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A Further Study on the Dietary-Regulated Biosynthesis of High-Sulphur Wool Proteins

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When the diet of sheep is supplemented by the infusion of sulphur-containing amino acids or casein into the abomasum, the newly synthesized wool shows characteristic changes in its amino acid composition, with significant increases in cystine, proline and serine and decreases in aspartic acid and phenylalanine. This modification seems to be due entirely to an alteration in the overall composition of the high-sulphur proteins and to an increase in their proportion in the fibre. These variations are not the result of a change in the composition of individual proteins, but are due to alterations in their relative proportions and to the initiation of the synthesis of 'new' proteins, many of which are extremely rich in cystine. It is suggested that the heterogeneity of the high-sulphur proteins may be due, in part, to similar changes in composition caused by natural variations in the nutrition of sheep.

It is now well established that wool is not a material of constant composition, but is quite variable, particularly in its content of sulphur (Reis & Schinckel, 1963, 1964). Genetic factors are probably involved to some extent, since individual sheep in the same environment can differ from one another in the sulphur content of the wool they produce (Reis, 1965*a*; Reis, Tunks, Williams & Williams, 1967), but variations in nutrition are the major cause of changes in the composition of wool grown by an individual sheep. Thus a change from a low to a high intake of feed, or an increase in the amount of feed available at pasture, results in quite large increases (up to 25%) in the sulphur content of wool (Reis, 1965*a*; Reis & Williams, 1965; Reis & Tunks, 1968). The infusion of small amounts (1–3g./day) of L-cysteine or DL-methionine directly into the abomasum also causes large increases in the sulphur content of wool; increases of as much as 35% have been obtained (Reis & Schinckel, 1963, 1964; Reis, 1967). Such wool is now referred to as sulphur-enriched, and amino acid analyses made in the present study have shown that its increase in sulphur content is solely due to a corresponding increase in cystine content.

The major cause of this increased cystine content is an increase in the total content of high-sulphur protein in the fibre and more specifically to an increased synthesis of a group of proteins extremely high in sulphur content, resulting in an increase in the average sulphur content of the high-sulphur proteins (Gillespie, Reis & Schinckel, 1964; Gillespie & Reis, 1966). Although no evidence has been found for changes in the low-sulphur proteins (Gillespie *et al.* 1964), these cannot be ruled out, particularly in the non-helical regions, which are similar in amino acid composition to certain of the high-sulphur protein components (Crewther & Harrap, 1967).

Preliminary studies on the new high-sulphur proteins present in sulphur-enriched wool showed that they differ from the usual group by their higher molecular weight and sulphur content and by containing no phenylalanine (Gillespie, 1967). In the present study results are presented that establish a quantitative relation between the proportion of these proteins in wool and the sulphur content of the wool.

When high-sulphur proteins, after conversion into *S*-carboxymethylkerateines, are studied by moving-boundary electrophoresis at pH 4·5, it has been found that the mobility of components is directly related to their content of SCMC* residues and hence to their sulphur content. The major peaks can be conveniently labelled peaks A–D in

* Abbreviation: SCMC, *S*-carboxymethylcysteine.

order of increasing mobility. The new high-sulphur proteins are found in peak D and can be resolved electrophoretically at pH 10–11 as a separate peak, peak D2 (Gillespie & Reis, 1966).

Further work has been carried out to ascertain whether other changes in the high-sulphur proteins occur during sulphur enrichment, and the results are reported here. However, owing to the great heterogeneity of the high-sulphur proteins (Gillespie, 1965; Swart, Joubert, Haylett & de Jager, 1966; Joubert, de Jager & Swart, 1968), it is difficult to determine whether the bulk of the high-sulphur proteins are the same as they were in the control (low-sulphur) wool. Changes in the well-characterized high-sulphur protein component, component SCMK–B2, which runs within peak D (Gillespie, 1963; Gillespie & Harrap, 1963; Lindley, Gillespie & Haylett, 1968), were looked for after sulphur enrichment of wool. This material was isolated from control and sulphur-enriched wool grown by one sheep, and comparisons were made between the two fractions, by using as criteria differences between their amino acid compositions and partial amino acid sequences. A search was also made for the existence of new components, or altered proportions of pre-existing ones, within the proteins of peaks A to C, by both moving-boundary and column electrophoresis under various conditions.

