# The Extents of Reduction of the Cystine Residues in the Low-and High-sulphur Proteins of Partially-reduced Wools in relation to the Microfibril-Matrix Structure of the Cortex

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(Received 3rd December 1978)

**Summary:** The disulphide bonds of two samples of Merino 70s wool were reduced to the extent of 38% and 56% respectively and S-carboxymethylated with iodo (2-$^{14}$C)-acetate. The disulphide bonds remaining intact after these first-stage reductions were reduced subsequently and S-carboxymethylated with non-radioactive iodoacetate. The two major low-sulphur proteins of wool (Components 7 and 8), three high-sulphur proteins (SCMKB2-A, -B and -C), and a α-helical polypeptide fraction were isolated from each of the two $^{14}$C-labelled wools, and the extents to which the half-cystine residues in all of them were reduced in the first stage determined by their contents of $^{14}$C, S-carboxymethylcysteine, cystine and cysteic acid. At both levels of first-stage reduction the low-sulphur proteins were reduced to a much higher degree than either the high-sulphur proteins or the α-helical protein fraction. However, although the *percentages* of half-cystine residues reduced at the 38% level were very different in the two types of protein, the *numbers* of half-cystine residues reduced per molecule were approximately the same. This finding, and other evidence, suggests that the disulphide bonds that are more easily reduced in wool are interchain disulphide bonds linking low- and high-sulphur proteins in the microfibril-matrix structure of the cortex.

During the period 1938-46 Phillips and his co-workers[1,2,3,4,5,6] showed that the disulphide bonds in wool could be divided into four subfractions, A, B, C, and D, distinguished by their different reactivities towards bisulphite, thioglycollate, formaldehyde and alkalis. Under optimum conditions rather more than half the disulphide bonds, fraction (A+B), were severed by bisulphite, the fraction (C+D) being severed only under much more severe reaction conditions. Lindley and Phillips[7] adduced evidence that fraction (A+B) probably owed its easier reactivity to the influence of neighbouring carboxyl groups provided by residues of glutamic and aspartic acids, and, much later, Lindley and Cranston[8] showed that the half-cystine residues of fraction (A+B) were in the main attached to amino-acid residues of polar character, whereas those of fraction (C+D) were attached to apolar residues.

When it became clear that the cortex of wool, comprising 90% of the fibre, was a microfibril-matrix structure in which the microfibrils were assembled from low-sulphur proteins and the matrix from high-sulphur proteins, the question arose as to whether the differences in disulphide bond reactivity were due to differences in disulphide bonding in these two types of protein, which have widely-different molecular weights, amino-acid compositions and secondary structures.[9,10,11,12] However, the results of experiments to test this hypothesis have so far proved negative and somewhat puzzling.

Gillespie and Springell[13] isolated SCMKA (low-sulphur) and SCMKB (high-sulphur) protein fractions from an S-carboxymethylated wool in which the cysteine residues of fraction (C+D) were alkylated with non-radioactive iodoacetate. The specific activities of the S-carboxy-methylcysteine in the hydrolysates of both protein fractions proved to be identical, indicating that the disulphide bonds of low- and high-sulphur proteins react, at the 50% level, by the same fractional amount. This unexpected result was substantiated by later work.[14, 15, 16] After considering several alternatives Springell and his co-workers[16] concluded that the most likely explanation is that the more-readily-reducible disulphide bonds are interchain bonds linking the low- and high-sulphur proteins. This explanation would accord with the work of Cecil and Lŏening[17] and of Frangione and Milstein,[18] who showed, respectively, that the intrachain disulphide bonds of insulin and of immunoglobulin – G may be reduced under conditions that leave the interchain disulphide bonds intact. But the evidence as it stands is equivocal, and the present work was undertaken in an attempt to resolve the issue.

Two wools were reduced to the extents of 38% and 56% respectively, using thioglycollate at pH 5.4, and alkylated with iodo(2-$^{14}$C) acetate. Two major low-sulphur proteins, three homologus high-sulphur proteins and an α-helical polypeptide fraction were isolated from each of the two $^{14}$C-labelled wools. The proportions of the half-cystine residues that carried a $^{14}$C-label both in the wools and in the protein fractions and individual proteins derived from them, were determined.

*Materials and Methods*

*Wool* The wool was taken from a fleece of Australian Merino 70s quality and purified by the method of Corfield and Robson.[19]

*Iodo(2-$^{14}$C) acetic acid*

This was supplied by the Radiochemical Centre, Amersham with a specific activity of 30 mCi/mmole. Non-radioactive potassium iodoacetate (1 mmole) was added to 250 μCi of iodo(2-$^{14}$C) acetic acid, and made to 10 ml with phthalate buffer (0.07M; pH 5.0). This stock solution was stored in the dark and diluted with additional carrier iodoacetate for use when required.

