Crosslinking Structure of Keratin. I. Determination of the Number of Crosslinks in Hair and Wool Keratins from Mechanical Properties of the Swollen Fiber

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Synopsis

Hair and wool keratin fibers which had been treated with an 11 *M* LiBr solution containing *N*-ethyl maleimide showed typical rubberlike elasticity in a solution composed of equal volumes of 8 *M* LiBr and diethylene glycol mono-*n*-butyl ether. Stress–strain curves and equilibrium force–temperature relations were measured for swollen hair and wool fibers. The non-Gaussian effects on deformation and the energy component in retractive forces were analyzed. On the basis of rubber elasticity theory, a method for estimation of the number of mechanically effective crosslinks in keratin fibers was proposed. A linear relationship between the crosslink density and the disulfide content was obtained from the data for a variety of keratin fibers (i.e., two different human hairs, horse hair, and 17 different wools). From the results of thermodynamical and non-Gaussian treatments for swollen keratin, it was suggested that the swollen fiber consists of a two-phase structure: a mechanically stable phase of higher crosslinked domains and rubbery phase with lower crosslink density. It was further found that considerable amounts of nondisulfide covalent crosslinks are present in wool and hair keratins.

INTRODUCTION

Chemical treatments of wool fabrics and hair fibers are an important aspect of the textile and cosmetic industries. The native disulfide (SS) bonds provide a high crosslink density and are principal covalent crosslinks in wool and hair keratins. New crosslinks are introduced into the fiber by the chemical treatments. Crosslinking as well as self-crosslinking, which is mainly concerned with SS linkages, may stabilize the structure. This leads to changes in mechanical properties.

A variety of physical measurements have been employed to assess crosslinking in keratins. These include swelling in formic acid,[1-3] urea-bisulfite solubility,[1,4] supercontraction in aqueous LiBr,[5,6] lateral compression,[7,8] torsional modulus,[9] load extension in formic acid,[1,10] and modulus of elasticity in aqueous LiBr.[11-13] A number of reports have reviewed crosslinking agents, mechanisms, and test methods for crosslinking as related to wool keratin but no reliable quantitative method for determining the crosslink density has been presented.[14,15]

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Recently, Menefee and Tillin[16] attempted to determine the crosslink density of wool keratin using a method of amino endgroup analysis after partial degradation, and indicated that theoretically,[17] about 40% of the crosslinks are intramolecular. They also reported that in a two-component model measuring the rate of hydrolysis and solubilization, that crosslinking could be entirely intermolecular.

Haly and Feughelman[11] first attempted to estimate the crosslink density of wool by measuring the modulus of elasticity of the supercontracted fiber in LiBr solution. Haly[12] suggested that the thiol-disulfide interchange reactions occur more or less in the native wool fibers at extensions in 8 *M* LiBr solution. According to Haly,[12] Beevers and McLaren[13] applied rubber elasticity theory for the determination of crosslink density of γ-irradiated wools and then studied the effect of radiation dose on crosslinking. However, application of rubber elasticity theory to determine the crosslink density of wool is limited, because interchanges occur easily in the swollen and stretched fibers.

One author previously reported[18,19] that for supercontracted wool which had been pretreated with *N*-ethyl maleimide, the following rubbery conditions can be fully satisfied in a swelling system containing concentrated aqueous LiBr and diethylene glycol mono-*n*-butyl ether: (i) high stability of the crosslinkages in swollen and stretched network, (ii) less interaction between the wool chains, and (iii) no crystallization during extensions of the fibers.

The purpose of this study is to establish a method for determination of the crosslink density of hair and wool keratins, to demonstrate the relations between the number of crosslinks obtained by chemical and physical methods, and to present a brief discussion on the crosslinking structure of keratin.

EXPERIMENTAL

Materials

Human hairs and wools were purified by Soxhlet extraction with acetone for about 24 h, followed by washing with cold water and air-drying. Tri-*n*-butyl phosphine (TBP) and *N*-ethyl maleimide (NEMI) used were special reagent grade. Acrylonitrile (AN) and diethylene glycol mono-*n*-butyl ether (BC) were obtained by distillation of commercial products under reduced pressure after dehydration with anhydrous sodium sulfate.

Preparation of Reduced and S-β-cyanoethylated Fiber

Fibers (0.1 g) of Lincoln wool and human hair were reduced with a 1% and a 0.4% TBP solution composed of 1-propanol (5 mL) and borate-phosphate buffer adjusted to pH 8.0 (5 mL) for 24 h at 25°C, washed with the same buffer containing 1-propanol (2 × 10 mL), and then treated with a 1% AN solution composed of 1-propanol (5 mL) and the same buffer (5 mL) for 24 h at 25°C, followed by washing with the same buffer solution as above, washing with water and air-drying.

