 15% strain. Both of the above observations point to the association of the Post-Yield region stiffness with the matrix structure of the cortical cells in the α-keratin fibers. The globular proteins which provide the extracts of SCMKB and the high-glycine tyrosine are in the matrix interacting with water at their hydrophilic surface, with their internal hydrophobic structure unaffected by the presence of water. The torsional

rigidity η of the wet fibers is primarily dependent on the mechanical properties of the matrix, which in turn result from the composite of water and matrix protein forming the matrix structure. The work of Bendit and Gillespie[55] on the properties of a range of α-keratin fibers suggests that in the biological formation of α-keratin fibers, the formation of the matrix protein limits that space available for water in a water-saturated fiber. Further, the matrix should be considered as (matrix protein + water), not as water interspersed throughout a protein. The volumetric limitation between the IFs of the cortex is a result of the presence of interfilament interactions spaced along the IFs at intervals of the order of 15-nm apart. These spacing data result from the particular model adopted for purposes of calculation. The calculations are based on the measurements of swelling behavior of wool fibers in varying concentrations of formic acid in water.[56] The extended two-phase model next presented depends on the large amount of information, primarily mechanical, discussed in the latter part of this article.

The Extended Two-Phase Model

In this proposed model of the α-keratin fiber cortex, the starting point of the consideration is not that of a polymer consisting of long random chains crosslinked and forming ordered crystalline regions in suitable regions throughout the fiber. The initiation of the formation of the α-keratin is within the wet swollen cortical cells, where the α-helical ropes are formed stabilized by the hydrophobic interactions between each of the pair of α-helices forming the rope. As the fiber grows, the spherical cortical cells elongate with the α-helical ropes becoming oriented in the fiber direction and forming the IFs spaced regularly apart from each other. It is at this stage the high sulphur protein is formed between the IFs, presumably into globules, which form in the wet cortical cell with the hydrophilic residues at the globule surface. A similar situation would exist for any high-glycine tyrosine globules formed. Figure 13(a, b) shows the proposed model with water-inpenetrable crystalline region of α-helices forming the IFs which each interact with neighboring IFs at regular positions along their length. The existence of these linkages between the IFs have been suggested by a number of authors[56,57] and explains the limited space available for water plus matrix protein within the α-keratin structure. The high-sulfur protein globules which correspond to the extractable SCMKB may or may not be directly attached to the inter-IF linkages or more directly by bonding as suggested by Crewther.[52] The latter factors, will be noted, make no difference to the control of the mechanical behavior of this proposed model, viewed as an extended version of the original two-phase model. When an α-keratin fiber is extended in water the α-helices unfold once the strain on the fiber exceeds the Hookean region, about 2%. The unfolding of the α-helices proceeds at almost a constant stress on the fiber to the end of the Yield region. The matrix "gel" consisting of the water and globular proteins extends in parallel with the unfolding α-helices resulting in only a small addition to the force extending the fiber. This force above a few percent strain reaches almost a constant level as the matrix goes from a "gel" to "sol" state. With the α-helices of the IFs transforming into extended β structures of over twice the length of the original α-helices, the IFs which are parallel to each other and the fiber direction move towards each other. This movement results at strains of the order of 25–30% in a jamming of the still-folded α-helices against the globular matrix proteins, making further unfolding of α-helices require a distortion in parallel of the globular matrix protein. Further extension is in the Post-Yield region, where the unfolding of the α-helices requires the increasing force to distort the matrix protein. The distortion of this matrix protein is clearly brought out in the comparison of the stress relaxation of fibers in water at 45% strain and 15% strain as shown in Figure 10. The difference between these two stress relaxation curves is the stress generated by the distortion of the matrix protein at the higher strain level.

