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STATE OF THE SULFUR IN OXIDIZED WOOL

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ABSTRACT

During treatment with oxidizing agents, the disulfide sulfur in wool appears to be changed to higher states of oxidation. The existence of oxidation derivatives of the disulfide compounds is indicated by the alkali-solubility determination, the lead acetate test, by reduction with hydrochloric acid-potassium iodide solutions, and by cystine analyses.

The intermediate oxidation derivatives of cystine are unstable under the conditions necessary for protein hydrolysis, and at least one of them, cystine disulfoxide, is partially converted to cystine. The results indicate that the values obtained for the cystine content of oxidized wool may be expected to be higher than the actual cystine content since they represent the amount of cystine formed from the partially oxidized cystine compounds as well as from unoxidized cystine groups.

CONTENTS

I. INTRODUCTION

Recent investigations relating to the oxidation of wool [1, 2, 3, 4] [2] indicate that the stability of the fiber is closely related to the state of the sulfur in the wool protein. The bulk of the sulfur in untreated wool is present as cystine sulfur [5, 6] and it appears that the maximum stability of the fiber is obtained when the sulfur is in this form. Changes in the state of this sulfur, such as are produced by oxidizing and reducing agents or by light, may result in the direct degradation of the fiber, or in incipient damage, which may be greatly aggravated in the subsequent processing of the fiber. It is apparent, therefore, that the proportion of the total sulfur in wool which exists in the disulfide form is very significant.

The present report is concerned with new methods which have been used in studying the state of the sulfur in oxidized wool and with the limitations of the methods for the determination of cystine when applied to wool in which the sulfur has been changed to a higher state of oxidation.

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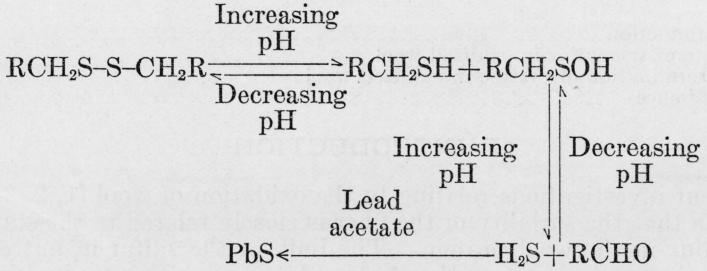
[2] The numbers in brackets throughout the text correspond to the literature references at the end of this paper.

II. STATE OF THE SULFUR IN OXIDIZED WOOL

When wool is treated with oxidizing agents, one of the main points of attack appears to be the disulfide linkage. Intermediate oxidation products, such as R–SO–S–R, R–SO_2–S–R, etc., may be formed.[3] Although methods for the identification of the individual intermediate oxidation derivatives of sulfur are not known, evidence given by four different methods indicates that the disulfide sulfur in wool is changed to higher states of oxidation during treatment with oxidizing agents. These tests are the alkali-solubility determination, the lead acetate test, the reduction method using mixtures of hydrochloric acid and potassium iodide, and the determination of the cystine content.

The alkali-solubility test [7] has been shown to be a measure of the extent to which wool is oxidized. Presumably as the number of oxygen atoms on the sulfur increases, the bond between the disulfide sulfur atoms becomes increasingly labile and more wool goes into solution. Analogous reactions are obtained with cystine and its intermediate oxidation derivatives. Cystine disulfoxide (RSO_2–SR or RSO–SOR) has been shown by Lavine [8] to be much less stable in alkaline solution than cystine, whereas Toennies [9] has recently found cystine disulfone (RSO_2–SO_2R) to be even less stable than the disulfoxide.

The lead acetate test [2], which distinguishes between unbleached wool and wool bleached with hydrogen peroxide, also appears to depend on the state of oxidation of the sulfur. When untreated wool is subjected to this test, hydrogen sulfide is liberated and reacts with lead acetate to form lead sulfide according to the following scheme:



Cystine appears to react similarly but requires a higher pH for formation of the lead sulfide. Oxidized wool and cystine disulfoxide, on the other hand, do not form lead sulfide under the conditions in which it is formed in untreated wool and in cystine.

The following mechanism is suggested for those cases in which the disulfide sulfur has been partially oxidized. Since in each disulfide group one sulfur atom is positively and one negatively charged, the negative oxygen atoms or hydroxyl groups react with the positive sulfur, whereas the positive groups such as hydrogen react with the negative sulfur. The hydrolytic cleavage of a partially oxidized disulfide compound may then be represented as follows:

$$RSO\text{–}SR \xrightarrow{H_2O} RSO.OH + RSH$$

[3] One sulfur atom in a disulfide compound is positively and the other negatively charged. It would be expected that only the positive sulfur would take up oxygen until it formed a sulfonic acid, the bond between the sulfur atoms then being broken. The remaining sulfur could then be oxidized to a sulfonic acid. This mechanism appears to be more tenable than one in which there is simultaneous oxidation of both sulfur atoms, with the formation of such intermediates as R–SO–SO–R, R–SO_2–SO–R, etc.