Determination of Disulfide Content

The disulfide (SS) contents of hair and wools were determined by polarographic method with methyl mercury iodide.[20] Polarographic analyzer used was a Yanako Polarograph Model P-900. All analyses were performed three times, and average value was taken.

Preparation of Swollen Fibers

The fibers (50 mg) were treated with an 11 *M* LiBr aqueous solution containing 1×10^{-2} *M* NEMI (2 mL) at 90°C for 1 h, and subsequently immersed in a solution composed of equal volumes of 8 *M* LiBr and BC at room temperature. A control sample was also prepared without NEMI. The swollen fibers thus obtained were subjected to mechanical tests.

Mechanical Tests for Swollen Fibers

The mechanical tests for the swollen fibers were carried out by using a model UTM-II Tensilon tester (Toyo Baldwin Co., Ltd.) equipped with an apparatus shown in Figure 1. The swollen fiber with ~ 20 mm length was set between clamps. The upper clamp was attached to a load cell (sensitivity 1 mg). The lower clamp could be moved downward so as to impose a certain elongation. The sample was placed in a double-jacket cell containing the solution composed of equal volumes of 8 *M* LiBr and BC. The temperature was controlled by circulating the water in the jacket at constant temperatures within ±0.1°C.

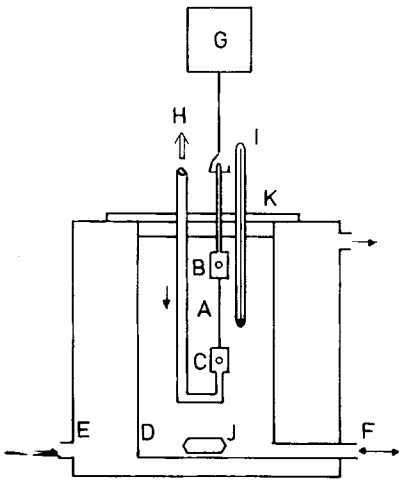

Fig. 1. Apparatus used for mechanical tests of the swollen fiber sample: (A) swollen sample; (B) and (C) clamps (quartz); (D) glass cell; (E) water jacket; (F) level regulation; (G) load cell; (H) crosshead; (I) thermometer; (J) magnet stirrer; and (K) glass cover.

Stress–Strain Curve

The stress–strain relations for the swollen samples were obtained at 40°C (wools) and 50°C (hairs) unless otherwise specified. Stresses refer to the average cross-sectional area of the swollen and unstrained fiber used to construct the stress–strain curve. Assuming a circular cross-section of the sample, although this assumption is not adequate for samples with an elliptical cross-section such as Merino wool, the average cross-sectional area was calculated from the average value of the swollen diameters D_s measured directly under a microscope.[12,18,19]

Stress-Temperature Relationship

The sample was elongated at a rate of ~ 1%/min to the maximum length and conditioned by repeated loading and unloading at the highest temperature used. To obtain the force-temperature relationships, equilibrium forces were measured at constant temperatures and at constant elongations. First the unstrained zero length at equilibrium L_o of the sample being conditioned at the maximum temperature was determined by extrapolating the forces under very small elongations to zero force. Strain ratios, α were calculated by the equation: $\alpha = 1 + L/L_o$. Next, the sample was elongated to length L_1, which corresponds to the strain ratio α_1 at this temperature and allowed to relax for t_1 (at least 15 min), until an equilibrium force f_1 in grams was obtained. The sample was further elongated to $L_2, (\alpha_2), L_3, (\alpha_3), \ldots$, and equilibrium forces $f_2, f_3, \ldots$, were obtained after relaxing for $t_2, t_3, \ldots$, respectively. Relaxation times required for measurement of equilibrium forces were different among the fibers under extension at different strain ratios. The longest relaxation time observed at maximum extension of swollen hair was ~ 30 min. The temperature was then lowered at intervals of 5°C to 20°C and the corresponding equilibrium forces were measured at constant strain ratios and at constant temperatures as described above. Finally, the temperature was raised again at 10°C to 20°C intervals.

After the mechanical tests for each swollen sample were completed, the solution was replaced with water. The zero length in water at room temperature L_w was also determined by the procedure described above. After length, L_w, was measured, average dry diameter D_d was obtained for the fiber released from the clamps and was dried in air on P_2O_5 desiccator.

The volume fraction of keratin materials in swollen sample, v_2 was calculated by the equation: $v_2 = (D_d/D_s)^2(L_w/L_o)$. Here, L_w was assumed to be equal to dry length.

