Aust. J. Biol. Sci., 1976, **29**, 513–24

Metabolism of Cystine by Merino Sheep Genetically Different in Wool Production IV.* Rates of Entry of Cystine into Plasma, Measured with a Single Intravenous Injection of L-[^{35}S]Cystine, and the Subsequent Incorporation of ^{35}S into Wool Fibres

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Abstract

Ten, 2-year-old Merino ewes from a flock selectively bred for high clean fleece weight (Fleece Plus) and ten from a flock bred for low clean fleece weight (Fleece Minus) were randomly divided between two dietary treatments: 600 or 1100 g/day of pelleted lucerne hay. After 14 weeks, each ewe received an intravenous injection of L-[^{35}S]cystine (66·4 μCi). Venous blood samples were collected at 15 specified times until 8 h after the injections, and wool fibres were plucked until 65–75 days after the injections.

Protein-free filtrates prepared from blood plasma were bulked within sample times for ewes from the same flock and dietary treatment. Equations relating the specific radioactivity of free cystine isolated from the bulked filtrates to time after injection contained three exponential terms. The entry rate and pool size of cystine estimated from these equations were greater in Fleece Minus than in Fleece Plus ewes (by 25 and 44% respectively for entry rate and pool size). Both traits were also higher in ewes offered 1100 g lucerne/day than in those offered 600 g/day (58·7 *v.* 33·9 mg/h for entry rate and 19·2 *v.* 11·8 mg for pool size). The concentration of free cystine in plasma was greater in ewes offered 1100 g lucerne/day (3·0 *v.* 2·1 mg/l; $P < 0{\cdot}05$), and greater in Fleece Minus ewes (3·0 *v.* 2·1 mg/l; $P < 0{\cdot}05$).

The percentage of the injected radioactivity recovered in the wool clipped to day 70 post-injection differed between genotypes and between dietary treatments ($P < 0{\cdot}05$), being greater in Fleece Plus than in Fleece Minus ewes, and greater in those offered 1100 g lucerne/day than in those offered 600 g/day.

The relationships between ^{35}S incorporated per 1000 fibres (R) and time after injection (t) were best fitted by equations of the form

$$R(t) = \sum_{i=1}^{n} X_i[1-\exp(-\alpha_i t)].$$

For all sheep, $n = 3$. The coefficient of the second term was significantly greater ($P < 0{\cdot}05$) in ewes offered 1100 g lucerne/day, whilst the constant of this term was significantly greater in Fleece Minus ewes.

The specific radioactivities of cystine incorporated into wool fibres (SR_f) during various intervals of time after injection were derived from these equations and from the measured rates of output of cystine in wool. The equations computed to relate SR_f to time after injection (t) were of the form

$$SR_f(t) = \sum_{i=1}^{n} B_i \exp(-\beta_i t).$$

Again there were three components. The coefficient of the third component was significantly greater ($P < 0{\cdot}05$) in ewes offered 1100 g lucerne/day, whilst the constant of the second term was significantly greater in Fleece Minus ewes.

* Part III, *Aust. J. Biol. Sci.*, 1973, **26**, 465–76.

Introduction

During intravenous infusions of L-[^{35}S]cystine into sheep from flocks selectively bred for high clean fleece weight (Fleece Plus) or low clean fleece weight (Fleece Minus), Williams *et al.* (1972) found that the mean rates of entry of cystine into the plasma were similar in sheep from these two flocks. The measured entry rates were, however, small and about equal to the presumed rate of absorption of cystine from the intestines. This result contrasted with the entry rate of lysine in humans which was approximately 50 times greater than the rate of absorption (Waterlow 1967). Again, the entry rates of cystine observed in the sheep were only about 10% of those determined for dogs by Crawhall *et al.* (1970) when both sets of data were expressed in terms of unit live weight. While it is generally considered that release of amino acids from tissues into the plasma contributes proportionately most to the entry rate of an amino acid, particularly in larger, older mammals (Waterlow 1967), recent evidence suggests that the entry rate of lysine in sheep is not greater than the presumed rate of absorption (Buttery *et al.* 1975).

