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The Distribution of Reactive Disulphide Bonds in Wool

REDUCTIVE AND OXIDATIVE STUDIES

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Elsworth & Phillips (1938) and Middlebrook & Phillips (1942) showed that about 50 % of the disulphide bonds of some types of wool appear to be more readily reduced than the rest. There has since been considerable speculation on the significance of this finding. Although it was generally thought that these disulphide bonds are restricted to particular protein components, Gillespie & Springell (1961) showed that the more-readily-reducible disulphide bonds are distributed throughout the major components of wool. This has since been confirmed by Blackburn (1961) and is consistent with other findings by Crewther & Dowling (1961) and Springell (1963). In addition, Gillespie & Springell (1961) recorded and commented on the unexpected finding that the disulphide groups of the high-sulphur and low-sulphur proteins of wool react by the same fractional amount when treated with mercaptoacetate at pH 5.0 and alkylated with iodoacetate at pH 8.5. This is in spite of the fact that these proteins have widely differing compositions and structures (Corfield, Robson & Skinner,

1958; Gillespie & Simmonds, 1960; Sikorski & Woods, 1960; Fraser, MacRae & Rogers, 1962). It may be a coincidence that the two protein components have equally reactive disulphide bonds. On the other hand, it is possible that one protein is less extensively reduced at pH 5.0 than the other, and that subsequent reactions obscure this. The reactions that may be involved are those which promote disulphide interchange or the interchange of *S*-carboxymethyl groups between the two proteins. We have investigated the possible extent of these reactions, and have extended the studies to Lincoln wool as well as to the least-reactive disulphide bonds in Merino wool. In an attempt to eliminate the possibility of interchange reactions, the reactivity of disulphide bonds towards oxidation with performic acid was also examined.

MATERIALS

Wool. Medium Peppin B type 64s Merino wool top (batch MW129) and loose Lincoln wool (batch MW114) had thiol-plus-disulphide contents (Leach, 1960) of 470 and 409 μ moles/g. dry wt. of wool respectively.

Iodo[2- 14 C]acetic acid. This was prepared, as described by Gillespie & Springell (1961), from bromo[2- 14 C]acetic acid

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(The Radiochemical Centre, Amersham, Bucks.) to which was added unlabelled carrier bromoacetic acid in amounts ranging from 20 to 1000 μ moles/ μ c.

METHODS

In principle the wool was modified by a two-cycle process. The first cycle consisted either of partial reduction and alkylation or of partial oxidation. In both cases the second cycle involved exhaustive reduction, extraction and alkylation.

First reduction of wool. Wools in 1 g. lots were reduced partially with 1 M-sodium mercaptoacetate at pH 5.0 (preparations I and V), as described by Gillespie & Springell (1961). More extensive reduction was achieved by the following method (Maclaren, 1962). Wool (1 g.) was mechanically shaken for at least 3 days in 100 ml. of 0.1 M-toluene- ω -thiol ($C_6H_5 \cdot CH_2 \cdot SH$) in aq. 20% (w/w) propan-1-ol, or (preparation VII only) in aq. 70% (w/w) propan-1-ol. After reduction, the mercaptoacetate-reduced wool was washed with distilled water, acetone and diethyl ether and then dried *in vacuo*. The toluene- ω -thiol-reduced wool was washed first with acetone and then with diethyl ether and dried *in vacuo*.

First alkylation of wool. Preliminary experiments showed that iodoacetate alkylates cysteine residues at pH 5.0, and that the reaction goes to completion. The reaction is, however, far slower at pH 5.0 than at pH 8.5. The time required for complete alkylation of fully reduced wool is about 24 hr. at pH 5.0 compared with about 4 hr. at pH 8.5. The quantities of iodoacetate referred to in the following sections were applied to the partially reduced product from 1 g. of wool. Except in two instances (preparations VII and VIII), the first alkylation was carried out with iodo[2- ^{14}C]acetate.

