The Dietary-Regulated Biosynthesis of High-Sulphur Wool Proteins

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(Received 12 July 1965)

1. When the diet of sheep is supplemented by the abomasal infusion of sulphur-containing amino acids or casein, a special group of proteins, with a very high content of sulphur (about 8.3%), is incorporated into the high-sulphur proteins of wool. These special proteins cannot be detected in control wool from the same sheep. 2. This is a naturally occurring process, as these special proteins are found in wool from sheep on a high level of nutrition under ordinary conditions of feeding, and in wool of an inherently high sulphur content. 3. This represents a control mechanism in protein synthesis that has not previously been observed, and may be further evidence that the high-sulphur proteins of wool are produced by an unusual synthetic route.

Reis & Schinckel (1963, 1964) showed that the rate of wool growth was substantially increased by infusing cysteine, methionine or casein directly into the abomasum of sheep. The sulphur content of wool was increased by as much as 35% as a result of such abomasal feeding. The observation that the sulphur content of wool is also altered substantially by changes in the nutritive status of sheep in controlled feeding experiments (Reis, 1965*a,b*) or under free-grazing conditions (Reis & Williams, 1965) implies that the mechanism for increased sulphur incorporation is a normal attribute of the animal and not a special property induced by the technique of abomasal feeding.

Wool keratins are complexes of two main types of protein: those with sulphur contents less than that of the parent wool (low-sulphur proteins) and a second fraction made up of a mixture of many proteins of sulphur content higher than that of the whole wool (high-sulphur proteins). An investigation of sulphur-enriched wool samples grown during abomasal feeding (Gillespie, Reis & Schinckel, 1964) showed that the increase in sulphur content was restricted to the high-sulphur proteins of the wool fibre. The proportion of the high-sulphur proteins was increased, and within this group of proteins there was increased formation of those protein components richest in sulphur. Within experimental error, these changes accounted for the increased sulphur content of the wool.

Electrophoresis by the moving-boundary technique at pH 4.5 and in starch gel at pH 8.0 gave no

evidence of new protein components in the sulphur-enriched wool samples and suggested that there had been an increased synthesis of the same proteins present in control wool. However, this was not entirely compatible with the evidence from amino acid analysis, which showed that the high-sulphur proteins from sulphur-enriched wool contained very much less aspartic acid, phenylalanine and leucine than did those from control wool. These differences could possibly be due to the production of the same protein components in both control and sulphur-enriched wool, but in different proportions, or more likely to the production of protein components that are not present in detectable amounts in control wool.

Further evidence is presented below which suggests that the second alternative is correct and that a special group of protein components is in fact formed during sulphur-enrichment. It is further suggested that this is a naturally occurring process as these special proteins are found in wool from sheep on a high level of nutrition and in wool of an inherently high sulphur content.

EXPERIMENTAL

Experimental procedures with sheep. Most sheep were kept indoors and were fed individually. The animals studied were English Leicester, Merino and English Leicester × Merino crosses. In some experiments the nutrition was varied by altering the amount or composition of the diet (Reis, 1965*a,b*), whereas in other experiments a basal diet

of equal parts of lucerne chaff and wheaten chaff was supplemented with sulphur-containing amino acids or casein, infused directly into the abomasum (Reis & Schinckel, 1963, 1964). Wool was collected at regular intervals, usually 3 weeks, from areas (approx. 10 cm. $\times$ 10 cm.) that were defined by tattooing. Control samples were obtained from these areas on each animal before the diet was altered

Fig. 1. Chromatography of high-sulphur proteins on DEAE-cellulose at pH 4.5. Experimental details are given in the text.

Fig. 2. Moving-boundary electrophoretic patterns of high-sulphur proteins from runs at pH 4.5 showing the differences between the proteins from control (i) and sulphur-enriched wools (ii) grown by three different sheep. (a) Merino (sheep SC 8): control wool, 2.84% of S; sulphur-enriched wool, 3.72% of S; abomasal infusion of 60g. of gelatin and 3.7g. of DL-methionine/day. (b) 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. (c) English Leicester $\times$ Merino (sheep 1391): control wool, 3.06% of S; sulphur-enriched wool 3.88% of S; abomasal infusion of 60g. of gelatin and 3.0g. of L-cysteine/day.

