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Investigation of Some Amino Acid Analogues and Metabolites as Inhibitors of Wool and Hair Growth

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Abstract

Sheep were given intravenous infusions of ethionine together with cycloleucine or reduced glutathione, in attempts to prevent the inhibition of wool growth by ethionine. Other sheep were given cycloleucine alone to measure effects on wool growth. Twenty-two compounds related to cystine, methionine, ethionine, lysine, phenylalanine and tyrosine were given as intravenous infusions to sheep to investigate their potential as depilatory agents. Nineteen of these compounds were also tested in mice during their first cycle of hair growth. The concurrent administration of cycloleucine with ethionine prevented the weakening of wool fibres caused by ethionine, but reduced glutathione was ineffective. Cycloleucine weakened wool fibres, as judged subjectively, and caused a small reduction in fibre diameter. Selenocystine and selenomethionine caused some hair loss in mice but selenocystine was also toxic. Both seleno-amino acids were toxic for sheep; selenocystine was lethal at 0·025 mmol $kg^{-0.75}$ and selenomethionine at 0·09 mmol $kg^{-0.75}$. Doses that permitted survival of sheep did not have depilatory effects. However, the presence of autophagic vacuoles in the cytoplasm of follicle bulb cells of sheep indicated that a toxic dose of selenocystine had potential depilatory activity. Other compounds investigated did not induce loss of wool or hair. Some compounds, notably 3-methylthiopropionic acid and *S*-(2-aminoethyl)-L-cysteine, were toxic to mice but not sheep. The methionine analogue, methoxinine (*O*-methyl-DL-homoserine), caused a substantial reduction in the strength of wool fibres and a prolonged alteration of the crimp pattern. It is suggested tentatively that cycloleucine inhibits methionine adenosyltransferase and thereby reduces or prevents the formation of S-adenosylethionine. The failure of various compounds related to methionine and ethionine to have any depilatory activity in sheep supports the view that ethionine influences wool growth via the formation of *S*-adenosylethionine.

Introduction

The growth rate and strength of wool fibres are particularly sensitive to the amounts and proportions of amino acids available to the wool follicle (Reis 1979). The omission of either methionine or lysine from a mixture of amino acids infused into the abomasum of sheep inhibits wool growth and weakens wool fibres (Reis and Tunks 1976, 1978). Reis and Tunks (1982) have shown that ethionine, an analogue of methionine, is an inhibitor of wool growth and may cause shedding of the fleece. However, in contrast to various compounds investigated by Panaretto *et al.* (1978), it did not induce hair loss in mice during their first cycle of hair growth. In the study reported in this paper, the effects of cycloleucine and reduced glutathione were investigated in sheep in an attempt to obtain further information relevant to the mode of action of ethionine as an inhibitor of wool growth. The inhibitory effects of amino acids and analogues on wool growth may have application in research aimed at new

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methods for harvesting wool from sheep (Reis and Panaretto 1979). Accordingly, experiments have been carried out to investigate various compounds related to lysine, methionine or ethionine as potential inhibitors of wool and hair growth. In addition to analogues of methionine and ethionine, two metabolites, 3-methylthiopropionic acid and 3-ethylthiopropionic acid, were investigated. The former compound is an intermediate in the metabolism of methionine via a transaminative pathway in mammalian tissues (Steele and Benevenga 1978), and the latter is likewise formed during the catabolism of ethionine (Steel and Benevenga 1979). In view of the different responses of sheep and mice to ethionine (Reis and Tunks 1982), the depilatory activity of most compounds was tested in mice as well as in sheep.

The selenium analogues of some sulfur-amino acids have been reported to have depilatory activity. Thus, selenocystathionine caused hair loss in humans and mice (Aronow and Kerdel-Vegas 1965; Palmer 1968), and alopecia has been reported following the administration of selenocystine to humans (Weisberger and Suhrland 1956). In the present studies, the effects of selenocystine and selenomethionine on wool and hair growth were investigated.

Many treatments that weaken wool have been shown to inhibit the synthesis of the high-tyrosine proteins in wool (Frenkel *et al.* 1974, 1975); these proteins are especially rich in the amino acids tyrosine, phenylalanine and glycine. Analogues of phenylalanine, such as 4-fluorophenylalanine, have been reported to inhibit cell division (Wheatley 1978) and might therefore be expected to influence wool growth. Another analogue of phenylalanine, β-2-thienylalanine, has been reported to inhibit feather growth in chick skin grown in culture medium (Fabiny 1959). In consequence, the effects of various analogues of phenylalanine and tyrosine have been investigated here in sheep and mice. These compounds are structurally related to mimosine, an effective depilatory agent in sheep (Reis *et al.* 1975) and mice (Panaretto *et al.* 1978).