RESULTS

Hysteresis Curve

Figure 2 shows hysteresis curves of the hair and wool fibers conditioned by three cycles of loading and unloading. The stress-strain behavior is similar to that of rubbers and elastomers for simple extension: (i) typical rubbery stress-extension curves, (ii) excellent elastic recoveries, and (iii) lower energy losses. Elastic modulus and energy loss for the hair are larger than the Lincoln

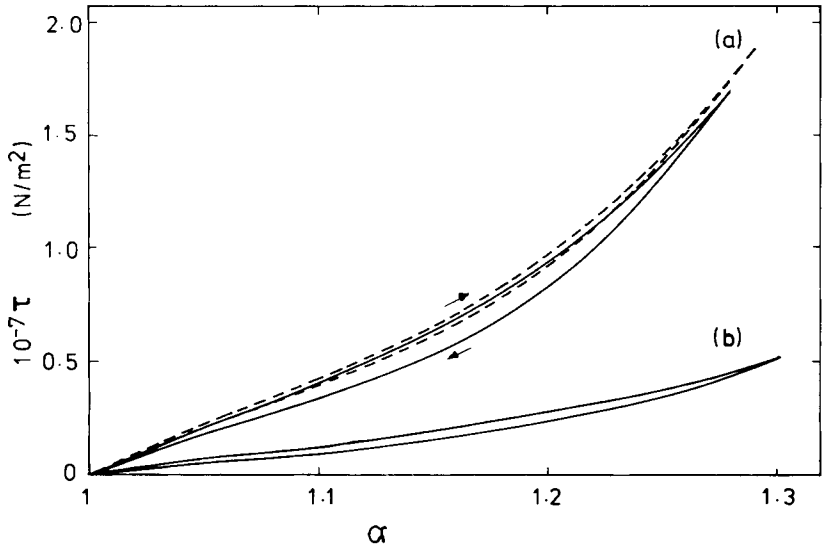

Fig. 2. Hysteresis curves of the swollen human hair (a) and the Lincoln wool (b): (—), 40°C; (---), 50°C.

wool. As the temperature is increased, however, a significant decrease in energy loss is observed for the hair.

Thermal Expansion Coefficient

Figure 3 shows the changes of the unstrained zero length (L_o) with temperature. Little or insubstantial changes were observed for either reduced or subsequent S-β-cyanoethylated hair and unreduced hair. It could be assumed, therefore, that the bulk coefficient of thermal expansion, $\beta = V_o^{-1}(\partial V/\partial T)_{p,\text{eq}}$ of the swollen fiber is substantially zero, where V_o is unstrained, swollen volume of the sample at equilibrium.

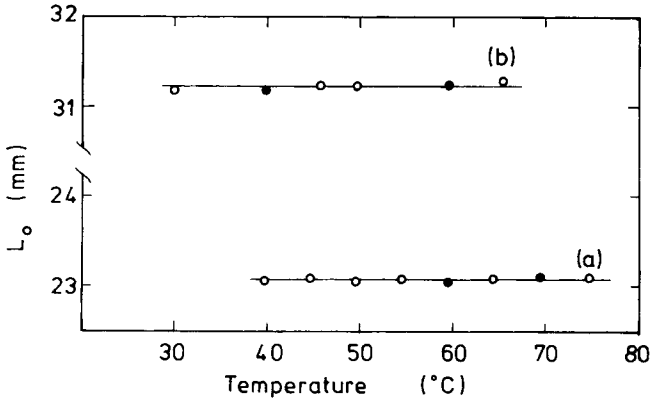

Fig. 3. Unstrained swollen length at equilibrium, L_o versus temperature curve for unreduced hair (a), and reduced and subsequently S-β-cyanoethylated human hair with SS = 91.6 μmol/g (b): (○), temperature descend; (●), temperature ascend.