Table 1. *Effect on wool composition of the abomasal infusion into sheep of cysteine and methionine, alone and in combination with gelatin or casein*

Sheep	Breed	Wool sample no.	Abomasal supplement	Sulphur content (%)	Content of high-sulphur protein (%)	Sulphur content of high-sulphur protein (%)
1390	English Leicester $\times$ Merino	63	None	3.04	22	4.9
		65	Casein and cysteine	3.98	31	5.7
1391	English Leicester $\times$ Merino	66	None	3.06	22	4.9
		68	Gelatin* and cysteine	3.88	30	6.5
SD 67	English Leicester $\times$ Merino	331	None	3.08	21	4.8
		332	Methionine	4.17	32	6.1
3307	English Leicester	333	None	3.04	24	4.8
		334	Methionine	3.96	33	6.0
SC 8	Merino	136	None	2.84	20	4.8
		137	Gelatin* and methionine	3.72	29	5.8

* Gelatin alone had no effect on the growth of wool or its composition.

or supplemented via the abomasum. Wool samples were also obtained from sheep in an experimental flock in the field by clipping at intervals of 6 weeks.

Preparation of wool samples. Each sample of wool was washed thoroughly in light petroleum (b.p. 30–80°), in ethanol and in water and was finally equilibrated in a conditioned room (20°; 60% relative humidity).

Sulphur analysis. The sulphur content of wool was measured by an oxygen flask technique as described by Reis & Schinckel (1963).

Isolation and estimation of wool proteins. Wool samples were extracted with a solution at pH 11.0 of 8M-urea containing potassium mercaptoacetate (0.2M) for 2 hr. at 40°. The extracted proteins, which represented about 90% of the fibre, were converted into *S*-carboxymethylkeratines by alkylation with iodoacetate and then dialysed. After removal of the low-sulphur fraction by precipitation with zinc acetate at pH 6.0 (final concn. 20mM), the high-sulphur proteins in the supernatant were recovered by dialysis and freeze-drying. Complete details of the extractions and of the procedures used to measure the proportions of proteins in the extracts have been described by Harrap & Gillespie (1963) and Gillespie (1964).

Chromatographic separation of high-sulphur protein components. Freeze-dried protein (1g.) dissolved in 60ml. of acetic acid–sodium acetate buffer, pH 4.5 and 1.02, was applied to a column (5cm. × 15cm.) of DEAE-cellulose (EK 7392) and eluted at room temperature with a linear gradient of 0.25–0.8M-NaCl in acetate buffer. The total volume of eluent was 1500ml. and 15ml. fractions were collected. The protein concentration of the column effluent was continuously monitored by measuring the extinction at 278m μ with an instrument designed for the purpose by Gilson Medical Electronics. Fig. 1 shows a typical elution profile. This degree of resolution was adequate for the

present purpose; however, it can be increased by using a shallower gradient and a lower load of protein (Gillespie, 1963*a,b*). Protein components running in electrophoretic peak D (see Fig. 2) were isolated by pooling the fractions eluted between the points indicated by arrows on Fig. 1, dialysing and freeze-drying. As some variation occurred in the position of eluted protein peaks from different protein preparations, the elution profile was plotted in each experiment and the position of the minimum used as the reference from which fractions were collected.