In order to determine whether the entry rate of cystine into the plasma was so unusual in Merino sheep, the entry rates were again measured in sheep from the Fleece Plus and Fleece Minus flocks, offered two quantities of a roughage diet, but using a single injection of labelled cystine rather than a continuous infusion. Both techniques are expected to yield similar results for the entry rate of a compound (Gurpide and Mann 1970).

The specific radioactivity of cystine in the wool fibres following the continuous infusion of [^{35}S]cystine was less in Fleece Plus ewes than in Fleece Minus ewes (Williams 1973), despite the similar mean specific radioactivities of cystine in plasma from the two flocks. An intermediate pool of cystine, causing dilution of the label during its passage from plasma into the wool fibre, apparently functioned differently in the two flocks. Because of this, the uptake of ^{35}S into wool fibres following the single injection of L-[^{35}S]cystine was serially monitored so that the curves relating both the ^{35}S accumulated and the specific radioactivity of sulphur incorporated into wool fibres to time after injection could be computed. The kinetic appearance of ^{35}S in wool could then provide further information on the operation of this intermediate pool of cystine and its influence in relation to genetic variation in wool production.

Materials and Methods

Sheep and Dietary Treatments

Ten 2-year-old ewes were chosen from the Fleece Plus flock and 10 from the Fleece Minus flock by stratified random sampling within each flock, based on clean fleece weight and body weight at 17–18 months. These ewes were randomly divided between the dietary treatments—ewes were offered either 600 or 1100 g of ground and pelleted lucerne hay once daily and any refusals were collected and weighed. During the 7 days before and after the injection of L-[^{35}S]cystine this feeding regime was modified, each ewe's daily ration being divided into three equal portions which were presented at 0800, 1200 and 1700 h. The dietary treatments continued from 14 March to 3 September 1968.

Measurement of Wool Growth and Sulphur Content in Wool

All ewes were shorn in December 1967, and extensive areas on both sides of the trunk were closely clipped soon after shearing. Wool was clipped from areas (*c.* 100 cm^2) on the right and left shoulder of each ewe on 14 March 1968, and three measurements of the patch areas were made on that day. Subsequently, wool was removed from these areas at intervals of 21 days, except at the fifth clipping when the interval was 28 days. Fourteen days after the final clipping (3 September 1969), the sheep were shorn and each fleece was weighed and sampled.

The wool clipped from the shoulder patches was cleaned in light petroleum (b.p. 60–80°C), ethanol and distilled water. The fleece samples were scoured as described by Chapman (Appendix to Fraser and Short 1960). Wool production per sheep between clippings was determined by apportioning the clean fleece weight according to the weights of clean wool clipped from the shoulder patches.

Skin was sampled by biopsy punch posterior to the shoulder patch of each ewe on the 14 March 1968, and follicle density was measured by the method of Carter and Clarke (1957).

The sulphur content of the clean wool from clippings 5, 6 and 7 was determined by the combustion method outlined by Reis and Schinckel (1963).

Injection of L-[^{35}S]*cystine*

L-[^{35}S]Cystine (Radiochemical Centre, Amersham: 50 mCi/mmol) was dissolved in a minimum volume of 0·1 M HCl before dilution with sterile physiological saline. Each ewe received a single intravenous injection of this solution at 1000 h during clipping period 5; two ewes were injected with labelled cystine (66·44 μCi) each day. Immediately after injection of L-[^{35}S]cystine, 10 ml of saline was drawn into the syringe and this was also injected. The amount of ^{35}S injected into each ewe was determined after subtraction of residual ^{35}S in each syringe.

Jugular blood samples were obtained from each ewe at 15 specified times after each injection (Fig. 3). Immediately after the collection of blood, plasma was separated and deproteinized with sulphosalicyclic acid (Block *et al.* 1966).

Determination of the Concentration and the Specific Radioactivity of Free Cystine in Plasma

The protein-free filtrate of each sample of plasma (2 ml) was collected, and to this was added the filtrates after two washings of the plasma proteins with 5 ml of sulphosalicyclic acid (2%, w/v). The protein-free filtrates were bulked for each treatment group of five sheep, within sampling times. For each sampling time, four samples were thus obtained, each containing the protein-free filtrate from 10 ml of plasma (2 ml/sheep).