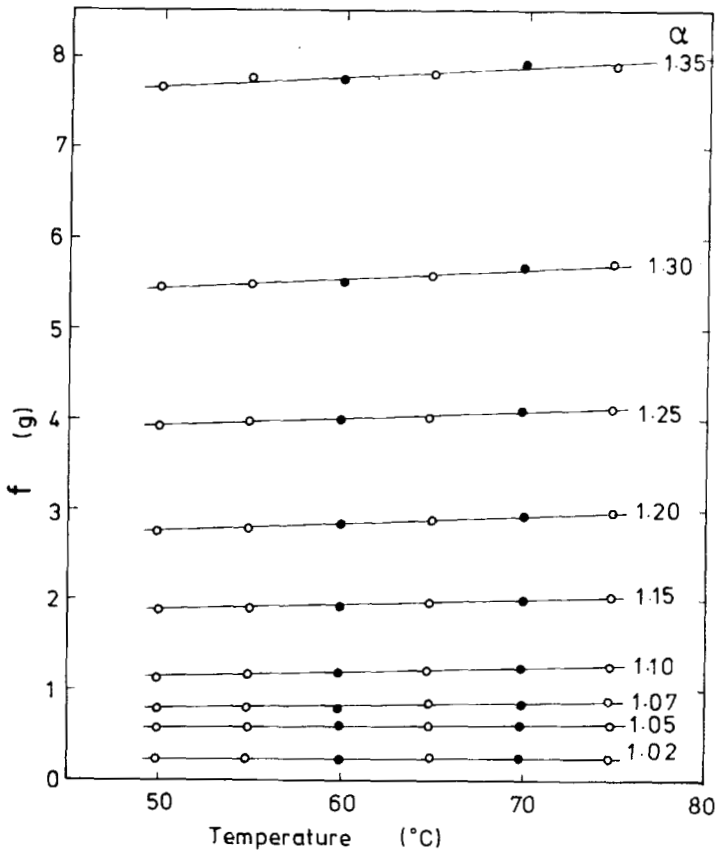

Fig. 4. Equilibrium force, f in gram versus temperature curves at different extension ratios, α for unreduced human hair: (○), temperature descend; (●), temperature ascend.

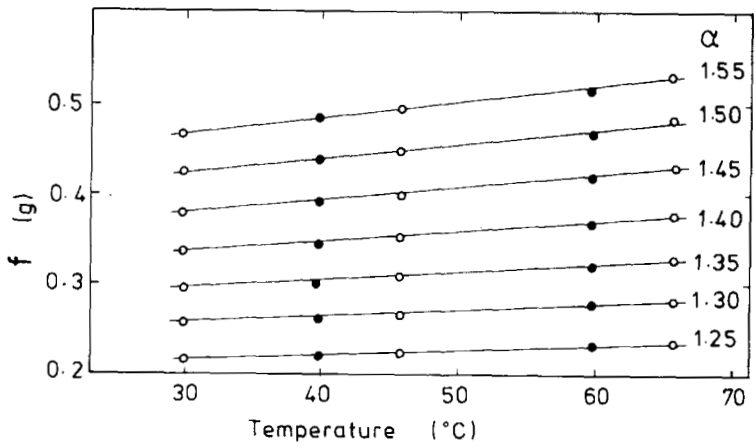

Fig. 5. Equilibrium force, f in gram versus temperature curves at different extension ratios, α for reduced and S-β-cyanoethylated human hair with SS = 91.6 μmol/g: (○), temperature descend; (●), temperature ascend.

Force-Temperature Relationship

Figures 4 and 5 show straight force-temperature graphs of the unreduced and the reduced hairs, respectively. The data taken at decreasing and increasing temperature coincided on the straight lines at constant extensions. For the reduced and S-β-cyanoethylated fiber, the data obtained for the lower strain levels below 1.20 were considered to be unreliable because the sensitivity of the load cell exceeded the limit for measuring the stress changes with temperatures, and were therefore omitted.

DISCUSSION

Force-Temperature Relationship of Hair Keratin

The force-temperature coefficient actually measured $(\partial f/\partial T)_{p,\alpha,\mathrm{eq}}$ can be taken[21,22] as equal to the corresponding constant volume coefficient $(\partial f/\partial T)_{V,L}$ because of the constancy of L_o with temperature (Fig. 3) and thus $\beta = 0$. The ratio of the energy component, $f_e = (\partial E/\partial L)_{V,T}$ to the total

TABLE I
Thermoelastic Data for Unreduced Human Hair Sample in Equilibrium Swelling with 8 *M* LiBr-BC Solution in the Temperature Range 50–70°C

$\alpha_{60°}$	f (g)	$10^3(\partial f/\partial T)_{p,\alpha,\mathrm{eq}}$	$(T/f)(\partial f/\partial T)_{p,\alpha,\mathrm{eq}}$	f_e/f
1.02	0.23	0.63	0.90	0.10
1.05	0.58	1.59	0.91	0.09
1.07	0.82	2.22	0.91	0.09
1.10	1.19	3.33	0.93	0.07
1.15	1.93	5.56	0.95	0.05
1.20	2.83	7.72	0.91	0.09
1.25	3.99	8.35	0.70	0.30
1.30	5.57	10.1	0.60	0.40
1.35	7.80	10.6	0.45	0.55

TABLE II
Thermoelastic Data for Reduced and S-β-Cyanoethylated Human Hair Sample in Equilibrium Swelling with 8 *M* LiBr-BC Solution in the Temperature Range 30–65°C

