A FURTHER STUDY ON THE EXTRACTION OF REDUCED PROTEINS FROM WOOL

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Summary

A study has been made on the extraction of reduced protein from wool by two methods. In one the aim has been to solubilize as much protein as possible and in the other to use the mildest conditions possible. In the first method extraction was made with potassium thioglycollate in the presence of urea and the variables temperature, pH, urea concentration, and thioglycollate concentration were studied. It was found that between 80 and 85% of wool can be solubilized at room temperature by 0·2M potassium thioglycollate at pH 10·5 in the presence of 6M urea. In the second method a total of more than 60% of the wool was solubilized; the high-sulphur protein was first preferentially extracted by 0·8M potassium thioglycollate at pH 10·5 and 0°C, and low-sulphur protein then released from the residue by plasmolysis by immersing the residue in ice-cold distilled water. The advantage of this method is that at no time does the temperature rise above 0°C or the pH above 10·5.

Although the low-sulphur proteins (i.e. proteins precipitable at pH 4·4 after alkylation with iodoacetate) prepared by both these procedures gave essentially single-boundaried patterns on electrophoresis at pH 11·0, amino acid analyses indicated that, in addition to the SCMKA1 and SCMKA2 proteins previously reported, these proteins also contain variable amounts of constituents rich in glycine and *S*-carboxymethyl cysteine.

I. INTRODUCTION

In previous papers from this Laboratory dealing with the properties of reduced wool extracts, the protein has been solubilized essentially by a development of the procedure of Goddard and Michaelis (1935) as described by Gillespie and Lennox (1953). This involved extracting the protein by the combined action of alkali (pH 9·8–11·5), heat (50°C), and a reducing agent (potassium thioglycollate). Under these conditions about 65% of the wool was obtained as a soluble derivative which was fractionated into two main groups of proteins, one of which had a sulphur content considerably higher and the other a sulphur content considerably lower than the wool from which it was derived.

In making comparisons between wools from different breeds of sheep and between wools from typical and atypical sheep within a breed it often is desirable to know the relative amounts of the high- and low-sulphur protein fractions in each. For this purpose a more complete extraction of wool would be desirable. It has been shown that higher yields can be obtained by using considerably more alkaline solutions, i.e. pH>12 (Jones and Mecham 1943; Gillespie and Lennox 1955); however, under these conditions considerable quantities of dialysable nitrogen appear in the system, suggesting peptide-bond breakage. Previous workers have indicated that the degree of extraction at a given pH is increased by the use of

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concentrated urea solutions in conjunction with reducing agents, although no systematic investigation of the optimum conditions appears to have been made (Jones and Mecham 1943; Ward 1952; Woods 1959). This paper reports on the effects of urea concentration, temperature, pH, and thioglycollate concentration on the percentage of protein extracted from wool.

Because of the increased possibility of damage to the protein (e.g. peptide-bond breakage, racemization) caused by the use of alkali at high temperatures, a method was also sought by which the reduced protein could be prepared under as mild conditions as possible.

II. Materials and Methods

(*a*) *Preparation of Wool*

The wool used in these experiments was sampled from Merino 64's quality solvent-scoured tops which were further degreased with petroleum ether, ethanol, and water. The two samples used were both from the Wintoc property in Victoria, MW127 from the 1959 shearing and MW148 from the 1961 shearing. Before use the wool was equilibrated at 68°F and 60% R.H.

(*b*) *Preparation of Wool Extracts*

(i) *Extraction with Solutions Containing Urea.*—Samples of wool (1·67 g) were treated with 50-ml aliquots of solutions containing the required quantities of thioglycollic acid, potassium hydroxide, and urea. The solutions were adjusted to the desired pH before the addition of urea. The "pH" of the urea solution as measured at the glass electrode was within 0·1 pH unit of that of the solution before the addition of urea.

The extraction mixture was shaken frequently during the incubation period and at the end of this time the undissolved wool was removed by filtration under vacuum. Two 15-ml aliquots of the filtrate were taken. One of these was used to determine the total quantity of protein extracted by refractive index measurements (see Section II(*c*)). The other 15-ml aliquot was alkylated with 1·2 g iodoacetic acid, the pH being maintained between 8 and 9 by the addition of 1M Tris. The alkylated solution was then quantitatively transferred to a dialysis bag and dialysed against several changes of acetic acid–sodium acetate buffer of pH 4·4 and ionic strength 0·5. Volume changes were determined and corrected for by weighing the contents of the dialysis bag. The supernatant containing the high-sulphur protein was separated by centrifugation and its protein concentration determined either by measuring the optical density at 276 mμ, assuming a value of 5·5 for $E^{1\%}_{1\,cm}$ (Gillespie 1962) or by measuring the refractive index increment (Δn) between the supernatant and the dialysis buffer. Values obtained by both methods were in good agreement.

