THE INFLUENCE OF SULPHUR-CONTAINING AMINO ACIDS ON THE BIOSYNTHESIS OF HIGH-SULPHUR WOOL PROTEINS

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Summary

The sulphur content of wool can vary within the range of about 2·7–4·2% depending on the diet of the sheep. The lower limit may represent a limiting fundamental structure for wool as it has not been possible to produce wool of sulphur content lower than 2·7% during sulphur-deprivation experiments. There is a highly significant linear relationship between the sulphur content of wool and its content of high-sulphur proteins. The major part of this variation in sulphur content is due to alterations in the extent of biosynthesis of proteins of extremely high sulphur content having about one-third of the amino acid residues present as half cystine. The biosynthesis of these proteins may be under separate metabolic control for they can be produced at maximum rate under conditions where the synthesis of other high-sulphur proteins is partly inhibited by a sulphur-deficient diet or by high levels of DL-methionine supplementation.

I. INTRODUCTION

The biosynthetic activities of wool follicles are influenced by the nutritional status of the sheep. This is manifested by major variations in the overall growth rate of the fibre and by changes in its composition, particularly its content of sulphur (Reis and Schinckel 1963, 1964; Reis 1965, 1967; Reis and Williams 1965; Reis and Tunks 1968). Most of the variation in sulphur content can be accounted for by the presence of varying amounts of certain proteins which are exceedingly rich in cystine residues, this residue accounting for about one-third of the total. The production of these proteins appears to be regulated by the amount of sulphur-containing amino acids (*S*-amino acids) available for metabolism in the sheep (Gillespie, Reis, and Schinckel 1964; Gillespie and Reis 1966; Gillespie, Broad, and Reis 1969). These sulphur-rich proteins will be referred to as the proteins of peak D2, from their position in moving boundary electrophoretograms (Gillespie and Reis 1966).

Both L-cysteine and DL-methionine given via the abomasum, stimulate the synthesis of D2 proteins (Gillespie and Reis 1966). In the present study, the synthesis of D2 proteins in response to varying amounts of these amino acids has been studied in more detail, with particular attention to the effect of large amounts (8–10 g/day) of cysteine and methionine, which in the latter case may inhibit overall synthesis of wool proteins (Reis 1967). Further, the effects of D-methionine and of the methionine hydroxy analogue (MHA)‡ on the synthesis of D2 proteins have been studied, as well

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‡ Calcium DL-2-hydroxy-4-methylthiobutyrate.

as the composition of wool proteins produced during the feeding of a sulphur-deficient diet.

In the present work an examination has been made of the relationship which exists between the sulphur content of wool and its content of high-sulphur proteins and of fractions of high-sulphur protein. These relationships have only been previously investigated semiquantitatively, but sufficient data have now been accumulated for a statistical analysis to be made. Sulphur-enriched wool is more resistant to solubilization by alkaline reducing solutions than is a control wool produced by the same animal and an explanation for this phenomenon has been sought.

No uniformly acceptable nomenclature has yet been devised for the high-sulphur protein components of wool. This is largely due to their extreme heterogeneity of composition and size. In this paper components resolved in moving boundary electrophoretic runs have been labelled, in order of increasing mobility and sulphur content, A, B, C, and D (at pH 4·5) and a, b, c, d, and D2 (at pH 10). In the text components are referred to as being of "lower sulphur content" (A,B), "higher sulphur content" (C,D), and highest sulphur content (D2), but at the moment no more specific identification is possible. Elsewhere the D2 fraction proteins, being in composition quite typical representatives of the high-sulphur proteins of many animal hairs and furs have also been referred to as the "ultra-high-sulphur proteins" (Gillespie and Broad 1969).

II. Materials and Methods

(a) *Experimental Procedures with Sheep*

The sheep (English Leicester × Merino crosses) were kept indoors and were fed individually. Wool was collected at intervals of 2 or 3 weeks from defined areas (*c.* 10 by 10 cm). Control wool was grown during the feeding of a diet of equal parts wheaten and lucerne chaff; sulphur-enriched wool was produced by supplementing this diet with *S*-amino acids infused directly into the abomasum (Reis and Schinckel 1963, 1964; Reis 1967).

Two sheep received a semipurified, sulphur-deficient diet consisting of alkali-extracted straw (60%), starch (22%), glucose (5%), molasses (5%), urea (6%), and a mineral mixture (2%), plus trace minerals and vitamins A and D3. The straw was prepared by soaking wheaten straw for 24 hr in sodium hydroxide (1·5 g/100 ml), followed by washing and drying. One sheep consumed 500 g, and the other 300 g/day, for 18 weeks; during the last 6 weeks of this period one sheep received a supplement of 5 g/day L-cystine in the diet and the other sheep received an equivalent amount of elemental sulphur (1·34 g/day) in the diet. The extracted straw contained 0·027% sulphur (0·053% before extraction) and supplied the sheep with about 80 and 50 mg/day sulphur respectively; an equivalent amount of sulphur may have been supplied by the molasses. Control wool from these sheep was grown during the feeding of 800 g/day of equal parts lucerne chaff and oats.

(b) *Preparation of Wool Samples*

The samples of wool were prepared by the procedures used by Gillespie and Reis (1966).

(c) *Sulphur Analysis*

The sulphur content of the wool samples was determined by an oxygen flask combustion technique (Reis and Schinckel 1963). The sulphur content of the alkali-extracted straw was determined by a modification of this technique, involving removal of interfering cations with Zeocarb 225 resin following combustion.

(d) Preparation of Soluble High-sulphur Proteins

High-sulphur proteins were extracted from the wool samples by alkaline reduction in the presence of urea as described previously (Harrap and Gillespie 1963; Gillespie 1964; Gillespie and Reis 1966).

High-sulphur proteins were also extracted by a two-stage procedure. The first step used a preferential extraction either at 0 or 40°C with 0·8M potassium thioglycollate at pH 10·3. The soluble proteins, largely high-sulphur components, were separated by vacuum filtration, alkylated, and then purified by the procedure of Gillespie (1962). In the second step the gelatinous residue was dispersed at 40°C in a solution adjusted to give final concentrations of 6M urea and 0·2M potassium thioglycollate at pH 11·0 by treatment in a Waring blender. This extract contained both low- and high-sulphur proteins and, after alkylation and dialysis, low-sulphur proteins were precipitated at pH 4·4 giving a second high-sulphur protein fraction in the supernatant, which was recovered by dialysis against running deionized water and freeze-drying. The proportion of high-sulphur proteins in each wool sample was measured by the procedure of Gillespie (1964). Because it is difficult to obtain reproducible results when this procedure is used a wool of known composition was included in each set of analyses as a control.