Materials and Methods

Experimental Animals

The experimental sheep were Merino wethers or non-pregnant ewes, consisting of 57 adult animals ranging in body weight from 29 to 49 kg, and three animals 5–6 months old and weighing 14–16 kg. Each animal was subjected to only one treatment schedule and was kept indoors in a metabolism cage in a room where the temperature varied from 20 to 25°C. All sheep received a ground and pelleted diet consisting of lucerne hay (three parts) and oat grain (two parts); the ration of 600 g for mature sheep and 400 g for the young sheep was offered once daily. Drinking water was available *ad libitum*.

Tests with sucking mice in their first hair-growth cycle were carried out, using Sydney White strain mice, as described by Panaretto *et al.* (1978). Most groups consisted of 10 mice with five serving as untreated controls. With selenocystine, groups of 10 treated mice were compared with an untreated control group. The mice were 6–7 days old when dosing began and the average weight of individuals in a group at this time was 3·5–5·5 g.

Chemicals

Ten of the 25 compounds administered to animals were purchased and used without further purification. The source of the purchased compounds was as follows: DL-ethionine, L-norleucine, seleno-DL-cystine, seleno-DL-methionine, *S*-(2-aminoethyl)-L-cysteine (as the hydrochloride), 4-chloro-DL-phenylalanine and 4-fluoro-DL-phenylalanine (Sigma Chemical Co., St Louis, Missouri, U.S.A.), 3,4-dihydroxy-L-phenylalanine (Koch-Light Laboratories, Colnbrook, Bucks, England), reduced glutathione (Calbiochem, San Diego, California, U.S.A.), cycloleucine (1-aminocyclopentane-1-carboxylic acid; Calbiochem and Sigma Chemical Co.). The other 15 compounds listed in Tables

1 and 2 were prepared essentially according to published procedures, with the exception of *O*-methyl-DL-homoserine (methoxinine) and *O*-ethyl-DL-homoserine. These compounds were prepared by aminolysis of the corresponding α-bromo acid in 60–70% yield. The bromo acids were obtained by a malonic ester synthesis with the corresponding 2-alkoxyethyl chloride, followed by saponification, bromination and decarboxylation.

Infrared and proton magnetic resonance spectra of all synthesized compounds were consistent with assigned structures. Purity of compounds was further checked by thin-layer chromatography on either cellulose or silica gel plates.

Preparation and Administration of Doses

Apart from one animal that received cycloleucine as an intravenous injection, all compounds were administered to sheep as continuous infusions via a catheter inserted into a jugular vein. A steady rate of infusion was maintained by means of a peristaltic pump for periods of 1–24 days. Where possible, compounds were dissolved directly in 0·9% (w/v) sodium chloride solution. Infusion volumes were 300–500 ml per 24 h for adult sheep and 100 ml per 24 h for the three younger sheep. To obtain a solution of some compounds (seleno-DL-cystine, *S*-propyl-DL-homocysteine, *S*-butyl-DL-homocysteine, *S*-benzyl-DL-homocysteine, 4-nitro-L-phenylalanine, 3,4-dihydroxy-L-phenylalanine, and 3,5-dibromo-L-tyrosine), it was necessary to acidify with hydrochloric acid; 3-nitro-L-tyrosine was dissolved by making the solution slightly alkaline with sodium hydroxide.

Mice were injected subcutaneously in the dorsal region with 100 μl of the test mixture, once daily for 3 days, unless toxic effects were observed before 3 days. As with sheep, compounds were dissolved in 0·9% (w/v) sodium chloride solution where possible. A solution of 3-nitro-L-tyrosine was prepared as described above, and the solutions of *S*-(2-aminoethyl)-L-cysteine hydrochloride were adjusted to near neutrality with sodium bicarbonate. Other compounds [β-(2-thienyl)-DL-alanine, 4-chloro-DL-phenylalanine, 3,4-dihydroxy-L-phenylalanine, 3,5-dibromo-L-tyrosine, 4-nitro-L-phenylalanine, and higher concentrations of L-norleucine, seleno-DL-cystine and 4-fluoro-DL-phenylalanine] were injected as a suspension prepared by adding carboxymethylcellulose (sodium salt) to 0·9% (w/v) sodium chloride solution.

Outline of Experiments

Six sheep were used to test the efficacy of reduced glutathione and cycloleucine in preventing the inhibition of wool growth by ethionine, by measuring changes in the force required to pluck staples of wool. Pairs of sheep were given intravenous infusions for 2 days, which provided DL-ethionine (25 mg per kg body weight), DL-ethionine (25 mg kg^{-1}) plus reduced glutathione (140 mg kg^{-1}), or DL-ethionine (25 mg kg^{-1}) plus cycloleucine (4 g; 110 and 120 mg kg^{-1}). The infusion of reduced glutathione and cycloleucine commenced 2 hr before the start of ethionine infusions. Three sheep were given cycloleucine either as an intravenous injection (2·4 g; 60 mg kg^{-1}) or as intravenous infusions over 4 days (total doses 8 and 16 g; 206 and 385 mg kg^{-1}, respectively). Wool growth was measured in the two infused sheep.

