ROLE OF CYSTINE IN THE STRUCTURE OF THE FIBROUS PROTEIN, WOOL

By Wilbur I. Patterson, Walton B. Geiger, Louis R. Mizell, and Milton Harris

ABSTRACT

A number of the important physical and chemical properties of wool are closely associated with the presence of disulfide cross-linkages between the polypeptide chains of the protein. This conclusion results from a study of the behavior of wool before and after alteration of the mode of linkage of the sulfur by a series of highly specific reactions.

The cystine in wool can be readily reduced to cysteine with thioglycolic acid. Strongly alkaline solutions of the reagent have been shown by previous investigators to dissolve the protein and destroy its fibrous structure. It is now shown that wool can be reduced with thioglycolic acid over a wide range of pH, and that when the reduction is carried out in neutral or acid solution, the fibrous structure of the wool is not destroyed when the disulfide groups are reduced to sulfhydryl groups. The sulfhydryl groups of fibers reduced in this way react readily with alkyl halides to form thioether groups.

Thus reaction of reduced wool with alkyl monohalides, such as methyl iodide, results in permanent rupture of disulfide linkages, and the fibers are greatly increased in extensibility and decreased in strength. Alkylation with aliphatic dihalides, such as methylene iodide or trimethylene dibromide, introduces hydrocarbon chains between pairs of sulfur atoms of cystine molecules in the fibers. Such fibers are very similar to untreated fibers in physical properties.

Wool in which the disulfide linkages have been broken by reduction, or by reduction and alkylation with alkyl monohalides, possesses much higher alkali-solubility than untreated wool, while wool in which the disulfide cross-linkages have been replaced by new covalent cross-linkages through reduction followed by alkylation with dihalides possesses much lower alkali-solubility. Since the susceptibility of wool to degradation by alkalies is one of its greatest disadvantages, processes that would make it stable toward alkalies should also enhance its durability.

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I. INTRODUCTION

Proteins, according to the hypothesis of Hofmeister [1] and Fischer [2], consist of amino acids joined through amide (peptide) linkages to form long chain structures called polypeptides. On the basis of this hypothesis, many of the similarities among proteins should be due to the large number of peptide linkages, while dissimilarities among them should be related to variations in the proportions and arrangement of the constituent amino acids, to differences in the lengths and arrangement of the polypeptide chains, and to the presence of linkages other than peptide. The present paper is concerned with the relationship of one such linkage, the disulfide linkage of the amino acid cystine, to the properties of the fibrous protein, wool.

Although cystine contains two amino and two carboxyl groups, it appears that all of these groups are involved in peptide links in most, if not all, of the cystine residues. If the cystine of wool were combined in a peptide chain only through amino and carboxyl groups attached to the same carbon atom (formula I), processes that break disulfide bonds should result in the elimination from the wool of one-half of the sulfur. This possibility is not supported by experiment since reduced wool contains as much sulfur as the original material. Were either (or both) of the amino groups of cystine uncombined (formula II), treatment of wool with nitrous acid would destroy at least half of the cystine in the former case and all of it in the latter. The investigations of Hess and Sullivan [3], as well as unpublished work in this laboratory, show that only a small portion is destroyed by this reagent. Evidence derived from titrations [4, 5] indicates that no considerable number of carboxyl groups of cystine in wool can be free (formula III) since the number of free carboxyl groups is essentially accounted for by the content of glutamic and aspartic acids. While these data do not afford complete proof, they strongly indicate that all the functional groups of all the cystine are combined, and that only a small proportion, if any, can be free.

1 Figures in brackets indicate the literature references at the end of this paper.
I.

\[
\begin{align*}
\text{NH} & \quad \text{CHR} \\
\text{CO} & \quad \text{NH}_2 \\
\text{CH} & \quad \text{CH}_2 & \quad \text{SS} & \quad \text{CH}_2 & \quad \text{CH} \\
\text{COOH} & \quad \\
\end{align*}
\]

II.

\[
\begin{align*}
\text{NH} & \quad \text{CHR} \\
\text{CO} & \quad \text{NH}_2 \\
\text{CH} & \quad \text{CH}_2 & \quad \text{SS} & \quad \text{CH}_2 & \quad \text{CH} \\
\text{CO} & \quad \\
\end{align*}
\]

III.