The concentration (mg/l) and specific radioactivity (nCi/mg cystine) of the free cystine in plasma were determined after chromatographic separation of the cystine (Williams *et al.* 1972).

Calculation of the Entry Rate, Pool Size and Space of Cystine

The equations describing the changes in the specific radioactivity of free cystine in plasma (SR_p) with time (t) were calculated using a computer program which minimized variance by iterative procedure, based on the simplex routine (Nelder and Mead 1965). Entry rates, pool sizes and spaces were calculated from these equations by standard procedures (Rescigno and Segre 1966; Baker and Rostrami 1969) with the usual assumptions (Baker *et al.* 1959). In addition, total entry rate (*TER*) of cystine was calculated using the formula

$$TER = Q\left(\sum\sum_{i=1}^{n} A_i' m_i\right),$$

where Q is the pool size of cystine, A_i' is the fractional intercept at $t = 0$ for the ith component, and m_i is the constant of the ith exponential component. The difference between total entry rate and entry rate represents the rate of re-entry into the sampled pool of cystine which has previously left the pool since the injection.

Procedures and Calculations Used to Determine the Incorporation of ^{35}S *into Fibres and the Specific Radioactivity of Cystine in Wool*

Staples of wool were plucked from the midsides of each ewe at various times after the injection of L-[^{35}S]cystine. The interval between pluckings was approximately 12 h during the first 3 days. Thereafter, staples were plucked daily to day 14, tri-weekly to day 30 and weekly to days 65–75. The ^{35}S in weighed portions of the clean dry wool in these staples was measured by direct liquid scintillation counting (Downes and Till 1963). Several samples of plucked fibres from each ewe were combusted in an oxygen flask and the radioactivity in aliquots of the absorption solution was measured to determine the efficiency of counting in the direct procedure. Radioactivity at each sampling was then expressed as nCi/mg clean dry wool.

The average weights of 1000 clean dry fibres plucked at the first two and final samplings were determined. A linear increase in fibre weight with time was assumed, and weights of 1000 fibres

at the various sampling times were calculated and used to estimate the radioactivity expressed as nCi/1000 fibres.

The data for each ewe were fitted to equations of the form

$$R(t) = \sum_{i=1}^{n} X_i[1-\exp(-\alpha_i t)],$$

where $R(t)$ is the ^{35}S incorporated per 1000 fibres at time t after injection, n is the number of exponential terms, X_i is the coefficient of the ith term, and α_i is the constant for the ith term.

From these equations, the ^{35}S incorporated (ΔR) into 1000 fibres between any two sampling times (t_1 and t_2) could be determined. From the rate of wool growth, its cystine content and follicle density, the output of cystine in 1000 fibres (ΔC) between t_1 and t_2 could also be calculated. Division of ΔR by ΔC estimated the specific radioactivity of cystine incorporated into wool fibres (SR_f) at the time $\frac{1}{2}(t_1+t_2)$ after injection.

The equations relating SR_f to t were calculated from these data similarly to those relating SR_p to t. The equations had the form

$$SR_f(t) = \sum_{i=1}^{n} B_i \exp(-\beta_i t),$$

with the same notation as previously described.

Statistical Analyses

The effects of genotypes and of dietary treatments on the variation associated with the various traits were tested by analyses of variance. When measurements were repeated on the same sheep, a 'period' term was included in the model, and the data were analysed using a split-plot design (Brown, in an Appendix to Dolling and Piper 1968).

The significance of differences between genotypes in the concentration of free cystine in plasma were tested within dietary treatments by using a t-test for unequal numbers of unpaired observations (Goulden 1952). A similar analysis was used to test the difference in this parameter between ewes offered 1100 g/day and those offered 600 g/day.

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

Results and Discussion

Dilution of ^{35}S in Plasma following a Single Injection of L-[^{35}S]*cystine*

Although all syringes were prepared to contain equal quantities of L-[^{35}S]cystine, variable small quantities (mean 0·049 μCi, s.d. 0·013 μCi) remaining in the syringes after the injections resulted in small differences among sheep in the quantity of ^{35}S actually injected.