(a) With excess of iodo[2- ^{14}C]acetate acting for a long time. A buffer of pH 5.0 was prepared by dissolving potassium hydrogen phthalate (14.0 g.) and sodium hydrogen carbonate (2.7 g.) in water. The solution was boiled to expel CO_2 , cooled and the volume adjusted to 1 l. The alkylating solution consisted of 50 ml. of this buffer, 50 ml. of propan-1-ol and 5 m-moles (50 μ c) of iodo[2- ^{14}C]acetic acid, and was adjusted to pH 5.0. The reaction was allowed to proceed for 24 hr. at room temperature. This treatment was given to Merino wool (preparation I) and to Lincoln wool (preparation V).

(b) With excess of iodo[2- ^{14}C]acetate acting for a short time. In a single experiment (preparation III) with Merino wool, 10 m-moles (10 μ c) of iodo[2- ^{14}C]acetic acid in 100 ml. of potassium hydrogen phthalate-propan-1-ol, pH 5.0 (as above), were allowed to react for 20 min. at room temperature.

(c) With limited amounts of iodo[2- ^{14}C]acetate acting for a long time. In one experiment (preparation IV) with Merino wool, 200 μ moles (10 μ c) of iodo[2- ^{14}C]acetic acid in 100 ml. of potassium hydrogen phthalate-propan-1-ol, pH 5.0, were allowed to react for 250 hr. at room temperature.

(d) With limited amounts of iodo[2- ^{14}C]acetate acting for a short time. The alkylating solution was prepared by dissolving 12.5 m-moles of boric acid and 200 μ moles (10 μ c) of iodo[2- ^{14}C]acetic acid in water, by the addition of 10 N-KOH to adjust the pH to 8.5, and then diluting to 25 ml.

The reaction was allowed to proceed for 30 min. at room temperature. This treatment was given to Merino wool (preparation II) and to Lincoln wool (preparation VI).

The extent of alkylation was determined by adding samples of the alkylating solution, before and after the reaction had occurred, to the 2 ml. aqueous component of the liquid-scintillation solution. The possible sources of error in this determination have been discussed by Gillespie & Springell (1961).

(e) Reaction with non-radioactive iodoacetate. In preparations VII and VIII, reduced Merino wool was first alkylated by treatment for 2 hr. with a solution prepared as follows: 25 m-moles of boric acid and 10 m-moles of iodoacetic acid were dissolved in water by adding 10 N-KOH to adjust the pH to 8.5 and then diluting to 50 ml.

The alkylated wools were in all cases exhaustively washed with water, with acetone and finally with diethyl ether before being dried *in vacuo*.

The methods of preparation are summarized in Table 1.

Reduction and extraction of partially-reduced and alkylated wool. A single long extraction (method L of Gillespie & Springell, 1961) was used except for preparations VII and VIII, where the wool was extracted for 3 hr. at 40° with 25 ml. of 0.2 M-mercaptoacetate-6 M-urea, adjusted to pH 11.0 with KOH. After extraction, the residual wool was filtered off. The yields of soluble protein were about 65% with method L and 90% for the mercaptoacetate-urea procedure.

Final alkylation of extracted wool proteins. Except for preparations VII and VIII, the extracts were alkylated for 15 min. by using 21.5 m-moles of non-radioactive iodoacetic acid with 1.5 g. of tris added in solid form to give a solution buffered at pH 8.5. The alkylated extract was dialysed against running tap water for at least 40 hr.

In preparations VII and VIII alkylation was also carried out at pH 8.5, but with 15 ml. of buffered solution containing 30 m-moles (200 μ c) of iodo[2- ^{14}C]acetic acid. The alkylated extract was dialysed first against running tap-water and then against 0.5 M-sodium acetate. The extent of alkylation in these two preparations was estimated from the specific activities of the soluble proteins.

Fractionation into proteins with high sulphur and low sulphur contents. The procedure employed was that of Gillespie, O'Donnell & Thompson (1962). The dialysed protein was adjusted accurately to pH 4.4 by the addition of sodium acetate-acetic acid (final 1:0.5). Under these conditions the low-sulphur protein was precipitated and was filtered off. The high-sulphur protein in the filtrate was dialysed against running tap-water overnight, then freeze-dried. The low-sulphur protein residue was dissolved in 100 ml. of saturated sodium borate and reprecipitated with acetate buffer at pH 4.4. The precipitate was collected by filtration, redissolved in borate, dialysed overnight against tap water and freeze-dried.

