A STUDY OF THE PROTEINS OF THE WOOL FOLLICLE

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Summary

A study has been made of the incorporation at different levels in the developing fibre of ^{35}S into the two main protein fractions of wool. The proteins have been studied as the *S*-carboxymethyl derivatives rather than as the oxidized derivatives previously investigated. The results obtained give further support for a mechanism of synthesis which involves two stages. However, the incorporation of some ^{35}S into the low-sulphur fraction of the keratinized fibre only 24 hr after the injection of [^{35}S]cystine is somewhat surprising and possible explanations for this have been considered. A detailed comparison has been made of the proteins extracted from the unkeratinized portions of wool roots by 8M urea with those which can be extracted from the keratinized residue with urea–thioglycollate. As might be expected the latter proteins were very similar to those isolated from wool itself. The group of urea-soluble, high-sulphur proteins was different in containing considerable amounts of protein lower in both molecular weight and sulphur content than the comparable fraction from the fully keratinized wool. The possibility is discussed that some of these urea-soluble, high-sulphur proteins may be precursors of those in the fully keratinized fibre, conversion taking place by a process of sulphur enrichment.

I. Introduction

Comparatively little is known about the chemical composition and properties of the proteins in wool follicles and in particular of the relation of these proteins to those in wool itself. Rogers (1959), making one of the few studies in this field, used wool roots isolated by the wax-sheet method (Ellis 1948) as a source of protein. He solubilized the prekeratins by treatment with 8M urea and, after conversion to *S*-carboxymethyl kerateines by reaction with iodoacetate, was able to separate them into two fractions which differed in sulphur content. The low-sulphur containing fraction was, in general, similar in composition to the analogous fraction obtained from reduced wool but the sulphur-rich fraction showed greater differences and, in particular, contained less sulphur than the corresponding high-sulphur proteins isolated from fully keratinized wool (Corfield, Robson, and Skinner 1958; Gillespie and Simmonds 1960).

The experiments described here were undertaken with two main aims. The first was to make more detailed comparisons of the proteins which can be isolated from wool roots with those isolated from wool grown by the same sheep just before the harvesting of its wool roots. Valid comparisons are otherwise impossible because of the variations which can occur in the relative amounts of low- and high-sulphur

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fractions and in the proportions of different components in the latter fraction even in wool grown by the same sheep at different times (Gillespie, Reis, and Schinckel 1964). The second aim was to test further the hypothesis of Downes, Sharry, and Rogers (1963) that soon after injecting [^{35}S]cystine into a sheep the specific activity of the sulphur should be higher in the high-sulphur than in the low-sulphur proteins of the newly synthesized labelled wool. This hypothesis follows from the assumption that keratinization in the wool follicle is a two-stage synthesis in which the proteins of the wool matrix (high-sulphur proteins) are the last to be synthesized (Mercer 1961). Downes, Sharry, and Rogers (1963) have already found supporting evidence for this hypothesis from the difference in the specific activity of the sulphur in the α- and γ-keratoses (oxidized low and high-sulphur proteins respectively) (Alexander and Earland 1950; Thompson and O'Donnell 1959) prepared from the urea-insoluble fraction of the wool roots. In the present work the reductive method of Harrap and Gillespie (1963) has been used because the proteins after alkylation are easier to characterize by physicochemical means.*

II. Materials and Methods

(*a*) *Chemicals*

L-[^{35}S]Cystine was obtained from the Radiochemical Centre, Amersham, England. The doses were prepared and the radiochemical purity was checked as described by Downes and Lyne (1961).

(*b*) *Preparation of ^{35}S-Labelled Wool Roots*

Wool roots were prepared from three English Leicester $\times$ Merino crossbred rams. The three preparations were studied separately, the data being referred to as experiments 1, 2, and 3. The sheep had been maintained with a constant food intake for several months before the experiments were performed. Thus, wool of reasonably constant composition was probably produced by each sheep during the few weeks preceding each experiment.

A dose (as given in Table 1) of L-[^{35}S]cystine was injected intravenously into each sheep. Then, in order to assess the extent of incorporation of ^{35}S into the keratinized wool root proteins, staples of wool were plucked 17–24 hr after injection. After measuring the total activity of the plucked fibres, unkeratinized proteins were removed by extraction with 8M urea and the residual activity determined. The data in Table 1 shows the proportion of ^{35}S which has been incorporated into keratinized proteins in the plucked fibres. Each sheep was then (within 10 min) anaesthetized by the intravenous administration of sodium pentobarbitone, killed, and immediately skinned. The wool was clipped from the skin and the remaining (short) fibres with their attached roots were harvested by the wax-sheet method (Ellis 1948; Rogers 1959). The complete operation took about 1·5 hr from the time the animal was killed. The relevant data for each of the three sheep involved are given in Table 1.

* Throughout this text the term high-sulphur protein refers to the material soluble at pH 4·4 at ionic strength 0·5, whereas the low-sulphur fraction is that which is insoluble under these conditions.

(c) *Preparation of Proteins*

For each of experiments 1, 2, and 3, three main protein preparations were made which were then further fractionated by precipitation at pH 4·4. The wool roots were extracted with 8M urea as shown in Table 2 following essentially the methods of Rogers (1959). The urea-soluble material, shown as L in Table 2, is very rich in –SH groups and appears to be derived from the fibrillation zone of the wool root whereas the urea-insoluble material, M in Table 2, contains very few –SH groups and consists mainly of keratinized shafts with some inner root sheath material (Rogers 1959). In order to compare in more detail the urea-insoluble material with the fully developed fibre, both it and the wool ($\simeq$ 1 mm) closest to the skin, M(ii) and N in Table 2 respectively, were extracted with 6M urea which was 0·2M with respect to potassium thioglycollate. In experiment 3, part of the urea-insoluble wool root material, M(i) in Table 2, was separately extracted with 0·05M NaOH according to the procedures of Mercer (1961) and Downes (1965). This preparation was also further fractionated by precipitation at pH 4·4.