*Preparation of partially-reduced and $^{14}$C-S-carboxymethylated wools*

Two wool samples (1 g) were reduced in 50 ml of 0.2M potassium thioglycollate at pH 5.4 for 2 hours and 24 hours respectively at room temperature. After thorough rinsing in deionized water the partially-reduced wools were S-carboxymethylated using a solution of 10 mmoles of iodo(2-$^{14}$C) acetate, of approximate specific activity 25 μCi/mmole, in 50 mm of n-propanol and 50 ml of phthalate buffer (0.07M; pH 5.0). The alkylation reaction was allowed to proceed for 24 hours. The two wools, *$^{14}$C-SCM-2h* and *$^{14}$C-SCM-24h,* were rinsed with deionized water and dried.

*Complete reduction and extraction of $^{14}$C-SCM-2h and $^{14}$C-SCM-24h wool and the preparation of their respective $^{14}$C-SCMKA-2h and $^{14}$C-SCMKB. protein fractions*

The two partially-S-carboxymethylated wools. $^{14}$C-SCM-2h and $^{14}$C-SCM-24h, were extracted for 3 hours at 40°C with 50 ml of 0.2M-potassium thioglycollate/6M urea adjusted to pH 11.0, following the procedure of Harrap and Gillespie.[20] The extracts were separated from the insoluble $^{14}$C-SCM-wool residues by filtrations, and S-carboxymethylated further by adding 5 mmoles of iodoacetate and adjusting the pH to 8.5. After 30 minutes the two extracts were dialysed against running tap water for 48 hours, their pHs were adjusted to 4.4 with 5N-acetic acid, and the precipitates, $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h, allowed to separate overnight. These precipitates were collected by filteration and retained for further purification, and the two supernatants dialysed against tap water for 48 hours and freeze-dried. These were the high-sulphur protein fractions, *$^{14}$C-SCMKB-2h* and *$^{14}$C-SCMKB-24h.* The two precipitates were each dissolved in 50 ml of 0.005M sodium metaborate, and re-precipitated by lowering the pH to 4.4. The process of re-dissolution and re-precipitation was carried out three times in an attempt to remove traces of high-sulphur proteins. Finally the *$^{14}$C-SCMKA-2h* and the *$^{14}$C-SCMKA-24h* preparations were thoroughly washed with deionized water and dried.

*Separation of the high-sulphur proteins $^{14}$C-SCMKB-F2A, -F2B and -F2C from $^{14}$C-SCMKB-2h and $^{14}$C-SCMKB-24h.*

The high-sulphur protein fractions $^{14}$C-SCMKB-2h and $^{14}$C-SCMKB-24h (100 mg of each) were subjected in turn to gel filtration on a 2.5 x 80 cm column of Sephadex G-100 by the procedure of Joubert and Burns.[21] The column was eluted with 0.3M-sodium acetate buffer (pH 4.5). Effluent fractions of 8 ml volume were collected at the rate of 35 ml/hr, their optical densities measured at 280 nm, and the data used

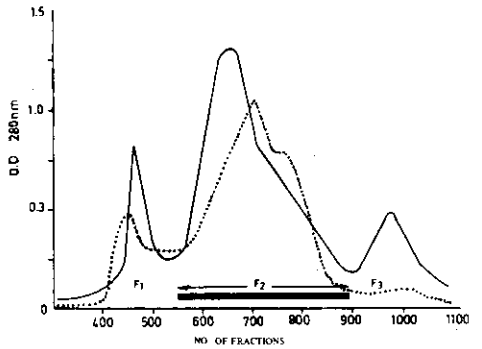


Fig. 1 Gel filtration of $^{14}$C-SCMKB-2h (100 mg approx.) on a 2.5 x 80 cm column of Sephadex G-100, by elution with 0.3M sodium acetate (pH 4.5). The flow rate was 35 ml/hr; 8 ml fractions were collected and their optical densities measured at 280 nm.

(---------- Joubert and Burns[21]).
(———— Present work).

to plot an elution profile as shown in Figure 1. The effluent fractions comprising the F2 peak (indicated by the black bar in Figure 1) were combined, dialysed against several changes of deionized water over a period of 48 hours, reduced in volume to 5 ml by rotary evaporation, and freeze-dried, to give the protein fractions *$^{14}$C-SCMKB-F2-2h* and *$^{14}$C-SCMKB-F2-24h*.

