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Effects of Methoxinine, an Analogue of Methionine, on the Growth and Morphology of Wool Fibres

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Abstract

Methoxinine (*O*-methyl-DL-homoserine) was given to 18 Merino sheep by intravenous infusion or injection in amounts of 52–100 mg/kg body weight. Effects on strength, morphology and growth rate of wool fibres were studied.

On average, methoxinine reduced the strength of bundles of wool fibres to about one-third of pretreatment values, but the magnitude of the effect varied considerably between sheep. Methoxinine also reduced the staple crimp frequency over a distance of *c.* 5–30 mm. A loss of cuticle scale pattern on wool fibres was observed following dosing; other abnormalities included grooved cuticle scales, degraded sections of fibre and distorted fibres. Wool growth was temporarily reduced after methoxinine administration to *c.* 60% of the pretreatment rate. Effects were greater and more rapid on length growth rate than on fibre diameter.

None of the above effects of methoxinine was prevented by the concurrent administration of cysteine and the efficacy of the concurrent administration of methionine was equivocal. A continuation of the infusion of methionine for 4 weeks following methoxinine administration prevented the depression of wool growth and either prevented or reduced the effects on wool fibres.

Following a dose of methoxinine (60 mg/kg) the concentration of methoxinine in blood plasma was 500–800 μmol/l, at the end of a 2-day infusion or 5 h after an injection. The concentration in plasma declined slowly thereafter and was 50–80 μmol/l after 5 weeks. The effects of methoxinine were not mediated via copper deficiency as judged by plasma copper concentrations and the failure to cause depigmentation of black wool.

Introduction

Reis *et al.* (1983) investigated various analogues of amino acids as potential inhibitors of wool growth. During these studies an analogue of methionine, methoxinine (*O*-methyl-DL-homoserine), was observed to cause a substantial reduction in the strength of wool fibres after intravenous infusion for 2 days. In addition, a prolonged alteration in the staple crimp frequency occurred following dosing.

The effects of methoxinine on sheep had not previously been studied. Methoxinine was shown to be a bacteriostatic agent and to reduce weight gain in rats and mice; these effects were alleviated by methionine (Roblin *et al.* 1945; Shaffer and Critchfield 1948; Travers and Cerecedo 1951). More extensive studies with methoxinine given to sheep by intravenous infusion and injection are now reported. The aims of the studies were to define the effects of methoxinine on wool fibres and to attempt to alleviate or prevent these effects by the administration of methionine or cysteine. In view of the fact that copper deficiency causes loss of crimp (steely wool) and reduced tensile strength of wool fibres (Underwood 1977), the possibility of an induced

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copper deficiency as a result of methoxinine dosing was investigated by monitoring plasma copper and by dosing a sheep which had black wool. Depigmentation of black wool is a sensitive test for depleted copper status (Underwood 1977).

Materials and Methods

Experimental Animals

The experimental sheep consisted of 18 Merinos, either castrate males or non-pregnant, non-lactating ewes, which ranged in body weight from 34 to 52 kg. One sheep, 7580, had black wool. 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. Throughout the period of observation the sheep received once daily 600 g of a ground and pelleted diet consisting of lucerne hay (three parts) and oat grain (two parts). In the diet of three sheep (7868, 7870 and 9784) the oat grain was replaced by sorghum grain. Drinking water was available *ad libitum*.

Chemicals and Dosing

Methoxinine was synthesized as described by Reis *et al.* (1983). DL-Methionine was obtained from BDH Chemicals Ltd, Poole, England and L-cysteine hydrochloride monohydrate from Ajinomoto Co. Inc., Tokyo, Japan.

Total doses of methoxinine ranged from 2·2 to 3·8 g depending on body weight and dose rate as indicated in Table 1. Methoxinine for injection was dissolved in *c.* 10 volumes of sterile 0·9% (w/v) sodium chloride solution and injected into a jugular vein over *c.* 5 min. Infusions of methoxinine were given via a catheter inserted into a jugular vein; the methoxinine was dissolved in sterile 0·9% (w/v) sodium chloride solution and infused at a steady rate by means of a peristaltic pump. Two-day infusions were given at a rate of 360–400 ml/24 h and eight-day infusions at a rate of 150–160 ml/24 h. Cysteine and methionine were usually dissolved in water and infused via an abomasal cannula in a volume of 700–760 ml/24 h. However, for infusion into two sheep, methionine was dissolved with the methoxinine in sterile 0·9% (w/v) sodium chloride solution and given via the intravenous catheter.

Wool Measurements

After a sufficient length of wool had grown following treatment (*c.* 2 cm), the staple strength was measured with an Instron 1026 tensile 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 staples from the midside of each sheep before and after dosing, and results were expressed as N $ktex^{-1}$. Pre-dosing measurements were made about 20 mm above the weakened zone. In addition, subjective assessments were made of the strength of wool fibres by estimating the force needed to break staples of about 1 ktex. The assessments were made by three observers and the wool was classified as normal, slightly weak or weak.

The effects of the treatments on wool growth were assessed in 17 sheep using one or more of three procedures; no measurements of wool growth were made on the black sheep (7580). First, the mass of wool grown per unit time was measured in 12 sheep (see Table 2) over 14-day periods before and after treatment. Wool was removed from a defined area of skin (150–220 cm^2) on the midside or shoulder with small animal clippers (Oster size 000) and was cleaned as described by Reis (1967). Secondly, wool growth was measured in 11 sheep (see Table 2) by the autoradiographic technique of Downes *et al.* (1967). An intravenous injection of a tracer dose of L-[^{35}S]cystine (70–80 μCi; 2·59–2·96 MBq) was given at intervals of 4–6 days, to enable wool growth to be measured for 10 days before the start of dosing with methoxinine and for up to 21 days thereafter. Fibre diameter at the front of each radioactive mark, and the distance between each radioactive mark, were measured. The distance between each mark was used to calculate the mean length of fibre grown per day. Fibre volume was calculated from fibre diameter and length growth rate by regarding each section of fibre as a frustum of a cone. Fibres were sampled from four sites along one side of each sheep. The total number of fibres measured from most sheep was 95–130 with approximately equal numbers from each site. With two sheep (7870 and 7873, Table 2) the fibres were very weak and a total of only 32 or 51 complete fibres respectively could be obtained for measurement. Thirdly, 20 fibres from a midside site on six sheep (see Fig. 4) were mounted on glass slides and fibre diameter was measured, before and after dosing, at intervals of *c.* 500 μm using a projection microscope (Reichert lanameter). The approximate start of

methoxinine treatment was indicated by a dyeband (Durafur Black R; Chapman and Wheeler 1963) applied when dosing commenced.