(e) Moving Boundary Electrophoresis

The electrophoretic runs were carried out in a Tiselius moving boundary apparatus (LKB Productor, Stockholm)—for experimental details see Gillespie and Reis (1966). The buffers consisted of acetic acid–sodium acetate (ionic strength 0·1) at pH 4·5 and glycine–NaOH (ionic strength 0·1) at pH 10·0. A 1·5% protein solution was dialysed for 16 hr against the appropriate buffer prior to electrophoresis. In the patterns from runs at pH 4·5, the main peaks have been labelled A–D (see Gillespie 1964) but in the runs at pH 10 only the fastest moving peak, labelled D2, can be unequivocally identified with pH 4·5 components (Gillespie and Reis 1966). Therefore, the other peaks resolved at pH 10 have been labelled a–d.

(f) Amino Acid Analysis

Amino acid analyses were carried out by the methods given in Gillespie and Reis (1966).

III. Results

(a) Relation between the Sulphur Content of Wool and Its Content of High-sulphur Proteins

Up to now the relationship between the sulphur content of wool and its content of high-sulphur protein has only been investigated semiquantitatively (Gillespie, Reis, and Schinckel 1964). In order to put this relationship on a more quantitative basis 27

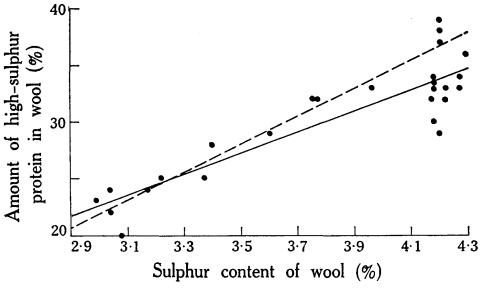


Fig. 1.—Relationship between the sulphur content of wool and its apparent content of high-sulphur proteins. —— Results for all wools. – – – Data for wools of sulphur content > 4% excluded.

wool samples with sulphur contents in the range of 3·0–4·2% S were solubilized and their content of total high-sulphur protein estimated. Figure 1 shows the relationship

found between the sulphur content of wool and amount of high-sulphur protein. Because of the large degree of scatter at high levels of sulphur content, two curves are shown, one including all the results and the other omitting the data for wools of sulphur content higher than 4%. The large scatter in the results at high levels of sulphur content is probably due to the greater difficulty of extracting protein from these wool samples [see Section III(*c*)]. Both sets of data have been analysed statistically and the curves drawn are the calculated regression lines. Both curves show a highly significant linear relationship ($P < 0{\cdot}001$) between the sulphur content of wool and its content of high-sulphur protein.

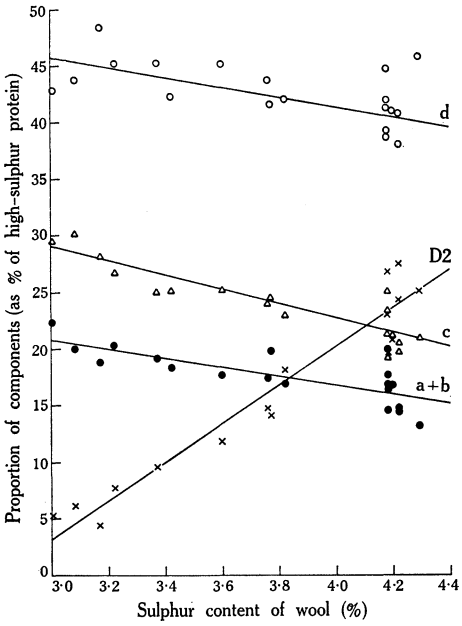


Fig. 2.—Relationship between the sulphur content of wool and the contribution which electrophoretic fractions a+b, c, d, and D2 make to the high-sulphur protein content of each wool sample.

(*b*) *Relation between the Sulphur Content of Wool and the Proportion of High-sulphur Protein Fractions*

The high-sulphur proteins isolated in the experiments of Section III(*a*) were run in moving boundary electrophoresis experiments at pH 10. The actual patterns are not shown here although some of them can be seen in Figures 5–12 associated with Sections III(*d*) and III(*e*). The proportion of each electrophoretic peak was estimated from the areas under the peaks (peak a was included with peak b because of its poor resolution). The proportion of each component is plotted against the sulphur content of the wool from which it was derived (Fig. 2). The curves are regression lines obtained by the statistical analysis of the data. The proportion of peak D2 proteins in the high-sulphur proteins increases substantially as the sulphur content of wool increases and this relationship is highly significant ($P < 0{\cdot}001$). In contrast, the proportions of the other components decrease slightly but significantly as the sulphur content of

wool increases, the levels of significance being: peak a+b, 0·1%; peak c, 0·1%; peak d, 5%. The greater degree of scatter for the relationship between D2 components and sulphur content (and between high-sulphur proteins and sulphur content, Fig. 1), at high levels of sulphur is probably due to variability in the extraction of this component from sulphur-enriched wool.