TABLE 1

DATA ON THE SHEEP AND EXPERIMENTAL CONDITIONS USED IN EXPERIMENTS 1–3

	Experiment 1	Experiment 2	Experiment 3
Sheep No.	1073	1096	1963
Body weight (kg)	47	57	94
Dose of L-[^{35}S]cystine	200 μc; 1·4 mg	25 μc; 0·8 mg	22 μc; 1·1 mg
Time of killing (hours after injection)	17	24	21
Mean percentage sulphur in wool	3·25	3·33	3·33
^{35}S in urea-insoluble components, as % of total ^{35}S in plucked fibres	37	56	34

(d) *Fractionation of the Extracted Proteins*

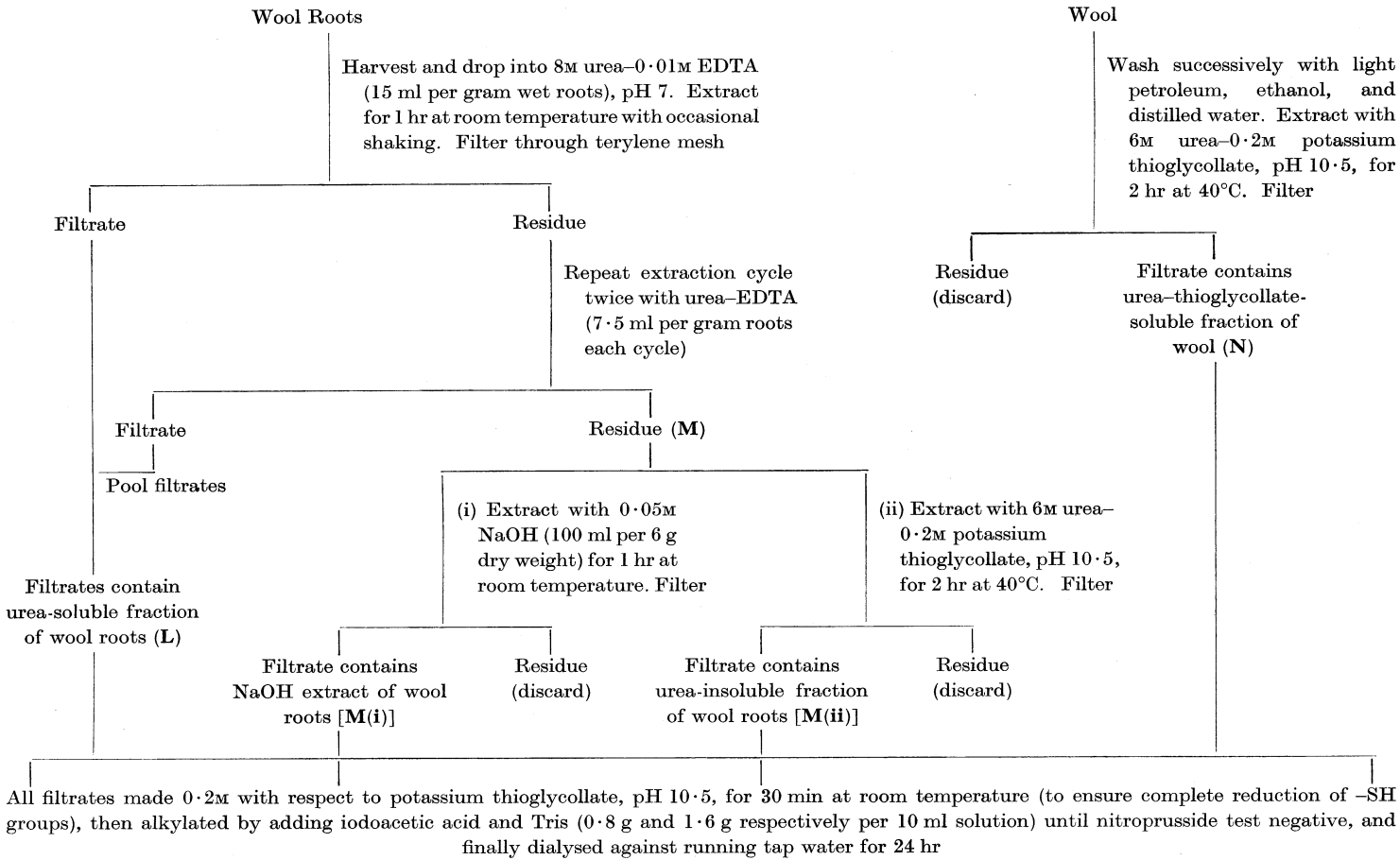
Low-sulphur proteins were precipitated from the dialysed extracts by adding an equal volume of acetic acid–sodium acetate buffer of ionic strength 1·0 at pH 4·4. High-sulphur proteins from the supernatant were dialysed free of salts and recovered by freeze-drying. The precipitates of low-sulphur proteins were reprecipitated three times by alternately dissolving them in saturated sodium borate and then precipitating with the pH 4·4 buffer. The final precipitate was dissolved in borate, dialysed, and freeze-dried.

(e) *Chromatography*

Freeze-dried high-sulphur protein (250 mg) was dissolved in acetic acid–sodium acetate buffer of ionic strength 0·01 at pH 4·5 and loaded onto a 3 $\times$ 8 cm column of DEAE-cellulose which had been equilibrated with this buffer. Fractions were eluted with a linear gradient of sodium chloride in the acetate buffer. In some cases the initial buffer was run through until any weakly bound protein was eluted and then the gradient was commenced. Although the gradient used sufficed to elute all the components of the high-sulphur proteins of fully keratinized wool (Gillespie 1963),

TABLE 2

PREPARATION OF SOLUBLE PROTEINS FROM WOOL AND WOOL ROOTS



the urea-soluble wool root proteins contained material which was not completely eluted, and in order to remove this completely, 1·0M NaCl was run through the column after the gradient had been completed. Fractions were collected in a fraction collector, and the elution profile was traced with a continuous recording ultraviolet absorption meter (Gilson Medical Electronics). The elution conditions are recorded on each profile.