$\alpha_{60°}$	f (g)	$10^3(\partial f/\partial T)_{p,\alpha,\mathrm{eq}}$	$(T/f)(\partial f/\partial T)_{p,\alpha,\mathrm{eq}}$	f_e/f
1.25	0.23	0.63	0.90	0.10
1.30	0.28	0.75	0.91	0.09
1.35	0.32	0.87	0.88	0.12
1.40	0.37	1.05	0.94	0.06
1.45	0.42	1.38	1.09	−0.09
1.50	0.47	1.53	1.09	−0.09
1.55	0.52	1.74	1.11	−0.11

equilibrium force, f can therefore be written as Eq. (1):

$$f_e/f = 1 - (T/f)(\partial f/\partial T)_{p,\alpha,\mathrm{eq}} \quad (1)$$

Table I shows the result obtained for unreduced hair with 662.5 μmol/g of disulfides. The energy component amounting to about +0.08 is obtained as average value in the strain range 1.02 to 1.20. It is notable that the retractive force arising from the swollen network is nearly entropic so far as the strain ratios do not exceed the values of 1.20.

Table II shows the result obtained for the reduced and S-β-cyanoethylated fiber with 91.6 μmol/g of disulfides. This result indicates that there is basically no energy component at all.

Stress-Strain Relation

It has been reported[19] that the swollen network of keratin fiber deviates from the ideal (Gaussian) network that the average force between any two adjacent crosslinks is same. To analyze the non-Gaussian effects, Mooney-Rivlin plot has been usually made according to Eq. (2) for swollen rubbers.[23]

$$\tau v_2^{-1/3}/2(\alpha - 1/\alpha^2) = C_1 + C_2/\alpha \quad (2)$$

where τ is the force per unit unstrained swollen area, v_2 the volume fraction of rubber in swollen state, α the strain ratio, C_1 and C_2 are the constants. The deviations from linearity with increasing strain (decreasing $1/\alpha$) represents the non-Gaussian region of strain.[23,24]

Figure 6 shows plots of the quantity ϕ defined as $\tau v_2^{-1/3}/2(\alpha - 1/\alpha^2)$ against $1/\alpha$ for different swollen fibers. For the human hair, approximately linear relation is obtained in lower strain range ($\alpha < 1.15$ or $1/\alpha > 0.87$) and parallel to the $1/\alpha$ axis. This means $C_2 = 0$, and suggest that the retractive forces arising from the network system are mainly entropic, which is the same conclusion as derived from the force-temperature relationships. At higher strains, the upturn takes place from the straight line. It is inferred, therefore, that as far as the initial extension regions are concerned, the Gaussian statistics in rubber network can be applied for the swollen keratin systems. For the reduced and subsequently S-β-cyanoethylated hair, linear relation is continued to higher strain range.

The ϕ value in the initial extension regions at $C_2 = 0$, which equals to C_1 as a function of network structure, is analogous to one-half the value of shear modulus, G in the statistical theory. As compared with ϕ value for the swollen human hair and the control hair prepared without NEMI, the quantity ϕ in the latter is lower by about half than the value in the former. This suggests that during treatment with 11 M LiBr, SH-SS interchange reactions occur and, as a result, modulus of network elasticity decreases.

Determination of the Number of Crosslinks

The crosslink density of Gaussian network or the number of chains in unit volume of dry polymer, ρ/M_c can be calculated by the following equation[23,25].