\[
\begin{align*}
\text{NH} & \quad \text{CHR} \\
\text{CO} & \quad \text{NH}_2 \\
\text{CH} & \quad \text{CH}_2 & \quad \text{SS} & \quad \text{CH}_2 & \quad \text{CH} \\
\text{COOH} & \quad \\
\end{align*}
\]

IV.

\[
\begin{align*}
\text{NH} & \quad \text{CHR} \\
\text{CO} & \quad \text{NH}_2 \\
\text{CH} & \quad \text{CH}_2 & \quad \text{SS} & \quad \text{CH}_2 & \quad \text{CH} \\
\text{CO} & \quad \\
\end{align*}
\]
On the basis of these considerations, at least the major portion of the cystine must form parts of either two separate polypeptide chains (formula IV) or of a single folded polypeptide chain, and in either case its disulfide group would serve as a connecting or a cross-link. Astbury and Street [6] were the first to suggest that such disulfide cross-linkages were present in wool protein; since then Astbury, and Speakman, with their collaborators, have extended this hypothesis.

This view suggests that modification of the mode of linkage of the sulfur in wool should lead to profound alterations in the properties of the fiber. Unfortunately, since many of the processes known to affect disulfide linkages, such as the action of light, of alkalies, and of oxidizing and of some reducing agents, involve side reactions with other groups in the protein, conclusions based on their use may be open to criticism. The action of a few reducing agents, however, appears to be solely with the disulfide groups and therefore it appeared possible to use these reagents in evaluating the relation of the disulfide groups to the structure and properties of the wool fiber. Of these reagents, only thioglycolic acid and certain other mercaptans can reduce each disulfide group to two sulfhydryl groups. Other reagents, such as potassium cyanide, sodium sulfide, and sodium bisulfite, react with disulfide groups to form not only sulfhydryl groups but others as well [7].

Goddard and Michaelis [7, 8] found that strongly alkaline solutions of thioglycolic acid rapidly converted the disulfide groups of wool to sulfhydryl groups, and that under these conditions, rapid dissolution of the wool occurred. These authors used alkaline solutions since they observed that wool was not dissolved by neutral or acid solutions of the reagent, an observation that led them to conclude that wool could be reduced only at a pH of 10 or higher. Such a conclusion is not compatible with the behavior of disulfides in general, which are known to be readily reduced over a wide range of pH values. In the present investigation, it is shown that the disulfide groups of wool can also be reduced by thioglycolic acid over a wide range of pH, and that if the reduction is carried out in neutral or acidic solutions, the fibrous structure of the wool is not destroyed. The use of neutral or faintly acidic solutions of the reducing agent also has the advantage of eliminating such secondary reactions as degradation of cystine, cysteine, peptide, or other linkages.

Wool that has been reduced in neutral or acid solution can be further modified without loss of fibrous structure by treatment with alkyl halides, which convert the sulfhydryl groups to thioether groups. The reactions may be presented as follows:

\[ W-S-S-W + 2HS-CH_2-COOH \rightarrow 2W-SH + (S-CH_2-COOH)_2 \]  
\[ W-SH + RX \rightarrow W-SR + HX, \]

where W represents the portions of the wool connected by the disulfide groups, R represents an alkyl group, and X, a halogen atom. Reactions of this type result in the permanent rupture of covalent cross-linkages. Wool fibers have been prepared in which the mode of

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1 Goddard and Michaelis [7, 8] and Pilemmer, Ecker and Wells [9, 10], also found that their amorphous wool protein derivative could be alkylated by treatment with alkyl monohalides to form other amorphous protein derivatives.
linkage of at least 95 percent of the cystine has been modified through these reactions.

Dihalides are also capable of reacting with the reduced wool, the reaction being represented by the equation

$$2W-SH+(CH_2)_nX_2 \rightarrow W-S-(CH_2)_n-S-W+2HX.$$ (3)

The reaction with the dihalides results in the formation of new cross-links in which the sulfur atoms of the cystine are connected by short hydrocarbon chains.

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

II. EXPERIMENTAL PROCEDURE

1. MATERIALS

The wool fibers used in the present study were part of the lot used in earlier investigations reported from this laboratory [12]. This material had been subjected to no mechanical or chemical treatment other than successive extractions with alcohol and with ether at room temperature for 5 hr each, followed by washing with water at 40°C.

Worsted yarn was also used for some experiments. It was prepared from raw wool that had been previously extracted with Stoddard solvent and washed with water. The yarn was further purified by extraction in a Soxhlet apparatus with alcohol and with ether for 6 hr each, and finally washed with distilled water.

