THE ISOLATION AND PROPERTIES OF SOME SOLUBLE PROTEINS FROM WOOL

IX. THE PROTEINS IN WOOLS OF INCREASED SULPHUR CONTENT

By J. M. Gillespie,* P. J. Reis,† and the late P. G. Schinckel†

Summary

The proteins in wools of increased sulphur content, grown during abomasal infusions of casein and sulphur-containing amino acids, have been compared with those from control wools from the same sheep. It has been found that casein, methionine, or cysteine administered directly into the abomasum of the sheep, besides increasing the rate of growth of wool, greatly altered the composition of the wool proteins. The proportion of the high-sulphur proteins in wool was increased and within the group of high-sulphur proteins there was increased formation of the components richer in sulphur. No change other than the expected decrease in relative amount can be detected with the low-sulphur proteins. In electron micrographs of the test wool increased amounts of osmiophilic material can be seen in the para segment of the fibre.

I. Introduction

It is now generally accepted that the sulphur content of wool can vary over a wide range (for a review of the relevant literature see Reis and Schinckel 1963). Our understanding of the causes of this variability has been put on a much firmer basis since the findings by Reis and Schinckel (1963, 1964) that they could alter the sulphur content of wool in a controlled way by infusing sulphur-containing amino acids (*S*-amino acids) or casein directly into the abomasum of sheep. With both Merino and English Leicester–Merino crossbred sheep a large increase in wool growth rate occurred during the infusions and the sulphur content of the wool was increased from about 3% to as much as 4%.

The purpose of the work described in the present paper is to compare the composition of the proteins in control wools and in wools of increased sulphur content from the same animal, in order to determine whether the changes are restricted to certain protein components. These investigations are similar to those reported on the proteins in steely wool from copper-deficient sheep in Part VIII of this series (Gillespie 1964).

II. Materials and Methods

Generally the procedures used for preparing the wool and wool proteins have been described by Gillespie (1964) and will only be briefly mentioned here.

* Division of Protein Chemistry, CSIRO Wool Research Laboratories, Parkville, Vic.

† Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

(*a*) *Origin and Preparation of Wool*

The sets of control and sulphur-enriched wools, each representing 3 weeks of growth, from each sheep were obtained from the same tattooed area on the animal. During the control period the sheep were fed a moderate intake of a diet consisting of equal parts of lucerne chaff and wheaten chaff. Then sulphur-enriched wools were obtained by supplementing the diet by infusing an *S*-amino acid, a protein, or a mixture of both directly into the abomasum (for full experimental details of this procedure see Reis and Schinckel 1963, 1964). Before extraction, each sample of wool was washed thoroughly in petroleum ether, in ethanol, and in water, and finally equilibrated in a conditioned room (68°F, 60% R.H.).

(*b*) *Estimation and Isolation of Proteins*

The content of extractable high-sulphur protein (as the *S*-carboxymethyl kerateine) in each sample of wool was estimated by a urea–thioglycollate procedure (Harrap and Gillespie 1963; Gillespie 1964).

Proteins for analysis were prepared from these extracts by alkylation with iodoacetate, dialysis, and fractionation by precipitation of low-sulphur fractions with zinc acetate at pH 6·0, leaving the high-sulphur group of proteins in the supernatant.

(*c*) *Moving Boundary Electrophoresis*

The proportions and mobilities of high-sulphur protein fractions were estimated in electrophoretic runs at pH 4·5 in a buffer containing acetic acid–sodium acetate of ionic strength 0·1. The characteristics of the low-sulphur proteins were measured in a β-alanine–NaOH buffer of ionic strength 0·1 at pH 11·0.

(*d*) *Amino Acid Analysis*

The proteins were hydrolysed with 6N HCl for 24 hr under reflux and the content of amino acids estimated using a Beckman–Spinco automatic amino acid analyser. When the analytical values for individual amino acids of proteins from control and sulphur-enriched wools differ by 10% or more then it is assumed that the proteins differ significantly in this respect. Estimation of *S*-carboxymethylcysteine (SCMC) was somewhat unsatisfactory because of variable destruction during hydrolysis. The sum of residual SCMC and $\frac{1}{2}$ cystine has been taken as an approximation to the SCMC content of the intact protein (Gillespie 1963*a*). Thompson and O'Donnell (unpublished data) have recently confirmed this relation and have shown that the destruction of SCMC can be eliminated by hydrolysing *in vacuo* as suggested by Crestfield, Moore, and Stein (1963).

