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# Effects of Mimosine, a Potential Chemical Defleecing Agent, on Wool Growth and the Skin of Sheep

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*Abstract*

Twenty-two Merino sheep were dosed with various amounts of L-mimosine, given either as an intravenous or an intraperitoneal injection, or as a continuous intravenous infusion for periods of 1–4 days.

Single injections of mimosine (1–16 g) had no effect on the strength of wool, and wool growth rates were not appreciably altered by injections of small amounts (4 g or less). Injections of larger amounts slightly reduced both length growth rate and diameter of fibres during the 4 days after dosing.

The effects of intravenous infusions of mimosine depended on the rate and the duration of administration. Small amounts (0·5 or 1 g/day given for 4 days) has no effects on the strength of wool or on wool growth rates. Infusions of a total of 8 g, either at the rate of 2 or 8 g/day, weakened the wool but not sufficiently to allow the sheep to be defleeced. Both these treatments caused a temporary reduction in length growth rate and in diameter of fibres, and transient degenerative changes were observed in wool follicles. A region of the fibres representing 1–2 days' growth was constricted to about half the pre-infusion diameter when 8 g was given for 1 day. Infusions of at least 8 g mimosine over a period of 1½–2 days were effective for defleecing all sheep dosed. This corresponded to a daily rate of infusion of about 80 mg/kg. No toxic effects were observed with infusions given for periods of 2 days.

Defleecing was judged to be possible by 6–7 days after the start of infusion, and was readily carried out by about 14 days. Defleecing was associated with follicle retrogression and an abrupt cessation of wool growth within 2 days of the start of the infusion. It was estimated that fibre growth stopped for about 10 days; regrowth was first observed 17–18 days from the beginning of dosing.

Low rates of infusion of mimosine (up to 2 g/day) resulted in plasma levels below 0·1 mmol/l. Infusion at the rate of 4 g/day or above, which produced defleecing, quickly resulted in levels of mimosine in plasma above 0·1 mmol/l; after 2 days the concentration was steady at about 0·2 mmol/l. Injections of 8 or 16 g mimosine resulted in very large, but transient, rises of the level in plasma.

## Introduction

Large amounts of the amino acid mimosine are present in the seeds and foliage of *Leucaena leucocephala* (Lam.) de Wit (previously called *Leucaena glauca* Benth.) (Hylin 1969; Thompson *et al.* 1969). Consumption of seeds or leaves of *L. leucocephala* causes alopecia and other toxic symptoms in various species of animals; the early literature was reviewed by Owen (1958) and in several studies mimosine was shown to be the toxic agent. The conflicting evidence on the toxicity of *L. leucocephala* to ruminants was reviewed by Hegarty *et al.* (1964) who showed that mimosine was the depilatory agent. In the experiments of Hegarty *et al.* (1964) the intravenous infusion of about 20 g mimosine or the abomasal infusion of about 35 g mimosine into two separate sheep over a period of 4–5 days resulted in shedding of the fleece but also in

death of the sheep. Mimosine appears to act as an antimitotic agent (Montagna and Yun 1963; Hegarty *et al.* 1964; Pritchard and Court 1968; Tsai and Ling 1971) but its precise mechanism of action is not known. Various possible biochemical effects of mimosine are reviewed by Hylin (1969) and Thompson *et al.* (1969).

Recently there has been a stimulus to research on compounds that have a depilatory action, with the practical aim of defleecing sheep. Cyclophosphamide has been studied as a chemical defleecing agent for sheep, following the original observations of Dolnick *et al.* (1969) and Homan *et al.* (1969). However, such anti-cancer drugs may not be acceptable in commercial practice, as toxic residues may remain in the tissues and other drawbacks have been noted (Roberts and McMahon 1972; Hohenboken 1972). As part of a search for alternative defleecing agents, a study has been undertaken on the use of mimosine for this purpose. A wide range of amounts of mimosine and times of administration have been studied and, in addition to depilatory effects, changes in skin histology, effects on wool growth rate and the amounts of mimosine circulating in blood have been measured. It was found possible to defleece sheep without any adverse side-effects being apparent.

## Materials and Methods

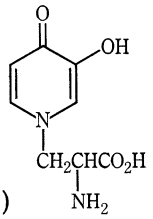
### (*a*) *Procedures with Sheep*

Mature Merino wethers (39–53 kg body weight) were kept indoors in metabolism cages in a room maintained at 20–24°C. The daily ration was 700 or 800 g of a ground and pelleted mixture of equal parts of chopped wheaten and lucerne hays. The sheep were fed once daily at about 10 a.m. Drinking water was available *ad libitum*.

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

Mimosine was administered to sheep as a solution of the sodium salt, which was prepared by dissolving recrystallized mimosine in 2N NaOH, adding sterile, isotonic saline and adjusting the pH to 7·8–8·0 with 2N HCl. Intravenous and intraperitoneal injections were given at a concentration of about 1 g mimosine per 15 ml solution. Intravenous infusions were given continuously, for periods of 1–4 days, via a catheter inserted into the jugular vein; the volume of the infusion was 400–500 ml per 24 h. Twenty-two sheep were treated with mimosine, as indicated in Table 1.

### (*b*) *Preparation and Identification of Mimosine*



(I)

Crude mimosine was isolated from seeds of *L. leucocephala* by the procedure of Hegarty and Court (1964), with the modifications for large-scale extraction described by Beyerman *et al.* (1964). The crude mimosine was recrystallized once from boiling water. Based on the evidence of Beyerman *et al.* (1964) this preparation can be regarded as pure mimosine, β-[*N*-(3-hydroxy-4-oxopyridyl)]-α-aminopropionic acid (I). Values obtained for elemental analysis (carried out by the Australian Microanalytical Service, Melbourne) of the mimosine preparation agreed well with calculated values on the basis of a composition of $C_8H_{10}N_2O_4$. The values found for C, H, and N respectively were 48·06, 5·25, and 13·96%, compared with calculated values of 48·48, 5·09, and 14·14%. The identity of the mimosine preparation was established by a variety of analytical techniques, including infrared, proton and $^{13}C$ spectroscopy.

### (*c*) *Wool Growth and Assessment of Fibre Strength*

Effects on wool growth were assessed by the autoradiographic technique of Downes *et al.* (1967). Intravenous injections of tracer doses of L-[$^{35}$S]cystine (*c.* 50 μCi/dose) were given at intervals of 4–7 days. The pattern of [$^{35}$S]cystine doses in relation to mimosine administration is shown in Fig. 1. Injections of mimosine were given and infusions were started 30–60 min after the second dose of

[$^{35}$S]cystine was given. Both length growth rate and diameter of wool fibres were measured, and fibre volume was calculated from these values assuming that the fibres were cylindrical. Fibre diameter was measured at the front of each radioactive zone. In addition, fibre diameter was measured at a point on some fibres where they were judged to be thinnest. Fibres were sampled from four sites along one side of each sheep, and measurements were made on approximately 20 fibres from each site. However, many of the fibres mounted from the two sheep that received 8 g mimosine for 1 day (Table 3b) were broken, and, while the total number of complete fibres measured was 80, the number per site varied.