2. METHODS

(a) REDUCTION OF WOOL WITH THIOGLYCOLIC ACID

Aqueous solutions of thioglycolic acid were used, and were adjusted to the desired pH by adding potassium hydroxide solution. The final concentration of thioglycolic acid was determined by titration with standard iodine solution [13]. It was found advisable to cool the thioglycolic acid solution in an ice bath during addition of the potassium hydroxide, since at higher temperatures the solutions often became colored. Although aqueous solutions of thioglycolic acid were used in all experiments reported in this paper, solutions of the reagent in organic solvents were also found effective.

The reduction was carried out by immersing a known weight of wool in a solution of thioglycolic acid at the required temperature, pH, and concentration. Wetting the wool was facilitated by evacuating the vessel with a filter pump. After the reduction had been allowed to proceed for the desired length of time, the wool was removed and washed several times with distilled water to remove excess thioglycolic acid and its oxidation products.

[4] Speakman [11] has suggested that divalent metal ions, such as that of barium, can form cross-linkages between sulfur atoms. Such linkages would be ionic and unstable, as compared with completely homopolar linkages formed according to eq 1 and 3.
(b) REOXIDATION OF SULFHYDRYL GROUPS OF REDUCED WOOL

Before the reduced wool was reoxidized, it was first washed thoroughly with 95-percent ethyl alcohol. Omission of this step led to products that contained amounts of sulfur in excess of that in the untreated wool, presumably by the formation of a mixed disulfide, one-half derived from cysteine, the other half from thioglycolic acid. The wool was then rinsed with distilled water, placed in 0.1 M potassium phosphate buffer solution at pH 7.0, and a stream of oxygen bubbled through the solution until the wool no longer gave a positive nitroprusside reaction [14]. Finally, the wool was again washed with distilled water, and dried in a stream of air.

(c) ALKYLATION OF SULFHYDRYL GROUPS OF REDUCED WOOL

Reduced wool that was to be alkylated was transferred immediately after washing to a 1.0 M phosphate buffer solution at pH 8.0, in which had been dissolved or suspended the desired alkylating agent. The rate of alkylation was increased by agitation of the mixture, particularly when the alkylating agent was not completely soluble. Ordinarily, 0.002 mole of alkylating agent and 100 ml of buffer solution were used for each gram of wool. The reaction was allowed to proceed until a negative nitroprusside test for sulfhydryl groups in the fiber was obtained.

The reactions take place not only in aqueous buffer solutions but also in organic solvents in the presence of soluble or insoluble substances capable of removing the acid formed in the reaction. For example, solutions of reagents in 50-percent aqueous methanol, 0.05 M with respect to NH₄OH and 0.1 M with respect to NH₄Cl, or in benzene in the presence of sodium bicarbonate, were found to be satisfactory.

(d) ANALYTICAL METHODS

The extent to which the sulfhydryl groups of the reduced wool had been alkylated was determined by analyzing the wool for cystine by the Sullivan [15] procedure, as previously used in this laboratory [16]. Neither thioglycolic acid nor thioethers derived from cysteine affect the color reaction of the cystine under these conditions. The adequacy of this method of determining the amount of reaction was checked in certain cases by alkylating the reduced wool with p-chlorobenzyl chloride, a reagent that introduces an atom of halogen for each sulfhydryl group with which it reacts. For example, one product that contained 2.2 percent of chlorine (0.00063 equivalent per gram), also was found to contain 4.7 percent of cystine. The original wool contained 12.4 percent of cystine, so allowing for the increase in weight due to introduction of p-chlorobenzyl groups during the reaction, the product contained a calculated total of 11.4 percent of reacted plus unreacted cystine. By subtracting the 4.7 percent of unreacted cystine from this total, it is seen that 6.7 percent, or 0.00056 equivalent, of cystine in each gram of wool had reacted. The amount of halogen introduced therefore corresponds, within analytical error, with the decrease in cystine content of the wool.

The amount of halogen was determined by the Pregl bead-tube method [17]. Sulfur determinations were made by the oxygen-bomb method [18].
The hygroscopicity of the wool was determined by keeping air-dried samples in a room at 21°C and 65-percent relative humidity for 24 hr, and determining the loss in weight on drying in a vacuum oven at 105°C for 2 hr. The values are calculated as percentages of the dry weights.

Alkali-solubility was measured by the method of Harris and Smith [19]. Samples were treated with 0.1 N sodium hydroxide solution for 1 hr at 65°C, and the percentage loss in weight was determined.

