

Synergistic Effects of Thiocyanate with Flumethasone in Inhibiting Wool Growth in Merino Wethers

B. A. Panaretto and A. L. C. Wallace

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

Abstract

Sheep bearing 5-cm fleeces and being fed a maintenance diet were infused subcutaneously with potassium thiocyanate (KSCN) ($0.7 \text{ g/kg}^{0.75}$) and/or flumethasone (0.66 or $1.3 \text{ mg/kg}^{0.75}$) over 8 days. Wool growth was evaluated by clipping defined patches of 100 cm^2 on the mid-sides of the animals. Plasma concentrations of flumethasone, thiocyanate, and thyroxine were measured. Food eaten by the animals was also monitored.

Thiocyanate treatment resulted in plasma concentrations in the range 4.1 – 11.8 mg/100 ml and caused some depression in circulating thyroxine, but had no effect on wool growth, fibre shedding or appetite.

Four of the five animals treated with flumethasone alone ($1.3 \text{ mg/kg}^{0.75}$) showed maximal flumethasone concentrations during the first 48 h of infusion followed by stable concentrations of 2.2 ± 0.4 to $10.6 \pm 2.3 \text{ ng/ml}$. Plasma flumethasone concentrations fluctuated throughout infusion in the fifth animal. Plasma thyroxine values declined from 4.5 to $1.5 \text{ } \mu\text{g/100 ml}$ during infusion. Only one of these animals refused substantial parts of its ration after treatment. Wool growth was depressed in this group to a mean of 62% of its pretreatment value 9–16 days after treatment and did not recover until 30 days. Wool fibre shedding was seen in only some of the wool fibres in these animals and the fleece could best be removed by the usual mechanical harvesting procedure.

The two sheep given flumethasone ($0.66 \text{ mg/kg}^{0.75}$) and KSCN ($0.7 \text{ g/kg}^{0.75}$) showed stable flumethasone concentrations of 2.4 and 4.0 ng/ml and thiocyanate concentrations of 8.8 and 5.0 mg/100 ml . Wool growth was depressed to about 35% of its control value 9 days after treatment and did not recover until 40–60 days. Fibre shedding was again partial and wool could best be removed in the conventional way.

Six sheep given 1.3 mg flumethasone plus $0.7 \text{ g KSCN/kg}^{0.75}$ showed peak flumethasone concentrations during the first 48 h of infusion and then had stable flumethasone concentrations of 1.4 – 9.4 ng/ml plasma. Thyroxine values were depressed to the same degree as in the sheep with flumethasone alone. Feed residues were significant after treatment in five of the animals; in many cases the animals left all their feed for about 7–10 days. Wool growth in these animals was depressed to a mean of 31.4% of control values on day 22 after treatment. In four out of the six sheep enough fibres were shed so that the fleece was removed, 30 days after treatment when regrowth was established, by breaking the few remaining continuous fibres. This result was different ($P = 0.045$) from that obtained by flumethasone alone.

The significance of the results is discussed from the point of view of wool harvesting. Discussion is also made of the possible bases of the synergism between thiocyanate and steroid.

Introduction

We have been studying the inhibitory effects of dexamethasone and flumethasone on wool fibre growth in order to develop a method of wool harvesting based on biological manipulations of fibre growth.

Dexamethasone and flumethasone inhibit wool growth in Merino wethers (Panaretto and Wallace 1978*a*, 1978*b*). A number of factors, including the dose of steroid analogue given, its route of administration, the time period over which it was given, and the state of wool growth in the recipients, appeared to influence the degree of wool growth inhibition in these experiments (Panaretto and Wallace 1978*b*).

Appropriate manipulation of these factors enabled the experimenters to cause the temporary cessation in growth of enough wool fibres of some animals and so ultimately to cause casting of the fleece. The intravenously administered dose of hormone analogue that caused complete shedding of the fleece did not do so when it was given intraruminally or subcutaneously. Instead varying proportions of wool fibres were shed but the shed portion of fleece was retained on the animals by the remaining continuous fibres. The fleeces could best be removed using conventional means. In some cases no shed wool fibres were observed following intraruminal or subcutaneous administration (Panaretto and Wallace 1978*b*).

In this paper the synergistic effects of subcutaneously administered thiocyanate (SCN^-) and dexamethasone or flumethasone on fibre growth are described. A synergist is defined here as a substance that increases the effects of another.

Attention was drawn to SCN^- for several reasons. Hollander *et al.* (1949) reported the loss of scalp hair in a human being who was treated with SCN^- for hypertension. Shinkai *et al.* (1974) and Reynolds *et al.* (1977) suggested the activation of latent collagenases by SCN^- *in vitro*, and Hatefi and Hanstein (1969, 1970) discussed the chaotropic properties of the ion.