$$\tau = G(\alpha - 1/\alpha^2) \quad (3)$$

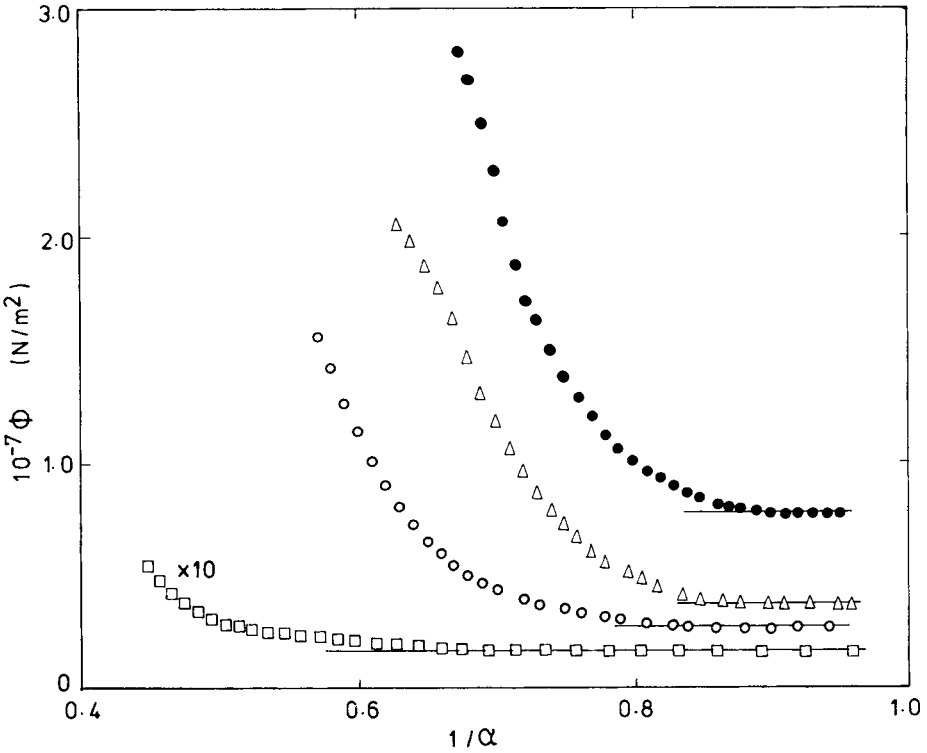

Fig. 6. Mooney-Rivlin plot for hair and wool: (●), human hair; (△), control human hair; (□), reduced and S-β-cyanoethylated human hair with SS = 91.6 μmol/g; (○), Lincoln wool.

where τ is the equilibrium stress referred to the swollen cross-sectional area, α the strain ratio, and G the shear modulus of the swollen fiber and represented by Eq. (4):

$$G = (\rho RT/M_c)v_2^{1/3}(1 - 2M_c/M) \tag{4}$$

where R is the gas constant, T the absolute temperature, V_2 the volume fraction of polymer in swollen sample, ρ the density of dry sample, M_c the number-average molecular weight between crosslinks and M the number-average molecular weight of primary molecule.

Application of Eqs. (3) and (4) to the swollen keratin fibers may be limited by the presence of the nonisotropic molecular structure and the nonuniformity arising from low-sulfur (crystalline) and high-sulfur (globular) regions throughout the fiber. Although the network chains behave like Gaussian chains in the initial extension range as described in preceding discussion, the swollen network may involve aggregates or globular regions which act as filler particles in rubbery phase. There is considerable evidence that the presence of fillers influences the overall elastic properties as "filler effect" of finely dispersed solids in conventional elastomers. Therefore, the calculated ρ/M_c values obtained by using Eqs. (3) and (4) apparently include the filler effects and also entanglement effects for the densely crosslinked keratins. These

structural effects in reduced keratin are thus considered to be considerably less than those of unreduced keratin, because disaggregation and disentanglement are enhanced by the reduction of disulfide crosslinkages. We can expect, therefore, that in a system with a low degree of crosslinking, the network is random and the crosslink density obtained from Eqs. (3) and (4) is absolute or near absolute value.

The ρ value for keratins is assumed to be 1.30 g/cm^3. A reasonable value of M for wool keratin[12] has been reported to be 2×10^4. For the other keratins, the same value as for the wool was assumed for our calculations. According to Eq. (4), the ρ/M_c value can be calculated from the slope of τ versus $(\alpha - 1/\alpha^2)$ plot. The results for SS content, ρ/M_c, and v_2 values of various keratin fibers are shown in Table III. An approximately linear relationship of ρ/M_c versus SS content is also shown in Figure 7. This indicates that the number of mechanically effective crosslinks increases proportionally with increase of the disulfide content of keratins. If it is assumed that all SS bonds act as elastically effective crosslinks,[19] corresponding crosslink density can be

TABLE III
Crosslink Density for Hairs and Wools

Keratin fiber samples	SS content (μmol/g)	v_2	Crosslink density[a] $10^4\rho/M_c$ (mol/cm^3)
Human hairs			
French female (15 age)	702.0	0.718	73.3
Japanese female (14 age)	662.5	0.623	56.4
Horse hair	480.0	0.473	25.7
Wools			
South Down	579.7	0.677	42.3
Suffolk	555.6	0.618	33.1
Bellah Speckled Face	553.5	0.680	41.4
Cymrieg	544.6	0.604	45.5
Merino Medium Sociality	543.4	0.608	41.5
Gramorgan Welsh	524.4	0.643	42.9
Improved Welsh	516.1	0.495	23.2
Hampshire Down	503.4	0.677	33.0
Shropshire Down	500.7	0.509	24.9
Welsh Mountain Sheep	500.7	0.504	28.0
Border Leicester	500.0	0.609	31.8
Dorset	500.0	0.498	24.3
Welsh Black Mountain	495.9	0.506	31.3
Leicester	482.9	0.540	30.4
Romney	451.6	0.518	24.1
Lincoln	450.3	0.607	24.9
Ryeland	444.0	0.559	28.9
Reduced and S-β-cyanoethylated			
Human hair, Japanese female	91.6[b]	0.250	2.7
Japanese female	~ 0[c]	0.242	1.8
Wool, Lincoln	~ 0[c]	0.248	1.5

[a]Average value was taken from the tests of 5–8 specimens for each sample.
[b]Reduction: 0.4% TBP.
[c]Reduction: 1% TBP; reduction-cyanoethylation, 2 cycles.