MATERIALS AND METHODS

Origin of wool samples. The sheep used in this study were kept in pens indoors at the C.S.I.R.O. Division of Animal Physiology, Prospect, N.S.W., Australia. They were given a basal diet of equal parts of chopped lucerne and wheaten hay in the control period, and this was supplemented in the sulphur-enriched period with sulphur-containing amino acids or proteins infused directly into the abomasum by the procedure described by Reis & Schinckel (1963, 1964). Wool was clipped every 2 weeks from areas that had been defined by tattooing.

Preparation of wool samples. Wool samples were thoroughly washed as described by Gillespie & Reis (1966).

Sulphur analyses. The sulphur content of the wool samples was determined by an oxygen-flask-combustion technique (Reis & Schinckel, 1963).

Preparation of soluble high-sulphur proteins. The wool samples were solubilized by alkaline reduction in the presence of urea, as described previously (Harrap & Glillespie, 1963; Gillespie, 1964; Gillespie & Reis, 1966). After alkylation with iodoacetate and dialysis against deionized water, the low-sulphur protein fraction was removed by precipitation at pH 4·4–4·5 with sodium acetate buffer at final ionic strength 0·5. The supernatant solution containing the high-sulphur proteins was dialysed against deionized water and freeze-dried.

Chromatographic separation of high-sulphur protein components. Chromatography of the high-sulphur protein components on DEAE-cellulose was carried out as described by Gillespie & Reis (1966). A typical elution curve is shown in Fig. 1. Components running in electrophoretic peaks A, B and C, referred to in the text as the ABC high-sulphur protein fraction, were recovered from tubes 25–52, and those in peak D from tubes 53–74. The eluates were first dialysed against deionized water and then freeze-dried.

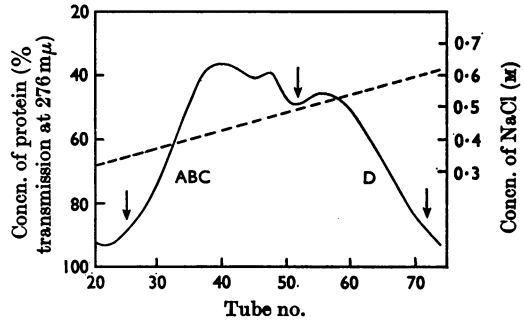

Fig. 1. Chromatographic separation of high-sulphur proteins into ABC and D fractions by gradient elution from DEAE-cellulose at pH 4·5. The experimental conditions were the same as those used by Gillespie & Reis (1966). ——, Protein; – – – –, concn. of NaCl. The arrows indicate the pools made for the ABC and D fractions.

Moving-boundary electrophoresis. Moving-boundary electrophoresis was carried out in a Tiselius apparatus (LKB Produkter, Stockholm, Sweden) as described by Gillespie & Reis (1966). The following buffers of ionic strength 0·1 were used in these experiments: H_3PO_4, pH 1·7; acetic acid–sodium acetate, pH 4·5; glycine–NaOH, pH 10·0. The protein solutions in buffers at pH 1·7 and 10·0 were kept close to 0° during preparation and dialysis to minimize hydrolysis.

Because of the difficulty of dissolving freeze-dried high-sulphur protein in dilute H_3PO_4, the protein (200 mg.) was first dissolved in 7·5 ml. of ice-cold 6 M-urea and then to this was added an equal volume of cold 0·1 M-H_3PO_4. Urea kept the protein soluble as the pH passed through the isoelectric region during the addition of the H_3PO_4, and was then removed from the system when the protein solution was dialysed against 2 l. of the electrophoresis buffer. A small amount of protein insoluble in the electrophoresis buffer was removed by centrifugation, and the final protein concentration was adjusted to 1·3–1·5%.

Electrophoresis was continued until the fastest-ascending peak had moved almost the full length of a standard long intermediate-cell section. The mobility and number of components in the high-sulphur protein mixture, and their relative proportions, were estimated from the patterns obtained.

