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IN THE BALL MILL AND AUTOCLAVE

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In the course of investigation of the microbiological degradation of wool, it is often necessary to treat wool either (a) by steam sterilization at 120° for 15 minutes, (b) by grinding in a ball mill, or (c) by a combination of these. Since we wish to study the degradation by a single microorganism, the sterilization procedure is required. Also, since wool has been shown to be a chemically heterogeneous substance both histologically and chemically, grinding was necessary in order to obtain a more uniform substrate and an increase in specific surface for the action of the enzymes liberated by the microorganism. Although it has been known for some time that such treatments induce changes in wool (1-10), the extent and nature of these changes, especially on the insoluble residue, have not been studied in detail. Since we are interested in the changes brought about by the microorganism under study, it is obviously of importance to know the chemical and physical characteristics of wool after being subjected to these treatments.

Routh (7), in studies on wool ground in a ball mill, found little change in the total nitrogen and sulfur content, but the cystine content decreased appreciably. Inorganic sulfates and intermediate oxidation products of cystine were found, and he concluded that the change was oxidative in nature.

Stirm and Rouette (8) make the statement that the moisture content is of the highest importance in the rate of destruction of wool due to heat. Among the decomposition products, they found carbon dioxide, sulfinic acid, alanine, and taurine. They noted that by the exclusion of oxygen the sulfur compounds of wool could not be oxidized to sulfate.

Cohen (9) observed a decrease in the nitrogen content of the water-soluble fraction of wool on successive grindings. He concluded that the production of a water-soluble protein is not primarily dependent on the splitting of the disulfide linkage, but on other linkages in addition to this one. Our own observations support this view.

EXPERIMENTAL

The wool used had been scoured and defatted, and had a moisture content of 9.9 per cent when in equilibrium with the room, which is kept at

50 per cent relative humidity. Portions of this sample were then either ground in a ball mill or chopped. After these basic treatments, 5 gm. portions of the samples were either heated 1 hour at 110° or autoclaved dry or wet. The chopping process consisted of one passage through a medium sized Wiley mill equipped with a 40 mesh sieve. Autoclaving was carried out at 15 pounds steam pressure (120°) for 20 minutes. A ceramic ball mill containing flint pebbles was used; this mill turned at a rate of 120 R.P.M. Unless otherwise specified, samples were milled for 4½ days or about 800,000 revolutions. All samples were analyzed on a moisture-free basis, having been dried in a vacuum oven at 60° for at least 18 hours. At least duplicate analyses were made in every case.

TABLE I
Effect of Grinding and Heat on Wool

Treatment of fiber	Analysis*		
	Cystine <i>per cent</i>	Sulfur <i>per cent</i>	Nitrogen <i>per cent</i>
Whole fiber.....	11.5	3.43	16.53
Chopped fiber.....	11.5	3.33	16.36
“ “ heated 1 hr. at 110°.....	10.4	3.16	16.54
“ “ autoclaved dry, 20 min. at 120°.....	8.2	2.90	16.61
“ “ “ wet, 20 “ “ 120°.....	8.0	2.59	16.67
Ball-milled fiber.....	8.4	2.84	16.27
“ “ heated 1 hr. at 110°.....	7.5	2.47	16.64
“ “ autoclaved dry, 20 min. at 120°.....	6.5	2.34	16.37
“ “ “ wet, 20 “ “ 120°.....	6.6	2.32	16.21

* Calculated on an ash-free, moisture-free basis.

Total sulfur was determined gravimetrically by the oxygen bomb method and cystine by the photometric method of Kassell and Brand (10). Cysteine was calculated as the difference after blocking the —SH groups by iodoacetic acid (11). The intermediate oxidation products of cystine were sought by the method of Lavine (12) and nitrogen was determined by the micro-Kjeldahl procedure. Qualitatively, sulfhydryl was detected by the nitroprusside reaction, aldehydes by the action of Schiff's reagent, and hydrogen sulfide by lead acetate paper.

Results

The effect of chopping, ball milling, and the subsequent autoclaving on the chemical constitution of wool is summarized in Table I. It is to be noted that these figures represent the composition of the water-insoluble residue. In addition to the changes in cystine, sulfur, and nitrogen, it has

been observed qualitatively that hydrogen sulfide is given off during the grinding process. Previous workers have noted that hydrogen sulfide is given off when wool is autoclaved.

Because a positive nitroprusside test was invariably obtained after ball milling, quantitative determinations for cysteine were made by blocking the —SH with iodoacetate. In a typical batch of ball-milled wool, the cystine content was 7.52 and the cysteine was 0.48 per cent. Vari-

TABLE II
Effect of Grinding on L-Cystine

Compound	Non-ball-milled cystine,*		Ball-milled cystine			
	Calculated	Found	Found*			
			Batch 1		Batch 2	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent loss</i>	<i>per cent</i>	<i>per cent loss</i>
Cystine.....	100.0	100.0	70.1	29.9	80.8	19.2
Cysteine.....	0	0	8.5		5.8	
Sulfur.....	26.68	26.70	24.60	7.9	25.65	4.0
Nitrogen.....	11.66	11.67	10.50	11.1	11.17	4.2

* Batch 1, about 1,400,000 revolutions; Batch 2, about 1,000,000 revolutions; calculated on an ash-free, moisture-free basis.