Twenty-two compounds related to cystine, methionine, ethionine, lysine, phenylalanine and tyrosine were investigated as potential depilatory agents in sheep; 19 of these compounds were also tested in mice (Tables 1 and 2). Compounds 7–11 (Table 1) were given to sheep on a comparative molar basis to ethionine in previous experiments (Reis and Tunks 1982). The lower dose of each of these five compounds was equivalent to the minimum effective dose of ethionine for inhibiting wool growth (20 mg kg^{-1} body weight). Effects on wool growth rate were measured with two compounds given for 24 days (Table 2).

Wool Measurements

After the sheep were dosed with various compounds, the force needed to pluck staples of wool was subjectively assessed. In a similar manner to that described by Reis *et al.* (1975), the wool was classified into four grades: normal, slightly weak, weak and very weak (the fleece could be readily removed from the sheep by hand). This test allowed any potential depilatory compounds to be identified. With eight sheep, the force required to pluck a staple of wool from the midback region was quantified before and after dosing. Five measurements were made on each occasion using a sensitive spring balance; the force was corrected for the cross-sectional area of the staple plucked to

give the depilation force in N ktex^{-1} (Gordon 1980). These values are well correlated with staple breaking force up to 5 N ktex^{-1} (Gordon and Donnelly 1970). The strength of staples of wool grown by two sheep that had been dosed with methoxinine was measured with an Instron 1026 tensile

Table. Dosages of compounds related to cystine, methionine, ethionine and lysine

Each entry represents one sheep or a group of five mice (10 mice for the groups given selenocystine). The doses shown for sheep are the total given by intravenous infusion over 2 days (1 day for the highest dose of selenocystine). The doses shown for mice were given over 3 days as described in the text (1 day only for 0·5 mmol kg$^{-0.75}$ of 3-methylthiopropionic acid)

Compound	Total dose given (mg kg^{-1})	Total dose given (mmol kg$^{-0.75}$)	
	Sheep	Sheep	Mice
Analogue of cystine			
1. Seleno-DL-cystine	0·8	0·006	0·007
HOOC.CH(NH_2).CH_2.Se.Se.CH_2.CH(NH_2).COOH	1·5	0·011	0·014
	3·4	0·025	0·024
	4·5	0·026	
Analogues of methionine			
2. Seleno-DL-methionine	4·4	0·044	0·013
CH_3.Se.CH_2.CH_2.CH(NH_2).COOH	6·0	0·074	0·030
	9·0	0·092	0·060
3. L-Norleucine	40	0·78	1·48
CH_3.CH_2.CH_2.CH_2.CH(NH_2).COOH	80	1·55	3·03
	120	2·27	
4. DL-Crotylglycine	50	0·96	2·34
CH_3.CH=CH.CH_2.CH(NH_2).COOH	50	0·92	
	112	2·12	
5. *O*-Methyl-DL-homoserine	20	0·37	1·45
CH_3.O.CH_2.CH_2.CH(NH_2).COOH	40	0·74	2·89
	52	0·94	
	60	1·16	
Analogues of ethionine			
6. *O*-Ethyl-DL-homoserine	20	0·34	1·23
CH_3.CH_2.O.CH_2.CH_2.CH(NH_2).COOH	40	0·67	2·24
	60	1·01	
7. *S*-Propyl-DL-homocysteine	22	0·33	
CH_3.CH_2.CH_2.S.CH_2.CH_2CH(NH_2).COOH	65	0·93	
8. *S*-Butyl-DL-homocysteine	23	0·32	
CH_3.CH_2.CH_2.CH_2.S.CH_2.CH_2.CH(NH_2).COOH	70	0·90	
9. *S*-Benzyl-DL-homocysteine	28	0·31	
C_6H_5.CH_2.S.CH_2.CH_2.CH(NH_2).COOH	83	0·91	
Metabolites of methionine and ethionine			
10. 3-Methylthiopropionic acid	15	0·31	0·50
CH_3.S.CH_2.CH_2.COOH	44	0·87	0·80
11. 3-Ethylthiopropionic acid	16	0·31	1·27
CH_3.CH_2.S.CH_2.CH_2.COOH	49	0·89	2·42
Analogues of lysine			
12. *S*-(2-Aminoethyl)-L-cysteine	20	0·30	1·55
NH_2.CH_2.CH_2.S.CH_2.CH(NH_2).COOH	41	0·62	2·41
	82	1·18	3·12
13. DL-2-Amino-6-hydroxyhexanoic acid	25	0·39	1·14
HO.CH_2.CH_2.CH_2.CH_2.CH(NH_2).COOH	100	1·64	2·38

tester, with clamps designed both to hold the staple and to measure its thickness (Caffin 1976). The gauge length was fixed at 20 mm. Ten measurements were made on each sheep before and after dosing, and results were expressed as N ktex^{-1}.

Measurements of wool growth rate were carried out on some sheep using the autoradiographic technique of Downes *et al.* (1967). An intravenous injection of a tracer dose of L-[^{35}S]cystine (50–60 μCi; 1·85–2·22 MBq) was given at intervals of 4 or 6 days. Fibre diameter at the front of each radioactive mark, and the distance between each radioactive mark were measured. The distances between two marks were used to calculate the mean length of fibre grown per day. Fibre volume was calculated from mean fibre diameter and length growth rate assuming that the fibres were cylindrical. Fibres were sampled from three or four sites along one side of each sheep; the total number measured per sheep varied from 35 to 120.

