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Inhibitory Effects of Ethionine, an Analogue of Methionine, on Wool Growth

P. J. Reis and D. A. Tunks

Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

Varying amounts of DL-, L- or D-ethionine were administered intravenously to sheep, either as a continuous infusion, usually over 2 days, or as a single injection. Groups of sucking mice and rats, in their first cycle of hair growth, were given subcutaneous injections of DL-ethionine at several dose rates.

Ethionine was a potent inhibitor of wool growth in sheep; the L- and D-isomers appeared equally effective. An infusion of 20 mg/kg DL-ethionine (*c.* 50 mg/kg$^{0\cdot75}$) given at a daily rate of 10 mg/kg for 2 days, or an injection of 40 mg/kg DL-ethionine (*c.* 100 mg/kg$^{0\cdot75}$), were sufficient to cause the growth of very weak wool and allow the fleece to be readily removed by hand within 3 weeks after dosing. The inhibition of wool growth was usually associated with a concentration of ethionine in blood plasma, during intravenous infusion, of 10 μmol/l or higher. An infusion of DL-ethionine at a daily rate of 1 mg/kg for 12 days caused the growth of weak fibres and substantially reduced both length growth rate and diameter of fibres.

The toxicity of ethionine to sheep was dependent on the total dose and the duration of administration. An infusion of 40 mg/kg (20 mg/kg daily for 2 days) produced severe effects, but the sheep recovered; a dose of 14 mg/kg (2 mg/kg daily for 7 days) was lethal. The effects of ethionine on wool growth were reduced or prevented by the concurrent infusion of methionine (10–15 mol/mol ethionine).

Doses of DL-ethionine as high as 460 mg/kg$^{0\cdot75}$ failed to cause hair loss in sucking mice. While body growth was severely retarded at this dose, no deaths occurred. Likewise, DL-ethionine failed to cause hair loss in sucking rats, but was lethal to some rats at a dose of 360 mg/kg$^{0\cdot75}$.

Introduction

The rate of wool growth is considerably influenced by the supply of sulfur-amino acids to the wool follicles (Reis 1979). While the major sulfur-amino acid required for the synthesis of wool proteins is cysteine, methionine is equally effective for stimulating wool growth in many circumstances (Reis 1979), although excessive amounts of methionine, given abomasally or parenterally, can depress wool growth (Reis 1979). In addition, wool growth is depressed, and the fibres are substantially weakened, by the abomasal administration of mixtures of amino acids lacking methionine (Reis and Tunks 1978). Apart from being an essential amino acid for the synthesis of proteins in mammals, methionine has several important biological functions. Thus, it plays a key role in the initiation of protein synthesis as *N*-formylmethionine-sRNA (Weissbach and Ochoa 1976), it is converted to cysteine via the transulfuration pathway, the first step of which is the formation of *S*-adenosylmethionine (Finkelstein 1970) and it is a major donor of methyl groups, via *S*-adenosylmethionine.

Ethionine, an antagonist of methionine (Meister 1965), has been shown to interfere with various aspects of methionine metabolism in mammals (Simpson *et al.* 1950; Fowden *et al.* 1967; Farber 1971). Consequently, the effects of ethionine on wool growth, and on hair growth in mice and rats during the first cycle of growth, have been investigated.

Materials and Methods

Experimental Animals

The experimental sheep were mature Merino wethers or ewes, ranging in body weight from 34 to 48 kg. Thirty-six sheep were used in the various experiments, each animal being subjected to only one treatment schedule. The sheep were kept in metabolism cages in a room where the temperature varied from 20 to 25°C. The ration, 600 g of a ground and pelleted mixture of lucerne hay (3 parts) and oat grain (2 parts), 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). Each group consisted of 10 mice, with five being untreated controls; the mice were 6–7 days old when dosing began and had an average weight of *c.* 5 g. Tests with rats in their first hair growth cycle were carried out with Wistar strain rats, in a similar manner to those for mice. Group size and numbers of untreated controls varied as indicated in Table 5. Rats were 5–7 days old when dosing began and the average weight varied from 12–16 g in different groups.

Chemicals

The source of the various compounds administered was as follows: DL-ethionine (Sigma Chemical Co., St. Louis, Missouri, U.S.A., or E. Merck, Darmstadt, Germany), D- and L-ethionine (Sigma Chemical Co.), DL-methionine (BDH Chemicals Ltd., Poole, England), L-cysteine hydrochloride monohydrate (Ajinomoto Co. Inc., Tokyo, Japan), adenine hemisulfate monohydrate and choline chloride (Calbiochem-Behring Corp., La Jolla, California, U.S.A.). L-Mimosine was prepared as described by Reis *et al.* (1975).