The products of this reaction appear to be stable under the conditions of these experiments and further decomposition to form hydrogen sulfide does not occur.

The third method is based on the method used by Lavine [8] in his studies on cystine disulfoxide. Samples of oxidized wool were treated for 1 hour with mixtures of equal volumes of solutions of 4 *M* hydrochloric acid and 4 *M* potassium iodide, the ratio of wool to solution being 1 to 100. The reductions were conducted in the dark at 22 to 23° C. The oxidizing value of some of the intermediate oxidation products was determined by direct titration of the iodine liberated with sodium thiosulfate. The value obtained has no quantitative significance with respect to the extent of oxidation of the wool since, as has been shown by Lavine, only sulfur which has been oxidized below the sufonic acid state is reduced.

The cystine content of wool has been shown [1] to decrease with increased oxidation. That the values for the cystine content of oxidized wool have no quantitative significance will be shown later.

Of the four methods described, the alkali-solubility test is the most satisfactory. A comparison of the results obtained by the different methods is given in table 1.

TABLE 1.—*Effect of treatment for different lengths of time with hydrogen peroxide on the sulfur in wool*

[The alkali-solubility and cystine contents were determined on wool which had been treated at 50° C with 0.6 percent (2 vol.) hydrogen peroxide, while the remaining 2 tests were made on wool similarly treated, except that 3 percent (10 vol.) peroxide was used.]

Duration of treatment	Alkali-solubility	Cystine content	Lead-acetate test [1]	Hydrochloric-acid-potassium iodide test [2]
Hours	*Percent*	*Percent*		*Milliequivalent of iodine per gram of wool*
0	9.7	11.6	Positive	0
1	10.3	10.7	Negative	0.015
3	12.4	10.1	do	.024
5	15.2	10.0	do	
7	18.2	9.9	do	.038
9	20.1	9.6	do	.051
16	40.0	8.6	do	.072

[1] These results are obtained visually. The lead sufide formed in the reaction darkens the wool.
[2] Similar experiments with oxidized silk showed no significant changes in iodine liberated with increased oxidation.

III. DETERMINATION OF CYSTINE IN OXIDIZED WOOL

The available methods for the estimation of disulfide compounds have been classified and evaluated elsewhere [10] and need not be further discussed here. In the work reported in this paper, cystine was determined by the methods of Sullivan [11], Okuda [12], and Folin-Morenzi [13]. The latter two were used as described in the literature, while the Sullivan procedure was modified only with respect to the measurement of the color developed in the determination. A few interesting points regarding this procedure are here noted.

The amount of the colored substance developed by the reaction of cystine with sodium β-naphthoquinone-4-sulfonate was measured photometrically at the wave length 501 millimicrons. A calibration curve, shown in figure 1, was obtained by determining the transmit-

tancy of the red solutions formed when known amounts of cystine were treated according to the Sullivan procedure.

When this method was first used, erratic results were obtained, especially during the summer when the temperature rose above 30° C. It was found that the reaction is markedly affected by temperature, as shown in figure 2. The initial rate of development of color increases with increasing temperature, but at equilibrium, the amount of colored substance developed decreases with increasing temperature. Analyses of solutions containing 100 mg of cystine per liter were made, the color being allowed to develop at different temperatures for 30

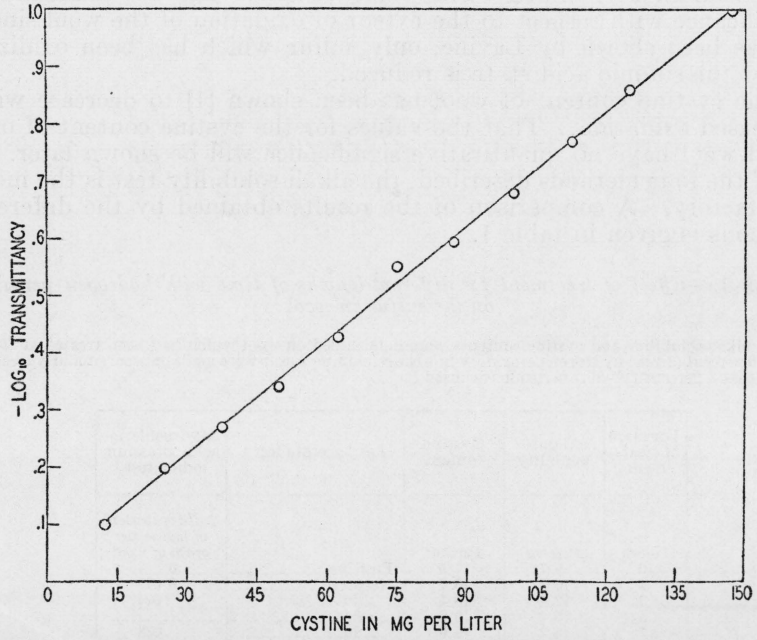


FIGURE 1.—*Transmittancy at wave length 501 millimicrons of the red solutions formed when known amounts of cystine were treated according to the Sullivan procedure.*

minutes. The results are shown in figure 3. There is practically no change between 24 and 30° C but above 30° C the apparent cystine content decreases rapidly with increasing temperature. The cystine determinations reported in this paper were made at 23 to 24° C.