(ii) *Extraction by Plasmolysis at Low Temperature.*—Following the original extraction method of Gillespie and Lennox (1955) involving the incubation of wool at 50°C, attempts were made to extract similar proteins at lower temperatures. Only small amounts of protein could be extracted at 0°C but if the undissolved wool were washed with water or placed in a large volume of water considerable swelling of

the fibres occurred together with the release of large quantities of protein.* During this swelling, rupture of the fibrous structure occurs (Plate 1). Since this disruption is caused by changing from a medium of high ionic strength to one of low ionic strength we have called the process "plasmolysis". The general procedure used in the further investigation of this effect was as follows: 5 g wool was incubated for 18 hr at 0°C with 150 ml of aqueous solutions of thioglycollic acid adjusted to the desired pH with potassium hydroxide. This mixture was filtered (filtrate = extract I) and the residue treated with a further 150 ml of the same solution for an additional 18 hr (filtrate = extract II). The residue from this second treatment was placed in a Waring Blendor jar containing sufficient volume of ice-cold plasmolytic medium so that the jar was completely filled. The jar was sealed and the suspension was blended for about 10 sec, then centrifuged for 30 min at 13,000 *g* at 0°C (supernatant = extract III). The protein concentration of an aliquot of the supernatant was determined by a semi-microKjeldahl technique. The remainder of the supernatant was alkylated at pH 9 by the addition of 8 g of iodoacetic acid and thoroughly dialysed. The percentage of high-sulphur protein was measured essentially as described in the following section.

(*c*) *Determination of Protein Concentrations in Wool Extracts*

In studies on extraction involving plasmolysis the amount of protein extracted was estimated in terms of the amount of nitrogen solubilized as a percentage of the total nitrogen in the whole wool. Nitrogen estimations were made by the semi-microKjeldahl procedure.

In the case of preparations containing urea this method is not easily applicable because of the difficulties associated with the complete removal of urea from the extracts. Consideration was given to the possibility of determining protein concentrations by means of optical density measurements at 276 mμ. However, in the case of total extracts, which contain both high- and low-sulphur protein components, difficulties arise because of the wide range covered by the extinction coefficients ($E\,{}^{1}_{1}\,{}^{\%}_{cm} = 5{\cdot}5$–$9$) of the various components. Thus the total amount of protein cannot be obtained from a measurement of optical density unless the proportions of each component in the mixture is known. On the other hand, when dealing with the separated high-sulphur protein mixture this method is more reliable since the extinction coefficients of known fractions lie within the range $E\,{}^{1}_{1}\,{}^{\%}_{cm} = 5{\cdot}5$–$5{\cdot}9$.

In order to determine the total amount of protein extracted by solutions containing urea, refractive index measurements have been found most suitable. Values of the specific refractive index increment (dn/dc) vary little from one protein to another, normally falling within the range $1{\cdot}8$–$1{\cdot}9 \times 10^{-3}$ at 546 mμ. The method used involved dialysis of an aliquot of the urea–thioglycollate extract against two changes each of 1l. of solution containing 0·02M potassium thioglycollate and 0·2M potassium chloride at pH 10·5, each for 18 hr. The protein concentration of

* This emphasizes the danger of using the weight of residue after washing as a measure of extent of extraction by any particular extracting medium. This procedure has frequently been used in tests for the estimation of damage to wool.

the solution was then determined from the difference in refractive index between the protein solution and solvent, assuming a value of $1 \cdot 85 \times 10^{-3}$ for dn/dc and making a correction for changes in volume during dialysis.

(*d*) *Electrophoresis*

Moving boundary electrophoresis was carried out in a Tiselius apparatus (made by LKB-Produkter, Stockholm) as previously described (Gillespie 1962). The high-sulphur proteins were run in acetic acid–sodium acetate buffer of ionic strength 0·1 at pH 4·5 whilst the low-sulphur proteins were run in a β-alanine–KOH buffer of ionic strength 0·1 at pH 11·0.

(*e*) *Amino Acid Analysis*

For amino acid analysis 50 mg of freeze-dried protein was hydrolysed for 24 hr under reflux with 6N HCl. The hydrolysate was freeze-dried and analysed by the Spinco automatic amino acid analyser as described by Spackman, Stein, and Moore (1958).

III. Results and Discussion

(*a*) *Extractions in the Presence of Urea*

(i) *Effect of Urea Concentration.*—The influence of variations in urea concentration on the extraction of protein by 0·2M potassium thioglycollate at pH 10·5 for 3 hr at 40°C is illustrated in Figure 2(*a*). The addition of urea produces a substantial increase in the total extraction, the maximum effect being achieved when the urea concentration reaches about 4M. In contrast to the increase in total extraction, the addition of urea produces relatively little increase in the extraction of high-sulphur protein. Thus, as the total extraction increases so the relative proportion of high-sulphur protein in the extract decreases. This situation is somewhat different from that which occurs with proteins from oxidized wool if, as O'Donnell and Thompson (1962) assume, the ratio of extracted high-sulphur proteins to low-sulphur proteins remains constant over a wide range of total extraction of the oxidized proteins.