For examination under the light microscope, wool fibres from all sheep were washed with light petroleum (Shell X4) and were stained with picric acid and eosin, prior to mounting on glass slides. For scanning electron microscope studies, fibres were washed twice with light petroleum. Twelve to 15 fibres from each of nine sheep were mounted on stubs, coated with gold in a sputter coater (Dynavac Model SC 150), and were examined with a model Super III A electron microscope (International Scientific Instruments Inc.).

Analytical

Methoxinine and methionine 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 adding sulfosalicylic acid crystals (30 mg/ml plasma), followed by mixing and centrifugation. Norleucine (0·25 μmol/ml) was added, with the sulfosalicylic acid, as an internal standard. The supernatant was stored at −10°C. After passing through a Millipore filter (0·45 μm pore size), the supernatant was reduced to dryness in a rotary evaporator and the residue was dissolved in 0·1 M hydrochloric acid containing 1% (w/v) thiodiglycol. The concentration of amino acid in plasma was measured with a TSM Amino Acid Autoanalyzer, by reference to norleucine as an internal standard, using an 8% cross-linked cation-exchange resin in a 41·5 by 0·5 cm column kept at 47°C and employing a flow rate of 0·45 ml/min. A sample of 100 μl was placed on the column for analysis. In this system, methoxinine separated between glutamic acid and proline.

Copper and zinc in blood plasma

Total copper and zinc in blood plasma were measured with a Varian Techtron (model AA6) atomic absorption spectrophotometer after dilution with water (Price 1972). Comparisons were made with aqueous standards; mean recoveries of copper and zinc added to plasma were 99 and 97% respectively in this system.

Results

General Effects of Methoxinine on Sheep

Ten of the 18 sheep dosed with methoxinine exhibited no adverse effects and continued to consume all their ration. Seven sheep (indicated in Table 1) showed temporary loss of appetite to a variable extent but no other effects were observed. With three of the above sheep feed intake was reduced for only 2 (9778) or 3 days (4362, 7868), commencing 6–10 days from the start of dosing; more than half the ration was refused during this period. Sheep 9778 received the highest dose of methoxinine (Table 1) but was not adversely affected. The other four affected sheep (4371, 7870, 9784 and 9792) exhibited significant feed refusals for a longer period; about two-thirds of the ration was refused for 10 (4371, 7870), 12 (9792) or 16 days (9784), commencing either 2 days (9792) or 1 week after the start of dosing. Sheep 4363 (not included in Table 1), which received 80 mg/kg methoxinine over 8 days, refused feed from 10 days after the start of treatment and died from an undetermined cause 6 days later.

Strength and Crimp Frequency of Wool

Data for 17 sheep are given in Table 1. Measurements of staple strength and fibre morphology could not be made on a further sheep (4363) which died before wool could be harvested (see above). Of the 10 sheep listed that received doses of methoxinine alone, ranging from 52 to 100 mg/kg either injected or infused over 2 or 8 days, all showed a reduction of staple strength by objective measurement.

Table 1. Effects of methoxinine on strength, crimp frequency and morphology of wool fibres

DL-Methoxinine was given by intravenous infusion over the period indicated or by injection. L-Cysteine and DL-methionine were given via an abomasal cannula except for sheep 9779 and 9781 which received DL-methionine via an intravenous catheter

Methoxinine treatment	Sheep No.	Subjective assessment of staple strength	Staple strength (N $ktex^{-1}$) ± s.e. Control region	Treated region	Staple crimp frequency reduction (approx. length affected, mm)	Changes in fibre morphology[C]
52 mg/kg; 2 days	9768	Weak	63·2±1·97	17·9±0·70	5	CSP(c); D(s)
60 mg/kg; 2 days	4371[B]	Weak	62·9±1·50	9·4±0·36	30	CSP(c); D(e)
	7580	Normal	55·9±2·21	41·1±1·00	5	CSP(c)
	9762	Weak	51·7±2·24	13·0±0·59	30	CSP(c); D(i)
	9784[B]	Weak	68·4±0·66	14·0±0·46	5	CSP(c); D(i)
	9793	Weak	59·9±2·64	33·1±1·27	5	CSP(s)
60 mg/kg; injected	4362[B]	Sl. weak	63·8±2·42	48·1±1·53	0	No obvious effects
	9792[B]	Weak	51·8±2·31	5·9±0·43	20	CSP(c); D(e)
80 mg/kg; 8 days	9794	Weak	71·0±1·87	19·3±0·70	30	CSP(c); D(e)
100 mg/kg; 2 days	9778[B]	Weak	60·9±2·66	8·5±0·29	20	CSP(c); D(i)
60 mg/kg for 2 days; plus cysteine (8 mol)[A]	7868[B]	Weak	66·9±3·44	10·1±0·48	30	CSP(i)
	7873	Weak	36·1±0·90	6·5±0·19	30	CSP(c); D(e)
60 mg/kg for 2 days; plus methionine (2 mol)[A]	9779	Sl. weak	54·8±1·50	42·0±1·44	20	CSP(c); D(s)
methionine (8 mol)[A]	7870[B]	Weak	71·0±2·56	1·8±0·24	20	CSP(c); D(e)
	7871	Normal	61·7±1·27	51·1±0·94	5	CSP(s)
methionine (8 mol for 2 days, then 1 mol for 26 days, see text)[A]	7869	Normal	62·4±2·43	67·2±1·35	0	No obvious effects
	9781	Sl. weak	49·6±1·43	29·6±2·12	0	No obvious effects

[A] Moles of cysteine or methionine per mole of methoxinine.
[B] Feed refusals following dosing (see text).
[C] Changes in fibre morphology described are: cuticle scale pattern (CSP) classified as complete loss (c), intermediate loss (i) or slight loss (s); distortion (D) classified as extreme (e), intermediate (i) or slight (s).