(*f*) *Moving-boundary Electrophoresis*

Freeze-dried high-sulphur protein (200 mg) was dissolved in acetic acid–sodium acetate buffer (15 ml) of ionic strength 0·1 at pH 4·5 and the solution dialysed against 2 litres of this buffer for 24 hr. Moving-boundary electrophoresis was carried out in a standard Tiselius apparatus (LKB, Stockholm) at a protein concentration of 1·0–1·2% with a voltage gradient of 7·2 V/cm for 3 hr.

(*g*) *Molecular Weight Determination*

Molecular weights were determined by equilibrium centrifugation employing the Spinco model E ultracentrifuge with schlieren optics. The sedimenting columns were 1 mm in length and the speeds selected were such that the concentration at the mid-point of the column equalled the initial concentration (C), enabling the determination of the apparent weight-average molecular weight ($M_{app.}$) at this point by the method of Yphantis (1960). By the use of a wedge cell, two concentrations could be run simultaneously. The buffer used was 0·0125M Na_2HPO_4–0·0125M NaH_2PO_4–0·3M NaCl, pH 6·9, and runs were made at 20°C. Values of molecular weight reported are those obtained by plotting $1/M_{app.}$ versus C and extrapolating to zero concentration. Solutions in experiment 1 were given a preliminary centrifugation for 30 min at 144,000 g in a Spinco model L centrifuge to remove very high-molecular weight aggregates, but in experiments 2 and 3 the whole solutions were used as prepared. Most solutions examined were obviously heterogeneous, containing some high molecular weight material which produces a sharp turn-up in the schlieren boundary near the bottom meniscus.

(*h*) *Amino Acid Analysis*

Freeze-dried protein (30 mg) was hydrolysed *in vacuo* in Thunberg tubes with 6M HCl (5 ml) for 24 hr at 108°C. The HCl was removed by freeze-drying and the hydrolysate analysed for its content of amino acids by chromatography using a Beckman-Spinco automatic amino acid analyser.

(*i*) *Sulphur Analysis*

The sulphur contents were determined by the Schöniger (1954) oxygen-flask method, as described by Reis and Schinckel (1963).

(*j*) *Radioactivity Measurements*

Infinitely thick samples (1 cm^2) of the protein fractions were prepared in duplicate and assayed with an end-window Geiger–Müller counter as described by Downes, Sharry, and Rogers (1963). Samples with a specific activity of 1 μc/g gave

TABLE 3

SPECIFIC ACTIVITIES AND SULPHUR CONTENTS OF VARIOUS PROTEIN FRACTIONS FROM WOOL ROOTS

See Table 2 for method of preparation of the various fractions

Preparation	Experiment 1			Experiment 2			Experiment 3		
	(A) Specific Activity*	(B) Sulphur Content (%)	Ratio A/B	(A) Specific Activity	(B) Sulphur Content (%)	Ratio A/B	(A) Specific Activity	(B) Sulphur Content (%)	Ratio A/B
Urea-soluble									
Low-sulphur fraction	5·3	1·5	3·6	4·6	1·5	3·0	3·5	1·6	2·2
High-sulphur fraction	15·1	3·6	4·2	8·8	3·8	2·3	9·1	2·9	3·1
Urea-insoluble									
Low-sulphur fraction	1·5	1·8	0·8	1·2	1·9	0·6	0·6	2·2	0·3
High-sulphur fraction	12·2	4·8	2·5	8·7	5·5	1·6	5·3	5·3	1·0
Urea-insoluble (NaOH extract)									
Low-sulphur fraction	—	—	—	—	—	—	2·2	1·2	1·8
High-sulphur fraction	—	—	—	—	—	—	21·4	5·0	4·3

* Counts per minute at infinite thickness (1 cm^2 samples) divided by the number of microcuries injected.

about 700 counts/min and the background rate was 12 counts/min. At least 2500 counts were registered for each sample. The proportion of ^{35}S present in 8M urea-insoluble material in the plucked fibres was determined by direct liquid-scintillation counting, as described by Downes and Till (1963).

(*k*) *Starch-gel Electrophoresis*

Starch-gel electrophoresis was carried out as described by Ferguson and Wallace (1963) and Ferguson (1964).

III. Results

(*a*) *Recovery of Extracted Protein*

From a typical sheepskin about 30 g (18 g dry weight) of wool roots were harvested, of which about 3–4 g were recovered as urea-soluble proteins (fraction L, Table 2). It was difficult to make a quantitative material balance in the presence of high urea concentrations in the extractant because during dialysis (18/32 Visking cellulose tubing), not only were there losses of salts, amino acids, and peptides, but also of some of the high-sulphur protein components of lower molecular weight. Thus the yield and composition of this fraction depends critically on the dialysis history of the preparation and showed considerable variation between the three experiments.

The urea-soluble, high-sulphur fractions were contaminated with nucleic acid, as indicated by the ratio of optical densities at 260 and 280 mμ. The contamination was variable among preparations and was particularly large in experiment 2.

The urea-insoluble wool root proteins [fraction M(ii)] showed much less variability in composition from one experiment to another than did the urea-soluble fractions.

(*b*) *Specific Activities of the Protein Fractions*

The specific activity measurements on the fibres plucked just before the sheep were killed showed that 34–56% of the ^{35}S in the plucked fibres was present in 8M urea-insoluble components (Table 1).

The specific activity and sulphur content of each protein fraction from the wax-sheet harvests are given in Table 3, which also shows the calculated specific activity of the sulphur (per microcurie injected) in each fraction. Since different amounts of ^{35}S were injected in the three experiments, the counting rates (corrected for decay) have been divided by the number of microcuries injected.

The ratio of the specific activities of sulphur in the urea-soluble (fraction L) low-sulphur proteins to those in the urea-soluble (fraction L) high-sulphur protein varied around unity in all three experiments (Table 3). However, the specific activities of sulphur in the urea-insoluble [fraction M(ii)] low-sulphur protein was only about one-third that in the urea-insoluble [fraction M(ii)] high-sulphur proteins regardless of whether they were solubilized by urea–thioglycollic acid or sodium hydroxide. In experiment 3 the fraction containing sulphur with the highest specific activity was the high-sulphur fraction extracted by sodium hydroxide [fraction M(i)] from the urea-insoluble material.