TABLE 1

AMINO ACID COMPOSITIONS OF HIGH-SULPHUR PROTEINS ISOLATED FROM CONTROL AND SULPHUR-ENRICHED WOOL SAMPLES PRODUCED BY ENGLISH LEICESTER×MERINO SHEEP SD67

Proteins were extracted with 0·2M potassium thioglycollate–6M urea at pH 11. The analytical data are presented as residues of each amino acid per 100 residues in the protein. The effects of level of methionine supplementation and yield of high-sulphur protein on amino acid composition are shown. To aid in interpretation, the analysis is given of a partially purified peak D2 material prepared by the method of Gillespie and Broad (1969)

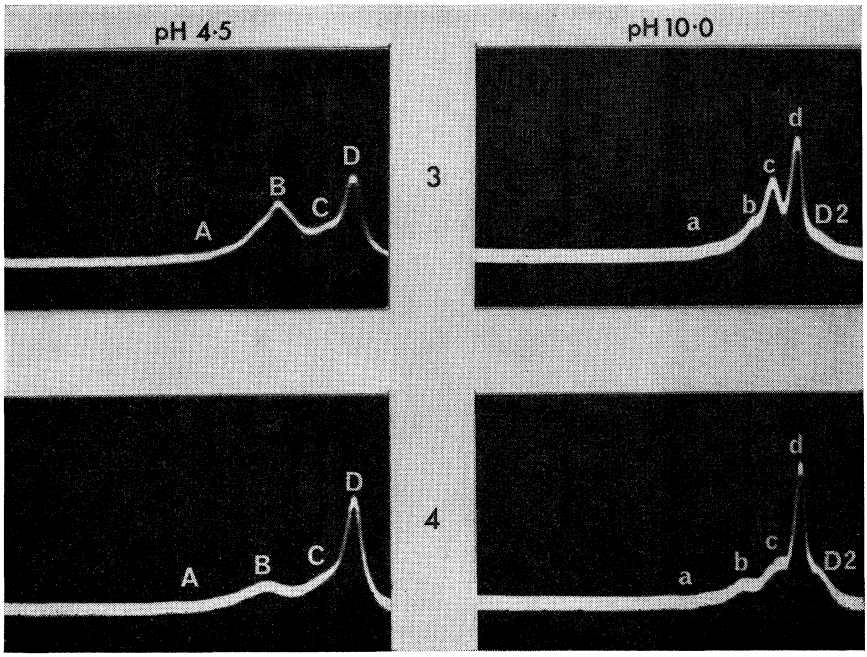
Amino Acid	Control Wool (3·08%S)	Sulphur-enriched Wool 1 (4·22%S). Good Extraction*	Sulphur-enriched Wool 2 (4·18%S)		Partially Pure D2 Preparation
			Poor Extraction†	Good Extraction‡	
Lys	0·58	0·58	0·63	0·57	0·89
His	0·63	0·71	0·70	0·74	1·29
Arg	5·84	6·37	6·26	6·22	6·90
SCMC	20·1	23·4	21·8	24·5	29·9
Asp	3·08	2·06	2·56	1·93	0·61
Thr	9·95	10·3	9·98	10·4	11·1
Ser	13·0	13·2	13·1	12·8	12·7
Glu	8·15	8·14	8·11	7·94	7·90
Pro	11·5	12·2	11·7	12·9	12·8
Gly	6·68	5·75	6·07	5·43	4·16
Ala	3·14	2·49	2·81	2·54	1·96
Val	5·78	5·19	5·49	5·15	4·34
Ile	3·19	2·68	2·78	2·57	1·74
Leu	4·11	2·87	3·34	2·80	1·33
Tyr	2·30	2·08	2·16	2·12	1·85
Phe	1·97	1·48	1·60	1·40	0·46

* DL-Methionine supplementation 2·46 g/day; protein yield 32%.
† DL-Methionine supplementation 4·18 g/day; protein yield 26%.
‡ DL-Methionine supplementation 4·18 g/day; protein yield 31%.

Further evidence in support of this conclusion comes from a comparison of the amino acid composition of high-sulphur proteins isolated during two different extractions on samples of the same wool—one, yielding 31% high-sulphur protein, was classed as a "good" extract, whilst the other yielded only 26% high-sulphur protein and was classed as a "poor" one. From the last three columns of Table 1 it can be seen that the differences in contents of *S*-carboxymethylcysteine (SCMC), aspartic acid, glycine, alanine, leucine, and phenylalanine are consistent with the concept that the high-sulphur proteins in the poor extract contain substantially less D2 proteins than in the good extracts.

(c) *Extractability of D2 Proteins*

The following experiment confirms that D2 proteins are apparently more difficult to extract from wool than are the other high-sulphur proteins. Sulphur-enriched wool (4·2% S) produced by sheep SD67 was extracted by the two-stage procedure and the high-sulphur proteins obtained were examined electrophoretically at pH 4·5 and pH 10·0 (Figs. 3 and 4). The two protein preparations appear



Figs. 3 and 4.—Moving boundary electrophoresis patterns from runs at pH 4·5 and 10·0 of high-sulphur proteins extracted from sulphur-enriched wool (4·18% S) produced by sheep SD67 during supplementation by the abomasal infusion of 2 g L-cysteine per day. *3*, Extracted with 0·8M potassium thioglycollate at 0°C pH 10·3 for 18 hr. *4*, Extracted from the residue from *3* in the presence of 0·2M potassium thioglycollate and 6M urea at pH 11 for 2 hr at 40°C.

substantially different at both pH values and it would seem that components A and B (those of lowest sulphur content) are richest in the initial extract whilst components C and D (those of higher sulphur content) are in greater concentration in the second extract. In agreement with these observations it also appears that the proteins of highest sulphur content of fraction D2 occur in greater concentration in the second extract, representing in some experiments between 65 and 75% of the total amount extracted (Table 2). Similar results have been obtained for multiple extractions of sulphur-enriched wool produced by sheep 1390. Raising the temperature of extraction of sulphur-enriched wool from 0 to 40°C made little difference to the amount of high-sulphur protein extracted by 0·8M potassium thioglycollate whereas with normal wool the amount of protein extracted would have increased to almost double (Gillespie 1962).

(*d*) *Relationship between the Amount of Cysteine and Methionine Given per Abomasum and the Proportion of D2 Proteins Appearing in Wool*

The effect on the protein composition of wool of stepwise increases in the level of L-cysteine and DL-methionine infused into the abomasum was studied with sheep SD67. There was a progressive increase in the amount of peak D proteins and peak D2 proteins as the amount of L-cysteine supplement was increased (Figs. 5–8). Similar results were obtained in experiments with DL-methionine (Figs. 9–12).