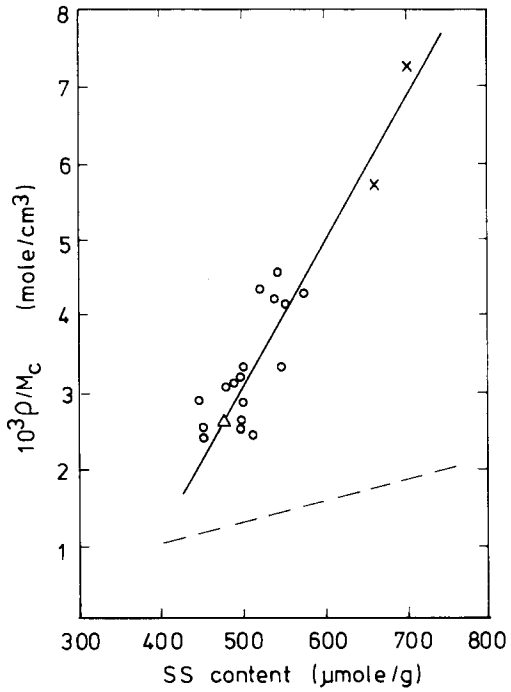

Fig. 7. Relationship of crosslink density versus disulfide (SS) content for a variety of keratins: (×), human hair; (△), horse hair; (○), wool; (---), $(\rho/M_c)_{chem}$ curve calculated by assuming all SS bonds are intermolecular links.

calculated by using the equation: $(\rho/M_c)_{chem} = 2 \cdot 10^{-6}\rho[SS]$ in mol/cm^3, where [SS] is the content of SS in μmol/g. The result obtained is shown in Figure 7 by the broken line. A remarkable difference is observed between the crosslink densities obtained by physical and chemical methods.

Interpretation of the Number of Crosslinks

It has been shown that in vulcanized natural rubber with relatively high crosslink density, the modulus is increased by a factor of about 2.5 for the entanglement effects.[23] Corresponding factors for keratin networks can be obtained from Figure 7; namely ~ 2.0 to 3.0 for wools and 3.5 to 4.0 for hairs. Feughelman[26] has pointed out the presence of the intermicrofibrillar linkages by the nonhelical "tails" of the low-sulfur microfibrillar proteins and their effects on fiber properties. These linkages may give rise to physical entanglements in the keratin systems. For the unreduced human hair, it seems difficult, however, to explain reasonably in terms of the entanglement effects.

The ρ/M_c value obtained from Eqs. (3) and (4) derived for random network of Gaussian chains may not be absolute value for real keratin network, but only relative one, since the structural nonuniformity arising throughout the fiber will remain more or less in swollen state.

In considering the network structure, it is important to characterize the nature of keratin molecules in the diluent. The segment length of the real keratin chains in the swelling media could be evaluated by the Langevin

approximation on the stress-extension curve obtained for the fiber with lower crosslink density, such as the reduced and S-β-cyanoethylated hair which contains only 91.6 μmol/g of SS bonds. Here, it should be noted that the retractive forces are mainly entropic at the whole extension range (Table II). The three-chain model[23] was applied, and non-Gaussian stress-extension relation fitted by using computer to all the extension regions of experimental data with $\rho/M_c = 2.17 \times 10^{-4}$ mol/cm^3 or $M_c = 6.0 \times 10^3$ and the number of segments equivalent to random chain, n = 3.5 as adjustable parameters is shown by solid line in Figure 8. The magnitude of ρ/M_c value evaluated from the non-Gaussian treatment corresponds to ~ 89% of the value (2.45×10^{-4} mol/cm^3 or $M_c = 5.3 \times 10^3$) from Eqs. (3) and (4). It is, thus, shown that as far as such a low crosslinking system is concerned, the Gaussian treatment for swollen keratin fibers is good approximation. This result coincides well with the consideration for Mooney-Rivlin plots.

The number of amino acid residues per segment, n_r can be calculated to be 13.7 by using the equation: $n_r = M_c/nM_o$, where M_o (= 125) is the average molecular weight of amino acid residues of hair protein.