(c) *Properties of Extracted Proteins*

The urea-insoluble high-sulphur protein fractions from both wool (fraction N) and wool roots [fraction M(ii)] were essentially identical and were similar to the previously characterized high-sulphur proteins from wool (Gillespie 1965). No distinction therefore will be made between these two fractions in the description which follows.

TABLE 4

AMINO ACID COMPOSITIONS AND MOLECULAR WEIGHTS OF HIGH-SULPHUR PROTEINS FROM WOOL AND WOOL ROOTS FROM THE SAME SHEEP

Analytical data given as amino acid nitrogen as percentage of total nitrogen

Amino Acid	Wool Root (expt. 1)		Wool (expt. 1)	Wool Root (expt. 2)		Wool Root (expt. 3)	
	Urea Soluble	Urea Insoluble	Urea Insoluble	Urea Soluble	Urea Insoluble	Urea Soluble	Urea Insoluble
Lysine	2·56	0·81	0·83	1·41	0·67	3·26	1·96
Histidine	1·40	1·56	1·43	0·97	1·00	1·40	1·35
Ammonia	9·64	7·82	7·67	8·26	6·20	10·6	7·41
Arginine	8·20	15·1	14·6	11·9	16·7	10·3	18·6
SCMC*	8·15	14·5	13·9	11·2	17·7	8·21	17·0
Aspartic acid	3·65	1·75	1·89	2·00	1·51	2·70	1·96
Threonine	5·82	7·23	7·16	6·01	7·32	4·80	6·56
Serine	7·95	9·25	8·87	8·10	8·62	6·86	7·92
Glutamic acid	5·42	5·49	5·67	5·43	5·30	5·24	5·35
Proline	6·62	9·19	9·25	6·77	9·49	5·76	9·24
Glycine	5·06	4·08	4·50	5·02	4·03	4·97	4·00
Alanine	2·54	1·85	1·99	2·14	1·95	2·69	2·14
Valine	3·71	3·72	3·91	3·50	3·87	3·33	3·87
Methionine	Trace	0·00	0·00	Trace	0·00	0·16	0·00
Isoleucine	2·00	2·00	2·09	2·02	2·18	1·95	2·18
Leucine	2·61	2·10	2·29	2·42	2·21	2·68	2·33
Tyrosine	0·58	0·56	0·69	1·32	1·24	1·16	1·41
Phenylalanine	1·21	1·00	1·15	1·22	0·92	1·14	1·08
Total	77	88	88	80	91	77	94
Molecular weight	11,000	24,500	—	18,500	28,000	17,000	21,000

* *S*-carboxymethylcysteine.

The urea-insoluble, high-sulphur proteins were found to have molecular weights of the order of 21,000 to 28,000 (Table 4), values which are slightly weighted by the presence of a small amount of highly aggregated protein in the solutions. The amino acid compositions are typical of high-sulphur proteins (Table 4) notably in their high content of *S*-carboxymethylcysteine (SCMC), proline, and serine, and their comparatively low content of lysine, histidine, aspartic acid, and phenylalanine. The electrophoretic components range in mobility (at pH 4·5) from *c.* $-3 \cdot 5$ to -7 cm^2 sec^{-1} V^{-1} and in molecular weight from about 16,000 to 28,000 (Gillespie 1965).

The urea-soluble, high-sulphur proteins are very different in many respects from those insoluble in urea. They contain electrophoretic components with mobilities which range from about 0 (as evidenced by the abnormally large δ anomaly) to about

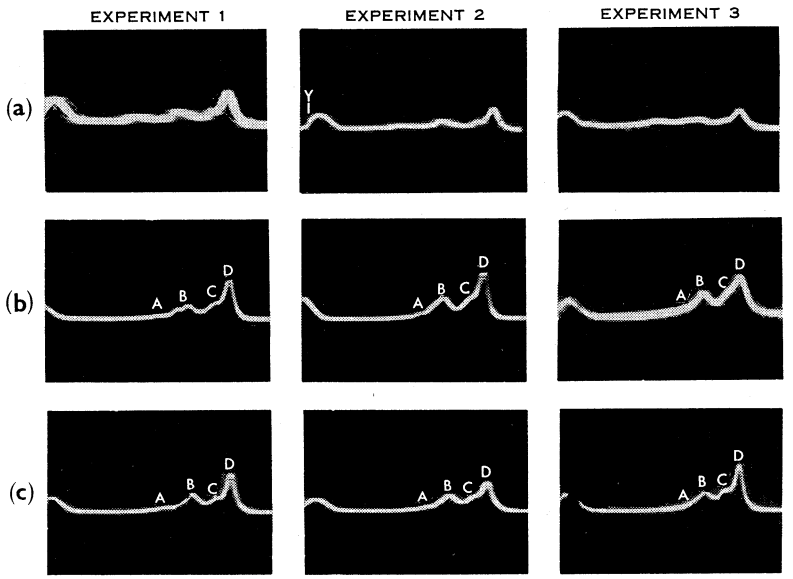


Fig. 1.—Electrophoretic patterns (ascending boundary) of high-sulphur protein run in acetic acid–sodium acetate buffer of ionic strength 0·1 at pH 4·5. Protein concentration 1·0–1·2%. Electrophoresis was for 180 min with a voltage gradient of about 7·2 V/cm. High-sulphur proteins originated from the following sources: (*a*) Urea-soluble fraction of wool roots; (*b*) urea-insoluble fraction of wool roots; (*c*) wool.