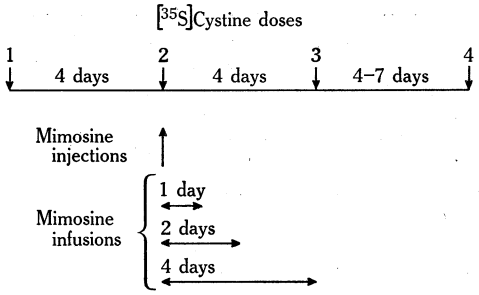



**Fig. 1.** Pattern of dosing with [$^{35}$S]cystine (*c.* 50 $\mu$Ci/dose) in relation to injections and intravenous infusions of mimosine.

All fibres from the sheep that were defleeced stopped growing between the second and third doses of [$^{35}$S]cystine. The assumption was made that the pretreatment length growth rate continued until fibre growth stopped following dosing with mimosine. On this basis, the distance from the front of the radioactive mark corresponding to the beginning of mimosine infusion to the ends of the fibres was converted to days of fibre growth. Also, the regrowth of wool after defleecing was collected to detect radioactivity as a result of the third and fourth doses of [$^{35}$S]cystine.

A subjective assessment was made of the strength of wool fibres following dosing with mimosine, in a similar manner to that described by Hegarty *et al.* (1964). The wool was classified into four grades: normal, slightly weak, weak, and very weak (sheep could be defleeced by hand).

(*d*) *Analysis of Mimosine in Blood Plasma*

Blood plasma was deproteinized by adding 30 mg sulphosalicylic acid crystals per millilitre of plasma, followed by mixing and centrifugation. The supernatant, which had a pH of 1·8–1·9, was stored at −10°C. A sample representing 1 ml plasma was analysed with a Technicon amino acid analyser (model NC-1, Technicon Co., New York), using a 25 by 0·6 cm jacketed column packed with 17 cm of Technicon type B resin. The amino acids were eluted with a sodium citrate buffer, pH 3·10, prepared by mixing 0·2M sodium citrate buffer (pH 2·875) and 0·8M sodium citrate buffer (pH 4·74) in the proportions of *c.* 38 : 1, and employing a flow rate of 0·66 ml per minute and a column temperature of 60°C. Mimosine concentration in plasma was calculated by reference to norleucine used as an external standard. Mimosine, added to plasma, was adequately separated in this system and was eluted after isoleucine and leucine (Fig. 2).

(*e*) *Skin Histology*

Skin biopsies, 1 cm diameter, were taken from the midside region of the sheep before administration of mimosine, and at times from 1 to 134 days after either injection or the start of infusion of mimosine. The skin samples were fixed in 10% buffered formalin, embedded in paraffin and sectioned at 8 $\mu$m thickness longitudinal to the follicles. The sections were stained with haematoxylin, eosin, and picric acid and were examined microscopically.

## Results

### (*a*) *Effects of Mimosine on the Strength of Wool*

Intravenous or intraperitoneal injections of from 1 to 16 g mimosine or intravenous infusions of small amounts (a total of 2 or 4 g given over 4 days) had no effect on the

strength of wool (Table 1). Infusions of larger amounts of mimosine (8 g or more) caused weakening of the wool, but the magnitude of the effect depended on the rate and the duration of administration. Infusions of a total of 8 g, either at the daily rates of 2 g/day (44 and 52 mg/kg) or 8 g/day (152 and 157 mg/kg), weakened the wool but not sufficiently to allow the sheep to be defleeced; infusion at the higher

**Table 1. Effect of mimosine on strength of wool**

All sheep were dosed with solutions of the sodium salt of mimosine

| Mimosine treatment | Total amount of mimosine given (g) | Daily dose rate of mimosine (mg/kg) | No. of sheep | Strength of wool after treatment | Adverse effects of treatment |
|---|---|---|---|---|---|
| Intravenous injections | 1 | 20 | 1 | Normal | Nil |
| | 2 | 43 | 1 | Normal | Nil |
| | 4 | 80, 92 | 2 | Normal | Nil |
| | 8 | 193 | 1 | Normal | Nil |
| | 16 | 350 | 1 | Normal | Nil |
| Intraperitoneal injections | 4 | 94 | 1 | Normal | Nil |
| | 8 | 154 | 1 | Normal | Nil |
| Intravenous infusions: | | | | | |
| 0·5 g/day, 4 days | 2 | 10 | 1 | Normal | Nil |
| 1 g/day, 4 days | 4 | 20 | 1 | Normal | Nil |
| 2 g/day, 4 days | 8 | 44, 52 | 2 | Slightly weak | Nil |
| 8 g/day, 1 day | 8 | 152, 157 | 2 | Weak | Nil |
| 8 g over 35 h | 8 | 104 | 1 | Very weak (defleeced) | Nil |
| 4 g/day, 2 days | 8 | 77–96 | 4 | Very weak (defleeced) | One sheep—feed refusals (4 days) |
| 4 g/day, 4 days | 16 | 77, 87 | 2 | Very weak (defleeced) | Feed refusals 3–6 days; excessive salivation |
| 6 g/day, 4 days | 24 | 147 | 1 | Very weak (defleeced) | Stopped eating after 2 days, excessive salivation, died after 7 days |

rate for 1 day had a greater effect (Table 1). Infusions of a total of 8 g mimosine over a period of 1½–2 days, or of larger amounts, consistently weakened the wool sufficiently to allow all sheep dosed to be defleeced (Table 1). Defleecing in these sheep was obtained with mimosine infused at the daily rate of 77 mg/kg or higher (Table 1).