It is of interest that the limiting value for the amount of high-sulphur protein extracted by 0·2M potassium thioglycollate in the presence of urea is about the same as that extracted by 0·8M thioglycollate in the absence of urea (Gillespie 1962).

The electrophoretic pattern of a high-sulphur protein mixture prepared by extracting wool at 40°C for 2 hr with a solution containing 6M urea and 0·2M potassium thioglycollate, followed by alkylation with iodoacetate and removal of the low-sulphur protein at pH 4·4, is shown in Figure 1 and appears to be identical with those of the high-sulphur protein mixtures previously reported (Gillespie 1960, 1962).

(ii) *Effect of pH.*—The influence of pH on the extraction in the presence of urea is shown in Figure 2(*b*). The general effect of urea is to displace the extraction curves in the direction of lower pH values. In the presence of 8M urea both the total extraction and the extraction of high-sulphur protein have almost reached a maximum at pH 9·5 whereas in the absence of urea this point is not reached until about pH 10·5.

(iii) *Effect of Temperature.*—Since increases in the concentration of urea above 4M had relatively little effect on the extraction (Fig. 2(*a*)) a concentration of 6M rather than 8M was selected for temperature studies so that investigations could be carried out at 0°C without the complication of crystallization of urea from solution. Figure 2(*c*) indicates that temperature has relatively little influence on the total extraction in the range 0–40°C, such effect as there is being confined almost entirely to the amount of high-sulphur protein extracted.

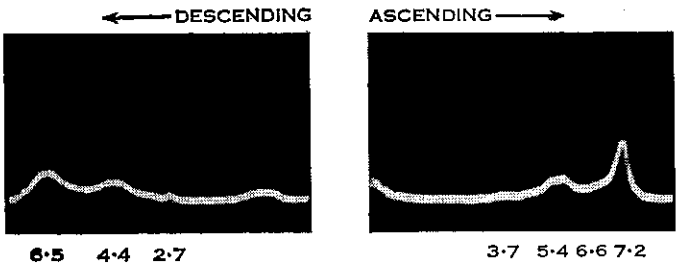

Fig. 1.—Electrophoretic pattern of the high-sulphur protein fraction prepared by urea–thioglycollate extraction of Merino 64's wool, sample MW148 (see text, p. 545), and run at pH 4·5 in acetic acid–sodium acetate buffer of ionic strength 0·1 for 180 min at a voltage gradient of 7·3 V/cm. Protein concentration approximately 1%. Mobilities recorded on the figure were calculated from the rate of movement of the peaks in descending boundaries.

(iv) *Effect of Thioglycollate Concentration.*—As the thioglycollate concentration is increased in the presence of 6M urea the total protein extraction decreases markedly between 0·5 and 1·0M (Fig. 2(*d*)). On the other hand, there is a steady increase in the amount of high-sulphur protein extracted as the thioglycollate concentration is increased. This general effect has previously been observed in the absence of urea (Gillespie 1962) and utilized to extract preferentially the high-sulphur protein fraction. However, whereas the extraction of low-sulphur protein is almost entirely suppressed at a thioglycollate concentration of 0·5M in the absence of urea, approximately twice the thioglycollate concentration is necessary in the presence of 6M urea to achieve the same effect.

(*b*) *Extractions by Plasmolysis at Low Temperature*

(i) *Effect of Thioglycollate Concentration at an Initial pH of* 10·5.—The data in Table 1 show that variations in the concentration of potassium thioglycollate over the range 0·1–0·8M have little effect on the overall amounts of either high- or low-sulphur protein extracted from extracts I, II, and III (Section II(*b*)(ii)). There is virtually no difference between 0·5 and 0·8M potassium thioglycollate in the yields of high- and low-sulphur proteins at the different stages of extraction, i.e. extracts I and II consist almost entirely of high-sulphur protein, whereas the protein released by plasmolysis (extract III) is mainly low-sulphur protein contaminated with about 10% of high-sulphur protein.

The situation is somewhat different in the case of 0·1M potassium thioglycollate. In extract I the pH dropped during extraction to about 9·8 because of the low buffering of the system and therefore, as expected from the results of Gillespie (1962), mainly high-sulphur protein was extracted. In extract II, the drop in pH during extraction was less and a mixture of both types of protein was extracted. Gillespie