Determination of specific radioactivity of soluble proteins. In a limited number of experiments up to 5 mg. of soluble protein was dissolved in 2 ml. of distilled water and made up with liquid-scintillation solution.

Isolation and determination of specific radioactivity of S-carboxy[^{14}C]methyleysteine. Hydrolyses with 6 N-hydrochloric acid (under reflux for 24 hr.) were performed on 50 mg. of high-sulphur protein and on 100 mg. of low-sulphur protein. The freeze-dried hydrolysates were dissolved in N-hydrochloric acid, chromatographed on Dowex

Table 1. Summary of conditions used for the reduction and alkylation of wool

Details are given in the text. M, Mercaptoacetate; T, toluene- ω -thiol.

Prepn. no.	Wool type	Treatment cycle	Reducing conditions		Alkylating conditions			Reaction time (hr.)	Percentage of <i>S</i> -carboxymethylcysteine labelled in extracted protein
			Agent	Solvent	pH	Amount of agent			
						(m-moles)	(μ c)		
I	Merino	First	M	Water	5.0	5.0	50	5.0	59
		Second	M	Water	11.0	21.5	—	8.5	—
II	Merino	First	T	Aq. (20%, w/w) propan-1-ol	—	0.2	10	8.5	16
		Second	M	Water	11.0	21.5	—	8.5	—
III	Merino	First	T	Aq. (20%, w/w) propan-1-ol	—	10.0	10	5.0	55
		Second	M	Water	11.0	21.5	—	8.5	—
IV	Merino	First	T	Aq. (20%, w/w) propan-1-ol	—	0.2	10	5.0	23
		Second	M	Water	11.0	21.5	—	8.5	—
V	Lincoln	First	M	Water	5.0	5.0	50	5.0	52
		Second	M	Water	11.0	21.5	—	8.5	—
VI	Lincoln	First	T	Aq. (20%, w/w) propan-1-ol	—	0.2	10	8.5	20
		Second	M	Water	11.0	21.5	—	8.5	—
VII	Merino	First	T	Aq. (70%, w/w) propan-1-ol	—	10.0	200	8.5	22
		Second	M	6M-Urea	11.0	30.0	—	8.5	—
VIII	Merino	First	T	Aq. (20%, w/w) propan-1-ol	—	10.0	—	8.5	20
		Second	M	6M-Urea	11.0	30.0	200	8.5	—

2 resin (X8; acetate form) and collected in 2 ml. fractions. Each fraction to be analysed was neutralized and 100 μ l. was withdrawn for development of colour with ninhydrin (Gillespie & Springell, 1961).

When the fractions containing *S*-carboxy-[14 C]methylcysteine had been located, 100 μ l. samples were withdrawn from five fractions closest to the peak. Distilled water (1.9 ml.) was added to make up the aqueous component of the liquid-scintillation solution. Specific radioactivities were determined for each of the five fractions and the mean values calculated.

Liquid-scintillation counting. The liquid-scintillation solution consisted of 2 ml. of aqueous component, 25 μ l. of 0.77M-tetraethylammonium hydroxide (Leach & Hill, 1963) and 15 ml. of liquid-scintillation mixture. The composition of the latter was: 120 g. of naphthalene, 4 g. of 2,5-diphenyloxazole, 50 mg. of *p*-bis-(5-phenyloxazol-2-yl)-benzene and dioxan to 1 l. (Butler, 1961). Measurements were made in a Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.). Quenching was corrected for where necessary by use of internal standards. A standard error of $\pm 1\%$ was generally not exceeded.

Partial oxidation of wool. Oxidations were carried out with performic acid by a modification of the method of Thompson & O'Donnell (1959). To prepare performic acid a mixture of 1 ml. of hydrogen peroxide (30%, w/v) and 9 ml. of formic acid (98–100%, w/v) was allowed to stand at 20° for 1 hr. (Toennies & Homiller, 1942). The experimental conditions are summarized in Table 2, but a more detailed account follows for preparation C. The products from duplicate small-scale (70 mg. of wool) preparations were used for the analyses given in Table 2. In preparation C, wool (1 g.) was cut into 2–3 mm. lengths and cooled to 0°. Cold formic acid (120.5 ml.) was added, and after 15 min. this was followed by 4.5 ml. of cold performic acid reagent. The reaction was allowed to proceed at 0° for 1 hr. with occasional shaking, then 350 ml. of iced water was added and the mixture was freeze-dried without delay.