*(b) Observations in Relation to Defleecing*

Some weakness in the wool of the eight sheep that were subsequently defleeced (Table 1) was first detected 3 or 4 days after the beginning of the mimosine infusion, and the wool was judged to be weak enough for defleecing by day 6 or 7. By day 14

or 15 the fleece was usually coming away from the skin, especially round the neck, and the sheep were then defleeced readily. The wool could be readily removed from the whole of the wool-bearing area at this time by pushing with the hand; there was no visible regrowth of wool after defleecing (Fig. 4). In all sheep that were defleeced wool growth appeared to stop abruptly; no tapering of the fibres prior to cessation of wool growth was observed (Fig. 5). In five sheep that were defleeced, measurements were

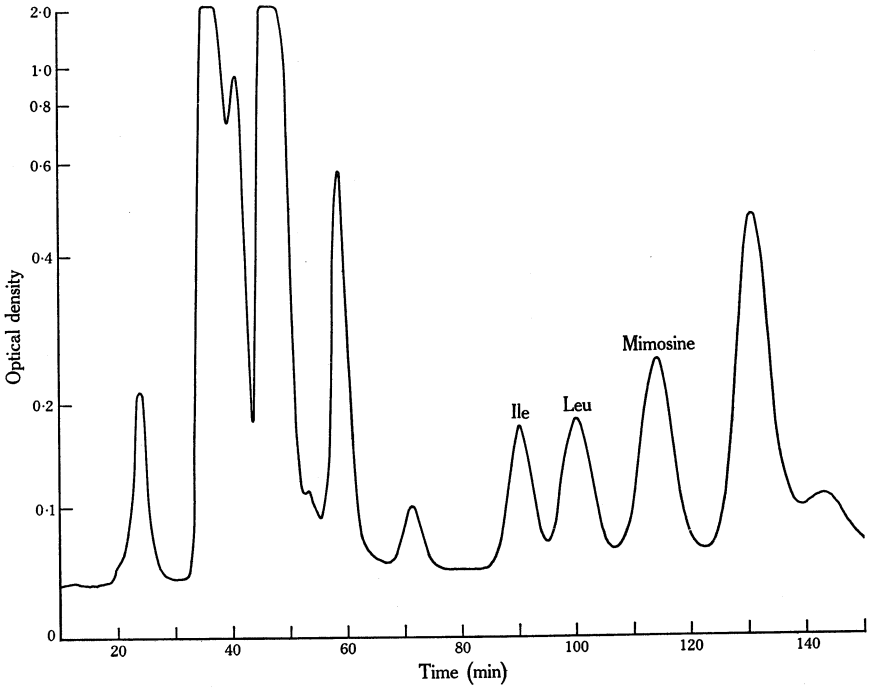



**Fig. 2.** Elution pattern of amino acids in plasma. Mimosine (0·1 μmol) was added to 1 ml of blood plasma from a sheep. Elution conditions are described in Materials and Methods, section (*e*).

made of the average length from the end of the fibres to the front of the radioactive mark that corresponded to the start of the infusion. These lengths corresponded to 2·0–2·4 days' length growth at the pretreatment rate (see section (*c*), Materials and Methods), indicating that fibre growth stopped within this period. Some information is available from four sheep defleeced with mimosine (4 g/day for 2 days, two sheep; 4 g/day for 4 days, two sheep) regarding the period during which wool follicles were inactive. No radioactive spots were detected in radioautograms of fibres in the regrowth after mimosine treatment from doses of [$^{35}$S]syctine given 8 days (one sheep) or 11 days (three sheep) after the beginning of mimosine infusion. This result indicates that all follicles were inactive for at least these periods. The first visual signs of regrowth of wool were seen in all defleeced sheep 17–18 days after the start of the infusion.

### (*c*) *Effects of Mimosine on Wool Growth Rate*

Measurements were made of fibre diameter and length growth rate of wool in 19 sheep that were dosed with mimosine.

Intravenous or intraperitoneal injections of small amounts of mimosine (4 g or less) given to five sheep (see Table 1) had no appreciable effects on wool growth rate. Larger amounts given as intravenous injections (8 and 16 g) or an intraperitoneal injection (8 g) did cause small reductions in both components of wool growth, and hence in fibre volume, during the 4 days after the injection (Table 2).

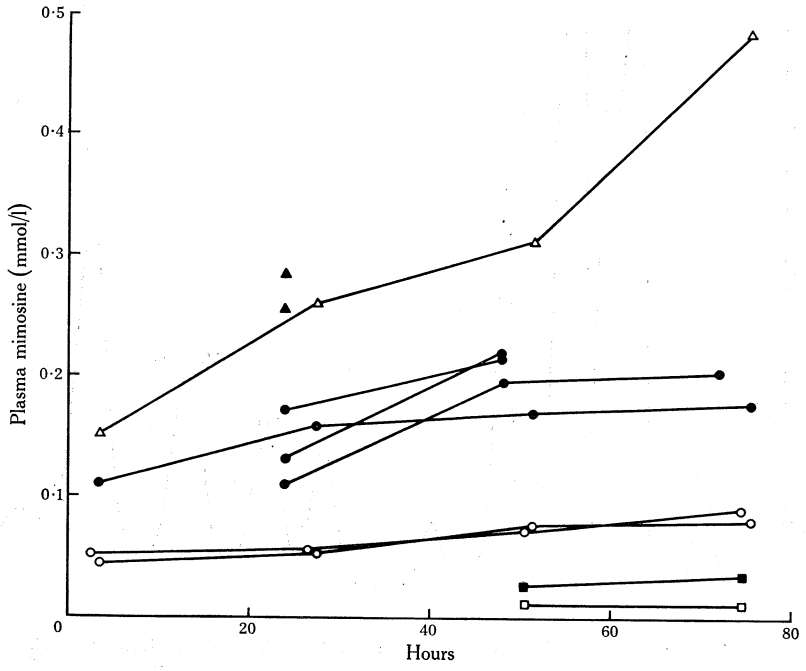



**Fig. 3.** Effect of intravenous infusions of mimosine on the concentration of mimosine in blood plasma. Solutions of the sodium salt of mimosine were infused continuously for periods of from 1 to 4 days, and the concentration of mimosine in plasma was measured at the times indicated after the start of the infusion. Each point is a value for one sheep; values for each sheep are joined. The rates of infusion of mimosine were: 0·5 g/day (□), 1 g/day (■), 2 g/day (○), 4 g/day (●), 6 g/day (△), and 8 g/day (▲).

The intravenous infusion of small amounts of mimosine (a total of 2 or 4 g during 4 days; see Table 1) had no appreciable effects on wool growth rate. Larger amounts of mimosine (a total of 8 g or more) defleeced several sheep (Table 1) and, as a result, wool growth was temporarily stopped and no measurements of wool growth rate could be made. Four sheep that received 8 g of mimosine (either 2 g/day for 4 days or 8 g for 1 day) continued to grow wool and measurements are reported for these sheep.

The intravenous infusion of 2 g mimosine per day for 4 days caused a small reduction in mean fibre diameter when measured at days 4 and 8 after the start of the infusion, and in length growth rate during these periods (Table 3*a*). The mean fibre volume growth rate was also reduced after mimosine infusion (Table 3*a*). However, inspection of the fibres from both sheep showed that these volumes were probably an overestimate of the true fibre growth following mimosine infusion.