The longitudinal swelling behavior of α-keratin fibers can be understood in terms of the extended two-phase model (ETPM). From dry (0% relative humidity) to wet (100% relative humidity), wool fibers extend by about 1.2% with almost all the extension occurring between 0 and 65% relative humidity. Above 65% relative humidity, the longitudinal swelling for a fiber in water is water negligible ($\sim$ 0.1%). Considering the behavior of the α-keratin fiber as it is dried from 100% relative humidity, initially on the basis of the ETPM as the water is removed from the fiber the IFs approach each other and remain parallel to the fiber direction until the IFs jam against the matrix protein globules. Further drying will cause distortion of the IFs, which results in the fiber contracting. Distortion of the orientation of the

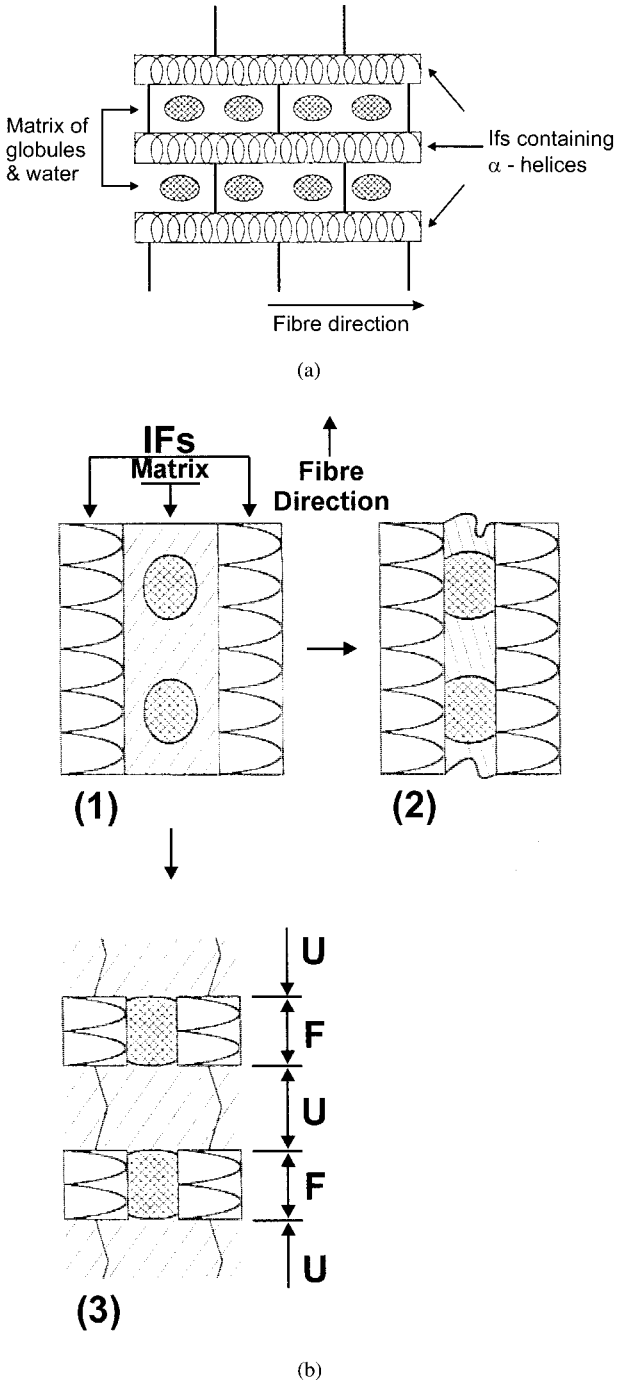

Figure 13 (a) Diagram showing IFs containing the α-helices, and interconnected at regular intervals along their length. The globular high sulphur proteins may or may not be attached to the inter-IF linkages or more directly by bonding to the IFs (see text). (b) 1. Enlarged segment from (a) for a wet unextended α-keratin cortex showing the relationship between the IF's and the matrix of globular proteins embedded in water. 2. As in 1 except the fiber has been dried to $\sim$ 65% relative humidity with the globular matrix protein jamming against the intact. 3. As in 1 except the fiber wet has been longitudinally extended to the end of the Yield region. IFs have unfolded in zones along each IF (U) and the extended unfolded chains are surrounded by water. The folded components of the IFs (F) are hindered in any unfolding by the jamming matrix protein between these components.