Table 2. Dosages of analogues of phenylalanine and tyrosine

Each entry represents one sheep or a group of five mice. The doses shown for sheep are the total given by intravenous infusion over 2 days, 4 days (for a dose of 100 mg kg^{-1} of compounds 14, 15 and 16), or 24 days (for a dose of 320 mg kg^{-1} of compounds 14 and 15). These latter doses were given at a rate of 10 mg kg^{-1} for 16 days followed by 20 mg kg^{-1} for 8 days. The doses shown for mice were given over 3 days as described in the text

Compound	Total dose given (mg kg^{-1}) Sheep	(mmol kg$^{-0.75}$) Sheep	Mice
Analogues of phenylalanine			
14. 4-Fluoro-DL-phenylalanine	25	0·34	0·79
	50	0·70	1·77
	100	1·39	
	320	4·10	
15. 4-Chloro-DL-phenylalanine	25	0·31	0·76
	50	0·63	1·78
	100	1·28	
	320	3·98	
16. β-(2-Thienyl)-DL-alanine	50	0·71	1·01
	100	1·45	2·22
17. 3,4-Dihydroxy-L-phenylalanine	160	1·99	0·89
18. 4-Amino-L-phenylalanine	144	1·98	0·87
19. 4-Nitro-L-phenylalanine	170	2·06	0·73
Analogues of tyrosine			
20. 3-Amino-L-tyrosine	157	1·96	0·81
21. 3-Nitro-L-tyrosine	182	2·05	0·82
22. 3,5-Dibromo-L-tyrosine	271	1·99	0·80

Skin Histology

Skin biopsies, 1 cm in diameter, were taken from the midside region of sheep that received compounds 1–9 (Table 1), both before dosing and at daily intervals for up to 4 days after the start of infusion. The biopsies were treated as described by Chapman and Rigby (1980) and were examined by light microscopy.

Results

Effect of Ethionine and Cycloleucine on Wool Fibres

An infusion of 25 mg kg^{-1} body weight of DL-ethionine, given over 2 days, caused the growth of very weak wool in two sheep. The force required to pluck a staple of wool from these sheep was in the range of 14–20 N ktex^{-1} before infusion, but fell rapidly after infusion to 2–3 N ktex^{-1} (Fig. 1). These latter values are well below the

minimum value of 8 N ktex^{-1} quoted by Gordon and Donnelly (1979) for their control sheep and are essentially a measure of staple breaking force (Gordon and Donnelly 1979). The concurrent infusion of reduced glutathione (140 mg kg^{-1}) to two sheep, which provided 3 moles per mole of ethionine, did not alter the result (Fig. 1). However, the concurrent administration of cycloleucine to two sheep (110 or 120 mg kg^{-1}) prevented the formation of very weak wool, and the force required to pluck a staple of wool after dosing remained in the range of 12–24 N ktex^{-1} (Fig. 1).

Fig. 1. Force required to pluck staples of wool following infusions of DL-ethionine (25 mg kg^{-1}), with and without reduced glutathione (140 mg kg^{-1}), or cycloleucine (4 g; 110 or 120 mg kg^{-1}). Each point is a mean of five measurements for one sheep. Infusions were ethionine (●, ○), ethionine plus reduced glutathione (▲, △), and ethionine plus cycloleucine (■, □).

An injection of 2·4 g cycloleucine (60 mg kg^{-1}) had no apparent effects on the strength of wool fibres and there were no adverse effects on the sheep. Infusions of a total of 8 or 16 g (206 or 385 mg kg^{-1}) cycloleucine over 4 days caused the growth of weak wool, as judged subjectively, and small reductions in fibre diameter (Table 3). Length growth rate and fibre volume were slightly reduced during the 4 days after the infusion finished (Table 3). However, interpretation of these latter effects is complicated by the refusal of both sheep to eat from day 4 onwards. They subsequently died at days 10 and 21 after infusions of 16 and 8 g respectively.

Selenium-containing Amino Acids

Selenocystine was toxic for both sheep and mice. Doses of 0·026 and 0·025 mmol kg$^{-0.75}$ (4·5 and 3·4 mg kg^{-1}, Table 1) killed sheep after 1 and 2 days, respectively; lower doses had no obvious effects on sheep apart from feed refusals for 2 days following a dose of 0·011 mmol kg$^{-0.75}$. Doses of 0·014 and 0·024

mmol $kg^{-0.75}$ reduced the body weight gain in mice when compared with an untreated control group. These animals became very disturbed and hyperactive by the second day of dosing, and the highest dose caused a mortality rate of 50%. Selenomethionine was less toxic than selenocystine. A dose of 0·092 mmol $kg^{-0.75}$ (Table 1) killed a sheep after 3 days, whereas 0·074 mmol $kg^{-0.75}$ caused a sheep to