Reduction and alkylation of partially oxidized wool and subsequent fractionation of the extracted proteins. Partial-oxidation products derived from cystine [CySO₂·SCy and CySO₂H, where Cy is NH₂·CH(CO₂H)·CH₂—] are readily reduced to cystine (Lavine, 1936). In oxidized wool, the partial-oxidation products are rapidly converted by alkali into cystine and cysteic acid (Maclaren, Leach & Swan, 1960) and are reduced by aqueous mercaptoacetic acid (J. A. Maclaren, unpublished work). On the other hand, cysteic acid is stable to both reducing agents and alkali.

The partially oxidized wool was reduced and extracted with urea-mercaptoacetate, as described above for preparations VII and VIII. The reduced products were then alkylated with iodoacetate and fractionated into high-sulphur and low-sulphur proteins, as described above. As a result of these treatments, the extracted proteins contained both cysteic and *S*-carboxymethylcysteic residues. The isolated protein fractions represent the bulk of the fibre, since about 90% of the wool is solubilized by the above procedure.

Determination of total sulphur and cysteic acid nitrogen. Analyses of total sulphur were carried out on samples of soluble protein by the method of Schöniger (1956). Cysteic acid nitrogen was determined in acid hydrolysates of proteins by a modified version (Ingliš, 1963) of an automatic

Table 2. *Summary of conditions used for the partial oxidation of wool*

The conditions were as described for preparation C in the Methods section, except that 70 mg. of wool was used and the variations given in columns 2 to 4 were applied. Preparations a to g represent small-scale versions of preparations A to G referred to in Table 5. The percentage oxidation was calculated from

$$100 \left[1 - \frac{\text{disulphide in oxidized wool}}{\text{disulphide in untreated wool}} \right]$$

Prep. no.	Oxidizing conditions			Percentage oxidation	
	H ₂ O ₂ in formic acid (% w/v)	Vol. of reagent (ml./g. of wool)	Reaction time (min.)	Hydrolytic method	Non-hydrolytic method
a	0.05	100	15	18	74
b	0.10	50	60	—	—
c	0.11	125	60	53	97
d	0.11	125	60	—	—
e	0.20	50	60	52	92
f	0.20	130	60	—	—
g	3.00	30	60	91	98

amino acid analyser (Simmonds & Rowlands, 1960). *S*-Carboxymethylcysteine was also determined, but the results were rather variable and have not been quoted. Partial destruction of *S*-carboxymethylcysteine during acid hydrolysis, particularly in the high-sulphur proteins (Gillespie, 1963), is presumably the reason for these variations.

Determination of the extent of oxidation in partially oxidized wool. Residual cystine in partially oxidized wool was estimated either by hydrolysing the wool and applying the method of Shinohara (1935*a, b*) or by using the method of Leach (1960) on the intact wool. Because partially oxidized cystine derivatives dismutate during acid hydrolysis to give cystine and cysteic acid (Maclaren *et al.* 1960), the difference between the amounts of cystine found by the two methods provides a measure of the amount of partially oxidized cystine in the wool.

RESULTS

Possible interchange of S-carboxy[¹⁴C]methylcysteine residues between the high-sulphur and low-sulphur proteins. Direct exchange of the *S*-carboxy[¹⁴C]methyl groups of the modified protein with mercaptoacetate or iodoacetate can be excluded because of the high recovery of protein-bound radioactivity. However, another possible reaction is the interchange of *S*-carboxy[¹⁴C]methyl residues between the two soluble wool proteins during their preparation. In separate experiments, labelled high-sulphur protein and labelled low-sulphur protein were added to wool, and the mixtures were extracted with alkaline mercaptoacetate. The soluble proteins were alkylated with iodoacetate and fractionated into their high-sulphur and low-sulphur components. If interchange of ¹⁴C occurs, then in both cases each of the extracted protein fractions should contain radioactivity. Extraction and fractionation of wool in the presence of labelled high-sulphur proteins causes no transfer of the radioactivity to the low-

Table 3. *Extent of interchange of S-carboxy[¹⁴C]-methylcysteine groups between the high-sulphur and low-sulphur proteins*

The radioactive proteins were added to wool before the usual extraction with mercaptoacetate at pH 11.0. The soluble proteins so obtained were alkylated with non-radioactive iodoacetate at pH 8.5 in the usual manner and fractionated into high-sulphur and low-sulphur proteins. Samples of each protein were weighed into scintillation vials and counted. Details of the procedures are given in the Methods section.