Column electrophoresis. The equipment for column electrophoresis was constructed and set up by the directions given by Porath (1956). The column was packed with cellulose powder (Munktell type 400) in suspension in water, and then equilibrated by pumping 500 ml. of 0·05 M-H_3PO_4 through it. Protein for application to the column (200–500 mg.) was dissolved in 2 ml. of ice-cold 6 M-urea, 1 ml. of 0·05 M-H_3PO_4 was added and the solution was dialysed against 1 l. of 0·05 M-H_3PO_4 for 18 hr. at 0°. A small amount of insoluble protein was removed by centrifugation and the solution was applied to the top of the

column, allowed to run down into the packing and then followed by 3ml. of buffer. The column was then attached to the remainder of the apparatus as described by Porath (1956) and the protein was subjected to electrophoresis down the column for 25hr. with a potential gradient of 800v. The column was jacketed and cooled with circulating water at 4°.

At the completion of the run, 0·05M-H_3PO_4 was pumped into the top of the column and the eluate was collected in fractions (3ml.). The protein concentration of these fractions was determined by measuring the extinction at 276mμ. The components of highest mobility, having moved furthest down the column, were the first to be recovered in this procedure.

Amino acid analyses. Samples of wool and wool proteins were hydrolysed *in vacuo* at 108° with 6N-HCl for 24hr. The HCl was removed by freeze-drying and the contents of amino acids in the hydrolysate were determined by ion-exchange chromatography with an automatic amino acid analyser (Beckman–Spinco).

Preparation of component SCMK–B2. Preparations of component SCMK–B2 were made from control and sulphur-enriched wool samples by the procedure of Gillespie (1963), modified by the use of urea–thioglycollate-extracted high-sulphur proteins as the starting material for the fractionation (Gillespie, Haylett & Lindley, 1968). From 5g. of high-sulphur protein, 200–250mg. of component SCMK–B2 was recovered.

Peptide 'mapping'. Digestions with chymotrypsin and trypsin were carried out at 37° in 2% (w/v) $(NH_4)_2CO_3$ solution, pH8·3, 0·10mg. of enzyme being used for each 10mg. of protein. The enzyme pretreatments and the subsequent handling of the digests and their resolution by high-voltage paper electrophoresis were carried out as described by Gillespie *et al.* (1968).

RESULTS

Changes in the amino acid composition of wool during sulphur enrichment. Comparative amino acid analyses of control and sulphur-enriched wools produced by sheep 1390 are shown in Table 1. The sulphur-enriched wool was grown during a period when the sheep was given a daily supplement of 3·7g. of DL-methionine into the abomasum. If changes of 10% or more are taken as significant, then in addition to the expected increase in cystine content there were also increases in histidine, threonine, serine and proline. There were decreases in the contents of aspartic acid, leucine and phenylalanine. These differences would be consistent with a simple increase in the proportion of high-sulphur proteins in wool after sulphur-enrichment, for high-sulphur proteins, compared with low-sulphur proteins, show variations in composition of the same kind, namely a higher content of SCMC, histidine, threonine, serine and proline and a lower content of aspartic acid, leucine and phenylalanine.

There were characteristic changes (Table 2), however, in the composition of the high-sulphur proteins themselves after sulphur enrichment, notably increases in histidine, arginine, SCMC and proline and decreases in aspartic acid, glycine, alanine, isoleucine, leucine, tyrosine and phenylalanine. Most of these changes can be accounted for by the existence of new high-sulphur proteins that can be resolved in electrophoretic runs at peak 10–11 as a peak (peak D2) of greater mobility than the normal components (Gillespie & Reis, 1966). The amino acid composition of a preparation of the D2 proteins is given in Table 2. However, the changes in the content of lysine and tyrosine in high-sulphur proteins cannot be explained by the presence of these new proteins.

Relation between the sulphur content of wool and its content of dietary-regulated proteins of peak D2. High-sulphur proteins were isolated from a number of control and sulphur-enriched wool samples that covered the range of sulphur contents from about 3·0 to 4·4%. The proportion of D2 proteins in these preparations was estimated from the area under the corresponding peak in moving-boundary-electrophoresis patterns from runs at pH10, similar to those shown by Gillespie & Reis (1966). The results (Fig. 2) show that there is a positive linear relationship between the sulphur content of wool and its content of dietary-regulated high-sulphur proteins that is significant at a level of $< 0·1\%$.

Table 1. *Amino acid composition of control and sulphur-enriched wool produced by English Leicester × Merino sheep* 1390

Results are expressed as amino acid N as a percentage of total N. Percentage differences of magnitude greater than 10% are indicated.