Moving-boundary electrophoresis. The proportions and mobility of high-sulphur protein fractions were estimated by electrophoresis in a Tiselius moving-boundary apparatus (LKB Produktor, Stockholm, Sweden). Freeze-dried proteins were dissolved in the appropriate buffer to give a 1.1–1.2% (w/v) solution and then dialysed for 24 hr. against 2l. of the same buffer. The following buffers of ionic strength 0.1 were used; acetic acid–sodium acetate buffer, pH 4.5; veronal buffer, pH 8.6; glycine–NaOH buffer, pH 10.0; β -alanine–NaOH buffer, pH 11.0. The experiments were continued until the fastest ascending peak traversed the full length of a standard long intermediate cell. The proportions of components in the proteins were estimated from the relative areas under peaks in the ascending patterns. On tracings, the peaks were arbitrarily defined by vertical lines, the horizontal distance covered under each peak being kept the same in control and sulphur-enriched wool patterns to make the results strictly comparable.

Amino acid analysis. Samples of protein (30mg.) were hydrolysed *in vacuo* at 108° for 24 hr. with 5ml. of 6N-HCl. The HCl was removed by freeze-drying and the content of amino acids in the hydrolysate estimated by ion-exchange chromatography with an automatic amino acid analyser (Beckman-Spinco).

Table 2. *Amino acid composition of high-sulphur proteins isolated from control and sulphur-enriched wool produced by Merino × English Leicester sheep 1390*

The results are expressed as amino acid N as percentage of total N. Where sulphur-enrichment has resulted in a change in amino acid composition exceeding 10% this has been indicated.

Amino acid	High-sulphur protein			Peak D fraction		
	Control	Sulphur-enriched	Difference (%)	Control	Sulphur-enriched	Difference (%)
Lysine	1.05	0.96		0.50	0.62	
Histidine	1.58	1.66		0.64	1.05	
Arginine	12.10	12.56		18.27	18.17	
<i>S</i> -Carboxymethylcysteine	14.37	16.16	+ 12	16.49	18.76	+ 14
Aspartic acid	2.43	1.94	– 20	1.08	0.77	– 29
Threonine	7.77	8.02		7.44	7.62	
Serine	9.39	9.79		10.20	10.50	
Glutamic acid	6.01	6.26		6.74	6.48	
Proline	9.42	9.96		9.18	9.23	
Glycine	5.23	4.61	– 12	5.11	4.54	– 11
Alanine	2.52	2.03	– 19	2.40	1.92	– 20
Valine	4.61	4.17		3.71	3.40	
Isoleucine	2.39	2.22		2.25	2.09	
Leucine	3.11	2.36	– 24	1.98	1.53	– 23
Tyrosine	0.59	0.42		1.68	1.62	
Phenylalanine	1.54	0.96	– 37	0.86	0.65	– 24

RESULTS

Protein composition of sulphur-enriched wool. Supplements of DL-methionine or L-cysteine, either alone or in combination with gelatin or casein, infused directly into the abomasum substantially increased the sulphur content of wool (Table 1). This was accompanied by an increase in the proportion of high-sulphur protein and an increase in the sulphur content of that fraction. Although the accurate estimation of the proportion of high-sulphur proteins is difficult, the evidence suggests that these changes in the high-sulphur proteins account for the increased sulphur content of the wool. Thus an examination of the electrophoretic patterns (Fig. 2) and the data in Table 1 indicate that the increase in the high-sulphur protein content of sulphur-enriched wools is entirely due to the increased synthesis of protein with mobilities in the ranges of peaks C and D. This conclusion applies irrespective of the breeding of the sheep (Merino, English Leicester or English Leicester $\times$ Merino). The present investigation is confined to an examination of the changes in the proteins within peak D: first, because changes in this region account for about 60% of the total change, and, secondly, because at present peak D proteins are more readily separated from the mixture by column chromatography.

Amino acid composition of high-sulphur proteins isolated from control and sulphur-enriched wool. Comparative amino acid analyses of high-sulphur proteins originating from control and sulphur-enriched wools are shown in Table 2. Besides showing the expected increase in *S*-carboxymethyl-cysteine content due to sulphur-enrichment, the unfractionated protein also shows large decreases in aspartic acid, glycine, alanine, leucine and phenylalanine. Similar changes occur in the composition of peak D protein as a result of sulphur-enrichment. These results suggest the synthesis of a protein high in cystine and low in aspartic acid, glycine, alanine, leucine and phenylalanine but in other respects similar in composition to high-sulphur proteins from control wool. It was decided to investigate whether this is a new synthesis of a novel protein or merely increased synthesis of a component present in control wool.