Preparation and Administration of Doses

Sheep

Ethionine, or ethionine plus methionine, cysteine, adenine or choline, were dissolved in 0·9% (w/v) sodium chloride solution. Intravenous infusions were usually given continuously for 2 days via a catheter, inserted into a jugular vein. Two sheep received infusions for 7 and 12 days respectively (see Table 1). Administration was maintained at a steady rate by means of a peristaltic pump. Infusion volumes ranged from 300 to 500 ml/24 h for 2-day infusions and were 90 ml/24 h for the longer infusions. Intravenous injections of ethionine were given into a jugular vein over a period of 3–5 min in a volume of 40–70 ml. Ethionine was dissolved in these small volumes of sodium chloride by the addition of two to four drops of 6 M HCl and warming.

Mice and rats

DL-Ethionine was dissolved in 0·9% (w/v) sodium chloride solution and was injected subcutaneously in the dorsal region, once daily for 2 or 3 days, in a volume of 50 or 100 μl for mice and 150 μl for rats. One group of rats was given 100 μl/day of a suspension prepared by adding 150 mg DL-ethionine and 20 mg carboxymethylcellulose (sodium salt) to 3 ml 0·9% (w/v) sodium chloride solution. A solution of L-mimosine was prepared as described by Reis *et al.* (1975); rats were injected with 150 or 200 μl/day.

Wool Measurements

Seven to 10 days after dosing with ethionine, a subjective assessment was made of the strength of wool fibres 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). The latter grade was equivalent to a complete break in the fleece as defined by Reis and Panaretto (1979), in which all or most fibres are shed from the follicle canal.

One sheep that received a daily infusion of ethionine for 12 days (1 mg/kg body weight) continued to grow wool, and measurements were made of wool growth rate by two methods. Firstly, the mass of wool grown per unit time was measured for a 10-day period prior to infusion and for 8-day periods for 32 days after infusion commenced. Wool was removed from a defined area of skin (*c.* 150 cm^2) with small animal clippers (Oster size 000), and was cleaned as described by Reis (1967). The rate of growth of clean dry wool, during and after administration of ethionine, was expressed relative to the pre-infusion rate. Secondly, wool growth was measured by the autoradiographic technique of Downes *et al.* (1967). An intravenous injection of a tracer dose of L-[^{35}S]cystine (*c.* 50 μCi) was given on the following days relative to the start of ethionine infusion: −10, −4, 0, 4, 8, 12, 16, 22 and 28 (see Fig. 2). Measurements were made of fibre diameter at the front of each radioactive mark, and of the distance between each radioactive mark. The distances between two marks were used to calculate the mean length of fibre grown per day. Fibre volume was calculated from fibre diameter and length growth rate for each period assuming that the fibres were cylindrical. Measurements were made of 105 fibres; there were approximately equal numbers from each of four sites along one side of the sheep.

For scanning electron microscope studies, fibres were washed twice with light petroleum (Shell X4). They were mounted on stubs, coated with gold in a sputter coater (Dynavac Model SC150), and were examined with a model Super IIIA microscope (International Scientific Instruments Inc.).

Analysis of Ethionine in Blood Plasma

Blood samples were collected from a jugular vein, using heparin as an anticoagulant. Plasma was separated by centrifugation and the samples were stored at −10°C pending analysis.

Blood plasma was deproteinized by the addition of 30 mg sulfosalicylic acid per millilitre of plasma, followed by mixing and centrifugation. A sample of the supernatant representing 1 ml of plasma was analysed with a Technicon amino acid analyser (model NC-1, Technicon Co., New York), using a jacketed column 0·6 cm in diameter with a packed length of 18 cm of Aminex A5 resin (Biorad, Richmond, California). The amino acids were eluted with 0·2 M sodium citrate buffers, employing a flow rate of 42 ml/h and a column temperature of 35°C. A buffer of pH 2·36 (plus 6% methyl cellosolve) was pumped for *c.* 190 min, followed by a buffer of pH 2·80 (plus 2% methyl cellosolve); ethione was eluted at *c.* 280 min. Ethionine added to plasma was adequately separated in this system and was eluted after leucine. Plasma concentrations were calculated using a standard curve constructed by the addition of varying amounts of ethionine to plasma.

Results

Effects of Ethionine on Wool Growth

It can be seen from Table 1 that ethionine is a potent inhibitor of wool growth. When DL-ethionine was administered as an intravenous infusion over 2 days, a dose of 2·5 or 5 mg/kg body weight had no apparent effects on wool growth. A dose of 10 mg/kg of DL-ethionine (*c.* 25 mg/kg$^{0\cdot75}$) caused the growth of very weak wool which allowed removal of the fleece in one out of two sheep; the wool was slightly weakened by a similar dose of D- or L-ethionine (Table 1). Infusions of 20 mg/kg (*c.* 50 mg/kg$^{0\cdot75}$) caused the growth of very weak wool in three out of four sheep dosed with DL-ethionine, and in four sheep that received D- or L-ethionine (Table 1).

The fleece was readily removed by hand from these sheep within 3 weeks after dosing; regrowth of new fibres was evident above the skin at 3 weeks. Similar effects were observed following an infusion of 40 mg/kg given to one sheep. A band of weak wool appeared in the fleece of the fourth sheep that received an infusion of 20 mg/kg (Fig. 1). The wool fibres could be readily broken at this point. A dose of 20 mg/kg ethionine, which approximates the minimum amount needed to cause the fleece to be shed, is equivalent to 0·122 mmol/kg.