(c) DETERMINATION OF PHYSICAL PROPERTIES OF THE WOOL

Two tests were utilized in determining the changes produced in the physical properties of the fiber during the various treatments. The first involved the measurement of the breaking strength of treated yarn by the single-strand method on a pendulum-type machine. The values reported are the averages of 10 or more determinations.

In the second test, the stress-strain characteristics of individual fibers were determined by the method described by Soolme and Harris [12]. This method, which is a modification of procedures used by other investigators, is much more sensitive to small changes and has greater accuracy than methods involving a determination of the breaking strengths. It is based upon the observation of Speakman [5] that when a wool fiber is immersed in water and stretched under load until it has been elongated 30 percent, it will return practically to its original length when the load is removed. If the fiber is allowed to relax in the unstretched state in water for about 24 hr., and then again stretched exactly as before, it is found that the same amount of energy (within about 1 percent) is required to stretch the fiber the second time. If the fiber after the first stretching is subjected to chemical treatments which break chemical bonds, less energy will be required to stretch it the second time. The ratio of the energy required to stretch the fiber the second time to that required the first time is called the 30-percent index. It follows that if the 30-percent index is less than 1, the fiber has been weakened by the treatment; if it is greater than 1, strengthened. The accuracy of this method depends on the fact that comparisons are always made between successive measurements on the same fiber. Since wool fibers are extremely irregular and vary considerably in shape and cross section, comparisons between different fibers would give rise to large errors, which could be only partially eliminated by the application of statistical methods to measurements on large numbers of fibers. With the present technique, as few as three fibers were found sufficient to provide adequate checks.

III. RESULTS AND DISCUSSION

1. COURSE OF THE REDUCTION REACTION

The reaction of wool with thioglycolic acid under a variety of conditions was investigated and is described in the following sections.

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Preliminary work showed that solutions of thioglycolic acid to which no alkali had been added reacted with wool, and resulted in the production of sulphydryl groups, which gave a positive nitroprusside reaction, and were capable of reacting with alkyl halides. These reactions proceeded without destruction of the fiber, whereas strongly alkaline solutions (pH 11.0 or above) of the reagent reacted with the wool, not only to reduce it, but also to dissolve it [7, 8]. The effect of various pH values was studied in greater detail by reducing several samples of wool with a 0.2 M solution of thioglycolic acid at 35° C for 20 hr., then alkylating with p-chlorobenzyl chloride, and finally determining the amount of unchanged cystine. The results are shown in figure 1. It will be noted that the amount of reduction was practically constant over the pH range of 2 to 6, somewhat greater at pH 8, and very markedly greater at pH 10. The decrease in cystine in the alkaline region is due not to destruction of cystine by alkali but to reduction, since wool that has been reduced for short periods (2 hr) under these conditions can be reoxidized to products containing nearly the original amounts of cystine and sulfur. Longer exposure (20 hr) of wool to thioglycolic acid at pH 10 resulted in material that contains an abnormally low amount of cystine after reoxidation, but because a considerable portion of the wool was dissolved under such conditions, the results were probably caused by extraction of cysteine-rich material. The increased reduction in alkaline solutions may be caused by ionization of sulphydryl groups. This could change the mechanism of action of the reducing agent, and also increase swelling and facilitate penetration of the reagent.

Wool reduced in acid solution maintained its original appearance, but wool reduced at pH values of 9.0 or above became gelatinous when wet, and brittle and resinous when dry.
(b) EFFECT OF THE DURATION OF THE TREATMENT

The amount of cystine reduced during treatment of wool for various lengths of time with 0.2 M thioglycolic acid at pH 4.5 and 35° C is shown in figure 2. Under these conditions the reaction proceeded to about one-half of its final extent in the first 2 hr, and to about 95 percent of its final extent in 8 hr.

(c) EFFECT OF THE CONCENTRATION OF THIOGLYCOLIC ACID

The amount of cystine reduced in 20 hr at 35° C and at pH 4.5 by various concentrations of thioglycolic acid is indicated in figure 3.
Sufficient reducing reagent to insure the presence of at least 20 times the amount of thioglycolic acid theoretically necessary to reduce all the disulfide groups of the wool was used in each experiment. The amount of reduction increased rapidly in the range 0.01 to 0.2 M. The effect was less marked between 0.2 and 0.5 M. Increasing the concentration of thioglycolic acid beyond 0.5 M had little further effect.