Amino acid	Amino acid composition of wool (% of total N) Control	Sulphur-enriched	Difference (%)
Lys	4·49	4·22	
His	1·50	1·65	10
Arg	21·7	21·4	
Asp	4·87	4·16	−15
Thr	4·27	4·74	11
Ser	7·10	8·02	13
Glu	8·57	8·16	
Pro	4·57	5·36	17
Gly	4·94	5·14	
Ala	4·13	3·79	
CyS	7·57	9·38	24
Val	4·08	4·05	
Met	0·40	0·36	
Ile	2·36	2·26	
Leu	5·60	5·03	−10
Tyr	2·26	2·29	
Phe	1·82	1·54	−15
Sulphur (%)	3·04	3·98	31

Table 2. *Amino acid composition of high-sulphur proteins isolated from control and sulphur-enriched wool produced by English Leicester × Merino sheep*

Results are expressed as amino acid N as a percentage of total N.

	Amino acid composition of high-sulphur proteins (% of total N)				
Sheep no.	1390			SD67	
Amino acid	Control*	Sulphur-enriched*	Dietary-regulated (peak D2)	Control	Sulphur-enriched
Lys	1·05	0·96	1·98	0·88	0·90
His	1·58	1·66	2·80	1·44	1·62
Arg	12·1	12·6	14·7	17·6	19·6
SCMC	14·4	16·2	24·5	15·1	17·9
Asp	2·43	1·94	0·35	2·33	1·59
Thr	7·77	8·02	9·57	7·50	8·00
Ser	9·39	9·79	9·81	9·80	10·1
Glu	6·01	6·26	6·65	6·14	6·25
Pro	9·42	9·96	9·33	8·69	9·40
Gly	5·23	4·61	3·44	5·04	4·44
Ala	2·52	2·03	1·41	2·37	1·90
Val	4·61	4·17	2·53	4·35	4·00
Met	0·00	0·00	0·00	0·00	0·00
Ile	2·39	2·22	1·11	2·40	2·06
Leu	3·11	2·36	0·76	3·10	2·21
Tyr	0·59	0·42	1·24	1·74	1·60
Phe	1·54	0·96	0·00	1·48	1·14

* Data from Gillespie *et al.* (1964).

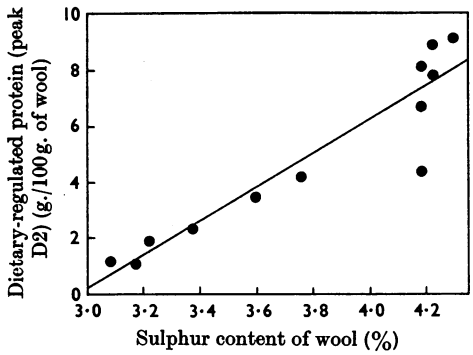

Fig. 2. Relation between the sulphur content of wool and its content of dietary-regulated proteins as estimated from the area under peak D2 in moving-boundary-electrophoretic runs at pH 10·0 of high-sulphur proteins.

Apparent constancy in composition of component SCMK–B2 during sulphur enrichment. The amino acid analyses of samples of component SCMK–B2 prepared from control and sulphur-enriched wool from sheep 1390 are given in Table 3. If lysine and histidine are excluded there were no significant differences between the two preparations, and with only three amino acids, aspartic acid, leucine and phenylalanine, did the differences even approach significance. Since component SCMK–B2 does not contain lysine or histidine (Gillespie, 1963), their presence in these preparations is indicative only of a lack of purity.

Further support for the complete identity of these preparations came from a study of the peptides produced by digestion of the proteins with trypsin and chymotrypsin. When these digests were run electrophoretically and stained no evidence for differences was seen (Fig. 3). Analyses of certain of the separated peptides also confirmed this identity of the preparations (Lindley *et al.* 1968; Gillespie *et al.* 1968).

Comparative study by moving-boundary electrophoresis of the ABC high-sulphur protein fraction prepared from control and sulphur-enriched wool samples. Conditions were sought for the maximum electrophoretic resolution of the ABC high-sulphur protein fraction, by running it in a series of buffers that covered the pH range from 1·7 to 10·0. The best resolution was obtained at pH 1·7, by using as buffer 0·1 M-orthophosphoric acid.