A single intravenous injection of DL-ethionine also resulted in the growth of very weak wool (Table 1). However, a dose of 40 mg/kg was required to achieve this effect; an injection of 20 mg/kg produced only a band of weak wool.

Table 1. Effects of ethionine on sheep

Isomer	No. of sheep	Total dose given (mg/kg)	Total dose given ($mg/kg^{0\cdot75}$)	Effects on fibre growth	Other effects observed
			Intravenous infusion of ethionine for 2 days		
DL-	1	2·5	6·4	Normal wool	Nil
	2	5	13, 13	Normal wool	Nil
	2	10	25, 26	Normal wool (1 sheep) Very weak wool (1 sheep)	Nil
	4	20	48, 50, 51, 52	Weak wool (1 sheep) Very weak wool (3 sheep)	Nil (1 sheep). Feed refusals for 2–7 days (3 sheep); complete refusal for 2 days (1 sheep)
	1	40	99	Very weak wool	Sheep sick. Feed refusals for 8 days; complete refusal for 4 days
	2	121, 124	306, 313		Both sheep died 4 days after start of infusion
L-	1	10	25	Slightly weak wool	Nil
	2	20	50, 52	Very weak wool	Feed refusals for 1–3 days; complete refusal for 1 day (1 sheep)
D-	1	10	24	Slightly weak wool	Nil
	2	20	51, 52	Very weak wool	Feed refusals for 6–9 days; complete refusal for 1–4 days
			Single intravenous injection of ethionine		
DL-	2	20	50, 51	Weak wool	Nil
	3	40	99, 101, 102	Weak wool (1 sheep) Very weak wool (2 sheep)	Nil (1 sheep). Feed refusals for 2–5 days (2 sheep)
			Daily intravenous infusion of ethionine (1 mg/kg) for 12 days		
DL-	1	12	30	Weak wool	Nil
			Daily intravenous infusion of ethionine (2 mg/kg) for 7 days		
	1	14	35	Weak wool before death	Sheep died 2 days after infusion terminated

When a sheep was given an intravenous infusion of DL-ethionine at a daily rate of 1 mg/kg for 12 days, fibre growth was maintained but a band of weak wool was produced. Both diameter and length growth rate of fibres were rapidly depressed during the infusion, but returned to pretreatment values 10 days after the treatment stopped (Fig. 2). Ethionine more than halved the rate of wool growth in this sheep, as judged by measurements of the volume or mass of wool grown (Fig. 2). The latter values showed a delayed effect due to the time taken for fibres to emerge above the skin.

Fibres from the sheep that exhibited a weak band in the fleece, following an infusion of 20 mg/kg DL-ethionine over 2 days (see Fig. 1), were examined in the scanning

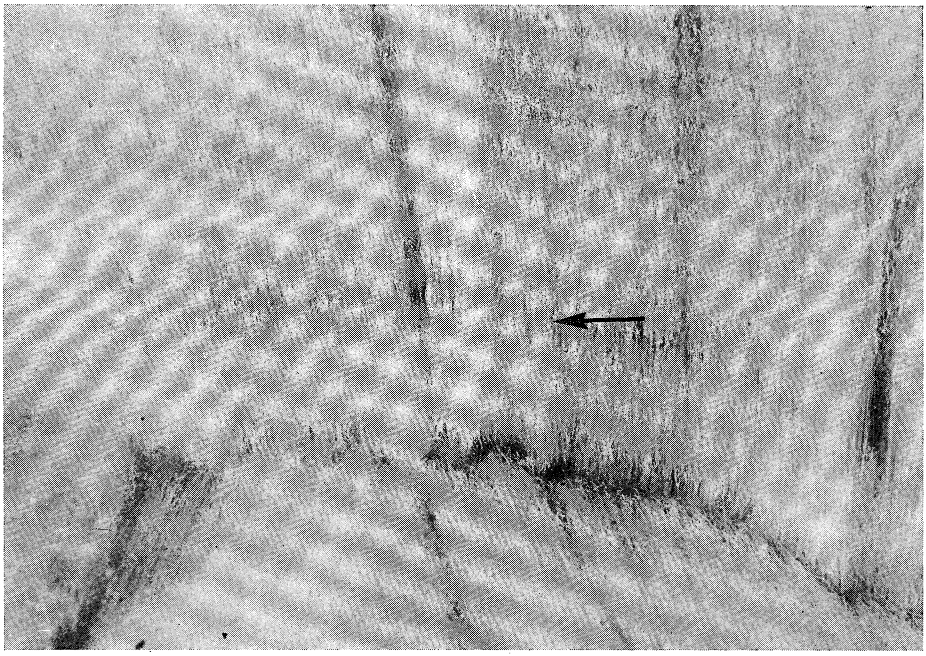


Fig. 1. A weak band (←) in the fleece of a sheep 7 weeks after an intravenous infusion of DL-ethionine (20 mg/kg over 2 days).