Additive to wool before extraction	Percentage distribution of radioactivity after extraction and fractionation	
	High-sulphur protein	Low-sulphur protein
<i>S</i> -Carboxy[¹⁴ C]methylcysteine-labelled high-sulphur protein (200 mg./g. of wool)	99.2	0.8
<i>S</i> -Carboxy[¹⁴ C]methylcysteine-labelled low-sulphur protein (160 mg./g. of wool)	2.0	98.0

sulphur protein (Table 3). Similarly, in the presence of labelled low-sulphur protein, all but 2% of the radioactivity is located in the subsequently isolated low-sulphur protein. The value 2% is a little higher than in the previous case, possibly because it is more difficult to get the labelled low-sulphur protein (originally added to the wool) free of the contaminating labelled high-sulphur proteins than vice versa. The findings not only demonstrate the absence of any interchange of *S*-carboxymethyl groups during the various preparative procedures but also provide quantitative information on the effectiveness of the protein fractionation.

Comparison of alkylations at pH 5.0 and at pH 8.5. Disulphide interchange between partially reduced proteins could also influence the distributions of label. It might be envisaged that, on raising the pH to 8.5 before and during alkylation, the more-extensively-reduced protein reduces the other protein by disulphide interchange, until both proteins have equal proportions of thiol groups exposed to alkylation. This has been tested by alkylating the reduced wool at pH 5.0, thus avoiding the pH shift and presumably lowering the rate of disulphide interchange (Spackman, Moore & Stein, 1960; Sluyterman, 1961), although admittedly this also lowers the rate of alkylation, both rates being dependent on the concentration of RS^- . The results (Table 4) show that reduction and alkylation of Merino wool with iodo[2- ^{14}C]acetate at pH 5.0 results in high-sulphur and low-sulphur proteins (preparations I, III and IV) which are labelled to an equal extent. This result is the same as that obtained after alkylation at pH 8.5 (preparation II), and indicates that the equal reactivity of the cystine in these two proteins is not an artifact caused by shifting the pH before or during alkylation.

Reactivity of thiol groups in high-sulphur and low-sulphur wool proteins with iodoacetate. There is another way in which disulphide interchange could influence the final distribution of radioactivity. If the thiol groups of the less-extensively-reduced protein were also the more reactive towards iodoacetate, then, as the protein containing the more reactive thiol groups becomes depleted in thiol, further reaction might be brought about by disulphide interchange. By treating the thiol groups in almost completely reduced wool with iodoacetate, but without allowing the reaction to go to completion, it should be possible to detect any major

difference in the reactivity of the thiol groups in the two proteins towards iodoacetate. This possibility was tested by reducing wool with toluene- ω -thiol (Maclaren, 1962) and then partially alkylating it with iodo[2- ^{14}C]acetate. If the thiol groups of the two component proteins have different rates of reaction with iodoacetate, then the proportion of label found in each should differ.

In each experiment only part of the thiol groups of reduced wool were allowed to react (Table 1). The wool was treated with a limited amount of iodo[2- ^{14}C]acetate for a short time (preparation II) and for a long time (preparation IV), whereas in another experiment (preparation III) wool was treated with an excess of iodo[2- ^{14}C]acetate for a short time. Table 4 shows that, within experimental error, thiol groups in both high-sulphur and low-sulphur proteins have a similar reactivity towards iodoacetate.