(*e*) *Electron Microscopy*

Sets of control and sulphur-enriched wools were reduced with 0·4M sodium thioglycollate at pH 5·5, treated with 2% osmium tetroxide (unbuffered) for 3–5 days, embedded in Araldite, and sectioned for examination under the electron microscope by the procedure of Rogers (1959*a*).

(*f*) *Starch-gel Electrophoresis*

Starch-gel electrophoresis was performed as described previously (Gillespie 1964; Thompson and O'Donnell 1964).

III. Results

(*a*) *Proportion of High- and Low-sulphur Proteins in Control and Sulphur-enriched Wools*

The control wools all contained between 21 and 24% of extractable high-sulphur protein calculated on the weight of original wool (Table 1). In every case supplementing the diet by abomasal infusion of cysteine, methionine, or casein very significantly increased the proportion of high-sulphur protein in the wool. The largest increase was found with a supplement of casein plus cysteine, in which there was an increase in proportion of high-sulphur protein amounting to 50%.

Table 1

Yield of total protein and of high-sulphur protein from control and sulphur-enriched wools

Sheep	Wool Sample No.	Abomasal Supplement	Control Wool		Sulphur-enriched Wool	
			Total Protein (%)	High-sulphur Protein (%)	Total Protein (%)	High-sulphur Protein (%)
SC8*	2–3	Cysteine	85	21	77	27
1390*	44–45	Cysteine	85	22	76	29
1392*	48–49	Cysteine	84	21	77	27
1391*	46–47	Methionine	88	22	74	31
SC8*	56–57	Casein	80	22	83	29
E122*	54–55	Casein	80	24	76	26
1391†	66–67	Gelatin	89	22	89	25
	66–68	Gelatin + cysteine	89	22	89	30
1393†	72–73	Gelatin	90	23	90	24
	72–74	Gelatin + cysteine	90	23	90	31
1390†	63–64	Casein	85	22	91	31
	63–65	Casein + cysteine	85	22	91	34
1392†	69–70	Casein	92	23	91	28
	69–71	Casein + cysteine	92	23	89	31

* For experimental details, see Reis and Schinckel (1963).

† For experimental details, see Reis and Schinckel (1964).

(*b*) *Sulphur Content of High-sulphur Proteins from Control and Sulphur-enriched Wools*

There is not only more high-sulphur protein in the sulphur-enriched wool but these proteins are also richer in sulphur than are those from the control wools (Table 2). By combining the data in Table 1 with that from Reis and Schinckel (1963, 1964) the contribution which the high-sulphur proteins make towards the increase in the sulphur content of the wool can be computed. Column 6 of Table 2 gives the increase in sulphur found by Reis and Schinckel in the enriched wools and column 7 the increase in sulphur

expected from the increase in high-sulphur protein and its increase in sulphur content. Considering the inaccuracies present in the estimation of the high-sulphur protein content of wool, the values of columns 6 and 7 generally agree quite well. As the means of columns 6 and 7 also are in agreement it is probable that differences between individual pairs of values are due to random errors. The conclusion to be drawn is that, within experimental error, all the increase in sulphur content in the enriched wools as compared with the controls is due to changes in the high-sulphur proteins.

TABLE 2

SULPHUR CONTENTS OF HIGH-SULPHUR PROTEINS FROM CONTROL AND SULPHUR-ENRICHED WOOLS

Sheep No.	Wool Sample No.	Abomasal Supplement	Sulphur Content of of Wool (%)	Sulphur Content of High-sulphur Protein (%)	Increase in Sulphur Content of Wool	
					%*	%†
SC8	2	None	2·90‡	4·8	0·78	0·56
	3	Cysteine	3·68‡	5·8		
1390	44	None	3·10‡	4·8	0·74	0·63
	45	Cysteine	3·84‡	5·8		
1391	46	None	3·07‡	5·0	0·73	0·70
	47	Methionine	3·80‡	5·8		
1391	66	None	3·06§	4·9‖	0·82	0·87
	68	Gelatin+cysteine	3·88§	6·5‖		
1393	72	None	3·38§	5·2‖	0·57	0·66
	74	Gelatin+cysteine	3·95§	6·0‖		
1390	63	None	3·04§	4·9‖	0·94	0·86
	65	Casein+cysteine	3·98§	5·7‖		
1392	69	None	3·15§	5·5‖	0·58	0·68
	71	Casein+cysteine	3·73§	6·2‖		
Mean					0·74	0·71

* Computed from differences in column 4.

† Computed from the values for amount of high-sulphur protein given in Table 1 and the sulphur content of the protein given in column 5 of this table.

‡ Values from Reis and Schinckel (1963).