The specific radioactivity of cystine in plasma (SR_p) from each of the four sets of pooled protein-free filtrates declined rapidly following the injection, with a linear relationship between the logarithm of SR_p and time becoming apparent after 200–300 min (Figs 1 and 2). The fit of each of the sets of data to the computed equations relating SR_p to time is also evident from these figures. Each of the curves was best fitted by equations containing three exponential terms (Table 1). The estimates derived from these parameters for entry rates, pool sizes and spaces of cystine are also presented in Table 1.

The cystine was apparently distributed in a volume equivalent to 15–18% of fleece-free live weight, with neither genotype nor dietary treatment influencing this volume. In so far as the extracellular fluid in sheep was determined to occupy a similar space (McDonald *et al.* 1954; English 1966), cystine in the plasma and the interstitial fluids probably represented a single, freely mixing compartment.

The pool sizes and entry rates of cystine varied considerably among the four treatment groups (Table 1), both traits being greater in those ewes offered the larger quantity of food. On the basis of the average intake of food on the day before and on the day of injection, the estimated entry rates per 100 g food were approximately 10–20% lower than estimates derived from continuous infusions of L-[^{35}S]cystine to older sheep consuming 500 or 1100 g daily of chaffed lucerne hay (Williams *et al.*

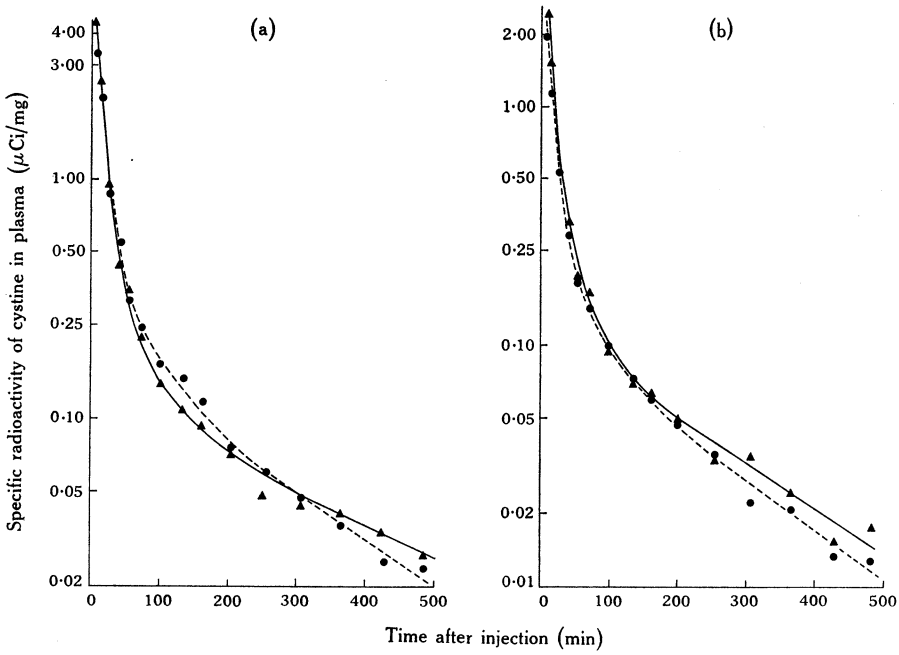

Fig. 1. Decreases in SR_p (log scale) following a single injection of L-[^{35}S]cystine to Fleece Plus (▲) and Fleece Minus (●) ewes offered (*a*) 600 g lucerne/day, and (*b*) 1100 g lucerne/day.

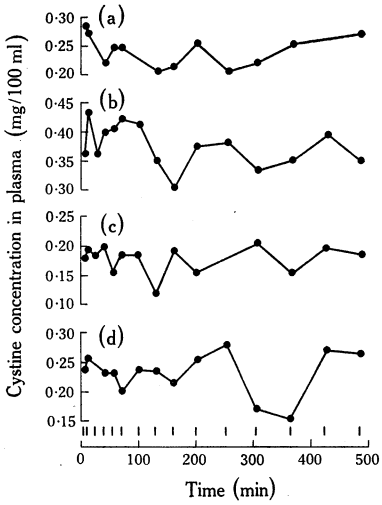

Fig. 2. Concentrations of cystine in plasma at various times after injection of L-[^{35}S]cystine for each treatment group. Sampling times are shown by vertical lines below (*d*). (*a*) Fleece Minus ewes offered 600 g/day; mean concentration ±s.d., 0·235±0·025 mg/100 ml. (*b*) Fleece Minus ewes offered 1100 g/day; mean, 0·373±0·035 mg/100 ml. (*c*) Fleece Plus ewes offered 600 g/day; mean, 0·184±0·016 mg/100 ml. (*d*) Fleece Plus ewes offered 1100 g/day; mean, 0·228±0·012 mg/100 ml.