ABC fractions prepared from control and sulphur-enriched wool samples from each of four sheep were run at pH 1·7, and examples of the results obtained are shown in Figs. 4(*a*) and 4(*d*). In each case the proteins were resolved into seven or eight components, which could be divided into

Table 3. *Amino acid composition of SCMK–B2 preparations isolated from control and sulphur-enriched wool produced by English Leicester × Merino sheep* 1390

Results are expressed as amino acid N as a percentage of total N.

Amino acid	Amino acid composition of component SCMK–B2 (% of total N)	
	Control	Sulphur-enriched
Lys	0·18	0·24
His	Trace	0·28
Arg	14·6	14·1
SCMC	19·4	19·0
Asp	0·65	0·60
Thr	8·32	8·22
Ser	12·0	11·8
Glu	9·07	8·82
Pro	8·57	8·12
Gly	6·33	6·33
Ala	2·61	2·47
Val	3·09	3·23
Met	0·00	0·00
Ile	2·71	2·85
Leu	1·63	1·48
Tyr	1·82	1·77
Phe	0·75	0·81

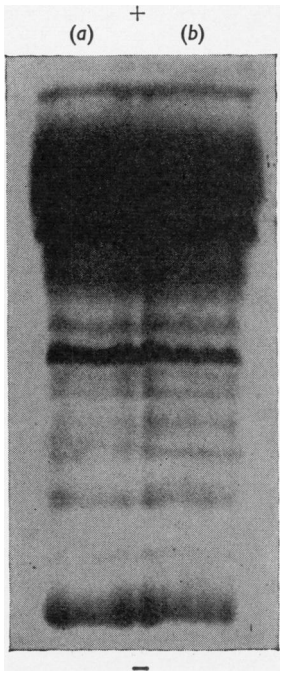

Fig. 3. Electrophoretograms showing comparative peptide 'maps' of chymotryptic digests of SCMK–B2 fractions isolated from control (*a*) and sulphur-enriched (*b*) wool, run at pH 3·5 and 50 v/cm. for 1 hr. The peptides were developed with a Cl_2–tolidine reagent.

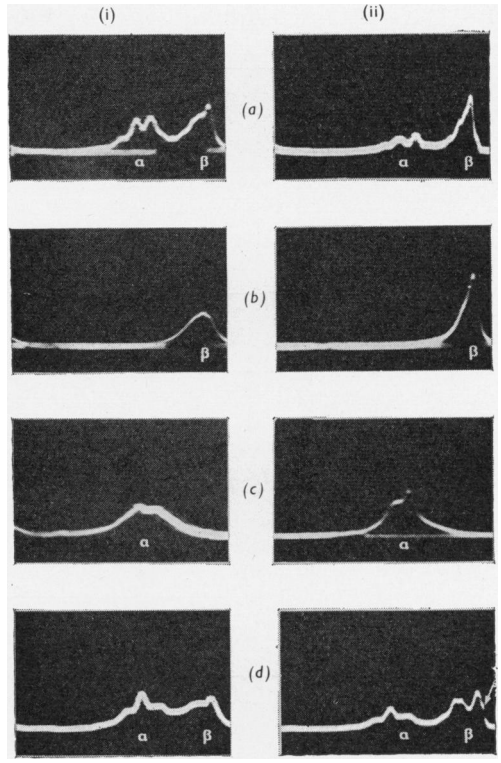

Fig. 4. Moving-boundary-electrophoresis patterns of high-sulphur protein fractions isolated from control (i) and sulphur-enriched (ii) wool produced by English Leicester × Merino sheep. (*a–c*) Sheep 1390: control wool, 3·04% of S; sulphur-enriched wool, 3·98% of S; abomasal infusion of 60g. of casein and 3·0g. of L-cysteine/day. (*d*) Sheep SD 67: control wool, 3·08% of S; sulphur-enriched wool, 4·18% of S; abomasal infusion of 2·0g. of L-cysteine/day. (*a*) ABC fraction; (*b*) β subfraction; (*c*) α subfraction; (*d*) ABC fraction. Electrophoresis was at pH 1·7. The arrow in (*d*) (ii) shows a shoulder present in the sulphur-enriched and not in the control proteins.

two well-defined groups labelled α and β in order of ascending mobility.

