METABOLISM OF CYSTINE BY MERINO SHEEP GENETICALLY DIFFERENT IN WOOL PRODUCTION

III.* THE INCORPORATION OF RADIOACTIVITY INTO WOOL FIBRES DURING AND AFTER INTRAVENOUS INFUSIONS OF L-[^{35}S]CYSTINE AND ITS RELATIONSHIP TO WOOL GROWTH AND EFFICIENCY OF CONVERSION OF FOOD INTO WOOL

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Abstract

Twelve mature ewes from a flock selected for high clean fleece weight (Fleece Plus) and twelve from a flock selected for low clean fleece weight (Fleece Minus) were randomly divided between two dietary treatments: 500 or 1100 g per day of chaffed lucerne hay. After the sheep experienced these dietary regimes for 40 days, each ewe was infused intravenously with L-[^{35}S]cystine for 12–14 hr. Incorporation of ^{35}S activity into fibres during the infusions, and into wool clipped subsequent to the infusions, was measured.

During the final 7–9 hr of infusion, the ^{35}S activity in plucked fibres increased linearly with time. The linear regression coefficients relating ^{35}S activity to time did not differ in the genetic or the dietary comparisons, regardless of whether the rate of incorporation of ^{35}S was expressed relative to a 1000 fibres, or to the number of fibres growing from unit area of skin. The mean specific radioactivity of cystine incorporated into wool proteins during the infusions was less in ewes consuming 1100 g per day of lucerne (23 *v.* 35 nCi/mg : $P < 0{\cdot}05$). This difference was less than the difference in the "plateau" specific radioactivity of cystine in the plasma, indicating that the ^{35}S was differentially diluted with "cold" cystine during its transfer from plasma into the wool fibre; dilution being greater in ewes consuming the lesser quantity of feed (4·0 *v.* 2·9 : $P < 0{\cdot}05$).

A similar effect of feeding level was evident on the specific radioactivity of cystine in wool clipped from the ewes subsequent to the infusions. In addition, the specific radioactivity of cystine in wool from Fleece Plus ewes was less than that from Fleece Minus ewes (3·2 *v.* 3·6 nCi/mg : $P < 0{\cdot}05$), indicating that greater dilution of ^{35}S with "cold" cystine was occurring in these ewes. As judged by the wool production per unit area of skin, the efficiency of conversion of food to wool would have been greater in Fleece Plus ewes and in those consuming 500 g per day of lucerne. Thus, mechanisms controlling the dilution of ^{35}S between plasma and fibre, presumably arising

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from differences in the size or turnover rates in an intermediate pool of cystine, were probably responsible for genetic and dietary differences in the efficiency of utilization of cystine for the growth of fibre.

The relative recovery of infused ^{35}S was greater in sheep consuming 1100 g per day of lucerne (0·185 *v.* 0·258 nCi cm^{-2} day^{-1} : $P < 0·05$), but this trait was similar in Fleece Plus and Fleece Minus ewes. Differences in the recovery of isotope probably represented differential partitioning of extracellular cystine between wool production and other metabolic functions; this partitioning depending upon the availability of cystine to the organism.

The differences observed between the treatments in the specific radioactivity of cystine in clipped wool were not caused by differences in the ratio of specific radioactivities of cystine in the high- and low-sulphur proteins extracted from the wool. The specific radioactivity in high-sulphur protein was 15–20% ($P < 0·05$) greater than that in the low-sulphur proteins, and this difference was maintained during the period of observation, 25 days post-infusion.

I. Introduction

In an earlier paper (Williams *et al.* 1972*a*), the entry rate of cystine into the plasma pool was shown to be similar in sheep from two flocks, one of which was selectively bred for high clean fleece weight (Fleece Plus), the other being selected for low clean fleece weight (Fleece Minus). Furthermore, the mean phenotypic difference in wool production between these two flocks when the sheep in them consumed sufficient food for the maintenance of body weight was less than that observed when the diet of these sheep was supplemented with sulphur-containing amino acids infused into the abomasum (Williams *et al.* 1972*b*). Hence, the proportion of the expressed genetic potential for wool production was limited by the availability of sulphur-containing amino acids to a greater extent in sheep genetically superior with respect to wool production.

Therefore, the differences which have been observed between these flocks, both in wool production (Dun 1958) and the efficiency of conversion of food to wool (Ahmed *et al.* 1963) must arise from genetic differences in the effectiveness with which the follicle populations utilize the similar quantities of cystine available.