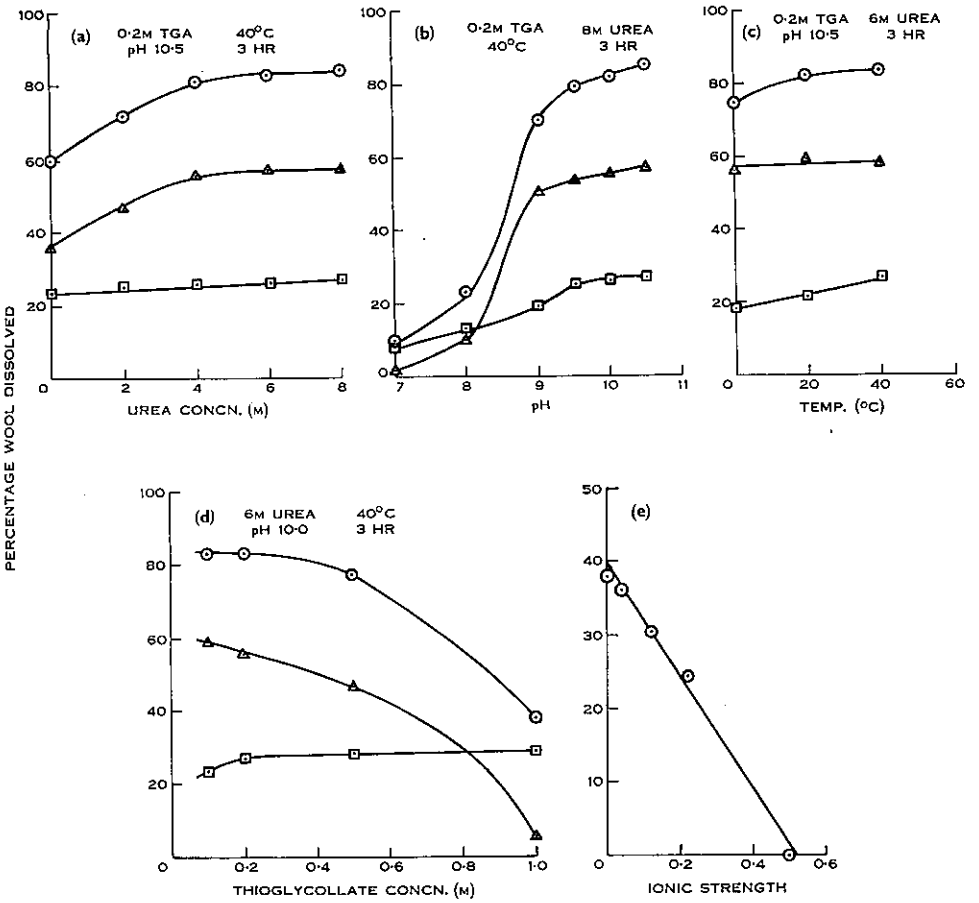

Fig. 2.—(*a*)–(*d*) Effect of (*a*) urea concentration, (*b*) pH, (*c*) temperature, and (*d*) potassium thioglycollate concentration on the extraction of protein from Merino 64's wool, sample MW148 Other conditions of extraction are given on the corresponding parts of the figure. ⊙ Total. protein. ⊡ High-sulphur protein. △ Low-sulphur protein. TGA, potassium thioglycollate. (*e*) Effect of ionic strength of the plasmolytic medium on the extraction of protein from Merino 64's wool, sample MW148, after treatment with 0·8M potassium thioglycollate, pH 10·5, at 0°C.

(1962) has shown that at this ionic strength the extraction of the low-sulphur protein is not repressed as much as at higher ionic strengths. The total extraction of high-sulphur protein by 0·1M potassium thioglycollate is somewhat higher than was previously observed by Gillespie (1962). This difference may be due to the different wools employed or to the fact that in using two successive extractions the liquor : wool

ratio is effectively 60 : 1; this increase in reductant : wool ratio may cause more complete reduction of the wool and lead to higher total extraction.

TABLE 1

INFLUENCE OF THIOGLYCOLLATE CONCENTRATION ON THE INITIAL EXTRACTION OF PROTEIN FROM WOOL SAMPLE MW127 AT pH 10·5 AND 0°C AND ON THE SUBSEQUENT PLASMOLYSIS

Thioglycollate Concn. for Extracts I and II	% Extraction		% Extraction on Plasmolysis* (≡Extract III)	Estimated Total High-sulphur Protein (%)	Estimated Total Low-sulphur Protein (%)
	Extract I	Extract II			
0·1	7·8†	15·2‡	37·1§	19	41
0·5	12·9†	4·0†	49·1§	22	44
0·8	13·5†	3·5†	48·0§	22	43

* Plasmolytic medium was distilled water in all cases.

† Almost entirely high-sulphur protein (i.e. protein soluble at pH 4·4).

‡ 50% of this extract was low-sulphur protein (i.e. protein insoluble at pH 4·4).

§ 8–10% of these extracts was high-sulphur protein.

The recovered volume of filtrate after the first extraction is only about 80–85% of the volume of extractant originally applied to the wool, the remainder being

TABLE 2

EFFECT OF pH ON THE INITIAL EXTRACTION OF PROTEIN FROM WOOL SAMPLE MW148 BY TREATMENT WITH 0·8M POTASSIUM THIOGLYCOLLATE AT 0°C AND ON THE SUBSEQUENT PLASMOLYSIS

pH of Thioglycollate for Extracts I and II	% Extraction		% Extraction on Plasmolysis* (≡Extract III)	Estimated High-Sulphur Protein in Extract III (%)
	Extract I	Extract II		
9·5	4·9	3·5	3·1	1·6
10·0	10·9	2·5	13·3	4·5
10·5	16·4	3·4	36·1	3·6

* Plasmolytic medium was 0·02M potassium thioglycollate, pH 10·3, in all cases.

entrained in the swollen fibres. If it is assumed that the concentration of soluble protein in the entrained liquid is the same as that in the filtrate, then it can be shown

from the data that the second extraction (with 0·5 or 0·8M thioglycollate) appears to remove only this entrained protein, very little, if any, additional protein being solubilized.

