CHEMISTRY OF KERATIN*

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Perhaps nothing in research today is more fascinating than the dependence of extremely diverse industries on a given field of basic science. An excellent example of this is found in a branch of chemistry which has been rapidly developing during the last fifteen years and which has come to be known as the chemistry of organic high polymers. This branch of modern science has been the basis for many of the outstanding developments on rubber, resins, fibers, and leather, materials which have all found an extremely wide range of application in industry. In addition and somewhat indirectly, it can be considered responsible for some of the more recent developments in the cosmetic industry. I refer particularly to the relationship of the chemistry of wool to that of human hair and to the applications of some of the principles of high polymer science to a number of present-day cosmetic developments. As a background for the present discussion, I should like to consider a few of these fundamental principles.

The great industrial importance of the organic polymeric systems stems from their wide range of mechanical properties. Thus, rubber is important because of its long-range elasticity; plastics can be molded into materials of considerable strength or modified so as to yield systems possessing considerable flexibility or elasticity; textile fibers have a great variety of properties which lend themselves well to the fabrication of materials ranging from women's sheer hose to heavy industrial fabrics.

A most interesting feature of all of these systems is that, in many cases, a small difference in chemical structure may determine whether a material is a rubber, plastic, or a fiber. An excellent example of this is found in the behavior of the polyamides of the type used for producing nylon. Polyamides such as those produced by the condensation of adipic acid and hexamethylene diamine make outstanding textile fibers. However, the substitution of an occasional isobutyl group for a hydrogen atom in the amide group results in a rubber-like material with an elastic extension which may

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reach several hundred percent.

In other words, the problems of those who are particularly interested in fibers are very similar to those of workers who are interested in other high polymer fields whether they deal with leather, human hair, skin, rubber, or plastics. From a chemical point of view, there is an over-all similarity among these systems in that they are all polymers of high molecular weights, most of which are linear although a few are branched or cross-linked. True, they differ in details of chemical structure which accounts for their different properties. And from an industrial point of view, all of these systems are important because of their diverse mechanical properties.

As a matter of fact one can go still farther in emphasizing these inter-relationships and put forth a set of qualitative rules for predicting the mechanical behavior of organic high polymers (1). For example, if the chemical structure of the molecules is such that they fit poorly into a lattice structure (in other words, an amorphous state is favored) and further if the intermolecular forces are weak, then the material should have rubber-like properties. In contrast, if the structure of the molecules is such as to provide for an easy fitting into a lattice (that is, a relatively high state of crystallinity is favored) and the intermolecular forces are strong, the material is a fiber. In intermediate cases, plastic systems are obtained.

If we now pass from the broad field of high polymers to a narrower section dealing with fibers, the same considerations are found to be applicable. In some respects, the classification just given is even more useful for classifying different fiber systems. For example, if the molecules fit readily into a lattice and the intermolecular forces are strong, a very strong fiber with low elongation and at best very short range elasticity is obtained (cotton, linen, nylon). If the molecules fit poorly into a lattice and the intermolecular forces are weak, a fiber of low strength but greater extensibility results; further, if the molecules are flexible, the fiber can exhibit relatively long range elasticity (wool, elastic, nylon). Again, for intermediate cases, fibers with intermediate properties may be obtained (silk). It is at once obvious that there will be a number of borderline cases but, nevertheless, this is a useful qualitative classification.

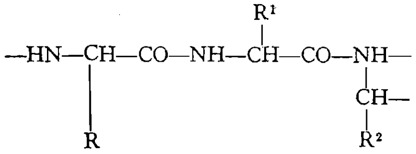
If we now narrow the section dealing with fibers still further and consider one type of fiber, the keratin fibers, as exemplified for example by wool, we find the same considerations to be applicable, and this will be borne out in the subsequent discussion.