In these experiments, the rates of incorporation of ^{35}S into fibres during intravenous infusions of L-[^{35}S]cystine and the ^{35}S contained in wool clipped subsequent to the infusions were measured in sheep from flocks selected for high or low clean fleece weight. The results are discussed in relation to the effectiveness of the follicle populations in utilizing cystine for the growth of wool.

II. Materials and Methods

Details concerning the sheep, experimental plan, and the conduct of the infusions of L-[^{35}S]cystine were presented in Part I of this series (Williams *et al.* 1972*a*). Briefly, 4-yr-old ewes from two flocks (Fleece Plus and Fleece Minus) were offered daily either 500 or 1100 g of chaffed lucerne hay. There were six ewes from each flock at each intake level. These dietary regimes continued for approximately 40 days. During the final 5–7 days, each ewe received its daily ration in equal quantities at hourly intervals over 24 hr. On the final day, L-[^{35}S]cystine, with a specific radioactivity of approximately 50 mCi/mmole, was infused intravenously for 12–14 hr. The solution of L-[^{35}S]cystine, containing approximately 600 nCi/ml, was infused at 6–7 ml/hr.

Each ewe was weighed before commencing the experimental diet. The mean body weights of Fleece Plus and Fleece Minus ewes were 35 and 37 kg respectively.

During the infusion of L-[^{35}S]cystine, samples of blood were collected and the specific radioactivities and concentrations of "free" cystine in plasma were assayed (Williams *et al.* 1972*a*).

(a) Rate of Wool Growth

Wool was clipped (Oster No. 40) from approximately 100 cm^2 of skin on the right and left midside of each ewe 14 days before the infusion of L-[^{35}S]cystine. Four days after the infusions, the wool grown on these patches was collected by clipping and the unstretched area of each patch measured.

The wool was washed successively with light petroleum (b.p. 60–80°C), alcohol, and distilled water. Wool growth during the 18 days was then expressed as the weight of clean dry wool per unit area of skin per day, the mean value from measurements on the right and left midside patches being used in the analyses.

Some months before these experiments, skin samples were collected, by trephine, from the midside of each ewe. The density of follicles in each sample was measured histologically (Carter and Clarke 1957).

(b) Incorporation of ^{35}S into Plucked Fibres

Small staples of fibre (4–8 cm long) were plucked (Downes 1965) from an area of skin anterior and dorsal to the midside patch. Fibres were first plucked approximately 200 min after the infusion of L-[^{35}S]cystine commenced, further samples being collected at intervals of 60–80 min until the end of each infusion.

These fibres were cleaned similarly to the clipped wool, and the radioactivity in 300–400 mg of clean dry wool was assayed by direct liquid scintillation counting (Downes and Till 1963). The fibres were recovered from the scintillation fluid and those plucked from each ewe were bulked and mixed before division into four subsamples. In two of these, 500 fibres were weighed for estimation of average fibre weight. The fibres in the remaining two subsamples were combusted in an oxygen flask to determine the efficiency of the direct counting procedure (see below).

The ^{35}S activity in fibres plucked at various times during each infusion was then expressed relative to unit weight of fibre. From this, two estimates were derived for comparisons among sheep:

(1) ^{35}S activity per 1000 fibres;

(2) ^{35}S activity in fibres growing from a unit area of skin, using the histological estimates of follicle density.

All measurements of ^{35}S incorporated into plucked fibres were corrected to a common rate of infusion of ^{35}S (4·5 μCi/hr).

(c) Incorporation of ^{35}S Activity into Clipped Wool

Eleven days after each infusion, the wool was again clipped from the midside patches, the areas of which had been increased at the clipping on day 4 post-infusion. Preliminary experiments indicated that very little radioactivity was present in clipped wool until 4 days after an infusion of L-[^{35}S]cystine. Thus, the radioactivity in the wool clipped at day 11 was assumed to represent the ^{35}S incorporated into the fibres during the 7 days after an infusion.

These samples of clipped wool were cleaned as described previously, and weighed portions of the clean, dry wool were combusted in an oxygen flask. The oxides of sulphur were absorbed in 15 ml of 6% H_2O_2. The ^{35}S in an aliquot (5 ml) of this solution was assayed as a gel, using toluene–Triton X (7 : 6 v/v) scintillation fluid (Patterson and Greene 1965).

The remaining solution was diluted with ethanol (4 : 1 v/v), and used for the estimation of the sulphur content of the wool, by the method outlined by Reis and Schinckel (1963). The cystine content of each wool sample was derived from its sulphur content, assuming cystine-sulphur represents 97% of the sulphur in wool (Reis and Schinckel 1964).