—7. There is also a small quantity of material which is positively charged (at pH 4·5) and which moves in the opposite direction to the bulk of the protein (peak Y, Fig. 1),

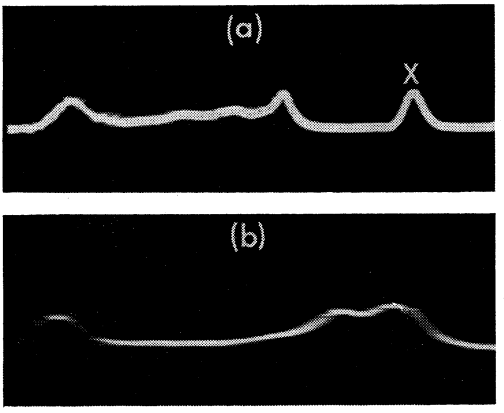


Fig. 2.—Electrophoretic patterns (ascending boundary) of high-sulphur protein run in acetic acid–sodium acetate buffer of ionic strength 0·1 at pH 4·5. Protein concentration 1·0–1·2%, voltage gradient about 7·2 V/cm. (*a*) Urea-soluble fraction of wool roots. Electrophoresis for 120 min. (*b*) Urea-insoluble fraction of wool roots prepared by solubilization with 0·1M NaOH. Electrophoresis for 180 min.

and a component with a very high negative charge which has moved out of the cell in the patterns of Figure 1 but which is marked as peak X in Figure 2(*a*). This material

moves faster than any component in wool and after isolation by preparative electrophoresis gives a spectral curve characteristic of nucleic acid.

Although there are many similarities in the amino acid compositions of the urea-soluble and urea-insoluble high-sulphur proteins (Table 4) the former may be distinguished by their higher content of lysine and aspartic acid and their lower content of SCMC.

The urea-soluble, high-sulphur fractions are also much lower in molecular weight (Table 4) than the urea-insoluble proteins. The three preparations range in molecular weight from 11,000 to 18,500. These values are dependent both on the level of contamination with high-molecular weight nucleic acid and on the extent to which low-molecular weight proteins have been removed by dialysis.

Major differences were also found between the high-sulphur proteins of the urea-soluble (fraction L) and urea-insoluble [M(ii) and N] fractions in their starch-gel electrophoresis patterns (Plate 1, Fig. 1). To facilitate comparison the gel patterns have been arbitrarily divided into six regions (α–ζ). The differences in intensities in these regions between the fractions have been subjectively assessed and are presented in the following tabulation:

	α	β	γ	δ	ϵ	ζ
Urea-insoluble fractions of wool and wool roots	Trace	++	−	+++++	Trace	+
Urea-soluble fraction of wool roots	+++++	+++	+	+	+	−

The urea-insoluble, high-sulphur protein fraction of wool roots (expt. 3) which was extracted with dilute NaOH [i.e. fraction M(i)] gave a rather diffuse moving-boundary electrophoretic pattern [Fig. 2(*b*)] with the mobility of the fastest component close to that of peak C in the pattern of a normal high-sulphur protein fraction from wool (Fig. 1). The weight-average molecular weight of this fraction (determined by equilibrium ultracentrifugation) was about 15,000.

(*d*) *Fractionation of Urea-soluble, High-sulphur Proteins from Wool Roots*

In order to characterize the components of the urea-soluble, high-sulphur proteins, the preparations from experiments 2 and 3 were chromatographed on DEAE-cellulose at pH 4·5. The elution profiles obtained and the fractions collected are shown in Figures 3(*a*) and 3(*b*) respectively. In order to increase the resolution of components in experiment 3, the middle fraction [II, Fig. 3(*b*)] was rechromatographed [Fig. 3(*c*)].

Fractions A–E cut as indicated by the arrows on the elution profiles [Figs. 3(*a*), 3(*c*)]* were characterized by the determination of molecular weights and amino acid compositions (Table 5). Fractions B, C, D, and E from experiment 3 were also characterized by electrophoresis in starch gel (Plate 1, Fig. 3).

A sufficient quantity of fraction A could not be prepared for a more complete study but as this material was not bound to the resin it is reasonable to suppose that

* These fractions A–E from experiments 2 and 3 would not be expected to be identical because of the somewhat different elution conditions used in their chromatographic separation.

it represents the proteins of zero and positive mobility observed in moving boundary runs at pH 4·5 (Fig. 1). Chromatographic peak F [Fig. 3(*a*)] contains material with a molecular weight of about 50,000, a value higher than that of any of the high-sulphur protein fractions found in wool, and also an extremely high extinction coefficient at 276 mμ. Moreover, since the E_{260}/E_{276} ratio of this fraction was greater than 2, it is probably rich in nucleic acid, as was the material in moving-boundary peak X [Fig. 2(*a*)].