The fibres broke at a point which corresponded approximately to the start of methoxinine dosing. On average, staple strength was reduced to about one-third of the pretreatment value. However, the extent of weakening was variable and was relatively small in two sheep (7580 and 4362) that received 60 mg/kg infused and injected respectively. Subjectively the wool from these two sheep was classed as normal and slightly weak (Table 1). All the above 10 sheep, apart from 4362, exhibited a reduction in staple crimp frequency after dosing, but the duration of the effect was variable (Table 1). Fig. 1 illustrates the crimp changes observed in four sheep, ranging from a prolonged alteration in staple crimp frequency to a brief alteration over a distance of *c*. 5 mm.

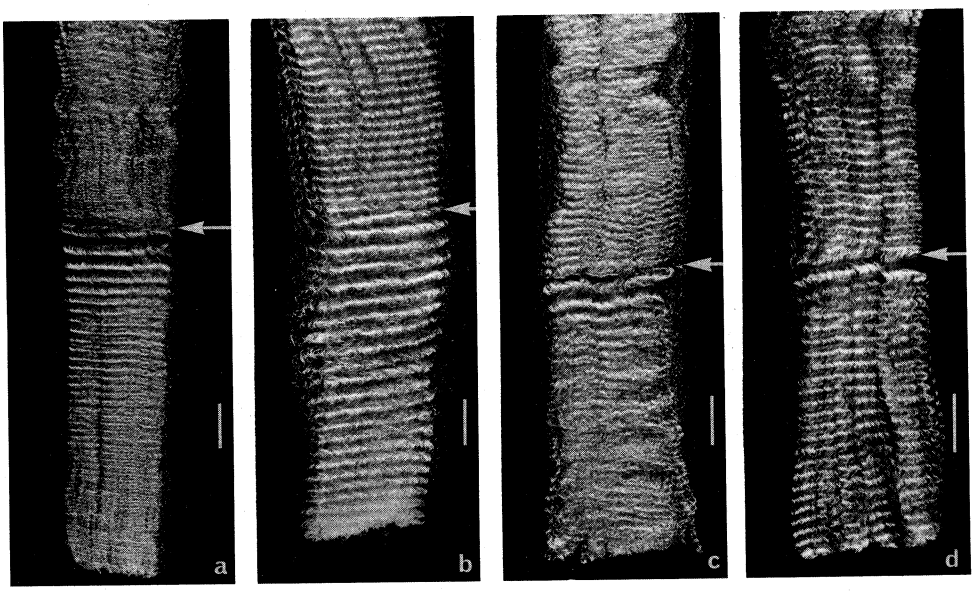

Fig. 1. Effects of methoxinine on staple crimp. All sheep received an intravenous infusion of methoxinine for 2 days: (*a*) 9762, 60 mg/kg; (*b*) 4371, 60 mg/kg; (*c*) 9778, 100 mg/kg; (*d*) 9768, 52 mg/kg. The start of dosing is indicated by an arrow, with pretreatment wool above the arrow. The bar lines are 10 mm.

The administration of cysteine (8 mol/mol methoxinine) with methoxinine during 2-day infusions had no influence on the ability of methoxinine to weaken wool fibres or to alter staple crimp frequency (Table 1). When methionine (2 or 8 mol/mol methoxinine) was given with methoxinine to three sheep during 2-day infusions, the change in crimp frequency was not prevented but the results with staple strength were variable (Table 1). In view of the results with methoxinine alone no conclusions can be drawn. However, when two sheep were given methionine for 4 weeks from the start of methoxinine administration, the crimp change was prevented in both sheep and staples were not weakened in sheep 7869 (Table 1). With sheep 9781 fibre strength was reduced (Table 1) but, in contrast to the other sheep with weakened fibres, the fibres broke at a point which corresponded approximately to the end of the 4-week methionine infusion. These two sheep were given methionine at a rate of 14·03 and 11·83 g/day respectively for 2 days during methoxinine infusion followed by 1·75 and 1·48 g/day respectively for a further 26 days; these amounts for the two periods correspond respectively to 8 and 1 mol/mol methoxinine given.