Additional samples of wool were collected from eight ewes (two from each flock at each level of food consumption), by clipping the midside patches at days 18 and 25 post-infusion. The ^{35}S activities and the sulphur contents in these samples were determined as above. The radioactivity in all clipped samples of wool was corrected for the efficiency of counting (Hendler 1964) and for adioactive decay subsequent to the infusions.

(d) ^{35}S Activity in High- and Low-sulphur Proteins extracted from Clipped Wool

Samples of wool clipped at day 11 post-infusion from the patches of 12 ewes (three per flock per intake level) were reduced in urea–thioglycollate at pH 11 and separated into high- and low-

sulphur protein fractions (Harrap and Gillespie 1963) with purification of the low-sulphur fraction by re-precipitation (Downes *et al.* 1966). The high-sulphur proteins were precipitated with trichloroacetic acid. The ^{35}S activity and sulphur content in each of the two fractions were determined as described for wool.

The cystine content of the low-sulphur proteins was estimated from the sulphur content, after correcting for methionine sulphur, based on the results of Gillespie *et al.* (1964).

For eight of these ewes, the specific radioactivity (nCi/mg cystine) of cystine in the high- and low-sulphur fractions was determined, as above, in wool clipped at days 18 and 25 post-infusion, as well as in that clipped at day 11.

(*e*) *Statistical Analyses*

The significance of the variation measured in traits due to flocks, levels of intake and periods was determined by analyses of variance. Where measurements were repeated, as in the case of traits associated with clipping at days 11, 18, and 25 post-infusion, the analyses of variance were conducted using a split-plot design to test the effects of flocks, levels of intake and sampling times [Brown, in an Appendix to Dolling and Piper (1968)].

Analyses of covariance were used to adjust treatment means for the specific radioactivity of cystine in plucked fibres and in clipped wool for differences among treatments in the "plateau" specific radioactivity of "free" cystine in plasma during the infusions.

Linear regressions were calculated to describe the relationships between ^{35}S activity in plucked fibres and sampling time for each sheep, and between the specific radioactivity of cystine in wool and the specific radioactivity of cystine in the high- and low-sulphur proteins extracted from the wool.

Results were considered significant when $P < 0{\cdot}05$.

TABLE 1

MEANS AND STANDARD ERRORS FOR THE RATE OF WOOL GROWTH, SULPHUR CONTENT, FOLLICLE DENSITY, AND THE RATE OF INCORPORATION OF CYSTINE INTO WOOL OF FLEECE PLUS AND FLEECE MINUS EWES AT TWO LEVELS OF FEEDING

Flock	Feed intake (g/day)	Wool production (μg cm^{-2} day^{-1})	Sulphur content (%)	Follicle density (No./mm^2)	Rate of incorporation of cystine into wool (μg cm^{-2} day^{-1})
Fleece Plus	500	456±48	2·95±0·04	72±6	49±6
	1100	835±80	3·32±0·07	72±7	102±10
Fleece Minus	500	370±9	3·37±0·11	54±7	45±3
	1100	588±35	3·68±0·09	56±5	78±5
Significance*		a, b, c	a, b	a	a, b, c

* a, significant difference between genotypes.
b, significant difference between feeding levels.
c, significant interaction between genotypes and feeding levels.

III. RESULTS

(*a*) *Wool Growth and Composition*

The rate of wool growth differed significantly between flocks and levels, but a significant interaction between these factors was evident for this trait (Table 1). The density of follicles was 31% greater in Fleece Plus ewes (Table 1).

The wool grown on the patches of Fleece Plus ewes between days 4 and 11 post-infusion had a lower sulphur content, and the difference was apparent at each feeding level (Table 1). When the rates of output of cystine in wool were derived from values for the rate of wool growth and the sulphur content of the wool, there was again a significant interaction between flocks and levels. The rate of output of cystine was 31% greater for Fleece Plus ewes consuming 1100 g/day, but only 9% greater for Fleece Plus ewes which consumed 500 g/day (Table 1).

(*b*) *Incorporation of* ^{35}S *Activity into Plucked Fibres*

During the infusion of L-[^{35}S]cystine, the rate of incorporation of ^{35}S into the plucked fibres appeared to increase at approximately the fifth hour of infusion (Fig. 1). After this time, linear relationships between ^{35}S activity and time of sampling accounted for more than 96% of the variation in radioactivity among samples from each of the ewes.