α-helices in a dried fiber has been shown to occur by the reduction of the infrared dichroism associated with the amide —NH bond of the crystalline α-helices.[58]

Based on the knowledge of matrix protein content of a fiber, and its water content at 65% relative humidity at which the IFs just jam the matrix protein, an estimate can be made of the turnover strain from the Yield to Post-Yield regions based on the ETPM.[59] For Corriedale wool fibers with a turnover strain measured directly on the fibers in water of 29.6%, the estimate is 32.7%, and for human hair with a measured value of 25.0%, the estimate is 28.2%. In both cases the estimate is above the measured value, but importantly in both bases by a similar amount of 3.1 and 3.2%, respectively. Other estimates still based on the ETPM and other knowledge of the matrix protein content of the fiber[59] have been performed with comparable estimates of the turnover strain from the Yield to Post-Yield region. The observed increase in the extent of the Yield region for fibers taken from equilibrium with 65% relative humidity to equilibrium at 100% relative humidity (wet) is of the order of 5–6%. This increase has been explained by the increase of water uptake resulting from the extension of $\alpha \rightarrow \beta$ structures in the extended fiber in the more wet environment.[60] The extended two-phase model has been successful in incorporating the known mechanical behavior in extension, torsion, and lateral properties of α-keratin fibers, together with an ability to predict quantitatively the other mechanical features mentioned above. The model gives consideration to the biological origin of the α-keratin cortex as a composite of cortical cells formed in equilibrium with a moist environment, and that the order in the fiber must be considered as initiated in that wet state. It is in this latter regard the ETPM differs basically from the earlier models. It should be noted that the diagrammatic discussion of the ETPM has been on a two-dimensional basis for ease of understanding. A recent three-dimensional analysis[61] has been developed, which produced an explanation for the well-known hexagonal packing of IFs which occur

in many α-keratin fibers. The model has been a useful and successful initial base for the consideration of α-keratin fibers in terms of IFs consisting of ordered helical ropes packed into continuous crystalline units held together by end groups containing cystine bonds as indicated by the analysis of the low-sulfur protein SCMKA. The matrix between the IFs consists of globular proteins of high-sulfur proteins and high-glycine tyrosine, which themselves do not contain water but interact with the water present in the matrix at their hydrophilic surface. The interactions directly between the IFs and spaced at intervals along the IFs probably arise from excess molecular material at each end of the ordered helical units.

Set in α-Keratin Fibers

Wool fibers in common with other fibers in the textile field need to be either temporarily or permanently distorted from their native configuration. This requirement also exists for human hair in the cosmetic field. Two conditions are required to be met by the setting procedure:

- In the distorted state bond breakdown must occur to reduce the forces of retraction of the fiber toward its original configuration.
- Bonds must be formed in the distorted structure in equilibrium with the distortion applied so that on release of the fiber from the setting distortion the newly formed bonds tend to maintain the structure as closely as possible to the distorted state.

A considerable body of literature on the above topic for α-keratin fibers has been published over many years.[62] The involvement of the disulfide bond has been basic to the consideration of any permanent setting procedure. The measurement of mechanical-property changes produced by various physical and chemical procedures has been performed revealing various aspects of the α-keratin structure.

Silk Fibroin

All natural fibrous proteins are biological polymers of polypeptide chains. Because of the presence of amino acid residues a large proportion of the bonding is inter- and intrahydrogen bonding. Silk fibroins are protein filaments spun by many types of insects and spiders. Biosynthesis[63] occurs in the silk gland cells and the proteins are stored