Behaviour of different wools. The experiments of Gillespie & Springell (1961) were performed on Merino 64s wool (Wintoc, batch MW 127), with a thiol-plus-disulphide content of $512 \mu\text{moles/g.}$, whereas in the present studies a different batch of Merino wool was used having a thiol-plus-disulphide content of $470 \mu\text{moles/g.}$ In spite of this difference the distribution of the readily reducible disulphide bonds was identical for the two wools. With Lincoln wool (Table 4, preparation V), radioactivity was similarly found to be evenly distributed between the *S*-carboxymethyl residues in the high-sulphur and low-sulphur proteins. The thiol groups of the two proteins derived from Lincoln wool also have the same reactivity towards iodoacetate (preparation VI), as was the case with Merino wool. It therefore appears that the present findings may be applicable to a wide range of wools.

Table 4. *Specific radioactivities of S-carboxy[^{14}C]methylcysteine isolated from high-sulphur and low-sulphur proteins derived from reduced and alkylated wool*

The conditions of reduction and alkylation are described in the Methods section and summarized in Table 1. The procedures for fractionation and for the isolation and determination of *S*-carboxymethylcysteine are also given in the Methods section. The numbering of preparations corresponds to those given in Table 1. Specific activity was calculated from the mean of five fractions closest to the peak.

Prep. no.	Mean sp. activity of <i>S</i> -carboxy[^{14}C]methylcysteine ($\mu\text{C/m-mole}$)		Standard error of differences between means	Sp. activity of high-sulphur protein/sp. activity of low- sulphur protein
	High-sulphur protein	Low-sulphur protein		
I	4.56	4.54	0.11	1.00
II	5.20	5.34	0.07	0.97
III	3.00	3.13	0.07	0.96
IV	5.89	5.29	0.11	1.11
V	2.21	2.31	0.07	0.96
VI	8.11	7.12	0.28	1.14
VII	1.68	1.27	0.06	1.32
VIII	0.89	0.80	0.06	1.11

Table 5. *Distribution of cysteic acid in proteins from partially oxidized wool which was subsequently reduced and alkylated*

Merino wool was partially oxidized, reduced, extracted, alkylated and then fractionated into the two soluble wool proteins. Sulphur analyses were performed on the unhydrolysed samples; cysteic acid (CySO_3H) nitrogen was determined in acid hydrolysates of the proteins. Preparations A to G represent large-scale versions of preparations a to g referred to in Table 2. Values are corrected for the observed ash content in preparations B, D and E. As the ash contents of the high-sulphur and low-sulphur proteins were reproducible, an average ash content was used for correcting the remaining preparations.

Prep. no.	Fraction	Total S (%) (A)	CySO_3H N (% of total N) (B)	B:A ratio	B:A ratio of high-sulphur protein/B:A ratio of low-sulphur protein
A	High-sulphur protein	4.73	1.65	0.35	0.96
	Low-sulphur protein	1.57	0.57	0.36	
B	High-sulphur protein	4.76	3.44	0.72	0.87
	Low-sulphur protein	1.58	1.32	0.84	
C	High-sulphur protein	5.02	5.63	1.12	1.02
	Low-sulphur protein	1.61	1.77	1.10	
D	High-sulphur protein	4.90	7.09	1.45	0.95
	Low-sulphur protein	1.62	2.48	1.53	
E	High-sulphur protein	4.80	7.33	1.53	0.92
	Low-sulphur protein	1.60	2.65	1.66	
F	High-sulphur protein	4.93	9.55	1.94	0.85
	Low-sulphur protein	1.67	3.79	2.27	
G	High-sulphur protein	4.88	10.95	2.24	1.00
	Low-sulphur protein	1.82	4.10	2.25	

Distribution of the least-reactive cystine residues. In two experiments (Table 4, preparations VII and VIII) the distribution of the least-readily-reducible cystine residues has been examined. Unfortunately, the results are subject to a relatively greater experimental error, because only a small amount of radioactivity could be incorporated into the wool proteins. It can, however, be concluded that the least-reactive disulphide bonds are not confined to one or other soluble protein fraction. From the oxidative studies described below, it appears, further, that these cystine residues are found in approximately equal proportions in the high-sulphur and low-sulphur proteins (Table 5, preparations F and G).