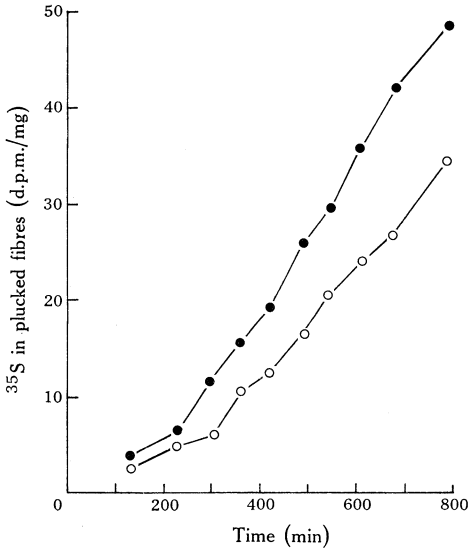

Fig. 1.—Examples of ^{35}S activity in fibres plucked at different times during continuous intravenous infusions of L-[^{35}S]cystine to two ewes.

● Ewe 8394; infusion rate 4·4 μCi/hr.
○ Ewe 8144; infusion rate 3·8 μCi/hr.

Two corrections were made to the regression coefficients of ^{35}S (nCi per milligram wool per unit time) on time so that regression coefficients were comparable among sheep. The regressions coefficients were expressed as ^{35}S per 1000 fibres per unit time and as ^{35}S in fibres growing from a unit area of skin per unit time. As follicle density is normally greater on areas of skin anterior and dorsal to the midside than at the midside (Young and Chapman 1958) at which site follicle density was measured, the latter adjusted coefficient would be a relative measure of the rate of incorporation of ^{35}S, provided treatment group × site interactions for follicle density were not large. The mean values of both adjusted coefficients did not differ between flocks or between feeding levels (Table 2).

The specific radioactivity of cystine being incorporated was estimated by division of the rate of incorporation of ^{35}S (nCi of ^{35}S in fibres from unit area of skin per unit time) by the rate of incorporation of cystine into wool (mg cystine in fibres

from unit area of skin per unit time). The mean specific radioactivity of cystine in fibres plucked from sheep consuming 1100 g of lucerne daily was 30% lower than that from sheep consuming 500 g daily, but the flock means were not significantly different (Table 2). Using the "plateau" specific radioactivity of free cystine in plasma (Williams *et al.* 1972*a*) as an independent variate in covariance analysis, the variation in the specific radioactivity of cystine in the plucked fibres was independent of the "plateau" specific radioactivity in plasma. These results indicated that labelled cystine was diluted with non-labelled cystine during its transfer from plasma into the follicle, the dilution being significantly greater in those sheep consuming 500 g of lucerne daily (Table 2).

TABLE 2

MEANS AND STANDARD ERRORS FOR DIFFERENCES BETWEEN MEANS (S.E.M.) FOR MEASUREMENTS ASSOCIATED WITH THE INCORPORATION OF ^{35}S INTO PLUCKED FIBRES DURING CONTINUOUS INFUSIONS OF L-[^{35}S]CYSTINE

All values for radioactivity have been adjusted to a common rate of infusion of 4·5 μCi/hr

Flock	Feed intake (g/day)	S.R. (plasma)* (nCi/mg)	Rate of incorporation of ^{35}S into plucked fibres†		S.R. (fibres)‡ (nCi/mg)	Dilution of ^{35}S between plasma and fibre
			A	B		
Fleece Plus	500	130	0·015	0·109	33·0	4·06
	1100	63	0·021	0·150	22·3	3·10
Fleece Minus	500	129	0·024	0·116	36·7	3·99
	1100	63	0·023	0·129	23·8	2·76
S.E.M.		±3	±0·005	±0·021	±4·2	±0·43
Significance§		b			b	b

* Specific radioactivity of "free" cystine in plasma. Values from Williams *et al.* (1972*a*).

† Expressed as nCi per 1000 fibres per 100 min (A) and as nCi in fibres from 1 cm^2 skin per 100 min (B).

‡ Specific radioactivity of cystine incorporated into fibres.

§ b denotes significant difference between feeding levels.

(*c*) *Radioactivity in Clipped Wool*

In the wool grown by the ewes during the 7 days following the infusions (i.e. clipped at day 11), the radioactivity per unit weight of wool (Table 3) differed between flocks and levels of intake ($P < 0·05$). The product of ^{35}S per unit weight of wool and the rate of wool production would have given a relative estimate of the recovery of the infused radioactive cystine in the wool, as differences in surface area would have made only a small contribution to the difference in wool production between these flocks (Dun 1958). The recovery was greater at the high intake, but there was a significant flock × level interaction (Table 3).