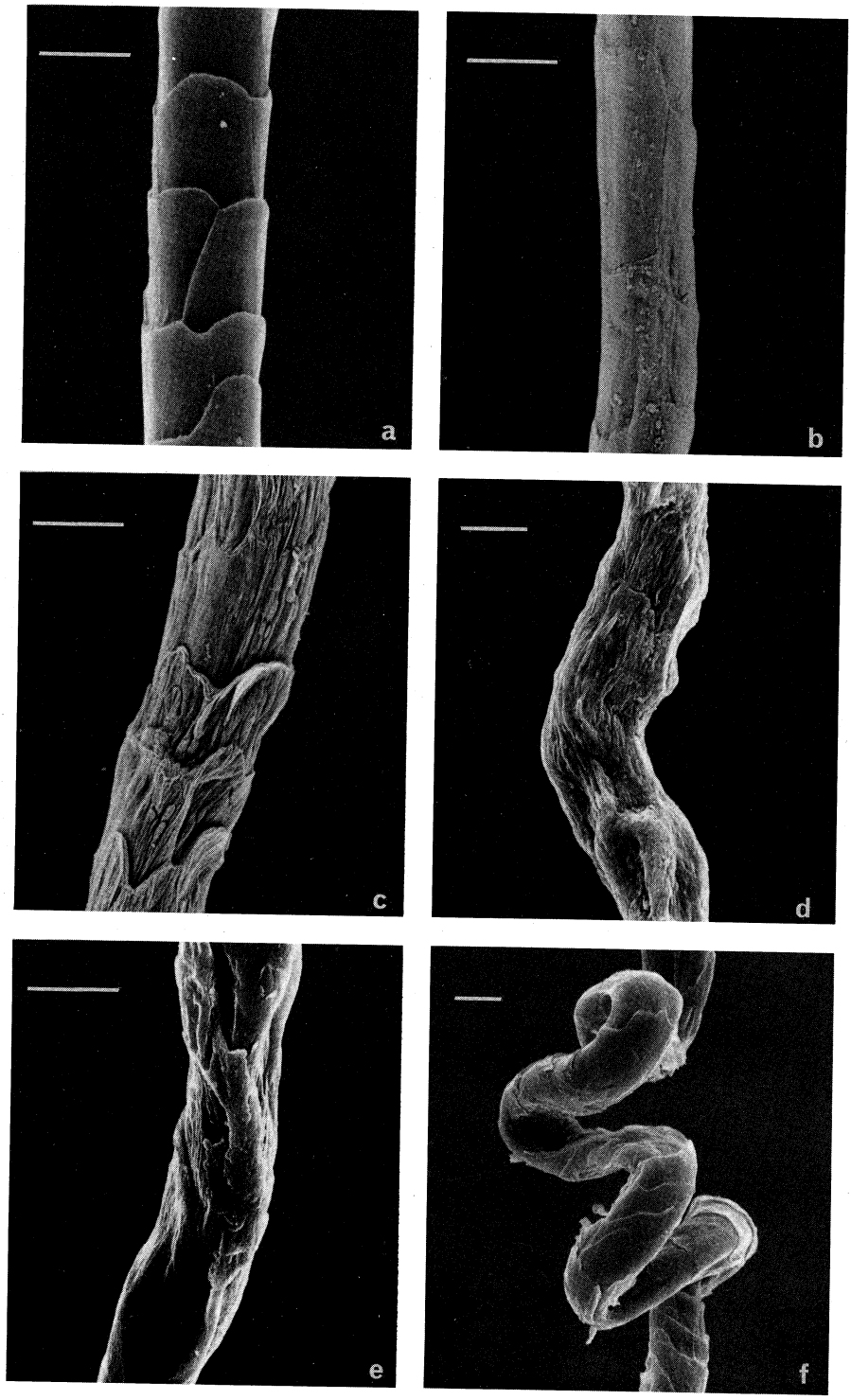

Fig. 2. Effects of methoxinine on fibre morphology. (*a*) 9762, pretreatment. (*b*) 9762, 60 mg/kg; loss of cuticle scale pattern. (*c*) 9762, 60 mg/kg; grooved cuticle scales. (*d*) 9778, 100 mg/kg; degraded section of fibre. (*e*) 7873, 60 mg/kg + cysteine; degraded section of fibre. (*f*) 7873, 60 mg/kg + cysteine; deformed section of fibre. The bar lines are 10 μm.

Morphology of Wool Fibres

Apart from sheep 4362 that received an injection of methoxinine, fibre abnormalities were observed with the light microscope in all sheep given methoxinine alone or with cysteine (Table 1), but the magnitude of the effects varied between sheep. Within a sheep, fibres were affected to different extents and over variable distances; some fibres appeared normal. The length of fibre over which abnormalities could be seen under the light microscope is recorded for six sheep in Fig. 4; changes were first observed 0·5–1·0 mm after the dyeband, applied at the start of dosing, and continued for a distance of 2·5–6·5 mm. In all affected sheep a loss of cuticle scale pattern was observed which ranged from short regions with a faint scale pattern to longer regions where no scale pattern was discernible; in addition most of these sheep exhibited fibre distortion which varied from slight kinking to extreme contortion (Table 1). No firm conclusions can be drawn regarding the effect of including methionine with methoxinine, but the administration of methionine for an extended period of 4 weeks prevented fibre abnormalities (Table 1).

Table 2. Effects of methoxinine on wool growth

DL-Methoxinine was given by intravenous infusion over the period indicated or by injection. L-Cysteine and DL-methionine were given via an abomasal cannula except for sheep 9781 which received DL-methionine via an intravenous catheter

Methoxinine treatment	Sheep No.	Wool growth (% of pretreatment rate) Mass[B]	Fibre volume[C]
60 mg/kg; 2 days	4371	46	48 (8–16 days)
	9784	37	34 (8–16 days)
	9793	89	70 (4–12 days)
60 mg/kg; injected	4362	72	70 (4–12 days)
	9792	43	–[D]
80 mg/kg; 8 days	4363	–[D]	77 (4–8 days)
	9794	64	55 (8–16 days)
60 mg/kg for 2 days; plus cysteine (8 mol)[A]	7868	65	49 (8–20 days)
	7873	54	50 (4–16 days)
60 mg/kg for 2 days; plus methionine (8 mol)[A]	7870	59	44 (8–16 days)
	7871	126	116 (4–16 days)
methionine (8 mol for 2 days, then 1 mol for 26 days, see text)[A]	7869	215	137 (12–21 days)
	9781	157	–[D]

[A] Moles of cysteine or methionine per mole of methoxinine.
[B] Clean wool growth for weeks 2–4 after the start of methoxinine administration.
[C] Calculated from diameter and fibre length data; values in brackets indicate the period after the start of methoxinine administration from which data were used to estimate depression or stimulation of wool growth (see Figs 3–5).
[D] Not measured.

The most common change seen in wool fibres under the scanning electron microscope was a loss of cuticle scale pattern (Fig. 2*b*). In some sheep this was the only change observed. This effect was observed in all fibres from sheep 9762 (Fig. 2*b*) and in at least 90% of fibres from all sheep examined. Other abnormalities seen included grooved cuticle scales (Fig. 2*c*), degraded sections of fibre (Figs 2*d*, 2*e*) and distorted fibres. Some severely deformed fibres (Fig. 2*f*) were observed from some of the sheep.

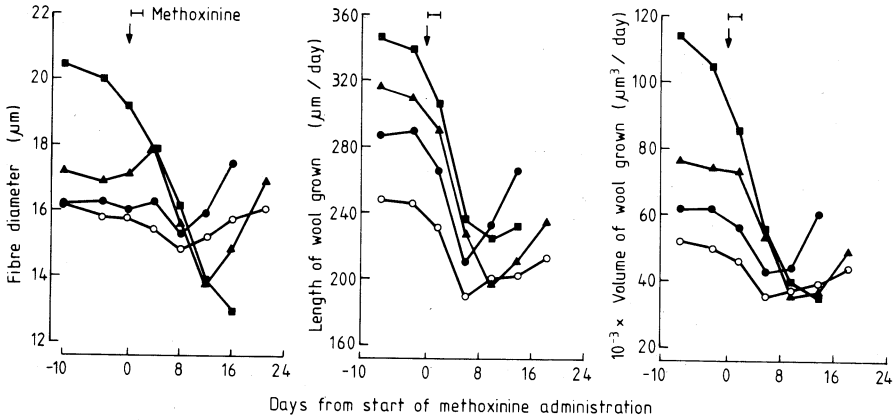

Fig. 3. Effects of methoxinine on wool growth. All sheep received 60 mg/kg methoxinine intravenously either by injection [↓; 4362 (○)] or infusion over 2 days [⊢⊣; 4371 (▲), 9784 (■), 9793 (●)]. Length growth and volumes are plotted at the mid-point of each measurement period.