(ii) *Effect of Variation in the pH of Potassium Thioglycollate.*—The pH of the thioglycollate solutions has a considerable influence at all three stages of extraction. The large increase in the extraction of low-sulphur protein over the pH range 10·0–10·5 has also been noted in previous studies at higher temperatures. The general effect of the plasmolytic procedure is that it allows the extraction temperature, for equal yield of low-sulphur protein, to be lowered from 50 to 0°C. However, in contrast to extractions in the presence of 8M urea where large amounts of low-sulphur protein can be extracted at pH values as low as 9, a pH of 10·5 is necessary for appreciable extraction of protein by the plasmolytic procedure.

TABLE 3

INFLUENCE OF IONIC STRENGTH OF THE PLASMOLYTIC MEDIUM ON THE EXTRACTION OF PROTEIN FROM WOOL SAMPLE MW 148 AFTER TREATMENT WITH 0·8M POTASSIUM THIOGLYCOLLATE (TGA), pH 10·3, AT 0°C

% Extraction		Plasmolytic Medium		% Extraction on Plasmolysis (≡Extract III)
Extract I	Extract II	Composition	Ionic Strength	
16·7	4·2	Distilled water	0	38·1
16·4	3·4	0·02M TGA, pH 10·3	0·04	36·1
17·3	3·4	0·02M TGA–0·08M KCl, pH 10·3	0·12	30·7
16·3	4·4	0·02M TGA–0·18M KCl, pH 10·3	0·22	24·2

The values referring to extraction with 0·8M potassium thioglycollate at pH 10·5 in Table 2 differ from the corresponding values in Table 1, although the extractions were done under the same conditions. This difference seems to be genuine and is attributed to a change in the type of wool used between the two experiments. Thus although the data in each of these two tables is internally consistent, comparisons cannot be made between them.

(iii) *Influence of Ionic Strength of the Plasmolytic Medium.*—In Table 3 the percentage of protein extracted in extracts I and II are replicates and so indicate the reproducibility of a particular set of extraction conditions. As the ionic strength of the plasmolytic medium increases from 0 to 0·22, the amount of protein extracted falls from 38 to 24%. This observation gives support to the idea that the protein is released by a plasmolytic action. The plot of percentage extraction (Fig. 2(*e*)) against the ionic strength of the plasmolytic medium is linear and indicates that at an ionic

strength of about 0·5 there would be no release of protein. Thus for maximum release of protein there should be a difference of 0·5 in ionic strength between the liquid inside and outside the fibre. The idea is supported by the data in Table 1 which show that there is virtually no difference in the extent of plasmolytic release of protein whether 0·5 or 0·8M potassium thioglycollate is used for the first two extractions.

(iv) *Effect of Variation in Volume of the Plasmolytic Medium.*—Changing the volume of the plasmolytic medium over the range 235–1020 ml did not significantly affect the percentage of extraction (Table 4). The final concentrations of thioglycollate, calculated from the carry over of about 20 ml of 0·8M potassium thioglycollate in

TABLE 4

EFFECT OF VOLUME OF THE PLASMOLYTIC MEDIUM ON THE EXTRACTION OF PROTEIN FROM WOOL SAMPLE MW148 AFTER TREATMENT WITH 0·8M POTASSIUM THIOGLYCOLLATE, pH 10·3, AT 0°C

% Extraction		Volume of Plasmolytic Medium* (ml)	% Extraction on Plasmolysis (≡Extract III)
Extract I	Extract II		
14·7	3·5	235	39·8
15·0	3·5	520	34·5
16·4	3·4	1020	36·1

* Distilled water in all cases.

the residue from extract II, are approximately 0·09, 0·05, and 0·035M. The data in Figure 2(*e*) show that if the ionic strength of the medium in which the wool is immersed is 0·09, there is a significant decrease in the percentage of extracted protein. Since there was no difference for the different volumes of plasmolytic medium (Table 4), it is likely that the initial difference in ionic strength between the inner and outer liquid and not the final ionic strength determines the extent of extraction. This observation gives further support to the idea of a plasmolytic release of protein.

(*c*) *Electrophoresis and Amino Acid Analysis of Extracted Proteins*

The electrophoretic patterns of the low-sulphur protein fractions extracted both by the plasmolytic and the urea–thioglycollate extraction procedures are shown in Figure 3, the mobilities of the components are given in Table 5, and the amino acid analyses in Table 6.

Electrophoretically these fractions always appear to consist of one major component although the mobilities vary somewhat from one preparation to another.