Materials and Methods

The Merino wethers used in these experiments were about 1 year and 9 months old and had fleeces approximately 5 cm long. Each animal was housed in an animal house in a cage and offered 600 g of a mixture of lucerne chaff and oats (1 : 1) daily. The body weights of the sheep included the weight of wool; body weight raised to the power 0.75 has been used in calculating dosages of chemicals. The body weights and treatments of the experimental animals are included in Table 1.

Polyethylene catheters were inserted subcutaneously for about 10 cm over the first three cervical vertebrae for infusion, and jugular venous catheters were used for blood sampling (Panaretto and Wallace 1978*b*).

Infusions were made over an 8-day period, using a Harvard Infusion pump (series 932, Harvard Apparatus Co. Inc., Mass., U.S.A.). Flumethasone ['Flucort'—a solution of flumethasone (6,9-fluoro-11,17,21-trihydroxy-16 α -methyl-pregna-1,4-diene-3,20-dione) in polyethylene glycol 400, containing several preservatives and anti-oxidants (Diamond Laboratories Inc., Des Moines, Iowa, U.S.A.)] and/or KSCN were contained in 45 ml infusate and infused at an even rate over 24 h. Blood samples were centrifuged and plasma stored at -20°C until analyses were made.

All infusion experiments could not be made simultaneously and the one group of uninfused controls was used for comparison with all treatment groups. Small adjustments in control values have been made with respect to time in order to compare them with appropriate treatment values.

Wool Growth

Wool growth was measured on defined areas of 100 cm² on the mid-sides of the sheep at 7–14-day intervals using the methods described by Panaretto and Wallace (1978*b*). The pretreatment control values were the means of at least four samples.

A shed fibre was defined as one no longer in the hair canal and a continuous fibre was still growing.

Analytical Methods

Flumethasone and thyroxine were measured in plasma using the methods described by Panaretto and Wallace (1978*b*). Thiocyanate was measured in plasma using the method of Bowler (1944).

Statistical Methods

Statistical analyses were made by the method of Paulson and Wallis (1947).

Results*Plasma Flumethasone Concentrations*

Plasma flumethasone concentrations in animals infused with flumethasone alone are illustrated in Fig. 1. Most animals showed peak flumethasone concentrations during the first 48 h of infusion and these were followed by the stable values shown in Fig. 1. Sheep 1887 showed fluctuating values throughout the infusion period with a mean \pm s.e.m. of 5.3 ± 0.8 ng/ml.

Table 1. Mean body weights and treatment doses of sheep infused subcutaneously for 8 days with flumethasone and KSCN

Treatment	Sheep No.	Body weight \pm s.e.m. (kg) (kg ^{0.75})		Flumethasone dose (mg) (mg/kg) (mg/kg ^{0.75})			KSCN (g/kg ^{0.75})
Controls	1264	34.7 ± 1.1	—	—	—	—	—
	1396						
	1562						
KSCN	1265	38.3 ± 0.6	15.4	—	—	—	0.71
	1267						
	1383						
Flumethasone	1360	35.2 ± 1.6	14.5	19.6	0.56	1.35	—
	1365						
	1508 ^A						
	1570 ^A						
	1887 ^A						
Flumethasone + KSCN	1388	34.3	14.2	9.4	0.26	0.66	0.70
	1600	40.5	16.1	10.6			
	1202	33.4 ± 0.9	13.9	18	0.54	1.30	0.70
	1262						
	1269						
	1542						
	1582						
1615							

^A These results were included in a previous paper (see Panaretto and Wallace 1978b).

Sheep infused with 0.66 mg flumethasone plus 0.7 g KSCN/kg^{0.75} had very stable concentrations following initial peaks that were measured during the first 24 h of infusion; sheep 1388 had 2.4 ± 0.2 ng flumethasone/ml and sheep 1600 had 4.0 ± 0.5 ng/ml for 7 days of infusion.

Sheep infused with 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75} had the plasma flumethasone profiles illustrated in Fig. 1. Plasma flumethasone concentrations again showed their maximal values during the first 48 h of infusion and these ranged from about 6 to 15 ng/ml. Stable flumethasone concentrations during the last 5 days of infusion then followed, ranging from 1.4 ± 0.1 to 9.4 ± 0.6 ng/ml.

Thus at the same dose rate of flumethasone (with or without thiocyanate) there were about 5–7:1 fold differences in stable plasma flumethasone concentrations between animals.

Plasma Thiocyanate Concentrations

Mean \pm s.e.m. plasma SCN^- concentrations are given in Table 2. Pre-infusion SCN^- concentrations were <1 mg/100 ml. During infusion these values were clearly elevated, ranging from approximately 4.1 to 11.8 mg/100 ml plasma (Table 2).