1972). Allowing for differences in the experimental conditions, the entry rates derived from the two techniques of isotope dilution were reasonably similar. Thus cystine apparently passes into the plasma of Merino sheep at a relatively low rate. If the portal intake of cystine contributed approximately 30% to the net entry rate (Reilly and Ford 1971), or, in quantitative terms, 12 mg/h (Wolff *et al.* 1972), only a small proportion of the 130 mg/h of cystine derived from the turnover of body proteins (Buttery *et al.* 1975) was evidently released into the plasma to augment the entry rate. Consequently, extensive recycling of cystine must occur within the tissues.

Table 1. Parameters for the equations relating SR_p to time after injection for each treatment group, and estimates of pool size, entry rate, and cystine space derived from these parameters

The equations were of the form

$$SR_p = A_1 \exp(-m_1 t) + A_2 \exp(-m_2 t) + A_3 \exp(-m_3 t)$$

Parameter	Fleece Plus ewes		Fleece Minus ewes	
	600 g/day	1100 g/day[A]	600 g/day	1100 g/day[A]
Coefficients (nCi/mg)				
A_1	6273	3052	3901	2262
A_2	597	956	666	537
A_3	89	115	198	113
Constants (min^{-1})				
m_1	0·106	0·138	0·096	0·110
m_2	0·020	0·038	0·026	0·030
m_3	0·002	0·004	0·005	0·005
Pool size (mg cystine)	9·6	16·0	14·0	22·8
Entry rate (mg/h)	30·0	52·2	37·7	65·2
Space (% body weight)	17	17	18	15
Total entry rate (mg/h)	55·7	106·6	69·1	124·6
Recycling (mg/h)	25·7	54·4	31·4	59·4

[A] The average intake of these Fleece Plus ewes on the penultimate and on the day of injection of L-[^{35}S]cystine was 1070 g/day; that of Fleece Minus ewes was 1030 g/day.

Whilst the differences between the dietary treatments were obvious for both pool size and entry rate, the significance of the consistent differences between the flocks in these traits was difficult to assess, in the absence of standard errors. Entry rate and pool size were less in Fleece Plus ewes on both diets than in Fleece Minus ewes (Table 1). This result contrasts with the earlier result which indicated that genotype for wool production had no effect on the entry rate of cystine, measured by a continuous infusion (Williams *et al.* 1972). Although continuous infusions and single injections of a labelled compound should give the same net entry rate to the compound (Gurpide and Mann 1970), it is possible that the continuous infusion of L-[^{35}S]cystine may underestimate the net rate of entry of cystine into the plasma, given the extent and the lability of disulphide binding of cystine to plasma proteins (Downes 1961*b*; Downes *et al.* 1965). The extent of the recycling, presumably due to exchange of cystine between the free and the disulphide-bound form, was evident in the present results (Table 1). In addition, there was an apparent difference between the flocks in the rate of recycling. If the net entry rate of cystine is, in fact, lower in Fleece Plus ewes, the ^{35}S derived from recycled cystine must have contributed proportionately more to the SR_p observed in Fleece Plus ewes than to that observed in Fleece Minus ewes during

the continuous infusions of L-[^{35}S]cystine. Further experiments, preferably including infusions of [^{35}S]cystine disulphide-bound to plasma proteins, are required to resolve the apparently anomolous conclusions. On the basis of the two sets of experimental results, the wisest conclusion that can be drawn at present appears to be that the net entry rate of cystine in genetically superior wool producers is not greater, and may even be less than that in sheep of the inferior wool producers.