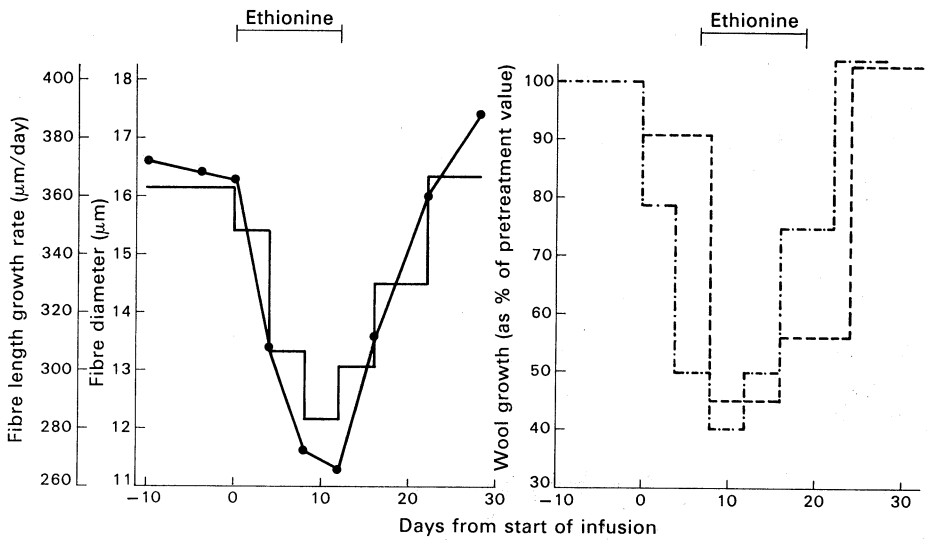
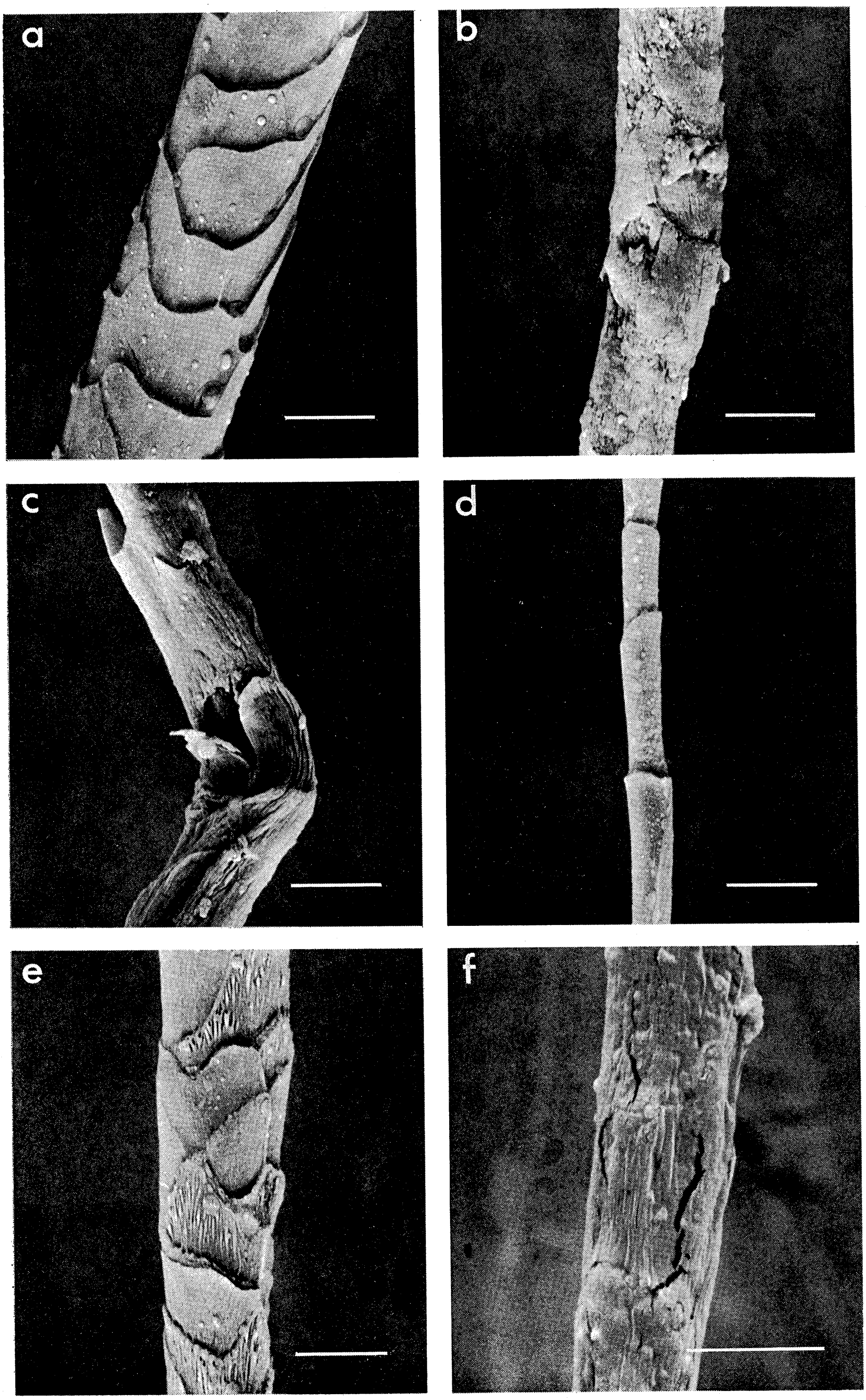