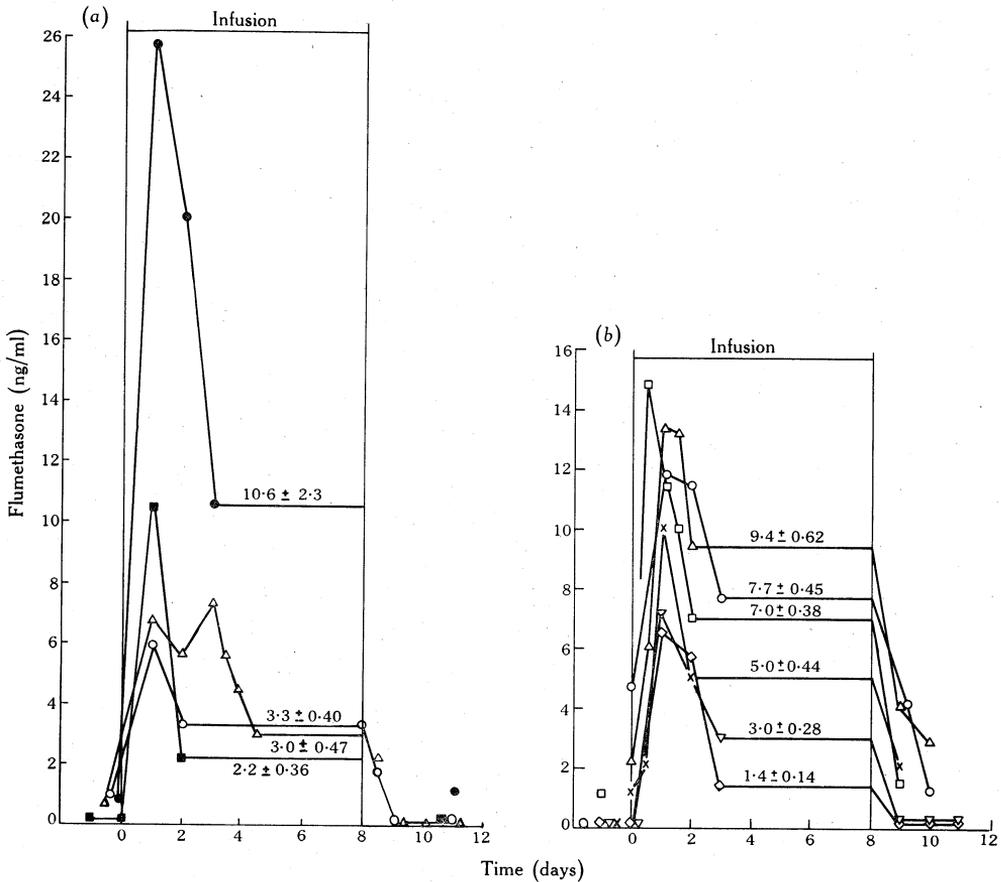


Fig. 1. Plasma flumethasone concentrations during the subcutaneous infusion of (a) 1.3 mg flumethasone/kg^{0.75}, and (b) 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75} over 8 days. Mean \pm s.e.m. concentrations are given by values close to the appropriate horizontal lines and apply for the times over which these lines extend. The following symbols have been used: (a) sheep 1360 (●), 1365 (■), 1508 (○) and 1570 (Δ); (b) sheep 1202 (Δ), 1262 (◇), 1269 (○), 1542 (□), 1582 (×) and 1615 (▽).

Plasma Thyroxine Concentrations

Mean plasma thyroxine concentrations did not vary greatly over a period of 1 week in uninfused controls—in three control animals mean (\pm s.e.m.) values of 4.4 ± 0.2 , 5.3 ± 0.2 and 5.1 ± 0.2 $\mu\text{g}/100$ ml were recorded. Animals infused with SCN^- alone showed some decline in plasma thyroxine concentrations from approximately 4.7 to 2.8 $\mu\text{g}/100$ ml at the lowest point on the 3rd day of infusion. Mean plasma thyroxine concentrations in the five animals given flumethasone alone fell from 4.5 to 1.5 $\mu\text{g}/100$ ml, a decline not dissimilar to that of 3.8 to 1.0 $\mu\text{g}/100$ ml

seen in the animals given flumethasone and SCN^- . In this last group the two animals, 1262 and 1582, with the lowest plasma SCN^- concentrations, 4.2 and 4.1 mg/100 ml (Table 2), had the highest concentrations of thyroxine, 1.9 and 2.1 ng/100 ml respectively, compared to a mean value of about ≤ 1 ng/100 ml in the other four sheep. However, while flumethasone concentration during the last 5 days in sheep 1262 was low (1.4 ± 0.1 ng/ml), that in sheep 1582 was higher (5.0 ± 0.4 ng/ml; see Fig. 1). In all three groups of animals thyroxine concentrations began to rise within 24 h after infusion finished.