(d) EFFECT OF TEMPERATURE

The amounts of cystine reduced during treatment of wool for various lengths of time with 1.0 M thioglycolic acid at pH 4.5 and at 10° and 50° C are shown in figure 4. At 50° the reaction was essentially complete after 1 hr, whereas at 10° the reaction proceeded more slowly. Increasing the temperature did not increase markedly the amount of cystine finally reduced.

![Figure 4](https://example.com/figure4.png)

**Figure 4.**—Cystine content of wool after reduction with 1.0 M thioglycolate at 10° C and at 50° C at pH 4.5 for various lengths of time, and alkylation with p-chlorobenzyl chloride.

2. ALKYLATION

Most of the alkyl halides that were investigated were found to react more or less readily with reduced wool. In general, the reaction took from 6 to 24 hr at 35° C to reach completion, depending on the amount of reduction of the wool and on the reagent used. It was found that iodides reacted faster than bromides, and bromides faster than chlorides. Tertiary alkyl halides, such as tertiary butyl chloride and triphenylmethyl chloride, were found to be almost completely unreactive.

Besides the reagents listed in table 1, the following were also found to be reactive: allyl chloride, 2-methylallyl chloride, chloroacetone, methyl chloromethyl ether, ethyl chloride, ethylene dichloride, ethylene oxide, epichlorohydrin, 1,3-dichloroisopropanol, and 1,3-dibromoisopropanol.
3. PROPERTIES OF REDUCED AND ALKYLATED WOOLS

The properties of wool fibers and yarn that had been modified by reduction and alkylation are summarized in the following sections. In each instance, the wool was reduced for 20 hr at 35° C and pH 4.5 with a large excess of 0.2 M thioglycolate. Duplicate experiments were found to lead to variations amounting to less than 0.02 in the 30-percent indices, 0.3 percent in hydrations, and 0.5 percent in alkali-solubilities. Analyses showed that the materials contained 6.5 ± 0.5 percent of cystine in every case; that is, the mode of linkage of approximately half of the sulfur atoms had been altered, since the untreated wool contained 12.4 percent of cystine.

Table 1.—30-percent indices and breaking strengths of wool reduced and alkylated with alkyl monohalides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30-percent index</th>
<th>Breaking strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.99</td>
<td>1,310</td>
</tr>
<tr>
<td>Reduction</td>
<td>0.97</td>
<td>1,310</td>
</tr>
<tr>
<td>Reduction and reoxidation</td>
<td>0.95</td>
<td>1,310</td>
</tr>
<tr>
<td>Reduction and CH₂I</td>
<td>0.92</td>
<td>1,310</td>
</tr>
<tr>
<td>Reduction and CH₂Br</td>
<td>0.95</td>
<td>1,380</td>
</tr>
<tr>
<td>Reduction and C₆H₅CH₂Cl</td>
<td>0.95</td>
<td>1,450</td>
</tr>
<tr>
<td>Reduction and p-C₆H₅ClCH₂Cl</td>
<td>.95</td>
<td>1,380</td>
</tr>
<tr>
<td>Reduction and C₆H₅Br</td>
<td>.95</td>
<td>1,340</td>
</tr>
<tr>
<td>Reduction and CI₆H₅COOH</td>
<td>.95</td>
<td>1,240</td>
</tr>
</tbody>
</table>

(a) PHYSICAL PROPERTIES

As indicated by table 1, the breaking of cross-linkages during reduction greatly decreased the 30-percent index of the fibers. Reduction followed by reoxidation resulted in the restoration of nearly the original 30-percent index, indicating practically complete reaction of the sulphydrol groups to rebuild disulfide cross-linkages. Fibers modified by reduction followed by alkylation with an alkyl monohalide of low molecular weight, such as methyl iodide or ethyl bromide, were only slightly less easily elongated than those reduced alone. Alkylation with chloroacetic acid introduced ionizable carboxyl groups (combined as —S—CH₂COOH) into the fiber. The data show that wool fibers treated in this manner have 30-percent indices of the same order as those of wool fibers reduced alone.

Reduction of wool followed by alkylation with monohalides of higher molecular weight, such as benzyl chloride, p-chlorobenzyl chloride, heptyl bromide, or dodecyl bromide gave products higher in 30-percent index than those obtained with methyl iodide or ethyl bromide. Such behavior appears to be due either to the action of intermolecular forces of the van der Waals type between alkyl groups on neighboring sulfur atoms, or between these groups and other parts of the polypeptide chains of the protein. Such secondary linkages would be expected to be weaker than the original covalent type. The data in table 1 offer support for such a hypothesis.