Table 3. Effects of cycloleucine on wool growth

Cycloleucine was given by continuous intravenous infusion for 4 days. Sheep 1 received a total of 8 g (206 mg kg^{-1}) and sheep 2 a total of 16 g (385 mg kg^{-1}). The values are means (±s.e.) calculated from 37 fibres (sheep 1) and 35 fibres (sheep 2)

No. of days from start of infusion	Sheep 1			Sheep 2		
	Fibre diameter (μm)	Fibre length growth (μm day^{-1})	10^{-3} × Fibre volume growth (μm^3 day^{-1})	Fibre diameter (μm)	Fibre length growth (μm day^{-1})	10^{-3} × Fibre volume growth (μm^3 day^{-1})
−6	18·4±0·43			19·8±0·31		
		249±6·0	67±4·1		326±7·6	103±4·7
0	17·9±0·51			20·0±0·39		
		256±7·7	64±4·5		323±8·0	97±4·2
4	16·9±0·48			18·8±0·36		
		230±8·6	48±3·2		298±6·1	82±3·2
8	15·1±0·40			18·3±0·32		

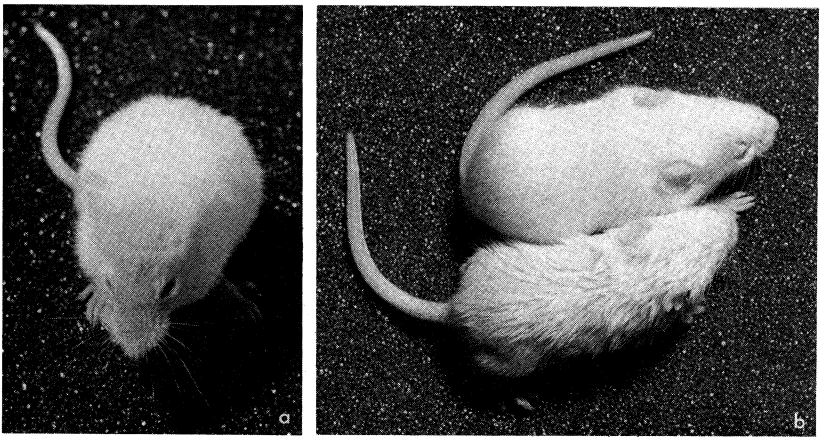

Fig. 2. Effects of amino acid analogues on sucking mice. (*a*) A mouse dosed with seleno-DL-methionine (0·06 mmol $kg^{-0.75}$), showing hair loss on the head. (*b*) A mouse dosed with *S*-(2-aminoethyl)-L-cysteine (2·41 mmol $kg^{-0.75}$) showing appearance of the coat, compared with a control (upper) mouse.

become ill but it appeared to be fully recovered within 3 weeks. No untoward effects were observed in mice given the two lower dose rates of selenomethionine. A dose of 0·06 mmol $kg^{-0.75}$ impaired growth rate and the animals became disturbed and hyperactive as with selenocystine. No deaths of mice due to dosing with selenomethionine occurred.

When mice were dosed with selenium-containing amino acids (Table 1), doses of 0·014 and 0·024 mmol $kg^{-0.75}$ of selenocystine and 0·06 mmol $kg^{-0.75}$ of selenomethionine caused partial hair loss in all mice, particularly on the head (Fig. 2*a*). In contrast, neither selenocystine nor selenomethionine proved effective for inducing