TABLE 2

EFFECT OF EXTRACTION CONDITIONS ON THE YIELD OF HIGH-SULPHUR PROTEINS FROM SULPHUR-ENRICHED WOOLS AND ON THEIR COMPOSITION AS MEASURED BY MOVING BOUNDARY ELECTROPHORESIS AT pH 10·0

	Sheep 1390		Sheep SD67		Sheep 1390	
	First Extract	Extract of Residue	First Extract	Extract of Residue	First Extract	Extract of Residue
	Conditions of extraction					
Temp. (°C)	40	40	0	40	0	40
Time (hr)	2	2	18	2	18	2
Thioglycollate concn. (M)	0·8	0·2	0·8	0·2	0·8	0·2
pH	10·3	11	10·3	11	10·3	11
Urea concn. (M)	0	6	0	6	0	6
	Yield of high-sulphur protein					
Approx. yield (%)	11*	17*†	14*	13*†	7*	15*†
Total yield (%)	28		27		22	
	Composition of high-sulphur proteins					
Peak a‡	5·8	5·4	5·5	4·4	4·7	7·1
Peak b‡	11·5	9·6	13·5	9·5	12·2	11·5
Peak c‡	35·8	25·2	27·4	18·3	34·6	22·1
Peak d‡	35·1	41·4	37·7	38·5	37·5	43·3
Peak D2‡	11·8	18·3	16·0	29·3	11·1	16·1

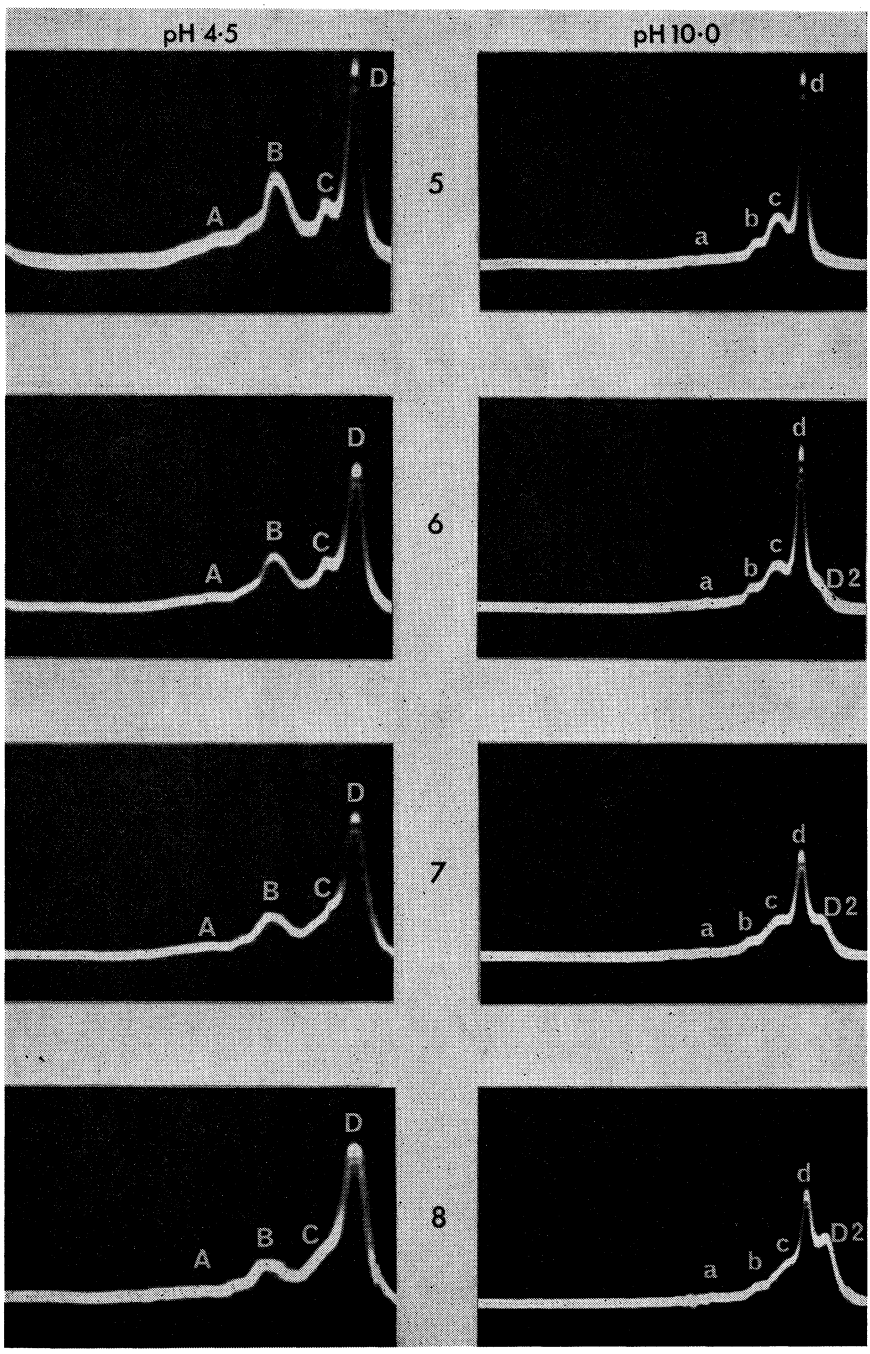
* Calculated from the weight of recovered freeze-dried protein.

† Corrected for about 10% lost in liquor absorbed in undissolved residue.