When aliphatic dihalides are used to alkylate reduced wool, groupings that may be represented as W—S—(CH₂)n—S—W appear to replace the disulfide bridges of the original wool, as eq 3 indicates. Analogous reactions of dihalides with simple mercaptans are known [20], and evidence that the reaction proceeds in this way is found both
in the properties of the products, and in the fact that they are practically free of halogen. The halogen contents of several samples of wool prepared by reduction and alkylation with trimethylene dibromide are shown in table 2. Included in the table are halogen contents calculated on the basis of the assumption that only one of the bromine atoms of the dihalide had reacted. It is readily seen that only a very small fraction of the dihalide molecules had reacted in this way. Extensibility of the wool prepared in this way would be expected to be slightly increased, presumably since the cross-links between polypeptide chains would be lengthened, and it should increase further as the number of methylene groups separating the two sulfur atoms is increased. The data obtained with fibers reduced and treated with methylene iodide, ethylene dibromide, trimethylene dibromide, and tetramethylene dibromide show such a trend (table 3).

**TABLE 2.—Bromine content of wool reduced and alkylated with trimethylene dibromide.**

Calculated results are based on the assumption that only one bromine atom of each dihalide molecule has reacted.

<table>
<thead>
<tr>
<th>Cystine reacted</th>
<th>Bromine (calculated)</th>
<th>Bromine (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>2.1</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>6.4</td>
<td>5.0</td>
<td>1.0</td>
</tr>
<tr>
<td>8.4</td>
<td>6.5</td>
<td>0.9</td>
</tr>
<tr>
<td>10.2</td>
<td>7.9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**TABLE 3.—30-percent indices and breaking strengths of wools reduced and alkylated with alkyl dihalides**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30-percent index</th>
<th>Breaking strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>.99</td>
<td>1,310</td>
</tr>
<tr>
<td>Reduction and CH₃I</td>
<td>.94</td>
<td>1,380</td>
</tr>
<tr>
<td>Reduction and (CH₂)₂Br₂</td>
<td>.92</td>
<td>1,420</td>
</tr>
<tr>
<td>Reduction and (CH₂)₃Br₂</td>
<td>.90</td>
<td>1,460</td>
</tr>
<tr>
<td>Reduction and (CH₂)₄Br₂</td>
<td>.88</td>
<td>1,380</td>
</tr>
</tbody>
</table>

The breaking strengths of yarn treated like the fibers show similar trends (tables 1 and 3). Reduced wool, reduced and methylated wool, and wool reduced and treated with chloroacetic acid were markedly lower in breaking strength than the untreated wool, while wool that had been reduced and alkylated with monohalides of high molecular weight, or with a dihalide, was not far different from untreated wool in this respect.

(b) HYGROSCOPICITY

The hygroscopicity of the modified wools varied depending on the chemical nature of the groups introduced into the wool, as shown in tables 4 and 5. Wools into which large groups have been introduced by alkylation with benzyl chloride, p-chlorobenzyl chloride, heptyl bromide, or dodecyl bromide were found to be less hygroscopic than untreated wool. By introducing hydrophobic groups of high molecular weight into the wool the percentage of hydrophilic structures, such as peptide bonds, is decreased, and as a result the products are less highly hydrated.
TABLE 4.—Alkali-solubility and hygroscopicity of wools reduced and alkylated with monohalides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alkali-solubility</th>
<th>Moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>10.5%</td>
<td>16.0%</td>
</tr>
<tr>
<td>Reduction</td>
<td>cir. 50.0%</td>
<td>16.9%</td>
</tr>
<tr>
<td>Reduction and reoxidation</td>
<td>12.5%</td>
<td>16.3%</td>
</tr>
<tr>
<td>Reduction and CH$_3$I</td>
<td>35.0%</td>
<td>16.9%</td>
</tr>
<tr>
<td>Reduction and CH$_3$Br</td>
<td>18.3%</td>
<td>15.6%</td>
</tr>
<tr>
<td>Reduction and CH$_3$CH$_2$Cl</td>
<td>15.4%</td>
<td>14.8%</td>
</tr>
<tr>
<td>Reduction and p-ClC$_6$H$_5$COOH</td>
<td>12.8%</td>
<td>14.0%</td>
</tr>
<tr>
<td>Reduction and CH$_3$I</td>
<td>18.0%</td>
<td>15.6%</td>
</tr>
<tr>
<td>Reduction and CH$_3$Br</td>
<td>18.2%</td>
<td>15.6%</td>
</tr>
<tr>
<td>Reduction and ClCH$_2$COOH</td>
<td>cir. 50.0%</td>
<td>16.9%</td>
</tr>
</tbody>
</table>