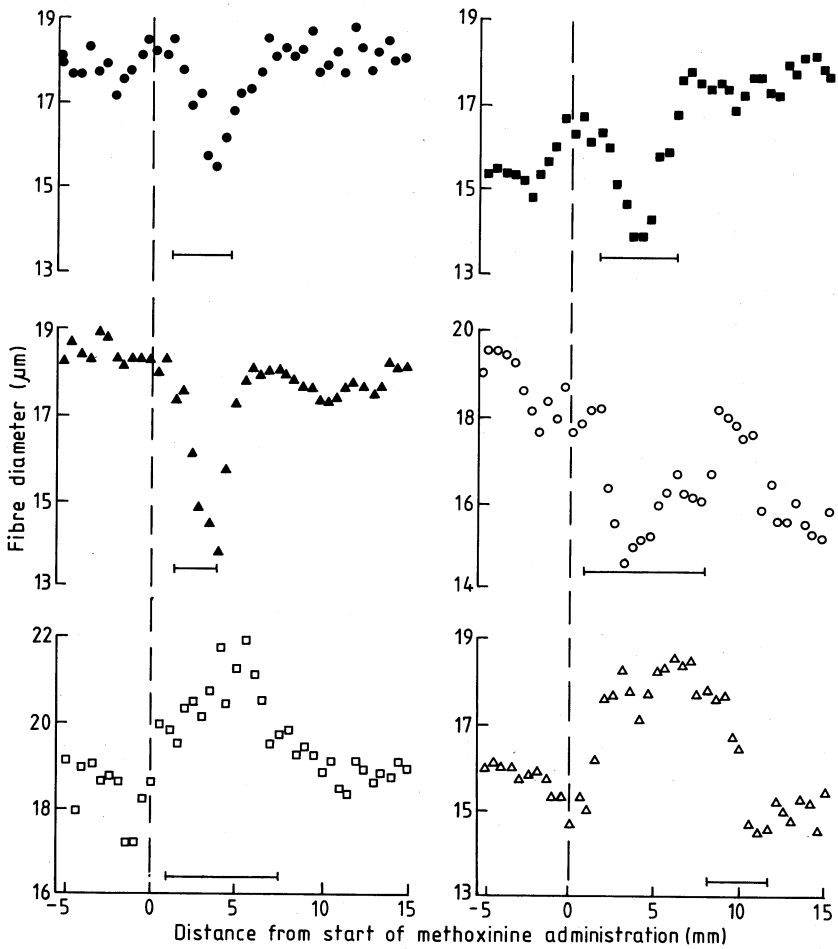

Fig. 4. Effects of methoxinine and methoxinine plus methionine on wool fibre diameter. The sheep received methoxinine (9762, ■; 9768, ●; 9778, ▲; 9792, ○) or methoxinine plus methionine (9779, □; 9781, △) intravenously (see Table 1). The bar lines indicate the length of fibre over which abnormalities could be seen under the light microscope.

Wool Growth

The administration of methoxinine alone caused a temporary depression of wool growth, but the extent of the change was variable. Wool growth was measured by both autoradiography and clipping in four of the seven sheep into which methoxinine was infused or injected at a dose rate of 60 mg/kg (Table 2). Wool growth after dosing, as a percentage of the pretreatment rate, was 37–89% (by mass) and 34–70% (by volume). The greater depression observed with sheep 9784 may have been influenced by appreciable feed refusals (see below). Both length growth rate and fibre diameter were depressed by methoxinine in these four sheep, but the effects were greater and more rapid on length (Fig. 3). Wool growth showed a variable recovery within the period of measurement (up to 21 days). The greater depression of wool growth and failure to recover in sheep 9784 (Fig. 3) may be attributed to feed refusals which began during the second week after treatment (see above).

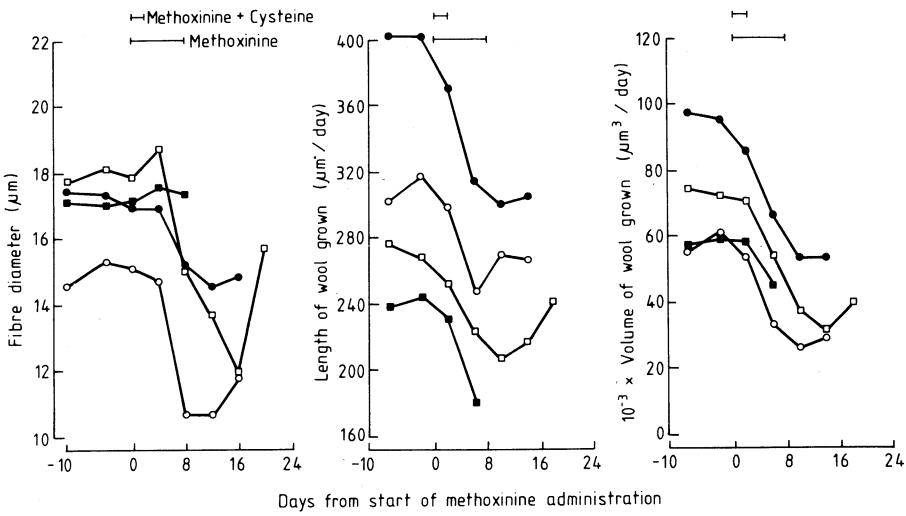

Fig. 5. Effects of methoxinine and methoxinine plus cysteine on wool growth. Sheep 4363 (■) and 9794 (●) received 80 mg/kg methoxinine intravenously over 8 days and sheep 7868 (□) and 7873 (○) received cysteine with methoxinine over 2 days as indicated by bar lines (see also Table 2). Length growth and volume are plotted at the mid-point of each measurement period.

Effects on wool growth were assessed in four other sheep that received methoxinine alone by measuring fibre diameter at intervals before and after dosing. Fibre diameter was appreciably reduced after doses of 52, 60 (two sheep) and 100 mg/kg, reaching a minimum about 4 mm after the dyeband applied at the commencement of dosing (Fig. 4). In view of the results mentioned above, these data indicate a large reduction in the rate of wool growth. This reduction was confirmed for sheep 9792 by the mass of wool clipped from a defined area of skin (Table 2) but this result may have been influenced by appreciable feed refusals after injection of methoxinine (see above).