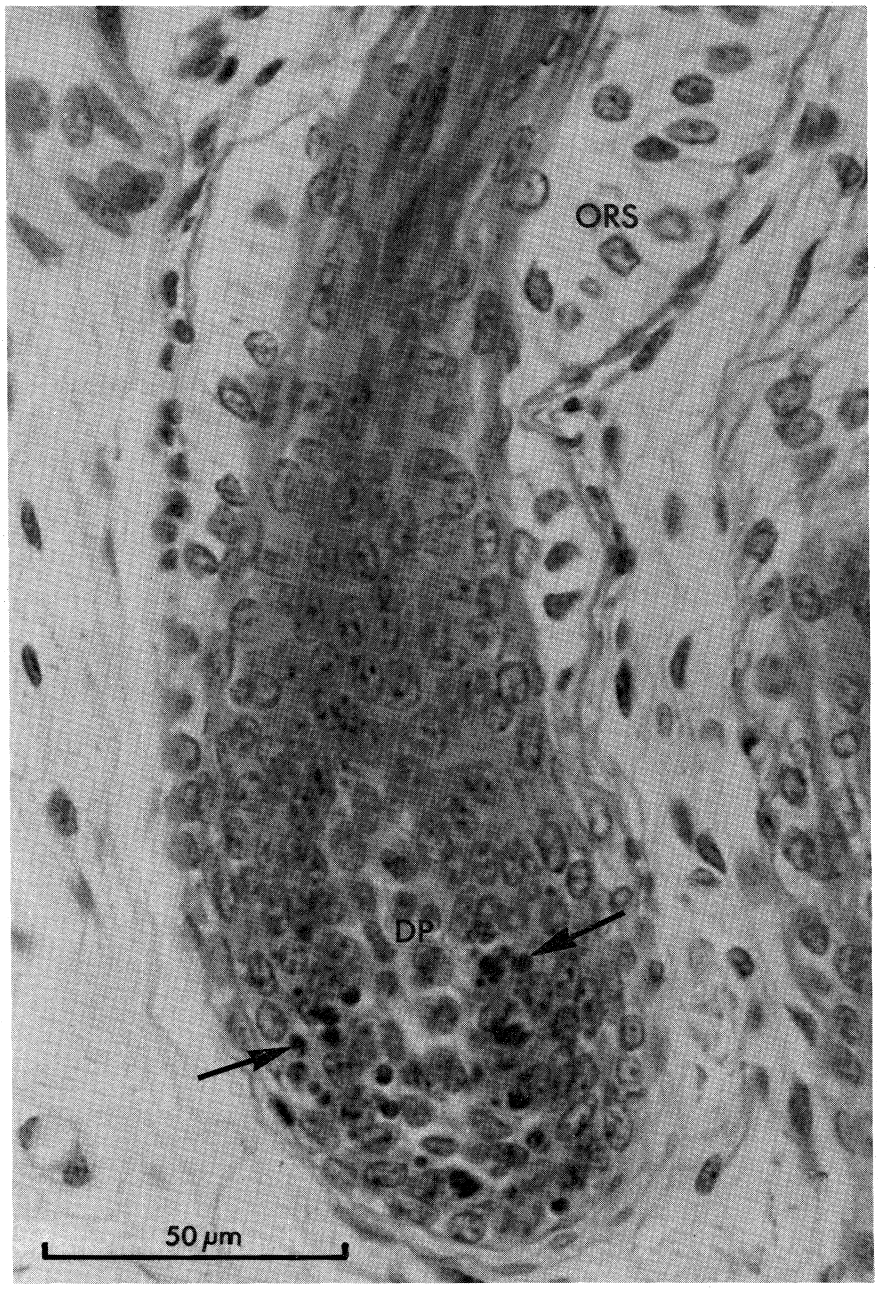

Fig. 3. A wool follicle bulb with dark-staining bodies (arrows, autophagic vacuoles). The skin biopsy was taken at the end of a 24-h intravenous infusion of selenocystine that provided 0·026 mmol $kg^{-0.75}$. *DP*, dermal papilla; *ORS*, outer root sheath.

the growth of weakened wool in suriving sheep, with doses up to 0·011 mmol $kg^{-0.75}$ of selenocystine and 0·074 mmol $kg^{-0.75}$ of selenomethionine (Table 1). However, examination of skin biopsies taken just before the death of the sheep that received 0·025 and 0·026 mmol $kg^{-0.75}$ of selenocystine showed the presence of numerous dark-staining bodies (autophagic vacuoles) in the cytoplasm of follicle bulb cells (Fig. 3). Such a finding is indicative of impending regression of wool follicles (Chapman 1980; Chapman and Rigby 1980). Also, a few autophagic vacuoles were seen in most bulb cells before the death of the sheep that received 0·092 mmol $kg^{-0.75}$ of selenomethionine.

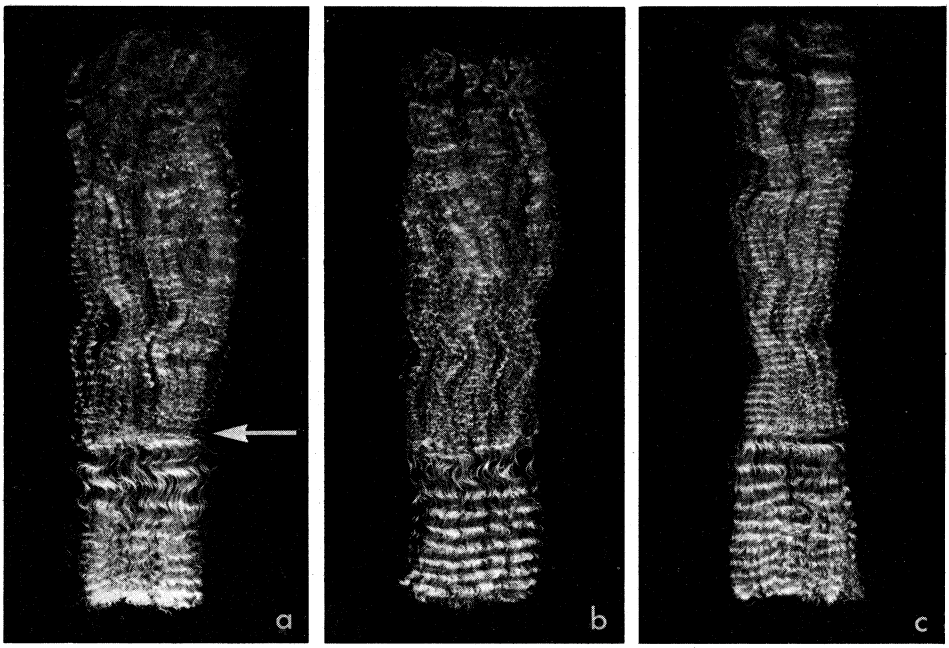

Fig. 4. Effects of methoxinine on staple crimp. A sheep received a dose of 60 mg kg^{-1} body weight of methoxinine (arrow). Staples were harvested 18 weeks later from sites on shoulder (*a*), midside (*b*) and thigh (*c*).