§ Values from Reis and Schinckel (1964).

‖ Sulphur content computed from amino acid analysis.

(c) *Variations in the Amino Acid Composition of High-sulphur Proteins*

Amino acid analyses of high-sulphur proteins from normal and sulphur-enriched wools (Table 3) showed very significant differences in a few amino acids, and smaller changes in some others. The addition of a protein to the *S*-amino acid supplement resulted in larger differences in composition than did a supplement of amino acid alone. Irrespective of the *S*-amino acid supplement given (cysteine, methionine, casein, or combinations of these), no methionine was found in these proteins. However, in all cases there was an increase in SCMC (mean 15%), and highly significant decreases in

TABLE 3

AMINO ACID ANALYSES OF HIGH-SULPHUR PROTEINS FROM CONTROL AND SULPHUR-ENRICHED WOOLS

Results are expressed as amino acid nitrogen as percentage of total nitrogen

Amino Acid	Sheep 1390			Sheep 1391			Sheep 1392		Sheep 1393	
	Control*	Cysteine	Casein + Cysteine	Control*	Methionine	Gelatin + Cysteine	Control	Casein + Cysteine	Control	Gelatin + Cysteine
Sample No.:	44/63	45	65	46/66	47	68	69	71	72	74
Lysine	1·05	0·96	0·96	0·88	0·86	1·18	0·82	0·84	0·87	0·98
Histidine	1·58	1·66	1·66	1·52	1·62	1·77	1·52	1·51	1·51	1·71
Arginine	12·10	12·47	12·56	15·63	16·72	15·09	17·81	18·48	11·89	12·59
Aspartic acid	2·43	2·05	1·94	2·15	1·63	1·41	2·14	1·66	2·20	1·64
Threonine	7·77	8·36	8·02	7·22	7·30	7·07	7·12	7·23	7·54	7·64
Serine	9·39	9·81	9·79	8·87	9·01	8·49	9·26	9·29	8·48	8·51
Glutamic acid	6·01	6·48	6·26	5·45	5·53	5·31	5·52	5·66	5·18	5·29
Proline	9·42	10·15	9·96	8·74	8·79	8·72	9·08	9·50	9·51	9·40
Glycine	5·23	5·08	4·61	4·56	4·23	4·07	5·16	4·27	4·75	4·28
Alanine	2·52	2·36	2·03	2·41	2·06	1·96	2·31	1·91	2·16	1·95
½ Cystine	4·48	4·32	4·94	2·66	1·30	0·94	2·49	2·79	3·38	2·56
Valine	4·61	4·25	4·17	4·43	4·01	3·85	4·45	4·10	4·36	3·96
Isoleucine	2·39	2·15	2·22	2·28	2·06	1·99	2·31	2·18	2·33	2·10
Leucine	3·11	2·52	2·36	2·79	2·08	2·00	2·76	2·35	2·73	2·32
Tyrosine	0·59	0·65	0·42	1·26	1·30	1·30	1·00	0·99	1·05	0·99
Phenylalanine	1·54	1·09	0·96	1·27	1·08	0·94	1·48	1·22	1·35	1·08
SCMC	9·89	11·83	11·22	11·72	14·75	17·57	13·26	15·00	11·42	14·44
SCMC+½ cystine	14·37	16·15	16·16	14·38	16·05	18·51	15·75	17·79	14·80	17·00

* Values for the control wools from sheep 1390 were the mean analyses of wool samples 44 and 63 and from sheep 1391 of wool samples 46 and 66.

aspartic acid, leucine, and phenylalanine, which averaged respectively 23, 21, and 25%. Valine and isoleucine decreased by 7 and 10% respectively. Glycine and alanine