The concentration of free cystine in the plasma of each of the treatment groups fluctuated randomly during the 8 h after the injections (Fig. 3). The lower concentrations of cystine in plasma from Fleece Plus ewes ($P < 0{\cdot}05$) and from ewes offered the smaller quantity of food ($P < 0{\cdot}05$) confirmed earlier results (Williams *et al.* 1972).

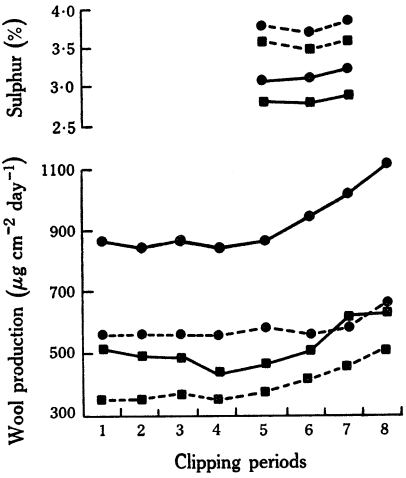

Fig. 3. Daily wool production per unit area of skin for each of the treatment groups during the eight clipping periods, and the sulphur content of the wool clipped in periods 5, 6 and 7.
—— Fleece Plus ewes. – – – Fleece Minus ewes. ■ 600 g/day. ● 1100 g/day.

Wool Production and its Content of Sulphur

The measurements associated with the wool collected at the eight clippings are given in Table 2 and in Fig. 4. The results generally confirmed previous findings with these flocks experiencing similar dietary treatments:

(1) Wool production per unit area of skin and wool production per sheep were greater in Fleece Plus ewes; the difference between the flocks was more pronounced as level of intake of food increased (Williams 1966).

(2) The wool grown by Fleece Plus ewes had a lower sulphur content, and the difference was independent of dietary intake (Williams 1973).

In the present experiment, the efficiency of conversion of food into wool was not influenced by the level of intake. Consequently, no interaction between genotype and diet was evident for efficiency.

Recovery of Injected Radioactivity in Clipped Wool

A significantly greater proportion of the injected radioactivity was recovered in wool clipped from Fleece Plus ewes, and from ewes offered the larger quantity of food (Table 2), confirming previous results (Williams 1973). There, the results were equivocal due to possible bias introduced by variations among treatment groups in

emergence times of the fibres (Downes and Sharry 1971). The large proportions of the injected ^{35}S recovered in the wool emphasize the major role of the follicle population in the metabolism of cystine in Merino sheep.

Whilst the smaller size of the cystine pool could be responsible for the greater recovery of injected ^{35}S in the wool grown by the Fleece Plus ewes, the greater recovery in the wool of the sheep consuming more food can not be explained on this basis. An increased rate of entry of cystine into the plasma pool due to dietary alterations was apparently associated with a proportionate increase in the availability of cystine for incorporation into the wool fibre.

Table 2. Mean values for measurements associated with wool production in each treatment group

Measurement	Fleece Plus ewes		Fleece Minus ewes		s.e.m.	Significance[A]
	600 g/day	1100 g/day	600 g/day	1100 g/day		
Wool production ($\mu g\ cm^{-2}\ day^{-1}$)	506	882	383	558	±99	a,b,c
Follicle density (No./mm^2)	72	64	63	58	±11	
Efficiency (g wool/kg food)	10·62	11·23	7·20	6·70	±1·93	a
Sulphur content (%)	2·84	3·16	3·53	3·78	±0·21	a,b
Recovery of injected ^{35}S in wool (%)	40·2	49·2	36·0	39·6	±3·6	a,b

[A] a, Significant difference between genotypes. b, Significant difference between dietary treatments. c, Significant interaction between genotype and dietary intake.

Incorporation of ^{35}S in Plucked Fibres

Following the injections of L-[^{35}S]cystine, ^{35}S rapidly accumulated in the plucked fibres. The form of the equations computed to relate this ^{35}S incorporation (R) to time after injection (t) was

$$R(t) = \sum_{i=1}^{n} X_i[1-\exp(-\alpha_i t)].$$

Curves containing three exponential terms gave the best relationship between R and t. Of the observed values of R, 87% were within 5% of the values predicted by the equations; less than 4% of the observations differed by more than 10% from the predicted values.