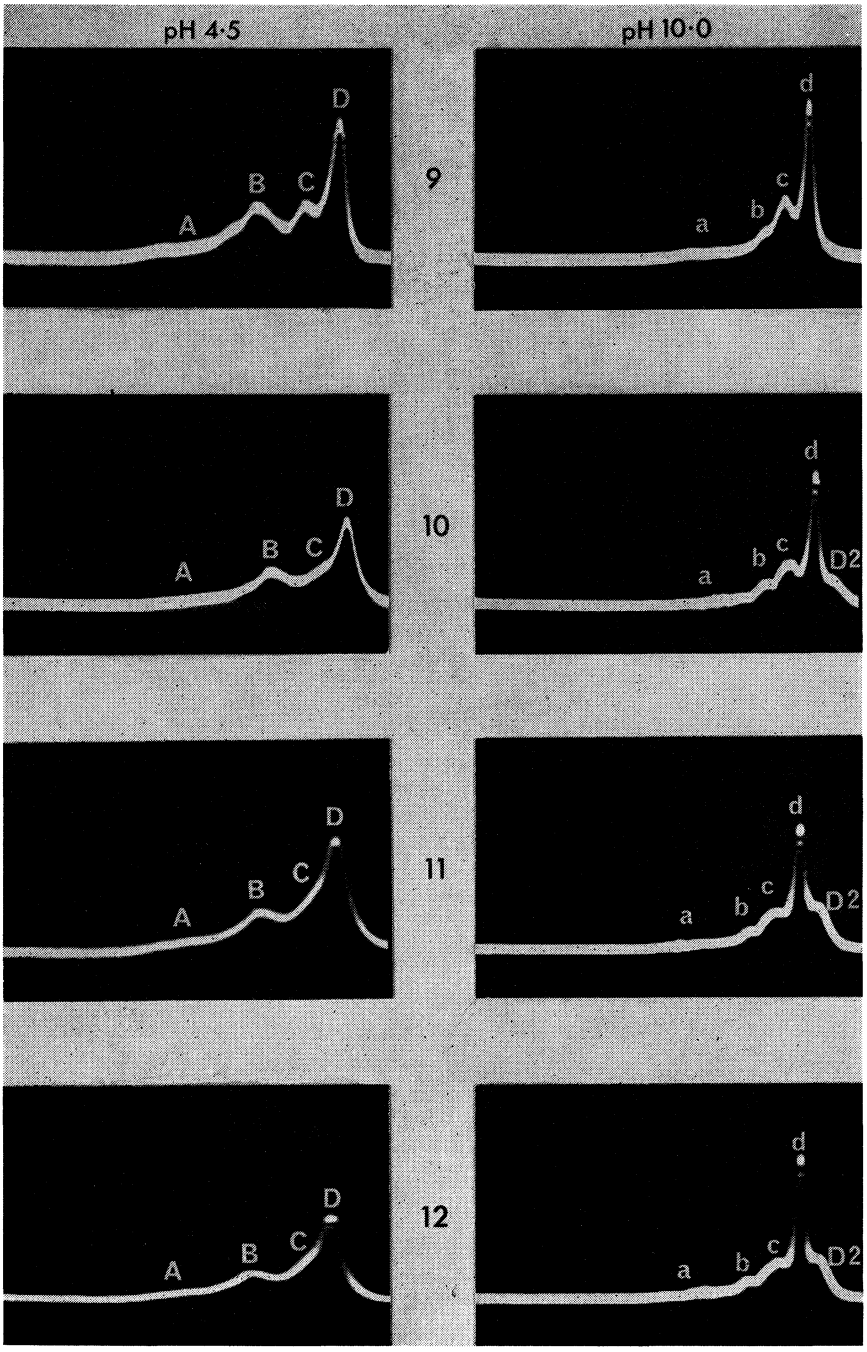
‡ The proportion of protein in these fractions was estimated from the area under electrophoretic peaks from runs at pH 10·0 and is given as a percentage of high-sulphur protein.

Although 9·84 g/day of DL-methionine usually inhibits wool growth (Reis 1967), there was no suppression of the formation of the proteins of the D2 fraction (Fig. 12). Furthermore the high-sulphur proteins produced under these conditions appear to be normal, for they have an amino acid composition which is essentially identical to that of the proteins produced during infusion with lower levels of methionine (Table 1, columns 3 and 5).

It is clear from these and many other results that there is a non-linear relationship between the amount of L-cysteine and DL-methionine (x) given per



Figs. 5–8.—Moving boundary electrophoretic patterns of high-sulphur proteins from runs at pH 4·5 and 10·0 showing the differences between the proteins isolated from wools grown by sheep SD67 during abomasal infusions with different amounts of L-cysteine: *5*, control wool (3·17% S); *6*, 0·5 g/day L-cysteine (sulphur content of wool 3·60%); *7*, 2·0 g/day (sulphur content of wool 4·18%); *8*, 8·0 g/day (sulphur content of wool 4·29%).



Figs. 9–12.—Moving boundary electrophoresis patterns from runs at pH 4·5 and 10·0 showing the differences between the proteins from wools grown by sheep SD67 during abomasal infusions with different amounts of DL-methionine: *9*, control wool (3·37% S); *10*, 0·615 g/day DL-methionine (sulphur content of wool 3·36%); *11*, 2·46 g/day (sulphur content of wool 4·20%); *12*, 9·84 g/day (sulphur content of wool 4·18%).

abomasum to sheep and the proportion of D2 proteins (y) in the wool they produce (Fig. 13). Two curves have been fitted to these data, the first having the logarithmic form

$$y = 17{\cdot}3+4{\cdot}3\log_e(x+0{\cdot}1),$$

with a residual standard error (of an estimate of y) of 3·50, and the second the polynomial relation

$$y = 7{\cdot}3+10{\cdot}7x-1{\cdot}64x^2+0{\cdot}0725x^3,$$

with a standard error of 2·44. Of the two curves the latter provided the best fit.

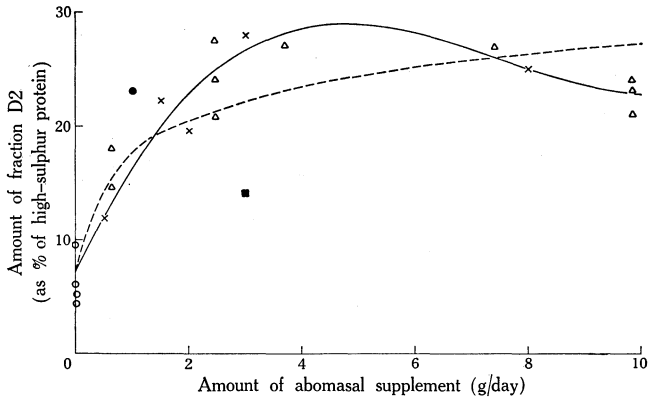


Fig. 13.—Relationship between the amount of L-cysteine (×) or DL-methionine (△) supplement (g/day) and the D2 content of wool produced. The data originated from experiments with seven English Leicester×Merino sheep. Data is also included for control wool from the same animals (○) and for supplements with D-methionine (●) and MHA (■). Curves have been fitted to the data following a logarithmic form (- - - -) and a polynomial form (——).