Analyses of six samples of the untreated wool used in this work showed an average cystine content of 11.5 percent, with an average deviation from the mean of 0.2 percent.

The lability of the intermediate oxidation derivatives of cystine, shown by Toennies [9] and Lavine [8], suggested that similar derivatives formed in wool during oxidation might partially revert to cystine during the hydrolytic process, which is used in the analyses of proteins for cystine. In order to test this possibility, 10-mg samples of cystine disulfoxide[4] were treated with 5-ml portions of 6 *N* hydrochloric acid

[4] The cystine disulfoxide used in this work was furnished by Drs. Toennies and Lavine of the Lankeman Hospital Research Institute, Philadelphia, Pa.

in an oil bath at 120 to 125° C for different lengths of time, and the amount of cystine in the resulting solutions was determined by the Sullivan and the Okuda methods. A similar set of experiments, using 6 *N* sulfuric acid, was made and the cystine determined by the Folin-Morenzi method. The results recorded in table 2 show that 1 mole of cystine is formed from 2 moles of cystine disulfoxide.

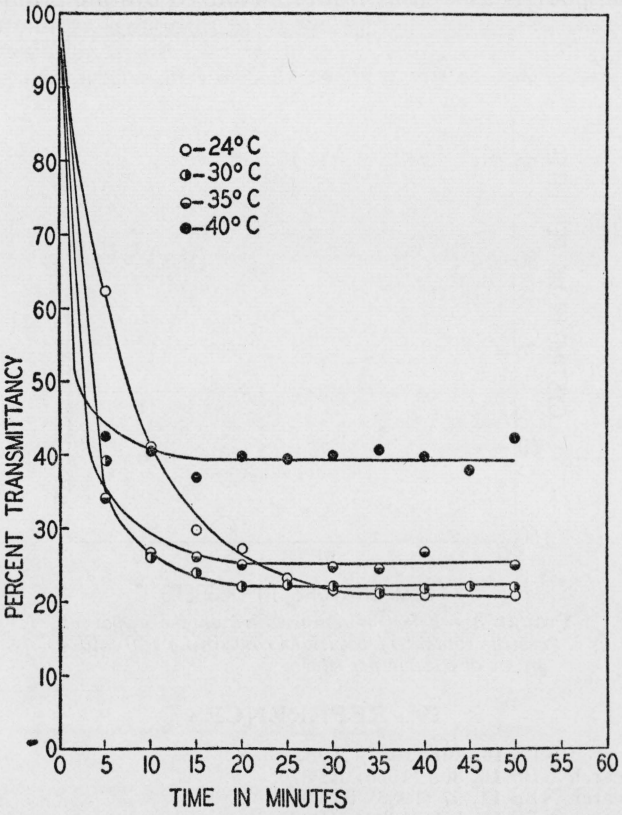


FIGURE 2.—*Effect of different temperatures on the rate of color development in the Sullivan method for the determination of cystine.*

TABLE 2.—*Decomposition of cystine disulfoxide during the hydrolysis used in cystine analyses*

The values represent the mole percentage of the cystine disulfoxide which has been converted to cystine.

Duration of hydrolysis	Cystine found		
	Sullivan	Okuda	Folin-Morenzi
Hours	*Percent*	*Percent*	*Percent*
1	46.5	53.8	45.7
3	48.3	54.6	50.0
5	46.1	52.0	47.5
7	48.6	54.3	47.0
10	47.5	45.8	45.8
20	52.0	56.4	46.7
50	45.3	52.4	49.1
75	52.3	51.4	48.2

The results of the above experiments indicate that under the conditions necessary for the hydrolysis of proteins, at least a portion of the oxidized disulfide groups revert to disulfide groups. Therefore, the values obtained for the cystine content of oxidized wool may be expected to be higher than the actual cystine content since they represent the amount of cystine formed from the partially oxidized cystine compounds as well as from unoxidized cystine groups.

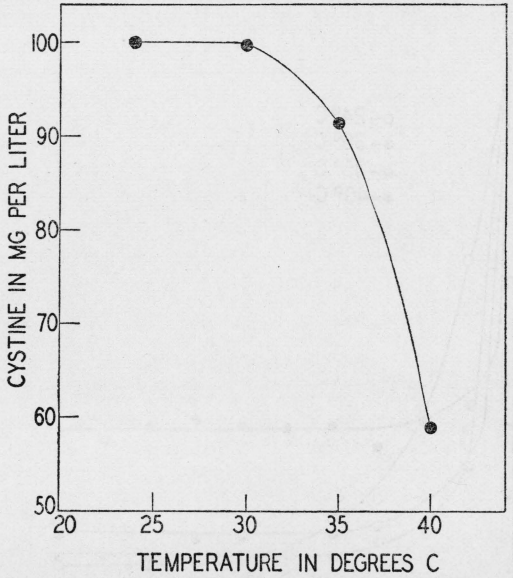


FIGURE 3.—*Effect of temperature on the apparent cystine content of solutions containing 100 milligrams of cystine per liter.*

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