Many fibres had a very thin region which represented about 2 days' growth; this thin region was most frequently just after the end of the 4-day infusion period (Fig. 6). The mean diameter at the position that was subjectively assessed to be the thinnest part of each fibre was only about 13 μm for each sheep and was considerably less than the mean diameters measured at days 4 and 8 (Table 3, footnote).

The intravenous infusion of 8 g mimosine for 1 day caused a substantial reduction in mean fibre diameter at day 4, but by day 11 fibre diameter was slightly above the pretreatment value (Table 3*b*). Length growth rate and fibre volume were also considerably reduced following mimosine treatment (Table 3*b*). The estimate of fibre volume between days 4 and 11 can only be approximate as the fibre diameter varied considerably during this period and the value used for calculation was the mean of the diameter at days 4 and 11. The mean diameter at the thinnest part of each fibre (in the region of day 4) was also measured; the values were very low at 9·8 and 11·4 μm (Table 3, footnote). The fibres from these two sheep were very weak and, of those that were mounted on slides for autoradiography, only 56% (80 fibres) remained intact and could be measured to obtain the data in Table 3*b*.

**Table 2. Effect of injections of mimosine on wool growth rate**

Solutions of the sodium salt of mimosine were injected either intravenously or intraperitoneally into three sheep as follows: sheep I received 8 g intravenously, sheep II 16 g intravenously, and sheep III 8 g intraperitoneally. The values are the means of measurements on 80 fibres

| Time after injection of mimosine (days) | Fibre diameter (μm) | | | Fibre length growth rate (μm/day) | | | $10^{-3}$ × Fibre volume growth rate (μm³/day) | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | I | II | III | I | II | III |
| −4 | 21·0 | 23·9 | 26·7 | 323 | 287 | 276 | 114 | 133 | 162 |
| 0 | 20·8 | 24·0 | 27·4 | 300 | 256 | 255 | 98 | 109 | 136 |
| 4 | 19·6 | 22·1 | 24·1 | 334 | 295 | 292 | 111 | 125 | 154 |
| 8 | 21·0 | 23·5 | 27·2 | | | | | | |

*(d) Concentration of Mimosine in Plasma*

No mimosine was present in plasma prior to administration of mimosine to sheep. The effect of the intravenous infusion of mimosine on the concentration of mimosine in plasma is shown in Figure 3. Plamsa mimosine increased as the rate of infusion of mimosine was increased. Low rates of infusion (up to 2 g/day) resulted in plasma levels below 0·1 mmol/l. Infusion at the rate of 4 g/day quickly resulted in levels of mimosine in plasma above 0·1 mmol/l; after 2 days the concentration was steady at about 0·2 mmol/l. Higher rates of infusion resulted in higher levels of mimosine in plasma, and it appeared that the concentration continued to rise instead of levelling off after 2 days of infusion at the rate of 6 g/day (Fig. 3).

Injections of large amounts of mimosine (8 or 16 g) resulted in very high levels of mimosine in plasma 3 h later, whereas 27 h after injection only very small amounts of mimosine were present in plasma. The respective concentrations of mimosine in

plasma (mmol/l) at 3 and 27 h after injection were: 0·99 and 'trace' (8 g, intraperitoneal), 0·99 and 0·01 (8 g, intravenous), and 1·70 and 0·03 (16 g, intravenous).

**Table 3. Effect of intravenous infusions of mimosine on wool growth rate**

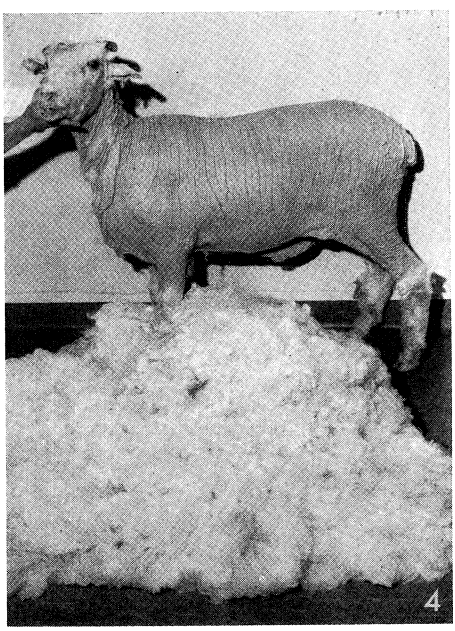
All sheep received a continuous intravenous infusion of a solution of the sodium salt of mimosine which provided either (*a*) 2 g mimosine per day for 4 days or (*b*) 8 g mimosine for 1 day. The values are means of measurements on 80 fibres

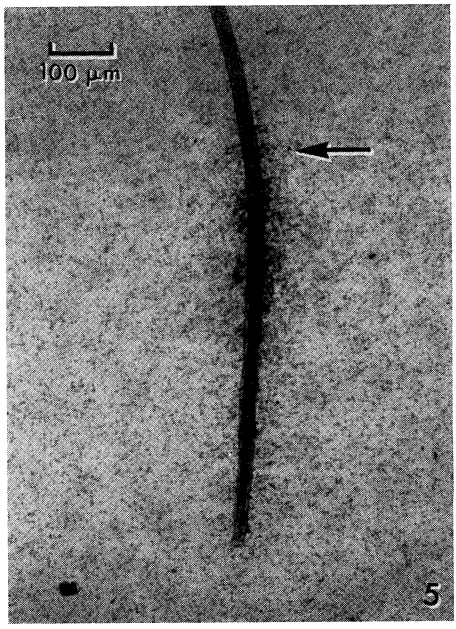
| Time after commencement of infusion (days) | Fibre diameter (μm)* | | Fibre length growth rate (μm/day) | | $10^{-3}$ × Fibre volume growth rate (μm³/day) | |
|---|---|---|---|---|---|---|
| | (a) 2 g mimosine per day for 4 days | | | | | |
| | Sheep A | Sheep B | Sheep A | Sheep B | Sheep A | Sheep B |
| −4 | 21·9 | 23·5 | | | | |
| | | | 275 | 317 | 107 | 139 |
| 0 | 21·6 | 23·0 | | | | |
| | | | 252 | 306 | 82 | 122 |
| 4 | 18·4 | 21·5 | | | | |
| | | | 255 | 292 | 74 | 109 |
| 8 | 19·4 | 21·3 | | | | |
| | (b) 8 g mimosine for 1 day | | | | | |
| | Sheep C | Sheep D | Sheep C | Sheep D | Sheep C | Sheep D |
| −4 | 20·1 | 22·9 | | | | |
| | | | 260 | 310 | 85 | 140 |
| 0 | 19·6 | 24·1 | | | | |
| | | | 191 | 240 | 45 | 75 |
| 4 | 14·1 | 14·8 | | | | |
| | | | 199 | 294 | 51 | 106 |
| 11 | 21·1 | 26·9 | | | | |

* Mean fibre diameter at thinnest point 12·8 and 13·1 μm respectively for sheep A and B [treatment (*a*)] and 9·8 and 11·4 μm respectively for sheep C and D [treatment (*b*)].