(e) *Effect of Variation in Type of Sulphur-containing Supplement on Proportion of D2 Proteins in Wool*

A comparison has been made of the effectiveness of L-cysteine, DL-methionine, D-methionine, and MHA for stimulating the synthesis of D2 proteins by the wool follicle. Equimolar amounts were given, except that less than half the molar

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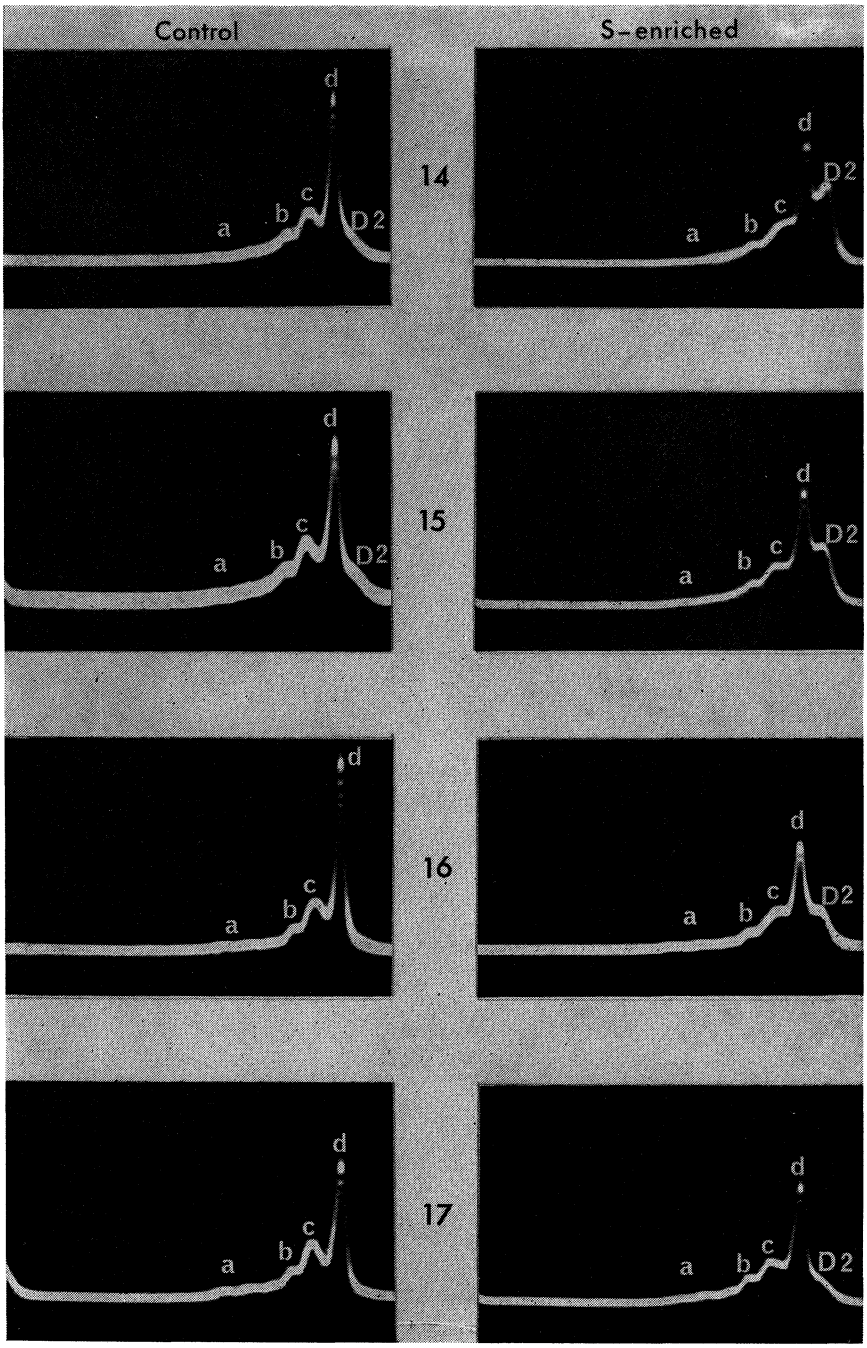
Figs. 14–17.—Moving boundary electrophoresis patterns of high-sulphur proteins run at pH 10·0 showing the differences between the proteins of control and sulphur-enriched wool grown by three sheep. Amounts of sulphur-containing supplements to the diet which were given by abomasal infusion are indicated in the following tabulation:

Fig. No.	Sheep No.	S Content of Control Wool (%)	S Content of S-enriched Wool (%)	Supplement
14	1038	3·37	4·22	DL-Methionine (2·46 g/day)
15	1038	3·42	4·18	D-Methionine (1·0 g/day)
16	SD67	3·17	4·18	L-Cysteine (2·0 g/day)
17	1024	2·99	3·77	MHA (3·0 g/day)


Control
S-enriched
14
15
16
17
a
b
c
d
D2

equivalent of D-methionine was used. In each case the sulphur content of the wool produced was increased during the supplementation and this was associated with an increase in the amount of D2 proteins contained in the wool (Figs. 14–17).

The data of Figures 13–17 suggest that MHA is significantly less effective in stimulating the formation of D2 proteins than L-cysteine and both optical isomers of methionine. The point in Figure 13 defining the response to MHA departs from both the logarithmic and polynomial curves at significance levels of 5 and 0·1% respectively.

TABLE 3

GROWTH RATE AND COMPOSITION OF WOOL PRODUCED BY SHEEP RECEIVING A SEMIPURIFIED DIET

The sheep received a diet of equal parts lucerne chaff and oats (800 g/day) for 12 weeks, followed by a semipurified sulphur-deficient diet for 18 weeks (sheep 1095 consumed *c.* 500 g/day and sheep 1100 *c.* 300 g/day). During the last 6 weeks of this period sheep 1095 received a supplement of 5 g/day L-cystine in the diet and sheep 1100 received an equivalent amount of elemental sulphur (1·34 g/day) in the diet. Values are expressed on the basis of clean dry wool. Wool analysed was that grown between weeks 3 and 12 and weeks 12 and 18 of the sulphur-deficient diets periods. Wool growth was the rate attained after sheep had received the sulphur-deficient diet for 9 weeks

Treatment	Sheep	Wool Growth (mg/cm²/day)	Sulphur Content of Wool (%)	High-sulphur Protein in Wool (%)
Normal diet	1095	0·97	2·82	19·8
	1100	0·72	3·04	21·7
Sulphur-deficient diet	1095	0·10	3·04	21·1
	1100	0·08	3·10	21·5
Sulphur-deficient diet plus supplement	1095	0·09	3·63	27·7
	1100	0·06	3·48	26·7