The distinguishing mechanical property of wool is its long-range elasticity, that is, the ability to recover from deformations of a magnitude considerably greater than that permitted by other types of fibers, regardless of whether the deformations have been produced by stretching, twisting, or crushing. The importance of this property becomes most apparent when we consider the uses to which wool is put in industry, namely, in the fabrication of such materials as blankets, clothing, and carpets. Thus, a wool blanket is warmer than a cotton blanket, but not because the wool fiber is intrinsically warmer than the cotton fiber. It is a well-known fact that if two blankets of similar construction and thickness, one of wool and one of cotton, are subjected to thermal transmission studies, they show closely similar insulation values. This is due to the fact that the insulation value of these systems is largely dependent on the large number of small air pockets trapped in the structures. However, empirical experience has indicated that the wool fabric is more satisfactory, and this appears to result from the fact that a cotton blanket during use, and especially when wet, tends to mat and, therefore, loses a portion of its insulating qualities; whereas the wool blanket, largely because of the elasticity of the wool fiber, tends to retain its essential thickness and thus maintain itself as a better insulator over a longer period of time. In clothing these same considerations are important, but in addition, the elasticity of the wool fiber results in a fabric which will recover its shape more satisfactorily after deformation than cotton, linen, rayon, etc.—fabrics which tend to crease readily on similar deformation. Finally, in the case of carpets, the important feature of the wool pile is that it recovers well after it has been deformed by applying a load.

From the foregoing discussion, it is not surprising that nature should have furnished the human and animal body with protective and insulative tissues such as hair, skin, and fingernails which are highly elastic. In view of the importance of this property of elasticity in keratin fibers it is particularly desirable to examine in more detail, those aspects of their molecular structure which contribute to their unique behavior as fibers.

Chemical Nature of Wool

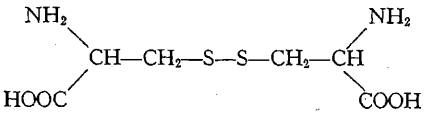
Keratin is composed principally of protein substances and as such is a polycondensation product of amino acids in which the different amino acids are linked together to form the polypeptide chain shown in the following scheme:



$$-HN-\underset{\displaystyle R}{\underset{|}{CH}}-CO-NH-\overset{\displaystyle R^1}{\overset{|}{CH}}-CO-\underset{\displaystyle R^2}{\underset{|}{\underset{\displaystyle CH-}{\underset{|}{NH-}}}}$$

It will be immediately obvious that two molecular properties of these chains are of importance in terms of the principles proposed earlier. First, because of free rotation about the carbon-carbon and carbon-nitrogen bonds, such chains exhibit great flexibility which makes possible a large number of configurations. The long-range extensibility of keratin fibers can be ascribed to the opening of these folds; the elastic properties to the tendency of the extended chain to assume a more probable or stable form which is the folded form. The second factor influencing the properties of such chains is the presence of relatively large size chains (R groups in the scheme of the polypeptide chain) which prevent close packing of the protein molecules. It is estimated that close to 50% of the weight of the fiber is in the side chains (2). In other words the combination of the flexible chains and a structure that would fit poorly into a lattice structure would be expected to yield a system with rubber-like properties.

Based on present knowledge of fiber structure, however, a system made up of flexible polar molecules which pack poorly would be expected to exhibit relatively low strength, especially in the wet state (3). Since this is not in accord with the known properties of keratin fibers, it is necessary to search further for additional factors accounting for the observed properties. A more detailed examination of the constitution of the keratin protein reveals the presence of a relatively high percentage of the double amino acid, cystine.



$$\begin{array}{ccc} \mathrm{NH_2} & & \mathrm{NH_2} \\ & \mathrm{CH{-}CH_2{-}S{-}S{-}CH_2{-}CH} & \\ \mathrm{HOOC} & & \mathrm{COOH} \end{array}$$

Although cystine contains two amino and two carboxyl groups it appears that all these groups are involved in peptide linkages (4). On the basis of this, at least the major portions of the cystine must form parts of two separate polypeptide chains and accordingly the disulfide group becomes a connecting or cross link between polypeptide chains. Keratin fibers must therefore be considered a three-dimensional network of polypeptide chains held together by the disulfide groups of the amino acid cystine. Such a concept suggests that the role of cystine in keratin must be an important one and indeed it can be shown that many of the chemical, physical, and biological properties of keratin are dependent on the presence of these cross links (2, 4, 5).