The specific radioactivity of cystine in clipped wool differed significantly between flocks and levels of intake (Table 3). Covariance analysis of these data with the "plateau" specific radioactivity of cystine in plasma during the infusions, indicated that the differences between flocks and levels for the specific radioactivity were independent of the "plateau" specific radioactivities. Thus, the mean ratios of the

specific radioactivity of cystine in plasma to that in wool differed significantly between flocks and levels, with no interaction (Table 3).

TABLE 3

^{35}S ACTIVITY IN WOOL CLIPPED AT DAY 11 POST-INFUSION

All values have been adjusted to a common total infusion of 63 μCi

Flock	Feed intake (g/day)	^{35}S per unit weight of wool (nCi/mg)	^{35}S recovered in wool per day (nCi/cm^2)	S.R. (wool)* (nCi/mg)	Dilution of ^{35}S between plasma and wool
Fleece Plus	500	0·378	0·178±0·028	3·58	36·5
	1100	0·336	0·285±0·042	2·81	22·5
Fleece Minus	500	0·518	0·193±0·014	4·22	29·2
	1100	0·406	0·230±0·014	3·06	20·1
S.E.M.		±0·042		±0·24	±1·6
Significance†		a, b	b, c	a, b	a, b

* Specific radioactivity of cystine in wool.

† a, significant difference between genotypes.
b, significant difference between feeding levels.
c, significant interaction between genotype and feeding level.

For the eight sheep from which wool was clipped from the patches at days 11, 18, and 25 post-infusion, the specific radioactivity of cystine in wool decreased rapidly with the successive samplings (Fig. 2). Analysis of the logarithms of values

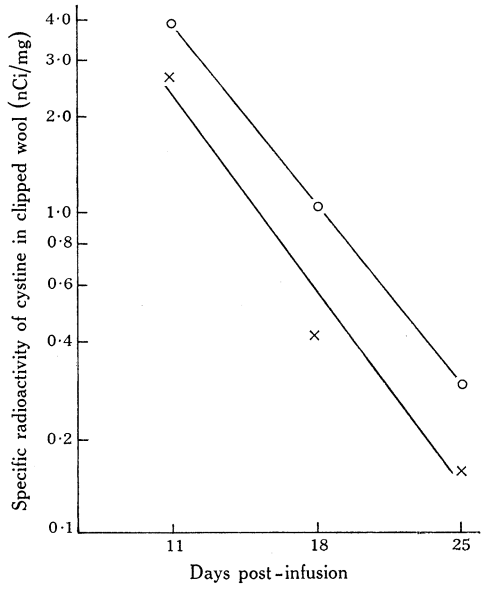

Fig. 2.—Specific radioactivity of cystine in wool clipped from ewes offered 500 g/day (○) or 1100 g/day of lucerne hay (×), at days 11, 18, and 25 post-infusion. The specific radioactivities have been plotted on logarithmic scale, and the lines drawn freehand.

of the specific radioactivity of cystine in clipped wool demonstrated significant differences between levels of intake and sampling times, but with a significant interaction

between these two variables. The relationship between specific radioactivity and sampling time was apparently log-linear.

(*d*) *Specific Radioactivity of Cystine in High- and Low-sulphur Proteins extracted from Clipped Wool*

For the wool samples clipped from 12 ewes at day 11 post-infusion, the regression equations relating the specific radioactivity of cystine in the high (Y) and low (Y') sulphur proteins to the specific radioactivity of cystine in clipped wool (x) were as follows:

$$Y = 0{\cdot}882x - 67 \quad (S_b = 0{\cdot}053),$$
$$Y' = 0{\cdot}773x - 80 \quad (S_b = 0{\cdot}052).$$

Both regression coefficients were less than unity ($P < 0{\cdot}05$), and neither intercept differed from zero. The regression coefficient of Y on x was significantly greater than that for Y' on x, indicating that the specific radioactivity of cystine in the high-sulphur proteins was 15% greater than that in the low-sulphur proteins. The size of this difference was not influenced by flock or feeding level.

Similar differences in the regression equations were obtained for wool clipped from eight of these ewes at days 11, 18, and 25 post-infusion, the specific radioactivity of cystine in the high-sulphur proteins being 18% greater than that in the low-sulphur proteins. The ratio between the two specific radioactivities did not differ significantly between flocks, levels, or times of sampling (Table 4).