These two protein fractions were chromatographed in turn on a 1.0 x 103 cm column of SP-Sephadex C-25 by elution with a linear gradient from 100 ml of 2% citrate (pH 4.6)/5M urea to a 100 ml of 4% tricine (pH 7.5)/5M urea. This is the separatory procedure used by Lindley and Elleman,[22] with SP-Sephadex C-25 substituted for the SE-Sephadex C-25 used by them, which was unobtainable. Effluent fractions of 5 ml volume were collected, and their optical densities at 280 nm used to plot the elution profile given in Figure 2. After examination of this profile the effluent fractions indicated by the bars A, B and C were pooled, dialysed against deionized water and freeze-dried. By this procedure the six high-sulphur proteins designated *$^{14}$C-SCMKB-F2A-2h*, *$^{14}$C-SCMKB-F2B-2h*, *$^{14}$C-SCMKB-F2C-2h*, *$^{14}$C-SCMKB-F2A-24h*, *$^{14}$C-SCMKB-F2B-24h* and *$^{14}$-C-SCMKB-F2C-2* were isolated as white powders.

*Separation of the low-sulphur proteins $^{14}$C-Component 7 and $^{14}$C-Component 8 from $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h*

Following the method of O'Donnell and Thompson[23] the two $^{14}$C-SCMKA preparations were chromatographed in turn on a 3.0 x 15 cm column of DEAE-Cellulose (DE32, microgranular grade) by stepwise elution, using four solutions of 8M urea/0.001 M-EDTA/0.01M-Tris buffer (pH 7.4) with KCl concentrations of 0.05M, 0.10M, 0.15M and 0.20M respectively. Effluent fractions (10 ml) were collected at 30 ml/hr, and their optical densities, measured at 280 nm, used to plot the elution profile given in Figure 3. The effluent fractions indicated by the black bars, 7 and 8, were combined, dialysed against deionized water, reduced to 5 ml in volume, and freeze-dried.

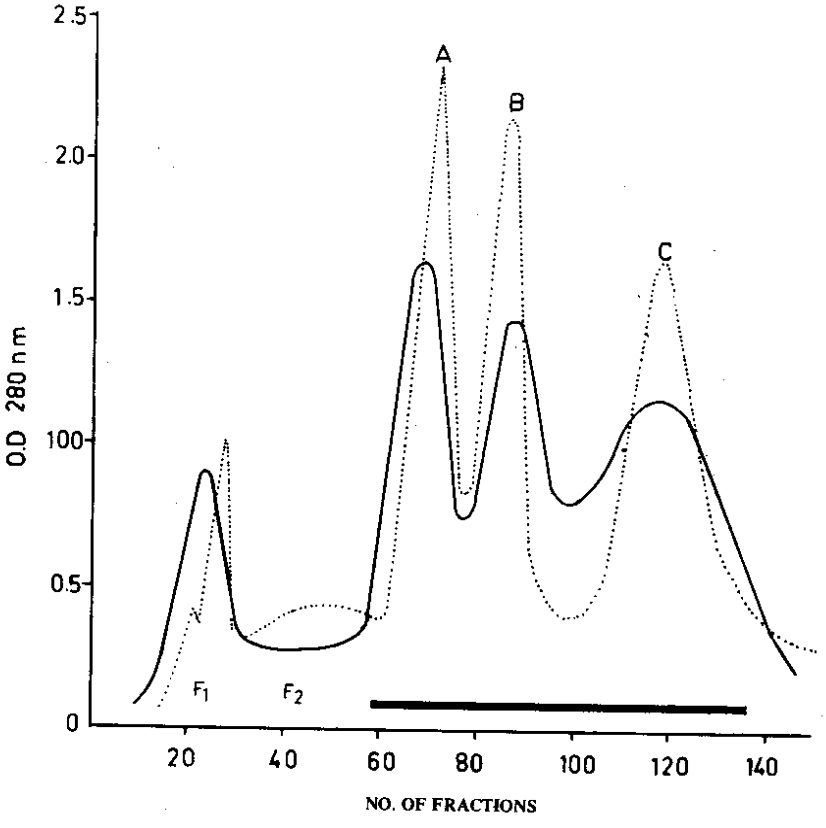


Fig. 2. Fractionation of $^{14}$C-SCMKB-F2-2h on a 1.0 x 103 cm cloumn of SP-Sephadex G-25, eluted with a linear gradient formed from 100 ml of 2% citrate (pH 4.6)/5 M-urea to 100 ml of 4 tricite (pH 7.5)/5 M-urea. Fractions of 5 ml volume were collected, and their optical densities measured at 280 nm).

(———— Lindley and Elleman[22]).
(- - - - - - - - Present work).