Such a structure suggests that the modification of the mode of linkage of the sulfur cross links should lead to profound alterations in the properties of the fiber. In subsequent investigations, it was found that the disulfide group was readily ruptured by a number of reducing agents, especially of the water-soluble type such as thioglycolic acid (4, 5). If the solution of such reducing agents was highly alkaline, the cross links were ruptured and in addition the freed polypeptide chains went into solution. This is today the basis for a number of depilating systems. On the other hand if a portion of the disulfide groups was ruptured and the pH was kept below about pH 10, the original fiber structure was retained and further the reduced fiber could be subsequently treated with alkyl dihalides to form a whole series of keratin derivatives (4, 5). The reactions may be presented as follows:

$$W—S—S—W + 2HS—CH_2—COOH \rightleftharpoons 2W—SH + (S—CH_2—COOH)_2 \quad (1)$$

$$WSH + RX \rightarrow W—SR + HX \text{ (where } X = \text{Cl, Br, etc.)} \quad (2)$$

$$2WSH + (CH_2)_nX_2 \rightarrow 2HX + W—S—(CH_2)_n—S—W \quad (3)$$

Reaction of the type shown in equations 1 and 2 result in permanent rupture of the cross links; reaction with dihalides as shown in equation 3 result in the formation of new cross links in which the sulfur atoms of the cystine are connected by short hydrocarbon chains.

The reaction in equation 2 and 3 offers a means of preparing a large number of wool derivatives. Thus it was not only possible to investigate the nature of the sulfur linkages and their relation to the structure of the fiber but also to study the effects on the properties of introducing into the fiber new groups having widely different properties.

ELASTIC BEHAVIOR OF WOOL AND CHEMICALLY MODIFIED WOOLS

Measurements of the elastic properties were made on individual fibers by a modification of the method described earlier (6). In this method the 30 per cent index is the energy required to stretch a fiber to 30 per cent elongation after a treatment, divided by the similar energy requirement prior to the treatment. This index is based on Speakman's demonstration that wool fibers can be elongated 30 per cent without permanent deformation or weakening, if the duration of the strain is short (7). In the former investigations from this laboratory, however, the stress-strain characteristics of fibers were determined only during the extension process. In the present work, this procedure was modified so that measurements of the behavior of the fiber during its retraction could also be obtained.

Figure 1 shows the behavior of a typical wool fiber during two successive stress-strain determinations. The fiber was allowed to relax for approximately 24 hours between the first and second extensions. It is noteworthy that the entire stress-strain cycle is reproducible. This fact makes it possible to compare the stress-strain characteristics of a particular fiber in retraction as

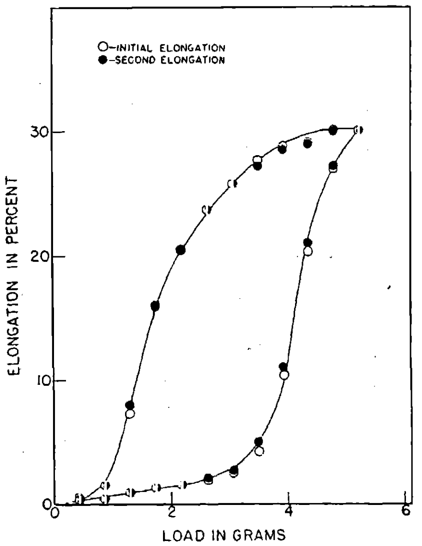


Figure 1.—Typical stress-strain cycle of an untreated wool fiber.

well as in extension, before and after a modification of its chemical structure.

When the cross linkages have been permanently ruptured by the reduction process, followed by alkylation of the sulfhydryl groups with an alkyl monohalide of low molecular weight, such as methyl iodide or ethyl bromide, the resistance to extension is greatly altered, as shown in Figure 2. Much less energy is required to elongate the fiber after the cross links have been split, the 30 per cent index dropping to the low value of 0.26. In this material the S-shaped curve, characteristic of the original wool fiber and of other highly elastic materials, is not observed in extension to only 30 per cent, but can be demonstrated in experiments involving higher extensions. The whole scale of the relations of stress to strain has been shifted toward greater extensions for smaller forces. It should be added that the recovery from 30 per cent extension to the original length is complete and rapid.

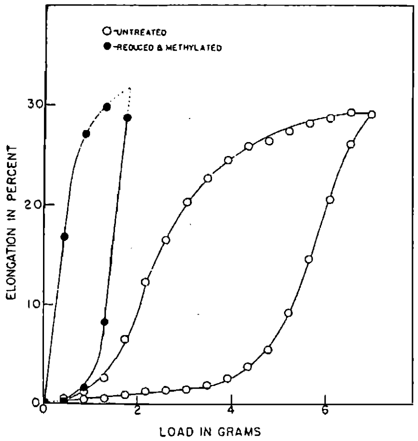


Figure 2.—Effect on stress-strain cycle of rupturing cross links.