The constants and coefficients for the exponential terms are shown in Table 3. Analyses of variance revealed that the coefficient of the second exponential term, X_2, was 24% greater ($P < 0{\cdot}05$) in the equations for ewes offered the smaller quantity of food. The constant of this exponential term was 78% greater ($P < 0{\cdot}05$) in equations for Fleece Minus ewes. In the analysis of α_3, the constant for the third exponential term, a significant interaction between flocks and dietary treatments was observed.

The sums of the coefficients ($X_1 + X_2 + X_3$) measured the proportions of the injected ^{35}S recovered in 1000 fibres. This parameter was not significantly affected by the experimental treatments. In view of the greater proportion of ^{35}S recovered in wool

clipped from Fleece Plus ewes, total follicle number, rather than the ability of individual follicles, would be a major factor determining the output of cystine in wool which was related to the proportion of ^{35}S recovered in clipped wool (Williams 1973). While differences in follicle number may account for the results in the genetic comparison, there would have to be 20–30% fewer follicles in the ewes offered 600 g lucerne/day to account for the similar values of $X_1+X_2+X_3$ at the two levels of intake, but with a greater recovery of ^{35}S in clipped wool among the ewes offered 1100 g lucerne/day. Shedding of fibres would not be expected to be sufficiently extensive to account for this difference. Sampling error is a more likely explanation.

Table 3. Parameters for the equations relating the accumulation of ^{35}S activity in plucked fibres to time after injection for each treatment group

The equations were of the form

$$R(t) = X_1[1-\exp(-\alpha_1 t)] + X_2[1-\exp(-\alpha_2 t)] + X_3[1-\exp(-\alpha_3 t)].$$

Standard deviations are shown in parentheses

Parameter	Fleece Plus ewes		Fleece Minus ewes	
	600 g/day	1100 g/day	600 g/day	1100 g/day
Coefficients (nCi/1000 fibres)				
X_1	0·098 (0·100)	0·099 (0·022)	0·069 (0·113)	0·085 (0·099)
X_2 [A]	0·237 (0·122)	0·319 (0·173)	0·234 (0·068)	0·289 (0·173)
X_3	0·192 (0·256)	0·112 (0·127)	0·227 (0·207)	0·133 (0·138)
Constants (h^{-1})				
α_1	0·391 (0·507)	0·657 (0·839)	0·574 (0·813)	0·805 (0·942)
α_2 [B]	0·019 (0·008)	0·018 (0·019)	0·029 (0·021)	0·035 (0·021)
α_3 [C]	0·0026 (0·002)	0·0015 (0·001)	0·0022 (0·001)	0·0033 (0·003)

[A] Significant difference between dietary treatments.
[B] Significant difference between genotypes.
[C] Significant interaction between genotypes and diets.

The exponential form of the equations relating the accumulated ^{35}S in plucked fibres to time resulted in the first fibres plucked after the injection having the highest SR_f values. If it is assumed that the cystine in the vascular space and that in the follicles are contained in different metabolic compartments, SR_f should be zero at $t = 0$. Due to the uncertainty of fitting equations with this constraint in the absence of early values less than the maxima, the data for each ewe were fitted to equations of the form

$$SR_f(t) = \sum_{i=1}^{n} B_i \exp(-\beta_i t).$$

Equations containing three exponential terms yielded the best fit of the data for all except four ewes (one in each treatment group). The equations for these four ewes contained four exponential terms. As the reduction in residual variance due to the additional term was marginal, the data for all ewes were fitted to equations containing three terms.

The parameters of the equations were extremely variable among ewes within treatment groups (Table 4). The process of 'curve-peeling' in the derivation of the equations leads to the interdependence of the parameters, thus inflating the variance. The

significant effect of dietary level on B_1, and the interaction between treatments for B_1 are questionable. B_1 for one Fleece Minus ewe offered 600 g/day was 20 times greater than B_1 for the other ewes of this treatment group. As this led to the means and variances of B_1 being related, analysis of the log-transformed data eliminated both the significant effect of treatment and the interaction.