TABLE 4

RATIOS OF SPECIFIC RADIOACTIVITY BETWEEN HIGH- AND LOW-SULPHUR PROTEINS EXTRACTED FROM WOOL CLIPPED AT DAY 11 POST-INFUSION (12 SHEEP) AND ON DAYS 18 AND 25 (8 SHEEP)

Flock	Food intake (g/day)	Day 11	Day 18	Day 25
Fleece Plus	500	1·22	1·22	1·24
	1100	1·13	1·19	1·28
Fleece Minus	500	1·12	1·19	1·17
	1100	1·15	1·13	1·13
Clipping means		1·15±0·02	1·17±0·02	1·21±0·03

IV. DISCUSSION

As the rate of wool growth and the cystine content of the wool can be increased by infusions of the sulphur-containing amino acids into the abomasum (Reis and Schinckel 1963; Gillespie *et al.* 1964), it is probable that protein synthesis within the follicle is regulated by the quantity of cystine or methionine which enters the follicle. As cystine is irreversibly incorporated into follicle proteins (Downes 1965), the rate of output of cystine in wool may serve as a comparative estimate of the entry rate of cystine into follicle proteins. In the present experiment, the greater rate of output of cystine in wool when the ewes consumed 1100 g of lucerne daily, probably reflected the greater availability of cystine, as measured by the entry rate of cystine into the

plasma pool (Williams *et al.* 1972). As the cystine content of the wool from sheep consuming 1100 g daily was greater, the efficiency with which incorporated cystine was utilized for the growth of fibre (i.e. rate of wool production per unit of cystine incorporated) obviously decreased as intake of feed or availability of cystine increased.

On the other hand, both a greater rate of incorporation and a more efficient utilization of incorporated cystine contributed to the greater wool production of the Fleece Plus ewes, despite similar mean entry rates of cystine into the plasma pools of both groups of ewes. In addition, the difference between the flocks in the rate of incorporation of cystine into follicle proteins increased as the availability of cystine increased. This greater capacity in Fleece Plus ewes could explain the more marked responses in wool growth of these ewes to infusions of cystine or methionine into the abomasum (Williams *et al.* 1972*b*).

Some insight into the mechanisms controlling the differences observed between the treatments may be gained from a study of the rates of incorporation of labelled cystine into the proteins of the follicle bulb during continuous infusions of L-[^{35}S]cystine, and examination of the specific radioactivities of cystine in the wool.

The linear regression coefficients relating ^{35}S in plucked fibres to time were variable among sheep, and no significant effects due to flocks or levels of feeding were revealed in the analyses. Two related factors may have been responsible for the insensitivity of the regression coefficients. Firstly, an apparently linear portion of an overall exponential function was used. Secondly, the ^{35}S assayed would have been heterogeneous (Downes 1965). The level of feeding did, however, exert a significant influence on the specific radioactivity of cystine being incorporated into the plucked fibres. The specific radioactivity of cystine was less in both the plasma and plucked fibres from sheep consuming the 1100 g of lucerne daily. As the difference between the dietary treatments was less in the plucked fibres than in the plasma, greater dilution of the ^{35}S with non-radioactive cystine during its transfer from plasma to follicle proteins was evident in ewes consuming the 500 g per day. Cystine which was not in isotopic equilibrium with the free cystine in plasma, must have been responsible for this dilution.

A similar effect of feeding level was observed in the specific radioactivity of cystine in the wool clipped at day 11 post-infusion. The specific radioactivity of cystine was also less in the wool clipped from Fleece Plus ewes, despite equal specific radioactivities of free cystine in plasma during the infusions. If the time taken for the ^{35}S to rise with the wool fibres from the follicle bulbs to a height above the skin where it could be harvested by clipping varied among the treatments, the validity of these estimates of the specific radioactivity would be questionable. Emergence time varies with nutritional level, and resultant rate of length increment of the fibres (Downes and Sharry 1971). Thus, a longer emergence time would be expected in those sheep consuming 500 g per day, resulting in underestimation of the specific radioactivity of cystine in clipped wool, relative to that measured in sheep with a greater consumption, and with shorter emergence times. The consistent and rapid decreases in the specific radioactivity of cystine observed in wool clipped at days 18 and 25 post-infusion suggest that the major portion of the ^{35}S was in the wool clipped at day 11, and that differences in emergence times did not seriously bias the comparison between feeding levels. Although length increment of fibres is 10–20% more

rapid in Fleece Plus ewes (Short 1964; Williams 1966), the follicles of these sheep are longer and more deeply embedded in the skin than those of Fleece Minus ewes (Nay 1966). Hence, the mean emergence times were probably similar in the genetic comparison.