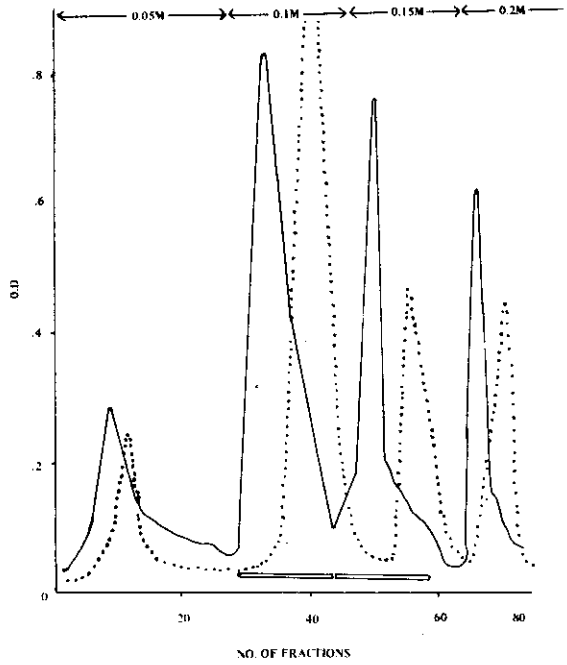


Fig. 3. Fractionation of $^{14}$C-SCMK A on 3 x 15 Cm column on DEAE-Cellulose eluted with stepwise elution: 8 M urea/0.00 M EDTA/0.01 M Tris buffer (pH 7.4) with KCl concentrations of 0.05 M, 0.10 M, 0.15 M and 0.2 M Fraction of 10 ml volume were checked and their optical densities measured at 280 nm.

(..... O'Donnell and Thompson[23])
(———— present work).

The four low-sulphur proteins isolated in this manner were designated *$^{14}$C-Component 7-2h*, *$^{14}$C-Component 7-24h*, *$^{14}$C-Component 8-2h* and *$^{14}$C-Component 8-24h.*

*Preparation of $^{14}$C-α-helical polypeptides from $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h.*

The procedure followed was that of Crewther and Harrap.[24] Portions (75 mg) of $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h were dissolved in 15.5 ml of 0.006M-sodium tetraborate and 8 ml of 0.15M-$CaCl_2$ added. The two solutions were adjusted to pH 8.4, and, with their temperatures maintained at 37°C, Pronase P (1 ml : 0.25 mg) was added to each. For the ensuing 30 minutes their pHs were held at pH 8.4 by the automatic addition of 0.2M-KOH, and subsequently lowered to pH 4.0 with HCl. After 30 minutes the precipitates which separated were removed by centrifugation, and re-dissolved in 15 ml of 0.006M-sodium tetraborate/0.15M-KCl. They were re-precipitated, recovered by centrifugation, washed with deionized water and freeze-dried. These two preparations were *$^{14}$C-α-helical polypeptide-2h* and *$^{14}$C-α-helical polypeptide-24h,* and were obtained in yields of 11 mg and 14 mg respectively.

*Protein hydrolysates*

All protein hydrolysates were prepared by heating 4-20 mg of material with 0.5 - 25 ml of 6N-HCl at 105°C for 24 hours. The HCl was removed by rotary evaporation, and the hydrolysates dissolved in 1.10 ml of deionized water, as appropriate, for future use.

*Amino-acid analyses*

Complete amino-acid analyses were carried out on 0.2-0.6 mg of hydrolysate using a Technicon Autoanalyser. Certain determinations of $^{14}$C-S-carboxymethylcysteine were made by high-voltage paper electrophoresis at pH 5.2. Duplicate analyses were made on all hydrolysates.

*Radiometric Assay of $^{14}$C*

The $^{14}$C contents of hydrolysates of $^{14}$C-labelled wools, wool protein fractions and polypeptides were determined with a Beckman model C.P.M. 200 Liquid Scintillation Spectrometer; appropriated corrections being made for loss of counts by quenching. Hydrolysates, 0.10 to 0.50 ml, were mixed with 5 ml of scintillation medium (composition: naphthalene, 60 g; 2:5 diphenyloxazole, 6 g; dioxane, 1 l). All samples were counted to give an error of no more than 1.5%, and were assayed in triplicate.

## Results

### *Amino-acid compositions*

The amino-acid compositions of $^{14}$C-SCMKA-2h and -24h, and of $^{14}$C-SCMKB-2h and -24h, and of the $^{14}$C-labelled protein and α-helical polypeptide fractions derived from them, were determined from duplicate analyses on single, 24-hour, acid hydrolysates, and no corrections were made for hydrolytic losses of Ser, Thr and Cys. Using this technique the errors in amino-acid analysis are probably no less than ± 5%. Within this margin of error it was found that the amino-acid compsitions of all the preparations from the $^{14}$C-SCM-2h wool were identical with the corresponding preparations from the $^{14}$C-SCM-24h wool.