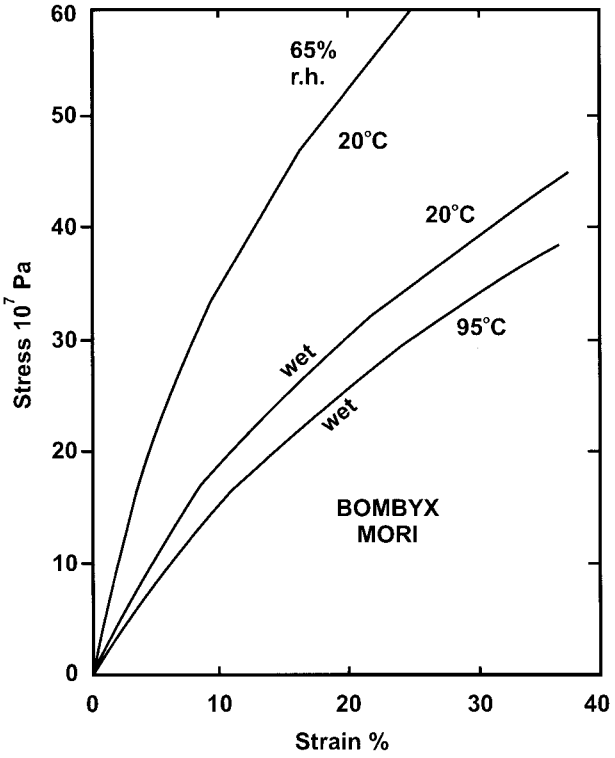

Figure 14 Stress–strain curves for silk (*Bombyx mori*) in water at 20 and 95°C compared with the one at 65% relative humidity and 20°C.

outside the cells in the lumen of the silk glands as a concentrated viscous solution. By extrusion through spinnerets the proteins are transformed into continuous filaments. It is important to note that silk fiber filaments are produced physically in a manner parallel to that of manmade fibers. Of over 100 species of silks, that have been examined the smaller amino acid residues of glycine, alanine, and serine usually form the major proportion of the proteins present. Commercial silk fibroin (*Bombyx mori*) has a simple highly crystalline component corresponding to extended β-chains. Glycine, alanine, and serine[33] represent more than 75% of the amino acid residues, with the residue of tyrosine at about 8% representing the only other residue of note. The molecular chains of silk fibroin (*Bombyx mori*) consist of a number of segment types. A repeating hexapeptide Ser-Gly-Ala-Gly-Ala-Gly corresponds to a common structural element of the main segment type. Also, a terminal group common to many segments is —Gly—Tyr, suggesting a possible discontinuity resulting from a large residue of tyrosine.[63]

In the case of long-chain fibers, the crystalline-amorphous model has been generally applied as

the initial means of interpretation of the relationship between the mechanical properties and the molecular structures. As with other long-chain molecular structures, X-ray diffraction studies of silk fibroin indicate the presence of highly ordered crystalline regions associated with less-ordered amorphous structures. The crystalline structure shows little change with considerable extension of the fiber. This indicates the main uptake of strain is in the far less organized and more mobile amorphous regions and results from the unfolding of the polypeptide chains and their orientation in the direction of extensions. The stress–strain behavior of commercial silk fibers[33,64] (see Fig. 14) is dependant both on the moisture content and temperature at which tests are performed. The amorphous regions become more mobile and extensible with increase of water uptake and temperature, as would be expected for a structure held together primarily by hydrogen bond interactions. The mechanical behavior of silk fibroin filaments in general do not reflect any detail of structure such as that forthcoming from a study of α-keratin fibers. These latter fibers, whether from horn or hair, reflect in their detail mechanical behavior an invariant complex structure based on their organized formation within a biological cell.

Collagen

In the second category of fibrous proteins are the relatively inextensible collagen fibers. Collagen is the most abundant and common of proteins in the animal kingdom. Skin, tendon and cartilage are formed from this fibrous protein. In bone, collagen forms the fibrous component of this composite providing a continuous network holding the structure together. Collagen forms the supporting framework protein of many body components. Whether they are derived from vertebrates or invertabrates, collagens, which are formed by fibroblasts, consist of molecules of tropocollagen.

Collagens are extracellular proteins, which once formed are relatively stable in most sites, unlike body proteins, which show a continuous molecular turnover. The basic tropocollagen[65] molecule consists of three polypeptide chains intertwined into a triple helix of approximate dimensions of 280 nm in length and 1.4 nm in cross-section with a molecular weight of 3×10^5 Daltons. Under the electron microscope fibrils of collagen often exhibit a banding pattern, which results from a staggered arrangement of the triple helical units.

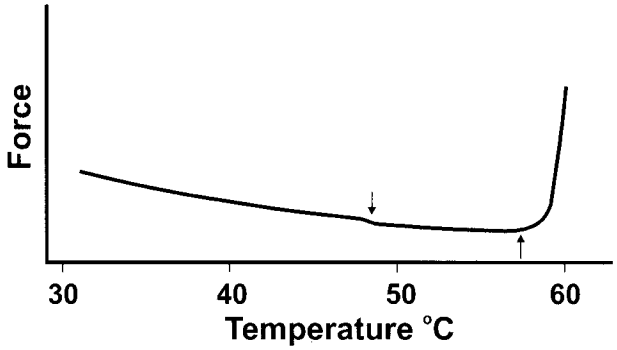

Figure 15 A force-temperature curve for kangaroo tail tendon immersed in 0.9% saline. The arrow pointing down indicates a glass transition T_g, and the one pointing up indicates the shrinkage temperature T_S.