Detection of new protein components. A search was made for new protein components by varying the conditions of electrophoresis. In terms of the number of visible components obtained, resolution of unfractionated high-sulphur proteins steadily worsens as the pH of electrophoresis is varied away from 4.5 (Gillespie, 1963*a,b*). However, on electrophoresis of sulphur-enriched high-sulphur protein in the pH range 10–11, there was evidence of a fast component not present, or present to only a small

extent, in the control high-sulphur protein (Fig. 3). To identify this material, peak D proteins were isolated chromatographically and run at pH11.0; a clear difference was seen between the patterns of the fractions from control and sulphur-enriched wool samples. Peak D protein from a control wool (sheep 1390) showed essentially only a single boundary at pH4.5, 8.6, 10.0 and 11.0, whereas peak D protein from sulphur-enriched wool from this sheep separated into two peaks at pH11.0 (D1 and D2; Fig. 4). The resolution at pH10.0 was not quite as good as at pH11.0 but, as there was some evidence of protein degradation on continued

Fig. 3. Moving-boundary electrophoretic patterns of high-sulphur proteins run at pH 11.0 showing the differences between control (i) and sulphur-enriched (ii) wools grown by a number of English Leicester $\times$ Merino sheep. (a) Sheep 1391: control wool, 3.06% of S; sulphur-enriched wool, 3.88% of S; abomasal infusion of 60g. of gelatin and 3.0g. of L-cysteine/day. (b) Sheep 1392: control wool, 3.15% of S; sulphur-enriched wool, 3.73% of S; abomasal infusion of 60g. of casein and 1.5g. of L-cysteine/day. (c) Sheep 1393: control wool, 3.38% of S; sulphur-enriched wool, 3.95% of S; abomasal infusion of 60g. of gelatin and 1.5g. of L-cysteine/day. (d) Sheep SD67: control wool, 3.08% of S; sulphur-enriched wool, 4.17% of S; abomasal infusion of 3.7g. of DL-methionine/day.

dialysis at pH11.0, electrophoresis at pH10.0 was employed in some experiments.

Peak D2 has been identified as the new peak in two ways. (1) Peak D fractions were isolated from wool samples enriched with sulphur to only a moderate degree and run electrophoretically at pH11.0. Peak D2 was still observable but was

present in diminished amount as compared with the fractions from fully enriched wool (Fig. 5). It is clear that the proportion of peak D2 increases with the degree of sulphur-enrichment of the wool. (2) When a mixture of equal parts of peak D protein from control and sulphur-enriched wool samples (sheep 1391) was run electrophoretically at pH11.0, the area under peak D2 (Fig. 6) decreased by about the expected 50%.

There seem to be ample grounds for identifying peak D2 with the fastest peak observed in runs at pH11.0 with unfractionated high-sulphur protein from sulphur-enriched wool (Fig. 3). It has therefore been possible to dispense with the preliminary fractionation and run the unfractionated protein at pH11.0 to assess the proportion of peak D2 material.

Properties of peak D2 protein. The proteins of peak D2 have not yet been completely separated from peak D1 proteins although a purity of about 90% has been obtained by fractional precipitation and chromatography. Even though this fraction is not completely pure the amino acid analysis is noteworthy (Table 3), for compared with other high-sulphur proteins this fraction has a strikingly high content of *S*-carboxymethylcysteine, a very low content of aspartic acid, leucine and phenylalanine and a comparatively low content of glycine, alanine and valine. This composition is extremely close to that which would be predicted from the observed differences in amino acid composition of the high-sulphur proteins and fractions isolated from control and sulphur-enriched wool (Table 3).