On sulphur enrichment there was a marked increase in the proportion of proteins in the β region, and this was true for each of the four preparations the average increase being about 25%. The relative proportions of individual components within the β region also altered on sulphur enrichment, and quite large changes were observed in the proteins of wool produced by sheep SD 67 when its diet was supplemented by moderate amounts of cysteine (Fig. 4*d*) and large amounts of methionine. Quantitatively, the changes in the β region differed between preparations, and this may have been a manifestation of variations in the treatment of

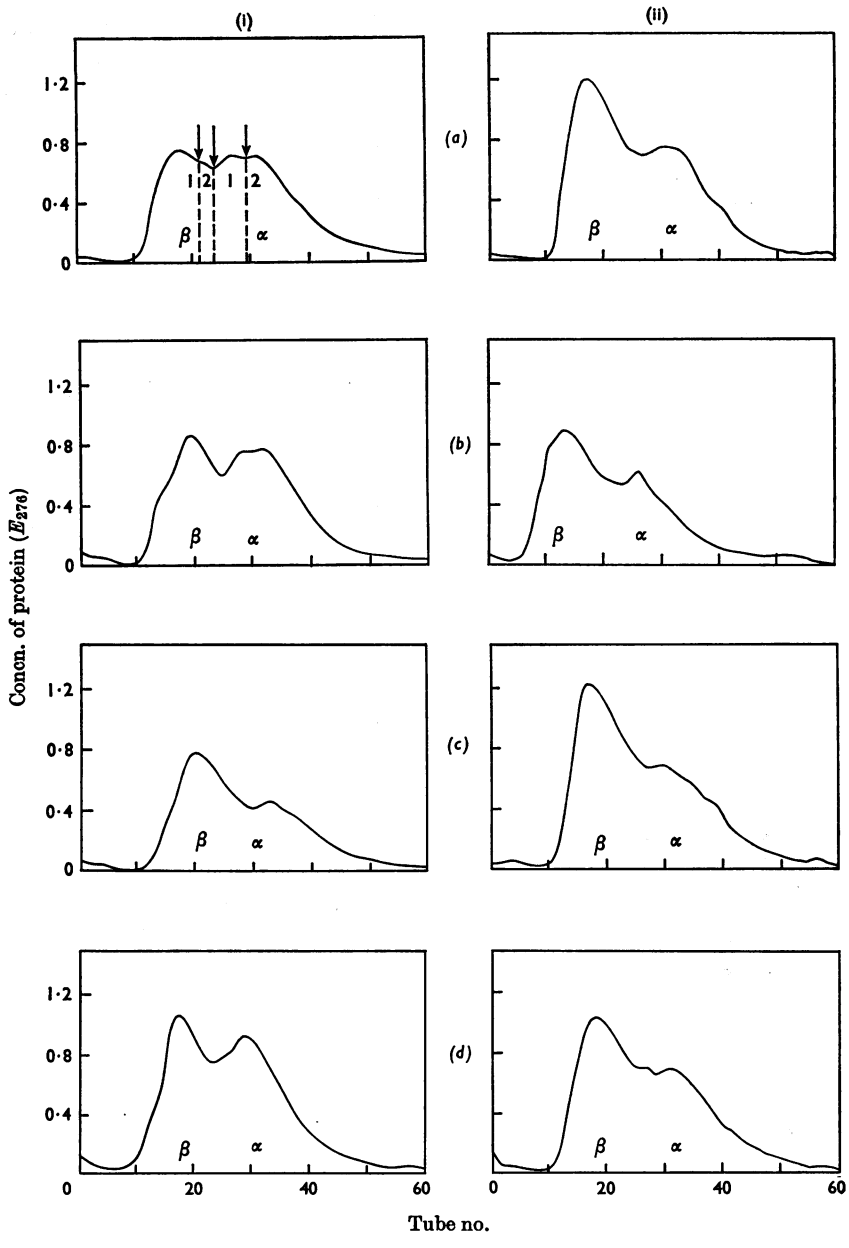

Fig. 5. Column-electrophoresis patterns of the ABC fraction of high-sulphur protein isolated from control (i) and sulphur-enriched (ii) wool produced by four different sheep. (*a*) English Leicester × Merino (sheep 1390): control wool, 3·04% of S; sulphur-enriched wool, 3·98% of S; abomasal infusion of 60g. of casein and 3·0g. of L-cysteine/day. (*b*) English Leicester × Merino (sheep SD67): control wool, 3·08% of S; sulphur-enriched wool, 4·17% of S; abomasal infusion of 3·7g. of DL-methionine/day. (*c*) English Leicester (sheep 3307): control wool, 3·04% of S; sulphur-enriched wool, 3·96% of S; abomasal infusion of 3·7g. of DL-methionine/day. (*d*) Merino (sheep SC8): control wool, 2·84% of S; sulphur-enriched wool, 3·72% of S; abomasal infusion 60g. of gelatin and 3·7g. of DL-methionine/day. Electrophoresis was at pH 1·7. The arrows in (*a*)(i) indicate the pools that were made for the subfractions $\alpha 1$, $\alpha 2$, $\beta 1$ and $\beta 2$.

sheep or of differing responses by sheep to a particular treatment.

Of the ABC fraction, 5–10% was found to be insoluble at pH 1·7 and was removed by centrifugation before the ABC fraction was submitted to electrophoresis. The composition of this insoluble material was the same whether it was isolated from control or sulphur-enriched wools, and it is therefore disregarded in the discussions below.

Column electrophoresis of the ABC high-sulphur fraction of control and sulphur-enriched wool samples. As no consistent differences were found between the mobilities of individual components within the α and β groups from control and sulphur-enriched wool, it is reasonable to suggest that the components were largely unchanged in composition. An alternative approach to this problem was to fractionate the preparations described in the previous section by preparative column electrophoresis at pH 1·7. By this means components were separated and a search was made for changes in their amino acid composition after sulphur enrichment. The electrophoretic patterns obtained in this way are shown in Fig. 5. Once again two main peaks were obtained, which were labelled, as before, α and β in order of increasing mobility. Resolution into components by this technique was somewhat poorer than that achieved in the moving-boundary experiments (cf. Fig. 4), and this limited the extent to which it was possible to isolate fractions and to make comparisons between them.