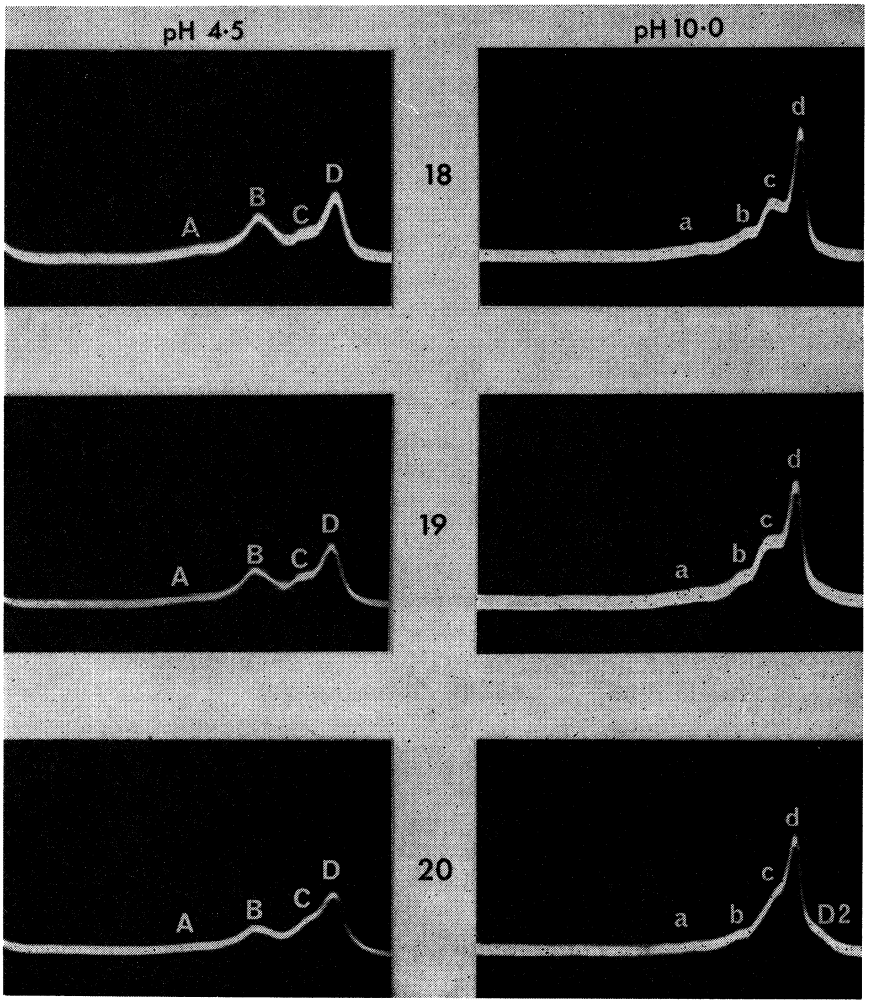
(*f*) *Differences between Sheep in the Capacity to Synthesize D2 Proteins*

Examination of the accumulated data shows that sheep differed in their ability to form D2 proteins during the control diet periods; this is indicated by the scatter of points relating D2 formation at zero supplementation levels (Fig. 13) and in the differing sulphur contents of the wool samples. However, the remarkably small scatter in the experimental data (Fig. 13) relating dosage of *S*-amino acid (both L-cysteine and DL-methionine) to D2 formation, which includes data from seven English Leicester × Merino sheep, suggests that if differences exist between sheep they are smaller than experimental errors.

(*g*) *Effect of a Restricted Supply of Sulphur on the Growth and Composition of Wool*

The feeding of a sulphur-deficient diet to two sheep for 12 weeks caused wool growth to decline to very low rates (Table 3). In contrast, the sulphur content and

proportion of high-sulphur protein in wool samples produced under these conditions was unaltered in one sheep and slightly increased in the other (Table 3). These high-sulphur proteins gave electrophoresis patterns at both pH 4·5 and 10·0, which were apparently identical to those given by proteins isolated from control wool; on this criterion therefore normal high-sulphur proteins were produced (Figs. 18 and 19) under conditions of dietary sulphur deficiency.



Figs. 18–20.—Moving boundary electrophoresis patterns of high-sulphur proteins from runs at pH 4·5 and pH 10·0 showing the differences between the proteins from wools grown by sheep 1095 during a control period (Fig. 18), during sulphur deprivation (Fig. 19), and during sulphur enrichment with 5·0 g/day of L-cystine in the diet (Fig. 20).

The subsequent addition of L-cystine or of sulphur to the sulphur-deficient diet of these same sheep for a period of 6 weeks did not influence wool growth, but there was a marked increase in the sulphur content of wool, and its content of high-sulphur

protein (Table 3). Electrophoresis of these high-sulphur proteins showed the appearance of some D2 proteins (Fig. 20) not previously observable.

IV. Discussion

Sulphur-enriched wools are in general more difficult to solubilize than are control wools produced by the same sheep, and it is probable that this is another manifestation of the difficult and variable solubilization of the D2 proteins. A preferential extraction with 0·8M potassium thioglycollate, although useful for preparing normal high-sulphur proteins (Gillespie 1962) is a poor method for obtaining D2 proteins. At present we can only obtain these proteins in good yield by extracting the wool with urea–thioglycollate which essentially solubilizes all the proteins of the fibre. There are a number of possible explanations for this difficulty. It has been observed (Gillespie, unpublished data) that sulphur-enriched wool swells far less in formic acid than does its control and this is probably related to its higher cross-linking density of disulphide bonds. Swelling has been postulated as an initial phase in the solubilization of wool by alkaline reductants (Fraser and Rogers 1953) and hence the poor extractability could be related to lower swelling, leading to poor penetration of the reagents and slower outward diffusion of the soluble proteins. Possibly this process is affected by minor procedural differences such as the effectiveness of wetting of the wool or the degree of stirring which may account for the variability that has been noted also in the extractability of the D2 proteins.