Table 2. Mean (\pm s.e.m.) plasma SCN^- concentrations in sheep during the subcutaneous infusion of KSCN at the rate of $0.7 \text{ g/kg}^{0.75}$ for 8 days

Plasma SCN^- concentrations are expressed as milligrams per 100 ml plasma

Sheep No.	Plasma SCN^-	Sheep No.	Plasma SCN^-
1265	6.1 ± 0.46	1262	4.2 ± 0.41
1267	7.1 ± 0.35	1269	8.0 ± 0.46
1383	7.2 ± 0.51	1542	7.4 ± 0.42
1388	8.8 ± 0.63	1582	4.1 ± 0.42
1600	5.0 ± 0.57	1615	9.9 ± 1.20
1202	11.8 ± 1.40		

Feed Residues

None of the animals treated with thiocyanate alone refused food. Of the five animals given flumethasone alone only sheep 1570 left residues of 61, 58, 76, 52.5 and 52% of the 600-g ration given on days 2-6 following infusion. Neither animal given 0.66 mg flumethasone plus $0.7 \text{ g KSCN/kg}^{0.75}$ refused feed. Feed residues for sheep given 1.3 mg flumethasone plus $0.7 \text{ g KSCN/kg}^{0.75}$ are illustrated in Fig. 2. Three sheep (1202, 1269 and 1262) left feed sporadically during the infusion period; sheep 1615 had a history of feed refusals during the pretreatment period that continued into the treatment period and beyond it and may have been exacerbated by the treatment. All sheep except 1582, which ate all food given, left substantial quantities of food for 10-11 days following treatment, after which time feed was again readily eaten.

Wool Growth

Clean dry wool harvested from patches of 100 cm^2 in the treatment groups are illustrated in Fig. 3. Potassium thiocyanate caused no depression in wool growth relative to untreated controls (Fig. 3). Flumethasone at $1.3 \text{ mg/kg}^{0.75}$ depressed wool growth in five sheep, leaving a mean residual wool growth of 62.5 ± 9.9 and $62.7 \pm 12.4\%$ relative to pretreatment values 9 and 16 days after treatment respectively; in other words wool growth was depressed by approximately 38%. The size of the s.e.m. reflected variability in wool growth depression; residual wool growth 16 days after treatment in sheep 1360 and 1365 was 93% of pretreatment values while in the other three sheep (Table 1) the mean value was $42.5 \pm 1.9\%$.

Recovery to pretreatment values and to equal wool growth in untreated controls was made by about 1 month after treatment.

The subcutaneous infusion of 0.66 mg flumethasone plus 0.7 g KSCN/kg^{0.75} depressed wool growth by 65 and 55% of pretreatment values in sheep 1600 and 1388 respectively (Fig. 3). Both animals had recovered their pretreatment wool growth by days 36–50 post-treatment (Fig. 3).

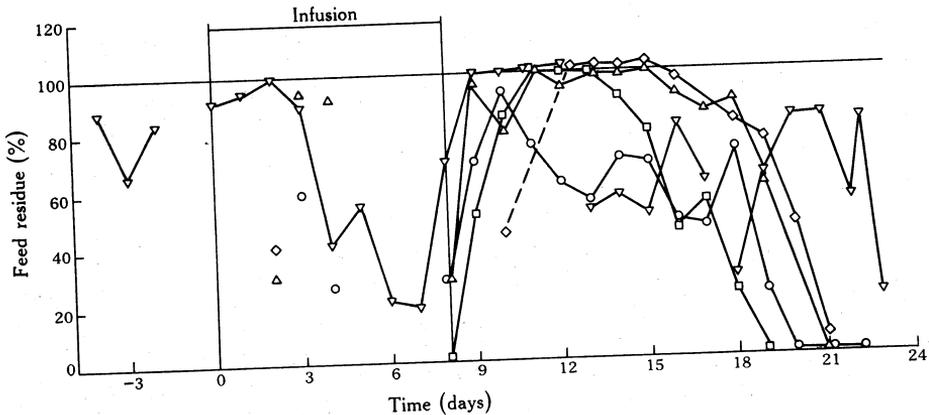


Fig. 2. The quantity of feed left daily expressed as a percentage of the 600 g offered. Sheep 1202 (Δ), 1262 (\diamond), 1269 (\circ), 1542 (\square) and 1615 (∇) were treated subcutaneously with 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75} during the infusion period marked.