Distribution of cysteic acid in protein fractions from wool that has been partially oxidized, reduced, extracted and alkylated. Disulphide interchange can be entirely eliminated when reactivity towards oxidizing agents is examined. Wool was partially oxidized with performic acid, reduced and extracted with alkaline mercaptoacetate-urea and finally alkylated with iodoacetate. Soluble proteins containing both cysteic acid and *S*-carboxymethylcysteine residues were thus obtained. The ratio of cysteic acid nitrogen to total sulphur in the two soluble proteins gives a measure of the relative reactivity of the cystine residues in each protein to oxidation. Values for total sulphur analyses in the soluble fractions show that a real separation into high-sulphur and low-sulphur proteins has been

effected (Table 5). The ratios of cysteic acid nitrogen to total sulphur are similar in both the high-sulphur and low-sulphur fractions, regardless of the extent of oxidation. This indicates that both soluble proteins contain about the same proportion of the more-easily-oxidizable as well as of the least-readily-oxidizable disulphide bonds. Thus the previous findings with reductive methods are confirmed by a procedure where disulphide interchange cannot introduce spurious effects.

DISCUSSION

The results presented above show, in confirmation of work by Gillespie & Springell (1961), that an equal proportion of the disulphide bonds in both the high-sulphur and low-sulphur proteins of Merino wool are reduced at pH 5.0 by mercaptoacetate. As these results have been obtained with two types of Merino wool and with one type of Lincoln wool, these findings may well apply to a wide range of wools. It has been shown, further, that this result is not an artifact arising out of: (1) exchange of carboxymethyl groups between the two soluble proteins (Table 3); (2) change in the distribution of thiol groups between the two proteins as a result of a change in pH from 5.0 to 8.5 on alkylation (Table 4, preparations I and V); or (3) disulphide interchange after preferential reaction of thiol groups of one protein with iodoacetate (Table 4, preparations II, III, IV and VI).

In partially oxidized wool the proportion of the total sulphur which is in the oxidized disulphide form is the same in the high-sulphur as in the low-sulphur proteins (Table 5). This is good evidence that disulphide interchange during alkylation does not determine the reactivity towards reduction. Gillespie & Springell (1961) concluded that the more-readily-reducible cystine residues are not confined to any particular protein component but that they are equally distributed throughout the morphological components of wool. Proteolytic (Springell, 1963) and radioautographic (G. E. Rogers & P. H. Springell, unpublished work) studies do in fact support the random distribution of the readily reducible disulphide bonds within the wool fibre.

Three possible explanations of our observations in terms of wool structure were presented previously: (1) the two soluble wool proteins contain disulphide bonds in a wide range of sequence situations and the mean reactivities of these bonds are approximately equal; (2) both proteins may be present in the microfibrils and the matrix, so that the cystine residues in each protein share a common environment; or (3) the disulphide bonds may cross-link the two proteins. The third explanation implies that a major proportion of the disulphide bonds must consist of interchain bonds, whereas the other explanations would permit both inter-chain and intrachain disulphide bonds within the wool fibre. For the second alternative to apply, it would be necessary to interpret the differential staining of the histological components with heavy metals (Birbeck & Mercer, 1957; Rogers, 1959) in terms of penetration of oriented and unoriented regions, rather than of differences in sulphur content. Unfortunately there is still no evidence on the relative proportions of the two types of disulphide bonds in wool. The results of Blackburn (1962) are open to the criticism that disulphide interchange may have altered the original pattern existing in native wool. For these reasons it is difficult to decide between the various alternatives at this stage. Nevertheless, some of the results quoted in the present work appear to be consistent with the third alternative.

The sulphur content of the low-sulphur proteins is subject to considerable fluctuations (Harrap & Gillespie, 1963), even in different preparations from the same wool. The proportions of high-sulphur and low-sulphur protein also show some variation. Nevertheless, the range of values is such as to permit a major part of the disulphide bonds to link the two proteins. Such an arrangement of disulphide bonds could be obtained either by distribution of both types of protein in the microfibrils and the matrix, or by an orientation of the half-cystine residues of the low-sulphur protein which

would permit the disulphide bonds to be at the interface between the high-sulphur and low-sulphur proteins.