It was desirable to establish that the column-electrophoretic α and β components were the same as those observed in moving-boundary experiments. To do this, α and β components from a column run (Fig. 5*a*i) were prepared by taking fractions from each side of the minimum on the curve at about tube 25. Both fractions were run at pH 1·7 in moving-boundary electrophoresis, and the results obtained are shown in Fig. 4 together with the comparable moving-boundary pattern of the parent ABC preparation. The α and β column fractions corresponded in mobility to the α and β components in the original ABC fraction. This indicates that column electrophoresis under these conditions separated components on the same basis as moving-boundary runs, and that strict comparisons can be made between peaks observed in moving-boundary runs and those isolated in the column-electrophoretic separations.

To make a comparison between the compositions of α and β components, four fractions prepared by column electrophoresis [labelled in order of increasing mobility in Fig. 5(*a*)(i) as α2, α1, β2 and β1] were hydrolysed, and each was analysed for its contents of amino acids. The results, shown in Table 4, indicate that there were substantial differences between the fractions in their contents of aspartic acid, leucine, isoleucine and the basic amino acids.

Examination for a relation between charge and relative contents of amino acids with ionizable side chains shows that as the mobility of a component increased from fraction α2 to fraction β1 there was a

Table 4. *Amino acid composition of subfractions of the ABC high-sulphur protein fraction of control wool produced by English Leicester × Merino sheep* 1390

Results are expressed as amino acid N as percentage of total N.

	Amino acid composition of subfraction (% of total N)			
Subfraction ... ... Amino acid	β1	β2	α1	α2
Lys	0·40	0·89	1·17	1·55
His	0·24	0·85	1·83	2·51
Arg	29·1	25·1	20·5	13·0
SCMC	15·5	14·4	12·6	12·8
Asp	1·23	1·58	2·57	3·72
Thr	7·45	7·13	7·82	7·70
Ser	7·06	8·19	9·22	9·65
Glu	4·46	4·75	4·56	4·74
Pro	9·53	9·26	8·71	9·19
Gly	3·31	3·60	3·78	4·76
Ala	1·77	1·80	1·84	2·54
Val	4·95	5·16	5·58	5·08
Met	0·00	0·00	0·00	0·00
Ile	1·64	1·96	2·59	3·25
Leu	1·73	2·14	3·07	4·19
Tyr	1·19	1·19	1·48	1·51
Phe	1·48	1·31	1·51	1·80

major increase in arginine content, with small decreases in lysine and histidine as well as changes in SCMC, aspartic acid and glutamic acid. At the pH of electrophoresis it is highly probable that the basic amino acids determine the charge and that the mobility differences between the α and β components are largely due to their different contents of arginine. It is not surprising therefore that, when run in moving-boundary experiments at pH 4·5, both have similar mobilities and each contains components that fall within the A, B and C groups.