Compounds Related to Methionine, Ethionine and Lysine

Apart from selenomethionine, three further analogues of methionine (compounds 3–5, Table 1) were investigated in sheep and mice. No autophagic vacuoles were seen in follicle bulb cells of sheep after dosing with these compounds. No effects on wool or hair fibres were observed with compounds 3 and 4, but methoxinine (compound 5, *O*-methyl-DL-homoserine) caused a prolonged alteration in the staple crimp pattern at the two higher doses given to sheep (Fig. 4). Hair growth in mice did not appear to be affected by methoxinine. The wool fibres were also weakened in the region grown immediately after dosing. The force required to break staples (in N $ktex^{-1}$, ±s.e.) was reduced from 63·2±1·97 to 17·9±0·70 and from 51·7±2·24 to 13·0±0·59 for doses of 52 and 60 mg kg^{-1}, respectively.

No effects on wool fibres were observed following doses of analogues of ethionine (compounds 6–9, Table 1), and no autophagic vacuoles were observed in follicle

bulb cells. Likewise, compound 6 did not influence hair growth in mice; compounds 7–9 were not tested. The metabolites of methionine and ethionine (compounds 10 and 11, Table 1) did not affect wool or hair growth. However, 3-methylthiopropionic acid was toxic for mice but not sheep. A single dose of 0·5 mmol $kg^{-0.75}$ killed four out of five mice, and 0·8 mmol $kg^{-0.75}$ given over 3 days caused the subsequent death of four out of five mice. 3-Ethylthiopropionic acid reduced growth rates in mice.

Neither analogue of lysine (compounds 12 and 13, Table 1) influenced wool growth nor caused untoward effects on the sheep. Likewise, hair loss was not induced in mice by either compound. Both compounds reduced body growth and killed some mice at all dose levels. *S*-(2-Aminoethyl)-L-cysteine (compound 12) killed four out of five mice at the two higher doses given (Table 1). This compound also altered the appearance of the coat in the surviving mice (Fig. 2*b*).

Analogues of Phenylalanine and Tyrosine

None of the analogues of phenylalanine or tyrosine tested in sheep and mice (Table 2) induced the growth of weakened wool or hair loss. Apart from some feed refusals for 4 days after a dose of 100 mg kg^{-1} 4-fluoro-DL-phenylalanine, no adverse effects were observed in sheep. Wool growth was measured before dosing and during two 4-day periods following the start of dosing in the six sheep that received compounds 17–22. No compound changed the rate of wool growth. When two sheep were given an infusion of either 4-fluoro-DL-phenylalanine or 4-chloro-DL-phenylalanine for 24 days (total dose 320 mg kg^{-1} body weight), neither compound changed the rate of wool growth nor the strength of wool fibres as estimated by the force required to pluck a staple of wool. No adverse effects were observed with either sheep. No untoward effects were observed in mice apart from some reduction in growth rate with compounds 14–17. In addition, 4-chloro-DL-phenylalanine (compound 15) killed one out of five mice at the higher dose given.

Discussion

Ethionine has been shown to be an inhibitor of wool growth (Reis and Tunks 1982), but its mode of action in this regard has not been elucidated. The failure of other analogues of methionine (norleucine, crotylglycine and methoxinine) to exert a depilatory action on sheep in the present studies, despite the fact that methoxinine weakened wool fibres, indicates that a specific function of ethionine is likely to be involved in the inhibition of wool growth. L-Ethionine is a substrate for methionine adenosyltransferase (EC 2.5.1.6) with the resultant formation of *S*-adenosylethionine, but the other analogues of methionine mentioned above cannot serve as substrates for this enzyme (Lombardini *et al.* 1970). These facts are consistent with a hypothesis that ethionine influences wool growth via the formation of *S*-adenosylethionine. Such a reaction could influence wool growth by interfering with important methylation reactions or with the biosynthesis of the polyamines, spermidine and spermine, which appear to have a role in nucleic acid and protein synthesis, especially in actively dividing tissues (Tabor and Tabor 1976; Williams-Ashman and Canellakis 1979). The failure of the analogues of ethionine, *S*-propyl-, *S*-butyl- and *S*-benzylhomocysteine, to influence wool growth is also in agreement with the suggestion that ethionine acts via the formation of *S*-adenosylethionine. *S*-Propylhomocysteine

cannot act as a substrate for methionine adenosyltransferase (Stekol *et al.* 1964; Lombardini *et al.* 1970), and the other analogues would also be expected to be inactive with this enzyme (Lombardini *et al.* 1970).