Differences between breeds of sheep. The proteins discussed so far have been extracted from the wool of English Leicester $\times$ Merino sheep. High-sulphur proteins have also been obtained from control and sulphur-enriched wool grown by Merino and English Leicester sheep and in each instance the proportion of protein in peaks C and D greatly increased during sulphur-enrichment (Fig. 2). Calculations showed that, as in the experiments above, changes in the high-sulphur proteins accounted for substantially all the increases in sulphur content of the wool. When peak D fractions from these wools were run

Fig. 4. Moving-boundary electrophoretic patterns of peak D fraction of high-sulphur proteins isolated from control (i) and sulphur-enriched (ii) wool produced by an English Leicester $\times$ Merino sheep (1390). Control wool, 3.04% of S; sulphur-enriched wool, 3.98% of S; abomasal supplement of 60g. of casein and 3.0g. of L-cysteine/day. (a) Electrophoresis at pH4.5; (b) electrophoresis at pH11.0.

Fig. 5. Moving-boundary electrophoretic patterns of peak D high-sulphur proteins run at pH11.0, showing the increase in proportion of D2 protein with increasing degree of sulphur-enrichment of wool. (a) Wool: 3.04% of S; no detectable peak D2; control wool. (b) Wool: 3.48% of S; 18% of peak D2; abomasal infusion with 60g. of casein/day. (c) Wool: 3.60% of S; 21% of peak D2; abomasal infusion with 60g. of casein/day. (d) Wool: 3.98% of S; 39% of peak D2; abomasal infusion with 60g. of casein and 3.0g. of L-cysteine/day. Samples (a), (c) and (d) were from English Leicester $\times$ Merino sheep 1390; sample (b) was from English Leicester $\times$ Merino sheep 1392.

Fig. 6. Moving-boundary electrophoretic patterns of peak D high-sulphur proteins isolated from (a) control, (c) sulphur-enriched wools (sheep 1390) and (b) a mixture (1:1) of these two fractions. Runs were made at pH11.0.

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Table 3. *Amino acid composition of partially purified peak D2 fraction of high-sulphur protein*

The results are expressed as amino acid N as percentage of total N. For comparison, predicted compositions are given; these have been calculated from the differences in amino acid composition between high-sulphur protein and fractions isolated from control and sulphur-enriched wool samples.

Amino acid	Amino acid composition of impure peak D2 protein	Predicted composition of peak D2 proteins	
		From differences in composition between high-sulphur protein isolated from six pairs of control and sulphur-enriched wool samples	From difference in composition between peak D proteins isolated from control and sulphur-enriched wool, assuming that peaks D1 and D2 contain equal amounts of protein (sheep 1390)
Lysine	1.23	—	0.74
Histidine	2.27	—	1.46
Arginine	16.1	—	18.1
S-Carboxymethylcysteine	22.1	21.2	21.1
Aspartic acid	0.49	0.5	0.46
Threonine	8.39	—	7.72
Serine	9.56	—	10.8
Glutamic acid	6.69	—	6.31
Proline	8.96	—	9.25
Glycine	3.01	3.1	3.97
Alanine	1.99	1.4	1.44
Valine	3.08	3.2	3.09
Isoleucine	1.27	1.6	2.00
Leucine	1.12	0.8	1.08
Tyrosine	1.10	—	1.60
Phenylalanine	0.22	0.2	0.44

at pH11.0 (Fig. 7) in each case peaks D1 and D2 were resolved, but the ratio of peak D2 to D1 was lower than found in the wool proteins from most of the cross-bred sheep (Figs. 3 and 7).

Presence of peak D2 proteins in wool from normally fed sheep. (a) Wool grown during periods of high nutritive status. In experiments with pen-fed sheep, Reis (1965*a,b*) found that increasing the level of nutrition of sheep increased the sulphur content of the wool they produced. It has been shown that, as found with abomasal supplements, this increase in sulphur content of the wool is caused by an increase in the level of high-sulphur protein in the wool, and by an increase in the proportion of the proteins in peaks C and D (Gillespie, 1965; and Fig. 8*a*). Electrophoresis at pH10.0 (Fig. 8*b*) shows that this increase in the sulphur content of wool, caused by high nutritional status, is paralleled by an increase in the proportion of peak D2 protein.