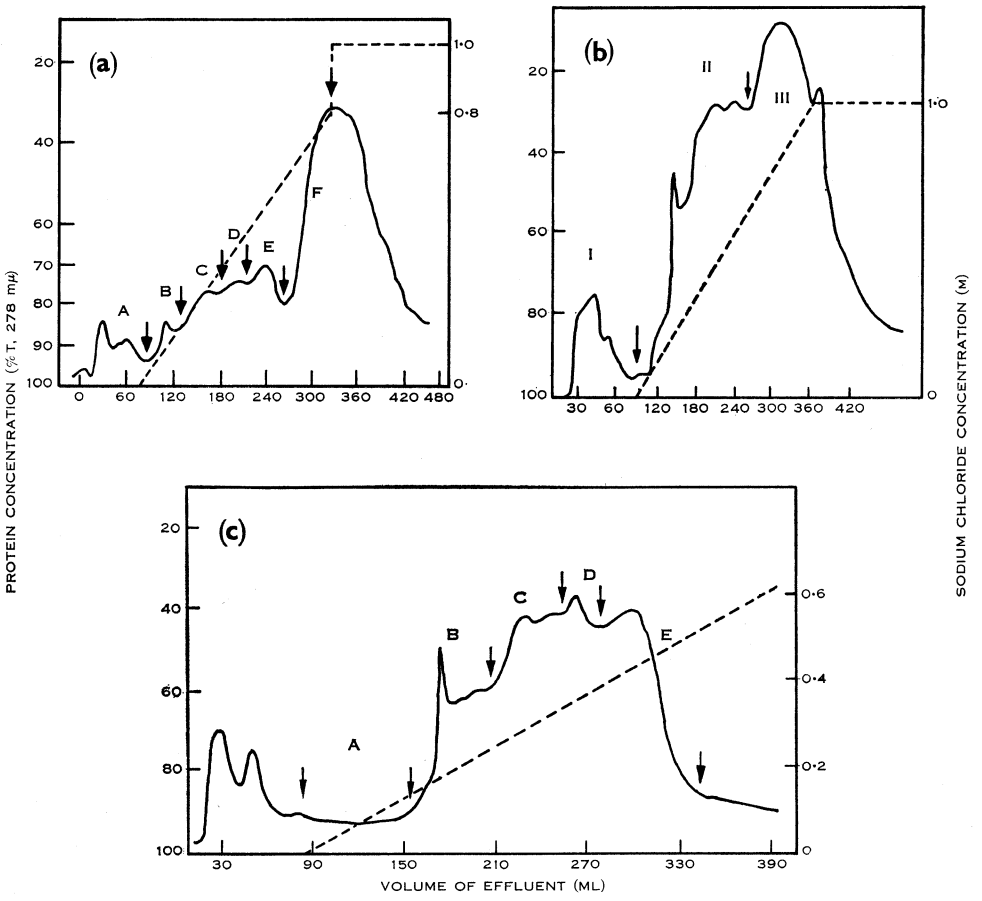


Fig. 3.—Elution profiles obtained by the chromatography of urea-soluble high-sulphur proteins of wool roots on DEAE-cellulose at pH 4·5 in sodium acetate–acetic acid buffer of ionic strength 0·01. The sodium chloride gradient employed is shown on each graph. (*a*) Preparation from experiment 2. (*b*) Preparation from experiment 3. (*c*) Refractionation of fraction II from (*b*).

IV. Discussion

There are very striking differences between the moving-boundary electrophoretic patterns of the urea-soluble (fraction L) and urea-insoluble [fraction M(ii)] high-sulphur proteins from the wool root (the latter being indistinguishable from the equivalent fraction of the fully developed fibre); this is particularly noticeable

in the very fast and very slow components in the urea-soluble fraction. The large δ anomaly (especially evident in experiment 1) suggests that at pH 4·5 there is a considerable amount of protein with zero net charge; the preparation from experiment 2 also contained material with a small net positive charge which migrated towards the cathode. Neither of these components nor the large quantity of material of intermediate mobility −2 to −3·5 $cm^2 sec^{-1} V^{-1}$ in the urea-soluble fraction has a counterpart in the urea-insoluble fraction of either wool or wool root.

TABLE 5

AMINO ACID COMPOSITIONS AND MOLECULAR WEIGHTS OF CHROMATOGRAPHIC FRACTIONS OF UREA-SOLUBLE, HIGH-SULPHUR PROTEINS FROM WOOL ROOTS

Analytical data given as amino acid nitrogen as percentage of total nitrogen

Amino Acid	Experiment 2 [see Fig. 3(*a*)]				Experiment 3 [see Fig. 3(*c*)]			
	A	B	D	E	B	C	D	E
Lysine	4·00	4·04	0·80	0·50	7·93	5·69	4·31	2·10
Histidine	1·89	1·56	0·90	0·73	2·93	2·28	2·99	0·74
Ammonia	—	9·48	5·71	8·71	7·09	7·31	7·39	10·9
Arginine	14·4	15·6	17·0	15·2	14·7	14·1	14·6	14·4
SCMC	3·59	6·24	12·3	16·9	4·26	6·83	9·87	16·4
Aspartic acid	2·31	3·31	2·65	1·04	4·46	4·41	4·46	1·91
Threonine	3·89	5·32	6·74	7·64	4·29	6·45	6·80	7·70
Serine	7·92	8·51	6·91	8·88	7·22	8·99	7·19	10·7
Glutamic acid	3·91	6·38	4·37	7·02	6·27	6·14	5·18	8·74
Proline	6·00	6·53	8·14	7·63	5·46	6·54	8·38	7·45
Glycine	7·61	6·00	3·57	5·61	6·41	4·81	3·86	6·00
Alanine	4·08	2·65	1·80	2·33	4·04	3·18	2·70	5·08
Valine	3·98	5·40	3·89	3·15	4·48	4·98	4·26	3·45
Methionine	0·35	0·23	0·00	0·00	0·49	0·21	0·00	0·00
Isoleucine	2·15	2·56	1·98	2·49	2·58	2·14	2·39	2·71
Leucine	3·00	3·66	2·82	1·58	4·48	4·31	4·13	1·97
Tyrosine	1·34	1·11	1·04	1·65	1·19	1·61	1·23	1·29
Phenylalanine	1·86	1·40	0·82	0·85	1·38	1·37	1·61	0·80
Total	72	90	81	92	87	89	89	90
Molecular weight	—	7,000	14,300	23,000	9,000	11,400	12,800	19,500

The proportions of these components in the wool root preparations were very variable from one experiment to another. Since there appears to be a correlation between electrophoretic mobility and molecular weight it is not surprising that the molecular weights of the preparations from different experiments were also very variable (Tables 4 and 5).

It can be seen that striking differences between the urea-soluble and urea-insoluble high-sulphur proteins from wool root are found in the regions α and δ of the gel electrophoresis patterns (Plate 1, Figs. 1 and 2; see also tabulation p. 328). The urea-soluble fraction is much richer in proteins running in the α region and the urea-insoluble fraction is very rich in components in the δ region. There are also

components in the β, γ, and ϵ regions which are confined to the urea-soluble fractions.

The results in Plate 1, Figures 1 and 2, illustrate the much greater complexity of the high-sulphur protein mixture in the unkeratinized (urea-soluble) wool root compared with that which may be isolated from wool.

Partial separations of the electrophoretic components have been made by DEAE-cellulose chromatography for preparations from experiments 2 and 3.