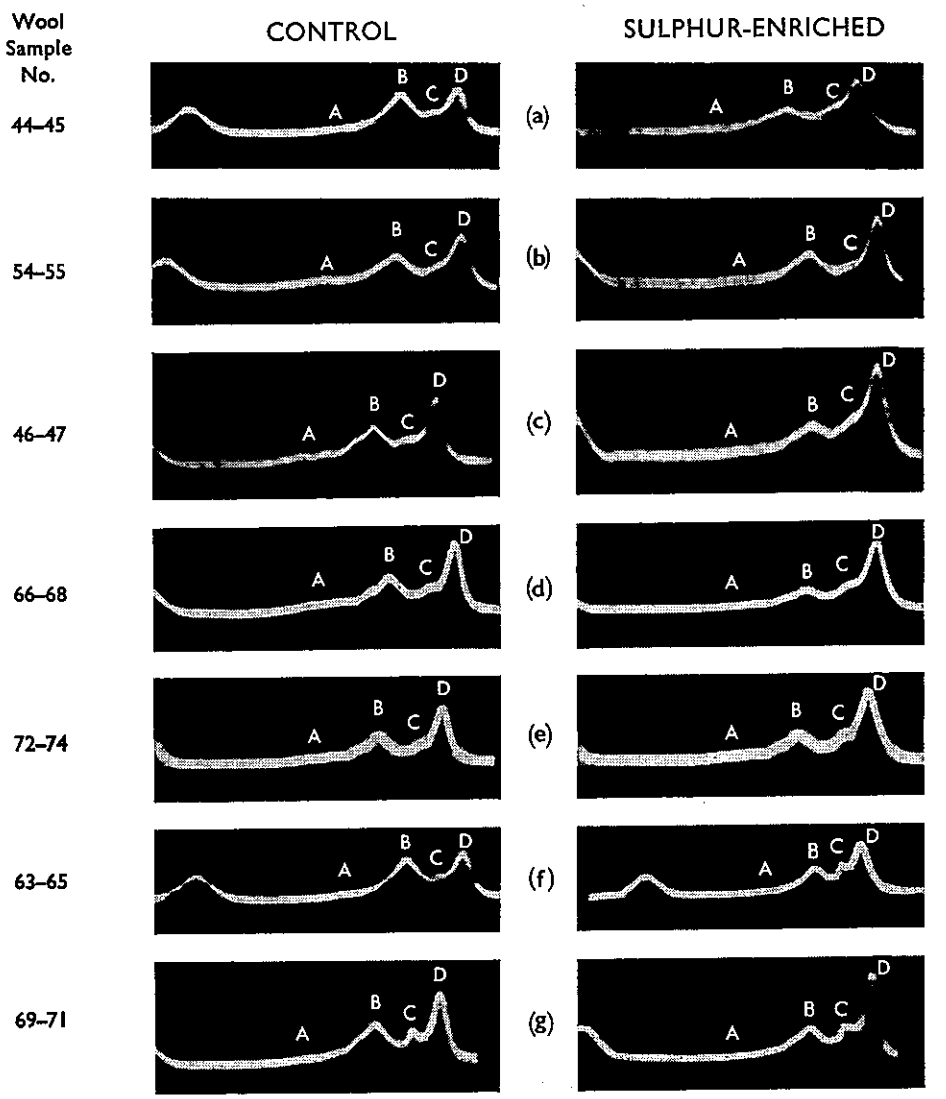


Fig. 1.—Moving boundary electrophoresis of high-sulphur proteins from control and sulphur-enriched wools run in acetic acid–sodium acetate buffer of ionic strength 0·1 at pH 4·5, with protein concentrations between 1·0 and 1·2%. The supplements infused into the abomasum were: (*a*) cysteine; (*b*) casein; (*c*) methionine; (*d*), (*e*) gelatin+cysteine; (*f*), (*g*) casein+cysteine.

also decreased but the amount depended on the type of abomasal supplement, whether it was amino acid alone or amino acid and protein. Glycine decreased on the average 5 and 14% and alanine 10 and 18% respectively for these two situations.

There were no obvious changes in serine, threonine, glutamic acid, and proline; and only slight changes in the basic amino acids, although there was a trend towards an increase in histidine and arginine. No trends were observable with tyrosine although the results for this amino acid and for arginine were very variable.

Analyses (not reported here) of high-sulphur proteins isolated from control wools harvested from the same sheep at different times showed no significant differences between them. However, there were major differences between control high-sulphur proteins from different sheep (Table 3).

TABLE 4

COMPARISON OF MOBILITIES OF RESOLVABLE COMPONENTS IN HIGH-SULPHUR FRACTIONS FROM CONTROL AND SULPHUR-ENRICHED WOOLS

Electrophoresis runs were made in acetic acid–sodium acetate buffer of ionic strength 0·1 at pH 4·5 and the calculations (to nearest 0·1 unit) of mobility were made from the descending boundaries. Protein concentration 1·0–1·2%

Sheep No.	Wool Sample No.	Abomasal Supplement	$10^5 \times$ Mobility ($cm^2 sec^{-1} volt^{-1}$) of Component in:			
			Peak A*	Peak B*	Peak C*	Peak D*
E122	54	None	3·1	4·6	†	6·6
	55	Casein	3·1	4·5	†	6·5
1391	46	None	3·3	4·9	†	6·8
	47	Methionine	3·7	4·9	5·9	6·7
1391	66	None	3·6	4·8	5·4	6·7
	68	Gelatin+cysteine	3·5	4·9	5·5	6·6
1393	72	None	3·6	4·7	†	6·7
	74	Gelatin+cysteine	3·5	4·6	†	6·6
1390	63	None	3·2	5·2	5·9	6·7
	65	Casein+cysteine	3·3	5·3	6·0	6·6
1392	69	None	3·6	4·8	†	6·8
	71	Casein+cysteine	3·5	4·7	5·9	6·7

* Corresponds to lettering of peaks of electrophoretic patterns in Figure 1.