Fig. 2. Effects of intravenous infusion of DL-ethionine on wool growth. Ethionine was given at a daily rate of 1 mg/kg body weight for 12 days. Fibre diameter (●—●) and length growth rate (—) were measured on 105 fibres. Changes in volume of wool (—.—.) were calculated from diameter and length, and changes in mass of wool (— — —) were obtained by clipping a defined area of skin.

Fig. 3



electron microscope. Effects were observed over 1–1½ mm of fibre and were variable both between and along fibres. The initial effect appeared to be a loss of definition of cuticle scale pattern, followed by the appearance of deformed regions, longitudinal ridges and cracks on some fibres (Fig. 3). Subsequently, the fibres became very fine (5–12 μm) and the scale pattern reformed (Fig. 3). In contrast, fibres from the sheep that received an infusion of ethionine for 12 days were appreciably reduced in diameter, but showed little malformation and no loss of cuticle scale pattern.

Table 2. Concentration of ethionine in blood plasma during intravenous infusion of ethionine
Infusions of DL-ethionine were given over 2 days. DL-Methionine was given to some sheep concurrently with ethionine (see Table 3). Each value is for one sheep. —, no samples taken

Total dose of DL-ethionine (mg/kg)	DL-Methionine (mol/mol ethionine)	Effects on fibre growth	Ethionine concn (μmol/l) after 22–23 h[A]	28 h	45–47 h
5	Nil	Normal wool	5	3	—
10	Nil	Normal wool	11	1	Trace
10	Nil	Very weak wool	6	4	3
20	Nil	Very weak wool	14	12	—
20	Nil	Very weak wool	19	17	—
20	Nil	Very weak wool	17	15	25
40	Nil	Very weak wool	30	28	24
20	2	Very weak wool	32	—	31
20	2	Weak wool; fleece removed	30	—	50
20	10	Slightly weak wool	39	—	31
20	10	Slightly weak wool	29	—	46
20	15	Normal wool	38	—	35
20	15	Slightly weak wool	28	—	40

[A]Hours after start of infusion.

Concentration of Ethionine in Plasma

The concentration of ethionine in plasma during 2-day infusions of various amounts of ethionine is shown in Table 2. In general, the concentration was higher when larger amounts of ethionine were infused; there also appeared to be a tendency for plasma levels to decline during the infusion when smaller amounts were given. The concentration associated with the inhibition of wool growth in most sheep was of the order of 10 μmol/l or higher.

Single injections of DL-ethionine resulted in very high concentrations of ethionine in plasma 30 min after dosing (Fig. 4). Earlier sampling times were not investigated. Thereafter the concentration in plasma declined rapidly. Following a dose of 40 mg/kg the concentration in plasma 30 h later was about 10 μmol/l whereas, 30 h after an injection of 20 mg/kg, concentrations of ethionine had declined to *c.* 1 μmol/l (Fig. 4).

Fig. 3. Fibres from a sheep that exhibited a weak band in the fleece, following an infusion of 20 mg/kg DL-ethionine over 2 days (see Fig. 1). Photographs (*a*) to (*d*) were taken sequentially along the same fibre, prior to treatment (*a*) and in the affected region (*b*, *c* and *d*). Photographs (*e*) and (*f*) show affected regions of two other fibres. The bar lines are 10 μm.