Extremes of this variability are exemplified in the plasmolytic preparations A and B (Fig. 3) which were both prepared from the same wool under similar conditions. When the mobilities of the main peaks of the low-sulphur proteins obtained by extraction with urea–thioglycollate at 40°C and by plasmolysis were compared with those of SCMKA1 and SCMKA2 (also shown in Fig. 3 and listed in Table 5) they were usually in the same range. However, the mobilities of these latter proteins also vary from one preparation to another (Woods 1959) so that, on the basis of electrophoresis, it is difficult to decide whether the low-sulphur fractions prepared by the urea–thioglycollate or plasmolytic procedures are related to the SCMKA1 or SCMKA2 fractions or, as is more likely, a mixture of both.

TABLE 5

ELECTROPHORETIC MOBILITIES OF LOW-SULPHUR PROTEIN PREPARATIONS FROM WOOL SAMPLE MW148

Electrophoresis was carried out in β-alanine–NaOH buffer of ionic strength 0·1 at pH 11. Mobilities are calculated from the rate of movement of the main peaks in the descending boundaries

Type of Preparation	$10^5 \times$Mobility ($cm^2\ volt^{-1}\ sec^{-1}$)	Figure Reference
Plasmolytic*		
Preparation A	−6·6	3(*a*)
Preparation B	−8·2	3(*b*)
Preparation B after heating at 50°C for 2 hr at pH 10·5	−6·6	3(*c*)
Urea–thioglycollate extract†	−7·4	3(*d*)
SCMKA2‡	−6·3	3(*e*)
SCMKA1 + "minor" components§	−4·2, −6·8, −8·2, −10·2	3(*f*)

* Prepared by two consecutive preliminary extractions with 0·8M potassium thioglycollate, pH 10·5, at 0°C for 24 hr each, followed by plasmolytic release of low-sulphur protein in distilled water.

† Prepared by extraction at pH 10·2 for 2 hr at 40°C with 0·2M potassium thioglycollate in presence of 6M urea.

‡ See Gillespie (1957) for details of preparation.

§ See Gillespie (1960) for details of preparation.

A possible cause of this variability in mobility may be found in the amino acid analyses of the plasmolysis preparation B and the urea–thioglycollate fraction, whose mobilities differed most from those of SCMKA1 and SCMKA2. It is clear that the contents of some residues in these fractions cannot be reconciled with the analysis of SCMKA1, SCMKA2, or any possible mixture of them. Thus the contents of aspartic and glutamic acids, alanine, and isoleucine are lower and those of glycine and *S*-carboxymethyl cysteine (SCMC) are higher than the corresponding values in either SCMKA1 or SCMKA2. On the other hand, the plasmolytic preparation A is similar in amino acid analysis to SCMKA1 and SCMKA2 and is also similar in electrophoretic mobility.

TABLE 6

AMINO ACID ANALYSES OF LOW-SULPHUR PROTEIN FRACTIONS OBTAINED FROM WOOL

Values are uncorrected for destruction on hydrolysis

Amino Acid	Amino Acid Nitrogen (as% total N)						
	Plasmolytic Prep. A	Plasmolytic Prep. B	Urea–Thioglycollate Extract	SCMKA1	SCMKA2	"Minor" Components*	α-Keratose†
Lysine	5·11	4·46	4·78	5·70	5·34	0·44	4·52
Histidine	1·46	1·19	1·13	1·30	1·23	0·99	1·49
Ammonia	9·49	8·92	7·80	8·77	8·97	6·91	7.24
Arginine	20·45	21·32	19·63	18·99	21·10	13·60	20·66
Aspartic acid	5·72	5·89	5·79	7·09	6·37	2·85	5·48
Threonine	3·04	3·42	3·27	3·02	3·29	3·40	3·05
Serine	5·48	6·61	6·23	5·03	5·89	8·34	6·26
Glutamic acid	9·61	9·79	9·62	14·41	10·76	2·52	9·16
Proline	2·56	2·71	2·70	1·73	2·33	4·39	—
Glycine	4·99	6·77	6·48	4·19	4·52	18·76	6·70
Alanine	4·38	4·54	4·21	4·91	4·93	1·43	4·44
½ Cystine	Trace	Trace	Trace	0·67	Trace	2·08	—
Valine	4·14	4·06	3·84	4·13	4·32	2·19	3·38
Methionine	0·37	0·32	0·31	0·39	0·34	0	—
Isoleucine	2·31	2·31	2·33	2·57	2·53	0·88	2·02
Leucine	6·57	6·77	6·78	8·66	7·13	4·28	6·47
Tyrosine	2·43	3·42	3·40	2·23	2·26	8·89	3·17
Phenylalanine	1·95	2·16	2·20	1·67	1·78	3·73	2·29
SCMC‡	4·02	5·57	4·84	2·96	4·32	6·03	0
Cysteic acid	0·49	—	—	—	—	0·22	5·3
Total	94·57	100·23	95·34	98·42	97·41	91·93	—
SCMC+½ cystine	4·02	4·06	3·84	3·63	4·32	8·11	—

* Obtained by partial precipitation of SCMKA1 with acetone. SCMKA1 content <30%.