The average amino-acid analyses for the $^{14}$C-SCMKA and $^{14}$C-SCMKB protein fractions are compared in Table 1 with those of similar fractions analysed by other workers. Bearing in mind that both the SCMKA and SCMKB fractions are known to be heterogeneous mixtures, whose compositions may differ because of slight differences in separatory methods, and also that the parent wools may have differed in sulphur content and structure, the measure of agreement between these compositions is good. However, there is considerably more Gly in the $^{14}$C-SCMKA preparations than is the SCMKA analysed by Crewther *et al*[25], and much less Pro in the $^{14}$C-SCMKB than would normally be expected.

Table 1. *Amino-acid composition of $^{14}$C-SCMKA and $^{14}$C-SCMKB prepared from $^{14}$C-SCM-2h and $^{14}$C-SCM-24h wools, compared with earlier analyses.*

(Compositions are expressed as amino-acid residues/1000 residues)

| Amino acid | SCMKA *Present work | SCMKA **Crewther et al. | SCMKB *Present work | SCMKB ***Lindley et al. |
|---|---|---|---|---|
| ǂCys | 68 | 71 | 194 | 179 |
| Asx | 78 | 80 | 33 | 41 |
| Thr | 58 | 47 | 110 | 104 |
| Ser | 85 | 94 | 117 | 119 |
| Glx | 133 | 141 | 87 | 64 |
| Pro | 32 | 40 | 93 | 136 |
| Gly | 111 | 88 | 68 | 54 |
| Ala | 65 | 63 | 35 | 29 |
| Val | 60 | 63 | 72 | 67 |
| Ile | 33 | 33 | 30 | 30 |
| Leu | 99 | 96 | 45 | 50 |
| Tyr | 41 | 42 | 21 | 19 |
| Phe | 32 | 28 | 17 | 24 |
| Lys | 30 | 34 | 6 | 7 |
| His | 6 | 6 | 7 | 9 |
| Arg | 68 | 74 | 63 | 67 |

*Average values of the compositions of $^{14}$C-SCMKA and $^{14}$C-SCMKB preparations from $^{14}$C-SCM-2h and $^{14}$C-SCM-24h wools.

**Crewther, Dobb, Dowling and Harrap (1968).[25]

***Lindley, Broad, Damoglou, Darskus, Elleman, Gillespie and Moore (1971).[26]

ǂ Includes S-carboxymethylcysteine, Cysteic acid and cystine.

In Table 2 the amino-acid compositions of the $^{14}$C-Component 7 and $^{14}$C-Component 8 preparations are given together with those of Components 7 and 8 isolated by O'Donnell and Thompson[27] using the same separatory technique that was employed in the present work. Although more recent work has shown that both of these low-sulphur proteins are mixtures of related proteins the close agreement between the two sets of analyses suggests that the method of preparation gives reproducible results. There is a close agreement also between the average composition of the two $^{14}$C-α-helical polypeptide preparations and that of the α-helical polypeptide prepared by Crewther *et al.*[25]

The average values for the amino-acid analyses of the three high-sulphur protein fractions $^{14}$C-SCMKA-F2A, -F2B and -F2C are compared in Table 3, with the analyses of the three homologous high sulphur proteins SCMKB-2A -2B and -2C which were purified, analysed and sequenced by Lindley and Elleman.[22] Whilst there is a fair measure of agreement between the two sets of analyses there are some notable differences, which most probably arise from the omission in the present work of the final stages of purification on DEAE-Cellulose used by Lindley and Elleman. Thus the comparatively-large amounts of Asx, and the traces of Lys and His, in the $^{14}$C-SCMKB-F2 preparations may be contributed by contaminating proteins of the 10,500-dalton SCMKB-III group of high-sulphur proteins, which are relatively-rich in Asp and Asn (about 65 residues/1000 on average) and are the only high-sulphur proteins with Lys and His in their primary structures.

### *Determinations of the extents of first-stage reduction of the half-cystine (Cys) residues*

Six analyses were made on the hydrolysates of the $^{14}$C-SCM-2h and -24h wools to determine the proportions of the $^{14}$C that was carried by the S-carboxymethylcysteine residues. For this purpose radioassays were made on suitable portions of the hydrolysates and on their S-carboxymethylcysteine($^{14}$C-Scmc) contents, which were determined after separations by high-voltage electrophoresis on paper at pH 5.2. The mean of these analyses showed that 96 ± 2% of the radioactivity in the hydrolysates was accounted for as $^{14}$S-Scmc. Since there is a possibility that this result may be low because of inefficient leaching of the $^{14}$C-Scmc from the paper with formic acid it has been assumed that alkylation of other amino-acid residues only occurred to a negligible extent.