† These peaks were not resolved in the descending boundary.

(d) *Moving Boundary Electrophoresis of High-sulphur Proteins from Control and Sulphur-enriched Wools*

Moving boundary electrophoresis of high-sulphur proteins from control and sulphur-enriched wools (Fig. 1) shows that qualitatively the proteins are identical, for no new peaks appear and the mobilities of the various components are, within experimental error, identical between sets of proteins (Table 4). There are, however, big changes in the relative proportions of the various components. From tracings of ascending patterns of each preparation estimates were made of the proportions of the protein under each peak (Table 5). Compared with the control, the sulphur-enriched protein contains more of the fast-moving peaks C and D and relatively less of the slower peaks A and B. As mobility increases proportionately with sulphur content (Gillespie 1963*b*), it is apparent that high-sulphur proteins from the sulphur-increased wools contain relatively more of the sulphur-rich components than do the control wools.

When the data from Tables 1 and 5 are combined, the percentage in wool of each of the four components (A, B, C, and D) can be calculated (Table 6). It is clear that

TABLE 5

COMPARISON OF THE PERCENTAGES OF RESOLVABLE COMPONENTS IN HIGH-SULPHUR FRACTIONS FROM CONTROL AND SULPHUR-ENRICHED WOOLS

Calculated from ascending electrophoretic patterns in runs in sodium acetate–acetic acid buffer of ionic strength 0·1 at pH 4·5

Sheep No.	Wool Sample No.	Abomasal Supplement	Percentage of Protein in			
			Peak A*	Peak B*	Peak C*	Peak D*
1390	44	None	12	43	13	32
	45	Cysteine	10	31	19	40
E122	54	None	17	35	15	33
	55	Casein	6	33	17	45
1391	46	None	12	37	17	34
	47	Methionine	3	26	21	50
1391	66	None	12	36	16	36
	68	Gelatin + cysteine	8	25	20	47
1393	72	None	19	27	12	42
	74	Gelatin + cysteine	11	20	16	53
1390	63	None	10	44	14	33
	65	Casein + cysteine	7	31	19	43
1392	69	None	9	36	21	34
	71	Casein + cysteine	7	29	24	40

* Corresponds to the lettering of peaks in Figure 1.

TABLE 6

ESTIMATED WEIGHT (G/100 G WOOL) OF THE MAJOR HIGH-SULPHUR PROTEIN COMPONENTS IN CONTROL AND SULPHUR-ENRICHED WOOLS

Calculated from the data of Tables 1 and 5

Sheep No.	Wool Sample No.	Abomasal Supplement	Peak A	Peak B	Peak C	Peak D
1390	44	None	2·6	9·5	2·9	7·0
	45	Cysteine	2·9	9·0	5·5	12
1391	46	None	2·7	8·1	3·7	7·5
	47	Methionine	0·9	8·1	6·5	16
1391	66	None	2·6	7·9	3·5	7·9
	68	Gelatin + cysteine	2·4	7·5	6·0	14
1393	72	None	4·3	6·2	2·8	9·7
	74	Gelatin + cysteine	3·4	6·2	5·0	16
1390	63	None	2·2	9·7	3·1	7·3
	65	Casein + cysteine	2·4	10	6·5	15
1392	69	None	2·1	8·3	4·8	7·8
	71	Casein + cysteine	2·2	9·0	7·4	12

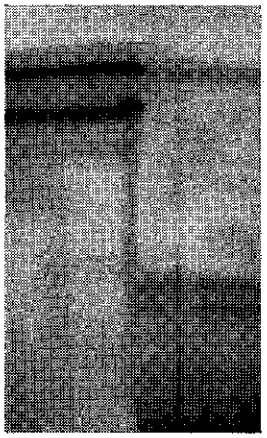
the protein in peak B stays constant in amount, whilst those in peaks C and D greatly increase in amount and usually by about the same percentage. Therefore the increase

in the high-sulphur protein content of sulphur-enriched wools (Table 1) is entirely due to the increased synthesis of protein with mobilities in the ranges of peaks C and D.