Mean residual wool growth in six sheep given 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75} was 34.8 ± 7.1 , 31.4 ± 5.6 and $70.1 \pm 10.4\%$ of pretreatment values 8, 22 and 36 days after infusion respectively. Thus maximal wool growth depression approximated 70% of pretreatment values. The four animals that could be manually defleeced (sheep 1202, 1542, 1582 and 1615) had mean residual maximal wool growth values of 26.7 ± 7.0 , 23.7 ± 2.0 and $55.5 \pm 2.8\%$ respectively 8, 22 and 36 days after treatment, i.e. wool growth depression exceeded 75% of the pretreatment values. Sheep 1262, whose fleece could be removed only by conventional mechanical shearing, had a maximal residual wool growth value of 37.7% of the pretreatment value on day 22 after treatment (Fig. 3). Sheep 1269, where significant amounts but not all of the wool could be removed manually, had a residual wool growth value of 42% of the pretreatment value on day 8 after treatment. These values were similar to those recorded in three of the sheep treated either with 1.3 mg flumethasone/kg^{0.75} alone or with 0.66 mg flumethasone plus 0.7 g KSCN/kg^{0.75} (Fig. 3).

Wool Shedding

Animals dosed subcutaneously with 1.3 mg flumethasone/kg^{0.75} or with 0.66 mg flumethasone plus 0.7 g KSCN/kg^{0.75} did not shed all their wool fibres. Approximately 1 month after treatment with flumethasone alone two sheep (1508 and 1570) showed a larger proportion of shed wool fibres than continuous ones, while sheep

1360, 1365 and 1887 showed the reverse situation. Sheep 1388 and 1600 treated with flumethasone and SCN^- had fleeces that resembled those in sheep 1508 and 1570. In all these animals the fleeces could best be harvested by the conventional means of mechanical shearing.

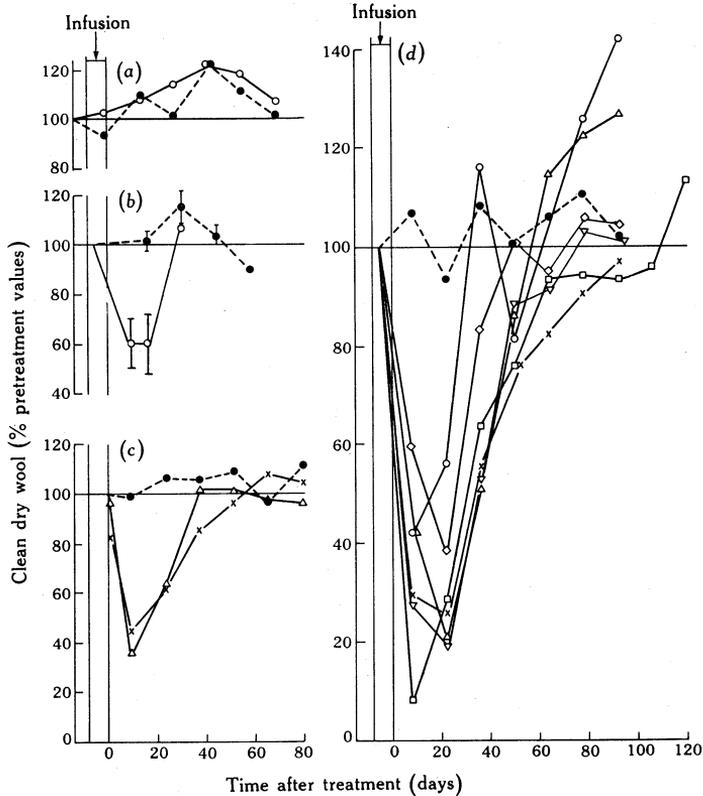


Fig. 3. Clean dry wool as a percentage of pretreatment values harvested from patches of 100 cm² on the mid-sides of experimental animals. Mean values for uninfused controls (●) are shown on all graphs. (a) Sheep subcutaneously infused with KSCN (0.7 g/kg^{0.75}) during the period shown. (b) Mean (± s.e.m.) values for five sheep subcutaneously infused with 1.3 mg flumethasone/kg^{0.75} during the period shown. (c) Sheep 1388 (×) and 1600 (△) were subcutaneously infused with 0.66 mg flumethasone plus 0.7 g KSCN/kg^{0.75} during the period indicated. (d) Individual wool growth values for sheep subcutaneously infused with 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75} during the period shown. The following symbols have been used: sheep 1202 (△), 1262 (◇), 1269 (○), 1542 (□), 1582 (×) and 1615 (▽).

A significantly different ($P = 0.045$) result to that above was obtained in most animals dosed with 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75}. In four out of six sheep inspection of the fleeces about 1 month after treatment revealed that the vast majority of wool fibres had been shed and new fibres were emerging at the skin's surface. The fleece as a whole was being retained on the animal by a small

number of continuous fibres. This is illustrated in Fig. 4. In these cases the continuous fibres were so few that it was possible to readily harvest the whole fleece manually by using the hand to disrupt the continuous fibres (Fig. 5). Fleeces weighing 2.6–4.0 kg were thus removed from sheep 1202, 1542, 1582 and 1615 30–35 days after dosing. The defleeced animals resembled conventionally shorn sheep. In the