† Values taken from Gillespie *et al.* (1960).

‡ *S*-carboxymethyl cysteine.

Further information from these departures in composition from SCMKA1 and SCMKA2 may be gained if we examine in more detail the mixture of proteins

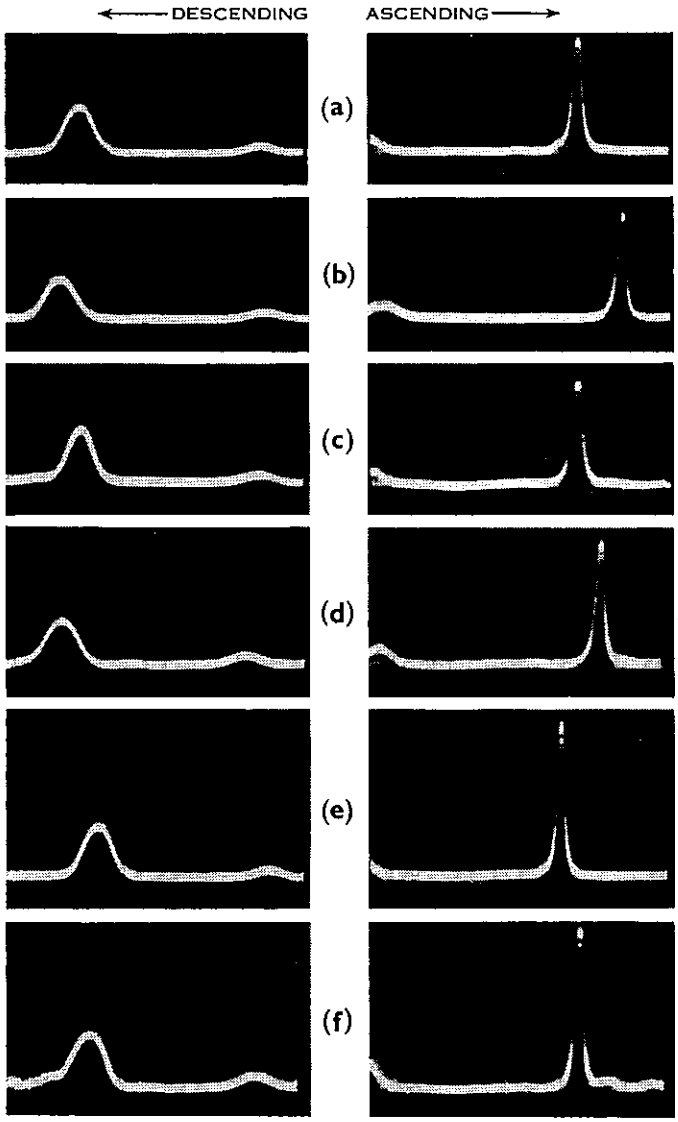

Fig. 3.—(*a*)–(*f*) Electrophoretic patterns of low-sulphur fractions from Merino 64's wool, sample MW148, run at pH 11·0 in β-alanine–NaOH buffer of ionic strength 0·1 for 180 min at a voltage gradient of 5·7 V/cm. Protein concentrations approximately 1%. Mobilities were calculated from the rate of movement of peaks in descending boundaries and are recorded, together with the methods of preparation of the fractions, in Table 5: (*a*) plasmolytic preparation A; (*b*) plasmolytic preparation B; (*c*) plasmolytic preparation B after heating; (*d*) urea–thioglycollate extract; (*e*) SCMKA2; (*f*) SCMKA1, together with "minor" components.

from which SCMKA1 was isolated (Gillespie 1960). Electrophoretically this mixture showed, in addition to the major component SCMKA1, three "minor" components

(Fig. 3(*f*)). By partial precipitation of SCMKA1 from the mixture with acetone a solution enriched in these minor components was prepared (SCMKA1<30%) and its amino acid analysis is listed in Table 6. The analysis of this fraction differs widely from the other low-sulphur fractions, especially in the residues in which there are large differences between the plasmolytic preparation B and the urea–thioglycollate fraction on the one hand and SCMKA1 and SCMKA2 on the other. The observation suggests that the former two preparations are composed of an admixture of the latter two with varying amounts of the minor components.

Also listed in Table 6 is the amino acid analysis of α-keratose, a low-sulphur fraction prepared from wool by oxidation with performic acid (O'Donnell and Thompson 1959; Gillespie *et al.* 1960). In composition it differs from SCMKA1 and SCMKA2 in much the same way as the plasmolysis preparation B and the urea–thioglycollate fraction. O'Donnell and Thompson (1962) have recently shown that, by repeated precipitation of performic acid-oxidized α-keratose with trichloroacetic acid, protein can be removed and then the purified α-keratose approaches SCMKA1 and SCMKA2 in composition. It is thus likely that the protein removed by precipitation with trichloroacetic acid is similar in nature to the minor components.*

It is not clear why the minor components are not resolvable by electrophoresis of the plasmolytic preparation B and the urea–thioglycollate fraction and also of performic acid-oxidized α-keratose. When the plasmolytic preparation B was heated at 50°C at pH 10·5 for 2 hr (i.e. under the conditions which the protein in Figure 3(*c*) was extracted) the mobility of the major peak decreased to within the normal range for SCMKA1 and SCMKA2 (Table 5) and yet only traces of components of higher mobility were resolved (Fig. 3(*c*)).