### (*e*) *Adverse Effects of Mimosine*

No adverse effects on the sheep were observed with injected or infused doses of mimosine that failed to defleece the sheep (Table 1). Of the five sheep defleeced with a total of 8 g mimosine infused over 35 h or 2 days no adverse effects were observed except for failure by one sheep to consume its complete ration for a period of 4 days. The infusion of mimosine at a rate of 4 g/day for 4 days caused feed refusals for 3 or 6 days and excessive salivation (Table 1). The sheep that received a total of 24 g mimosine stopped eating after 2 days, showed excessive salivation, and died after 7 days. At this time the hooves were inflamed at the base and were starting to shed. Post-mortem examination revealed considerably enlarged adrenal glands; the total wet weight of the two glands was 7·5 g compared with normal weights of 2·0–2·5 g (B. A. Panaretto, personal communication). Histological examination showed gross changes from the normal in tissue from kidney, adrenals, liver, and lung.



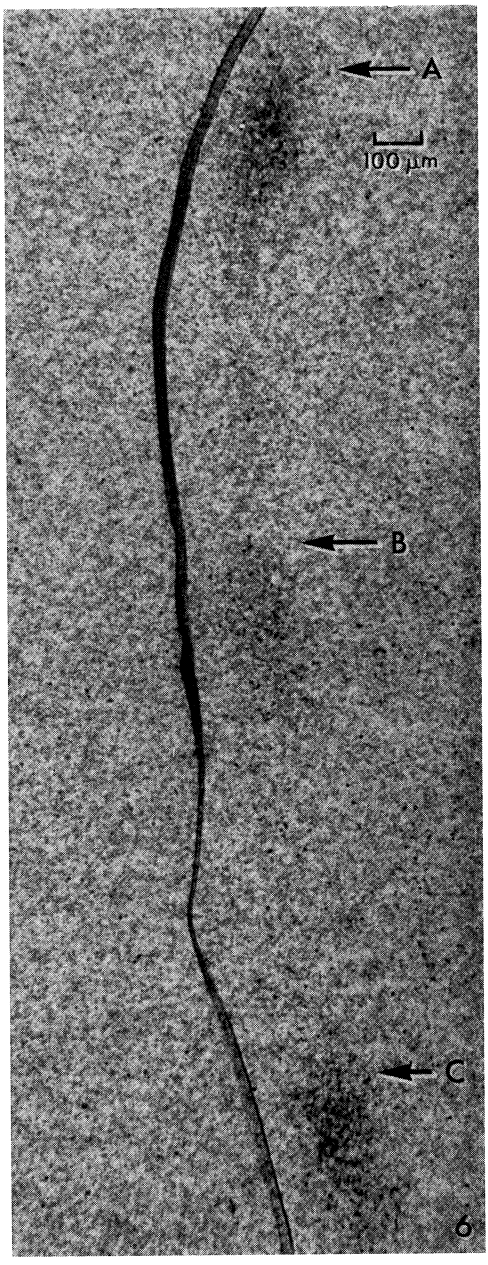




**Fig. 4.** A sheep defleeced 15 days after the start of an intravenous infusion of mimosine (4 g/day for 2 days). No regrowth of wool had emerged from the skin.

**Fig. 5.** The end of a fibre shed following an infusion of mimosine for 4 days (4 g/day). The arrow indicates the front of the mark from a dose of [$^{35}$S]cystine given at the start of the infusion.

**Fig. 6.** The constricted region of a fibre resulting from the infusion of mimosine for 4 days (2 g/day). The fronts of the marks from doses of [$^{35}$S]cystine given at intervals of 4 days are indicated by arrows. The first mark (arrow A) corresponds to the start of the infusion period. The second faint mark (arrow B), indicates the end of the infusion; the third mark (arrow C) corresponds to 4 days after the infusion stopped. The autoradiogram was slightly displaced when the film was remounted over the fibre.

*(f) Effects of Mimosine on Wool Follicles*

Histological sections of skin, prepared from biopsies taken at various times after dosing, were examined from 11 sheep that received mimosine. Before treatment, less than 1% of the wool follicles in any sheep were inactive.

Skin biopsies were taken at intervals, up to 8 days after dosing, from two sheep that received intravenous injections and from two sheep that received an intravenous infusion of mimosine at the rate of 2 g/day for 4 days. No effects on wool follicles were observed following an injection of 4 g mimosine. Some pycnotic cells were present in follicle bulbs 1 day after the injection of 16 g mimosine, but the bulbs appeared normal 1 day later. Subsequently, fibre growth appeared normal except for slight irregularities in the thickness of fibres in the lower parts of many follicles. During the infusion of mimosine (2 g/day) it was observed, 3–4 days after treatment started, that the fibres were eosinophilic distal to the keratogenous zone instead of being picrophilic, indicating that keratinization was abnormal. Concomitant reductions occurred in follicle bulb size and in fibre diameter (Fig. 6).

Skin biopsies were taken at intervals, up to 134 days after dosing, from seven sheep that received intravenous infusions of mimosine at the rate of 4–8 g/day for periods of 1–4 days. The intravenous infusion of mimosine at the rate of 4–8 g/day caused degenerative changes in the cells of the follicle bulbs after 1 day (Fig. 7). In the two sheep that received an infusion of 8 g mimosine for only 1 day, the cells of the fibre and inner root sheath withdrew from around the proximal half of the dermal papilla in many of the follicles, during the following day. However, very few follicles proceeded to retrogress and by 4 days after dosing at least 95% of the follicles had reformed bulbs. Thus, in most follicles, fibre growth was continuous, although irregularities were present along the fibres (Fig. 8). After 17 days, when sampling was stopped, only 1–2% of follicles were still inactive in these sheep.

When mimosine was infused at the rate of 4 g/day for 2 or 4 days, follicle retrogression began on the second day with the cells of the fibre and inner root sheath withdrawing from around the dermal papilla (Fig. 9). By the third day the cells of the fibres and inner root sheaths had moved about 50 $\mu$m away from the resting dermal papilla to form unkeratinized, rounded ends on the fibres (Fig. 10). By the sixth day the fibre ends were about one-quarter of the distance up the follicles, and appeared to have hardened (Fig. 11). There was no sign of any follicle regeneration at this stage. Infusion of mimosine at the rate of 6 g/day for 4 days, which was lethal to the sheep, inactivated all follicles slightly more rapidly than did infusion at the rate of 4 g/day.