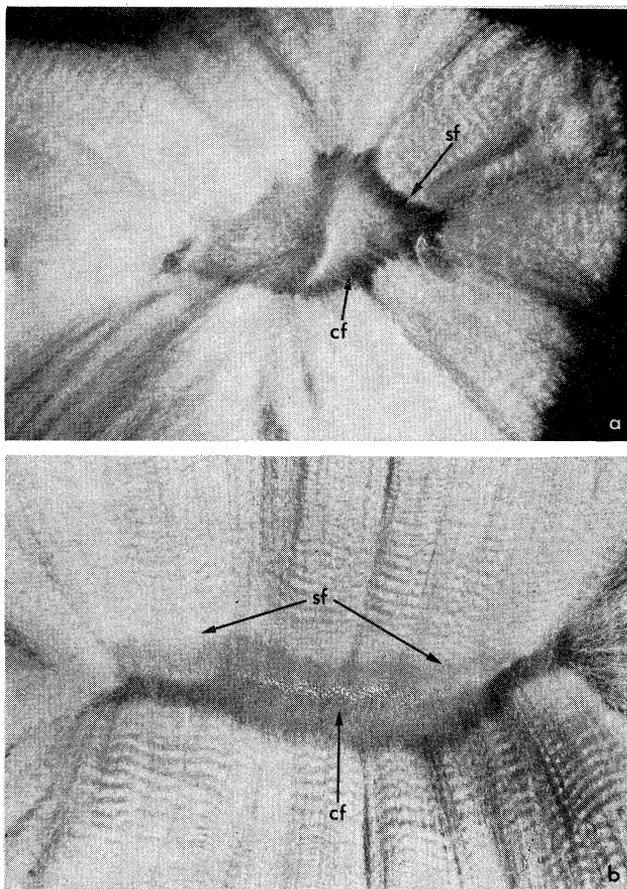


Fig. 4. The opened fleece of (a) sheep 1202 and (b) sheep 1262 30 days after they had been subcutaneously infused with 1.3 mg flumethasone plus 0.7 g KSCN/kg^{0.75}. In (a) almost all wool fibres have been shed (*sf*) and new fibres are emerging at the skin's surface. The small number of continuous fibres (*cf*) retained the fleece on the animal but could easily be disrupted in order to harvest the fleece manually (see Fig. 5). In (b), on the other hand, although shed fibres (*sf*) were obvious, continuous fibres (*cf*) were too numerous to disrupt by hand in order to harvest the fleece.

case of sheep 1269 wool was removed from the trunk region but it was not possible to manually harvest the wool from the cervical and brisket regions because the continuous wool fibres were too numerous or too strong there to be easily disrupted. The majority of wool fibres were also shed all over the body in sheep 1262 but the continuous fibres did not allow manual harvesting of any wool.

Discussion

There was no direct relationship between plasma flumethasone concentrations and wool growth depression in the animals treated with flumethasone alone. Panaretto and Wallace (1978*b*) suggested that 8 ng flumethasone/ml was required to obtain casting of the fleece. This was not the case for sheep 1360. Another example can be found in the sheep treated with flumethasone and SCN^- .

The mean plasma SCN^- concentration in sheep here (7.2 mg/100 ml) lay close to what was once considered to be the therapeutic range of the drug (8–12 mg/100 ml) in human beings when it was used in the treatment of hypertension (Nickerson 1965).

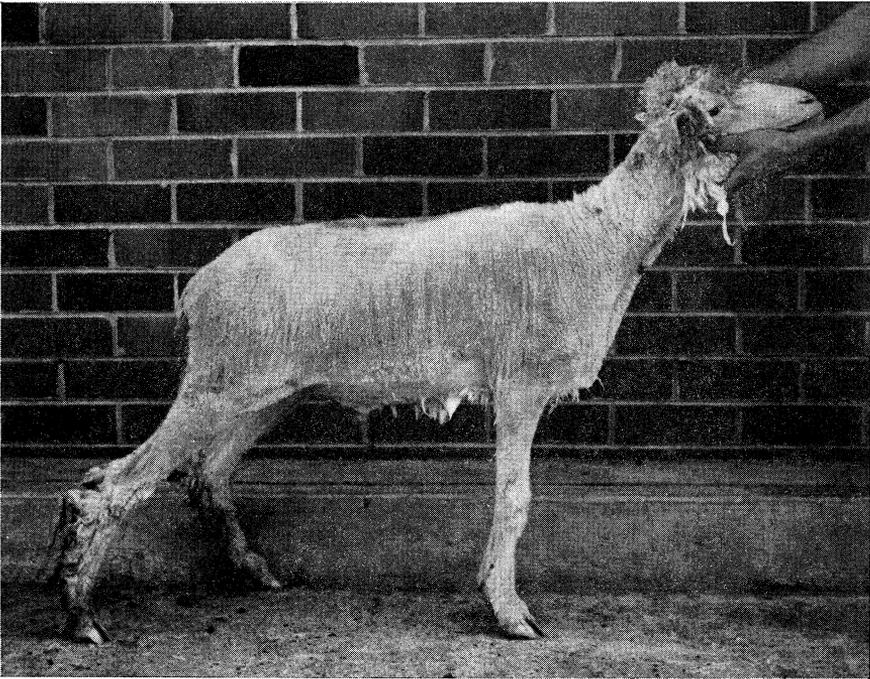


Fig. 5. Sheep 1202 after its fleece had been removed by breaking the continuous fibres illustrated in Fig. 4.