(b) Wool of different sulphur content produced by sheep on equal levels of nutrition. Wool samples were obtained from two sheep pen-fed at the same level of nutrition (Reis, 1965*a,b*). One sheep

(E 113; a high-producer of wool) grew wool of a low sulphur content (3.00%), whereas the other (E 123; a low-producer of wool) provided wool of comparatively high sulphur content (3.65%). It was found that the latter wool contained more high-sulphur protein (25% compared with 20%) and specifically more of the proteins in peaks C and D than did the wool of lower sulphur content (Fig. 9*a*). Electrophoretic analysis at pH11.0 of peak D protein isolated from these wool samples (Fig. 9*b*) showed that wool from sheep E 113 contained almost none of peak D2 protein whereas wool from sheep E123 contained a substantial amount.

(c) Seasonal variation in sulphur content of wool from grazing sheep. Wool samples, clipped from grazing animals at 6-weekly intervals throughout a year, were analysed for sulphur content. Wool from the same animal showed considerable variations in this respect, the range for an individual animal being from 3.06 to 3.74%. These seasonal variations are most probably due to variable nutrition (Reis & Williams, 1965). High-sulphur proteins were isolated from wool samples at the

Fig. 7. Moving-boundary electrophoretic patterns of peak D high-sulphur proteins run at pH11.0 showing the differences between control (i) and sulphur-enriched (ii) wools produced by (a) Merino sheep SC8, (b) English Leicester sheep 3307 and (c) English Leicester $\times$ Merino sheep 1390. The abomasal supplements provided and the sulphur contents of the wools are given with Figs. 2 and 4.

Fig. 8. Moving-boundary electrophoretic patterns of high-sulphur proteins isolated from wools of different sulphur contents produced by Merino sheep E109 under different levels of nutrition: (i) low-nutrition wool (3.04% of S); (ii) high-nutrition wool (3.65% of S). (a) Electrophoresis at pH4.5; (b) electrophoresis at pH10.0.

extremes of the ranges and were run electrophoretically at pH4.5 and 10.0 (Fig. 10): the increase in sulphur content in wool is paralleled by an increase

Fig. 9. Moving-boundary electrophoretic patterns of high-sulphur proteins isolated from wools of different sulphur content produced by two Merino sheep receiving the same level of nutrition. The sheep had equal food intakes but differed in having a high production of wool of 3.00% S content (sheep E113) (i) and a low production of wool of 3.65% S content (sheep E123) (ii). (a) Electrophoresis at pH4.5; (b) electrophoresis of peak D fraction at pH11.0.

Fig. 10. Moving-boundary electrophoretic patterns of high-sulphur proteins isolated from wool samples of different sulphur content clipped from Merino sheep 773 at different times during the year: (i) wool of 3.06% S content; (ii) wool of 3.74% S content. (a) Electrophoresis at pH4.5; (b) electrophoresis at pH10.0.

in the proportion of peaks C and D and by an increased proportion of peak D2 protein.

DISCUSSION

As the result of this and previous work (Gillespie *et al.* 1964) it is now well established that the increased sulphur content of wool produced during

the abomasal infusion of sulphur-containing amino acids into sheep can be accounted for by an increased synthesis of those high-sulphur proteins that fall within the mobility ranges of peaks C and D. In most of the English Leicester $\times$ Merino sheep used in the present study the increased synthesis of peak D protein, accounting for about 60–70% of the total, is largely confined to the formation of the proteins of peak D2 that are not present, or present to only a limited extent, in the controls.

The protein material in peak D2 differs from the other high-sulphur proteins by its higher content of *S*-carboxymethylcysteine, its lower content of glycine, alanine and leucine and its extremely low content of phenylalanine and aspartic acid. The presence of peak D2 proteins in sulphur-enriched wool, produced during abomasal infusions, is sufficient to account for most of the changes that take place in the amino acid composition of the high-sulphur proteins (Table 3). That these proteins are not produced by an abnormal process is shown by their occurrence in wool of high-sulphur content produced by sheep on normal diets, both indoors and in the field. The nature of the changes in the proteins in peak C has not yet been examined.