For the unreduced human hair with 662.5 μmol/g in SS content, the ρ/M_c value obtained is 56.4×10^{-4} mol/cm^3 (Table III) and the calculated M_c is 230 ($= 10^4 \cdot 1.30/56.4$), which corresponds to only 1.8 amino acid residues. These calculations show that the average chain length of the real network is significantly less than the length of equivalent random-chain segment n_r (= 13.7). This means that the real chain should not be Gaussian, and the network elasticity probably be energetic rather than entropic. Contrary to this, the experimental results show that the network chains behave like Gaussian chains (Fig. 6) and the energy components are very small in the

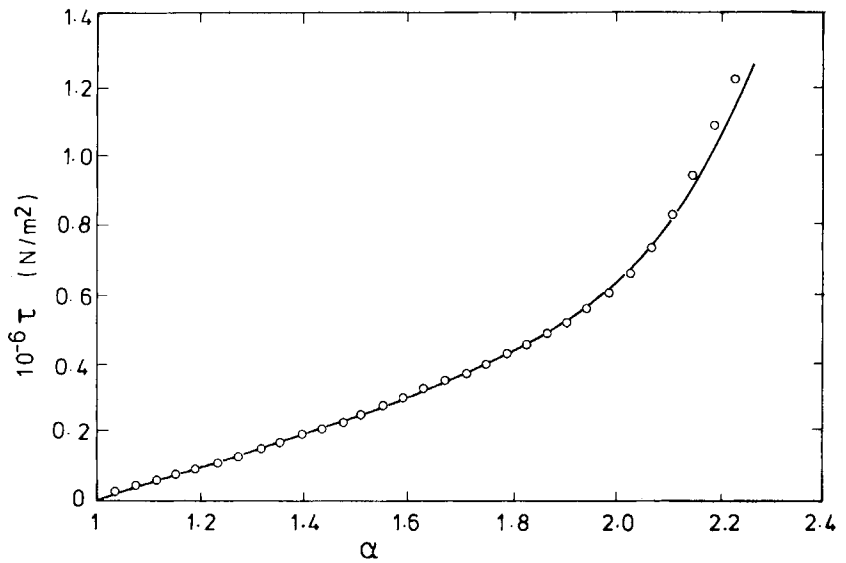

Fig. 8. Non-Gaussian stress-extension relation fitted to all the extension regions of experimental data obtained from reduced and S-β-cyanoethylated human hair with SS = 91.6 μmol/g: (○), experimental data; (—), calculated curve with $M_c = 6.0 \times 10^3$ and the number of segments equivalent to random chain, n = 3.5.

range of 20% elongation (Table I). It is suggested, therefore, that the typical rubbery behavior observed on the swollen keratin arises from the deformation of rubbery phase with a lower crosslink density. It is considered that the swollen fiber is composed of a two-phase structure similar to the block copolymer forming stable domains and rubbery phase. Despite the unusually large v_2 value (Table III), the swollen hairs and wools are highly elastic (Fig. 2). This may be ascribed to the occurrence of a localized swelling at microseparated regions in fiber.

In the two-phase system, a larger elongation is resulted in rubbery phase than the sample elongation and the deformation of the network chains may be varied as a function of the volume fraction of rubbery phase in swollen sample.[27] As a consequence, strain ratios for actually elongated rubbery phase must be taken to calculate the number of crosslinks in the corresponding phase, and the crosslink density referred to the volume of sample could be expected to be less than the calculation from Eq. (4). Matrix regions rich in cystine which give rise to nonuniformity throughout the fiber structure may probably be concerned with mechanically stable domains. Validity of this structure awaits further investigations.

Number of the Crosslinks Other Than SS Links

The reduced and S-β-cyanoethylated wool and hair are likely to have a randomly crosslinked network elasticity as seen from Figures 6 and 8. Therefore, by using Eqs. (3) and (4), the crosslink densities of the wool and hair fibers with no disulfide groups can be estimated to be 1.5×10^{-4} and 1.8×10^{-4} mol/cm^3 (Table III), respectively, which correspond to about 60 and 70 μmol in the number of crosslinks per gram of keratin. This result shows that the amounts of nondisulfide interchain crosslinks present in both keratins are somewhat considerable.

Crewther[28,29] reported that the wool fiber contains crosslinkages, other than disulfides, which are hydrolyzed by acids and alkalis, and ester crosslinkages are more probable than amide crosslinkages. A quantitative treatment of supercontraction of wool in solutions of LiBr showed that there are approximately 200 μmol disulfide/g and about 150 μmol of acid-labile crosslinkages/g of the low-sulfur (α-helical) protein which constitute about 60% of the fiber. Crewther[29] showed that the content of the acid-labile bonds would be about 90 μmol/g in whole fiber if the high-sulfur proteins do not contain such crosslinkages.

It has been reported that the lanthionine and lysinoalanine crosslinks cannot be regarded as normal constituents of intact wool proteins,[15] while very small amounts of lanthionine are formed in reduced and alkylated wool.[30] Asquith,[31] Schmitz,[32,33] and Zahn[34] and their co-workers demonstrated the presence of isopeptide linkages, ϵ-(γ-glutamyl)lysine from the enzymatic digests of fetal wool, and native wool and hair. It is uncertain, however, whether these links are really responsible for elastically effective crosslinks and associate with intermolecular crosslinkages between protein chains.

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