TABLE III
Qualitative Tests on Wool and on Cystine Ground in Ball Mill

Material	Test	Result
Water-insoluble residue	Nitroprusside, for —SH	Positive
“ “	Schiff's reagent, for aldehydes	“
Gases present after milling	Hydrogen sulfide	“
“ “ “ “	Ammonia	“
Water-soluble fraction	Sulfates	“
“ “	Intermediate oxidation products of cystine	Negative

tions were within ± 5 per cent of the above figures. The formation of cysteine was also noted with ball-milled cystine, as shown in Table II.

Since the major change in wool keratin due to the degree of comminution appears to involve the disulfide linkage or cystine content, the effect of grinding on L-cystine was also studied. The results are summarized in Table II.

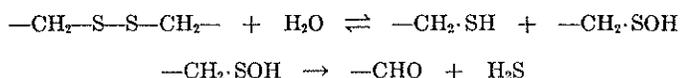
Qualitative tests on both ball-milled wool and cystine gave the results shown in Table III.

DISCUSSION

The available data are inadequate for a detailed explanation of the mechanism of decomposition of wool or cystine due to grinding. The results, however, suggest generalizations which appear useful in interpreting some of these reactions. That the reactions are complex is indicated by the multiplicity of breakdown products identified, such as cysteine, aldehyde, hydrogen sulfide, ammonia, and sulfates. Although no oxidation products of cystine such as cystine disulfoxide or cysteinesulfinic acid were detected, it does not necessarily follow that they do not appear in the course of the degradation. Other results in these Laboratories indicate that these compounds may appear only momentarily as intermediate steps in the oxidation of cystine to sulfate.

As Cohen (9) suggests, other bonds are broken in addition to the disulfide linkage. This process produces only a partially soluble substance as contrasted to a completely insoluble product obtained from the reduction of wool by means of thioglycolic acid or sodium sulfide. Our own experiences corroborate those of Routh (7) and Cohen (9) that proteolytic enzymes such as pepsin and trypsin readily digest reduced wool and only slowly and partially digest ball-milled wool. This suggests differences in the chemical nature of the two substrates. Possibly the chemical reduction transforms the wool protein to long, insoluble polypeptide chains, whereas ball milling produces small, soluble, dialyzable fragments.

Since it is very difficult to remove all the water from wool, it may be postulated that a hydrolytic cleavage of the disulfide group in wool occurs during the ball milling.



Detailed studies by Schöberl (13) on the above reaction have shown that it occurs with surprising ease. Simple calculations will show that the small amount of water necessary to change the cystine content of wool from 11.5 to 8.4 per cent would be readily available. It is possible that hydrogen sulfide may be oxidized to sulfuric acid, especially in the presence of trace metals (14) which are probably present in the pebbles, accounting in part for the sulfate found present. Although no oxidation products of cystine were actually detected in this study, the further oxidation of sulfinic acid ion to sulfate ion is quite possible. That deamination of wool or cystine was accelerated by ball milling is partially substantiated by the fact that ammonia was given off and a suspension of either wool or cystine became increasingly acid with increasing the length of time of ball milling. It is known that the impact of pebbles in the rotating mill

generates heat, which may materially catalyze the various decompositions (5). Thus it would appear that during the ball milling both hydrolytic and oxidative processes operate simultaneously.

SUMMARY

When either wool or cystine has been subjected to ball milling and autoclaving or either procedure alone, ammonia, hydrogen sulfide, aldehydes, cysteine, and sulfate can be detected. The suggestion is made that these compounds result from simultaneous hydrolytic and oxidative reactions on the wool proteins, the rate of reaction being increased by heat. Quantitative cystine, sulfur, and nitrogen values on wool and cystine under the above conditions support this hypothesis.

BIBLIOGRAPHY

1. Reychler, A., *Bull. Soc. chim. Belg.*, **29**, 291 (1920).
2. Humfeld, H., Elmquist, R. E., and Kettering, J. H., *U. S. Dept. Agr., Tech. Bull.* **588** (1937).
3. Geiger, W. B., Patterson, W. I., Mizell, L. R., and Harris, M., *J. Res. Nat. Bur. Standards*, **27**, 459 (1941).
4. Hock, C. W., Ramsay, R. C., and Harris, M., *J. Res. Nat. Bur. Standards*, **27**, 181 (1941).
5. Newell, G. W., and Elvehjem, C. A., *J. Nutr.*, **33**, 673 (1947).
6. Meecham, D. K., and Olcott, H. S., *Ind. and Eng. Chem.*, **39**, 1023 (1947).
7. Routh, J. I., *J. Biol. Chem.*, **135**, 175 (1940).
8. Stirn, V. K., and Rouette, P. L., *Melliand Textilber.*, **16**, 4 (1935).
9. Cohen, H. R., *Arch. Biochem.*, **4**, 145 (1944).
10. Kassell, B., and Brand, E. J., *J. Biol. Chem.*, **125**, 115 (1938).
11. Sanford, D., and Humoller, F. L., *Anal. Chem.*, **19**, 404 (1947).
12. Lavine, T. F., *J. Biol. Chem.*, **113**, 583 (1936).
13. Schöberl, A., and Eck, H., *Ann. Chem.*, **522**, 97 (1936).
14. Krebs, H. A., *Biochem. Z.*, **204**, 343 (1929).