Less peak D2 protein was produced by English Leicester (3307) and Merino (SC8) sheep than might be expected from the extent of sulphur-enrichment of the wool, although electrophoresis at pH 4.5 showed the expected proportional increases in the proteins of peaks C and D. Although the reason for this is unknown, two explanations seem feasible. During sulphur-enrichment these sheep may produce mostly additional normal peak C and D proteins; it is also possible that peak D1 and D2 proteins are produced, but with mobilities so close that they cannot be adequately resolved by electrophoresis at pH 11.0. The different response of these two sheep is probably a reflection of individual differences rather than differences between breeds. Thus Merino sheep E123 (Fig. 9b), although producing wool enriched to only a moderate degree, produced as much D2 fraction as did an English Leicester $\times$ Merino sheep (1390) whose wool was enriched to a similar extent (Fig. 5c). However, peak D2 proteins are always produced during the process of sulphur-enrichment, regardless of the type of abomasal supplement given (methionine, cysteine or casein) or the breed of sheep, although there may be individual differences in the magnitude of the response.

In the three experiments involving normally fed sheep, the higher the sulphur content of the wool, the greater was the amount of peak D2 protein it contained. It is thus reasonable to suppose that this is a generally occurring phenomenon. To what extent changes in this protein fraction quantitatively account for the normally occurring variations

in the sulphur content of wool cannot yet be answered.

It has been shown above that when Merino sheep of different wool-producing capacity were provided with near identical food intake and hence of sulphur-containing amino acid intake, the faster-growing wool contained much less sulphur and peak D2 protein than the slow-growing wool. A possible hypothesis to explain this would be that there is a lower limit to the sulphur content of wool provided by a basic structure of microfibrillar protein plus a minimal amount (about 20%) of high-sulphur protein. The balance between the rate of wool growth and the amount of cysteine available to the follicles will determine the amount of sulphur in excess of the minimum required for this essential wool structure. It is probable that this excess of sulphur can be incorporated into wool as additional high-sulphur components, and of these the proteins of peak D2 provide a major fraction. We thus have evidence for the existence of an unusual biological control mechanism that may play a part in the formation of α -keratins generally.

The follicle presumably has all the necessary synthetic mechanisms for making D2 proteins but these do not function, at least to a detectable extent, until the sulphur-containing amino acid concentration reaches a particular value. It is not possible at present to define this concentration or the site where it is measured. However, this is an unusual control mechanism in protein synthesis and appears to be of a type not observed before. The control may be exercised either at the follicle or elsewhere in the body, perhaps on the formation of a hormone. If the site of action is at the follicle level, an inductive process or other direct control on the protein-synthetic mechanism may be involved. Alternatively, two not mutually exclusive ways in which mass action effects could occur can be visualized: (1) It is thought that cysteine penetrates the suprabulbar region of the follicle and then diffuses distally (Downes, Lyne & Clarke, 1962). A concentration gradient of cysteine would thus be set up, the slope of which would depend on the level of cysteine uptake by the follicle and the rate of metabolism. The proteins of peak D2 are probably among the last to be synthesized as the fibre moves along the follicle (Downes, Ferguson, Gillespie & Harrap, 1966). It is reasonable to suppose therefore that, at low levels of cysteine uptake or at high rates of metabolism, only traces of cysteine may reach this synthetic region. (2) If, as has been suggested (Gillespie, 1963b, 1965), high-sulphur proteins from keratins are synthesized by the stepwise addition of sulphur-rich peptides to precursors, then it might be expected that under conditions of abundant supply of cysteine this addition reaction would be pushed through to

produce the very sulphur-rich components of the D2 fraction.

We thank Miss C. M. Tomlinson and Mr D. A. Tunks for their skilled technical assistance.

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