Assuming that differences in emergence time were small and have not biased the measurement of radioactivity in the dietary or the genetic comparisons, the results indicated that recovery of infused ^{35}S in the wool within each flock was related to the intake of food, or the availability of cystine. Thus, when the availability of cystine was reduced, a greater proportion of the infused ^{35}S was apparently utilized preferentially for functions other than wool growth. From the results for both the rate of output of cystine in wool and the recovery of infused ^{35}S in wool, it appears that Fleece Plus ewes have a greater capacity to incorporate cystine into follicle proteins, but this greater capacity can only be expressed when the availability of cystine is high; the metabolic requirements for cystine having presumably been met. The greater follicle density of the Fleece Plus ewes may contribute to the greater capacity of these ewes to incorporate cystine into wool proteins, when the availability of cystine is high.

If the wool production per unit area reflected the fleece production of these sheep, the efficiency of conversion of food into wool was greater in the Fleece Plus ewes, and decreased as consumption of food was increased. It is apparent that recovery of infused ^{35}S in the wool was not related to the efficiency of conversion, but this trait was related to the dilution of ^{35}S with non-radioactive cystine during its passage from the plasma through intermediate pools into the wool fibre. Dilution was greater in Fleece Plus ewes, and was less in those ewes consuming 1100 g of lucerne daily. The source and location of this intermediate pool (or pools) of cystine (Downes 1961) which mixes with and dilutes the ^{35}S, cannot be ascertained from the results. Its operation does appear to be associated with a relatively depressed rate of synthesis of high-sulphur proteins in the matrix of the cortical cells.

In the genetic comparison, the dilution of ^{35}S between plasma and wool was approximately 20% greater for Fleece Plus ewes at both feeding levels. These ewes had also a 20% lower concentration of free cystine in the plasma, and this difference was independent of feeding level (Williams *et al.* 1972*a*). The quantitative similarity of these two differences may be fortuitous, or it may reflect the operation of mechanisms causing a genetic difference in the plasma concentration of cystine, from which the differences in the transfer of cystine through intermediate compartments, and the synthesis of high-sulphur proteins result in the genetic differences in efficiency of conversion of food to wool. Further speculation is unwarranted in the absence of more experimental data.

The differences in the mean specific radioactivity of cystine in wool between feeding levels and flocks were not due to differences in the ratios of specific radioactivities of cystine in the high- and low-sulphur proteins in these clipped samples. Both Downes *et al.* (1963) and Fraser (1969*b*), although proposing different mechanisms for the incorporation of cystine into keratin, concluded that the specific radioactivity of cystine in the high-sulphur proteins was initially higher than that in the low-sulphur proteins following a single injection of L-[^{35}S]cystine. However, the difference was eliminated with time. The present results do not confirm this pat-

tern; rather the specific radioactivity of cystine in the high-sulphur proteins remained 10–20% greater until 25 days post-infusion.

In both fractions, the specific radioactivities were lower than that in the wool. This indicated that the material not dissolved by the urea–thioglycollate had a relatively high specific radioactivity of cystine. As this insoluble material would probably include largely high-sulphur proteins (Broad *et al.* 1970) the recorded difference in specific radioactivity between the two fractions may be minimal. No satisfactory explanation can be advanced why these results differ so greatly from those of Downes *et al.* (1963) and Fraser (1969*b*). The results would indicate that the two protein fractions were synthesized from cystine derived from separate compartments. This compartmentation could occur within the follicle if the cystine derived from the extensive trans-sulphuration of methionine (Downes *et al.* 1964) was preferentially incorporated into low-sulphur protein fractions. This suggestion is directly contrary to that made by Rogers (1959) that the methionine may provide cystine for the synthesis of high-sulphur proteins. The mode of incorporation of cystine into keratin remains a controversial problem (Fraser 1969*a*, 1969*b*).

V. Acknowledgments

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VI. References

Ahmed, W., Dun, R. B., and Winston, R. J. (1963).—The efficiency of conversion of feed to wool in Merino flocks selected for or against fleece weight. *Aust. J. exp. Agric. Anim. Husb.* **3**, 269.

Broad, A., Gillespie, J. M., and Reis, P. J. (1970).—The influence of sulphur-containing amino acids on the biosynthesis of high-sulphur wool proteins. *Aust. J. biol. Sci.* **23**, 149.

Carter, H. B., and Clarke, W. H. (1957).—The hair follicle group and skin follicle population of Australian Merino sheep. *Aust. J. agric. Res.* **8**, 91.

Dolling, C. H. S., and Piper, L. R. (1968).—Efficiency of conversion of food to wool. III. Wool production of ewes selected for high clean wool weight and of random control ewes on restricted and unrestricted food intake in pens. *Aust. J. agric. Res.* **19**, 1009.