Synergism with respect to inhibiting fibre growth is discussed below. SCN^- and flumethasone, contrary to treatment with flumethasone alone, caused almost all animals to leave feed residues. A similar result was seen following the intravenous infusion of flumethasone in sheep (Panaretto and Wallace 1978*b*). It was suggested then, and now, that these feed refusals were probably due to metabolic disturbances due to treatment. The fasting observed in our experimental animals would have tended to decrease wool growth and to exacerbate the inhibitory effects on fibre growth by steroids (Chapman and Bassett 1970; Thwaites 1972). There was, however, no direct relationship between steroid treatment, fasting and fibre shedding here or previously (Panaretto and Wallace 1978*b*). In the present experiments sheep 1582, for example, ate all its food throughout the experiment and shed enough fibres to enable us to harvest the fleece manually. Sheep 1262, on the other hand, showed

the lowest plasma flumethasone concentrations (Fig. 1), refused virtually all feed on five consecutive days after infusion (Fig. 2) but its fleece had to be removed by conventional machine shearing.

Although we have not investigated the matter further, we suspect that the feed refusals observed were due to metabolic disorders caused by flumethasone. Steroid-induced enlargements and structural changes in the hepatocytes have been reported in rats (Wiener *et al.* 1968) and dogs (Thompson *et al.* 1971). We have observed significant hepatic enlargement in steroid-treated sheep (Panaretto, unpublished data) but have not made any morphological or functional studies in these situations. We speculatively suggest that the transitory feed refusal observed in our steroid-treated sheep may prove to be related to functional changes in the liver.

Residual wool growth in the six sheep given the higher flumethasone dose and SCN⁻, $31.4 \pm 5.6\%$ relative to pretreatment values, and especially in the four animals that were defleeced manually, $23.7 \pm 2.0\%$, compared well with residual wool growth of $21 \pm 5\%$ of pretreatment values 20 days after intravenous infusion of flumethasone (Panaretto and Wallace 1978*b*). Residual wool growth in the five animals treated with flumethasone alone was $62.5 \pm 9.9\%$ relative to pretreatment values.

An important feature of the experiments was the relationship of wool growth inhibition to the extent of shedding of wool fibres and hence the ability of experimenters to harvest the fleeces. Shortcomings of the predictive ability of this parameter for wool harvesting are discussed below but as an initial step in predicting the likelihood of success in the manual removal of wool the measurement has some merit. Too great a proportion of continuous fibres of normal strength to shed ones will militate against success in manual wool harvesting, i.e. without recourse to conventional mechanical shearing. The complete shedding of all wool fibres under some field conditions can also be undesirable (see below).

Results obtained in sheep 1202, 1582, 1542 and 1615 when compared to sheep 1262, 1269, 1388 and 1600 indicated that a degree of wool growth depression, less than 30% of pretreatment values, was required to produce a great enough proportion of shed fibres while at the same time leaving enough continuous ones to retain the shed fleece on the animal until the wool was harvested by disrupting the continuous fibres. It is probable that wool growth depression should lie in the range greater than 15 and less than 30% in order to obtain manual wool harvesting in *most* animals treated. We have depressed wool growth in several animals to a residual value of about 10–20% of pretreatment values but have not been able to remove the fleeces manually even though the vast majority of wool fibres were clearly shed (Panaretto, unpublished data). Thus we do not yet understand the problem clearly, but the important measurements that need to be made are the proportions of shed to unshed fibres and their strength. This is not clearly represented by residual wool growth values in our data.

If cells within the wool follicle constitute targets for glucocorticoid hormones then where might these targets lie? In that fibroblasts are putative targets for steroids, it may be suggested that the fibroblasts in the collagenous hyaline membrane surrounding the epithelial cells of the wool follicle (Parakkal 1969) constitute a receptor for the hormone analogues. Perhaps like other classical glucocorticoid targets, lymphocytes, these too are lysed (Kaiser and Edelman 1977). During natural fibre shedding in mammals extensive changes have been observed in the hyaline membranes of hair