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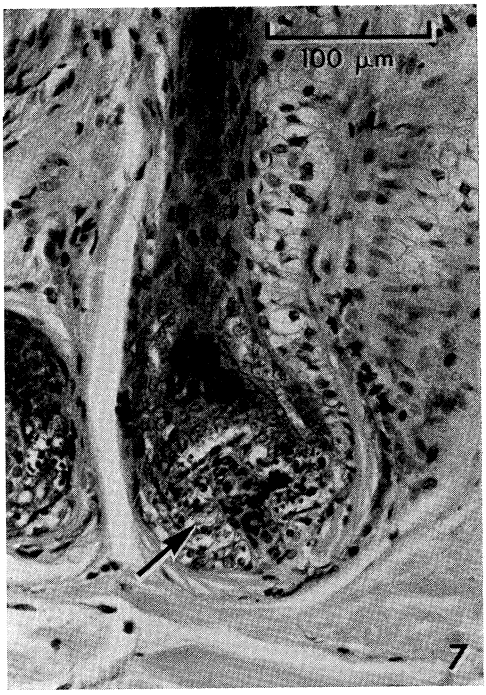
**Figs 7–10.** Changes in wool follicles of Merino sheep resulting from intravenous infusions of mimosine.

**Fig. 7.** Degeneration and vacuolation of cells (arrow) in the mitotic region of a follicle bulb at the end of the first day of an infusion of mimosine (4 g/day).

**Fig. 8.** Irregularities (arrows) in a wool fibre 3 days after the end of a 1-day infusion of 8 g mimosine.

**Fig. 9.** Withdrawal of the cells of the fibre and inner root sheath (arrow) from around the dermal papilla (*P*) of a follicle at the end of the second day of an infusion of mimosine (4 g/day). The papilla is surrounded by epithelial cells derived from the outer root sheath.

**Fig. 10.** An unkeratinized, rounded end of a fibre (arrow) approximately 50 $\mu$m from the resting papilla (*P*) of a follicle at the end of the third day of an infusion of mimosine (4 g/day).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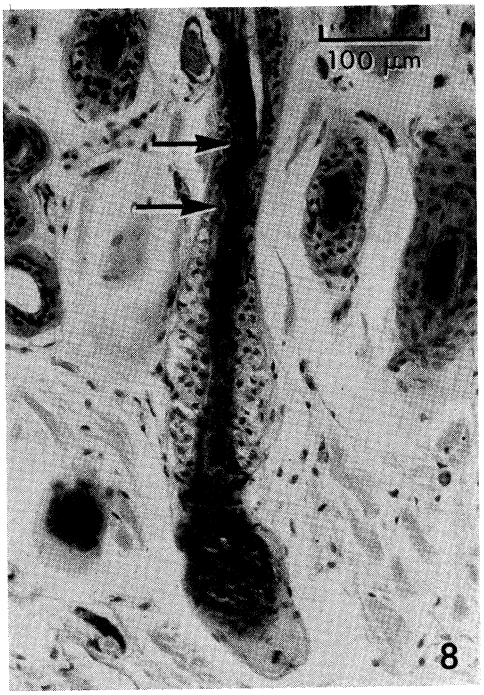
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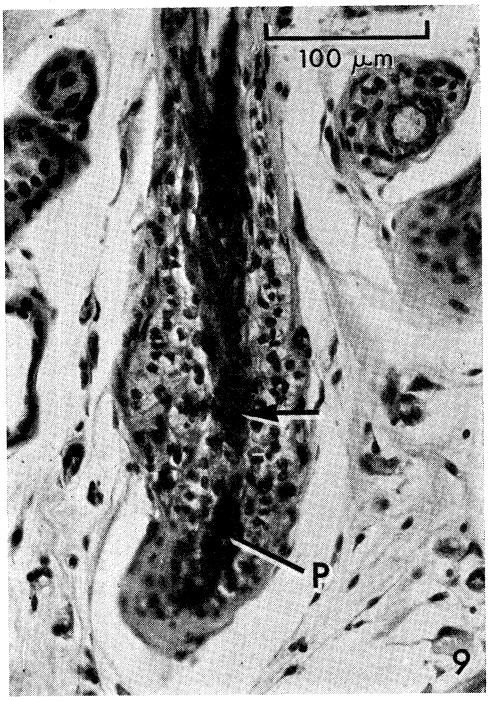
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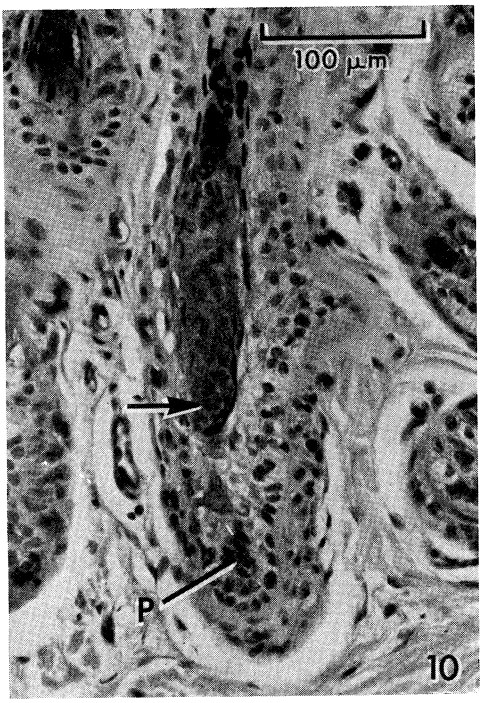
9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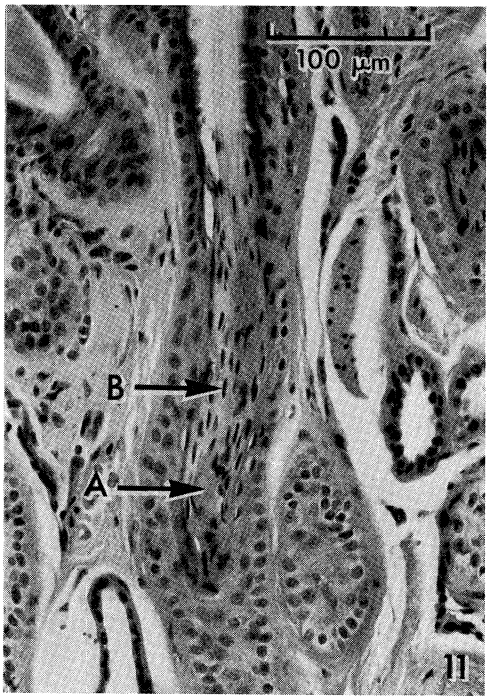
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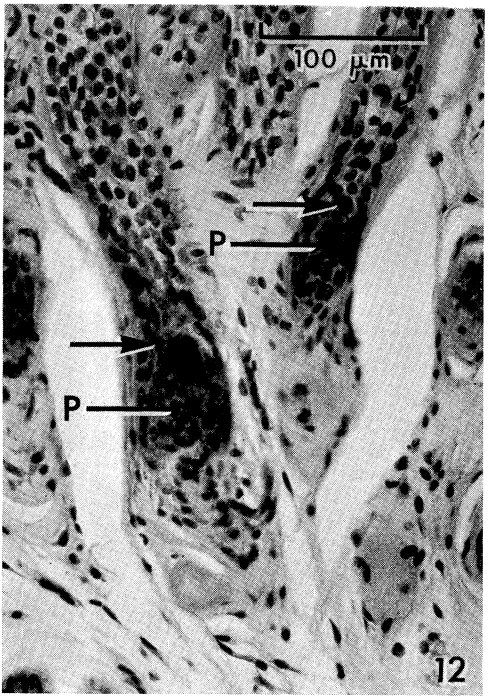
B
A
11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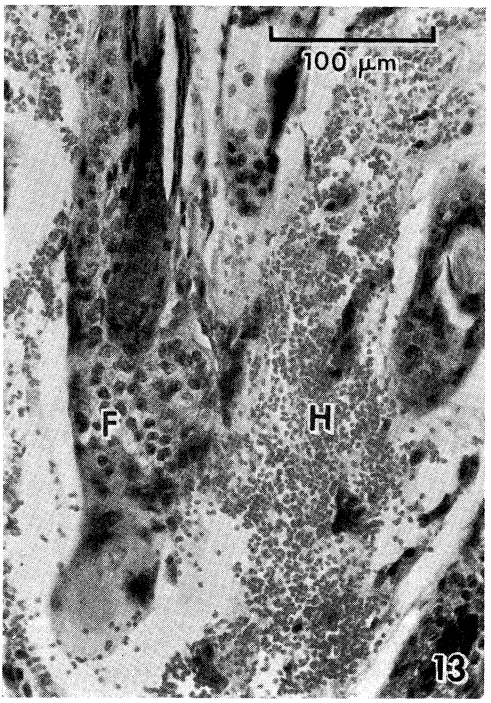
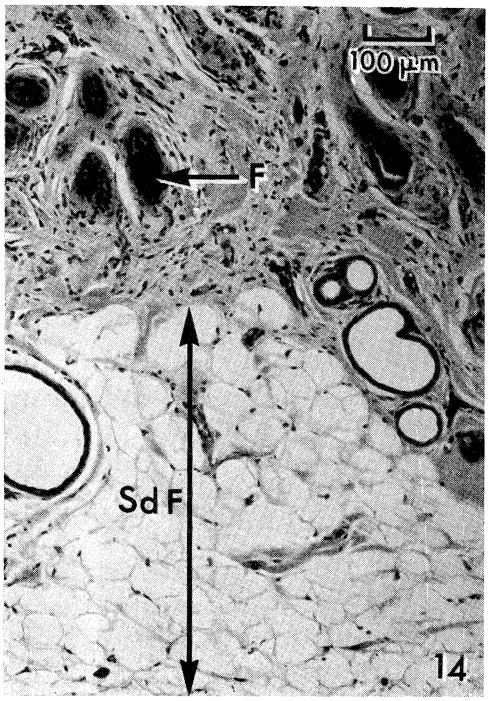
P
12