Examination of the column-electrophoretic patterns of Fig. 5 shows that the major effect of sulphur enrichment was to cause an increase in the proportion of β components, as was found in the moving-boundary studies. A number of α and β fractions were analysed for their amino acid compositions and in each case there was a major difference in the arginine contents of the α and β fractions, whether these fractions arose from the control or the sulphur-enriched wools (see Table 4). There were also small differences between the comparable pair of proteins isolated from control and sulphur-enriched wools, and these may indicate a compositional change in these fractions.

DISCUSSION

In earlier work, Gillespie & Reis (1966) suggested that changes in the sulphur content of wool after sulphur enrichment could be accounted for largely by changes in the amount of proteins present in electrophoretic peak D2. In the present work this has been confirmed and it is now evident that there is a highly significant quantitative relationship connecting these two parameters. Further, the overall changes in amino acid composition after sulphur enrichment can also be accounted for by the presence of D2 proteins in the wool.

Of the other proteins that run in electrophoretic peak D one, component SCMK–B2, seems to be unchanged in both composition and arrangement of amino acids. If this is also true for the other components an important generalization can be made about the mechanism of sulphur enrichment. This process, in drastically changing the composition of wool and its constituent high-sulphur proteins, does so not by altering the composition of individual proteins but by varying the proportion of pre-existing ones and by initiating the synthesis of 'new' components.

It is more difficult to draw definite conclusions about the changes that occur in the proteins in the ABC electrophoretic peaks. Clearly on sulphur enrichment there is an overall increase in the synthesis of the arginine-rich proteins of the β electrophoretic fraction, the greatest increase being in the components of highest net charge at pH 1·7 (Figs. 4*a* and 4*d*). Simultaneously, significant changes occur in the amino acid composition of proteins within both the α region and the β region, which can be accounted for by considering the alterations that occur in the relative proportions of α and β subcomponents, without the necessity for postulating the synthesis of major new components. This is supported by the absence of consistent changes in the mobility of the components after sulphur enrichment. There is, however, good evidence for the synthesis of small amounts of new components, which can be seen as small shoulders on the leading edges of the fastest moving peaks of sulphur-enriched preparations [marked with an arrow in Fig. 4(*d*)(ii)].

The effects of sulphur enrichment can be summarized as follows. As the amount of sulphur-containing amino acids available to the tissues of the sheep increases, large changes take place in the synthetic activities of the follicle. First, there is a large increase in the overall growth rate of the fibre, and this means that there must be an increased synthesis of high-sulphur proteins generally. There is an optimum for this, as an excess of methionine, for example, decreases synthetic activity (Reis, 1967). Accompanying these changes there is an alteration in the pattern of synthesis of the high-sulphur proteins. A 'new' synthetic process produces the proteins of the D2 peak in direct response to the amount of available sulphur-containing amino acids. There is an increased synthesis of certain of the β components of the ABC high-sulphur protein fraction, but it is unlikely that major new components are produced.

The evidence presented here strongly suggests that part at least of the heterogeneity of the high-sulphur proteins is due to the effects of variable nutrition. This could be due to variations in sulphur content along the length of single fibres as a result of periods of variable nutrition (Reis, 1965*b*) or perhaps to trace-element deprivation, e.g. copper deficiency (Marston, 1946). There may also be differences in the availability of nutrients between follicles or between cells within a follicle, perhaps dependent on relative accessibility to sources of sulphur-containing amino acids. Superimposed on these effects of nutrition are the large individual differences in sulphur content of wool, so that genotype also affects the heterogeneity of high-sulphur proteins.

This work provides further evidence of the large number of components found in the high-sulphur group of proteins and of their variable nature, and makes most improbable the single-protein theory of keratin structure (Corfield, 1962). But as yet no insight has been obtained into the reason for this heterogeneity, into the protein-synthetic mechanism involved or into the control mechanisms regulating

the proportions of various components that are synthesized.

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