XXVII. NOTE UPON THE SULPHUR LINKAGE IN WOOL.

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ALTHOUGH the greater part of the sulphur present in wool-keratin can be accounted for as cystine [see Rimington, 1929], the actual state of combination of this amino-acid is still a matter of considerable uncertainty. Thus, if cystine were in simple polypeptide linkage in the protein, it would be expected that suitable treatment of the wool fibre would reveal the presence of free disulphide (—S.S—) or sulphydryl (—SH) groups. That this expectation is not realised will be clear from the experiments recorded here. It seems necessary to conclude that the cystine-sulphur groups in wool are involved in some complex ring system or otherwise obscured so that they cannot react as does free cystine.

EXPERIMENTAL.

A sample of 50's crossbred wool was degreased in warm benzene and then washed thoroughly in many changes of distilled water. Portions of about 0.05 g. were weighed out and subjected to various treatments recorded in Table I. Unless otherwise stated the duration of each test was 24 hours, the tubes being stoppered and left in a dark cupboard. After washing, the lock of wool was divided and tested for free —S.S— and —SH groups in the way recommended by Walker [1924, 1925] using 10 cc. of saturated ammonium sulphate as suspending fluid, and also for general damage by the Pauly reagent. It has been shown by Burgess [1928] and by Burgess and Rimington [1929] that undamaged wool remains uncoloured when immersed in the Pauly reagent, whereas fibres which have suffered mechanical or chemical injury are stained a brownish-red colour proportional to the extent of the injury.

The results are given in Table I, one or more plus signs being used to indicate the intensity of a positive reaction.

An examination of these results warrants the following statements.

(1) Treatment to which free cystine responds readily has little effect upon the sulphur groups in wool. (Compare the effect of reducing agents.)

(2) Considerable alteration of, or "damage" to, the fibre may take place, as indicated by the intensity of the Pauly stain, without any parallel increase occurring in the nitroprusside —S.S— or —SH reactions. If, as is generally

Table I.

Treatment	Duration	—S.S.—	—SH	Pauly reaction	Remarks
Control	—	—	—	+	—
				(tips only)	
Boiling water	20 mins.	—	—	+	No change in appearance
				(patchy)	
„	45 „	—	—	++	Yellowish. Characteristic smell appears
				(patchy)	
„	1 hr.	—	—	+++	Brownish. Pauly stains most intense on discoloured portions
				+++	Pauly stain general
„	3 hrs.	+	—	+++	
		(faint)			
Absolute alcohol	24 „	±	—	+	—
				(tips only)	
Formalin 5 %	18 „	—	—	±	—
Saturated H ₂ S solution	18 „	++	—	—	—
Ultra-violet radiation	80 „	++	—	++++	Pauly stain general
ENZYMES:					
3.3 % trypsin (<i>p_H</i> 8.0)	48 „	++	—	+++	Pauly stain general
2.02 % pepsin (<i>p_H</i> 1.2)	48 „	±	—	+	—
0.1 <i>N</i> NaOH alone	24 „	++	±	++	—
BACTERIA:					
<i>B. subtilis</i> (saline)	5 days	—	—	++	Growth poor
<i>B. subtilis</i> (broth)	5 „	+	—	+++	Pauly stain general
		(tips only)			
<i>B. mesentericus ruber</i> (saline)	5 „	—	—	+	—
<i>B. mesentericus ruber</i> (broth)	5 „	±	—	+++	Pauly stain intense in places
REDUCING AGENTS:					
Sodium sulphite 1 %	18 hrs.	.	+	.	—
			(tips)		
„	66 „	±	±	+	—
				(tips)	
Sodium sulphite 4 %	18 „	.	+	.	—
			(tips)		
„	66 „	+	+	++	Pauly stain fairly general
		(tips)	(tips)		
Sodium sulphite 1 % in 0.1 <i>N</i> sodium hydroxide	18 „	.	+++	.	—
„ „	66 „	++	+++	++++	Pauly stain strongest at tip
Sodium hydrosulphite 1 %	18 „	.	±	.	—
„	66 „	—	—	+	Fibres have a white, bleached appearance
				(tips)	
Sodium hydrosulphite 4 %	18 „	.	±	.	—
„	66 „	—	±	+	—
				(tips)	
Sodium hydrosulphite 1 % in 0.1 <i>N</i> sodium hydroxide	18 „	.	+++	.	—
„	66 „	++	+++	+++	Fibres very yellow; staining patchy
5 % suspension of calcium oxide in 1 % sodium sulphite	18 „	.	++	.	—
„ „	66 „	+++	+++	+++	Fibres have a soapy feel
CYSTINE SOLUTION (0.3 % IN WATER PLUS SUFFICIENT HCl):					
10 cc. of solution used, to which the following reagents were added to produce solutions of the concentrations indicated.					
Sodium sulphite 1 %	18 hrs.	.	+++	.	—
„ 4 %	18 „	.	++	.	—
Sodium hydrosulphite 1 %	18 „	.	+++	.	—
„ 4 %	18 „	.	+	.	—
5 % suspension of calcium oxide in 1 % sodium sulphite	18 „	Faint pink changing immediately to yellow			

supposed, the behaviour of the wool fibre towards the Pauly reagent is conditioned solely by the state of the epidermal scales, acting as an impenetrable sheath unless mechanically or chemically disrupted, then it would be expected that also in the case of the nitroprusside reaction, where damage was extensive, staining of the cortex would occur. That this is not so seems to show that the failure of the nitroprusside reaction can only be interpreted as indicating the absence of —S.S— and —SH groups.

(3) With the exception of exposure to ultra-violet radiation, only one treatment can be recorded—the action of alkaline solutions—which has been found to have any marked effect upon the sulphur groups of wool. Ultra-violet radiation causes the appearance of —S.S— groups only, whereas after immersion in alkalis the fibre shows positive reactions for both —S.S— and —SH.

DISCUSSION.

Whilst so little is known concerning the structure of proteins, it is impossible to offer a precise explanation of the results recorded in this paper. The keratins, especially, are a group which exhibit many characteristics not met with in typical proteins. The stability and chemical inertness typical of their class have for various reasons been ascribed to the high proportion of sulphur which they contain. The present results show that the sulphur groupings are much less reactive than might have been expected when the lability is recalled of such compounds as cystine peptides and anhydrides.

It would appear that in wool the cystine molecules are bound in some manner involving the sulphur groups and thereby depriving them of reactive freedom. Undenatured egg-albumin presents a somewhat similar case, giving no nitroprusside reaction; on denaturation, however, by whatever means this is accomplished, a strong —SH reaction develops [Harris, 1923]. Blood-serum, normally non-reactive, develops —S.S— groups on denaturation.

Further work is in progress directed towards the elucidation of the sulphur linkage in wool.

SUMMARY.

1. The action of a variety of reagents upon wool has been studied and the conclusion drawn that the sulphur groups of wool are less simple than those of free cystine.

2. Free —S.S— or —SH groups are not present in undamaged wool and can only be induced by the action of alkalis or of ultra-violet radiation.

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