An infusion of 80 mg/kg over 8 days reduced both length growth rate and fibre diameter in sheep 9794, and markedly depressed both the volume and mass of wool grown (Fig. 5; Table 2). Wool growth remained depressed up to day 16 when measurements ceased (Fig. 5). With a second sheep, 4363, wool growth was assessed only by autoradiography up till the end of the infusion on day 8. At this time, length growth, but not diameter, had been depressed (Fig. 5). In summary, there was evidence of a reduction in wool growth rate in 10 sheep that received methoxinine alone.

The administration of cysteine with methoxinine did not prevent the inhibition of wool growth in two sheep; wool growth was approximately halved in both sheep (Table 2; Fig. 5). When methionine was administered for 2 days with methoxinine

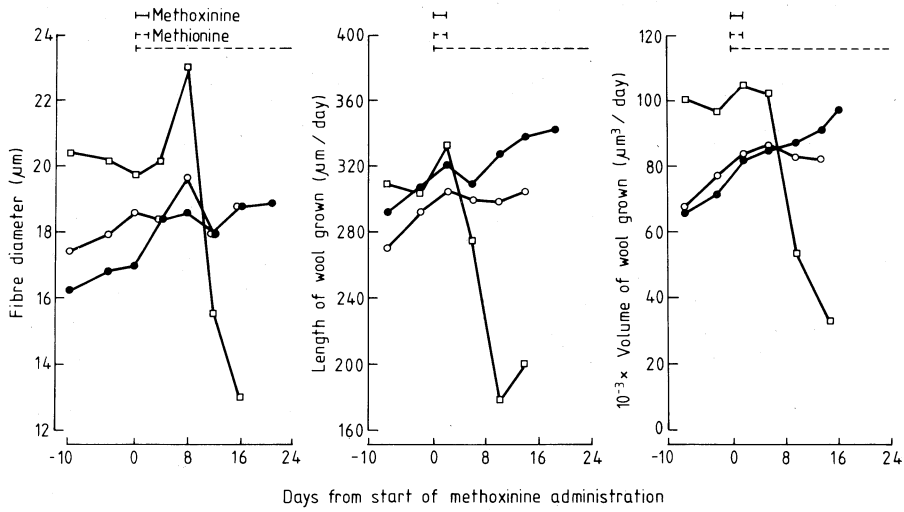

Fig. 6. Effects of methoxinine plus methionine on wool growth. All sheep received 60 mg/kg methoxinine intravenously plus methionine as indicated by bar lines and in Table 2: 7869 (●), 7870 (□), 7871 (○). Length growth and volume are plotted at the mid-point of each measurement period.

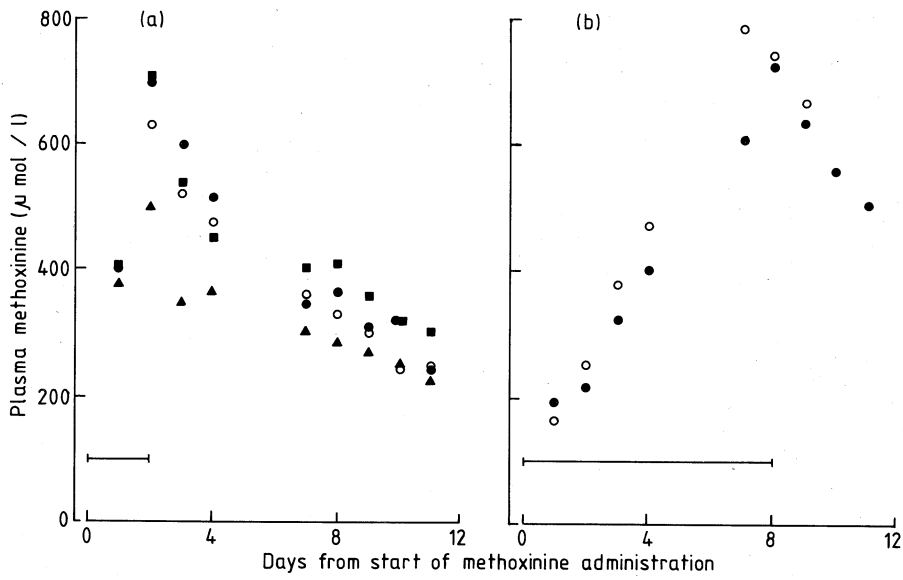

Fig. 7. Effect of methoxinine administration on the concentration of methoxinine in plasma. (*a*) The sheep received 60 mg/kg methoxinine over 2 days (bar line): 7580 (○), 7871 (▲), 7873 (■), 9793 (●). In addition, 7873 and 7871 received cysteine or methionine respectively for 2 days (see Table 1). (*b*) The sheep received 80 mg/kg methoxinine over 8 days (bar line): 4363 (○), 9794 (●) (see Table 2).

(2 or 8 mol/mol methoxinine) wool growth was increased in two out of three sheep (9779, Fig. 4; 7871, Table 2 and Fig. 6). With sheep 7870, methionine (8 mol/mol methoxinine) failed to prevent the inhibition of wool growth (Table 2 and Fig. 6).

However, this result may have been influenced by feed refusals (see above). The administration of methionine for 4 weeks from the start of methoxinine dosing prevented inhibition of wool growth in two sheep and actually enhanced wool growth (Table 2 and Fig. 6).