Table 4. Parameters for the equations relating the changes in SR_f to time after injection for each treatment group

The equations were of the form

$$SR_f = B_1 \exp(-\beta_1 t) + B_2 \exp(-\beta_2 t) + B_3 \exp(-\beta_3 t).$$

Standard deviations are shown in parentheses

Parameter	Fleece Plus ewes		Fleece Minus ewes	
	600 g/day	1100 g/day	600 g/day	1100 g/day
Coefficients (nCi/mg cystine)				
B_1[A]	175 (155)	95 (86)	460 (1564)	84 (93)
B_2	18·4 (17·2)	12·1 (8·7)	21·4 (12·0)	19·4 (26·4)
B_3[B]	1·54 (1·92)	0·36 (0·50)	1·59 (0·80)	1·10 (0·92)
Constants (h^{-1})				
$\beta_1 \times 10^3$	447 (441)	483 (379)	461 (669)	609 (599)
$\beta_2 \times 10^4$[C]	199 (76)	229 (81)	290 (224)	367 (200)
$\beta_3 \times 10^5$	295 (264)	182 (149)	254 (120)	348 (312)

[A] Significant difference between dietary treatments, and significant interactions between genotypes and diets.
[B] Significant difference between dietary treatments.
[C] Significant difference between genotypes.

The significantly lower mean coefficient of the terminal exponential (B_3) in the equations for ewes offered 1100 g/day indicated that the total quantity of cystine with which the injected ^{35}S equilibrated was considerably greater in these ewes than in those offered 600 g/day. The value of B_3 for the better-fed sheep indicated that the ^{35}S mixed with 93 g cystine. Assuming that tissue proteins contain a constant 1·5% cystine (Block and Bolling 1951), this quantity of cystine would have been associated with approximately 6 kg of protein—a reasonable estimate of the protein content of the body of a well-fed Merino sheep weighing 35–40 kg (Keenan *et al.* 1969). On the other hand, the 2·9 kg of protein calculated as the protein content of the body of the sheep offered 600 g/day appears improbable. A lowered intake of food may have induced a response which effectively reduced the quantity of cystine with which injected ^{35}S mixed.

The constants of the exponential terms were not influenced by the dietary level, yielding half-times of 1·9, 29 and 319 h for β_1, β_2 and β_3 respectively. Whilst these half-times were considerably shorter than those estimated by Downes (1961*a*) for an individual sheep, the $t_{\frac{1}{2}}$ values estimated for β_1 and β_2 were in close agreement to the estimates of Downes (1965) for the times taken for ^{35}S to pass through water-soluble and urea-soluble fractions (1–2 h and 16–20 h respectively) in the follicle bulb tissue. Downes (1961*a*) had earlier postulated the existence of a cystine pool with a half-life of a few days, and in the later paper, considered that the urea-soluble fraction of the wool root tissue represented this pool. If, in fact, the $t_{\frac{1}{2}}$ of the second exponential

term relates to the time taken for conversion of ^{35}S into a urea-insoluble form in the follicle, the significantly smaller β_2 observed in Fleece Plus ewes (Table 4) would indicate that the process of keratinization would take longer for ewes in this flock.

Whilst the approach adopted in this work has demonstrated a difference in the rate of dilution of isotope in one of the pools supplying cystine to the follicles of sheep genetically different in wool production, more precise techniques are needed to measure follicle function. The use of chemical extracts of wool root tissue (Downes 1965) offers many advantages for these studies, as the specific radioactivity of the proteins being synthesized can be measured directly. Again, further progress in this field probably depends on measurement of the specific radioactivity of a precursor amino acid within the follicle. As the quantity of free cystine in wool root tissue is very small (Rogers 1959), the use of a labelled amino acid whose specific radioactivity can be measured in wool root tissue is required. Micro-techniques, as suggested by Airhart *et al.* (1973), would need to be adopted for these studies, with the difficulty of harvesting wool root tissue from Merino sheep being borne in mind.

Acknowledgments

The competent technical assistance of Mr R. Winston in collecting blood and wool samples is gratefully acknowledged. The late Mr L. Harris, University of New England Computing Centre, greatly assisted with the computing of the equations. This work was financed in part by the Australian Wool Corporation.

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Manuscript received 1 March 1974, revised manuscript received 31 May 1976