(*d*) *General Remarks on the Extraction of Proteins from Wool*

It has been demonstrated in this paper that large amounts of protein can be extracted from wool at low temperatures in the presence of urea at pH 9 and significant amounts even at pH 8. It is interesting to consider these results in the light of recent papers by Corfield (1962) and Blackburn (1962).

Corfield (1962) studied the properties of keratoses prepared by oxidizing wool with peracetic acid, using essentially the methods described by Alexander and Smith (1956). Corfield found that the low-sulphur α-keratose which was initially non-dialysable became dialysable through "Cellophane" after chromatography on hydroxyapatite even at neutral pH. On the basis of these results Corfield contends that peptide-bond splitting occurs even under the pH conditions used in his chromatography and fractionation procedures and thus under the conditions in which proteins have normally been extracted from wool. On the basis of these conclusions Corfield suggested that peptide-bond breakage is a necessary preliminary to wool solubilization. These ideas support the conclusions of Blackburn (1962) in his study on the extraction of proteins from wool with their cystine linkages still intact. This extraction was achieved by mild hydrolysis of wool with dilute acid solutions followed by solubilization in dilute alkali. The proteins extracted were fractionated into two groups of markedly

* Further studies on the fractionation, purification, and composition of the minor components are now in progress.

differing sulphur contents and with amino acid compositions which were similar in many respects to the corresponding groups of high- and low-sulphur proteins extracted from wool after breakage of cystine cross-links. Blackburn suggested that, in the wool fibre, there are regions of high- and low-sulphur content linked together end-to-end in the same polypeptide chain; when protein is solubilized by rupture of cystine cross-links, he contends that peptide-bond breakage also occurs at the peptide linkage between the high- and low-sulphur regions.

An explanation of many of the effects observed by Corfield may lie in the fact that his keratoses were prepared by oxidation of wool with peracetic acid according to the method of Alexander and Smith (1956). It is likely that this treatment produces *peptide-bond breakage* since the resultant γ-keratose is largely dialysable through "Cellophane" whereas the high-sulphur fraction prepared by reduction methods or by oxidation with performic rather than peracetic acid (Thompson and O'Donnell 1959) is not significantly dialysable. Furthermore the experience in this Laboratory has been that neither α-keratose (prepared by performic acid oxidation) nor the low-sulphur protein (prepared by reduction and alkylation) is dialysable either before or after chromatography.

A possible explanation of the dialysability of Corfield's α-keratose after chromatography may be that, following peptide-bond breakage during oxidation with peracetic acid, the liberated peptides remain attached to the parent molecule by non-covalent bonds. These may be subsequently broken down under the conditions of chromatography in urea or of precipitation with ethanol. Similar non-covalent linkages involving polypeptide chains have been reported for ribonuclease (Richards 1958) after the breakage of a single peptide bond by the enzyme subtilisin and for γ-globulin after reduction of the fragments produced by enzyme digestion (Grossberg, Stelos, and Pressman 1962). This seems a more likely explanation of Corfield's results than peptide-bond breakage at the mild pH conditions used during chromatography and fractionation. It should be noted that Woods (1952) reported the extraction of significant amounts of protein from wool with urea–bisulphite at pH 5·7 and Maclaren (personal communication) has extracted at pH 7 high- and low-sulphur fractions from wool which has been both reduced and alkylated also at pH 7.

Corfield has further suggested that high- and low-sulphur fractions have no separate existence in the fibre and cannot be identified with any histological component in wool. The following comments are pertinent and appear to have been overlooked by both Blackburn and Corfield:

(1) Rogers (1959*a*) in an electron-microscopic study has shown that the high-sulphur protein extracted from oxidized wool originates in the matrix of the fibre.

(2) Rogers (1959*b*) has been able to isolate proteins in the –SH form from the wool root simply by extraction with urea alone (not involving the use of oxidants or reductants) at pH 7. After alkylation a low-sulphur protein can be separated by acid-precipitation which is very similar in amino acid composition to the low-sulphur protein SCMKA2 extracted from whole wool. This appears to have a definite histological significance and does not arise simply from a splitting of peptide bonds.

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EXTRACTION OF REDUCED PROTEINS FROM WOOL

Fig. 1.—Wool fibres after treatment in 0·8M potassium thioglycollate at 0°C, pH 10·3, for 18 hr. × 200.
Fig. 2.—The same fibres after immersion in distilled water (plasmolysis). × 200.