There does not seem to be any uniformity in the fate of the protein in the several minor peaks collectively referred to as peak A. This may be more a reflection of the difficulties in estimating the proportions of these materials than of any change in their proportions. It is likely that as with peak B these materials do not change greatly in amount.

(*e*) *Starch-gel Electrophoresis*

The high-sulphur proteins from pairs of control and sulphur-enriched wools have been compared by starch-gel electrophoresis (Fig. 2). No difference can be seen either in intensity or number of bands and therefore judged by this method the proteins are identical. Apparently the method is not sensitive enough to detect the differences in relative concentrations of proteins observed by the moving boundary method. It appears therefore that in the shift in synthesis of the high-sulphur proteins in sulphur-enrichment the regular proteins are produced but just in different amounts.



(*a*) (*b*) (*c*) (*d*)

Fig. 2.—Starch-gel electrophoresis of proteins from control and sulphur-enriched wools run at pH 8·6: (*a*) and (*b*) high-sulphur proteins from control and sulphur-enriched wools, respectively; (*c*) and (*d*) low-sulphur proteins from control and sulphur-enriched wools, respectively.

(*f*) *Electron-microscopical Examination of Fibres*

Electron micrographs of cross-sections of control and sulphur-enriched wools showing the ultrastructure of the para segment can be seen in Plate 1. In the sulphur-enriched wool there is a greater proportion of dense-staining matrix and the inter-microfibrillar distance is increased. These changes can be seen only in the para segment of the fibre.

(*g*) *Amino Acid Analysis of Low-sulphur Proteins from Control and Sulphur-enriched Wools*

The analytical values for the amino acid content of hydrolysates of low-sulphur proteins from control and sulphur-enriched wools (Table 7) suggest that there are no significant differences between them that cannot be explained by the presence of imperfectly separated minor protein contaminants. Except for histidine, glycine,

tyrosine, and phenylalanine in sample No. 1393 all other differences are within the generally accepted analytical error of ±3–4%.

(*h*) *Electrophoresis of Low-sulphur Proteins*

Moving boundary electrophoresis at pH 11·0 of low-sulphur proteins from control and sulphur-enriched wools showed in each case single peaks, the mobilities of which did not differ significantly.

A comparison of these proteins by starch-gel electrophoresis showed that the major bands also had identical mobilities (Fig. 2).

TABLE 7

AMINO ACID ANALYSIS OF LOW-SULPHUR PROTEINS ISOLATED FROM CONTROL AND SULPHUR-ENRICHED WOOLS

The analytical values are expressed as amino acid nitrogen as percentage of total nitrogen in the hydrolysate

Amino Acid	Sheep 1391			Sheep 1393	
	Control*	Methionine	Gelatin + Cysteine	Control	Gelatin + Cysteine
Sample No.:	46/66	47	68	72	74
Lysine	4·65	4·71	4·66	4·86	4·98
Histidine	1·39	1·41	1·44	1·45	1·32
Arginine	19·72	20·11	20·02	18·60	19·00
Aspartic acid	6·05	5·97	5·56	5·90	5·83
Threonine	3·37	3·42	3·30	3·24	3·14
Serine	6·48	6·63	6·35	6·32	5·98
Glutamic acid	9·77	10·22	9·42	10·10	9·89
Proline	2·53	2·57	2·66	2·29	2·39
Glycine	6·22	6·38	6·70	6·96	6·02
Alanine	4·46	4·28	4·14	4·35	4·37
½ Cystine†	0·43	0·50	0·31	0·46	0·26
Valine	4·09	3·90	3·98	4·08	3·98
Methionine	0·31	0·29	0·28	0·31	0·29
Isoleucine	2·37	2·34	2·26	2·40	2·35
Leucine	6·83	6·63	6·53	6·81	6·77
Tyrosine	2·54	2·59	2·84	1·97	2·22
Phenylalanine	2·09	1·86	2·16	2·16	2·01
SCMC	4·84	4·49	5·18	4·47	4·41
SCMC + ½ cystine	5·28	4·99	5·49	4·93	4·67

* The mean of analyses of protein from wool samples 46 and 66.

† The intact protein contained no cystine.

IV. DISCUSSION

The increase in the sulphur content of wool caused by abomasal administration of *S*-amino acids to sheep has been shown to be due, within experimental error, to changes within the high-sulphur group of proteins. With present techniques no

detectable changes could be found in the low-sulphur proteins. Thus it seems likely that although the rate of production of the low-sulphur proteins can be varied, increased by supplements of *S*-amino acids, and decreased by copper deficiency (Gillespie 1964), their composition is fixed.