The introduction of small groups by alkylation with methyl iodide, ethyl bromide, or an alkyl dihalide of low molecular weight should lead to introduction of only a small percentage of hydrophobic groups. As would be expected, these products were found to be very little different from untreated wool in hygroscopicity. The introduction of a polar group, as by reduction and alkylation with chloroacetic acid, yielded products that were somewhat more hygroscopic than untreated wool.

(c) ALKALI-SOLUBILITY

Reduction of wool, followed by alkylation with an alkyl monohalide may be expected to render the wool more readily soluble in alkali, since this process breaks disulfide bonds which form links between polypeptide chains, and therefore decreases the average molecular weight of the wool proteins. The high alkali-solubilities of wool that has been reduced and treated with alkyl monohalides illustrate such a behavior. The extremely high alkali-solubility of wool alkylated with methyl iodide may be due to the formation of sulfonium groupings according to the equation

\[ \text{WSCH}_3 + \text{CH}_3\text{I} \rightarrow \text{W-S(CH}_3)_2^- + \text{I}^- \]

Methyl iodide is known to be exceptionally reactive in this respect [21]. Sulfonium compounds are highly ionized and would be expected to increase the alkali-solubility.

Reduction breaks disulfide cross-linkages, a process which lowers the molecular weight and at the same time introduces ionizable sulfhydryl groups into the wool. Each of these changes should tend to increase the solubility of the wool in alkali, and observations confirmed this expectation. In view of these results, it is apparent why strongly alkaline solutions of thioglycolic acid, as observed by Goddard and Michaelis [7, 8], are capable of dissolving wool. Reduction followed
by alkylation with chloroacetic acid should also yield products that possess high alkali-solubility, since carboxyl groups also are ionized in alkaline solution. Such wool was found to be very readily dissolved by alkali.

Reduction of wool, followed by treatment with polymethylene dihalides such as methylene iodide, ethylene dibromide, trimethylene dibromide or tetramethylene dibromide, is presumed to maintain the original molecular weight of the protein, and also replaces disulfide cross-linkages, which are unstable toward alkali [22], with stable bis-thioether \(^7\) cross-linkages as 3 indicates. Such a process was found greatly to increase the resistance of wool to the action of alkali. The alkali-solubilities (table 5) of these samples were much lower than those of untreated wool, or of any of the wools reduced and then alkylated with alkyl monohalides.

(d) EFFECT OF VARYING THE EXTENT OF REDUCTION AND ALKYLATION

The results described in the preceding sections were obtained from wool that had been reduced to nearly the same cystine content and then alkylated. The relation between the extent of reduction and alkylation, as judged from the decrease in content of unchanged cystine and the properties of the products, has also been studied, using two of the alkylating agents, methyl iodide and trimethylene dibromide. The experimental results are summarized in table 6.

It is apparent that in both cases the 30-percent index decreased as the extent of reduction and alkylation increased. Fibers that had been reduced and methylated, however, were decidedly more changed in this respect than those which had been reduced and treated with trimethylene dibromide. The breaking strengths of yarn show the same trend as the 30-percent indices of fibers treated in the same way, but are a much less sensitive means of detecting the changes that have taken place.

<table>
<thead>
<tr>
<th>Cystine Percent</th>
<th>30-percent index</th>
<th>Breaking strength</th>
<th>Alkali-solubility Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.2</td>
<td>0.99</td>
<td>1,310</td>
<td>10.5</td>
</tr>
<tr>
<td>(B) Alkylated with (CH(_2))(_2)Br(_2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.3</td>
<td>0.99</td>
<td>1,338</td>
<td>5.3</td>
</tr>
<tr>
<td>6.0</td>
<td>.86</td>
<td>1,318</td>
<td>3.7</td>
</tr>
<tr>
<td>4.0</td>
<td>.72</td>
<td>1,272</td>
<td>2.6</td>
</tr>
<tr>
<td>2.2</td>
<td>.68</td>
<td>1,012</td>
<td>2.5</td>
</tr>
<tr>
<td>(C) Alkylated with CH(_3)I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.3</td>
<td>0.73</td>
<td>1,287</td>
<td>13.2</td>
</tr>
<tr>
<td>6.0</td>
<td>.48</td>
<td>1,230</td>
<td>31.5</td>
</tr>
<tr>
<td>4.0</td>
<td>.27</td>
<td>1,240</td>
<td>43.5</td>
</tr>
<tr>
<td>2.2</td>
<td>.18</td>
<td>989</td>
<td>83.0</td>
</tr>
</tbody>
</table>