A study of the properties of these fractions helps to clarify the differences between the urea-soluble and urea-insoluble high-sulphur proteins, for it is apparent that the former preparations contain low-molecular weight proteins of such SCMC content and high lysine content that they can be classified with neither the high-sulphur nor the low-sulphur proteins. Thus fractions A and B [experiment 2, Fig. 3(*a*)] and B and C [experiment 3, Fig. 3(*c*)] represent a class of proteins which are not present in fully keratinized wool. They also appear to be the slow-moving components observed in moving-boundary electrophoresis experiments. On the other hand, fractions D and E [experiment 2, Fig. 3(*a*)] and E and probably D [experiment 3, Fig. 3(*c*)] have SCMC contents, amino acid compositions, and molecular weights which are more characteristic of typical high-sulphur fractions from wool.

The low-molecular weight, low-sulphur fractions are probably the starch-gel components which run in region α, as these gel components are not present in wool. This is supported by the starch-gel electrophoretograms of wool root high-sulphur fractions B–E of experiment 3 (Plate 1, Fig. 3) since fractions B and C, which are of low molecular weight (Table 5), contain much material running in region α.

These low molecular weight proteins which run in region α, together with others running in regions β, γ, and ϵ disappear as the fibre is keratinized [Fig. 3(*a*)]. At the same time new proteins apparently appear, most notably those which run in position δ. As the sulphur-rich proteins of moving-boundary peak D (Fig. 1) run mainly in the δ region of the starch-gel patterns (Plate 1, Fig. 2) it can be suggested that these sulphur-rich proteins are the last to be synthesized and are only incorporated at the level of keratinization. However, this conclusion could be invalidated if these proteins were in fact produced in a urea-insoluble form.

What is the fate of the proteins running in the starch-gel position α? They are low both in molecular weight and in sulphur content but they resemble the high-sulphur proteins in their high serine and proline contents. It is therefore possible that they are precursors which are converted to high-sulphur proteins by some sulphur-enrichment process (Rogers 1959; Gillespie 1963). However, it is also possible that they are cytoplasmic proteins which are withdrawn from the maturing cortical cells.

The experiments of Mercer (1961) and Downes (1965) suggest that the sodium hydroxide-extracted proteins contain all the protein components of the completed wool but that keratinization itself is not a complete at the level of the fibre from which they are extracted. Since these proteins are insoluble in urea some disulphide bonds have probably been formed. It is not certain whether the sodium hydroxide treatment solubilizes the lightly cross-linked fibres by breaking the disulphide bonds or whether

a small number of peptide bonds are also split. Large-scale breakage of peptide bonds is unlikely since only traces of protein can be extracted from the fully keratinized fibre under these conditions (Downes 1965). Moreover, the relatively high molecular weight of the sodium hydroxide-solubilized high-sulphur protein (about 15,000) also argues against large-scale breakage of peptide bonds.

A consequence of a two-stage mechanism of synthesis (Downes, Sharry, and Rogers 1963) would be that the sulphur in the sodium hydroxide-soluble high-sulphur proteins should have a higher specific activity than the sulphur in the urea-soluble proteins. The results (Table 2) show that this is so.

It does not seem profitable to compare the specific activities of these fractions with those of the proteins isolated by urea-thioglycollic acid extraction of the urea-insoluble residues because the values obtained for the latter depend on the amount of fully keratinized wool in the particular wax-sheet harvest. However, the relative specific activities of high-sulphur to low-sulphur fractions increased in the order: urea-soluble; sodium hydroxide-soluble; urea-insoluble; that is as one proceeds up the follicles.

In comparing these results (Table 2) with those of Downes, Sharry, and Rogers (1963), the relative specific activities of the proteins were of the same order (for wool roots prepared about 20 hr after injecting labelled cystine) even though in the present experiments reduced and alkylated proteins were used rather than oxidized protein. The similarity of the results after two such different separations provides further support for the two-stage synthetic mechanism.

A consequence of the hypothesis of Downes, Sharry, and Rogers (1963) that the low-sulphur proteins are entirely synthesized in the lower regions of the follicle and remain in the cells as they move up the follicle is that the urea-insoluble, low-sulphur protein should contain no radioactivity even 24 hr after the administration of labelled cystine. However, it is found that they have an appreciable radioactivity (Table 2). Possible explanations for this are:

(1) Triply reprecipitated low-sulphur protein may still contain labelled high-sulphur protein.

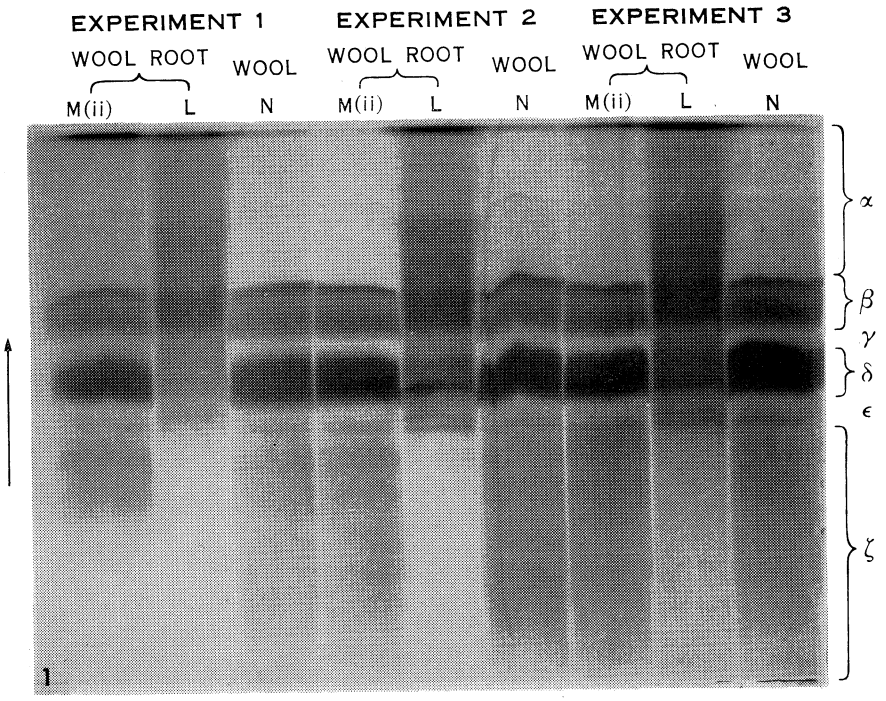
(2) The low-sulphur proteins are a complex mixture some of which may indeed be synthesized in the upper regions of the root.