The commercial interest in collagen primarily arises from its use as the basic material for the manufacturer of leather products. It is the relative inextensibility and stiffness of the individual collagen fibers, which form the leather composite and which control the mechanical properties of the leather.

Because collagen in the native state exists in equilibrium with tissue fluid, the most significant mechanical tests probing the molecular and near-molecular structure are performed in an appropriate physiological solution. For test purposes, collagen fibers, such as rat tail and kangaroo tail tendons, are removed directly from their native state into the physiological saline solution to avoid any possible modification of the fibers by the transition. Simple mechanical extension tests show that the collagenous materials extensibility limit is $\sim$ 4% strain. This is a result of the complete jamming of the intertwined long-pitched triple helical structure of the tropocollagen molecules. Any unfolding of these helices is severely limited and cannot occur without the breaking of the covalent bonds resulting in a complete mechanical failure of the fiber. The most informative data, that result from mechanical measurements, is the relationship between thermal transitions within the collagen structures, the environment in which the structures are formed and aging effects produced within the collagen structures of a living animal.

When a specimen of collagen from a warm-blooded animal such as rat tail tendon or skin is slowly heated in an appropriate physiological solution there is little evidence for change in the collagen material below 60°C.[66] At about this latter temperature the specimen under test shrinks

quite suddenly and in the case of rat tail tendon to a measurable one quarter of its original length. This well-known transition in collagen is referred to as the shrinkage temperature T_S. T_S is best detected by a simple apparatus developed by Rigby,[65] in which the specimen is held just taut with the force on the specimen slight and only just detectable by a force transducer. On heating when the specimen's dimensions suddenly reduces at T_S the force detected will increase rapidly indicating the transition in the collagen. T_S corresponds to a first-order transition change in the bulk collagen, a result of the crystalline units of molecules melting into random coils. This is not the transition temperature T_D of the rod-like collagen molecules alone going through the helix → coil transition but the macroscopic result of the crystalline aggregate of molecules going through the transition. The temperature of the helix → coil melting of a single collagen molecule T_D was obtained using the glass transition temperature T_g indicated by the force-temperature measurements made after a partial melting of the collagen fiber (see Fig. 15).[66]

The values of T_D were obtained for various collagen sources.[67] T_D for warm-blooded animals (human, beef, rat, etc.) is close to their normal body temperatures of about 38°C. In the case of cold-blooded animals such as fish, T_D corresponds to the upper limit of their environment temperature. As an extreme example,[67] the Antarctic "icefish," which lives in an environment range of −1°C to 3°C, has a value of T_D at 4°C. The value of T_D corresponds to the temperature at which the collagen molecules are laid down (in the case of warm-blooded animals), or the upper-limit temperature at which the molecules are laid down (in the case of cold-blooded animals).

Collagens have a high proline plus hydroxyproline content[66] ranging from 150 to 300 residues per 1000. These amino acid residues play an important role in the thermal stability of all collagens, and in particular, there is a well-defined correlation between the shrinkage temperature, T_S, and the sum of proline plus hydroxyproline for a fiber.

The measurement of both T_S and T_D has been successful in detecting the action of ageing in live animals, and extracted collagen maintained in physiological solutions. Changes in crosslinking and crystallinity are both reflected in changes of T_S and T_D. These simple mechanical tests with parallel data from other techniques combine to clarify the link between macro- and near-molecular structures and events.

SUMMARY

In this work, an emphasis has been placed on the mechanical measurements applied to different protein fibers. It has been done both to demonstrate the ability of these measurements to be related to near-molecular structural events, but also to place in perspective the roles of the many and varied techniques that have been also applied to solve the same structural problems. Fibers are primarily used for their mechanical properties and a clear understanding of these properties is essential, if modification and variation of these properties are contemplated.

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