The specific activity of the $^{14}$C-Scmc in the two hydrolysates was also determined as $2.66 \times 10^{-3}$ μCi/μmole for the $^{14}$C-SCM-2h wool and $2.57 \times 10^{-3}$ μCi/μmole for the $^{14}$C-SCM-24h wool. Since the same

Table 2. *Amino-acid compositions of $^{14}$C-Components 7 and 8 and of $^{14}$C-α-helical polypeptide isolated from $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h, compared with the compositions of these wool fractions determined by previous workers*

(Compositions are expressed as amino-acid residues/1000 residues)

| Amino acid | Component 7 | | Component 8 | | α-helical polypeptide | |
|---|---|---|---|---|---|---|
| | ‡ Present* work | O'Donnell & Thompson | ‡ Present* work | O'Donnell & Thompson | ‡ Present** work | Crewther et al. |
| +Cys | 52 | 62 | 41 | 60 | 33 | 35 |
| Glx | 146 | 156 | 165 | 182 | 209 | 218 |
| Leu | 106 | 99 | 103 | 118 | 139 | 138 |
| Asx | 86 | 90 | 84 | 108 | 93 | 107 |
| Ala | 73 | 78 | 66 | 60 | 77 | 79 |
| Arg | 68 | 75 | 79 | 78 | 69 | 68 |
| Ser | 76 | 82 | 75 | 76 | 61 | 66 |
| Val | 67 | 64 | 58 | 63 | 64 | 53 |
| Lys | 35 | 44 | 38 | 31 | 54 | 59 |
| Ile | 36 | 39 | 45 | 38 | 40 | 40 |
| Thr | 51 | 44 | 38 | 53 | 41 | 36 |
| Tyr | 38 | 29 | 23 | 26 | 36 | 34 |
| Gly | 111 | 74 | 86 | 42 | 50 | 28 |
| Phe | 30 | 24 | 30 | 21 | 24 | 19 |
| Pro | 26 | 29 | 33 | 37 | 11 | 11 |
| Met | – | – | – | – | – | 12 |
| His | 6 | 5 | 5 | 5 | 8 | 8 |

*O'Donnell and Thompson (1964).[27]

**Crewther, Dobb, Dowling and Harrap (1968).[25]

+Includes S-Carboxymethylcysteine, cysteic acid and cystine.

‡Average values of compositions of $^{14}$C-Component 7, $^{14}$C-Component 8 and $^{14}$C-α-helical polypeptide obtained from $^{14}$C-SCMKA-2h and $^{14}$C-SCMKA-24h.

Table 3. *Amino-acid compositions of the three high-sulphur proteins isolated from $^{14}$C-SCMKB-2h and $^{14}$C-SCMKB-24h, compared with the compositions of the three SCMKB2 proteins purified and sequenced by Lindley and Elleman[22]*

(The designations of all the proteins listed below should carry the prefix SCMKB-. Compositions are expressed as amino-acid residues/1000 residues).

| Amino acid | *-2A | ** -F2A | * -2B | ** -F2B | * -2C | ** -F2C |
|---|---|---|---|---|---|---|
| ‡ Cys | 220 | 231 | 217 | 186 | 225 | 168 |
| Ser | 147 | 128 | 153 | 139 | 150 | 132 |
| Thr | 100 | 113 | 92 | 121 | 104 | 112 |
| Glx | 120 | 105 | 113 | 101 | 103 | 102 |
| Pro | 101 | 81 | 90 | 90 | 89 | 80 |
| Gly | 92 | 90 | 101 | 82 | 96 | 94 |
| Ile | 46 | 29 | 45 | 26 | 32 | 26 |
| Ala | 31 | 42 | 33 | 43 | 41 | 53 |
| Val | 34 | 51 | 44 | 54 | 38 | 52 |
| Arg | 37 | 39 | 39 | 51 | 48 | 56 |
| Tyr | 20 | 13 | 12 | 15 | 25 | 16 |
| Leu | 19 | 33 | 20 | 25 | 22 | 45 |
| Phe | 15 | 12 | 25 | 15 | 13 | 11 |
| Asx | 9 | 23 | 8 | 33 | 7 | 37 |
| Trp | 6 | – | 6 | – | 2 | – |
| Lys | – | 4 | – | 6 | – | 10 |
| His | – | 5 | – | 8 | – | 10 |

*Calculated from data given in Table 1 by Lindley and Elleman (1972).[22]

**Average values of compositions of $^{14}$C-SCMKB-F2A, -F2B and -F2C preparations from $^{14}$C-SCMKB-2h and $^{14}$C-SCMKB-24h.

‡ Includes amounts of cysteic acid, S-carboxymethyl cysteine and cystine.

solution of iodo (2-$^{14}$C) acetate was used to alkylate both wools after the first stage of reduction these specific activities should be the same. The difference between them is a measure of the analytical errors involved, and for all subsequent calculations of the analytical errors involved, and for all subsequent calculations the mean value of $2.61 \times 10^{-3}$ $\mu$Ci/$\mu$mole was used to determine the amount of Cys residues reduced in the first stage of reduction.