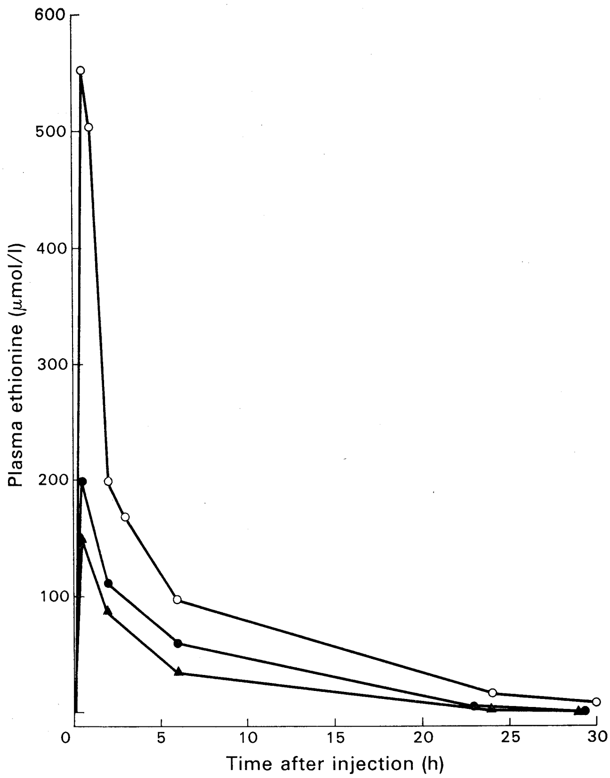


Fig. 4. Concentration of ethionine in blood plasma of three sheep following an intravenous injection of DL-ethionine. Doses given were 20 mg/kg body weight (●, ▲) and 40 mg/kg body weight (○).

Table 3. Influence of various compounds on the effects of ethionine on sheep

All sheep were given an intravenous infusion of DL-ethionine (20 mg/kg over 2 days). Other compounds were given concurrently with ethionine, except where indicated. Values in parenthesis are the number of moles per mole of ethionine

Treatment given with ethionine (mol/mol ethionine)	No. of sheep	Effects observed
DL-Methionine (2)	2	Weak wool, fleece removed by hand (1 sheep); very weak wool (1 sheep). Feed refusals for 2–8 days
DL-Methionine (10)	2	Slightly weak wool
DL-Methionine (15) plus pre-infusion for 3 days (10)	2	Normal wool (1 sheep); slightly weak wool (1 sheep)
L-Cysteine (10)	2	Weak wool (1 sheep); very weak wool (1 sheep). Feed refusals for 9 days with complete refusal for 4 days (1 sheep)
Choline (10)	1	Very weak wool. Feed refusals for 2 days
Adenine (0·5)	1	Weak wool
Adenine (1)	1	Very weak wool. Feed refusals for 7 days; complete refusal for 1 day

Adverse Effects of Ethionine on Sheep

No adverse effects were observed with doses of ethionine, either infused or injected, that were insufficient to cause the growth of very weak wool. An intravenous infusion of 20 mg/kg of DL-, D- or L-ethionine given over 2 days, or an injection of 40 mg/kg of DL-ethionine, usually caused partial or complete loss of appetite for several days

Table 4. Effects of ethionine on sucking mice

Each group consisted of 10 mice, of which five were uninjected controls. DL-Ethionine was injected subcutaneously on consecutive days as indicated, in a volume of 50 or 100 μl per dose

Group	Daily dose (mg)	No. of doses	Total dose (mg)	Total dose (mg/kg$^{0\cdot75}$)	Extent of depilation	Other effects observed
1	0·25	2	0·5	27	Nil	Nil
2	0·5	2	1·0	51	Nil	Nil
3	1·0	3	3·0	143	Nil	Reduced growth rate
4	2·0	3	6·0	270	Nil	Reduced growth rate
5	3·0	3	9·0	461	Nil[A]	Severely reduced growth rate

[A] Coat appeared slightly rough in some mice.

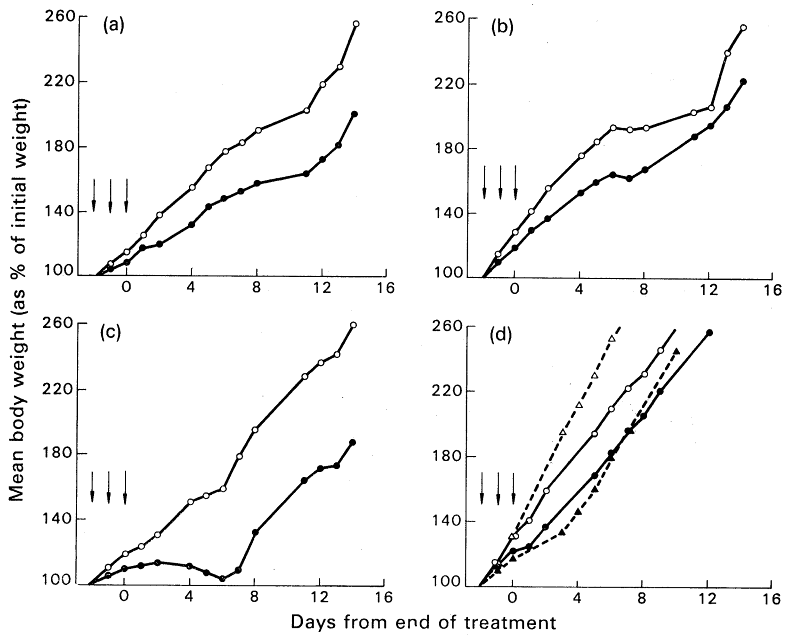


Fig. 5. Mean body weight of groups of sucking mice and rats following administration of DL-ethionine. Treated mice (●) received injections of (*a*) 1 mg, (*b*) 2 mg and (*c*) 3 mg of ethionine as indicated (↓) on 3 successive days (groups 3, 4 and 5, Table 4); control mice are shown as (○). (*d*) Treated rats [groups 1(●) and 2(▲), Table 5] received injections of 4 mg of ethionine as indicated (↓) on 3 successive days; control rats are shown as (○, △).

after dosing (Table 1), and some sheep appeared listless during this period. An infusion of 40 mg/kg over 2 days caused feed refusals for 8 days and the sheep was obviously sick and declined to stand for 3 days from the end of the infusion; it subsequently recovered fully.