100 µm
F
H
13


100 µm
F
Sd F
14

Follicle regeneration was more rapid in the two sheep that received mimosine at the rate of 4 g/day for 2 days than in the two sheep that received mimosine at this rate for 4 days. Following a 2-day infusion, 50–70% of the follicles were at the pre-hair cone stage of development by the eighth day (Fig. 12), whereas following a 4-day infusion no regenerating follicles were evident after 8 days and only about 30% had commenced regeneration by the eleventh day. After 17 days about 90% of the follicles from the four sheep were in various stages of regeneration. Following a 2-day infusion, 1–2% of follicles had atrophied after 44 days when sampling ceased. In contrast, in one sheep that received a 4-day infusion, remnants of about 7% of atrophied follicles were present 134 days after dosing.

### (*g*) *Effects of Mimosine on Skin*

In some of the sheep that received mimosine infusions, enlargement of the sebaceous glands was evident 11–17 days after treatment started, but these changes were not related to dosage. Considerable amounts of wax accumulated on the skin of those sheep which were defleeced and which had sebaceous gland enlargement. The glands gradually returned to their pretreatment size after 17 days.

Dilatation of the sweat glands and thickening of their walls, due to hypertrophy of the secretory cells, occurred in the sheep that received a 4-day infusion of mimosine at the rate of 4 g/day. The glands had returned to near pretreatment size after 17 days, but thickening of the gland walls was still evident at the last sampling 44 or 134 days after treatment began.

No changes were observed in the epidermis following dosing with mimosine.

Dilatation of dermal capillaries was evident 1 day after the injection of at least 4 g mimosine, or after the start of an infusion of mimosine at the rate of 2 g/day. Infusion at the rate of 4 g/day or greater produced areas of haemorrhage in the dermis, which persisted for the duration of the infusion but subsequently cleared. In the sheep infused with 6 g/day for 4 days, and which died after 7 days, the haemorrhages extended throughout the dermis (Fig. 13).

Depots of subdermal fat were present for up to 17 days after the start of an infusion of mimosine at the rate of 4 g/day for either 2 or 4 days (Fig. 14). These fat depots subsequently disappeared when fibre growth had resumed in most follicles.

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**Figs 11–14.** Changes in wool follicles and skin of Merino sheep resulting from intravenous infusions of mimosine.

**Fig. 11.** A relatively blunt, keratinized fibre end (arrow A) surrounded by inner root sheath cells (arrow B) about one-quarter the distance up a follicle 6 days after the start of a 4-day infusion of mimosine (4 g/day). The resting dermal papilla, attached to the fibre end by epithelial cells, is beyond the bottom of the figure.

**Fig. 12.** Early signs of regeneration of bulb cells (arrows) around the dermal papillae (*P*) of two follicles 8 days after the start of a 2-day infusion of mimosine (4 g/day).

**Fig. 13.** Haemorrhage (*H*) alongside an inactive follicle (*F*) at the end of the third day of an infusion of mimosine (6 g/day).

**Fig. 14.** Accumulation of subdermal fat(*SdF*) beneath inactive follicles (*F*) 11 days after the start of a 4-day infusion of mimosine (4 g/day).