follicles (Parakkal 1970). Furthermore, thinning of the dermis in rat skin during catagen has been reported from several sources (see Griesemer 1956). Shinkai *et al.* (1974) and Reynolds *et al.* (1977) have shown the dissociation of collagenase inhibitor-complex by SCN^- *in vitro*. Reddick *et al.* (1974) have demonstrated collagenase in dermal fibroblasts and in what the authors suggested to be an extracellular location in the dermis of human skin; the extracellular location of collagenase has also been reported by Gross (1976). Thiocyanate is thought to be largely extracellular in its distribution and has been used for the measurement of extracellular fluid volume (Elkinton and Danowski 1955). Prolonged glucocorticoid treatment in rats resulted in the formation of underhydroxylated collagen, due to decreased prolyl hydroxylase activity, and a specific depression on collagen biosynthesis. These effects were suggested to contribute to the abnormal physical properties of skin after steroid treatment (Cutroneo and Counts 1975). Thus it was possible that there was decreased collagen biosynthesis and increased collagenase activity in the hyaline membrane and dermal tissue of our sheep.

Wool shedding in steroid-treated sheep has, 30 days after treatment, some resemblances to seasonal shedding of wool fibres in that the fleece at that time usually showed, to some degree, the situation depicted in Fig. 4. Although it may be tempting to suggest that steroid-treated sheep mimicked shedding in such breeds as the Soay, a breed that exhibits seasonal shedding of fibres (Ryder 1971*a*), it remains to be seen whether the similarity will be sustained. 'Brush-end' fibres were seen both in shedding sheep (e.g. Ryder 1971*a*) and in glucocorticoid-induced shedding in Merino crossbreds (Chapman and Bassett 1970) and Merinos (Chapman and Panaretto, unpublished data).

Another locus of change during natural fibre shedding in rats was in the cells of outer root sheath about half-way down the anagen follicle (Parakkal 1970). It was here that outer root sheath cells were described as transforming to germ cells and the appearance of autophagic vacuoles, which played their part in the resorption of epithelial cells between the mid-follicle and the dermal papilla, was seen. Although this region of the wool follicle may be involved in steroid-induced shedding of wool fibres, such involvement is yet to be shown.

There are, however, differences between our sheep here and those showing natural shedding in that follicles of the cervical and brisket regions were least susceptible to treatment whereas in natural shedding this does not appear to be the case. In Soay sheep Ryder (1971*a*) has recorded that the 'brush-end' fibres are not shed *until* new fibres have grown and that the shedding of wool fibres tended to follow the sequence of foetal development across the body, i.e. the fibres of the head and neck are first shed. Ryder (1974) has emphasized, as we have above for steroid-treated sheep, that in natural shedding the animal is *never naked* as a result of the moult. The importance of continuous fibres in preventing the casting of shed fibres in sheep has been emphasized by Ryder (1971*b*). The possibility that these continuous fibres are primaries needs to be considered since Lyne (1964) showed a very much greater shedding among secondary than primary follicles in undernourished Merino sheep. Whatever the causal mechanisms may prove to be, the steroid-treated sheep resembled, to some degree, 30 days after treatment, the events recorded in naturally shedding sheep.

On the other hand, in those cases where sufficiently large doses of mimosine have been given to sheep to cause the shedding of the whole fleece 10–15 days after treatment (Reis *et al.* 1975), shedding has occurred *before* regrowth has reached the surface

of the skin. Sheep that were nude on the wool growing regions resulted (for example see fig. 4 in Reis *et al.* 1975) and would probably have required protection from the environment. These points were exemplified in the case of sheep treated with cyclophosphamide, which at appropriate doses also caused shedding of the whole fleece. Roberts and McMahon (1972) emphasized the loss of wool in the paddocks where treated ewes were held, and 73 out of 75 sheep had no regrowth 3 weeks after treatment. The 19 deaths recorded in the 48 h after wool harvesting were tentatively ascribed by Roberts and McMahon (1972) to exposure of the (nude) animals to cold weather.

To what mechanism can the synergism between SCN^- and flumethasone reported here be attributed? The effects of SCN^- on thyroxine concentrations can probably be dismissed. The effects of SCN^- and flumethasone on collagenases and collagen, discussed above, may have to be investigated further. The activation of collagenases to attack structural collagen might be important in the thinning of the skin seen in steroid treatments and in the attacking of the structure of the hyaline membrane of the follicles, but it would be unlikely to have important direct effects on epithelial structures within follicles.

Finally the reputed chaotropic effects of SCN^- ions might have helped in causing wool fibre shedding; these include *inter alia* disaggregation of cell membranes by lipid auto-oxidation, oxidative changes to collagen, and increasing water solubility of small organic molecules (in this case flumethasone) (Hatefi and Hanstein 1970). These effects of the ion need further research. In addition the consequences of the possible conversion of SCN^- to CN^- also may need to be considered.

Acknowledgments

We are indebted to Miss R. Jackson for analytical work and to Mr S. G. Humphreys for his help with many aspects of the experiments.

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