If, after reduction, the cross links are largely rebuilt by reoxidation of the sulfhydryl groups to the disulfide form, the wool recovers to a large extent its original properties, as shown in Figure 3. The small discrepancy may arise either from the possibility that reoxidation has not been complete or that a few SH groups had reacted, during the reoxidation, with sulfhydryl groups other than those with which they were combined in the original fiber.

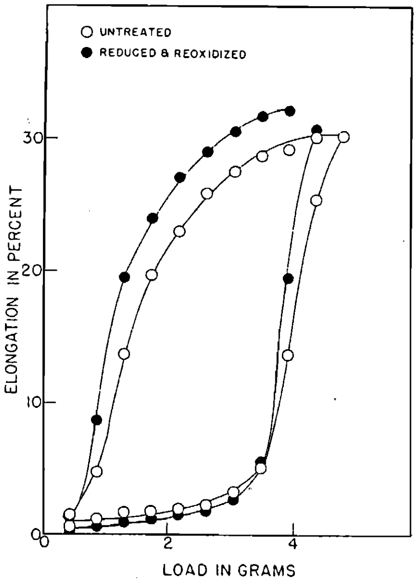


Figure 3.—Effect on stress-strain cycle of reduction of cross links followed by reoxidation with oxygen.

Alkylation of the reduced wool with an alkyl dihalide, such as methylene bromide or trimethylene bromide, results in the re-formation of cross linkages, but in this case, the disulfide linkage has been replaced by a bis-thioether linkage of the type —$S(CH_2)_nS$—. While the position of the stress-strain curve for the fiber after such treatment has been slightly shifted from that of the original fiber, the shape of the curve and the recovery are very similar to those of the original fiber. A slight shift in the position of the curve is to be expected, since obviously the lengths of the cross linkages have been increased and one would expect a slightly greater ease of extension of the fiber.

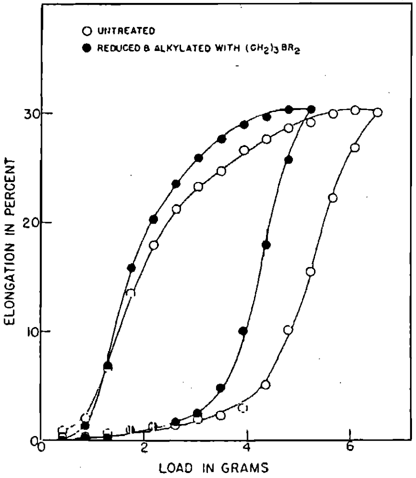


Figure 4.—Effect on stress-strain cycle of rebuilding cross links by reduction and alkylation with an alkyl dihalide.

These experiments lead to a better understanding of the molecular basis of the elastic properties of keratin fibers. The long range elastic characteristics probably result from the ability of the flexible chain molecules to contract from the less probably stretched state to a more random form. The function of the cross links is to strengthen the fibers and to suppress plastic flow.

INFLUENCE OF CROSS LINKS ON OTHER PROPERTIES OF KERATIN FIBERS

From the foregoing discussion it is apparent that the disulfide cross links have a marked influence on the mechanical properties of keratin fibers. It can be also demonstrated that these cross links have a profound effect on certain chemical and biological properties of these materials. For example, keratin fibers are distinguished from most protein systems by a resistance to attack by biological agents such as bacteria and enzymes. The resistance to digestion by enzymes is probably due to the unique structure of peptide chains joined by disulfide cross links to form a three-dimensional network of extremely high molecular weight. Rupture of the cross links results in a material which is readily digested in solutions of the proteolytic enzyme pepsin (8, 9, 10).

It is interesting to note that although keratin as a protein contains most of the essential amino acids, this resistance to attack by enzymes makes it worthless as a food source for humans or most animals. Rupture of the cross links, however, does yield a digestible material. A notable exception, however, is found in the case of the moth larvae which encounters little difficulty with keratin materials. Apparently moth larvae can digest keratin but only after breaking its disulfide cross linkages by the action of an alkaline reducing agent present in their intestinal tracts (11). For this reason it would appear probable that modified wools containing stable cross linkages such as shown in equation (3) would be far more stable to biological agents and such stability was actually observed in experiment (5).

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