Downes, A. M. (1961).—On the mechanism of incorporation of [^{35}S]cystine into wool. *Aust. J. biol. Sci.* **14**, 109.

Downes, A. M. (1965).—A study of the incorporation of labelled cystine into growing wool fibres. In "The Biology of Skin and Hair Growth". (Eds. A. G. Lyne and B. F. Short.) p. 345. (Angus and Robertson: Sydney.)

Downes, A. M., Ferguson, K. A., Gillespie, J. M., and Harrap, B. S. (1966).—A study of the proteins of the wool follicle. *Aust. J. biol. Sci.* **19**, 319.

Downes, A. M., and Sharry, L. F. (1971).—Measurement of wool growth and its response to nutritional changes. *Aust. J. biol. Sci.* **24**, 117.

Downes, A. M., Sharry, L. F., and Rogers, G. E. (1963).—Separate synthesis of fibrillar and matrix proteins in the formation of keratin. *Nature, Lond.* **199**, 1059.

Downes, A. M., Sharry, L. F., and Till, A. R. (1964).—The fate of intradermal doses of labelled amino acids in sheep. *Aust. J. biol. Sci.* **17**, 945.

Downes, A. M., and Till, A. R. (1963).—Assay of tritium, carbon-14 and sulphur-35 in wool by liquid scintillation counting. *Nature, Lond.* **197**, 449.

DUN, R. B. (1958).—The influence of selection and plane of nutrition on the components of fleece weight in Merino sheep. *Aust. J. agric. Res.* **9**, 802.

FRASER, I. E. B. (1969*a*).—Proteins of keratin and their synthesis. 1. Proteins of pre-keratin and keratin. *Aust. J. biol. Sci.* **22**, 213.

FRASER, I. E. B. (1969*b*).—Proteins of keratin and their synthesis. II. Incorporation of [^{35}S]cystine into pre-keratin and keratin proteins. *Aust. J. biol. Sci.* **22**, 231.

GILLESPIE, J. M., REIS, P. J., and SCHINCKEL, P. G. (1964).—The isolation and properties of some soluble proteins from wool. IX. The proteins in wools of increased sulphur content. *Aust. J. biol. Sci.* **17**, 548.

HARRAP, B. S., and GILLESPIE, J. M. (1963).—A further study on the extraction of reduced proteins from wool. *Aust. J. biol. Sci.* **16**, 542.

HENDLER, R. W. (1964).—Procedure for simultaneous assay of two β-emitting isotopes with the liquid scintillation counting technique. *Analyt. Biochem.* **7**, 110.

NAY, T. (1966).—Wool follicle arrangement and vascular pattern in the Australian Merino. *Aust. J. agric. Res.* **17**, 797.

PATTERSON, M. S., and GREENE, R. C. (1965).—Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. *Analyt. Chem.* **37**, 854.

REIS, P. J., and SCHINCKEL, P. G. (1963).—Some effects of sulphur-containing amino acids on the growth and composition of wool. *Aust. J. biol. Sci.* **16**, 218.

REIS, P. J., and SCHINCKEL, P. G. (1964).—The growth and composition of wool. 2. The effect of casein, gelatine, and sulphur-containing amino acids given per abomasum. *Aust. J. biol. Sci.* **17**, 532.

ROGERS, G. E. (1959).—Electron microscopic studies of hair and wool. *Ann. N.Y. Acad. Sci.* **83**, 378.

SHORT, B. F. (1964).—Staple length: fibre length ratios in the Peppin Merino fleece under selection. *Proc. Aust. Soc. Anim. Prod.* **5**, 350.

WILLIAMS, A. J. (1966).—The efficiency of conversion of feed to wool during limited and unlimited feeding of flocks selected on clean fleece weight. *Aust. J. exp. Agric. Anim. Husb.* **6**, 90.

WILLIAMS, A. J., LENG, R. A., and STEPHENSON, S. K. (1972*a*).—Metabolism of cystine by Merino sheep genetically different in wool production. I. Comparison of the entry rates of cystine in sheep from flocks selectively bred for high and low fleece weight. *Aust. J. biol. Sci.* **25**, 1259–68.

WILLIAMS, A. J., ROBARDS, G. E., and SAVILLE, D. G. (1972*b*).—Metabolism of cystine by Merino sheep genetically different in wool production. II. The responses in wool growth to abomasal infusion of L-cystine or DL-methionine. *Aust. J. biol. Sci.* **25**, 1269–76.

YOUNG, S. S. Y., and CHAPMAN, R. E. (1958).—Fleece characters and their influence on wool production per unit area of skin in Merino sheep. *Aust. J. agric. Res.* **9**, 363.