## Discussion

It is apparent that mimosine has a potential use as a chemical defleecing agent if a practical means of administration could be found to obtain a controlled rate of release into the bloodstream. The amounts of mimosine given by Hegarty *et al.* (1964) as intravenous or abomasal infusions, which resulted in death of the sheep, were much greater than the amounts needed to obtain complete defleecing. On the basis of total amounts given the margin between an effective dose and a toxic dose of mimosine appears to be about 1 : 3, which is at least as safe as cyclophosphamide (Dolnick *et al.* 1969). On the basis of the rate of administration per kilogram body weight this margin may appear to be narrower. However, toxic effects were observed only with infusions given for periods of 4 days; infusions at the rate of about 150 mg/kg for 1 day caused no obvious toxic effects, whereas this rate of infusion for 4 days caused death of the sheep.

It can be concluded from the results with intravenous infusions that a total of 8 g mimosine, infused at a daily rate of about 80 mg/kg over a period of 36–48 h, is sufficient to obtain consistent and complete defleecing of sheep. These effective doses always resulted in the complete cessation of wool growth for a period. Larger amounts of mimosine may produce toxic effects, while smaller amounts or shorter periods of administration do not stop fibre growth but may cause weakening of the fibres, slight irregularities along the fibres, and a reduction in both fibre diameter and length growth rate. It was also observed that the extent of incorporation of cystine (as judged by the intensity of the autoradiograms) was appreciably reduced following mimosine infusions that reduced wool growth rate. Fibre growth continues even when there has been partial withdrawal of some of the fibre and inner root sheath cells from around the dermal papillae. It is only when there has been complete withdrawal of these cells from around the papillae that the follicles retrogress and defleecing ensues. It would appear that substantial weakening of the fibres without cessation of wool growth does not allow easy manual defleecing.

The effectiveness of mimosine for defleecing sheep appears to be related to the concentration of mimosine in blood plasma. Following intravenous or intraperitoneal injections, mimosine is cleared rapidly from the body, probably mainly by direct excretion in the urine (Hegarty *et al.* 1964). With intravenous infusions, a concentration of mimosine in blood plasma of at least 0·1–0·2 mmol/l for about 36 h is required to stop wool growth and allow defleecing.

Hegarty *et al.* (1964) found that shedding of the fleece occurred 6–7 days after sheep began eating *L. leucocephala*, provided the daily intake of mimosine was 200–300 mg/kg. Indirect evidence indicated substantial breakdown of mimosine to 3,4-dihydroxypyridine in the rumen when *L. leucocephala* was fed. The time required for shedding after the feeding of *L. leucocephala* began (6–7 days) was similar to the period required for positive shedding after the beginning of intravenous infusions in our experiments. These results suggest that the daily oral administration of mimosine at 200–300 mg/kg for no longer than 2–3 days should also be effective for defleecing sheep.

Estimates can be made of the time taken for fibres to stop growing in those sheep that were defleeced following administration of mimosine, from the autoradiographic results and from the skin histology studies. It was calculated from the autoradiographic data that the average length of fibre from the radioactive mark, indicating

the start of the infusion, to the end of the fibre corresponded to 2–2½ days' growth at the pretreatment rate. The time taken for fibres to stop growing would be at least 1 day less than this period (i.e. 1–1½ days), if allowance is made for the spread of the [$^{35}$S]cystine dose as discussed by Reis and Chapman (1974). The start of degenerative changes in the follicle bulb cells 1 day after the start of the infusion, and follicle retrogression after 2 days, also indicate that fibre growth stopped within 2 days. These estimates are in line with the observations that a 24-h infusion of 8 g mimosine failed to cause complete cessation of wool growth, whereas the infusion of 8 g for 36–48 h caused complete cessation of fibre growth and subsequent defleecing.

The degenerative changes in the follicles of the sheep that were defleeced were essentially similar to those described by Hegarty *et al.* (1964). However, the retrogression of follicles in the present study was more rapid than in the sheep to which Hegarty *et al.* (1964) infused mimosine intravenously, even though they used a high daily dose rate of 125 mg/kg. This faster retrogression may account for the relatively blunt ends on the fibres in the inactivated follicles in the present study, compared with the tapering before cessation of growth reported by Hegarty *et al.* (1964), particularly in sheep fed *L. leucocephala.*

An important question to be answered is how long the follicles remain inactive after fibre growth has been stopped by administration of mimosine. Hegarty *et al.* (1964) reported that the first signs of follicle regeneration were noted at 72 h, but in the present study there was no sign of regeneration of follicle bulbs until 8–11 days after the infusions commenced. An estimate of the period during which fibre growth ceased can be made in those sheep that received doses of [$^{35}$S]cystine. As the doses given 11 days after the start of the infusions were not detected in the radioautographs, at least 10 days' wool growth must have been lost, allowing 1 day for fibre growth to stop. The first regrowth of wool was seen 17 days after the start of the infusion; this would indicate a loss of about 12 days' growth if allowance is made for the time for fibres to stop growing (1 day) and emergence time (4 days). This loss of wool growth would be comparable to that observed following dosing with cyclophosphamide (Reis and Chapman 1974).

Provided the amount of mimosine infused and the duration of the infusion are just sufficient to achieve defleecing, few follicles are permanently damaged. Although small haemorrhages occur in the dermis, they clear without apparent untoward effects. Prolongation of the infusion time increases the percentage of follicles that subsequently atrophy, and causes a persistent thickening of the walls of the sweat glands. The significance of this and of the transient enlargement of the sebaceous glands, in relation to the suint and wax contents of the wool regrown after defleecing, has yet to be ascertained. Information is also needed on the wool growth rate after defleecing with mimosine. The measurements following the infusion of 8 g mimosine for 24 h showed an increase in fibre diameter 11 days after dosing, which may indicate a slight stimulation of wool growth.

The accumulation of subdermal fat during either depressed activity or inactivation of the follicles may be due to a redirection of nutrients normally utilized by the follicles. A similar feature occurs in sheep skin during follicle retrogression induced by an increase in the concentration of cortisol in plasma (Chapman and Bassett 1970). The amount of subdermal fat in fur-bearing animals also increases when the follicles are in the resting phase and the skin is ready for pelting (Dolnick 1965).

The effects of mimosine on wool growth are very similar to those of cyclophosphamide, which casues a constriction of the fibres when small doses are given (Hourihan *et al.* 1971) and the complete cessation of fibre growth, and defleecing, when larger amounts are given (Reis and Chapman 1974). In our experience fleeces can be removed by hand as readily following mimosine treatment as after dosing with cyclophosphamide at 30 mg/kg. It was possible to remove the wool readily from all wool-bearing regions on all sheep that were defleeced following treatment with mimosine. Mimosine may be superior to cyclophosphamide in this respect.

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