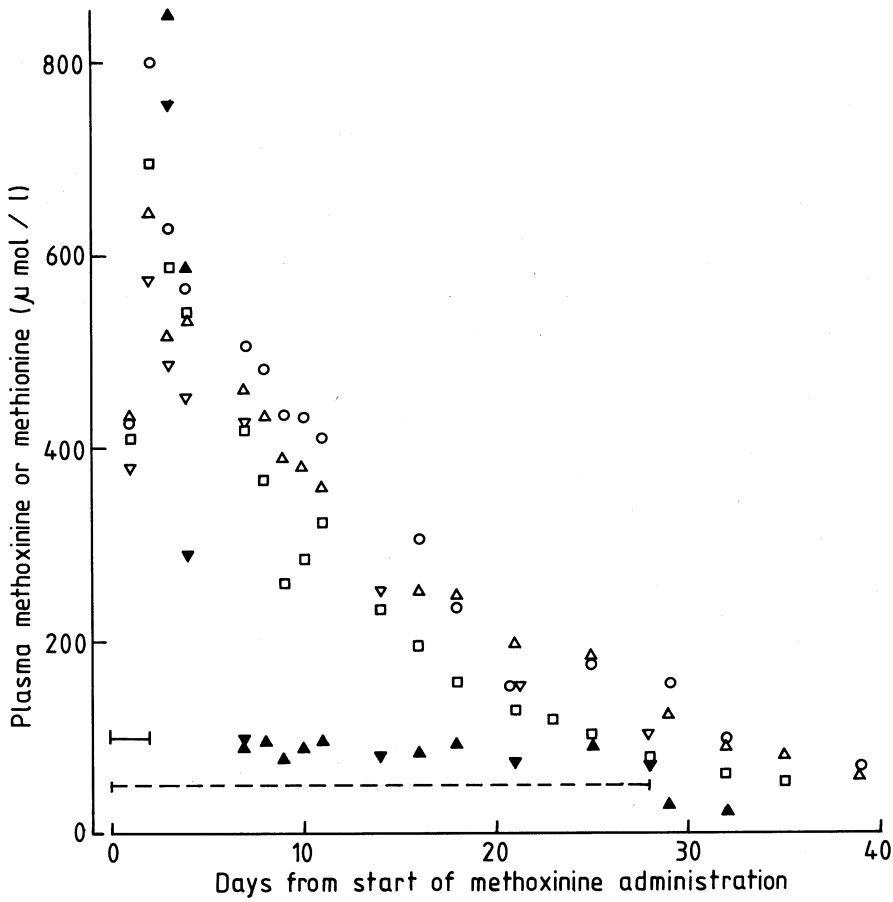

Fig. 8. Methoxinine and methionine concentrations in plasma. All sheep received 60 mg/kg methoxinine over 2 days (⊢⊣). In addition, 7868 received cysteine for 2 days and 7869 and 9781 received methionine (⊢ – – ⊣) for 28 days (see Table 1). Methoxinine concentrations are shown as: 4371 (○), 7868 (□), 7869 (△), 9781 (▽); methionine concentrations are shown as: 7869 (▲), 9781 (▼).

Concentration of Methoxinine in Plasma

Methoxinine concentrations in plasma were measured in eight sheep that received a 2-day infusion of 60 mg/kg methoxinine, either alone or with cysteine or methionine. Plasma values were monitored for 11 days in four of the sheep (Fig. 7*a*) and for 4–6 weeks in the other sheep (Fig. 8). At the end of the 2-day infusion the concentration of methoxinine was 500–800 μmol/l; methoxinine was removed only slowly from plasma and by 11 days the concentration was 200–400 μmol/l, falling exponentially to 50–80 μmol/l by about 5 weeks (Figs 7*a*, 8). The concentration of methoxinine in plasma was not influenced by the administration of cysteine or methionine

(Figs 7*a*, 8). Two sheep (7869, 9781; Fig. 8) received methionine for a total of 28 days (see above). Infusion of methionine for the first two days at a rate of *c*. 14 or 12 g/day respectively resulted in plasma methionine values of *c*. 1500 μmol/l. Thereafter, a daily infusion of 1·75 or 1·48 g methionine respectively for a further 26 days maintained a concentration of methionine in plasma in the region of 80–90 μmol/l, which declined to 20–30 μmol/l when the infusion stopped (Fig. 8). Fig. 7*b* shows that methoxinine in plasma rose steadily when 80 mg/kg methoxinine was infused over a period of 8 days, to reach a concentration of 700–800 μmol/l.

Fig. 9 shows plasma methoxinine concentrations following injections of 60 mg/kg given to two sheep. The concentration declined rapidly during the first hour after injection from an initially high value of 2200–3000 μmol/l and by 5 h was 600–700 μmol/l, a value similar to those found at the end of a 2-day infusion. The subsequent pattern of decline in methoxinine concentration was as observed in the infused sheep, reaching a value of 50–60 μmol/l after 5 weeks.

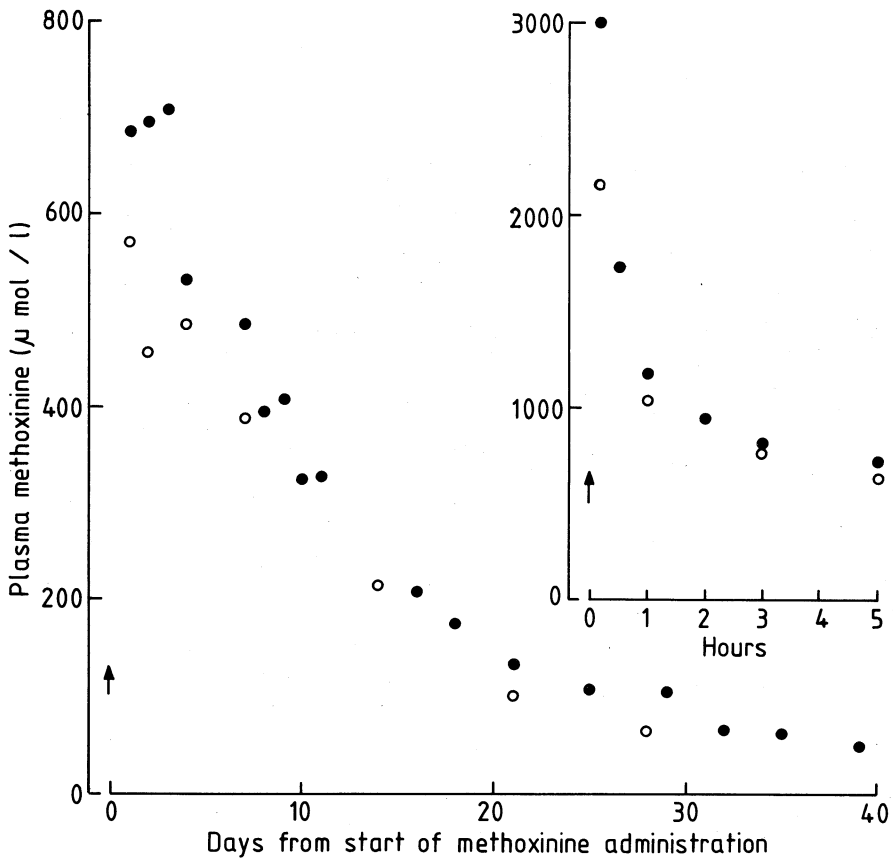

Fig. 9. Effect of methoxinine injection (60 mg/kg) on the concentration of methoxinine in plasma. Sheep 4362 (●), 9792 (○). The inset figure shows changes during the 5 h after injection.