\(^7\) This terminology is in accord with the suggestions of Patterson and Curran [23].
Of particular interest is the fact that, while the alkali-solubility of the reduced and methylated wool increased as its content of unchanged cystine decreased, that of the wool reduced and treated with trimethylene dibromide decreased. This contrasting behavior furnishes further evidence that reduction followed by methylation breaks disulfide cross-linkages and decreases the average molecular weight of the wool protein, while reduction followed by treatment with trimethylene dibromide maintains the molecular weight by rebuilding cross-linkages of a type that are extremely stable to alkali.

Susceptibility to degradation by alkali, which has been one of the most important practical disadvantages of wool, can now be overcome to a large extent through conversion of relatively unstable disulfide cross-linkages to extremely stable thioether cross-linkages. The stability of wools of this sort, under a variety of conditions of practical interest, is now being investigated in this laboratory.

IV. REFERENCES


WASHINGTON, March 27, 1941.
Attention is invited to a series of publications which is being prepared by the Project for the Computation of Mathematical Tables conducted by the Federal Works Agency, Work Projects Administration for the City of New York, under the sponsorship of the National Bureau of Standards.

To date six tables have been made available through the National Bureau of Standards. These are listed below:

MT1. Table of the First Ten Powers of the Integers from 1 to 1000:
(1938) VIII + 80 pages; heavy paper cover. 50 cents.

MT2. Tables of the Exponential Function $e^x$:
The ranges and intervals of the argument and the number of decimal places in the entries are given below:

<table>
<thead>
<tr>
<th>Range of $x$</th>
<th>Interval of $x$</th>
<th>Number of decimals given</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-2.5000$ to $1.0000$</td>
<td>0.0001</td>
<td>18</td>
</tr>
<tr>
<td>$1.0000$ to $2.5000$</td>
<td>0.0001</td>
<td>15</td>
</tr>
<tr>
<td>$2.500$ to $5.000$</td>
<td>0.001</td>
<td>15</td>
</tr>
<tr>
<td>$5.00$ to $10.00$</td>
<td>0.01</td>
<td>12</td>
</tr>
</tbody>
</table>

(1939) XV + 535 pages; bound in buckram, $2.00.

MT3. Tables of Circular and Hyperbolic Sines and Cosines for Radian Arguments:
Contains 9 decimal place values of $\sin x$, $\cos x$, $\sinh x$ and $\cosh x$ for $x$ (in radians) ranging from 0 to 2 at intervals of 0.0001.
(1939) XVII + 405 pages; bound in buckram, $2.00.

MT4. Tables of Sines and Cosines for Radian Arguments:
Contains 8 decimal place values of sines and cosines for radian arguments ranging from 0 to 25 at intervals of 0.001.
(1940) XXIX + 275 pages; bound in buckram, $2.00.

MT5. Tables of Sine, Cosine, and Exponential Integrals, Volume I:
Values of these functions to 9 places of decimals from 0 to 2 at intervals of 0.0001.
(1940) XXVI + 444 pages; bound in buckram, $2.00.

MT6. Tables of Sine, Cosine, and Exponential Integrals, Volume II:
Values of these functions to 9, 10, or 11 significant figures from 0 to 10 at intervals of 0.001, with auxiliary tables.
(1940) XXXVII + 225 pages; bound in buckram, $2.00.

Payment is required in advance. Make remittance payable to the “National Bureau of Standards”, and send with order, using the blank form on the page facing this one for the purpose.

Above prices are for delivery in the United States and its possessions and in countries extending the franking privilege. To other countries the price of MT1 is 65 cents and that of MT2, MT3, MT4, MT5, and MT6 is $2.50 each; remittance to be made payable in United States currency.

Copies of these publications have been sent to various Government depositories throughout the country, such as public libraries in large cities, and colleges and universities, where they may be consulted.

A mailing list is maintained for those who desire to receive announcements regarding new tables as they become available. A list of the tables it is planned to publish will be sent on request.