The two observable changes in the high-sulphur proteins, namely an increase in relative amount in wool and an increase in sulphur content, are both caused by an increased synthesis of proteins whose electrophoretic mobilities lie within the limits of peaks C and D, i.e. the protein components richest in sulphur. The proteins in peaks A and B probably do not change in amount.

Thus for the first time, variations in the amount and composition of wool proteins have been observed as a result of the controlled treatment of sheep. If, as seems likely, the properties of a wool fibre are determined by the proteins of which it is composed, then the way lies open to the controlled production of fibres with altered properties, some of which may be desirable in a textile fibre. Structural differences caused by the increase in the relative proportions of the matrix may result in observational changes in torsional and elastic moduli (Feughelman 1959; Feughelman and Haly 1960; Feughelman, Haly, and Mason 1962) and in the relative wet and dry strengths of the fibre (Crewther and Dowling 1960). Values of plasticity (Ripa and Speakman 1951; LeRoux and Speakman 1957; Whitely and Speakman 1959), of dye uptake (Speakman 1955), and of maximum regain and lateral swelling under standard conditions (Alexander and Hudson 1954) should also reflect these changes.

Recently several workers (Blackburn 1962; Corfield 1962, 1963) have challenged the theory of wool structure that the microfibrils contain the low-sulphur proteins and the matrix the high-sulphur proteins (Birbeck and Mercer 1957; Rogers 1959*b*), proposing instead that no histological localization of proteins occurs. Corfield (1963) stated this as follows: "the unchanging chemical composition of wool can reasonably be accounted for in terms of a single keratin precursor in the developing cells of the follicle" and "the postulation of two structures in wool with such widely different compositions as the low- and high-sulphur fraction isolated from wool is untenable . . .". These ideas have been challenged already on a number of grounds (Harrap and Gillespie 1963; Rogers 1964).

The results in this and previous papers make it impossible to believe that wool has an unchanging composition, for besides the large changes in sulphur content found in wools by Reis and Schinckel (1963, 1964), variations in this element have been repeatedly observed in steely wool from copper-deficient sheep (Marston 1946; Burley and Horden 1959; Gillespie 1964), in wool sampled from individual sheep at different times over the course of the year (Ross 1961), and in pen-fed sheep with variation in diet (Reis, unpublished data). In addition, several findings in the present work are in even greater conflict with the hypothesis of unchanging composition. If there was a single keratin precursor containing both high- and low-sulphur moieties (Corfield 1963) then presumably some sort of stoichiometry between these moieties might be expected. It can be seen (Table 1) that the ratio of high-sulphur to low-sulphur protein in the extractable protein can vary at least from 0·32 to 0·62, an almost twofold variation which alone would make stoichiometry impossible.

Furthermore, the finding that an increase in high-sulphur protein is accompanied by an increase in matrix protein (i.e. osmiophilic material) provides some of the best support for the localization theory. At present therefore the evidence is still overwhelmingly in favour of the microfibril–matrix theory of wool structure.

Electrophoresis by both the moving boundary and starch-gel technique indicates that the same high-sulphur components are present in both control and sulphur-enriched wools and the evidence thus favours the concept of the increased synthesis of normal proteins. However, this is not entirely compatible with the evidence from amino acid analysis. For example, it is unlikely that there is an increased synthesis of SCMKB2 [a major sulphur-rich fraction devoid of lysine and histidine (Gillespie 1963*a*)] for there is no decrease in the amount of lysine and histidine in the sulphur-enriched high-sulphur proteins; if anything, there is a slight increase in these amino acid residues. Furthermore, the large decreases in some amino acids—for example, aspartic acid, phenylalanine, and leucine—are difficult to explain. However, until preparations of peak C and peak D proteins from each pair of control and sulphur-increased wools are separated and analysed, no certain answer to this problem can be given. Nevertheless, the implications are quite important, for the synthesis of at least part of the high-sulphur protein mixture must be controlled by the availability of *S*-amino acids.

Because the proteins in peaks A and B do not vary in amount with variation in supplementary feeding, it is reasonable to assume that they are invariable and are required in certain amount for a particular structure within the fibre. As the proteins in peaks C and D do vary in amount they must occur in the fibre in a structure in which a certain amount of latitude in composition is permitted, and this would appear to be the para region of the fibre. Since the new synthesis of high-sulphur protein in sulphur-enriched wools appears to be confined to the para region of the fibre and because this protein is located in the fast-moving electrophoretic peaks, it is now possible to suggest very tentatively that at least part of the proteins located in the slow-moving peaks A and B occur in the ortho region and the fast-moving sulphur-rich material in the para region.