The percentage extent of first-stage reduction of the Cys residues in all $^{14}$C-labelled wool protein fractions and polypeptides was calculated using the expression:

$$\text{Percentage of Cys residues reduced in first-stage reduction} = \frac{^{14}\text{C-Scmc} \times 100}{\text{Scmc} + \text{Cya} + \text{Cys}}$$

where $^{14}$C-Scmc is the amount of the Cys residues reduced in the first stage (assayed as $^{14}$C), and Scmc, Cya and Cys are the amounts of S-carboxymethylcysteine, cysteic acid and cystine present in the hydrolysates. This method of calculation had to be used because the hydrolysates of all the protein fractions contained traces of Cya (2 to 5%) and of Cys (5 to 25%). The highest amounts of Cys residues, assayed as cystine, were present in the $^{14}$C-SCMKB-F2A, -F2B and -F2C preparations. It should be pointed out here that all previous work on the determination of the extent of firststage reduction of Cys residues, using alkylation with iodo(2-$^{14}$C) acetate to label the cysteine residues produced in the first stage and alkylation with non-radioactive iodoacetate for the cysteine residues produced in the second (complete) reduction stage, has relied on the measurements of the specific activities of the $^{14}$C-Scmc in the preparations. Calculations based solely on measurement of specific activity are only valid if it can be shown that all the Cys residues have been converted quantitatively into Scmc residues. In the present investigation, despite much preliminary work involving changes in the conditions for the second stage of reduction, this was never achieved. The results of the measurements of the precentage extent of reduction in all the materials examined are given in Table 4.

Table 4. *Comparisons of the extents of first-stage reduction of the Cys residues low-sulphur and high-sulphur proteins and α-helical polypeptides derived from $^{14}$C-SCM-2h and $^{14}$C-SCM-24h wools. (The two wools were prepared by first reducing them with 0.2M-thioglycollate at pH 5.4 and 20°C, and alkylating them with iodo (2-$^{14}$COacetate) at pH 5: then reducing them further with 0.2M-thioglycollate 6M-urea at pH 11 for 3 hours at 40°C, and alkylating with non-radioactive iodoacetate at pH 8.5. For preparative details of the protein fractions listed see text).*

| | $^{14}$C-SCM-2h wool | | | $^{14}$C-SCM-24h wool | |
|---|---|---|---|---|---|
| | Number of Cys residues per molecule | Percentage of Cys residues Reduced | Number of Cys residues reduced per molecule | Percentage of Cys residues reduced | Number of Cys residues reduced per molecule |
| Wool | – | 38% | – | 55% | – |
| SCMKA | – | 48% | – | 56% | – |
| SCMKB | – | 33% | – | 55% | – |
| SCMKB-F2A | * 40 | 17% | 6.8 | 36% | 14.4 |
| SCMKB-F2B | * 34 | 26% | 8.8 | 23% | 7.8 |
| SCMKB-F2C | * 33 | 20% | 6.6 | 19% | 6.3 |
| Component 7 | ** 28 or 32 | 37% | 10.4 or 11.8 | 57% | 16.0 or 18.2 |
| Component 8 | ** 20 | 45% | 9.0 | 59% | 11.8 |
| α-helical polypeptide | – | 27% | – | 26% | – |

*Based on the assumption that these protein fractions are identical with SCMKB-2A, -2B and -2C respectively.[22]

**Based on the known amino-acid compositions of these Components (see Table 2) and on molecular weights of 51,000 (or 58,000) daltons for Component 7 and 40,000 daltons for Component 8.

## Discussion

The data in the fourth column of Table 4 confirms the findings of previous workers; that when wool is given prolonged treatment at room temperature with thioglycollate at pH 5 about 55% of its disulphide bonds are reduced. In the present work the cysteine residues thus produced were distinguished from unchanged Cys residues by reaction with iodo(2-$^{14}$C) acetate to give a modified wool, $^{14}$C-SCM-24h. The low- and high-sulphur protein fractions prepared from this, $^{14}$C-SCMKA-24h and $^{14}$C-SCMKB-24h, both had 55-56% of their Cys residues reduced during the first-stage reduction of the parent wool. This result is also in accord with previous work.[13,16] However, this finding that the Cys residues in the low- and high-sulphur proteins of wool react to the same extent at the 55% level of reduction has always been difficult to interpret in view of the widely divergent characteristics of the two types of protein.