SUMMARY

1. Wool (Merino or Lincoln) was reduced to various extents with toluene- ω -thiol in aqueous propan-1-ol or with mercaptoacetate at pH 5.0, alkylated with iodo[2- 14 C]acetate under a variety of conditions and then extracted with alkaline mercaptoacetate and alkylated with non-radioactive iodoacetate. In this way, the more-readily-reducible cystine residues were labelled.

2. The first alkylation was also carried out with non-radioactive iodoacetate and the extracted proteins were alkylated with iodo[2- 14 C]acetate. In this way the least-reactive cystine residues were labelled.

3. The soluble proteins were separated into fractions with high sulphur and low sulphur contents and then hydrolysed. The specific radioactivity of *S*-carboxy[14 C]methyleysteine from the hydrolysates is a measure of the extent of reduction, under the specified conditions, in each protein.

4. Wool was partially oxidized with performic acid, and then reduced, alkylated and fractionated into proteins with high-sulphur and low-sulphur contents. The ratio of cysteic acid nitrogen to total sulphur is a measure of the extent of oxidation of each protein.

5. Equal proportions of the more reactive disulphide bonds of each protein fraction are ruptured by partial reduction or by partial oxidation. Hence the disulphide bonds in high-sulphur and low-sulphur fractions are equally reactive.

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Isolation and Study of Rabbit Antibodies Specific for Certain of the Antigenic Groups of Human Serum Albumin

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A fragment of human serum albumin called 'inhibitor' was shown by Lapresle & Webb (1960a) to carry only some of the antigenic sites of the albumin molecule. In the present work, we have studied the isolation and properties of rabbit anti-(human serum albumin) antibodies specific for these sites. The antibodies were isolated by specific adsorption and subsequent elution from insoluble 'inhibitor'-benzylcellulose conjugates.

MATERIALS

Polyaminopolystyrene was kindly given by the Norsk Hydro Elektrisk, Oslo (Norway).

p-Aminobenzylcellulose was obtained from Touzart et Matignon, Paris, France.

Diethylaminoethylcellulose (DEAE-cellulose) and carboxymethylcellulose (CM-cellulose) (Selectacel) were obtained from Brown and Co. and were used in columns provided with a cooling jacket kept at 4°.

Sephadex G-200 and Sephadex G-100 were obtained from Pharmacia, Uppsala, Sweden. They were used in columns at room temperature.

The molarity of the potassium phosphate buffers used is expressed with respect to phosphate and the pH was measured with a pH-meter.

Human serum albumin (Squibb fraction V), prepared by ethanol fractionation, was kindly given by the American Red Cross.

Degraded human serum albumin was prepared as follows. A portion (2.5 g.) of human serum albumin was degraded (in the presence of 1 mm-cysteine) for 6 hr., at pH 3.5

and 37° by 1250 proteolytic units of rabbit cathepsin D prepared as described by Lapresle & Webb (1960b, 1962).

Rabbit anti-(human serum albumin) sera were prepared as follows. Rabbits were immunized by intravenous injections of human serum albumin adsorbed on alum and the sera were collected after either 3 weeks or 6 weeks of immunization, as described by Lapresle & Webb (1960a).

METHODS

Preparation of 'inhibitor'. The fragment of human serum albumin called 'inhibitor' can be detected by electrophoresis in agar gel, as shown by Lapresle & Webb (1960a). This property was used to detect the presence of 'inhibitor' in the various fractions obtained during the following purification.

Step I. A solution (50 ml.) containing 2.5 g. of degraded human serum albumin was dialysed against 5 mm-potassium phosphate buffer, pH 8.0, and passed through a column (50 cm. long × 2 cm. diam.) of 15 g. of DEAE-cellulose equilibrated with the same buffer. Under these conditions 'inhibitor' is eluted by the passage of the same buffer, but most of the degraded albumin stays on the column. All eluates having an extinction at 280 mμ of more than 0.1 were pooled and the pH of the mixture was adjusted to 6.55.

Step II. The pooled eluates obtained in step I were passed through a column (30 cm. long × 2 cm. diam.) of 6 g. of CM-cellulose equilibrated with 5 mm-potassium phosphate buffer, pH 6.55. The column was washed with the same buffer and a first fraction not containing the inhibitor was discarded. A second fraction containing