No attempt was made to determine minimum toxic levels of ethionine for sheep, but intravenous infusions of *c*. 120 mg/kg over 2 days, and 14 mg/kg given over 7 days, were lethal (Table 1).

Fig. 6. Effect of DL-ethionine on sucking mice. The photograph was taken 5 days after the last injection of the highest dose of ethionine (group 5, Table 4). Upper mice were injected; lower mice were controls.

Prevention of Effects of Ethionine on Sheep

Various treatments were given with an infusion of DL-ethionine (20 mg/kg for 2 days) as attempts to prevent the inhibition of wool growth. The effects of ethionine on wool growth were either reduced or prevented if sufficient DL-methionine was given concurrently (Table 3). However, a considerable excess of methionine (10–15 mol/mol ethionine) was needed to protect against ethionine and protection was not complete. Methionine also appeared to prevent adverse side-effects of ethionine; only the sheep that received the lowest level of methionine showed any adverse effects (Table 3). Other compounds tested (L-cysteine, choline and adenine) were ineffective for preventing the inhibition of wool growth by ethionine (Table 3).

The concurrent infusion of methionine with ethionine resulted in concentrations of ethionine in plasma in excess of 30 μmol/l, in contrast to a mean value of 17 μmol/l during the infusion of 20 mg/kg ethionine alone (Table 2). However, the concentration of ethionine in plasma of sheep that received methionine was not related to the amount of methionine given (Table 2).

Effects of Ethionine on Mice and Rats

No hair loss was observed in mice following dosing with DL-ethionine (Table 4), even after a dose of nine times (on the basis of metabolic body weight) that which caused the growth of very weak wool and loss of the fleece in sheep. The growth rate of mice was retarded with total doses of 3 or 6 mg ethionine, 143 and 270 mg/kg$^{0\cdot75}$ respectively, and was severely retarded for a week after a dose of 9 mg, 461 mg/kg$^{0\cdot75}$ (Fig. 5). No animals died as a result of treatment. The lack of effects on hair growth, and the reduced body growth, after the highest dose of ethionine are demonstrated in Fig. 6.

Table 5. Effects of ethionine and mimosine on sucking rats

Doses were given subcutaneously once daily for 3 days. DL-Ethionine was given in solution in a volume of 150 μl per dose (groups 1 and 2) or as a suspension in a volume of 100 μl (group 3). L-Mimosine was given in solution in a volume of 150 or 200 μl per dose

Group	No. of control animals	No. of animals treated	Daily dose (mg)	Total dose (mg)	Total dose (mg/kg$^{0\cdot75}$)	Extent of depilation	Other effects observed
			Rats treated with ethionine				
1	3	3	4·0	12·0	245	Nil	Reduced growth rate
2	4	4	4·0	12·0	306	Nil	Reduced growth rate
3	3	4	5·0	15·0	360	Nil	Toxic; 3 out of 4 rats died
			Rats treated with mimosine				
4	0	4	1·2	3·6	79	Slight (3 rats) Advanced (1 rat)	Nil
5	3	4	2·4	7·2	167	Advanced	Nil

No hair loss was observed in rats following dosing with DL-ethionine, but the compound was toxic at a dose rate of 360 mg/kg body weight$^{0\cdot75}$ (Table 5). As with mice, body growth was temporarily retarded by ethionine (Fig. 5). As the procedure of dosing sucking rats with potential depilatory compounds had not previously been tried, it was established that mimosine, which is effective for depilating mice (Panaretto *et al.* 1978), was also effective for depilating rats (Table 5).

Discussion

These studies have shown that ethionine is a potent inhibitor of wool growth; both the L- and D-isomers appear equally effective. However, it is conceivable that D-ethionine is converted to L-ethionine in the sheep's tissues, as occurs with methionine, and no conclusions can be drawn regarding the activity of the two isomers with respect to specific reactions involved in the inhibition of wool growth. There are several examples of differences in the effects of L- and D-isomers. In mice, L-ethionine was a more potent inhibitor of RNA synthesis than D-ethionine (Berry and Friedman 1976), but D-ethionine was much more toxic (Friedman *et al.* 1977). D-Ethionine was less effective than L-ethionine for inhibiting DNA synthesis in mouse and human lymphocytes (Zabos *et al.* 1978), while L-ethionine arrested division of cells of *Saccharomyces cerevisiae* whereas D-ethionine was inactive (Singer *et al.* 1978).