Copper and Zinc Status in Relation to Methoxinine

One of the sheep dosed with methoxinine (7580, Table 1) had black wool, but no depigmentation of the wool was observed following dosing. Plasma copper concentrations were measured in five sheep (7580, 7871, 7873, 9793 and 9794; see Table 1 for the treatments given). The mean concentrations (μg/ml), with the number of analyses in brackets, before, during and after methoxinine infusion were 0·86 (15), 0·87 (13) and 0·93 (23) respectively, indicating no effect of methoxinine on total copper values. The mean concentrations of zinc in the same plasma samples before, during and after methoxinine infusion were 0·66, 0·72 and 0·67 μg/ml respectively.

Discussion

Although the effects of methoxinine were somewhat variable in different sheep that received the same treatment, it is clear that methoxinine had considerable effects on wool fibres. It reduced the rate of fibre production, caused a prolonged alteration of the staple crimp frequency and produced morphologically abnormal and weakened wool fibres. While the effect on crimp appears to be unique to methoxinine, the fibre abnormalities observed, including loss of cuticle scale pattern, were similar to those induced by various wool-weakening agents such as mimosine (Reis *et al.* 1976), methionine with a wheat diet (Reis and Downes 1980) and ethionine (Reis and Tunks 1982). However, it would be of interest to see whether methoxinine has had any effects on the internal structure of fibres. The protein composition of wool is also considerably altered following dosing with methoxinine; the major effect is a reduction in the content of high-tyrosine proteins (Reis and Gillespie 1985). Methoxinine also had general effects as judged by feed refusals in some sheep, but there was no clear evidence of toxicity despite the death of one sheep 8 days after the end of dosing.

The data from the two sheep that received methoxinine injections indicate similar effects to those seen after infusions of methoxinine, even though wool from one injected sheep (4362) was not appreciably weakened. The similarity of effects would be expected in view of the slow removal of methoxinine from blood plasma after infusion or injection. The injected sheep were each given *c.* 2·8 g methoxinine and 10 min later the plasma concentrations were 2·2 and 3·0 mmol/l respectively. Assuming a plasma volume of 2 litres this represents an average of about 700 mg methoxinine circulating in plasma. This value fell to *c.* 180 mg at 5 h, but declined very slowly thereafter. It would thus appear that methoxinine is rapidly distributed throughout the body fluids but that its subsequent removal from the body is very slow. This result is in contrast to the rapid removal from the circulation of other 'foreign' amino acids that have been shown to influence wool growth, such as mimosine (Reis *et al.* 1975) and ethionine (Reis and Tunks 1982). Five weeks after the start of methoxinine administration, the concentration of methoxinine in plasma was 50–80 μmol/l. These concentrations exceed the concentration of methionine normally circulating in sheep's plasma of 10–30 μmol/l (Reis *et al.* 1973).

None of the effects of methoxinine was prevented by the concurrent administration of cysteine while the effectiveness of the administration of methionine with methoxinine for 2 days was equivocal. However, the effects of methoxinine were largely prevented by the administration of methionine for a period of 4 weeks from the start of methoxinine dosing. It would appear that the maintenance of a concentration of methionine in plasma above 80 μmol/l, by infusion of methionine

for this period, was sufficient to prevent the adverse effects of methoxinine. This protective effect of methionine is analogous to that observed in rats and mice, where reduced weight gain due to methoxinine was alleviated by providing additional methionine (Shaffer and Critchfield 1948; Travers and Cerecedo 1951). The result with methionine indicates that methoxinine is interfering with some aspect of methionine metabolism in relation to wool growth. However, the manner in which methoxinine produces its various effects is a matter of speculation. It has previously been pointed out (Reis *et al.* 1983) that, in the absence of a sulfur or selenium atom, methoxinine would not act as a substrate for methionine adenosyltransferase (Lombardini *et al.* 1970) and therefore would not act on wool fibres in the manner postulated for another methionine analogue, ethionine (Reis and Tunks 1982). Nevertheless, it is possible that methoxinine could interfere with the transulfuration pathway of methionine metabolism by virtue of the fact that L-methoxinine has been shown to be an inhibitor of methionine adenosyltransferase (Sufrin *et al.* 1982).

The wool grown after administration of methoxinine bore some resemblance to 'steely' wool induced by copper deficiency (Underwood 1977). However, no evidence for the involvement of copper deficiency in the effects of methoxinine was obtained. Moreover, methoxinine does not cause a reduction of the high-sulfur proteins in wool (Reis and Gillespie 1985) as was observed in steely wool produced by copper deficiency (Gillespie 1965).

It is possible that methoxinine may exert some of its effects by interfering with the incorporation of methionine into biologically active proteins, including enzymes. In this connection it has been shown that the biological activities of octapeptides of cholecystokinin-pancreozymin are appreciably altered when methoxinine replaces methionine in one or both positions in the molecule (Gillessen *et al.* 1979; Meyer *et al.* 1980). It is conceivable that the unique effects of methoxinine on staple crimp frequency may be related to an interference of incorporation of methionine into proteins of the inner root sheath. Nagorcka (1981) has proposed a role for the inner root sheath in crimp formation and these proteins are appreciably richer in methionine than are the proteins of wool fibres (Rogers 1964). It is difficult to explain the prolonged effect on crimp frequency observed in some sheep, although this difficulty is obviated in part by the finding that methoxinine is cleared very slowly from blood plasma over a period of 5–6 weeks after dosing. It should be noted that, at least with the depression of wool growth following methoxinine dosing, the effects are not clearly related to the concentration of methoxinine circulating in plasma. Thus, wool growth had largely recovered in some sheep while appreciable amounts of methoxinine were still present in plasma. The various effects of methoxinine may be mediated via more than one mechanism with different threshold levels of methoxinine required at the site of action.

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