More direct evidence for the involvement of *S*-adenosylethionine formation was obtained by the demonstration that the concurrent administration of cycloleucine prevented the inhibition of wool growth by ethionine. Cycloleucine is an inhibitor of methionine adenosyltransferase (Lombardini *et al.* 1970; Lombardini and Talalay 1973), and it can be postulated that it has acted by preventing the formation of *S*-adenosylethionine. The slight inhibition of wool growth by cycloleucine alone (Table 3) could be related to an inhibition of methionine adenosyltransferase by cycloleucine. However, cycloleucine is also known to influence the transport of some amino acids (Craan and Bergeron 1975) and to cause a substantial aminoaciduria, especially of cystine, lysine, arginine and ornithine (Brown 1967; Goyer *et al.* 1969). It may thus modify the effects of ethionine on wool growth, or influence wool growth directly, by alternative mechanisms.

The attempt to modify the effects of ethionine on wool growth by the concurrent administration of reduced glutathione was based on the observations of Hsu *et al.* (1968) and Glaser and Mager (1974) that hepatic concentrations of reduced glutathione are depleted for up to 5 h after dosing rats with ethionine. However, the lack of protection afforded by reduced glutathione in these experiments indicates that the effects of ethionine on wool growth are not related to a depletion of reduced glutathione.

Ethionine can be metabolized in rats via a transaminative pathway (Steele and Benevenga 1979), and it is possible that products of such a pathway in sheep could inhibit wool growth. The failure of 3-ethylthiopropionic acid to influence wool fibres does not support this mode of action, but before it can be ruled out 4-ethylthio-2-oxobutyric acid, a previous metabolite on this pathway, should be tested for activity. On a comparative molar basis, the higher dose of 3-ethylthiopropionic acid (Table 1) was three times the minimum effective dose of ethionine for inhibiting wool growth (Reis and Tunks 1982).

The seleno-amino acids, selenocystine and selenomethionine, had effects at much lower doses than the other compounds investigated. Both compounds exhibited some depilatory activity in mice. Doses of selenocystine and selenomethionine that permitted survival of the sheep were insufficient to either stop wool growth or weaken fibres. The presence of large numbers of autophagic vacuoles in follicle bulb cells after dosing sheep with a variety of compounds is associated with subsequent regression of follicles and casting of the fleece (Chapman 1980; Chapman and Rigby 1980). The finding of numerous autophagic vaculoles in follicle bulb cells of sheep given the higher doses of selenocystine thus indicates potential depilatory activity, which cannot be observed due to the toxicity of these doses. Autophagic vacuoles were not observed in follicle bulb cells of sheep given compounds 3–9 (Table 1), which failed to cause casting of the fleece. Selenomethionine can serve as a substrate for methionine adenosyltransferase (Stekol 1963; Pan and Tarver 1967; Lombardini *et al.* 1970) and might therefore be expected to interfere with *S*-adenosylmethionine formation as postulated with ethionine, with the resultant inhibition of wool growth. However, the highest dose of selenomethionine that allowed survival of the sheep provided only about one-quarter of the dose (on a molar basis) of ethionine required to inhibit wool growth (Reis and Tunks 1982).

The depilatory activity of seleno-amino acids in mice is in accord with the previously reported activity of selenocystathionine in mice and humans (Aronow and Kerdel-Vegas 1965; Palmer 1968) and of selenocystine in humans (Weisberger and Suhrland 1956). The present data indicate that selenocystine is about equally toxic for mice and sheep when doses are calculated on the basis of metabolic body weight, with a lethal dose in the region of 8 mg $kg^{-0.75}$. Recalculation of the data of Palmer (1968) indicates that a lethal dose of selenocystathionine for mice is of the same order, namely about 10 mg $kg^{-0.75}$. In contrast, selenocystine appears to be much less toxic for humans (Weisberger and Suhrland 1956). Precise calculations cannot be made, but some patients in these studies received a total dose in the region of 250–300 mg $kg^{-0.75}$ over an extended period without lethal effects.

Although it does not act as a depilatory agent, it is apparent that methoxinine (*O*-methyl-DL-homoserine) influences wool fibres as judged by the reduction in strength and the prolonged alteration of staple crimp. This compound has not previously been studied in sheep, but the effects on wool fibres warrant further investigation. Methoxinine has been reported to reduce weight gain in rats and mice; these effects are alleviated by methionine (Shaffer and Critchfield 1948; Travers and Cerecedo 1951). In the absence of a sulfur or selenium atom, methoxinine would not act as a substrate for methionine adenosyltransferase (Lombardini *et al.* 1970). It must, therefore, act on wool fibres by a different mechanism from that postulated for ethionine.

No evidence was obtained in the present studies for any potential depilatory compounds amongst analogues of lysine, phenylalanine or tyrosine. The failure of 4-fluorophenylalanine to influence wool growth, even after prolonged administration, was somewhat surprising in view of its reported activity as an inhibitor of cell division (Wheatley 1978).

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