Ethionine has been shown to have a variety of effects in animals, and many mechanisms may be involved (Farber 1963, 1971; Fowden *et al.* 1967). Many of the effects of ethionine in animals are attributable to the fact that L-ethionine is a substrate for the enzyme methionine adenosyltransferase (EC 2.5.1.6), with the resultant formation of *S*-adenosylethionine (Lombardini *et al.* 1970; Farber 1971). D-Ethionine is not a substrate for this enzyme (Lombardini *et al.* 1970). One consequence of the formation of *S*-adenosylethionine is the induction of a deficiency of ATP, especially in liver (Shull *et al.* 1966; Farber 1971). Most of the effects of ethionine on animals can be prevented by the concurrent administration of methionine (Farber *et al.* 1964; Meister 1965). Adenine can alleviate effects due to trapping of ATP in *S*-adenosylethionine (Farber 1971), and choline also alleviates some effects of ethionine (Meister 1965). Our attempt to prevent the inhibition of wool growth by ethionine have indicated that methionine can be effective if a large excess over ethionine is given; even then, protection is not complete. No firm conclusions can be drawn from the failure of adenine or choline to prevent inhibition of wool growth. The failure of cysteine to prevent inhibition of wool growth indicates that ethionine is unlikely to influence wool growth by stopping the conversion of methionine to cysteine (Simpson *et al.* 1950). Overall, the attempts to prevent inhibition of wool growth by ethionine indicate that its mode of action may differ from many reported effects in other species. However, detailed biochemical studies are clearly needed.

No evidence was obtained that the inhibition of wool growth was closely related to the concentration of ethionine in plasma. While intravenous infusions of amounts of ethionine that consistently produced very weak wool resulted in concentrations of ethionine in plasma above 10 μmol/l, very weak wool was produced by one sheep given an infusion that sustained a concentration of ethionine in plasma of about half this value (see Table 2). The protective effect of methionine appeared to result in concentrations of ethionine in plasma somewhat above the values obtained when ethionine was given alone. This may be a reflection of a reduced utilization of ethionine in reactions such as the formation of *S*-adenosylethionine. A concentration of 10–20 μmol/l ethionine is some 100 times lower than that required to inhibit cell division in lymphocytes (Zabos *et al.* 1978), and is also lower than the concentration (60–200 μmol/l) required to inhibit cell division in *Saccharomyces cerevisiae* (Colombani *et al.* 1975; Singer *et al.* 1978). The concentration in plasma may not, of course, reflect the concentration at the site of action in the animal.

The inhibitory effects of ethionine on wool growth are relevant to research into chemical defleecing as a procedure for harvesting wool from sheep (Reis and Panaretto 1979). Dosing with ethionine has produced similar effects (complete breaks and weakened wool) to those observed with other potentially useful compounds (Reis and Panaretto 1979). However, the narrow margin between an effective and a toxic dose, and the reported carcinogenicity of ethionine during chronic administration (Farber 1963), indicate that it would not be an acceptable compound in practice, even though it is conceivable that the short period of dosing required to inhibit wool growth may not produce carcinogenic effects.

The observed effects of ethionine on wool growth are similar to those of other potential defleecing compounds studied, such as cyclophosphamide (Dolnick *et al.* 1969), mimosine (Reis *et al.* 1975), and *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Tunks *et al.* 1980). As observed with these compounds, there was individual variation in the response to a particular dose. Detailed studies have not been undertaken of the time required to inactivate wool follicles, or for fibre growth to recommence following dosing, as ethionine is not likely to be a practical defleecing agent. A prolonged infusion of a small amount of ethionine caused a marked reduction in fibre diameter as observed with mimosine, but, in contrast to mimosine (Reis *et al.* 1976), there was no loss of cuticle scale pattern. Ethionine differs from mimosine (Reis *et al.* 1975) in that a single intravenous injection is effective for inhibiting wool growth, even though the concentration in plasma falls rapidly after injection. Ethionine is about 10 times more potent than mimosine on a weight basis, or about eight times on a molar basis (Reis *et al.* 1975). Ethionine also differs from mimosine (Panaretto *et al.* 1978) in not depilating sucking mice. This result indicates that a test system employing sucking mice may not be a reliable screening procedure for potential depilatory compounds for sheep (Panaretto *et al.* 1978).

The differences in the effects of ethionine on sheep and rodents are particularly interesting. Ethionine was less toxic for rodents, especially mice, than for sheep and failed to cause any depilation in mice or rats. However, both mice and rats exhibited the expected depression of body growth following dosing with ethionine (Farber 1963; Meister 1965). One possible explanation for the failure of ethionine to depilate rodents is that a metabolite of ethionine, formed in sheep but not in rodents, is responsible for the inhibition of wool growth. Alternatively, there may be a biochemical reaction, specific to sheep's skin and associated with wool growth, which is influenced by ethionine. An understanding of the mode of action of ethionine on wool growth may provide information on the role of methionine, or other factors, in controlling the activity of wool follicles.

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