An understanding of the control mechanism by which the production of high-sulphur proteins is regulated by the level of *S*-amino acids is dependent on an adequate knowledge of the mode and site of synthesis of these proteins. Although there is still a controversy concerning this mechanism (Mercer 1961; DeBersaques and Rothman 1962; Downes, Sharry, and Rogers 1963), nevertheless the consensus of opinion is that these proteins are synthesized or "completed" in the keratogenous zone. As a positive relation has been found between the molecular size of each high-sulphur component and its content of sulphur (Gillespie 1963*b*; Haylett *et al.* 1963) it is not inconceivable that the final act of synthesis is the stepwise addition of sulphur-rich peptides to low-molecular weight low-sulphur precursors. It then follows that, because of the limited time the developing fibre has in the keratogenous zone (Marston 1952), the rate of supply of sulphur-rich peptides could determine the extent to which the addition reaction proceeds.

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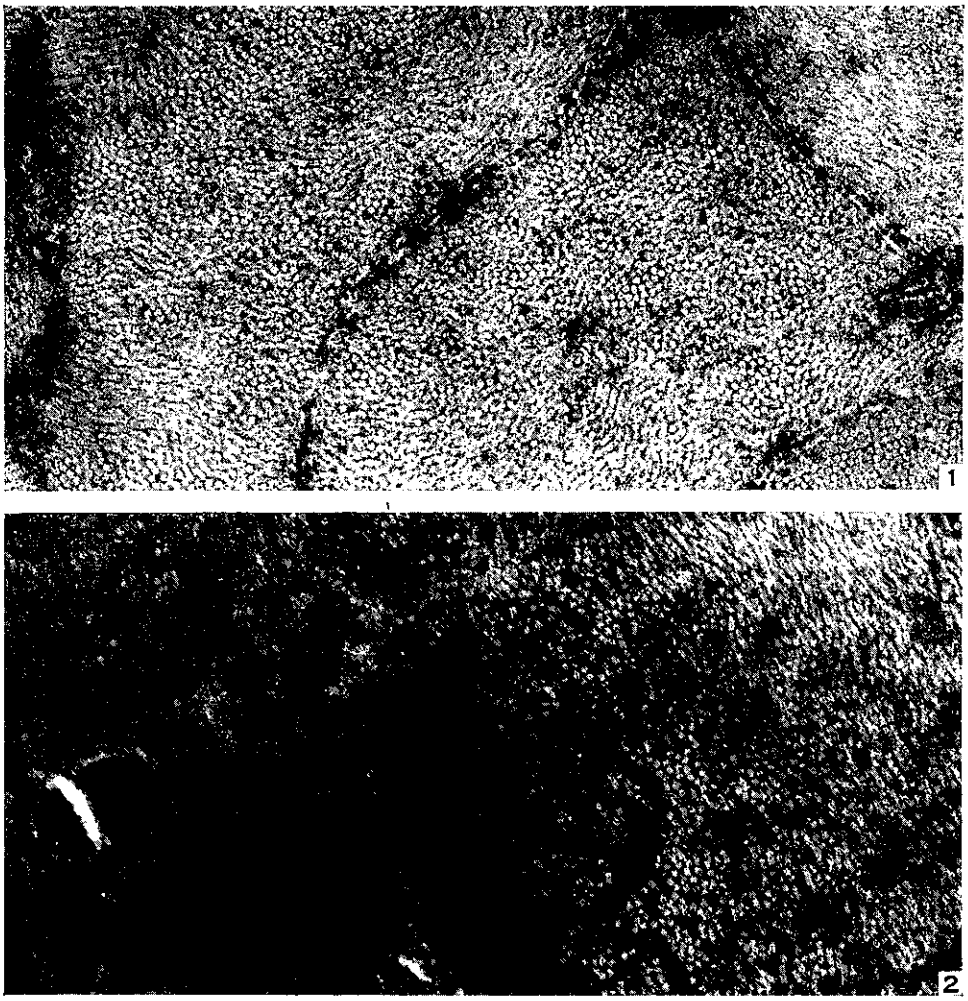
ISOLATION OF SOME SOLUBLE PROTEINS FROM WOOL. IX



Figs. 1 and 2.—Electron micrographs (× 33,000) of cross-sections of control wool (Fig. 1) and of sulphur-enriched wool from sheep No. 1391 with abomasal supplement of gelatin + cysteine (Fig. 2).