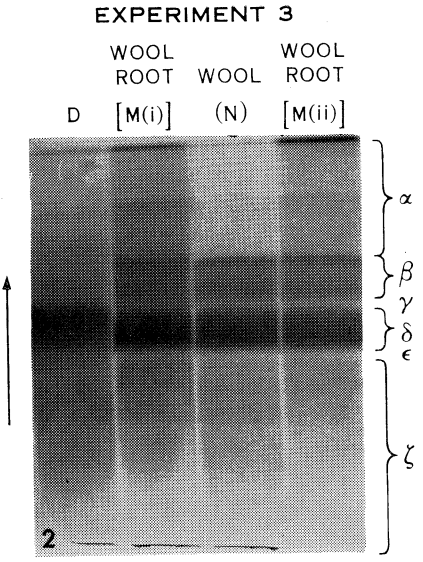
(3) The two-stage synthesis should perhaps be pictured as a merging of two different synthetic processes, the synthesis of the low-sulphur proteins diminishing as the follicle is ascended while the synthesis of the high-sulphur proteins increases to a maximum in the keratogenous zone, there being no sharp line of demarcation between them.

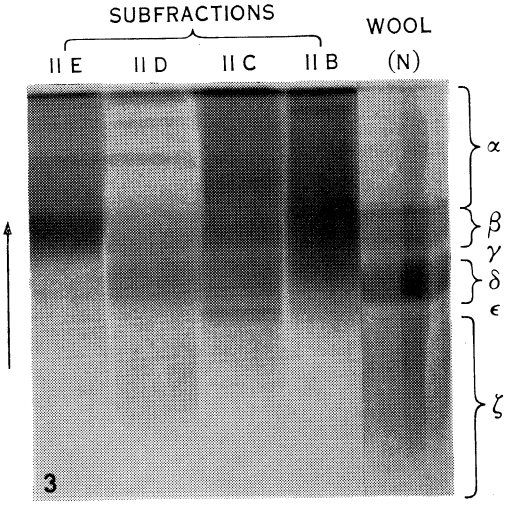
(4) There are indications that the distribution of sulphur in the low-sulphur proteins is such that there are sequences of amorphous high-sulphur protein-like material (Crewther and Harrap 1965 and unpublished data). It is possible that this is incorporated into the low-sulphur protein after the synthesis of the helical region.

Additional experimental data will be needed before a decision can be made between these alternative hypotheses.

PROTEINS OF THE WOOL FOLLICLE







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VI. References

Alexander, P., and Earland, C. F. (1950).—*Nature, Lond.* **166**: 396.
Corfield, M. C., Robson, A., and Skinner, B. (1958).—*Biochem. J.* **68**: 348.
Crewther, W. G., and Harrap, B. S. (1965).—*Nature, Lond.* **207**: 295.
Downes, A. M. (1965).—In "The Biology of the Skin and Hair Growth". (Eds. A. G. Lyne and B. F. Short.) (Angus and Robertson Ltd.: Sydney.)
Downes, A. M., and Lyne, A. G. (1961).—*Aust. J. Biol. Sci.* **14**: 120.
Downes, A. M., Sharry, L. F., and Rogers, G. E. (1963).—*Nature, Lond.* **199**: 1059.
Downes, A. M., and Till, A. R. (1963).—*Nature, Lond.* **197**: 449.
Ellis, W. J. (1948).—*Nature, Lond.* **162**: 957.
Ferguson, K. A. (1964).—*Metabolism* **13**: 985.
Ferguson, K. A., and Wallace, A. L. C. (1963).—*Recent Prog. Horm. Res.* **16**: 1.
Gillespie, J. M. (1963).—*Aust. J. Biol. Sci.* **16**: 259.
Gillespie, J. M. (1965).—In "The Biology of the Skin and Hair Growth". (Eds. A. G. Lyne and B. F. Short.) (Angus and Robertson Ltd.: Sydney.)
Gillespie, J. M., Reis, P. J., and Schinckel, P. G. (1964).—*Aust. J. Biol. Sci.* **17**: 548.
Gillespie, J. M., and Simmonds, D. H. (1960).—*Biochim. Biophys. Acta* **39**: 538.
Harrap, B. S., and Gillespie, J. M. (1963).—*Aust. J. Biol. Sci.* **16**: 218.
Mercer, E. H. (1961).—"Keratin and Keratinization: An Essay in Molecular Biology." (Pergamon Press: Oxford.)
Reis, P. J., and Schinckel, P. G. (1963).—*Aust. J. Biol. Sci.* **16**: 218.
Rogers, G. E. (1959).—*Ann. N.Y. Acad. Sci.* **83**: 408.
Schöniger, W. (1954).—*Mikrochim. Acta* **1954**: 74.
Thompson, E. O. P., and O'Donnell, I. J. (1959).—*Aust. J. Biol. Sci.* **12**: 282.
Yphantis, D. A. (1960).—*Ann. N.Y. Acad. Sci.* **88**: 58.

Explanation of Plate 1

In Figures 1–3, L, M(i), M(ii), and N refer to fractions of soluble proteins from wool and wool roots prepared as outlined in Table 2. The regions α–ζ are arbitrarily defined regions to facilitate comparison of the starch-gel patterns—see tabulation, p. 328

Fig. 1.—Comparison of starch-gel electrophoretic patterns of high-sulphur proteins from wool and wool roots obtained in three experiments.

Fig. 2.—Comparison of moving-boundary electrophoretic peak D protein from wool with the total urea-insoluble, high-sulphur proteins from both wool (N) and wool roots [M(ii)] and also the sodium hydroxide extract of urea-insoluble protein from wool roots [M(i)].

Fig. 3.—Starch-gel electrophoresis patterns of subfractions of urea-soluble, high-sulphur proteins from wool roots obtained by chromatography as indicated in Figure 3(*c*), compared with the total high-sulphur proteins from wool.