There are of course more disulphide bonds in the sulphur-enriched wool than in the control wool and the new disulphide bonds may be more difficult to reduce due to some special spatial arrangement or due to the situation in which they are held by the tertiary structure of the proteins. It is well known that intra-chain disulphide bonds may be more difficult to reduce than those between chains and that many proteins can only be completely reduced following complete disruption of their tertiary structure, for example with urea (Cecil and McPhee 1959). Furthermore, whilst it may be coincidental that the release of the D2 proteins is accompanied by the solubilization of the low-sulphur proteins, it is also possible that the two are intimately associated in the fibre structure.

There is a linear relationship between the sulphur content of wool and its content of high-sulphur proteins over the range of concentrations encountered. Of the individual high-sulphur proteins, only the D2 fraction shows significant increases in amount with increasing sulphur content when this increase is calculated either as proportion of the total high-sulphur protein or as proportion of wool. The proportion of fractions a+b, c, and d in wool remain essentially constant irrespective of sulphur content; however, an inverse relationship is obtained when sulphur content is expressed as a fraction of high-sulphur protein.

L-Cysteine and both optical isomers of methionine were apparently equally effective in stimulating the formation of D2 proteins at all levels. The response was a non-linear one, best fitted by a polynomial relation which reached a plateau with wool which contained about 4·2% S and 10% D2 proteins. Reis (1967) found marked differences in the responses of individual sheep to dietary supplements of *S*-amino acids both in respect of wool growth and wool sulphur content. If the sheep used in these experiments showed similar variations in their capability to synthesize D2 proteins, this was within the experimental error of our measurements.

Several pieces of evidence suggest that the synthesis of the D2 proteins is under metabolic control separate from that of the other high-sulphur proteins. At high levels of methionine (e.g. 9·84 g/day) which are partly inhibitory to wool growth (Reis 1967) maximum levels of D2 protein are still reached. It has been found that the growing areas of ovine horn and hoof, although capable of producing much the same spectrum of high-sulphur proteins as those of control wool, are apparently unable to produce D2-like proteins (Gillespie 1968). The increase in the proportion of high-sulphur proteins obtained when cystine or sulphur were added to the sulphur-deficient diet is further evidence that D2 proteins can be synthesized when synthesis of other wool proteins is inhibited. These supplements increased the supply of cysteine available to the follicle but the overall synthesis of wool proteins was still low, presumably due to dietary inadequacy of other amino acids.

The results of the experiment in which the sheep received a restricted supply of sulphur suggest that there may be a lower limit for the sulphur content of wool. This level varies with individual sheep but is of the order of 2·7% (Reis 1965) and would correspond with a microfibrillar framework packed with about 18% high-sulphur protein. This might be considered as a limiting fundamental structure for wool. The upper limit for sulphur content of wool seems to be about 4·2–4·3% (Reis 1965, 1967), which corresponds to about 35% high-sulphur protein.

So far there is no evidence that wool of increased sulphur content possesses more desirable characteristics than wool having the limiting fundamental structure, and, in fact, only the smallest differences in any mechanicochemical parameters can be found between wools with very different sulphur contents (Feughelman and Reis 1967; Armstrong and Feughelman 1969). Whilst further experiments may alter this conclusion or may indicate advantages in properties not yet studied, at present it seems that any sulphur in excess of about 3·0% is incorporated into D2 proteins, which do not seem to be needed for effective fibre formation and which do not contribute any additional desirable properties to the wool. What this implies in terms of loss of sulphur can be indicated by the following calculation. A sheep producing 5 kg of wool per year, of sulphur content 3·6%, would utilize about 30 g of sulphur (or about 0·08 g per day) in synthesizing D2 proteins. If this were available to supplement the *S*-amino acids in the diet it would be equivalent to about 0·4 g of methionine a day which, on the basis of the results of Reis (1967), could cause a substantial increase in wool growth rate. With some sheep this increase could approach 100%. Merino sheep selected for high wool production grow wool of a lower sulphur content than those selected for low wool production (Reis *et al.* 1967). Thus in selecting for high wool growth it is probable that selection has been made against the synthesis of D2 proteins and this has had no adverse effects on the physical properties of the wool. It would be a valuable aid to the conservation of sulphur if the metabolic regulation of the synthesis of D2 proteins could be subject to direct experimental control.

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VI. References

Armstrong, L. D., and Feughelman, M. (1969).—*Text. Res. J.* **39**, 261, 267.

Cecil, R., and McPhee, J. R. (1959).—*Adv. Protein Chem.* **14**, 255.

Feughelman, M., and Reis, P. J. (1967).—*Text. Res. J.* **37**, 334.

Fraser, R. D. B., and Rogers, G. E. (1953).—*Biochim. biophys. Acta* **12**, 484.

Gillespie, J. M. (1962).—*Aust. J. biol. Sci.* **15**, 262.

Gillespie, J. M. (1964).—*Aust. J. biol. Sci.* **17**, 282.

Gillespie, J. M. (1968).—*Proc. Aust. Biochem. Soc.* p. 29.

Gillespie, J. M., and Broad, A. (1969).—*Proc. Aust. Biochem. Soc.* p. 76.

Gillespie, J. M., Broad, A., and Reis, P. J. (1969).—*Biochem. J.* **112**, 41.

Gillespie, J. M., and Reis, P. J. (1966).—*Biochem. J.* **98**, 669.

Gillespie, J. M., Reis, P. J., and Schinckel, P. G. (1964).—*Aust. J. biol. Sci.* **17**, 548.

Harrap, B. S., and Gillespie, J. M. (1963).—*Aust. J. biol.* Sci. **16**, 542.

Reis, P. J. (1965).—In "Biology of the Skin and Hair Growth". (Eds. A. G. Lyne and B. F. Short.) pp. 365–75. (Angus and Robertson Ltd.: Sydney.)

Reis, P. J. (1967).—*Aust. J. biol. Sci.* **20**, 809.

Reis, P. J., and Schinckel, P. G. (1963).—*Aust. J. biol. Sci.* **16**, 218.

Reis, P. J., and Schinckel, P. G. (1964).—*Aust. J. biol. Sci.* **17**, 532.

Reis, P. J., and Tunks, D. A. (1968).—*Proc. Aust. Soc. Anim. Prod.* **7**, 402.

Reis, P. J., Tunks, D. A., Williams, O. B., and Williams, A. J. (1967).—*Aust. J. biol. Sci.* **20**, 153.

Reis, P. J., and Williams, O. B. (1965).—*Aust. J. agric. Res.* **16**, 1011.