The proteins of the low-sulphur group have molecular weights from 40,000 to 58,000 daltons, approximately half of their molecular chains are α-helical in secondary structure, and most of their Cys residues are concentrated in their non-helical chain segments. These proteins assemble in a manner not yet known with certainty to form the microfibrils of the wool cortex. By contrast, the proteins of the high-sulphur type comprise four families of closely-related proteins with molecular weights of approximately 10,500, 16,000, 19,000 and 23,000 daltons respectively, whose Cys contents increase, with increasing molecular weight, from 1 residue in 5 to 1 residue in 4. All the high-sulphur proteins whose aminoacid sequences have been determined have a common C-terminus, Cys, and most of them have a common N-terminal sequence, Acetyl-Ala-Cys-Cys-, and the sequence -Cys-Cys- repeats many times in their primary structures. Nothing is known precisely about the locations of these different high-sulphur proteins in the wool fibre, but there is no doubt that a large proportion of them constitute the matrix phase of the microfibril/matrix structure of the cortex.

With protein fractions of such diverse characteristics one might expect to find some difference in the reactivities of their disulphide bonds under a given set of reducing conditions. To obtain further information on this the present work has been concerned primarily with an investigation of the extents of reduction of the Cys residues in the two principal low-sulphur proteins, three of the high-sulphur proteins and an α-helical polypeptide derived from the low-sulphur proteins of two wools partially-reduced to the 38% and 55% extents. The extents of first-stage reduction of the Cys residues in Component 7 (57%) and in Component 8 (58%) were very little different from that of their parent wool (55%) But the extents of first-stage reduction of the Cys residues in the three high-sulphur proteins were very much lower; $^{14}$C-SCMKB-F2A, -F2B and -F2C were reduced to the extent of 36%, 23% and 19% respectively.

The low- and high-sulphur protein fractions isolated from the wool which was partially-reduced to the extent of 38%, had their Cys residues reduced to the extents of 48% for SCMKA and 33% for SCMKB. The corresponding low-sulphur proteins, Components 7 and 8 had their Cys residues preferentially reduced to the 37% and 45% extents, whilst the high-sulphur proteins SCMKB-F2A, -F2B and -F2C had first-stage Cys reductions of 17%, 26% and 20%.

Similarly the figures for the numbers of Cys residues at the 55% level of overall reduction seem to indicate that the numbers of Cys residues in individual proteins are reduced, some to the same and others to a greater extent than they were at the 38% level. In turn this may be interpreted in terms of a gradual reduction of intrachain disulphide bonds following the completion of reduction of the interchain bonds, but such a conclusion is purely speculative and not entirely justified on the evidence presented.

Nevertheless we believe that the results, especially at the 38% level of disulphide-bond reduction, are consistent with the view that most of the bonds broken in the early stages are interchain bonds between microfibrillar and matrix proteins. The data also gives encouragement to the idea that it may be possible to devise techniques to distinguish between the two types of disulphide bond in keratins, as in immunogloblin-G and also offer a prospect of identifying specific disulphide bonds. For example, Cys residues associated with α-helical segments of the low-sulphur proteins, Components 7 and 8, are much less easily reduced than those associated with non-helical segments.

However, before such prospects can be opened up with confidence it is essential that the present work should be extended in an attempt to determine whether specific disulphide bonds are broken in the initial stages of reduction of wool keratin, especially in the proteins of the cortex. To this end cortical cells might be used instead of the whole fibre, and much greater attention paid to methods for purifying individual proteins.

The α-helical polypeptides from both partially-reduced and $^{14}$C-carboxymethylated wools were apparently reduced to about the same extent (26%), a level of reduction much lower than the general levels of 38% and 55%, and of the corresponding Components 7 and 8.

Taking account of a probable error of at least ± 6% in the measurements of the percentage extents of reduc-

tion of Cys residues in the two wools, and in the proteins and polypeptides extracted from them, it is clear that there are very much larger differences between low- and high-sulphur proteins in this respect than have been suspected hitherto.

The number of Cys residues reduced per molecule, for all the proteins and polypeptides extracted from the two wools, can be calculated by multiplying the number of Cys residues for each protein molecule (given in column 1 of Table 4) by the percentage of Cys reduced at the first-stage of reduction (from columns 2 and 4 of Table 4). When the results are examined in the light of the numbers of Cys residues per protein molecule reduced at the first-stage of reduction they assume a new significance, despite the fact that these may be regarded as only approximate because the protein fractions isolated were impure. With respect to the wool reduced at the 38% level, the numbers of Cys residues reduced per molecule are approximately the same for both the low- and high-sulphur proteins. If these low- and high-sulphur proteins were the only ones present in the microfibril/matrix structure the result would strongly suggest that the disulphide bonds preferentially-reduced at 38% level are interchain bonds between the two types of protein. However, such a conclusion must be treated with caution since there are known to be high-sulphur proteins, of the 10,500 and 23,000 dalton groups, and probably high-glycine-tyrosine proteins, also in the cortex of the fibre.

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